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PHYTOHORMONES DURING GROWTH AND DEVELOPMENT OF POLYPODIOPHYTA

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Abstract. The review highlights the basic classes of phytohormones, their localization and involvement in the regulation of sporophyte and gametophyte growth and development in pteridophytes. Gibberellins and gibberellin-like substances (antheridiogen and antheridiogen-B) involvement in the sexual polymorphism determination has been discussed. Antheridiogen that has a gibberellin skeleton in its structure is produced by a physiologically active meristem. At high antheridiogen concentrations there is formed the male gametophyte while its deficiency results in the female one. Publications dealing with studies on the content and possible involvement in the regulation of physiological processes of abscisic acid (ABA) (in regulation of spore dormancy breaking), auxins and cytokinins (in frond formation), ethylene and jasmonic acid (in resistance formation) in pteridophytes have been analyzed. A current state of research of the basic phytohormones complex components in the Polypodiophyta division is summarized in the table.

Keywords: Polypodiophyta, gametophyte, sporophyte, phytohormones.

The number of pteridophytes species – the most numerous group of higher vascular spore-bearing plants amounts to more than 12 thousands and they are one of the main components of the terrestrial vegetation cover [6, 53]. In the flora of Ukraine the *Polypodiophyta* division has 60 species [11]. Ferns are found in most parts of the world and occur starting with deserts to swamps, lakes and salty waters. The greatest diversity of ferns is typical for tropic forests where they grow not only on soil, under trees but also as epiphytes on tree trunks [6].

Adaptation to living conditions resulted in the emergence of fern various life forms, which differ in their structure and physiological characteristics. Thus, the water fern *Salvinia natans* has 2 cm long solid leaves. Water species of the genus *Azola* that contain symbiotic nitrogen-fixing cianobacteria in hollows situated close to the frond base are more similar to pteridophytes. Among woody ferns of the *Cyathea* genus there are observed 24 m high plants with leaves whose size is about 5m. Their trunk diameter reaches 30 cm but they are formed of tissues primary in origin. Cambium occurs only in the grassy species *Botrychum* [57]. According to the modern concepts the *Polypodiophyta* division combines clades of Ophioglossales, Psilotales, Equisetales (horsetails), Marattiales and

actually leptosporangiate ferns, attributing each of those clades to the subclass [21, 15, 61].

Evolutionarily, pteridophytes belong to the group of the most ancient plants. Their age is exceeded only by that of rhinophytes and club mosses and is almost equal to a geological age of horsetails [57]. Various paleobotanical evidences indicate that the first monilophytes emerged in the Devonian period (416-360 million years ago), while all five evolution lines are known from the end of the Carbon (≈ 299 million years ago) [66]. The Permian (299-251 million years ago) and Triassic (251-200 million years ago) fossils testify to the heyday of most groups of leptosporangiate ferns. However, despite an ancient origin most of the modern fern diversity evolved during the Cretaceous period (146-66 million years ago) and Cainozoic Era (66-0 million years ago) [50].

The fern quantitative biodiversity was found to have a pulsating nature. There are known three surges in the number of species: the first happened in the Paleozoic period and caused the development of multiple carbonic forms; the second occurred in the late Permian period and in the beginning of the Mesozoic Era – it gave rise to many modern fern families; and the third – late Cretaceous-Paleogene period when there evolved the most progressive families and most of the modern genera. The last surge in the number of fern species took place at the time of flowering plants domination and reduction in the number of other vascular plant groups [54, 55]. The reasons why the number of fern species has reduced are as follows: 1) extinction due to significant climatic, tectonic and floristic events in past geologic epochs; 2) competitive impact by flowering plants that have a selective advantage in using the environment resources; 3) fern narrow limits of tolerance to effects of environment abiotic factors that is caused by the lack of some morphological, anatomic and physiological mechanisms typical for flowering plants as well as by various ecological requirements to the two phases of the reproduction cycle – sporophyte and gametophyte; 4) genetic simplicity associated with the lack of prezygotic mechanism of species incompatibility (unlike in flowering plants) as a result of external fertilization. This leads to some increase in the rate of genetic drift between genetically compatible individuals, a low speciation rate and evolution of an insignificant number of species; 5) considerable, as compared to the tropics, genetic identity of sister taxa in temperate latitudes that slows down fern speciation there and that was demonstrated for the two species groups (temperate *Polypodium sibiricum* group and tropic *Pleopeltis* group) [31, 32, 52].

Competition of ferns with flowering plants for resources turned out to be an evolution factor that resulted in ecological niche diversification, species specialization, significant biodiversity expansion and rise of an adaptive level in many taxa. The main reasons that limit a wide spread of ferns compared to flowering plants as well as advantages that enable to win a competition for habitats have been identified. According to the author the first include the availability of gametophyte phase, necessity of water involvement in the realization of sex processes, lack of reproduction control, limited and slow sporophyte growth, inability to exist in a wide range of conditions, insufficient control of transpiration. Among the advantages there are in particular the ability

to carry out photosynthesis at a low light intensity, a high resistance to intensive moistening, tolerance to a substrate with a poor mineral content, spore resistance to air quality injuries, poikilohydry of gametophytes in some species, potential longevity of sporophytes, mycotrophy polyplodium [45].

