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ENDOGENOUS AUXIN AND ABSCISIC ACID IN REGULATION OF *EQUISETUM ARVENSE* L. SPOROPHYTE GROWTH AND DEVELOPMENT

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Background. Phytohormones are natural regulators of plant growth and development, with their content and distribution varying across organs and tissues throughout the plant's life cycle. Indole-3-acetic acid (IAA) regulates organogenesis, delays aging, and is involved in responses to environmental stresses. Abscisic acid (ABA), a stress hormone, controls transpiration, root growth, and plant aging. While extensive research exists on the role of IAA and ABA in the growth and morphogenesis of higher flowering plants, their roles in vascular spore-bearing plants remain poorly understood.

Materials and Methods. This study examined the dynamics and distribution of endogenous IAA and ABA in the organs of reproductive and sterile plants of the sporophyte generation of *Equisetum arvense* L. across nine ontogenetic phases, using HPLC-MS analysis.

Results. The study found that during the growth of shoots, rhizomes, and reproductive structures, the active form of IAA accumulates. As growth slows down, organs age, and spores mature, the content of endogenous ABA increases. Across all development phases, hormone levels were higher in the organs of sterile summer plants than in reproductive spring plants, except during the germination phase for IAA and the semi-open and open strobile phases for ABA. The accumulation of free ABA in strobiles during the massive spore shedding indicated its role in regulating spore maturation and

strobile aging. Hormone levels in sterile shoots of varying heights increased following the formation and growth of second-order lateral branches. In spring rhizomes, IAA and ABA accumulation occurred during the open strobile phase, while in the rhizomes of summer plants, IAA (due to the bound form) and ABA (due to the free form) accumulated in 40- and 50-cm tall plants. Upon cessation of growth, IAA levels in the rhizomes of 70-cm tall plants decreased, while ABA levels remained unchanged.

Conclusions. Active growth processes in both above-ground and underground organs as well as the development of reproductive structures were associated with the accumulation of the active form of IAA. In contrast, the slowing of growth, aging of organs and maturation of spores were accompanied by increased ABA content. The study also revealed similarities in the patterns of IAA and ABA accumulation in the ontogeny of higher spore-bearing and flowering plants, contributing to the fundamental understanding of phytohormonal regulation of plant growth and development.

Keywords: *Equisetum arvense* L., abscisic acid, indole-3-acetic acid, sporophyte, growth, development

INTRODUCTION

Phytohormones are natural regulators of plant growth and development, whose content and distribution change in organs and tissues throughout the life cycle (Bajguz, Piotrowska-Niczyporuk, 2023; Ljung, 2013). Indole-3-acetic acid (IAA), the most studied auxin, regulates processes such as cell division, elongation, differentiation, tropic responses, flowering, apical dominance, the formation of the conducting system, organogenesis, aging, and responses to environmental stresses (Korver *et al.*, 2018; Wakeman & Bennett, 2023). IAA is primarily synthesized in the cells of the apical meristem, young leaves, and flower buds, from where it is transported through the phloem to other plant organs (Fàbregas & Fernie, 2022). Local synthesis of auxins also occurs in roots (Zhang *et al.*, 2022). IAA homeostasis is maintained by converting the free active form of the hormone into the conjugated form (Favre *et al.*, 2024).

Abscisic acid (ABA), a well-known stress hormone (Sharma & Sharma, 2023), plays a crucial role in regulating plant physiological and metabolic processes during ontogenesis (Humplik *et al.*, 2017). ABA controls stomatal activity, root growth, cuticular wax formation, seed development and germination, organ senescence, and growth inhibition (Chen *et al.*, 2020; Kavi Kishor *et al.*, 2022; Sakata *et al.*, 2014). The fine-tuning of ABA-mediated signaling is considered a key evolutionary event that helped vascular plants conquer land (Chen *et al.*, 2020; Sun *et al.*, 2019). ABA is synthesized in leaves, roots, stems, and fruits, but the main site of hormone formation is chloroplasts, the vascular system, and stomatal closing cells. ABA accumulates mainly in vacuoles (Cardoso *et al.*, 2020). It mediates both rapid responses and relatively longer changes in gene expression related to stress response, leading to the formation of protective substances, including dehydrin proteins and various antioxidant compounds (Sharma & Sharma, 2023; Yang *et al.*, 2022).

Among modern vascular spore plants, horsetails form the smallest group. However, in terms of the number and diversity of fossil species, they hold a leading place (Husby, 2013). The division Equisetophyta is represented by one genus *Equisetum*, which includes 15 to 60 species (Hauke, 1990). In Ukraine, this genus comprises nine species in two subgenera, *Equisetum* and *Hippochaete* (Milde) Baker. (Mosyakin & Fedoronchuk, 1999).

Horsetails are capable of rapid colonization, facilitated by an effective reproductive and vegetative strategy (Mosyakin & Tyshchenko, 2010). The most common species in Ukraine is field horsetail (*Equisetum arvense* L.), a noxious weed found in fields with slightly acidic soil among crops, as well as in wet meadows, riverbanks and reservoirs. *E. arvense* is a 10–50 (100) cm tall herbaceous plant, with a long blackish rhizome forming spherical nodules filled with starch at the nodes. Its life cycle is dominated by a perennial sporophyte characterized by dimorphic shoots (Tymchenko *et al.*, 2019).

