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Influence of Quercetin-Ferrum Complex on the Biochemical Profile of Berry Crops In Vitro

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ABSTRACT

The purpose of the research was to study the effect of the quercetin-Ferrum complex on the synthesis of plastid pigments and secondary metabolites in berry crops. In the research, biotechnological, physiological, biochemical and statistical methods were used. The ability of quercetin, which is one of the most common flavonol aglycons, to create a chelated complex with Fe^{2+} and regulate physiological processes associated with oxidation-reduction reactions, synthesis of pigments and metal enzymes when being a supplement of nutrient medium is shown. With the addition of quercetin-Ferrum complex with a fraction of Fe^{2+} in a concentration equivalent to the base nutrient media composition optimized for the cultivation of berry crops *in vitro*, regenerated plants showed a sufficiently high regeneration capacity. Based on the indicators of the content of chlorophylls and carotenoids in leaves, the physiological availability of Ferrum in the quercetin-Ferrum complex was established. The concentration of chlorophylls *a* and *b* in raspberry and strawberry leaves increased by 20–25%, and the content of carotenoids increased by 30–40%. On the contrary, in black currant, the content of chlorophyll *a* in leaves decreased by 18–20% and chlorophyll *b* by almost 75%. It was found that quercetin is a biologically active phenolic chelating agent capable of chemically binding Fe^{2+} ions and participating in the regulation of growth processes, in particular in the induction of callusogenesis. Metal-flavonol complex is advisable to use in micro clonal reproduction of plants sensitive to oxidative stress in conditions of Fe^{2+} ions deficiency under the condition of individual selection of the components of the chelate complex and adjusting its concentration in the nutrient medium.

Keywords: quercetin, regenerated plants, pigments, phenolic compounds, berry crops.

INTRODUCTION

The content of flavonoids, which are products of the secondary metabolism in the majority of plant species, plays important role in the plant regulatory system. Flavonoids provide a wide range of physiological functions due to the presence of oxygen groups in the aromatic rings A and B, the degree of their glycosylation, location and nature of hydrocarbon residues, configuration of glycosidic bonds and nature of the bond of the glycosidic part with aglycon [Panche et al., 2016; Dias et al., 2021; Rothwell et al., 2017; Perez-Vizcaino and Fraga, 2018; Hayat, 2017]. Flavonoids are classified into the following subgroups: anthocyanins, proanthocyanidins, flavonols, flavones, flavanones, dihydroflavonols, chalcones, dihydrochalcones, isoflavonols, aurones, etc. [Amer, 2018]. They cause pigmentation of plants, participate in cell signaling processes and act as messengers of chemical signals [Oldroyd, 2013; Mathesius, 2018; Zhao et al., 2020].

Flavonoids are regulators of auxin transport. Those with o-hydroxyls in the nucleus B (quercetin, myricetin, luteolin) are synergists of auxins and stimulate plant growth, in particular, due to

the inhibition of IAA oxidase, while flavonoids with p-hydroxyls (apigenin, naringenin) are co-factors of IAA oxidase and act as antagonists of auxins [Li et al., 2020; Gayomba, 2017]. Flavonoids involved in plant defense mechanisms are often localized in tissue barriers, where they can play the role of signaling molecules or be directly involved in defense mechanisms [Albuquerque et al., 2021; Klyachenko et al., 2018].

The biological effect of plant flavonoids is associated with the regulation of oxidation-reduction processes, stabilization of cell membranes, and modulation of enzyme activity and receptors [Makarenko and Levitskij, 2013]. In the presence of oxidoreductases, namely polyphenol oxidases, flavonoids are oxidized by oxygen and converted into the corresponding quinones, which are reduced by hydrogen atoms of the respiratory substrate and turn into available forms by polyphenol oxidase. Thus, the 'flavonoid-polyphenol oxidase' system acts as a hydrogen carrier at the end stages of the respiratory process. Such a system allows the plant cell to oxidize many compounds (amino acids, ascorbic acid, cytochrome C, malic and citric acids, and polyphenols with a linear arrangement of oxygen groups) in a non-enzymatic way [Klessig et al., 2018; Heldt and Piechulla, 2021; Makarenko and Levitskij, 2013]. According to the analysis of the literature, the antioxidant properties of flavonoids are based on their ability to be 'traps' for free radicals, as well as to chelate metal ions involved in peroxide oxidation [Yin et al., 2014; Nguyen et al., 2022].

The aim of the research was to study the effect of the quercetin-Ferrum complex on the synthesis of plastid pigments and the secondary metabolites in berry crops.