Such adaptation advantages would not have been possible without the formation of an evolutionarily perfect mechanism of metabolic process regulation. It is the complex, multi-component hormonal system that provides the coordination and regulation of such basic physiological processes as growth, development, photosynthesis, respiration, tolerance to environmental factors. Phytohormones act at very low concentrations of 10^{-6}-10^{-12} M, but are not directly involved in biochemical reaction triggered by them [23]. As is now understood, a decisive factor in individual hormones effects is their concentration and localization in plant specific organs and tissues [12, 20, 22, 35].

A cascade pattern of phytohormones effects in plant organism when one hormone affects the synthesis, degradation or inactivation of the other, may be regulated by synthesis *de novo*, changes in synthesis pathways, and, in case of using a common predecessor, by interconversion and catabolism of their forms [10, 35, 44]. Due to the hormonal regulation and exogenous factors effects, the rate of internal biological time may change that is, one or the other steps of genetic program realization in plant organisms accelerate or slow down depending on the level of influence [13]. To date all basic phytohormone groups of the *Polypodiophyta* division have been identified (Table 1).

Gibberellins. Gibberellins unite a large group of phytohormones possessing a wide range of responses involved in the life cycle of plants, fungi, algae, lichen mosses and bacteria [69]. Gibberellins (gibberellic acids – GA) regulate seed germination, coordinate cells division and their elongation, determinate the sex, induce florescence of flowering plants etc.

Studies on the hormone control of plant sex formation in various systematic groups whose life cycle has the gametophyte phase have been fragmentary [29, 65, 77]. It was shown that a key role in sex polymorphism formation in pteridophytes belongs to gibberellins and gibberelin-like substances (antheridiogen and antheridiogen-B). The antheridiogen structure contains a gibberellin skeleton. This hormone was discovered in pteridophytes that synthesize GA_7. Studies on the two fern species of the *Dennstaedtiaceae* genus revealed that antheridiogen is produced by a physiologically active meristem [27]. A high antheridiogen concentration leads to the formation of the male gametophyte and its lack – female one. A low antheridiogen content causes the development of a bisexual seedling that contains both antheridium and archegonium. In studies of antheridiogen influence on the gametophyte formation in *Blechnum spicant* L. there were revealed proteins associated with the sex development [67]. The GA_7 and GA_{73} synthesis pathway of the *Anemia* Sw genus that leads to antheridiogen production was identified [37, 38]. Antheridiogen-B being absent in *L. japonicum* (Thunb.) Sw. was isolated from the fern *Lygodium circinatum* Sw. Pteridiophytes were found to differ in the gibberellin composition [25, 41]. In the leptosporangiate fern *Ceratopteris richardii* Brongn. the male sex gametophyte was shown to be determined by the exogen antheridiogen

epigenetically. Antheridiogen is secreted by female or bisexual organisms and induces the development of spores in the male gametophyte. To understand a role of gibberellins in determining the gametophyte sex of the fern *Blechnum spicant* (L.) Roth, an exogenous GA_{4+7} and synthesis inhibitor were used within the range from entkauren to kaurenoic acid – flurprimidol . It was shown that the impact of GA_{4+7} on the antheridium and anthegonium formation was low. The antheridium formation was inhibited by – flurprimidol and that is confirmed by the gibberellins involvement in this process [41]. By means of antheridiogen application there was established a chemical interaction of gametophytes in fern populations. Such inter-population actions resulted in the polyvariant pattern of gametophyte ontogenesis and complex age- and sex-related structure of populations [60]. Gibberellins control fern spore germination [2, 79]. Gibberellin-like substances were identified in spores of the fern *Matteucia struthiopteris* (L.) Tod. Comparing gibberellins from plant spores *in situ* with those that had been kept for the whole year at the temperature of +18-20° C, we found that the quantity of gibberellins-like substances in spores of stored samples decreased almost two times. Findings concerning gibberellins interactions with other phytohormones and metabolites that are able to stimulate or inhibit their effects in the regulation of formation and development of fern gametophytes are fragmentary. It was established that abscisic acid (ABA) blocks antheridiogen effects [8, 26, 29, 65, 76, 77,] on the gametophyte *Anemia phyllitidis*, it was found that along with gibberellins the regulation of antheridia development involves ethylene. Cytomorphological analysis demonstrated that phytohormone effects are manifested only in the location of gametophyte antheridia and antheridium mother cells. Ethylene was revealed to stimulate the nuclear chromatin reorganization and induce the DNA synthesis in the nucleus of the antheridium part of the human gametophyte [36]. We analyzed the gibberellin-like substances accumulation and localization in organs of heterosporous annual water fern *Salvinia natans* at the various stages of ontogenesis. For the first time, gibberellin GA_3, which dynamics and localization allow to classify it as `working` gibberellin, was identified in the fern organs using the high-performance chromatography – mass-spectrometry (HPLC-MS). The largest amount of free GA_3 was found in floating fronds while submerged ones showed insignificant accumulations of bound forms. At the stages of sporophyte growth and formation of sporocarps there was observed some increase in bound GA_3 forms content. Sporocarp accumulation was characterized by almost a fourfold increase in bound forms content. Predominance of gibberellins free forms over bound ones was reported for all organs and at all phenological phases while submerged fronds contained higher quantities of free forms (Fig.1). Dynamics of changes in gibberellins content in organs of *S. natans* corresponds with the fern development stages and indirectly indicate that the phytohormone is involved in regulation of growth and reproduction processes [70].

