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AVKSENTIEVA O. O., BATUIEVA Y. D.✉, FESENKO M. O.
V. N. Karazin Kharkiv National University,
Ukraine, 61022, Kharkiv, Svobody square, 4, ORCID: 0000-0003-2532-7141, 0000-0002-3274-3410, 0009-0005-9520-3779
✉ *batuieva@karazin.ua*

THE INFLUENCE OF RL (660 nm) AND BL (450 nm) ON THE PROCESSES OF CALLUSOGENESIS AND MORPHOGENESIS *IN VITRO* OF TOMATO VARIETIES OF CONTRARY PRECOCITY

Aim. To study the effect of RL (660 nm) and BL (450 nm) photoirradiation on the growth and morphogenetic reactions of callus of different origin of two tomato varieties contrasting in precocity. ***Methods***. Two tomato varieties differing in growth type and precocity – ACE 55 VF and Kremenchutskyi – were used as plant material. Primary callus was obtained through the stage of aseptic seedlings, and three types of organs were used as explants: hypocotyl segments, cotyledon leaves, and apical parts of roots. Callus was grown on basal medium MS + 5 mg/l 2.4 D, in the dark at +26°C. The photoactivation with RL (660 nm) and BL (450 nm) was performed using Korobov LEDs. The frequency of callus proliferation, growth and morphogenetic reactions of the callus culture *in vitro* were analysed. ***Results***. It was found that hypocotyl segments and cotyledon leaves of both studied varieties are more effective explants for obtaining primary callus. The ACE 55 VF variety, characterised by indeterminate growth type, forms mature callus tissue more efficiently than the determinate Kremenchutskyi variety. The photoexposure with RL (660 nm) stimulates the growth response of callus of ACE 55 VF variety. It is shown that irradiation with RL and especially BL activates the manifestation of morphogenetic reactions in callus of both varieties. ***Conclusions.*** To implement different pathways of morphogenesis *in vitro*, it is necessary to take into account the type of growth *in vivo*. Activation of the photoreceptor systems with RL and BL in the mature callus culture stimulates the processes of morphogenesis *in vitro*.

Keywords: *Lycopersicon esculentum* Mill., growth rate and type, callus culture, light irradiation, RL (660 nm) and BL (450 nm), growth index, morphogenesis *in vitro*.

It is known that light is the most important environmental factor that affects the plant organism and ensures the processes of photosynthesis and photomorphogenesis. Plants perceive information about light signals (light intensity, photoperiod, spectral composition) using several photoreceptor systems and transduce it to regulate growth and development processes. The most important photoreceptor systems in plants are the phytochrome system, which is activated by red/far-red light, and the cryptochrome system with phototropins, that are activated by blue light [1]. Phytochromes Phy A and Phy B regulate a wide variety of photomorphogenetic and physiological reactions in the plant organism, such as seed germination, etiolation/de-etiolation reactions, shade avoidance, metabolic activity, flowering, fruiting and other processes [2]. According to current information, phytochromes also act as thermoreceptors [1] and have a protective effect under the influence of abiotic and biotic factors [3]. Cryptochromes and phototropins regulate chlorophyll biosynthesis, stomatal movements, anthocyanin synthesis, flowering time, and many other reactions. It has also been shown that different photoreceptor systems interact with each other and are connected to other plant signalling pathways, such as phytohormonal, trophic, and pro/antioxidant systems [1,4]. Tomato is one of the most widespread and valuable vegetable crops in the world and in Ukraine. In addition, *Lycopersicon esculentum* Mill. is also a convenient biological model for molecular genetic and physiological-biochemical studies of economically valuable traits of vegetable crops. This is due to a number of characteristics of tomato, including a small (12 chromosomes with more than 1300 genes) and fully sequenced genome, a rather short growing season and high reproductive potential, the ability to vegetatively reproduce, pronounced photoperiodic sensi-

