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PROFILING OF HORMONES IN PLANT TISSUES: HISTORY, MODERN APPROACHES, USE IN BIOTECHNOLOGY

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The review analyzes and summarizes the latest literature on the history of development and the current state of methodological approaches to the identification and quantification of phytohormones in plant tissues. Phytohormones play a key role in the regulation of physiological processes throughout the life cycle of plants — from seed germination to aging. In tissues of plants, their concentrations are very low (from 10^{-9} M to 10^{-6} M), so the development of fast, highly efficient, comprehensive, and at the same time reliable approaches to the determination of phytohormones is extremely urgent. A brief description of the main classes of phytohormones was given in the article and it was described their functional activity. The substantiated and checked sequence of procedures on the extraction of plant hormones, their separation from interfering substances, quantification methodology for indol-3-acetic, abscisic, gibberellic (GA_3), salicylic acids and five cytokinins that combines a high-performance liquid chromatography with mass spectrometry are presented. Four chromatographic methods of profiling were described in the article. The conditions for detecting phytohormones of different classes and ionization of these substances in a mass spectrometer were given. The importance of phytohormones profiling in plant tissues for application in biotechnological approaches, in particular in metabolic engineering, for the formation of stress resistance in crops were discussed.

Key words: high-performance liquid chromatography, mass spectrometry, phytohormones.

Phytohormones are signaling biomolecules of different chemical structures and physicochemical properties producing their effects in nanomolar concentrations and regulate most physiological and metabolic processes in plants. The dominant classes of plant hormones include auxins, cytokinins, gibberellins (GA_n), abscisic acid (ABA) and ethylene. Phytohormones include salicylic acid (SA), jasmonates, brassinosteroids, polyamines, and strigolactones as well. Phytohormones play a key role in regulating all stages of the plant life cycle — from seed germination to aging, so determining their content and localization sites is important to find ways to control the rate of growth and development and the formation of stress tolerance.

The main active endogenous auxin — indole-3-acetic acid (IAA) is involved in the regulation of most growth processes, among which are apical dominance, phototropism, and root growth [1]. Up to 90–95% of IAA is bound to amino acids and sugars forming an auxin depot. Also, IAA can be metabolized to indole butyric acid [2].

Cytokinins are important components of the hormonal complex regulating cell division, meristem formation, responses to negative environmental effects, photosynthesis, aging, absorption of macro- and microelements [3]. The main physiologically active forms of cytokinins are *trans*-zeatin (Z) and zeatin riboside (ZR). The main reserve form of the hormone is *O*-glucoside, which easily hydrolyzed to the active forms [2].

Gibberellins number comprices more than 130 forms involved in the regulation of seed germination, coordinate cell division, and elongation, sex determination, and flowering. Gibberellic acid (GA_3) is major number comprices more than gibberellin that functions *in vitro* and is applied in exogenous plant treatment [4]. The key hormone that defends plants against abiotic stresses is ABA. It mediates both quick responses (stomata closing) and long changes in expression of genes involved in stress responses [5]. The most active is the *cis*-isomer of ABA; the less active is *trans*-isomer. The inactive hormone is preserved after being bound to glucose [2]. Cytokinins and ABA have an antagonistic effect on overcoming stress. High content of ABA and a low level of active cytokinins inhibit growth and lead to a redistribution of limited energy resources in the formation of defensive reactions. At the same time, due to high levels of cytokinins, photosynthesis in tissues is stabilized and that contributes to the mobilization of plant metabolism and energy accumulation, especially in the case of milder or shorter stresses [3].

Salicylic acid is a phytohormone of phenolic nature which is involved in the regulation of growth and development, photosynthesis, respiration, transpiration, plays an important role in the formation of stress resistance [6]. The exogenous SA effects on growth depend on the type of plant, stage of development, and hormone concentration that indirectly indicates the relationship of SA with the hormonal status of the whole plant [7]. SA and ABA are involved in many responses to stress, and the nature of their interactions can be antagonistic or synergistic [8, 9].

The hormonal regulation is characterized by the complexity of hierarchical relationships and the dynamism of response to environmental factors. The content and localization of phytohormones in organs and tissues determine the dynamics of plant development *in vitro* and *in vivo* [10], so the availability of a sensitive and rapid physicochemical method of a simultaneously quantitative assessment of various phytohormones is extremely important in the study of signaling systems. In our review, we analyzed and summarized the latest literature on the history of development and the current state of methodological approaches to identification and quantification of phytohormones in plant tissues, highlight the results of our own methodological achievements, discussed the importance of phytohormones profiling for further application in biotechnological approaches.

Extraction and quantification of phytohormones

Assessing the content of phytohormones in plant tissues is a difficult task due to their extremely low content (in the range of picomoles per gram of fresh weight). However, plant tissues are rich in potential contaminants, among which primary and secondary metabolites occur in much higher concentrations (micro- and millimoles per gram of fresh weight).

The first methods of relative assessment of hormone content in plant tissues were bioassays [11]. They were based on the use of isolated cells, tissues, and organs of plants completely devoid of their hormone or with its very low concentration and high sensitivity to the applying of exogenous hormone. The physiological response of plant test objects to a solution of exogenous hormone depended on its amount and was proportional to the logarithm of its concentration. After contact between the test system and the hormone solution from the test plant material, as well as the solution with the standard of this hormone, the value of the physiological response of the test system was compared and the amount of hormone in the sample was evaluated. The bioassay method is characterized by high selectivity and specificity in the identification of hormones by appropriate receptors. The presence of cytokinins, gibberellins, IAA, and ABA in plant extracts was established by bioassays.