MATERIALS AND METHODS

The research was carried out in the years 2018–2020 at the Laboratory of Biotechnology and Cell Engineering of the National University of Life and Environmental Sciences of Ukraine.

Aseptic cultures of raspberry variety 'Brusviana', black currant variety 'Raduzhna' and garden strawberry variety 'Alina' were used in the study. Regenerated plants of garden strawberry, currant and raspberry were cultivated in the Murashige-Scoog medium, supplemented with 0.1 g/L of mesoinosite, 0.5 mg/L of BAP, 1.0 mg/L of IAA and 0.25 mg/L of quercetin. The plant material was cultivated according to the generally accepted method in a growth chamber at a temperature of +25±1˚C, lighting 2000–3000 Lux, 16-hour photoperiod and relative humidity of 75% [Melnychuk and Klyachenko, 2014].

Determination of photosynthetic pigments

The content of chlorophylls a, b and carotenoids in the leaves of regenerated plants (mg/g of raw substance) was determined in methanol extracts, which were prepared at a ratio of 1:10 (leaves: methanol, g/g). Optical density was measured at a wavelength of 662 and 664 nm (chlorophyll *a* and chlorophyll *b*), and 440 nm (carotenoids) using Optizen POP spectrophotometer (South Korea). Methanol served as the control [Petchsomrit et al., 2023].

Determination of the total content of phenolic compounds

The determination of total phenolic compounds was carried out by the Folin-Chikolteu method modified by Singleton and Rossi [Ahmad et al., 2015]. 50 µL of the leaf methane extract was diluted with 450 µL of methanol and then 2.5 mL of Folin-Chicolteu reagent and 2.0 mL of 7% sodium carbonate were added. The solution was then incubated at room temperature for two hours. Measurements were performed using the spectrophotometer at a wavelength of 765 nm.

Determination of the antioxidant activity of phenolic compounds

The antioxidant activity of phenolic compounds was determined by the modified Blois method [Brand-Williams, 1995]. A stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used to determine the antioxidant activity of plant extracts. 400 µL of 0.2 mM DPPH solution and 350 µL of methanol were added to 50 µL of plant extract. After 30 min, absorbance was measured at a wavelength of 517 nm and converted to a percentage of antioxidant activity (AA) by the following formula:

$$AA(\%) = \\ = 100 - \left[\frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}}\right] \quad (1)$$

Determination of the content of flavonoids

The content of flavonoids in the plant material was determined by the spectrophotometric method at λ = 419 nm. 300 μL of the extract was supplemented with 200 μL of 0.1M aluminum chloride solution ($AlCl_3$) and 300 μL of 1M sodium acetate 73(CH_3COONa). The calibration graph was built using quercetin (Sigma, Germany).

Statistical data processing was carried out with the use of the software for PC Statistica 6.0.

RESULTS AND DISCUSSION

The ability of one of the most common flavonol aglycon – quercetin – to create a chelated complex with Fe^{2+} and regulate physiological processes associated with the oxidation-reduction reactions, synthesis of pigments and metal enzymes when being added to the nutrient medium was tested.

Quercetin-Ferrum complex (with a fraction of Fe^{2+} in a concentration equivalent to the base media) added to the nutrient media optimized for berry crops caused sufficiently high regenerative capacity. Moreover, the leaf blades developed without visual anomalies and deformations. According to the external morphological features, the vegetative organs of plants cultivated in media supplemented with quercetin-Ferrum complex did not differ from those grown with the addition of a standard chelate. An increase in the concentration of metal-flavonol in nutrient media led to intensive formation of non-morphogenic callus in regenerated plants of raspberry and garden strawberry on the basal parts of the stems. Over time the callus was filled with oxidation products, and the external cells darkened and gradually died. Thus, it can be stated that quercetin and its chelated form at concentrations above 25 mg per 100 mL of medium create favourable conditions for callusogenesis. This effect may be associated with blocking the outflow of endogenous auxins through the system of live tissues into the nutrient medium [Guo et al., 2007; Kejík et al., 2021. The accumulation of auxins in the cells of the basal part of stem activates the proliferation of cortical cells and cambium. Intensive cell division in the absence of their differentiation leads to the formation of calluses [Singh et al., 2021]. It was found that the intensity of callus formation in regenerated plants of raspberry linearly increased with the increase in the concentration of quercetin-Ferrum complex from 25 to 75 mg per 100 mL of nutrient medium.