Abscisic acid (ABA) is considered to be one of the basic phytohormones involved in the adaptation syndrome formation [79]. Increase in the tissue ABA content causes stomata closing followed by some decrease in respiration level and, accordingly, in water content increase that mitigates the stress effects. By

stimulating the expression of some genes and synthesis of new polypeptides, ABA contributes to the formation of defense responses and promotion of plant tolerance [9, 57,64]. To date, fern ABA has been studied insufficiently. In ferns ABA was for the first time identified in spores and protonema of *Anemia phyllitidis*L. Sw.[18]. Later the hormone was discovered in spores of *Lygodium japonicum* [78], in young fronds of woody ferns *Cibotium glaucum* and *Dicksonia antarctica* [77]. Findings concerning ABA involvement in the regulation of fern physiological processes are few and diverse. Most studies are focused on studying exogenous ABA influence on fern growth and development. Thus, there was analyzed the ABA impact on spore germination and protonema growth in the fern *Mohria caffrorum* (L.) Desv., as well as the ABA relation with other hormones (GA, IAA, kinetins) in the regulation of these processes. It was found that ABA does not affect the initial division of spore protoplasts that leads to the formation of rhizoids and protenema, but inhibits a protenoma further growth. Elimination of ABA inhibiting effects on a protenoma filament extension by GA treatment resulted most probably from some stimulation of protenoma growth rather than a competitive interaction of GA and ABA. However, IAA and kinetin that do not promote protenoma growth, somewhat neutralize an ABA inhibiting effect on its growth [58]. In the water fern *Marsilea quadrifolia* L. ABA changed an aquatic development to aerial one that led to roots elongation, frond surface expansion and internodes reduction. This is an indication of an ABA decisive role in the formation of the phenotype plasticity mechanism in vascular spore-bearing plants-hydrophytes that exist on the border of the air and water environments. Some ABA-sensitive genes (ABRH) were shown to be expressed at the initial stage of such a «reprogramming». The application of pure ABA isomers showed that natural S-(1)-ABA and synthesized R-(2)-ABA preparations can induce the operation of «switch» and control the ABRH-gene expression. Comparison of these two preparations effects at the similar concentrations indicated that a morphogenetic effect of synthetic ABA exceeds that of natural one. Application of deuterium-labeled ABA showed that native ABA is metabolized to dihydrophaseic acid while R-(2)-ABA is slowly metabolized to 7-9- hydroxy abscisic acid. Following an initial stimulation of leaf and root growth, further ABA-treatment resulted in ageing, suspended development and stimulated transition to the reproductive phase, tissue yellowing, reduction in the internodes number and sporocarp formation [39].

Ethylene – gas-like phytohormone that plays an important role in the regulation of plant growth and its tolerance to stresses [73]. Despite its simple two-carbon structure, the olefin ethylene is a potent modulator of plant growth and development. Data on ethylene effects on plant growth are contradictory. It is preferably thought that ethylene acts as an inhibitor of plant growth [1]. However, it was found out that depending on concentrations, plant species and growth conditions ethylene is able to stimulate growth [43, 48]. Different ways of ethylene effects on plants are associated with its interactions with other hormones [68], in particular, with ABA, whose antagonist ethylene is [75].

It was established that the fern *Onoclea sensibilis* L., kept in darkness and whose gametophyte was treated by ethylene showed a rapid elongation of

filament, inhibition of cell division and rhizoid growth slowdown. An optimal ethylene concentration was 0,01-0,1 mM. The filament elongation was stable up to an ethylene high concentration effect and 1 mM of ethylene had no inhibiting effect on growth. Keeping gametophyte cultures in a small chamber simulated ethylene effect but no such effect was observed after plants transfer to a chamber with copper perchlorate. Ethylene production by gametophyte was recorded using gas-liquid chromatography. *Pteridium sp.* seedlings exogenously treated with 2,4-dichlorophenoxyacetic acid and indoleacetic acid (IAA). IAA in culture *in vitro* showed some rise in ethylene synthesis through influence on the activity of a key enzyme of its biosynthesis – methylthioadenosine-lyase [37, 38, 40].