Further improvement of analytical approaches allowed more accurately measure the content of phytohormones. However, the first quantitative estimates of phytohormones were characterized by low selectivity and sensitivity. Fig. 1 shows the regular procedure of isolation, purification, fractionation, and analysis of auxins, gibberellins, cytokinins, abscisic acid in plant tissues. We have added our generalizations and supplements to the main stages set out in the publication "Methodical Recommendations" [12]. According to this procedure, hormonal analysis lasted a long time. The main advantage consisted in finding hydrolytic (reserve) forms of IAA and ABA in the sample. The analysis required a lot of plant material (up to 9 g) and included several complex stages of sample purification. The results of the isolation procedure were often unpredictable, purification was time-consuming and costly.

The frozen and centrifuged extract was divided into three aliquots to detect IAA and ABA, gibberellin-like substances, cytokinins;

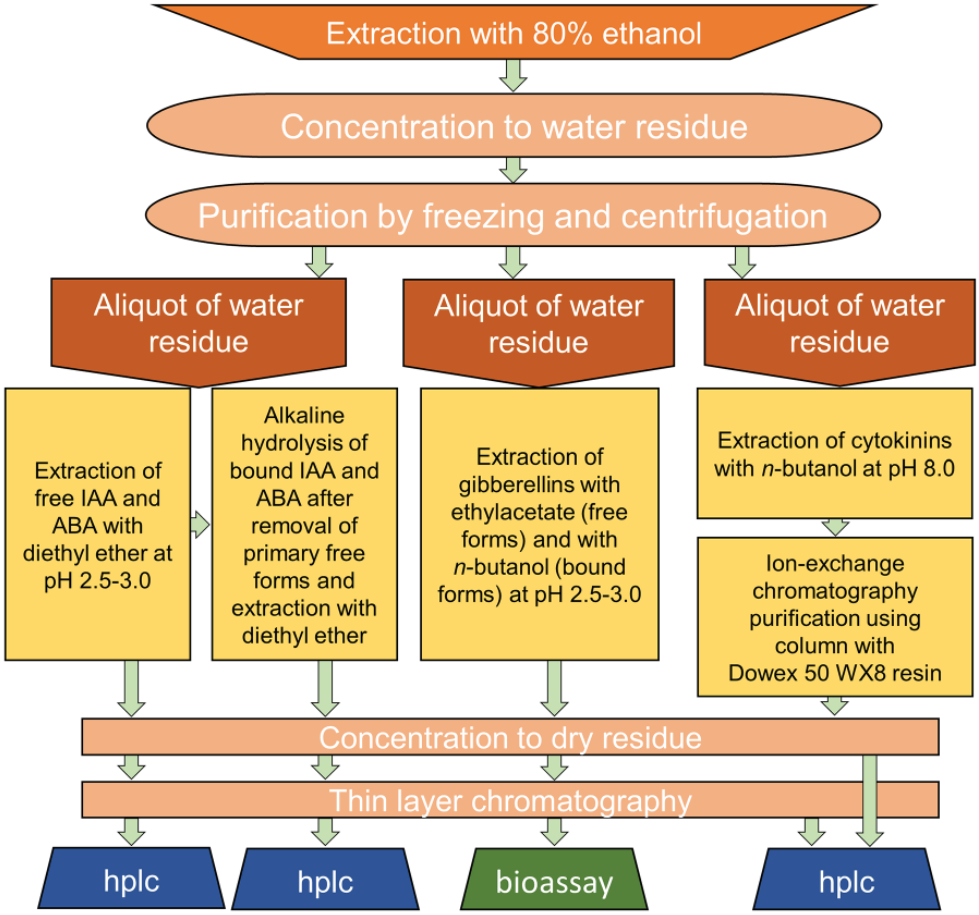


Fig. 1. **The regular procedure of isolation, purification, fractionation, and analysis of auxins, gibberellins, cytokinins, abscisic acid in plant tissues:**
HPLC — high-performance liquid chromatography

The next stage in the elaboration of methods for assessing of phytohormones content is associated with the production of hormone-specific antibodies [13]. The determination of concentration in samples is grounded on the competition for a limited number of antibodies. An immunochemical method combines high sensitivity (extraction of substances at a concentration of 10–100 pg per ml) and selectivity, enables to simultaneously analyze several dozen samples; it does not require large portions of plant material and complex long-term purification of the extract. Limitations of immune methods are the low specificity of antibodies, as well as the presence of interfering substances in samples. Due to the relatively small size of hormone molecules, antibodies can bind only to a hormone conjugate or high-molecular "carriers". Antibodies have a fairly low level of recognition of the molecular part that is used to bind to the protein. The immune analysis is still applied to detect ABA because the structure of the inactivation products of the substance is sufficiently different from that of the active molecule, but this method may give erroneous results in the case of cytokinins. Numerous cytokinins inactivation products, cytokinin glucosides, show significant cross-reactivity ability. Therefore, during the detection of cytokinins, preparative fractionation of samples using preparative HPLC with the subsequent immunological determination of individual substances in individual fractions is more often applied. This approach is complicated by the difference in delay time caused by other compounds present in the sample (the number of which significantly exceeds the hormone) or their effect on the hormone-antibody interaction (this phenomenon can be both negative and positive).

