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ORIGINAL PAPER

Petunia × *hybrida* during transition to flowering as affected by light intensity and quality treatments

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Abstract *Petunia* × *hybrida* was grown under high (H), medium (M) and low (L) light intensity [photoperiod; 16 h d^{-1}, photosynthetic photon flux density (PPFD); 360, 120 and 40 μmol m^{-2} s^{-1}, respectively] as well as under end-of-day (EOD) red (R) and far-red (FR) light quality treatments [photoperiod; 14.5 h d^{-1}, PPFD; 30 μmol m^{-2} s^{-1} EOD; 15 min, Control (C) light; without EOD light treatment]. Shoot growth, leaf anatomical and photosynthetic responses as well as the responses of peroxidase (POD) isoforms and their specific activities following transition to flowering (1–6 weeks) were evaluated. Flower bud formation of *Petunia* × *hybrida* was achieved at the end of the 4th week for H light treatment and on the end of the 6th week for FR light treatment. No flower bud formation was noticed in the C and R light treatments. H and M light treatments induced lower chlorophyll (Chla, Chlb, Chla+b) concentrations in comparison to L light. On the other hand R and FR light chlorophyll content were similar to C light. Photosynthetic parameters [CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) values] were higher in the H light treated plants in comparison to M and L light treated plants. A, E and g_s values of R and FR light were similar to C light plants. Leaf anatomy revealed that total leaf thickness, thickness of the contained tissues (epidermis, palisade and spongy parenchyma) and relative volume percentages of the leaf histological components were differently affected within the light intensity and the light quality treatments. POD specific activities increased from the 1st to the 6th week during transition to flowering. Native-PAGE analysis revealed the appearance of four anionic POD (A_1–A_4) isoforms in all light treatments. On the basis of the leaf anatomical, photosynthetic and plant morphological responses, the production of high quality *Petunia* × *hybrida* plants with optimal flowering times could be achieved through the control of both light intensity and light quality. The appearance of A_1 and A_2 anionic POD isoforms could be also used for successful scheduling under light treatments.

Keywords Far-red light · Leaf anatomy · Peroxidase · Photosynthesis · Red light

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Abbreviations

A	CO_2 assimilation rate
BSA	Bovine serum albumin
C	Control light
DMAB	3-dimethylamino benzoic acid
E	Transpiration rate
EOD	End-of-day
FR	Far-red
g_s	Stomatal conductance
H	High
L	Low

LDP	Long day plant
M	Medium
MBTH	3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate
PAGE	Polyacrylamide gel electrophoresis
PMSF	Phenyl methyl sulfonic fluoride
POD	Peroxidase
PPFD	Photosynthetic photon flux density
PVPP	Polyvinyl polypyrolidone
R	Red
TEMED	Tetramethyl ethylene diamine
Tris	Tris-hydroxymethyl-aminomethane

Introduction

Light intensity and light quality changes cause a high degree of responses on several plant anatomical, physiological, morphological, and biochemical parameters (Boardman 1977; Caesar 1989; Barreiro et al. 1992; Schuerger et al. 1997; Hanba et al. 2002; Feng et al. 2004; Oguchi et al. 2005; Thomas 2006). The time of flowering of *Petunia* × *hybrida* is a complex function of temperature, light integral and photoperiod; flowering can be hastened by high temperatures, high light intensity and long days (Adams et al. 1999). Recently, results were obtained about the effect of red and far-red light deficiency on *Petunia* × *hybrida* growth and flowering (Kubota et al. 2000; Fletcher et al. 2005; Ilias and Rajapakse 2005a, b). Additionally, several studies have approached genetical and biochemical features of *Petunia* × *hybrida* transition to flowering (Souer et al. 1998; Snowden and Napoli 2003; Ben-Nissan et al. 2004).

Flower differentiation is an exciting aspect of plant morphogenesis, with respect to the perception of environmental signals and the switching of a developmental process from a vegetative to a reproductive pathway (Zhou et al. 2007). Floral organs are homologous to foliage leaves and genetic studies show that the floral organs are modified leaves (Smyth 2005). Since the floral organs are modified leaves, physiological and biochemical responses of the leaves as a concept can also be applied to predict the timing of flower bud formation during morphogenesis of floral organs from shoot meristems (Goto et al. 2001).

