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Physiological dissection of blue and red light regulation of apical dominance and branching in M9 apple rootstock growing *in vitro*

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Abstract

This paper presents the results of the interaction of red light (R) with blue light (B), applied to shoots of M9 apple (*Malus pumila* paradisiaca Schmid) rootstock, on the regulation of stem growth, apical dominance and branching. These results are compared with the active form of phytochromes (PHYs) under monochromatic and dichromatic light treatments. The inhibition of internode elongation was determined by PHY photoequilibrium, with the additional effect of B, by means of a separate photoreceptor. The development of phytomers appeared to be primarily due to the active form of PHY, with a marginal effect from B. Shoot growth, which combines internode elongation and development of the phytomer, was highest under R and lowest under B and far red light (FR), showing the largely positive role of PHY photoequilibrium. Under FR, reduced stem elongation was due to the very small number of phytomers formed. Apical dominance was inhibited, while branching was increased under R, corresponding to the highest values of PHY photoequilibrium determined. Apical dominance was increased and branching was reduced by B, indicating a complex interaction between PHY and B receptor. In the shoot cluster system, photomorphogenic behavior was dependent on the time of exposure to the different light qualities. The information gained from the study will be helpful in improving the set up of *in vitro* growth light conditions, and in providing useful insights into research of the development of the woody plant canopy, an important factor in ecological plant communities.

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Introduction

The shoot is a modular system, the base unit of which is formed by the phytomer, consisting of an internode, a leaf and an axillary meristem (AM) (Carles and Fletcher, 2003). The interaction between internal and external factors influences shoot apical meristems, regulates apical dominance, and consequently, the outgrowth of AM and branching (McSteen and Leyser, 2005). According to classical theory, both an apical source, usually young apical leaves synthesizing auxin, and basipetally polar auxin transport are required to influence the supply of an acropetally moving cytokinin, and to stabilize apical dominance (Ljung et al., 2001; Leyser, 2003). This theory is supported by experiments where the inhibitory effect of the correlative inhibition of the apex is removed by decapitation, while correlative inhibition can be restored by the application of auxin (Cline, 1996). In *Arabidopsis*, Chatfield et al. (2000) confirmed the influence of both auxin and cytokinin. Auxin polar transport is affected in a light-dependent manner (Jensen et al., 1998).

Light quality regulates a variety of plant development pathways from germination to flower induction and fruit development (Smith, 1994). The plant translates the complex set of light quality signals into biochemical signals, by means of a discrete number of photoreceptors, such as the UV-A/blue light (B) receptors, the cryptochrome (CRY) and phototropin (PHOT) families (Banerjee and Batschauer, 2005), and the red/far red light (FR) receptors, the phytochrome (PHY) family (Banerjee and Batschauer, 2005). In addition, PHYA has also been found to mediate various B responses (Lin, 2000). PHYs act in detecting mutual shading through the changed quality of natural radiation and change in R:FR ratio, and in appropriately redirecting growth and development according to survival strategies (Aphalo and Ballaré, 1995; Gilbert et al., 2001), modulation of apical dominance and of AMs (Ballaré, 1999). CRY1 is thought to be the CRY responsible for the blue high-irradiance response, inhibiting stem plant growth and reducing internode elongation, whereas CRY2 is likely responsible for the inhibition due to the blue low-irradiance response (Lin, 2000). PHOT1 and PHOT2 are involved in auxin polar transport, modulation of auxin sensing and phototropism (Esmon et al., 2005).

Research carried out during the last few years has shown that light quality regulates several morphological characters of plants grown *in vitro*, such as leaf anatomy and leaf size in birch; leaf growth and chlorophyll content in *Cymbidium* (Sæbø et al., 1995; Tanaka et al., 1998); stem elongation in chrysanthemum, strawberry, tomato and *Pelargonium* (Mortensen and Stromme, 1987; Appelgren, 1991; Nhut et al., 2003); axillary shoot formation in grapevine (Chéé, 1986); and rhizogenesis in pear (Bertazza et al., 1995). In plum, the dynamics of shoot branching appeared to be influenced to differing extents by B and R (Muleo et al., 2001). B increased the formation of phytomers and decreased bud outgrowth, while R decreased phytomer formation but reduced the strength of apical dominance, thus increasing bud outgrowth. Some *in vitro* studies, aimed at increasing shoot proliferation, have highlighted the interaction between growth regulators and light quality. For example, on plum clone GF 655-2, 6-benzylaminopurine promoted proliferation only in the presence of light (Baraldi et al., 1988). In *Spiraea nipponica* the interaction between cytokinins and R resulted in an enhancement of the shoot proliferation rate (Norton et al., 1988). Further, when explants were exposed to combinations of R+FR, shoot proliferation was greater (Herrington and McPherson, 1993).

Little is known about the functional role of the photoreceptors of woody fruit crop plants, mainly due to the lack of mutants. In this article, we present a report on the interaction between B and R and their photoreceptors in the photoregulation of M9 apple (*Malus pumila* paradisiaca, Schmid) shoots cultured *in vitro*. The primary objective of the present study was to determine whether different photomorphogenic mechanisms regulate apical dominance, phytomer formation, outgrowth of AMs and branching, while maintaining the equivalent irradiance density of B, R and FR.

Materials and methods

Plant material and culture conditions

Shoot tips of the M9 apple (*M. pumila* paradisiaca, Schmid) clonal rootstock, about 1 cm long and uniform in vigor, with three phytomers and mean fresh weight of about 10 mg, were collected from cultures that had been established to *in vitro* conditions for roughly 1 year. The culture medium used was DKV (Driver and Kuniyuki, 1984) supplemented with 20 g L^{-1} of sucrose and 10 g L^{-1} of sorbitol, 8.86 μM benzyladenine, 0.53 μM gibberellic acid and 0.3 μM indol-3-butyric acid. The medium was sterilized at 120 °C for 20 min after addition of 5 g $L^{-1}$of pectine and 4.5 g L^{-1} Bacto Agar (Difco) and pH tritation to 5.7. Experiments were performed in growth cabinets maintained at a temperature of 21 ± 1 °C and a 16 h photoperiod. The temperature in each growth cabinet was controlled by an air flux system with the exception of