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EFFECT OF ZEATIN ON in vitro EMBRYOGENESIS AND PLANT REGENERATION FROM ANTHER CULTURE OF HEXAPLOID TRITICALE (× *Triticosecale* Wittmack)

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Abstract

To achieve results sooner, cereal crop selection programs usually combine conventional methods, such as selection of parents and large-scale cross-breeding with haploid technology, a methodology which allows obtaining homozygous lines from the F_1 hybrids. Methods of androgenesis (anther culture and isolated microspore culture techniques) have gained widespread use for selection of wheat and triticale. Currently, the main issue for the androgenesis in Triticale is the low efficiency of green plant regeneration. The present work, for the first time ever, utilizes cytokinin zeatin as an exogenic phytohormone in the induction medium, and determines its concentration optimal for improving embryo formation and green plant regeneration from the triticale anther culture. The aim of this research is to increase efficiency of the triticale anther culture, and study the effects of adding cytokinin zeatin to the nutrient medium on embryogenesis induction and regeneration. Two lines of spring triticale, YaTKh-327-11 and Zernokormovoye 5 (facultative), and two lines of winter triticale, T-968 and T-45, were used. Donor plants for the haploid technology were grown in the irrigated field of Kazakh Research Institute of Agriculture and Plant Growing LLP (Kazakhstan, Almaty Region). Cut spikes were subjected to low temperature (4 °C for 14 days), and then the anthers, after they were isolated, to high temperature (32 °C for 3 days). The spikes were sterilized with 0.1 % solution of mercuric chloride. Modified mW14 medium was used as the basic nutrient medium for embryogenesis induction. Five variants of nutrient medium were studied, with concentration of phytohormone zeatin gradually increasing in each subsequent variant (0.2 mg/l, 0.4 mg/l, 0.6 mg/l, 0.8 mg/l, 1.0 mg/l), and medium without zeatin served as control. The study conducted on 4 genotypes of triticale has shown that addition of zeatin to the nutrient mediums in concentrations of 0.2-0.8 mg/l increased the rate of androgenic structure formation by 42.3-65.2 %. Maximal effect on the androgenic structure formation was achieved at 0.4 mg/l concentration of zeatin, with 112 androgenic structures (AS) per 100 anthers on average compared to 67.8 AS per 100 anthers in control group. In the embryogenesis inducing nutrient mediums with 0.4-0.6 mg/l zeatin concentrations the rate of embryogenesis was 16.9-24.1 % higher compared to the control, with embryos having bipolar structure, and producing stem and roots during the regeneration, which indicates positive effect of zeatin on differentiation and organogenesis of the dividing microspore cells. All the variants in the experiment showed a significant increase in the rate of regeneration compared to the control with no zeatin added. In embryos transplanted from the medium containing 0.6 mg/l zeatin the rate of green plant regeneration was the highest reaching 6.3 pcs/100 anthers. It has been established that addition of zeatin and the effect of genotype were the statistically significant factors for androgenic structure formation and regeneration. Efficiency of spontaneous chromosome doubling in triticale amounted to 26.5 %, which has allowed producing 97 double haploid lines from the promising lines of triticale without colchicination.

Keywords: triticale, anther culture, zeatin, embryo, regeneration, albino plants, green plants, spontaneous doubling

Triticale (× *Triticosecale* Wittmack) is a species designed by crossbreeding the wheat (*Triticum* spp.) and rye (*Secale cereale* L.). Combination of alleles of both ancestors enables the plants to adapt to the environment that is less favorable for the wheat but ensures better production of biomass and fodder quality. Triticale possesses considerable potential for production of grain and fodder, although the research for improvement of yield of this species remains behind the similar works in respect of other cereals. It also becomes popular as a cover crop for improvement of soil and reduction of depletion of nutrients. Triticale, just as rye, is suitable for both linear and hybrid breeding methods. Achievements in molecular biology and diversity of genetic resources of wheat and rye may be used to improve triticale [1].