The analysis of enzyme isoform patterns has been well established in studies of plant physiology as a useful genetic and/or biochemical tool (Gaspar et al. 1975; Karege et al. 1982; Goto and Hamada 1988; Nakanishi and Fujii 1992; Bernal et al. 1993; Monerri and Guardiola 2001). Peroxidases (POD; EC 1.11.1.7) belong to a large family of enzymes able to oxidize several different substrates in the presence of H_2O_2. These enzymes are involved in several physiological and biochemical processes, such as cell growth and expansion (Simonovicova et al. 2004; Syros et al. 2005a), differentiation and development (Gaspar et al. 1982), lignification (Chen et al. 2002; Syros et al. 2004a), as well as in abiotic and biotic stresses (Syros et al. 2004b, 2005b; Molassiotis et al. 2006) and high light intensity responses (Cakmak et al. 1995; Asada et al. 1998). Changes in POD activities and isoform patterns during transition to flowering have been defined for several plants e.g. *Spinacia oleracea* (Gaspar et al. 1975; Karege et al. 1982), *Arabidopsis thaliana* (Goto and Hamada 1988), *Pharbitis nil* (Nakanishi and Fujii 1992), *Capsicum annuum* (Bernal et al. 1993) and *Citrus unshiu* (Monerri and Guardiola 2001).

The aim of this study was to introduce, based on peroxidase activities and isoform patterns during flower bud formation, the starting point of a more biochemically-based model during transition to flowering of high quality *Petunia* × *hybrida* plants.

Materials and methods

Plant growth

Seeds of *Petunia* × *hybrida* grandiflora 'Ultra Blue' (Goldsmith®) were sown into plug-trays containing a peat-based soilless mixture (Agrohoum, GR). They were watered and held for seed germination at 23°C in a growth chamber (Conviron®) providing a photosynthetic photon flux density (PPFD) of 20 μmol m^{-2} s^{-1} from cool white fluorescent bulbs, with a 16 h d^{-1} photoperiod. The light level was kept with a LI-1000 datalogger (LI-COR, Inc., Lincoln, Nebr.). Two weeks after sowing, uniform seedlings were transplanted individually into 0.5 L plastic pots containing a commercial soilless potting mixture (peat/perlite, 4:1 v/v). Plants were acclimatized for 1 week in the abovementioned conditions and then moved to growth chambers in order to receive different light intensity and light quality treatments.

Light intensity treatments

In the case of the light intensity treatments, three photosynthetic photon flux densities were applied; low (L) light: PPFD; 40 μmol m^{-2} s^{-1}, medium (M) light: PPFD; 120 μmol m^{-2} s^{-1}, high (H) light: PPFD; 360 μmol m^{-2} s^{-1}. Light was provided from cool white fluorescent bulbs, 40 W standard GRO-LUX light bulbs and two 400 W high intensity discharge (HID) high-pressure sodium bulbs (Sylvania's Lumalux) for a 16 h d^{-1} photoperiod.

Light quality treatments

In the case of the light quality treatments, plants were grown in growth chambers at 14.5 h d^{-1} photoperiod, in a photon flux density (PPFD) of 30 μmol m^{-2} s^{-1} provided from cool white fluorescent bulbs. At the end of the photoperiod (EOD, end-of-day), plants were exposed for 15 min to red (R) light (16.5 μmol m^{-2} s^{-1}) or far-red (FR) light (0.49 μmol m^{-2} s^{-1}) light treatments. Control (C) light corresponded to plants without EOD light treatment.

General plant culture

Petunia × *hybrida* plants received the abovementioned light treatments in the growth chambers for a six-week period. They were watered as necessary and fertilized with 1.0 g L^{-1} of 9N-15P-27K + 3Mg water soluble fertilizer as required. For both light treatments, temperature was set to 23°C day and night.

Chlorophyll determination

Chlorophyll concentration (Chla, Chlb and Chla+b) was determined according to Wintermans and Demots (1965). Samples (consisting of five disks) were taken from mature leaves of light intensity and light quality treated plants at the end of the six-week period in the growth chambers and were extracted with ethanol 95% in water-bath at 80°C. Full extraction of chlorophyll was achieved when the sample was discoloured. The absorption was measured at 649 and 665 nm. The results were expressed as μg mL^{-1} mg^{-1} f.w.