Previous studies have shown that spring spore-bearing and summer vegetative shoots of *E. arvense* differ in morphometric parameters and physiological functions. The reproductive buds consisted of 90 % embryonic strobili and 10 % meristematic embryonic stem with close internodes, nodes, and rings of leathery leaves. Spore-bearing, unbranched, articulated-annular reproductive shoots grow intensively from April to early May (semi-open strobilus stage). When photosynthetic internode growth ceases, the strobili axis lengthens, and the tightly closed hexagonal shields covering the sporangia diverge. Mass shedding of spores begins after 80 % opening of the strobile at the opened strobile stage. Once the spores mature, the spore-bearing shoots die, and by late May or early June, the development of green branched vegetative, or assimilation, articulated-ring shoots begins. Significant variability in organ size is observed within one range, depending on lighting and surrounding plant species. Analysis of the growth curves of various organs of the assimilating shoot reveals uneven elongation in May, while in June–July organ sizes level off, and growth and development slow down. From mid-July to autumn, a long stationary stage follows, during which growth stops and nutrients accumulate in the rhizome (Voytenko *et al.*, 2016). Cells of the intercalary meristem in vegetative shoots are characterized by electron-dense cytoplasm. During active cell growth, the vacuole expands, reaching 80 % of the protoplast volume in differentiated cells. High photosynthetic activity in internodes is associated with a dense membrane system of chloroplasts (Voytenko *et al.*, 2016). A correlation between lipoxygenase (LOX) activity and seasonal changes in physiological and metabolic processes in the rhizome has also been found. In winter, LOX activity is low and stable, but with spring warming, lipid metabolism and LOX activity increase. During the rhizome's emergence from dormancy, starch grains in amyloplasts decrease, forming characteristic clusters near the plasmalemma (Babenko *et al.*, 2015). The high mechanical strength and rigidity of the stem and side shoots are provided by silica plates located in a thin uniform dense layer on the epidermis surface of stem internodes (Stakhiv *et al.*, 2013).

In contrast to numerous studies on the role of phytohormones in regulating growth and morphogenesis in flowering plants, the phytohormones of vascular spore plants remain understudied (Dathe *et al.*, 1989; Kosakivska *et al.*, 2019). Using the HPLC-MS method, we have established the dominance of the bound forms of IAA and ABA in the ontogeny of the sporophyte of the evergreen rough horsetail (*Equisetum hyemale* L.). IAA is involved in the regulation of the development of stem organs, lateral roots and storage nodules, while ABA activates adaptation processes during the autumn vegetation period (Voytenko, 2021). In the complex system of the plant organism, various phytohormones interact intricately. In some growth and development processes, one hormone's effect can be dominant or at least predominant. The effects of different phytohormones often intersect multiple times. Therefore, the aim of our work was to investigate the dynamics and localization of endogenous IAA and ABA in the organs of *E. arvense* at different stages of ontogenesis to clarify these phytohormones' possible role in regulating sporophyte growth and development processes.

MATERIALS AND METHODS

Plant Materials. The study focused on field horsetail plants (*Equisetum arvense* L.), growing on loamy soils in well-lit forest glades in Kyiv and Zhytomyr regions. Material collection occurred during the following periods: autumn-winter (November and December) during the phases of autumn and winter dormancy; spring (March–May) in the phases of spring awakening, germination, active growth, when plants reached 4–5 cm in height and closed, semi-open and open strobilus; and summer-autumn (June–October) during the summer vegetation phases. The temperature regime and humidity during collection were consistent with the statistical average for the Forest Steppe climatic zone. The study examined rhizomes, reproductive germ buds, sprouted buds, internodes 1–6 (from the rhizome), leaf rings, strobili of the reproductive shoot, and internodes 1–6 lower and 8(9)–13(14) upper (from the rhizome) from a ring of I and II-order branches of vegetative photosynthetic shoots of different lengths (18, 21, 26, 40, 50 and 70 cm) and age (**Fig. 1**).

