



# **Knjiga sažetaka**

*Book of Abstracts*

## **Simpozij HDBB**

*HDBB Symposium*

**1. prosinca 2023. / December 1<sup>st</sup> 2023**

**Osijek, Hrvatska / Croatia**



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## **Simpozij HDBB / *HDBB Symposium***

### **Izdavač/*Publisher:***

Hrvatsko društvo za biljnu biologiju, Rooseveltov trg 6, 10 000 Zagreb,  
Hrvatska / *Croatian Society of Plant Biologists, Rooseveltov trg 6, 10 000  
Zagreb, Croatia*

**Učestalost izlaženja/*Publication frequency:*** godišnje / yearly

### **Urednice/*Edited by:***

Selma Mlinarić, Lidija Kalinić, Martina Šrajer Gajdošik

**Naslovnica/*Cover design by:*** Selma Mlinarić

**Fotografija/*Photo:*** *Centaurea jacea* L.

ISSN 3043-7334 (Online)

Zagreb, 2024.

### **Organizatori/Organizers:**

Hrvatsko društvo za biljnu biologiju, Zagreb / *Croatian Society of Plant Biologists, Zagreb*

Odjel za biologiju, Sveučilište Josipa Jurja Strossmayera u Osijeku, Osijek / *Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek*



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## Fitotoksični odgovor nanočestica i iona bakrovog oksida u mikroalgi *Chlorella vulgaris*

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**Ključne riječi:** *Chlorella vulgaris*, CuO, Cu<sub>2</sub>O, nanočestice, oksidacijski stres

**Sažetak:** Nanočestice bakra se sve više koriste u raznim industrijama, a kako bi se istražili učinci nanočestica CuO i Cu<sub>2</sub>O te iona bakra na algu *Chlorella vulgaris*, stanice su tretirane koncentracijama koje omogućuju 75% preživljenja stanica nakon 72 sata. Radi utvrđivanja fitotoksičnosti nanočestica bakrovih oksida, analizirana je razina reaktivnih kisikovih vrsta (ROS), količina bakra na i u stanicama, razina lipidne peroksidacije, oštećenja proteina i molekule DNA te ultrastruktura stanica, dok se antioksidacijski potencijal analizirao mjerenjem aktivnosti antioksidacijskih enzima i sadržajem neenzimskih antioksidansa te razinom ekspresije proteina Hsp70 i Hsp90. Rezultati pokazuju da se nanočestice bakrovih oksida nakupljaju na površini te da ulaze u stanice. Svi tretmani su uzrokovali povećanje sadržaja ROS-a, što je dovelo do povećanog oštećenja lipida i molekule DNA. S druge strane, jedino je CuO tretman rezultirao značajnim oštećenjem proteina, ali i jedinom povećanjem aktivnosti antioksidacijskog enzima, superoksid dismutaze. Dok niti jedan tretman nije povećao sadržaj prolina ili promijenio ekspresiju Hsp70 proteina, svi su rezultirali povećanjem razine GSH, ali i smanjenjem ekspresije Hsp90 proteina. Zaključno, sve nanočestice bakrovog oksida i ionski bakar su narušili integritet alge *C. vulgaris* induciranjem oksidativnog stresa dok se smanjenje aktivnosti antioksidativnog mehanizma može objasniti prekomjernom proizvodnjom ROS-a, što može promijeniti strukturu enzima.



## Phytotoxic responses of different copper oxide nanoparticles and ions in *Chlorella vulgaris*

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**Keywords:** *Chlorella vulgaris*, CuO, Cu<sub>2</sub>O, nanoparticles, oxidative stress

**Abstract:** To study the effects of CuO and Cu<sub>2</sub>O nanoparticles and copper ions on the alga *Chlorella vulgaris*, cells were treated with concentrations that enable 75% cell survival after 72 hours. To determine the phytotoxicity of copper oxide nanoparticles, the level of reactive oxygen species (ROS), the amount of copper on and in the cells, the degree of lipid peroxidation, the damage to proteins and DNA molecules and the ultrastructure of the cells were analyzed, while the antioxidant potential was investigated by measuring the activity of antioxidant enzymes and the level of non-enzymatic antioxidants as well as the expression level of Hsp70 and Hsp90 proteins. The results show that copper oxide nanoparticles accumulate on the surface and penetrate into the cells. All treatments increased the ROS content, leading to increased damage to lipids and DNA molecules. In contrast, only the CuO treatment led to significant protein damage, but also to the only increase in the activity of the antioxidant enzyme superoxide dismutase. While none of the treatments increased the proline content or altered the expression of the Hsp70 protein, all treatments led to an increase in GSH content and a decrease in the expression of the Hsp90 protein. In conclusion, all copper oxide nanoparticles and ionic copper damaged the integrity of *C. vulgaris* algae by inducing oxidative stress, while the decrease in the activity of the antioxidant mechanism can be explained by the excessive production of ROS, which can alter the enzyme structure.

## Toksičnost nanočestica srebra s omotačima u mikroalgi *Chlorella vulgaris*

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**Ključne riječi:** površinski omotači, oksidativni stres, slatkovodne alge

### Sažetak:

Nanočestice srebra (AgNP) su od velikog interesa zbog svojih antimikrobnih svojstava, ali njihova reaktivnost i toksičnost predstavljaju značajan rizik za vodene ekosustave. U biološkim sustavima AgNP su sklone oksidaciji i agregaciji, zbog čega se često stabiliziraju sredstvima koja utječu na njihova fizikalno-kemijska svojstva. U ovoj studiji, mikroalga *Chlorella vulgaris* korištena je kao modelni organizam za procjenu učinaka AgNP u vodenim staništima. Alge su bile izložene AgNP koje su stabilizirane s dva omotača: citratom i cetiltrimetilamonijevim bromidom (CTAB) te s AgNO<sub>3</sub> u koncentracijama koje su omogućile preživljenje stanica od 75% nakon 72 sata. Akumulacija srebra, otopljeni organski ugljik, sadržaj ROS-a, oštećenje biomolekula, aktivnost antioksidacijskih enzima, sadržaj neenzimskih antioksidansa i promjene u ultrastrukturi analizirani su kako bi se istražio odgovor algi. Rezultati su pokazali da su svi tretmani izazvali oksidacijski stres i nepovoljno utjecali na stanice algi. AgNO<sub>3</sub> rezultirao je najbržom smrću algi u usporedbi s oba AgNP-a, ali je opseg oksidacijskog oštećenja i antioksidacijske enzimske obrane bio sličan AgNP-citratu. Uz to, AgNP-CTAB pokazao je najmanje toksični učinak i uzrokovao najmanje oksidacijsko oštećenje. Ovi rezultati naglašavaju važnost površinskih omotača u određivanju fitotoksičnosti AgNP i temeljnih mehanizama koji utječu na vodene organizme.

## The toxicity of coated silver nanoparticles in the microalga *Chlorella vulgaris*

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**Keywords:** surface coatings, oxidative stress, freshwater algae

### Abstract:

Silver nanoparticles (AgNPs) are of great interest due to their antimicrobial properties, but their reactivity and toxicity pose a significant risk to aquatic ecosystems. In biological systems, AgNPs are prone to oxidation and aggregation, which is why they are often stabilized by agents that affect their physicochemical properties. In this study, the microalgae *Chlorella vulgaris* was used as a model organism to evaluate the effects of AgNPs in aquatic habitats. The algae were exposed to AgNPs stabilized with two coatings: citrate and cetyltrimethylammonium bromide (CTAB) and to AgNO<sub>3</sub> at concentrations that allowed 75% cell survival after 72 hours. Silver accumulation, dissolved organic carbon, ROS content, damage to biomolecules, activity of antioxidant enzymes, content of non-enzymatic antioxidants and changes in ultrastructure were analyzed to investigate the response of the algae. The results showed that all treatments induced oxidative stress and adversely affected the algal cells. AgNO<sub>3</sub> resulted in the fastest death of algae compared to both AgNPs, but the extent of oxidative damage and antioxidant enzymatic defense was similar to AgNP-citrate. In addition, AgNP-CTAB showed the least toxic effect and caused the least oxidative damage. These results emphasize the importance of surface coatings in determining the phytotoxicity of AgNPs and the underlying mechanisms affecting aquatic organisms.