It has been reported that gas chromatography can be used in tandem with a multi quadrupole mass spectrometer (GC-MS/MS) for the simultaneous analysis of IAA, ABA, and SA in *Arabidopsis thaliana* [14]. However, GC-MS/MS analysis is limited to volatile

compounds, so hormones must be purified and derivatized before analytical work. Another disadvantage of gas chromatography is the high temperature of the column thermostat, at which the separation of the sample components should take place. As a result, thermally labile compounds can be destroyed during analysis [15].

Today, the use of physicochemical methods of analysis is preferred, including reversed-phase high-performance liquid chromatography in combination with mass spectrometry. This method is based on the separation of pre-purified plant extract using columns filled with lipophilic-modified silica gel, followed by the determination in the detector unit. The chromatogram contains a series of peaks that characterize the test substance and impurities. Accurate identification of the peak obtained by the spectrophotometric detector (DAD-matrix) can be performed using a single-quadrupole mass spectrometer, which shows the ratio of molecular mass to charge of a particular substance to which the peak corresponds. There are also used standards — chemically synthesized phytohormones.

Liquid chromatography combined with mass spectrometry (HPLC/MS) is a successful alternative to the GC/MS/MS method. Modern liquid chromatography can efficiently and rapidly separate complex mixtures of compounds with a wide range of polarity without the need for their derivatization. A method of high-performance liquid chromatography in tandem with mass spectrometry under electrospray ionization (HPLC/EC-MS/MS) for the simultaneous analysis of plant hormones of four different classes (auxins, cytokinins, GA and ABA) and their metabolites has been reported. That made it possible to thoroughly analyze the hormonal regulation of lettuce seed dormancy, simulated by temperature conditions [16]. HPLC/EC-MS/MS method to identify seven major classes of plant hormones, including auxins, cytokinins, GA, ABA, jasmonates, brassinosteroids, and SA in *Arabidopsis thaliana* tissues, has been worked out [17]. The method of detecting phytohormones (cytokinins, auxins, ABA and GA) in rice tissues using ultra-high-performance chromatography (ultra-high pressure) in tandem with mass spectrometry under the conditions of "electrospray" ionization (HPLC/EC-MS/MS) is described [18]. To increase the detection level of analytes, which are usually ionized in the conditions of negatively charged compounds, IAA, ABA, and GA_n were derivatized with bromocholine and all compounds were analyzed in the conditions of positive ionization. However, this method cannot be applied to detect other plant hormones, in particular SA and JA.

Modern mass spectrometers are ideal detectors of high sensitivity, selectivity, and speed. Mass spectrometers can detect even a few picograms of the analytes. The sensitivity of mass spectrometers can significantly reduce the amount of material required for analysis. The minimum portion for analysis may be one hundred milligrams of fresh plant material, or even less. This simplifies the sampling procedure and allows to analyze materials in limited quantities, such as fragments of individual plant parts or tissues of plants having a small size and weight. The selectivity of mass spectrometry is based on the ability to measure a specific parameter of a compound, its molecular mass (mass to charge ratio — m/z). During the mode of selection and monitoring (SIM) of the particular analyte mass, the instrument filters out of all other masses. Thus, the recorded chromatogram shows only compounds of the same mass, isomers, or isobars (unrelated compounds of the same mass, which, however, are separated by a chromatographic column). An even more sensitive variant of analytical equipment is the tandem known as MS/MS. The first quadrupole of the mass detector enables to measure the ratio of mass to charge m/z of one substance (precursor, actually m/z of the analyte), which is then "broken" into specific fragments. Then the formed fragments are separated, and only the fragments marked by the operator are scanned by the device. Due to this, only compounds with pre-selected mass transformations of the precursor-fragments are detected. The MS/MS tandem has such a high selectivity that the chromatogram of even an imperfectly purified extract may contain only one peak of the target analyte compound. High selectivity of mass detectors increases the accuracy of compound identification and simplifies the purification procedure to only a few stages. In turn, progress in the production of electronic components has made the performance of mass detectors extremely high and that made it possible to instantly measure and switch between many masses pre-set in the software interface of the device. The speed of modern mass detectors allows the simultaneous analysis of tens and hundreds of compounds in one sample, which leads to a significant expansion of metabolic profiling.

Thus, the improvement of analytical instruments has increased the efficiency, selectivity, sensitivity, and throughput for a large number of substances, including phytohormones. The optimal analytical method for the analysis of phytohormones is HPLC or UHPLC (ultra-high-pressure chromatography) in tandem with a three-quadrupole mass spectrometric detector (MS/MS). However, this is quite expensive equipment and not all laboratories have it. Analytical chromatographs with a spectrophotometric detector and a mass spectrometer with a single quadrupole HPLC/MS are much more common.