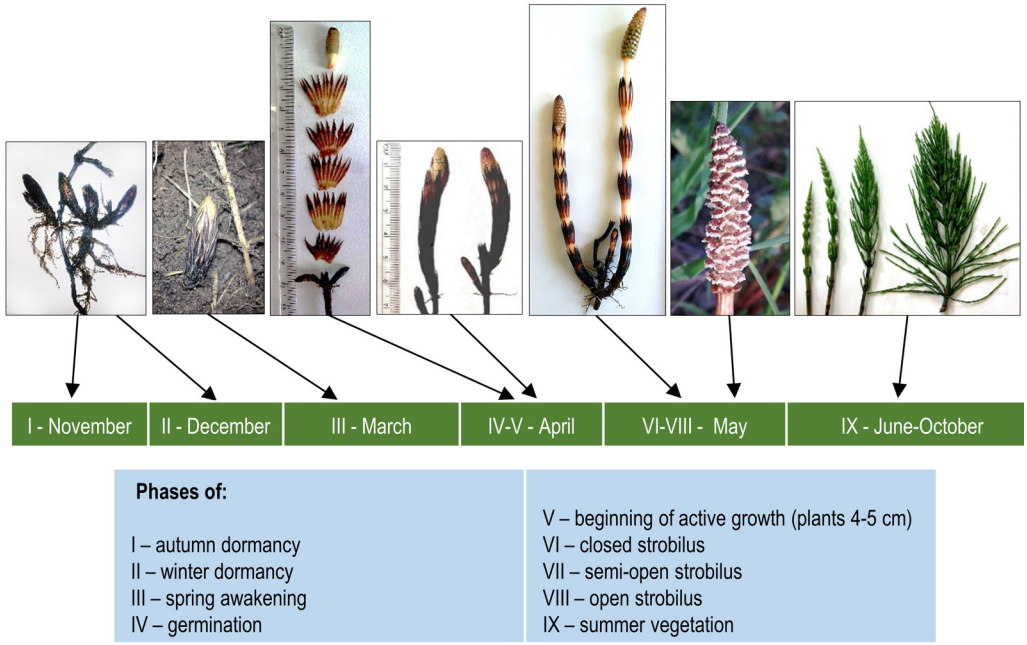

Fig. 1. Phenospectrum of the annual cycle of *Equisetum arvense* sporophyte

Extraction and determination of phytohormones. The plant material (10 g) was ground in liquid nitrogen and homogenized in 30 mL of chilled 80% ethanol with 1–2 drops of an antioxidant (0.02% sodium diethyldithiocarbamate) and extracted for 24 hours. The alcoholic extracts were evaporated to an aqueous residue and frozen at -18 °C for 12 hours to remove proteins and pigments. An aliquot of the thawed aqueous residue was adjusted to pH 3.0 with 2 N HCl and centrifuged at 10,000 rpm for 20 minutes on a K-24 centrifuge (Janetski, Germany). The free form of the total fraction of IAA and ABA was extracted three times with diethyl ether from the supernatant. The free form of IAA and ABA was further purified by acid-alkaline reextraction and thin-layer chromatography (TLC) on Silica gel 60 F254 plates (Merck, Germany) using a solvent

system chloroform : ethyl acetate : glacial acetic acid (70:30:5). Chromatogram zones corresponding to the Rf of the IAA and ABA standards were eluted with 96% ethanol, and the eluates were evaporated to a dry residue using a vacuum rotary evaporator (type 350 r, Poland) at a temperature not exceeding +40 °C. The bound form of both hormones was determined after hydrolyzing the aqueous residue (post-extraction of free forms with diethyl ether) with 1 N NaOH in 30% ethanol in a water bath. Further purification followed the same process as for the free form determination described above (Methodological recommendations, 1988).

Quantitation of IAA and ABA was performed by high performance liquid chromatography – mass spectrometry (HPLC-MS) on Agilent 1200 LC/MS liquid chromatograph with diode-array G1315B (DAD) and single-quadrupole mass-selective G6120A (MSD) detectors on Eclipse XDB-C18 column 4.6×250 mm with a particle size of 5 μm with a mobile phase rate of 0.5 mL/min in the solvent system, methanol water, acetic acid in a volume ratio of 40: 59.9: 0.1. Unlabeled IAA and ABA from Sigma (USA) were used to identify plant hormones. Recording of IAA spectrograms on DAD was performed in the UV range at a maximum absorption at a wavelength of 280 nm, followed by identification by mass spectrum equipped with a combined ionization source (MM-ES-APCI). IAA detection on a mass-selective detector was performed in SIM and Scan modes in Negative Polarity (registration of negatively charged ions) in the mass range of 100–300. Elution of ABA was performed at an analytical wavelength of detection of 254 nm. Analysis and processing of chromatograms was performed using ChemStation software version B.03.01 offline.

Statistical analysis. Experiments were performed in three biological replicates, each with five analytical replicates. Data analysis was performed using Microsoft Excel 2016 and Python 3.12.6. A one-way analysis of variance (ANOVA) was employed to assess statistical differences between average values. The significance level was set at $P \leq 0.05$. Post hoc comparisons were conducted using the Bonferroni correction to identify specific groups with significant differences. Significant differences between individual data groups are denoted by letters in the accompanying figures.

RESULTS and DISCUSSION

Accumulation and distribution of endogenous IAA in *Equisetum arvense* reproductive sporophyte shoots and rhizomes. During the autumn and winter dormancy phases, IAA accumulated predominantly in primordia, with the total hormone content being 1.3 to 7.5 times higher than that in the rhizome. The bound form of the IAA exceeded the free form by 85.7 % and 42.1 %, respectively. As the rhizomes transitioned into the winter dormancy phase, the IAA levels decreased, primarily due to a reduction in the bound form, which reached trace levels (**Fig. 2**).

In the spring awakening phase, with no above-ground parts present, the IAA level in the rhizome increased solely due to the accumulation of the hormone's free form, reaching 4.6±0.23 ng/g of fresh weight (FW). During the germination phase, as the reproductive bud developed and intercalary meristems activity increased in the internodes, IAA levels in the rhizomes dropped to trace values. In contrast, the hormone content rose significantly in above-ground organs (reproductive bud with formed internodes and leaf rings), reaching 150.9±7.55 ng/g FW. At this stage, the free form of IAA exceeded the bound form by 37.8 %. At the onset of active growth, when shoots reached a height of the 4–5 cm, IAA was predominantly found in the shoots (six internodes with leaf rings),

where its total content was 2.2 times higher than that in the rhizome and stems. The free form of IAA dominated in the shoot by 58.9 %. In rhizomes and strobilus, only the bound form of the hormone was present, with contents of 7.4±0.37 and 7.8±0.39 ng/g FW, respectively (**Fig. 2**).