Photosynthetic parameters determination

A portable photosynthesis system (LC*i*, Leaf Chamber Analysis System), equipped with a square (6.25 cm^2) chamber, was used for CO_2 assimilation (*A*), transpiration rate (*E*) and stomatal conductance (g_s) measurements. Data collections were performed on eight fully expanded, randomly selected leaves of light treated plants at the end of the six-week period in the growth chambers for both light treatments.

Microscopy

Small pieces of fully expanded leaves from the light treatments were initially fixed for 3 h with 5% glutaraldehyde in 0.05 M phosphate buffer (25°C, pH 7.2). After washing in buffer, the specimens were post-fixed for 2 h with 2% osmium tetroxide, similarly buffered. Samples were dehydrated in an alcohol series followed by propylene oxide. The samples were then embedded in Spurr's (1969) resin. Semi-thin sections for light microscopy were obtained in a Reichert OM U_2 ultramicrotome, stained with toluidine blue O and photographed in a Zeiss III photomicroscope.

Morphometry

For the morphometrical assessment of the relative volume of the leaf histological components, a transparent sheet bearing a square lattice of point arrays, 10 mm apart, was laid over light micrographs of leaf cross sections (×800) and then the point-counting analysis technique was applied (Steer 1981). Similar sections were used to estimate the thickness of the leaf lamina and the contained tissues.

Extraction of soluble peroxidases-enzyme assays-protein determination

At the end of the 1st– 6th week during transition to flowering, apical immature leaves from each light treatment were weighed (about 2.0 g) and 50 mg of lyophilised tissue was extracted with ice cold buffer in the presence of liquid nitrogen (at a tissue to medium ratio of 1:5) consisting of 100 mmol L^{-1} K-phosphate buffer pH 6.5, containing 1.0 mmol L^{-1} PMSF and 10% (w/v) PVPP. When plants were in the flowering stage, the bract leaves were used as starting plant material and treated as above. The homogenate was clarified by centrifugation (20 min; 10,000×*g*). The supernatant was dialyzed against extraction buffer (except for PVPP) and was used as a soluble enzymes source.

Peroxidase activity was measured at 590 nm in a reaction mixture containing 0.1 M Na-phosphate buffer (pH 4.5), 1 μmol H_2O_2, 30 μmol 3-dimethylamino-benzoic acid (DMAB), 0.6 μmol 3-methyl-2-benzothiazolinone-hydrazone-hydrochloride monohydrate (MBTH) and enzyme extract (Ngo and Lenhoff 1980).

Protein content was determined according to Bradford (1976), using BSA (bovine serum albumin) as standard.

Polyacrylamide gel electrophoresis and activity staining

Extracts of leaves from the light treatments (4 μg protein per slot) were loaded on native polyacrylamide gel electrophoresis (PAGE) with a mini vertical slab gel [10% (w/v) separating gel (5.0 cm × 1.5 mm) and 5% stacking gel (3.0 cm × 1.5 mm)] according to Laemmli (1970) for anionic peroxidases and Reisfeld et al. (1962) for cationic peroxidase isoforms. Staining of anionic and cationic POD isoforms was conducted by incubating the gels in 50 mM sodium acetate buffer (pH 5.0), with 3-amino-9-ethyl-carbazole in the presence of 10 mM H_2O_2 (Syros et al. 2004a).

Gel development was stopped with distilled water. Gels were photographed over a white light box with Kodak T-Max 100 film.

Statistical analysis

Each light treatment consisted of 40 (4 × 10) plant replicates. The experiment was conducted twice and the reported data are the means of the two experiments. The experimental plan employed was the completely randomized block design. Analyses of variance were performed on the data using the SPSS-10 (SPSS, Chicago) program. The standard error or standard deviation of means and significant differences according to Duncan's multiple range tests at a significance level of $P \leq 0.05$ were calculated with the same software. For biochemical data, each value (carried out on complete two different extracts) is the mean of three replicates.

Results

Plant morphological parameters

Flower bud formation of *Petunia* × *hybrida* was achieved at the end of the 4th, 5th and 6th week after H, M and L light treatments, respectively, in the case of light intensity treatments. On the other hand, flower bud formation occurred at the end of the 6th week after FR light treatment, while no flower bud formation was noticed after the R and C light treatments. The shortest shoot length was noticed in the H light in comparison with the M and the L light treatments. In the case of H light treatment, plants were multi-branching in the flowering stage (results not shown). On the other hand, in the case of light quality treatments R light treated plants were shorter, while FR light treated plants were taller, in comparison with the C light treated plants (results not shown).

