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# Physio-morphological traits and drought stress responses in three wild Mediterranean taxa of Brassicaceae

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| **Abstract:** | Crop wild relatives (CWRs) have extremely relevant roles in biodiversity conservation, in investigating phylogeny and improving abiotic stress tolerance of crop plants. We screened the variability in leaf functional traits of three CWRs of kale crops (Brassica oleracea) from Sicily, Italy, grown in pots under well-watered and drought conditions. Our aim was to highlight traits in the different genotypes of endemic Sicilian threatened taxa.<br>We measured several structural/anatomical traits (stomatal size, density and stomatal pore index – SPI, leaf mass per area – LMA) and leaf functional traits (stomatal conductance - gs, leaf water potential – ΨL, leaf temperature (TL), leaf relative water content - RWC) at pre-dawn and midday of leaves of three wild taxa: B. macrocarpa, B. rupestris subsp. rupestris and B. villosa subsp. bivoniana. Pressure-volume curves were constructed to obtain leaf water potential at turgor loss point (Ψtlp ), osmotic potential at full rehydration (Ψπ100), relative water content at turgor loss point (RWCtlp), elastic bulk modulus (εmax) and leaf area specific capacitance at full turgor (Cft*).<br>Several significant differences were found among the taxa: under water deficit, B. macrocarpa had the less negative Ψtlp and showed the smallest ΔΨL between prem dawn and midday. B. villosa subsp. bivoniana showed the highest SPI and had significantly higher gs under water availability, while under drought it had the most negative ΨL. Each of the taxa investigated possessed traits that confer particular stress tolerance, offer competitive advantage in their natural environment and may be exploited for crop improvement. |

**TITLE:**

# Physio-morphological traits and drought stress responses in three wild Mediterranean taxa of Brassicaceae

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**Keywords:** Crop wild relatives, Sicily, leaf water potential, stomatal conductance, LMA, SPI

## Author contributions

AG and EO conceived and designed the experiments. SI, AG and EO performed the experiments and analyzed the data. AG and EO wrote the manuscript.

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## Abstract

Crop wild relatives (CWRs) have extremely relevant roles in biodiversity conservation, in investigating phylogeny and improving abiotic stress tolerance of crop plants. We screened the variability in leaf functional traits of three CWRs of kale crops (*Brassica oleracea*) from Sicily, Italy, grown in pots under well-watered and drought conditions. Our aim was to highlight traits in the different genotypes of endemic Sicilian threatened taxa.

We measured several structural/anatomical traits (stomatal size, density and stomatal pore index – SPI, leaf mass per area – LMA) and leaf functional traits (stomatal conductance – $g_s$, leaf water potential – $\Psi_L$, leaf temperature ($T_L$), leaf relative water content – RWC) at pre-dawn and midday of leaves of three wild taxa: *B. macrocarpa*, *B. rupestris* subsp. *rupestris* and *B. villosa* subsp. *bivoniana*. Pressure-volume curves were constructed to obtain leaf water potential at turgor loss point ($\Psi_{tlp}$), osmotic potential at full rehydration ($\Psi_{\pi 100}$), relative water content at turgor loss point ($RWC_{tlp}$), elastic bulk modulus ($\varepsilon_{max}$) and leaf area specific capacitance at full turgor ($C_{ft}$*).

Several significant differences were found among the taxa: under water deficit, *B. macrocarpa* had the less negative $\Psi_{tlp}$ and showed the smallest $\Delta\Psi_L$ between pre-dawn and midday. *B. villosa* subsp. *bivoniana* showed the highest SPI and had significantly higher $g_s$ under water availability, while under drought it had the most negative $\Psi_L$. Each of the taxa investigated possessed traits that confer particular stress tolerance, offer competitive advantage in their natural environment and may be exploited for crop improvement.

## Introduction

The genus *Brassica* L. (Brassicaceae) comprises a large number of extremely variable taxa, both from the morphological and genetic point of view, many of which are crops widely used for human nutrition as health-promoting vegetables, condiments and edible oils.

Selection of traits included in the so-called domestication syndrome (Hammer 1984) have often led to a decrease in the fitness of a cultivated species in natural environments or under changing climatic conditions (Gepts 2004). Furthermore, following the 'Green Revolution' traditional cultivars and local varieties have been substituted with uniform, widespread cultivars, reducing the genetic diversity of modern crops and making them more susceptible to abiotic stresses, particularly drought, which affect plant growth and development causing large yield losses (Fita et al. 2015).

Crop wild relatives (CWRs) have been playing increasingly relevant roles for the study of plant genomes and the genetic improvement of the cultivated relatives. This aspect is present in the Convention on Biological Diversity (CBD) and is highlighted in the Global Strategy for Plant Conservation 2011-2020. Objective II, target 9, states that "70 per cent of the genetic diversity of crops including their wild relatives and other socio-economically valuable plant species conserved [...]" ( Research on CWRs has contributed to the investigation on both the evolutionary and phylogenetic relationships, and the inheritance of genes of several crop plants (Kole 2011). Several donor genes from CWRs have already been used to improve domesticated relatives, increasing the yields and nutritional quality of crops, providing cultivars with resistance against pests and diseases, and improving tolerance to abiotic stresses (Hopkins and Maxted 2010; Kole 2011).

*Brassica* species are moderately salt-sensitive (François 1994; Hayat et al. 2007) and this genus shows a certain variability with regard to drought and salt tolerance (Ashraf and Sharif 1997; Beltrao et al. 2000; Maggio et al. 2005). Wild species, particularly those living in harsh environments such as cliffs and coastal areas, are well adapted to tolerate abiotic stress and the genetic diversity of wild relatives may be an useful source of genes to improve drought and salt tolerance in cultivated *Brassica* (Siddiqui et al. 2008; Zhang et al. 2014). Sicily is one of the main centers of diversification of wild species belonging to the same cytodeme of *Brassica oleracea* L. (*Brassica* sect. *Brassica*, $2n = 18$), that comprises several crop species, such as cabbage, cauliflower, broccoli, Brussels sprouts and kale. The Sicilian wild taxa can hybridize with cultivated forms so they represent a useful genetic resource for the improvement of cultivated varieties (Snogerup 1990; Von Bothmer et al. 1995; Geraci et al. 2001; 2004; Branca and Cartea 2011). Indeed, Sicilian *Brassica* wild species have been used for their resistance/tolerance to biotic and abiotic stress and to obtain new cultivars with high levels of glucosinolates (Faulkner et al. 1998; Mithen et al. 2003; Branca et al. 2012).

Identifying the CWRs' functional traits that confer tolerance to abiotic stress is an important step for the selection of genotypes that may be used for crop improvement. Plant functional traits (e.g. leaf stomatal conductance, leaf mass per area, leaf water potential) are related to plants' ecological strategies and determine how plants respond to environmental factors such as drought or salinity (Kattge et al. 2011; Pérez-Harguindeguy et al. 2013; Heschel et al. 2017). Assessment of fundamental plant functional traits is being increasingly used as a potential tool to detect and predict responses to drought, for example in the case of forest mortality (O'Brien et al. 2017). Leaf water relation traits are particularly important since water is the main factor limiting plant growth and crop productivity (Schulze et al. 1987; Zaghdoud et al. 2013). Among these, turgor loss point, i.e. the leaf water potential when turgor reaches zero, is a key functional trait that quantifies plant drought tolerance and is associated with stomatal closure, wilting and loss of hydraulic conductance (Bartlett et al. 2014), which in turn influence plant productivity.

Functional traits have been genetically correlated at the intraspecific level in *Brassica rapa* L. providing combinations of traits useful for crop improvement (Edwards et al. 2011).

The ecophysiology and functional traits of most of the wild Sicilian taxa relatives of cultivated *Brassica* have not yet been characterized, so the aim of this study was to screen the variability in leaf functional traits of three taxa, *B. macrocarpa* Guss., *B. rupestris* Rafin. subsp. *rupestris* and *B. villosa* Biv. subsp. *bivoniana* (Mazzola et Raimondo) Raimondo et Mazzola*,* grown under the same environmental conditions, comparing well-watered and drought treatments.

## Material and Methods

### Plant material

Three taxa with differences in leaf morphology, *B. macrocarpa*, *B. rupestris* subsp. *rupestris* and *B. villosa* subsp. *bivoniana*, were selected (Table A1). The habitats of all taxa occurring in Sicily and in the small surrounding islands consist of limestone cliffs, mostly facing north, from sea level to 1000–1200 m a.s.l.