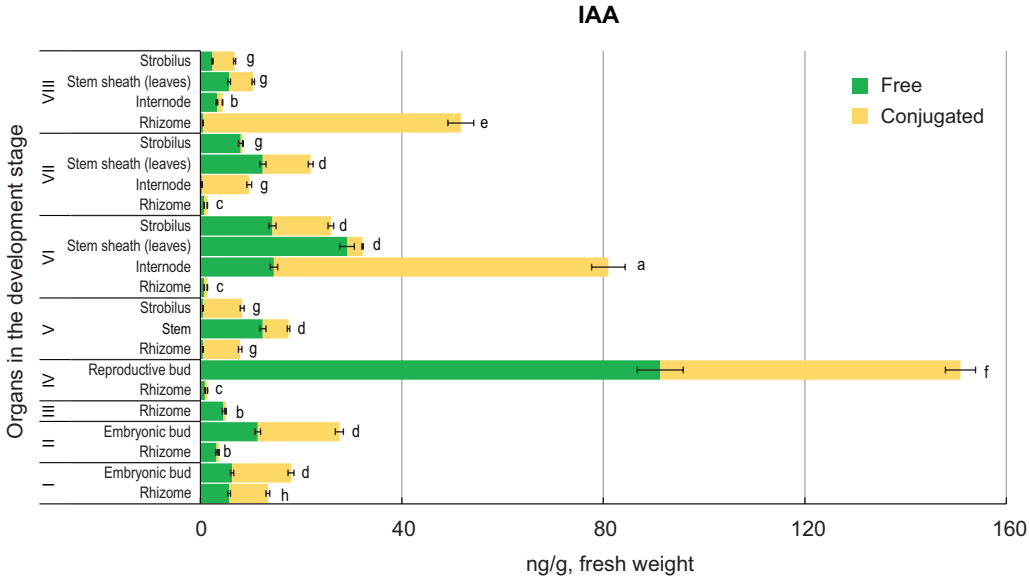

Fig. 2. IAA accumulation and distribution in *Equisetum arvense* reproductive shoots and rhizomes in the phases of autumn (I) and winter (II) dormancy, spring awakening (III), germination (IV), the beginning of active growth (V), when the plants reached 4–5 cm in height, closed (VI), semi-open (VII) and open (VIII) strobilus

Note: n = 5; x ± standard error (SE), different letters beside the bars indicate statistically significant differences between means assessed by Bonferroni's test at P ≤0.05. a – Internode 6; b – Rhizome 2, Rhizome 3, Internode 8; c – Rhizome 4, Rhizome 6, Rhizome 7; d – Embryonic bud 1, Embryonic bud 2, Stem 5, Stem sheath (leaves) 6, Strobilus 6, Stem sheath (leaves) 7; e – Rhizome 8; f – Reproductive bud 4; g – Rhizome 5, Strobilus 5, Internode 7, Strobilus 7, Stem sheath (leaves) 8, Strobilus 8; h – Rhizome 1

During the development of reproductive structures (closed strobili phase), IAA accumulated in the strobili, although the total hormone content in these structures was 3 and 1.2 times lower than in internodes and leaf rings. The highest IAA levels were found in internodes, where active growth was starting to decelerate. At this point, the bound form of IAA was 355 % lower than the free form. In contrast, the free form of IAA predominated in leaf rings and closed strobili by 89 % and 19 % (**Fig. 2**).

In the semi-open (pre-sporulation) and open (posy-80 % sporulation) strobili phases, the total IAA content in the strobili decreased from 8.4 to 6.8 ng/g FW, respectively. During the mass shedding of spores, the bound form of IAA predominated in the strobili, being 1.8 times greater than the free form. As the growth of internodes and leaf rings ceased during the following phases, a gradual reduction in IAA content was observed, with changes being more pronounced in internodes than in leaves. The total content of IAA in internodes during the open strobilus phase was 94.6 % and 67.4 % lower, respectively, compared to closed strobilus phase. At this stage, the bound and later the free form of IAA predominated in internodes by 9 and 3 times, respectively.

Conversely, the free form of IAA prevailed in the leaves by 1.3 and 1.2 times (**Fig. 2**). In the rhizomes, IAA accumulation (mainly in the bound form) was detected only during the open strobilus phase at the onset of summer vegetation.

Thus, in the reproductive above-ground organs of *E. arvense*, IAA accumulation occurred during the active growth phase of spring fertile shoots (germination phase) and the strobilus development and spore formation phases (closed strobilus phase). IAA content in rhizomes was significantly lower than in above-ground organs, with accumulation occurring only during the transition from reproductive to vegetative development of the sporophyte (open strobilus phase).

Accumulation and distribution of endogenous IAA in *Equisetum arvense* vegetative sporophyte shoots and rhizomes. After the spores ripen, the spore-bearing shoots die, and at the end of May to early June, green branched vegetative, or assimilation, shoots begin to develop. These shoots are characterized by an articulated-ring structure and grow intensively until mid-July, reaching heights of 50 cm or more, after which growth stops and nutrients storage in the rhizome is activated. There is significant variability in organ sizes within the same range of *E. arvense*. In well-lit areas without other plant types, the stems of *E. arvense* become thin (1–3 mm in diameter), short (up to 50 cm), and sprawling. However, in environments with intensive surrounding vegetation growth, mature rhizomes of the field horsetail clone produce powerful erect unbranched stems 70–80 cm long and 4 mm in diameter, with massive first-order branches of up to 30 cm long and second-order branches up to 6 cm long (Voytenko *et al.*, 2016).

As a result, vegetative shoots of different lengths and ages develop simultaneously on one rhizome. We studied 1–6 lower and 8(9)–13(14) upper internodes with branch rings of the first and second order in summer sterile horsetail shoots of different lengths and ages (18, 21, 26, 40, 50 and 70 cm). As the length of vegetative shoots increased, the total IAA content rose, reaching a maximum of 105.0±5.24 ng/g FW in 50 cm high plants. Upon cessation of growth in 70 cm tall shoots, IAA level decreased by 3.8 times, amounting to 27.2±1.35 ng/g FW (**Fig. 3**).

In 18 cm high hoots, hormone accumulation was observed in the upper internodes with first-order branches, where the free form of IAA exceeded the bound form by three times. In the lower internodes, IAA content was 2.4 times lower, with the bound form dominating at 4.3±0.22 ng/g FW. With the initiation and formation of second-order branches in the nodes of lower first-order branches of 21 cm tall plants, the free and bound forms of IAA increased by 8 and 2.4 times in the lower metameres, while in the upper metamers, they decreased by 2.2 and 3 times, respectively. The free form of the hormone remained dominant in the upper metamers, while bound IAA accumulated in the lower metamers. In 26 cm tall plants, IAA content remained almost unchanged in the shoot's upper organs, amounting to 4.2±0.21 ng/g FW, with the free form accumulating and the bound form present in trace amounts. In the lower metamers, both IAA forms decreased, totaling 3.2±0.16 ng/g FW, which was 1.3 times lower than in the upper metamers (**Fig. 3**).

