Photomorphogenetic and Physiological Responses of *In Vitro* Pomegranate *Punica granatum* L. cv. Malase Saveh) to Varying Light Spectra and Intensities

Kawther Sahib Ahmed Aljasim[1]*, Nargees Moayad Jaseem[2], Abdullah Dhiaa Mohammed[3], Ali Salih Mahdi[4], Mustafa Hameed Al-Majdi[5]

[1-5]Department of Horticulture and Landscape, College of Agriculture, Al Qasim Green University, Babylon, 51001, Iraq.

Corresponding author Email[1]: kawther.sahib@agre.uoqasim.edu.iq, Email[2]: nargus.muaid@agre.uoqasim.edu.iq, Email[3]: abdulla.deyaa@agre.uoqasim.edu.iq, Email[4]: ali.salih@agre.uoqasim.edu.iq, Email[5]: Mustafa.hameed@agre.uoqasim.edu.iq

Abstract

The experiment determined how the light spectrum and light intensity impacted the morphogenesis and physiology of *Punica granatum* L. cv. Malase Saveh under in vitro conditions. The experimental design was a factorial study that was to have two light spectra (Red and Blue LEDs) and three white light intensities (25, 50, and 100 µmol $m^{-2}s^{-1}$). Morphological characteristics were determined, such as the quantity of the explants, the length of the explant (in cm) and the mean leaves per explant. A number of physiological characteristics were also evaluated such as fresh weight per explant (g), dry weight per cluster (g). The findings showed that the most prominent explant number and leaf initiation were generated by the blue light at 100 µmol $m^{-2}s^{-1}$. The difference was quite significant at almost all intensities of light at 100 µmol m^{-2} s^{-1} and blue spectrum, there being no effect at 25 µmol m^{-2} s^{-1} and under red-light treatment. The findings highlight the importance of the optimization of light regimes to advance morphogenetic responses and physiological quality of in vitro Pomegranate culture.

Keywords: Light Intensity, Light Spectra, Malase Saveh, Micropropagation, Pomegranate.

Introduction

Pomegranate (*Punica granatum L.*) is a perennial fruit crop with great economic, nutritional, and medicinal value, which is extensively grown in arid and semi-arid areas [1]. Iran is regarded as a key source of origin and genetic variation, and the type of cultivars has been known, like the Malase saveh, due to their drought-resistant and high-temperature characteristics [2]. Due to resistance, this cultivar has prospects of growing in the central parts of Iraq.

Micropropagation *in vitro* has proven to be a credible method of high-rate multiplication of elite cultivars with genetic uniformity [3]. Light quality and light intensity are some of the key environmental factors that control morphogenic, photosynthetic activity, and secondary metabolism during tissue cultures [4]. Blue light has been linked with enhanced shoot growth and chlorophyll development, whereas red light frequently has an effect on elongation and biomass development [5]. However, little is known about the optimal light regimes for micropropagation of *P. granatum* cv. Malase Saveh

The present study aimed to evaluate the effect of two LED spectra (red and blue) in combination with three intensities of white light morphogenetic and physiological parameters of *P. granatum* cv. Malase Saveh cultured *in vitro.*

Materials and Methods

Plant Material and Culture Conditions

Plantlets (10 cm high) originating from *Punica granatum* cv. Malase Saveh was obtained from virus-free mother plants maintained *in vitro* at the Tissue Culture Laboratory, Al-Razi University, Iran. These were transferred to the Tissue Culture Laboratory, College of Agriculture, Al-Qasim Green University, Iraq, where they were subdivided and cultured in 250 ml sterilized jars containing Murashige and Skoog (MS) basal medium [6] supplemented with 1 mg L^{-1} 6-benzylaminopurine (BAP), 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA), 3% sucrose, and 0.7% agar.

Light Treatments

A factorial arrangement of treatments was used, consisting of two LED light spectra, red (660 nm) and blue (450 nm), combined with three white light intensities (25, 50, and 100 µmol m^{-2} s^{-1}). Cultures were maintained at 25 ± 2 °C under a 16 h light/8 h dark photoperiod (Figure 1).