tivity, and identified photoreceptors (5 phytochromes, as in the model object Arabidopsis thaliana) [3]. In addition, it can be successfully cultivated in greenhouses [5] and is also a convenient object for *in vitro* cultivation [6, 7]. Cultivation of plants under controlled conditions requires a certain lighting regime, which has traditionally been maintained by fluorescent lamps and sodium lamps. LEDs, as an alternative to traditional lamps, certainly have a number of advantages: energy efficiency, absence of thermal radiation, and a monochromatic spectrum of light flux [8]. All of this contributes to their widespread use in the cultivation of plants in closed ground conditions, including *in vitro* culture, and to the intensification of research on the effect of various effects of light irradiation for the purpose of programmed regulation of the growth and development of cultivated plants [9]. Studies on tomato seedlings cultivated in greenhouses showed that a certain ratio of red and blue light (10:1) stimulated the processes of photosynthesis, biomass growth, increase in the content of photosynthetic pigments, etc. [5]. It was also experimentally established for tomato seedlings that additional illumination in the morning with LED lamps at red and blue spectra of 7:2 ratio led to the activation of photosynthetic enzymes, including RuBisCo, and maximum synthesis of assimilates [6]. *In vitro* culture is known to be a tool that is widely used in modern phytobiotechnology – genetic transformation of plants, microclonal reproduction, production of valuable metabolites, preservation of gene pool, plant material recovery, etc. In addition, *in vitro* culture is a convenient biological model for studying fundamental biological processes. The use of light to improve the quality of *in vitro*-grown plants has been confirmed by numerous researchers in the field of plant tissue culture [9]. Tomato is quite easy to introduce into *in vitro* culture. Optimisation of cultivation conditions for callusogenesis and tissue regeneration of different tomato genotypes has been the subject of many studies [6, 7, 10]. The study of photoactivation by red and blue light of morphogenetic reactions of tomato genotypes under *in vitro* culture conditions is promising in this regard. Thus, the aim of this study was to investigate the effect of photoreceptor activation on the growth and morphogenetic responses of callus of different origins of two tomato varieties contrasting in growth rate and type.

Materials and methods

The study was carried out on the basis of the laboratory 'Morphogenesis of higher plants *in vitro'* of the Department of Physiology and Biochemistry of Plants and Microorganisms of V. N. Karazin Kharkiv National University. The plant material used in the study was tomato cultivars *L. esculentum* that differ in maturity groups and growth type: ACE 55 VF (USA), a mid-season variety with indeterminate growth type, and Kremenchutskyi (Ukraine), an ultra-early variety with determinate growth type. The primary callus was obtained from aseptic seedlings that were cultured on hormone-free Murashige and Skoog medium (MS) for 7 days. Sections of hypocotyls, roots, and cotyledon leaves were used as explants for primary callusogenesis, which were placed in Petri dishes with MS medium + 5 mg/L 2.4 D (2,4-dichlorophenoxyacetic acid) under sterile conditions (Laminar Box Bionom V). After the formation of the primary callus, after 4 weeks of cultivation in the dark at +26 °C (thermostat TCO-80), the photoreceptor systems were photoactivated by irradiating the experimental callus with red (RL, λ = 660 nm) and blue (BL, λ = 450 nm) light using Korobov LEDs for 2 weeks for 30 minutes each day. Control callus was grown without irradiation. Subsequently, to analyse the aftereffects of photoactivation on *in vitro* morphogenesis, experimental and control callus were subcultivated onto MS regeneration medium + 2 mg/L NAA (naphthylacetic acid) + 1.5 mg/L BAP (6-benzaminopurine) + 2 mg/L kinetin and grown in luminostat at 2 lux with a photoperiod of 16 h/8 h (day/night) for 5 weeks. The efficiency of primary callusogenesis, growth index (GI), and the manifestation of different pathways of morphogenetic reactions were analysed. The frequency of callusogenesis and *in vitro* morphogenesis was calculated as the ratio of explants that formed certain morphogenetic structures to the total number of cultured explants [11]. The graphs show the mean values and their standard deviations; the significance of differences between the control and experimental variants was calculated by pairwise comparison using the Tukey's test at $P \leq 0.05$ using Statistica 5.0 software.

