The influence of water stress on growth, ecophysiology and ornamental quality of potted Primula vulgaris 'Heidy' plants. New insights to increase water use efficiency in plant production.

Published version:

DOI:10.1007/s10725-017-0301-4

Matteo Caser, Claudio Lovisolo, Valentina Scariot

The influence of water stress on growth, ecophysiology and ornamental quality of potted Primula vulgaris 'Heidy' plants. New insights to increase water use efficiency in plant production

Plant Growth Regulation, 2017, 1-14, DOI 10.1007/s10725-017-0301-4

The influence of water stress on growth, ecophysiology and ornamental quality of potted *Primula vulgaris* 'Heidy' plants. New insights to increase water use efficiency in plant production.

Matteo Caser[1*], Claudio Lovisolo[1], Valentina Scariot[1]

[1]Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini, 2, 10095, Grugliasco (TO), Italy

*Corresponding author: Matteo Caser, [1]Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini, 2, 10095, Grugliasco (TO), Italy; +390116708935; matteo.caser@unito.it

Abstract

Efficient irrigation practices are required to reduce the amount of water used. In this study, the effects of different irrigation regimes on changes in growth, ecophysiology and ornamental traits of potted *Primula vulgaris* ‘Heidy’ plants were investigated. Three experiments were carried out. In the first, the plants were either fully irrigated (100% of container capacity) or not. In the second, plants were watered to full irrigation (control), to 50% of the control (moderate water stress), to 25% of the control (severe water stress), or not irrigated and followed by a rehydration phase. Both experiments were conducted under controlled growth conditions. The third experiment was performed under common nursery conditions in an unheated and shaded greenhouse where plants were either irrigated with common irrigation practices (control), or with 66% of the control amount (moderate water stress), or with 33% of the control (severe water stress). In general, the percentage of senescent plants, the growth index, the number of leaves, and the aerial fresh and the dry weight were not affected by moderate water stress treatments. As expected, increasing water stress resulted in a general decrease in all studied gas exchange parameters. However, stressed plants were more efficient in using water than control plants, suggesting that stomata closed to cope with drought conditions without damaging photosynthesis events. The number of fully opened flowers during the growing season was highest in both control and moderately water stressed plants. In conclusion, moderate, but not severe, water stress could be imposed in *Primula vulgaris* ‘Heidy’ pot production to reduce the water consumption, still maintaining plant ecophysiological performances and ornamental quality.

Keywords Flower, Gas exchange parameters, Instantaneous water use efficiency, Midday leaf water potentials, Plant stress, Primrose

Introduction

Managing global water resources is one of the most pressing challenges of the 21st century. In many areas of the world there is a considerable pressure to produce crops more efficiently by reducing the water use (Fulcher et al. 2016). In floriculture, 100–350 L of water are needed to produce 1 kg of plant dry matter, with differences related to species, variety, cultivation system, and plant growing season (Fornes et al. 2007). Surprisingly, irrigation needs of ornamental pot plants have not been much investigated, although they constitute a major part of horticultural production (Henson et al. 2006). In the last decades, the interest in irrigation procedures was mainly centered on fruit and vegetable crops (Goldhamer and Beede 2004; Mo et al. 2016; Pérez-Jiménez et al. 2016).

In flower farming, the irrigation is generally based on personal experience and is rarely managed to match the effective water needs (Grant et al. 2012). Water and nutrients are often applied in excess, resulting in water wastage and environmental pollution due to the leaching of fertilisers and herbicides (ARPAT 2007). The controlled application of the water may be used in potted plants also to improve quality (Cameron et al. 2006), but precise scheduling is required to minimise the risk of excessive drying of the substrate. In this sense, different works have demonstrated that the plant quality decreases as the severity of water deficit increases (Sanchez-Blanco et al. 2009; Bolla et al. 2010; Bernal et al. 2011; Caser et al. 2012). Plant responses to drought are multiple and interconnected (Efeoğlu et al. 2009; Ali et al. 2017) and their capacities to adapt to this stress may vary considerably within genera and species (Sánchez-Blanco et al. 2002; Torrecillas et al. 2003). Water scarcity can delay and reduce the flowering and the new leaves sizes and the quality (Sanchez-Blanco et al., 2002; Augé et al., 2003) as a physiological consequence to drought (Davies et al. 2002; Zollinger et al. 2006; Alvarez et al. 2009; Caser et al. 2016). Thus, understanding morphological and physiological responses of plants to water management is critical for optimizing a sustainable high-quality production without compromising the economic value of the crop (Cameron et al. 2006; Franco et al. 2006).

Primula vulgaris (primrose, syn. *P. acaulis* (L.) Hill) is a perennial herbaceous native species of western and southern Europe, northwest Africa, and southwest Asia, frequently cultivated as a garden or potted flowering plant all around the world. Effect of water stress on morphology and physiology of *Primula* species have been reported only in *P. palinuri* (Dietz and Heber 1983) and in *P. veris* (Whale 1984) as high tolerant species. A high tolerance to adverse conditions, namely strong light intensity (photosynthetically active radiation > 1500 µmol $m^{-2}s^{-1}$) and heat stress at 38°C and 42°C was assessed within the genus by Liu et al. (2006) and Ceriani et al. (2009) and Hu et al. (2010). Cloned small heat shock proteins (sHSPs) gene, PfHSP17.1, was obtained by *P. forrestii* plants exposed to thermal stress (42 °C for 2 h) and used to higher resistance to salt and drought in transgenic

Arabidopsis thaliana plants (Zhang et al. 2013). Moreover, further work is needed to characterize and quantify the responses to water shortage.

The present research investigated the effects of different irrigation regimes on changes in the growth, the physiology and the ornamental traits of *P. vulgaris* 'Heidy', both under controlled growth conditions and common greenhouse practices. Such knowledge might help to optimise water use in primrose production and contribute to develop irrigation protocols for potted ornamental plants.

Materials and methods

Plant material

Plants of *P. vulgaris* 'Heidy' were provided in November 2013 by Planta s.s. (Bressanone, Bolzano, Italy) and immediately potted into vases of 11 cm in diameter, filled with a mixture of peat, conifer bark and clay (Turco Silvestro TS2, Albenga, Italy), and amended with 2 g L^{-1} of Osmocote Plus (14:13:13 N, P, K plus microelements). Their cultivation occurred till April 2014 in an unheated and shaded high tunnel at the Floricoltura Lagomarsino nursery (Genova, Italy) (44°23'06.5''N, 9°02'47.6''E). The weather conditions during greenhouse cultivation are listed in Supplementary Table 1. Plants were used in three different experiments as described below.