## Profiliranje ekspresije proteina iz biljaka duhana nakon tretmana nanočesticama srebra s različitim omotačima i ionima srebra

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**Ključne riječi:** *Nicotiana tabacum*, L. nanočestice srebra, fitotoksičnost, stabilizirajući omotači, ekspresija proteina

### Sažetak:

Nanočestice srebra (AgNP) imaju veliku primjenu u potrošačkim proizvodima te predstavljaju rastući ekološki problem. Zbog sklonosti agregaciji i disocijaciji, dodaju im se stabilizirajući omotači koji mogu modulirati njihov toksični učinak. U ovom radu istražen je utjecaj AgNP stabiliziranih polivinilpirolidonom (PVP) i cetiltrimetilamonijev bromidom (CTAB) na promjene u ekspresiji proteina korijena i lista duhana. Usporedna analiza uključila je i izlaganje istoj koncentraciji AgNO<sub>3</sub>. *In vitro* uzgojene biljke duhana tretirane su 7 dana u tekućoj podlozi s dodatkom 100 μM AgNP ili AgNO<sub>3</sub>, sa ili bez 500 μM cisteina, snažnog liganda iona Ag<sup>+</sup>. Ukupni topivi proteini razdvojeni su dvodimenzionalnom elektroforezom, nakon čega su proteinske mrlje, koje su u tretmanima pokazale razlike u odnosu na kontrolu, analizirane spektrometrijom masa. Promjene u ekspresiji zabilježene su za ukupno 34 proteina korijena i 20 proteina lista u tretiranim u odnosu na kontrolne biljke duhana. Najveći broj diferencijalno eksprimiranih proteina korijena bio je uključen u odgovor na stres, dok su u listu to bili proteini fotosinteze. Sljedeći po zastupljenosti u oba organa bili su proteini uključeni u procese transkripcije i translacije. Dodatak cisteina svim tretmanima doveo je većinu različito eksprimiranih proteina na razinu ekspresije u kontroli, što pokazuje da fitotoksičnost AgNP barem djelomično potječe od disociranih iona Ag<sup>+</sup>.



## Protein expression profiling of tobacco plants upon treatment with differently coated silver nanoparticles and silver ions

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**Keywords:** *Nicotiana tabacum*, L. silver nanoparticles, phytotoxicity, stabilizing coatings, protein expression

### Abstract:

Growing environmental concerns arise from the increasing usage of silver nanoparticles (AgNPs) in consumer products. Since AgNPs readily aggregate and dissociate, stabilizing coatings are added that may modulate their toxic effects. In this work, the effect of AgNP stabilized with polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB) on changes in protein expression of tobacco roots and leaves was investigated. Comparative analysis included exposure to AgNO<sub>3</sub> at the same concentration. *In vitro* grown tobacco plants were treated for seven days in liquid medium supplemented with 100 μM AgNP or AgNO<sub>3</sub>, with or without the addition of 500 μM cysteine, a strong ligand of Ag<sup>+</sup> ions. Total soluble proteins were separated by two-dimensional electrophoresis, after which, protein spots that showed differences in treatments compared to control were analyzed by mass spectrometry. Changes in expression were recorded for a total of 34 root proteins and 20 leaf proteins in treated compared to control tobacco plants. The largest number of differentially expressed proteins in root were involved in stress response, whereas in leaf it was proteins involved in photosynthesis. Next in terms of representation in both organs were proteins involved in transcription and translation processes. The addition of cysteine to all treatments equalized the expression level with that of control for most of the detected proteins, indicating that the phytotoxicity of AgNPs at least partially derives from dissociated Ag<sup>+</sup> ions.

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## Učinak mikroplastike na pojavu oksidacijskog stresa u biljkama

**Biljana Balen<sup>1\*</sup>, Karla Košpić<sup>1</sup>, Renata Biba<sup>1,2</sup>, Luka Kobelščak<sup>1</sup>, Bruno Komazec<sup>1</sup>, Petra Cvjetko<sup>1</sup>, Mirta Tkalec<sup>1</sup>**

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**Ključne riječi:** *Allium cepa*, *Lemna minor*, ROS, antioksidacijski enzimi

### Sažetak:

Zagađenje plastikom jedan je od najvećih ekoloških problema svjetskih razmjera zbog brzo rastuće proizvodnje jednokratnih proizvoda od plastike, njihovog usitnjavanja i dugog zadržavanja u okolišu, što utječe na žive organizme, uključujući i biljke. U ovom istraživanju korjenčići luka (*Allium cepa*) izloženi su djelovanju komercijalnih mikročestica polistirena (PS-MPs) i polimetil metakrilata (PMMA-MPs) koncentracija 0,01, 0,1 i 1 g L<sup>-1</sup>, dok su biljke vodene leće (*Lemna minor*) tretirane istim mikročesticama koncentracija 0,01, 0,05 i 0,1 g L<sup>-1</sup>, nakon čega su u obje vrste spektrofotometrijski analizirani parametri oksidacijskog stresa i aktivnost antioksidacijskih enzima. Povećani sadržaj vodikovog peroksida (H<sub>2</sub>O<sub>2</sub>) te malondialdehida i proteinskih karbonila, pokazatelja oksidacijskog stresa, izmjereni su u korjenčićima luka nakon tretmana s PS-MPs, dok je u vodenoj leći jači učinak zabilježen nakon izlaganja česticama PMMA-MPs. Značajno povećana aktivnost enzima za uklanjanje H<sub>2</sub>O<sub>2</sub>, askorbat i pirogalol peroksidaze, izmjerena je nakon izlaganja PS-MPs u oba modelna organizma, dok je PMMA-MPs doveo do povećanja vrijednosti samo u luku. Dobiveni rezultati pokazuju da oba tipa mikroplastike izazivaju pojavu oksidacijskog stresa i aktivaciju antioksidacijskih enzima, ali je specifični učinak ovisan o vrsti biljke.

## Microplastics effects on the occurrence of oxidative stress in plants

**Biljana Balen<sup>1\*</sup>, Karla Košpić<sup>1</sup>, Renata Biba<sup>1,2</sup>, Luka Kobelščak<sup>1</sup>, Bruno Komazec<sup>1</sup>, Petra Cvjetko<sup>1</sup>, Mirta Tkalec<sup>1</sup>**

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**Keywords:** *Allium cepa*, *Lemna minor*, ROS, antioxidant enzymes

### Abstract:

Plastic pollution has become one of the most pressing environmental issues due to rapidly increasing production of disposable plastic products, their fragmentation into smaller pieces, and long persistence in the environment, which affects all living organisms, including plants. In this study, onion (*Allium cepa*) roots were exposed to commercial microparticles of polystyrene (PS-MPs) and polymethyl methacrylate (PMMA-MPs) at concentrations of 0.01, 0.1 and 1 g L<sup>-1</sup>, while duckweed (*Lemna minor*) plants were treated with the same microparticles at concentrations of 0.01, 0.05 and 0.1 g L<sup>-1</sup>, after which oxidative stress parameters and antioxidant enzymes activity were spectrophotometrically analyzed in both species. Increased levels of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as well as malondialdehyde and protein carbonyls, oxidative stress indicators, were measured in onion roots after treatment with PS-MPs, while a stronger effect was observed in the duckweed after exposure to PMMA-MPs. A significantly increased activity of H<sub>2</sub>O<sub>2</sub> removal enzymes, ascorbate and pyrogallol peroxidase, was measured after exposure to PS-MPs in both model organisms, while PMMA-MPs resulted in increased levels only in the onion. The results obtained show that both types of microplastics induce oxidative stress and activation of antioxidant enzymes, but the specific effect is dependent on the plant species.

## Selen u vodenom okolišu - primjer Drave, Dunava i povezanih vodenih biotopa

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**Ključne riječi:** voda, sediment, plankton, makrofite, poplavno područje

### Sažetak:

Istraživanje distribucije selena (Se) u vodi, sedimentu, planktonu i makrofitama provedeno je na šest odabranih postaja na Dravi, Dunavu, u poplavnom jezeru i melioracijskim kanalima tijekom sušnog i poplavnog razdoblja u 2016. godini. Rezultati su ukazali na nedostatak Se u sedimentu i vodi, a na fizikalno-kemijske uvjete, uključujući distribuciju Se, primarno su utjecale hidrološke promjene, ali i biogeokemijske i morfološke specifičnosti pojedinog biotopa. Više koncentracije Se u vodi utvrđene su u uvjetima plavljenja, a u sedimentu u sušnom razdoblju (osim u rijekama). Iako su bile niže od graničnih ekotoksičnih vrijednosti, koncentracije Se su se povećavale prema sljedećem nizu: voda (0,021-0,187  $\mu\text{g Se L}^{-1}$ ) < sediment (0,005-0,352  $\text{mg Se kg}^{-1}$ ) < makrofite (0,010-0,413  $\text{mg Se kg}^{-1}$ ) < plankton (0,044-0,518  $\text{mg Se kg}^{-1}$ ) što upućuje na moguću biomagnifikaciju pri dnu hranidbenog lanca. Planktonske i makrofitske zajednice bile su karakterizirane vrstama s visokim potencijalom za akumulaciju Se te predstavljaju dobre pokazatelje za praćenje prisutnosti Se u vodenim biotopima.