Sample preparation. To obtain reliable, physiologically relevant data on the content of phytohormones, in addition to the availability of high-performance analytical equipment it is necessary to take precautions during sampling. The content of phytohormones fluctuates significantly during the day, so sampling should be carried out at the same time. It should also be borne in mind that hormone levels change throughout the year. Thus, the samples taken in winter are significantly different from those taken in spring and summer (even in the case of plants grown in vegetation chambers at the same temperature without daylight access) [2]. The intensity of light and its spectrum significantly affect the hormonal balance that complicates the reproduction of the experiment results in different laboratories. The levels of phytohormones in individual tissues of the whole plant (leaves, roots, stems) differ. Quite a large difference in concentration is between individual leaves and even within a single leaf blade — higher levels of cytokinins have parts that are actively growing. This is observed, for example, in the basal part of the leaf blades of monocotyledons [2]. The analysis procedure includes sampling, extraction of phytohormones, purification of the obtained extracts, and quantification. In the articles [2, 19]. methodological approaches that enable the detection of cytokinins, IAA, and ABA in one sample were described. By using these approaches, we made some modifications and developed our own method, the analytical component of which is quite original. The acid fraction, which contains IAA and ABA, during sample preparation includes gibberellins, in particular GA_3, and SA. Their quantity can be determined. The presented method is focused on the preparation of samples for analysis using liquid chromatography with a diode-array detector unit and a single-quadrupole mass detector (HPLC/MS). This equipment is used in most biological research laboratories.

Solutions for extraction and standards. The extraction solution contains methanol, double distillation water, and formic acid (15:4:1 in volume). It is stored at −20 °C. To make calibration tables and verify method losses, phytohormones chemical standards are dissolved in methanol with water (1:1 in volume). The solution for loading columns of solid-phase extraction (SPE) is 1M formic acid (37.7 ml of 99% formic acid to make up to 1000 ml with double distillation water, pH about 1.4). The first solvent for elution is 100% methanol. The second solvent for elution is 0.35 M NH_4OH (2.5 ml of 26% ammonium hydroxide solution add to 60 ml of methanol and add double distillation water to 100 ml with, pH about 11). The third solvent for elution is an alkaline solution of methanol (add 2.5 ml of 26% ammonium hydroxide solution to 60 ml of methanol and add double distillation water to final volume in 100 ml). To determine the bound forms of cytokinins, an incubation buffer is prepared for reaction with calf alkaline phosphatase (0.1 M ammonium acetate solution, pH about 10). The enzyme concentration is 0.2 U per 20 µl for 1 g of fresh sample weight (fresh solution should be prepared each time).

Sampling. The plant material cut into thin strips is weighed and immediately frozen in liquid nitrogen. The portions of plant material 0.5–2 g of fresh weight are for HPLC/MS and 0.05–0.2g for HPLC/MS/MS. The weight accuracy of each sample must be within ± 1% and must significantly exceed the minimum phytohormones detection limit. The central vein is removed from the leaf blade of dicotyledonous plants. In monocotyledonous plants, the upper third of the leaf must be removed. Leafs of the same phase of ontogenesis are analyzed. If necessary, the samples are taken to determine the dry weight. The root system is thoroughly washed from the substrate with cold water, dried with filter paper, weighed, and frozen in liquid nitrogen.

Extraction. The purpose of extraction is to release analyte compounds from plant tissue cells without chemical destruction. The ratio of extraction solvent to sample is 5:1 in volume, extraction is performed twice. Degradation of analytes should be avoided by working at low temperatures with an extraction solution that contains a significant proportion of organic solvent and has a low pH. The extraction step involves centrifugation, which precipitates cellulose, nucleic acids, and proteins. The extraction of plant hormones should be carried out following the procedure shown in the Fig. 2.