Chlorophyll concentration

Under various light treatments, Chla, Chlb and Chla+b concentrations were lower in the case of H and M light treatments as compared to L light treatment, while the above mentioned concentrations were similar to the C plants in the R and FR light treatments (Table 1).

Leaf anatomy

Leaves of L light treated plants were significantly thinner than those of H light treated plants and this was due to their thinner palisade parenchyma, spongy parenchyma and lower epidermis (Table 2, Fig. 1). In light quality treatments, leaves of both C and FR light treated plants were significantly thinner than those of R light treated plants (Table 2, Fig. 2). The latter had a thicker spongy parenchyma than leaves of both the C and the FR light treated plants and also a thicker palisade parenchyma than leaves of the FR light treated plants (Table 2, Fig. 2). The assessment of the relative volume percentages (%) of the leaf histological components (cross-section) revealed significant differences between H and L light treated plants (Table 3, Fig. 1). Leaves of the plants treated in H light were more compact (less voluminous intercellular spaces) with a higher volume of spongy parenchyma and a lower volume of upper epidermis than leaves of plants treated in L light (Table 3, Fig. 1). In the light quality treatments, leaves of the R light treated plants had higher relative volumes of both palisade and spongy parenchymas than those of the C and FR light treated plants. On the other hand, they had the lowest relative volume values of intercellular spaces, being the most compact (Table 3, Fig. 2).

Table 1 Chlorophyll concentration (Chla, Chlb, Chla+b; μg mL^{-1} mg^{-1} f.w.) of *Petunia* × *hybrida* in the end of the 6th week of light treatments

Treatment		Chla	Chlb	Chla+b
Light intensity	High	0.53 b	0.61 c	1.09 c
	Medium	0.66 b	0.85 b	1.50 b
	Low	1.36 a	1.42 a	2.76 a
Light quality	Control	1.17 a	1.48 a	2.65 a
	Red	1.30 a	1.39 a	2.68 a
	Far-red	1.29 a	1.42 a	2.71 a

Values are the mean of eight replicates. In each treatment column, the values represented by the same lower-case letters are not significantly different at $P \leq 0.05$ according to the Duncan's test

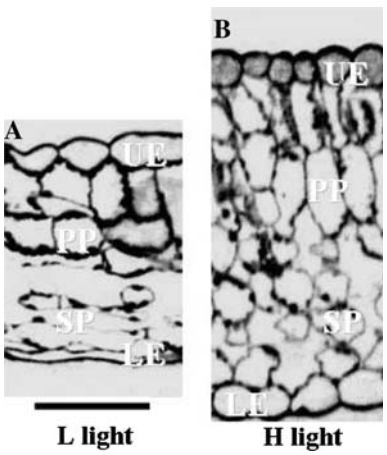

Fig. 1 Leaf cross-sections of *Petunia* × *hybrida* from the low (L) light (**a**) and high (H) light (**b**) intensity treatments. *UE* upper epidermis, *PP* palisade parenchyma, *SP* spongy parenchyma, *LE* lower epidermis. Bar equals 100 μm

Table 2 Thickness (μm) of the leaf and the contained tissues (cross section) of *Petunia* × *hybrida* as affected by light treatments (end of 6th week, $n = 8$, ±SD)

Treatment	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Lower epidermis	Total leaf thickness
Low light	28.2 ± 7.7 a	78.2 ± 11.0 b	95.1 ± 11.4 b	18.0 ± 5.7 b	219.5 ± 20.5 b
High light	28.3 ± 5.7 a	121.0 ± 6.3 a	132.7 ± 8.8 a	22.5 ± 6.0 a	304.5 ± 10.8 a
Control light	24.6 ± 11.4 a	84.3 ± 10.8 a	88.8 ± 14.8 b	23.5 ± 10.8 a	221.2 ± 15.4 b
Red light	23.4 ± 3.8 a	89.8 ± 15.1 a	121.7 ± 12.5 a	22.6 ± 7.0 a	257.5 ± 15.0 a
Far-red light	29.2 ± 8.5 a	73.1 ± 13.2 b	96.2 ± 18.3 b	26.5 ± 5.9 a	225.0 ± 6.8 b

