EXPERIMENTAL BOTANY

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CALLUSOGENESIS AND *IN VITRO* MORPHOGENESIS OF BEAN PLANTS WITH CONTRAST PHOTOPERIODIC REACTION BY RED LIGHT EXPOSURE [1]

Batuieva Y. D.
Postgraduate Student at the Department of Physiology and Biochemistry of Plants and Microorganisms
V. N. Karazin Kharkiv National University

Avksentieva O. O.
Candidate of Biological Sciences, Associate Professor,
Associate Professor at the Department of Physiology and Biochemistry of Plants and Microorganisms
V. N. Karazin Kharkiv National University
Kharkiv, Ukraine

In vitro culture is an adequate modern biological model for the study of the plant organism. Plant tissue cells of *in vitro* culture are able to retain a number of properties that are typical for *in vivo* conditions, including photoperiodic reaction (PPR), which is a genetically determined property. Transduction of photoperiodic signal in the plant organism is carried out with the participation of the main photoreceptors – the phytochrome system [2, 6], which is activated by irradiation with monochromatic red light – RL (660 nm). The phytochrome system controls the course of individual development of the plant organism from seed germination to flowering and fruitage [3], coordinates circadian rhythms, regulates the transition to flowering and other metabolic, physiological and ontogenetic processes [4, 7]. It is known that the morphogenesis of plants in *in vitro* culture has a wider range of manifestations in different ways than *in vivo*. Activation of the phytochrome system i*n vitro* could be a factor of stimulating different

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pathways of morphogenetic reactions, but this process remains poorly understood. The aim of our work was to study the effect of irradiation with monochromatic red light (660 nm) on the efficiency of the main stages of *in vitro* culture and morphogenetic reactions of calluses of legumes with contrast photoperiodic reaction.

Materials and methods

The research 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 KhNU. As plant material in the work we used plants of the legume family (*Fabaceae*), contrasting by photoperiodic reaction: long-day plants (LDP) of pea (*Pisum sativum L.)* Maecenat variety, short-day plants (SDP) of soybean (*Glycine max (L.) Merr.)* Korsak variety and photoperiodically neutral plants (PPN) of soybean (*Glycine max (L.) Merr.)* Diadem Podillya variety. The main stages of *in vitro* culture introduction were analyzed – the efficiency of sterilization processes, primary callusogenesis and the direction of morphogenetic reactions. Sterilization of plant material – seeds of the studied crops – was performed in stages according to the previously developed protocol: detergent → 70% ethanol → 15% NaClO solution → sterile water [1]. Cultivated in Petri Dishes for 5-7 explants on Murashige Skug medium for induction of primary callusogenesis (MS + 10 mg/l 2,4 D) in a thermostat at 26 ° C. The Petri Dishes were irradiated with RL using an LED array for 30 minutes for 7 days. Control samples were cultured without irradiation. The sterilization efficiency and the frequency of primary callusogenesis were analyzed. After 4 weeks of cultivation, the formed primary callus tissues were passivated on the regeneration medium MS + 3 mg/l BAP + 0.5 mg/l NAA and cultured in luminescent under conditions: light 2 klk, temperature 22 ° C, photoperiod 16/8 hours (day / night). In the first week of cultivation, the RL was also irradiated with experimental samples for 30 min every 7 days (at the same time of day) using an LED matrix. For 4 weeks, observations were made on the manifestation of various pathways of morphogenetic reactions – chlorophyll genesis, hemogenesis, rhizogenesis.

Results and discussion

The results of experiments showed that the studied plant objects was quite successfully introduced into *in vitro* culture using this sterilization protocol. The sterilization efficiency was 75-96%, but irradiation with the RL (660 nm) slightly reduced the efficiency of introduction into culture *in vitro* by 5-10% in all studied plant objects, regardless of their photoperiodic reaction (table 1). We observed in our experiments that legumes were

successfully introduced into the culture and formed the typical callus tissues. The frequency and rate of primary callus formation faster and in bigger quantity occurred in the LDP of pea Maecenat variety; in PPN and SDP soybean Diadem Podillya variety and Korsak variety occurred less efficient processes of callusogenesis, that may not be associated with their photoperiodic reaction, and is determined by the species characteristics of objects. Activation of the phytochrome system by irradiation with RL (660 nm) slightly stimulated callusogenesis in the LDP, did not affect the SDP and inhibited this process in the PPN of soybean.

