IDENTIFICATION OF INTERVARIETAL AFFINITY OF *RUBUS IDAEUS* L. PLANTS ON BIOCHEMICAL PROFILES OF PHENOLIC COMPOUNDS

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The biochemical profiling of phenolic compounds in leaves of nine raspberry cultivars of Ukrainian selection is conducted. The localization of the optically active substances in the tissues of raspberry stems and specific of varieties features in the structure of the endoderm, the development of bast fibers and medullary rays are investigated. It is established that phenolic complexes of leaves before flowering exhibit close genetic links between similar origin raspberry cultivars under the same conditions of plant cultivation.

Keywords: *raspberry, cultivar, stem, leaves, phenolics, flavonoids, tannins, autofluorescence*

In recent years, a concept of the roles of phenolic compounds in the regulation of vital processes of plant organisms significantly expanded [2]. It is demonstrated that flavonoids are synthesized in cells in response to environmental signals, and then perform diverse functions [2, 7]. Recent reports explain an influence mechanisms of flavonoids on auxin transport. It is shown that high content of certain flavanones inhibits a basipetal movement of hormones, which is easily restored in conditions of increasing the of flavonols concentration [7, 9].High concentrations of naringenin flavanone slow down the development of the fibers in cotton tissues (*Gossypium hirsutuma* L.). The phenomenon of inhibition of stretching cells occurs against the oppression of the gene expression responsible for the synthesis of the enzyme flavonone-3-hydroxylase (F3H), which has naringenin as a substrate [9]. Phenolic acids and flavonoids play an important role in immune responses of plants to inhibition of pathogens and fungi [6]. For example, leaves and fruits of apple (*Malus domestica* Borkh), infected with the

pathogen (*Venturia inaequalis* (Cooke) G. Winter) have a higher content of chlorogenic acid and quercetin conjugates (rutin, quercitrin and isoquercitrin) In the leaves of raspberry (*Rubus idaeus* L.) terpenoids, glycosides of quercetin and kaempferol, anthocyanins (cyanidin-3-sophoroside, cyanidin-3-glycosyl rutinoside and cyanidin-3-glucoside) were found, as well as a significant amount of hardening agents and tannin which contain gallic and ellagic acid (ellagitannins, gallotannins) [3,6]. Ellagitannins and anthocyanins have strong antioxidant properties and protect tissues and organs of plants from the negative impact of internal and external factors. In raspberry fruits basic phenolic compounds are represented by ellagotannins and anthocyanins [4,5]. However, their content and qualitative composition have varietal differences and depend on seasonal variations and growing conditions of plants. Biochemical indicators of raspberry fruit genotypes are classified into three groups: with high content of flavonoids (anthocyanins and quercetin glycosides), high amounts of organic acids and low pool of flavonoids and plants with high content of soluble solids in fruits [5]. Considering the high level of functionality, and varietal specificity of the secondary metabolites of the genus *Rubus* L., **the aim of our research** was to conduct biochemical profiling of raspberry varieties of Ukrainian selection, examine the localization of certain optically active substances in the tissues of vegetative organs and identify potential links between the qualitative composition of phenolic complexes, plant genotypes and parent forms which were used in the selection of studied cultivars.

Materials and methods. The plant material for research was selected from ten varieties of raspberries from the collection of NUBiP Ukraine: Prumara, Kozachka, Halynka, Kosmichna, Osinnya, Brusvyana, Barabashka, Promin, Ulyublena Sycha, Vidbirna. Samples of leaves collected from plants at the beginning of blossom and extracted (1:10) with 80% MeOH. The extracts were stored in a freezer at a temperature -15-20°C.

