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Preliminary Study on the Effect of LED Light and Cytokinin on the Growth of Pear Plants In Vitro

Nataliya Dimitrova[1*], Lilyana Nacheva[1], Yulia Kyoseva[2], Małgorzata Berova[2]

1 - Fruit Growing Institute, Department of Breeding, Genetic Resources and Biotechnology,
12 Ostromila Str., 4004 Plovdiv, BULGARIA
2 - Agricultural University, Faculty of Agronomy, Department of Plant Physiology, Biochemistry and Genetic, 12 Mendeleev Str., 4000 Plovdiv, BULGARIA
[*]Corresponding author: natali.dimitrova@yahoo.com

Abstract. In the past two decades, light emitting diodes (LED) have become an alternative source of light for plant tissue culture, due to their low energy consumption, low heat emission, specific wavelength irradiation etc. The effect of three LED lights (white, blue and mixed) and two cytokinins (6-benzylaminopurine or *meta*-topolin) on the growth of pear (*Pyrus communis* L. 'OHF 333') *in vitro* was studied. The plantlets were cultivated in microboxes on a modified MS solid medium supplemented with 2.5 µM 6-benzylaminopurine (BAP) or *meta*-Topolin (mT). The plantlets were grown in controlled room using Philips GreenPower LED research module. Three groups of LEDs emitting in white (W), blue (B), mixed (W:R:B:far-red=1:1:1:1) (BR) lights were applied. Biometric parameters, content of photosynthetic pigments and gas-exchange analysis of the plants were measured after three four weeks passages under corresponding light/cytokinin treatment. The results obtained indicated that different LEDs and cytokinin specifically affected the growth and development of *in vitro* cultured pear plants. The highest fresh and dry mass distinguished the plants grown under white LED light with both cytokinins studied. The maximum values for plant height was achieved in plants grown under white LED light with BAP and blue LED light with mT. The leaf sizes of plants grown on mT enriched medium were larger than those grown on BAP enriched medium, regardless of light and the largest were the leaves of plants grown under white LED light. Also, plants grown with mT in the nutrient medium showed more intensive photosynthesis, with the difference between the white and mixed LED light being insignificant.

Key words: micropropagation, shoot culture, *in vitro*, light quality, blue light, photosynthetic pigments, *meta*-Topolin.

Introduction

One of the most important factors influencing the growth and morphogenesis of plant cells *in vitro* is light and the nutrient medium, as well as phytohormones composition.

Fluorescent lamps (FL) are the most commonly used light source in *in vitro* cultivation of plant cells and tissues. But in the past two decades, light emitting diodes (LED) have become an alternative source of light for plant tissue culture, due to their low energy consumption, low heat emission, specific wavelength irradiation etc. (Bourget, 2008; Morrow, 2008). Many authors reported the successful applications of LEDs in promoting *in vitro* growth and morphogenesis from various plant species (Gupta & Jatothu, 2013). Under various LED treatments improvement in shoot organogenesis, *ex vitro* survival rate and biomass yield have been demonstrated (Hahn et al., 2000; Nhut et al., 2003; Jao et al., 2005; Shin et al., 2008; Li et al., 2010; Gupta & Sahoo, 2015). According to Muneer et al. (2018) red and blue LEDs play a significant role in overcoming hyperhydricity in carnation often observed under fluorescent light.

Several authors reported better *in vitro* responses of different species when a combination of red and blue LED light was used (Nhut et al., 2003; Azmi et al., 2014; Ferreira et al., 2017). However, the spectral composition and photosynthetic photon flux density (PPFD) specifically affect the growth and morphogenesis of different plant species. Furthermore, the research with woody species and in particular fruit species are too limited - *Populus* (Kwon et al., 2015), *Castanea* (Park & Kim, 2010) as the studies mainly refer to somatic embryogenesis - *Pinus* (Merkle et al., 2005; Kim & Moon, 2014), coffee (Mai et al., 2016). The stimulating effect of red LED light on the length of the shoots and leaf area in pear multiplication has recently been reported (Lotfi et al., 2019). The growth and development

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of plant cells in *in vitro* culture is largely determined by the plant growth regulators used, in particular cytokinins (CKs) due to their importance for cell division and cell expansion (Howell et al. 2003, Aremu et al. 2012). Currently N^6-benzyladenine (BAP) is the most widely used aromatic cytokinin in the micropropagation industry because of its effectiveness and affordability (Bairu et al., 2007; Aremu et al., 2012). But at a high concentration (BAP) has disadvantages such as hyperhydricity (Leshem et al. 1988, Teramoto et al. 1993, Magyar-Tabori et al. 2010), stunted growth, epigenetic and somaclonal variation in some crops (Harrar et al. 2003, Werbrouck, 2010, Smulders & De Klerk 2011).

