Biofortifikacija lisnatog povrća i soje selenom

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Selen (Se) je esencijalan mikroelement prisutan u prirodi, od vitalnog je značaja za različite organizme, uključujući ljude, životinje, mikroorganizme, dok je za biljke beneficijalan. Nedostatak selena u prehrani predstavlja globalni problem čiji intenzitet često ovisi o koncentracijama i raspoloživosti selena u tlu. Selen ima ključnu ulogu u antioksidacijskim procesima, regulaciji funkcija reproduktivnog i imunološkog sustava u zdravlju ljudi. Kao odgovor na ovaj izazov, biofortifikacija postaje ključnom strategijom za povećanje sadržaja selena u biljkama. Iz provedene meta-analize, istraživanja sugeriraju da folijarna primjena selena u obliku selenata često postiže bolje rezultate od primjene u tlu, izuzevši rižu gdje je efikasniji selenit. Iz terenskih istraživanja u Bosni i Hercegovini, Srbiji i Hrvatskoj dobiveni su podaci o dostupnosti selena u tlu analizama kemijskih svojstava tala i o utjecaju na prisutnost selena u vodenoj fazi tla. Temeljem dobijenih rezultata razvijen je model parcijalnih najmanjih kvadrata (eng. Partial least squares regression - PLS) za planiranje biofortifikacije u poljoprivrednoj proizvodnji. Istraživani su fiziološki parametri (askorbinska kiselina, ukupni fenoli, prolin, ukupna antioksidativna aktivnost i lipidna peroksidacija) kao odgovor na osmotski stres izazvan polietilen glikolom (PEG tretmanom) klijanaca soje (*Glycine max* L. Merr.) čije je sjeme u prethodnoj vegetaciji biofortificirano selenom, te su utvrđene različite reakcije dva kultivara soje (Sonja i Lucija). Istraživanjem utjecaja različitih supstrata (treset, vermikompost i njihove mješavine) na učinkovitost biofortifikacije selenom i prinos u uzgoju matovilcu (*Valerianella locusta* L.), utvrđena je pogodnost smjese vermikomposta i tresetnog medija za uzgoj i biofortifikaciju matovilca. Također, u istraživanju biofortifikacije natrijevim selenatom (Na_2SeO_4) i nanočesticama selena u hidroponskom uzgoju dvije vrste "*baby leaf*" povrća (matovilac i amarant – *Amaranthus caudatus* L.), analizama morfoloških i fizioloških značajki "*baby leaf*" povrća utvrđena je različita učinkovitost ovih pristupa biofortifikaciji.

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Biofortification of leafy vegetables and soybean with selenium

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Thesis performed at Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer in Osijek Supervisor: prof. dr. sc. Zdenko Lončarić

Selenium (Se) stands out as an essential trace element in nature, vital for various organisms, including humans, animals, microorganisms, while being beneficial in plants. Selenium deficiency in diet poses a global problem, with severity often depending on selenium concentrations in the soil. Selenium plays a crucial role in antioxidant processes and regulates reproductive and immune system functions in human health. In response to this challenge, biofortification becomes a key strategy for increasing selenium content in plants. Meta-analysis suggests that foliar application of selenium in the form of selenates often yields better results than soil application, except for rice, where selenite proves more effective. Field studies in Bosnia and Herzegovina, Serbia, and Croatia provided data on selenium availability in soil, using soil samples for chemical analysis to explore connections between chemical properties and selenium presence in the soil's water phase. Based on these data, a Partial Least Squares (PLS) regression model was developed, offering guidelines for planning biofortification in key agricultural production. Physiological parameters (ascorbic acid, total phenols, proline, total antioxidant activity, and lipid peroxidation) were examined in response to osmotic stress induced by polyethylene glycol (PEG treatment) in germinating soybeans (*Glycine max* L. Merr.), whose seeds were biofortified with selenium in the previous season, investigating differences in reactions of two soybean cultivars (Sonja and Lucija). Furthermore, the impacts of different growth substrates, including peat, vermicompost, and their mixtures, on the efficiency and yield of selenium biofortification in lamb's lettuce (*Valerianella locusta* L.), were studied, emphasizing peat reserve preservation. Finally, a comparative study explored the influence of biofortification with sodium selenate (Na_2SeO_4) and selenium nanoparticles on various "baby leaf" vegetables (lamb's lettuce and amaranth – *Amaranthus caudatus* L.) in a hydroponic system, examining potential benefits and differences between these approaches through morphological and physiological features of "baby leaf" vegetables.

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ZAHVALE

KAZALO

1. UVOD

Selen je element u tragovima esencijalan za ljude i životinje, dok je za biljke beneficijalan (Ullah i sur., 2019.). Nedostatak selena u ljudskom organizmu može dovesti do ozbiljnih medicinskih komplikacija kao što su siva mrena, endotelnu disfunkciju, kardiovaskularne bolesti, kardiomiopatiju, slab imunološki sustav, pa čak i rak (Zhou i sur., 2020.). Utvrđene su suboptimalne koncentracije selena u hrani i vodi širom Europe, Velike Britanije i na Bliskom istoku, što potvrđuje i naglašava nedostatak selena u ljudskoj populaciji (Stoffaneller i Morse, 2015.). Točnije, 1 milijarda ljudi biva pogođena nedostatkom selena u prehrani (Schiavon i sur., 2020.). Široka rasprostranjenost pothranjenosti rezultira negativnim utjecajem na socio-ekonomski status pojedinca, zajednice, te se na nacionalnoj razini ovaj problem, koji je poznat kao „skrivena glad“, smatra jednom od najozbiljnijih globalnih izazova čovječanstva (Carvalho i Vasconcelos, 2013.). Selen ima malu granicu između toksičnog efekta i deficita, te se doza od 55 µg po danu smatra optimalnom, a 400 µg selena po danu toksičnom dozom (Zafeiriou i sur., 2022.). Sadržaj selena u hrani jako je ovisan o koncentracijama selena u tlu, o bioraspoloživosti i o mogućnosti biljaka da usvajaju i akumuliraju selen u jestive dijelove biljke (Bañuelos i sur., 2015.), te je stoga potrebno implementirati selen u tla s nedostatkom (Leija-Martínez i sur., 2018.). Biljke akumuliraju selen i zbog toga predstavljaju značajan izvor selena prehranom (Schiavon i sur., 2020.), što se postiže biofortifikacijom ili „biološkom fortifikacijom“. Cilj biofortifikacije je nutritivno poboljšanje prehrambenih usjeva povećanjem biodostupnosti ljudskoj populaciji, što se postiže suvremenom biotehnologijom, konvencionalnim oplemenjivanjem bilja i agronomskom praksom (Garg i sur., 2018.). Za provedbu valjane i ekonomski opravdane biofortifikacije neophodno je poznavati kemijska svojstva selena i fizikalno-kemijska svojstva tla na kojemu se uzgaja usjev uz biofortifikaciju. Koncentracije selena značajno variraju u različitim okolišnim uvjetima i distribucija selena je uglavnom heterogena i specifična za određeno područje (Bañuelos i sur., 2015.). U većina tala koncentracije selena su između 0,01 i 2,0 $mgkg^{-1}$ i u prirodi je prisutan u četiri oksidacijska stanja: +6 (selenat), +4 (selenit), 0 (elementarni selen) i -2 (selenid) (White, 2018.a). Ciklus raspoloživosti selena započinje i završava u tlu, a osim kemijskog oblika i koncentracije selena u tlu, fizikalno-kemijska svojstva i mehanički sastav tla također određuju bioraspoloživost selena. Općenito, Se je snažno imobiliziran u kiselim i reduktivnim tlima uz smanjenu pokretljivost (Dinh i sur., 2017.). Selenat (SeO_4^{2-}) je glavni vodotopivi oblik selena u oksidnim tlima (pH + pE > 15), dok selenit (SeO_3^{2-}, $HSeO^{3-}$, H_2SeO_3) prevladava u anaerobnim

tlima s neutralnom do kiselom reakcijm (pH + pE = 7,5–15) (White, 2018.a). Selenat je relativno pokretljiv u otopini tla, ali je selenit snažno apsorbiran željeznim i aluminijevim oksidima/hidroksidima te, u manjoj mjeri, česticama gline i organskom tvari. Selenidi (Se^{2-}) su prisutni samo u izrazito anaerobnim, a često i kiselim tlima (pH + pE < 7,5). Organoselenijevi spojevi, kao što su selenometionin (SeMet), selenocistin (SeCys2), prisutni su u metaneleninskoj kiselini (MeSOOH) i trimetilselenijevom ionu (Me_3Se^+) u malim koncentracijama u otopini tla kao rezultat razgradnje organskih komponenata tla (White, 2018.a). Međutim, većina studija razmatrala je selen (IV) i selen (VI) kao jedine i najzastupljenije oblike selena raspoložive za usvajanje biljkama. Mobilnost i topljivost različitih oblika selena ovise o reakcijama sorpcije/desorpcije, pH, redoks potencijalu, organskim i anorganskim kompleksima i procesima otapanja u tlima i sedimentima (Natasha i sur., 2018.). Stanice korijena mogu usvajati selenat, selenit i organoselenijeve spojeve, kao što su SeCys i SeMet, ali ne mogu usvajati selenide ili koloidni elementarni selen (White, 2018.b). Selenat je dominantan bioraspoloživi oblik selena u tlima, pa je stoga to tipičan oblik selena koji biljka usvaja. Može se usvojiti i transportirati između biljnih organa proteinima transportera sulfata (SULTR). Put reduktivne asimilacije sulfata može metabolizirati selenat u selenocistein (SeCys), a rezultirajući SeCys može se ugraditi u protein ili se može dalje metabolizirati kao selenometionin (SeMet) u hlapljivi dimetilselenid (DMSe) (Pilon-Smits, 2019.; Santiago i sur., 2020.). Većina biljaka nije razvila selektivne mehanizme usvajanja i metabolizma selena i sumpora, te ne mogu ugraditi selen u sve sumporne spojeve. Iznimka su hiperakumulatori selena, koji mogu razlikovati selen i sumpor, te preferencijalno usvajati selen umjesto sumpora (Pilon-Smits, 2015.). Anorganski oblici selena razlikuju se u smislu apsorpcije i mobilnosti unutar biljaka, selenat se lakše prenosi do vršnih dijelova biljke, dok se selenit nakuplja u korijenu biljaka. Zbog toga se u nekim programima biofortifikacije selenom preporučuje korištenje selenata umjesto selenita (Ramos i sur., 2010.; Ligowe i sur., 2020.). Također, veća je pokretljivost selenata u alkalnom tlu, a samim time je intenzivnije usvajanje i translokacija selenata iz korijena u vršne dijelove biljke (Zafeiriou i sur., 2022.). Utvrđen je značajan utjecaj transportnog sustava fosfata u kretanju selenita u biljci pošto povećane koncentracije fosfata smanjuju unos selenita kod različitih vrsta biljaka (Schiavon i Pilon-Smits, 2017.). Utjecaj selena u biljkama je pozitiavn jer povećava toleranciju na potencijalno toksične elemente, stres i patogene te u odgovarajućim koncentracijama i povećava prinos (Zafeiriou i sur., 2022.). Ramos i sur., 2010. utvrdili su da biofortifikacija zelene salate selenom pogoduje rastu i povećava masu mladih biljaka.

Lisnato povrće značajan je izvor esencijalnih elemenata i vitamina u prehrani ljudi i hranidbi životinja (Tomasi i sur., 2015.). Industrija svježe rezanog lisnatog povrća je sektor s intenzivnim razvojem na tržištu voća i povrća (Tomasi i sur., 2014.). Većina lisnatog povrća tijekom pakiranja je minimalno obrađena, rashlađena i konzumira se u roku od 5-10 dana nakon berbe. Bilo bi vrlo korisno povećati svijest potrošača o konzumaciju svježeg povrća bogatog fitokemikalijama sa zaštitnom ulogom u prehrani ljudi (Tomasi i sur., 2014.). U posljednje vrijeme, najsuvremenije tehnologije omogućuju upotrebu selena u obliku selenovih nanočestica (SeNPs) kao zamjenu za konvencionalna selenova gnojiva s ciljem povećanja razine organskih spojeva selena u usjevima (El-Ramady i sur., 2015.; Kumar i Prasad, 2020.; Márquez i sur., 2020.). Nanooblik selena privlači povećanu pozornost zbog izvrsne biodostupnosti i smanjene toksičnosti u usporedbi s anorganskim i organskim oblicima (Hosnedlova i sur., 2018.). Postoje različiti pristupi sintezi SeNps, poput fizičkih, kemijskih i bioloških (Alam i sur., 2019.). S obzirom da će globalna populacija doseći otprilike 9 milijardi do 2050. godine, sigurnost hrane je jedno od ključnih pitanja novoga tisućljeća i izazov za poljoprivrednu proizvodnju, što klimatske promjene dopunski otežavaju (Adams i sur., 1999.; Sambo i sur., 2019.). Održiva poljoprivredna proizvodnja usmjerava agronomski pristup prema novim metodama i sustavima proizvodnje koji smanjuju potrošnju i optimiziraju gospodarenje prirodnim resursima. U proizvodnji u zaštićenim prostorima, hidroponski sustavi nude održivu alternativu tlu, omogućujući uzgoj usjeva u okolinama gdje je tradicionalna poljoprivreda neizvediva (Vernieri i sur., 2005.). Plutajući sustav je tehnika uzgoja bez tla gdje se biljke uzgajaju na različitim kontejnerima, posudama ili drugim nosačima koji plutaju u spremnicima s otopinom hranjivih tvari (Lenzi i sur., 2011.). Plutajući sustav predstavlja jedan od najjednostavnijih sustava uzgoja (Ferrarese i sur., 2012). Uzgoj biljaka u takvim sustavima sve je interesantniji, posebno u proizvodnji "*baby leaf*" povrća. Rastuća je potražnja za proizvodima vrhunske kvalitete uz stroge higijenske standarde (Gonnella i sur., 2003.), a prednost sustava uzgoja bez tla je što nema potrebe za dezinfekcijom tla, smanjena je mogućnost pojave bolesti koje se prenose tlom, što povećava sigurnost pakiranog povrća i smanjuje probleme s kemijskim ostatcima (reziduima) (Alberici i sur., 2008.).

2. PREGLED LITERATURE

Dall'Acqua i sur. (2019) proveli su istraživanje na dvije vrste rukole: *Eruca sativa* (salata rukola) i *Diplotaxis tenuifolia* (divlja rukola). Ove dvije vrste rukole uzgajane su u hidroponskom sustavu gdje su istraživači htjeli utvrditi kakav utjecaj i koju efikasnost imaju koncentracije od 0 do 40 µM selena u obliku selenata (Na_2SeO_4). Ispitivan je sadržaj sumpornih spojeva u biljkama i druge fitoaktivne komponente. Rezultati istraživanja su pokazali da je *D. tenuifolia* akumulirala više selena i selenocisteina od *E. sativa* u suhoj tvari biljke. Sadržaj aminokiselina se smanjio sa selenom u *E. sativa*, ali povećao u *D. tenuifolia*, a količina fenola je više smanjena u *D. tenuifolia*. Zaključno, primjena selenata u hidroponskom uzgoju omogućila je obogaćivanje rukole selenom i pri niskoj koncentraciji od 5 µM gdje nije značajno utjecao na nakupljanje fitokemikalija i obrambenih sumpornih metabolita.

Tomasi i sur. (2015.) su koristili dvije sorte (Gala i Baron) matovilac salate (*Valerianella locusta* (L.) Laterr.) u istraživanju mogućnosti povećanja sadržaja selena u jestivim dijelovima (listovima) matovilca. Rezultati su pokazali da je matovilac podnosio koncentracije selenata (Na_2SeO_4) u rasponu od 10 do 40 µM u hranjivoj otopini, s biljkama koje akumuliraju selen na razinama sukladnim potrebama u prehrani ljudi uz koncentraciju 10 µM selenata. Selenom tretirane biljke pokazale su neke prednosti kao smanjenje koncentracije nitrata i povećanje sadržaja pigmenata (klorofila i karotenoida). Pri 10 µM selenata, proizvedeni su selenocistein i selenometionin, bez utjecaja na neproteinske tiole ili sadržaj cisteina i metionina.

Santiago i sur. (2016.) usporedili su učinke selena i njegovih analognih tretmana sa sumporom na rast biljaka i biokemijske odgovore između akumulatora selena (rukola) i neakumulatora (zelena salata). Rukola je pokazala povećanu proizvodnju biomase u usporedbi s netretiranim kontrolama pri višoj koncentraciji selenata od zelene salate (20 µM prema 10 µM Na_2SeO_4), pokazujući bolju toleranciju na selen. Pod istim tretmanima rukola je nakupila 3 puta više selena i sumpora od biljaka salate. Međutim, asimilacija selen/sumpor procijenjena aktivnostima ATP sulfurilaze i O-acetilserin(tiol)liaze bila je usporediva između biljaka rukole i salate. Približno 4 puta veće koncentracije selena u proteinima s istim tretmanima selenom uočene su u rukoli nego u salati, što ukazuje na to da akumulatori selena imaju bolju toleranciju na seleno-aminokiseline u proteinima. U rukoli je utvrđena 6 puta veća aktivnost askorbat peroksidaze i preko 5 puta više glutationa i neproteinskih tiola od biljaka salate, što ukazuje na kritičnu ulogu antioksidansa u toleranciji selena. Rezultati pokazuju da je povećana tolerancija na selen kod rukole u usporedbi sa salatom

najvjerojatnije posljedica učinkovitijeg antioksidativnog obrambenog sustava. Ova studija pruža daljnji uvid u razumijevanje razlike u toleranciji i metaboliziranju/akumulaciji selena između akumulatora i neakumulatora selena.

Leija-Martínez i sur. (2018.) su u svom istraživanju koristili selen u obliku biopolimernih kompleksa. Salata, *Lactuca sativa* var. Great Lakes uzgojena je u posudama sa supstratom i tretmanima sa SeO_2 (5 mg Se biljci^{-1}), kompleks hitozan-poliakrilna kiselina + selen (Cs-PAA + Se) (5 mg Sebiljci^{-1}) i kompleks hitozan-poliakrilne kiseline (Cs-PAA). Upotreba biopolimernih kompleksa Cs-PAA + selen povećala je koncentraciju selena do 24 mg kg^{-1} suhe tvari u tkivu salate. Rezultati pokazuju da kompleksi s Cs-PAA mogu biti korisni u procesu biofortifikacije, zbog povećanja apsorpcije selena i povećanja aktivnosti katalaze (CAT) i glutation peroksidaze (GPX), bez utjecaja na razvoj usjeva. Uočeno je da su selen i Cs-PAA kompleksi bili učinkoviti za povećanje otpornosti biljaka, kao i nutricionističke kvalitete zelene salate (Leija-Martínez i sur., 2018.).

Ramos i sur. (2010.) proveli su eksperiment na salati (*Lactuca sativa* L. cv. Vera) u stakleniku s posudama koje su sadržavale 3 L nutritivne otopine u potpuno randomiziranom faktorskom dizajnu, sa sedam koncentracija selena (0, 2, 4, 8, 16, 32 i 64 μM L^{-1}) i dva oblika selena (natrijev selenat – Na_2SeO_4 i natrijev selenit – Na_2SeO_3), u šest ponavljanja. Primjena selena kao selenata u niskim koncentracijama prikladnija je za biofortifikaciju salate jer pogoduje rastu biomase mladih biljaka i razinama selena u biomasi izdanaka. Selen je u oba oblika imao dva učinka na metabolizam biljaka salate: u malim dozama djelovao je kao antioksidans i pospješio rast biljaka, dok je u višim razinama smanjio prinos.

Suša izaziva niz fizioloških promjena u biljkama soje, uključujući oksidativna oštećenja. U istraživanju Seminario i sur. (2017.) je utvrđeno da stres uzrokovan sušom rezultira smanjenjem biosinteze askorbinske kiseline u biljkama soje.

U istraživanju Basal i sur. (2020.) zabilježene su značajne razlike kod oba kultivara soja na vodljivosti puči na primjenu PEG-a (eng. polyethylene glycol); povećanje koncentracije PEG-a rezultiralo je smanjenjem vodljivosti puči u svim rasta. Može se zaključiti da suša ima različite učinke na fiziologiju ta dva kultivara soje; međutim, negativni učinci bili su očitiji u kasnim fazama životnog ciklusa biljke kod oba kultivara, što će vjerojatno utjecati na komponente prinosa i, posljedično, očekivani prinos.

U istraživanju na soji je utvrđeno da kombinacija selena sa salicilnom kiselinom ili u tretmanu bez nje, ima značajno inducirajuće učinke na enzimski (peroksidaza, katalaza i superoksid dismutaza) i neenzimski (askorbat) antioksidativni sustav. Na temelju dobivenih rezultata može se zaključiti da folijarna primjena selena u kombinaciji sa salicilnom kiselinom može ublažili solni stres (Ardebili i sur., 2014.).

Ligowe i sur. (2020.) koristili su izotopski označeni selenat (> 99% obogaćen 77Se) za procjenu usvajanja i raspoloživosti selena u uzgoju *Brassice napus* (uljana repica) i *Amaranthus retroflexus* (šćir) na tri različita tla u Malavima: vertisol (vapnenasto), alfisol (umjereno kiselo) i oxisol (kiselo). Biljke su uzgajane u stakleničkim uvjetima (4 ponavljanja; 6 kg tla po posudi) uz primjenu selenata u dozama 0, 10 i 20 g ha^{-1}. Listovi su ubirani u razmacima od dva tjedna, na kon čega su biljke nastavile rasti. Uzorci listova analizirani su masenom spektrometrijom induktivno spregnute plazme (ICP-MS) za izotope selena (77Se i 78Se). Izotopski podaci obrađeni su kako bi se kvantificirao doprinos koncentraciji selena u biljkama selenom iz gnojiva i tla. U početku je bioraspoloživost selena iz gnojiva bila tri reda veličine veća nego selena u tlu, ali se to smanjilo na isti red veličine do kraja ispitivanja. Biofortifikacija selenom (*A. retroflexus* i *B. napus*) na oxisolu, alfisolu i vertisolu povećala je koncentracije selena u biljci i, potencijalno, unos selena u prehrani ljudi. Aplicirani selen brzo je bio usvojen u uzgoju na sva 3 tipa tla, ali je također brzo fiksiran u tlu, što ga čini nedostupnim za biljku nakon 28 dana od aplikacije 77Se (Ligowe i sur., 2020.).

Zafeiriou i sur. (2022.) proveli su biofortifikaciju salate selenom u dvije koncentracije (5 i 10 mg kg^{-1} tla) bilo selenata ili selenita uz dodavanje biougljena (5 % w/w). Sadnice salate bile su uzgajane u posudama s 1 kg vapnenačkog tla. Nakon dvanaest tjedana, biljke su ubrane i izmjerene su koncentracije selena, fosfora i sumpora u glavicama i korijenu salate. Primjena biougljena smanjila je usvajanje selenita pri visokim dozama i štiti biljke od toksičnosti. No pri visokoj koncentraciji selenata, biougljen nije zaštitio biljke od toksičnog utjecaja na rast, vjerojatno zbog mnogo veće mobilnosti selenata i dostupnosti u alkalnom tlu u usporedbi sa selenitom. U tretmanima selenatom koncentracija selena u salati bila je i do 10 puta veća nego u tretmanima selenitom, potvrđujući veću pokretljivost selenata u alkalnim tlima, te veću translokaciju selenata iz korijena u izdanak. Većina selena u tretmanima selenitom ostala je u korijenu, bez obzira na aplikaciju biougljena. U tretmanima selenatom uočeno je suprotno, tj. većina selena prenesena je u izdanak, također bez obzira na primjenu biougljena (Zafeiriou i sur., 2022.).

Istraživanje koju su proveli Zhang i suradnici (2020.) pruža dokaze da selenom biofortificirani proteini soje mogu učinkovito ublažiti oštećenja kože kod četrdeset ženskih miševa izazvana UVB zračenjem, sugerirajući da selen može biti zanačaj agens za obranu kože od UVB zračenja (Zhang i sur., 2020.). Rady i suradnici (2020.) utvrdili su da je folijarna suplementacija selenovim nanočesticama imala značajnu ulogu u ublažavanju štetnih učinaka solnog stresa na različite aspekte rasta graha (*Phaseolus vulgaris)*, uključujući masu svježe tvari, fiziološke i biokemijske pokazatelje. Stoga su zaključili da se folijarna biofrtifikacija selenovim nanočesticama može predložiti kao značajan strateški pristup poboljšanju rasta i produktivnosti biljaka graha na tlima povećanog saliniteta (Rady i sur., 2020.). Slično tome, u istraživanju na sjemenkama *Hordeum vulgare* uz uporabu selenovih nanočestica utvrđen je učinak selena kao promotora rasta i razvoja sjemenke u stresnim uvjetima uz poboljšanjima morfofunkcionalnih svojstava (Nagdalian i sur., 2023.). Rezultati istraživanja Asghari-Paskiabi i suradnika (2018.) otkrivaju da *Fusarium oxysporum* može proizvoditi selenove nanočestice na siguran i ekonomičan način.

Nanoselen nalazi primjene u području medicine, gdje je također predmet istraživanja. Kemijski sintetizirane selenove nanočestice proučavane su zbog njihovog potencijala kao antibakterijskih agensa u liječenju oboljelih od bakterijskih bolesti uzrokovanih prominentnim patogenim bakterijama (Ananth i sur., 2019.).

Amarant je vrlo interesantna biljna vrsta čiji se listovi konzumiraju kao "*baby leaf*" povrće, a zrno se konzumira kao i zrno žitarica (Rastogi i Shukla, 2013.). Amarantove stabljike i listovi sadrže prehrambena vlakna, proteine s esencijalnim aminokiselinama, vitamine, karotenoide, minerale, razne antioksidanse i fitokemikalije poput betacijanina, antocijana, karotenoida i askorbinske kiseline (Pasko i sur., 2015.; Sarker i Oba, 2020.). Mogu se konzumirati svježi u salatama ili kuhani kao špinat. Zbog komzumacije i zelenih dijelova i zrna amaranta, vrlo je interesantna i mogućnost biofortifikacije amaranta, što su u svojim istraživanjem amaranta uspješno proveli Mala i sur., (2017,).

3. CILJEVI I HIPOTEZE ISTRAŽIVANJA

Ciljevi istraživanja su:

1. utvrditi mogućnost kreiranja modela (regresijski model i multivarijatne metode) raspoloživosti selena u tlu na temelju izmjerenih ukupnih koncentracija i fizikalno-kemijskih svojstva tla,
2. utvrditi postoji li različitost fiziološkog odgovor klijanaca soje u stresnim uvjetima (suša) uslijed biofortifikacije soje selenom,
3. utvrditi efikasnost biofortifikacije lisnatog povrća selenom u hidroponskom i supstratnom načinu uzgoja.

Hipoteze:

1. Moguće je modelirati i predvidjeti utjecaj svojstava tla na topivost i raspoloživost selena.
2. Selenom biofortificirano sjeme soje utječe na fiziološki odgovor klijanaca na stresne uvjete (sušu).
3. Biofortifikaciju je moguće provesti dodavanjem selena u obliku selenata u različite medije za uzgoj biljaka.
4. Lisnato povrće učinkovito akumulira različite koncentracije i oblike selena u hidroponskom načinu uzgoja.

4. MATERIJAL I METODE RADA

4.1. Analiza medija za uzgoj

Izmjerena su sljedeća svojstva: pH vrijednost, električna vodljivost (EC), organska tvar (OM), sadržaj pepela, ukupna koncentracija dušika, ukupne koncentracije mikroelemenata (Zn, Cu, Mo i Ni) i toksičnih elemenata (Cd, Pb, Cr, Hg i As), i ukupna koncentracija selena. Laboratorijske analize i određivanje elemenata provedene su u laboratoriju Fakulteta agrobiotehničkih znanosti Osijek.

1. Elektrometrijsko mjerenje reakcije (pH vrijednosti) provedeno je s pH-metrom kojim su mjerene razlike u električnom potencijalu (EN 2011). Elektrometrijsko određivanje pH vrijednosti medija za uzgoj izveden je prema europskom standardu 13037:2011 u suspenziji od 60 mL svježeg uzorka u 300 mL deionizirane vode, tj. volumni omjer 1:5 (uzorak:voda), nakon miješanja 60 minuta.

2. Električna vodljivost (EC) je mjerena prema europskoj normi EN 13038: 2009 (EN 2009) u suspenziji od 60 mL svježeg uzorka mućkanog na mućkalici 60 minuta u 300 mL deionizirane vode, tj. u volumnom omjeru 1:5 (uzorak:voda). Električna provodljivost je pokazatelj udjela vodotopivih elektrolita u analiziranom uzorku.

3. Ukupni sadržaj organske tvari i pepela određeni su sušenjem 5 g uzorka na 103 ± 2 °C najmanje 4 h, i uzastopnim žarenjem uzorka na 450 ± 10 °C na najmanje 6 h (EN 2011). Uzorak je žaren u peći za žarenje, s prvim vaganjem mase uzorka nakon 6 h, a zatim nakon svakog dodatnog sata žarenja do konstantne masa, tj. kada je razlika između dva uzastopna vaganja bila <0,01 g.

4. Analiza sadržaja organskog ugljika provedena je vlažnom destrukcijom, pri čemu je izvagano 50 mg suhog uzorka, preliveno s 5 mL 0,27 M dm^{-3} $K_2Cr_2O_7$ i 7,5 mL koncentrirane H_2SO_4 i razarano 30 minuta na bloku za razaranje na 135 °C (ISO, 1998.). Nakon razaranja, uzorak je kvantitativno prenesen u odmjerne tikvice, dopunjene deioniziranom vodom do 100 mL, prebačene u epruvete, centrifugirane 10 minuta na 2000 rcf i filtrirane. U uzorcima i nizu standardne otopine glukoze, mjerene su vrijednosti transmisije na 585 nm spektrofotometrom, a sadržaj (koncentracija) organskog ugljika izražen je u %.

5. Određivanje dušika temelji se na prevođenju dušika iz svježeg uzoraka u amonijski oblik i destilaciji u predložak borne kiseline. Količina dušika u analiziranom uzorku je izračunata iz potrošnje klorovodične kiseline u titraciji (ISO, 1995., modificirana metoda) i izražena u % N u svježoj tvari.

6. C/N omjer je izračunat iz podatke o sadržaju ukupnog ugljika u suhoj tvari i ukupnog dušika u svježoj tvari prema sljedećim formulama:
% C u svježoj tvari = (% C u suhoj tvari x % suhe tvari) ÷ 100
C/N = % C u svježoj tvari ÷ % N u svježoj tvari

7. Za određivanje koncentracije teških metala (Zn, Cu, Ni, Mo, Cr, Cd, Hg, i Pb) korištena je metoda prema Matusiewicz i sur. (1989.), pripremom osnovne otopine razaranjem suhog uzorka digestijom sa smjesom koncentrirane dušične i klorovodične kiseline u omjeru 1:3. Koncentracije teških metala su izmjerene pomoću Perkin Elmer Optima 5300 DV induktivno spojene plazme optičkom emisijskom spektrometrijom (ICP-OES, Waltham, MA, SAD). Koncentracije ovih elemenata izražene su u mg kg^{-1} suhe tvari uzorka.

8. LOI (eng. loss on ignition) je metoda koja se koristi u analizi tla i drugih materijala za određivanje gubitka mase pri žarenju na visokim temperaturama. Ova metoda pomaže identificirati količinu organske tvari i karbonata u uzorku, jer se pri žarenju ovi sastojci razgrađuju ili gube. LOI je određen u uzorcima tla koji su prethodno osušeni u pećnici, te zatim žareni u zatvorenoj pećnici na temperaturi od 550 ± 25 °C tijekom minimalno 3 sata. Nakon žarenja, uzorak je hlađen 30 minuta u desikatoru prije mjerenja mase. Vrijednosti organskog udjela izračunate su na temelju udjela gline (Nelson i Sommers, 1982.). Rezultata LOI analize izražava se u postotocima, a veći postotak znači veći gubitak mase pri žarenju što ukazivuje na veću koncentraciju organske tvari i karbonata u uzorku tla.