Spiked cereals acreage in Kazakhstan was 14209.3 thousand hectares in 2018 [2]. For Kazakhstan, cultivation of spring and winter triticale forms is important, although the relevant breeding programs are just developing. Cereal crop selection programs usually combine conventional methods, i.e. selection of parent pairs, large-scale cross-breeding and haploid technology (anther and isolated microspore cultures), a methodology which allows obtaining homozygous lines from the F_1 hybrids. The effectiveness of the main methods of triticale anther culture and isolated microspore culture depends on genotype, growing conditions, time of harvesting of donor plants, preliminary treatment (cold, warmth, carbohydrate deficiency), composition of nutrient medium for induction of embryogenesis and plant regeneration. Most progress in studying triticale anthers was achieved due to development of wheat haploid technology [3].

For now, fairly high performance was achieved through use of two androgenesis methods [4-7]. Isolated microspore culture is more effective as compared to the anther culture although this method is more labor-intensive and requires fine manipulations in density-gradient fission of microspores, which may be difficult when handling a large number of hybrid combinations.

Growth regulators are one of the important factors in androgenesis. Embryogenesis induction can be manipulated through use of various types and concentrations of exogenic phytohormones and regulating their presence in the nutrient medium [8]. Auxins are introduced to the anther and isolated microspore cultures of cereals for the purpose of initiation of microspore fission. Addition of 2,4-dichlorophenoxyacetic acid (2,4-D) allows for high results in obtaining androgenic structures and green plants (9). Use of 2,4-D auxin is described in many reports of success [4, 10, 11]. As growth regulators acting in combination with auxin, 6-benzylaminopurine (6-B) and kinetin are used the most [12-14]. However, in some studies where zeatin was used, fairly high results have been achieved in the frequency of embryoids (53-68%) and green plant formation (20-22 plants/100 anthers) [15, 16].

Zeatin is a cytokinin class phytohormone extracted from unripe corn seeds. In cultural plant media, it is a widely use alternative to kinetin, 6-benzylaminopurine or isopentenyl-adenosine [17]. Zeatin participates in in vitro differentiation of callus tissue and in organogenesis [18-21], successfully used for experimental androgenesis of pepper and eggplant [22, 23].

Despite effective protocols and continuous optimization of nutrient media, culture and pre-treatment conditions and other factors increasing the dihaploid line production, there is still an issue of reproducibility of results, low production of green plants and dependence of the result on the genotype. For large-scale application and production of double haploid, triticale needs optimization of existing tested protocols.

In our experiments involving triticale anther cultures, cytokinin zeatin was for the first time used as exogenic phytohormone of induction medium. Its optimal concentration was established and the process of embryoids and regeneration of green plants was improved.

The purpose of this research was the improvement of efficiency of the triticale anther culture technology and studying the effect of adding cytokinin zeatin to nutrient medium for embryogenesis induction and green plant regeneration.

Techniques. Spring (YaTKh-327-11 and Zernokormovoye 5) and winter (T-968 and T-45) triticale varieties and lines responsive to embryogenesis induction and anther culture regeneration were used in the experiment [24, 25].

Donor plants for haploid technology were grown in 2017 (irrigated field, Kazakh Research Institute of Agriculture and Plant Growing LLP, Republic of Kazakhstan, Almaty Region, Karasarai district). Unripe inflorescences were harvested from donor plants during the phase of flag leaf still in leaf sheath with microspores at medium and late uninuclear development stages. Microspore development stage was assessed according to the generally accepted methodology (light microscopy of temporary squash preparations) [26].

All cut donor plants were cured in the refrigerator at 4 °C for 14 days [27]. Cold-treated spikes were sterilized with 0.1% mercury dichloride for 6 minutes on the shaker, than flushed for three minutes thrice with sterile distilled water.

