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Effects of Exogenous Cytokinins on Spore Germination and Gametophyte Morphogenesis of *Dryopteris filix-mas* (L.) Schott in vitro Culture

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Abstract—The impact of exogenous cytokinins (kinetin, zeatin, 6-benzylaminopurine, and N6-2-isopentenyladenine) on the pattern of *Dryopteris filix-mas* (L.) Schott spore germination, gametophyte growth and morphology in vitro have been studied. It was found that all studied cytokinins significantly retarded spore germination, inhibited gametophyte growth, caused deformations and decrease in the thallus size, and suppressed the development of reproductive structures and sporophyte growth at the concentration of 10^{-5} M. The reduction of the hormone concentration to 10^{-8} M stimulated the gametophyte development, induced cell divisions, particularly in the apical zone, due to which some of thalli were deformed, promoted the production of rhizoids, affected the formation of antheridia and archegonia, and slowed the sporophyte development.

Keywords: Dryopteris filix-mas, gametophyte, spores, prothallium, thallus, cytokinins, kinetin, zeatin, 6-benzylaminopurine, N^6-2-isopentenyladenine

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INTRODUCTION

A characteristic feature of ferns is the alternation of two generations during the life cycle, which implies the independent development of sporophyte and gametophyte. The processes of growth and development of gametophyte and sporophyte in ferns, as well as in representatives of other taxa, are controlled by a multicomponent hormonal system [1]. Phytohormones are low molecular weight organic compounds that coordinate genetically determined growth and development of plants as well as the continuous integration of environmental signals [2, 3]. As a rule, they act in low concentrations, and the place of these compounds' action is often separated from the place of their biosynthesis [4, 5]. Transport of phytohormones for short and long distances creates the necessary morphogenetic gradients for each hormone in different tissues and organs [6].

A spore is the first cell of the gametophyte—a sexual phase in the life cycle. Spores of ferns can stay in a state of dormancy from several weeks to a year and even decades maintaining the ability to germinate. It is known that spore germination in more than 200 fern species is affected by various environmental factors, including light conditions, temperature, the presence of nutrients, and gravity [7–12]. It is also shown that exogenous phytohormones, such as gibberellins, antheridiogen, ethylene, brassinosteroids, jasmonic acid, and cytokinins, are capable to regulate the process of spore germination in ferns [12–17]. However, it turned out that exogenous phytohormones also affect morphogenesis, development, and sex polymorphism of gametophytes. In particular, high concentrations of gibberellic acid (GA_3) inhibits prothallium growth of *Lygodium japonicum* (Thunb.) Sw., whereas low concentrations do not affect its development [18] or pro-

mote elongation of cells [19]. In the apical and antheridial zones of the prothallium of *Anemia phyllitidis* (L.) Sw., GA_3 induces cell division and cell size increasing [20]. Jasmonic acid stimulates the transition of gametophytes of *Platycerium bifurcalum* (Cav.) C. Chr. from a filamentous to a spatulate shape, increases the number of rhizoids, and activates cell division [14]. For gametophytes of *Anemia tomentosa* (Savigny) Swartz var. *anthriscifolia* (Schrader) Mickel, jasmonic acid promotes the development of sporophytes [21]. Exogenous indole-3-acetic acid, by contrast, does not affect the development of gametophyte in this fern [21]. It has been shown that synthetic auxins promote cell elongation and induce the development of filamentous gametophytes [19, 22]. But this effect is lost in combination with abscisic acid, resulting in the development of laminar gametophytes [19].

The vast majority of ferns are homosporous plants, that is, they produce one type of spores, which give rise to potentially bisexual gametophytes [7]. However, it is reported that male, female, and even asexual individuals are developed in both natural and laboratory population [23]. It was found that gibberellins and antheridiogen (gibberellin related compound) play a key role in the formation of sexual polymorphism of ferns [24]. Exogenous treatment with gibberellins in most cases activates the formation of antheridia and slows down the development of archegonia [18, 20, 24–26]. Exogenous auxins induce apogamic development of sporophytes from sterile (asexual) gametophytes in vitro [27].

Cytokinins are used to regulate growth for microclonal propagation and rooting of sporophytes in vitro [28–34]. These phytohormones control cell division, stimulate formation and activity of shoot meristems, delay the process of leaf aging, inhibit growth and branching of the root, participate in the regulation of seed germination and the formation of response to stress, etc. [35–37]. The class of cytokinins includes derivatives of adenine, compounds that are similar in structure but have different biological activity and heterogeneous functions. The hormone molecules with certain variations in the side chain structure are likely to mediate different biological signals: the participation of trans-zeatin and isopenthenyladenine in the transmission of long-distance signals in the acropetal and basipetal directions is now established [38]. Cytokinins are involved in the formation of angiosperms' sex. Thus, male sterility of corn can be overcome by the use of exogenous phytohormones of this class [39]. Cytokinins are necessary for the development of anthers and pollen [40].