4.2. Analiza biljnog materijala

U uzorcima biljnog materijala (soja, matovilac i amarant) izmjerene su koncentracije selena i koncentracije bioaktivnih komponenti (prolin, ukupni fenoli, lipidna peroksidacija i vitamin C).

1. Sadržaj selena izmjeren je nakon digestije u koncentriranoj ultra čistoj HNO_3 i H_2O_2 (omjer 3:1) postupnim zagrijavanjem do 250 °C pomoću Milestone Ultra clave peći 1 h i 15 min. Ekstrakcija je provedena prema modificiranoj metodologiji Matusiewicza i sur. (1989.). Koncentracija selena određena je pomoću Perkin Elmer Sciex Elan induktivno spojene plazme masenom spektrometrijom (ICP-MS). Korišten je standardni referentni materijal (SRM) SRM 2709 (NIST - National Institute of Standards & Technology and Certificate 2011).

2. Sadržaj slobodnog prolina (PRO) u biljnom tkivu određen je prema Batesa i sur. (1973.). Tkivo je homogenizirano u tekućem dušiku i izvagano (oko 0,2 g) u plastične epruvete. Prolin je ekstrahiran iz tkivica s 10 mL sulfosalicilne kiseline (3 %). Tkivo je odvojeno od supernatanta centrifugiranjem na 3500 rcf na 4 °C tijekom 15 minuta. 2 mL supernatanta, 2 mL kiselog ninhidrinskog reagensa (2,5 %) i 2 mL ledene octene kiseline je bilo dodano. Tako pripremljena smjesa se miješa na vrtložnoj mućkalici i grije se 1 h u vodenoj kupelji na 95–98 °C. Nakon zagrijavanja, smjesa je stavljena u ledenu vodu i svakom uzorku je dodano 4 mL toluena. Uzorci su miješani 20 s i ostavljeni su na sobnoj temperaturi dok se gornji sloj toluena nije odvojio od prolina, donjeg, vodenog sloja. Standardna krivulja napravljena je pomoću osnovnih standardnih otopina L-prolina koncentracije 20 µg PRO mL^{-1} u rasponu koncentracije od 0-20 µg PRO mL^{1}. Koncentracija prolina u toluenu je određena mjerenjem apsorbancije na 520 nm, te je izračunata iz standardne krivulje s poznatim koncentracijama prolina, koje su tretirane na isti način kao što su bili uzorci. Konačni rezultati izraženi su kao µM prolina g^{-1} svježe tvari.

3. Sadržaj ukupnih fenola u biljnom tkivu određen je spektrofotometrijskom metodom s Folin–Ciocalteu reagensom prema Singletonu i Rossiju (1965.) Fenoli su ekstrahirani s 2,5 mL etanola (95 %) na 20 °C tijekom 48 sati s oko 0,1 g tkiva maceriranog u tekućem dušiku. Nakon ekstrakcije, homogeniziraju se i bivaju centrifugirani na 10000 rcf na 4°C 10 minuta. Na određeni volumen supernatanta (ovisno o očekivanim vrijednostima koncentracije fenola), oko 1,5 mL destilirane vode (ukupni volumen supernatanta i vode je 1,6 mL), 100 µL Folin–Ciocalteu reagensa, i dodano je 300 µL Na_2CO_3 (zasićene otopine). Ukupno 2 mL reakcijske smjese je miješana na vrtložnoj mućkalici i inkubirana u vodenoj kupelji na 37 °C tijekom 60 minuta. Sadržaj fenola u inkubiranoj i ohlađenoj smjesi određen je mjerenjem apsorbancija na

spektrofotometru na valnoj duljini od 765 nm. Koncentracija fenola izračunata je iz standardne krivulje s poznatim koncentracijama galne kiseline (GA) u raspon od 0,05 do 0,5 mg GA mL^{-1}. Konačni sadržaj fenola izražen je kao mg GA g^{-1} svježe tvari. Uzorci standardne otopine, kao i otopine uzorka, su pripremljene u tri primjerka.

4. Lipidna peroksidacija provedena je prema Heathu i Packeru (1968.). Biljno tkivo je usitnjeno tekućim dušikom u fini prah i odvagano ko 0,2 g. Biljno tkivo ekstrahirano je s 1 mL trikloroctene kiseline (0,1 %). Nakon centrifugiranja na 6000 rcf pri 4 °C tijekom 5 minuta, 1 mL tiobarbiturne kiseline (0,5 %) u trikloroctenoj kiselini (20 %) dodana je u 0,5 mL supernatanta. Smjesa je zagrijana u vodenoj kupelji na 95 °C 30 min, a zatim ohlađen. Nakon hlađenja, supernatant je izoliran centrifugiranjem na 18 000 rcf tijekom 15 minuta na 4 °C. Apsorbancija supernatanta uzorka mjeren je spektrofotometrijski na valnim duljinama od 532 i 600 nm. Tiobarbiturna kiselina (0,5 %) u trikloroctenoj kiselini (20 %) korištena je kao slijepa proba. Koncentracija produkata lipidne peroksidacije izračunata je pomoću koeficijenta molarne ekstinkcije (ε = 155 mM^{-1} cm^{-1}) i izraženo u ekvivalentima tiobarbiturne kiseline u jedinicama nM tiobarbiturne kiseline g^{-1} svježe tvari.

5. Koncentracija vitamina C određena je prema modificiranom Roe i Kuether protokolu (1943.). Korištene su topina TCA 13,3 % (trikloroctena kiselina), H_2SO_4 65 % (sumporna kiselina), DNPH 2 % 2,4 dinitrofenilhidrazin (2 g DNPH, 230 mg tiouree, 270 g CuSO4 i 100 mL 5M H_2SO_4) i askorbinska kiselina (osnovna otopina) za izradu kalibracijskih krivulja (0,1 mL^{-1}). Biljno tkivo je macerirano tekućim dušikom i odvagano 0,2 g usitnjenog tkiva u plastične epruvete od 15 mL. Zatim je 250 µL biljnog soka pipetirano u 15 mL plastične epruvete s čepon na navoj, te je izvagana masa pipetiranog soka. Zatim je dodano 10 mL destilirane vode odnosno do 15 mL do oznake u epruveti. Nakon ekstrakcije, uzorci su centrifugirani na 15 minuta na 4000 rcf pri 4 °C. Nakon toga, 150 µL je pipetirano u dvije epruvete (2 mL) za uzorak i praćenje. Za potrebe epruveta, 175 µL destilirane vode, 100 µL 13,3 % TCA i 75 µL uzorka DNPH, a pripremljeni ekstrakti su dodani inkubirano na 37 °C 3 h. Nakon inkubacije uzorka i sljedeće probe, dodano je 1000 µL H_2SO_4 i 75 µL DNPH. Standardi su pripremljeni na isti način kao i uzorak, pa je prije inkubacije dodan DNPH. Pripremljene su otopine askorbinske kiseline u koncentracijama od 0, 25, 50, 75, 100, 125,

150, 175, 200, 250 i 275 g mL^{-1}. Svi uzorci su vorteksirani te je izmjerena apsorbancija na 520 nm u staklenoj kiveti 1x1 cm širine na Varianu Cary 50 UV-Vis spektrofotometru.

6. Metoda određivanja antioksidativne moći putem redukcije željeza - FRAP (eng. Ferric Reducing Antioxidant Power), a koristi za određivanje ukupne antioksidativne aktivnosti. Provedena je prema Benzie i Strain (1996.) s modifikacijama. Liofilizirano biljno tkivo je izvagano (0,2 g) u plastičnu epruvetu i dodano 10 mL 96 % etanola. Uzorak se zatvori i mućka 20 minuta na mućkalici, a zatim centrifugira 15 minuta na 6000 G ispod 4 °C. Supernatant je prenesen Pasteurovom pipetom, stavljen u odmjernu tikvicu od 10 mL i zatim napunjena metanolom do oznake. Sadržaj odmjerne tikvice prebačen je u posude i odmah zamrznut. Otopljeni pelet s 4 mL n-heksana je centrifugiran, a supernatant je odvojen u odmjernoj tikvici od 10 mL i zamrznut. Pripremljen je FRAP reagens s 200 mL acetatnog pufera, 20 mL otopine TPTZ i 20 mL otopine $FeCl_3$. Pomiješane otopine stavljene su u vodenu kupelj na 37 °C. Za spektrofotometrijsko mjerenje na 593 nm, pripremljena je otopina s 1 mL FRAP reagensa i 100 µL metanola te standardi sa 100 µL standardne otopine + 1 mL FRAP otopine, promiješani vortex mučkalicom, inkubirani 4 minute na 37 °C u vodenoj kupelji.

4.3. Uzgoj "*baby leaf*" povrća u plutajućem hidroponskom sustavu

Za plutajući hidroponski sustav korišteni su bazeni dimenzije 80 x 120 cm i dubine 30 cm, s dubinom hranjive otopine 20 cm, rezultirajući ukupnim volumenom od 200 litara hranjive otopine po bazenu. U bazenima su korištene zračne pumpe za dodotok zraka. Hranjiva otopina za plutajući hidroponski sustav pripremljena je prema Hoaglandovom receptu, pri čemu je koncentracija svih esencijalnih elemenata smanjena za 50 %, kako je preporučeno za uzgoj lisnatog povrća. U eksperimentu su korišteni sjemenke matovilca (*Valerianella locusta*) od nizozemskog proizvođača RIJK ZWAAN, sorte STYLUS RZ i amaranta (*Amaranthus caudatus*) od REIN SAAT (Austrija). Za pripremu osnovne hranjive otopine korištene su različite soli od tvrtki Gram-mol d.o.o. (Hrvatska), TTT d.o.o. (Hrvatska) ili Haifa Chemicals (Haifa, Izrael) . Vegetacijski eksperiment bio je dizajniran prema potpuno nasumičnom dizajnu s 4 biološke replikacije. Svaka biološka replikacija sastojala se od 20 biljaka, rezultirajući multifaktorijalnim eksperimentom. U svako sadno mjesto posađene su tri sjemenke matovilca ili amaranta, a nakon klijanja izvršeno je

prorjeđivanje kako bi se zadržale dvije biljke po sadnom mjestu. To je rezultiralo s ukupno 80 biljaka po spremniku, što je u 2 spremnika činilo 160 biljaka po eksperimentalnoj varijanti. Spremnici od polistirena prethodno su napunjeni smjesom vermikulita i komercijalnog supstrata (Klassmann Potgrond P) u volumnom omjeru 1:1. Amarant je proklijao 3-4 dana nakon sjetve s ukupnim razdobljem klijanja od 6-7 dana. Matovilac je počeo klijati 5-6 dana nakon sjetve, a klijanje se nastavilo do 10. dana nakon sjetve. Spremnici s biljkama preneseni su na plutajući hidroponski sustav 16. dana nakon sjetve. Tijekom eksperimenta, temperatura u stakleniku održavana je u rasponu od 19,5 do 22 °C tijekom dana i od 14,5 do 16 °C noću.

4.4. Sinteza selenovih nanočestica

Selenove nanočestice su obložene ili stabilizirane polisorbatom (PS) i huminskom kiselinom (HA). Veličina nanočestica dobivena korištenjem polisorbata bila je 72,6 ± 8,1 nm (nanometara), sa zeta potencijalom od -29,1 ± 2,4 ζ mV^{-1}. Nasuprot tome, čestice sintetizirane s huminskom kiselinom imale su radijus od 74,20 ± 5,2 nm, a njihov zeta potencijal bio je -31,2 ± 0,6 ζ mV^{-1}. Sinteza selenovih nanočestica temelji se na redukciji natrijevog selenita (Na_2SeO_3) u njegov elementarni oblik dodatkom askorbinske kiseline kao redukcijskog sredstva. Sinteza obuhvaća tri faze: redukciju, nukleaciju i rast te stabilizaciju. U fazi nukleacije ioni selena (Se^{+4}) reduciraju se u elementarni oblik Se (0), koji potom formira jezgre. Te jezgre služe kao temelj za daljnji rast nanočestica. Površinski agensi zatim vežu i stabiliziraju nanočestice, pri čemu se huminska kiselina (HA) i polisorbat (Tween 20 ili PS) koriste kao površinski stabilizatori. Nanočestice obložene huminskom kiselinom stabilizirane su putem elektrostatskih interakcija, dok polisorbat (Tween 20) sterički stabilizira selenove nanočestice (unatoč slabom negativnom naboju).

1. Sinteza selenovih nanočestica pomoću polisorbata (PS/Tween 20). Sinteza selenovih nanopartikula, stabiliziranih polisorbatom, slijedila je modificirani postupak prema Vahdati i Moghadam (2020.). U odmjernu tikvicu ukupnog volumena reakcijske smjese 100 ml pažljivo je dodano 90 ml natrijevog selenita (Na_2SeO_3) koncentracije 0,0012 M. Reakcijska smjesa miješana je na magnetskoj miješalici pri 400 o min^{-1}. Nakon toga, kap po kap dodano je 2 ml askorbinske kiseline. Nakon toga, u reakcijsku smjesu dodano je 0,01 mL (10 µL) polisorbata (Tween 20). Ovaj postupak dodavanja askorbinske kiseline i polisorbata (Tween 20) ponovljen je dok nije

dodano ukupno 10 mL askorbinske kiseline. Smjesa je miješana 20 minuta. Nakon toga, reakcijska smjesa podvrgnuta je centrifugiranju tijekom 20 minuta pri 19802 rcf (12000 o/min) u *Falcon* epruvetama od 50 mL. Kao rezultat toga, nanočestice su se istaložile na dnu *Falcon* epruvete. Nastali precipitat nanočestica zvađen je pipetom. Bistar i bezbojan supernatant značio je da nema preostalih nanočestica. U suprotnom, preostala supernatantna tekućina bila je podvrgnuta dodatnoj centrifugi kako bi se omogućila potpuna izolacija nanočestica.

2. Sinteza selenovih nanočestica pomoću huminske kiseline (HA). Sinteza selenovih nanočestica stabiliziranih huminskom kiselinom provedena je prema modificiranim metodama Ye i sur. (2017.), Vahdati i Moghadam (2020.) i Selmani i sur. (2020.). Ukupni volumen reakcijske smjese bio je 30 mL. U odmjernu tikvicu je dodano 10 ml natrijevog selenita (Na_2SeO_3) koncentracije 0,001 M zajedno s 10 mL huminske kiseline (0,001 %). Smjesa je miješana 5 minuta na magnetskoj mješalici pri 450 o min^{-1}. Nakon toga dodavana je kap po kap askorbinska kiselina (0,01 M) uz kontinuirano miješanje. Nakon 20 minuta reakcije, smjesa je centrifugirana 15 minuta pri 15000 rcf u *Falcon* epruvetama od 50 mL. Nastali precipitat nanočestica prikupljen je pipetom. Supernatant je bio podvrgnut dodatnoj centrifugi sve dok nije bio poptuno bezbojan i proziran, što je značilo da u supernatantu nije više bilo nanočestica.

4.5. Statistička obrada podataka

1. Statistička analiza provedena je programskim jezikom R (verzija 4.0.4) (Team R Core 2021). Dvosmjerna analiza varijance tipa II (ANOVA) provedena je s dva glavna efekta, a pretpostavljena je dvosmjerna interakcija. Razlike između tretmana smatrane su značajnim na razini vjerojatnosti $p < 0,05$ u Fisherovom LSD testu. Također je provedena trosmjerna analiza varijance tipa III (ANOVA) s tri glavna efekta i sve pretpostavljene interakcije. Razlike između srednjih vrijednosti tretmana smatralo se značajnim na razini vjerojatnosti $p < 0,05$ u Fisherovom LSD testu. Provedena je jednosmjerna ANOVA kako bi bio prikladan prikaz svih kombinacija efekata u stupčastim grafikonima. Razlike između srednjih vrijednosti uspoređeni su korištenjem Fisherove najmanje značajne razlike pri $p = 0,05$ razini vjerojatnosti.

2. Provedena je meta-analiza postavljanjem linearnog mješovitog modela (Bates i sur., 2015.). Za postavljanje modela korišteni su podaci normalizirani kao porast sadržaja Se za svaki 1 g porasta primjene Se. Ukupno osam modela postavljeno je u R programskom paketu lme4 (Bates i sur., 2015.) sa svim nasumičnim (*random*) učincima i prekid toka linije regresije (*intercept*), uz pretpostavku nestrukturirane varijance greške (homogena varijanca reziduala). Analizirani modeli imali su osam kombinacija sljedećih čimbenika: vrsta primjene Se, biljna vrsta, oblik Se i njihove interakcije. Konačni model odabran je na temelju najnižih ocjena Akaikeovog i Bayesijskog informacijskog kriterija (AIC i BIC). Akaikeov i Bayesijski informacijski kriteriji koriste se za objektivni odabir modela prema porastu informativnosti u odnosu na objašnjenu varijancu. Tri tipa modela korištena su za interpretaciju rezultata. Prvi je tip modela bio s prekidom toka linije regresije koji se mijenjao u zavisnosti od načina aplikacije Se i oblika Se, drugi je bio s prekidom toka linije regresije koji se mijenjao kao funkcija biljne vrste i oblika Se, a treći tip je bio tip eksperimenta s terminom slučajnog prekida toka linije regresije s fiksnom srednjom vrijednosti. Koeficijenti pojedinih modela ekstrahirani su pomoću naredbe lme4 coef().

3. PCA (Principal Component Analysis) analize podataka provedene su pomoću R softvera verzije 4.0.2. (Team R Core 2021). Analiza glavnih komponenti (PCA) korištena je za pregled skupa podataka pronalaženjem latentnih (sintetičkih) varijabli, tj. glavnih komponenti (PC) napravljenih od linearnih kombinacija varijabli iz izvornog skupa podataka. Pojedinačne PC-a predstavljaju linearne statističke modele s rezultatima (udaljenost od početka PC-a za svaku podatkovnu točku) opterećenja (varijabilni doprinosi za svaki PC) i rezidualima. Podaci korišteni za PCA bili su podaci analize tala sa 6 različitih lokaliteta (Sarajevo, Banja Luka, Novi Sad, Mostar, Osijek i Prud). Prikupljeni su rezultati za: pH, KIK, LOI, DOC, LOI/TC, TC, TN, ukupne koncentracije Na, K, Ca, Mg, te kupne koncentracije i vodotopive frakcije Se, Cd, Zn, Ni, Fe, Cr.

4. Iste varijable kao za PC analizu također su korištene u modelu penalizirane regresije u okviru parcijalnih najmanjih kvadrata (PLS), implementiranom u R programskom paketu pls. Cilj PLS analize je, slično kao i PCA, objasniti varijabilnost u skupu podataka projekcijama na latentne varijable. Međutim, postoji značajna razlika u PLS pristupu, koji istodobno objašnjava varijabilnost u nezavisnim kao i u zavisnim varijablama. PLS model je kalibriran u postupku validacije izostavljanjem (unakrsna validacija), pri čemu se za parametrizaciju modela uzima n-i

uzoraka, dok se I koristi za predviđanje. Proces se ponavlja sve dok ne postoji n predviđenih vrijednosti, koje se zatim povezuju s izvornim podacima i koriste za izračunavanje srednje kvadratne pogreške predviđanja (RMSEP). Broj komponenti korištenih u modelu odabran je na temelju najniže vrijednosti RMSEP u postupku validacije. Dodatno, latentne varijable (komponente) iz kalibriranog modela koji je pokazao najbolje rezultate korištene su u mješovitom modelu kao fiksne kovarijante, zajedno s lokacijom, tretirane kao slučajni učinak uz pretpostavku homogene varijance u paketu R/lme4 (Bates i sur., 2015.).

5. REZULTATI ISTRAŽIVANJA S RASPRAVOM

Ciljevi ovog istraživanja usmjereni su prema tri glavna područja. Prvi cilj istraživanja fokusiran je na utvrđivanje mogućnosti kreiranja modela raspoloživosti selena u tlu. Ovo uključuje razvoj regresijskog modela i upotrebe multivarijatnih metoda kako bi se analizirala ovisnost ukupnih koncentracija selena u tlu i fizikalno-kemijskih svojstva tla. Realizacija ovog cilja ostvarena je rezultatima istraživanja prikazanih u znanstvenim radovima "Važnosti agronomske biofortifikacije selenom kod žitarica – meta-analiza" i "Modeliranje utjecaja različitih svojstava tla na vodotopivost selena u tlima jugoistočne Europe".

Drugi cilj istraživanja usmjeren je na fiziološki odgovor klijanaca soje pod uvjetima stresa izazvanog sušom nakon naklijavanja sjemena koje je u prethodnoj vegetaciji biofortificirano selenom. Rezultati ovog istraživanja doprinose razumijevanju adaptivnih mehanizama biljaka pod stresom, a opisani su u radu "Utjecaj osmotskog stresa na fiziološki odgovor klijanaca soje iz sjemena biofortificiranog selenom".

Treći cilj istraživanja bio je utvrditi efikasnost biofortifikacije lisnatog povrća selenom u hidroponskom i supstratnom načinu uzgoja što je uključilo analizu biofortifikacije matovilca uz upotrebu vermikomposta kao medija za uzgoj, što je prikazano u radu "Biofortifikacija selenom uz istovremenu upotrebu vermikomposta kao medija u uzgoju matovilca (*Valerianella locusta* L. Laterr)." Istraživanje biofortifikacije matovilca i amaranta natrijevim selenatom i nanoselenom u hidroponskom uzgoju opisano je u rukopisu "Biofortifikacija matovilca (*Valerinaella locusta* L.) i amaranta (*Amaranthus caudatus* L.) natrijevim selenatom i nanoselenom u hidroponskom uzgoju".

5.1. Razvoj modela raspoloživosti selena u tlu na temelju izmjerenih ukupnih koncentracija i fizikalno-kemijskih svojstava tla

5.1.1. Važnost agronomske biofortifikacije selenom kod žitarica – meta-analiza

Selen (Se) je bitan mikroelement neophodan većini živih organizama te se pojavljuje u obliku organskih i anorganskih spojeva u vodenim sustavima, tlima, biomasi i atmosferi. Osim što je esencijalan za ljude i životinje, Se ima korisne učinke i na biljke te uglavnom sudjeluje u antioksidacijskim procesima i poticanju rasta kod biljka. Kod ljudi Se sudjeluje u mehanizmima antioksidativnog odgovora organizma, detoksifikaciji teških metala i regulaciji reproduktivnog i

imunološkog sustava, te osigurava ispravno djelovanje štitnjače. Nedostatak selena u prehrani predstavlja globalni problem, a razina Se u tlima generalno odražava njegovu prisutnost u hrani i dostupnost ljudima. Biljke su značajan izvor Se u prehrani ljudi, a biofortifikacija je ključna strategija za povećanje sadržaja Se u jestivim dijelovima biljaka. Agronomska biofortifikacija pruža učinkovit pristup povećanju sadržaja Se u jestivim poljoprivrednim proizvodima primjenom gnojiva obogaćenih selenom u tlo ili putem folijarne aplikacije. Najčešće konzumirane žitarice u prehrani ljudi su pšenica, riža, kukuruz i ječam, stoga su one najznačajnije za agronomsku biofortifikaciju. Ovaj pregled se usredotočuje na sažetak najučinkovitijih oblika i metoda primjene Se na najčešće konzumiranim žitaricama putem agronomske biofortifikacije, temeljenih na meta-analizi iz dostupne literature. Vrsta primjene je prvi važan faktor koji značajno povećava sadržaj selena u biljkama, a većina istraživanja je pokazala da je folijarna aplikacija učinkovitija metoda gnojidbe, iako je gnojidba tla popularnija (Winkel i sur., 2015.; Gupta i Gupta, 2017.; Lyons, 2018.; Petković i sur., 2019.; Izydorczyk i sur., 2020.). Folijarna primjena dovodi do efikasnije apsorpcije selena u usporedbi s aplikacijom na tlo, niti ima ostataka selan u tlu. Tehnike folijarne aplikacije koriste minimalne količine soli selena i najučinkovitiji su, sigurni i ekonomski prihvatljiviji način povećanja sadržaja selena u usjevima poput pšenice (Pezzarossa i sur., 2012.). Folijarna aplikacija selena je učinkovitija metoda biofortifikacije jer nema prijenosa iz korijena u nadzemni dio biljke, a tlo može djelovati kao značajan rezervoar selena (Winkel i sur., 2015.). Također, dostupnost primijenjenog selena biljkama može se brzo smanjiti u tlu (Wang i sur., 2013.). Oblik selena je drugi važan parametar za učinkovitu biofortifikaciju. Većina istraživanja je pokazala da je selenat (gdje se selen nalazi u najvišem oksidacijskom stanju, +6) najučinkovitiji oblik kada se primjenjuje na tlo i obično je učinkovitiji od selenita (Se+4) kada se primjenjuje folijarno (Lyons, 2018.). Veća učinkovitost selenata proizlazi iz brže apsorpcije Se(VI) i translokacije iz korijena u stabljiku i listove, kao i brže transformacije u organski oblik, dok je selenit(IV) lakše adsorbiran u tlo, što otežava njegovu apsorpciju (Izydorczyk i sur., 2020.). Selenat se lako raspoređuje od korijena prema nadzemnom dijelu biljke, dok se selenit ili njegovi metabolički produkti obično nakupljaju u korijenu (Li i sur., 2008.). Može se očekivati da većina primijenjenog selenata ostaje u anorganskom obliku u nadzemnom dijelu biljke, dok se većina dodanog selenita ugrađuje kao organski selen, odnosno u obliku selenskih aminokiselina i selenskih proteina (Cartes i sur., 2005.). Jedno istraživanje je pokazalo da je učinkovitost folijarne gnojidbe u biljkama riže veća s natrijevim selenitom u usporedbi sa selenatom (Lidon i sur., 2018.).

Koncentracije Se u zrnu riže u pravilu su veće u usporedbi s kukuruzom i pšenicom *(Dinh i sur.*, 2018.), a uz to se sorte riže mogu podijeliti na one s većim i manjim sadržajem i akumulacijom Se (Zhang i sur., 2019.). Kao osnovna hrana, riža je izvrstan izvor energije, s prevladavajućom potrošnjom u više od 30 zemalja, što je oko 80 % dnevnog unosa kalorija za otprilike tri milijarde ljudi (Lidon i sur., 2018.), što je čini značajnom za agronomsku biofortifikaciju Se. Studije su pokazale da folijarna aplikacija selenata i selenita povećava sadržaj Se u zrnu pšenice (Poblaciones i sur., 2014.; Ducsay i sur., 2016.; De Vita i sur., 2017.; Wang i sur., 2020.). Folijarna aplikacija Se dala je dobre rezultate i najčešće je korišten način biofortifikacije pšenice. Primjena Se u tlo rezultira nižim povećanjem Se u jestivim dijelovima biljaka. Dugotrajna uporaba može biti toksična za okoliš, stoga uporabu gnojiva sa Se trebala provoditi pažljivo bez štetnog utjecaja na okoliš (Gupta i Gupta, 2017.). Agronomska biofortifikacija učinkovito povećava i sadržaj Se u zrnu kukuruza (Chilimba i sur., 2012.; Nawaz i sur., 2016.; Bocchini i sur., 2018.). Translokacija, nakupljanje, distribucija i metabolizam Se u biljkama kukuruza ovise o obliku Se koji se koristi (Longchamp i sur., 2015). Ječam je također značajna žitarica, po zastupljenosti odmah nakon riže, pšenice i kukuruza (Narwal i sur., 2020.). Dvoredi ječam mogao bi biti prikladan za biofortifikaciju sa Se (Rodrigo i sur., 2014.) folijarnom aplikacijom natrijevog selenata odmah nakon cvatnje ili tijekom faze klijanja u procesu sladovanja, što rezultira nakupljanjem Se u hrani (Gibson i sur., 2006.). Analiza rezultata istraživanja biofortifikacije Se pokazala je značajnu varijaciju u metodama, vremenima i oblicima primjene kod četiri glavne žitarice (Tablica 1). Zbog pozitivnog otklona od srednje vrijednosti, uz manji broj koji su imali jako negativan otklon od srednje vrijednosti, pa je zbog toga, raspodjela vrijednosti pomaknuta prema desno kod riže zbog ograničenog broja studija nakon Premaranthe i sur. (2012.). Međutim, povećanje koncentracije Se se kretalo od 0,025 μg Se po 1 g primijenjenog selena (Premarathna i sur., 2012.) do 0,42 μg Se po 1 g primijenjenog Se (Reis i sur., 2018.) u poljskim pokusima i do 6,34 μg Se po 1 g primijenjenog Se u eksperimentu u kontroliranim uvjetima (Wang i sur., 2013.). U kukuruzu, povećanja koncentracija u μg Se po 1 g primijenjenog Se varirale su od 0,091 (Wang i sur., 2012.) do 0,92 (Ngigi i sur., 2019.). Rezultati na ječmu pokazali su znatno niže učinkovitosti kada se koristio selenit (Winter i sur., 1993.; Rodrigo i sur., 2014.), s vrijednostima od 0,0422 do 0,78 μg Se po 1 g primijenjenog Se. Kod pšenice, najniža vrijednost od 0,1125 μg Se po 1 g primijenjenog Se utvrđena je za selenit u istraživanju Ducsay i sur.. (2016.), dok je najviša vrijednost od 0,853 ostvarena u istraživanju De Vita i sur. (Ducsay i sur., 2016.; De Vita i sur., 2017.).

Tablica 1. Različiti oblici selena (Se) i metode primjene kod glavnih žitarica (riža, ječam, pšenica i kukuruz)

Biljna vrsta	Vrsta eksperimenta	Način primjene	Vrijeme primjene	Oblik Se	g Se ha^{-1}	Se u zrnu (kontrola) µg kg^{-1}	Se u zrnu (tretman) µg kg^{-1}	Povećanje	Povećanje po 1 g dodanog Se	Literatura
Riža	poljski pokus	tlo	formiranje cvati	selenit	30	76	59	0,78	0,03	(Premarathna i sur., 2012.)
Riža	Poljski pokus	tlo	formiranje cvati	selenat	30	76	79	1,04	0,03	(Premarathna i sur., 2012.)
Riža	Poljski pokus	tlo	formiranje cvati	selenit	30	86	85	0,99	0,03	(Premarathna i sur., 2012.)
Riža	poljski pokus	tlo	formiranje cvati	selenat	30	86	92	1,07	0,04	(Premarathna i sur., 2012.)
Riža	poljski pokus	tlo	formiranje cvati	selenit	30	97	82	0,85	0,03	(Premarathna i sur., 2012.)
Riža	poljski pokus	tlo	formiranje cvati	selenat	30	97	92	0,95	0,03	(Premarathna i sur., 2012.)
Riža	poljski pokus	folijarno	formiranje cvati	selenit	30	76	273	3,59	0,12	(Premarathna i sur., 2012.)
Riža	poljski pokus	folijarno	formiranje cvati	selenat	30	76	150	1,97	0,07	(Premarathna i sur., 2012.)
Riža	poljski pokus	folijarno	formiranje cvati	selenit	30	86	122	1,42	0,05	(Premarathna i sur., 2012.)
Riža	poljski pokus	folijarno	formiranje cvati	selenat	30	86	105	1,22	0,04	(Premarathna i sur., 2012.)
Riža	poljski pokus	folijarno	formiranje cvati	selenit	30	97	136	1,4	0,05	(Premarathna i sur., 2012.)
Riža	poljski pokus	folijarno	formiranje cvati	selenat	30	97	176	1,81	0,06	(Premarathna i sur., 2012.)