In each column, within light intensity and light quality treatments, the values represented by the same lower-case letters are not significantly different at $P \leq 0.05$ according to Duncan's test

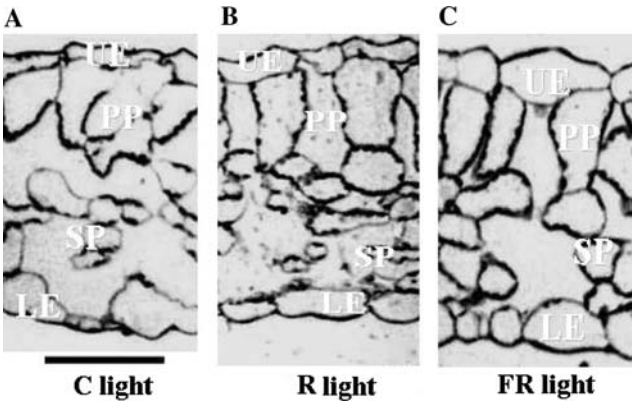

Fig. 2 Leaf cross-sections of *Petunia* × *hybrida* from the C light (**a**), R light (**b**) and FR light (**c**) quality treatments. *UE* upper epidermis, *PP* palisade parenchyma, *SP* spongy parenchyma, *LE* lower epidermis. Bar equals 100 μm

Photosynthetic parameters

A, *E* and g_s values in the case of the light intensity treatment were higher in the H light in comparison with the M and L light treatments (Table 4), while the above mentioned values of R and FR light were similar to C light in the light quality treatments (Table 4).

Peroxidase activity and PAGE

Peroxidase specific activity increased for both light intensity and light quality treatments from the 1st to the 6th week during transition to flowering (Fig. 3a, b). In detail this activity increased considerably from the 2nd–6th week after the H light treatment and from the 5th–6th week after both L and M light treatments (Fig. 3a). On the other hand, POD specific activity increased slightly from the 1st–6th week in all cases of light quality treatments (Fig. 3b). This activity was accompanied by the appearance of four (4) anionic (A_1–A_4) peroxidase isoforms (Figs. 4 and 5). The slow migrating A_1 isoform appeared slightly from the 1st to 2nd week after H, M and L light intensity treatments and appeared strongly from the 3rd–6th week after the H light treatment as well as from the 5th–6th week after the M light and at 6th week after the L light treatment (Fig. 4). The fast migrating A_2–A_4 isoforms appeared from the 3rd–6th week after the H light treatment as well as from the 5th–6th week and at the 6th week after the M and the L light treatments, respectively (Fig. 4). On the other hand, in the light quality treatments, all the abovementioned A_1–A_4 isoforms were only noticed under R light treatment (Fig. 5). The slow migrating A_1 isoform as well as the faster migrating A_2 and A_3 isoforms appeared from the 5th– 6th and at 6th week after the R and FR light treatments, respectively (Fig. 5). With regard to the response of A_4 isoform in light quality treatment, this appeared only after FR light treatment at 6th week. The above isoforms (A_1–A_4) did not appear in the C light treatment up to 6th week (Fig. 5). With regard to the cationic peroxidase isoforms, four (4) very faint isoforms (C_1−C_4) were noticed during transition to flowering after light intensity and light quality treatments (results not shown).

Table 3 Relative volume percentages (%) of the leaf histological components (cross section) of *Petunia* × *hybrida* as affected by light treatments (end of 6th week, $n = 8$, ±SD)

Treatment	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Lower epidermis	Mesophyll intercellular spaces
Low light	12.8 ± 1.2 a	35.6 ± 2.2 a	21.8 ± 2.9 b	9.0 ± 1.6 a	20.8 ± 3.5 a
High light	9.4 ± 0.9 b	32.4 ± 3.2 a	34.8 ± 2.3 a	9.0 ± 1.1 a	14.4 ± 5.3 b
Control light	11.7 ± 1.2 b	26.0 ± 2.0 b	18.3 ± 3.4 b	11.7 ± 1.2 a	32.3 ± 1.6 a
Red light	11.3 ± 2.6 b	33.0 ± 5.2 a	25.2 ± 1.4 a	10.0 ± 2.0 a	20.5 ± 5.7 b
Far-red light	14.6 ± 1.0 a	21.9 ± 1.3 b	20.9 ± 1.0 b	12.5 ± 2.2 a	30.1 ± 2.9 a