There are multiple studies dedicated to the effect of cytokinins on sporophyte morphogenesis of ferns [34, 41–45]. It has been shown that cytokinins inhibit the gametophyte development, reduce the size or cause multiple overgrowth and deformation of the heart-shaped thallus, and affect the formation of gametes [46–48]. It has been established that cytokinins induce photo-morphogenesis of gametophytes grown without light, and affect the growth rate, division, elongation, and differentiation of cells [49]. However, the role of cytokinins in regulation of morphogenesis and the appearance of sexual dimorphism in gametophytes in vitro, as well as in optimization of the process of germination of fern spores, remains poorly investigated. Nevertheless the fern's gametophytes are the useful model organisms sutable for studying the influence of exogenous factors. They are distinguished by the simple structure and ease of in vitro cultivation [7, 33]. Isolated gametophytes of ferns are successfully used to study the genetic and physiological mechanisms of growth and development regulation, which contributes to obtaining new knowledge about the evolution of land plants [50]. As was reported early [30, 33, 48, 51] cytokinins are available exogenous growth regulators for in vitro cultivation of ornamental and rare fern species. The study of the effect of exogenous cytokinins can determine the role of these hormones in the regulation of growth and development of gametophytes [47]. Therefore, the objective of our work was to invesigate the effect of cytokinins on the spore germination, morphology, and characteristic features of *Dryopteris filix-mas* (L.) Schott gametophytes to clarify the possibilities for further use of exogenous cytokinins for the regulation of these processes in vitro.

MATERIALS AND METHODS

The spores were obtained from plants of leptosporangyate fern of Ukrainian flora (*Dryopteris filix-mas* (L.) Schott, family Dryopteridaceae) growing on the expositional site of vascular spore bearing plants in the O.V. Fomin Botanical Garden in Kyiv. Spores were collected from the end of June to the middle of July. Fertile leaves were cut and stored in paper bags under dry conditions until sporing (usually after a week). Spores were separated from the fragments of leaves and sporangia by shaking and stored at +20°C. Before sowing, spores were washed with sterile distilled water, centrifuged for 15 min at 9000 rpm, kept at 40% ethanol for 1.5 min, washed three times with sterile distillate, and then sown on the sterile liquid Knop medium in Petri dishes. Spores grew at +22–25°C, the photon flux density was 35–40 $\mu M/m^{-2}\ s^{-1}$, the photoperiod was 16:8 (day:night), and pH of the medium was 5.8–6.0. Observations were carried out in accordance with the stages of gametophyte development, which were determined in two terms: from the appearance of the first specimens until the maximum number of specimens that were in a certain phase of development. Exogenous phytohormones from the group of cytokinins, namely kinetin, zeatin, 6-benzylaminopurine (BAP), and N^6-2-isopentenyladenine (iP), were added into the Knop's medium immediately prior to the sowing of spores at the concentrations of 10^{-5}, 10^{-6}, 10^{-7}, and

10^{-8} M. Knop's medium without phytohormones used as control.

Since germination of *D. filix-mas* spores in vitro starts on the seventh to eighth day from the sowing, and the full germination of viable spores ends on the 13th day, the effect of phytohormones was measured precisely at this time period. The number of germinated and ungerminated spores was counted using a Bogorov chamber. The germination percentage was calculated as the ratio of the number of germinated spores to the total number of spores. Morphological changes in the development of gametophytes under the influence of exogenous phytohormones were estimated at three stages of development: 1, spatulate prothallium (30 days from the moment of sowing spores); 2, heart-shaped thallus (80 days after sowing spores); 3, sporophyte formation (120 daysafter sowing spores).

Spore germination and gametophyte development were observed using the an MBS-9 binocular microscope (Soviet Union). The morphology of spores and gametophytes was investigated using a JEOL JSM-6060 LA scanning electron microscope (Japan) and Carl Zeiss Primo Star light microscope (Germany). Sizes of the thallium of the gametophyte were determined with the AxioVision Rel. 4.8program. All experiments were carried out in three biological and analytical repetitions. The obtained results were statistically processed in the Excel program of the standard package Microsoft Office 2013. The significance of differences was estimated using Student's **t**-test using a 5% significance level ($P < 0.05$).

RESULTS AND DISCUSSION

1. Effects of exogenous cytokinins on spore germination. Spores of *D. filix-mas* are bilateral, monolete, reniform in polar view, depressed ovate to reniform in longitudinal equatorial view. The perisporium is wrinkled, wavy-folded, with verrucous outgrowths and single crests. In the lateral view, the spore with the perisporium was 49.5 ± 0.9 μm long and 37.1 ± 0.6 μm wide (Fig. 1). Taking into account the morphological features of the periosporium, spores of this species were referred to the verrucous-folded type [52].