Experiment 1

Plants at the foliar period were transferred and maintained at the Department of Agriculture, Forest and Food Sciences of the University of Torino (Grugliasco, Italy) (45°03'59.73''N, 7°35'24.72''E) in a growth chamber at 20 °C, 60% of relative humidity and 16 h of photoperiod, with a photosynthetically active radiation (PAR) of 157 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the top of the canopy, provided by high pressure sodium lamps. The experimental design was a split-plot design with two treatments and three replications per treatment. Thirty-six plants were randomly divided in two groups (18 plants each) and subjected to fully irrigation (100% of container capacity), while the others were not irrigated. The water content was kept constant throughout the experiment. Gravimetric determinations of the water content was made by weighing soil samples before and after oven-drying to constant weight at 80 °C for one week. These values were used to calibrate all measurements of the moisture content of the substrate in the container. The container capacity was determined 48 h after irrigation and was calculated according to the equation of Paquin and Mehuys (1980). The soil moisture levels were maintained by manual irrigation and checked by weighing individual container every two days. This experiment lasted until the not irrigated plants reached the complete leaf turgor loss. These lasts were then recovered by the application of fully irrigation for one week.

Experiment 2

Plants at the beginning of the blooming stage were cultivated under the same growth conditions of the Experiment 1 and divided into four lots. The experimental design was a split-plot design with four treatments and three replications per treatment. Seventy-two plants were randomly divided in four groups (18 plants each) and subjected to fully irrigation (100% of container capacity) as control treatment, 50% of the control (moderate water stress), 25% of the control (severe water stress) or no irrigation till the complete leaf turgor loss. To evaluate their recovery attitude, these lasts were irrigated as control after three days from the complete leaf turgor loss. The gravimetric determinations of water content was conducted as described in Experiment 1.

Experiment 3

The experimental design was a split-plot design with three treatments and three replications per treatment. One hundred and eighty plants at the beginning of the foliar period were randomly divided in into three lots (60 plants each) and irrigated using a drip irrigation system under common nursery conditions at the Floricoltura Lagomarsino nursery (Genova, Italy). In the common irrigation practice, used as control treatment (100% of container capacity), plants were watered with three emitters per plant for a total of 4.824 l h^{-1} so that 15% (v/v) of the applied water was leached. Plants subjected to moderate water stress were watered with two emitters (66% of the control) and plants subjected to severe water stress with one emitter (33% of the control). The gravimetric determinations of water content was conducted as previously described. This experiment lasted the entire growing season from November 2013 to April 2014.

Measurements

Growth and ornamental quality evaluation

The described parameters were periodically measured over the growing season on the basis of the specific experiment. Height and diameter of each plant per treatment were measured to calculate the growth index (GI; $\Pi \times \{[(D'+D'')/2]/2\}^2 \times H$, where D' is the widest width, D'' is the perpendicular width and H is the height; Hidalgo and Harkess 2002). The number of leaves per plant and their length and width were measured to assess growth variation leaf area (*k* x length x width; *k* = 0.75; Ruget et al. 1996). The relative quantity of the chlorophyll present in leaf tissue was measured on four leaves of each plant per treatment using the Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc., Osaka, Japan). At the end of the experiments, six plants per treatment were harvested and the aerial part was collected. After recording the fresh biomass (FW), the plant aerial parts were oven-dried at 65 °C for one week and the dry biomass (DW) was weighted. The DW:FW ratio was then calculated.

The The root quality was visually evaluated and rated using five classes of roots covering (0= 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% of vase surface; Larcher et al. 2011).

To assess the ornamental quality, the leaf damage was visually evaluated and rated using five classes (visual damage class: 0 = 0%; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100% leaf area; Caser et al. 2013). The number of flowered plants, the number of completely opened flowers per plant and their diameter, and the height of peduncles were determined throughout the experiments. The petal color (L^*, a^*, b^* space) was measured on four flowers of six plants per treatment at the beginning and at the end of the blooming stage using a Spectrophotometer CM-2600 (Konica Minolta Sensing Inc., Osaka, Japan). Chroma (C^*) and hue angle (h°) was calculated according to Onozaky et al (1999) and Scariot et al (2008).

Ecophysiological measurements

Based on the specific experiment, one hour before the beginning of the physiological measurements (10 - 12 a.m.), plants were transferred in the lab for the adaptation to the ambient light intensity, the temperature and the relative humidity. Midday leaf water potentials (MLWP, MPa) were determined in three adult leaves of six plants per treatment using a Scholander-type pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA) (Scholander et al. 1965). The measurement of the internal CO_2 concentration (C_i), the transpiration rate (E), the stomatal conductance (GH_20), and the net photosynthetic rate (A) were performed in three adult leaves of six plants per treatment, using a portable infrared gas analyzer ADC-LCPro+ (The Analytical Development Company Ltd, Hoddesdon, UK). The instantaneous water use efficiency (WUE) was calculated as the ratio between A and E. Leaves were clamped in the leaf chamber, where the light source was set at 1200 μmol photons m^{-2} s^{-1} and the temperature (25 °C) kept constant. The environmental concentration of CO_2 (450-470 ppm) and the vapour pressure deficit (about 2.3 kPa) were maintained during the experiments.

Statistical analysis

An arcsine transformation was performed on all percent incidence data before statistical analysis in order to improve homogeneity of variance. All the measured and the derived data were then subjected to the homogeneity of the variances and then post-hoc tested using Ryan-Einot-Gabriel-Welsch-F test (REGW-F). The critical value for statistical significance was $P < 0.05$. All the data were computed by means of the SPSS statistical package (version 21.0; SPSS Inc., Chicago, Illinois).

