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

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12

13 **Abstract**

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

32

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

35

36 **Introduction**

37 Managing global water resources is one of the most pressing challenges of the 21st century. In many
38 areas of the world there is a considerable pressure to produce crops more efficiently by reducing the
39 water use (Fulcher et al. 2016). In floriculture, 100–350 L of water are needed to produce 1 kg of
40 plant dry matter, with differences related to species, variety, cultivation system, and plant growing
41 season (Fornes et al. 2007). Surprisingly, irrigation needs of ornamental pot plants have not been
42 much investigated, although they constitute a major part of horticultural production (Henson et al.
43 2006). In the last decades, the interest in irrigation procedures was mainly centered on fruit and
44 vegetable crops (Goldhamer and Beede 2004; Mo et al. 2016; Pérez-Jiménez et al. 2016).

45 In flower farming, the irrigation is generally based on personal experience and is rarely
46 managed to match the effective water needs (Grant et al. 2012). Water and nutrients are often applied
47 in excess, resulting in water wastage and environmental pollution due to the leaching of fertilisers
48 and herbicides (ARPAT 2007). The controlled application of the water may be used in potted plants
49 also to improve quality (Cameron et al. 2006), but precise scheduling is required to minimise the risk
50 of excessive drying of the substrate. In this sense, different works have demonstrated that the plant
51 quality decreases as the severity of water deficit increases (Sanchez-Blanco et al. 2009; Bolla et al.
52 2010; Bernal et al. 2011; Caser et al. 2012). Plant responses to drought are multiple and
53 interconnected (Efeoğlu et al. 2009; Ali et al. 2017) and their capacities to adapt to this stress may
54 vary considerably within genera and species (Sánchez-Blanco et al. 2002; Torrecillas et al. 2003).
55 Water scarcity can delay and reduce the flowering and the new leaves sizes and the quality (Sanchez-
56 Blanco et al., 2002; Augé et al., 2003) as a physiological consequence to drought (Davies et al. 2002;
57 Zollinger et al. 2006; Alvarez et al. 2009; Caser et al. 2016). Thus, understanding morphological and
58 physiological responses of plants to water management is critical for optimizing a sustainable high-
59 quality production without compromising the economic value of the crop (Cameron et al. 2006;
60 Franco et al. 2006).

61 *Primula vulgaris* (primrose, syn. *P. acaulis* (L.) Hill) is a perennial herbaceous native species
62 of western and southern Europe, northwest Africa, and southwest Asia, frequently cultivated as a
63 garden or potted flowering plant all around the world. Effect of water stress on morphology and
64 physiology of *Primula* species have been reported only in *P. palinuri* (Dietz and Heber 1983) and in
65 *P. veris* (Whale 1984) as high tolerant species. A high tolerance to adverse conditions, namely strong
66 light intensity (photosynthetically active radiation > 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$) and heat stress at 38°C and
67 42°C was assessed within the genus by Liu et al. (2006) and Ceriani et al. (2009) and Hu et al. (2010).
68 Cloned small heat shock proteins (sHSPs) gene, PfHSP17.1, was obtained by *P. forrestii* plants
69 exposed to thermal stress (42 °C for 2 h) and used to higher resistance to salt and drought in transgenic

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

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

76

77 **Materials and methods**

78 **Plant material**

79 Plants of *P. vulgaris* 'Heidy' were provided in November 2013 by Planta s.s. (Bressanone, Bolzano,
80 Italy) and immediately potted into vases of 11 cm in diameter, filled with a mixture of peat, conifer
81 bark and clay (Turco Silvestro TS2, Albenga, Italy), and amended with 2 g L⁻¹ of Osmocote Plus
82 (14:13:13 N, P, K plus microelements). Their cultivation occurred till April 2014 in an unheated and
83 shaded high tunnel at the Floricoltura Lagomarsino nursery (Genova, Italy) (44°23'06.5''N,
84 9°02'47.6''E). The weather conditions during greenhouse cultivation are listed in Supplementary
85 Table 1. Plants were used in three different experiments as described below.