Auxins. IAA is involved in plant cell division, directly affects the mitotic cycle, cell transfer from dormancy to active proliferation [28, 30]. Ethylene and IAA growth-regulation effects in filament and rhizoid germination were found to be independent. Thus, the inhibition of rhizoid elongation resulting from IAA high concentration effects is not associated with an auxin-induced synthesis of ethylene [42]. Studies on the phytohormone involvement in apical dominance in the fern *Davallia trichomanoides* Blume indicated that lateral buds may be activated through the removal of main stem crowns. An auxin treatment did not substitute a decapitation effect. Bioassays and chromatography revealed no auxin in crowns. Developing fronds of this fern synthesize auxin but it does not control lateral bud growth [24, 42]. In the water fern *Salvinia molesta* Mitchell IAA was identified in an amount of 393 пмоль/g of dry substance, but the reason why this hormone concentration was so high the authors failed to explain [3]. Exogenous auxins (α-naphthaleneacetic end 2,4,5-dichlorophenoxyacetic acid) were revealed to induce the development of lateral meristems in gametophyte of *Ceratopteris richardii* Brongn. and generate the development of male plants. Auxin antagonist – β-chlorophenoxyisobutyric acid – suppresses the development of lateral and apical meristems and generates the emergence of a ball-shaped gametophyte. Disturbances in auxin transport as a result of 2,3,5-iodine benzoic acid action cause a temporary delay in lateral meristem growth while N-(1-naphthyl)phthalamic acid - blocked auxin transport provokes the production of different forms of plants from secondary lateral meristems. It is thought that in the fern *Ceratopteris richardii* Brongn. auxin mediates the activation of lateral meristem development [33, 34]. Auxin transport from the lateral meristem to other gametophyte parts was shown to occur during the fern growth and development. Auxin controls the production of rhizoids on gametophyte and affects the manifestation of totipotent cells. The conducted studies enabled to create a model for the auxin regulation of lateral buds and meristem activity during gametophyte development [28]. In *Dryopteria affinis* an exogenous auxin and gibberellin caused a female sterility [41]. Almost all ferns exhibited a so-called leaf folding (snail-shaped leaf rolling) due to an IAA increased synthesis on the abaxial surface of young fronds. Auxin synthesis on both surfaces gradually equalizes and fronds straighten [23].

Using HPLC-MS we analyzed the pattern of indole-3-acetic acid (IAA) accumulation and localization in organs of the sporophyte *S. natans*. It was shown for the first time that at the phase of a fern intensive growth (June) the total IAA

content of floating and submerged fronds was similarly high and reached 182 ng/g of fresh weight. At the phase of submerged fronds active growth and pubescence the endogenous IAA content was 546.3 ng/g of fresh weight while the quantity of this hormone in floating fronds remained at the level of the previous phase. A considerable reduction of this hormone content in ageing floating and submerged fronds was observed following the reproductive phase beginning and also during sporocarp formation and spore afterripening. At the stage of late sporogenesis (September) the IAA content in soporocarps that contained mature spores was 193 ng/g of fresh weight. a free hormone form dominated. The free hormone form dominated at the stage of an active and steady-state growth of floating and submerged fronds. Conjugated IAA was localized mostly in submerged fronds in quantity which considerably exceeded that of floating ones. The dynamics of content changes of free and conjugated IAA in *S. natans* organs corresponded to the phenological phases of the sporophyte fern development and indirectly indicates that the phytohormone is involved in regulation of growth and reproductive processes (Table 2). Distribution of this hormone between floating and submerged fronds shows that during the steady-state growth phase IAA is localized in submerged fronds while at the phase of late sporogenesis – in sporocarps [72]

Cytokinins. Studies on fern cytokinins began in the eighties of the twenties century. A significant number of obtained data described exogenous cytokinins effects on plant growth in culture. Thus, it was found that kinetin did not affect protonema growth in *Mohria caffrorum* Sw., but it substantially smoothed inhibitory effects of ABA on it [19]. Cytokinins are a necessary component of the cultural medium in microclonal reproduction of the fern *Rumohra adiantiformis*, they are required for the development of new rhizome mass [17]. Benzyl adenine, which extends the lifespan for many cut plants, reduces it in the fern *Lycopodium cernuum* [47].

A cytokinin role in the photo morphogenesis regulation was studied during *Ceratopteris richardii* growth [56, 62]. Even very low, subnamolar concentrations of benzyl-amino purine (BAP), kinetin and isopentenyl adenine can change the rate of cell growth, division, elongation and differentiation. Higher concentrations induce the formation of rhizoidal embryos below apical meristems. In dark-grown plants they also induce processes mediated by red and blue light that is evidence of phytochrome and cryptochrome signal stimulation. However, a cytokinin treatment fails to substitute light effects completely, it does not at least induce hermaphrodite development or germination spores in darkness.

