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# ФИТОГОРМОНАЛЬНАЯ РЕГУЛЯЦИЯ ФОРМИРОВАНИЯ *IN VITRO* АНДРОГЕННЫХ СТРУКТУР ПШЕНИЦЫ

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**Аннотация**
Явление андрогенеза *in vitro* активно используется в биотехнологических исследованиях пшеницы. При решении конкретных задач важно знать, по какому именно пути морфогенеза *in vitro* будет идти формирование андрогенных растений из инициальных клеток–микроспор. Исследование посвящено разработке способа фитогормональной регуляции формирования *in vitro* определенного типа андрогенных структур (эмбриоидов и каллусов) пшеницы. С использованием метода твердофазного иммуноферментного анализа показано, что индукция конкретного пути морфогенеза *in vitro* микроспоры зависит как от содержания эндогенного ауксина ИУК в пыльниках перед инокуляцией их на индукционную питательную среду, так и от концентрации экзогенного ауксина 2,4-Д в этой среде. Полученные данные подтверждают принципиальную возможность регуляции способов получения андрогенных растений *in vitro* путем подбора оптимального баланса эндогенных и экзогенных ауксинов.
**Ключевые слова:** андрогенез *in vitro*; фитогормоны; морфогенез; эмбриоид; каллус; пшеница.

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# PHYTOHORMONAL REGULATION OF IN VITRO FORMATION OF WHEAT ANDROGENIC STRUCTURES

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**Abstract**
The phenomenon of androgenesis *in vitro* is widely used in biotechnological investigations of spring wheat. When solving the specific biotechnological problems it is important to know what

kind of morphogenesis pathway *in vitro* will result in androgenic plants. This research is devoted to developing a method of phytohormonal regulation of *in vitro* formation of a certain type of wheat androgenic structures. Using the method of ELISA it was shown that the induction of certain sporophytic morphogenesis pathway *in vitro* of anther haploid cells – microspores depends on both the content of endogenous auxin IAA in anthers before inoculating them onto induction medium, and the concentration of exogenous auxin 2,4-D in this medium. The obtained data confirms the principle possibility of regulation of ways of getting androgenic regenerants *in vitro* by selecting the optimal balance of endogenous and exogenous auxins.
**Key words:** androgenesis *in vitro*; phytohormones; morphogenesis; embryoid; callus; wheat.

**INTRODUCTION**

The biotechnological method of anther culture *in vitro* is widely used in breeding wheat programs. This method is based on the phenomenon of androgenesis *in vitro* – the formation of haploid plants from microspores, which development switches from normal gametophytic pathway to a fundamentally different one – sporophytic [3, 8, 9, 18, 23].

Haploid plants can be obtained from androgenic structures developing by morphogenesis pathways *in vitro* – embryoidogenesis or callusogenesis [5, 8, 14, 25, 26]. When solving the certain biotechnological problems it is important to know what kind of morphogenesis pathway *in vitro* will result in androgenic plants and what are the conditions inducing microspore development by this pathway.

In cereals, one of the main factors inducing sporophytic development of microspore is addition into the culture medium a synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) at a certain concentration [3, 8, 20]. However selection of the optimal concentration of hormones often is mostly random. Also it does not take into consideration the content of endogenous phytohormones, particularly indole-3-acetic acid (IAA) which to a large extent determines the explants morphogenetic competence [4, 10, 11–13, 21].

**MAIN PART**

The aim of the research was to study the phytohormonal peculiar properties of formation of wheat androgenic structures by different morphogenesis pathways in anther culture *in vitro* depending on the balance of endogenous (in anthers) and exogenous (in culture medium) auxins.

**Materials and methods**

The objects were for the first time introduced in biotechnological practice spring wheat cultivars Salavat Yulaev, Zhnitsa, Duet, Skala, Bashkirskaya 26 and Omskaya 35. These cultivars demonstrate a high response in the culture *in vitro* and also are promising for the climatic zone of the Southern Ural. Donor plants were grown in the field conditions (Ufa region).

Anthers cultured *in vitro* according method described in [15] on induction nutrient medium Potato II supplemented by 0,2 mg/l kinetin and different concentrations of 2,4-D (from 0,0 to 2,5 mg/l at interval of 0,5 mg/l ).