The chromatographic profiling of the phenolic compounds' complex in raspberry leaves performed by thinlayer chromatography method on Kieselgel plates F254 (Merck, Germany) in a solvent system: ethyl acetate - formic acid -

acetic acid - water (v / v / v / v - 100: 11: 11: 26). The samples of leaves' methanol extracts of nine varieties of raspberries of 7 (mcl) were deposited at the start line. After drying, the chromatograms were treated with 5% EtOH solution of AlCl3, heated for 5 minutes at 105°C and examined in UV (365 nm). Spectrophotometric analysis of phenolic compounds was performed on a scanning spectrophotometer Optizen Pop (South Korea). Histological analysis of the stems, and the autofluorescence of cells and tissues studied with the fluorescence microscope AxioScope A -1 Carl Zeiss with five optical filters (420-470, 460-500, 505-555, 546-575, 640-690 nm). Photo documentation was made and morphometric parameters were analyzed in specialized programs Image Pro - Premier 9.1 AxioVision Carl Zeiss. Statistical processing of data, multiple correlation and cluster analysis was performed using Statistica 6.

Results. Based on our chromatographic profiling products in raspberry leaves of studied varieties hydrolyzed tannins - derivatives of gallic and ellagic acids, including their conjugates (wine, cinchona and shikimic with hydroxy-cinnamic acids), caffeic, p-cumaric and ferulic (depsides with the type of chlorogenic, isochlorogenic acid neochlorogenic acids); condensed tannins and catechins (proanthocyanidins); and flavonoids (quercetin and kaempferol glycosides mainly) were identified. In addition, as a part of plant material nor-, di- and triterpenoids, and sterols with their esters were presented. Value of biochemical components depend on the type, growth stage, impact on plant of abiogenic and biogenic stress factors. Relatively to these rates common condition of plant and quality indicators of seedballs were changed.

Secondary metabolites of raspberry leaf of studied varieties according to the qualitative composition separated in two main groups - phenols and terpenoids. In the initial stages of development in young leaves a significant amount of terpenoids was accumulated. Over time, their contents decreased against the background of the total pool of flavonoids and hydroxy-cinnamic acids increasing, including caffeic, p-cumaric and ferulic. It should be noted that in raspberry leaves there was not detected salicylic acid, but in Prumara and Halynka varieties its

methylated form was identified - methyl salicylate, which is due to the high lipophilicity easily overcomes the barrier of membrane organelles, cytosolic membrane and has alarm functions on intercellular level.

The analysis of stem tissue autofluorescence showed that a high concentration of hydroxy-cinnamic acids, including ferulic, which is characterized by intense luminescence in the blue spectrum and revealed in secondary cell walls of xylem elements at the beginning of tissues woodiness, and in cells of peripheral area in sclerenchyma fibers (Fig. 1). In raspberry plants distribution of optically active metabolites of vertical and radial transport in stems has some sort of difference. Topologically, in the primary cortex of the raspberry stem, exocortex, which created from several layers of slightly lignified obliterated cells, is appeared, mesocortex, which is represented with parenchyma cells and large endocortex, consisting of one, two or sometimes three layers of endoderm cells, which are located between the rows of cells with high starch content. According to morphology, endoderm cells have tabular shape, plain elongated, with tightly closed radial walls. This structure creates necessary conditions for the effective regulation of radial transport of substances between the primary cortex and stele. Endoderm cells glowed brightly in the range of 420-470 nm wavelengths.

The concentration of optically active components in the cell walls of tissue was spotted. The most intense autofluorescence specific to phenolcarbonic acid in the anticline (radial) and internal plain (transverse) walls was detected. Results of anatomical stems analysis of nine raspberry varieties showed that the most plastic elements in their structure is medullary rays and endoderm, the main differences are related to the morphology, total development of tissues and concentration in protoplasts, and cell walls of optically active compounds. In such way, endoderm of Osinnya raspberry variety was represented with three rows of tabular cells with lignified walls, and varieties Brusvyana and Osinnya - with one- or two-row. Cell walls autofluorescenced in intensely blue (420-470 nm) spectrum.