Results and discussion

Experiment 1

The changes in physiological traits and growth were investigated in fully or not irrigated plants to characterize the responses to water stress and to determine the minimal water needs of *P. vulgaris* 'Heidy'. Significant differences in midday leaf water potential between treatments were observed starting from the day 2 in not irrigated plants with a constant decrease till day 8, followed by a strong decline to the complete loss of the leaf turgor which occurred at day 10 (MLWP = -0.71 MPa in not irrigated plants, Figure 1A). Similarly, a general decrease was observed in all the studied gas exchange traits (Figure 1B-C-D-E). Stomatal limitation is the first major event that occurs in response to the drought stress (Grassi and Magnani 2005). In fact, the stomatal conductance is extremely sensitive to physiological and environmental factors (e.g. leaf water status). In drought conditions, water deficit stress leads to a progressive limitation of photosynthesis, which is a consequence of an alteration in carbon assimilation (Siddique et al. 2016). The opening and closing of stomata is regulated by changes in turgor pressure in the guard cells that are present in epidermis and, hence, this process protects plants from the dehydration and death during fluctuating environmental conditions. Hence, a complex set of factors is involved in stomatal response to drought stress (Lawlor 2002). Here, a stomatal limitation to photosynthesis occurred. In fact, as stomatal conductance (Figure 1B) closes starting from day 2, the amount of carbon dioxide (Figure 1C) present in mesophyll spaces in leaves also decreases which results in the decline of carbon dioxide to oxygen ratio and to a constant decrease in photorespiration rate (Figure 1D) during mid-time period (from day 2 to day 8) of applied water stress with a mean value of *E* equal to 1.30 mmol m^{-2} s^{-1}. In the same period, net assimilation rate (Figure 1E) kept constant values (mean *A* = 6.04 µmol m^{-2} s^{-1}), perhaps because internal CO_2 concentration was utilized in photosynthesis (Faußer et al. 2016). Later stomatal close completely during severe drought at days 9 and 10, which causes both photosynthesis and photorespiration rates to lower (Athar and Ashraf 2005). However, midday leaf water potential equal to -0.40 MPa (day 8) could represent the minimum threshold to maintain the ornamental quality of the studied cultivar. Figure 1F shows how instantaneous water use efficiency (*A*/*E*) changed during the experiment. In general, stressed plants resulted equally efficient in using water than control plants, with the exception at days 1, 2, 8 and 9. In the lasts time points, in particular, we hypothesize that plants start to dissipate energy and to close the stomata to survive under severe drought conditions, inducing a consequent increase of WUE at the end of the experiment. Drought-stressed plants with higher WUE are more efficient in utilizing energy captured by photosynthesis per unit of water transpired. Similar trends were observed in *Callistemon laevis* (Alvarez et al. 2011), *Hybanthus floribundus* (Kachenko et al. 2011), *Rosa* x *hybrida* 'RADrazz' (Cai et al. 2012), and in *Zea mays* (Zhang et al. 2015). An experiment carried out on *Erica multiflora* and *Globularia alypum* confirmed that plants exposed to drought conditions show low gas exchange rates compared to plants grown in normal environmental conditions (Llorens et al. 2004).

Regarding morphological changes, the growth index, the leaf area and SPAD values were measured at the end of the experiment (day 10) and after one week of recovery (Table 1). At day 10, fully irrigated plants were bigger and showed wider leaves than not irrigated plants (1004.80 cm^3 and 207.63 cm^3 G.I.; 35.19 and 30.75 cm^2 in full irrigation and no irrigation, respectively). While, no differences in SPAD values were recorded (data not shown). Similar results were observed by Alvarez et al (2009) in *Dianthus caryophyllus* and by Caser et al (2012) in *Salvia dolomitica* subjected to severe water stress conditions. This is in agreement with the common plant responses to water stressed conditions leading to the decrease in cell enlargement and leaf size due to the low turgor pressure (Martinez et al. 2007). After one week of recovery, no more differences were observed with the exception for the leaf area with 38.10 and 32.15 cm^2 in full irrigated and not irrigated plants, respectively. Similar trend was observed in *Echinacea purpurea*, *Gaillardia aristata*, *Lavandula angustifolia*, *Leucanthemum* x *superbum*, *Penstemon barbatus* and *Penstemon* x *mexicali* irrigated every 4 weeks (Zollinger et al. 2006).

Experiment 2

The morphological responses to the imposed water stress and consequently the ornamental quality of *Primula vulgaris* 'Heidy' were characterized by the application of four different irrigation regimes under controlled growth conditions. Water stress significantly affected leaf damage values, SPAD values, leaf area, growth index, number of fully opened flowers and flower damages as reported in Figure 2. Only few damages (dead leaves or yellowing and wilted leaves) appeared starting from 21 days of cultivation (leaf damage = 1.0) in fully irrigated plants. Starting from this time point, moderate and severe stressed plants presented always superior leaf damage values than control. These achieved the complete leaf damage (class = 4.0) at day 42. Not irrigated plants reached the complete leaf damage after eleven days of cultivation. But imposed re-watering of them after three days (day 14) lead to the restoration of their morphological traits. The application of the recovery reduced the leaf damage of these plants already after three days (day 21 = 2.0) but, it could not reinstate as the control and, at day 49, resulted in the highest leaf damage values as well as half irrigated plants. Zollinger et al (2006) explained that the excellent visual quality of *Penstemon barbatus* after severe drought may be related to the ability to regulate the water loss by stomata closure.

Regarding SPAD values, here, no differences were counted between control and moderate stressed plants, showing that a half irrigation not affected the health plant status. On the opposite, the application of severe water stress and no irrigation treatments significantly reduced this parameter.

Several studies reported that water stress leads to a decreased level of chlorophylls in plant leaves (Reddy et al. 2004; Kaminska-Rozek and Pukacki 2004; Guerfel et al. 2009). Here, the reduction in SPAD value could be identified as a drought response mechanism in order to minimize the light absorption by chloroplasts (Pastenes et al. 2005). The application of the recovery induced an increase in SPAD values similarly to control and moderate water stress treatments already from day 14 till the end of the experiment with the exception at day 35, showing that *P. vulgaris* 'Heidy' has an excellent desiccation tolerance of their pigment apparatus (Netto et al. 2005).

Plant can adapt to environmental change usually by morphological and anatomical modifications. In the present study, fully irrigated plants showed the widest leaves and the highest growth index. While, surprisingly plants subjected to moderate water stress showed more narrow leaves, starting from day 8, and reduced growth, after 21 days of cultivation, as well as severe water stressed plants. Not irrigated plants showed the lowest values for both traits at day 11. The recovery phase was effective to increase the studied traits starting from the day 14 onward. The effect of water stress on plant growth reduction has been described in several crops species such as *Cistus albidus, Cistus monspeliensis*, *Petunia* x *hybrida*, *Pelargonium* x *hortorum* and *Callistemon citrinus* (Sanchez-Blanco et al. 2002; Niu et al. 2006; Vernieri et al. 2006; Alvarez and Sanchez-Blanco 2013). During severe water stress, the total leaf area and the stem length commonly significantly decreased in many ornamentals such as potted *Dianthus caryophyllus* and *Petunia* x *hybrida* (Alvarez et al. 2009; Van Iersel et al. 2010), resulting from the decline in the cell enlargement and the leaves senescence (Bañon et al. 2004).