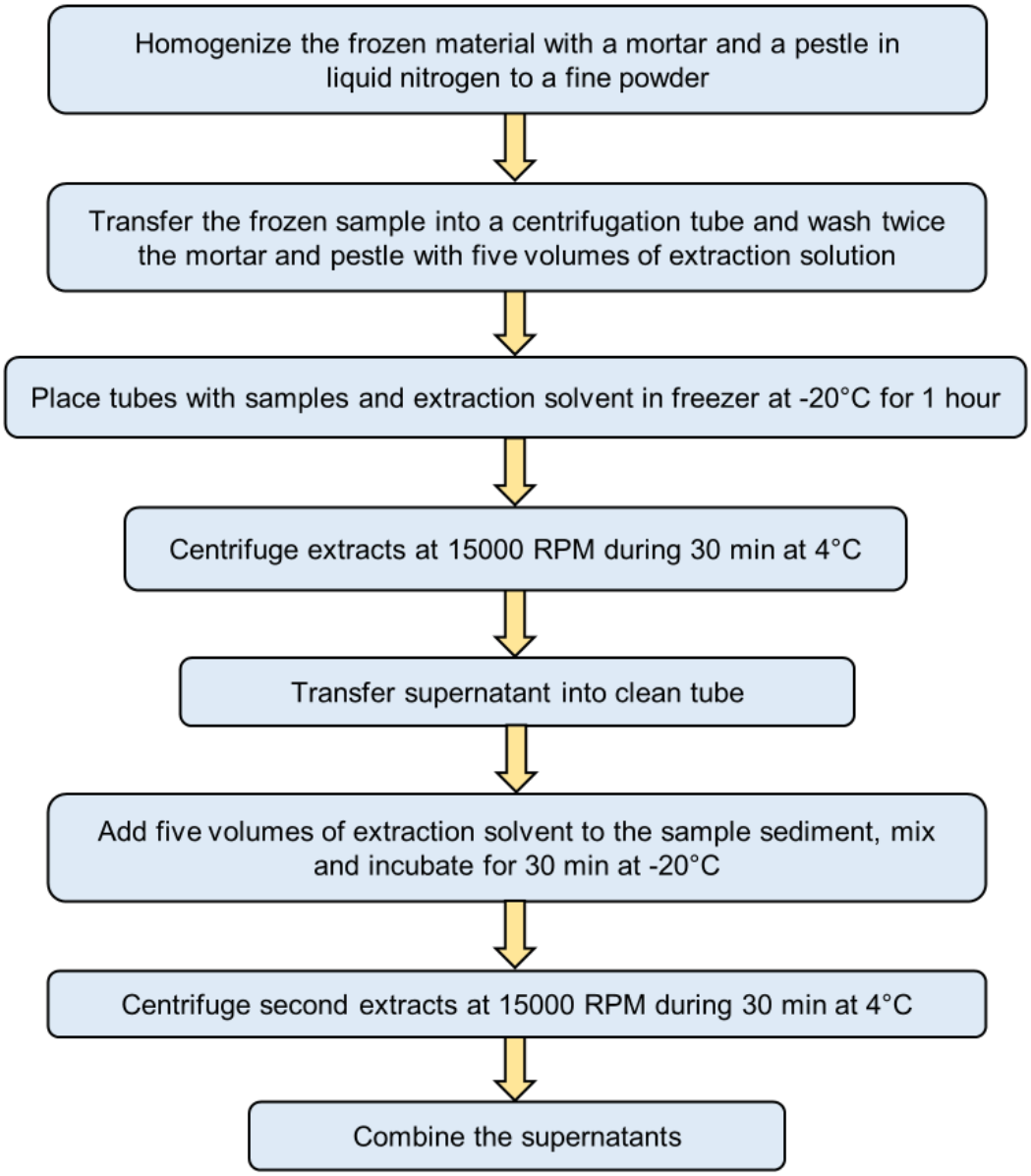


Fig. 2. **The procedure steps for extraction of phytohormones from plant tissue**
RPM — rotations per minute

Purification of the obtained extracts. Purification aims to remove interfering substances from the extract. Solid-phase extraction (SPE) cartridges are used for this purpose. The cartridge with solid silica gel phase C18 serves as a filter for removing lipophilic substances (Fig. 3), while phytohormones pass through it freely. The second cartridge with solid MCX 6 phase binds the hormones, which are then sequentially eluted with appropriate solvents (Fig. 4). The pollutants are washed with an alkaline solution and double distillation water. After purification and separation, we obtain two fractions that contain different groups of hormones. If alkaline phosphatase is used, three fractions are obtained. The sequence of purification is presented in details published in the work [2].

The purified fractions were analyzed using the HPLC system equipped with a diode array detector in tandem with a mass spectrometer, isolating the analyte compounds as peaks on the chromatograms. The amount of phytohormones is estimated by comparing the detector responses to

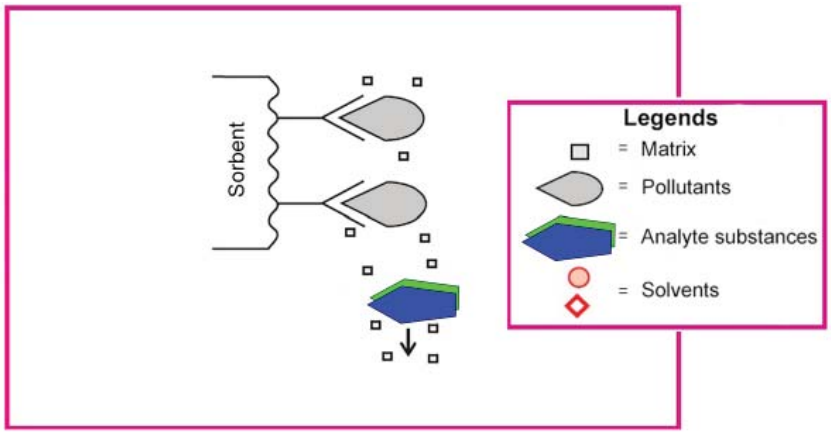
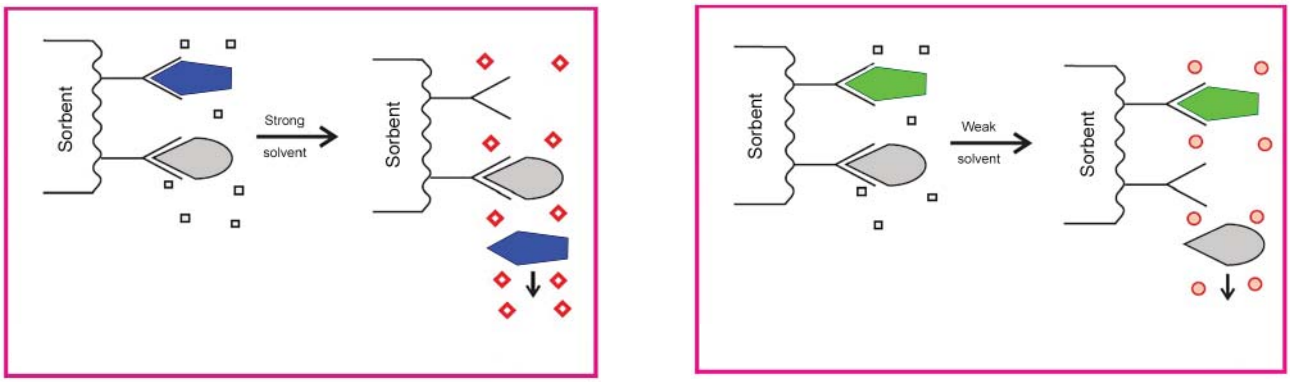


Fig. 3. **The process of selective extraction in the cartridge SPE C18, Sep-Pak Plus, Waters**



A *B*

Fig. 4. **A simplified scheme of the sample substances separation in the cartridge SPE Oasis MCX, 6 cc/150 mg Waters:**
A — selective elution of acidic analytes; *B* — the leaching of pollutants
Quantification with HPLC/MS

the endogenous hormone in an aliquot of the sample to the recorded responses to known amounts of the substance (external standard) entered in the calibration tables, which are pre-formed in the interface of the program. The amount of endogenous phytohormones is calculated according to the area of peaks. The chromatographic separation procedure, determination and ionization parameters for HPLC/MS depend on the specific instrument.