Recently monomethoxy derivatives of 6-benzyladenine and 6-benzyladenosine were also isolated and identified from several different plant sources and their high cytokinin activity has been confirmed (Tarkowska et al. 2003). Podwyszyńska et al. (2012) reported that *meta*-methoxytopolin (MemT) and its riboside (MemTR), improved the micropropagation and shoot quality of smoke bush (*Cotinus coggygria* Scop.).

In our previous study, a good multiplication rate and high quality shoots were found at 6-9 µM mT treatment of pear rootstock OHF 333 (Dimitrova et al., 2016). The use of *meta*-topolin resulted in improvement of the leaf gas exchange and low content of phenols, as well as in the total antioxidant activity. These results indicate that the slight structural difference between BAP and mT could have a profound impact on plants during micropropagation and *meta*-methoxytopolins could be considered an alternative to other commonly used cytokinins in micropropagation of recalcitrant species. In this study, the effect of three LED lights (white, blue and mixed) and two cytokinins (6-benzylaminopurine or meta-Topolin) on the *in vitro* growth of pear (*Pyrus communis* L. 'OHF 333') was studied.

Materials and Methods

Plant material and experimental conditions

The experiment was carried out on pear rootstock (*Pyrus communis* L. 'Old Home' x 'Farmingdale' 333), which was characterized by good compatibility with most of the pear cultivars, high yields and a moderate degree of resistance to fire blight (Lombard and Westwood, 1987; Wertheim, 2002). *In vitro* culture was maintained at 3-week subculture intervals as described previously (Nacheva et al., 2009). Briefly, shoots were grown in microboxes with green filter ($SacO_2$, Belgium) on a modified MS (Murashige & Skoog, 1962) solid medium with ½ concentration of NH_4NO_3 and $CaCl_2$ and 1000 mg L^{-1} $Ca(NO_3)_2$, supplemented with 2.5 µM 6-benzylaminopurine (BAP), 0.05 µM IBA, 30 g L^{-1} sucrose, 6.5 g L^{-1} Phyto agar (Duchefa, The Netherlands). The medium (pH 5.6) was autoclaved at 121°C for 20 min. In each container on 100 mL of culture medium 10 shoot tips with a length of 7-8 mm with two leaves were set. The cultures were incubated in the growth room at an air temperature of 22±2°C with 16/8 h hours photoperiod supplied by cool-white fluorescent lamps (OSRAM 40W; 50 µmol $m^{-2}s^{-1}$ PPFD).

For the purpose of the present experiment the plantlets were cultivated *in vitro* on above mentioned basal nutrient medium, supplemented with 2.5 µM 6-benzylaminopurine (BAP) or 2.5 µM *meta*-Topolin (mT) at 22±2°C using an illumination system based on Philips GreenPower LED research module (16-h photoperiod with 80-95 µmol m^{-2} s^{-1} PPFD). Three groups of LEDs emitting in white (W), blue (B), mixed (1:1:1:1 far-red) lights (BR) were applied.

Growth parameters

Data on fresh (FW) and dry (DW) mass, number and length of shoots, leaf characteristics (the length and width of the first fully developed leaf), content of photosynthetic pigments was evaluated in six passages of three weeks of culture.

Photosynthetic pigments content

The photosynthetic pigment (chlorophyll *a*, chlorophyll *b* and total carotenoids) content in plantlets was determined. For pigment extraction, 250 mg of fresh material was extracted in the dark with 10 ml 85% acetone cooled up to 4°C. The pigment content was determined spectrophotometrically, and calculated according to the formulae of Lichtenthaler & Wellburn (1983).

Gas-exchange analysis

Gas-exchange analysis was performed on the all ten plants in one vessel. Measurements were taken with a LCpro + portable gas exchange system (ADC, UK) at a light intensity of about 180 µmol m^{-2} s^{-1} PPFD and a temperature of 25°C. Net photosynthesis rate (A, µmol CO_2 m^{-2} s^{-1}), transpiration intensity (E, mmol H_2O m^{-2} s^{-1}) were determined.

Statistical analysis

For each light treatment three replications, each containing ten shoots was tested and the experiment was repeated three times. Statistical analysis of physiological parameters was performed using a one-way ANOVA using the Tukey test to validate the different significance at $p \leq 0.05$.