Riža	poljski pokus, bez obrade tla	tlo	prilikom sjetve	selenat	25	30	320	10,67	0,43	(Reis i sur., 2018.)
Riža	u komori za rast biljaka	folijarno	7 puta tokom vegetacije	selenit	0,53	30	100	3,33	6,35	(Wang i sur., 2013.)
Riža	u komori za rast biljaka	folijarno	7 puta tokom vegetacije	selenit	10,5	30	1540	51,33	4,89	(Wang i sur., 2013.)
Riža	u komori za rast biljaka	folijarno	7 puta tokom vegetacije	selenit	21	30	1560	52	2,48	(Wang i sur., 2013.)
Kukuruz	poljski pokus	tlo	prije sjetve	selenit	150	3,7	51	13,78	0,09	(Wang i sur., 2013.)
Kukuruz	poljski pokus	folijarno	metličanje i tjedan nakon svilanja	selenit	11	11	96	8,73	0,79	(Wang i sur., 2013.)
Kukuruz	poljski pokus	tlo	po tlu prije sjetve	selenat	5	34	41,66	1,23	0,25	(Ngigi i sur., 2019.)
Kukuruz	poljski pokus	tlo	po tlu prije sjetve	selenat	10	34	68,33	2,01	0,2	(Ngigi i sur., 2019.)
Kukuruz	poljski pokus	tlo	po tlu prije sjetve	selenat	20	34	92,66	2,73	0,14	(Ngigi i sur., 2019.)
Kukuruz	poljski pokus	folijarno	izduživanje stabljike	selenat	5	34	156,66	4,61	0,92	(Ngigi i sur., 2019.)
Kukuruz	poljski pokus	folijarno	izduživanje stabljike	selenat	10	34	205,33	6,04	0,6	(Ngig i sur., 2019.)
Kukuruz	poljski pokus	folijarno	izduživanje stabljike	selenat	20	34	305,66	8,99	0,45	(Ngigi i sur., 2019.)
Ječam	poljski pokus	tlo	prije sjetve	selenit	20	45	57	1,27	0,06	(Winter i sur., 1993.)
Ječam	poljski pokus	tlo	prije sjetve	selenat	20	33	391	11,85	0,59	(Winter i sur., 1993.)
Ječam	poljski pokus	tlo	prije sjetve	selenit	40	45	76	1,69	0,04	(Winter i sur., 1993.)
Ječam	poljski pokus	tlo	prije sjetve	selenat	40	33	959	29,06	0,73	(Winter i sur., 1993.)

Ječam	poljski pokus	folijarno	završetak busanja	selenat	10	111,7	880	7,88	0,79	(Rodrigo i sur., 2014.)
Ječam	poljski pokus	folijarno	završetak busanja	selenat	20	111,7	1113,9	9,97	0,5	(Rodrigo i sur., 2014.)
Ječam	Poljski pokus	folijarno	završetak busanja	selenit	10	111,7	270	2,42	0,24	(Rodrigo i sur 2014.)
Ječam	poljski pokus	folijarno	završetak busanja	selenit	20	111,7	345,5	3,09	0,15	(Rodrigo i sur., 2014.)
Pšenica	poljski pokus	folijarno	busanje	selenit	10	66,6	153,6	2,31	0,23	(Poblaciones i sur., 2014.)
Pšenica	poljski pokus	folijarno	busanje	selenit	20	66,6	254,8	3,83	0,19	(Poblaciones i sur., 2014)
Pšenica	poljski pokus	folijarno	busanje	selenit	40	66,6	430,4	6,46	0,16	(Poblaciones i sur., 2014.)
Pšenica	poljski pokus	folijarno	busanje	selenat	10	66,6	266,8	4,01	0,4	(Poblaciones i sur., 2014.)
Pšenica	poljski pokus	folijarno	busanje	selenat	20	66,6	820	12,31	0,62	(Poblaciones i sur., 2014.)
Pšenica	poljski pokus	folijarno	busanje	selenat	40	66,6	1383,2	20,77	0,52	(Poblaciones i sur., 2014.)
Pšenica	poljski pokus (mala parcela)	folijarno	pojava 2. nodija	selenit	10	32	51	1,59	0,16	(Ducsay i sur., 2016.)
Pšenica	poljski pokus (mala parcela)	folijarno	pojava 2. nodija	selenit	20	32	72	2,25	0,11	(Ducsay i sur., 2016.)
Pšenica	poljski pokus (mala parcela)	folijarno	pojava 2. nodija	selenat	10	32	190	5,94	0,59	(Ducsay i sur., 2016.)
Pšenica	poljski pokus (mala parcela)	folijarno	pojava 2. nodija	selenat	20	32	350	10,94	0,55	(Ducsayi sur., 2016.)
Pšenica	poljski pokus	folijarno	prije cvatnje	selenit	20	120	610	5,08	0,25	(Wang i sur., 2020.)
Pšenica	poljski pokus	folijarno	prije cvatnje	selenat	20	120	1340	11,17	0,56	(Wang i sur., 2020.)

Pšenica	poljski pokus	folijarno	nakon oplodnje	selenit	20	120	970	8,08	0,4	(Wang i sur., 2020.)
Pšenica	poljski pokus	folijarno	nakon oplodnje	selenat	20	120	1590	13,25	0,66	(Wang i sur., 2020.)
Pšenica	poljski pokus	folijarno	tokom stadija GS 31 i GS 49	selenat	5	150	640	4,27	0,85	(De Vitai sur., 2017.)
Pšenica	poljski pokus	folijarno	tokom stadija GS 31 i GS 49	selenat	25	150	2390	15,93	0,64	(De Vita i sur., 2017.)
Pšenica	poljski pokus	folijarno	tokom stadija GS 31 i GS 49	selenat	50	150	2820	18,8	0,38	(De Vita i sur., 2017.)
Pšenica	poljski pokus	folijarno	tokom stadija GS 31 i GS 49	selenat	80	150	3930	26,2	0,33	(De Vita i sur., 2017.)

Izvor: Galić i sur. (2021.a)

Analiza objavljenih rezultata različitih istraživanja provedena je meta-analizom postavljanjem linearnog mješovitog modela (Bates i sur., 2015.). Mješoviti linearni modeli postavljeni su s normaliziranim podacima o povećanju Se po 1 g primijenjenog selena kao varijable odgovora. Ulazni podaci bile su vrijednosti iz Tablice 1. Ukupno je postavljeno osam modela u biblioteci R lme4 prema Bates i sur. (2015.) sa svim slučajnim učincima i presjecima (eng. "*intercept*") za svaki pojam (činitelj), uz pretpostavku nestrukturirane pogreške varijance. Modeli su uključivali osam kombinacija sljedećih činitelja: način primjene, biljna vrsta, oblik Se i njihovo međudjelovanje. Konačni model odabran je na temelju najnižih vrijednosti Akaikeova i Bayesovog informacijskog kriterija (AIC i BIC). Tri tipa modela korištena su za interpretaciju rezultata. Prvi je tip modela bio s prekidom toka linije regresije koji se mijenjao u zavisnosti od načina aplikacije Se i oblik Se, drugi je bio s prekidom toka linije regresije koji se mijenjao kao funkcija biljne vrste i oblik Se, a treći tip je uzimao tip eksperimenta s terminom slučajnog prekida toka linije regresije s fiksnom srednjom vrijednosti. Koeficijenti modela ekstrahirani su pomoću naredbe lme4 coef().

Tablica 2. Varijanca objašnjena načinom primjene × oblikom selena, biljnom vrstom × oblikom selena i vrstom eksperimenta, praćena koeficijentima slučajnih učinaka (najboljim linearnim nepristranim procjenama) za svaku razinu činitelja iz 11 znanstvenih radova (n.a., nije primjenjivo, eng. *not applicable*).

Faktor	**Tip aplikacije × oblik Se**	**biljna vrsta × oblik Se**	**Tip eksperimenta**	**Rezidual**
Varijanca	0,012352	0,006132	10,624367	0,213215
Koeficijent nasumičnih efekata				
tlo:selenat	-0,007177254	–	–	
folijarno:selenat	0,095549031	–	–	
tlo:selenit	-0,060080039	–	–	
folijarno:selenit	-0,022608397	–	–	
ječam:selenat	–	0,031985788	–	n.a.
kukuruz:selenat	–	0,012493402	–	
riža:selenat	–	-0,039315129	–	

pšenica:selenat	–	0,038706521	–
ječam:selenit	–	-0,013442636	–
kukuruz:selenit	–	0,010163323	–
riža:selenit	–	-0,028004366	–
pšenica:selenit	–	-0,009765509	–
poljski pokus	–	–	0,2971243
uzgoj u komori	–	–	4,5913276

Izvor: Galić i sur. (2021.a)

Analiza komponenti varijance (Tablica 2) pokazala je najmanji udio objašnjene varijance povezan s vrstom primjene, zatim interakcijom biljna vrsta × oblik selena, dok je vrsta eksperimenta objasnila najznačajniji dio varijance. Očekivano, utvrđena je niska vrijednost reziduala varijance. Analiza koeficijenata slučajnih učinaka pokazala je manju učinkovitost primjene selena u tlo u usporedbi s folijarnom primjenom. Kod analize interakcije biljna vrsta × oblik primjene (Tablica 2), utvrđene su niže učinkovitosti selenita u svim vrstama usjeva, dok su samo kod riže veličine izračunatih koeficijenata bile usporedive. Eksperiment s uzgojem u plastičnim posudama u komori za rast pokazao je višestruko povećanje nakupljanja selena, vjerojatno zbog nedostatka atmosferskih čimbenika koji utječu na ispiranje i isparavanje selena (Winkel i sur., 2015.). Kod biljaka tretiranih selenitom utvrđene su niže koncentracije selena u ksilemskom soku nego pri tretiranju selenatom (Shanker i sur., 1996.), a isto je potvrđeno i analizom miješovitog modela (Tablica 2) za zrna žitarica. Drugi najznačajniji udio varijance objasnio je interakciju načina primjene x oblika selena s folijarnom primjenom selenata, koja je najbolja kombinacija za uspješnu agronomsku biofortifikaciju. Primjena selenata u tlo predstavlja drugu najbolju opciju za povećanje razine selena u žitaricama. Primjene selenita u tlo ili folijarno predstavljaju manje učinkovite opcije, s tim da je folijarna primjena selenita ipak bolji izbor za povećanje koncentracije selena u biljkama. Pšenica je najvažnija žitarica za agronomsku biofortifikaciju zbog važnosti za prehranu ljudi (Guerrero i sur., 2014.) i veće apsorpcije selena u zrno u usporedbi s ostalim proučavanim žitaricama (Tablica 2). Općenito, selenit se obično jače adsorbira na čvrstu fazu tla i stoga je manje topljiv od selenata u otopini tla (Li i sur., 2008.), iako i genotip također utječe na povećanje koncentracija selena u zrnu (Cubadda i sur., 2010.). Najbolji rezultati u analizi biljna vrsta x oblika selena bili su: pšenica:selenat > ječam:selenat > kukuruz:selenat > kukuruz:selenit

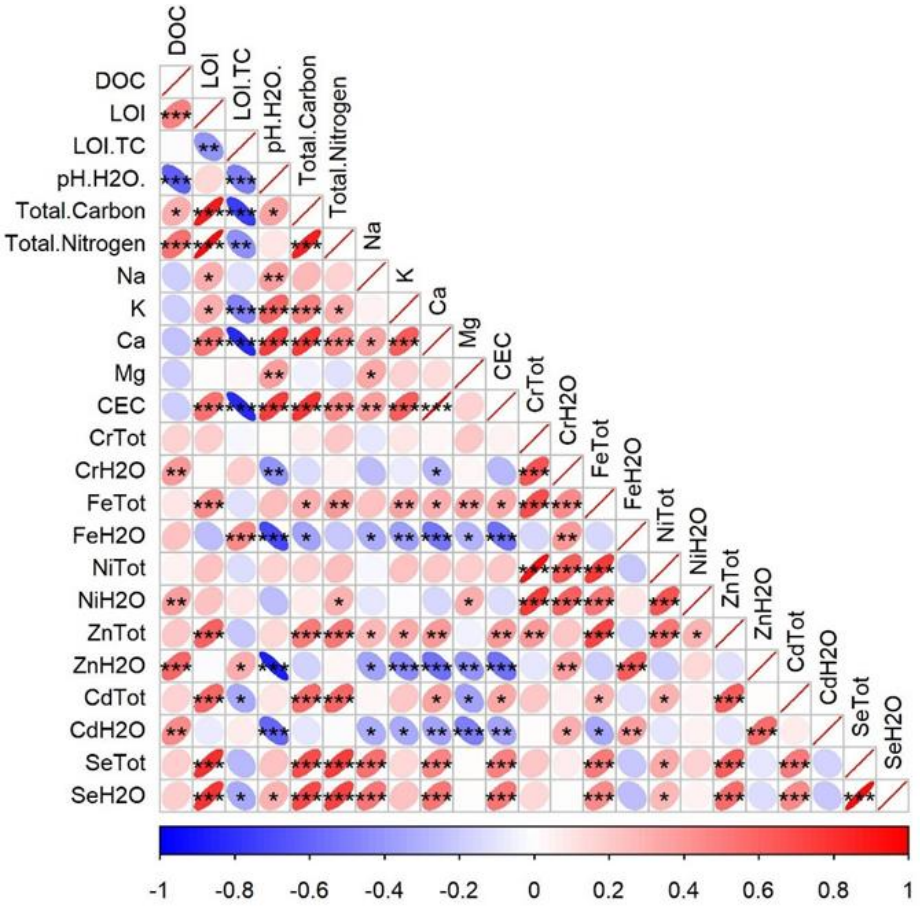
> pšenica:selenit > ječam:selenit > riža:selenit > riža:selenat, redom od najučinkovitijeg do manje učinkovitog. Veći prijenos selena iz selenata u zrno (Tablica 2) može se stoga pripisati njegovoj većoj biološkoj dostupnosti biljkama u usporedbi sa selenitom, koji je jače adsorbiran za površinu tla (Cartes i sur., 2005.). Nadalje, u biljkama, selenit i fosfat konkuriraju za apsorpciju jer dijele zajedničke transportere (Schiavon i Pilon-Smits, 2017.), dok je prijenos selenata iz korijena u izdanke izvjesniji nego prijenos selenita (Sors i sur., 2005.). Ječam je također pokazao učinkovitost u usvajanju selena (Gibson i sur., 2006.), a primjena selenata rezultirala je povećanim nakupljanjem selena u zrnu ječma (Narwal i sur., 2020.) u usporedbi sa selenitom. Chilimba i sur. (2012.) izvještavaju da se agronomska biofortifikacija kukuruza sa selenom čini izvedivom opcijom za povećanje sadržaja selena u hrani (Chilimba i sur., 2012.). Folijarna primjena selenita u uzgoju riže rezultirala je višim ukupnim sadržajem selena u usporedbi s primjenom u tlu (Yin i sur., 2019.), a selenit je učinkovitiji oblik selena za biofortifikaciju rižekod koje se akumulacija selena razlikuje kod različitih genotipova (Lidon i sur., 2019.). Analizom objavljenih rezultata istraživanja, možemo zaključiti da pšenica kao najuzgavanija žitarica ima i najveću učinkovitost u usvajanju selena i njegovom prijenosu u zrno. Ječam je pokazao veću učinkovitost u biofortifikaciji selenom u usporedbi s rižom i kukuruzom, ali se uzgaja u manjem opsegu. Od proučavanih žitarica, jedino je kod riže selenit pokazao veću učinkovitost od selenata kao oblik selena. Najveći udio varijance u analizi miješovitog modela pripisuje se interakciji načina primjene x oblika Se (Tablica 2), što predstavlja najvažniji čimbenik u odabiru prave strategije agronomske biofortifikacije selnom zajedno s izborom odgovarajuće biljne vrste.

5.1.2. Modeliranje utjecaja različitih svojstava tla na vodotopivost selena u tlima jugoistočne Europe

Selen je prisutan u četiri različita oksidacijska stanja u vodenim sustavima i u tlu, odnosno II, 0, IV i VI, te lako tvori spojeve s metalima, što potvrđuje činjenica da se pojavljuje u otprilike 50 minerala (Goh i Lim, 2004.). Kemijski je selen sličan sumporu (S), a u geosferi je povezan s nalazištima sumpora i ugljena (Sharma i sur., 2015.). Selenat i sulfat očito dijele mehanizam usvajanja u biljkama, što je vidljivo iz međusobne konkurencije (Birringer i sur., 2002.). Biološka dostupnost selena regulirana je fizikalno-kemijskim uvjetima tla poput pH vrijednosti, redoks uvjeta, slanosti, organske tvari u tlu itd (Roman i sur., 2014). Selen u tlima postoji u anorganskim oblicima kao što su selenat (SeO_4^{2-}) i selenit (SeO_3^{2-}), ali i u organskim oblicima (Nowak i sur., 2004.), poput dimetilselenida (DMSe) i dimetildiselenida (DMDSe), koji mogu volatizirati iz tla u plinovitom obliku. Selenat, koji je topljiv u vodi, predstavlja najdostupniji oblik selena za biljke u dobro prozračenim, neutralnim do alkalnim tlima (Galić i sur., 2021.), dok je selenit gotovo potpuno nedostupan zbog snažne adsorpcije na čestice tla u gotovo svim tipovima tala (Nakamaru i sur., 2005.; Sharma i sur., 2015.). Budući da je značajan sastojak tla i sedimenata, organska tvar u tlu igra važnu ulogu u specijaciji i pokretljivosti selena (Sharma i sur., 2015.). Sadržaj selena u tlu uglavnom ovisi o matičnom supstratu i klimi, pri čemu su suha i polusušna područja bogatija selenom, dok u vlažnim i navodnjavanim područjima tla pokazuju niži sadržaj selena zbog ispiranja (Kang i sur., 1990.). Alkalna tla imaju više dostupnog selena, pri čemu je većina prisutna u obliku selenata. S druge strane, u kiselim i slabo prozračenim tlima, selen se uglavnom pojavljuje kao netopivi selenidi i elementarni selen povezan sa željezovim (Fe) oksidima (Manojlović i Lončarić, 2017.). Topivi selen predstavlja glavni izvor selena dostupnog za usvajanje biljkama (Supriatin i sur., 2016.), a njegove koncentracije obično su <0,05 μg g^{-1} selena (Manojlović i Lončarić, 2017.). Organska tvar u tlu može sadržavati i do 50 % ukupnog selena u tlu, od čega se značajan dio može mobilizirati u tlu nakon što biljke usvoje otopljeni selen (Wang i sur., 2018.). Organska tvar ima ključnu ulogu u predviđanju dostupnosti selena u tlu jer značajno utječe na mobilnost selena (Coppin i sur., 2006.). Točna procjena statusa selena u poljskim uvjetima zahtijeva informacije o brzinama transformacije selena (Guo i sur., 2000.). Sadržaj selena u tlu može varirati, ali većina europskih tala siromašna je sa selenom (Antanaitis i sur., 2008.). Razine selena u većini tala s područja Balkana niske su, s koncentracijama između 0,024 i 0,45 μg g^{-1} selena (Manojlović i Lončarić, 2017.). Normalne razine selena u tlu kreću se od 0,1 do 2,0 μg g^{-1},

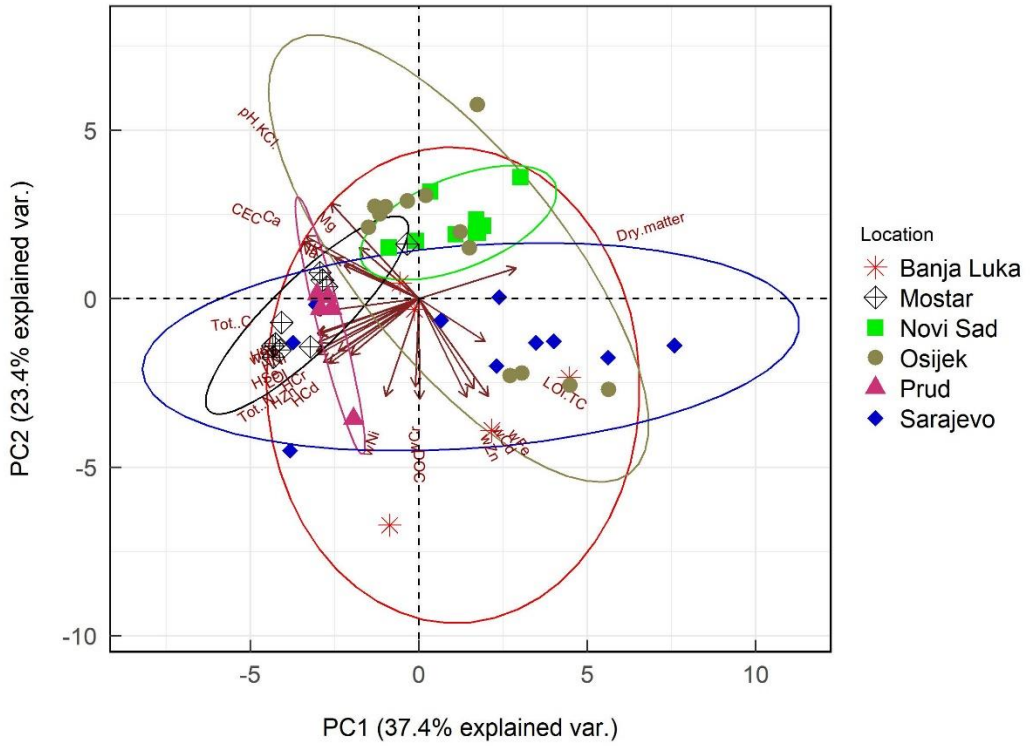
Girling (1984.) navodi da "zdrava" tla sadrže oko 2 μg g^{-1}. S druge strane, visoke koncentracije seelna u tlu, uvtrđene između 30 i 324 μg g^{-1}, mogu djelovati toksično. Općenito, ukupne razine selena u tlima ispod 0,5 μg g^{-1} smatraju se nedostatnima (Manojlović i Lončarić, 2017.). U prediktivnim modelima ponašanja selena u tlu, svojstva tla općenito se odabiru kao nezavisne varijable (Gu i sur., 2019.). Tako su Jones i sur. (2017.) predložili korištenje prediktivnih modela i izradu karata za identifikaciju područja s nedostatka selena u tlu. Biofortifikacija selenom može biti važna strategija za osiguravanje dovoljnog statusa selena u usjevima gdje bi prediktivni modeli mogli imati veliki doprinos (Danso i sur., 2023.). Cilj istraživanja u ovom radu je ispitati geokemijske čimbenike koji kontroliraju topljivost selena u vodenoj fazi tala prikupljenih sa značajnih i produktivnih poljoprivrednih područja Hrvatske (Osijek), Bosne i Hercegovine (Mostar, Prud, Sarajevo i Banja Luka) te Srbije (Novi Sad). Hipoteza je bila da je topljivost selena uglavnom kontrolirana sadržajem organske tvari u tlu, kationskim izmjenjivačkim kapacitetom (KIK) i pH vrijednošću tla. U tlima iz okolice Mostara utvrđen je najveći postotak (9,7 %) gubitka mase žarenjem (odnosno *LOI*), dok su u drugim područjima vrijednosti za LOI varirale između 5,2 % i 6,8 %. Srednja vrijednost pH tla za sve istraživane lokalitete bila je blago alkalna (7,18). Također, u okolici Mostara utvrđen je najveći udio ukupnog ugljika (TC - eng. *total carbon*) (4,7 %) i ukupnog dušika (TN - eng. *total nitrogen*) (0,36 %). Vrijednosti otopljene organske tvari (DOC – eng. *dissolved organic cabon*) kretale su se od 156 mg kg^{-1} u okolici Pruda do 352 mg kg^{-1} u okolici Banja Luke. Omjer LOI/TC prosječno je iznosio 2,66. KIK je imao značajnu varijabilnost, s najvećim vrijednostima u području Mostara (101,607 cmolc kg^{-1}) i najnižim vrijednostima na lokalitetima u Banja Luci (30,336 cmol kg^{-1}). Također su izmjerene ukupne koncentracije i vodotopiva frakcija nekih esencijalnih (Fe, Zn, Ni, Se) i toksičnih (Cr, Cd) elemenata ekstrakcijom s vodom (Cr_{H2O}, Fe_{H2O}, Ni_{H2O}, Se_{H2O}, Cd_{H2O} i Zn_{H2O}) ili ultra-čistom HNO_3 (Cr_{Tot}, Fe_{Tot}, Ni_{Tot}, Zn_{Tot}, Se_{Tot}, Cd_{Tot}). Koncentracije Cr_{Tot} bile su iznad smjernica WHO-a u okolici Sarajeva, Banja Luke, Mostara i Pruda. Koncentracije Ni_{Tot} bile su iznad razine 35 mg kg^{-1} na svim područjima osim u Osijeku. Koncentracije Zn_{Tot} bile su ispod 50 mg kg^{-1} na svim istraživanim lokacijama. Razine Cd_{Tot} bile su ispod najvećih dopuštenih koncentracija (0,8 mg kg^{-1}) na svim ispitnim mjestima. Uzorci iz okolice Mostara pokazali su koncentracije Se_{Tot} iznad razine deficita, dok je deficit utvrđen na svim drugim lokalitetima. Koncentracije SeO_4^{2-} slijede sličan obrazac, s najvišim vrijednostima u Mostaru i najnižim u Sarajevu i Osijeku kako slijedi: Mostar > Prud > Banja Luka = Novi Sad > Sarajevo = Osijek. Udio vodotipovog selena (Se_{H2O}) u

ukupnom selenu (Se_{Tot}) bio je prilično konzistentan prema područjim aistraživanja: Sarajevo 3,5 %; Banja Luka 3,26 %; Novi Sad 4,12 %; Mostar 2,72 %; Osijek 3,9 %; i Prud 3,1 % (prosječno 3,1 %, n = 52). Se_{Tot} i Se_{H2O} bili su linearno povezani u svim tlima, a najveće količine utvrđene su na loklaitetima područja Mostara (Se_{Tot} = 0,643 mg kg^{-1} i Se_{H2O} = 0,0175 mg kg^{-1}). Nasuprot tome, najniže zabilježene razine Se_{Tot} bile su u području Osijeka (0,228 mg kg^{-1}), a niske razine Se_{H2O} u području oko Sarajeva (0,00856 mg kg^{-1}) i Osijeka (0,0089 mg kg^{-1}). Koncentracija Se_{Tot} za ostala područja je kako slijedi, od više prema nižoj: Prud > Banja Luka > Novi Sad, a isto vrijedi i za Se_{H2O}. Analiza korelacije otkrila je snažne veze između vodom ekstrahiranih metala i ukupnih koncentracija u tlu (Slika 1). LOI, TC i TN pokazali su snažne korelacije te značajne pozitivne korelacije s drugim svojstvima tla i koncentracijama metala. pH tla uglavnom je imao značajne pozitivne korelacije s alkalnim i zemnoalkalnim metalima, ali negativne korelacije s koncentracijama teških metala ekstrahiranih vodom. Se_{Tot} i Se_{H2O} pokazali su vrlo snažnu korelaciju, a oboje su snažno pozitivno korelirali s LOI, TC i TN, kao i umjereno do snažno pozitivno korelirali s veličinom kationskog izmjenjivačkog kapaciteta (KIK), koncentracijama Na i Ca i ukupnim koncentracijama svih teških metala.

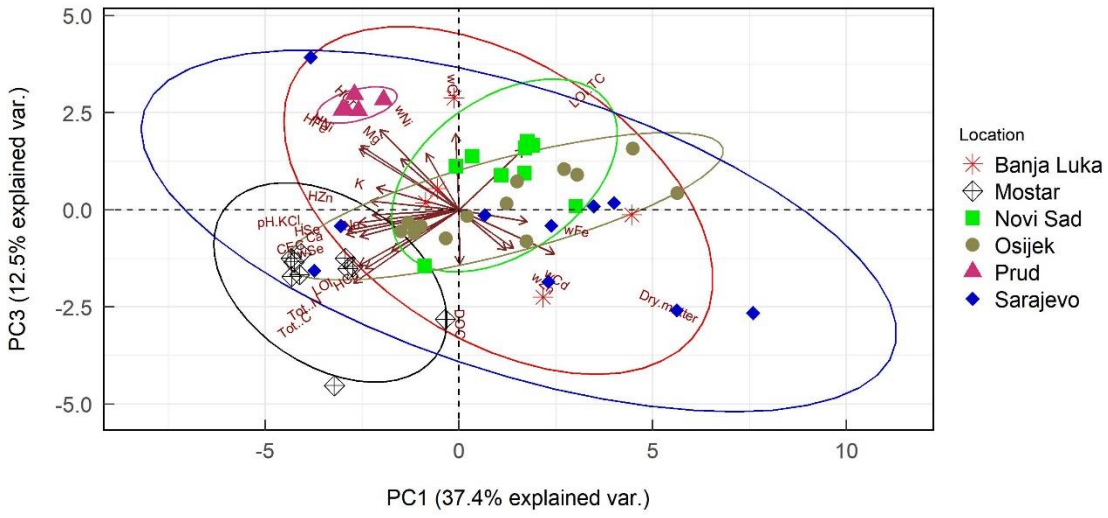


Slika 1. Ilustracija prikazuje negativne (-, plave) i pozitivne (+, crvene) korelacije između svojstava tla i ukupnih koncentracija elemenata u tlu te koncentracija ekstrahiranih vodom. Značajnost korelacije označena je sa * (α = 0,05), ** (α = 0,01) i *** (α = 0,001). Izvor: Galić i sur., 2023.

Kako bi se procijenilo grupiranje tala prema analiziranim svojstvima, provedena je PC analiza (*eng. principal component analysis*). Prve tri glavne komponente objasnile su 73,6 % varijacije u skupu podataka, pri čemu su prve dvije komponente objasnile 61,1% (36,9 % od strane PC1 i 24,2 % od strane PC2, Slika 2). Pokazano je da je PC1 uglavnom bio koreliran s LOI/TC u pozitivnom smjeru i TC, KIK, koncentracijama Ca, Mg, Na i K u negativnom smjeru. PC2 je bio pozitivno koreliran s DOC i ekstraktima teških metala vodom, te negativno koreliran s pH vrijednošću i Mg (Slika 2).



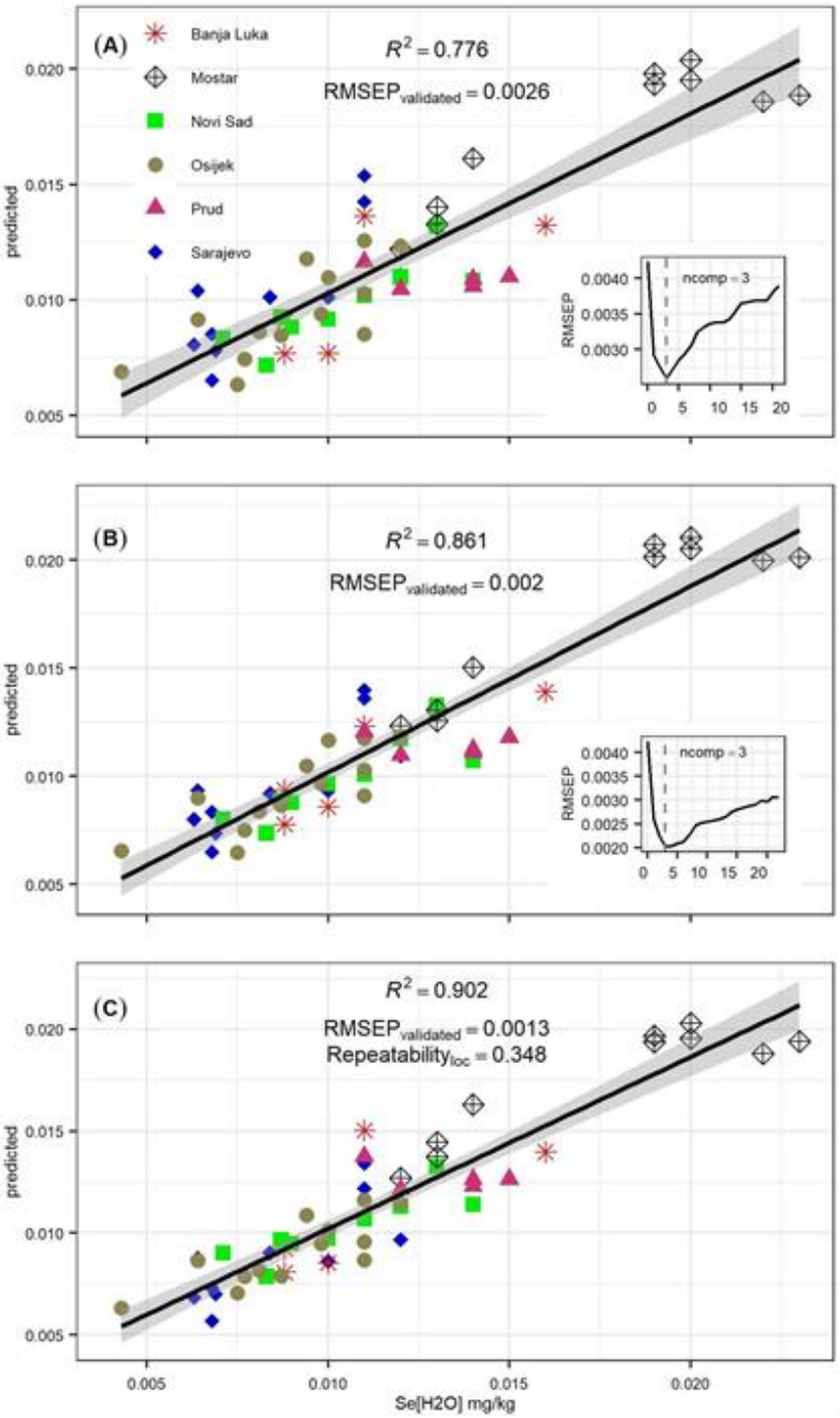
Slika 2. Biplot glavnih komponenti 1 i 2 iz PC analize varijacija među šest lokacija s različitim svojstvima tla. Strelice predstavljaju svojstvene vrijednosti (*eng*. "*eigenvalues*") svakog od 24 odabrana parametra tla. Izvor: Galić i sur., 2023.