Quantitation of endogenous IAA content in anthers was performed by ELISA [27]. Morphological identification of androgenic structures was performed with a stereomicroscope Technival 2 (Carl Zeiss, Germany). The frequencies of developing androgenic structures were calculated as a percentage of formed structures to total number of inoculated anthers.

Data were analyzed with Microsoft Office Excel 2010. Student *t* test was used to test significant differences.

**Results and discussions**

The ELISA data on endogenous auxin IAA content in anthers just before inoculating them on the induction medium Potato II are shown in table (data for 2014–2015 years of study). The results showed significant differences of anthers of studied cultivars on the content of this hormone. According to the criteria developed by [10], cultivars Skala, Bashkirskaya 26 and Omskaya 35 are highly-auxin genotypes, and cultivars Salavat Yulaev, Zhnitsa and Duet –low-auxin ones.

In anther culture *in vitro* of all studied wheat cultivars have been observed following morphogenesis pathways *in vitro* of microspores – embryogenesis and callusogenesis.

Embryoidogenesis is the formation of two types of embryo like structures - embryoids: (1) bipolar, similar to the zygotic embryos – proper embryoids and (2) with multiple shoot apexes (SA) and one common root – polyembryoids. The first type of embryoids in cereals, including wheat, well studied

[3, 8, 9, 14, 18, 23, 24]. The second type of embryoids in wheat was revealed by us for the first time [26]. Callus is a heterogeneous mass of cells having different morphogenetic potentials. In anther culture *in vitro* of cereals two types of calli are present – morphogenic and non-morphogenic – able and enable to form androgenic plants, respectively [2, 8, 14, 16, 25].

*Table*

**Content of endogenous auxin IAA in anthers of the studied cultivars of spring wheat before their inoculation on Potato II induction medium (values are means ± standard errors)**

| Cultivar | Content of endogenous auxin IAA, ng/g of dry weight | |
|---|---|---|
| | 2014 | 2015 |
| Salavat Yulaev | 71,4±6,5* | 66,5±11,3$^{ns}$ |
| Skala | 324,8±38,1* | 390,7±21,5* |
| Omskaya 35 | 429,5±6,3* | 402,9±3,9* |
| Bashkirskaya 26 | 276,9±3,7* | 234,7±8,5* |
| Zhnitsa | 59,4±10,3* | 70,3±13,2* |
| Duet | 45,8±2,3$^{ns}$ | 54,6±3,2$^{ns}$ |