Results and discussion

The first stage of our study was to obtain primary callus using aseptic seedlings *in vitro* culture. Three types of meristematic tissues were used as explants: hypocotyl segments (1–1.5 cm in size), cotyledon leaves, and apical root segments (1–1.5 cm in size). Tomato is easily introduced into *in vitro* culture using a variety of explants [10]. According

to the results of our experiments, it was found that the primary callus is formed quite quickly – within the first 7 days we observed the formation of callus using sections of hypocotyls and cotyledon leaves, only apical sections of roots formed callus later - at this time (7 days after passage) the formation of callus tissues was not yet observed. The most effective explants were hypocotyl segments, which formed callus with a maximum frequency of 80–100 %. Primary cotyledon leaves were also quite effective as explants, the frequency of callusogenesis was lower, but quite high at 71–90 %. Apical segments of roots were characterised by the lowest rates – the frequency of callusogenesis was 40–78 %. The fastest primary callusogenesis occurred during the first 14 days regardless of the type of explant, and then the growth slowed down or reached a "plateau" (Fig. 1). It should be noted that varieties that differed in speed and type of growth differed in terms of frequency of callusogenesis – early ripe variety Kremenchutskyi was characterised by slightly higher frequency of callusogenesis than mid-season indeterminate by type of growth ACE 55 VF – by about 10–25 % when using all types of explants. No differences were found in the morphological characteristics of callus when using different types of explants and different genotypes of varieties.

All formed calluses were heterogeneous, heavily watered, actively growing, and darkly coloured. Thus, in the study of primary callusogenesis, it was found that all types of explants are capable of forming callus tissues, but with varying degrees of efficiency. The most effective is the use of hypocotyl segments and cotyledon leaves. The genotype of the variety and the type of explant do not affect the morphological characteristics of primary callus, but the ultra-early variety is characterised by higher rates of callusogenesis regardless of the type of explant.

Since the maximum rates of primary callusogenesis were characterised by callus where hypocotyl segments and cotyledon leaves were used as explants, further studies of influence of light irradiation were carried out on callus of this origin. The analysis of the growth response of callus tissue of two tomato varieties under photoexposure with RL and BL light showed that there are certain differences in the response of genotypes that differ in type and growth rate under *in vivo* conditions. In general, the callus culture of the indeterminate variety ACE 55 VF is characterised by growth index values in the range of 224–288 %, which significantly exceeds the GI of callus of the ultra-early variety Kremenchutskyi, which is 88–185 % for all experimental variants regardless of the origin of the explant (Fig. 2). A significant stimulation of the growth response was observed in callus of the indeterminate variety ACE 55 VF of hypocotyl and cotyledon origin under photoinduction of CS. Similar effects were shown in the works of other researchers on many plant crops [9, 12]. Activation of callus growth can occur due to intensification of cell proliferation or growth by "stretching" of callus cells or both processes simultaneously. The photoactivation with BL by light did not significantly affect the growth of callus cultures of different origin of ACE 55 VF variety (Fig. 2). In callus of the determinant variety Kremenchutskyi, a significant inhibition of the growth response was shown under RL irradiation, and under BL photoexposure – inhibition of GI in cotyledonous callus and no significant effect in hypocotyl callus (Fig. 2). Photoreactions in cotyledonous callus of both varieties are more pronounced than in hypocotyl callus.

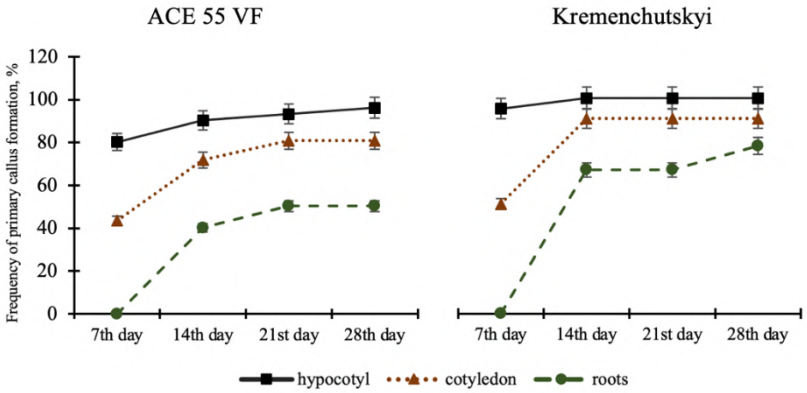

Fig. 1. Frequency of primary callusogenesis in tomato varieties contrasting in precocity depending on the type of explant, % (m±SD, n = 14–21).