As a basic medium for embryogenesis induction in studying the effect of zeatin phytohormone, modified mW14 medium [28] was used with addition of 90 g/l of maltose (TM Media, India), 1 000 mg of glutamine-L (AppliChem GmbH, Germany) [12], 2 mg/l of synthetic auxin 2,4-D (Aldrich Chemistry, USA), 50 g/l of ficoll 400 (Sigma Life Science, Sweden). For removal of inhibition of embryogenesis with phenolic compounds egested from obsolescent anthers, nutrient medium was supplemented with ascorbic acid (4 mg/l) (24). Experiment variants differed in zeatin (Sigma-Aldrich, India) concentration in nutrient medium (I, II, III, IV, V — 0.2; 0.4; 0.6; 0.8 and 1.0 mg/l, respectively), the control was zeatin-free medium.

Anthers were extracted from spikes under aseptic conditions and placed on plastic Petri dishes with a diameter of 60 mm (100 anthers per dish containing 6 ml of liquid nutrient medium for embryogenesis induction) [29]. In each variant, 500 anthers were used. To prevent contamination, an antibiotic (cefatoxime) in a concentration of 200 mg/l was added to the nutrient medium. Anthers were incubated in the dark at 32 °C for the first 72 hours, whereafter they were moved to the incubator with a temperature of 25-28 °C until new formations appeared.

In the process of extraction and after inoculation to culture medium, the state of microspores was monitored using MT4000 microscope (Meiji Techno, Japan; ×40-×1000 magnification).

Androgenic structures (AS) that reached the size of 2.0-2.5 mm, were re-inoculated on nutrient medium for regeneration on Petri dishes with a diameter of 90 mm (20-30 androgenic structures per dish). Smaller AS were left on the medium for further growth. After every such inoculation, a medium was added 1 ml of similar fresh medium.

Material inoculated on nutrient medium for plant regeneration was incubated at 16-hour photoperiod, 10 000 lux illumination and temperature of 24-26°C. For regeneration, a premix of Murashige and Skoog (MS) nutrient medium components (Sigma Life Science, USA) with addition of 2 mg/l of zeatin (Sigma-Aldrich, India), 30 g/k of sucrose (AppliChem GmbH, Germany) and 6 g/l of agar (B&V srl, Italy) was used. For root formation, a premix of MS nutrient medium

with addition of 0.5 g/l of casein hydrolysate (Fluka Analytical, USA), 20 g/l of sucrose, 2 mg/l of indolebutyric acid-3 (IBA) (Sigma Life Science, China), 6 g/l of agar was used.

Ploidy of plants obtained was measured on Cy Flow Ploidy Analyser (Sysmex Partek GmbH, Germany). Samples for analysis were prepared using CyStain® UV Precise P kit (Sysmex Partek GmbH, Germany).

Adaptation of regenerant plants to soil was carried out in a climatic chamber KBWF 720 (Binder GmbH, Germany) where the temperature of 23-24 °C, illumination of 8 000-10 000 lux and humidity of 80% were maintained. During the first 2 weeks (adaptation period), the regenerant plants were sprinkled with a phytohormone solution (0.5 mg/l kinetin, 2 mg/l gibberellic acid, 3 mg/l nicotinamide). Regenerant plants of winter lines of triticale were vernalized in a refrigerated chamber for 6 weeks at 3-4 °C and constant illumination.

Statistical processing was carried out in open-source R programming language, R version 3.2.3 (2015-12-10) (Wooden Christmas-Tree) (https://www.r-project.org/alt-home/). Mean (*M*) and standard deviations (±SD) were calculated. Standard parametric tests were run using integrated libraries and extra suites (dplyr, gglot, pisch and others): regression analysis, analysis of variance (ANOVA) and pairwise comparison of means by Tukey test.

Results. Microspore development and androgenic structure formation was monitored throughout the period of anthers cultivation. Escape of microspores from the anther sac into liquid nutrient medium happened very fast and made 70-80%. Emergence of the first androgenic structures was registered 18-25 days after commencement of cultivation depending on triticale genotype. The majority of androgenic structures was forming of separate microspores in the process of direct embryogenesis and possessed all structures typical for a normal embryo. In YaTKh-327-11 genotype, AS emergence was registered in the middle of the 3rd week of culture in all experiment variants. In three other samples, visible AS started to emerge on the 4th week of cultivation.