Slika 3. Biplot glavnih komponenti 1 i 3 iz PCA analize varijacija među šest lokacija s različitim svojstvima tala. Strelice predstavljaju svojstvene vrijednosti (*eng*. "*eigenvalues*") svakog od 24 odabrana parametra tla. Izvor: Galić i sur. (2023.)

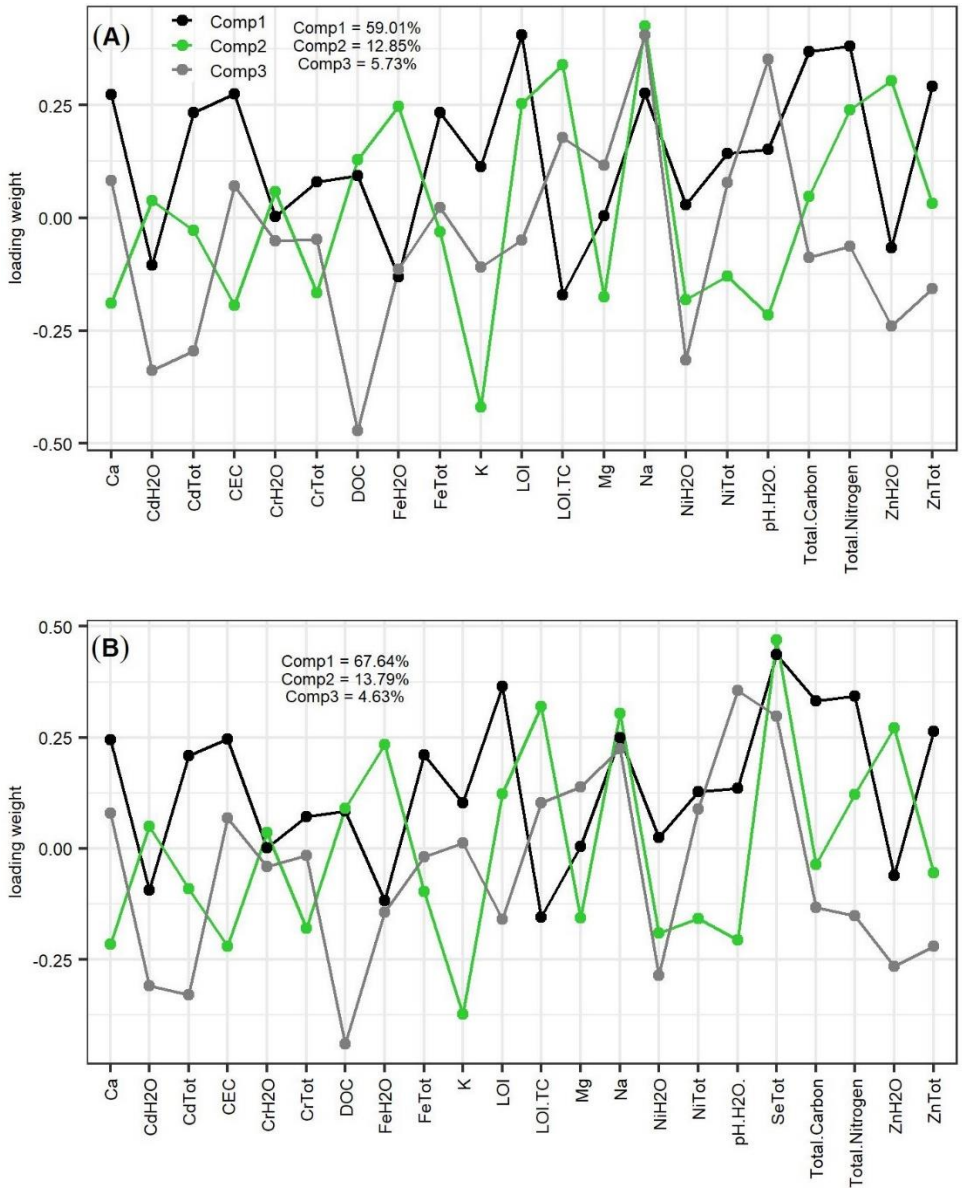
Prva i treća glavna komponenta (PC1 i PC3) zajedno su objasnile 49,9 % varijacije u skupu podataka, pri čemu je 12,5 % objašnjeno od strane PC3 (Slika 3).

Općenito, tla iz okolice Banja Luke, Sarajeva i Osijeka pokazala su značajnu varijabilnost svojstava, što se može primijetiti na Slikama 2 i 3, gdje su elipse raspršene po svim kvadrantima. Na Slici 2, svojstva tala iz okolice Pruda, Novog Sada i Mostara su snažnije grupirana. Uzorci lokaliteta NoviSad također su se grupirali blizu početka PC1, dok su tla lokaliteta Prud i Mostar uglavnom bila na pozitivnoj strani PC2. Tla s područja lokaliteta Osijek oblikovala su dvije različite skupine u PC2, s kontrastnim svojstvima povezanim s tom komponentom, dok su uzorci lokaliteta Banja Luka i Sarajevo pokazali visoku raznolikost i bili raspršeni po procijenjenim PC-ima. Prud, Mostar i Novi Sad održali su svoju razliku i čvrsto grupiranje u PC3 (Slika 3), slično PC2. Između PC1 i PC3, jasne skupine uzoraka lokaliteta Osijek nisu bile vidljive, dok su uzorci lokaliteta Banja Luka i Sarajevo pokazali slične rasporedne uzorke kao u PC2. Na oba biplota, uzorci u gornjem lijevom kvadrantu pokazali su najviše vrijednosti Se_{Tot}. Na temelju složenih odnosa između Se_{H2O} i ostalih analiziranih svojstava tla i koncentracije elemenata (Slika 1), zajedno s velikom varijabilnošću analiziranih tala (Slike 2 i 3), izrađena su i kalibrirana tri prediktivna modela za Se_{H2O}. Prvi PLS model (*eng*. "*partial least squares model*") uključivao je sva analizirana kvantitativna svojstva osim Se_{Tot} (Slika 4A), dok je drugi model uključio Se_{Tot} (Slika 4B). Treći model uključivao je latentne varijable iz modela 2 (Slika 4B) zajedno s učinkom slučajne lokacije u mješovitim modelom (Slika 4C). Nekalibrirani modeli 1 i 2 objasnili su 89,78 % i 93,82 %, redom, varijance u Se_{H2O} (nije prikazano), dok su kalibrirani modeli objasnili 77,60 %, 86,06 % i 90,20 % (Slika 4). Kao što se i očekivalo, model bez Se_{Tot} (Slika 4A) imao je veću pogrešku predikcije u usporedbi s modelom koji ga je uključivao (Slika 4B). Pogreška predikcije bila je najmanja u modelu 3, koji je uključivao učinak slučajne lokacije (Slika 4C).



Slika 4. Model parcijalnih najmanjih kvadrata (PLS - partial least squares) regresije za Se_{H2O}, uključujući svojstva tla i koncentracije drugih elemenata (A), Se_{Tot} (B) i PLS rezultate iz (B) zajedno s glavnim učinkom lokacije u mješovitom modelu (C). Odgovarajuće validacijske pogreške, odabrani broj komponenata i ponovljivost slučajnog učinka lokacije prikazani su unutar grafova. Izvor: Galić i sur. (2023.)

Opterećenje (weight) prve komponente u modelima 1 i 2 (Slika 5) većinom je sličilo korelacijama između Se_{H2O} i drugih svojstava tla te koncentracijama drugih elemenata, pri čemu su najjače korelacije prenesene u apsolutne težine > 0,25 (Se_{Tot}, TC, TN, LOI, CEC).



Slika 5. Opterećenja (weights) iz kalibriranog PLS modela (3 komponente, Slika 4) za Se_{H2O}, uključujući svojstva tala i koncentracije drugih elemenata (A) te Se_{Tot} (B). Izvor: Galić i sur. (2023.)

Istraživanje je otkrilo snažnu pozitivnu korelaciju između Se_{H2O} i LOI, što ukazuje da se vodotopljivi dio selena povećava s većim udjelom organske tvari u tlu. Ovo otkriće je u skladu s prethodnim istraživanjima koja su pokazala da se vodotopljivi i izmjenjivi selen tendenciozno povećavaju s ubrzanim procesima trošenja, budući da argilne gline, željezni oksidi i organski spojevi pružaju više mjesta za razmjenu selena (Imran i sur., 2020.). Vodotopivi dio selena u tlu ovisi o svojstvima tla i varira s biološkim reakcijama (Wang i Gao, 2001.). Ovo je također potvrđeno značajnom slabom do srednjom pozitivnom korelacijom između pH vrijednosti tla i Se_{H2O}. Pokazano je da alkalnost i slanost mogu izazvati precipitaciju nekih elemenata i utjecati na adsorpciju kationskim izmjenjivačkim kompleksom (Singh i sur., 1981.). Nadalje, u ovom istraživanju, Se_{H2O} i KIK su pokazali značajnu umjerenu pozitivnu korelaciju (0.53). Kalcij i natrij također su pokazali umjerene do jake korelacije s Se_{H2O}, vjerojatno zato što su kationi koji su adsorbirani na KIK alkalnih tala. Analiza prediktivne sposobnosti geokemijskih svojstava tla uz konfuzijske klimatološke učinke u ovom istraživanju podudara se s rezultatima Liu i sur. (2021.), koji su koristili prediktivni model za procjenu dostupnosti selena (Liu i sur., 2021.). Njihovo istraživanje je identificiralo organsku tvar u tlu (SOM) i pH vrijednost kao ključne čimbenike koji utječu na prediktivnost modela (Liu i sur., 2021.). Rezultati ovog istraživanja ukazuju da bi modeliranje Se_{H2O} iz geokemijskih parametara tla pomoću penaliziranog modela moglo biti vrijedno, s obzirom na raspon tala koji su zastupljeni u studiji; tla s blago kiselom do blago alkalnom pH vrijednosti, s umjerenim sadržajem organske tvari u tlu (LOI/TC/TN/DOC), sa ili bez informacija o ukupnom selenu (Se_{Tot}) (Slika 4A,B). Dodavanjem informacija o Se_{Tot} očekuje se povećanje prediktivne točnosti modela zbog uspostavljene korelacije između selena ekstrajiranog vodom i ukupnog selena (Xiao i sur., 2020.), što je također potvrđeno u ovom istraživanju (Slike 4B i 5B). Također, istraživanje je pokazalo da su ukupni željezo (Fe_{Tot}) i kalcij (Ca) ključni u predviđanju koncentracija selena, što je također potkrepljeno studijom koju su proveli Xu i sur. (2020.). Iz navedenih pokazatelja o dostupnosti i bioraspoloživosti selena u tlu, može se zaključiti da je diljem svijeta značajan udio tala s nedostatkom selena, uključujući sva istražena područja u ovom istraživanju, osim lokaliteta Mostar. Ovo istraživanje pruža vrijedne informacije za biofortifikaciju selenom na jugoistoku Europe, gdje se fizikalno-kemijska svojstva značajno razlikuju ovisno o lokaciji. Se_{H2O}, koji je dostupan biljkama, pokazao je pozitivne korelacije s LOI (organska tvar tla), CEC (kationski izmjenjivački kapacitet), ukupnim ugljikom, ukupnim dušikom, ukupnim koncentracijama kalcija, natrija, željeza, cinka, kadmija i ukupnim

selenom. Tla iz lokaliteta Mostar, koji ima mediteransku klimu, imala su najviši sadržaj selena, dok su tla s kontinentalnom klimom na drugim lokacijama imala niže razine selena. Učinkovitost penaliziranih modela u predviđanju Se_{H2O} iz dostupnih geokemijskih informacija naglašava ovisnost koncentracije selena o geokemiji tla, klimatskim uvjetima i specifičnim fizikalno-kemijskim svojstvima tla. Stoga je biofortifikacija selenom nužan korak u održavanju zdravlja ljudi u regiji. Ovaj prediktivni model ima veliki potencijal za upotrebu u drugim regijama koje karakterizira nedostatak selena u tlu. Upotrebom osnovnih analiza tla kao što su organska tvar, kationski izmjenjivački kapacitet, ukupni dušik, ukupni ugljik, ukupni kalcij i natrij, prediktivna sposobnost modela može se značajno poboljšati, što omogućuje dobivanje značajnih informacija o razinama selena u tlu. Konačno, ovaj pristup ima potencijal olakšati optimalnu primjenu biofortifikacije selenom, osiguravajući adekvatnu dozu selena za biofortifikaciju usjeva, a time i za prehranu ljudi.

5.2. Utjecaj osmotskog stresa na fiziološki odgovor klijanaca soje iz sjemena biofortificiranog selenom

Poljoprivredni proizvodni sustavi izloženi su različitim intenzitetima suše, što je sve veći problem diljem svijeta. Nedostatak vode, ekstremne temperature i niska atmosferska vlaga dovode do suše, koja je jedan od najlimitirajućih čimbenika za bolji biljni porast i veće poljoprivredne prinose (Hamayun i sur., 2010.; Makbul i sur., 2011.) te klimatske promjene predstavljaju ozbiljnu prijetnju poljoprivrednoj proizvodnji. Sušni stres dovodi do nakupljanja reaktivnih kisikovih radikala (*eng.* "*reactive oxygane species*" - ROS) i povećane peroksidacije lipida (Khan i Komatsu, 2016.). Oksidativni stres uzrokovan različitim aktivnim kisikovim vrstama koje se formiraju pod utjecajem sušnog stresa oštećuje mnoge stanične komponente kao što su ugljikohidrati, lipidi, nukleinske kiseline i proteini, što na kraju smanjuje rast biljke, respiraciju i fotosintezu (Ahmad i sur., 2016.). Na staničnoj razini, osmotski stres rezultira dehidracijom, što izaziva promjene u sastavu i svojstvima membrane lipida (Aziz i Larher, 1998), signali suše potiču proizvodnju metabolita koji štite od stresa, kao što je prolin (Gupta i sur., 2020.). Prolin ima četiri glavne uloge tijekom stresa, tj. kao osmotski regulator - osmoprotektant, kelator metala, molekula antioksidativne obrane i signalna molekula (Hayat i sur., 2012.). Prolin doprinosi stabilizaciji sub-staničnih struktura (npr. membrana i proteina), uklanjanju slobodnih radikala i održavanju staničnog oksidacijskog potencijala u stresnim uvjetima (Hayat i sur., 2012). Fenolni spojevi su vrlo reaktivni u neutralizaciji slobodnih radikala doniranjem vodikova atoma ili elektrona te keliranjem metalnih iona u vodenim otopinama. Osim toga, imaju višestruka biološka svojstva poput antitumorskih, antimutagenih i antibakterijskih svojstava, a ta bi aktivnost mogla biti povezana s njihovom antioksidativnom aktivnošću (Song i sur., 2010.). Askorbinska kiselina (AA), poznata i kao vitamin C, jedan je od najobilnijih vodotopivih antioksidativnih spojeva prisutnih u biljnim tkivima, koji također djeluje kao donator elektrona u brojnim reakcijama (Seminario i sur., 2017.). Dokazano je da askorbat ima višestruke uloge u rastu biljaka, poput sudjelovanja u diobi stanica, ekspanziji stanične stijenke i drugim razvojnim procesima (Biosci i sur., 2014.). Očekivano je da sušni stres potakne povećanu biosintezu glavnog antioksidativnog spoja kao što je AA, te bi biljke s povišenim razinama AA-a mogle imati poboljšanu toleranciju na takve stresove (Seminario i sur., 2017.). Askorbinska kiselina poboljšava biljnu reakciju na stres smanjujući proizvodnju štetnih tvari (Biosci i sur., 2014.). Selen je poznat kao sudionik u reakciji biljaka na biotski i abiotski stres metaboličkim, strukturnim i fiziološkim procesima u

višim biljkama. Selen je esencijalni element za životinje i ljude, no njegova važnost za biljke još je predmet istraživanja (Józwiak i Politycka, 2019.). Toksičnost ili korisnost selena izrazito ovise o količini primijenjenog selena i njegovoj koncentraciji (Camelina i sur., 2021.). Niske razine selena mogu potaknuti antioksidativne mehanizme u biljkama, ali on djeluje kao prooksidans pri visokim razinama (Nawaz i sur., 2015.). Neki od pozitivnih učinaka selena na biljke su poticanje rasta, smanjenje oksidativnih oštećenja uzrokovanih UV zrakama, obnavljanje klorofila nakon izlaganja svjetlosnom stresu, povećanje antioksidativnih kapaciteta starijih biljaka i regulacija vodnog statusa biljaka izloženih suši (Yao i sur., 2009.). Generalno, selen može poticati rast i razvoj biljaka, povećavati njihovu toleranciju i antioksidativni kapacitet na okolišne stresove (Iqbal i sur., 2015.; Nawaz i sur., 2015.), čime pridonosi postizanju većih prinosa (Hasanuzzaman i sur., 2014.). Stoga se u pripremi istraživanja pretpostavilo da bi biofortifikacija selenom kod soje mogla utjecati na metaboličke puteve sinteze biološki aktivnih spojeva i antioksidativnu aktivnost klica koje su rasle iz biofortificiranih sjemenki soje. Cilj ovog istraživanja bio je istražiti fiziološke odgovore klica dva kultivara soje (Sonja i Lucija) obogaćenih selenom na osmotski stres induciran tretmanom polietilen glikolom 6000 (PEG 6000).

Tablica 3. Srednje vrijednosti ± standardne devijacije sadržaja selena u zrnima soje nakon folijarne biofortifikacije sa 30 g Se ha^{-1} u 2020. godini. wSe označava tretman bez selena (eng. without Se), a Se je kratica za tretman sa selenom. Različita slova označavaju značajnost razlika na razini $p < 0,05$.

Treatman	**Kultivar**	**µg Se g^{-1}**
wSe	Lucija	64,02 ± 36,04 b
	Sonja	101,42 ± 65,87 b
Se	Lucija	2091,67 ± 97,29 a
	Sonja	2315,33 ± 331,8 a
LSD 0.05	333,09	

Izvor: Galić i sur. (2021.b)

Biofortifikacija koja je provedena 2020. godine rezultirala je značajnim povećanjem sadržaja selena u zrnu kod kultivara Lucija i Sonja. Utvrđene su značajne razlike između izdanaka kultivara soje (Tablica 3) koje su obogaćene selenom u prethodnoj vegetaciji. Analiza varijance pokazala je

značajne učinke kultivara na sve analizirane karakteristike osim na koncentraciju prolina (PRO) (Tablica 4). Tretman sa selenom (biofortificirano sjeme selenom i sjeme bez selena)nije značajno utjecao na bilo koju od analiziranih fizioloških karakteristika, dok su visoko značajni učinci uočeni za glavni učinak PEG-2,5 (tretman suše). Interakcija između kultivara i selena pokazala je značajne učinke na sve karakteristike osim na ukupne fenole (TP), dok je interakcija između kultivara i tretmana PEG-2,5 bila značajna samo za PRO i askorbinsku kiselinu (AA). Interakcija između selena i tretmana PEG-2,5 značajno je utjecala samo na lipidnu peroksidaciju (LP) i TP. Trosmjerna interakcija nije značajno utjecala na bilo koju od analiziranih karakteristika.

Tablica 4. Rezultati ANOVA-e za produkt lipoperoxidacije (LP), sadržaj prolina (PRO), ukupne fenole (TP), ukupnu antioksidativnu aktivnost (FRAP) i askorbinsku kiselinu (AA). Značajnost učinaka označena je sa * ($\alpha = 0,05$), ** ($\alpha = 0,01$) i *** ($\alpha = 0,001$), dok su p-vrijednosti prikazane za čimbenike koji nisu pokazali značajnost.

	LP	**PRO**	**TP**	**FRAP**	**AA**
Kultivar	**	0,345	*	**	***
Se	0,485	0,783	0,356	0,064	0,061
PEG-2,5	***	***	***	***	***
Kultivar * Se	**	**	0,061	*	*
Kultivar * PEG-2,5	0,492	*	0,086	0,923	*
Se * PEG-2,5	*	0,813	*	0,788	0,103
Kultivar * Se * PEG-2,5	0,937	0,103	0,225	0,625	0,399

Izvor: Galić i sur. (2021.b)

Srednje vrijednosti za glavne učinke PEG-2,5 i selena prikazane su u Tablici 5. U skladu s rezultatima ANOVA-e (Tablica 4), uočeno je značajno povećanje LP i TP u kombinaciji s PEG-2,5 i sortama obogaćenim selenom u odnosu na obje sorte.

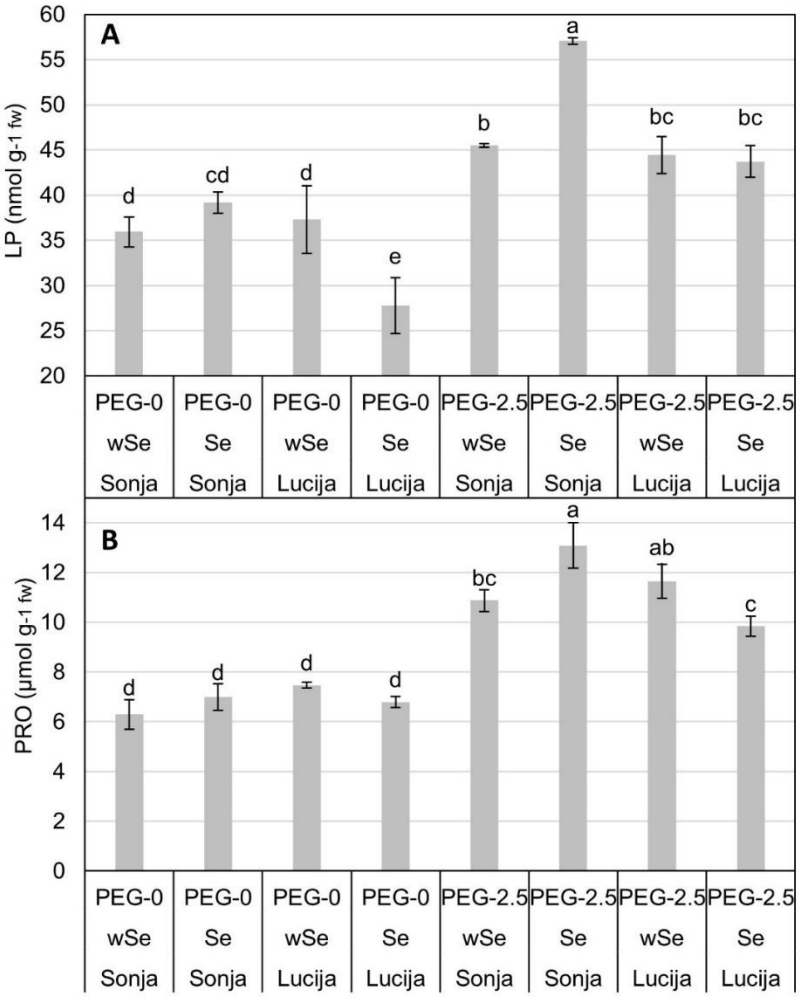
Tablica 5. Srednje vrijednosti ± standardna pogreška srednje vrijednosti za produkt lipidne peroksidacije (LP), sadržaj prolina (PRO), ukupni sadržaj fenola (TP), ukupnu antioksidativnu aktivnost (FRAP) i askorbinsku kiselinu (AA) preko tretmana PEG (suša) i selena (wSe i Se). Različita slova označavaju značajnost razlika na razini $\alpha = 0,05$.

PEG	Se	LP	PRO	TP	FRAP	AA
PEG-0	wSe	36,64 ± 1,86b	6,88 ± 0,38b	0,69 ± 0,02b	3,35 ± 0,22b	32,19 ± 1,45c
	Se	33,47 ± 2,95b	6,89 ± 0,26b	0,65 ± 0,02b	4,16 ± 0,49b	32,69 ± 1,91bc
PEG-2,5	wSe	44,97 ± 0,94a	11,26 ± 0,4a	0,87 ± 0,02a	6,47 ± 0,42a	41,04 ± 2,46ab
	Se	50,39 ± 3,09a	11,46 ± 0,85a	0,95 ± 0,05a	7,08 ± 0,59a	47,62 ± 4,54a
LSD0.05	-	7,01	1,54	0,094	1,32	8,39

Izvor: Galić i sur. (2021.b)

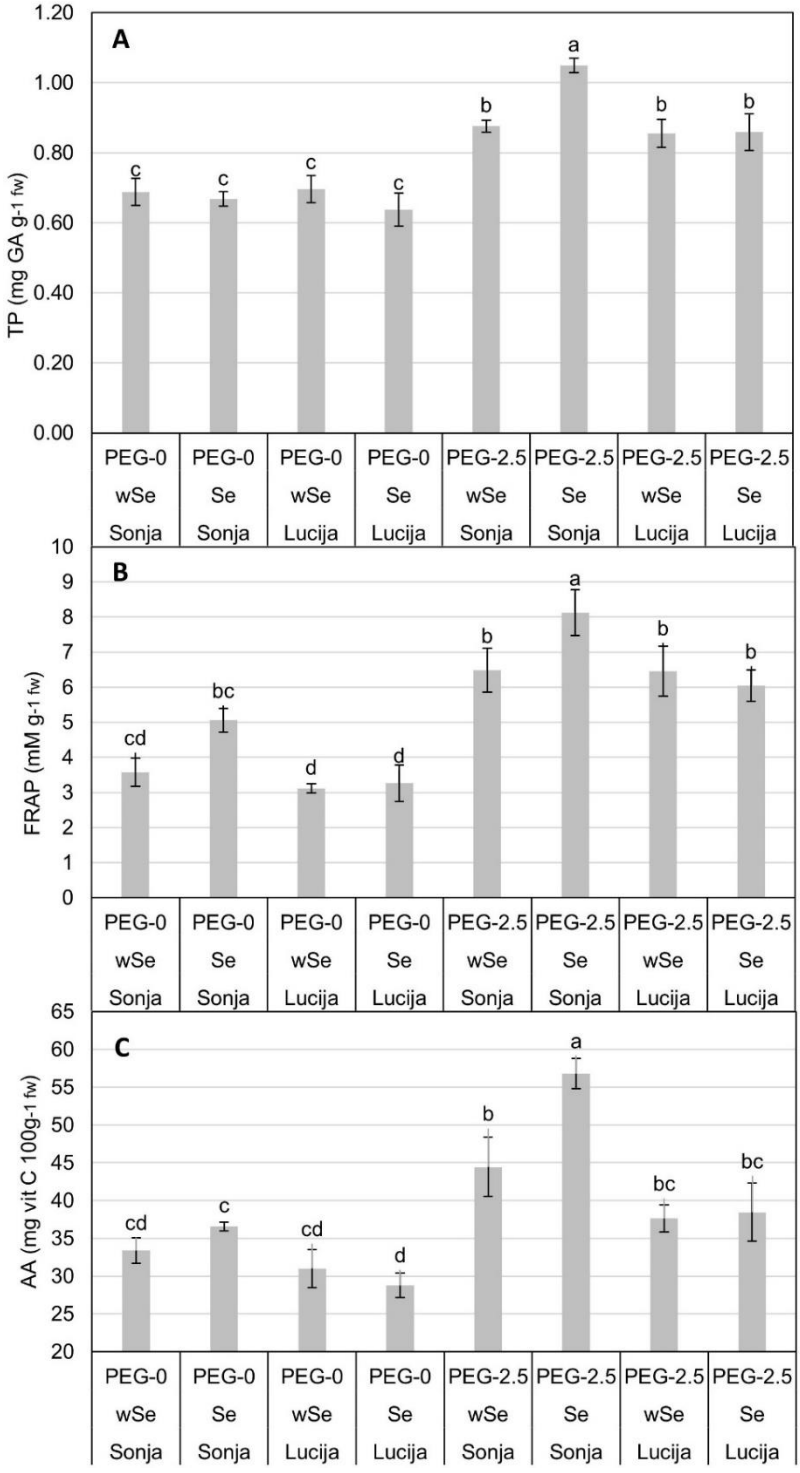
Utvrđeno je povećanje sadržaja LP-a od 26 % (Slika 6A) u biljkama pod tretmanom PEG-2,5. Utvrđene su značajne razlike između sadržaja LP-a u biljkama tretiranim s PEG-om i u onima bez dodatka PEG-a. Značajno najviša razinu LP-a u tretmanu bez PEG-a zabilježena je kod kultivara Sonja biofrtificirane sa selenom (39,161 nM g^{-1}), dok se sadržaj LP-a nije značajno razlikovao između kultivaru Lucija (37,325 nM g^{-1}) i Sonja (35,95 nM g^{-1}) u biofrtificiranom sjemenu. U tretmanu bez dodatka PEG-a, kultivar Lucija pokazala je značajnu reakciju LP-a na selen s najnižom vrijednošću od 27,769 nM g^{-1}.

Sadržaj PRO-a (Slika 6B) kod biljaka tretiranih s PEG-2,5 bio je 40,38 % viši u usporedbi s onima bez dodatka PEG-a. Nisu utvrđene značajne razlike između biljaka u tretmanu bez dodatka PEG-a. Kultivar Sonja saselenom u tretmanu PEG-2,5 pokazala je značajno najvišu razinu PRO-a (13,083 µM g^{-1}). Značajno najniži sadržaj PRO u skupini s PEG-2,5 i selenom zabilježen je kod kultivara Lucija s 9,84 µM g^{-1}.



Slika 6. Srednje vrijednosti i standardne pogreške (SE) srednjih vrijednosti za PRO (prolin) (**A**) i LP (produkt lipoperoxidacije) (**B**). Značajnost učinaka označena je različitim slovima na razini α = 0,05. Izvor: Galić i sur. (2021.b)

Nisu otkrivene značajne razlike između biljaka u tretmanu bez dodatka PEG-a u sadržaju TP (Slika 7A). Biljke tretirane s PEG-2,5 imale su viši sadržaj TP-a za 27,17 % u odnosu na biljke bez dodatka PEG-a. Jedina značajna razlika u tretmanu s PEG-2,5uočena je kod kultivara Sonja sa selenom, s vrijednošću od 1,049 mg GA g^{-1} svježe tvari, dok u tretmanu bez selena nije bilo značajnih razlika. Vrijednosti FRAP-a (Slika 7B) kod tretmana s PEG-2,5 bile su veće za 45,29 % u usporedbi s tretmanom bez dodatka PEG-a. U tretmanu bez dodatka PEG-a uočene su značajne razlike između sorata i biofortifikacije selenom.