In the upper and lower metameres of second-order branches in 40 cm tall plants, IAA content increased by 6.4 and 9.2 times, respectively (**Fig. 3**). In the lower metamers, the amount of free and bound IAA forms was equal. Conversely, in the upper metamers, the free form content was 2.5 times higher than the bound form. During the transition to stationary growth in 50 cm tall plants, the bound form of IAA accumulated in both shoot parts, with the hormone amount being 1.3 timed higher in the lower

metamers. In contrast, the free form level was equal in the upper and lower metamers and lower than the bound IAA content. During growth slowing/stopping in of 70 cm tall plants, there was a decrease in both IAA forms, with amounts remaining within close limits. During the summer-autumn growth period, IAA levels in the rhizome gradually increased. In 40 cm tall plants, hormone content peaked at 65.4±3.27 ng/g FW, then decreased to 9.8±0.49 ng/g FW in 70 cm tall plants. During stationary growth, the bound hormone form dominated in rhizomes, except for 50 cm tall plants (**Fig. 3**).

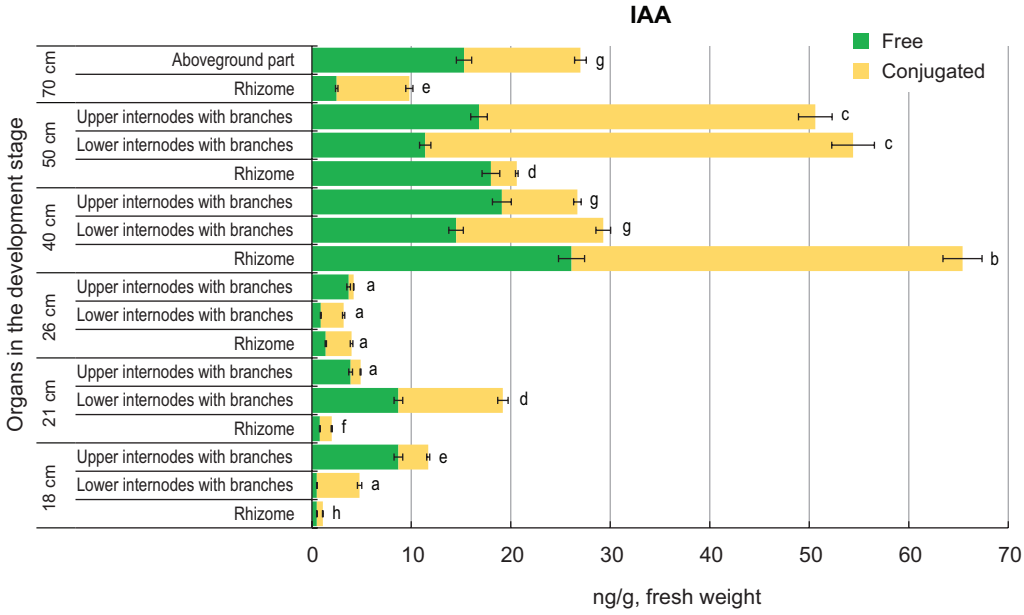

Fig. 3. IAA accumulation and distribution in upper and lower internodes with branch rings of the first and second order of *Equisetum arvense* assimilation shoots and rhizomes of different height and age

Note: n = 5; x ± standard error (SE), different letters beside the bars indicate statistically significant differences between means assessed by Bonferroni's test at P ≤0.05. a – Lower internodes with branches 18, Upper internodes with branches 21, Rhizome 26, Lower internodes with branches 26, Upper internodes with branches 26; b – Rhizome 40; c – Lower internodes with branches 50, Upper internodes with branches 50; d – Lower internodes with branches 21, Rhizome 50; e – Upper internodes with branches 18, Rhizome 70; f – Rhizome 21; g – Lower internodes with branches 40, Upper internodes with branches 40, Aboveground part 70; h – Rhizome 18

Thus, the growth of metamers in the assimilating summer-autumn shoots of *E. arvense* was accompanied by an increase in the free form of IAA, while growth slowdown corresponded with a decrease in hormone levels, with the bound form becoming dominant.

Accumulation and distribution of endogenous ABA in *Equisetum arvense* reproductive sporophyte shoots and rhizomes. During the autumn dormancy phase, ABA accumulation occurred primarily in the rhizomes, with a total hormone content of 4.9±0.24 ng/g FW. In the winter dormancy phase, ABA was predominantly found in the primary buds, with a total content of 8.6±0.42 ng/g FW. In autumn, ABA accumulated in buds primarily in its bound form, while the free form was present only in trace amounts. The free form of ABA became more prominent in overwintering buds, with its content

being 2.7 times higher than that of the bound form. In rhizomes during the autumn period, the levels of both ABA forms were similar, however both decreased to trace amounts during the phase of winter dormancy (**Fig. 4**).