The present experiment was conducted at the beginning of the blooming stage and is known that flowering is the most sensitive phase to water stress in ornamental plants such as in potted geranium (Alvarez et al. 2013). Here, significant differences between treatments in the number of fully opened flowers were observed (Fig. 2E). At day 8, fully irrigated plants presented more opened flowers (11 flowers per plant) than the others. At day 21, plants subjected to control, moderate water stress, and recovery treatments had more flowers than severe water stressed plants (16, 14, 11 and 7, respectively). In fact, these lasts anticipated the flowering peak at day 14. Later, a general decrease in all the treated plants was observed. As indicated in the previous experiment, *P. vulgaris* 'Heidy' subjected to drought stress maintain active photosynthesis, suggesting that no metabolic impairment occurred in this cultivar. In fact, in this experiment we can observe a flower production strictly related to the severity of imposed water stress and not a flowering peak induction caused by severe water stress. Figure 2F shows the evolution of flower damage (wilted flowers) during the experiment. In general no significant differences were observed, even if, fully and half irrigated plants had a similar dynamic. The recovered plants showed a restoration already after the first week of recovery, showing the lowest damages from day 14 till day 35. Later, a strong increase occurred, reaching the highest class of flower damage (4.0) at the end of the experiment as well as for severe water stressed plants. As reported by different authors, water stress application may increase flower damages and reduce flowering intensity such as in *Antirhinum majus* (Asrar et al. 2012) and in *Callistemon citrinus* (Alvarez and Blanco 2013; 2015).

At the end of the experiment the mean flower diameter, dry weight and fresh weight ratio, and the midday leaf water potential were evaluated (Table 2). Fully irrigated plants showed the highest values in flower diameter, without significant differences with half irrigated and recovered plants, and in MLWP. No differences with recovered plants were observed also in DW:FW ratio. On the opposite, plants subjected to severe water stress showed the lowest values in all the traits (1.9 cm, and -0.46 MPa, respectively) with exception for DW:FW ratio (0.98). These results confirmed that controlled irrigation practices could be possible and can be a useful tool to affect ornamental and morphological characteristics.

Experiment 3

Plant growth and ornamental traits

Water use in the nursery is an increasingly important factor due to limited water supply and drought is one of the main adverse factors for seasonal plants, especially for plants grown in pots (Alvarez et al. 2013). In the present experiment three irrigation regimes were applied during the common growing cycle of potted *P. vulgaris* ‘Heidy’ in nursery conditions.

The percentage of senescent plants, the aerial dry and fresh weight ratio, the growth index, the number of leaves and the SPAD values were not affected by control and moderate water stress treatments (Table 3; Figure 3). At the opposite, severe water stress significantly increased the percentage of senescent plants starting from day 29 (21.7%) until the end of the experiment, when the 83% of plants were completely senescent. While the application of the same irrigation regime resulted in the lower values in the G.I., starting from day 29, in the leaf area, from day 46, and in the number of leaves from day 85. A significant increase in SPAD values occurred at day 163 (32.7) in comparison with control and moderate water stress (20.0 and 21.1, respectively). A peak in SPAD values in plants close to the full senescence was observed also in aromatic plants such as *Salvia sinaloensis*, *S. dolomitica* and *Helicrhysum petiolare* treated with 20% of container capacity as showed by Caser et al (2012). This result could be explained by the fact that the leaf water status of a plant may interfere with the SPAD 502 Chlorophyll Meter measurements. Martinez and Guiamet (2004) also described that SPAD values increased as leaf water content decreased in wheat leaves. Taken together these data highlighted the possibility of water safe of ca. 35% without compromise the survival of plants. With the same purpose to reduce water irrigation, similar results were observed also in other nursery potted species such as *Dianthus caryophyllus* and *Pelargonium* x *hortorum* subjected to 35% and 40% of control irrigation as described by Alvarez et al (2009) and Sanchez-Blanco et al (2009), respectively. Moreover, as shown in Table 3, the irrigation with 33% of the control induced significant higher aerial dry /fresh weight ratio. While, no differences in the root quality (data not shown) was observed between treatments, indicating that shoots and roots react differently to drought (Bacelar et al. 2007). The effect of water stress is usually greater on aerial growth than on root growth as indicated by Navarro et al (2009). As reported by Bradford and Hsiao (1982) and by Sanchez-Blanco et al (2009), this is probably due to the plants need to maintain root surface area under drought conditions in order to absorb water from the substrate. This criterion can be considered as a plant adaptation to drought conditions and could promote a quicker establishment in gardening (Franco et al. 2011). Within the genus *Primula*, also Noda et al (2004) found out that water stress increased the root weight in *P. sieboldii*. Thus, the application of controlled deficit irrigation during nursery production can be used as a technique to save water without reducing morphological traits (Morvant et al. 1998). However, plants may lose their ornamental characteristics and reduce and delay flowering to save assimilates (Augé et al. 2003; Cameron et al. 2006; Alvarez et al. 2009). Flower colour parameters (lightness, chroma and hue angle) variation during the blooming stage were not affected by imposed treatments (data not shown). This suggested that the petal colour was not modified by the applied water stress conditions and meaning that plants can cope with water shortage without losing their ornamental value (Brawner 2003). Similar results were observed in potted *Pelargonium* x *hortorum* (Sanchez-Blanco et al. 2009; Alvarez et al. 2013), *Dianthus caryophyllus* (Alvarez et al. 2009) and in *Callistemon citrinus* (Alvarez and Sanchez-Blanco 2013) plants in which deficit irrigation treatments did not affected flower colour parameters. Instead, plant quality in terms of percentage of leaf damage (wilted leaves) was significantly affected by moderate and severe water stress starting from day 113 onward (Fig. 3E). Only at the end of the experiment moderate water stressed plants showed a similar leaf damage to the controlled (2.73 and 2.40, respectively). Concerning the number of fully opened flowers during the growing season, control and moderate water stressed plants showed a similar trend (Fig. 3F). The blooming peak was observed at day 113 with 6.7 and 5.9 flowers per plants, respectively. On the opposite, at day 142 and 163 the highest number of flowers per plants was observed in severe stressed plants (6.4 and 6.5 flowers, respectively). These results highlighted that this condition extend and delay the flowering period in *P. vulgaris* ‘Heidy’. Unlike the Experiment 2, here, in common growing condition, we observed that severe water stress significantly influenced a delay of flowering in the studied plants, implying that the different growing conditions may influence the flower development. Several studies further indicate that drought delays the onset of flowering and shortens the length of flowering (Prieto et al. 2008; Jentsch et al. 2009) such as in *Genista tinctoria* and *Calluna vulgaris* subjected to recurring weather extremes (Nagy et al. 2013). Control and moderate water stress induced also the highest peduncles and the largest flowers in comparison with the severe water stress conditions (5.9, 6.1 and 4.4 cm and 5.1, 5.2 and 3.7 cm, respectively). These data suggested that *P. vulgaris* 'Heidy' requires at least 66% of control irrigation to maintain acceptable plant and flower quality. Similarly, another season plant such as potted geranium, cultivated in nursery, needs at least 75% of control irrigation as reported by Henson et al (2006) and Sanchez-Blanco et al (2009). Nevertheless, further research is required to determine the most appropriate degree of water stress in order to optimise the growth and health of the plants and flower development. Similarly, Ma and Gu (2012) successfully applied severe water stress to extend flowering in *Bougainvillea spectabilis* 'Raspberry ice'.