## Selenium in the freshwater environment - A study of the Drava, Danube and associated aquatic biotopes

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**Keywords:** water, sediment, plankton, macrophytes, floodplain

### Abstract:

The research focused on selenium (Se) distribution among different aquatic compartments (water, sediment, plankton and macrophytes) at six selected sites of the Drava and Danube rivers, the floodplain lake and the melioration channels in two sampling periods (flooding and drought) in 2016. The study results revealed Se deficiency in sediments and water, while the physicochemical environment, including Se distribution, was primarily influenced by hydrology rather than site-specific biogeochemical and morphological features. The flooding period was characterized by higher Se content in water compared to drought conditions, while the opposite was found for the sediments (except for the river sites). Although below the threshold for aquatic ecotoxicity, Se concentrations increased in the following order: water (0.021-0.187  $\mu\text{g Se L}^{-1}$ ) < sediments (0.005-0.352  $\text{mg Se kg}^{-1}$ ) < macrophytes (0.010-0.413  $\text{mg Se kg}^{-1}$ ) < plankton (0.044-0.518  $\text{mg Se kg}^{-1}$ ) indicating possible biomagnification of Se at the bottom of the food chain. Plankton and macrophyte communities were characterized by species with high Se accumulation potential, which can be a more suitable steady-state compartment for Se assessment in freshwater biotopes.

## Sekundarni metaboliti lišajeva kao pokazatelji kvalitete zraka

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**Ključne riječi:** onečišćenje zraka, Slavonski Brod, HPLC

### Sažetak:

Problem onečišćenja zraka je od rastućeg značaja u cijelom svijetu, s utjecajem na biološku raznolikost i ljudsko zdravlje. Lišajevi se često koriste kao bioindikatori jer ove zajednice organizama reagiraju na kompleksne utjecaje kvalitete zraka, ali i mikroklimatskih uvjeta. Veliki broj različitih sekundarnih metabolita koje lišajevi sintetiziraju većinom su specifični isključivo za lišajeve, a uz mnogostruke biološke aktivnosti, pripisuju im se i uloga u toleranciji na onečišćenje. U okviru većeg istraživanja u kojem su lišajevi korišteni kao bioindikatori kvalitete zraka, postavljeno je 20 ploha s dvije vrste lišajeva, *Flavoparmelia caperata* i *Evernia prunastri*, u radijusu od 20 km od rafinerije nafte preko puta Slavenskog Broda, koja predstavlja veliki izvor onečišćenja zraka. Uzorci su prikupljeni na različitim udaljenostima od rafinerije, u dvije vremenske točke, nakon tri te šest mjeseci izlaganja. Sekundarni metaboliti su analizirani pomoću tekućinske kromatografije visoke djelotvornosti – HPLC. Identificirano je i kvantificirano šest različitih metabolita, međutim rezultati nisu pokazali jasnu vezu niti s duljinom izlaganja niti s udaljenošću od rafinerije. Kako bi se dodatno istražila veza onečišćenja zraka i metabolizma lišajeva, potrebno je modelirati dobivene rezultate u prostoru, odnosno sagledati odnos s podacima o vjetru za šire područje Slavenskog Broda, te usporediti s drugim fiziološkim promjenama.

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## Lichen's secondary metabolites as indicators of air quality

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**Keywords:** air pollution, Slavonski Brod, HPLC

### Abstract:

The problem of air pollution is of increasing importance throughout the world, with an impact on biodiversity and human health. Lichens are often used as bioindicators because these communities of organisms react to the complex effects of air quality, but also microclimate conditions.

Many different secondary metabolites synthesized by lichens are mostly specific only for this group of organisms, and in addition to multiple biological activities, they are also attributed to a role in pollution tolerance. As part of a larger study in which lichens were used as bioindicators of air quality, 20 plots with two lichen species, *Flavoparmelia caperata* and *Evernia prunastri*, were placed within a radius of 20 km from the oil refinery across from Slavonski Brod, which is a major source of air pollution. The samples were collected at different distances from the refinery, at two time points, after three and six months of exposure. Secondary metabolites were analysed using high performance liquid chromatography - HPLC. Six different metabolites were identified and quantified, however, the results did not show a clear relationship with either the length of exposure or the distance from the refinery. In order to further investigate the connection between air pollution and lichen metabolism, it is necessary to model the obtained results in space, that is, to look at the relationship with wind data for the wider area of Slavonski Brod, and to compare it with other physiological changes.

## Divlji kupus (*Brassica incana*): eksperimentalni model za istraživanje mehanizama tolerancije na abiotički stres

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**Ključne riječi:** abiotički stres, *Brassica incana*, divlji kupus, mehanizmi tolerancije

### Sažetak:

Kupusnjače (*Brassica* spp.) spadaju među najčešće konzumirano povrće zbog visoke nutritivne vrijednosti i obilja spojeva s pozitivnim učinkom na ljudsko zdravlje (polifenoli, glukozinolati, karotenoidi itd.). Klimatske promjene imaju značajan utjecaj na biološku raznolikost i poljoprivrednu proizvodnju diljem svijeta, negativno utječući na rast i razvoj biljaka, a posljedično i na prinos i kvalitetu usjeva. Suša, visoke temperature i povećana slanost tla glavni su čimbenici abiotičkog stresa za mnoge usjeve, uključujući kupusnjače, osobito u Sredozemlju. Divlji predstavnici kupusnjača koji prirodno rastu u ekstremnim uvjetima okoliša mogu poslužiti kao izvrsne modelne biljke i potencijalni genski fond za uzgoj kupusnjača prilagođenih, često, nepovoljnim okolišnim uvjetima. Kako bi se istražili mehanizmi otpornosti na abiotički stres u kupusnjača, šest populacija divljih srodnika (*B. incana*) identificiranih na jadranskim otocima (Kosor, Obljak, Sušac, Stupe) bilo je izloženo suši, visokoj temperaturi, povećanom salinitetu i kombiniranim stresovima. U osjetljivim ekopopulacijama faktori stresa rezultirali su jačim odgovorom i značajno većom promjenom izmjerenih molekularnih markera nego u rezistentnim ekopopulacijama. Preliminarni rezultati su diskutirani u odnosu na prijašnje rezultate dobivene na raštici, bijelom kupusu i kineskom kupusu.

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## Wild cabbage (*Brassica incana*): an experimental model to investigate mechanisms of tolerance to abiotic stress

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**Keywords:** abiotic stress, *Brassica incana*, wild cabbage, tolerance mechanisms

### Abstract:

Brassica crops (*Brassica* spp.) are among the most commonly consumed vegetables due to their high nutritional value and abundance in various valuable health-promoting compounds (polyphenols, glucosinolates, carotenoids etc.). Climate change has a significant impact on biodiversity and agricultural production worldwide, negatively affecting plants' growth and development and, consequently, crop yield and quality. Drought, heat and increased soil salinity are major abiotic stressors for many crops, including brassicas, particularly in the Mediterranean. Wild representatives growing naturally at extreme environmental conditions may serve as excellent model plants and potential gene pools for breeding strategies of brassica crops adapted to, often, unpleasant environments. To explore the mechanisms of abiotic stress tolerance in brassicas six populations of wild relatives (*B. incana*) naturally growing at Adriatic islands (Kosor, Obljak, Sušac, Stupe) were exposed to drought, heat, increased salinity and combined stresses. In the sensitive ecopopulations, the applied stress factors resulted in a stronger response and a significantly greater change in the measured molecular markers than in the resistant ecopopulations. Preliminary results are discussed with previous data obtained with brassica crops such as kale, white cabbage and Chinese cabbage.

## Od klijanja do cvatnje – molekularno-biokemijski odgovori rajčice na povišenu temperaturu

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**Ključne riječi:** biljni hormoni, *de novo* DNA metilacija, ekspresija gena, rast korijena, toplinski stres

### Sažetak:

Rajčice imaju važno mjesto u poljoprivrednoj proizvodnji te smo stoga istražili kako podnose povišene temperature. Ispitali smo klijavost sjemena rajčice pri povišenim temperaturama i utjecaj produljene izloženosti klijanaca i odraslih biljaka temperaturi od 37 °C ili toplinskom valu od 45 °C. Mjerena je akumulacija prolina, malondialdehida, biljnih hormona IAA, ABA i prekursora etilena 1-aminociklopropan-1-karboksilne kiseline (ACC). Kako bi se rasvijetlio odgovor na toplinski stres, pratili smo ekspresiju gena za transkripcijske faktore HSF, DREB i NAC te gena važnih za *de novo* DNA metilaciju metodom qPCR. Izloženost temperaturama 37 °C ili 45 °C različito je utjecala na rast korijena klijanaca i izazvala je značajno nakupljanje IAA te smanjila razinu ABA. Za razliku od tretmana na 45 °C, izlaganje temperaturi 37 °C povećalo je nakupljanje ACC, koji bi mogao biti uključen u promjenu rasta korijena. Općenito, drastičnije fenotipske promjene (kloroza i venuće lišća te savijanje stabljike) pronađene su i kod klijanaca i kod odraslih biljaka nakon izlaganja toplinskom valu. To se također odrazilo na nakupljanje prolina, malondialdehida i proteina toplinskog šoka HSP90. Genska ekspresija transkripcijskih faktora povezanih s toplinskim stresom bila je poremećena i pokazalo se da je DREB1 najkonzistentniji marker toplinskog stresa. Toplinski stres je značajno inducirao ekspresiju gena *RDM1\_480* koji bi mogao imati ulogu u *de novo* metilaciji DNA.