Moreover, cytokinins do not stimulate the chlorophyll synthesis in gametophyte that grows in darkness and is able, unlike flowering plants, to produce mature chloroplasts without light. These findings confirm the evolutionary conservatism among flowering plants and pteridophytes concerning a cytokinin role in the photomorphogenesis regulation [41, 62]. The cytokinin qualitative composition in *Azolla filiculoides* Lamarck and *S. Molesta* was identified. In *A. filiculoides* bioassays revealed an activity that corresponded to zeatin, zeatin riboside, dihydrozeatin, isopentenyladenin and isopentenyladenosin, while in *S. molesta* there were detected only the first three [63]. Ferns, green algae

and mosses contain only some conjugated cytokinins – isopentenyladenin and zeatin, while higher plants have a more complex composition of them, particularly, conjugate of dihydroseatin. The conjugation pattern is thought to become more complex along with higher plant development [4].

A significant quantity (81,62 pM/g of dry weight) of 16 isoprenoid cytokinins was isolated and identified in *S. molesta*. Studies of *S. molesta* as a cytokinin source revealed that under conditions of culture *in vitro* only small quantities of four cytokinins move to a nutrient medium. Fern composting for 14 days resulted in a cytokinin content decrease by 17% [3].

Using HPLC methods, we have for the first time analyzed the pattern of cytokinin accumulation and localization in organs of the annual fern-hydrophyte *Salvinia natans* (L.) All. at the stages of sporophyte intensive and steady-state growth, sporocarp formation and vegetative organs die-off (Fig. 2). We detected all basic cytokinins typical for most plants – zeatin (trans- and cis-forms), zeatin riboside, zeatin glucoside, isopentenyladenin and isopentenyladenosin [71]. It was discovered that the cytokinin spectrum and content varied according to a stage of sporophyte development and growth intensity. The highest total content of free cytokinins was in floating fronds during sporophyte intensive growth. A conjugated zeatin form occurred at the final stage of fern development in sporangia during spore formation and maturing. The cytokinin dynamics and arrangement between floating and submerged fronds indicated that functionally, these organs are unequal and the key role in phytohormone production belongs to floating fronds (Fig. 3). Studies of endogenous phytohormones in *Marsilea drummondii* A.Br. were associated with the investigation of the apical dominance phenomenon. In apex buds the zeatin and zeatin riboside content is much higher than that of younger inhibited buds. A subapical bud was found to have abnormally high isopentenyladenin content. It was this bud, which intensively developed following decapitation and researchers regard isopentenyladenin as a reserve form for hormones of the zeatin line [49]. In *Plagiomnium cuspidatum* cultivated *in vitro* kinetin, IAA and benzyl adenine treatment of lateral buds in decapitated gametophytes resumes an inhibiting effect in the apical bud and that involves the production of plants similar to intact controls concerning the structure and anatomic morphology.

Jasmonic acid. Jasmonates belong to the group of physiologically active substances that affect plant growth and development [6, 72]. In ferns jasmonates were detected using the radio-immunoassay techniques with jasmonate-specific antiserums [46]. (JA) was shown to be involved in the early development of gametophyte and sporophyte protoplast culture in the fern *Platycerium bifurcatum* (Cav.) C. Chr. JA has no direct effect on spore germination and primary rhizoid production and that contributes to an early gametophyte development confirmed by the emergence of longer primary rhizoids as well as by their quantitative increase and rise in the number of gametophyte cells [14]. JA concentration of 1 μM stimulates the gametophyte transition from a filamentary protonema to flat shoot. An optimal elongation of primary rhizoids and the highest rate of gametophyte cell division occurred at JA concentration of 0,01-1 μM, while the greatest number of rhizoids on gametophyte is observed at JA