Water relations

Midday leaf water potential and gas exchange traits were measured at the beginning of the blooming stage (Fig. 4). Moderate and severe water stressed plants showed significant lower MLWP (-0.31 and -0.45 MPa, respectively) than control (-0.10 MPa; Figure 4A). The data on MLWP are in agreement with the previous experiments conducted under controlled growth conditions. In particular, under severe water stress the decrease in the leaf water potential could be the cause of morphological adaptations previously described such as lower growth index, number of leaves, leaf area, and dry weight, which could contribute to reduce the total water consumption (Kang et al. 2000). Moreover, we can confirm that a MLWP equal to -0.40 MPa could be considered as a critic threshold for the survival of the studied plants. On the opposite, differences with the previous experiment were highlighted on gas exchange traits. We observed that after about 80 days of cultivation under common nursery conditions, no differences between treatments were measured, and the levels of transpiration rate (E), stomatal conductance (GH_2O), and net photosynthetic rate (A) were lower than at the end of the Experiment 1 (almost 1/5 in E and GH_2O and 1/10 in A). This fact could be explained by the completely different growing conditions and by the longest cultivation period in comparison to the previous experiment. While, both moderate and severe water stress induced significant higher content of internal CO_2 concentration (C_i). Within the genus *Primula*, Dietz and Heber (1983) reported that water stressed *Primula palinuri* in field showed a complete wilting with MLWP equal to -4.0 MPa, much lower than in our study and that the complete stomatal closure was reach with at least a water loss of the 20% of turgid leaves. Here, a shift from stomatal limitation to non-stomatal limitation was observed as described by Siddique et al (2016). We can hypothesize that studied plants impaired carbon assimilation to cope with drought by conserving the internal CO_2 and blocking photosynthesis.

Hasibeder et al (2015) demonstrated that in plants under severe drought regimes the usage of fresh photosynthates is transferred from metabolic activity to osmotic adjustment and storage compounds. Taken together all these results suggested that there is variability within *Primula* species and that *P. vulgaris* ‘Heidy’ is a not a drought tolerant cultivar under severe water stress.

Conclusions

In controlled growth conditions, the mechanism of drought tolerance of *Primula vulgaris* ‘Heidy’ was described by the physiological dynamics, that show a long-term decline in stomatal conductance and transpiration rate also under extreme drought condition. The application of recovery resulted in a restoration of all the morphological and ornamental traits and in the leaf water potential, thus the decrease in leaf water potential during the water stress could be reversed by the application of control irrigation.

Under common nursery cultivation practices, moderate water stress could be successfully applied in potted *Primula vulgaris* ‘Heidy’ production, allowing a reduction of ca. 40% (ca. 1.64 L h^{-1} per plant) of irrigation water and in the meanwhile maintaining plant health and ornamental quality comparable with common irrigation practice. Also severe deficit irrigation did not affect ornamental quality in terms of flower colour, number and size, but it deeply compromised plant survival. Overall, the studied cultivar appears to be a not drought tolerant cultivar reacting to deficit irrigation by reducing leaf water potential and stomata, activating photosynthesis and maintaining the water use efficiency similarly to control plants. In both growth conditions, the parameters that best allow determining the water use of the studied species were leaf water potential, gas exchanges, leaves traits, growth index, and the percentage of senescent plants.

In conclusion, the degree of the imposed water stress is critical to reveal the responses of the different species at the different phenological stages. The knowledge of physiological dynamics and morphological responses to water stress of a potted seasonal ornamental plant along the cultivation cycle could allow floriculture companies to schedule a sustainable irrigation plan, which match the effective species water needs.

Acknowledgement

This work has been supported by “PSR 2007-2013-Misura 1.1.1. - Progetti dimostrativi semplici - Risparmio Idrico in Floricoltura (R.ID.inFlor.)” project. The authors gratefully acknowledge Alessia Aru, Eugenio Lagomarsino and Andrea Sampietro for providing plant material and practical help.

References

Ali F, Bano A, Fazal A (2017) Recent methods of drought stress tolerance in plants. Plant Growth Regul 82:363-375

Álvarez S, Navarro A, Bañón S, Sánchez-Blanco MJ (2009) Regulated deficit irrigation in potted *Dianthus* plants: effects of severe and moderate water stress on growth and physiological responses. Sci Hortic 122:579-585

Álvarez S, Navarro A, Nicolas E, Sanchez-Blanco MJ (2011) Transpiration, photosynthetic responses, tissue water relations and dry mass partitioning in Callistemon plants during drought conditions. Sci Hortic 129(2):306-312

Álvarez S, Sanchez-Blanco MJ (2013) Changes in growth rate, root morphology and water use efficiency of potted *Callistemon citrinus* plants in response to different levels of water deficit. Sci Hortic 156:54-62

Álvarez S, Bañón S, Sanchez-Blanco MJ (2013) Regulated deficit irrigation in different phenological stages of potted geranium plants: water consumption, water relations and ornamental quality. Acta Physiol Plant 35:1257-1267

Álvarez S, Sanchez-Blanco MJ (2015) Comparison of individual and combined effects of salinity and deficit irrigation on physiological, nutritional and ornamental aspects of tolerance in *Callistemon laevis* plants. J Plant Physiol 185:65-74

ARPAT (2007) Programma di monitoraggio dell'ambiente marino costiero della toscana attività luglio 2006 - gennaio 2007, pp. 85.