## From germination to flowering - molecular and biochemical response of tomato to elevated temperature

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**Keywords:** *de novo* DNA methylation, gene expression, heat stress, plant hormones, root growth

### Abstract:

Tomatoes are one of the most important vegetables for human consumption. We investigated tomato seed germination at elevated temperatures and the impact of prolonged exposure to 37 °C and heat waves at 45 °C on seedlings and adult plants. Accumulation of proline, malondialdehyde, plant hormones IAA, ABA and ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were measured. To shed light on heat stress-induced plant response, the expression of transcription factor genes HSF, DREB and NAC, and genes important for *de novo* DNA methylation was quantified by qPCR. Exposure to 37 °C or 45 °C affected root growth differently, induced significant accumulation of IAA, and reduced ABA levels. As opposed to the heat wave treatment, exposure to 37 °C increased the accumulation of ACC, which may have been involved in the root architecture modification of seedlings. Generally, more drastic phenotypic changes (chlorosis and wilting of leaves and bending of stems) were found in both seedlings and adult plants after the heat wave-like treatment. This was also reflected by proline, malondialdehyde and heat shock protein HSP90 accumulation. The gene expression of heat stress-related transcription factors was perturbed, and DREB1 was shown to be the most consistent heat stress marker. Heat stress significantly induced the *RDM1\_480* gene which may have function in *de novo* DNA methylation.

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## Metabolizam ugljikohidrata u odgovoru pšenice na biotički stres uzrokovan fuzarijskom paleži klasa

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**Ključne riječi:** *Fusarium* spp, glikoliza, metabolizam saharoze, metabolizam škroba, *Triticum aestivum*

### Sažetak:

Fitopatogene gljive roda *Fusarium* uzročnici su fuzarijske paleži klasa (FHB), jedne od najraširenijih gljivičnih bolesti pšenice na našim prostorima. Pri interakciji biljke i patogena dolazi do promjena u metabolizmu biljke-domaćina što rezultira povećanjem energije i proizvodnje spojeva koji su izvor ugljika. Osim što je glavni transportni oblik ugljikohidrata u biljaka, saharoza kao i njene sastavne komponente fruktoza i glukoza, su važne signalne molekule uključene u signalne puteve koji dovode do aktivacije obrambenog odgovora biljke. Cilj ovog istraživanja bio je odrediti ulogu metabolizma ugljikohidrata u obrambenom odgovoru pšenice na FHB. Istraživanje je provedeno na listu zastavičaru šest genotipova ozime pšenice inokulirane vrstama *Fusarium culmorum* i *F. graminearum*, pri čemu su analizirani enzimi uključeni u glikolizu, metabolizam škroba, metabolizam saharoze i pentoza-fosfatni put. Inokulacija klasa pšenice gljivama roda *Fusarium* uzrokovala je promjene metabolizma ugljikohidrata u listu zastavičaru, koje su bile sortno-specifične. Tako su genotipovi koji su opisani kao otporniji na FHB, 'Vulkan' i 'Galopper', imali povećane aktivnosti većine istraživanih enzima, dok su osjetljiviji genotipovi, 'Golubica' i 'Tika Taka', imali smanjene aktivnosti enzima glikolitičkog puta i metabolizma saharoze. Rezultati istraživanja upućuju na važnu ulogu metabolizma ugljikohidrata u obrambenom odgovoru pšenice na vrste roda *Fusarium*, te će doprinijeti selekciji i razvoju otpornijih genotipova pšenice.



## Carbohydrate metabolism in wheat response to biotic stress caused by fusarium head blight

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**Keywords:** *Fusarium* spp., glycolysis, starch metabolism, sucrose metabolism, *Triticum aestivum*

### Abstract:

Phytopathogenic fungi of the *Fusarium* genus are the causative agents of fusarium head blight (FHB), one of the most widespread fungal diseases of wheat in our region. During plant-pathogen interactions, the host plant's metabolism is modified, resulting in energy increase and the production of carbon-source compounds. In addition to being the main transport form of carbohydrates in plants, sucrose and its constituent components, fructose and glucose, are important signalling molecules involved in signalling pathways that activate the plant's defence response. This research aimed to determine the role of carbohydrate metabolism in wheat defence response to FHB. The study was conducted on flag leaves of six winter wheat genotypes inoculated with *Fusarium culmorum* and *F. graminearum*, where the enzymes of glycolysis, starch metabolism, sucrose metabolism as well as of pentose-phosphate pathways were analysed. Inoculation of wheat spikes with *Fusarium* species caused a modification of carbohydrate metabolism in the flag leaves, and the changes were genotype-specific. Thus, the genotypes characterised as FHB resistant, 'Vulkan' and 'Galopper', had increased activities of most metabolic enzymes analysed, while the more susceptible genotypes, 'Golubica' and 'Tika Taka', showed decreased activities of glycolysis and sucrose metabolism enzymes. The research results indicate the important role of carbohydrate metabolism in the wheat defence response to *Fusarium* species and will contribute to the selection and development of more resistant wheat genotypes.

## Interakcija proteina TROL i FNR uključena je u procese redoks homeostaze kod biljke *Arabidopsis thaliana* (L.) Heynh.

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**Ključne riječi:** fotosinteza, linearni tok elektrona, ROS, askorbat peroksidaza, glutation

### Sažetak:

Protein TROL (Thylakoid RhOdanese-Like) integralni je protein fotosinteskih membrana većine vaskularnih biljaka. TROL sa svojim C-terminalnim dijelom tranzijentno interagira s flavoenzimom feredoksin:NADP<sup>+</sup> oksidoreduktaza (FNR) te omogućuje efikasan prijenos elektrona s reduciranog feredoksina (Fd) na molekulu NADP<sup>+</sup>. U odsustvu interakcije TROL-FNR elektroni se s fotosustava I (PSI) vrlo efikasno prenose na alternativne primatelje te se time sprečava propagacija ROS-ova i posljedično oštećivanje fotosintetskog aparata. Korištenjem tandemskog afinitetnog pročišćavanja i spektroskopije masa utvrdili smo da TROL vjerojatno stvara i komplekse s tilakoidnom formom enzima askorbat peroksidaze (APX). Elektronskom mikroskopijom ultratankih prereza listova imunokemijski obilježenih s koloidnim zlatom vezanim za antitijelo na glutation, utvrdili smo da mutante biljke *Arabidopsis thaliana* (L.) Heynh. koje ne posjeduju TROL (KO, *knock-out* linija) i one koje ga prekomjerno nakupljaju (OX, *over-expression* linija) imaju, u usporedbi s divljim-tipom, znatno izmijenjen sadržaj glutationa u različitim staničnim odjeljcima. Nadalje, elektronskom paramagnetnom rezonancijom utvrdili smo da za razliku od divljeg-tipa i KO linije, OX linija nakuplja značajno više ROS-a. Zaključujemo da je interakcija TROL-FNR uključena u procese održavanja redoks homeostaze ne samo u kloroplastima već i čitavim biljkama.

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H D B B

## TROL-FNR interaction is integrated into processes of plant redox homeostasis in *Arabidopsis thaliana* (L.) Heynh.

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**Keywords:** photosynthesis, linear electron flow, ROS, ascorbate peroxidase, glutathione

### Abstract:

TROL (Thylakoid Rhodanese-Like) protein is an integral membrane protein found in photosynthetic membranes of many vascular plants. TROL with its C-terminal domain interacts with the flavoenzyme ferredoxin:NADP<sup>+</sup> oxidoreductase and enables efficient transfer of photosynthetic electrons from the reduced ferredoxin (Fd) to NADP<sup>+</sup>. In the absence of this interaction electrons generated by the photosystem I (PSI) flow very rapidly towards alternative acceptors and therewith prevent the propagation of ROS, and the consequent damage to the photosynthetic apparatus. By using tandem affinity purification followed by the MS analysis, we have determined that TROL very likely also forms complexes with the thylakoid form of the enzyme ascorbate peroxidase (tAPX). Electron microscopy of the ultrathin leaf sections immunodecorated with the gold conjugated antibody against glutathione, revealed that the mutants of *Arabidopsis thaliana* (L.) Heynh., which do not accumulate TROL (KO, *knock-out* line), and those that over-accumulate it (OX, *over-expression* line) have drastically altered glutathione content in various cellular compartments in comparison to the wild-type. Further, by using electron paramagnetic resonance, we have determined that, in contrast to the wild-type and the KO line, the OX line accumulates significantly more ROS. We conclude that the TROL-FNR interaction is not only integrated into processes of chloroplast redox homeostasis, but also in the redox homeostasis of the entire plant. \*

H D B B

## Uloga disulfidne veze u biljnoj citosolnoj seril-tRNA-sintetazi

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**Ključne riječi:** seril-tRNA-sintetaza, translacija, disulfidna veza, oksidacijski stres