The physiological availability of Fe^{2+} for plant tissues during the period of active regeneration of organs is confirmed by sufficiently high activity of peroxidase, which includes metal and a balanced pigment complex. At the same time, the content of chlorophylls in the leaves of the regenerated plants of *Rubus idaeus* L.*, Ribes nigrum* L. and *Fragaria ananassa* Duch. was relatively leveled (Figure 1 a-c).

Electronic spectra of methanol extracts of raspberry, black currant and garden strawberry leaves in terms of absorbance maxima were relatively stable (Table 1). Quantitative indicators of the content of chlorophylls and carotenoids in leaves indicate the physiological availability of Ferrum in the quercetin-Ferrum complex. The concentration of chlorophylls *a* and *b* in raspberry leaves increased by 20−25% and the content of carotenoids increased by 30−40%. A similar tendency of chlorophyll *a* accumulation in the leaves of garden strawberry was found. The content of chlorophyll *b* in the studied plants decreased almost twice, while the total pool of carotenoids remained unchanged.

On the contrary, in black currant plants, the content of chlorophyll *a* in leaves decreased by 18−20%, and chlorophyll *b* by almost 75%. This can be explained by the features of the secondary metabolism of black currant: raspberry plants are characterized by a significant content and variability of the flavonoid complex. Unlike raspberry, black currant plants with relatively low flavonoid concentrations contain significant amounts of catechins, leukoanthocyanidins and chlorogenic acid conjugates [Jarret et al., 2018]. Thus, the enzymatic system of raspberry is better balanced for quercitin transformation and glucosylation with the formation of a wide range of diverse glucosides. Therefore, when using flavonol-metal chelates, it is worth considering the specifics of the secondary metabolism of a crop, which is confirmed by the data of phytochemical analysis of the phenolic complex of the berry crops we studied. By Da Silva's et al. (2020) studies it was revealed that the quercetin-Fe complex presented the best antioxidant and antiacetylcholinesterase actions [Da Silva et al., 2020]. In raspberry leaves *in vitro*, the total