We performed analytical determination of phytohormones using HPLC on Agilent 1200 LC liquid chromatography system with diode-array matrix detector G1315B (USA) in tandem with a single-quadrupole mass spectrometric detector G6120A. Analysis and calculation of phytohormone content were performed using Agilent OpenLAB CDS ChemStation Edition software (rev. C.01.09). The procedures to perform the analysis were as follows (Fig. 5):

To quantify phytohormones in the obtained samples, we developed four chromatographic methods. Chromatographic separation was carried out in an Agilent ZORBAX Eclipse Plus C18 column 4.6×250 mm, which was filled with a lipophilic-modified sorbent with a particle size of 5 µm (reverse phase chromatography).

Profiling of IAA and ABA. 20 µl aliquots were chromatographically separated using a system of solvents (methanol, ultrapure water — distilled and then deionized, acetic acid in a volume ratio of 45: 54.9: 0.1) and IAA and ABA were detected in the UV absorption spectrum at analytical wavelengths of 280 and 254 nm. The flow speed of the mobile phase was 0.7 ml/min.

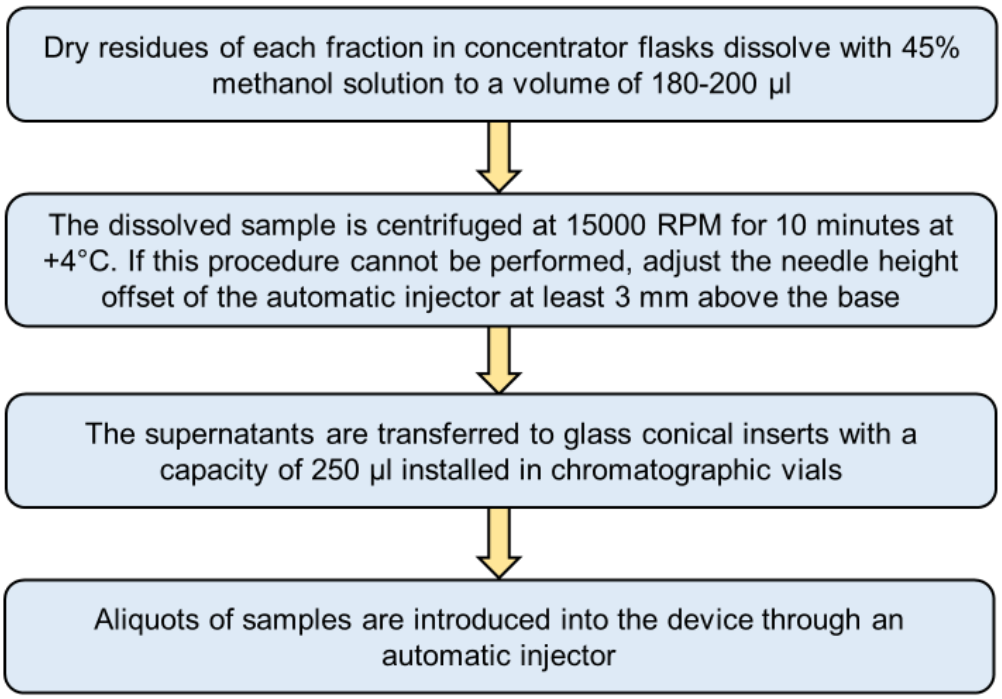


Fig. 5. **The procedure steps for performing HPLC/MS analysis**

Determination of the SA content. 10 µl aliquots were separated using a solvent system (acetonitrile, ultrapure water, acetic acid in a volume ratio of 45:54.9:0.1) and SA was detected at an analytical wavelength of 302 nm. The flow speed of the mobile phase was 0.8 ml/min.

GA_3 detection. After separating the 20 µl aliquots with a system of solvents (acetonitrile, ultrapure water, acetic acid — 30:69.9:0.1) GA_3 was quantitatively detected by the signal of the mass detector. The flow speed of the mobile phase was 0.5 ml/min.

Cytokinins detection. The 20 µl aliquots of the fraction with cytokinins were separated using a system of solvents (methanol, ultrapure water, acetic acid), the detection was performed at wavelength of 269 nm. A step gradient system was used to elute the cytokinins, namely: 0 min: CH_3OH/0.5% solution of CH_3COOH in deionized water (37/63) — 25 min: CH_3OH/0.5% solution of CH_3COOH (70/30) — 35 min: CH_3OH/0.5% CH_3COOH solution (100/0) at a constant flow rate of 0.5 ml per minute. The duration of the column equilibration after analysis (post-run) was 15 min.