### Sažetak:

Seril-tRNA sintetaza (SerRS) esencijalni je enzim koji kovalentno povezuje serin i tRNA<sup>Ser</sup>, pripremajući tako supstrat za ribosom tijekom translacije. U kristalnoj strukturi citosolne SerRS iz biljke *Arabidopsis thaliana* uočena je disulfidna veza u svakom monomeru između cisteina Cys213 i Cys244, što je bilo neočekivano s obzirom da, općenito, citosolni proteini nemaju disulfidne veze zbog redukcijske prirode citosola. Cisteini uključeni u disulfidnu vezu evolucijski su očuvani u svim citosolnim SerRS iz zelenih biljaka, uključujući jednostanične zelene alge, što ukazuje na njihovu biološku važnost. Dizajnirane su mutirane inačice proteina (C213S, C244S i C213S/C244S) te je uspoređena njihova toplinska stabilnost, aktivnost i struktura s proteinom divljeg tipa. Uočeno je da su oba cisteina važna za stabilnost SerRS, ali sa suprotnim učincima. Identificirane su strukturne značajke proteinskog mikrokoliša, koje utječu na reaktivnost Cys213 i Cys244 te potiču stvaranje disulfidne veze u oksidacijskim uvjetima. U prisutnosti oksidirajućeg reagensa H<sub>2</sub>O<sub>2</sub>, aminoacilacijska aktivnost proteina divljeg tipa nije bila smanjena, dok je aktivnost mutiranih inačica bila ozbiljno narušena. Rezultati pokazuju da je disulfidna veza u biljnoj SerRS važna za otpornost proteina na oksidaciju, što može biti korisno tijekom translacije u stanju oksidacijskog stresa u biljkama.

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H D B B

## The role of disulfide bond in the plant cytosolic seryl-tRNA synthetase

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**Keywords:** seryl-tRNA synthetase; translation, disulfide bond; oxidative stress

### Abstract:

Seryl-tRNA synthetase (SerRS) is an essential enzyme that covalently links serine and tRNA<sup>Ser</sup>, thus preparing substrate for ribosome during translation. The crystal structure of *Arabidopsis thaliana* cytosolic SerRS revealed a disulfide bond in each monomer involving cysteines Cys213 and Cys244, which was unexpected considering that, in general, cytosolic proteins do not have disulfide bonds due to the reductive nature of cytosol. Cysteines involved in the disulfide bond are evolutionarily conserved in all cytosolic SerRSs from green plants, including unicellular green algae, implying their biological importance. We have designed mutant protein variants (C213S, C244S and C213S/C244S) and compared their thermal stability, activity, and structure with the wild-type protein. Both cysteines are important for SerRS stability, but with opposite effects. We have identified structural features of the protein microenvironment, which influence the reactivity of Cys213 and Cys244 and promote the formation of the disulfide link in the oxidizing conditions. In the presence of the oxidizing reagent H<sub>2</sub>O<sub>2</sub>, the aminoacylation activity of the wild-type protein was not abolished, while the activity of mutants was severely impaired. Results indicate that disulfide bond in plant SerRS is important for protein resistance to oxidation, which may be beneficial during translation in oxidative stress conditions in plants.

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## Biofortifikacija zrna soje selenom utječe na fiziološke odgovore klijanaca na osmotski stres

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**Ključne riječi:** biljna fiziologija, tretmani suše, funkcionalna hrana

### Sažetak:

Klimatske promjene ugrožavaju poljoprivrednu proizvodnju, posebice jer uzrokuju nedostatak vode u tlu. Ovo istraživanje proučava fiziološke odgovore klijanaca soje biofortificiranih selenom na osmotski stres primjenom polietilen glikola (PEG). U komori za rast uzgajana su biofortificirana zrna dvije sorte soje („Lucija“ i „Sonja“) iz 2020. godine te podvrgnute dvama tretmanima (kontrola zalijevana vodom i tretman PEG-2,5 %). Rezultati su pokazali različit sadržaj selena i različite fiziološke odgovore u dvije istraživane sorte, pri čemu je u sorti „Lucija“ pri tretmanu PEG-2,5 % uočeno ublažavanje štetnih učinaka stresa, što ukazuje na antioksidacijski potencijal selena. Nasuprot tome, u sorti „Sonja“ primijećeni su negativni učinci osmotskog stresa, kao što je povećanje lipidne peroksidacije, unatoč biofortifikaciji selenom koji u ovoj sorti djeluje kao prooksidans. Međutim, aktivacija antioksidacijskih puteva u ovoj sorti može povećati vrijednost klijanaca soje kao funkcionalne hrane. Zaključno, različite sorte soje pokazuju različite fiziološke reakcije na osmotski stres i selen, ističući složenu interakciju između okolišnih stresora i biljnih odgovora.

## Selenium biofortification of soybean seeds influences physiological responses of seedlings to osmotic stress

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**Keywords:** plant physiology, drought treatments, functional food

### Abstract:

Climate change threatens agricultural production, especially through soil water deficits. This study investigates the physiological responses of selenium-biofortified soybean seedlings biofortified with selenium to osmotic stress using polyethylene glycol (PEG). In the growth chamber, biofortified grains of two soybean cultivars ("Lucija" and "Sonja") from 2020. biofortification program were grown and subjected to two treatments (watered control and PEG-2.5% treatment). The results showed different content of selenium and different physiological responses in the two investigated varieties, where in "Lucija" cultivar, when treated with PEG-2.5%, mitigation of the harmful effects of stress was observed, which indicates the antioxidant potential of selenium. In contrast, negative effects of osmotic stress, such as an increase in lipid peroxidation, were observed in the cultivar "Sonja", despite biofortification with selenium, which in this variety acts as a prooxidant. However, the activation of antioxidant pathways in this variety can increase the value of soybean seedlings as a functional food. Overall, different soybean cultivars show diverse physiological responses to osmotic stress and selenium, underscoring the intricate interplay between environmental stress and plant responses.

## Učinak biofortifikacije selenom na fotosintetsku učinkovitost mikrozelenja

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**Ključne riječi:** fotosustav II, protok energije, maksimalni kvantni prinos, Brassicaceae

### Sažetak:

Biofortifikacija mikrozelenja selenom je obećavajuća, isplativa i održiva metoda unosa selena za konzumente koji imaju ograničen pristup raznolikoj prehrani. Mikrozelenje je nezrelo zeleno povrće, koje se može jednostavno i brzo uzgojiti, a u odnosu na zrelo povrće ima povećani sadržaj bioaktivnih tvari. Cilj ovog istraživanja je odrediti utjecaj različitih koncentracija selena na fotosintetsku učinkovitost četiriju vrsta mikrozelenja iz porodice Brassicaceae (azijska raštika, kelj, brokula i koraba) nakon biofortifikacije selenom. Tri dana stare biljke su zalivene otopinom selena u koncentracijama od 2 i 5 mg/L, dok su biljke zalivene vodovodnom vodom služile kao kontrola. Osam dana starim biljkama je izmjerena fluorescencija klorofila *a*. Rezultati su pokazali da ni jedna koncentracija selena kod kelja, brokule i korabe nije utjecala na fotosintetsku učinkovitost. S druge strane, najveća koncentracija selena je uzrokovala smanjenje maksimalnog kvantnog prinosa fotosustava II ( $TR_0/ABS$ ) kod azijske raštike. Uz to, došlo je i do povećanja protoka apsorpcije ( $ABS/RC$ ), hvatanja ( $TR_0/RC$ ) te otpuštanja suvišne energije po aktivnom reakcijskom središtu ( $RC$ ), kao i elektronskog transporta ( $ET_0/RC$ ). Ovakvi rezultati ukazuju na to da je kod azijske raštike povećana koncentracija selena dovela do transformacije dijela reakcijskih središta u  $Q_A$ -nereducirajuće uz istovremeno smanjenje funkcionalne veličine antena fotosustava II.

## The effect of selenium biofortification on the microgreens photosynthetic efficiency

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**Keywords:** photosystem II, energy fluxes, maximum quantum yield, Brassicaceae

### Abstract:

Biofortification of microgreens with selenium is a promising, cost-effective, and sustainable method of selenium intake for consumers who have limited access to a diverse diet. Microgreens are immature green vegetables which can be grown easily and quickly, and compared to mature vegetables, show increased content of bioactive substances. The aim of this research is to determine the influence of different concentrations of selenium on the photosynthetic efficiency of four types of microgreens from the Brassicaceae family (pak choi, kale, broccoli and kohlrabi) after biofortification with selenium. Three-day-old plants were watered with a selenium solution in concentrations of 2 and 5 mg/L, while plants watered with tap water served as a control. The fluorescence of chlorophyll *a* was measured in eight-day-old plants. The results showed that none of the selenium concentrations in kale, broccoli, and kohlrabi affected the photosynthetic efficiency. On the other hand, the highest concentration of selenium caused a decrease in the maximum quantum yield of photosystem II ( $TR_0/ABS$ ) in pak choi. In addition, there was an increase in the absorption ( $ABS/RC$ ), trapping ( $TR_0/RC$ ) fluxes, and dissipation of excess energy per active reaction center ( $RC$ ), as well as in electron transport ( $ET_0/RC$ ). These results indicate that in the pak choi, the increased concentration of selenium led to the transformation of part of the  $RCs$  into non- $Q_A$ -reducing ones with a simultaneous decrease in the functional size of the antennae of the photosystem II.