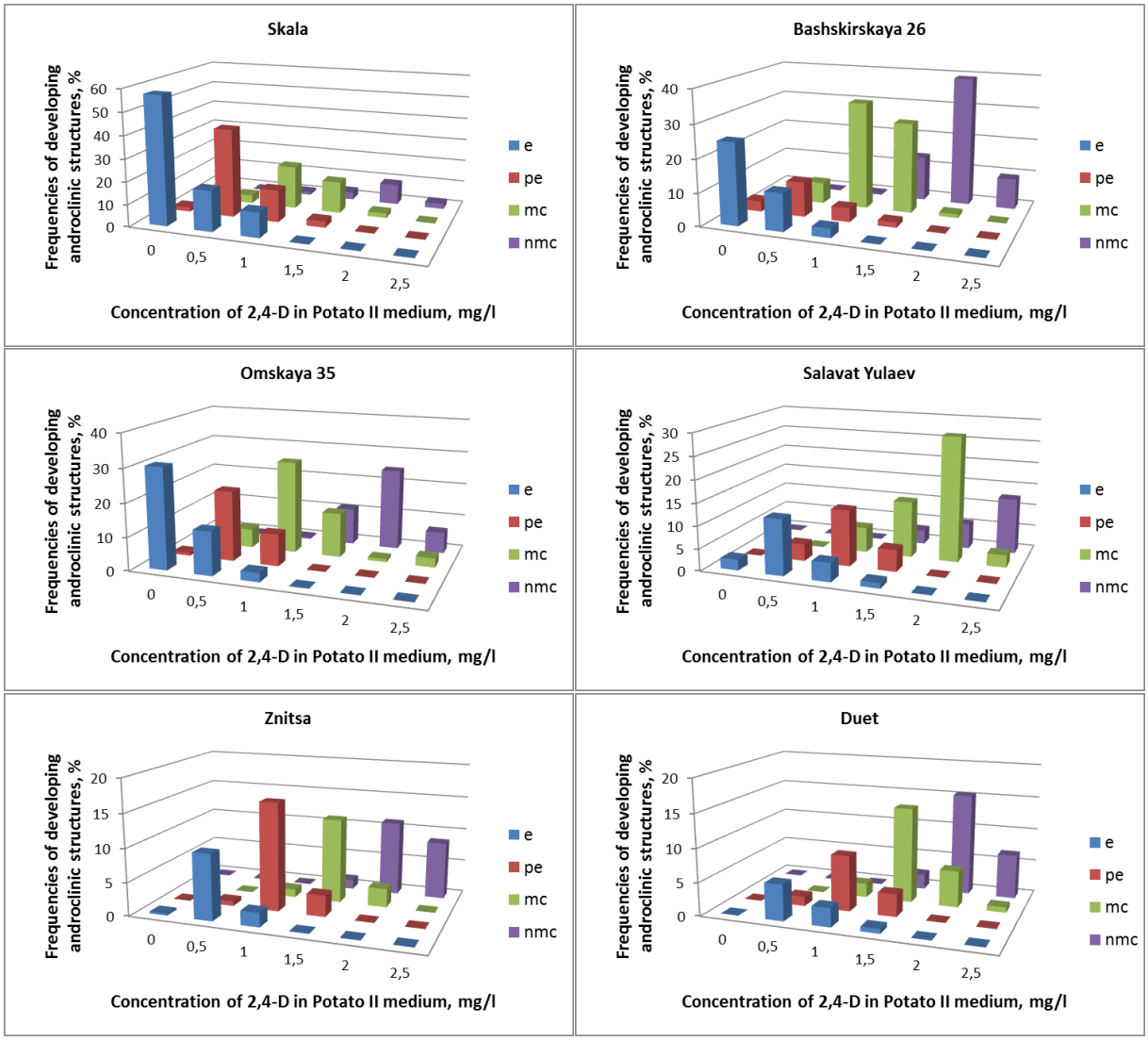
* significant at $p < 0,05$, $^{ns}$ not significant

Morphogenesis in the anthers of each of studied wheat cultivars was characterized by its own peculiarities (fig.). So, in highly-auxin cultivars the maximum frequency of embryoids formation observed on 2,4-D free medium. Increasing of 2,4-D concentration led to lessening of embryoids number and the beginning of polyembryoids formation. The maximum frequency of polyembryoid formation observed on medium with 0,5 mg/l 2,4-D. On medium with 1,0 mg/l 2,4-D lessening of polyembryoids happened and began the formation of morphogenic callus. Stable morphogenic callus formation in anthers of Omskaya 35 occurred on medium with 1,0 mg/l and in anthers of Skala and Bashkirskaya 26 – on medium with a wide range of 2,4-D concentrations (1,0–1,5 mg/l). Increasing of 2,4-D concentration to 2,0–2,5 mg/l led to formation of non-morphogenic callus. A further increase of 2,4-D concentration does not lead to the formation of any identified androgenic structures in anthers of these wheat cultivars.

When anthers of low-auxin cultivars were cultured on 2,4-D free medium the formation of a small number of embryoids was observed only for Salavat Yulaev; cultivars Zhnitsa and Duet did not form any androgenic structures. Concentration of 2,4-D optimal for formation of maximum number of embryoids and polyembryoids in these cultivars was 0,5 and 1,0 mg/l, respectively. The preferential formation of morphogenic calli in cultivars Zhnitsa and Duet was observed on medium with 1,5 mg/l 2,4-D; in the cultivar Salavat Yulaev concentration range of 2,4-D was wider – 1,5–2,0 mg/l. Increase of the 2,4-D concentration to 2,5 mg/l resulted in the formation of primarily non-morphogenic calli. At the concentration of 2,4-D more than 3,0 mg/l were no any androgenic structures.

A comparison of the obtained results revealed the following rule: regardless of the contents of endogenous IAA induction morphogenesis pathways at gradually increasing concentrations of exogenous 2,4-D is always characterized by a certain sequence: embryoids formation – polyembryoids formation – morphogenic callus formation – non-morphogenic callus formation. Thus, different hormonal status of anthers of studied wheat cultivars causes their various ability to form androgenic structures on nutrient media having different concentrations of 2,4-D.