*B. macrocarpa* is an endemic taxon restricted to two small islands, Favignana and Marettimo (Egadi archipelago), off the west coast of Sicily (Snogerup et al. 1990, Raimondo et al. 1991). The species is characterized by wide siliqua, with thick valves, stout and conical beak, yellow flowers, glabrous and petiolate leaves (Fig. 1*a*). It grows on limestone cliffs and rocky slopes from 0 to 300 m a.s.l., very close to the sea where it can be reached by seawater spray. This species is assessed as Critically Endangered because it has a highly fragmented habitat limitated to two isolated subpopulations. The quality and the extension of the habitat is declining due to fires, grazing, reforestation and other anthropic activities (Geraci and Mazzola 2012; Branca and Tribulato 2013).

*B. rupestris* s.l. is endemic to Sicily and Calabria (Mazzola and Raimondo 1997). The plants have leaves with long petiole, hairs with bulbose base, light yellow flowers, long and thin siliqua (Fig. 1*b*). *B. rupestris* subsp. *rupestris* is the more widespread subspecies in north-west Sicily, except for one population found near Roccella Valdemone (eastern Sicily). Their populations are often threatened by human activities, grazing and fires, therefore the taxon is included in the national and regional Red Lists as Low Risk (LR) and Near Threatened (NT) according to the IUCN categories (Conti et al. 1997; Scoppola and Spampinato 2005; Branca and Donnini 2013).

*B. villosa* comprises a group of intraspecific taxa endemic of central and west Sicily (Mazzola and Raimondo 1997; Raimondo and Geraci 2003) characterized by hairy leaves without wings at the base of the petiole, light yellow flowers (Fig. 1*c*). *B. villosa* subsp. *bivoniana* consists of several populations located in western Sicily. This taxon is also included in the national and regional Red Lists as LR or as NT in the IUCN list (Conti et al. 1997; Scoppola and Spampinato 2005; Branca and Donnini 2013).

**Growth conditions**

Seeds were collected from 3-5 wild specimens per taxon in June 2014. In October of the same year, seeds were sown in 5 L pots containing garden soil and grown in the open at the University of Palermo, Italy (Fig. 1d). Plants were watered to field capacity three times a week up to approximately 220 d from sowing, after which they were randomly divided in two treatment groups of 9 plants per taxon. One group of plants continued to be irrigated regularly for measurements under watered conditions (W). To impose drought conditions (D), in the second group of plants irrigation was suspended for 5 days, after which measurements were taken on fully expanded, healthy leaves. Soil water potential was estimated from the pre-dawn leaf water potential measured as described below. All measurements were taken between the end of May and June 2015, on clear sunny days, approximately 230 days after sowing. Measurements were replicated in three days, on three plants per day. Average air temperature and relative humidity on the days measurements were taken were 28.5± 0.4 °C and 68.3± 8.4 %, respectively.

**Leaf surface anatomy**

Stomatal size and density were measured from scanning electron microscope (SEM) micrographs, on ten replicates per taxon, collecting fully expanded, healthy leaves. Air-dried small portions of the leaves were placed on double-sided transparent tape on the surface of a polished aluminum stub. The samples were sputter coated with a 225 Å thick gold layer, and examined with a LEO 420 SEM Microscope (LEO Electron Microscopy Ltd.) using an acceleration voltage of 10-15 KV, at magnifications between 400 – 5500 ×.

Stomatal pore index (SPI) was calculated as the product of the stomatal density by the square of the mean stomatal pore length (Sack et al. 2003).

**Functional traits measurements**

Approximately 220 d from sowing, before imposing drought stress treatment, five fully expanded leaves per taxon were collected from three different plants grown under well watered conditions and used to construct pressure-volume (PV) curves by the bench dehydration method (Tyree and Hammel 1972; Savi et al. 2017). From the analysis of the PV curves we obtained the main water relation traits related to drought tolerance: leaf water potential at turgor loss point ($\Psi_{tlp}$), osmotic potential at full rehydration ($\Psi_{\pi100}$), relative water content at turgor loss point ($RWC_{tlp}$), bulk modulus of elasticity ($\varepsilon_{max}$) and leaf area specific capacitance at full turgor ($C_{ft}$*).

Daily changes in leaf water potential ($\Psi_L$) were measured with a pressure chamber (PMS 1505D, PMS Instrument Company, Albany, OR, USA) on a total of four fully expanded leaves collected from two plants per taxon per measurement time. The evening before measurements, plants were prepared for "pre-dawn" leaf water potential (PD$\Psi_L$) measurements, when values are assumed to be in equilibrium with soil water potential (Sellin 1999; Kangur et al. 2017). Selected leaves were wrapped in plastic film and aluminum foil and bagged plants were kept in the dark until measurement at 07:30 h, in order to avoid predawn disequilibrium due to night time transpiration (Oddo et al. 2011). Preliminary measurements showed that $\Psi_L$did not change significantly in the time interval between "pre-dawn" and 08:00 h. After cutting off the leaves for estimation of PD $\Psi_L$, plants were placed in the open and allowed to reach steady state (30 minutes) before measuring, at 08:00 h, abaxial stomatal conductance to water vapor ($g_s$) and leaf temperature ($T_L$) with a porometer (SC-1 Leaf Porometer, Decagon, Pullman, WA) on 5 fully expanded leaves per plant. At 12:00 h $g_s$, $T_L$ and $\Psi_L$measurements were repeated. Midday (MD) $\Psi_L$ values of W and D plants were used to calculate $\Delta\Psi_{MD}$ and describe drought strategy according to Farrell et al. (2017). Immediately after measuring $\Psi_L$, two leaf discs per leaf were sampled with a cork borer (0.7 $cm^2$) and used to calculate relative water content (RWC). The leaf disc fresh weight (FW) was recorded with an analytical balance (AL 104, Mettler Toledo, Greifensee, Switzerland); leaf discs were floated on distilled water in plastic tubes for 20 h, after which turgid weight was recorded (TW). Dry weight (DW) was recorded after drying the samples at 70 °C for 48 h. RWC was then calculated as

$$RWC\ (\%) = 100 \times (FW - DW)/(TW - DW)$$

Leaf mass per unit area (LMA) was calculated for W plants at three different intervals during the experiment: 190, 210 and 230 d from sowing. On the first two sampling dates, leaf discs were cut out from fully expanded leaves with a cork borer, oven dried at 70 °C for 48 h and DW was determined by dividing leaf DW over leaf disc area. For the 230 d data, LMA was calculated from the same leaf discs used for RWC determination.

## Data analysis

Data are presented as mean values ± standard deviation. After testing for equal variance and for normality (Shapiro-Wilk), one-way ANOVA was applied to test for significant differences among the means for stomatal traits and for PV parameters. Two-way ANOVA was applied to test the effects of factors species (S) and days from sowing (D) on LMA data. Differences in functional trait means (gs, $\Psi_{L}$,RWC, $T_{leaf}$) were tested using a three-way ANOVA, with species (S), irrigation regime (I), time of day (T) and their interactions as fixed effects. , Tukey's post hoc test ($\alpha = 0.05$) was used to compare means when ANOVA was significant. All analyses were carried out using the software package SigmaPlot 13 (Systat Software, Inc., San Jose, USA).

## Results

### Stomatal characteristics and SPI

All species showed stomata both on adaxial and abaxial sides, so the leaves may be considered amphistomatous. The values of stomatal density, length and SPI differed among the taxa (Table 1). *B. macrocarpa* and *B. rupestris* showed about the same stomatal density on both adaxial and abaxial sides, similar to that of the adaxial side of the leaves of *B. villosa* subsp. *bivoniana*. On the abaxial side of the leaf of *B. villosa* subsp. *bivoniana*, instead, stomatal density was about twice that of the adaxial surface, significantly higher than in the other two taxa. Stomatal pore length was never significantly different between adaxial and abaxial sides, while differences between taxa were significant only for *B. villosa* subsp. *bivoniana*; the largest stomata were found in *B. rupestris,* followed by *B. macrocarpa*, while the smallest stomata were found in *B. villosa* subsp. *bivoniana* (Fig. 2).

Differences in SPI among the taxa were always significant. SPI was lowest in *B. macrocarpa*, while *B. rupestris* showed higher values than *B. macrocarpa* due to slightly longer stomata. *B. villosa* subsp. *bivoniana* had the highest total SPI value of the three taxa, due to the higher stomatal density of the abaxial side that compensated for the smaller size of stomata.

### Functional traits

Data obtained from the analysis of PV curves (Table 2) showed that *B. rupestris* and *B. villosa* subsp. *bivoniana* had significantly more negative $\Psi_{tlp}$ values than *B. macrocarpa*, with correspondingly more negative $\Psi_{\pi100}$ values. Average

$C_{ft}$* was larger for *B. macrocarpa* than for the other two taxa, though this difference was not significant. The differences found in $RWC_{tlp}$ and $\varepsilon_{max}$, were also non-significant among species.