## Promjene fenolnog sastava uzrokovane visokom temperaturom kod klijanaca brokule

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**Ključne riječi:** fitokemikalije, globalno zagrijavanje, klimatske promjene, kupusnjače

### Sažetak:

Fenolni spojevi poput flavonoida i fenolnih kiselina su biljni metaboliti pozitivnih bioloških učinaka na organizam čovjeka. Njihova biosinteza ovisi o okolišnim faktorima, uključujući temperaturu, što utječe na prehranbenu vrijednost biljaka. Stoga je u okviru znanstvenog istraživačkog projekta Hrvatske zaklade za znanost (IP-2020-02-7585) „Indirektni utjecaj globalnog zagrijavanja na fiziološke parametre sisavaca prehranom biljkama uzgojenim pri visokoj temperaturi (TEMPHYS)“ proučavan je utjecaj visoke temperature uzgoja na udio različitih fenolnih skupina u klijancima brokule (*Brassica oleracea botrytis* var. *cymosa*). Istraživanje je uključivalo tri biološke replike klijanaca uzgojenih pri visokoj temperaturi (38 °C dan/33 °C noć) i tri replike uzgojenih pri sobnoj temperaturi (23 °C dan/18 °C noć) kao kontrolnu skupinu. Nakon sakupljanja nadzemnih dijelova biljaka i njihove liofilizacije, pripremljeni su ekstrakti i spektrofotometrijski kvantificirani ukupni fenoli, flavonoidi, flavonoli, fenolne kiseline i hidroksicimetne kiseline. Rezultati su pokazali značajan porast ukupnih fenola pri visokoj temperaturi (+45%), no smanjenje ukupnih flavonoida (-9%), flavonola (-23%), fenolnih kiselina (-22%) i hidroksicimetnih kiselina (-14%). Ovi rezultati naglašavaju specifičan odgovor različitih fenolnih skupina u klijancima brokule na visoku temperaturu okoliša. Iako je udio ukupnih fenola povećan, sadržaj specifičnih skupina fenolnih spojeva se smanjio, ističući potrebu za daljnjim istraživanjima kako bi se razumjele primjene visokih temperatura uzgoja na ukupnu nutritivnu vrijednost klijanaca brokule.

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## High temperature-driven alterations in phenolic compounds of broccoli seedlings

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**Keywords:** Brassicaceae, climate change, global warming, phytochemicals

### Abstract:

Phenolic compounds, such as flavonoids and phenolic acids, are plant metabolites with positive biological effects on the human body. Their biosynthesis depends on environmental factors, including temperature, which affects the nutritional value of plants. The influence of high growing temperature on the composition of different phenolic groups in broccoli seedlings (*Brassica oleracea botrytis* var. *cymosa*) was investigated as a part of Croatian Science Foundation project (IP-2020-02-7585) “Indirect Effect of Global Warming on Mammals Physiological Parameters via High-Temperature Stressed Plant Diet (TEMPHYS)”. The research involved three biological replicas of seedlings grown at high temperature (38 °C day/33 °C night) and three at room temperature (23 °C day/18 °C night) as a control group. After collecting and lyophilizing the aboveground parts of the plants, extracts were prepared and spectrophotometrically quantified total phenols, flavonoids, flavonols, phenolic acids, and hydroxycinnamic acids. The results showed a significant increase in total phenols at high temperature (+45%), but a decrease in total flavonoids (-9%), flavonols (-23%), phenolic acids (-22%), and hydroxycinnamic acids (-14%). These findings highlight the specific response of different phenolic groups in broccoli seedlings to high environmental temperature. Although the proportion of total phenols increased, specific groups of phenolic compounds decreased, emphasizing the need for further research to understand the implications of high growing temperature on the overall nutritional value of broccoli seedlings.

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## Utjecaj bisfenola na vodene leće

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**Ključne riječi:** bisfenoli, bisfenol A, *Lemna minor*, test toksičnosti, procjena rizika

### Sažetak:

Plastični otpad perzistentan je u okolišu i kontinuirano pod utjecajem okolišnih uvjeta otpušta bisfenole. Cilj ovog rada je utvrditi učinke bisfenola na vodenu leću (*Lemna minor* L.). Vodena leća, primarni producent i modelni organizam u standardnim testovima toksičnosti, tretirana je sedam dana s 1, 5, 7 i 10 mg/L tri analoga bisfenola (BPA, BPS i BPF) u standardnom testu inhibicije rasta (OECD). Utvrđena je inhibicija rasta vodene leće ovisna o primijenjenom analogu i dozi. Prema efektivnoj koncentraciji koja uzrokuje 50%-tnu inhibiciju rasta ( $EC_{50}$ ), BPA i BPS imali su sličan utjecaj na vodenu leću ( $EC_{50}(BPA)=13,8\pm 1,5$  mg/L;  $EC_{50}(BPS)=14,4\pm 1,1$  mg/L), dok je utjecaj BPF bio znatno slabiji ( $EC_{50}(BPF)=17,1\pm 4,2$  mg/L). Prema rezultatima akumulacije svježe biomase biljaka analozi bisfenola mogu se svrstati u sljedeći niz toksičnosti: BPS > BPA > BPF. Sva tri testirana bisfenola imala su negativan utjecaj na koncentraciju fotosintetskih pigmenata, pri čemu je BPA imao najizraženije negativno djelovanje. Poznati su negativni učinci BPA dok se ostali analozi smatraju „sigurnim“ alternativama. Međutim, iako analozi bisfenola imaju sličnu molekulsku strukturu, ipak se razlikuju u kemijskim karakteristikama te pokazuju značajnu analog-ovisnu specifičnost učinaka na vodenu leću. Identifikacija i karakterizacija specifičnih pokazatelja toksičnosti doprinosi procjeni rizika i efektivnijim mjerama zaštite vodenih ekosustava od negativnih učinaka svih analoga bisfenola.

## Effects of bisphenols on duckweeds

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**Keywords:** bisphenols, bisphenol A, *Lemna minor*, toxicity test, risk assessment

**Abstract:**

Plastic waste is persistent in the environment and continuously releases bisphenols under environmental conditions. This work aims to determine the effects of bisphenol on duckweeds (*Lemna minor* L.). The duckweed, a primary producer and model organism for standard toxicity tests, was treated with 1, 5, 7 and 10 mg/L of three bisphenols (BPA, BPS and BPF) for seven days in a standard growth inhibition test (OECD). Analogue-dependent and dose-dependent inhibition of growth was determined, and according to the effective concentration causing 50% growth inhibition ( $EC_{50}$ ), BPA and BPS had a similar effect ( $EC_{50}(\text{BPA})=13.8\pm 1.5$  mg/L;  $EC_{50}(\text{BPS})=14.4\pm 1.1$  mg/L), while the influence of BPF was significantly weaker ( $EC_{50}(\text{BPF})=17.1\pm 4.2$  mg/L). According to the fresh biomass accumulation, bisphenol analogues can be classified in the following order of toxicity: BPS > BPA > BPF. All three bisphenols negatively affected the concentration of photosynthetic pigments, with BPA having the most pronounced effect. The adverse effects of BPA are known, while other analogues are considered “safe” alternatives. Although bisphenols have similar molecular structures, they differ in chemical characteristics and show significant analogue-dependent specificity of effects on duckweed. Identifying and characterizing specific toxicity indicators contributes to risk assessment and more effective measures for protecting aquatic ecosystems from the harmful effects of bisphenol analogues.

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## Utjecaj vodnog stresa na morfološka svojstva zelene salate (*Lactuca sativa* L.)

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**Ključne riječi:** kristalka salata, vodni stres, morfološka svojstva

### Sažetak:

Istraživanje je postavljeno u plasteniku na pokušalištu Fakulteta agrobiotehničkih znanosti Osijek 2023. godine. Dvočimbenično istraživanje se sastojalo od genotipova salate: ljubljanska (k1) i Zagrebačka (k2) te navodnjavanja: n1 - 100 % Rkv; n2 - 75 % Rkv; n3 - 50 % Rkv. Promatrana su sljedeća morfološka svojstva: masa, visina i promjer glavice te broj listova, masa, duljina i promjer korijena. U prosjeku su veća svojstva izmjerena kod k2 genotipa. Dvofaktorijalnom analizom varijance je utvrđen značajan utjecaj genotipa ( $p < 0,01$ ) na visinu glavice (cm; k1 = 13,77; k2 = 18,33), promjer glavice (cm; k1 = 27,72, k2 = 31,6), broj listova (n; k1 = 16,89, k2 = 20,77), masu (g; k1 = 36,83, k2 = 20,17) i promjer korijena (mm; k1 = 6,78, k2 = 11,78) salate. U pogledu tretmana navodnjavanja, najveće izmjerene varijable su zabilježene na n1 (100 % Rkv) tretmanu, s iznimkom duljine korijena. Kod n1 tretmana je zabilježena značajno veća ( $p < 0,01$ ) masa (g; n1 = 95,3, n2 = 76,67, n3 = 65,67) i visina glavice (cm; n1 = 95,3, n2 = 16, n3 = 13,1), broj listova (n; n1 = 21,67, n2 = 17,5, n3 = 17,17) te promjer korijena (mm; n1 = 10,5, n2 = 9,0, n3 = 8,3).