1. Embryogenesis and plant regeneration in spring and winter lines of triticale (× *Triticosecale* Wittmack) with various zeatin concentrations in the induction medium of (*M*±SD)

Genotype	Group					
	control	I	II	III	IV	V
Androgenic structures per 100 anthers						
Spring lines:						
YaTKh-327-11	74.0±15.2	143.0±8.4	160.0±15.8	122.0±41.4	126.0±25.1	76.0±35.0
Zernokormovoye 5 (variety)	50.0±15.8	55.0±12.2	60.0±15.8	56.0±23.0	54.0±18.2	46.0±20.7
Winter lines:						
T-968	64.0±16.7	93.0±8.3	98.0±11.4	98.0±19.2	100.0±11.4	110.0±29.1
T-45	83.0±12.0	108.0±14.8	130.0±15.8	110.0±23.8	110.0±16.4	84.0±35.1
M±SD	67.8±12.2	99.8±31.5	112.0±37.6	96.5±24.8	97.5±26.7	79.0±22.8
Green plant regenerants per 100 anthers						
Spring lines:						
YaTKh-327-11	1.4±1.4	2.2±0.8	2.6±1.9	3.6±2.1	2.4±1.3	2.8±1.1
Zernokormovoye 5 (variety)	2.2±0.8	4.4±1.5	7.0±1.6	7.8±2.2	7.4±1.5	8.6±2.9
Winter lines:						
T-968	2.2±0.8	4.0±0.7	5.4±1.1	7.0±1.0	4.4±7.7	5.8±2.1
T-45	2.6±1.1	4.2±1.4	6.4±2.1	6.8±0.4	5.0±2.3	5.8±2.1
M±SD	2.1±0.4	3.7±0.9	5.4±1.7	6.3±1.6	4.8±1.7	5.7±2.1
Albino plant regenerants per 100 anthers						
Spring lines:						
YaTKh-327-11	33.4±13.0	50.2±7.5	25.8±14.1	18.6±6.1	33.4±4.7	28.8±8.3
Zernokormovoye 5 (variety)	34.6±7.5	26.8±2.9	23.6±4.0	28.2±4.9	24.4±3.7	21.0±3.8
Winter lines:						
T-968	34.2±4.0	26.7±2.9	22.3±4.0	29.0±6.5	23.8±7.4	20.4±5.0
T-45	31.6±7.7	26.9±2.9	32.2±13.9	26.0±8.1	21.0±5.6	19.4±4.8
M±SD	33.4±1.2	32.6±10.1	25.9±3.80	25.4±4.1	25.6±4.7	22.4±3.7

Note. For groups, see *Techniques* section.

In control group, the average of 67.8 AS per 100 anthers formed in all samples, in experimental group it was 96.5-112 AS per 100 anthers (Table 1). The largest formation of androgenic structures was registered in the variant II of experiment (zeatin concentration of 0.4 mg/l), where the average value was 112 AS per 100 anthers. On some Petri dishes, up to 300 AS has formed. The results substantially exceeded the values obtained in anther culture by other authors: over 50 embryo-like structures per 100 anthers on CHB-3 and NPB99 media [30], 5.8-20.7 embryo-like structures per 100 anthers on 190-PAA and 190-D/K media [13], and 47.2-55.5 embryo-like structures per 100 anthers on mW14 medium [4]. It should be noted, however, that in some studies where the isolated microspore cultures were used, a fairly high degree of formation of androgenic structures, up to 500 embryo-like structures per 100 anthers, was observed in responsive model genotypes [4, 7].

To determine the dependence of embryogenesis on genotype and zeatin phytohormone, the analysis of variance was conducted (Table 2). Zeatin and genotype turned out to be statistically significant factors in formation of androgenic structures and regeneration.