The retention time tR of each substance by the chromatographic column and other details of the presented methods are listed in the table below. Unlabeled IAA, ABK, SA, GA_3, *trans*-zeatin-*O*-glucoside (t-ZG), *trans*-zeatin (t-Z), *trans*-zeatinribozid (t-ZR), isopentenyladenine (IP) and isopentenyladenosine (IPA) were used as standards for making calibration tables manufactured by Sigma-Aldrich (USA). In some cases, internal standards were used for identification. The occurrence of analytes in the samples was determined through a combined mode of operation of the mass spectrometer "multimode" (electrospray and chemical ionization at atmospheric pressure) for IAA, ABA, SA, GA_3 with the negative polarity of ionization of analyte molecules and positive — for cytokinins. To analyze GA_3 quantitatively, the signal of the mass detector MSD SIM by setting 50% of the scanning time of the device indicator m/z 345 [346-H] — was applied.

The sensitivity of the methods is slightly lower compared to more modern techniques [2, 20], when, after chromatographic separation of aliquots, a mass spectrometer with three quadrupoles (MS/MS) is used as the main detector. However, due to the rather incomplete provision of domestic biological laboratories with modern high-tech equipment, the presented method may well be used. Besides, the presented sample preparation procedures and individual chromatographic techniques with certain adjustments make the basis for use on the latest equipment.

Retention time (t_R) of plant hormones by the chromatographic column according to the proposed methods, m/z values, ionization conditions of their molecules in the mass detector, and elution mode

Phytohormone	t_R, min	m/z	Ionization conditions	Elution mode
The first method — duration 30 min				
IAA	11.075	174	Negative $-H^+$	Izocratic
ABA	18.955	263		
The second method — duration 20 min				
GA_3	8.072	345	Negative $-H^+$	Izocratic
The third method — duration 20 min				
SA	7.153	137	Negative $-H^+$	Izocratic
The fourth method — duration 35 min				
ZG	6.746	382	Positive $+H^+$	Gradient
Z	7.504	220		
ZR	10.232	352		
IP	18.290	204		
IPA	22.673	336		

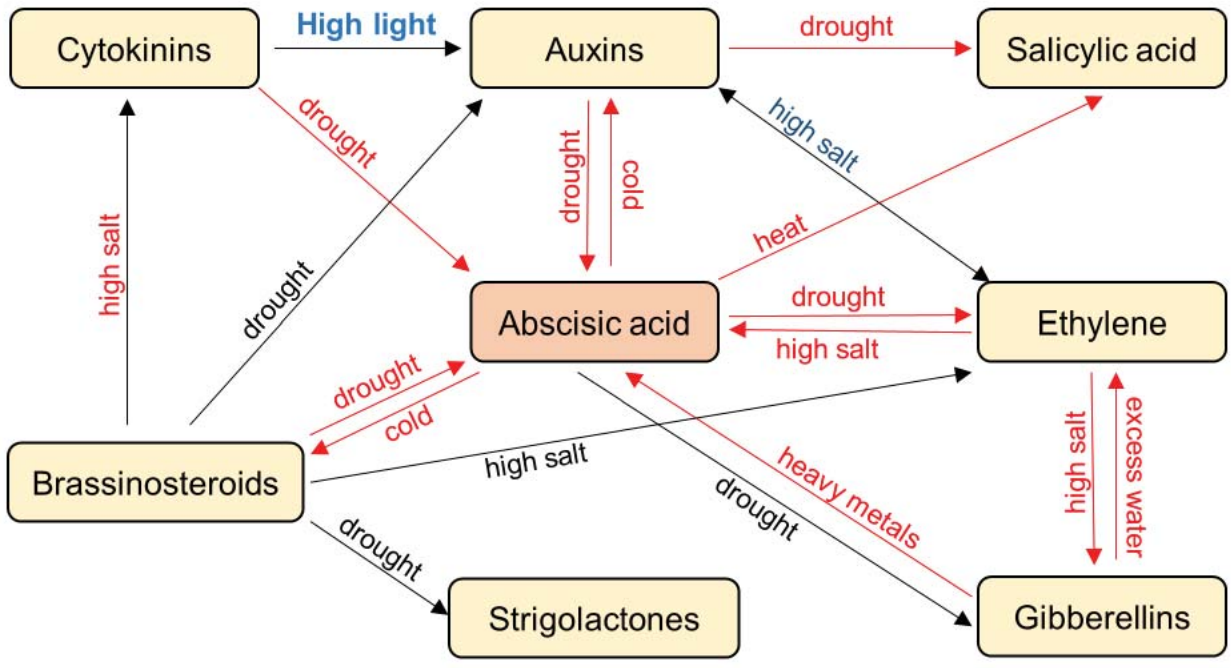


Fig. 6. **The phytohormones in the regulation of plant abiotic stress tolerance and crosstalk between phytohormone signaling, according to [24] with modifications and adaptations**

Phytohormonal biotechnology

To overcome stresses, plants form adaptive mechanisms that combine morphological, physiological, and biochemical responses, and set conditions for growth success and productivity [21]. Phytohormones play a leading role in modulating physiological and molecular responses that ensure plant survival under adverse conditions, and these responses include modifications of endogenous phytohormone levels. Plants respond synchronously to stressors by crosstalk between various hormonal signaling pathways. Changes in the content and ratio of phytohormones are among the first plant responses to stresses (Fig. 6).