Asrar AA, Abdel-Fattah GM, Elhindi KM (2012) Improving growth, flower yield, and water relations of snapdragon (*Antirhinum majus* L.) plants grown under well-watered and water-stress conditions using arbuscular mycorrhizal fungi. Photos 50:305-316

Athar HR, Ashraf M (2005) Photosynthesis under drought stress. In: Pessarakli M (Ed), Handbook of photosynthesis, CRC Press, Taylor and Francis Group, New York, pp. 793–809.

Augé RM, Stodola AJW, Moore JL, Klingeman WE, Duan X (2003) Comparative dehydration tolerance of foliage of several ornamental crops. Sci Hortic 98:511-516

Bacelar EA, Moutinho-Pereira JM, Gonçalves BC, Ferreira HF, Correia CM (2007) Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Env Exp Bot 60:183-192

Bañón S, Fernández JA, Franco JA, Torrecillas A, Alarcón JJ, Sánchez-Blanco MJ (2004) Effects of water stress and night temperature pre-conditioning on water relations and morphological and anatomical changes of *Lotus creticus* plants. Sci Hort 101:333–342

Bernal M, Estiarte M, Peñuelas J (2011) Drought advances spring growth phenology of the Mediterranean shrub *Erica multiflora*. Plant Biol 13:252-257

Bolla A, Voyiatzis D, Koukourikou-Petridou M, Chimonidou D (2010) Photosynthetic parameters and cut-flower yield of rose 'Eurored' (H.T.) are adversely affected by mild water stress irrespective of substrate composition. Sci Hortic 126:390-394

Bradford KJ, Hsiao TC (1982) Physiological responses to moderate water stress. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (Eds) Encyclopedia of plant physiology. New series. Physiological plant ecology II: water relations and carbon assimilation. Springer, Berlin, pp 263–324.

Brawner F (2003) Geraniums. The Complete Encyclopedia, Schiffer Publishing Ltd., Atglen, p. 13.

Cai X, Starman T, Niu G, Hall C, Lombardini L (2012) Response of selected garden rose to drough stress. HortSci 47(8):1050-1055

Cameron RWF, Harrison-Murray RS, Atkinson CJ, Judd HL (2006) Regulated deficit irrigation – a means to control growth in woody ornamentals. J Hortic Sci Biotech 81:435-443

Caser M, Ruffoni B, Scariot V (2012) Screening for drought tolerance in *Salvia* spp. and *Helichrysum petiolare*: a way to select low maintenance ornamental plants. Acta Hortic 953:239-246

Caser M, Scariot V, Gaino W, Larcher F, Devecchi M (2013) The effects of sodium chloride on the aesthetic value of *Buxus* spp. Europ J Hortic Sci 78(4):153-159

Caser M, D'Angiolillo F, Chitarra W, Lovisolo C, Ruffoni B, Pistelli Lu, Pistelli La, Scariot V (2016) Water deficit regimes trigger changes in valuable physiological and phytochemical parameters in *Helichrysum petiolare* Hilliard & BL Burtt. Ind Crops Prod 83:680-692

Ceriani RM, Pierce S, Cerabolini B (2009) The survival strategy of the alpine endemic *Primula glaucescens* is fundamentally unchanged throughout its climate envelope despite superficial phenotypic variability. Plant Ecol 204:1-10

Davies WJ, Wilkinson S, Loveys B (2002) Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phyt 153:449-460

Dietz KJ, Heber U (1983) Carbon dioxide gas exchange and the energy status of leaves of *Primula palinuri* under water stress. Planta 158:349-356

Efeoğlu B, Ekmekçi Y, Çiçek N (2009) Physiological responses of three maize cultivars to drought stress and recovery. South Afri J Bot 75:34-42

Faußer AC, Dušek J, Čížková H, Kazda M (2016) Diurnal dynamics of oxygen and carbon dioxide concentrations in shoots and rhizomes of a perennial in a constructed wetland indicate down-regulation of below ground oxygen consumption. AoB PLANTSplw025 doi: 10.1093/aobpla/plw025

Fornes F, Belda RM, Carrión C, Noguera V, García-Agustín P, Abad M (2007) Pre-conditioning ornamental plants to drought by means of saline water irrigation as related to salinity tolerance. Sci Hortic 113:52–59

Franco JA, Martinez-Sánchez JJ, Fernández JA, Bañón S (2006) Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. J Hortic Sci Biotech 81:3-17

Franco JA, Bañón S, Vicente MJ, Miralles J, Martinez-Sánchez JJ (2011) Root development in horticultural plants grown under abiotic stress conditions – a review. J Hortic Sci Biotech 86:543-556

Fulcher A, LeBude AV, Owen Jr JS, White SA, Beeson RC (2016) The next ten years: strategic vision of water resources for nursery producers. HortTech 26:121-132

Goldhamer D, Beede R (2004) Regulated deficit irrigation effects on yield, nut quality and water-use efficiency of mature pistachio trees. J Hortic Sci Biotech 79:538-545

Grant SB, Saphores JD, Feldman DL, Hamilton AJ, Fletcher TD, Cook PLM, Stewardson M, Sanders BF, Levin LA, Ambrose RF, Deletic A, Brown R, Jiang SC, Rosso D, Cooper WJ, Marusic I (2012) Taking the “waste” out of “wastewater” for human water security and ecosystem sustainability. Science 337(6095):681-686

Grassi G, Magnani F (2005) Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell Env 28:834-849

Guerfel M, Ouni Y, Boujnah D, Zarrouk M (2009) Photosynthesis parameters and activities of enzymes of oxidative stress in two young ‘Chemlali’ and ‘Chetoui’ olive trees under water deficit. Photosynthetica 47(3):340-346

Hasibeder R, Fuchslueger L, Richter A, Bahn M (2015) Summer drought alters carbon allocation to roots and root respiration in mountain grassland. New Phyt 205:1117-1127

Henson DY, Newman SE, Hartley DE (2006) Performance of selected herbaceous annual ornamentals grown at decreasing levels of irrigation. HortSci 41:1481-1486

Hidalgo PR, Harkess RL (2002) Earthworm castings as a substrate for *Poinsettia* production. HortSci 37:304–308