Slika 7. Srednje vrijednosti i standardne pogreške srednje vrijednosti (SEM) za ukupni sadržaj fenola (A), FRAP (ukupna antioksidativna aktivnost) (B) i AA (askorbinska kiselina) (C). Značajnost učinaka označena je različitim slovima na razini $\alpha = 0,05$. Izvor: Galić i sur. (2021.b)

Najviša vrijednost bez dodatka PEG-a zabilježena je kod kultivara Sonja biofortificirane selenom (5,06 mM g^{-1}), dok kod kultivara Lucija nije bilo značajnih razlika između tretmana bez dodatka PEG-a i selena, iako su generalno vrijednosti bile značajno niže u usporedbi sa kultivarom Sonja. U tretmanu s PEG-2,5, utvrđena je značajna razlika FRAP vrijednosti između kultivara Sonja biofortificirane selenom (8,127 mM g^{-1}) i svih ostalih tretmana. Biljke u tretmanu s PEG-2,5 pokazale su viši sadržaj AA za 27,41% u odnosu na tretman bez dodatka PEG-a (Slika 7C). U tretmanu bez dodatka PEG-a utvrđene su značajne razlike kod kultivara Lucija biofortificirane selenom, s najnižom koncentracijom od 28,78 mg AA 100 g^{-1}, i kultivara Sonja biofortificirane selenom (56,794 mg AA 100 g^{-1}). U tretmanu s PEG-2.5, kultivar Sonja biofortificirana selenom pokazala je najveću i značajno veću vrijednost od 56,794 mg AA 100 g^{-1}. Nisu utvrđene ostale značajne razlike u tretmanu s PEG-2,5.

Značajne razlike između tretmana i kultivara pokazale su kontrastne reakcije izdanaka soje na biofortifikaciju selenom u PEG-2,5 tretmanu (Slika 6A). Slično ovim rezultatima, PEG-2,5 tretman je izazvao blagi stres u soji u studiji Basal i sur. (2020.). Suša ili toplinski stres mogu rezultirati povećanjem peroksidacije lipida (Jiang i Huang, 2001.), dok uporaba selena u manjim dozama može potaknuti antioksidativnu sposobnost krastavca (*Cucumis sativus* L.) i smanjiti peroksidaciju lipida (Józwiak i Politycka, 2019.). U ovom istraživanju, biljke PEG-0 biofortificirane Se u Luciji pokazale su smanjenu peroksidaciju lipida od 29,09 % u usporedbi sa Sonjom biofortificiranom selenom u istom tretmanu, dok biljke bez biofortifikacije nisu pokazale značajne razlike. Ovi rezultati u sorti Lucija usklađeni su s rezultatima u bijeloj djetelini (*Trifolium repens* L.) (Wang, 2011.), salati (*Lactuca sativa* L.) (Xue i sur., 2001.) i engleskom ljulju (*Lolium perenne* L.) (Hartikainen i sur., 2000.) gdje je selen smanjio peroksidaciju lipida. Smanjenje peroksidacije lipida pripisuje se povoljnim učincima selena na antioksidativni potencijal biljaka (Iqbal i sur., 2015.). Međutim, povećanje peroksidacije lipida u kultivaru Sonja biofortificiranoj selenom (PEG-2,5) uzrokovano je različitim fiziološkim reakcijama u usporedbi sa kultivarom Lucija, što je potvrđeno rezultatima istraživanja provedenih na engleskom ljulju gdje su veće doze selena poticale nakupljanje produkata peroksidacije lipida (Hartikainen i sur., 2000.). U skladu s tim, Jóźwiak i Politycka (2019.) proveli su istraživanje koje potvrđuje da veće doze selena mogu povećati peroksidaciju lipida; stoga je selen u nižim koncentracijama antioksidans (Ardebili i sur., 2014.), dok visoke doze mogu imati štetne učinke (Hartikainen i sur., 2000.). Kultivar Sonja u PEG-2,5 tretmanu s biofortifikacijom selenom pokazao je 24,79 % povećanje prolina u tkivu

izdanaka u usporedbi s kultivarom Lucija u istom tretmanu (Slika 6B). Osmoliti, poput prolina i glicinbetaina, nakupljaju se uslijed nedostatka vode kako bi pomogli u očuvanju vode u tkivima i zaštitili proteine i stanične membrane od osmotskog i oksidativnog stresa (Anjum i sur., 2012.). Akumulacija prolina generalno se smatra funkcionalnom prilagodbom protiv osmotskog stresa (van Heerden i de Villiers, 1996.). Povećanje nakupljanja prolina s povećanjem intenziteta i trajanja suše pomaže biljkama održavati hidratiziranost tkiva i izbjeći oštećenja uzrokovana sušom (Anjum i sur., 2012.). U kultivaru Sonja u PEG-2,5 biofortificiranom selenom detektirano je povećanje ukupnih fenola (TP) (Slika 7A). Povećanje TP pod stresnim uvjetima povezano je s genotipom i okolišnim uvjetima (Zahedi i sur., 2020.). Antioksidativna aktivnost fenolnih spojeva uglavnom je uzrokovana njihovim redoks svojstvima, koja im omogućuju djelovanje kao reducirajućih agensa, donatora vodika i hvatača singletnog kisika, zajedno s njihovim potencijalom za kelaciju metala (Kähkönen i sur., 1999.). Pannico i sur. (2020.) utvrdili su da se uz biofortifikaciju selenom povećavaju koncentracije fenolnih spojeva u korijandru (*Coriandrum sativum* L.), bosiljku (*Ocimum basilicum* L.) i tatsoiju (*Brassica rapa* var. rosularis). Fenoli imaju sposobnost povećanja antioksidativnog kapaciteta i poboljšanja sposobnosti biljaka da ublaže oksidativni stres (Puccinelli i sur., 2020.; Camelina i sur., 2021.). Kultivar Sonja je pokazao najvišu razinu FRAP kako u PEG-2,5, tako i u PEG-0 tretmanu (Slika 7B). Prethodna istraživanja također su pokazala povećanje antioksidativne aktivnosti u biljkama tretiranim selenom pod različitim abiotičkim stresovima (Rady i sur., 2020.). U istraživanju Puccinelli i sur. (2020.), najviši sadržaj antioksidativnog kapaciteta, ukupnih fenola i rosmarične kiseline detektiran je u biljkama tretiranim selenom. To bi moglo biti povezano s reakcijom biljaka na potencijalno toksične učinke selena u bosiljku (Puccinelli *i sur.*, 2020). Kultivar Sonja sa selenom u PEG-2,5 tretmanu pokazao je najviši sadržaj AA (askorbinske kiseline) (Slika 7C).AA je organska kiselina koja pod utjecajem suše povećava proces disanja u biljkama, stoga ove kiseline djeluju ujedno i kao supstrat u tom procesu. (Zahedi i sur., 2020.). Povećanje sadržaja AA u biljkama može imati trostruko pozitivan učinak: proizvodnja hrane s visokim sadržajem AA za zdravu prehranu, povećanje roka trajanja proizvoda i povećanje otpornosti biljaka na razne vrste stresa (Paciolla i sur., 2019.). Iz provedenog istraživanja zaključujemo da bi biofortifikacija selenom u kultivaru Lucija mogla biti prikladna mjera za poboljšanje otpornosti, što bi trebalo dalje testirati u poljskim uvjetima. Što se tiče kultivara Sonja, trebalo bi provesti daljna istraživanja u pravcu proizvodnje funkcionalne hrane u obliku klica ili izdanaka soje jer je selen u biofortificiranom zrnu izravno utjecao na veću

proizvodnju ukupnih fenola (TP) i ukupne antioksidativne aktivnosti (FRAP), te udvostručio koncentraciju vitamina C (AA). Rezultati istraživanja pokazuju kako je biofortifikacija u oba kultivara značajno povećala količinu selena u zrnu, što je od velike važnosti za ostvarivanje ciljeva biofortifikacije.

5.3. Efikasnost biofortifikacije lisnatog povrća selenom u hidroponskom i supstratnom načinu uzgoja

5.3.1. Biofortifikacija selenom uz istovremenu upotrebu vermikomposta kao medija u uzgoju matovilca (*Valerianella locusta* L. Laterr)

Listnato povrće je značajno za prehranu ljudi diljem svijeta, a istodobno, postoji globalni problem s nedostatkom selena u prehrani ljudi, uglavnom zbog niskog sadržaja selena u tlu. Budući da biljke predstavljaju značajan izvor ovog elementa u prehrani ljudi, biljna hrana koja sadrži povećane koncentracije selena može biti učinkovita u povećanju unosa selena u prehrani ljudi i u hranidbi životinja. S druge strane, proizvodnja supstrata za uzgoj povrća temelji se na ograničenim rezervama treseta, a potrošnja takvih supstrata može se smanjiti djelomičnom supstitucijom s kompostima ili vermikompostima . Vermikompostiranje je učinkovit proces recikliranja hranjivih tvari koji uključuje korištenje gujavica kao svestranih prirodnih bioreaktora za razgradnju i stabilizaciju organske tvari. Drugim riječima, gujavice su sposobne pretvoriti smeće u "zlato" (Pathma i Sakthivel, 2012.). Vermikompostiranje je netermofilni biološki proces oksidacije u kojem se organski materijali pretvaraju u vermikompost, što je materijal po doređenim svojstvima sličan tresetu s visokom poroznošću, prozračnošću, zadržavanjem vode i bogatom mikrobiološkom aktivnošću (Pathma i Sakthivel, 2012.). Biljke su značajan segment recikliranja selena u prehrambenom lancu. Stoga, povećanje koncentracije selena u usjevima biofortifikacijom koristan je način povećanja unosa selena u hranidbeni lanac životinja i prehrani ljudi (Ramos i sur., 2010.). Kod ljudi je apsorpcija selena iz proizvoda biljnog podrijetla puno jednostavnija u usporedbi s apsorpcijom iz proizvoda životinjskog podrijetla (Galić i sur., 2021.). Nedostatak selena u prehrani ima negativne učinke na ljudsko zdravlje, a s nedostatkom selena povezano je više od 40 vrsta bolesti, poput Keshanove bolesti, Kashin-Beckove bolesti, kardiovaskularnih bolesti, bolesti jetre, određenih vrsta raka i katarakte (Galić i sur., 2021.). Slično cinku, dodatak selena zaraženim pacijentima s niskim razinama selena u krvi može biti opcija kao prirodno liječenje protiv bolesti uzrokovane koronavirusom 2019 (COVID-19) (Galić i sur., 2021.). Svježe narezano ili minimalno obrađeno voće i povrće igraju važnu ulogu u ljudskoj prehrani, za razliku od visokokaloričnih dijeta bogatih lipidima i šećerima. Stoga se ljudska prehrana usmjerava prema gotovoj hrani poput predkuhanog i minimalno obrađenog povrća ili voća (Ferrante i sur., 2009.). S relativno dobrom kvalitetom skladištenja, matovilac je u sve većoj potražnji ne samo kao lisnata

salata već i kao sastojak u svježe narezanim proizvodima i gotovim mješavinama salata (Enninghorst i Lippert, 2003.). Matovilac ima skromne zahtjeve za toplinom, tako da se uglavnom uzgaja tijekom hladnijeg dijela godine, s optimalnom temperaturom rasta između 5 i 10 ºC (Fabek i sur., 2011.). Matovilac je povezan s pozitivnim utjecajem na određene bolesti, poput dijabetesa, kardiovaskularnih poremećaja i raka (Ramos-Bueno i sur., 2016.). Cilj ovog istraživanja bio je istražiti utjecaj tri različita medija za uzgoj (komercijalni treseti, vermikompost i njihova smjesa u omjeru 1:1) na učinkovitost biofortifikacije selenom i prinos matovilca. Biofortifikacija selenom provedena je dodatkom natrijevog selenata (Na_2SeO_4). Utvrđene su značajne razlike u svojstvima ispitivanih uzgojnih medija. Iz Tablice 6 vidljivo je da je komercijalni supstrat (KS) imao najveći sadržaj organske tvari i najveći omjer C:N u usporedbi s druga dva uzgojna medija. KS je imao najnižu električnu vodljivost (EC), sadržaj ukupnog dušika i udio pepela u usporedbi s druga dva supstrata. Vermikompost je imao najveći udio pepela, najveću pH vrijednost, konduktivitet (EC) i sadržaj ukupnog dušika, a u usporedbi s drugim supstratima sadržavao je najmanje organske tvari i imao najuži omjer C:N. Svojstva smjese komercijalong supstrata i vermikmposta u omjeru 1:1, očekivano su bila unutar raspona vrijednosti kod komercijalnog supstrata i vermikomposta.

Tablica 6. Osnovna svojstva medija za uzgoj

Medij za uzgoj	**organska tvar (%)**	**pepel (%)**	**pH_{H2O}**	**EC ($mS\ m^{-1}$)**	**ukupni N ($g\ kg^{-1}$)**	**C/N odnos**
KS	39,6	51,4	6,34	42,1	2,2	180:1
Vermikompost	12,6	87,4	9,23	67,9	5,4	23,3:1
Smjesa 1:1	22,4	78,6	7,55	59,5	3,1	72,3:1

Izvor: Galić i sur. (2021.c)

KS je imao najveće koncentracije Cd, Se i Pb, te najmanje sadržaje Zn, Cu, Mo, Ni, Cr, Hg i As u usporedbi s druga dva analizirana supstrata. Vermikompost je imao najveće razine Zn, Cu, Mo, Ni, Cr, Hg i As, dok je s druge strane imao najniže koncentracije Cd, Se i Pb. Koncentraciej navedenih elemenata u smjesi 1:1 bile su između vrijednsoti komercijalnog supstrata i vermikomposta.

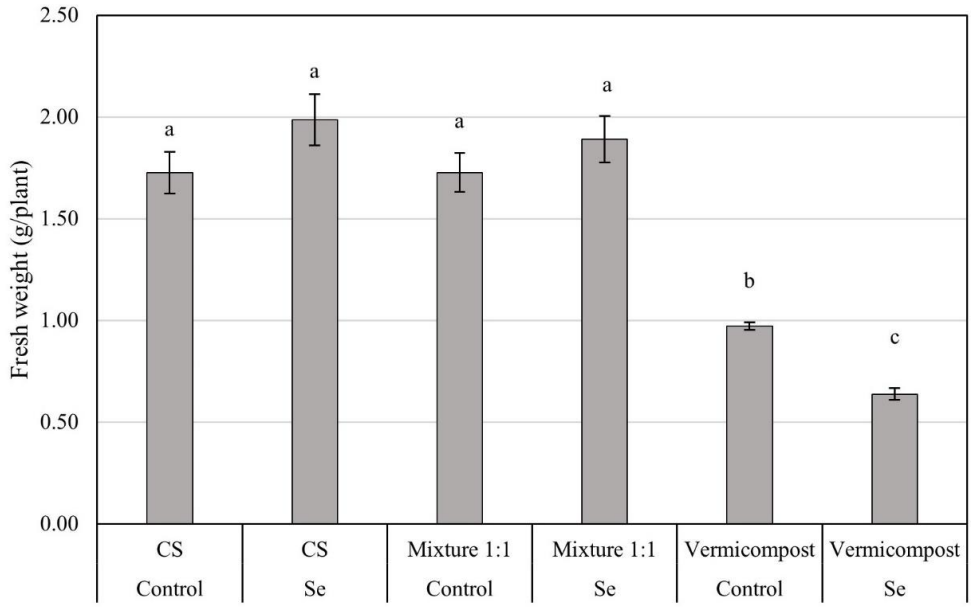
Glavni učinak medija za uzgoj pokazao je značajan utjecaj na svježu i suhu masu po biljci i sadržaj selena u svježem biljnom materijalu, dok je glavni učinak biofortifikacije selenom pokazao značajan utjecaj samo na koncentraciju selena u svježoj i suhoj tvari matovilca (Tablica 7).

Tablica 7. Analiza varijance za svježu i suhu masu po biljci te koncentracije selena. *označava značajnost na $p < 0,05$, *** označava značajnost na $p < 0,001$. U slučaju nedostatka značajnih učinaka, prikazane su p-vrijednosti.

	Masa svježe tvari po biljci	**Masa suhe tvari po biljci**	**Se mg kg^{-1} svježa tvar**	**Se mg kg^{-1} suha tvar**
Medij za uzgoj	***	***	*	0.122
Tretman selenom	0,6735	0,129	***	***
Medij za uzgoj : Tretman Se	*	*	*	0,121

Izvor: Galić i sur. (2021.c)

Prinos svježe mase po biljci (g) značajno je varirao s obzirom na interakciju tretmana i uzgojnih medija. Nisu uočene značajne razlike u prinosu između uzgojnih medija komercijalni supstrat i smjese 1:1 u usporedbi s kontrolom i tretmanom selenom. Međutim, uočene su značajne razlike između tretmana selenom i kontrole u vermikompostu. Značajno veći prinos uočen je u kontroli u odnosu na tretman selenom (Slika 8).



Slika 8. Srednje vrijednosti ± standardna pogreška svježe mase po biljci (g) prema glavnom učinku tretmana biofortifikacije selenom i uzgojnim medijima komercijalni supstrat (KS), vermikompost i smjesa 1:1. Različita slova predstavljaju značajnost razlika na razini = 0,05. Izvor: Galić i sur. (2021.)

Tablica 8. prikazuje sadržaj selena u svježoj i suhoj masi matovilca. Tretman selenom značajno je utjecao na povećanje sadržaja selena u suhoj i svježoj biljnoj tvari u sva tri uzgojna medija. U svježoj tvari matovilca sadržaj selena povećan je 172,86 puta u usporedbi s kontrolom. Sadržaj selena u suhoj tvari povećan je 177,45 puta u usporedbi s kontrolom.

Se treatman		
	Se u svježoj tvari (mg kg^{-1})	**Se u suhoj tvari (mg kg^{-1})**
Kontrola	0,0065 ± 0,0019 ^{b}	0,0691 ± 0,0131 ^{b}
Se	1,1236 ± 0,5914 ^{a}	12,2615 ± 4,3845 ^{a}
LSD 0.05	0,2794	2,629
Uzgojni medij		
KS	0,532 ± 0,584 ^{ab}	6,7207 ± 7,3576 ^{a}
Vermikompost	0,848 ± 1,01 ^{a}	7,4656 ± 9,0653 ^{a}
Smjesa 1:1	0,314 ± 0,361 ^{b}	4,3096 ± 4,6585 ^{a}
LSD 0.05	0,342	3,221

Izvor: Galić i sur. (2021.c)

Cilj ovog istraživanja bio je ispitati učinkovitost biofortifikacije selenom u tri različita uzgojna medija: KS (komercijalni supstrat), vermikompost i mješavinu ova dva medija u omjeru 1:1. KS je imao najveći udio organske tvari, ali i najširi omjer C:N (Tablica 6). Omjer C:N trebao bi biti između 1:20 i 1:30, što se smatra najpovoljnijim omjerom C:N jer ne dovodi do nedostatka dušika. Vermikompost je imao optimalan omjer C:N (23,3:1) i najveću količinu N, iako je imao najmanji udio organske tvari. Električna vodljivost (EC) može poslužiti kao mjera topivih hranjivih tvari, kako kationa tako i aniona (Smith i Doran, 2015.), što može značiti da je vermikompost bogatiji,

a KS siromašniji otopljenim hranjivim tvarima. Niža EC vrijednost mogla bi rezultirati smanjenim sadržajem kationa u tlu (Smith i Doran, 2015.). Kod mješavine 1:1 utvrđene su vrijednosti između vermikomposta i KS-a, što je za neka svojstva značilo poboljšanje u odnosu na preostala dva medija. Biofortifikacija selenom bila je uspješna u sva tri ispitana uzgojna medija. KS i mješavina 1:1 nisu pokazali značajne razlike u prinosu i koncentraciji selena u svježem i suhom listu matovilca. Utvrđeno je da je biofortifikacija povećala sadržaj selena tako da je masa od samo 48,9 g svježih listova sadržavala dovoljno selena za preporučeni dnevni unos u ljudskoj prehrani (55 µg selena dan^{-1}), što predstavlja značajan potencijal za rješavanje problema nedostatka selena u prehrani. Nadalje, korištenje smjese vermikomposta i komercijalnog supstrata u omjeru 1:1 pokazalo je slične rezultate kao i korištenje komercijalnog uzgojnog medija, što može doprinijeti očuvanju rezervi treseta. Prema rezultatima ovog istraživanja, mješavina komercijalnog supstrata i vermikomposta u omjeru 50:50 pokazala je sličnu uspješnost kao i komercijalni supstrat u uzgoju matovilca. Izravne uštede bi u ovom scenariju bile 50 % vrijednosti komercijalnog supstrata, umanjeno za troškove proizvodnje vermikomposta, koji mpogu biti vrlo niski jer se vermikompost može proizvesti od otpadnih organskih tvari i organskih nusproizvoda. Korištenje vermikomposta kao samostalnog medija za uzgoj rezultiralo je smanjenim prinosima, ali u matovilcu uzgojenom u vermikompostu izmjerene su najveće koncentracije Se, vjerojatno zbog visoke pH vrijednosti i upotrebe selena u obliku natrijevog selenata. Zbog pozitivnih rezultata, možemo predlažiti vermikompost kao dodatak komercijalnim supstratima u omjeru 50:50, čime se smanjuje upotreba komercijalnih supstrata za 50 %, uz održavanje prinosa. Zbog izuzetne važnosti selena u prehrani ljudi, biofortifikacija uz primjena vermikomposta u uzgoju lisnatog povrća mogu predstavljati značajno poboljšanje proizvdonje.

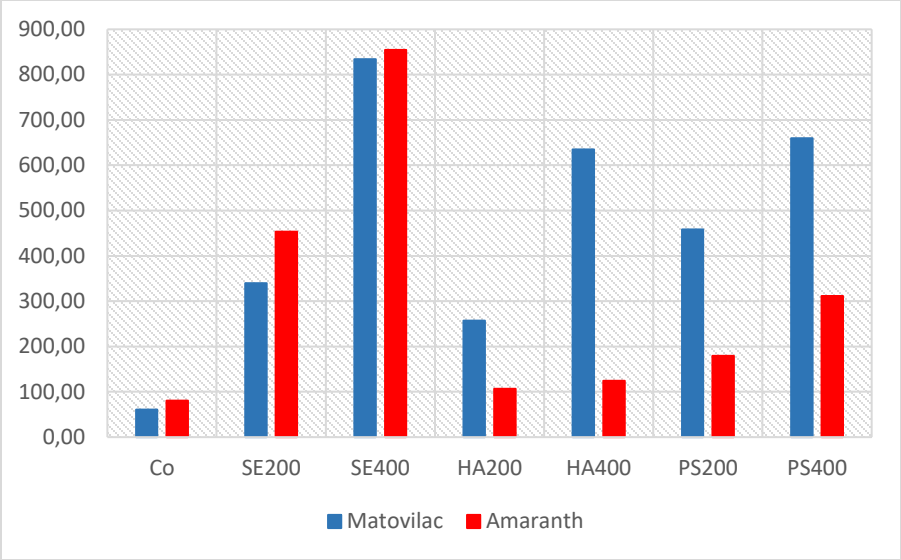
5.3.2. Biofortifikacija matovilca (*Valerinaella locusta* L.) i amaranta (*Amaranthus caudatus* L.) natrijevim selenatom i nanoselenom u hidroponskom uzgoju

Veliki značaj selena u prehrani ljudi i hranidbi životinja rezultirao je stvaranjem različitih metode biofrtifikacije selenom uz upotrebu različitih oblika selena ili načina uzgoja. Cilj ovog istraživanja bio je istražiti utjecaj oblika selena na prinos, morfološke karatkeristike, fiziološki odgovor "*baby leaf*" povrća (matovilca i amaranta) i uspješnost biofortifikacije u hidroponskom uzgoju. Pošto nedostatak selena zahvaća između 0,5 do 1 milijardu ljudi, uglavnom zbog unosa selena ispod preporučene dnevne doze od 50-70 µg Se dnevno (Schiavon i sur., 2016.), vrlo su značajna daljnaj istraživanja povećanja koncentracija selena u jestivim dijelovima biljaka. U agronomskim praksama selen se u biofortifkaciji najčešće primjenjuje u obliku natrijevog selenata (Na_2SeO_4) na tlo ili izravno folijarno na biljku (Rayman, 2008.; Garg i sur., 2018.; Ngigi i sur., 2019.; Prom-u-thai i sur., 2020.). Pri tome se selen prenosi u jestive dijelove biljke metabolizmom svog analognog sumpora (Schiavon i sur., 2020.). Rezultati Ramosa i sur. (2010.) sugeriraju da bi, u programima biofortifikacije s matovilcem, uporaba selena u obliku natrijevog selenata u niskim koncentracijama mogla biti korisna jer potiče rast biomase izdanaka, translokaciju selena i razinu selena u biomasi izdanaka (Ramos i sur., 2010.). Nedavno su napredne tehnologije ukazale na upotrebu selena u obliku selenovih nanočestica (SeNPs) kao zamjene za konvencionalna gnojiva selenom kako bi se povećala razina seleno-organskih spojeva u usjevima (El-Ramady i sur., 2015.; Kumar i Prasad, 2020.; Márquez i sur., 2020.). Nanočestice selena privlače sve veću pažnju zbog izvrsne biološke dostupnosti i smanjene toksičnosti u usporedbi s anorganskim i organskim oblicima (Hosnedlova i sur., 2018.). U literaturi su opisani različiti pristupi sintezi selenovih nanočestica, kao što su fizički, kemijski i biološki (Alam i sur., 2019.). U istraživanju Rady i suradnika iz 2021. godine utvrđeno je da folijarna suplementacija s nanočesticama selena igra značajnu ulogu u ublažavanju štetnih učinaka soli na različite aspekte rasta graha (*Phaseolus vulgaris* L.), uključujući fiziološka i biokemijska svojstva te prinos zelene mase. Zaključili su da se folijarna aplikacija nanočestica selena može predložiti kao vrijedan strateški pristup za poboljšanje rasta i produktivnosti biljaka graha na zaslanjenim staništima (Mostafa i sur., 2021.). Slično tome, Nagdalian i suradnici (2023.) istraživali su uporabu nanočestica selena kao stimulansa za rast i razvoj sjemena ječma (*Hordeum vulgare*) u stresnim uvjetima, uz poboljšanje morfofunkcionalnih svojstava (Nagdalian i sur., 2023.).

Rezultati istraživanja Asghari-Paskiabi i suradnika (2018.) otkrivaju da *Fusarium oxysporum* može proizvoditi selenove nanočestice na siguran i ekonomičan način. Nanoselen nalazi primjene u području medicine, gdje je zakođer predmet istraživanja. Kemijski sintetizirane selenove nanočestice proučavane su zbog njihovog potencijala kao antibakterijskih agensa u liječenju oboljelih od bakterijskih bolesti uzrokovanih prominentnim patogenim bakterijama (Ananth i sur., 2019.).

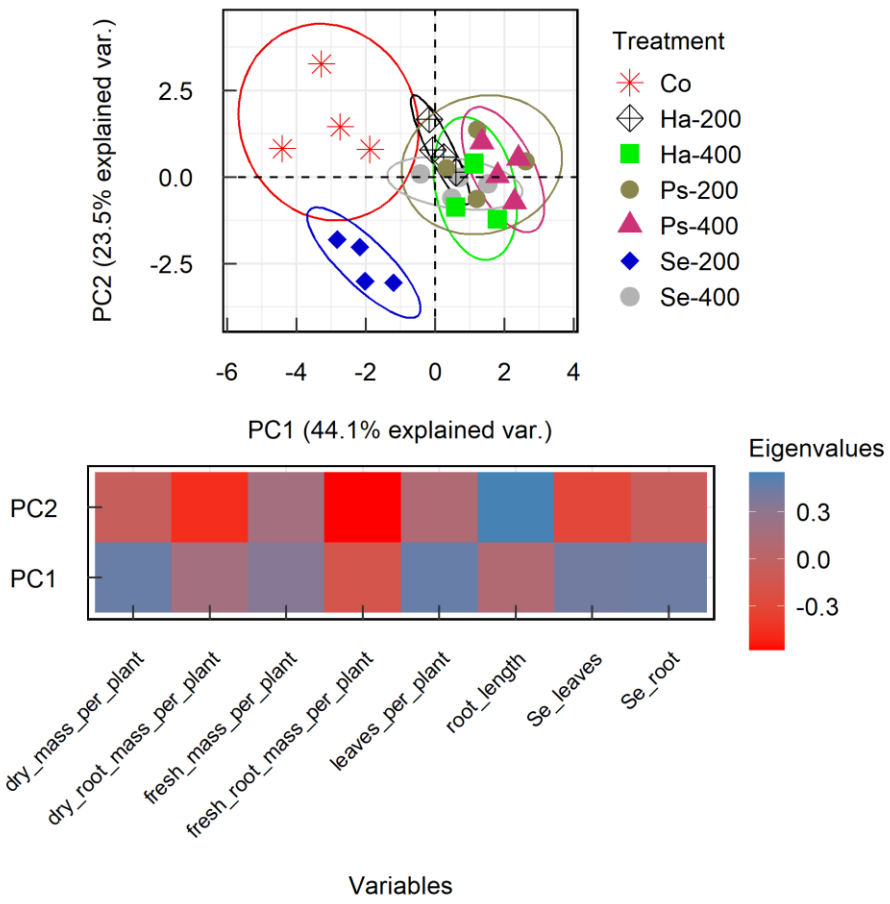
S obzirom na predviđeni globalni rast ljudske populacije na otprilike 9 milijardi do 2050. godine, postaje očito da je sigurnost hrane jedno od ključnih pitanja novog tisućljeća i, argumentirano, najhitniji izazov za poljoprivredni sektor gdje su klimatske promjene također značajan izazov (Adams i sur., 1999.; Sambo i sur., 2019.). Potreba očuvanja prirodnih resursa usmjerava agronomski pristup prema novim metodama proizvodnje uz optimizaciju potrošnje prirodnih resursa. U proizvodnji u staklenicima, hidroponski sustavi nude održivu alternativu tlu, omogućujući uzgoj usjeva u raznim okruženjima, uključujući područja gdje je tradicionalna poljoprivreda neizvediva (Vernieri i sur., 2005.). Plutajući hidroponski sustav je tehnika uzgoja bez tla u kojoj se biljke uzgajaju na stiropornim pločama u spremnicima s hranjivim otopinama (Lenzi i sur., 2011.) i predstavlja jedan od najjednostavnijih sustava uzgoja (Ferrarese i sur., 2012.). Uzgoj biljnih kultura u ovim sustavima sve je zanimljiviji proizvođačima, posebno u proizvodnji "*baby leaf*" povrća. Rastuća je potražnja za proizvodima vrhunske kvalitete uz stroge higijenske standarde (Gonnella i sur., 2003.), a prednost sustava uzgoja bez tla je što nema potrebe za dezinfekcijom tla, smanjena je mogućnost pojave bolesti koje se prenose tlom, što povećava sigurnost pakiranog povrća i smanjuje probleme s kemijskim ostatcima (reziduima) (Alberici i sur., 2008.). U hidroponskom uzgoju, provode se različita istraživanja u vezi s temperaturom otopine (Cortella i sur., 2014.) i raznim metodama za pripremu hranjive otopine (Tomasi i sur., 2014.; Sambo i sur., 2019.). Zbog klimatskih promjena, rastućih problema gladi i promjena u prilagođenosti usjeva u Europi i drugim zemljama diljem svijeta, postaje poželjno potražiti nove biljne vrste ili genotipove s visokim potencijalom u pogledu nutritivne vrijednosti i utjecaja na zdravlje (Baraniak i Kania-Dobrowolska, 2022.). Matovilac u Europi ima sve veći značaj kao salata, često prisutna u gotovim mješavinama za salatu (Enninghorst i Lippert, 2003.). Kao odgovor na prehranu s visokim kalorijskim sadržajem, pojavljuju se nove tehnologije proizvodnje i prerade hrane, pa tako i u pripremi "*ready to eat*" salate (Gonnella i sur., 2004.; Ferrante i sur., 2009.). Još jedna značajna i vrijedna "*baby leaf*" povrtnica je amarant, sve interesantniji

prehrambeni usjev. Ova jedinstvena biljka konzumira se zbog svojih listova kao povrće i zbog sjemenki kao žitarica (Rastogi i Shukla, 2013.). Stabljike i listovi amaranta nude povoljna i obilna vlakna, bjelančevine s esencijalnim aminokiselinama, vitamine, karotenoide, minerale i različite antioksidanse i fitokemikalije poput betacijanina, antocijana, karotenoida i askorbinske kiseline (Pasko i sur., 2015.; Sarker i Oba, 2020.). Osim toga, istraživanja poput istraživanja Hawrylak-Nowak i suradnika (2018.) i Puccinelli i suradnika (2021.) potvrđuju uspjeh biofortifikacije matovilca selenom (Hawrylak-Nowak i sur., 2018.; Puccinelli i sur., 2021.). U istraživanju koje su proveli Munandar i suradnici (2019.), također su ostvareni uspješni rezultati u biofortifikaciji listova amaranta jodom u hidroponskom uzgoju (Herlinda i sur., 2019.). Svrha ovog istraživanja bila je procijeniti uspješnost klasične i nano biofortifikacijematovilca i amaranta selenom u hidroponskom uzgoju. Istraživanje je uključivalo analizu prinosa matovilca i amaranta, koncentracija selena u navedenom "*baby leaf*" povrću te prateće morfološke karateristike i neka fiziološka svojstva. Na Slici 9 prikazana je koncentracija selena s obzirom na tretman u matovilcu i amarantu. Specifično je to što je amarant pokazao slabije usvajanje selenovih nanočestica sintetiziranih huminskom kiselinom (HA200 i HA400 tretmani) ili polisorbatom (PS200 i PS400 tretmani). Neki od mogućih obješnjenja su da se apsorpcija odvija kroz unutarstanične i izvanstanične puteve u biljnim tkivima sve do ksilema. Točan proces kojim nanočestice prolaze kroz Kasparijev pojas ostaje nejasan, no moguće je da se to događa pomoću meristemskih zona. Iako stanična stijenka služi kao fizička barijera, sadrži pore promjera od 5 do 20 nm, omogućavajući slobodan prolaz nanočesticama manjim od tog opsega (Márquez i sur., 2020.). U istraživanju koje su proveli Bai i suradnici (2021.), utvrđeno je da je usvajanje nanočestica veće u C3 vrstama nego u C4 vrstama, pri čemu su u tom istraživanju kukuruz i amarant primjeri za tip C4 biljke (Bai i sur., 2021.).