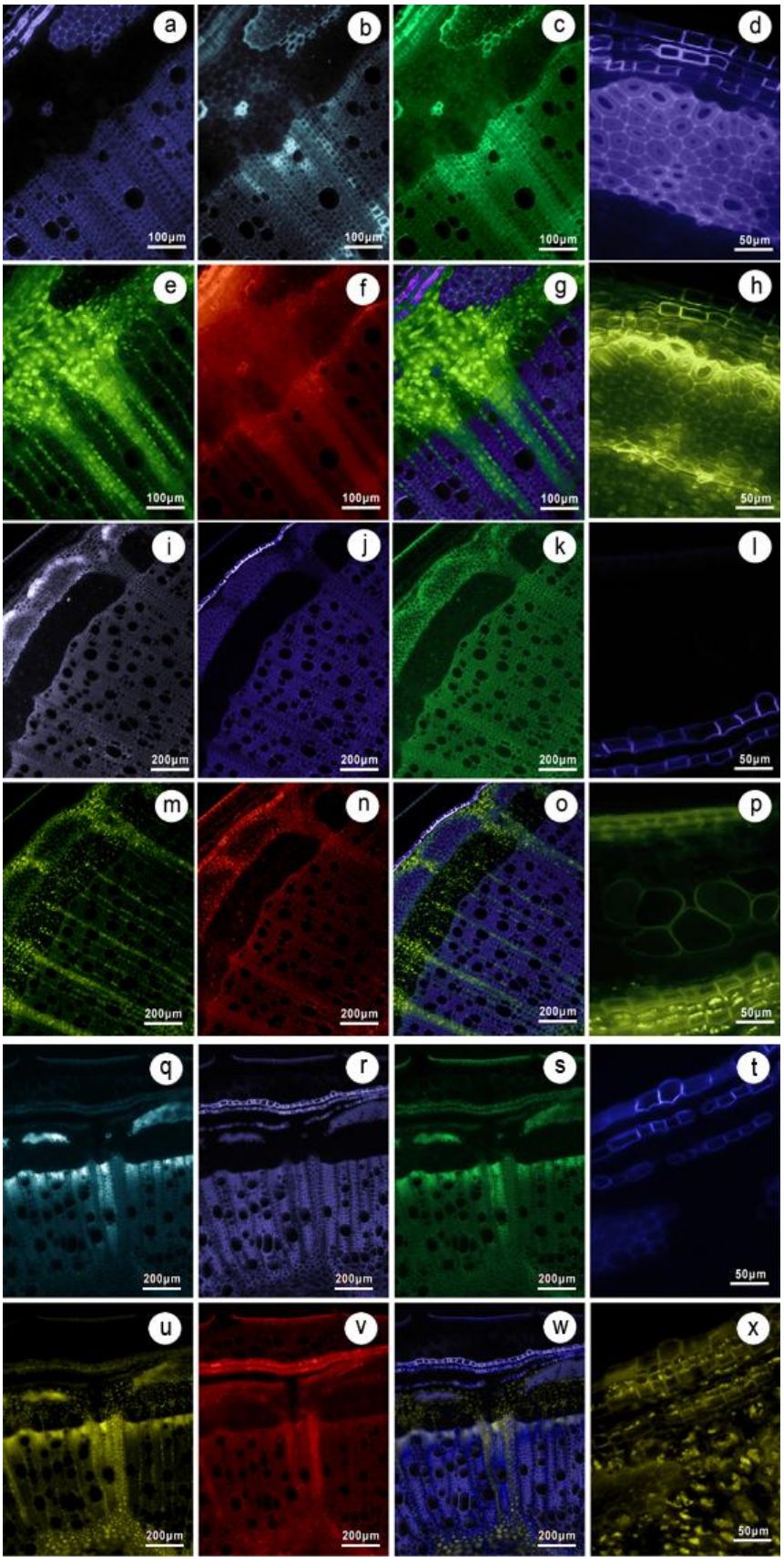

Fig. 1. Autofluorescence of secondary metabolites in stem tissues of raspberry varieties Osinnya (a-h), Halynka (i-p), Brusvyana (q-x): optical filters (a,

d, j, l, r, t) - 420-470 nm; (b, i, q) - 460-500 nm; (c, k, s) - 505-555 nm; (e, h, m, p, u, x) - 546-575 nm; (f, n, v) - 640-690 nm

The bright glow in the blue spectrum (480 nm), which is common for phenolcarbonic acids, observed in phloem fiber and xylem in the cambium area. Luminescence of phenolic compounds before and after treatment with chromogenic reagents simplified the possibility of fresh cuts identification.