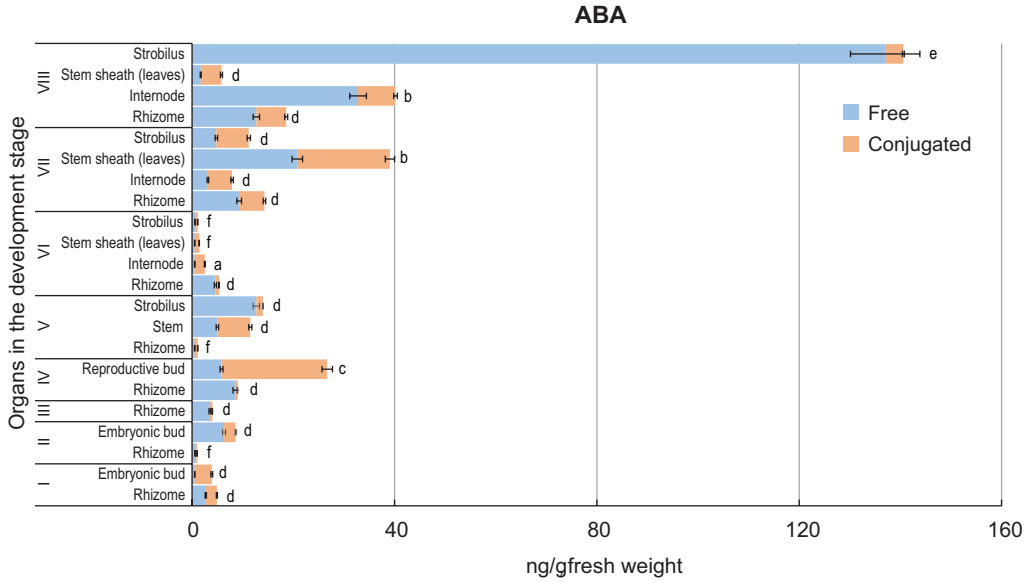

Fig. 4. ABA accumulation and distribution in *Equisetum arvense* reproductive sporophyte shoots and rhizomes in the phases of autumn (I) and winter (II) dormancy, spring awakening (III), germination (IV), the beginning of active growth (V), when the plants reached 4–5 cm in height, closed (VI), semi-open (VII) and open (VIII) strobilus

Note: n = 5; x ± standard error (SE), different letters beside the bars indicate statistically significant differences between means assessed by Bonferroni's test at P ≤0.05. a – Internode 6; b – Stem sheath (leaves) 7, Internode 8; c – Reproductive bud 4; d – Rhizome 1, Embryonic bud 1, Embryonic bud 2, Rhizome 3, Rhizome 4, Stem 5, Strobilus 5, Rhizome 6, Rhizome 7, Internode 7, Strobilus 7, Rhizome 8, Stem sheath (leaves) 8; e – Strobilus 8; f – Rhizome 2, Rhizome 5, Stem sheath (leaves) 6, Strobilus 6

During the phase of spring awakening and germination, there was a gradual increase in ABA levels in the rhizomes, mainly due to the accumulation of its free form. In the germination phase, the total ABA content in the sprouted reproductive buds, which included six internodes with leaf rings and a rudimentary strobilus, was three times higher than in the rhizome. In contrast, reproductive buds accumulated the bound form of ABA, which was 3.6 times higher than the free form. At the onset of active growth, ABA levels in the rhizomes decreased to trace values. In the shoots, the total hormone content was half that of the stems, with the bound form predominating in the shoots by 1.3 times, while the free form predominated in the stems by 9.8 times (**Fig. 4**).

As the growth of above-ground organs slowed and spores matured, ABA levels increased significantly, peaking during open strobile phase and reaching their lowest during the closed strobila phase. During the half-open strobila phase, ABA accumulated in leaf rings, where the hormone content was 4.9 and 3.5 times higher than that in internodes and strobiles, respectively.

In above-ground organs, the bound form predominated. At the open strobila phase, ABA accumulation shifted to the strobili, reaching 140.6±7.03 ng/g FW, which was

3.5 and 24.2 times higher than in the internodes and leaves, respectively. The free form predominated in internodes and strobili (77.4 % and 97.4 %, respectively), while the bound form dominated in the leaves by 192.9 %. During the development of reproductive shoots, ABA content in rhizomes increased from 5.3±0.26 ng/g FW in the closed strobila phase to 18.6±0.93 ng/g FW in the open strobila phase, with the free form of ABA being dominant in all investigated phases (**Fig. 4**).

In summary, ABA accumulation in rhizomes exhibited jump-like changes during growth and development, while a gradual increase was observed in mature above-ground organs. ABA levels in rhizomes decreased at the end of autumn dormancy, but increased during the germination stage. Following a second reduction in ABA content at the beginning of active growth, another increase occurred, peaking during the open strobilus phase. The free form of ABA was dominant in rhizomes. After the germination of above-ground organs, ABA gradually accumulated in reproductive buds, and, as the growth of reproductive shoots slowed, in leaves and internodes. The maximum accumulation of ABA was recorded in strobiles at the end of the spring reproductive shoot's vegetation, coinciding with the mass dispersal of mature spores.

Accumulation and distribution of endogenous ABA in *Equisetum arvense* vegetative sporophyte shoots and rhizomes. In the rhizomes of vegetative sterile summer shoots of field horsetail, the total content of ABA reached maximum values of 47.3±2.36 ng/g FW and 50.6±2.53 ng/g FW in 50 and 70 cm tall plants. Trace amounts of the hormone were detected in 18, 21, and 26 cm tall plants. In 40 cm tall plants, the content of both free and bound forms of ABA increased fivefold, with both forms present in equal amounts. The content of ABA was within the same limits and increased by 5 times. In plants measuring 50 and 70 cm, the free form dominated, exceeding the bound forms by factors of 10.3 and 4.9, respectively (**Fig. 5**).

In 18 cm tall plants, the total ABA content in the upper metamers was 1.8 times higher than in the lower metamers. The free form of ABA predominated in the upper part of the shoot, whereas both forms were equally present in the lower part. In 21 cm tall plants, during the formation of second-order branches, ABA accumulation shifted to the lower metamers, where its content increased fivefold. Meanwhile, ABA levels remained unchanged in the upper metamers, with the free form dominating in the lower metamers, and the bound form in the upper metamers. In 26 cm tall plants a 2.3-fold decrease in total ABA content was recorded in the lower metameres, mainly due to a reduction in the free form. The ABA content in the upper metamers remained constant, but the balance between forms shifted towards the free form, which reached 4.1±0.21 ng/g FW, while the bound form decreased to trace levels (**Fig. 5**).