Results and Discussion

The results obtained in this study indicated that LED sources with different light and cytokinin applied specifically affect the growth and development of *in vitro* cultured pear plants (Fig. 1, Table. 1). Due to the relatively low concentration of applied cytokinin (2,5), no significant differences were observed between the studied variants in the number of newly formed shoots. The plantlets grown in blue light with BAP had the longest shoots, followed by those in white light with BAP and mixed light (BR) with mT, but the differences between them were not statistically proven.

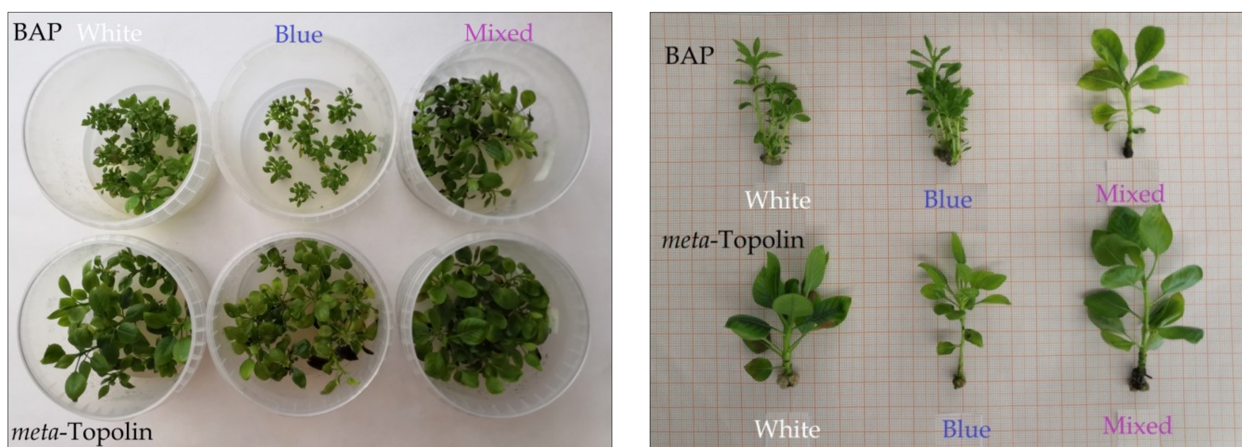

Fig. 1. Appearance of pear plantlets grown with BAP or mT in the nutrient medium at different light treatments. **W** white LEDs, **B** – blue LEDs, **BR** – mixed LEDs.

The highest number of leaves was reported in plants cultured in mixed light in the presence of BAP in nutrient medium (10.4) and the lowest – with mT under blue light (5.7). Despite some fluctuations in the fresh mass (FW) of the plants, there was no significant difference between the different treatments. However, the highest values of DW were reported in white LED light in both types of cytokinins used.

Table 1. Growth indices measured in pear plants grown with BAP or mT in the nutrient medium at different light treatments. **W** white LEDs, **B** – blue LEDs, **BR** – mixed LEDs. For each column, different letters indicate significant differences at $p \le 0.05$.

LED Light	Cytokinin	Shoot length /mm/	Number of shoots	Number of leaves	FW /g/	DW /g/
White	BAP	20.76 [a]	1.2 [a]	7.3 [ab]	1.20 [a]	0.34 [ab]
BR	BAP	11.98 [c]	1.6 [a]	10.4 [a]	0.67 [a]	0.15 [b]
Blue	BAP	21.96 [a]	1.6 [a]	9.1 [ab]	1.17 [a]	0.29 [ab]
White	mT	15.96 [b]	1.0 [a]	9.2 [ab]	1.55 [a]	0.47 [a]
BR	mT	20.58 [a]	1.0 [a]	7.5 [ab]	0.98 [a]	0.18 [b]
Blue	mT	15.90 [b]	1.1 [a]	5.7 [b]	1.28 [a]	0.34 [ab]

The plants at mixed light, regardless of the cytokinin applied in the nutrient medium, had the lowest dry biomass. The leaves of plantlets, grown with mT in nutrient medium, were larger regardless of the type of light (Fig. 2, 3). The combination of the four types of light (BR) favorably affected the growth of leaf lamina and is a prerequisite for more intense photosynthesis.