In the thin-layer chromatogram the separation of biochemical components of phenolic compounds in nine raspberry varieties was showed (Fig. 2). From methanol leaf extracts 19 compounds were identified with Rf rates ranging from 0.05 to 0.98. Hydroxy-cinnamic and hydroxybenzoic acids showed intense luminescence in blue and light blue spectra (upper and middle part of the chromatogram). Tannins (lower part) on the contrary absorbed UV light and was easily observed through eclipse, and flavonoids had intense luminescence in orange, yellow, green and blue colors after processing of the plate with aluminum chloride.

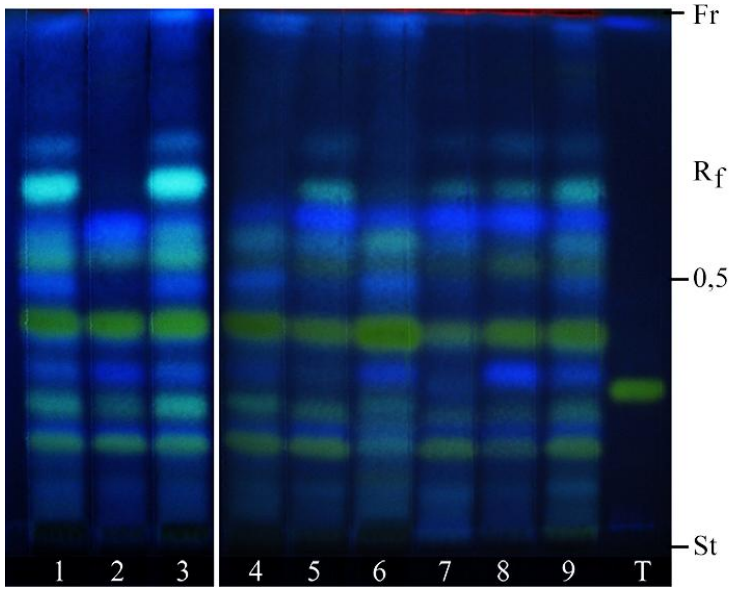

Fig. 2. Chromatogram of leaves flavonoids of different raspberry varieties: 1-Halynka; 2 - Prumara; 3 - Promin; 4 - Kosmichna; 5 - Vidbirna; 6 - Uliublena Sycha; 7 - Brusvyana; 8 - Kozachka; 9 - Barabashka; T - standards: rutin and salicylic acid

Important information about the phenolcarbonic acids and flavonoids content gave spectrophotometric analysis. Flavonoids characterized with absorption in UV and visible spectrum (210-600 nm). The absorption spectrum of flavonoids, usually, has two strips: the first (I) in the long-wave (320-380 or 490-540 nm for anthocyanidins), second (II) - in the short (210-290 nm) part of the spectrum. Position of the absorption strips is a characteristic feature of certain flavonoid groups. Flavanones and flavanonols differ from other flavonoids with position of strip II in the 270-290 nm region and presence of strip I in the form of a shoulder in 310-330 nm. Specific feature of flavones and flavonols was position of strip I in 320-355 nm and 340-385 (F). Spectrophotometry of isolated from raspberry leaves phenolic compounds showed that the spectral characteristics of secondary metabolites UV absorption divided into four groups: ellagic acid and ellagitannins (B); hydroxy-cinnamic acid (C), flavonoids (F) and (D) - condensed tannins, including proanthocyanidins (Fig. 3).

In these formed raspberry leaves synthesis and metabolism of terpenoids slowed and gradually switched to increase the pool of phenols, amount of which comparing to a period of leaves formation increased several times. However, for some varieties (Brusvyana, Kozachka) accumulation of phenols is not typical.