During the active growth of second-order branches and the slowing of internodal growth, ABA content increased significantly (**Fig. 5**). In 40 cm tall plants, total hormone content increased by 9.1 and 26.8 times in the lower and upper metamers, reaching 59.8±2.98 ng/g FW and 121.8±6.09 ng/g FW, respectively. The upper metamers were dominated by the free form (53.6 %), while the bound form dominated in the lower metamers (160.2 %). In 50 cm tall plants, hormone accumulation occurred in the lower metameres, with the bound form dominating by 1.7 times. Total ABA content in the upper metamers remained unchanged, with the bound form dominating by 15.4 times. In 70 cm tall plants, ABA accumulation was primarily in the rhizomes, while total hormone content in the shoots decreased significantly to 22.4±1.12 ng/g FW, with the bound form dominating by 11.4 times (**Fig. 5**).

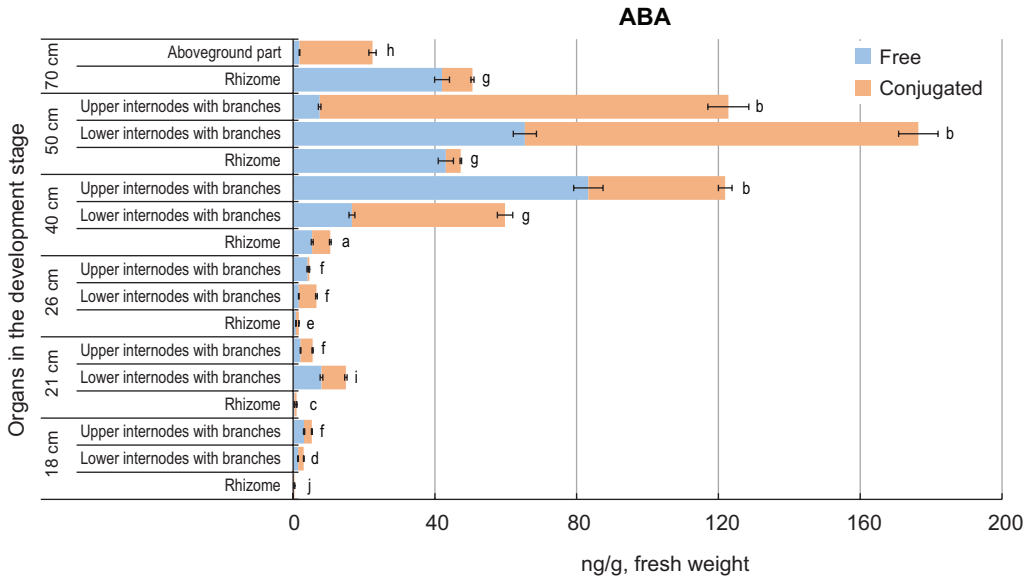

Fig. 5. ABA accumulation and distribution in the upper and lower internodes with branch rings of the first and second order of *Equisetum arvense* assimilation shoots and rhizomes of different height and age

Note: n = 5; x ± standard error (SE), different letters beside the bars indicate statistically significant differences between means assessed by Bonferroni's test at P ≤0.05. a – Rhizome 40; b – Upper internodes with branches 40, Lower internodes with branches 50, Upper internodes with branches 50; c – Rhizome 21; d – Lower internodes with branches 18; e – Rhizome 26; f – Upper internodes with branches 18, Upper internodes with branches 21, Lower internodes with branches 26, Upper internodes with branches 26; g – Lower internodes with branches 40, Rhizome 50, Rhizome 70; h – Aboveground part 70; i – Lower internodes with branches 21; j – Rhizome 18

Overall, ABA accumulation in the vegetative shoots of field horsetail occurred during the slowing and stopping of growth in both above-ground and underground organs. Active growth of organs coincided with significant reduction (almost tenfold) in ABA levels. The results suggest that endogenous IAA and ABA play a role in the regulating the growth processes of the sporophyte generation of field horsetail.

IAA is involved in regulating most plant growth and development reactions (Weijers & Wagner, 2016), affecting growth and development by regulating cell division and elongation (Perrot-Rechenmann, 2010). While IAA is considered a “growth hormone,” ABA is often referred to as a “stress hormone”, involved in the formation of stress responses (Vishwakarma *et al.*, 2017). Additionally, ABA regulates plant growth and development under non-stress conditions (Yoshida *et al.*, 2019), while IAA responds to various stress factors (Gray *et al.*, 1998). The signaling pathways of these two phytohormones intersect in growth regulation and stress response formation (Rowe *et al.*, 2016). In the phase of spring awakening, the onset of active growth, and the closed strobilus phase, the free form of IAA dominated in the above-ground organs of the reproductive sporophyte of field horsetail, while ABA was mainly in its bound form. In rhizomes, bound IAA and free ABA accumulated at the end of spring vegetation, while in previous phases, these phytohormones were present in trace amounts, except during autumn dormancy. Previously we established that in the sporophyte organs of rough horsetail (*Equisetum hyemale*) with an evergreen phenorhythm type, the maxima in the content of endogenous plant

hormones IAA and ABA also occurred during periods of active growth and development of reproductive shoots and development and maturation of strobili with spores. The accumulation of IAA (mainly the free form) in the rhizome occurred during the formation of storage tubers, when the plants were preparing for the transition to dormancy, the maximum of ABA occurred during the dormancy. A specific feature of *E. hyemale* compared to *E. arvense* is the predominance of the conjugated form of IAA and ABA in the sporophyte organs at in the organs of the sporophyte at almost all investigated stages of development (Voytenko, 2021).