2. Statistical analysis (ANOVA) **of effect of genotype and zeatin in the induction medium on embryogenesis and plant regeneration in spring and winter triticale (× *Triticosecale* Wittmack) lines**

Factor	df	MS	*F*-value	P-value
Androgenic structure formation				
Genotype	3	22328.0	39.2	< 2.2e – 16**
Zeatin	5	4885.7	8.6	6.766e – 07**
Residuals	111	570.0		
Green plant regeneration				
Genotype	3	73.8	26.6	4.87e – 13**
Zeatin	5	47.4	17.1	1.70e – 12**
Residuals	111	2.78		
Albino plant regeneration				
Genotype	3	219.1	3.2	0.027067*
Zeatin	5	387.5	5.6	0.000119**
Residuals	111	69.0		

Note. df — number of degrees of freedom, MS — mean square, *F*-value — F-test statistics, P-value — significance.
* and ** Effect is statistically significant at $p \leq 0.01$ and $p \leq 0.0001$.

3. Regression analysis of effect of genotype and zeatin in the induction medium on embryogenesis and plant regeneration in spring and winter triticale (× *Triticosecale* Wittmack) lines

Parameter	β-Factor of multiple regression	
	androgenic structure formation	green plant regeneration
Zeatin concetration, mg/l		
0.0 (Intercept)	29.4***	3.6***
0.2	32.0***	1.6**
0.4	43.3***	3.2***
0.6	28.3**	4.2***
0.8	29.5**	2.7***
1.0	11.2	3.7***
Genotype		
Zernokormovoye 5 (variety) (Intercept)	29.4***	3.6***
YaTKh-327-11	63.3***	–3.7***
T-968	39.8***	–1.4**
T-45	50.0***	–1.1*

*, ** and *** Factor value is statistically significant at $p \leq 0.01$; $p \leq 0.001$ and $p \leq 0.0001$.

After determining a substantial difference between the groups as a whole, we have applied regression analysis for determining a quantitative correlation between the indicators and factors (zeatin, genotype). For formation of androgenic structures, the highest β-regression factor (43.3) was detected in the variant II where zeatin concentration was 0.4 mg/l (Table 3). Genotypes

demonstrated distinct response to androgenic technology (see Table 3): highest β-regression factors were typical for YaTKh-327-11 and T-45, 63.3 and 50.0, respectively.

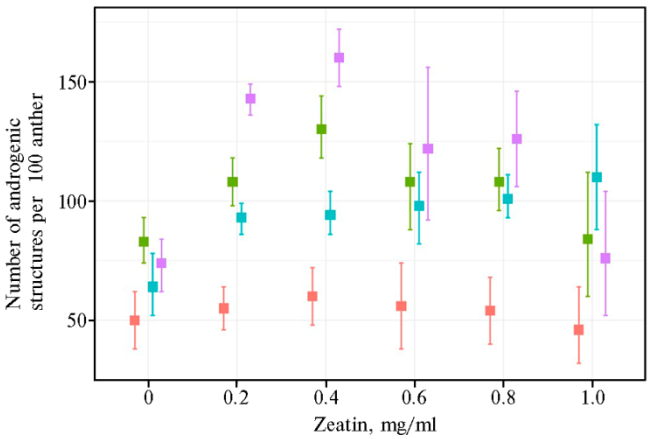

Fig. 1. Formation of androgenic structures in spring and winter triticale (× *Triticosecale* Wittmack) lines at different concentrations of zeatin in the induction medium and depending on genotype: : — Zernokormovoye 5 (variety), — T-45, — T-968, — YaTKh-327-11.

Tukey test for pairwise comparison of the average count of formed androgenic structures at different concentrations of zeatin and in control variant demonstrated statistically significant difference ($p_{adj} < 0.01$) for concentrations of 0.2-0.8 mg/l. The largest difference between group means was observed between control group and variant II, 43.2 (Tukey test results). In general, the results obtained correlate with the visual assessment of differences presented in the box plot (Fig. 1).