86

87 **Experiment 1**

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

103

104 **Experiment 2**

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

113

114 **Experiment 3**

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

125

126 **Measurements**

127 **Growth and ornamental quality evaluation**

128 The described parameters were periodically measured over the growing season on the basis of the
129 specific experiment. Height and diameter of each plant per treatment were measured to calculate the
130 growth index (GI; $\Pi \times \{[(D'+D'')/2]/2\}^2 \times H$, where D' is the widest width, D'' is the perpendicular
131 width and H is the height; Hidalgo and Harkess 2002). The number of leaves per plant and their length
132 and width were measured to assess growth variation leaf area ($k \times \text{length} \times \text{width}$; $k = 0.75$; Ruget et
133 al. 1996). The relative quantity of the chlorophyll present in leaf tissue was measured on four leaves
134 of each plant per treatment using the Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc.,
135 Osaka, Japan). At the end of the experiments, six plants per treatment were harvested and the aerial
136 part was collected. After recording the fresh biomass (FW), the plant aerial parts were oven-dried at
137 $65 \text{ }^\circ\text{C}$ for one week and the dry biomass (DW) was weighted. The DW:FW ratio was then calculated.

138 The The root quality was visually evaluated and rated using five classes of roots covering (0= 0%,
139 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% of vase surface; Larcher et al. 2011).

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

148

149 **Ecophysiological measurements**

150 Based on the specific experiment, one hour before the beginning of the physiological measurements
151 (10 - 12 a.m.), plants were transferred in the lab for the adaptation to the ambient light intensity, the
152 temperature and the relative humidity. Midday leaf water potentials (MLWP, MPa) were determined
153 in three adult leaves of six plants per treatment using a Scholander-type pressure chamber (Soil
154 Moisture Equipment, Santa Barbara, CA, USA) (Scholander et al. 1965). The measurement of the
155 internal CO_2 concentration (C_i), the transpiration rate (E), the stomatal conductance (GH_2O), and the
156 net photosynthetic rate (A) were performed in three adult leaves of six plants per treatment, using a
157 portable infrared gas analyzer ADC-LCPro+ (The Analytical Development Company Ltd,
158 Hoddesdon, UK). The instantaneous water use efficiency (WUE) was calculated as the ratio between
159 A and E . Leaves were clamped in the leaf chamber, where the light source was set at $1200 \mu\text{mol}$
160 $\text{photons m}^{-2} \text{s}^{-1}$ and the temperature (25°C) kept constant. The environmental concentration of CO_2
161 ($450\text{-}470 \text{ ppm}$) and the vapour pressure deficit (about 2.3 kPa) were maintained during the
162 experiments.

163

164 **Statistical analysis**

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

170

171 **Results and discussion**

172 **Experiment 1**

173 The changes in physiological traits and growth were investigated in fully or not irrigated plants to
174 characterize the responses to water stress and to determine the minimal water needs of *P. vulgaris*
175 ‘Heidy’. Significant differences in midday leaf water potential between treatments were observed
176 starting from the day 2 in not irrigated plants with a constant decrease till day 8, followed by a strong
177 decline to the complete loss of the leaf turgor which occurred at day 10 (MLWP = -0.71 MPa in not
178 irrigated plants, Figure 1A). Similarly, a general decrease was observed in all the studied gas
179 exchange traits (Figure 1B-C-D-E). Stomatal limitation is the first major event that occurs in response
180 to the drought stress (Grassi and Magnani 2005). In fact, the stomatal conductance is extremely
181 sensitive to physiological and environmental factors (e.g. leaf water status). In drought conditions,
182 water deficit stress leads to a progressive limitation of photosynthesis, which is a consequence of an
183 alteration in carbon assimilation (Siddique et al. 2016). The opening and closing of stomata is
184 regulated by changes in turgor pressure in the guard cells that are present in epidermis and, hence,
185 this process protects plants from the dehydration and death during fluctuating environmental
186 conditions. Hence, a complex set of factors is involved in stomatal response to drought stress (Lawlor
187 2002). Here, a stomatal limitation to photosynthesis occurred. In fact, as stomatal conductance (Figure
188 1B) closes starting from day 2, the amount of carbon dioxide (Figure 1C) present in mesophyll spaces
189 in leaves also decreases which results in the decline of carbon dioxide to oxygen ratio and to a constant
190 decrease in photorespiration rate (Figure 1D) during mid-time period (from day 2 to day 8) of applied
191 water stress with a mean value of E equal to $1.30 \text{ mmol m}^{-2} \text{ s}^{-1}$. In the same period, net assimilation
192 rate (Figure 1E) kept constant values (mean $A = 6.04 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$), perhaps because internal CO_2
193 concentration was utilized in photosynthesis (Faußer et al. 2016). Later stomatal close completely
194 during severe drought at days 9 and 10, which causes both photosynthesis and photorespiration rates
195 to lower (Athar and Ashraf 2005). However, midday leaf water potential equal to -0.40 MPa (day 8)
196 could represent the minimum threshold to maintain the ornamental quality of the studied cultivar.
197 Figure 1F shows how instantaneous water use efficiency (A/E) changed during the experiment. In
198 general, stressed plants resulted equally efficient in using water than control plants, with the exception
199 at days 1, 2, 8 and 9. In the lasts time points, in particular, we hypothesize that plants start to dissipate
200 energy and to close the stomata to survive under severe drought conditions, inducing a consequent
201 increase of WUE at the end of the experiment. Drought-stressed plants with higher WUE are more
202 efficient in utilizing energy captured by photosynthesis per unit of water transpired. Similar trends
203 were observed in *Callistemon laevis* (Alvarez et al. 2011), *Hybanthus floribundus* (Kachenko et al.
204 2011), *Rosa x hybrida* ‘RADrazz’ (Cai et al. 2012), and in *Zea mays* (Zhang et al. 2015). An
205 experiment carried out on *Erica multiflora* and *Globularia alypum* confirmed that plants exposed to