Hu WJ, Zhang QX, Pan HT, Dong LL (2010) Differences in ultrastructure of mesophyll cell between two *Primula* species with different thermotolerance under heat acclimation and heat stress. J South China Agric Univ 2010-03

Jentsch A, Kreyling J, Boettcher-Treschkow J, Beierkuhnlein C (2009) Beyond gradual warming: extreme weather events alter flower phenology of European grassland and heat species. Glob Change Biol 15:837-849

Kachenko AG, Bhatia N, Singh B (2011) Influence of drought stress on the nickel-hyperaccumulating shrub *Hybanthus floribundus* (Lindl.) F. Muell. Subsp. floribundus. Int J Plant Sci 172(3):315-322

Kaminska-Rozek E, Pukacki PM (2004) Effect of water deficit on oxidative stress and degradation of cell membranes in needles of Norway spruce (*Picea abies* (L.) Karst.). Acta Physiol Plant 26:431–442

Kang S, Shi W, Zhang J (2000) An improved water-use efficiency for maize grown under regulated deficit irrigation. Field Crops Res 67:207-214

Larcher F, Berruti A, Gullino P, Scariot V (2011) Reducing peat and growth regulator input in camellia pot cultivation. Hort Sci 38:35-42

Lawlor DW (2002) Limitation to photosynthesis in water-stressed leaves: stomatal vs. metabolism and the role of ATP. Ann Bot 89:871-885

Liu FH, Hou SMH, Liang XN (2006) Gas exchange characteristics of four domesticated *Primula* species. New Zeal J Crop Hortic Sci 34:403-411

Llorens L, Peñuelas J, Estiarte M, Bruna P (2004) Contrasting growth changes in two dominant species of a Mediterranean shrubland submitted to experimental drought and warming. Ann Bot 94:843-853

Ma S, Gu M (2012) Effects of water stress and selected plant growth retardants on growth and flowering of ‘Raspberry ice’ boungainvillea (*Boungainvillea spectabilis*). Acta Hortic 937:237-242

Martinez D, Guiamet J (2004) Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 24:41-46

Martinez JP, Silva H, Ledent JF, Pinto M (2007) Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.). Europ J Agr 26:30-38

Mo Y, Yang R, Liu L, Gu X, Yang X, Wang Y, Zhang X, Li H (2016) Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. Plant Growth Regul 79:229-241

Morvant JK, Dole JM, Cole JC (1998) Irrigation frequency and system affect *Poinsettia* growth, water use and runoff. Hort Sci 33:42–46

Nagy L, Kreyling J, Gellesch E, Beierkuhnlein C, Jentsch A (2013) Recurring weather extremes alter the flowering phenology of two common temperate shrubs. Int J Biometeorol 57:579 doi:10.1007/s00484-012-0585-z

Navarro A, Sanchez-Blanco MJ, Morte A, Bañón S (2009) The influence of mycorrhizal inoculation and paclobutrazol on water and nutritional status of *Arbutus unedo* L. Env Exp Bot 66:362-371

Netto AT, Campostrini E, de Oliveira JG, Bressan-Smith RE (2005) Photosynthetic pigments, nitrogen, chlorophyll *a* fluorescence and SPAD-502 readings in coffee leaves. Sci Hortic 104:199-209

Niu G, Rodriguez DS, Wang YT (2006) Impact of drought and temperature on growth and leaf gas exchange of six bedding plant species under greenhouse conditions. HortSci 41:1408-1411

Noda H, Muraoka H, Washitani I (2004) Morphological and physiological acclimation responses to contrasting light and water regimes in *Primula sieboldii*. Ecol Res 19:331-340

Onozaky T, Mato M, Shibata M, Ikeda H (1999) Differences in flower colour and pigment composition among white carnation (*Dianthus caryophyllus* L.) cultivars. Sci Hortic 82:103–111

Paquin R, Mehuys GR (1980) Influence of soil moisture on cold tolerance of alfalfa. Can J Plant Sc. 60:139–147

Pastenes C, Pimentel P, Lillo J (2005) Leaf movements and photoinhibition in relation to water stress in field-grown beans. J Exp Bot 56:425–433

Pérez-Jiménez M, Pazos-Navarro M, Piñero MC, Otálora-Alcón G, López-Marin J, del Amor FM (2016) Regulaytion of the drought response of sweet pepper (Capsicum annuum L.) by foliar-applied hormones, in Mediterranean-climate greenhouse conditions. Plant Growth Regul 80:159-169

Prieto P, Peñuelas J, Ogaya R, Estiarte M (2008) Precipitation-dependent flowering of *Globularia alypum* and *Erica multiflora* in Mediterranean shrubland under experimental drought and warming, and its inter-annual variability. Ann Bot 102:275-285

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161(11):1189-1202

Ruget F, Bonhomme R, Chartier M (1996) Estimation simple de la surface foliaire de plantes de mais en croissance. Agronomie 16:553-562

Sánchez-Blanco MJ, Rodriguez P, Morales MA, Torrecillas A (2002) Comparative growth and water relations of *Cistus albidus* and *Cistus monspeliensis* plants during water deficit conditions and recovery. Plant Sci 162:107-113

Sánchez-Blanco MJ, Álvarez S, Navarro A, Bañón S (2009) Changes in leaf water relations, gas exchange, growth and flowering quality in potted geranium plants irrigated with different water regimes. J Plant Phys 166:467-476

Scariot V, Seglie L, Caser M, Devecchi M (2008) Evaluation of ethylene sensitivity and postharvest treatments to improve the vase life of four *Campanula* species. Europ J Hortic Sci 73(4):166-170

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. Negative hydrostatic pressure can be measured in plants. Science 148:339–346

Siddique Z, Jan S, Imadi SR, Gul A, Ahmad P (2016) Drought stress and photosynthesis in plants. In: Ahmad P (Ed), Water Stress and Crop Plants: A Sustainable Approach, 2 Volume Set. Wiley Blackwell, Chichester, West Sussex, pp. 1-11

Torrecillas A, Rodriguez P, Sánchez-Blanco MJ (2003) Comparison of growth, leaf water relations and gas exchange of *Cistus albidus* and *C. monspeliensis* plants irrigated with water of different NaCl salinity levels. Sci Hortic 97:353-368

Van Iersel MW, Dove S, Kang JG, Burnett SE (2010) Growth and water use of *Petunia* as affected by substrate water content and daily light integral. HortSci 45:277-282

Vernieri P, Mugnai S, Borghesi E, Petrognani L, Serra G (2006) Non-chemical growth control of potted *Callistemon laevis* Anon. Agr Med 160:85-90