Irrigation regime, time of day and species all strongly affected leaf gas exchange (Fig. 3, Table 3). Under well-watered conditions, at 08:00 h $g_s$ was similar in *B. macrocarpa* and *B. rupestris*, while it was significantly higher in *B. villosa* subsp. *bivoniana*. At 12:00 h, the latter taxon reached the highest levels of $g_s$ (about 1000 mmol $m^{-2}$ $s^{-1}$) that were about twice those reached at the same time by the other two taxa. Five days after suspending irrigation, $g_s$ dropped on average to 45 mmol $m^{-2}s^{-1}$in all three taxa, both in the early morning and at midday.

The interaction between time of day, irrigation regime and species on $\Psi_L$ was significant (Table 3).Under well-watered conditions, $\Psi_L$ at pre-dawn did not differ among taxa (Fig. 3); MD $\Psi_L$ decreased significantly in all taxa, reaching about -1.5 MPa in *B. macrocarpa* and *B. villosa* subsp. *bivoniana*. In *B. macrocarpa* this value was definitely below turgor loss point, while in *B. villosa* subsp. *bivoniana* $\Psi_{tlp}$ was barely reached. The drop in MD $\Psi_L$ was greater for *B. rupestris*, and also in this case the turgor loss point threshold was passed. Under drought stress, none of the taxa managed to recover from water stress during the night, and all values measured were far below turgor loss point. *B. macrocarpa* showed the less negative values, at both pre-dawn and midday, and the smallest difference between pre-dawn and midday values (12% reduction). *B. rupestris* showed the most negative PD $\Psi_L$ value, which decreased a further 30% at midday. *B. villosa* subsp. *bivoniana* reached extremely negative $\Psi_L$ values at midday, -6.2 MPa. The $\Delta\Psi_{MD}$ values calculated as the difference between MD $\Psi_L$ under drought and under well-watered conditions were -1.8, -3.5 and -4.5 MPa for *B. macrocarpa*, *B. rupestris* and *B. villosa* subsp. *bivoniana* respectively.

Under well-watered conditions, leaf relative water content was on average 90% at pre-dawn for all taxa. This value was maintained at midday for *B. macrocarpa* and *B. rupestris*, while it decreased slightly but not significantly in *B. villosa* subsp. *bivoniana* (Fig. 3). Drought stress resulted in a significant decrease in RWC, down to 60% in *B. macrocarpa*, 50% in *B. rupestris* and 30% in *B. villosa* subsp. *bivoniana*. No significant differences were found between pre-dawn and midday samples, and no significant interaction between time of day and irrigation regime or species (Table 3).

Watered plants at 08:00 h showed the lowest leaf temperatures, 27.8 °C on average with no significant differences at species level (Fig. 3). Watered plants of *B. villosa* subsp. *bivoniana* maintained significantly cooler leaves at midday (29.1°C). Under drought stress, leaf temperatures increased significantly at 08:00 h in all taxa. Leaves of *B. macrocarpa* remained significantly cooler than those of the other two taxa (29.9 °C). At midday leaf temperatures increased in all plants, showing significant differences among all taxa, with *B. macrocarpa* reaching the lowest temperature (31.7 °C) compared to *B. villosa* subsp. *bivoniana* and *B. rupestris,* which reached the highest leaf temperature (34 °C). A significant interaction was found between irrigation regime and species (Table 3).

Leaf mass per area increased during the experiment (Fig. 4). Over a six-week period, all three taxa reached a similar LMA value, 90-95 g $m^{-2}$, but with a different pattern. *B. macrocarpa* and *B. rupestris* showed an initial rapid increase in LMA, from 190 to 210 d from sowing, after which there was no significant increase. *B. villosa* subsp. *bivoniana* showed constant LMA during the same period, and a rapid increase in this trait between 210 and 230 d from sowing, when drought stress was imposed and measurements were taken. There was a significant interaction between days from sowing and species (Table 3)

Visual assessment of plant conditions after the 5-day drought period showed in plants of *B. macrocarpa* and *B. rupestris* moderate wilting of the leaves, and yellowing of 2-3 leaves per plant. In *B. villosa* subsp. *bivoniana*, leaves were very strongly wilted, but did not show yellowing. On rewatering, leaves of *B. villosa* subsp. *bivoniana* recovered turgor rapidly, within 3-4 hours, while the other two species recovered turgor more gradually, within 12-18 hours.

## Discussion

The wild *Brassica* relatives occurring in Sicily generally grow on cliffs with little soil, a very arid habitat. For this reason, they are usually found on north-facing limestone cliffs, where exposure to direct sunlight is less and the evapotranspirational demand is lower. All taxa also rely on their root architecture and growth, with roots penetrating and extending deeply into rock cracks and crevices , and indeed rooting depth plays an important role in controlling the progression of drought responses (Nardini et al. 2016). Here we present data on the morpho-physiological traits of the three taxa investigated in this study, under experimental conditions in which cultivation in pots limited the possibility to form an extensive root system. To the best of our knowledge these are the first reports for *B. villosa* subsp. *bivoniana* and *B. macrocarpa*, providing information that could be of great interest to aid in the selection of genotypes that may be used for crop improvement.

One of the adaptations crucial to drought resistance is the reduction of water loss through stomata. The ability to control stomatal conductance depends on stomatal density, stomatal size and the regulation of stomatal aperture (Pérez-López et al. 2012; Zaghdoud et al. 2013), and stomatal traits can show substantial variability at the interspecific level, as reported for example for poplar (Pearce et al. 2006; Cirelli et al. 2016). The inverse relation commonly found between stomatal density and guard cell length (Wood 1934; Sack et al. 2003) was evident only for *B. villosa* subsp. *bivoniana*. The positive relation between SPI and guard cell length reported by several authors (e.g. Sack et al. 2003; Abrams and Kubiske 1990) was maintained for *B. macrocarpa* and *B. rupestris*. However, this relation was no longer valid for *B.*

*villosa* subsp. *bivoniana*, where the significant increase in SPI was driven by stomatal density, which on the abaxial surface was twice that of the other taxa. The high SPI in *B. villosa* subsp. *bivoniana* was likely the factor driving its high $g_s$ under well-watered conditions, as SPI has been shown to correlate tightly both with maximum stomatal conductance and leaf hydraulics in several temperate woody species (Sack et al. 2003, 2005). *B. villosa* subsp. *bivoniana* showed also the most rapid recovery of leaf turgor after rewatering, which could be related to a more efficient water transport due to a higher leaf lamina hydraulic conductance (Sack et al. 2003) and hypothetically a rapid recovery of leaf vein embolism (Trifilò et al. 2003).

Maintaining lower leaf temperature may positively affect carbon assimilation and decrease heat stress effects on cell metabolism (Yamori et al. 2014; Feller 2016). Transpirational canopy cooling has been previously suggested as a tool to screen *Brassica* genotypes for drought tolerance (Singh et al. 1985). When water was available, *B. villosa* subsp. *bivoniana* maintained the lowest leaf temperatures, due both to high $g_s$ allowing high evapotranspiration and to the presence of hairs that increased reflectance of the leaf surface (Holmes and Keiller 2002). The tomentose leaf surface could also be a main factor contributing to lowering cuticular transpiration when water supply was low. Under drought stress, instead, *B. macrocarpa* maintained the lowest leaf temperatures, possibly explained by a combination of leaf reflection due to higher presence of waxes on the epidermis that reduces also cuticular water loss (Holmes and Keiller 2002; Laila et al. 2017) and the higher water content, confirmed by higher RWC and less negative $\Psi_L$.

Several functional traits describing plant water relations have been proposed as predictors of drought tolerance, such as osmotic adjustment, osmotic potential at full turgor, MD $\Psi_L$, $\Delta\Psi_{MD}$, $\Psi_{tlp}$ and leaf RWC. Osmotic potential at full turgor can be a powerful indicator of drought tolerance, as it is the main driver of $\Psi_{tlp}$ (Bartlett et al. 2012; Mart et al. 2016) and more negative values generally indicate greater drought resistance. There is consistent evidence that solute accumulation maintains crop yield under water deficit conditions (Blum 2017) also in several genotypes of *Brassica napus* L. and *B. juncea* L. Czern. (Niknam et al. 2003; Ma and Turner 2006; Ma et al. 2006; Norouzi et al. 2008). Osmotic potential at full turgor was most negative in *B. villosa* subsp. *bivoniana*, significantly more negative than in *B. macrocarpa*. *B. villosa* subsp. *bivoniana* also showed the greatest overnight recovery in $\Psi_L$ during drought, compared to the other two taxa, with a $\Delta\Psi_L$ of 2.9 MPa, while *B. rupestris* had a $\Delta\Psi_L$ of 1.3 MPa and *B. macrocarpa* of only 0.3 MPa. During the night, plants generally tend to equilibrate leaf water status with water available in the soil, and PD $\Psi_L$ has been widely used as a proxy for soil water potential (e.g. Palmer et al. 2008; Petruzzellis et al. 2018). Though pre-dawn disequilibrium has sometimes been found under certain conditions (e.g. Donovan et al. 2001) this has most commonly occurred in field measurements, mainly due to night time transpiration and high levels of vapour pressure deficit (Kangur et al. 2017), which were avoided in our controlled experimental conditions.