206 drought conditions show low gas exchange rates compared to plants grown in normal environmental
207 conditions (Llorens et al. 2004).

208 Regarding morphological changes, the growth index, the leaf area and SPAD values were
209 measured at the end of the experiment (day 10) and after one week of recovery (Table 1). At day 10,
210 fully irrigated plants were bigger and showed wider leaves than not irrigated plants (1004.80 cm³ and
211 207.63 cm³ G.I.; 35.19 and 30.75 cm² in full irrigation and no irrigation, respectively). While, no
212 differences in SPAD values were recorded (data not shown). Similar results were observed by Alvarez
213 et al (2009) in *Dianthus caryophyllus* and by Caser et al (2012) in *Salvia dolomitica* subjected to
214 severe water stress conditions. This is in agreement with the common plant responses to water
215 stressed conditions leading to the decrease in cell enlargement and leaf size due to the low turgor
216 pressure (Martinez et al. 2007). After one week of recovery, no more differences were observed with
217 the exception for the leaf area with 38.10 and 32.15 cm² in full irrigated and not irrigated plants,
218 respectively. Similar trend was observed in *Echinacea purpurea*, *Gaillardia aristata*, *Lavandula*
219 *angustifolia*, *Leucanthemum x superbum*, *Penstemon barbatus* and *Penstemon x mexicali* irrigated
220 every 4 weeks (Zollinger et al. 2006).

221

222 **Experiment 2**

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

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

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

247 Plant can adapt to environmental change usually by morphological and anatomical
248 modifications. In the present study, fully irrigated plants showed the widest leaves and the highest
249 growth index. While, surprisingly plants subjected to moderate water stress showed more narrow
250 leaves, starting from day 8, and reduced growth, after 21 days of cultivation, as well as severe water
251 stressed plants. Not irrigated plants showed the lowest values for both traits at day 11. The recovery
252 phase was effective to increase the studied traits starting from the day 14 onward. The effect of water
253 stress on plant growth reduction has been described in several crops species such as *Cistus albidus*,
254 *Cistus monspeliensis*, *Petunia x hybrida*, *Pelargonium x hortorum* and *Callistemon citrinus*
255 (Sanchez-Blanco et al. 2002; Niu et al. 2006; Vernieri et al. 2006; Alvarez and Sanchez-Blanco 2013).
256 During severe water stress, the total leaf area and the stem length commonly significantly decreased
257 in many ornamentals such as potted *Dianthus caryophyllus* and *Petunia x hybrida* (Alvarez et al.
258 2009; Van Iersel et al. 2010), resulting from the decline in the cell enlargement and the leaves
259 senescence (Bañon et al. 2004).