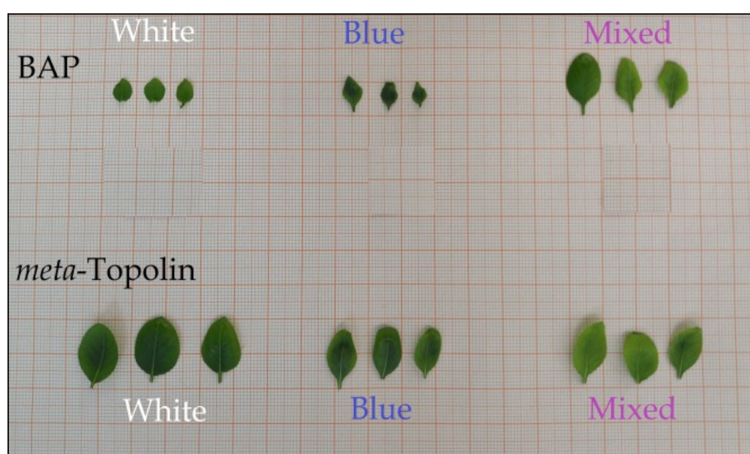

Fig. 2. Leaves of pear plantlets (*Pyrus communis* L. OHF 333) grown with BAP or mT in the nutrient medium at different light treatments. **W** white LEDs, **B** – blue LEDs, **BR** – mixed LEDs.

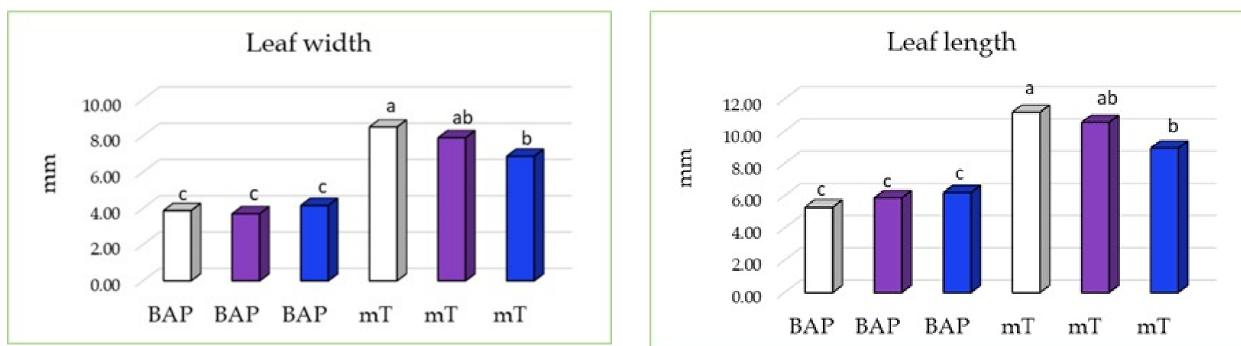

Fig. 3. The length and width of the first fully developed leaf of pear plantlets, grown with BAP or mT in the nutrient medium at different light treatments. **W** white LEDs, **B** – blue LEDs, **BR** – mixed LEDs.

Table 2. The content of photosynthetic pigments (mg g^{-1} FW) in the pear plantlets cultivated with BAP or mT in the nutrient medium at different light treatments. **W** white LEDs, **B** – blue LEDs, **BR** – mixed LEDs. For each column, different letters indicate significant differences at $p \leq 0.05$.

LED Light	Cytokinin	Chl *a*	Chl *b*	Chl (*a*+*b*)	Car	Chl *a*/*b*	Chl(a+b)/Car
White	BAP	0.52 [a]	0.17 [a]	0.69 [a]	0.23 [a]	3.05 [ab]	2.95 [b]
BR	BAP	0.25 [cd]	0.09 [bc]	0.34 [c]	0.15 [b]	2.58 [c]	2.31 [d]
Blue	BAP	0.39 [b]	0.12 [b]	0.51 [b]	0.16 [b]	3.15 [ab]	3.26 [a]
White	mT	0.26 [cd]	0.09 [bc]	0.35 [c]	0.14 [b]	2.96 [b]	2.58 [c]
BR	mT	0.21 [d]	0.08 [c]	0.30 [c]	0.11 [b]	2.61 [c]	2.74 [bc]
Blue	mT	0.32 [bc]	0.10 [bc]	0.42 [bc]	0.16 [b]	3.29 [a]	2.63 [b]

The highest content of chlorophyll *a*, *b* and carotenoids was reported in plants cultivated with BAP in the nutrient medium under white LED light, and the lowest in plants grown with mT under mixed LED light (Table 2). The lowest values of total chlorophyll content were reported under mixed LED light in both cytokinin tested. No deviations from the norm in the ratios between the photosynthetic pigments were found. Our results are similar to results with *Lippia filifolia* reported by Chaves et al. (2020) that white LEDs increased chlorophylls and carotenoids contents.