According to the FAO (Food and Agriculture Organization of the United Nations), the world`s population will grow to 10 billion by 2050 [22]. Therefore, the main task of agricultural production will be to meet the food needs of the population, while avoiding a negative impact on the environment. To date, the degree of intensification of agricultural production has reached a critical point, resulting in irreversible global climate change. In the recent years, scientists have focused on the study of molecular mechanisms of hormones synthesis regulation, their signaling, and activity. Phytohormonal engineering is becoming increasingly important in the formation of stress tolerance, opens up new opportunities to increase yields and food supply. Phytohormones stimulate tolerance and help plants adapt to various stressors. Phytohormonal engineering is seen as an important promising approach to overcoming the negative influences caused by adverse external factors [23].

The progress in phytohormonal engineering and further implementation of this biotechnology requires an in-depth study of the plant endogenous hormonal system that invokes the introduction and further improvement of analytical methods for the study of hormones. Hormonal balance control will make it possible to increase ecologically the tolerance and yield of crops, in particular, such as wheat, corn, rice, and others.

The quantitation of plant hormones is a technically laborious procedure since they are contained in plant tissues in extremely low concentrations. This requires plant hormone isolation from a mixture of concomitant substances that is achieved by purification and subsequent chromatographic separation. Determination of hormones is preceded by the preparation of plant material for analysis, namely fixation sampling, extraction, purification from impurities, fractionation, and identification. The method of material fixation, methods, and degree of sample purification depend on the physicochemical properties of the phytohormone, the stability of its molecule, and the method of quantification.

Four chromatographic methods of separation of sample substance aliquots and their detection, as well as ionization conditions of analyte substances in a mass-selective detector, are described. Our methods for quantifying the content of endogenous phytohormones IAA, ABA, GA_3, SA, and five forms of cytokinins combine high-performance liquid chromatography with mass spectrometry. This is a sufficiently sensitive and suitable approach to determine the dynamic changes in hormone concentrations during plant growth and development. In-depth study of plant hormones, identification of sites of their synthesis and localization, determination of quantitative modulations under the action of stressors will promote the development of phytohormonal engineering — a new biotechnological approach to increase the tolerance and yield of crops.

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ВИЗНАЧЕННЯ ГОРМОНІВ У РОСЛИННИХ ТКАНИНАХ: ІСТОРІЯ, СУЧАСНІ ПІДХОДИ, ВИКОРИСТАННЯ У БІОТЕХНОЛОГІЇ

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В огляді проаналізовано й узагальнено відомості про історію розвитку та сучасний стан методичних підходів якісного та кількісного визначення фітогормонів у рослинних тканинах. Фітогормони відіграють ключову роль у регуляції фізіологічних процесів упродовж усього життєвого циклу рослин — від проростання насіння до старіння. У рослинних тканинах вони присутні в дуже низьких концентраціях (від 10^{-9} М до 10^{-6} М), тому розроблення швидких, високоефективних, комплексних і, водночас, надійних підходів із визначення фітогормонів є надзвичайно актуальним. У статті наведено коротку характеристику головних класів фітогормонів, стисло описано їхню функціональну активність. Подано обґрунтовану і перевірену послідовність процедур з екстракції рослинних гормонів, очищення їх від речовин, що інтерферують, методологію кількісного визначення індоліл-3-оцтової, абсцизової, гіберелінової ($ГК_3$), саліцилової кислот та п'яти форм цитокінінів, яка поєднує високоефективну рідинну хроматографію з мас-спектрометрією. У статті описано чотири хроматографічні методи аналізу, умови детектування фітогормонів різних класів та умови іонізації цих речовин у мас-спектрометрі. Обговорено важливість методів ідентифікації фітогормонів у рослинних тканинах для використання в біотехнологічних підходах, зокрема в метаболічній інженерії з метою формування стресостійкості в аграрних культурах.

Ключові слова: високоефективна рідинна хроматографія, мас-спектрометрія, фітогормони, біотехнологія.

ОПРЕДЕЛЕНИЕ ГОРМОНОВ В РАСТИТЕЛЬНЫХ ТКАНЯХ: ИСТОРИЯ, СОВРЕМЕННЫЕ ПОДХОДЫ, ИСПОЛЬЗОВАНИЕ В БИОТЕХНОЛОГИИ

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В обзоре проанализированы и обобщены сведения об истории развития и современном состоянии методических подходов качественного и количественного определения фитогормонов в растительных тканях. Фитогормоны играют ключевую роль в регуляции физиологических процессов на протяжении всего жизненного цикла растений — от прорастания семян до старения. В растительных тканях они присутствуют в очень низких концентрациях (от 10^{-9} М до 10^{-6} М), поэтому разработка быстрых, высокоэффективных, комплексных и, одновременно, надежных подходов к определению фитогормонов является чрезвычайно актуальной. В статье приведена краткая характеристика основных классов фитогормонов, описана их функциональная активность. Представлена обоснованная и проверенная последовательность процедур по экстракции растительных гормонов, их очистке от интерферирующих веществ, методология количественного определения индолил-3-уксусной, абсцизовой, гиббереллиновой ($ГК_3$), салициловой кислот и пяти форм цитокининов, которая сочетает высокоэффективную жидкостную хроматографию с масс-спектрометрией. В статье дано описание четырех методов хроматографического анализа. Определены условия детектирования фитогормонов различных классов и условия ионизации этих веществ в масс-спектрометре. Обсуждается важность методов идентификации фитогормонов в растительных тканях для использования в биотехнологии, например, в метаболической инженерии с целью формирования стрессоустойчивости у аграрных культур.

Ключевые слова: высокоэффективная жидкостная хроматография, масс-спектрометрия, фитогормоны, биотехнология.