260 The present experiment was conducted at the beginning of the blooming stage and is known
261 that flowering is the most sensitive phase to water stress in ornamental plants such as in potted
262 geranium (Alvarez et al. 2013). Here, significant differences between treatments in the number of
263 fully opened flowers were observed (Fig. 2E). At day 8, fully irrigated plants presented more opened
264 flowers (11 flowers per plant) than the others. At day 21, plants subjected to control, moderate water
265 stress, and recovery treatments had more flowers than severe water stressed plants (16, 14, 11 and 7,
266 respectively). In fact, these lasts anticipated the flowering peak at day 14. Later, a general decrease
267 in all the treated plants was observed. As indicated in the previous experiment, *P. vulgaris* 'Heidy'
268 subjected to drought stress maintain active photosynthesis, suggesting that no metabolic impairment
269 occurred in this cultivar. In fact, in this experiment we can observe a flower production strictly related
270 to the severity of imposed water stress and not a flowering peak induction caused by severe water
271 stress. Figure 2F shows the evolution of flower damage (wilted flowers) during the experiment. In
272 general no significant differences were observed, even if, fully and half irrigated plants had a similar
273 dynamic. The recovered plants showed a restoration already after the first week of recovery, showing

274 the lowest damages from day 14 till day 35. Later, a strong increase occurred, reaching the highest
275 class of flower damage (4.0) at the end of the experiment as well as for severe water stressed plants.
276 As reported by different authors, water stress application may increase flower damages and reduce
277 flowering intensity such as in *Antirrhinum majus* (Asrar et al. 2012) and in *Callistemon citrinus*
278 (Alvarez and Blanco 2013; 2015).

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

287

288 **Experiment 3**

289 **Plant growth and ornamental traits**

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

294 The percentage of senescent plants, the aerial dry and fresh weight ratio, the growth index, the
295 number of leaves and the SPAD values were not affected by control and moderate water stress
296 treatments (Table 3; Figure 3). At the opposite, severe water stress significantly increased the
297 percentage of senescent plants starting from day 29 (21.7%) until the end of the experiment, when
298 the 83% of plants were completely senescent. While the application of the same irrigation regime
299 resulted in the lower values in the G.I., starting from day 29, in the leaf area, from day 46, and in the
300 number of leaves from day 85. A significant increase in SPAD values occurred at day 163 (32.7) in
301 comparison with control and moderate water stress (20.0 and 21.1, respectively). A peak in SPAD
302 values in plants close to the full senescence was observed also in aromatic plants such as *Salvia*
303 *sinaloensis*, *S. dolomitica* and *Helicrhysum petiolare* treated with 20% of container capacity as
304 showed by Caser et al (2012). This result could be explained by the fact that the leaf water status of
305 a plant may interfere with the SPAD 502 Chlorophyll Meter measurements. Martinez and Guiamet
306 (2004) also described that SPAD values increased as leaf water content decreased in wheat leaves.
307 Taken together these data highlighted the possibility of water safe of ca. 35% without compromise

308 the survival of plants. With the same purpose to reduce water irrigation, similar results were observed
309 also in other nursery potted species such as *Dianthus caryophyllus* and *Pelargonium x hortorum*
310 subjected to 35% and 40% of control irrigation as described by Alvarez et al (2009) and Sanchez-
311 Blanco et al (2009), respectively. Moreover, as shown in Table 3, the irrigation with 33% of the
312 control induced significant higher aerial dry /fresh weight ratio. While, no differences in the root
313 quality (data not shown) was observed between treatments, indicating that shoots and roots react
314 differently to drought (Bacelar et al. 2007). The effect of water stress is usually greater on aerial
315 growth than on root growth as indicated by Navarro et al (2009). As reported by Bradford and Hsiao
316 (1982) and by Sanchez-Blanco et al (2009), this is probably due to the plants need to maintain root
317 surface area under drought conditions in order to absorb water from the substrate. This criterion can
318 be considered as a plant adaptation to drought conditions and could promote a quicker establishment
319 in gardening (Franco et al. 2011). Within the genus *Primula*, also Noda et al (2004) found out that
320 water stress increased the root weight in *P. sieboldii*. Thus, the application of controlled deficit
321 irrigation during nursery production can be used as a technique to save water without reducing
322 morphological traits (Morvant et al. 1998). However, plants may lose their ornamental characteristics
323 and reduce and delay flowering to save assimilates (Augé et al. 2003; Cameron et al. 2006; Alvarez
324 et al. 2009). Flower colour parameters (lightness, chroma and hue angle) variation during the
325 blooming stage were not affected by imposed treatments (data not shown). This suggested that the
326 petal colour was not modified by the applied water stress conditions and meaning that plants can cope
327 with water shortage without losing their ornamental value (Brawner 2003). Similar results were
328 observed in potted *Pelargonium x hortorum* (Sanchez-Blanco et al. 2009; Alvarez et al. 2013),
329 *Dianthus caryophyllus* (Alvarez et al. 2009) and in *Callistemon citrinus* (Alvarez and Sanchez-Blanco
330 2013) plants in which deficit irrigation treatments did not affected flower colour parameters. Instead,
331 plant quality in terms of percentage of leaf damage (wilted leaves) was significantly affected by
332 moderate and severe water stress starting from day 113 onward (Fig. 3E). Only at the end of the
333 experiment moderate water stressed plants showed a similar leaf damage to the controlled (2.73 and
334 2.40, respectively). Concerning the number of fully opened flowers during the growing season,
335 control and moderate water stressed plants showed a similar trend (Fig. 3F). The blooming peak was
336 observed at day 113 with 6.7 and 5.9 flowers per plants, respectively. On the opposite, at day 142 and
337 163 the highest number of flowers per plants was observed in severe stressed plants (6.4 and 6.5
338 flowers, respectively). These results highlighted that this condition extend and delay the flowering
339 period in *P. vulgaris* 'Heidy'. Unlike the Experiment 2, here, in common growing condition, we
340 observed that severe water stress significantly influenced a delay of flowering in the studied plants,
341 implying that the different growing conditions may influence the flower development. Several studies