Whale DM (1984) Habitat requirements in *Primula* species. New Phyt 97:665–679

Zhang C, Zhang J, Zhang H, Zhao J, Wu Q, Zhao Z, Cai T (2015) Mechanisms for the relationships between water-use efficiency and carbon isotope composition and specific leaf area of maize (*Zea mays* L.) under water stress. Plant Growth Regul 77:233-243

Zhang L, Gao Y, Pan H, Hu W, Zhang Q (2013) Cloning and characterisation of a *Primula* heat shock protein gene, *PfHSP17.1*, which confers heat, salt and drought tolerance in transgenic *Arabidopsis thaliana*. Acta Physiol Plant 35:3191-3200

Zollinger N, Kjelgren, R, Cerny-Koenig T, Kopp K, Koenig R (2006) Drought responses of six ornamental herbaceous perennials. Sci Hortic 109:267-274

Tables

Table 1. Values of the growth index (G.I., cm^3) and the leaf area (cm^2) in potted *Primula vulgaris* 'Heidy' plants subjected to full irrigation (100% of container capacity) or no irrigation at day 10 and after the recovery phase during Experiment 1.

	G.I. (cm^3)		Leaf area (cm^2)	
Treatments	day 10	Recovery	day 10	Recovery
Full irrigation	1004.80	1140.12	35.19	38.10
No irrigation	207.63	930.74	30.75	32.15
P	**	ns	*	*

The statistical relevance of 'Between-Subjects Effects' tests (* = P ≤ 0.05, ** = ≤ 0.001, ns = not significant) was evaluated.

Table 2. Influence of water stress (full irrigation, control, 100% of container capacity; 50% of the control, moderate water stress, MWS; 25% of the control, severe water stress, SWS; no irrigation water followed by recovery at day 14) on flower diameter (cm), dry weight/fresh weight rate (DW:FW), and midday leaf water potential (MLWP, MPa) on *Primula vulgaris* 'Heidy' plants at the end of the Experiment 2.

Treatments	Flower diameter	DW:FW	MLWP
Control	3.2 a[§]	0.29 c	-0.07 a
MWS	3.0 a	0.50 b	-0.15 b
SWS	1.9 b	0.98 a	-0.46 c
No water + r	3.0 a	0.28 c	-0.27 b
P	**	**	**

[§] Different letter indicates significant differences at the 0.05 level, Ryan-Einot-Gabriel-Welsch (F) post hoc test (* = P ≤ 0.05, ** = ≤ 0.001, ns = not significant).

Table 3. Effect of different irrigation regimes (full irrigation, control, 100% of container capacity; 66% of the control, moderate water stress, MWS; 33% of the control, severe water stress, SWS) on the mean percentage (%) of senescent plants of *Primula vulgaris* ‘Heidy’ during the pot cultivation and the aerial dry weight/fresh weight ratio (DW:FW) calculated at the end of the Experiment 3

	Senescence (%)							
Days / Treatments	29	46	71	85	113	142	163	DW:FW
Control	0 b[§]	0 b	0 b	0 b	0 b	0 b	0 b	0.19 b
MWS	0 b	1.7 b	3.3 b	3.5 b	3.8 b	4.0 b	5.3 b	0.19 b
SWS	21.7 a	36.7 a	38.6 a	41.7 a	59 a	66.7 a	83 a	0.29 a
P	*	*	*	*	**	**	**	*

[§] Different letter indicates significant differences at the 0.05 level, Ryan-Einot-Gabriel-Welsch (F) post hoc test (* = P ≤ 0.05, ** = ≤ 0.001, ns = not significant).

Figure captions

Fig. 1 Dynamics of midday leaf water potential (MLWP, MPa) (A), stomatal conductance (GH_2O) (B), internal CO_2 concentration (C_i) (C), transpiration rate (*E*) (D), net photosynthetic rate (*A*) (E), and instantaneous water use efficiency (WUE, *A*/*E*) (F) in *Primula vulgaris* ‘Heidy’ plants subjected to full irrigation (100% of container capacity, control, solid black line) or no irrigation (solid grey line) during the Experiment 1. Each time point represents the mean values of six replicas and the vertical bars indicate standard errors. The statistical relevance of ‘Between-Subjects Effects’ tests (* = $P \leq 0.05$, ** = ≤ 0.001) was evaluated.

Fig. 2 Effect of irrigation treatments (100% of container capacity, control, solid black line), 50% of the control (moderate water stress, solid dark grey line), 25% of the control (severe water stress, solid light grey line), or no irrigation followed by recovery phase at day 14 (dotted black line) on leaf damage (class values) (A), SPAD values (B), leaf area (cm^2) (C), growth index (G.I., cm^3) (D), number of opened flowers (E), and flower damages (F) based on five class of flower withered (0= 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% of flower area) of *Primula vulgaris* ‘Heidy’ plants during the Experiment 2. The arrow indicate the begin of the recovery phase. Means were subjected to Ryan-Einot-Gabriel-Welsch (F) post hoc test (* = $P \leq 0.05$, ** = ≤ 0.001).

Fig. 3 Growth index (cm^3) (A), number of leaves (B), leaf area (cm^2) (C), SPAD values (D), leaf damages based on five classes of wilted damages (0 = 0%; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100% leaf area) (E), and number of opened flowers (F) variation in potted *Primula vulgaris* ‘Heidy’ plants under full irrigation (100% of container capacity, control, solid black line), moderate water stress (66% of the control, solid dark grey line), or severe water stress (33% of the control, solid light grey line). Each time point represents the mean values and the vertical bars indicate standard errors. Means were subjected to Ryan-Einot-Gabriel-Welsch (F) post hoc test (* = $P \leq 0.05$, ** = ≤ 0.001).

Fig. 4 Values of midday leaf water potential (MLWP, MPa) (A), transpiration rate (*E*) (B), stomatal conductance (GH_2O) (C), net photosynthetic rate (*A*) (D), and internal CO_2 concentration (C_i) (E) at the beginning of the blooming phase (day 85) of *Primula vulgaris* ‘Heidy’ plants. Black histograms referred to full irrigation (100% of container capacity, control), dark grey histograms to moderate water stress (66% of the control), and light grey histograms to severe water stress (33% of the control). Each histogram represents the mean values, the vertical bars indicate standard errors and similar letters indicate not significant differences ($P \leq 0.05$) according to Ryan-Einot-Gabriel-Welsch (F) post hoc test.