It was established that all exogenous cytokinins at the concentrations of 10^{-5} M significantly inhibited spore germination (Fig. 2). The most powerful inhibitor was iP, whose inhibitory effect was directly proportional to the concentration. Kinetin also inhibited spore germination, where both its highest (10^{-5} M) and lowest (10^{-8} M) concentrations showed the maximum inhibitory effect, while intermediate concentrations (10^{-6} M and 10^{-7} M) exhibited less activity. The BAP concentration of 10^{-8} M did not significantly affect the spore germination, while the germination became inhibited when its concentration was increased. Zeatin at low concentrations had a positive effect on spore germination; the concentration of 10^{-7} M was the most effective (Fig. 2).

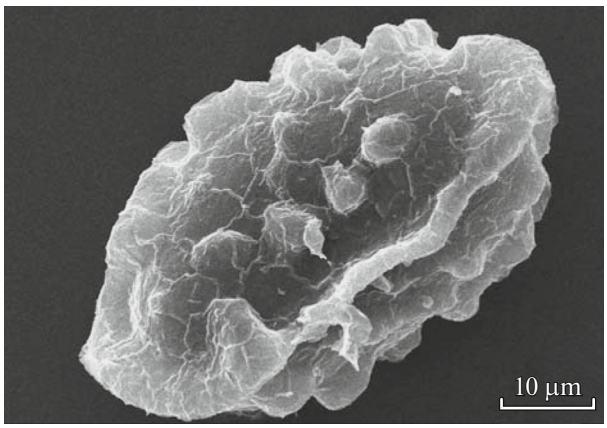

Fig. 1. General view of *D. filix-mas* spore (SEM).

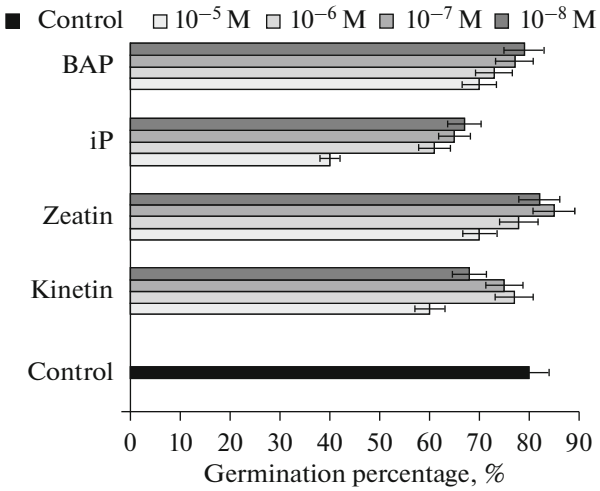

Fig. 2. Effects of exogenous cytokinins on spore germination of *D. filix-mas*.

2. Morphological changes in gametophytes under the influence of exogenous cytokinins at different stages of development. 2.1. The spatulate prothallium stage. Control samples formed asymmetric prothallium (Fig. 3a). The number of cells of the thallus was 20–45, the number of rhizoids 1–4, more often 2 (Table 1). The prothallium developed according to the *Aspidium*-type typical for ferns from the family Dryopteridaceae [53].

Exogenous iP, BAP, and zeatin at the concentration of 10^{-5} M blocked the formation of a normal protonema (Figs. 3b, 3c, 3d). The most pronounced effect was shown for iP: protonemata consisted of 3–12 cells, mainly with lateral branching from the first prothallial cell; no rhizoids were formed andthere were only their rudiments in 2–3th prothallial cells (Fig. 3b, Table 1). Under the effect of BAP and zeatin, protonemata acquired the filamentous shape, consisting of