In each column, within light intensity and light quality treatments, the values represented by the same lower-case letters are not significantly different at $P \leq 0.05$ according to Duncan's test

Table 4 Photosynthetic parameters of *Petunia* × *hybrida* in the end of the 6th week of the light treatments

Treatment		A (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	g_s (mol.m^{-2} s^{-1})
Light intensity	High	5.34 a	3.24 a	0.18 a
	Medium	2.46 b	2.82 b	0.15 b
	Low	1.56 c	2.04 c	0.10 c
Light quality	Control	1.75 a	1.68 a	0.07 a
	Red	1.94 a	1.76 a	0.08 a
	Far-red	1.63 a	1.84 a	0.08 a

Values are the mean of eight replicates. In each treatment column, the values represented by the same lower-case letters are not significantly different at $P \leq 0.05$ according to the Duncan's test

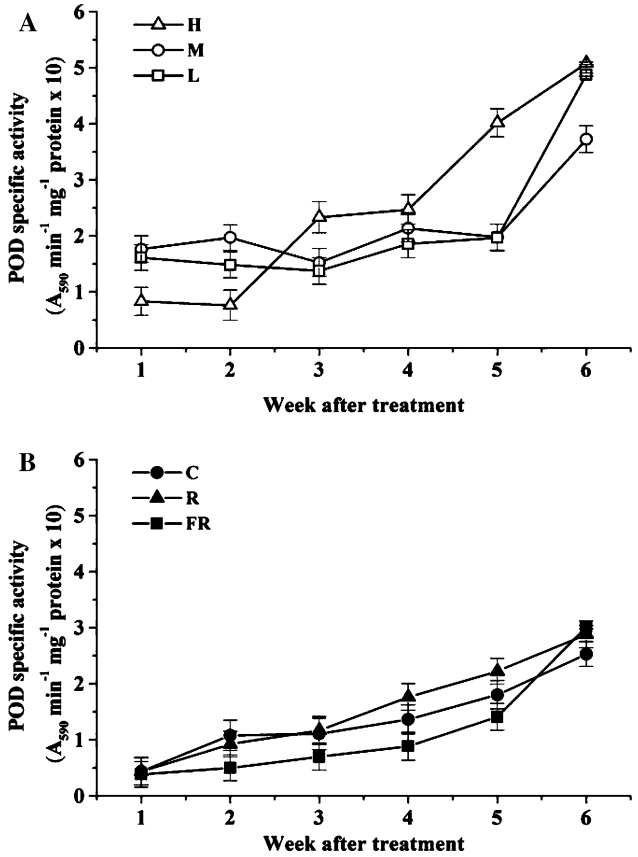

Fig. 3 Soluble peroxidase specific activity of apical immature leaf extracts during transition to flowering (from the 1st to the 6th week) in *Petunia* × *hybrida* from the light intensity (**a**) and light quality (**b**) treatments: The *vertical bars* indicate the standard error ($n = 6$)

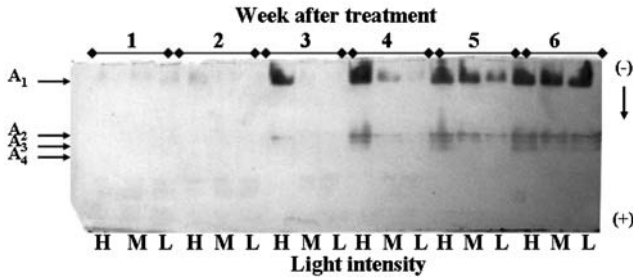

Fig. 4 Native-PAGE of soluble anionic peroxidase isoforms of apical immature leaf extracts during transition to flowering (from the 1st to the 6th week) in *Petunia* × *hybrida* from the light intensity treatments (*H* high light, *M* medium light, *L* low light). The isoforms appeared after staining the gels with 3-amino-9-ethyl-carbazole in the presence of H_2O_2

