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Light emitting diodes (LEDs) affect morphological, physiological and phytochemical characteristics of pomegranate seedlings

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ABSTRACT

The impact of light-emitting diodes (LED) on developmental, physiological, and phytochemical characteristics of pomegranate (*Punica granatum* L.) seedlings was investigated. Five LED lights [L20AP67 (moderate blue and red, high green), AP673L (moderate blue, high red and red:far-red), G2 (low blue, high red and far-red), AP67 (moderate blue, high red), and NS1 (high blue and green, low red, high red:far-red, 1% UV) with wide and continuous radiation spectra were used. Fluorescent [FL (high blue and green, low red)] tubes constituted the control treatment. Among treatments, seedlings grown under L20AP67 exhibited the best morphological and growth characteristics (rapid height increase, longer roots, greater fresh and dry weight, greater leaf area). Root activity was comparable among the various treatments, with FL exhibiting the lowest arithmetic value. A variant effect on pigments and secondary metabolites was recorded with the highest chlorophyll and carotenoid content detected under FL, flavonoid under G2 and AP67, and anthocyanin under G2, AP67 and NS1. In addition, NS1 produced the highest total phenolic compound content. Root growth capacity (RGC) was monitored in specialized chambers, then assessed to evaluate the transplanting capacity. Transplanted seedlings of AP67 had the longest, of L20AP67 the heaviest newly formed roots, thus securing a successful transplantation. The herein undertaken study demonstrates that certain LEDs promoted developmental characteristics in pomegranate more efficiently than conventional FL. In particular, the use of L20AP67 was proven a promising tool for producing robust pomegranate seedlings, while some of the other LEDs tested apparently imposed a stressful situation on plants.

1. Introduction

In order to increase photoperiod and subsequently photosynthesis in protected crops, various lamp types such as fluorescent (FL), high-pressure sodium, incandescent and metal halides are used as supplementary light sources. These lamp types increase the photosynthetic active radiation (PAR) levels, yet they are not as energetically efficient as desired. Further, they do not offer the option of spectral manipulation which is of uttermost importance for plant growth and development (Schuerger et al., 1997). For some years, light-emitting diodes (LEDs) facilitated photobiology studies and were proven an efficient light source for commercial crop production (e.g. Nhut et al., 2003). Compared to a conventional light source, a LED has smaller weight and volume and longer lifespan (about 5×10^4 versus $7–15 \times 10^3$ h of conventional lights). Its spectral efficiency is continuously increased (Bourget, 2008; Morrow, 2008). In addition, heat produced by LEDs can be dissipated through an external source, thus these lights can be placed close to the canopy without increasing the plant temperature or the risk of causing burns (Bourget, 2008; Massa et al., 2008; Morrow, 2008). A number of plant species has already been tested using LED lights, in particular tree species (e.g. wild cherry, holm oak and beech; Astolfi et al., 2012), but also fruits, vegetables and ornamental plants such as lettuce (Johkan et al., 2010), cucumber (Hogewoning et al., 2010), *Phalaenopsis*, roses, chrysanthemums and campanulas (Ouzounis et al., 2014a, 2014b).

It has been shown that plant responses to light are modulated by a signal transduction through various photoreceptors (e.g. phytochrome, cryptochrome and phototropins). These photoreceptors perceive,

Abbreviations: B, blue light; G, green light; R, red light; FR, far red light; W, white light; FWL, FWS, FWR, leaf, shoot, root fresh weight; DWL, DWS, DWR, leaf, shoot, root dry weight; NRDW, new root dry weight; NRL, new root length; RGC, root growth capacity; R:S, root: shoot ratio; TPC, total phenolic content

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interpret, and transduce light signals via distinct intracellular signaling pathways, regulating photoresponsive nuclear gene expression and ultimately leading to adaptive changes at the metabolic, the cell and the whole organism level (Li et al., 2011). Among these changes, alterations in phytochemical content are mostly affected by the light quality and intensity. In particular, UV-A increased anthocyanin biosynthesis in grape (Kataoka et al., 2003) and lettuce (Tsormpatsidis et al., 2008), blue (B) light positively influenced carotenoid and anthocyanin content in lettuce and anthocyanin biosynthesis in tomato (Giliberto et al., 2005), red (R) light increased phenolic and anthocyanin content in cranberry fruits (Zhou and Singh, 2002), while supplemental far red (FR) LED decreased carotenoid and anthocyanin accumulation in lettuce (Li and Kubota, 2009). With respect to chlorophyll content, both B and R light promoted the content in lettuce (Chen et al., 2016) and B light in *Phaleanopsis* (Ouzounis et al., 2014a). Also carotenoids:chlorophyll (car:chl) ratio seems to be highly affected by light intensity, being higher in sun exposed leaves (Valladares et al., 2003).

Chlorophylls along with carotenoids and xanthophylls are the main contributors to photosynthesis, and any alteration of these phytochemicals caused by light spectrum changes predominately affects the photosynthetic process. Actually, plants grown under a combination of R (660 nm) and B (470 nm) LEDs sustained higher leaf photosynthetic rates than did leaves from plants grown under R LEDs alone (Matsuda et al., 2004) or other monochromatic source. Moreover, changes in the B and R regions could trigger changes of photosynthetic performance, which in turn has an impact on growth and development (Ouzounis et al., 2014a). Indeed, processes such as germination, stem elongation, stomatal opening, phototropism, vegetative growth and flowering, root development and leaf enlargement, are found to be differentially affected by different light spectra (Massa et al., 2008).