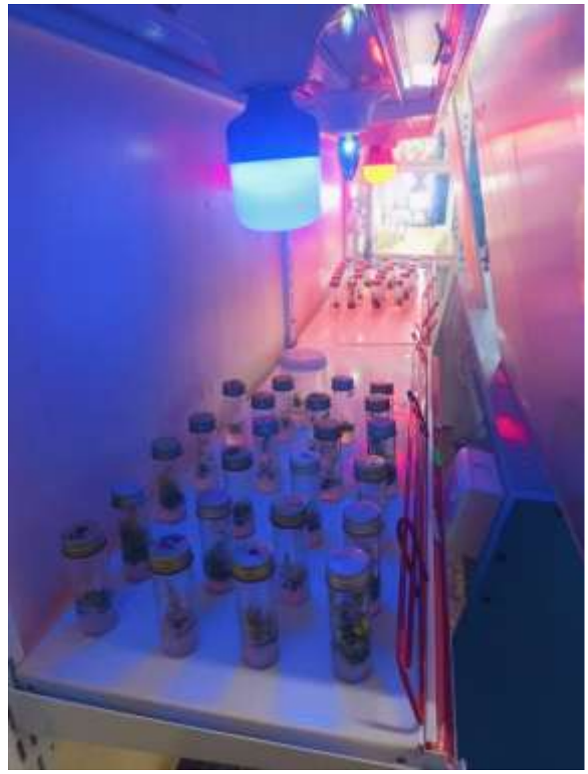

Figure 1. The pomegranate plantlets under different light treatments.

Data Collection

After eight weeks of incubation, with one subculture, the following parameters were evaluated:

- Number of Explants per jar.
- Length of Explants (cm).
- Average Number of Leaves Per Explant
- Fresh Weight Per Explant (g)
- Dry Weight Per Cluster (g).

Statical Analysis

Data were analyzed using a factorial experiment in a completely randomized design (CRD). Mean separation was performed using the Least Significant Differences (LSD) test at $p \leq 0.05$.

Results

Number of Explants

Blue light significantly increased explant proliferation across all intensities (Table 1). The highest number of explants (14.33) was obtained under blue light 100 µmol m^{-2} s^{-1}, while the lowest (8.00) was recorded under red light at 25 µmol m^{-2} s^{-1}.

Table (1): Effects of Light Spectrum and Intensity on the Number of Explants of *Punica granatum* L. cv. Malase Saveh Propagated In Vitro.

Light Intensity	Light Spectrum		
	Red	Blue	Average
25 µmol m^{-2} s^{-1}	8.00	13.33	10.66
50 µmol m^{-2} s^{-1}	9.67	13.00	11.33
100 µmol m^{-2} s^{-1}	13.67	14.33	14.00
Average	10.44	13.55	
LSD 0.05	Red 1.205	Blue 1.887	Interaction 3.268

Length of Explants

Light intensity had a great bearing on the elongation of the explants (Table 2, Figure 2). Explants cultivated at 100 µmol m^{-2} s^{-1} attained an average of 11.16 cm, whereas for 25 µmol m^{-2} s^{-1}, the average was 8.50 cm. The elongation (10.66 cm) was generally higher under the blue light compared with the red light (8.99 cm).

Table (2): Effects of Light Spectrum and Intensity on the Explant Length (cm) of *Punica granatum* L. cv. Malase Saveh Propagated In Vitro.

Light Intensity	Light Spectrum		
	Red	Blue	Average
25 µmol m^{-2} s^{-1}	7.33	9.67	8.50
50 µmol m^{-2} s^{-1}	8.33	11.33	9.83
100 µmol m^{-2} s^{-1}	11.33	11.00	11.16
Average	8.99	10.66	
LSD 0.05	Red0.854	lue 1.20	Interaction 2.088

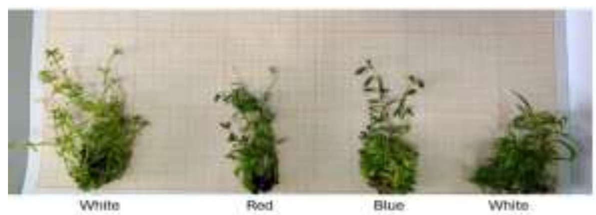

Figure 2. The pomegranate plantlets were examined after four weeks of growth and development under different light spectra.

Number of Leaves

The highest (15.25) and the lowest (8.33) of leaf formation occurred in blue and red light, respectively, under 50 µmol m^{-2} s^{-1} and 25 µmol m^{-2} s^{-1}, respectively (Table 3). The general trend of blue light was that it was found to be effective in the initiation of leaves as opposed to red light.

Table (3): Effects of Light Spectrum and Intensity on the Average Number of Leaves Per Explant of *Punica granatum* L. cv. Malase Saveh Propagated In Vitro.