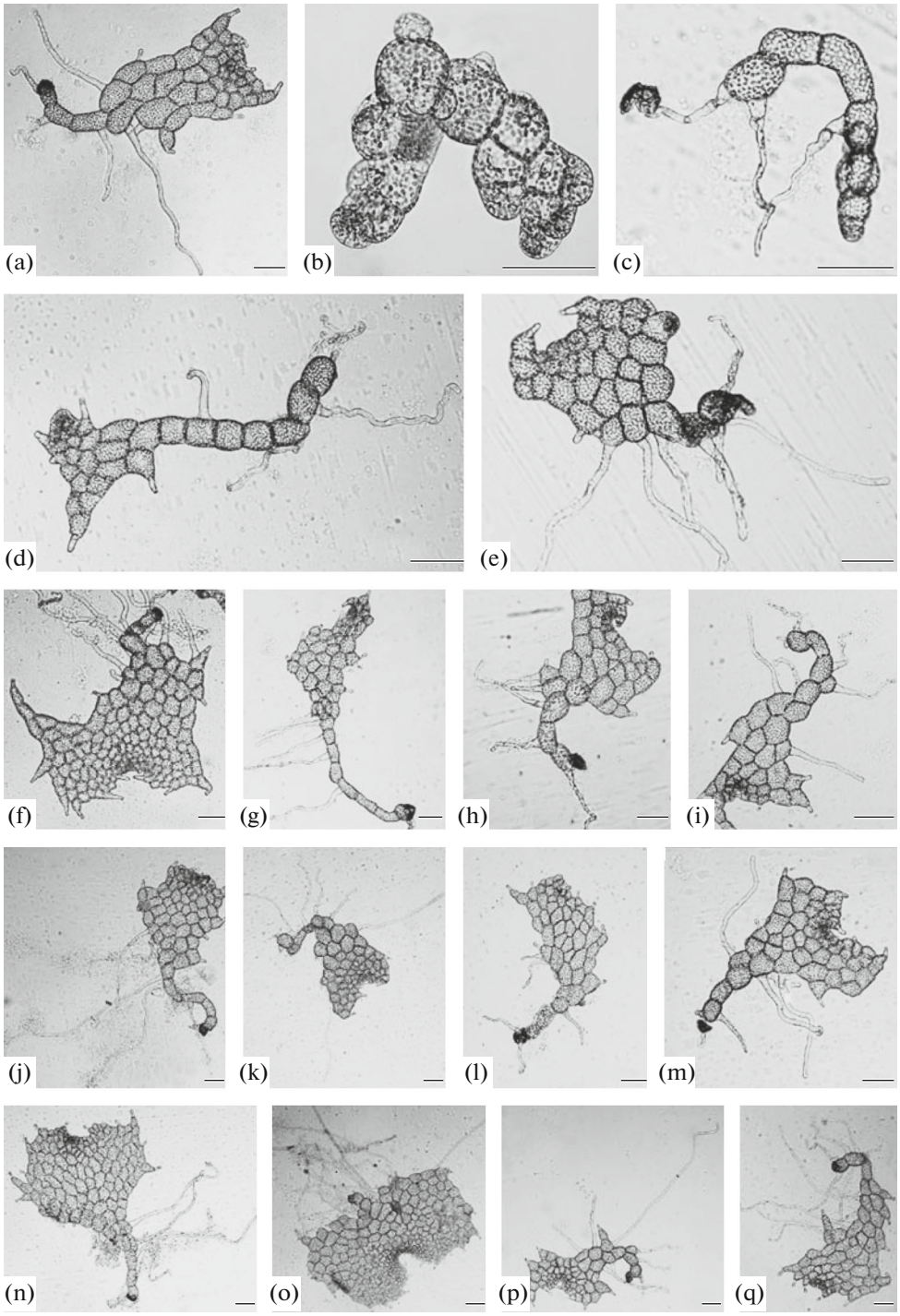

Fig. 3. Effects of exogenous cytokinins on spatulate prothallium development of *D. filix-mas* under varying concentrations: (a) control, (b) iP (10^{-5} M), (c) BAP (10^{-5} M), (d) zeatin (10^{-5} M), (e) kinetin (10^{-5} M), (f) zeatin (10^{-6} M), (g) BAP (10^{-6} M), (h) kinetin (10^{-6} M), (i) iP (10^{-6} M), (j) zeatin (10^{-7} M), (k) kinetin (10^{-7} M), (l) iP (10^{-7} M), (m) BAP (10^{-7} M), (n) iP (10^{-8} M), (o) zeatin (10^{-8} M), (p) kinetin (10^{-8} M), (q) BAP (10^{-8} M). Scale is 100 μm.

10–25 cells. Spatulate protonemata with lateral branches were rarely observed. Small rhizoids were formed in large numbers (Figs. 3c, 3d). Kinetin activated the development of rhizoids and reduced the number of cells of the prothallium, which made the "spatula" look more compact (Fig. 3e, Table 1).