Apart from changes on growth and development, phytochemical accumulation is linked to the plant adaptation to biotic and abiotic environmental regimes. In particular, carotenoids and xanthophylls extend chlorophyll life through hindering chlorophyll photooxidation, obtained via energy dissipation and radical detoxification when plants are exposed to excessive light intensities. As a result, they limit membrane damage and leaf bleaching (Deleris et al., 2006). Moreover anthocyanins, being members of the flavonoid group, act as defensive and signaling compounds as well as protective agents against UV radiation and oxidative damage by neutralizing reactive oxygen species (ROS) (Kefeli et al., 2003; Lattanzio et al., 2006). Thus, oscillations in their accumulation in plant tissues in response to different spectral manipulations reveal their importance for plant adaptation under various environmental cues and especially under different lights, and also unveil whether a light combination imposes stressful conditions to plant growth. This is crucial information for defining the proper light combination for growth and production in crop plants.

Pomegranate (*Punica granatum* L.) is a shrub or small tree obtaining a height from 5 to 10 m. It is cultivated all around the world, yet the main centre of its cultivation is the Mediterranean region, followed by Asian and former USSR countries (Texeira da Silva et al., 2013). Parts of the tree have been used for dyeing fabrics and as a tannin source for repairing leather. Pomegranate fruits are eaten fresh and used as well for the production of juice that is consumed worldwide. They are a good source of anthocyanins (Fischer et al., 2011) and are characterized by high phenolic content and antioxidant activity (Madrigal-Carballo et al., 2009; Ozgen et al., 2008; Qu et al., 2010; Zaouay et al., 2012). Our interest is thus focused on pomegranate since, thanks to its multiple usages, it holds a significant place among the various farming systems.

As mentioned above, exposure to different wavelengths induces a number of responses in angiosperms. The signaling pathway components for phytochromes and cryptochromes vary, leading to different responses in different plant organs and species (Weller et al., 1997, 2000). Consequently, it is generally accepted that the effects of specific wavelengths on plant morphology, physiology and probably on secondary metabolism are species-dependent. Since both, plant species and light quality affect this variability, special studies are needed in order to elucidate the optimal LED combinations which can enhance productivity in high value crops and /or improve special managing procedures, such as robust seedling production. There is, therefore, an important scope in examining the effect of light spectra on plant behaviour, in our case on pomegranate, the ultimate goal being the pinpointing of the most beneficial one for growth. The imposed effect of light is even more crucial during plant's juvenile stage, as production of robust seedlings shall minimize infections and subsequently maintain productivity in the field. Consequently, any manipulation of light that might satisfy the above requirement is of uttermost importance.

The present study centers on the effects of varying light spectra on growth and on a number of the aforementioned parameters of pomegranate seedlings, information that, to our knowledge, is currently unavailable. Nowadays there is growing interest toward the use of LEDs in agriculture (Cocetta et al., 2017). The research hypothesis under consideration was to test if LED artificial lighting can improve developmental characteristics of pomegranate seedlings to a greater extend than FL lights. To this end, the herein undertaken study focused on the following three objectives:

1) The investigation of the effects of LED light sources with different emission spectra on growth and physiology of pomegranate upon germination as compared to FL (control),
2) The determination of the most efficient among the tested light treatments in the pre-cultivation of pomegranate seedlings and,
3) The influence of LED lighting on pomegranate transplantation capacity.

To this aim, six different light spectra are evaluated for the purpose and their effectiveness is gauged by monitoring a comprehensive list of plant morphological, physiological and phytochemical parameters.

2. Materials and methods

2.1. Plant material, growth conditions and light treatments

The study was carried out at the Forest Research Institute in the provenance of Thessaloniki, Greece, during 2013–2014. Ripe pomegranate (*Punica granatum* var. Wonderful) fruits were collected from Riza, Chalkidiki provenance (40°30′214″N, 23°26′612″E), Greece, in October 2013. The fruits were depulped and the seeds were stored in polyethylene bags at 4 °C. For breaking dormancy, pomegranate seeds were hydrated for 24h and placed at 4 °C for two months in Petri dishes containing moist sand. Seeds were afterwards transferred for three weeks in a phytotron chamber (20 °C, 70% RH, 8h photoperiod) for warm stratification (Piotto, 2003). The germination percentage after three-weeks maintenance in the phytotron chamber was up to 46%. Pre-germinated seeds were then placed in plastic mini-plug container trays (QP D 104 VW, Herkuplast Kubern GmbH, Germany). The trays with identical dimensions (310 × 530 mm, density: 630 seedlings m^{-2}, 27 cc), were filled with enriched peat (pH 6.0). This substrate is suitable for growing young seedlings and transplanting material (TS1, Klassmann-Deilmann GmbH, Geeste, Germany).

The mini-plug containers with the pre-germinated pomegranate seeds, were transferred to environmentally controlled growth chambers set at a 14 h photoperiod, 20 °C and 80 ± 10% air relative humidity. Photosynthetic photon flux density (PPFD) was maintained at around 200 ± 20 $\mu mol\ m^{-2}\ s^{-1}$ at plant height. Automated water sprinklers were used every day for watering. The containers were rotated once per day in order to ensure similar growth conditions. FL and LED lighting was applied for an experimental period that lasted six weeks (42 days). LED lights, manufactured by Valoya (Valoya Oy, Helsinki, Finland), generated a wide continuous spectrum which consisted of a mixture of ultraviolet (UV, < 400 nm), blue (B, 400–500 nm), green (G, 500–600 nm), red (R, 600–700 nm) or far-red (FR, 700–800 nm). The