Slika 9. Koncentracije selena (µg/kg) u jestivom dijelu biljke (list) po tretmanu u matovilcu i amarantu. Izvor: Galić, neobjavljeni rezultati (2024.)

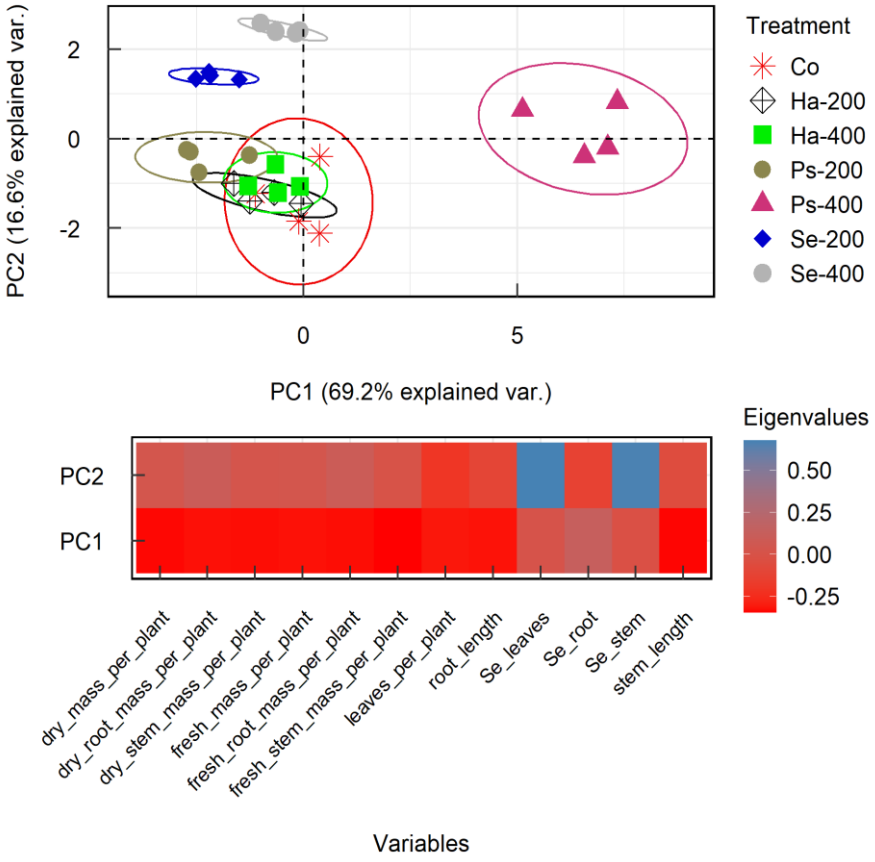
Analiza glavnih komponenata (Principal component analysis – PC) objasnila je 67,6 % ukupne varijance u morfološkim karakteristikama i koncentracijama selena u različitim dijelovima matovilca (Slika 10). PC1 je bio pozitivno povezan s ukupnom masom suhe tvari po biljci, brojem listova po biljci i koncentracijama selena u listovima i korijenju. PC2 je pokazao negativnu korelaciju s ukupnom svježom masom korijena po biljci i ukupnom masom suhe tvari korijena po biljci, te pozitivnu s duljinom korijena. Stoga je pozicioniranje kontrolne skupine i skupine SE200 na negativnoj strani PC1 ukazivalo na manju ukupnu suhu masu po biljci i niže koncentracije selena u listovima i korijenju. Suprotno tome, svi ostali tretmani grupirali su se na pozitivnoj strani PC1. Svi tretmani osim kontrolne i SE200 grupirale su se oko ishodišta PC2, ukazujući na prosječne reakcije u većini karakteristika. Međutim, grupiranje kontrolne i SE200 tretmana na pozitivnim i negativnim stranama, redom, ukazivalo je na razlike u ukupnoj svježoj masi korijena po biljci, ukupnoj masi suhe tvari korijena po biljci i duljini korijena između tretmana.



Slika 10. Analiza glavnih komponenata učinaka (Principal component analysis – PC) obrade na morfološke karakteristike i koncentracije selena u različitim dijelovima matovilca (gore) te varijabilni faktori (korelacije između PC-a i originalnih varijabli, dolje). Izvor: Galić, neobjavljeni rezultati (2024.)

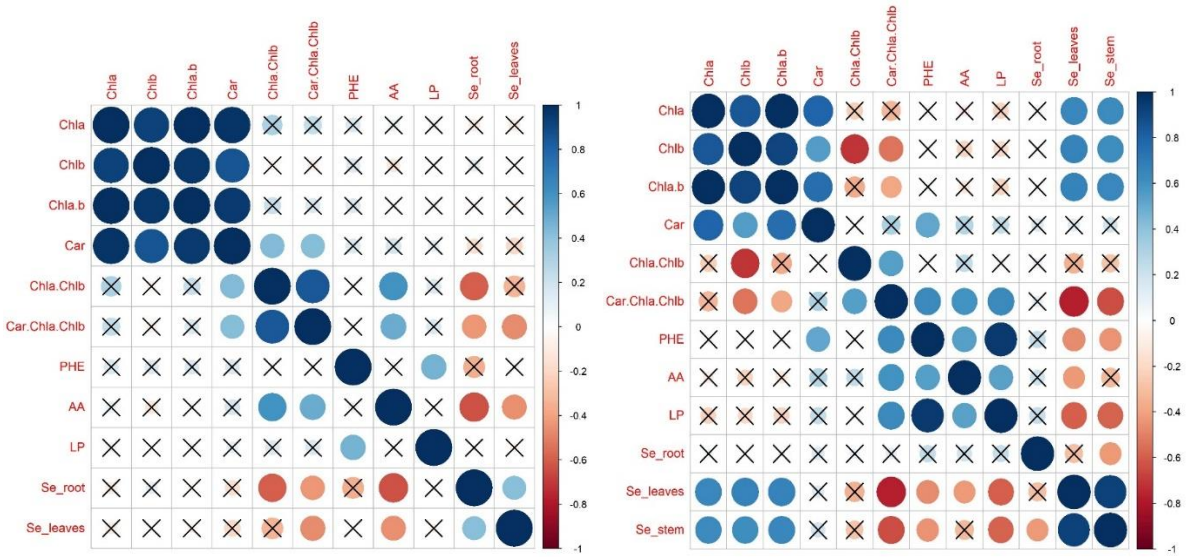
Analiza glavnih komponenata kod amaranta (Slika 11) objasnila je 85.8 % ukupne varijacije u skupu podataka u prve dvije PC-a. Analiza opterećenja ukazala je na negativne korelacije između svih morfoloških karakteristika i PC1, dok su korelacije s koncentracijama selena u različitim dijelovima biljke izostale. Suprotno tome, PC2 bila je pozitivno povezana s koncentracijama selena u listovima i stabljikama. Razmještaj različitih tretmana duž PC1 ukazao je na nadprosječne performanse biljaka u tretmanu PS400 u svim morfološkim karakteristikama, dok su se svi ostali tretmani grupirali blizu početne točke PC-a i njegove negativne strane. Grupiranje u PC2 ukazivalo je na najviše koncentracije selena u tretmanu SE400 uz performanse koje su bile blizu prosječnim vrijednostima morfoloških karakteristika.U tretmanu SE200 biljke su pokazale ispodprosječne rezultate u morfološkim karakteristikama s koncentracijama selena višim od prosjeka. . Svi ostali

tretmani grupirali su se na negativnoj strani PC2 s performansama koje su bile blizu prosječnim ili ispodprosječnim. U skladu s jednostrukom analizom varijance, multivarijantna analiza, posebice analiza glavnih komponenata (PCA) u ovom slučaju (Slika 10 i Slika 11), pokazala je da je tretman PS200 u matovilcu dao najpovoljnije rezultate za gotovo sve promatrane karakteristike, dok su kod amaranta svi tretmani bili relativno slični, s izuzetkom nanočestica selena u tretmanu PS400 kod amaranta. Rezultati ovog istraživanja u skladu su s drugim istraživanjima učinka selenata, selenita i nanoselena na morfološke karakteristike, kao što je vidljivo u istraživanju na bobu (*Vicia faba* L.) koje su proveli Sindireva i suradnici (2023.), gospinoj travi (*Hypericum perforatum* L.) u istraživanju Nazari i suradnika (2022.) te istraživanju na pelinu (*Artemisia annua* L.) od Logvinenko i suradnika (2022.).



Slika 11. Analiza glavnih komponenata učinaka tretmana na morfološke karakteristike i koncentracije selena u različitim dijelovima biljke kod amaranta (iznad) i opterećenja varijablama (korelacije između glavnih komponenata i izvornih varijabli, ispod). Izvor: Galić, neobjavljeni rezultati (2024.)

Na Slici 12 (lijevo) prikazana je korelacija između fizioloških parametara i koncentracija selena u listovima i korijenu matovilca. Pigmenti pokazuju snažnu međusobnu pozitivnu korelaciju, dok su s drugim parametrima poput askorbinske kiseline (AA) u slaboj do umjerenoj pozitivnoj korelaciji. U korijenima matovilca postoji umjerena negativna korelacija između pigmenata i selena, dok je negativna korelacija selena s koncentracijama u listovima matovilca nešto slabija. Također, uočena je slaba pozitivna korelacija između lipidne peroksidacije (LP) i fenola. U amarantu (Slika 12 – desno) posebice karotenoidi imaju slabu pozitivnu korelaciju s fenolima (PHE). Konačno, selen u listovima i korijenima pokazuje umjerenu pozitivnu korelaciju s pigmentima klrofila, klorofil b i kerotenoidi (Chl.a, Chl.b i Car), dok selen u listovima pokazuje snažnu negativnu korelaciju s omjerima pigmenata (Chl.a/Chl.b/Car), gdje su se omjeri kretali od 3.97 do 4.36. Lipidna peroksidacija (LP) umjereno negativno korelira s selenom u listovima i stabljikama amaranta. Utvrđena je vrlo visoka pozitivna korelacija između selena u listovima i selena u stabljikama, dok se u korijenima pokazuje slabom negativnom korelacijom sa selenom u stabljikama. Usklađeno s podacima prikazanim u korelacijskim grafikonima (Slika 12), može se zaključiti da su ovi tretmani selena, u različitim oblicima, imali ograničeni utjecaj na fiziološke parametre. Selen nije značajno utjecao na druge fiziološke parametre, uključujući fenole, askorbinsku kiselinu i lipidnu peroksidaciju. Ovi nalazi su u skladu s istraživanjem Neysanian i suradnika (2020.), koji su pokazali poboljšan rast, produktivnost i kvalitetu biljaka rajčice primjenom selenata i selena u obliku nanočestica, potvrđujući time rezultate ovog istraživanja gdje su Neysanian i sur., 2020.) analizirali askorbat, neproteinske tiole, prolin i topljive fenole. Rezultati Puccinelli i suradnika (2021.) također su dokazali da istodobno dodavanje selena u dozama od 13 μM i joda od 5 μM u i, povećava sadržaj ova dva mikroelementa u listovima salate bez negativnih interakcija (Puccinelli i sur., 2021.). Iz dostupnih podataka jasno je da selen u obliku nanočestica ima potencijal za poboljšanje morfoloških karakteristika, kao što su prinos biljaka i broj listova po biljci, dok ne izaziva fiziološke reakcije. Ovo je značajan parametar u razvoju suvremenih praksa uzgoja biljaka, s potencijalom za širu komercijalnu primjenu, posebice u pogledu upotrebe nanočestica selena.



Slika 12. Korelacijski dijagrami (korrelogram) prikazuju negativne (-, crvene) i pozitivne (+, plave) korelacije između fizioloških parametara i ukupnih koncentracija selena u listovima i korijenu matovilca (LIJEVO) i amaranta (DESNO). Izvor: Galić, neobjavljeni rezultati (2024.)

6. ZAKLJUČCI

- Agronomska biofortifikacija žitraica vrlo je značajna za smanjenje nedostatka selena u prehrambenim proizvodima jer su žitarice najčešća hrana u ljudskoj prehrani diljem svijeta, a biofortifikacija se može provoditi vrlo uspješno.
- Prema meta-analizi, folijarna primjena selana je pokazala veću učinkovitost u odnosu na primjenu u tlo. Oblik primijenjenog selena također igra važnu ulogu u povećanju sadržaja selena u zrnu žitarica, pri čemu se selen u oblik selenata pokazao učinkovitijim za agronomsku biofortifikaciju žitarica, osim riže kod koje je učinkovitija upoteba selenita.
- Značajne površine tala diljem svijeta karakterizira nedostatka selena, uključujući lokalitete u područjima na jugoistoku Europe koja su ispitana u istraživanju (Osijek, Prud, Sarajevo, Banja Luka, Novi Sad), osim Mostara. Za utvrđivanje potrebe biofortifikaciju selenom na jugoistoku Europe potrebno je poznavati fizikalno-kemijska svojstva tala jer se ona mogu značajno razlikovati, a utječu na dostupnost selena biljkama.
- Vodotopivi selen u tlima, koji je dostupan biljkama, u pozitivnoj je korelaciji s organskom tvari, kationskim izmijenjivačkim kapacitetom, koncentracijama ukupnih ugljika, dušika, kalcija, natrija, željeza, cinka, kadmija i ukupnog selena.
- Prediktivni model ima veliki potencijal optimizacije biofortifikacije selenom na istraživanim lokalitetima, ali i za druge regije koje karakterizira nedostatak selena u tlu. Korištenjem osnovnih analiza tla poput organske tvari, kationskog izmijenjivačkog kapaciteta, ukupnog dušika, ukupnog ugljika, kalcija i natrija, prediktivna točnost modela može se značajno poboljšati.
- Iz rezultata istraživanja na soji vidljivo je da bi biofortifikacija sojinog sjemena sa selenom mogla pomoći ublažiti učinak nedostatka vode kod kultivara Lucija, dok su kod kultivara Sonja utvrđene visoke koncentracije lipidne peroksidacije, prolina, ukupnih fenola, ukupne antioksidativne aktivnosti i askorbinske kiseline što ukazuje na to da je tretman biofortifikacije sjemena kultivara Sonja selenom potaklo snažan mehanizam obrane.
- Kultivar Sonja osjetljiviji je na selen i ima snažniji fiziološki odgovor ili su obrambene reakcije kod kultivara Sonja sporije i nisu bile utvrđene postavkama provedenog istraživanja. Rezultati istraživanja također ukazuju na genotipsku varijabilnost soje u

reakciji na primjenu selena, što znači da je potrebno odrediti koje koncentracije selena su optimalne za određeni genotip u smislu ublažavanja stresnog učinka.

- Biofortifikacija selena kod kultivara Lucija mogla bi biti prikladna za poboljšanje otpornosti usjeva, što bi trebalo dalje istražiti. Sorta Sonja je interesantnija za istraživanja u pogledu proizvodnje funkcionalne hrane (npr. u obliku sojinih klica) jer je selen direktno utjecao na veću proizvodnju ukupnih fenola i ukupne antioksidativne aktivnosti te je udvostručio koncentraciju vitamina C.
- Vermikompost bi u uzgoju matovilca mogao smanjiti upotrebu komercijalnih supstrata zbog odličnih rezultat uzgoja na 1:1 smjesi vermikomposta i komercijalnog supstrata.
- Biofortifikacijom matovilca selenom može se povećati koncentracija selena u listu više od 170 puta te bi dnevna konzumacija 50-ak grama matovilca bil dovoljna za unos dnevne preporučene količine selena.
- Uporaba nanočestica selena s polisorbatom (tretmani PS200 i PS400) poboljšava prinos i broj listova matovilca u hidroponskom uzgoju.
- Istraživanja ukazuju na visoke prinose matovilca i amaranta tretiranih selenatom i nanoselenom, osim amaranta u tretmanu sa polisorbatom (PS400), što otvara mogućnost biofortifikacije u realnom uzgoju pošto tretman selenom nije rezultirao negativnim fiziološkim odgovorm matovilca ili amaranta.
- Različito usvajanje nanočestica selena matovilcem (C3 tip biljaka) i amaranto (C4 tip biljaka) sugerira potrebu za daljnjim istraživanjima uključujući i kombinirane primjene konvencionalnog selena i nanočestica.

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Review

Agronomic Biofortification of Significant Cereal Crops with Selenium—A Review

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Abstract: Selenium (Se) is an important micronutrient which is essential for most living organisms and occurs in both organic and inorganic forms in the water system, soils, biomass, and the atmosphere. In addition to being essential for humans and animals, Se is beneficial for plants and is mostly involved in antioxidant activity/response, as well as a growth promoter. Se deficiency in the diet is a global problem, and Se levels in soils generally reflect its presence in food and, thus, availability to humans. Se participates in the antioxidant response mechanisms of the organism, heavy-metal detoxification, and regulation of the reproductive and immune system, as well as ensures the proper function of the thyroid gland. Plants are the main dietary source of Se for humans. Biofortification is a key strategy to increase Se in edible parts of plants. Agronomic biofortification provides an effective route to increase Se content in edible crop products via application of Se-enriched fertilizers to soil or by foliar application. The most common cereals in the human diet are wheat, rice, maize, and barley, making them the most suitable targets for agronomic biofortification. This review focuses on summarizing the most efficient form and method of Se application via agronomic biofortification corroborated by a meta-analysis of the literature reports. In the assessed literature, foliar application showed better results compared to application in soil. The selenate form appears to be the more efficient form of Se for biofortification than selenite in the most common cereals in human diet: wheat, rice, maize, and barley.

Keywords: agronomic biofortification; selenate; selenite; cereals; meta-analysis

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1. Introduction

Selenium (Se) is a nonmetal with atomic number 34 in group 16 [1]. The Se atom is slightly larger than sulfur (S) (the radius of Se^{2+} is 0.5 Å whereas the radius of S^{2+} is 0.37 Å), and, like S, Se can exist in five valence states, selenide (2^-), elemental Se (0), thioselenate (2^+), selenite (4^+), and selenate (6^+) [2,3]. Se is a mineral micronutrient essential for the adequate and healthy life of humans, animals, archaea, and some other microorganisms [4]. Se exists in the lithosphere layer of the earth crust, i.e. water, soil, and in open environments; therefore, its distribution differs throughout the globe [5,6]. Se levels in European soils are low, particularly in eastern Europe [6]. Plants absorb Se from soil primarily as selenate and translocate it to the chloroplasts, where it follows the sulfur assimilation pathway [7]. Se is essential for lower plants, but its essentiality in higher plants is still under investigation [8]. Se can have physiological benefits for plants, which may be related to its tendency to upregulate plant antioxidant metabolites and enzymes, leading to a better capacity to scavenge reactive oxygen species (ROS) that impede plant performance, especially under stress conditions [9].

Dietary Se deficiency has negative effects for human health, and more than 40 types of diseases have been associated with Se deficiency, such as Keshan disease, Kashin–Beck disease, some types of cancer, cardiovascular diseases, liver diseases, and cataracts [10]. Selenium deficiency occurs in several parts of the world, especially where Se concentration in soils is low, leading to poor concentration in agricultural plants [11]. The process of increasing the bioavailable concentrations of essential elements in edible parts of crop plants through agricultural intervention or genetic selection through breeding process is called biofortification [12]. Se biofortification substantially increases Se contents in agricultural food products and can help alleviate Se malnutrition, affecting more than one billion people worldwide [13]. Dietary deficiencies of Se can be improved through dietary diversification, food fortification, supplementation, or crop biofortification, including fertilization (agronomic biofortification) or crop improvement (genetic biofortification) as different approaches [14]. Cereal grains are grown and consumed by humans in higher quantities than any other crop and provide most of the food energy consumed worldwide [15], thus representing the most significant plant candidates suitable for biofortification with additional possible positive effects on plant yield [16], although differences in nutritional profiles and selenium content between wheat species were not observed [17].

Cereals are the most important source of Se in the Western diet with major representatives being rice, wheat, maize, and barley [18], while Se-enriched wheat has long been recognized as a source of supplemental Se for Se-deficient populations [7]. Many studies confirmed the positive effects of Se agronomic biofortification on Se content in rice, wheat, maize, and barley grains [16,19–35]. Agronomic biofortification is based on applying fertilizers with mineral elements lacking in diet to increase their concentrations in crops through soil or foliar application [30]. The two most important factors in achieving the increased Se content in grains are the type of application and the form of Se, although systematic reports on these questions are still missing [36]. Plants uptake Se more readily when applied in a foliar manner, compared to soil application, with the additional benefit of having no residual effects in the soils [37]. The foliar technique includes minimal consumption of Se salts and represents the most effective, safe, and economically justified measure of improving Se content in agricultural crops [38]. The reason for the higher efficiency of foliar application is that there is no root-to-shoot translocation, accompanied by the finding that soils can act as considerable sinks for Se [39]. The form of applied Se is the second most important factor for effective agronomic biofortification [40], with the most commonly used forms of Se being selenate and selenite. However, nanoselenium (nSe) is being used more and more frequently [8]. The synthesis and use of nSe as a nutrient and biofortifier has been proven as an interesting strategy [40], although reports of wider use are still missing. Studies have shown selenate (Se(VI)) to be the most effective form of Se when applied to the soil and usually more effective compared to selenite (Se(IV)) when applied in a foliar manner [41]. Selenate is more efficient due to the more rapid uptake of Se (VI) and translocation from root to stem and leaves, as well as quicker transformation into its organic form, while selenite is more easily adsorbed to the soil, which makes its uptake by roots more difficult [42]. Selenite has characteristics similar to phosphate, corroborating its easy adsorption to the soil surfaces compared to selenite [43].

In the context of the Se biofortification complexity and differences in the methodology found in literature survey, the aim of this study was to review the present knowledge on the basis of studies dealing with Se agronomic biofortification as the most feasible way of increasing the Se content in the food, as well as to identify the best practices for accomplishing efficient Se biofortification. The former aim was supported by a meta-analysis of the data found in different research reports by using a linear mixed modeling approach. Research reports for meta-analysis were selected on the basis of consistency in methodology and availability of important data needed for adjustment in the linear mixed model. Generally, the methodology found in the research articles addressing Se biofortification was not harmonized and uniform.

2. Selenium in the Environment

Selenium (Se) is a nonmetal belonging to the same group of elements (group 16) as oxygen and sulfur (S). Se is rarely considered a metalloid due to its intermediate properties between a metal and a nonmetal. It is stable and does not oxidize at ordinary temperatures [2,3]. Naturally, it exists in five different oxidation forms in the environment, such as Se(VI) (selenate), Se(IV) (selenite), Se(0) (elemental Se), and Se(−II) (selenide), along with various organic species [1]. Many elements can be combined with Se, such as hydrogen, fluorine, chlorine, bromine, and phosphorus, and Se can form compounds analogous to sulfur [3]. The distribution of different species of Se may vary in the environment depending on the prevailing redox conditions. Principally, selenium oxyanions (SeO_4^{2-} and SeO_3^{2-}) are highly soluble, stable, and potentially mobile in oxic natural environments [44]. Se can be found in all components of the agroecosystem including soils, plants, rocks, and water [5,28]. The biogeochemical cycle of Se begins from weathering of Se-rich rocks, soils, and sediments, moving toward the different water bodies. Later on, from water, it arrives in plants, animals, or humans by various means. The Se cycle is completed by the degradation and different activities of organisms, which allow Se accessibility in the soil sediments and, ultimately, the rock depositaries [1]. Distribution processes of Se through the environment, thus, involve a variety of physical, chemical and biological activities [4]. The presence of Se is linked to natural activities such as soil erosion, volcanism, and forest fires, with the atmosphere playing an important role in the biogeochemical cycle of Se. The Se content in ambient air is mainly low, and it varies from 1 to 10 ng $Se \cdot m^{-3}$ [3], while coal and oil burning are the primary sources of considerable emissions of Se compounds in the air. In natural waters, the dissolved selenium concentrations are reported to be in the range of <0.1 to 100 $\mu g \cdot L^{-1}$ [44], and the average Se concentration in the Earth's crust is around 0.05 $mg \cdot kg^{-1}$ [39].

2.1. Selenium in Soils

Se is a rare element, with an average concentration in igneous bedrock of only 0.05 $mg \cdot kg^{-1}$, less than any other nutrient element [45]. The largest reservoirs of Se are sulfide ores, pyrite, and high-sulfur coals [44]. Se occurs naturally in soils, at highly variable concentrations dependent on soil type [46]. Most soils have Se concentrations between 0.01 and 2.0 $mg \cdot kg^{-1}$ [47] with a worldwide mean of 0.4 $mg \cdot kg^{-1}$ [48]. However, in some soils associated with particular geological formations or climatic conditions, concentrations of Se up to 1200 $mg \cdot kg^{-1}$ have been reported [47]. Water-soluble selenate has been reported in seleniferous areas of the world at concentrations of around 38 mg $Se \cdot kg^{-1}$ [49] In such soils, Se is often lacking (for example, in China and USA, mainly derived from sedimentary rocks originating from the Cretaceous Period), while other soils are thought to derive much of their Se from atmospheric depositions [47]. The soils originating from sedimentary rocks, with high organic matter content, can contain high and potentially toxic concentrations of Se. In contrast, soils formed from magmatic rocks typically have low Se concentrations [48]. Generally, Se levels in European soils are low, particularly in eastern Europe [6]. Mountainous countries such as Finland, Sweden, and Scotland are generally deficient in soil Se content, while countries such as UK, France, India, Belgium, Brazil, Serbia, Slovenia, Spain, Portugal, Turkey, Poland, Germany, Denmark, Slovakia, Austria, Ireland, Greece, Netherlands, Italy, China, Nepal, Saudi Arabia, Czech Republic, Croatia, Egypt, Burundi, and New Guinea are reported to have Se-deficient areas. Some known Se-rich regions are the northeast region of Punjab in India, the Enshi district in Hubei province in China, the state of Para in the Brazilian Amazon, Japan, Greenland, USA, Venezuela, and Canada [13]. Se exists in soil in different forms including selenate (Se^{6+}), selenite (Se^{4+}), elemental selenium (Se^0), selenide (H_2Se), and organic selenide, among which selenate, partially selenite, and organic selenide are per se available for plant uptake [50]. Inorganic Se occurs in three phases: soil-phase-fixed, adsorbed to soil, and soluble, with only adsorbed/soluble forms of Se being available for plant uptake [51]. Se bioavailability

in soil varies greatly with different soil properties and composition. Soil pH and redox potential are the key factors reflecting Se bioavailability. Principally, Se is more strongly immobilized in acid and reductive soils [52]. In acid soils, Se is immobilized by sesquioxides [53], and, in organic soil fractions, it is more weakly bound to fulvic acids (FA) compared to humic acids (HA) [54]. Under strongly reductive soil conditions (pH <4 and Eh <0), selenate and selenite are easily reduced to selenide or even to elemental selenium, which are less available to plants [55] and show lower mobility [48]. Adsorption of selenite on goethite produces two type of complexes: the protonated selenite anion ($HSeO_3^-$) with the active site on the goethite surface, and the bivalent selenite anion (SeO_3^{2-}) reacting with the surface site. The proportion of each complex depends on the pH of the suspension [53]. Se speciation in the soil is basically controlled by three main mechanisms: oxidation vs. reduction, mineralization vs. immobilization, and volatilization. The rate coefficients of these processes vary depending on Se species, microbial activity, pH, and redox conditions, along with other soil properties [52]. Under aerobic conditions, selenium slowly oxidizes to selenite or selenate, depending on the soil pH. Alkaline soils favor the formation of selenate; however, in moist soils, most of the selenate is readily leached from the surface layers. In acidic soils, iron in colloidal material and sesquioxides render the selenite relatively unavailable as ferric selenite [56–58]. There is a general consensus that organic matter interacts with Se via a variety of mechanisms that can immobilize or release Se in soil [59] depending on the type of organic compounds present in soil, with some organic acids having the opposite effect on plant Se availability [60]. In addition to leaching, Se can be lost by volatilization in the form of dimethylselenide (DMSe), dimethyldiselenide (DMDSe), and dimethylselenone or methylmmethylselenite [61]. Strategies for crop biofortification with essential elements for humans aim to increase their accessibility in the soil. The soils lacking in essential elements are usually supplemented by the use of fertilizers. The amount of Se in the rhizosphere, its availability for plant uptake, and the physicochemical soil properties are usually considered for efficient agronomic biofortification.