Androgenic structures that reached the size of 2.0-2.5 mm were re-inoculated on nutrient medium for regeneration (Fig. 2, D). Plant regeneration took place for 3-14 days after passage. In YaTKh-327-11 and T-968 genotypes, plant regeneration was partially observed on embryogenesis induction medium in the dark. From among the androgenic structures inoculated in the control variant, the average of 2.1 green plants per 100 anthers regenerated for different genotypes. The largest frequency of regeneration of green plants (6.3/100 anthers) was registered in the variant III. Maximum green plant regeneration frequency was registered for Zernokormovoye 5 genotype (8.6/100 anthers) (see Table 1). The results of our research correlate with the data obtained by Eudes et al. [30] and Tuvesson [31] (6 or more green plants per 100 anthers). Higher values (10.8-16.8 green plants per 100 anthers) were obtained by Hungarian scientists [4, 13].

The regression analysis has shown positive impact of increase in zeatin concentration on green plant regeneration (see Table 3). The highest regression factor ($\beta = 4.2$) was obtained in variant III (see Table 3). Presence of statistically significant difference ($p_{adj} < 0.05$) between the experimental and control groups was identified in all zeatin concentrations. The largest difference (4.2) was established between control group and experience variant III.

Regeneration of albino (chlorophyll-free) seedlings in control variant made 33.4/100 anthers on the average for the lines. As zeatin concentration increased, the number of albino seedlings decreased, and their least number was registered in experiment version V (22.4/100 anthers).

Haploid lines may be obtained from isolated anthers through direct regeneration of somatic embryos (embryoidogenesis) and through callusogenesis. Effectiveness of embryoid formation depends on the genotype and may vary considerably from 0 to 95% [32]. Only few researchers pay attention to differentiating the formed androgenic structures as calluses and embryoids [30].

Fig. 2. Main stages of obtainment of regenerants in triticale (× *Triticosecale* Wittmack) anther culture: A — Zernokormovoye 5 donor spikes in the field; B — YaTKh-327-11 line microspore at late unicellular stage (×1000 magnification, MT4000 microscope, Meiji Techno, Japan); C – T-968 line androgenic structures on embryogenesis induction media, experiment version II; D – T-45 line embryoid plant regeneration, experiment version III; E – T-45 line green plants on nutrient medium for acceleration, experiment variant II; F – results of ploidy analysis using Cy Flow Ploidy Analyser (Sysmex Partek GmbH, Germany); G – T-968 line dihaploid plants, experiment variant II in greenhouse; H – T-968 line dihaploid plant blossoming, experiment variant III; I- T-968 line fertile dihaploid plant seeded spikes, experiment variant II. For variant description, see *Techniques* section.

Upon re-inoculation on agarized medium for regeneration, all androgenic structures in our experiments were differentiated as embryoid (Fig. 3) with bipolar structure and calluses. From the control zeatin-free medium, 949 AS were re-inoculated, 57.2% of which were embryoids (Table 4). In all variants where cytokinin was present in the medium, higher percentage of embryoid formation (60.8-71.0%) was registered, except for variant I (45.0%). Maximum percentage of embryoids has formed in variant III (71.0%). Embryoid regeneration of plants occurred in 92% of cases, resulting in formation of sprouts and roots.

4. Formation of embryoids, green plants and dihaploid lines in spring and winter triticale (× *Triticosecale* Wittmack) depending on zeatin concentration in the induction medium

Variant	Reinoculated androgenic structures	Embryoids, total (%)	Calluses, total (%)	Green plants	Plants, total (%)		Spontaneously doubled dihaploid lines, total (%)
					replanted in ground	acclimatized to ground	
Control	949	543 (57.2)	406 (42.8)	45	36 (80.0)	29 (80.5)	7 (24.1)
I	1397	630 (45.0)is	767 (54.0)	79*	48 (60.7)	42 (87.5)	12 (28.5)
II	1554	1040 (66.9)**	514 (33.1)	110**	87 (79.1)	68 (78.2)	21 (30.8)
III	1366	970 (71.0)**	396 (29.0)	114**	95 (83.3)	86 (90.5)	24 (27.9)
IV	1344	860 (64.0)**	484 (36.0)	93**	68 (73.1)	60 (88.2)	14 (23.3)
V	1185	720 (60.8)**	465 (39.2)	117**	89 (76.1)	81 (91.0)	19 (23.4)
Всего	7795	4793 (61.0)	3002 (39.0)	558	423 (75.8)	366 (86.5)	97 (26.5)

N o t e. For variant description, see *Techniques* section.
* and ** Effect is statistically significant at $p \le 0.001$ и $p \le 0.0001$ (ANOVA); is — impact is statistically insignificant.