Exogenous zeatin at the concentration of 10^{-6} M caused significant overgrowth of the prothallium in

Table 1. Effect of exogenous cytokinins on gametophyte morphogenesis of *D. filix-mas*

Features of gametophyte development in vitro	Control	Zeatin				Kinetin				iP				BAP			
		10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
Prothallium stage																	
Prothallium shape	Sp	Fil, Sp w/d	Sp w/d	Sp	H	Sp	Sp	Sp	Sp		Sp	Sp	Sp	Fil, Sp w/d	Sp	Sp	Sp
Number of cells	20–45	12–25	25–60	30–65	≥100	12–28	35–45	35–60	35–60	3–12	15–35	18–45	≥80	6–23	17–38	24–42	35–50
Number of rhizoids	1–4	3–6	4–8	5–8	7–18	4–6	4–6	5–7	5–9	–	3–6	3–8	5–9	2–5	2–6	4–7	4–9
Thallus stage																	
Thallus shape	H w/o/d	H w/d	H w/d	H w/d	H	H w/d	H w/d	H, Sp w/d	H	Br	Sp, Rib w/d	Sp w/d	Sp, H w/d	H, Sp w/d	H, Sp, Rib w/d	H, Sp	H
Antheridia	+	–	–	+	+	–	–	+	+	–	+	+	+	–	+	+	+
Archegonia	+	–	+	+	+	–	–	+	+	–	–	–	+	–	–	–	+
Sporophyte development	+	–	–	+	+	–	–	+	+	–	–	–	+	–	–	+	+

Br, branched shape; H, heart-like shape; Sp, spatulate shape; Rib, ribbon-like shape; Fil, filamentous shape; w/d, with deformations; w/o/d, without deformations.

width (Fig. 3f), whereas BAP, kinetin, and iP of the same concentrations did not significantly affect the gametophyte morphogenesis (Figs. 3g, 3h, 3i). Under the effect of all exogenous cytokinins, there was active development of rhizoids that were shorter than control but quantitatively prevalent (Figs. 3f–3i, Table 1).

Exogenous zeatin and kinetin at the concentration of 10^{-7} M, in contrast to iP and BAP, accelerated the overgrowth of the "spatula" of the prothallium by activating the initial cell (Figs. 3j, 3k), which was located in the central part of its apex. Such gametophytes were formed from a larger number of cells (Table 1). Zeatin, kinetin, and BAP at the concentration of 10^{-7} M stimulated the development and growth of rhizoids (Figs. 3j, 3k, 3m). However, iP at the same concentration activated appearing of rhizoids but slowed down their growth (Fig. 3l).

All exogenous cytokinins at the concentration of 10^{-8} M activated divisions of the initial cell at the apex of the spatulate prothallium, due to which the vast majority of prothallia were formed from a larger number of cells (Figs. 3n–3q, Table 1). Under the effect of iP at the concentration of 10^{-8} M, a heart-shaped thallus and a apical notch were formed (Fig. 3n), where a deformed prothallial "spatula" was also often observed. Zeatin at the lowest concentration showed a strong stimulating effect, which manifested itself in the overgrowth of the heart-shaped prothallial plate, the formation of an apical notch, and the beginning of the formation of a two-dimensional meristem (Fig. 3o). All cytokinins at the concentration of 10^{-8} M activated the growth and development of rhizoids, where BAP and zeatin were the most effective (Table 1).

2.2. The heart-shaped thallus stage. Control samples had the shape of a heart-like plate with symmetrical wings (Fig. 4a, Table 1). Typically, *D. filix-mas* gametophytes are bisexual: both antheridia and archegonia are developed at their thallus [54]. In the control, the archegonial cushion was well formed, and the first archegonia began to develop on it. Single antheridia were located along the edge of the thallus.

Exogenous iP at the concentration of 10^{-5} M blocked the development of a normal prothallial plate. The thallus was considerably branching due to the fact that the hormone stimulated multiple apical dominance with the formation of multiple initial cells. Multiple lateral proliferations were formed after divisions of initial cells that gave rise to the highly branched protonema (Fig. 4b). Zeatin at the concentration of 10^{-5} M contributed to the overgrowth of the prothallial plate, due to which the heart-shaped thallus was strongly deformed (Fig. 4c). It was accompanied by the formation of the apical notch and the multicellular meristem, but the development of the archegonial cushion was suppressed. Exogenous 10^{-5} M kinetin and BAP also caused deformity of the heart-like thallus: prothallial plates acquired a spatulate shape with barely noticeable apical notch or significant lateral overgrowths (Fig. 4d, 4e). Under the effect of 10^{-5} M of all the studied cytokinins, no reproductive organs (archegonia and antheridia) were formed (Table 1). In all the experimental variants, with the exception of iP, gametophytes had developed rhizoids.