concentration of 0,1-1 μM. JA (0,01 μM) stimulates the beginning of protoplast division as well. An exception is experiments on studying JA influence on spore germination, the results of which indicated that concentrations being higher than 1 μM suppressed cell elongation and division [14]. We identified for the first time the lipoxygenase (LOX) activity in higher vascular spore-bearing plants of *Equisetum arvense* L. and water fern *S. natans* (L.) All.[5, 7]. JA are specific cyclopentane derivatives of the lipoxygenase pathway of polyunsaturated fatty acid oxidation. Because the LOX activity present in these Pteridophytes we can talk about the presence of jasmonic acid in them.Pteridophytes are among the most widespread plants on the planet but despite their abundance, animals eat ferns much less than flowering plants. How can this be explained? Affection of leaves in *Pteridium aquilinum* (L.) by Kuhn *Spodoptera littoralis* and *Strongylogaster multifasciata* as well as mechanical injuries cause a very low emission of attractants of volatile organic compounds mixture (VOC) that contains primarily terpenoids. Release of such substances may be stimulated by treatment with exogenous JA that induces attractant synthesis in flowering plants. Mechanically injured plants of kidney bean, corn, cotton, poplar, tobacco, potato, arabidopsis and other angiosperms are known to synthesize great amount of JA in leaves [16]. Fronds exposed to exogenous JA intensively release VOC. Similarly, exposure to JA predecessors –12-oxo-phytodienoic acid and α-linolenic acid also results in VOC emission although a less intensive one compared to a direct JA treatment. Release of terpenoids associated with JA treatment may be blocked by fosmidomycin and mevinolin, which are inhibitors of mevalonic and non-mevalonic pathways in angiosperms. That is, like other higher plants, terpenoid VOC are formed in ferns via similar JA-sensitive pathways. However, a very small number of terpenoids produced in mechanically injured or plant-feeder injured ferns contrasts sharply with the situation among higher plants. It suggests that such pests as *C. multifasciata* and *C. littoralis* do not cause production of sufficient JA levels required to activate of mevalonic and non-mevalonic - pathways and improve further secretion of VOC in ferns. Pteridophytes do not probably need any additional defense involving VOC [51]. Ferns contain cyanogenic glycoside and tanning agents. Is the presence of highly toxic agents in fern fronds a sufficient condition to avoid injuries resulting from animal eating? The significance of quick and adequate responses to external damages to an organism existing throughout its life on one the same place and unable to avoid dangers cannot be overestimated. We may only guess but it is clear that even without a perfect indirect defense mechanism of angiosperms, ferns will successfully colonize our planet. [51].

Thus, in ferns the most studied phenomena are the cytokikin role in photomorphogenesis regulation, hormone regulation of sex determionation (by gibberellins and gibberellins-like substances), ABA ability to induce a heterophyl shifting from an aquatic to terrestrial phenotype and also JA-dependent defense mechanisms similar to those in angiosperms. However, problems of gibberellins interaction with other phytohormones in the regulation of sex determination are yet to be more completely studied, mechanisms of endogenous gibberellins effects during organ gametophyte formation also require further investigation.

Phytohormone regulations of metabolic processes, involvement of some phytohormone groups in the ontogenesis of fern various life forms remain insufficiently studied (Table. 1)

Table. 1. Current state of studies on the basic phytohormone classes of the subdivision *Polypodiophyta*

Hormone	Plant object	Functions	Source
Antheridiogene	Genus *Anemia* Sw.	Sex Determination	Furber et al., 2008
	Ceratopteris richardii Brongn.	Sex Determination	Banks, 2010
Antheridiogene-B	*Lygodium circinatum* Sw.	Sex Determination	Menendez et al., 2006
GA_{4+7}	*Blechnum spicant* L. Roth.	Antheridia and archegonia formation	Menendez et al., 2006
GA_3	*Salvinia natans* (L.) All.	Identified	Vasyuk et, al, 2016
ABA	*Mohria caffrorum* L. Desv.	Spore germination and protonema growth	Shih-Gee et al., 2006
	Marsilea drummondii A.Braun	Identified	Pilate et al., 1989
	Marsilea quadrifolia L.	Change in development type from aqueous to aerial (root elongation, frond surface expansion, internode shortening)	Bai-Ling Lin et al., 2005
Ethylene	*Onoclea sensibilis* L.	Filament elongation, inhibition of cell division and rhizoid growth	Pierik et al., 2006
IAA	*Davallia trichomanoides* Blume	Apical dominance	Miller et al., 2001
	Marsilea drummondii A. Braun	Apical dominance regulation	Pilate et al., 1989
	Ceratopteris richardii Brongn	Induction of gametophyte lateral meristem development	Gregoric, Fisher, 2006
	Salvinia natans (L.) All.	Identified	Voytenko et al., 2016
Cytokinins	*Davallia trichomanoides* Blume	Identified	Croxdale, 1976
Zeatin, zeatin riboside, isopentenyladenin	*Marsilea drummondii* A. Braun	Apical dominance regulation	Miller et al., 2001
16 isoprenoid cytokinins	*Salvinia molesta* D.S. Mitch.	Identified	Arthur et al., 2007