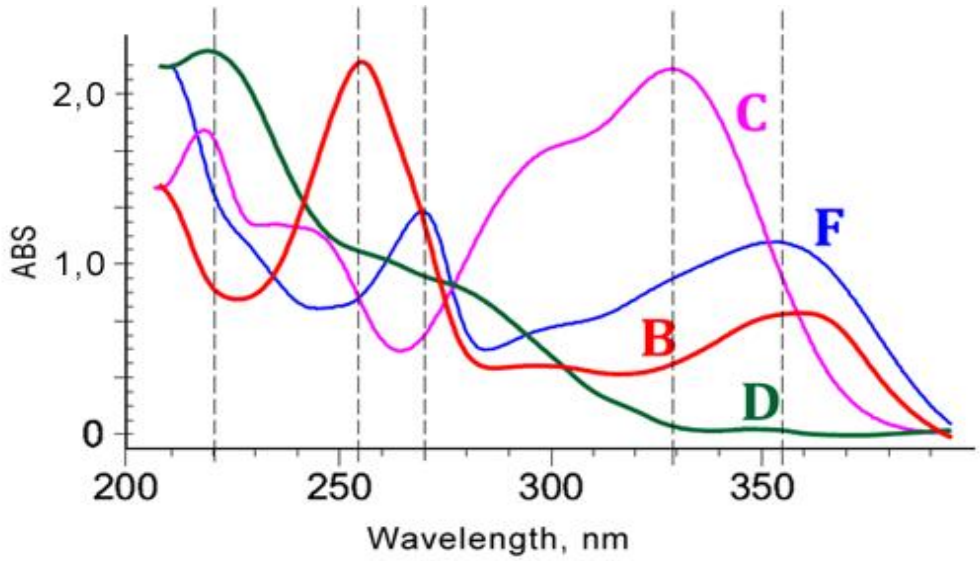

Fig. 3. Absorption spectra of phenolic compounds of methanol extracts in *Rubus idaeus* (wild form) leaves: B - ellagic acid and ellagitannins; C - hydroxy-cinnamic acid; F - flavonoids; D - condensed tannins.

As a result of biochemical profiling of raspberry leaves it appeared that some varieties of Ukrainian selection (breeders Sherenhoviy P.Z, Sherenhoviy V.P Andrusik Y.Y) can be identified by the presence or absence of certain secondary metabolites that acted as markers and may be related to their morphological and physiological characteristics. This assumption was confirmed by the results of cluster analysis and was based on the fundamental principle of varietal differences, presence in formation of isozyme complexes, responsible for phenylpropanoid and flavonoid blocks of phenolic compounds synthesis [2]. Obtained biochemical affinity dendrogram of raspberry varieties of Ukrainian selection consisted of two main clusters (Fig. 4). The first consisted of seven grades: Promin, Halynka, Barabashka, Kosmichna and Uliublena Sycha, Brusvyana and Kozachka (cluster Ia), the second one – Vidbirna and Prumara (Cluster Ib).