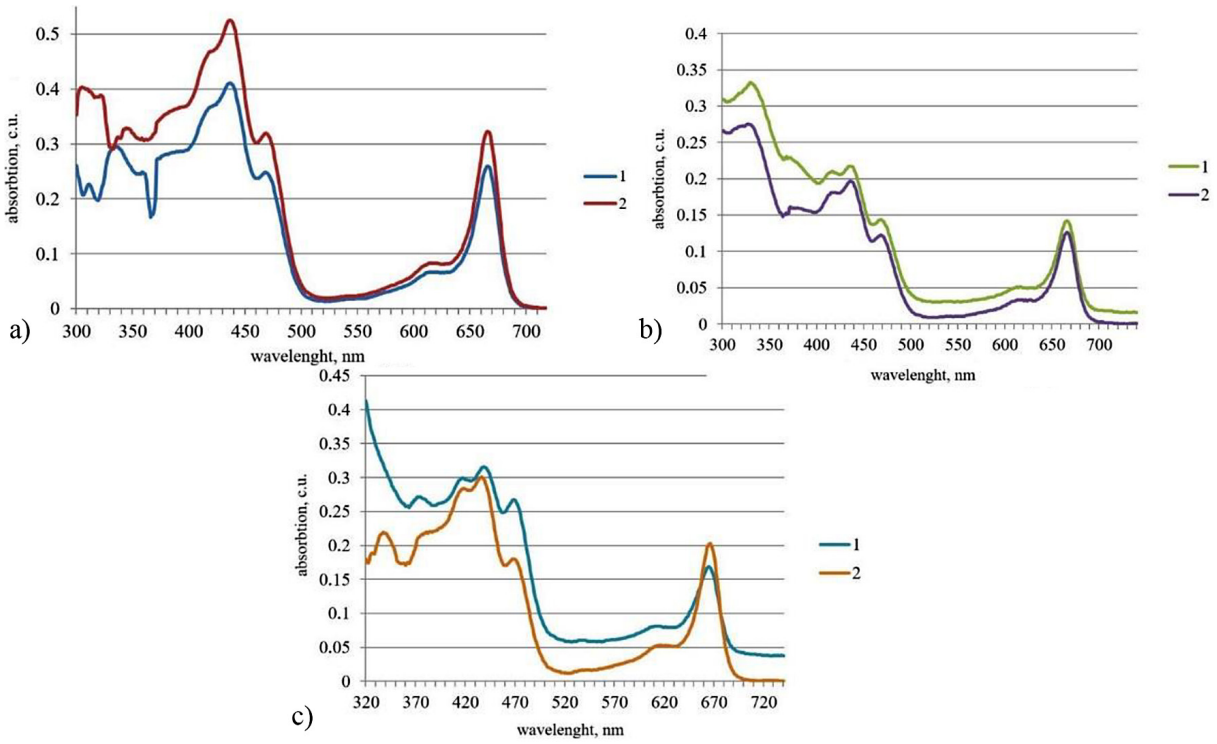

Figure 1. Absorbance spectra of a) *Rubus idaeus* L., b) *Ribes nigrum* L. and c) *Fragaria ananassa* Duch. leaf extracts; the plants cultivated *in vitro* in nutrient media with quercetin (concentration 25 µg/100 mL); 1 – control, 2 – quercetin

content of phenols and, in particular, flavonoids and catechins compared to control plants was sufficiently aligned. The content of phenols and flavonoids in the treatment with a quercetin-Ferrum complex was slightly higher (Table 2).

The data given in Table 2 show that in black currant plants, the total pool of phenols and catechins increased, although the content of flavonoids remained quite stable. In garden strawberry, the content of flavonoids slightly increased but the differences did not exceed 10%. Quercetin, rutin and (+) catechin exhibited strong antioxidant properties toward Fe which were found by Cherrak et al. (2016). Noticeable is the general antioxidant potential of plants. In the study, this indicator significantly decreased in strawberry plants, while in black currant and raspberry it almost did not change. A significant decrease in the antioxidant potential against the background of a sufficiently leveled content of phenolic compounds in the experiment and control can be explained by the qualitative ratio of phenolic compounds with different antioxidant potentials and substances of a non-phenolic nature capable of neutralizing free radicals.

Table 1. Influence of quercetin-Ferrum complex on the content and ratio of plastid pigments in leaves of berry crops (M±m; n=4)

Crop	Medium modifier	Chlorophyll (mg/g)				Car (mg/g)	$\frac{Chl\ a+b}{Car}$
		a	b	a+b	a/b		
Rubus idaeus	C	2.3 ± 0.07	0.9 ± 0.03	3.2 ± 0.09	2.7	1.0 ± 0.05	3.04
	Q	2.9 ± 0.09	1.1 ± 0.04	4.1 ± 0.08	2.7	1.4 ± 0.07	2.88
Ribes nigrum	C	1.3 ± 0.04	0.7 ± 0.02	2.0 ± 0.06	1.7	0.5 ± 0.02	4.30
	Q	1.1 ± 0.03	0.4 ± 0.01	1.5 ± 0.05	2.7	0.5 ± 0.03	3.15
Fragaria ananassa	C	1.5 ± 0.06	1.3 ± 0.04	2.8 ± 0.08	1.2	0.7 ± 0.04	3.91
	Q	1.8 ± 0.05	0.6 ± 0.02	2.4 ± 0.07	2.9	0.7 ± 0.05	3.31

Note: C – control; Q – quercetin; Car – carotenoids.

Table 2. Effect of quercetin on the content and ratio of the secondary metabolites in leaves of berry crops (M±m; n=4)

Crop	Medium modifier	Phenols (mg/g)	Flavonoids (mg/g)	Phenols / flavonoids	Catechins (mg/g)	Antioxidant activity (mg/g)
Rubus idaeus	C	4.1 ± 0.12	1.8 ± 0.09	2.29	1.1 ± 0.05	4.4 ± 0.05
	Q	5.2 ± 0.16	1.9 ± 0.07	2.71	1.5 ± 0.08	4.5 ± 0.09
Ribes nigrum	C	5.4 ± 0.11	1.0 ± 0.05	5.32	6.1 ± 0.10	7.7 ± 0.08
	Q	5.6 ± 0.17	1.0 ± 0.04	5.57	4.1 ± 0.08	9.1 ± 0.09
Fragaria ananassa	C	10.7 ± 0.11	1.1±0,05	9.80	1.2 ± 0.06	21.0 ± 0.19
	Q	9.2 ± 0.10	1.2 ± 0.06	7.42	1.1 ± 0.04	12.9 ± 0.13

Note: C – control; Q – quercetin.

CONCLUSION

It was determined that quercetin is a biologically active phenolic chelator capable of chemically binding Fe^{2+} ions. It participates in the regulation of growth processes, in particular in the induction of callusogenesis. Metal-flavonol complex is advisable to use in micro clonal propagation of plants sensitive to oxidative stress in conditions of Fe^{2+} ions deficiency under the condition of the individual selection of the components of the chelate complex and adjusting concentration in the nutrient medium.

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