During the day leaf water potential declines below soil water potential, reaching minimum values around midday that can provide an index of drought tolerance for the species (Pérez-Harguindeguy et al. 2013). The hypothesis that the steeper midday decrease in $\Psi_L$ measured under well-watered conditions in *B. rupestris* could be related to more rigid cell walls (highest average $\varepsilon_{max}$ values ), was not supported by statistical analysis. High $C_{ft}$* can contribute to reduce the fluctuations in leaf water potential under changing water supply and transpirational demand (Sack et al. 2003), however differences among the taxa were not significant, so could not justify the less negative $\Psi_L$ values shown by *B. macrocarpa* both under well-watered and drought conditions.

One of the traits allowing the maintenance of cell turgor under water deficit is a low $\Psi_{tlp}$. Since $\Psi_{tlp}$ integrates osmotic adjustment, $\varepsilon$ and other individual water relations traits, it is one of the fundamental traits to select for in breeding programs aimed at improving drought tolerance, even though the determination of $\Psi_{tlp}$ by PV curves is time-consuming (Mart et al. 2016). It must however be kept in mind that the use of $\Psi_{tlp}$ alone to predict drought resistance could be misleading if whole plant drought strategies are not considered (Farrell et al. 2017). *B. rupestris* and *B. villosa* subsp. *bivoniana* showed significantly lower $\Psi_{tlp}$ than *B. macrocarpa*. Considering $\Delta\Psi_{MD}$ as a descriptor of drought strategy in terms of the degree of isohydry and anisohydry (Farrell et al. 2017), *B. macrocarpa* showed a greater degree of isohydry than the other two genotypes.

Leaf relative water content is another trait that describes plant water status and is well correlated with physiological responses to water supply (Slatyer 1962; Munne-Bosch and Penuelas 2004). Taking the appropriate precautions in leaf sampling and measurements (Tanentzap et al. 2015), it is a helpful and often used tool for screening for drought tolerance in different genotypes (Rachmilevitch et al. 2006). Differences in RWC among the genotypes were not significant under well-watered conditions, but under drought stress *B. macrocarpa* had significantly higher RWC, even if midday $g_s$ was extremely low and similar to that of *B. rupestris*.

Leaf mass per area – or its reciprocal, specific leaf area (SLA) – is still another of the key traits related to plant function and performance, and is implicated in responses to several environmental factors, most importantly light availability and drought (Valladares and Sanchez-Gomez 2006; Poorter et al. 2009). A lower LMA is often related to greater leaf water loss, while higher LMA is associated with adaptation and resistance to water stress (Poorter et al. 2009; Bussotti et al. 2015). The values of LMA of the cabbage wild relatives we examined were in the same range as that reported in another study on two subspecies of *B. rupestris* (Crescente and Gratani 2013), and for *B. oleracea*, 80 g/m$^2$(Sage et al. 1989), while they were higher than those reported for *B. napus*, 30-50 g/m$^2$(Shengxin et al. 2016). LMA may vary with environmental conditions and with plant age, reflecting variations in plant plasticity related to genotype (Jullien et al. 2009). The change in LMA with time for our taxa was similar to that reported by Biemond et al. (1995) for leaves of

Brussels sprouts, which increased up to 90 days after planting and then remained stable for the rest of the experiment, and for leaves of turnip, and green cabbage (Li et al. 1999). The different LMA profile with time shown in *B. villosa* subsp. *bivoniana* could be related to source/sink relationships within the plant, as suggested by Jullien et al. (2009) for oilseed rape.

Though it is time consuming to obtain some of these functional traits, combining information from more than one trait results in the most complete picture of the stress tolerance of a particular genotype, which is necessary when screening CWRs for breeding purposes.

Among allotetraploid hybrids of Brassica U-Triangle species, Baker et al. (2017) found significant effects of species for morphological, anatomical and physiological traits, though there was no significant correlations between these traits in the diploid parents. This evidence shows that novel trait correlations could arise in the polyploid hybrids. The breeding of the ancestors examined in this study with cultivated varieties of the same cytodeme could give rise to hybrids with greater resistance to drought or disease, higher productivity under stress, and these CWRs are therefore of great interest for artificial selection and crop improvement.

## Conclusions

CWRs offer the opportunity of finding new sources of variation to face changing environmental conditions (Esquinas-Alcázar 2005). Each of the taxa examined possessed traits conferring particular stress tolerance. *B. rupestris* subsp. *rupestris* and *B. villosa* subsp. *bivoniana* suffered the most under the applied drought stress, however all three species were able to recover. Their particular functional traits could be therefore exploited for crop improvement.

*B. macrocarpa,* which grows on cliffs overhanging the sea exposed to high salinity due to sea spray, among the taxa examined was the one subject to the harshest conditions in its natural habitat. It's functional traits, such as thick, leathery leaves with a thick wax layer, a high leaf succulence index (data not shown) and greater degree of isohydry under drought stress – compared to the other two taxa – are all traits that favour adaptation to drought and/or salt stress. For several of the functional traits ($\Psi_{tlp}$, $\Delta\Psi_{MD}$, $\Psi_{\pi100}$), *B. macrocarpa* was significantly different from the other two genotypes. *B. rupestris* had the greatest capacity of lowering $\Psi_L$, and consequently the highest water extraction capacity when water was available. The slight opening of stomata in the early morning under drought, compared to the other two taxa, could avoid carbon starving under stress conditions. Crescente and Gratani (2013) comparing some functional traits of *B. rupestris* subsp. *rupestris* with those of *B. rupestris* subsp. *hispida* found that the former was more competitive, as it was able to maintain higher photosynthetic rate, stomatal conductance and water use efficiency under water stress. Indeed, *B. rupestris* is the most widely distributed of these taxa as for population numbers, arriving to Calabria, on the Italian mainland.

*B. villosa* subsp. *bivoniana* had very high $g_s$ under water availability, maintained the lowest leaf temperatures and barely reached turgor loss point, all traits that correlate positively with net photosynthesis and therefore result in high productivity, a highly desirable trait for crop improvement.

## References

Abrams MD, Kubiske ME (1990) Leaf structural characteristics of 31 hardwood and conifer tree species in central Wisconsin: influence of light regime and shade-tolerance rank. For Ecol Manage 31:245–253.

Ashraf M, Sharif R (1997) Does salt tolerance vary in a potential oil seed crop *Brassica carinata* at different growth stages? J Agron Crop Sci 181:103-115.

Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecol Lett 15:393-405.

Bartlett MK, Zhang Y, Kreidler N, Sun S, Ardy R, Cao K, Sack L (2014) Global analysis of plasticity in turgor loss point, a key drought tolerance trait. Ecol Lett 17:1580-1590.

Beltrao J, Faria J, Miguel G, Chaves P, Trindade D (2000) Cabbage yield response to salinity of trickle irrigation water. Acta Hortic 537:641-645.

Biemond H, Vos J, Struik PC (1995) Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 1. Brussels sprouts. Neth J Agr Sci 43:419-433.

Blum A 2017 Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ 40:4-10.

Bothmer R von, Gustafsson M, Snogerup S (1995) *Brassica* sect. *Brassica* (Brassicaceae) II. Inter- and intraspecific crosses with cultivars of *B. oleracea*. Genet Resour Crop Ev 42:165–178.

Branca F, Argento S, Tribulato A 2012 Assessing genetic reserves in Sicily (Italy): the *Brassica* wild relatives case study. In: Maxted N, Dulloo ME, Ford-Lloyd BV, Frese L, Iriondo JM, Pinheiro de Carvalho MAA (eds) Agrobiodiversity conservation: securing the diversity of crop wild relatives and landraces, CABI, Wallingford, UK, pp 52-58.

Branca F, Cartea E (2011) Brassica. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, Vol. Oilseeds, Springer, Heidelberg, Dordrecht, London, New York, pp 17-36.

Branca F, Donnini D (2013) *Brassica rupestris*. The IUCN Red List of Threatened Species 2013: e.T170114A6719025. http://dx.doi.org/10.2305.

Branca F, Tribulato A (2013) *Brassica macrocarpa*. The IUCN Red List of Threatened Species 2013: e.T162139A5548195. http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T162139A5548195.en.

Bussotti F, Pollastrini M, Holland V, Brüggemann W (2015) Functional traits and adaptive capacity of European forests to climate change. Environ Exp Bot 111:91-113.

Cirelli D, Equiza MA, Lieffers VJ, Tyree MT (2016) *Populus* species from diverse habitats maintain high night-time conductance under drought. Tree Physiol 36:229-242.