2.2. Selenium in Plants

Plants mainly take up nutrients via their roots, and the local conditions in the rhizosphere can influence the bioavailability of Se to the plants [62]. The uptake of Se by plants is governed by many factors in the soil and plants. The most important factors determining uptake are the form and concentration of Se in the soil. Other important factors in determining the accumulation of Se by plants include soil properties such as pH, clay content, soil mineralogy, and the concentration of competitive anions [57]. Moreover, the uptake of Se by the plant can be greatly inhibited by the simultaneous occurrence of a high soil content of organic matter, Fe hydroxides, and clay minerals, all of which can adsorb or bind Se [63]. Se can adsorb on positively charged sites of Al-octahedral sheets in clay minerals, such as kaolinite, and it varies greatly with soil pH. Interactions of Se with soil components are either via electrostatic attraction or via complex formation on soil mineral surface [48]. Therefore, the plant-available Se in the soil, such as water-soluble and exchangeable (adsorbed) Se, consists of mobile fractions that are readily taken up by the plants. Plants are the main driving force for nutrient movement from the non-rhizosphere to rhizosphere soil layer [64]. There are no studies describing Se essentiality in plants, although numerous researches have reported the beneficial effects of Se on plant growth especially under biotic and abiotic stress conditions [65,66]. Se and sulfur (S) compete for the same transporters, and Se uptake is generally limited by high S levels [65]. Plants can be classified as hyperaccumulators, secondary-accumulators, and non-accumulators depending on Se accumulation inside their cells [13]. Se non-accumulators act against Se uptake relative to sulfate, whereas Se hyperaccumulator species preferentially absorb Se over S [67], such as *Stanleya pinnata* [68], *Xylorhiza* spp., and *Symphyotrichum* spp. [69]. Hyperaccumulators accumulate higher amounts of Se in their cells (i.e., >1000 mg Se·kg^{-1} dry weight (DW)) and thrive well in Se-rich regions of the world. Secondary accumulators accumulate Se in the range of 100–1000 mg Se·kg^{-1} DW, such as *Brassica juncea* [69], and

non-accumulators, such as tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*) [68], are those plants which accumulate less than 100 mg Se·kg^{-1} DW [13]. Most crop plants are low rather than high Se accumulators [70]. Se typically stimulates growth and stress resistance at 1–10 mg Se·kg^{-1} DW, while the tissue concentration at which toxicity occurs is over 100 mg Se·kg^{-1} DW [9]. Of the two inorganic forms of Se, selenate and selenite, selenate is much more mobile and, thus, more plant-available in soils compared to selenite, which is tightly bound to positively charged binding sites in the soil [60]. Inorganic forms of Se absorbed by plants are transported from the root to the shoot through the xylem with the transport process dependent of the form of externally supplied Se. Se(IV) can be easily absorbed and transported by the xylem, before being distributed further to the reproductive organs by the phloem [62]. There are considerable differences present in the mechanisms of uptake and transport of selenate and selenite in plants [71]. Selenate, which is more soluble than selenite, can pass directly into plant roots [72], whereas selenite is probably transported by phosphate transporters [73]. Selenate is accumulated in plant cells against the gradient of electrochemical potential through a process of active transport [2]. The uptake of selenate across the plasma membrane of root cells is catalyzed by the high-affinity H^+/sulfate symporters, homologous to AtSULTR1;1 and AtSULTR1;2 of the model brassicaceous plant *Arabidopsis thaliana* L. [47]. On the contrary, selenite uptake is carried out through passive diffusion. Moreover, it was reported that it is mediated by active transport, as the uptake of selenite was significantly inhibited by a metabolic inhibitor [13]. Plants cannot directly take up metallic selenide and elemental Se because these forms of Se are water-insoluble, while organo-Se compounds such as seleno-amino acids have relatively higher phytoavailability [48]. The two Se-amino acids produced in the S assimilation pathway are selenocysteine (SeCys) and selenomethionine (SeMet), which are analogues of the S-amino acids cysteine (Cys) and methionine (Met) [74]. Selenocysteine (SeCys) and Selenomethionine (SeMet) are both taken up at rates that were up to 20-fold higher than those observed for selenate or selenite [62]. SeMet is one of the most effectively accumulated Se species in different organs [75]. The distribution of Se in various parts of the plant differs according to species, phase of plant development, and plant physiological condition [2]. Se concentrations tend to be the greatest in the younger leaves of plants and generally increase to a maximum during seedling growth, prior to declining before or upon flowering, when Se is translocated from leaves to reproductive organs [76]. In Se accumulators, Se is accumulated in young leaves during the early vegetative stage of growth. During the reproductive stage, high levels of Se are found in seeds, while the Se content in leaves is drastically reduced. Non-accumulator cereal crop plants, when mature, often show about the same Se content in grain and roots, with smaller amounts in the stem and leaves [2]. Following uptake by root cells, selenate moves rapidly through the root symplast to the stele and is translocated to the shoot, whereas selenite is converted to organoselenium compounds, which often remain within the root [77]. The conversion of selenate to selenite involves the consecutive action of two enzymes. ATP sulfurylase (APS) couples selenite to ATP, forming adenosine phosphoselenate (APSe). APSe is subsequently reduced to selenite by APS reductase (APR). There are isozymes for APS and APR in both chloroplast and cytosol, but most of the selenite reduction likely takes place in the chloroplasts. The further reduction of selenite to selenide may happen exclusively in the chloroplast if it is mediated by sulfite reductase, in analogy with sulfite reduction [78]. Se has dual effects on plant physiology depending on its concentration in plant tissues. At low doses, it can stimulate the growth of plants and counteract many types of environmental stresses [10]. At low concentration, Se enhances plant growth and can act as an antisenescent agent, assisting the upholding of cellular constituents and activities, thus helping to improve plant performance [5]. Se from plants has a negative effect on Se-sensitive ecological partners, which may protect plants from pathogens and herbivores, and which have allelopathic effects on neighboring plants [79]. Se was found to help to increase K^+ accumulation in plants and also mediate the increase in chlorophyll *a*, chlorophyll *b*, and total chlorophyll content by 65%, 39%, and 56%, respectively, as Se uptake is

linked to enhancing the uptake of Mg and Fe [5]. Many studies suggested the role of Se in the following plant physiological mechanisms: the regulation of reactive oxygen species (ROS) and antioxidants, the inhibition of uptake and translocation of heavy metals (HMs), changes in the speciation of HMs, rebuilding of the cell membrane and chloroplast structure, and recovery of the photosynthetic system [10]. Selenium addition to growing media or nutrient solution also increased the net photosynthesis rate, stomatal conductance, and transpiration rate of different plants [5]. Plants vary considerably in their physiological and biochemical response to Se, and a revision of the physiological responses of plants to Se was presented, especially in growth, uptake, transport, and interaction of Se with other minerals [80]. Beneficial soil microorganisms associated with plant roots via symbiotic association are rhizobia, mycorrhizal fungi, actinomycetes, and diazotrophic bacteria that protect plants by various means such as the promotion of nutrient mineralization and production of plant growth hormones [81]. The role of mycorrhizal fungi in enhancing Se uptake in plants [82], as well as the use of Se-tolerant bacteria (*Pseudomonas aeruginosa, Bacillus* spp., *Enterobacter* spp., *Stenotrophomonas* spp., *Acinetobacter* spp., and *Klebsiella* spp.) appears to be a possible alternative for Se enhancement of cereals grown on soils with low Se concentration [83]. The uptake of Se by agricultural crops is also dependent on the plant species [57]. The biogeochemical behavior of Se in soil–plant systems is considered to be the basis of Se cycling in living organisms. In fact, the Se content of edible plant parts is often closely related to soil Se content where the crops are being cultivated [48].

2.3. The Impact of Selenium Bioavailability on Human Health

Food safety and nutritional quality represent a priority to improve the health status of the global population [42]. Malnutrition is the main cause of global human mortality, with over 50% of deaths attributed to diet-related diseases [41]. Micronutrient deficiencies in human body mainly result from low concentrations and low availability of micronutrients in daily diet [84]. Although, Se is one of the most significant micronutrients for all forms of life, high levels of Se can be toxic, and the redox chemistry of Se can significantly influence its toxicity, mobility, and bioavailability [1]. Many agricultural areas in the world contain low Se levels, and it is estimated that more than one billion people are suffering from Se deficiency [46]. Se intake from drinking water and other nonfood sources is also minimal in most areas [14]. Individual dietary Se intakes across the world are estimated to range from 3 to 7000 μg per day, whereas, for European countries where estimates are available, mean intake is typically <50 $\mu g \cdot day^{-1}$ per person [51]. Daily intakes are high in Venezuela, Canada, USA, and Japan, but much lower in Europe, particularly in its eastern parts [85,86]. The minimal Se requirements depend on the form of Se ingested and the properties of the rest of the diet, as well as on the content of α-tocopherol, which seems to reduce the amount of required Se [87]. Recommended daily intake (following international guidelines) is 40 μg for adult women and 50 μg for men [45]. It has been suggested that approximately 100 selenoproteins may exist in mammalian systems [88]. Selenoproteins include glutathione peroxidases and thioredoxin reductases, which have a variety of functions including protection from oxidative damage, regulation of intracellular redox state, and thyroid hormone metabolism [39]. There is evidence that Se deficiency can adversely affect human health in a number of ways, such as suppressing immune functions, making the organism prone to viral infections, lowering reproduction success (especially male fertility), negatively regulating thyroid function, and causing asthma and various inflammatory conditions [65,89]. Additionally, selenium deficiency causes dilated cardiomyopathy (Keshan disease) and endemic osteoarthropathy (Kashin–Beck disease) [90]. A US study found Se-deficient HIV patients to be 20 times more likely to die from HIV-related causes than those with adequate levels of Se [91]. Se also acts as a cofactor of the GPx family of isoperoxidases which protect human cells against oxidative stress [92]. Recently, an epidemic caused by a novel coronavirus (COVID-19 or 2019-CoV) has spread worldwide while threating human health and threatening the world economy.

Supplementary natural treatments should be considered to reduce the viral load in hosts and enhance their immune system, such as Se supplementation. Similar to Zn, Se supplementation to COVID-19 infected patients with low Se blood levels could be an option as a natural treatment against the virus [93]. Previous studies reported that Se concentrations in plants and animals are closely correlated to Se contents in soil. It is also true that, in natural environments, some crop wild relatives have a high percentage of microelements useful to humans, such as Zn and Fe, which are contained in high quantities, e.g., in *Aegilops ventricose* Tausch [94] which is a progenitor of cultivated wheat (*Triticum* sp.). Some authors [95] have proposed to start its cultivation in cooperation with local farmers, because, unlike cultivated wheat varieties, *Ae. ventricose* has a higher quantity of microelements such as Fe and Zn, and it can be use as a natural alternative to conventional medicine to help people with deficiencies in these microelements. Therefore, it should also be verified if some wild species are rich in Se. Humans and animals ingest Se by consuming agricultural products derived from plants that absorb Se from soil [59,63,96]. Generally, human blood Se levels follow the same geographical pattern around the world as those of livestock in the same regions [96]. Se content of foods varies as follows: organ meats and seafood, 0.4 to 1.5 $\mu g \cdot g^{-1}$; muscle meats, 0.1 to 0.4 $\mu g \cdot g^{-1}$; most agricultural crops, <1 $\mu g \cdot g^{-1}$ dry weight, cereals and grains, <0.1 to greater than 0.8 $\mu g \cdot g^{-1}$; dairy products, less than 0.1 to 0.3 $\mu g \cdot g^{-1}$; fruits and vegetables, less than 0.1 $\mu g \cdot g^{-1}$ [63]. In humans, Se absorption from products of plant origin is much easier than Se absorption from products of animal origin. Therefore, researchers are mostly interested in analyzing Se speciation in plant-derived fortified foods [97]. Se concentration in foods, such as rice and wheat grain, can vary greatly across countries and regions. Thus, to avoid Se deficiency and toxicity, it is important to monitor and optimize Se concentrations in various crops [98]. Improving Se uptake by different crops during growth through biofortification can provide additional supplementation of Se in the human diet [70].

3. Agronomic Biofortification

The efficacy of food fortification has been demonstrated consistently considering different micronutrients and different foods [99]. Biofortification was firstly defined by Bouis (1996) [84] and evolved to be thought of as a process of increasing the bioavailable concentrations of essential elements in edible portions of crop plants through agricultural intervention or genetic selection [12]. Different approaches can be explored to enrich plants with Se [93]. Dietary deficiencies of Se can be improved through dietary diversification, food fortification, supplementation, or crop biofortification, including fertilization (agronomic biofortification) and crop improvement (genetic biofortification) [14]. Agronomic biofortification consists of applying fertilizers of mineral elements lacking in the diet in order to increase their concentrations in crops through soil or foliar application [30]. Se-enriched fertilizers for soil or foliar application for agronomic biofortification of cereals provide the best short-term solution for increasing Se concentrations in crops [55]. Compared to foliar application, soil application of Se introduces confounding factors, such as adsorption to soil colloidal surfaces, resulting in less Se available to the plants [70]. Cereals are grown in the greatest quantities and provide more food energy worldwide than any other type of crops; they are, therefore, staple food crops [15]. The availability of soil Se to crops can be affected by irrigation, aeration, liming, and Se fertilization [91]. Generally, mean concentrations of Se in grains are higher in countries having arid climates than in countries having humid climates [80]. Se uptake also varies with the rate of plant growth, soil type, soil concentration of Se, and its oxidation state, along with the rate and method of Se application, as well as concentrations of other anions in soil, especially sulfate [100]. The theory of mineral nutrition suggests that differences in Se concentration in grains occur as a result of different transport and absorption mechanisms, and that the processes are mainly under genotypic influence [64]. Accordingly, plant breeding for improved Se uptake and/or retention by plants may be an effective and sustainable strategy. Numerous findings suggest that it should be possible to breed cultivars with enhanced Se uptake

and/or retention or to use genetic engineering to enhance Se levels (and even specific Se metabolites) in food crops [91]. Plant breeding, as the most powerful agricultural approach, may not work effectively in regions where soils have very low plant-available pools of micronutrients due to very adverse soil chemical and physical conditions [101]. Another shortcoming of the breeding approach is the long breeding cycles combined with expensive phenotyping of segregating individuals. Maintaining the stability of target micronutrient traits across diverse types of environments and finding sufficient and promising genotypic variation may also be difficult [101]. Previous investigations suggested that genetic biofortification may be suitable for increasing the amounts of available Fe, carotenoids, and vitamin A, while agronomic biofortification is good for iodine (I) and Se [66]. Agronomic biofortification refers to the application of fertilizer through soil, as a foliar spray, or as seed treatment to enhance the status of a specific micronutrient in edible plant parts [66]. It is worth mentioning that the materials best suitable for Se fertilization include selenate, selenite, slow-release Se fertilizer, Se-enriched yeast, nano-Se, and amino acid-chelated Se [102]. Nano-Se is a relatively new approach to enrich food with Se. Selenium nanoparticles have been regarded as a promising material for many applications due to their unique properties such as high biological activity, bioavailability, low toxicity, high particle dispersion, and large surface area [103]. They represent a promising alternative to other forms of Se, where a reduction in application complexity may be achieved, and this leads to important results in the potentiation of antioxidant metabolism, the promotion of agronomic sustainability, and a reduction in waste [40]. The success of biofortification to enrich plants with Se depends on several factors, such as Se species, the mode of Se fertilization, and the crop species [93]. Investigations indicate that agronomic biofortification of wheat by Se fertilization may be the best approach to increase the Se intake by humans [23]. The average Se content in crop plants from non-seleniferous soils varies between about 0.01 and 1.0 mg·kg^{-1} DW [71]. Recommended Se contents in grain, used as fodder and food, are 0.2–0.3 mg Se·kg^{-1} dry matter (DM) and 01–0.2 mg Se·kg^{-1} DM, respectively [60]. Studies have shown that, in adequate concentrations, Se can be also beneficial to plants by increasing their productivity [104]. It has been reported that Se improves the yield of food crops such as wheat, barley, rice, and maize [16]. Therefore, type of application, form of Se, and time of application play a critical role in achieving prosperous biofortification in cereal crops.

4. Selenium Content Affected by the Method and Form of Selenium: Meta-Analysis

The type of application is the first important factor that significantly increases Se content in plants, and most studies have demonstrated that foliar application is a more efficient method of fertilization, although soil fertilization is more popular [13,39,41,42,105]. Foliar spraying leads to more efficient uptake of Se compared to soil application corroborated by the lack of soil residuals. Foliar techniques use the minimum amount of Se salts and are the most effective, safe, and economically acceptable way of improving Se contents in crops such as wheat [37]. Foliar application of Se is a more efficient method of biofortification because there is no root-to-shoot translocation and soils can act as considerable sinks for Se [39]. Accordingly, the plant availability of applied Se can decrease rapidly in soils [106]. Se form is the second important parameter for effective biofortification. Most studies have shown selenate (where Se exists in its highest oxidation state, +6) to be the most effective form when applied to the soil and usually more effective than selenite (Se+4) when applied in a foliar manner [41]. The higher efficiency of selenate results from the more rapid uptake of Se(VI) and translocation from root to stem and leaves, as well as quicker transformation into the organic form, while selenite(IV) is more easily adsorbed to the soil, which makes its uptake difficult [42]. Selenate is easily distributed from roots to shoots, whereas selenite or its metabolic products tend to accumulate in roots [58]. It can be expected that most of the applied Se as selenate remains in an inorganic form in shoots, whereas most of the added selenite is incorporated as organic Se, i.e., in Se-amino acids and Se-proteins [43]. One study showed that the efficiency of foliar fertilization in

rice plants becomes higher with sodium selenite compared to selenate [107]. The Se concentrations in rice grains tend to be higher compared to maize and wheat grains [108], possibly because rice cultivars can be differentiated into high-Se and low-Se cultivars [64]. As a staple food, rice is an excellent source of energy, with prevailing consumption in over 30 countries, providing about 80% of daily caloric intake to ca. three billion people [107], making it a suitable target for agronomic Se biofortification. Studies have shown that foliar application with selenate and selenite increased the Se content in wheat grains [20,33–35]. Foliar application of Se obtained good results, and it is a most commonly used method in wheat biofortification. The use of Se fertilizers in soil leads to low rates of Se enrichment in edible plant parts. Moreover, long-term use can be toxic to the nearby ecosystem; hence, the use of Se fertilizers should be carried out carefully to avoid toxicity [13]. Despite the cons of fertilizer application, agronomic biofortification is a justified measure to achieve increased Se content in maize grain [16,109,110]. The absorption, accumulation, distribution, and metabolization of Se in mature maize plants depend on the form of Se supplied [27]. Barley is the major small grain cereal after rice, wheat, and maize [18]. Two-rowed barley might be a suitable candidate to be included in Se biofortification programs [32], with foliar application of sodium selenate immediately after anthesis or during the germination stage of the malting process, resulting in the accumulation of Se in the foods [111]. The analysis of results of studies considering Se biofortification showed considerable variation in methods, times, and forms of application in the grains of four major cereal crops (Table 1). In rice, the distribution of values was skewed toward the right due to the limited number of studies after Premarantha et al. (2012). However, the increase varied from 0.025 μg Se per 1 g of applied Se [19] to 0.42 μg Se per 1 g of applied Se [28] in field experiments and up to 6.34 μg Se per 1 g of applied Se in a pot experiment in controlled conditions [29]. In maize, the reported values in μg Se per 1 g of applied Se varied from 0.091 [112] to 0.92 [30]. The results of barley showed considerably lower efficiencies when selenite was used [31,32] with values varying from 0.0422 to 0.78 μg Se per 1 g of applied Se. In wheat, the lowest value of 0.1125 μg Se per 1 g of applied Se was reported for selenite in a study by Ducsay et al. (2016), while the highest value of 0.853 was reported by De Vita et al. (2017).

Table 1. Different selenium (Se) forms and application methods in major cereal crops.

Specie	Type of Experiment	Application	Time of Application	Se Form	g Se/ha	Control μg·kg^{-1}	Se content in Grain μg·kg^{-1} DW	Increase	Increase by 1g of Added Se	References
Rice	Field experiment	Soil	At heading	Selenite	30	76	59	0.78	0.03	[19]
Rice	Field experiment	Soil	At heading	Selenate	30	76	79	1.04	0.03	[19]
Rice	Field experiment	Soil	At heading	Selenite	30	86	85	0.99	0.03	[19]
Rice	Field experiment	Soil	At heading	Selenate	30	86	92	1.07	0.04	[19]
Rice	Field experiment	Soil	At heading	Selenite	30	97	82	0.85	0.03	[19]
Rice	Field experiment	Soil	At heading	Selenate	30	97	92	0.95	0.03	[19]
Rice	Field experiment	Foliar	At heading	Selenite	30	76	273	3.59	0.12	[19]
Rice	Field experiment	Foliar	At heading	Selenate	30	76	150	1.97	0.07	[19]

Rice	Field experiment	Foliar	At heading	Selenite	30	86	122	1.42	0.05	[19]
Rice	Field experiment	Foliar	At heading	Selenate	30	86	105	1.22	0.04	[19]
Rice	Field experiment	Foliar	At heading	Selenite	30	97	136	1.4	0.05	[19]
Rice	Field experiment	Foliar	At heading	Selenate	30	97	176	1.81	0.06	[19]
Rice	Field experiment, no till	Soil	At sowing	Selenate	25	30	320	10.67	0.43	[28]
Rice	Plastic pots in growth chamber	Foliar	Seven times through vegetation	Selenite	0.53	30	100	3.33	6.35	[29]
Rice	Plastic pots in growth chamber	Foliar	Seven times through vegetation	Selenite	10.5	30	1540	51.33	4.89	[29]
Rice	Plastic pots in growth chamber	Foliar	Seven times through vegetation	Selenite	21	30	1560	52	2.48	[29]
Maize	Field experiment	Soil	Before sowing	Selenite	150	3.7	51	13.78	0.09	[106]
Maize	Field experiment	Foliar	Tasseling and one week after silking	Selenite	11	11	96	8.73	0.79	[106]
Maize	Field experiment	Soil	Before seeding	Selenate	5	34	41.66	1.23	0.25	[30]
Maize	Field experiment	Soil	Before seeding	Selenate	10	34	68.33	2.01	0.2	[30]
Maize	Field experiment	Soil	Before seeding	Selenate	20	34	92.66	2.73	0.14	[30]
Maize	Field experiment	Foliar	During the stem elongation stage	Selenate	5	34	156.66	4.61	0.92	[30]
Maize	Field experiment	Foliar	During the stem elongation stage	Selenate	10	34	205.33	6.04	0.6	[30]
Maize	Field experiment	Foliar	During the stem elongation stage	Selenate	20	34	305.66	8.99	0.45	[30]
Barley	Field experiment	Soil	Before seeding	Selenite	20	45	57	1.27	0.06	[31]
Barley	Field experiment	Soil	Before seeding	Selenate	20	33	391	11.85	0.59	[31]
Barley	Field experiment	Soil	Before seeding	Selenite	40	45	76	1.69	0.04	[31]
Barley	Field experiment	Soil	Before seeding	Selenate	40	33	959	29.06	0.73	[31]
Barley	Field experiment	Foliar	End of tillering EC-39	Selenate	10	111.7	880	7.88	0.79	[32]
Barley	Field experiment	Foliar	End of tillering EC-39	Selenate	20	111.7	1113.9	9.97	0.5	[32]
Barley	Field experiment	Foliar	End of tillering EC-39	Selenite	10	111.7	270	2.42	0.24	[32]
Barley	Field experiment	Foliar	End of tillering EC-39	Selenite	20	111.7	345.5	3.09	0.15	[32]
Wheat	Field experiment	Foliar	Tillering state	Selenite	10	66.6	153.6	2.31	0.23	[33]
Wheat	Field experiment	Foliar	Tillering state	Selenite	20	66.6	254.8	3.83	0.19	[33]

Wheat	Field experiment	Foliar	Tillering state	Selenite	40	66.6	430.4	6.46	0.16	[33]
Wheat	Field experiment	Foliar	Tillering state	Selenate	10	66.6	266.8	4.01	0.4	[33]
Wheat	Field experiment	Foliar	Tillering state	Selenate	20	66.6	820	12.31	0.62	[33]
Wheat	Field experiment	Foliar	Tillering state	Selenate	40	66.6	1383.2	20.77	0.52	[33]
Wheat	Small-plot field experiment	Foliar	Growth stage of 2nd node on the main stem	Selenite	10	32	51	1.59	0.16	[34]
Wheat	Small-plot field experiment	Foliar	Growth stage of 2nd node on the main stem	Selenite	20	32	72	2.25	0.11	[34]
Wheat	Small-plot field experiment	Foliar	Growth stage of 2nd node on the main stem	Selenate	10	32	190	5.94	0.59	[34]
Wheat	Small-plot field experiment	Foliar	Growth stage of 2nd node on the main stem	Selenate	20	32	350	10.94	0.55	[34]
Wheat	Field experiment	Foliar	Preflowering	Selenite	20	120	610	5.08	0.25	[35]
Wheat	Field experiment	Foliar	Preflowering	Selenate	20	120	1340	11.17	0.56	[35]
Wheat	Field experiment	Foliar	Pre-grain filling stages	Selenite	20	120	970	8.08	0.4	[35]
Wheat	Field experiment	Foliar	Pre-grain filling stages	Selenate	20	120	1590	13.25	0.66	[35]
Wheat	Field experiment	Foliar	During GS 31 and GS 49 stage	Selenate	5	150	640	4.27	0.85	[20]
Wheat	Field experiment	Foliar	During GS 31 and GS 49 stage	Selenate	25	150	2390	15.93	0.64	[20]
Wheat	Field experiment	Foliar	During GS 31 and GS 49 stage	Selenate	50	150	2820	18.8	0.38	[20]
Wheat	Field experiment	Foliar	During GS 31 and GS 49 stage	Selenate	80	150	3930	26.2	0.33	[20]

To further analyze the data patterns from other studies, a meta-analysis was conducted by setting a liner mixed model [113]. Mixed linear models were set with normalized data of Se increase per 1 g of applied Se as response variable. Input data were values from Table 1. Eight models in total were set in the R *lme4* library [113] with all random effects and intercepts for each term, assuming unstructured error variance. Models included eight combinations of the following factors: application type, species, form of Se, and their respective interactions. The final model was chosen on the basis of the lowest scores of Akaike and Bayesian information criteria (AIC and BIC), and it included three random terms. The first term was with the intercept varying as a function of application type and type of Se, the second was with the intercept varying as a function of species and type of Se, and the third was the random intercept term type of experiment with a fixed mean. Model coefficients were extracted using the *lme4 coef()* command.

The variance component analysis (Table 2) showed the lowest amount of variance explained by the application type, followed by the species × Se form interaction, while the type of experiment explained the most considerable portion of variance. Expectedly, a low value of residual variance was determined. The analysis of coefficients of random effects showed lower efficiency of Se application in soil compared to foliar application. In the analysis of species × form of application interaction, lower efficiencies of selenite were

determined in all crop species, while the sizes of calculated coefficients were comparable only in rice. A plastic pot experiment in a growth chamber showed a multifold increase in Se accumulation, probably due to the lack of atmospheric factors affecting the leaching and volatilization of Se [39]. Field growing represents a more feasible biofortification method compared to plastic pots for growing cereals due to the low amount of food that can be produced. Plants, when supplied with selenite, had selenium concentrations in the xylem exudate lower than selenate [114]. The same pattern was confirmed by the mixed model analysis (Table 2) for grains. The second largest proportion of variance was explained by the interaction of application type × Se form with the foliar application of selenate being the best combination to achieve successful agronomic biofortification. Application of selenate to soil represents the second-best option to increase Se level in cereals. Application of selenite to the soil or in a foliar manner represents the least effective options, and foliar application of selenite is a better choice for increasing Se in plants. Wheat is the most important cereal for agronomic biofortification because of its importance for human diet [115], and it had a higher uptake of Se in the grain compared to all other studied cereals (Table 2). Generally, selenite is more strongly adsorbed by the soil solid phase and, thus, less soluble than selenate in soil solutions [58], while genotype also influences the increase in Se in the grain [116]. The best results observed in the species × Se form analysis were wheat/selenate > barley/selenate > maize/selenate > maize/selenite > wheat/selenite > barley/selenite > rice/selenite > rice/selenate, in the order from more efficient to less efficient. The higher translocation of selenate into grains (Table 2) might, therefore, be due to its higher bioavailability to plants than selenite, which is more strongly adsorbed to the soil surfaces [43]. Furthermore, in plants, selenite and phosphate compete for uptake because they share common transporters [69], while the translocation of selenate from roots to shoots occurs more readily than selenite [67]. Barley was also shown to be efficient in Se uptake [111], and selenate application resulted in enhanced accumulation of Se in barley grains [18] compared to selenite. Chilimba et al. [109] reported that agronomic biofortification of maize with Se appears to be a feasible option for increasing dietary Se [109]. Foliar application of selenite in rice plants resulted in higher total Se content compared to soil application [117], and selenite is a more effective Se form for rice biofortification with differing accumulation across genotypes [118], as discussed in this paper. Considering the analyzed data and reviewed literature, we can see that wheat is the most cultivated cereal and has the highest efficiency of Se uptake and translocation toward its edible parts. Barley showed higher efficiency regarding Se biofortification compared to rice and maize, although it is not cultivated as much. From the studied cereals, selenite was shown only in rice to be a more efficient Se form than selenate. The largest proportion of variance in the mixed model analysis was attributed to application type × Se form (Table 2), representing the most important factor in choosing the right strategy of Se agronomic biofortification, along with the choice of right plant species.

Table 2. Variance explained by application type × Se form, species × Se form, and type of experiment, followed by coefficients of random effects (best linear unbiased predictions) for each level of factors from the 11 scientific papers evaluated (n.a., not applicable).

Factor	Application Type X Se Form	Species X Se Form	Type of Experiment	Residual
Variance	0.012352	0.006132	10.624367	0.213215
Coefficients of random effects				
Soil/selenate	−0.007177254	–	–	
Foliar/selenate	0.095549031	–	–	
Soil/selenite	−0.060080039	–	–	
Foliar/selenite	−0.022608397	–	–	
Barley/selenate	–	0.031985788	–	n.a.
Maize/selenate	–	0.012493402	–	
Rice/selenate	–	−0.039315129	–	

Wheat/selenate	–	0.038706521	–
Barley/selenite	–	−0.013442636	–
Maize/selenite	–	0.010163323	–
Rice/selenite	–	−0.028004366	–
Wheat/selenite	–	−0.009765509	–
Field experiment	–	–	0.2971243
Plastic pots in growth chamber	–	–	4.5913276

5. Conclusions and Future Perspectives

Cereals are the most common food in the human diet worldwide. Over one billion people have an Se deficiency, which leads to various disorders in human health. Recently, the necessity of a functioning immune system has been emphasized. Connecting these facts, we conclude that selenium agronomic biofortification should be done on the most widely used foodstuff to reduce Se malnutrition. According to a meta-analysis, foliar application has been shown to be a more efficient method than soil application. Foliar application is also a more cost-effective method with numerous advantages. The form of applied Se also plays an important role in increasing Se content in the cereal grain, with the selenate form being more effective for agronomic biofortification. For further research, it is necessary to determine the exact doses of selenium for individual crops that will ultimately have the best outcome for plants, humans, and soil. According to the available data and results of the meta-analysis, there is a possibility to design a computational model for predicting the efficiency of applied selenium with respect to crop and soil type, selenium form, and method of application. There are several papers that do not have yield data, and this would be crucial in the development of a model; thus, there is a niche for the improvement of scientific papers on agronomic Se biofortification. An interesting challenge would be to determine if some wild plant species have high concentrations of Se, starting from segetal weed species, which are often exclusive to these environments, sometimes have medicinal properties, and may be at risk of extinction due to intensive agricultural practices [119,120].

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Modelling Leverage of Different Soil Properties on Selenium Water-Solubility in Soils of Southeast Europe

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Abstract: Selenium (Se) is a nonmetal that is essential for humans and other animals, and is considered beneficial for plants. The bioavailability of Se strongly influences its content in the food chain. Soils are the main source of Se, and their Se content primarily influences its availability, along with other soil properties. A field survey was conducted on soils of Southeast Europe, specifically in Croatia (Osijek), Bosnia and Herzegovina (Sarajevo, Banja Luka, Mostar, and Prud), and Serbia (Novi Sad). Soil samples were taken from the arable soil layer (0–30 cm depth), and two types of Se availability were measured: Se extracted using pure HNO_3 (Se_{Tot}) and Se readily extracted in water (Se_{H_2O}). Only soils from the Mostar area had Se concentrations above deficit levels (0.5 mg kg^{-1}), with the highest values of cation exchange capacity (CEC), soil organic matter (SOM) measured as loss of ignition (LOI), total C, total N, Zn_{Tot} and Cd_{Tot}. The connections between the chemical characteristics of the soil and Se_{H_2O} were investigated. Principal component analysis (PCA) explained 73.7% of the variance in the data set in the first three principal components (PCs). Using the provided data, we developed a partial least squares (PLS) regression model that predicted the amount of Se_{H_2O} in the soil, with an accuracy ranging from 77% to 90%, depending on the input data. The highest loadings in the model were observed for LOI, CEC, total C, total N, and Se_{Tot}. Our results indicate the need for biofortification in these key agricultural areas to supplement the essential dietary requirements of humans and livestock. To efficiently and economically implement biofortification measures, we recommend utilizing regression models to accurately predict the availability of Se.

Keywords: selenium; soil properties; deficiency; regression model/analysis; Southeast Europe; biofortification

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1. Introduction

Selenium (Se) is an essential nonmetallic trace element for humans and animals [1] with a narrow range between deficiency and toxicity (40–400 µg per day). Selenium has numerous functions in the human body such as in the antioxidant defense system and oxidative metabolism, thyroid hormone metabolism, the immune system, male fertility, the prevention of cancer and cardiovascular diseases [2,3]. Furthermore, it was reported that in humans, Se stimulates the uptake of Fe in combination with Mn, Zn, and Cr [4]. In general, higher animals and humans directly acquire organic Se compounds, but they can also internalize inorganic Se [5]. Se deficiency has even been linked with Parkinson's disease mortality in the present day [6]. Selenium deficiency in soils often results in low Se concentrations in foods and negatively affects human and animal health [7]. Globally, Se deficiency is a more common problem from a human health point of view than toxicity [8].