The accumulation of endogenous IAA and ABA during autumn dormancy of *E. arvense* may be related to forming dormant lateral buds laid in rhizome nodes. IAA and ABA actively interact in regulating various processes of growth and development in higher plants, including root elongation, lateral root formation, seed germination, and cotyledon growth. In higher plants, IAA activity peaks during dormancy release, seed germination, and organ growth, whereas ABA activity increases during seed maturation, dormancy transition, and organ senescence (Emenecker & Strader, 2020).

The accumulation of IAA in strobiles decreased from the closed strobila phase to the open strobila phase, while ABA content increased. The accumulation of free ABA and bound IAA in strobili during mass spore shedding suggests ABA's involvement in spore maturation and strobili senescence. Similar hormonal fluctuations occur in higher plants during seed maturation and organ senescence/retardation (Shu *et al.*, 2016).

ABA accumulation in rhizomes of sterile plants (70 cm tall) occurred at the end of the growing season, while in the active growth phase (40 cm tall plants), endogenous IAA content was highest. This distribution suggests that during the active growth of above-ground organs, rhizomes are the main site of IAA biosynthesis, while ABA primarily controls storage nodule formation in rhizome nodes. In sterile summer vegetative shoots (40 and 50 cm tall), IAA and ABA accumulation coincided with the growth of second-order lateral branches. IAA accumulated in the lower metameres, where second-order branches developed in the nodes of the first-order branches. Conversely, ABA content increased in the upper metamers, where only second-order branches formed. As the growth slowed, IAA and ABA content decreased significantly in 70 cm tall plants. Free and bound IAA levels were equal, but ABA was mainly in its bound state. Overall, IAA and ABA content was higher in organs of sterile field horsetail plants than in reproductive plants, except during the germination phase for IAA and the semi-open and open strobilus phases for ABA.

CONCLUSION

The dynamics and distribution of endogenous IAA and ABA during the ontogeny of the sporophyte generation of *Equisetum arvense* were analyzed for the first time. It was established that active growth processes in both above-ground and underground organs, as well as the development of reproductive structures, occur alongside the accumulation of the active form of IAA. In contrast, the slowing of growth, aging of organs and maturation of spores are accompanied by an increase in the content of endogenous ABA. The results revealed similarities in the accumulation patterns of endogenous IAA and ABA in the ontogeny of both higher spore and flowering plants, contributing to the fundamental knowledge of plant growth and development regulation.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: this article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceptualization, [I.K.; L.V.]; methodology, [L.V.; M.S.; O.P.]; validation, [L.V.; M.S.; O.P.]; formal analysis, [L.V.; I.G.; O.Ts.]; investigation, [L.V.; I.G.]; resources, [L.V.; I.G.]; data curation, [L.V.; I.G.; I.K.]; writing – original draft preparation, [L.V.; I.K.; M.S.]; writing – review and editing, [L.V.; I.K.]; translating, [I.K.]; visualization, [L.V.; M.S.] supervision, [I.K.].

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ЕНДОГЕННІ АУКСИН І АБСЦИЗОВА КИСЛОТА В РЕГУЛЯЦІЇ РОСТУ ТА РОЗВИТКУ СПОРОФІТУ *EQUISETUM ARVENSE* L.

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Вступ. Фітогормони – природні регулятори росту й розвитку рослин, вміст і розподіл яких змінюються в органах і тканинах упродовж життєвого циклу. Індоліл-3-оцтова кислота (ІОК) регулює органогенез, сповільнює старіння, задіяна у відповідях на екологічні стреси. Гормон стресу абсцизова кислота (АБК) контролює транспірацію, ріст коренів, старіння рослин. На відміну від численних даних про участь ІОК і АБК у регуляції ростових і формотворчих процесів вищих квіткових рослин, роль цих гормонів у судинних спорових рослин є малодослідженою.

Матеріали та методи. Упродовж дев'яти фаз онтогенезу методом ВЕРХ-МС вивчено динаміку та розподіл ендогенних ІОК й АБК в органах репродуктивних і стерильних рослин спорофітного покоління *Equisetum arvense* L.

Результати. Встановлено, що під час росту пагонів, кореневища та розвитку репродуктивних структур накопичується активна форма ІОК. За уповільнення росту, старіння органів і дозрівання спор зростає вміст ендогенної АБК. На всіх фазах розвитку рівні гормонів в органах стерильних літніх рослин були вищими, ніж у репродуктивних весняних, за винятком фази проростання для ІОК і фаз напіввідкритого та відкритого стробілу для АБК. Накопичення вільної АБК у стробілах у період масового висипання спор засвідчило участь гормону в регуляції дозрівання спор і старіння стробілу. Рівні гормонів у стерильних пагонах різної довжини зростали після формування та росту бічних гілок другого порядку. У весняних кореневищах накопичення ІОК й АБК відбувалось у фазу відкритого стробілу. У кореневищах літніх рослин накопичення ІОК (переважно зв'язаної форми) і АБК (здебільшого вільної форми) виявлено у рослин висотою 40 і 50 см. З припиненням росту у 70 см рослин вміст ІОК у кореневищах зменшився, а рівень АБК залишився без змін.

Висновки. Активні ростові процеси у надземних і підземних органах та розвиток репродуктивних структур відбувалися на тлі накопичення активної форми ІОК, натомість уповільнення росту, старіння органів і дозрівання спор супроводжувалося зростанням вмісту АБК. Виявлено риси подібності у характері накопичення ІОК і АБК в онтогенезі вищих спорових та квіткових рослин, що доповнює фундаментальні знання з питань фітогормональної регуляції росту й розвитку рослин.

Ключові слова: *Equisetum arvense* L., абсцизова кислота, індоліл-3-оцтова кислота, спорофіт, ріст, розвиток

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