Conti F, Manzi A, Pedrotti F (1997) Liste Rosse Regionali delle Piante d'Italia. Associazione Italiana per il World Wildlife Fund & Società' Botanica Italiana, Camerino.

Crescente M, Gratani L (2013) Differences in Morphological, Physiological and growth traits between two endemic subspecies of *Brassica rupestris* Raf.: implications for their conservation. Am J Plant Sci 4:42-50.

Donovan LA, Linton MJ, Richars JH (2001) Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. Oecol 129:328–325.

Edwards CE, Ewers BE, Williams DG, Xie Q, Lou P, Xu X, McClung CR, Weinig C (2011) The Genetic Architecture of Ecophysiological and Circadian Traits in *Brassica rapa*. Genetics 189:375–390.

Esquinas-Alcázar JT (2005) Protecting crop genetic diversity for food security: political, ethical and technical challenges. Nat Rev Genet 6:946–953.

Farrell C, Christopher S, Arndt SK (2017) Does the turgor loss point characterize drought response in dryland plants? Plant Cell Environ 40:1500–1511.

Faulkner K, Mithen R, Williamson G (1998) Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. Carcinogenesis 19(4):605–609.

Feller U (2016) Drought stress and carbon assimilation in a warming climate: Reversible and irreversible impacts. J Plant Physiol 203:84-94.

Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O (2015) Breeding and domesticating crops adapted to drought and salinity: A new paradigm for increasing food production. Front Plant Sci 6:978. doi: 10.3389/fpls.2015.00978.

Francois LE (1994) Growth, seed yield and oil content of canola grown under saline conditions. Agron J 86:233-237.

Gepts P (2004) Crop domestication as a long-term selection experiment. Plant Breeding Review 24:1–44.

Geraci A, Chèvre AM, Divaret I, Eber F, Raimondo FM (2004) Isozyme analysis of genetic diversity in wild Sicilian populations of *Brassica* sect. *Brassica* in view of genetic resources management. Genet Resour Crop Ev 51:137–146.

Geraci A, Divaret I, Raimondo FM, Chèvre AM (2001) Genetic relationships between Sicilian wild populations of *Brassica* analysed with RAPD markers. Plant Breeding 120:193–196.

Geraci A, P Mazzola (2012) *Brassica macrocarpa* Guss. In: Rossi G, Foggi B, Gennai M, Gargano D, Montagnani C, Orsenigo S, Pedrini S (eds) Schede per una Lista Rossa della Flora vascolare e crittogamica Italiana. Italian Botanist 44(2):417-420.

Gomez-Campo C (1999) Biology of Brassica Coenospecies, Elsevier.

Greenwood S, Ruiz-Benito P, Martínez-Vilalta J, Lloret F, Kitzberger T, Allen CD, Fensham R, Laughlin DC, Kattge J, Bönisch G, Kraft NJB, Jump AS (2017) Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area. Ecol Lett 20:539–553.

Hammer K (1984) Das Domestikationssyndrom. Kulturpflanze 32:11-34.

Hayat S, Ali B, Hasan SA, Ahmad A (2007) Effect of 28-homobrassinolide on salinity-induced changes in *Brassica juncea*. Turk J Biol 31:141-146.

Heschel MS, Dalton K, Jamason M, D'Agnese A, Ruane LG (2017) Drought response strategies of *Clarkia gracilis* (Onagraceae) populations from serpentine and nonserpentine soils. Int J Plant Sci 178:313-319.

Holmes MG, Keiller DR (2002) Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. Plant Cell Environ 25:85–93.

Hopkins JJ, Maxted N (2010) Crop Wild Relatives: Plant conservation for food security. Natural England Research Reports 037. Natural England, Sheffield.

Jullien A, Allirand JM, Mathieu A, Andrieu B, Ney B (2009) Variations in leaf mass per area according to N nutrition, plant age, and leaf position reflect ontogenetic plasticity in winter oilseed rape (*Brassica napus* L.). Field Crop Res 114:188-197.

Kangur O, Kupper P, Sellin A (2017) Predawn disequilibrium between soil and plant water potentials in light of climate trends predicted for northern Europe. Reg Environ Change 17:2159-2168.

Kattge S, Díaz S, Lavorel IC, Prentice P, Leadley G, Bönisch E, Garnier M, Westoby PB, Reich IJ, Wright JH et al. (2011) TRY – a global database of plant traits. Global Change Biol 17:2905–2935.

Kole C (2011) Wild Crop Relatives: Genomic and Breeding Resources. Oilseeds Springer-Verlag, Berlin Heidelberg.

Li B, Suzuki JI, Hara T (1999) Competitive ability of two *Brassica* varieties in relation to biomass allocation and morphological plasticity under varying nutrient availability. Ecol Res 14:255–266.

Ma Q, Niknam SR, Turner DW (2006) Responses of osmotic adjustment and seed yield of *Brassica napus* and B.*juncea* to soil water deficit at different growth stages. Crop Pasture Sci 57:221–226.

Ma Q, Turner DW (2006) Osmotic adjustment segregates with and is positively related to seed yield in F3 lines of crosses between *Brassica napus* and *B. juncea* subjected to water deficit. Anim Prod Sci 46:1621–1627.

Maggio A, De Pascale S, Ruggiero C, Barbieri G (2005) Physiological response of field-grown cabbage to salinity and drought stress. Eur J Agron 23:57-67.

Mart KB, Veneklaas EJ, Ramley HEB (2016) Osmotic potential at full turgor: an easily measurable trait to help breeders select for drought tolerance in wheat. Plant Breeding 135:279–285.

Mithen R, Faulkner K, Magrath R, Rose P, Willianson G, Marquez L (2003) Development of isothiociante-enriched broccoli and its enhanced ability to induce phase 2 detoxification in mammalian cells. Theor Appl Genet 106:727-734.

Nardini A, Casolo V, Dal Borgo A, Savi T, Stenni B, Bertoncin P, Zini L, McDowell NG (2016) Rooting depth, water relations and non-structural carbohydrate dynamics in three woody angiosperms differentially affected by an extreme summer drought. Plant Cell Environ 39:618–627.

Niknam SR, Ma Q, Turner DW (2003) Osmotic adjustment and seed yield of *Brassica napus* and *B. juncea* genotypes in a water-limited environment in south-western Australia. Anim Prod Sci 43:1127–1135.

Norouzi M, Toorchi M, Salekdeh GH, Mohammadi SA, Neyshabouri MR, Aharizad S (2008) Effect of water deficit on growth, grain yield and osmotic adjustment in rapeseed. J Food Agric Environ 6:312–318.

O'Brien MJ, Engelbrecht BMJ, Joswig J, Pereyra G, Schuldt B, Jansen S, Kattge J, Landhäusser SM, Levick SR, Preisler Y, Väänänen P, Macinnis-Ng C (2017) A synthesis of tree functional traits related to drought-induced mortality in forests across climatic zones . J Appl Ecology, 54(6):1669-1686. doi:10.1111/1365-2664.12874.

Oddo E, Inzerillo S, La Bella F, Grisafi F, Salleo S, Nardini A (2011) Short-term effects of potassium fertilization on the hydraulic conductance of *Laurus nobilis* L. Tree Physiol 31:131-138.

Palmer AR, Fuentes S, Taylor D, Macinnis-Ng C, Zeppel M, Yunusa I, February E, Eamus D (2008) The use of pre-dawn leaf water potential and MODIS LAI to explore seasonal trends in the phenology of Australian and southern African woodlands and savannas. Aust J Bot 56:557–563.

Pearce DW, Millard S, Bray DF, Rood SB (2006) Stomatal characteristics of riparian poplar species in a semi-arid environment. Tree Physiol 26:211–218.

Pérez Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte SM, Cornwell WK, Craine JM, Gurvich DE et al. (2013) New handbook for standardised measurement of plant functional traits worldwide. Aust J Bot 61:167–234.

Pérez-López U, Mena-Petite A, Muñoz Rueda A (2012) Interaction between salinity and elevated $CO_2$: a physiological approach. Progress Botany 73:97–126.

Petruzzellis F, Nardini A, Savi T, Tonet V, Castello M, Bacaro G (2018) Less safety for more efficiency: water relations and hydraulics of the invasive tree *Ailanthus altissima* (Mill.) Swingle compared with native *Fraxinus ornus* L., Tree Physiol, tpy076, https://doi.org/10.1093/treephys/tpy076

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol 182:565–588.

Rachmilevitch S, Da Costa M, Huang B (2006) Physiological and biochemical indicators for stress tolerance. In: Huang B (ed) Plant-environment interactions, 3rd ed. CRC Press, Boca Raton, FL, pp 321–356.

Raimondo FM, Geraci A (2003) A new taxonomic arrangement in Sicilia *Brassica* sect. *Brassica* (Cruciferae). Fl Medit 12: 439-441.