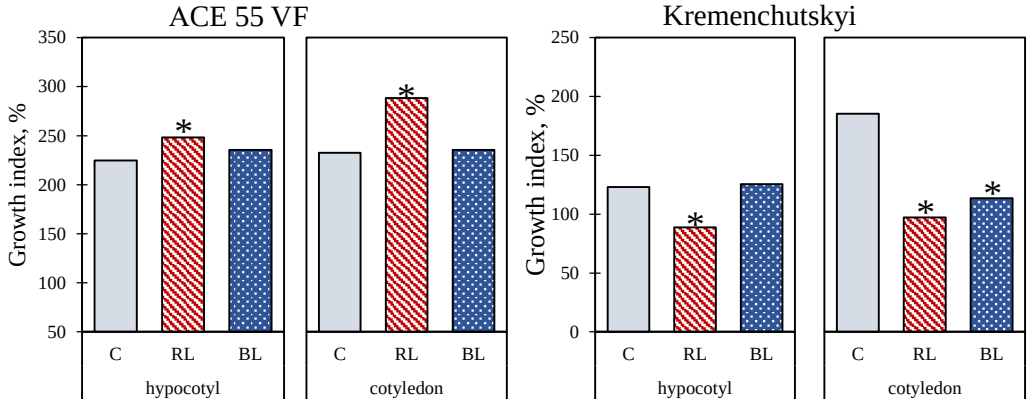

Fig. 2. Influence of irradiation with RL and BL on the growth index of primary callus of tomato varieties contrasting in precocity depending on the type of explant, % (m±SD, n = 14–21). C – control (darkness without irradiation), RL – irradiation with red light λ = 660 nm, BL – irradiation with blue light λ = 450 nm.

Photoactivation of photoreceptor systems in mature callus led to certain changes in their morphological structure (Fig. 3). An increase in morphological heterogeneity was observed; compact and loose areas, light and dark coloured parts, morphogenic foci in the form of separate numerous inclusions or morphogenic structures were simultaneously formed in callus.

A greater degree of heterogeneity and stimulation of morphogenic structures formation was observed under the influence of BL in callus of both varieties. In further experiments, we investigated the aftereffect of photoactivation of photoreceptor systems on the manifestation of morphogenetic reactions in callus cultures of two tomato varieties contrasting in growth rate and type. Callusogenesis in the mature callus culture of the indeterminate variety ACE 55 VF was most intensive in all experimental variants of callus of different origin. Callus cultures of hypocotyl and cotyledonous origin actively increased new callus tissues and the frequency of callusogenesis in all variants was 100 % (Fig. 4). The processes of callusogenesis in Kremenchutskyi variety, which is determinant by growth type, were significantly inhibited by phytochromes activation and occurred in different directions under the influence of BL in callus of different origin. In hypocotyl callus, the photostimulation with BL did not show a significant effect, and in cotyledonary callus it was significantly inhibited (Fig. 4). The same reaction was observed in the analysis of GI in primary callus of this variety. The frequency of callusogenesis in the determinate variety Kremenchutskyi was significantly lower than in mature callus of the indeterminate variety ACE 55 VF. Morphogenetic reactions under photoexposure with RL and BL were significantly stimulated in callus cultures of both varieties. The general response is a more significant stimulation of morphogenic structures formation under activation of cryptochrome photoreceptor systems. The stimulation of various pathways of morphogenesis *in vitro* under the influence of RL and BL or cultivation under monochromatic light irradiation has also been shown in a number of experimental studies [12–14]. In our experiments, the opposite effect of photoactivation with RL and BL on callusogenesis and morphogenesis *in vitro* was found in the determinant variety Kremenchutskyi callusogenesis is inhibited by photoirradiation, while morphogenesis is stimulated. This reaction may indicate that the processes of callusogenesis and morphogenesis are determined by different genetic systems. Nowadays, the analysis and search for candidate genes for stimulating callusogenesis is becoming a new area of molecular genetic research [15]. The indeterminate variety ACE 55 VF shows a greater ability to form and grow transplant callus culture under *in vitro* conditions than the Kremenchutskyi variety with a determinant type of growth and faster development under *in vivo* conditions. It is possible that the original donor plants characterised by a longer vegetative or ontogenetic period are more efficient in forming callus cultures, which may indicate certain genetic determinants that are unidirectionally manifested both *in vivo* and *in vitro*.