Light Intensity	Light Spectrum		
	Red	Blue	Average
25 µmol m^{-2} s^{-1}	8.33	13.33	10.83
50 µmol m^{-2} s^{-1}	10.87	15.25	13.06
100 µmol m^{-2} s^{-1}	15.39	14.29	14.84
Average	11.53	14.29	
LSD 0.05	Red 1.806	Blue 2.541	Interaction 4.401

Fresh Weight

Fresh weight per explant was generally stable across light intensities, ranging from 0.937 to 1.057 g (Table 4). Blue light produced slightly higher fresh weight (1.044 g) compared with red light (0.964 g).

Table (4): Effects of Light Spectrum and Intensity on the Fresh Weight Per Explant (g) of *Punica granatum* L. cv. Malase Saveh Propagated In Vitro.

Light Intensity	Light Spectrum		
	Red	Blue	Average
25 µmol m^{-2} s^{-1}	0.963	1.053	1.008
50 µmol m^{-2} s^{-1}	0.993	1.023	1.008
100 µmol m^{-2} s^{-1}	0.937	1.057	0.997
Average	0.964	1.044	
LSD 0.05	Red 0.0411	Blue 0.068	Interaction 0.117

Dry Weight

Dry weight per cluster showed clear spectrum- and intensity-dependent differences (Table 5). Under red light, dry weight increased progressively with intensity, from 0.539 g at 25 µmol m^{-2} s^{-1} to 0.895 g at 100 µmol m^{-2} s^{-1}. Blue light, however, produced relatively stable dry weights (0.931-0.993 g) across intestines, with higher overall averages compared to red light.

Table (5): Effects of Light Spectrum and Intensity on the Dry Weight Per Cluster (g) of *Punica granatum* L. cv. Malase Saveh Propagated In Vitro.

Light Intensity	Light Spectrum		
	Red	Blue	Average
25 µmol m^{-2} s^{-1}	0.539	0.983	0.761
50 µmol m^{-2} s^{-1}	0.673	0.931	0.802
100 µmol m^{-2} s^{-1}	0.895	0.993	0.944
Average	0.702	0.969	
LSD 0.05	Red 0.0882	Blue 0.1395	Interaction 0.2416

Discussion

Light quality and intensity are among the main environmental factors regulating growth and differentiation in in vitro cultures [5]. In the present work, most traits responded more positively to blue light than to red light. This agrees with [4], who reported that blue wavelengths enhance morphogenic responses and stimulate chlorophyll accumulation.

The improvement in the number of explants, length, and leaf initiations under blue light suggests that this spectrum has a stronger influence on shoot proliferation and organ development. Other woody species have also been reported to respond in the same way, with blue light preferring the growth of leaves and the development of shoots [3]. Conversely, the growth of explants in low intensity red light was minimal and this could be attributed to the low intensity of stimulation of the stomatal activity and photosynthetic processes in those environmental conditions [7; 8].

The intensity of light was also a major factor, especially in the growth of the shoot and development of dry matter. Explants that were cultured in the most intense (100 µmol m^{-2} s^{-1}) had longer shoots and higher dry weight, which suggests that increased photon supply favors cell growth and carbohydrate storage. Fresh weight was, in contrast, comparatively unchanged, which implies that the levels of water retention did not change notably across the treatments [9].

The effect of light intensity and light spectrum showed some clear trends, with moderate levels of blue light being enough to stimulate aggressive development of the shoot and leaves, and red with greater levels of light to increase dry biomass. The variations in these responses could be attributed to the differences in photoreceptors and phytochromes and cryptochromes or phytotropins reacting to red and blue light respectively [10].

Conclusion

The current study has shown that morphogenesis and physiology of Punica granatum cv. Malase Saveh grown in vitro are both affected by the light magnitude and light spectrum. Moderate to high levels of blue light stimulated shoot proliferation, foliage formation and extended the length of explants, and high-intensity red light increased the accretion of dry matter. There is no statistical difference in fresh weight among treatments, implying that structural changes in water content were the major motivator to biomass gain.

Such data suggest that light regime optimization is an important part of the optimization of tissue culture of pomegranate. In the case of massive propagation of Punica granatum cv. Malase Saveh, blue light at higher intensities is ideal in morphogenetic characteristics, whereas red could be utilized strategically to increase biomass. The knowledge gives a basis for how to come up with effective and affordable micropropagation systems that are capable of assisting in the conservation and commercial cultivation of elite pomegranate genotypes.

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