In the samples with an iP concentration of 10^{-6} M, thalli were not heart-shaped and gametophytes usually acquired a strongly elongated, spatulate, or ribbon-like shape with numerous lateral overgrowths (Fig. 4f). Under the effect of 10^{-6} M zeatin, kinetin, and BAP, gametophytes were similar to the control (Figs. 4g–4i). The heart-like shape of the thallus predominated. Under the effect of 10^{-6} M zeatin and kinetin, single deformed gametophytes with lateral branches were

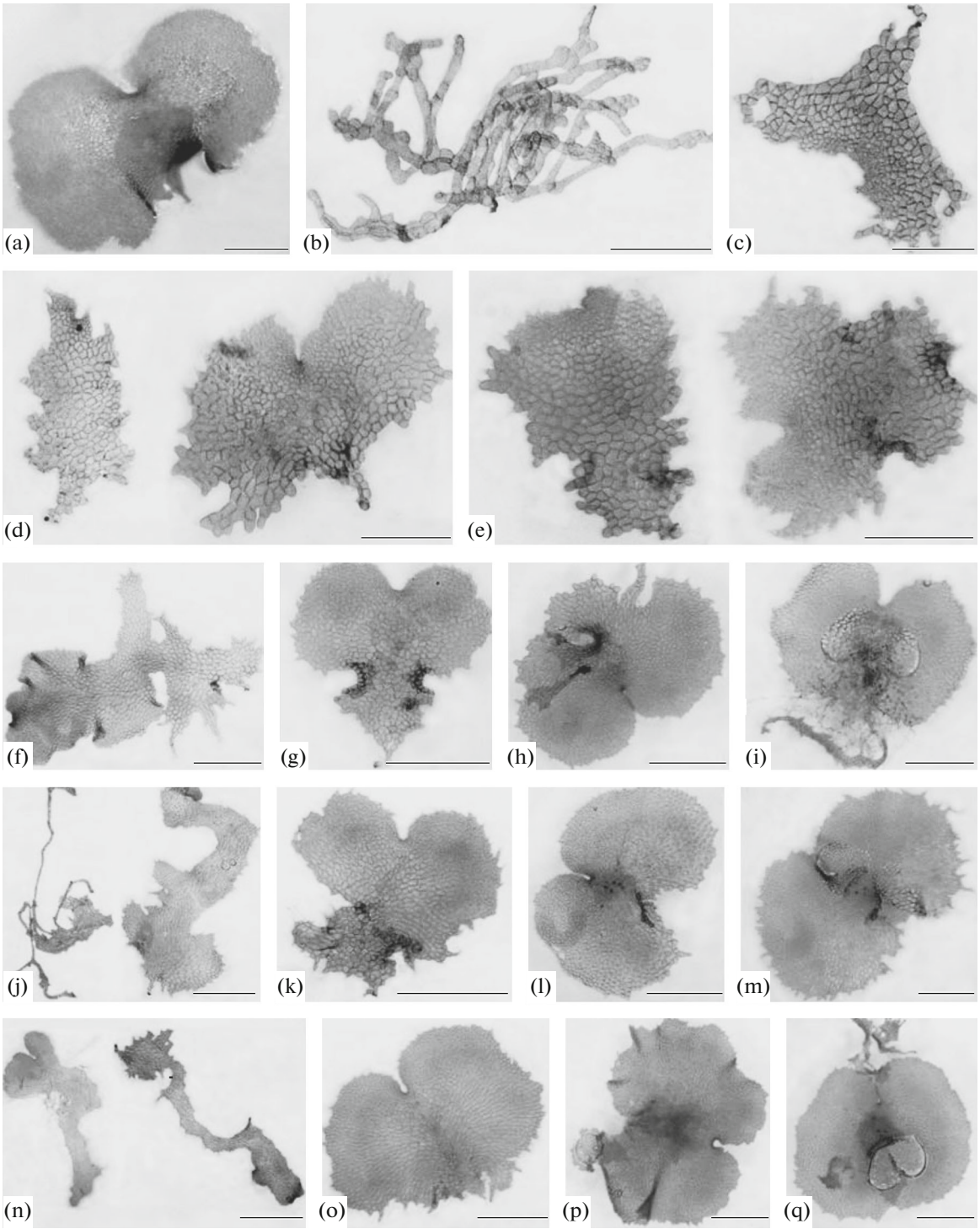

Fig. 4. Effects of exogenous cytokinins on heart-shaped thallus development of *D. filix-mas* under varying concentrations: (a) control, (b) iP (10^{-5} M), (c) zeatin (10^{-5} M), (d) BAP (10^{-5} M), (e) kinetin (10^{-5} M), (f) iP (10^{-6} M), (g) zeatin (10^{-6} M), (h) kinetin (10^{-6} M), (i) BAP (10^{-6} M), (j) iP (10^{-7} M), (k) zeatin (10^{-7} M), (l) kinetin (10^{-7} M), (m) BAP (10^{-7} M), (n) iP (10^{-8} M), (o) zeatin (10^{-8} M), (p) kinetin (10^{-8} M), (q) BAP (10^{-8} M). Scale is 100 mm.