Raimondo FM, Mazzola P (1997) A new taxonomic arrangement of the Sicilian members of *Brassica* sect. *Brassica*. Lagascalia 19:831–838.

Raimondo FM, Mazzola P, Ottonello D (1991) On the taxonomy and distribution of *Brassica* sect. *Brassica* (Cruciferae) in Sicily. Fl Medit 1:63-86.

Sack L, Cowan PD, Jaikumar N, Holbrook NM (2003) The ‘hydrology’ of leaves: co-ordination of structure and function in temperate woody species. Plant Cell Environ 26:1343-1356.

Sack L, Tyree MT, Holbrook NM (2005) Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. New Phytol 167:403–413.

Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated $CO_2$ in five $C_3$ species. Plant Physiol 89:590-596.

Savi T, Love VL, Dal Borgo A, Martellos S, Nardini A (2017) Morpho-anatomical and physiological traits in saplings of drought-tolerant Mediterranean woody species. Trees 31:1137-1148.

Schulze ED, Robichaux RH, Grace J, Rundel PW, Ehleringer JR (1987) Plant water balance. BioScience 37:30-37.

Scoppola A, Spampinato G (2005) Stato delle conoscenze sulla flora vascolare d’Italia – Atlante delle specie a rischio di estinzione. Versione 1.0. In: Scoppola A, Blasi C (eds) Stato delle conoscenze sulla flora vascolare d’Italia. Palombi Editori, Roma.

Sellin A (1999) Does pre-dawn water potential reflect conditions of equilibrium in plant and soil water status? Acta Oecol 20:51-59.

Shengxin C, Chunxia L, Xuyang Y, Song C, Xuelei J, Xiaoying L, Zhigang X, Rongzhan G (2016) Morphological, photosynthetic, and physiological responses of rapeseed leaf to different combinations of red and blue lights at the rosette stage. Front Plant Sci 7:1144. http://doi.org/10.3389/fpls.2016.01144

Siddiqui ZS, Khan MA, Gi Kim B, Huang JS, Kwon TR (2008) Physiological responses of *Brassica napus* genotypes to combined drought and salt stress. Plant Stress 2:78–83.

Singh DP, Singh P, Kumar A, Sharma HC (1985) Transpirational cooling as a screening technique for drought tolerance in oil seed Brassicas. Ann Bot 56:815–820.

Snogerup S, Gustaffson M, Bothmer RV (1990) *Brassica* sect. *Brassica* (Brassicaceae). I. Taxonomy and variation. Willdenowia 19:271-365.

Tanentzap FM, Stempel A, Ryser P (2015) Reliability of leaf relative water content (RWC) measurements after storage: consequences for in situ measurements. Botany 93:535-541.

TrifilòP, Gascó A, Raimondo F, Nardini A, Salleo S (2003) Kinetics of recovery of leaf hydraulic conductance and vein functionality from cavitation-induced embolism in sunflower. J Exp Bot 54:2323–2330.

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J Exp Bot 23:267–282.

Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94:1103–1116.

Wood JG (1934) The physiology of xerophytism in Australian plants: the stomatal frequencies, transpiration and osmotic pressures of sclerophyll and tomentose-succulent leaved plants. J Ecol 22:69–87.

Yamori W, Hikosaka K, Way DA (2014) Temperature response of photosynthesis in C3, C4, and CAM plants: temperature acclimation and temperature adaptation. Photosynth Res 119:101-117.

Zaghdoud C, Mota-Cadenas C, Carvajal M, Muries B, Ferchichi A, Martínez-Ballesta M (2013) Elevated $CO_2$ alleviates negative effects of salinity on broccoli (*Brassica oleracea* L. var. *italica*) plants by modulating water balance through aquaporins abundance. Environ Exp Bot 95:15– 24.

Zhang X, Lu G, Long W, Zou X, Li F, Nishio T (2014) Recent progress in drought and salt tolerance studies in*Brassica* crops. Breeding Sci 64:60–73.

Laila R, Robin AHK, Yang K, Park J-I, Suh MC, Kim J, Nou I-S (2017) Developmental and genotypic variation in leaf wax content and composition, and in expression of wax biosynthetic genes in *Brassica oleracea* var*. capitata.* Front Plant Sci 7: 1972. https://doi.org/10.3389/fpls.2016.01972

S. Munné-Bosch, J. Peñuelas, Drought-induced oxidative stress in strawberry tree (Arbutus unedo L.) growing in Mediterranean field conditions, Plant Science, Volume 166, Issue 4, 2004, Pages 1105-1110, https://doi.org/10.1016/j.plantsci.2003.12.034.

Baker, RL, Yarkhunova, Y, Vidal, K, Ewers, BE, Weinig, C. Polyploidy and the relationship between leaf structure and function: implications for correlated evolution of anatomy, morphology, and physiology in Brassica. *BMC Plant Biol.* 2017; 17: 3.

## Table Captions

**Table 1** Stomatal characteristics of adaxial and abaxial leaf sides. Different letters within the same column indicate significant differences (Tukey's post-hoc test, $P < 0.05$).

**Table 2** Functional traits of the investigated taxa derived from pressure-volume curves analysis. Different letters within the same column indicate significant differences (Tukey's post-hoc test, $P < 0.001$).

**Table 3** Results of two- or three-way ANOVA on plant functional traits (stomatal conductance, leaf water potential, relative water content and leaf mass per area) depending on interacting factors time of day (T), irrigation regime (I), species (S) and days from sowing (D).

## Figure Captions

**Fig. 1** Wild specimens of: *a*, *B. macrocarpa*, *b*, *B. rupestris* subsp. *rupestris* and *c*, *B. villosa* subsp. *bivoniana*. *d*, Potted specimens of the three taxa used for the experimental treatment (*B. r., B. m., B. v.*, left to right)

**Fig. 2** Scanning electron micrographs of the adaxial leaf surface showing stomata of: a, *B. macrocarpa*, b, *B. villosa* subsp. *bivoniana* and c*, B. rupestris* subsp. *rupestris*

**Fig. 3** Stomatal conductance ($g_s$), water potential ($\Psi$), relative water content (RWC) and temperature (T) of leaves of *B. macrocarpa*, *B. rupestris* subsp. *rupestris* and *B. villosa* subsp. *bivoniana*, measured at 8:00 h and 12:00 h under watered or drought conditions. According to a three-way ANOVA with time of day, irrigation regime and species as factors, different letters indicate significant differences among bars within each vertical set of graphs ( Tukey's post-hoc test, $P < 0.05$)

**Fig. 4** Variation in leaf mass per area (LMA) during the study period in *B. macrocarpa*, *B. rupestris* subsp. *rupestris* and *B. villosa* subsp. *bivoniana*, measured under watered conditions. Asterisks indicate significant differences within sampling date (two-way ANOVA, Tukey's post-hoc test, $P < 0.05$)

## Supplementary Material Captions

**Table A1** Seed collection localities, taxonomic and habitat characteristics of the taxa selected for the study. Meteorological data from Servizio Informativo Agrometeorologico Siciliano referred to the period 1976-1996.