Conclusions

According to the results of our experiments, it was found that the most effective explants in both varieties for obtaining primary callus culture are hypocotyl segments and cotyledon leaves.

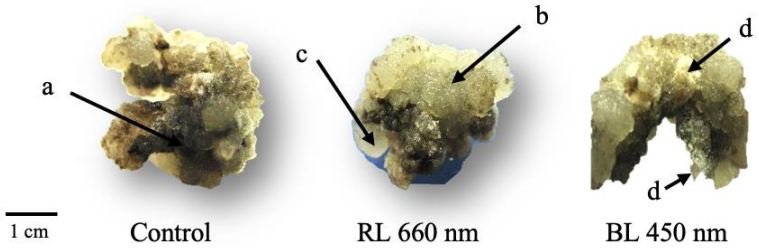

Fig. 3. Influence of irradiation with RL and BL on the morphological characteristics of mature callus tissue of tomato variety ACE 55 VF (a – darkly coloured areas; b – loose callus, c – matte, compact callus, d – morphogenic structures).

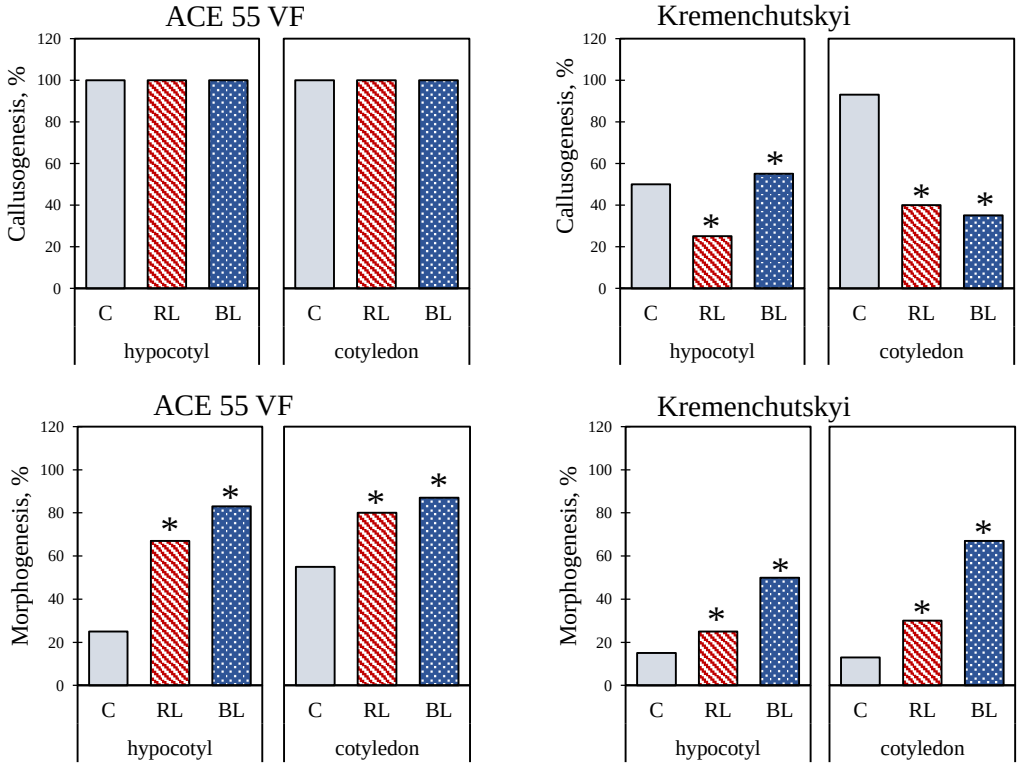

Fig. 4. Influence of irradiation with RL and BL on morphogenetic reactions *in vitro* of tomato varieties contrasting in precocity depending on the type of explant, % (m±SD, n = 14–21). C – control (darkness without irradiation), RL – irradiation with red light λ=660 nm, BL – irradiation with blue light λ = 450 nm.

The variety ACE 55 VF, characterised by an indeterminate type of growth *in vivo*, forms mature callus with a significantly higher frequency compared to the variety of determinate growth type. Photo-irradiation with RL (660 nm) light has a positive effect on the processes of callusogenesis and stimulates the growth index of callus of ACE 55 VF. The activation of photoreceptor systems through photoexposure with RL (660 nm) and BL (450 nm) stimulates the manifestation of morphogenetic reactions in the mature callus culture of both varieties, which is promising in the development of biotechnological protocols for *in vitro* regeneration of callus of the valuable vegetable crop *L. esulentum*.

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АВКСЕНТЬЄВА О. О., БАТУЄВА Є. Д., ФЕСЕНКО М. О.
Харківський національний університет імені В. Н. Каразіна,
Україна, 61022, м. Харків, майдан Свободи, 4