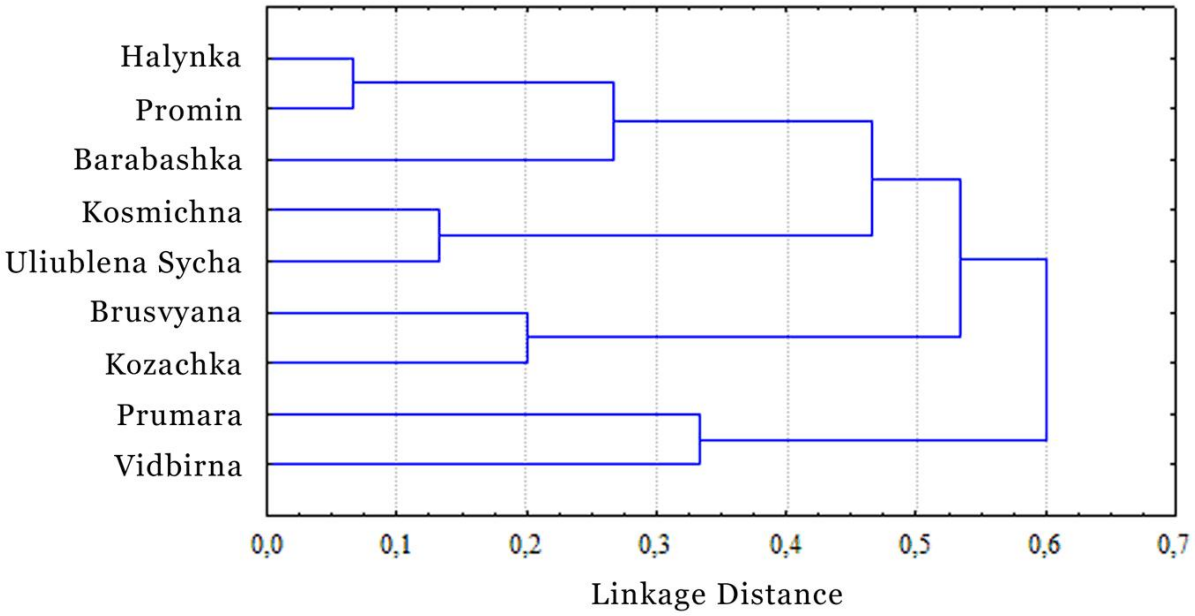

Fig. 4. Dendrogram of biochemical affinity of raspberry varieties, which is based on the qualitative composition of the phenolic compounds polymorphism in leaves

With the gradual reduction of the divergence level in the phenolic complexes, qualitative composition in leaves cluster Ia decomposed into two subclasters (IIa and IIb) in which varieties were consolidated into two groups. The first (IIIa) was represented with varieties - Promin, Halynka, Barabashka. According to the

divergence rate the most close were varieties Promin and Halynka (IP=0.06), created by crossing of varieties Novokytayivska, Sonce Kieva and Blagorodna (Fig. 5).

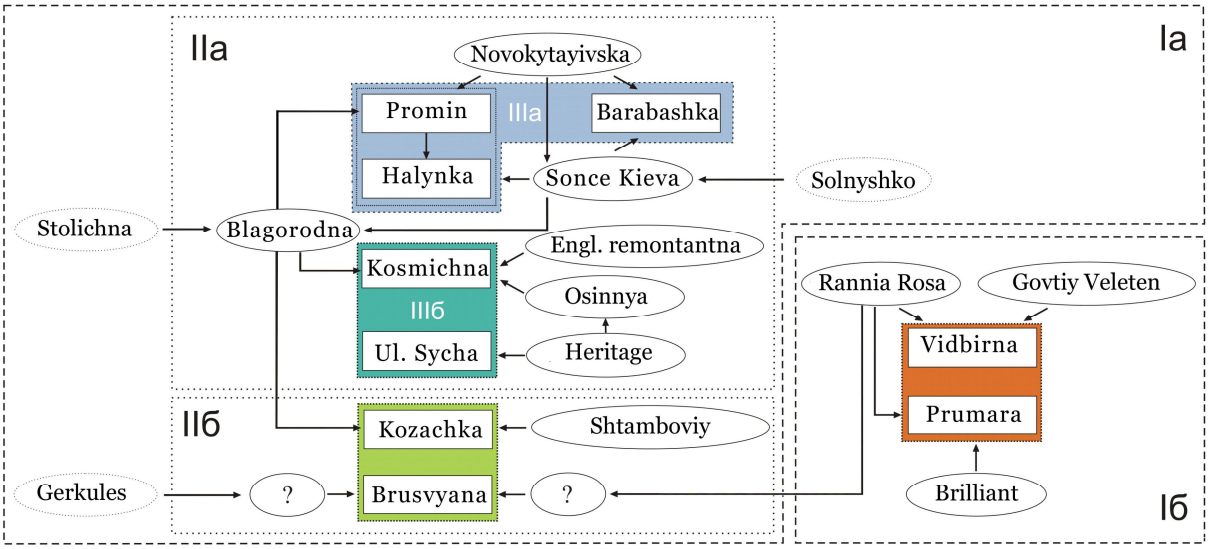

Fig. 5. Intervarietal links of raspberry plants of Ukrainian selection and their matching with clusters of biochemical affinity and with initial breeding material (ellipses encircled initial parental varieties)