Figure 1

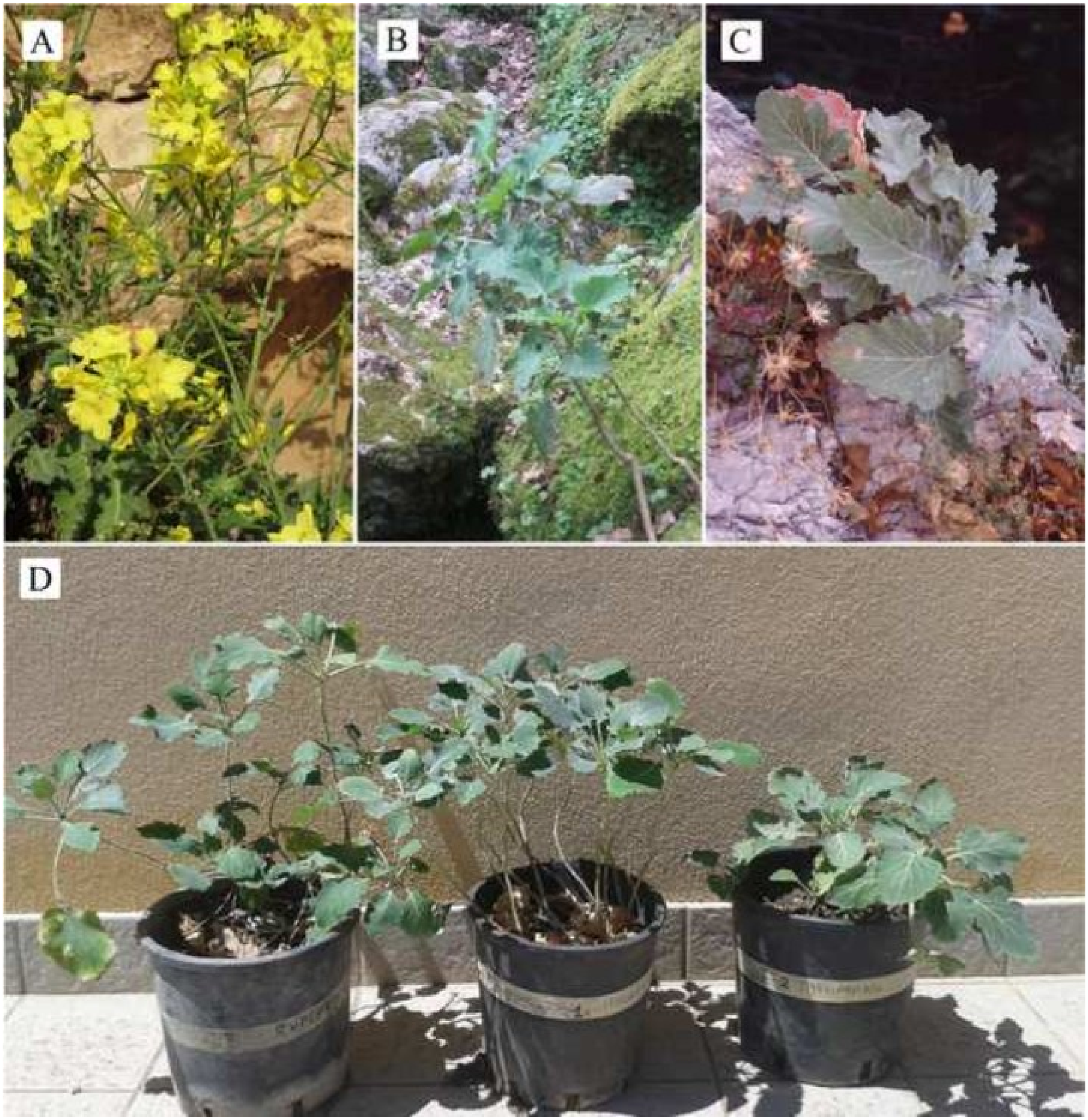


Figure 2

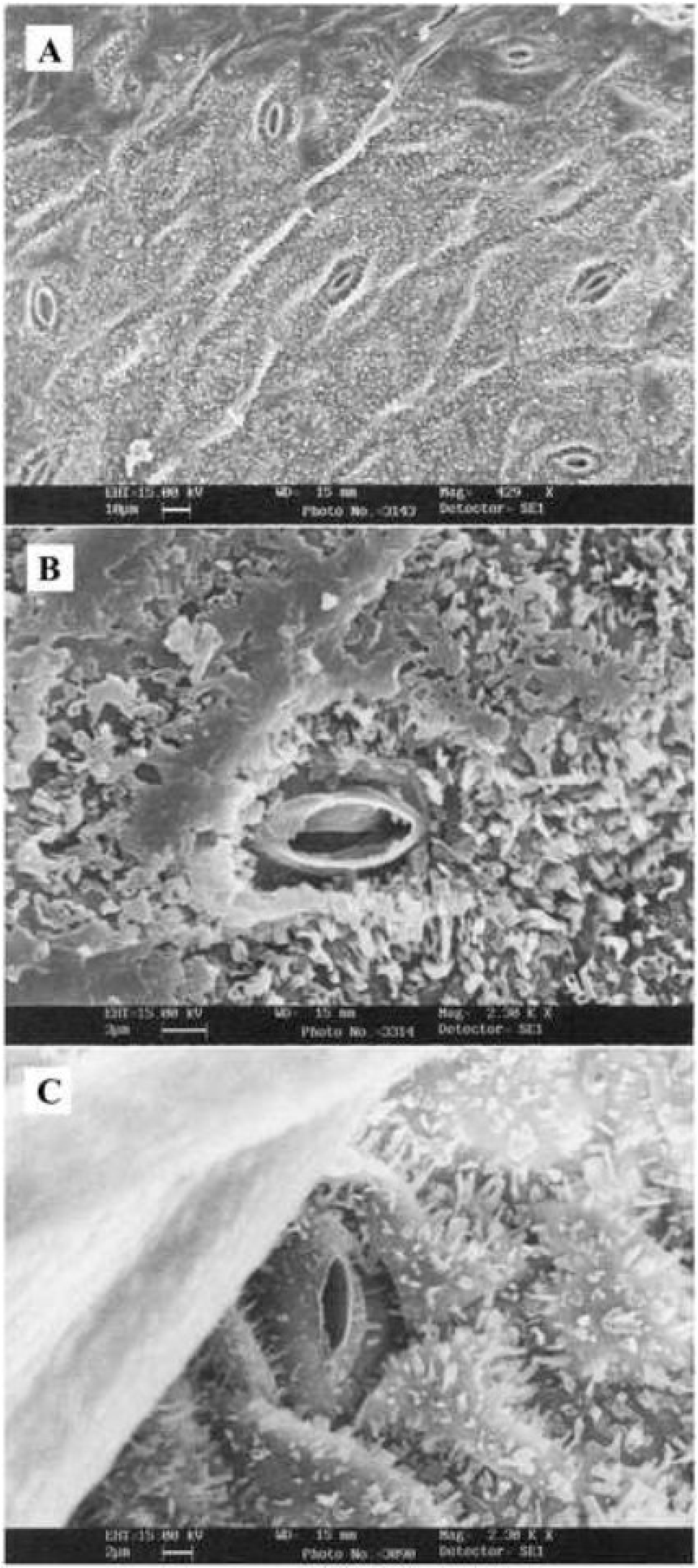
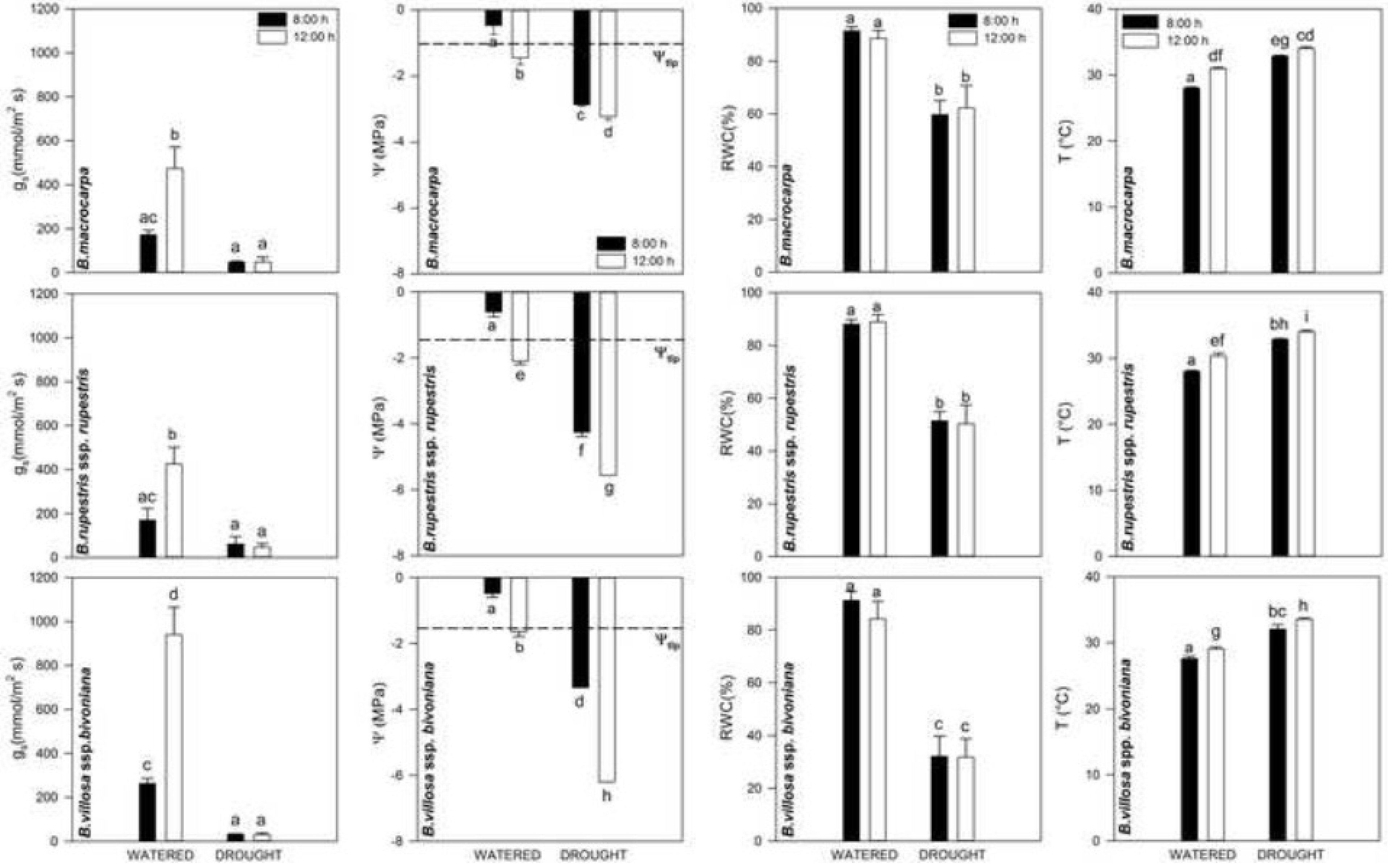