It is well known that auxin plays a key role in the growth and development processes, many of which depend on the polar auxin transport in organs and tissues [19]. Auxins are weak lipophilic acids that dissociate in aqueous solutions. This means that a decrease in pH of the solution result in increase of the proportion of undissociated auxin molecules. Usually the pH of the cytoplasm greatly exceeds the pH of the extracellular solution. Under these conditions, undissociated auxin molecules diffuse through the plasmalemma inside the cell and dissociate intracellularly, in accordance with the pH of the cytoplasm, thereby maintaining a gradient of concentrations and a neutral molecules flow in the cytoplasm. Because of the low permeability of dissociated auxin molecules they accumulate in the cell [22]. In culture *in vitro* synthetic auxin 2,4-D, apparently, also diffuses according the concentration




gradient from medium in microspores through plasmalemma.

Fig. Frequencies of androgenic structures formation in anthers of studied spring wheat cultivars under culture in vitro on Potato II induction medium, containing different concentrations of 2,4-D (data of 2015 year).
e – embryoids, pe – polyembryoids, mc – morphogenic calli, nmc – non-morphogenic calli

According to our observations preferential formation of embryoids in cultured anthers of highly-auxin cultivars noted at 2,4-D free medium. It is suggested that auxin gradients plays a major role in the establishment of embryo symmetry [6]. Thus auxin flows create positional information and act as a powerful morphogenetic factor and determine the differentiation of the embryo organs. Preferential formation of embryoids on 2,4-D free medium suggests that the amount of endogenous IAA entering the microspores from anther tissues (a kind of polar transport) is enough for microspore development *in vitro* by the such pathway as embryoidogenesis. Probably anthers with high endogenous auxin content are able to self-regulate morphogenic processes even in the absence of exogenous stimulants. Low-auxin cultivars, which microspores form embryoids only in the presence of exogenous 2,4-D, apparently insufficient endogenous auxin to induce and maintain morphogenesis. So microspores require exogenous auxin.

Formation of polyembryoids with increasing concentrations of 2,4-D in the medium may be explained by the accumulation of 2,4-D in the cells of forming androgenic structures. It is shown that the initial stages of embryoids and polyembryoids development (prior to organogenesis) are similar. The differences begin to appear from the time of SA initiation [24, 26]. Fischer et al. [7] found that in undifferentiated wheat zygotic embryo the site of the

SA emergence is not strictly determined, but all cells of embryo apical part competent to form SA. *In vivo* auxin from the basal part of the embryo is transported polar in two directions – to the sites of SA and scutellum differentiation and is not available for other cell groups of embryo. However auxin transport disturbance can result in auxin accumulation in cells in which its content is usually low. These cells may already be morphogeneticaly competent and auxin may push them to form additional SA and hence polyembryos. Since in a culture *in vitro* exogenous auxin influx in the cells is not controlled, it appears, in this case multiple SA of polyembryoids is formed as result of 2,4-D accumulation in the cells of apical parts of embryoids.

Morphogenic callus formation in further increasing of 2,4-D concentration can be explained as follows. It was found that the initial stage of the calli development (as well as embryoids and polyembryoids) is the formation of multicellular structures (MCSs) [8]. Apparently metabolism of synthetic auxin 2,4-D done by enzymes, which in microspores are intended for other purposes (e.g., detoxification of xenobiotics), therefore it carried out very slowly and its concentration in MCSs cells is high. In turn, this leads to a proliferation of the MCSs and the formation of calli. More high concentrations of 2.4-D (kind of stress) led to accumulation in anthers stress hormone – abscisic acid [8] and to suppression further MCSs development and non-morphogenic callus.

**CONCLUSION**

Thus, obtained data confirms the possibility of regulation of morphogenesis pathways induction in wheat anther culture *in vitro* in desired direction by selecting adequate balance of endogenous auxin IAA in anther of donor plants and exogenous auxin 2,4-D in the induction nutrient medium Potato II.

This approach makes the intricate processes of wheat microspore morphogenesis in culture *in vitro* controlled – depending on the goals of the certain biotechnological research.

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