Uliublena Sycha variety was obtained through the free pollination of raspberry seedlings of remontant variety Heritage. The composition of phenolic complexes were similar to varieties Kozachka (obtained by crossing of hybrids Blagorodna and Shtamboviy) and Brusvyana (data source about initial selection material unconfirmed). According to our data, these varieties accumulated in leaves the least amount of phenolic compounds and contained the lowest pool of salicylic acid. However, we found out that plants of Brusvyana raspberry variety on the stage of plant-regenerants adaptation, after in vitro culture, significantly increased the content of methylated form of salicylic acid - methyl salicylate in leaves. This compound is volatile, lipophilic and, therefore, easily overcame barriers and had cellular signaling function in the starting of a cascade mechanisms of the induced resistance formation in plant against pathogens. In addition, in vitro conditions in the plants Brusvyana variety significantly higher content of sterols and terpenoids, which are valued for pharmacological properties.

By the phenolic compounds complex of investigated raspberry varieties Vidbirna and Prumara (Cluster Ib) were distinguished in separated group. The first was obtained through the crossing of Rannia Rosa and Govtiy Veleten varieties, the second - Rannia Rosa and Brilliant varieties. These varieties accumulated in leaves significant number of hydroxy-cinnamic acids, flavonoids and proanthocyanidins.

Conclusions. 1. Biochemical profiling of raspberry varieties of Ukrainian selection showed that phenolic secondary metabolites, their qualitative and quantitative composition correlated with other biochemical and morphological, and physiological characteristics, assigned to the criteria for the varieties identification.

2. The facts of the close link between biochemical composition of phenolic compounds and phenotypic characteristics of plants confirmed the position about regulatory role of secondary metabolites in the formation of adaptive responses complex of plant to environment.

3. During identical growing conditions and active spring growth quality of phenolic compounds in raspberry leaves were high indicators which characterized the general condition of plants, their hereditary properties and origin varieties.

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ВИЗНАЧЕННЯ МІЖСОРТОВОЇ СПОРІДНЕНОСТІ РОСЛИН *RUBUS IDAEUS* L. ЗА БІОХІМІЧНИМИ ПРОФІЛЯМИ ФЕНОЛЬНИХ СПОЛУК

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Проведено біохімічне профілювання фенольних речовин листків дев'яти сортів малини української селекції. Показано особливості локалізації оптично активних сполук у тканинах стебел малини та виявлені сортоспецифічні особливості у будові ендодерми, розвиненості луб'яних волокон та серцевинних променів. Встановлено, що за однакових умов вирощування рослин фенольні комплекси листків до початку цвітіння

виявляють тісні генетичні зв'язки між близькими за походженням сортами малини.

Ключові слова: *малина, флавоноїди, фенольні речовини,таніни, стебло, листки, автофлуоресценція*

Определение межсортового родства растений *Rubus idaeus* L. по биохимическим профилям фенольных соединений

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Проведено биохимическое профилирования фенольных соединений листьев девяти сортов малины украинской селекции. Показаны особенности локализации оптически активных веществ в тканях стеблей малины и обнаружены сортоспецифические особенности в строении эндодермы, развитости лубяных волокон и сердцевинных лучей. Установлено, что при одинаковых условиях выращивания растений фенольные комплексы листьев до начала цветения обнаруживают тесные генетические связи между близкими по происхождению сортами малины.

Ключевые слова: *малина, сорт, стебель, листья, фенольные вещества, флавоноиды, таннины, автофлуоресценция*