also observed (Figs. 4g, 4h), and elongated spatulate or ribbon-shaped lateral overgrowths were observed under the effect of BAP (Fig. 4i). Under the effect of 10^{-6} M BAP and iP, the formation of single antheridia was noted (Table 1). At concentrations of 10^{-6} M, kinetin did not contribute to the formation of male and female sexual organs on the surface of the thallus. Whereas zeatin at the same concentration stimulated

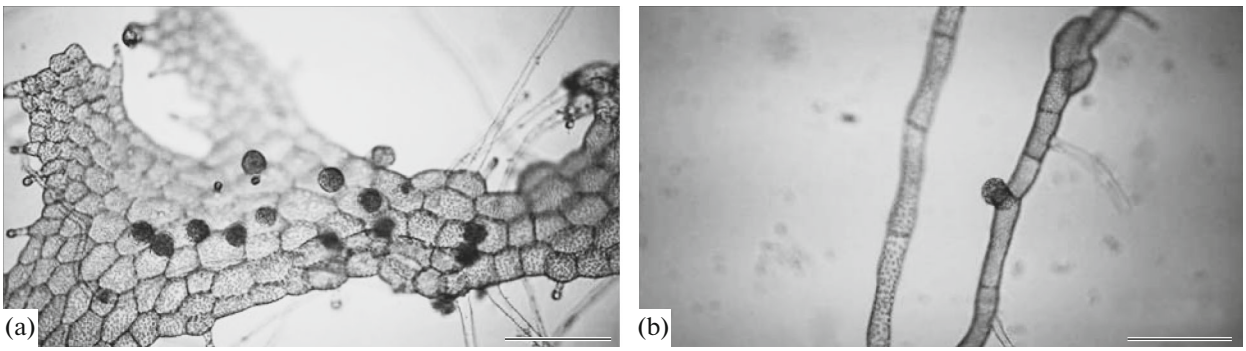

Fig. 5. Development of antheridia under the effect of isopentenylidenidine at the concentration of 10^{-7} M on the surface of the (a) ribbon-like thallus and (b) filamentous appendage.

the formation of single archegonia exclusively on the formed heart-shaped thalli (Table 1).

In the experiment with exogenous 10^{-7} M iP, no heart-shaped thalli were formed, instead strongly elongated spatulate thalli predominated, often expanded at the apex; there were deformed thalli with filamentous branched appendages (Fig. 4j). In the variant with 10^{-7} M zeatin, a heart-like shape of thalli was observed with large overgrowth at the base (Fig. 4k). At the concentration of 10^{-7} M of kinetin and BAP, the thallus shape was similar to the control but often with a ruptured disrupted (Figs. 4l, 4m). However, elongated spatulate thalli were also observed in both experiments. In the variants with 10^{-7} M iP, antheridia were actively developing both on the surface of the ribbon-like thallus (Fig. 5a) and on the lateral filamentous appendages (Fig. 5b). No development of archegonia was observed. On the elongated thalli of gametophytes in the experiments with 10^{-7} M BAP, single antheridia were found; no archegonia at the heart-shaped and spatulate thalli were found. At the thalli of gametophytes under the effect of 10^{-7} M zeatin and kinetin, an insignificant amount of female and male gametangia was found (Table 1).

Under the effect of 10^{-8} M iP, thalli of a very elongated spatulate or heart-shaped form were developed (Fig. 4n). Gametophytes with elongated heart-shaped thalli had a well-developed apical notch and an archegonial cushion, where archegonia were developing, whereas antheridia were actively forming on the elongated part of the thalli, closer to the base. Gametophytes under the effect of 10^{-8} M zeatin, kinetin, and BAP were similar to controls; single heart-shaped thalli with irregular edges were observed (Figs. 4o, 4p, 4q). The active development of archegonia and slow development of antheridia was noted in experiments using zeatin and kinetin (10^{-8} M), while BAP at the lowest concentration contributed to the active formation of antheridia exclusively (Table 1).

2.3. The sporophytes formation stage. At the 120th day from the moment of spore sowing, the development of the first sporophytes on the surface of the heart-shaped thalli in the control was noted. It was found that the highest concentrations (10^{-5} and 10^{-6} M) of all cytokinins used in the experiment suppressed the formation of sporophytes due to the delay in the formation of normal thallus and the inhibition in the development of the reproductive structures as well as inhibition in the growth of the formed sporophytes. The appearens of individual sporophytes was observed only when concentrations of all cytokinins (with the exception of iP) were reduced to 10^{-7} M (Table 1). At the smallest concentration of cytokinins (10^{-8} M), there were formation and development of sporophytes. Compared to the control, all sporophytes were smaller in size with retardation of their growth and development. Only in the experiment with zeatin the development of sporophytes did not differ from the control, and the number of young sporophytes was higher, than in the control samples.