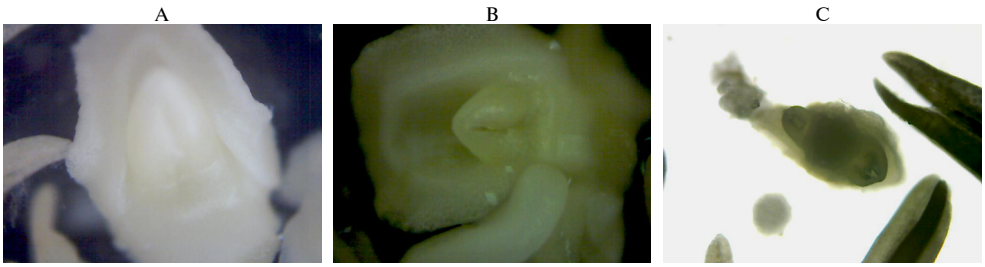

Fig. 3. Triticale (× *Triticosecale* Wittmack) embryoids obtained in anther culture: A — T-45 line, experiment variant II; B — YaTKh-327-11 line, experiment variant II; C — YaTKh-327-11 line, experiment variant III (×40 magnification, MT4000 microscope, Meiji Techno, Japan). For variant description, see *Techniques* section.

For adaptation to the ground, green plants that had well-formed root system and leaves were selected. In 24% of plants, lack of roots, poor development, leaf curl due to insufficient formation of mechanical tissue were found. During the first 2 weeks of transplanting (adaptation period), regenerant plants were sprinkled with phytohormone solution and irrigated with water solution of macro- and microelement salts, MS iron chelate. Soil acclimatization was carried out in the climatic chamber at high humidity (80%). Adaptation to ground was passed by 366 plants, which made 86.5% (see Table 4).

The final result of the described triticale anther culture technology is the obtainment of dihaploid plants. According to the tested protocols, the microspores are exposed to high and low temperatures promoting spontaneous doubling of chromosomes during the early stages of cultivation. Spontaneous doubling allows us to omit the colchicination process which the regenerant plants withstand poorly. In our experiment, spontaneous doubling was registered in 26.5% of 366 regenerant plants. This is the average for triticale which correlates with the data of other researchers [4, 7, 9], in some experiments it reached 57% [33]. Haploid plants in which spontaneous dihaploidization did not occur were subjected to colchicination during the tillering stage after sufficient rooting and development. All dihaploid plants obtained were raised to seeds under greenhouse conditions (see Fig. 2, H, I).

Thus, adding zeatin phytohormone in concentrations of 0.2-0.8 mg/l to the liquid nutrient medium mW14 for embryogenesis induction in spring and summer triticale results in 42.3-65.2% higher formation of androgenic structures (AS). The strongest effect on emergence of androgenic structures was achieved by adding 0.4 mg/l of zeatin (average formation of 112 AS per 100 anthers). In nutrient medium where zeatin concentration was 0.4-0.6 mg/l, higher frequency, as compared to control group, of formation of embryoids (by 16.9-24.1%) with

bipolar structure and producing the sprout and roots during organogenesis was established. That is, zeatin in the induction medium improves the differentiation of fissile microspore cells and organogenesis and promotes formation of embryoids. We have also registered significant increase in formation of green plants in all experiment variants (3.7-6.3/100 anthers) against control (2.1/100 anthers). The highest green plant regeneration frequency (6.3/100 anthers) was observed in embryoids replanted from nutrient medium that contained zeatin in 0.6 mg/l concentration. Effectiveness of spontaneous dihaploidization in triticale was 26.5%, which enabled us to obtain 91 dihaploid triticale lines without injurious colchicination process.

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