A
EHT=15.00 kV
WD= 15 mm
Mag= 429 X
10µm
Photo No.=3143
Detector= SE1
B
EHT=15.00 kV
WD= 15 mm
Mag= 2.50 K X
3µm
Photo No.=3314
Detector= SE1
C
EHT=15.00 kV
WD= 15 mm
Mag= 2.30 K X
2µm
Photo No.=3898
Detector= SE1

Figure 3


g$_s$(mmol/m$^2$ s)
0
200
400
600
800
1000
1200
8:00 h
12:00 h
B.macrocarpa
ac
b
a
a
B.rupestris ssp. rupestris
ac
b
a
a
B.villosa ssp.bivoniana
c
d
a
a
WATERED
DROUGHT
Ψ (MPa)
0
-2
-4
-6
-8
B.macrocarpa
a
b
c
d
Ψ$_{tlp}$
8:00 h
12:00 h
B.rupestris ssp. rupestris
a
e
f
g
Ψ$_{tlp}$
B.villosa ssp. bivoniana
a
b
d
h
Ψ$_{tlp}$
WATERED
DROUGHT
RWC(%)
0
20
40
60
80
100
8:00 h
12:00 h
B.macrocarpa
a
a
b
b
B.rupestris ssp. rupestris
a
a
b
b
B.villosa ssp. bivoniana
a
a
c
c
WATERED
DROUGHT
T (°C)
0
10
20
30
40
8:00 h
12:00 h
B.macrocarpa
a
df
eg
cd
B.rupestris spp. rupestris
a
ef
bh
i
B.villosa spp. bivoniana
a
g
bc
h
WATERED
DROUGHT

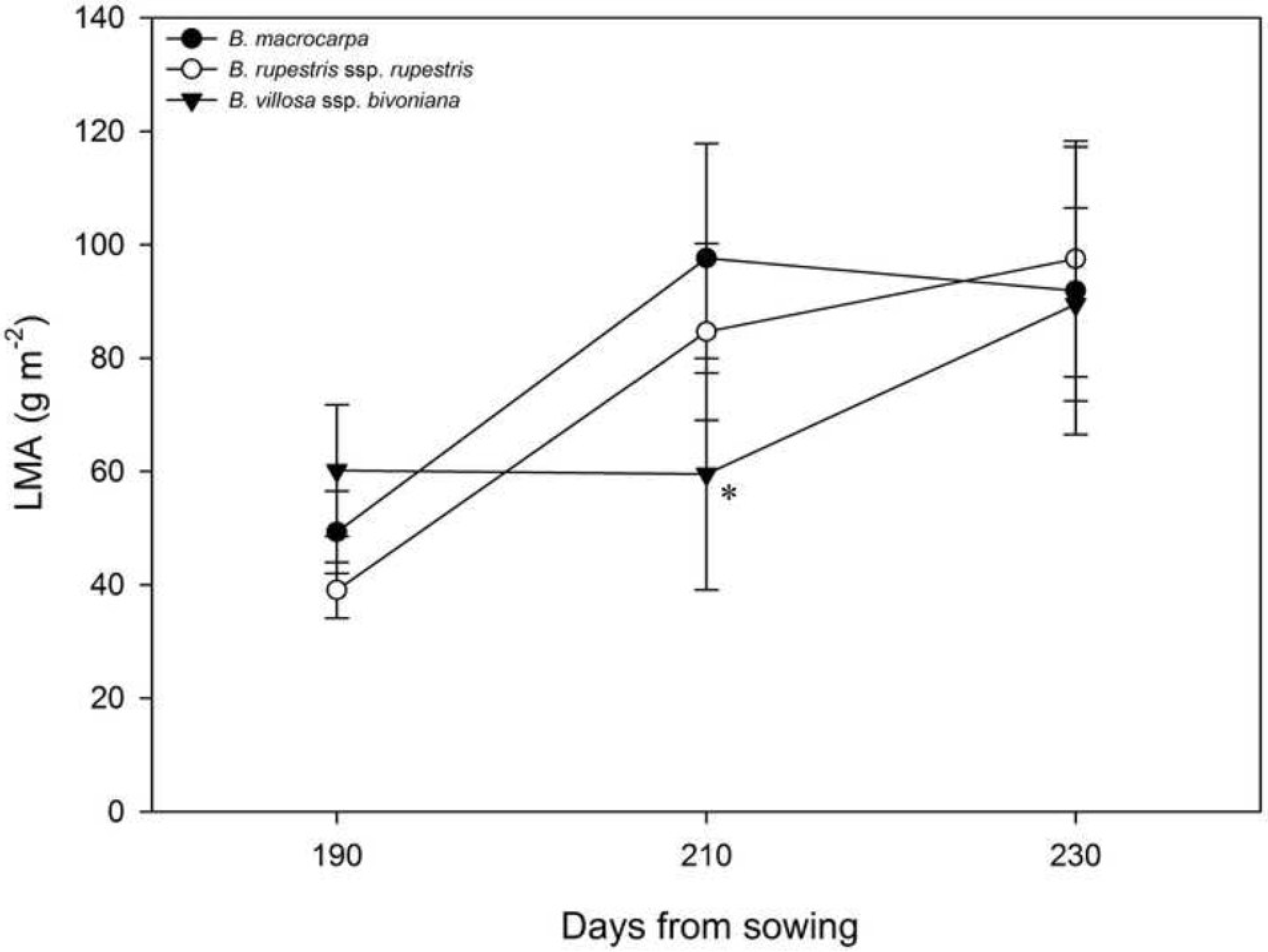

B. macrocarpa
B. rupestris ssp. rupestris
B. villosa ssp. bivoniana
LMA (g m-2)
0
20
40
60
80
100
120
140
190
210
230
Days from sowing
*

Table 1

| **Taxa** | **Stomatal density** (mm$^{-2}$) | | **Stomatal length** (µm) | **SPI** (x10$^{-2}$) |
|---|---|---|---|---|
| | Adaxial (mean ± SD) | Abaxial (mean ± SD) | adax+abax (mean ± SD) | Total adax+abax (mean ± SD) |
| *B. macrocarpa* | 298.5 ± 15.1 a | 304.4 ± 16.3 a | 8.20 ± 0.2 a | 4.05±0.21 a |
| *B. rupestris* subsp. *rupestris* | 295.5 ± 10.9 a | 307.0 ± 18.3 a | 8.69 ± 0.7 a | 4.55±0.22 b |
| *B. villosa* subsp. *bivoniana* | 297.5 ± 9.4 a | 602.0 ± 12.9 b | 7.43 ± 0.5 b | 4.97±0.12 c |

Table 2

| **Species** | **$\Psi_{tlp}$ (MPa)** | **$\Psi_{\pi100}$ (MPa)** | **RWC$_{tlp}$ (%)** | **$\varepsilon_{max}$ (MPa)** | **$C_{ft}$* (mol m$^{-2}$MPa$^{-1}$)** |
|---|---|---|---|---|---|
| ***B. macrocarpa*** | -0.979 ± 0.142 a | -0.721 ± 0.217 a | 92.06 ± 3.81 a | 11.304 ± 6.359 a | 1.324 ± 0.53 a |
| ***B. rupestris* ssp. *rupestris*** | -1.531 ± 0.296 b | -1.220 ± 0.305 b | 91.44 ± 2.18 a | 15.334 ± 3.336 a | 0.869 ± 0.23 a |
| ***B. villosa* ssp. *bivoniana*** | -1.584 ± 0.210 b | -1.270 ± 0.196 b | 87.68 ± 2.14 a | 12.061 ± 2.755 a | 0.989 ± 0.33 a |

Table 3

| Variable | $g_s$ | $\Psi_L$ | RWC | $T_{leaf}$ | LMA |
|---|---|---|---|---|---|
| T | *** | *** | NS | *** | |
| I | *** | *** | *** | *** | |
| S | *** | *** | *** | *** | NS |
| D | | | | | *** |
| T ×I | ** | *** | NS | NS | |
| T × S | | *** | NS | NS | |
| I × S | *** | *** | *** | *** | |
| D × S | | | | | ** |
| T ×I ×S | ** | *** | NS | NS | |

*, ** and *** significant at $P < 0.05$, 0.01 and 0.001 respectively. NS non-significant.

Table A1