Table 1

The effect of irradiation with RL (660 nm) on the efficiency of introduction into the culture *in vitro* by cultivation on the medium MS + 10 mg/l 2.4 D

Object	PPR	Sets of the experiment	Efficiency, %	
			sterilization	callusogenesis
Pea Maecenat variety	LDP	control	93,0±4,7	86,6±4,2
		RL (660 nm)	86,6±4,3	93,0±4,5*
Soybean Korsak variety	SDP	control	68,8±3,4	31,3±1,7
		RL (660 nm)	53,3±2,7*	33,0±1,4
Soybean Diadem Podillya variety	PPN	control	80,2±3,9	46,6±2,1
		RL (660 nm)	75,0±3,7	20,3±0,9*

**) note – the difference with the control is significant for P≤0.05, n = 15-21*

According to the literature and the results of our previous studies, the reactions of photomorphogenesis in *in vitro* culture of plants depend on the light spectrum and the genetic potential of plants – PPR, type and rate of their development [1, 5]. According to the results of the study of morphogenetic reactions in *in vitro* culture of legumes with contrast photoperiodic reaction, it was found that there are no morphogenetic reactions of rhizogenesis and manifestations of callus tissue necrosis in the LDP of pea Maecenat variety (table 2). This fact indicates an effective callus culture capable of regenerating plants. When the RL was irradiated (660 nm), the processes of hemogenesis and chlorophyll genesis were stimulated. All studied forms of morphogenetic reactions – rhizo-, hemo-, chlorophyllogenesis and manifestations of necrosis were found in SDP soybean explants, which may indicate significant tissue totipotency of *in vitro* culture and a certain complexity of regenerating plant formation. Irradiation with the RL (660 nm) inhibited the processes of rhizogenesis, stimulated

hemogenesis, did not affect chlorophyllogenesis and inhibited the processes of callus tissue necrosis. All the studied forms of morphogenetic reactions were also detected in PPN explants of soybean plants of the Diadem Podillya variety. In addition, a fairly high level of necrosis is shown, that indicates a low efficiency of *in vitro* cultivation. Irradiation with RL (660 nm) stimulated all manifestations of morphogenesis – rhizogenesis, hemogenesis and chlorophyllogenesis and inhibited necrosis processes.

Table 2

The effect of irradiation with RL (660 nm) on the morphogenetic reactions of transplanted callus culture of legumes with contrast PPR for cultivation on a medium MS + 3mg/l BAP +0.5 mg/l NAA

Object	Sets of the experiment	Morphogenetic reactions, %			
		Rhizogenesis	Hemogenesis	Chlorophyll genesis	Necrosis
Pea Maecenat variety	control	0	33,4±1,9	66,2±4,8	0
	RL (660 nm)	0	51,2 ±2,3*	71,2±6,9*	0
Soybean Korsak variety	control	33,2±0,9	50,4±2,9	100±1,0	33,1 ±0,8
	RL (660 nm)	10,1±0,3*	75,3 ±4,5*	100±1,0	0
Soybean Diadem Podillya variety	control	13,2±0,4	45,2±3,2	33,4±0,9	80,2 ±7,2
	RL (660 nm)	50,4±2,9*	75,1 ±5,9*	100±1,0*	20,4 ±0,7*

**) note – the difference with the control is significant for P≤0.05, n = 15-21*

Thus, during the experiments it was found that the influence of monochromatic RL (660 nm) on the process of primary callusogenesis depends on the PPR of plants, but the morphogenetic reactions of hemogenesis and chlorophyll genesis are stimulated by RL in all studied plants regardless of their photoperiodic reaction. In addition, irradiation with RL (660 nm) of callus cultures inhibits the processes of rhizogenesis and necrosis, which increases the potential ability of callus to form regenerating plants.

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