Kinetin (exogenous treatment)	*Mohria caffrorum* Sw.	Protenoma growth regulation	Chia, Raghavan, 1982
	Rumohra adiantiformis (G. Forst.) Ching.	Rhyzoid production	Chen, Read, 1983
Benzyladenin	*Lycopodium cernuum* L.	Inhibition of growth in culture *in vitro*	Paull, Chantrachit, 2001
BAP, kinetin, isopentenyladenin (exogenous treatment)	*Ceratopteris richardii* Brongn.	Induction of rhizoid embryos below apical meristem	Spiro et al., 2004
Zeatin, zeatin riboside, dihydrozeatin, isopentenyladenin isopentenyladenosin	*Azolla filiculoides* Lam.	Identified	Stirk, Van Staden, 2003
Zeatin, zeatin riboside, dihydrozeatin	*Salvinia molesta* Mitchell	Identified	Stirk, Van Staden, 2003
Zeatin (trans- and cis-forms), zeatin riboside, zeatin glucoside, isopentenyladenin, isopentenyladenosin	*Salvinia natans* (L.) All.	Identified	Vedenichova et.al., 2016
Jasmonic acid	*Plagiomnium cuspidatum* T. Koponen	Identified	Parthier, 1993
	Platycerium bifurcatum (Cav.) C. Chr.	Involvement in gametophyte development at the early stages	Camloh, 2010
	Pteridium aquilinum (L.)	Induction of attractant syntheis	Radhika et al., 2012

Table 2. Content of free and conjugated IAA in *S. natans* organs at the different phases of sporophyte development (ng/g fresh weight)

Organ	Form IAA	Phases of sporophyte development			
		Intensive growth (June)	Stationary growth (July)	Formation of sporocarps and maturation of spores (August)	Withering away of the vegetative organs (September)
floating fronds	free	149,5±7,5	166,6±8,3	13,7±0,7	
	conjugated	10,1±0,5	0,6±0,03	13,2±0,7	
submerged fronds	free	138,2±6,9	510,0±25,5	7,0±0,3	
	conjugated	43,5±2,2	36,3±1,8	20,1±1,0	
sporocarps	free			0,6±0,02	161,6±8,1
	conjugated			0,5±0,02	21,4±1,1

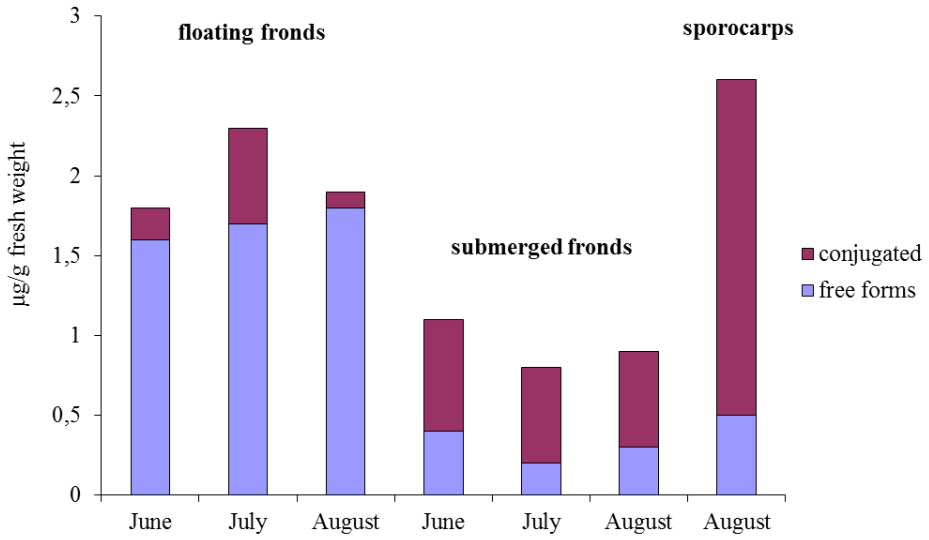

Fig. 1. The content of GA_3 in floating and submerged fronds and of *S. natans* at the different stages of sporophyte development (µg/g fresh weight).