## The impact of water stress on the morphological traits of lettuce (*Lactuca sativa* L.)

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**Keywords:** iceberg lettuce, water stress, morphological traits

### Abstract:

The research was conducted in a greenhouse at the experimental site of the Faculty of Agrobiotechnical Sciences Osijek in 2023. The two-factor research consisted of lettuce genotypes: Ljubljana iceberg (k1) and Zagreb iceberg (k2) and irrigation: n1 - 100 % WRC; n2 - 75 % WRC; n3 - 50 % WRC. The following morphological traits were observed: mass, height and head diameter and number of leaves, mass, length and diameter of the root. On average, higher properties were measured in the k2 genotype. Two-factor analysis of variance revealed a significant influence of genotype ( $p < 0.01$ ) on head height (cm; k1 = 13.77; k2 = 18.33), head diameter (cm; k1 = 27.72, k2 = 31.6), number of leaves (n; k1 = 16.89, k2 = 20.77), mass (g; k1 = 36.83, k2 = 20.17) and root diameter (mm; k1 = 6.78, k2 = 11.78). Regarding irrigation treatments, the highest measured variables were recorded in the n1 (100 % WRC) treatment, with the exception of root length. In n1 treatment, a significantly higher ( $p < 0.01$ ) mass (g; n1 = 95.3, n2 = 76.67, n3 = 65.67) and head height (cm; n1 = 95.3, n2 = 16, n3 = 13.1), number of leaves (n; n1 = 21.67, n2 = 17.5, n3 = 17.17) and root diameter (mm; n1 = 10.5, n2 = 9.0, n3 = 8.3) were observed.



## Učinak abiotičkih čimbenika na aktivnost nekoliko proteina povezanih sa stresom u biljci brokule

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**Ključne riječi:** metaboliti, *Brassica oleracea*, HSP, proteini

### Sažetak:

Ovo istraživanje je rađeno s ciljem utvrđivanja povezanosti između metabolizma i aktivnosti određenih enzima i reakcije mladih biljaka brokule na stresore – visoka temperatura, suša i poplava. Biljke su dobivene od firme ISP GmbH, Quedlinburg i uzgajane na supstratu Stender (GmbH) Blue u fitotronu. Kontrolne biljke uzgajane su 18 h na 23°C – dan te 6 h na 18°C – noć, zalijevane vodom svaki drugi dan, a biljke u tretmanu sušom uzgajane su na 38°C – dan te 33°C – noć i zalijevane vodom svaki sedmi dan. Za uvjete poplave, biljke su zalijevane svaki dan s vodom u suvišku i uzgajane u istim uvjetima kao kontrola. Metode korištene u eksperimentu uključivale su izolaciju i mjerenje produkta primarnog i specijaliziranog metabolizma (HPLC i spektrometrija), te izolacija proteina i mjerenje aktivnosti enzima peroksidaze. Proteini su iz uzoraka razdvojeni SDS-PAGE elektroforezom na 12% gelu, te prikazani korištenjem Coomassie boje CBB (G-250). Razdvojeni proteini su prebačeni na membranu te detektirani i vizualizirani antitijelima za HSP70 (mitohondrij) i Lhcb6 (kloroplast). Sadržaj prolina, otopljenih šećera i klorofila je bilo viši kod biljaka tretiranih visokom temperaturom, u odnosu na nižu temperaturu. Aktivnost enzima peroksidaze je bila povišena pod utjecajem poplave i povišene temperature, a snižena pri uvjetima suše. Količine proteina HSP70 i Lhcb6 su bile povećane pri uvjetima suše i povišene temperature, a smanjene pod utjecajem poplave. Mlade biljke su pokazale promjene u metabolizmu nakon svih eksperimenata, a rezultati pokazuju, u skladu, da je brokula osjetljivija na poplavu i na višu temperaturu.

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## The effect of abiotic factors on the activity of several proteins associated with stress in the broccoli plant

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**Keywords:** metabolites, *Brassica oleracea*, HSP, proteins

### Abstract:

This research was done to determine the relationship between metabolism and the activity of some enzymes and the reaction of young broccoli plants to stressors – high temperature, drought and flood. Plants were obtained from ISP GmbH, Quedlinburg and grown on Stender (GmbH) Blue substrate in a phytotron. The control was grown for 18 hours at 23 °C - day and 6 hours at 18 °C - night, and water every other day. On drought 38 °C - day and 33 °C - night and water every seventh day. For flooding, the plants were watered every day with excess water and in the same system as the control. Methods used in the experiment included isolation and measurement of products of primary and secondary metabolism (HPLC and spectrometry), as well as protein isolation and measurement of peroxidase enzyme activity. Proteins from the samples were separated by 12% SDS-PAGE gel electrophoresis, and visualized using Coomassie stain CBB (G-250). Separated proteins were transferred to the membrane and detected and visualized with antibodies for HSP70 (mitochondrion) and Lhcb6 (chloroplast). The amount of proline, dissolved sugars and chlorophyll was higher at high temperature, compared to lower temperature. The activity of the peroxidase enzyme was increased in flood stress and elevated temperature, and decreased in drought. The amounts of HSP70 and Lhcb6 proteins were increased during drought and elevated temperature, and the amount of HSP70 and Lhcb6 decreased during flooding. Young plants showed changes in metabolism after all experiments, and the results show, in agreement, that broccoli is more sensitive to flooding and higher temperatures.

## Oplemenjivanje ozime pšenice na fuzarijsku palež klasa praćeno fiziološkim analizama

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**Ključne riječi:** fuzarijska palež klasa, fiziologija, pšenica

### Sažetak:

Fuzarijska palež klasa je opasna gljivična bolest koja pogađa ozimu pšenicu u Hrvatskoj. Simptomi se prvo uočavaju na klasićima koji poprimaju bijelu ili žutu boju te se mogu proširiti na cijeli klas dok zdravo tkivo ostaje zeleno. Posljedica ove bolesti je smežuranost zrna male mase, što smanjuje urod i kvalitetu zrna. Epidemični uvjeti ove bolesti ovise o vremenskim uvjetima tijekom cvatnje. Nadalje, vrlo je opasna pojava mikotoksina koje proizvode *Fusarium* vrste, čija koncentracija može biti visoka, čak i kada su simptomi bolesti manji. Proizvođači najčešće odabiru genotipove pšenice koje imaju određenu razinu otpornosti na fuzarijsku palež klasa jer je to najučinkovitija kontrola bolesti. Obrana biljke ovisi o koordiniranoj regulaciji metabolizma biljke i gena izazvanih napadom patogena. Naši rezultati pokazali su povećanu aktivnost enzima askorbat-glutationskog ciklusa (AsA-GSH) u klasovima nakon inokulacije *Fusarium* vrstama kod genotipova pšenice koje su deklarirane kao otpornije na fuzarijsku palež klasa. Također, kod tih genotipova fotosustav II bio je očuvaniji.

## Winter wheat breeding for FHB resistance accompanied by physiological techniques

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**Keywords:** fusarium head blight, physiology, wheat

### Abstract:

Fusarium head blight (FHB) is a serious fungal disease affecting winter wheat in Croatia. Symptoms are first noticed in the spikelets that are bleached white or yellow that can spread through whole spike while healthy tissue remains green. As a result of disease grains become shriveled and of low weight thus decreasing grain yield and quality. Epidemic conditions of FHB are highly dependent on weather conditions around anthesis. Further, very dangerous is occurrence of mycotoxins produced by *Fusarium* spp., whose concentration can be high, even when disease symptoms are low. Producers most often select wheat genotypes that have some level of FHB resistance as this is the most efficient control of the disease. Plant defense depends on the coordinated regulation of plant's metabolism and genes induced upon pathogen attack. Our results showed that ascorbate-glutathione metabolism (AsA-GSH) increased in spikes of wheat genotypes, which were declared as FHB resistant, after inoculation with *Fusarium* species. Also, photosystem II was more preserved in those genotypes.