342 further indicate that drought delays the onset of flowering and shortens the length of flowering (Prieto
343 et al. 2008; Jentsch et al. 2009) such as in *Genista tinctoria* and *Calluna vulgaris* subjected to
344 recurring weather extremes (Nagy et al. 2013). Control and moderate water stress induced also the
345 highest peduncles and the largest flowers in comparison with the severe water stress conditions (5.9,
346 6.1 and 4.4 cm and 5.1, 5.2 and 3.7 cm, respectively). These data suggested that *P. vulgaris* 'Heidy'
347 requires at least 66% of control irrigation to maintain acceptable plant and flower quality. Similarly,
348 another season plant such as potted geranium, cultivated in nursery, needs at least 75% of control
349 irrigation as reported by Henson et al (2006) and Sanchez-Blanco et al (2009). Nevertheless, further
350 research is required to determine the most appropriate degree of water stress in order to optimise the
351 growth and health of the plants and flower development. Similarly, Ma and Gu (2012) successfully
352 applied severe water stress to extend flowering in *Bougainvillea spectabilis* 'Raspberry ice'.
353

353

354 **Water relations**

355 Midday leaf water potential and gas exchange traits were measured at the beginning of the blooming
356 stage (Fig. 4). Moderate and severe water stressed plants showed significant lower MLWP (-0.31 and
357 -0.45 MPa, respectively) than control (-0.10 MPa; Figure 4A). The data on MLWP are in agreement
358 with the previous experiments conducted under controlled growth conditions. In particular, under
359 severe water stress the decrease in the leaf water potential could be the cause of morphological
360 adaptations previously described such as lower growth index, number of leaves, leaf area, and dry
361 weight, which could contribute to reduce the total water consumption (Kang et al. 2000). Moreover,
362 we can confirm that a MLWP equal to -0.40 MPa could be considered as a critic threshold for the
363 survival of the studied plants. On the opposite, differences with the previous experiment were
364 highlighted on gas exchange traits. We observed that after about 80 days of cultivation under common
365 nursery conditions, no differences between treatments were measured, and the levels of transpiration
366 rate (E), stomatal conductance (GH_2O), and net photosynthetic rate (A) were lower than at the end of
367 the Experiment 1 (almost 1/5 in E and GH_2O and 1/10 in A). This fact could be explained by the
368 completely different growing conditions and by the longest cultivation period in comparison to the
369 previous experiment. While, both moderate and severe water stress induced significant higher content
370 of internal CO_2 concentration (C_i). Within the genus *Primula*, Dietz and Heber (1983) reported that
371 water stressed *Primula palinuri* in field showed a complete wilting with MLWP equal to -4.0 MPa,
372 much lower than in our study and that the complete stomatal closure was reach with at least a water
373 loss of the 20% of turgid leaves. Here, a shift from stomatal limitation to non-stomatal limitation was
374 observed as described by Siddique et al (2016). We can hypothesize that studied plants impaired
375 carbon assimilation to cope with drought by conserving the internal CO_2 and blocking photosynthesis.