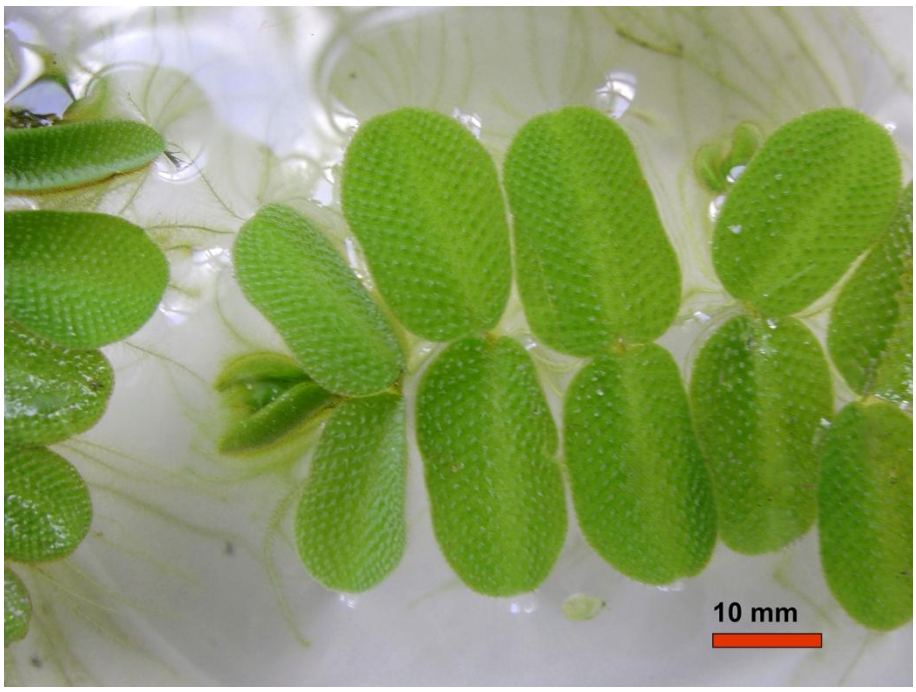

Fig. 2. Sporophytes of fern *Salvinia natans* (L.) All. on surface of water

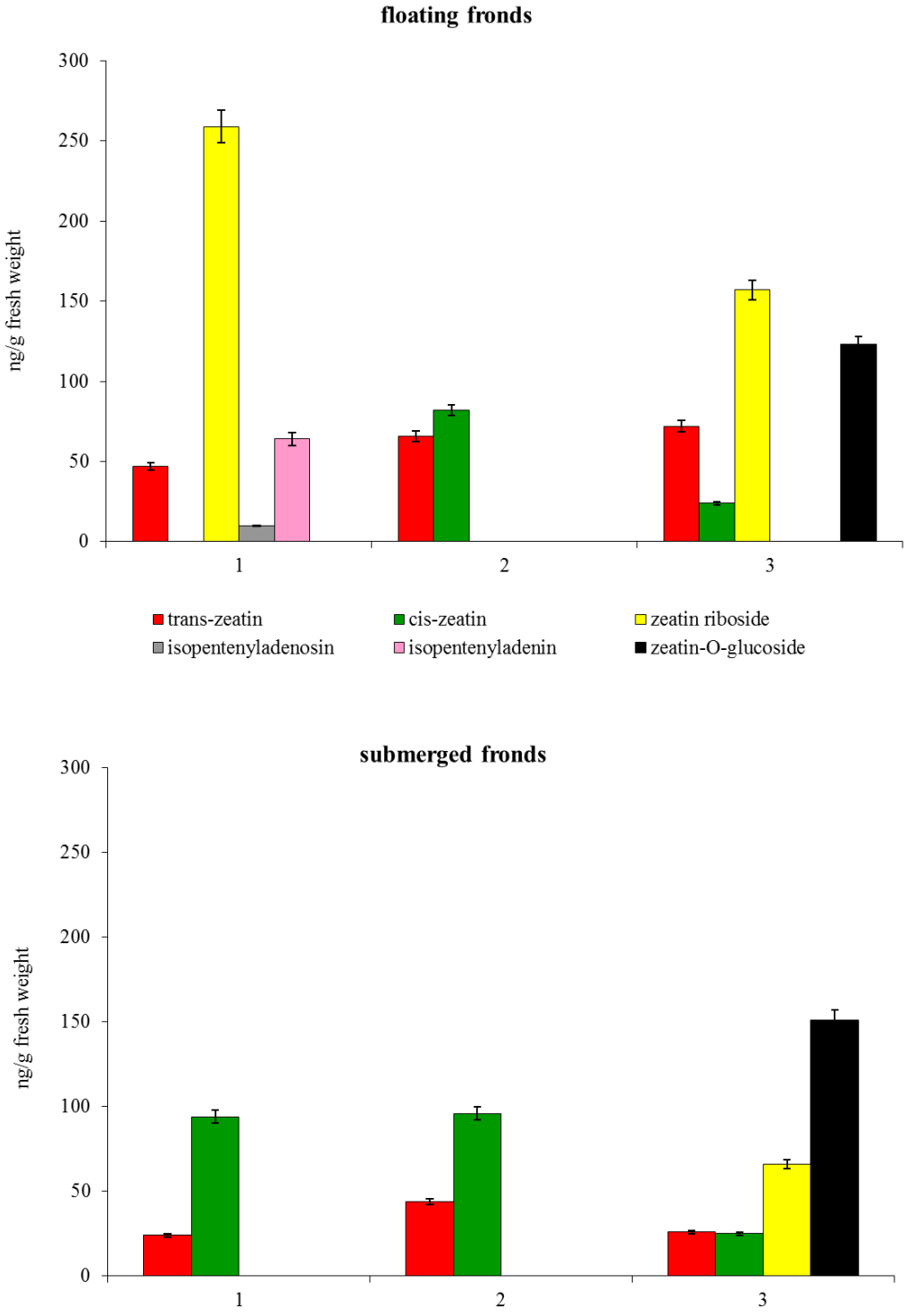

Fig. 3. The content of cytokinins in floating and submerged fronds and of *S. natans* at the different stages of sporophyte development (ng/g fresh weight): 1 – intensive growth (June), 2 – stationary growth (July), 3 – formation of sporocarps and maturation of spores (August)

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