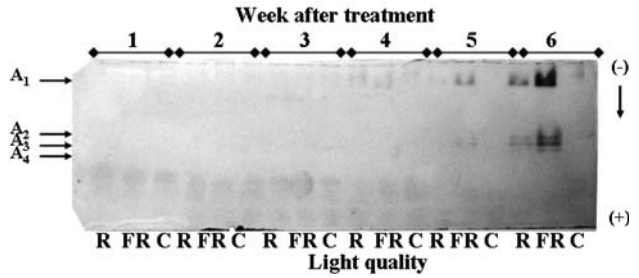

Fig. 5 Native-PAGE of soluble anionic peroxidase isoforms of apical immature leaf extracts during transition to flowering (from the 1st to the 6th week) in *Petunia* × *hybrida* from the light quality treatments (*C* control light, *R* red light, *FR* far-red light). The isoforms appeared after staining the gels with 3-amino-9-ethyl-carbazole in the presence of H_2O_2

Discussion

A fundamental objective of most commercial establishments growing bedding plants is to produce a flowering crop that meets the market quality standards in the shortest time possible. Growers may manipulate irradiance, photoperiod, temperature and other factors separately or in combination to achieve the desired results. The light treatments used in the present study played a crucial role in the flowering of *Petunia* × *hybrida*. The time for flowering is in the order: H < M < L light, while in light quality (R, FR and C light) treatments the time needed for flower bud formation is in the order: FR < C ≤ R light.

R and FR light receptors (phytochromes) and blue ultraviolet A light receptors (cryptochromes) regulate the shift from vegetative growth to floral development (Guo et al. 1998). During transition to flowering these photoreceptors, as well as the endogenous circadian clock, send signals which are integrated at the level of CONSTANS

(CO), a transcriptional regulator influencing positively the mRNA levels of FLOWERING LOCUS T (FT), which in turn activates the transition from vegetative growth to flowering (Valverde et al. 2004). Concerning the responses to light quality in LDP, flowering is most promoted when the reception of FR light occurs towards the end of the photoperiod and of R light during the early part (Thomas and Vince-Prue 1996). Light quality regulates FT via phytochromes B, D and E, acting independently of the circadian system. On the other hand, although it is considered that increased light levels have a positive effect on flowering (as in our experiments), the plant response to light quantity is very variable regarding flowering (Thomas 2006). Additionally, in light intensity treatments, H light corresponds to multi-branching plants with the shorter shoot length in comparison to the M and the L light, while in the case of light quality treatments, FR light treated plants were taller in comparison to R and C light (results not shown). Additionally, EOD FR light hastened flowering, while R light delayed it (results not shown). Similar observations after R or FR light treatments were reported (Ilias and Rajapakse 2005a). According to our results a compact plant shape with optimal flowering times could be achieved after H light treatment.

Light irradiation has been found to cause physiological and metabolic changes in leaves (Boardman 1977; Barreiro et al. 1992; Hanba et al. 2002; Oguchi et al. 2005; Liao, et al. 2006). Leaf Chl is an important component in plant physiological acclimation to different light intensities (Caesar 1989; Feng et al. 2004). H and M light treatments correspond to lower chlorophyll concentration (Chla, Chlb, Chla+b; Table 1) and a thicker leaf (Fig. 1, Table 2 and results not shown) in comparison to L light treated plants. This may be attributed to the ability of plants to activate several mechanisms involved in acclimatization to changing light levels, even more at very low light intensity treatments (Fisahn et al. 1995). Leaves treated with FR light may have lower Chl content, both on an area or on a dry weight basis (Caesar 1989). Similar results were reported for leaves of *Acer* species and other deciduous trees treated under high and moderate light intensity (Hanba et al. 2002; Oguchi et al. 2005). Spectral quality affects leaf anatomy, while physiological responses to spectral changes vary among plant species (Boardman 1977). Leaf thinning under shaded conditions has been attributed to an increase in the ratio of R:FR light (Boardman 1977; Barreiro et al. 1992) or to a decrease in the total photosynthetic photon flux (PPF) (Oguchi et al. 2005), while spectral quality appears to have the greatest impact during leaf expansion (Schuerger et al. 1997).