## Uređivanje *VvPDS* gena vinove loze (*Vitis vinifera L.*) korištenjem biolistike na somatskim embrijima

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**Ključne riječi:** uređivanje genoma, *VvPDS*, CRISPR, vinova loza

### Sažetak:

Uzgoj vinove loze je svjetska industrija, poznata po visokoj potrošnji pesticida. Nadalje, zbog različitih klimatskih i ekonomskih pritisaka, konvencionalni uzgoj za dobivanje novih kultivara nije isplativ niti vremenski učinkovit. Stoga postoji potreba za poboljšanjem postojećih sorti genetskim inženjerstvom s ciljem postizanja bolje otpornosti na biotički/abiotički stres te smanjene potrošnje pesticida. Primjena genetičkog inženjerstva kod drvenastih biljaka kao što je vinova loza otežana je zbog njene klonske propagacije i nepoželjnosti spolnog razmnožavanja. U ovom istraživanju, upotrijebili smo transformaciju somatskih embrija vinove loze putem biolistike u svrhu ciljane mutageneze gena *VvPDS* CRISPR-Cas9 vektorom. Nakon transformacije biolistikom, iz somatskih embrija vinove loze dobivene su kimerne biljke. Detektirali smo promjene u sekvenci ciljane regije, iako ne i bialelnu inaktivaciju gena *VvPDS*, koja bi bila vidljiva u obliku bijelih sektora na inače zelenoj biljci. Utvrdili smo nužnost inicijalne genotipizacije ciljane genomske regije u odabranoj sorti vinove loze, koja može otežati bialelnu inaktivaciju gena zbog svoje sortno-specifične heterozigotnosti.



## Genome editing of the *VvPDS* gene by using biolistics on the grapevine (*Vitis vinifera* L.) somatic embryos

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**Keywords:** genome editing, *VvPDS*, CRISPR, grapevine

### Abstract:

Grapevine cultivation is a worldwide industry, known for its high pesticides inputs. Furthermore, due to various climatic and economic pressures, conventional breeding for obtaining new cultivars is not cost-effective nor time-efficient. Therefore, there is a need to improve existing varieties by genetic engineering in terms of better biotic/abiotic stress tolerance and a decrease in pesticide application. Genetic manipulations are difficult in woody plants like grapevine due to their clonal propagation and undesirability of sexual reproduction. In this study, we used biolistic-mediated targeted mutagenesis of the reporter gene *VvPDS* in grapevine somatic embryos using a single CRISPR-Cas9 vector. Chimeric plants were regenerated from grapevine somatic embryos after particle bombardment. We detected sequence alterations in the targeted region, although not in a homozygous state, which would be visible in the form of white sectors in case of a successful *VvPDS* knockout. We demonstrate the necessity of the initial genotyping of the targeted genomic region in grapevine, which may hinder biallelic targeting due to its heterozygosity.

## Evolucija komponenti signalne mreže hormona salicilne kiseline

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**Ključne riječi:** salicilna kiselina, biljna imunost, duplikacije genoma

### Sažetak:

Salicilna kiselina (SA) je jedan od glavnih hormona biljne obrane od stresa. Prilikom napada patogena, biljke induciraju biosintezu SA te ona zatim pokreće procese biljne imunosti PTI (*pattern-triggered immunity*) i ETI (*effector-triggered immunity*). U modelnoj vrsti *Arabidopsis thaliana* poznato je da je izokorizmat sintaza (ICS1) glavni enzim u biosintezi SA u sklopu odgovora na patogene. Međutim, u drugim vrstama dominantan je alternativni put biosinteze SA preko enzima PAL (fenilalanin-amonijak lijaza). Ovdje smo istražili evoluciju gena uključenih u biosintezu SA: ICS1 i transportera EDS5. Čini se kako su se ovi geni pojavili relativno nedavno u evoluciji vrste *A. thaliana* te nisu prisutni izvan obitelji Brassicaceae. Duplikacija gena ICS1 i neofunkcionalizacija gena EDS5 vremenski odgovaraju duplikaciji cijelog genoma poznatoj kao duplikacija alpha u liniji kupusnjača koja se dogodila prije 49 Mya. Signalna mreža odgovora na stres je pod jakim selekcijskim pritiskom, jer su biljke neprestano izložene promjenama u okolišu. Ovi rezultati pokazuju kako *Arabidopsis* i druge krstašice imaju specifičan mehanizam indukcije biosinteze SA tijekom odgovora na patogene, koji se razvio relativno nedavno u njihovoj evolucijskoj prošlosti.

## Evolution of components of salicylic acid signaling network

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**Keywords:** salicylic acid, plant immunity, whole genome duplication

**Abstract:**

Salicylic acids (SA) is one of the main hormones in plant stress response. During pathogen attack in plants, SA biosynthesis is induced, which in turn activates the processes of PTI (pattern-triggered immunity) and ETI (effector-triggered immunity). In model species *Arabidopsis thaliana*, the main enzyme in SA biosynthesis during pathogen response is isochorismate synthase (ICS1). However, in other plant species an alternative biosynthesis pathway appears to be dominant – synthesis through PAL enzyme (phenylalanine ammonia lyase). Here, we have investigated the evolution of genes involved in SA biosynthesis: ICS1 and a transporter named EDS5. It seems these genes have evolved only recently and are not present outside of Brassicaceae family. ICS1 gene duplication and neofunctionalization of EDS5 correspond to whole genome duplication event in evolutionary history of Brassicas named alpha duplication, which has occurred 49 Mya. Signaling network of stress response is under a strong selection pressure, since plants are constantly exposed to environmental changes. These results show how *Arabidopsis* and other Brassicaceae have a specific mechanism of SA biosynthesis induction during pathogen response, which has evolved only recently in their evolutionary lineage.

## Istraživanje uloge biflavonoida u biljkama: rezultati biološke aktivnosti *in vitro* te distribucija u različitim tkivima ginka (*Ginkgo biloba* L.)

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**Ključne riječi:** antioksidacijska aktivnost, antifungalna aktivnost, polifenoli, HPLC-DAD

### Sažetak:

Polifenoli su skupina specijaliziranih metabolita koji imaju niz uloga u interakciji biljaka s okolišem, no njihova uloga značajno ovisi o strukturi. Flavonoidi su u nekim biljnim vrstama prisutni u obliku dimera – biflavonoida. Za razliku od monomernih flavonoida, uloga biflavonoida u biljkama je slabo istražena. Stoga je u okviru projekta Hrvatske zaklade za znanost „Uloga biflavonoida u biljkama: *Ginkgo biloba* L. kao modelni sustav“ (HRZZ-UIP-2019-04-1018) određena biološka aktivnost pet različitih biflavonoida mjerenjem antioksidacijske aktivnosti metodom DPPH te određivanjem antifungalne aktivnosti prema plijesnima *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Fusarium graminearum* i *Fusarium verticillioides*. Iako monomerni flavonoidi djeluju kao antioksidansi, biflavonoidi nisu pokazali antioksidacijsku aktivnost, no pokazali su antifungalnu aktivnost. Budući da distribucija flavonoida unutar biljke također može ukazivati na njihovu ulogu razvijena je metoda HPLC-DAD za simultanu kvantifikaciju pet biflavonoida (amentoflavona, isoginkgetina, ginkgetina, bilobetina i sciadopitisina) te je napravljen njihov profil u različitim tkivima ginka. Rezultati upućuju na lokalizaciju biflavonoida u dijelovima biljke koji su u direktnom doticaju s okolinom što ukazuje na njihovu ulogu u interakciji biljke i okoliša. No distribucija je ovisila i o tipu biflavonoida pa je tako amentoflavon bio najzastupljeniji u kori dok je u ostalim dijelovima bio prisutan u značajno nižim koncentracijama.

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## Investigation of the role of biflavonoids in plants: Results of *in vitro* biological activity and distribution in different tissues of ginkgo (*Ginkgo biloba* L.)

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**Keywords:** antioxidant activity, antifungal activity, polyphenols, HPLC-DAD

### Abstract:

Polyphenols are a group of specialized metabolites that have a variety of functions in the interaction of plants with the environment, whereby their role depends largely on their structure. Flavonoids occur in some plant species in the form of dimers - biflavonoids. In contrast to monomeric flavonoids, the role of biflavonoids in plants has been studied very little so far. To determine the biological activity of five different biflavonoids, as a part of Croatian Science Foundation project "Biflavonoids role in plants: *Ginkgo biloba* L. as a model system" (UIP-2019-04-1018), the antioxidant activity was measured using the DPPH method and the antifungal activity against the fungi *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Fusarium graminearum* and *Fusarium verticillioides* was determined. Although monomeric flavonoids have antioxidant properties, biflavonoids did not show antioxidant activity, but did show antifungal activity. Their distribution within the plant could also indicate their role. Therefore, an HPLC-DAD method was developed for the simultaneous quantification of five biflavonoids (amentoflavone, isoginkgetin, ginkgetin, bilobetin and sciadopitysin) and their profile in different tissues of ginkgo was established. The results suggest that the biflavonoids are localized in the parts of the plant that are in direct contact with the environment, indicating their role in plant-environment interaction. However, the distribution also depended on the type of biflavonoid. For example, amentoflavone was most abundant in the bark, while it was found in much lower concentrations in other parts of the plant.

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