Our data clearly showed that plants grown in plastic vessels with mT had more efficient photosynthesis, with the difference between the white and mixed light treatments being insignificant (55 µmol vessel^{-1} s^{-1} and 64 µmol vessel^{-1} s^{-1}, respectively) – (Fig. 4).

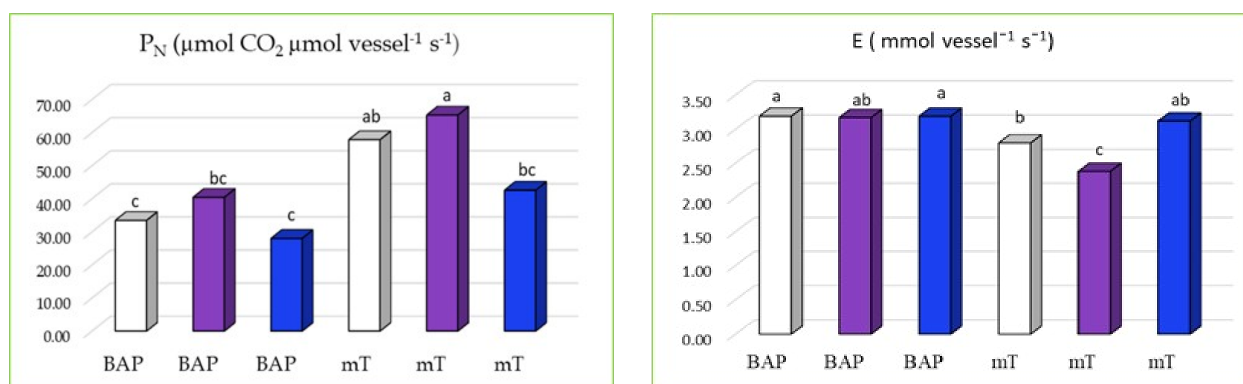

Fig. 4. Effect of the light and cytokinin on the net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and transpiration intensity (E, mmol H_2O m^{-2} s^{-1}) of pear plantlets; Different letters within column indicated difference at ($p<0.05$).

In regard to transpiration rate, the lowest values were reported in the treatment with mT under mixed LED light (Fig. 4). Comparing these results with the content of the photosynthetic pigments, it can be assumed that the changes found in the rate of CO_2 assimilation were not affected by the pigment content.

Few studies are reported on *in vitro* cultivation of plants under LED lamps (Batista et al., 2018). The results show that the effects of spectral quality on photosynthetic competence vary according to the plant species, with LED lights being more efficient compared to fluorescent lights in *in vitro* culture. In addition, the combination of different color LEDs can overcome the limitations of individual colors.

More and more scientists are paying attention to combining red and blue LED lights in different ratios. According to Nhut et al. (2002), the banana plantlets *in vitro* were enhanced under 80% red + 20% blue LED. In the absence of blue LEDs, plantlets were abnormal. Normal plantlet growth is clearly related to the presence of blue LEDs and plant quality is a function of the amount of blue LEDs. (Nhut et al., 2002). The combination of red and blue lights using LED lamps resulted in a higher number of shoots per explant in *Acer saccharum* (Singh et al., 2017) and *Eucalyptus urophylla* Souza et al. (2020) indicating the importance of this light combination for *in vitro* culture.

There is extensive data on the effect of light quality on *in vitro* herbaceous species, while there is less data concerning woody species (Batista et al., 2018) . One reason may be the reduced effects of light quality on woody species compared to that of herbaceous species (Morini & Muleo, 2003).

Conclusions

Pear plantlets grown under white LED light had higher values of fresh (FW) and dry (DW) biomass regardless of the cytokinin applied. Plants grown on the nutrient medium enriched with mT in polypropylene vessels with green gas permeable filter under white light had the largest fresh and dry mass, large leaf size and maximum net photosynthesis rate. The combination of the four types of LED light (BR) favorably affected the growth of leaf lamina and is a prerequisite for more intense photosynthesis. In addition, the better performance of plants grown under mixed LED light in terms of leaf size and photosynthetic performance showed that it is necessary to optimize the *in vitro* growing conditions of the pear rootstock OHF 333 by appropriately combining the light regime and cytokinin in the nutrient medium.

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