The highest values of A in H light in comparison to M and L light treated plants (Table 4) could be due to both larger volume of the photosynthetic tissue (thicker mesophyll; palisade and spongy parenchyma) (Table 3, Fig. 1) and higher light intensity. Decline of A from H to L light can be partly related to stomatal closure (Passioura 1988) and/or to non-stomatal factors (Shangguan et al. 1999). Although R light in comparison to C and FR light treated plants displayed larger volume of photosynthetic tissue (Table 3, Fig. 2) and had a more voluminous photosynthetic tissue and less mesophyll intercellular spaces (Table 3, Fig. 2), those *A* values were not different (Table 4). This could be due to the fact that the light intensity in our light quality treatments was low (see materials and methods; light quality treatments). There were no differences in photosynthesis in tobacco plants irradiated with R or FR light at the EOD (Kasperbauer and Peaslee 1973), while leaf anatomical changes under high light intensity are reflected in higher assimilation rate values (Barreiro et al. 1992; Schuerger et al. 1997). Decline of *E* values from H to L light treated plants (Table 4) could be related to a higher vapor pressure, since *E* is primarily a function of vapor pressure gradient and leaf stomatal conductance (Alexander et al. 1995). With regard to g_s values, they were lower in the L light treatment in comparison to M and H light (Table 4). This decline in g_s may have derived from the direct and/or the indirect effect of water limitation on the stomatal guard cells at low light intensity. Similar to *A* values (see above), *E* and g_s values were almost similar in all light quality treatments.

In all cases of light treatments, an increase of POD specific activity was noticed during transition to flowering (Fig. 3). In any case, the time of flower bud formation on *Petunia* × *hybrida* under light intensity and light quality treatments could also be related with the appearance of four (A_1–A_4) anionic POD isoforms (Figs. 4 and 5). Upon gel electrophoresis of leaf extracts from *Petunia* × *hybrida*, peroxidase *a* (PRX*a*) is visualized as a group of three slow migrating anionic POD isoforms (van den Berg and Wijsman 1981). PRX*a* occurs in a single form PRX*a*1.1, whereas the more anodal forms, PRX*a*1.2 and PRX*a*1.3, appear in the course of the leaf development. Additionally, Hendriks and van Loon (1990) reported that the above-mentioned *Petunia* × *hybrida* peroxidase *a* (PRX*a*) is a soluble extracellular glycoprotein, most prominently present in the epidermis of leaves and stems. Generally, among POD isoforms, anionic PODs are most likely involved in lignification (Chen et al. 2002; Syros et al. 2004a; Syros et al. 2005a), while cationic PODs have been reported to be able to degrade IAA (Gazaryan et al. 1996). Since PODs displayed low substrate specificity in vitro, a specific function for a particular peroxidase has only been proven in few cases (Hiraga et al. 2001). For example, in *Pharbitis nil* the appearance of cationic peroxidase isoforms in shoot apices has been shown to be correlated with flowering (Nakanishi and Fujii 1992). Changes in POD isoform

patterns in the leaves and the shoot apex were found during flower induction on several herbaceous species (Gaspar et al. 1975; Karege et al. 1982; Goto and Hamada 1988; Nakanishi and Fujii 1992; Bernal et al. 1993). In contrast, neither the enzyme activities nor the isoform patterns were useful markers for the developmental stages of the buds and leaves in relation to flowering in *Citrus unshiu* (Monerri and Guardiola 2001). According to our results, the first appearance of both A_1 and A_2 anionic POD isoforms from the end of the 3rd–5th week for H, M and L light, respectively (Fig. 4) and on the end of the 5th and 6th week for R and FR light treatments, respectively, (Fig. 5) could be used as potent biochemical markers of flower bud formation and it seems to correspond to the abovementioned PRX*a* (Van den Berg and Wijsman 1981; Hendriks and van Loon 1990). The information about how light quality (R or FR light) affects the oxidative metabolism is very scarce but POD isoforms may be also taken as evidence for an enhanced antioxidative capacity of high light acclimated leaves against reactive oxygen species (ROS) which might be formed in the chloroplast under high light conditions (Mishra et al. 1995; Burritt and Mackenzie 2003). With regard to cationic peroxidase isoforms, their intensities were very faint and the electrophoretic patterns were unchanged during transition to flowering (results not shown) and consequently were not related with flower bud formation.

The results report in this paper suggest that the production of high quality *Petunia* × *hybrida* plants with optimal flowering times would be approached, by the scheduling of light intensity and light quality treatments, on the basis of morphological, leaf anatomical and photosynthetic responses and the appearance of A_1 and A_2 anionic POD isoforms on the immature leaves of the apical shoots.

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