ФОТОВПЛИВ ЧС (660 нм) ТА СС (450 нм) НА ПРОЦЕСИ КАЛЮСОГЕНЕЗУ ТА МОРФОГЕНЕЗУ *IN VITRO* СОРТІВ ТОМАТУ, КОНТРАСТНИХ ЗА СКОРОСТИГЛІСТЮ

Мета. Вивчення впливу фотоопромінення ЧС (660 нм) та СС (450 нм) на ростову та морфогенетичну реакції калюсів різного походження двох сортів томатів, контрастних за скоростиглістю. ***Методи.*** У якості рослинного матеріалу використовували два сорти томату, що різняться типом росту та скоростиглістю – ACE 55 VF та Кременчуцький. Первинні калюси отримували через стадію асептичних проростків, у якості експлантів використовували три типи органів: частини гіпокотилів, сім'ядольні листки та апікальні частини коренів. Калюси культивували на базальному середовищі МС + 5 мг/л 2,4 Д, в темряві за температури +26°С. Фотоактивацію ЧС (660 нм) та СС (450 нм) світлом проводили за допомогою світлодіодних матриць Коробова. Аналізували частоту проліферації калюсів, ростову та морфогенетичні реакції калюсної культури *in vitro*. ***Результати.*** Встановлено, що ефективнішими експлантами для отримання перинного калюсу є відрізки гіпокотилів та сім'ядольні листки в обох досліджуваних сортів. Сорт ACE 55 VF, що характеризується індетермінантним типом росту ефективніше формує зрілу калюсну тканину, ніж детермінантний сорт Кременчуцький. Фотовплив ЧС (660 нм) стимулює ростову реакцію калюсів сорту ACE 55 VF. Показано, що опромінення ЧС та особливо СС активують прояв морфогенетичних реакцій у калюсах обох сортів. ***Висновки.*** Для реалізації різних шляхів морфогенезу *in vitro* необхідно враховувати тип росту *in vivo*. Активація фоторецепторних систем ЧС та СС у пересадковій калюсній культурі стимулює процеси морфогенезу *in vitro*.
Ключові слова: *Lycopersicon esculentum* Mill., швидкість та тип росту, калюсна культура, опромінення світлом, ЧС 660 нм та СС 450 нм, ростовий індекс, морфогенез *in vitro*.