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

380

381 **Conclusions**

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

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

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

403

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408

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598

599 **Tables**

600 **Table 1.** Values of the growth index (G.I., cm³) and the leaf area (cm²) in potted *Primula vulgaris*
 601 ‘Heidy’ plants subjected to full irrigation (100% of container capacity) or no irrigation at day 10 and
 602 after the recovery phase during Experiment 1.

Treatments	G.I. (cm ³)		Leaf area (cm ²)	
	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
<i>P</i>	**	ns	*	*

603 The statistical relevance of ‘Between-Subjects Effects’ tests (* = $P \leq 0.05$, ** = ≤ 0.001 , ns = not
 604 significant) was evaluated.

605

606 **Table 2.** Influence of water stress (full irrigation, control, 100% of container capacity; 50% of the
 607 control, moderate water stress, MWS; 25% of the control, severe water stress, SWS; no irrigation
 608 water followed by recovery at day 14) on flower diameter (cm), dry weight/fresh weight rate
 609 (DW:FW), and midday leaf water potential (MLWP, MPa) on *Primula vulgaris* ‘Heidy’ plants at the
 610 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
<i>P</i>	**	**	**

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

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621 **Table 3.** Effect of different irrigation regimes (full irrigation, control, 100% of container capacity;
 622 66% of the control, moderate water stress, MWS; 33% of the control, severe water stress, SWS) on
 623 the mean percentage (%) of senescent plants of *Primula vulgaris* ‘Heidy’ during the pot cultivation
 624 and the aerial dry weight/fresh weight ratio (DW:FW) calculated at the end of the Experiment 3

		Senescence (%)							
Treatments	Days	29	46	71	85	113	142	163	DW:FW
	Control		0 b [§]	0 b	0 b	0 b	0 b	0 b	0 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
<i>P</i>		*	*	*	*	**	**	**	*

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

627

628 **Figure captions**

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

636
637 **Fig. 2** Effect of irrigation treatments (100% of container capacity, control, solid black line), 50% of
638 the control (moderate water stress, solid dark grey line), 25% of the control (severe water stress, solid
639 light grey line), or no irrigation followed by recovery phase at day 14 (dotted black line) on leaf
640 damage (class values) (A), SPAD values (B), leaf area (cm^2) (C), growth index (G.I., cm^3) (D),
641 number of opened flowers (E), and flower damages (F) based on five class of flower withered (0=
642 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% of flower area) of *Primula vulgaris* 'Heidy'
643 plants during the Experiment 2. The arrow indicate the begin of the recovery phase. Means were
644 subjected to Ryan-Einot-Gabriel-Welsch (F) post hoc test (* = $P \leq 0.05$, ** = ≤ 0.001).

645
646 **Fig. 3** Growth index (cm^3) (A), number of leaves (B), leaf area (cm^2) (C), SPAD values (D), leaf
647 damages based on five classes of wilted damages (0 = 0%; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 =
648 76-100% leaf area) (E), and number of opened flowers (F) variation in potted *Primula vulgaris*
649 'Heidy' plants under full irrigation (100% of container capacity, control, solid black line), moderate
650 water stress (66% of the control, solid dark grey line), or severe water stress (33% of the control, solid
651 light grey line). Each time point represents the mean values and the vertical bars indicate standard
652 errors. Means were subjected to Ryan-Einot-Gabriel-Welsch (F) post hoc test (* = $P \leq 0.05$, ** = \leq
653 0.001).

654
655 **Fig. 4** Values of midday leaf water potential (MLWP, MPa) (A), transpiration rate (E) (B), stomatal
656 conductance (GH_2O) (C), net photosynthetic rate (A) (D), and internal CO_2 concentration (C_i) (E) at
657 the beginning of the blooming phase (day 85) of *Primula vulgaris* 'Heidy' plants. Black histograms
658 referred to full irrigation (100% of container capacity, control), dark grey histograms to moderate
659 water stress (66% of the control), and light grey histograms to severe water stress (33% of the control).
660 Each histogram represents the mean values, the vertical bars indicate standard errors and similar

661 letters indicate not significant differences ($P \leq 0.05$) according to Ryan-Einot-Gabriel-Welsch (F)
662 post hoc test.