Our studies have shown that high concentrations of cytokinins inhibit germination of spores and the development of *D. filix-mas* gametophytes, contribute to the formation of deformed thalli, and inhibit the formation of reproductive organs and sporophytes. Low concentrations of cytokinins generally accelerate the gametophyte development, which manifests itself in the early formation of heart-shaped thallus. However, cytokinins affect the nature of deformation and sexual differentiation differently, depending on the hormone isoform. It has been established that exogenous cytokinins activate the growth and development of rhizoids. The degree of stimulation increases simultaneously with the concentration of hormones in the growth medium. Exogenous cytokinins adversely affected the bisexual gametophytes formation. In a previous study, we analyzed the effect of exogenous BAP on spore germination and gametophyte morphogenesis in *Polystichum aculeatum* (L.) Roth. It was shown that high concentrations of the hormone significantly inhibited spore germination and slowed the gametophyte development at the protonema stage due to removing the apical dominance. The extent of the

effect depended on the hormone concentration in the medium [16]. It was reported that the effect of 0.01, 0.1, and 1.0 mg/L of BAP on the *in vitro* spore germination of *Alsophila odonelliana* (Alston) Lehnert was weakly expressed [48]. A significant decrease the size of the prothallium *Ceratopteris richardii* Brongn., and inhibition of the formation of heart-shaped thallus and gametangia of *Blechnum spicant* (L.) Sm. were detected after exogenous treatment of gametophytes by micro-, nano-, and subnanomolar BAP concentrations [46, 49]. Under higher concentrations of kinetin (10^{-8}, 10^{-5}, and 10^{-3} M), there was a decrease in the size of the heart-like thallus of the *Osmunda regalis* L. gametophyte and a deformation of the apical notch between its wings [47]. At the same time, BAP, kinetin, and iP induced photomorphogenesis of *Ceratopteris richardii* gametophytes grown without light [49]. At concentrations of 10^{-8}, 10^{-9}, and 10^{-12} M, each of these phytohormones changed the rate of cell division, promoted cell elongation and differentiation, induced the transition from filamentous to prothallial growth, which occurs under the action of light, and activated the formation of an apical notch in the apical zone of meristem and rhizoids in its lower zone [49]. The cultivation of *Alsophila odonelliana* gametophytes in vitro showed that BAP, irrespective of its concentration, affected the formation of filamentous gametophytes and contributed to the formation of branched thallus at the spatulate thallus stage and subsequently induced the formation of numerous thallus proliferations [48]. It is known that BAP and iP induce the development of numerous axillary shoots of vascular plants [55]. In the case of gametophytes of ferns, these hormones activate the multiple apical dominance of the meristem of the thallus.

Thus, our research and analysis of bibliography have shown that the use of exogenous cytokinins in high concentrations inhibits the fern spore germination and gametophyte growth at all stages of development, causes the deformation of thalli and reduces their sizes, and inhibits the development of sexual organs and sporophytes. Lower concentrations of cytokinin phytohormones promote the gametophyte development, induce cell division, especially in the apical zone leading to the formation of deformed thalli, activate the formation of rhizoids, but differently affect the formation of antheridia and archegonia and the development of sporophytes.

CONCLUSIONS

The effect of exogenous cytokinins (zeatin, kinetin, benzylaminopurine, and isopentenylidenidine) with various concentrations on in vitro spore germination, gametophyte morphogenesis, and sporophyte formation of the leptosporangiate fern *D. filix-mas* has been studied for the first time. It was found that high (10^{-5} and 10^{-6} M) and low (10^{-7} and 10^{-8} M) concentrations of kinetin, BAP, and iP inhibited the spore germination. The strongest inhibitor turned out to be iP. Zeatin at concentrations of 10^{-7} and 10^{-8} M positively affected the spore germination. Under the effect of high concentrations of cytokinins, the development of protonema was slowed down, deformed heart-shaped thalli were produced, and the development of reproductive organs and sporophytes was completely suppressed. Low concentrations of cytokinins contributed to an increase in the number of prothallial cells and, depending on the hormone isoform, had a different effect on the deformation of the heart-shaped thallus and sex differentiation. It was found that exogenous cytokinins activated the growth and development of rhizoids. The degree of stimulation was positively correlated to the concentration of hormones in the nutritional medium. It was found that cytokinins in general inhibited the growth and development of sporophytes due to a decrease of the number of the formed bisexual gametophytes. Exogenous iP in all concentrations had the most intense effect on the gametophyte morphogenesis at all stages of development: it blocked the protonema development at the highest concentration and the heart-shaped thallus development at the lowest concentration. Zeatin at the lowest concentrations stimulated the gametophyte development, minimized the emergence of thallus deformations, activated the growth and development of multiple rhizoids, and accelerated the formation of reproductive structures and first sporophytes.

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The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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