Deficiencies in Se occur in numerous countries across Europe, Asia, South America, and Africa. Specifically, countries including Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Poland, Portugal, Serbia, Slovakia, Slovenia, Spain, Turkey, and the United Kingdom in Europe; China, India, Nepal, and Saudi Arabia in Asia; Brazil, Argentina, and Uruguay in South America; Burundi and Egypt in Africa; and Papua New Guinea in Oceania, have reported instances of Se deficiency [9]. Agricultural soils frequently experience selenium deficiency, which is expected to worsen due to changes in weather patterns, including longer periods of drought and more heatwave days [10]. Although not yet proven essential as a plant nutrient, Se has a positive impact on plant growth and quality by aiding in plant antioxidant pathways. As a crucial component of human and animal diets, selenium is primarily sourced from the soil and transferred to the food chain through plants [11]. Se intake varies mostly depending on the Se content in the soil [12] and other site-specific characteristics [13]. Lui et al. (2021) utilized a predictive regression model spanning 55,500 km^2 to demonstrate that the distribution of soil Se content is influenced by a variety of factors, including topography, parent material, and climate, which in turn contribute to the development of distinct soil physicochemical properties [14]. As detailed below, the bioavailability of soil Se is related to soil Se content, crop species, and genotype, and soil physicochemical properties such as soil texture, redox potential, pH, and SOM status [15]. Understanding Se bioavailability in soils and its dependence on environmental factors is thus of crucial importance to prevent Se deficiency and improve Se availability in human diets [16]. Hence, experts suggest utilizing the current understanding of selenium behavior in soil to develop predictive models and maps, which can aid in the identification of regions with low selenium availability in soil [10]. Accordingly, the special biological function of Se as a trace element has received attention from researchers in the fields of geochemistry and environment, health, and agricultural sciences [15]. However, limited attention has been paid to the relationship between soils and human health by soil scientists and medical professionals [17]. Therefore, the term geomedicine has been coined to designate the "influence of ordinary natural processes on the health of humans and animals" [17].

Selenium is present in four different oxidation states in aqueous and subsurface system, namely II, 0, IV, and VI, and can easily form compounds with metals, corroborating the fact that it occurs in about 50 minerals [18]. Selenium chemically resembles sulfur and in the geosphere, and it is associated with sulfur deposits and coal [16]. Selenate and sulfate apparently share the uptake system in plants as is evident from their mutual competition [19]. The bioavailability of Se is regulated by the physicochemical conditions of the soils such as the pH, redox conditions, salinity, SOM, etc. [20]. Se in soils occurs in inorganic forms as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), as well as in organic forms [21], such as dimethylselenide (DMSe) and dimethyldiseledide (DMDSe), which can volatize from soils [22]. Selenate, which is water-soluble, represents the most available species of Se for plants in well-aerated, neutral to alkaline soils [23], while selenite is almost entirely unavailable due to its strong adsorption onto soil particles under relevant environmental pH values in nearly all soil types [16,24]. Being a substantial component of soils and sediments, soil organic matter plays an important role in the Se speciation and mobility [16]. Selenium content in soil is influenced mainly by the parent material and climate, with arid and semiarid areas containing larger amount of Se, whereas in humid and irrigated areas, soils show a lower Se content due to leaching [25]. Alkaline soils have more available Se, where it is mostly present in the form of selenate. On the other hand, in acidic, poorly aerated soils, Se occurs mainly as insoluble selenides and elemental Se bound to Fe oxides [26]. Typical Se concentrations of 0.13, 0.05, and 0.5 mg kg^{-1} are reported for ultramafic rocks, mafic rocks and granites, and shales/clays, respectively [16]. Selenium binds to organic and clay soil fractions and is found in phosphates, uranium ore, fossil coal, oil, and shale with a high organic carbon content [26]. Soluble Se is the main source of Se available for plant uptake [27] and its concentrations are usually <0.05 Se μg g^{-1} [26]. SOM can harbor as much as 50% of the total Se in soils, from which a substantial fraction can be mobilized

into soil solution following plant uptake of dissolved Se [28]. SOM plays a crucial role in predicting the availability of Se in soil, as it governs its mobility consistently [29]. The retention of Se in soil is a multifaceted process that is influenced by not only the surface charges of the soil, but also by the presence of anions such as sulfate, nitrate, or phosphate. This is due to the displacement of Se from the soil's adsorption complex [30,31]. As a result, the application of fertilizers can reduce Se retention in the soil as it is substituted by other anions and cations on the soil's adsorption complex [7]. An accurate assessment of Se's fate under field conditions requires information on the rates of Se transformation [32]. Selenium content in soil can vary, but the majority of European soils are poor in Se [33]. The levels of Se in most soils from the Balkans region are low, with concentrations between 0.024 and 0.45 μg Se g^{-1} [26]. Normal soil Se levels range from 0.1 to 2.0 μg g^{-1}, while toxicity is exerted between 30 and 324 μg g^{-1}, and healthy soils contain around 2 μg g^{-1} [34]. Generally, total Se levels below 0.5 μg g^{-1} are considered as deficient [26]. Because dietary intake is the most practical pathway providing sufficient human Se supplies, the biofortification of Se in agriculture through Se fertilization, breeding, or the genetic manipulation of crops has been proposed as an effective and safe measure [35]. Previous research and meta-analyses have determined that biofortification is an effective method for increasing the Se concentration in the most widely cultivated cereals, including wheat, barley, corn, and rice [23]. Biofortification practices have therefore gained increasing attention worldwide in the science-based development of selenate-enriched agricultural products [35,36]. Hence, it is essential to understand soil properties and mechanisms affecting Se uptake by plants to enable accurate predictions of Se status in soils, and develop effective management practices and fertilization recommendations to avoid Se deficiency, Se toxicity, and potential negative environmental impacts [27]. Studies have shown that several soil properties, such as pH, total organic carbon (TOC), CaO, Mn, Mo, and S, significantly affect the uptake and bioaccumulation of heavy metals and Se in rice. Thus, soil properties are generally chosen as independent variables in predictive models [37]. Furthermore, there has been a recent proposal to generate maps using predictive models to identify regions that suffer from Se deficiency in their soils [10].

Biofortification with Se may be an important strategy to provide sufficient Se status in crops [38]. This study was designed to investigate the geochemical factors controlling Se solubility in the water extracts of soils collected from highly productive agricultural regions of Croatia, Bosnia and Herzegovina, and Serbia. We hypothesized that the solubility of Se is mostly controlled by the SOM, CEC, and the pH reaction.

2. Materials and Methods

2.1. Study Area

This work represents a geochemical study of soils sampled from agricultural fields in Croatia (Osijek), Serbia (Novi Sad), and Bosnia and Herzegovina (Sarajevo, Mostar, Banja Luka and Prud), all located in Southeast Europe. The dominating soil types in Osijek are Chernozems, Eutric Cambisosl, Luvisols, and Gleysols [39], and in Banja Luka and Prud are Stagnic Podzoluvisols, Fluvisols, Umbric Gleysols and Eutric Gleysols [40]. In Sarajevo, the predominant soils types are Chromic Luvisols, Eutric Cambisol, Leptosols × Rendzic Leptosols, and Vertisols, and in Mostar, they are Lithic Leptosols, Regosols, Leptosols − Rendzic Leptosols, Chromic Cambisols, Fluvisols in the river valleys, but Umbric and Eutric Gleysols in the karst fields [40]. The whole part of Novi Sad area lies on a fluvial terrace with the autochthonous soil type, Fluvisol [41].

2.2. Sampling and Pretreatment of Soil Samples

In 2015, we conducted field sampling to collect soil samples from the arable soil layer, which was 0–30 cm deep. A diagonal sampling method was employed to collect 20 to 25 punctures on homogeneous plots using a soil probe. We selected 52 sampling sites, comprising 10 from Sarajevo, 5 from Banja Luka, 9 from Novi Sad, 10 from Mostar, 13 from Osijek, and 5 from Prud. These sites were chosen to represent the primary granaries of the region. Samples were dried and sieved through a 2 mm mesh for the determination of soil pH and the trace metal water extraction of Fe, Ni, Cr, Cd and Zn. For the determination of SOM using LOI and total metal extraction using ultra-pure HNO_3, samples were further ground to a finer particle size using agate mortar.

2.3. Chemical Analysis

The SOM content was estimated by calculating the LOI. The process involved placing oven-dried soil samples in a crucible, which was then ignited in a muffled oven at a temperature of 550 +/− 25 °C for a minimum of 3 h. After ignition, the crucible and sample were cooled for 30 min in a desiccator before being weighed. The SOM values obtained were then corrected for clay content [42]. For the determination of dissolved organic carbon (DOC) in soil, air-dry soil samples were weighted into 50 mL centrifuge tubes and 40 mL ultra-pure water (UP-H_2O, MilliQ H_2O, electric conductivity < 18.2 MS cm^{-1}) was added. The tubes were shaken on a linear shaker for two days and centrifuged at 3000 rpm for 30 min. The suspension was passed through 0.45 µm polyethersulfone membrane filters to poly propylene (PP) test tubes. The C concentrations were then determined using a Shimadzu TOC-5000 analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). The determination of C using the dry C combustion method was based on the thermal decomposition of carbonate minerals in a furnace at a temperature of approximately 1000 °C. In this process, the sample was burned in a purified O_2 gas stream, and other gases produced during the combustion were removed before the CO_2 absorption lamp reached the sample [43]. The nitrogen determination was performed by placing a soil sample weighing < 200 mg in a tin cuvette. The sample was then burned in an oven at 950 °C using oxygen gas. After the gases stabilized, they passed through two infrared detectors set up to read CO_2 and H_2O. The nitrogen was reduced, and the CO_2 and H_2O were removed, yielding the amount of N_2 in the sample [43–45]. The pH and concentrations of Cr, Ni, Se, Cd, Fe, and Zn were determined in the same water extracts. Soil pH also was determined in a soil to water solution ratio of 1:2.5 [46]. CEC was determined using the barium chloride method where 3 g of soil was added to 40 mL of 0.1 M $BaCl_2$, making the soil to solution ratio 1:13 [47]. The total heavy metal (HM) and Se concentrations in soil were determined after digesting the soil in concentrated ultra-pure HNO_3 (1:15 solid:solution ratio) via stepwise heating up to 250 °C using a Milestone Ultra clave for 1 h and 15 min. The Cr, Ni, Se, and Cd concentrations in the prepared water and acid extracts were determined using a Perkin Elmer Sciex Elan Inductively Coupled Plasma Mass Spectrometer (ICP-MS), and Fe and Zn using a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES). The certified reference material (CRM) used was the SRM 2709 [48]. Safe and toxic concentrations of trace elements were determined according to the World Health Organization paper, Trace elements in human nutrition and health [49]. All analyses were conducted at the Norwegian University of Life Sciences (NMBU, Aas).

2.4. Data Analysis

Data analyses were carried out using R software version 4.0.2 [50]. Principal component analysis (PCA) was used to screen the dataset by finding the latent (synthetic) variables, i.e., principal components (PCs) made from linear combinations of variables from the original dataset. Individual PCs represent linear statistical models with the scores (distance from the PC origin for every data point), the loadings (variable contributions for each PC), and the residuals. Input parameters were: LOI, pH (H_2O), total carbon (TC), total nitrogen (TN), DOC, LOI/TC, Mg (from the CEC), Cr_{H_2O}, Fe_{H_2O}, Ni_{H_2O}, Se_{H_2O}, Cd_{H_2O}, Zn_{H_2O}, Cr_{Tot}, Fe_{Tot}, Ni_{Tot}, Zn_{Tot}, Se_{Tot}, and Cd_{Tot}. Variables showing correlations stronger than 0.91 were considered for discarding from the analysis as redundant. All variables were scaled, centered, and log transformed. The components explaining at least 10% of the variation present in the dataset were analyzed. The same variables were also used in a penalized regression model in a partial least squares (PLS) framework, implemented into the R/pls library. Briefly, PLS aims, similarly to PCA, to explain variability in the dataset by making projections to latent variables. However, there is a considerable difference in the PLS approach, aiming to simultaneously explain the variability in predictors as well as in responses. The model was calibrated in the leave-one-out validation procedure, where $n - i$ samples are taken to calculate the model, while the ith observation is used to perform the predictions. The process is repeated until there are n predicted values, which are then correlated to original data, and used to calculate the root mean square error of predictions (RMSEP). The number of components used in the model was selected based on the lowest value of RMSEP in the validation procedure. Additionally, the latent variables (components) from a calibrated best-performing model were used in a mixed model as fixed covariates, along with location, treated as a random effect with an assumption of homogenous variance in the R/lme4 package [51].

3. Results

3.1. Physical and Chemical Soil Properties of the Analyzed Soil Samples

Table 1 shows means and standard deviations of chemical properties of samples from all studied areas. The soil organic matter (SOM/LOI) content was highest in Mostar soils (9.7%), while the content at other sites ranged from 5.2% to 6.8%. The mean value of pH (H_2O) for all locations was slightly alkaline, 7.18. The Mostar area had the highest value of total carbon (4.7%) and total nitrogen (0.36%). The highest total carbon (TC) values were accompanied by the highest values of LOI. Dissolved organic carbon (DOC) varied by location, from the lowest value in Prud (156 mg kg^{-1}) to the highest observed value in Banja Luka (352 mg kg^{-1}). The mean value for the LOI/TC (total carbon) ratio across all locations was 2.66. The CEC varied considerably by location, with the highest values around Mostar (101,607 cmolc kg^{-1}), and the minimum values observed in Banja Luka sites (30,336 cmolc kg^{-1}). Table 1 also shows the total concentrations in water extraction (Cr_{H_2O}, Fe_{H_2O}, Ni_{H_2O}, Se_{H_2O}, Cd_{H_2O}, and Zn_{H_2O}) as well as in ultra-pure HNO_3 (Cr_{Tot}, Fe_{Tot}, Ni_{Tot}, Zn_{Tot}, Se_{Tot}, Cd_{Tot}). Sarajevo, Banja Luka, Mostar, and Prud had Cr_{Tot} concentrations above the concentrations prescribed by the WHO (WHO 1996). All sample sites had a higher concentration of Ni_{Tot} than the recommended safe level, which is 35 mg kg^{-1}, except the Osijek area [52]. The Zn_{Tot} concentrations in all studied locations were lower than the recommended (50 mg kg^{-1}) values in soil [52]. In the area of all sample sites, it was found that Cd_{Tot} kg^{-1} amounts were below the maximum permissible concentrations (MPC—0.8 mg kg^{-1}) [52]. Samples from the Mostar area showed concentrations of Se_{Tot} (0.5 mg kg^{-1}) above deficiency levels [26], while Se_{Tot} deficiency was determined at all other sampling locations. The calculated SeO_4^2 concentrations (n = 52) varied, along with the Se_{Tot} and Se_{H_2O}, being the highest in soils from Mostar and lowest in soils from Sarajevo and Osijek: Mostar > Prud > Banja Luka = Novi Sad > Sarajevo = Osijek.

Table 1. The table shows mean values including ± standard deviation (SD) of important soil parameters measured in soils from Sarajevo, Banja Luka, Novi Sad, Mostar, Prud, and Osijek (n = 52).

	Sarajevo	Banja Luka	Novi Sad	Mostar	Osijek	Prud
pH (H_2O)	6.58 ± 0.722	6.14 ± 1.215	7.65 ± 0.200	7.67 ± 0.101	7.16 ± 0.922	7.68 ± 0.548
DOC (mg kg^{-1})	260 ± 77.888	352 ± 263.001	162 ± 28.185	225 ± 49.272	175 ± 40.541	156 ± 41.593
LOI (%)	6.535 ± 2.062	6.822 ± 2.303	5.201 ± 0.2	9.755 ± 1.847	5.352 ± 1.189	6.016 ± 0.731
Total Carbon (%)	2.45 ± 1.326	2.55 ± 1.016	1.78 ± 0.931	4.71 ± 0.898	2.29 ± 0.771	2.32 ± 0.089
LOI/TC	2.878 ± 0.469	2.781 ± 0.522	3.177 ± 0.599	2.081 ± 0.253	2.572 ± 0.838	2.589 ± 0.223
Total Nitrogen (%)	0.24 ± 0.099	0.25 ± 0.116	0.16 ± 0.048	0.36 ± 0.115	0.17 ± 0.04	0.21 ± 0.038
Na (cmol(Na^+) kg^{-1})	0.082 ± 0.023	0.076 ± 0.01	0.159 ± 0.114	0.196 ± 0.094	0.12 ± 0.067	0.106 ± 0.032
K (cmol(K^+) kg^{-1})	0.599 ± 0.351	0.484 ± 0.22	0.584 ± 0.132	0.668 ± 0.147	0.646 ± 0.125	0.734 ± 0.08
Ca (cmol(1/2Ca^{2+}) kg^{-1})	31.44 ± 39.708	24.02 ± 26.971	37.44 ± 22.897	98.2 ± 6.629	54.138 ± 36.994	63.2 ± 23.636
Mg (cmol(1/2Mg^{2+}) kg^{-1})	2.324 ± 1.868	1.512 ± 0.646	4.189 ± 1.158	2.69 ± 0.44	4.046 ± 1.847	4.12 ± 1.657
CEC (cmol$^+$ kg^{-1})	35.364 ± 39.23	30.336 ± 24.2	42.428 ± 22.79	101.607 ± 6.87	59.045 ± 37.73	68.03 ± 21.96
Cr_{Tot} (mg kg^{-1})	139.0 ± 188.75	205.4 ± 118.70	76.55 ± 11.74	108.89 ± 23.96	76.71 ± 6.198	272 ± 30.33
Cr_{H_2O} (mg kg^{-1})	0.0103 ± 0.009	0.0204 ± 0.017	0.0066 ± 0.002	0.0065 ± 0.002	0.0105 ± 0.009	0.0272 ± 0.004
Fe_{Tot} (g kg^{-1})	30.0 ± 10.31	32.8 ± 3.90	32.8 ± 3.57	34.8 ± 5.57	30.08 ± 2.32	41.2 ± 1.095
Fe_{H_2O} (g kg^{-1})	0.00122 ± 0.0007	0.0012 ± 0.0009	0.00026 ± 0.0003	0.00028 ± 0.00008	0.0019 ± 0.002	0.00034 ± 0.0004
Ni_{Tot} mg kg^{-1}	83.9 ± 115.09	97.6 ± 58.50	37 ± 6.22	76.54 ± 23.68	34.67 ± 2.71	242 ± 35.64
Ni_{H_2O} (mg kg^{-1})	0.0873 ± 0.131	0.0682 ± 0.055	0.0335 ± 0.012	0.0375 ± 0.014	0.0359 ± 0.024	0.0944 ± 0.093
Cd_{Tot} mg kg^{-1}	0.44 ± 0.1134	0.316 ± 0.101	0.195 ± 0.030	0.511 ± 0.067	0.2431 ± 0.036	0.386 ± 0.051
Cd_{H_2O} (mg kg^{-1})	0.00048 ± 0.0005	0.00093 ± 0.0008	0.000083 ± 0.00005	0.000172 ± 0.0001	0.000254 ± 0.0003	0.0001 ± 0.000008
Zn_{Tot} mg kg^{-1}	97.9 ± 0.03	85.2 ± 0.021	67.1 ± 0.006	120.7 ± 0.033	68.1 ± 0.007	116 ± 0.005
Zn_{H_2O} (mg kg^{-1})	0.0557 ± 0.039	0.1016 ± 0.089	0.0041 ± 0	0.0255 ± 0.014	0.0496 ± 0.061	0.0182 ± 0.022
Se_{Tot} mg kg^{-1}	**0.243 ± 0.12**	**0.334 ± 0.011**	**0.25 ± 0.08**	**0.643 ± 0.237**	**0.228 ± 0.081**	**0.426 ± 0.035**
Se_{H_2O} mg kg^{-1}	**0.0085 ± 0.002**	**0.0109 ± 0.003**	**0.0103 ± 0.002**	**0.0175 ± 0.004**	**0.0089 ± 0.002**	**0.0132 ± 0.001**

Concentrations of Se_{Tot} and Se_{H_2O} are shown in Table 1. The % Se_{H_2O} of the Se_{Tot} by location was fairly consistent: Sarajevo, 3.5%; Banja Luka, 3.26%; Novi Sad, 4.12%; Mostar, 2.72%; Osijek, 3.9%; and Prud, 3.1% (mean 3.1%, n = 52). Se_{Tot} and Se_{H_2O} were linearly related across all soils, and the highest amounts of both were found around Mostar (Se_{Tot} = 0.643 mg kg^{-1} and Se_{H_2O} = 0.0175 mg kg^{-1}). In contrast, the lowest observed levels of Se_{Tot} were in the vicinity of Osijek (0.22808 mg kg^{-1}), and low Se_{H_2O} levels were found around Sarajevo (0.00856 mg kg^{-1}) and Osijek (0.0089 mg kg^{-1}). The concentration of Se_{Tot} for the rest of the locations are as follows, from higher to lower: Prud > Banja Luka > Novi Sad as well as Se_{H_2O}.

3.2. Correlation and Principal Component Analysis (PCA) of Analyzed Soil Properties

Correlation analysis showed a strong relationship between all metal water extracts and their total soil concentrations (Figure 1). As expected, LOI, TC, and TN were strongly correlated, also showing a large number of significant positive correlations with other soil properties and metal concentrations. Correlations between soil pH and alkaline/earth metals were mostly significantly positive, while with water extracts of heavy metals were mostly negative. Except for a very strong correlation between Se_{Tot} and Se_{H_2O}, both Se_{Tot} and Se_{H_2O} were most strongly positively correlated with LOI, TC, and TN, along with medium to strong positive correlations with CEC, Na, Ca, and the total concentrations of all heavy metals.

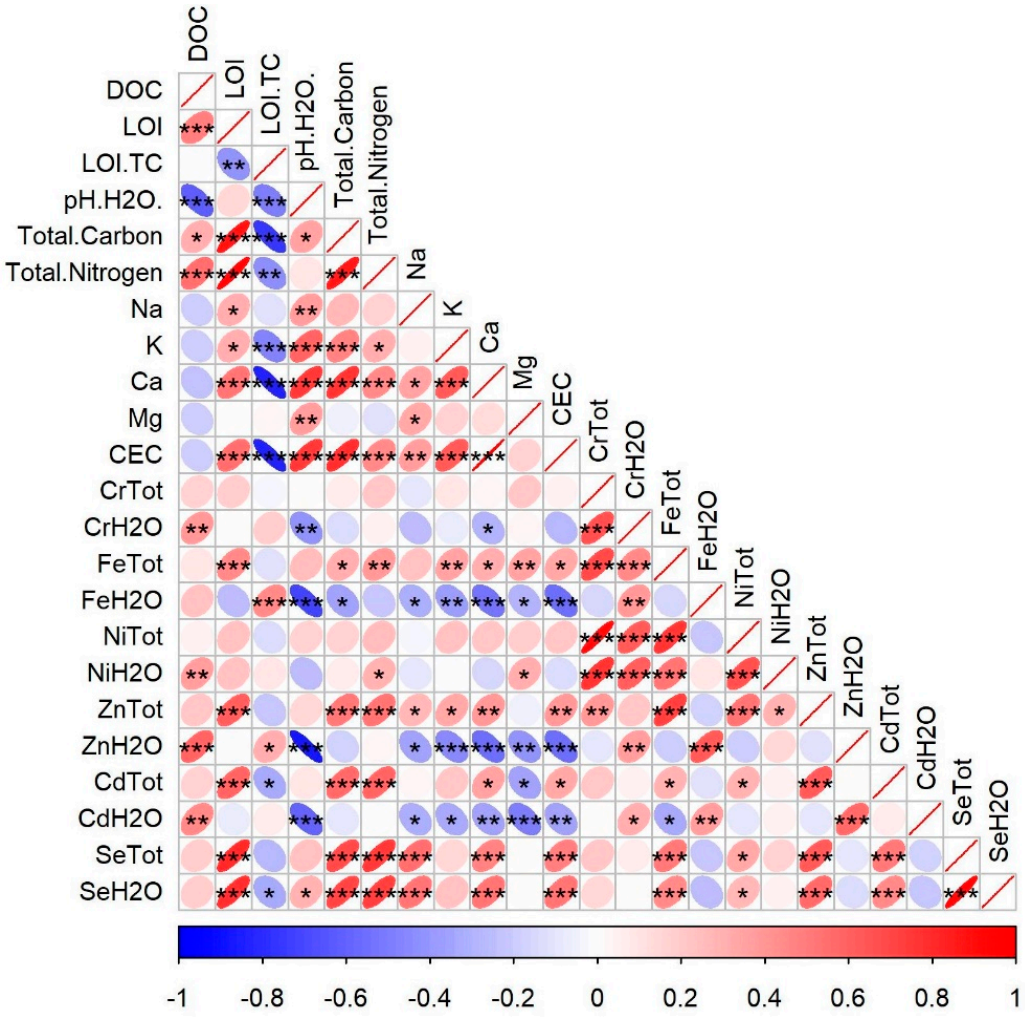

Figure 1. The illustration shows the negative (−, blue) and positive (+, red) correlations between soil properties and total soil and total water-extractable concentrations of several elements. Significance of correlation is denoted with * (α = 0.05), ** (α = 0.01), and *** (α = 0.001).

To assess the grouping of soils according to the analyzed properties, PC analysis was carried out. The first three principal components explained 73.6% of the variation in the data set, with 61.1% in the first two components (36.9% by PC1 and 24.2% by PC2, Figure 2). An inspection of the biplot (for communalities see Supplementary Table S1) showed that PC1 was mainly correlated with LOI/TC in the positive direction and TC, CEC, Ca, Mg, Na, and K in the negative direction. PC2 was in a positive correlation with DOC and the water extracts of heavy metals, and in a negative correlation with pH and Mg (Figure 3).

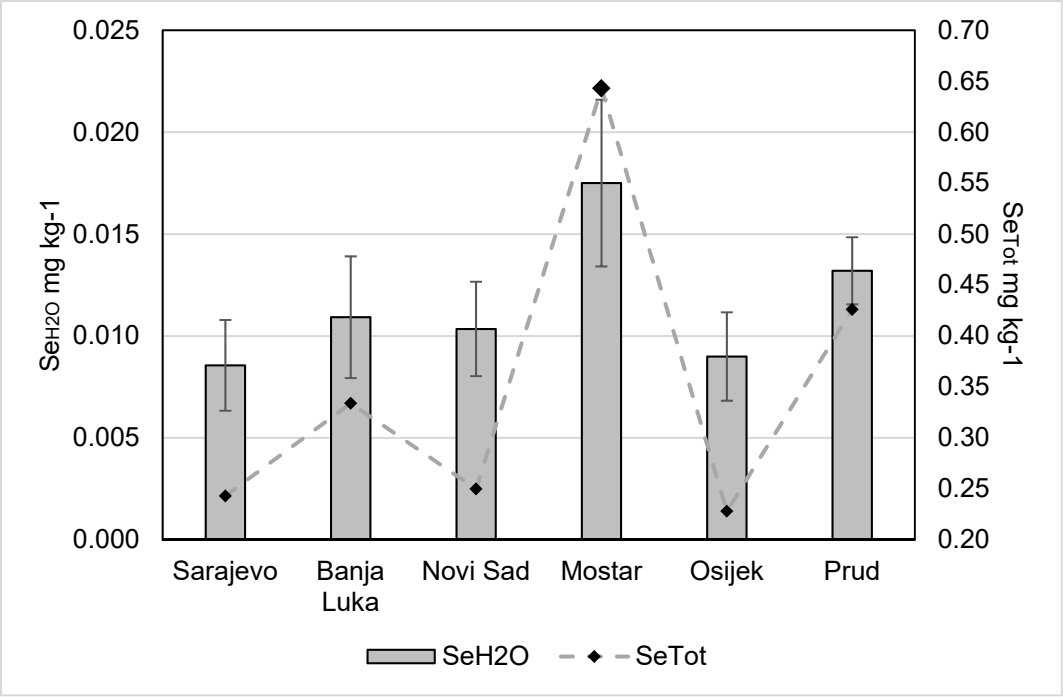

Figure 2. Se_{H_2O} concentrations are shown on the left and Se_{Tot} on the right side through studied locations. Location effects were significant in both analyzed parameters at $p < 0.001$ in one-way analysis of variance.

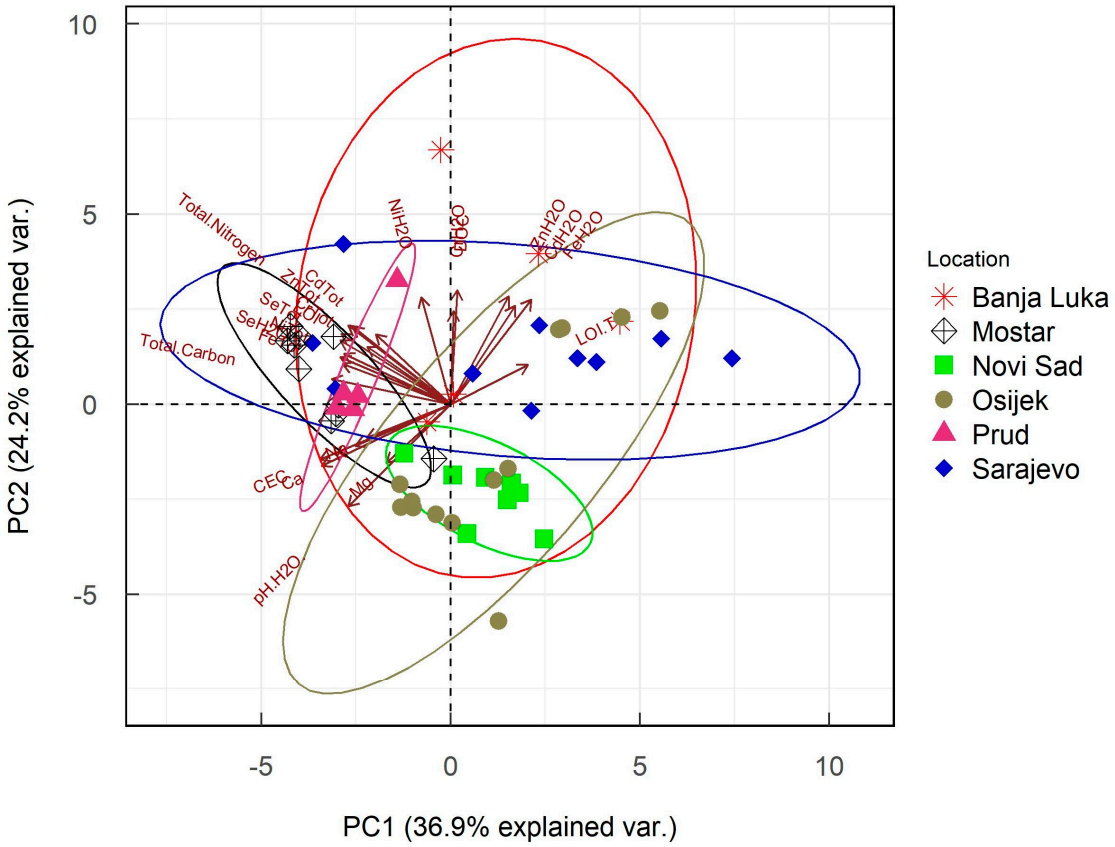

Figure 3. Biplot of principal components 1 and 2 from PC analysis of variation among six locations with different soil properties. Arrows represent the eigenvalues of each of the 24 selected soil parameters.

The first and third principal components (PC1 and PC3) explained, together, 49.9% of the variation in the data sets, 12.5% of which was explained by PC3 (Figure 4). An inspection of the biplot (Figure 4) and Supplementary Table S1 shows that PC3 was mainly positive correlated with DOC and negatively with Cr_{H_2O}.

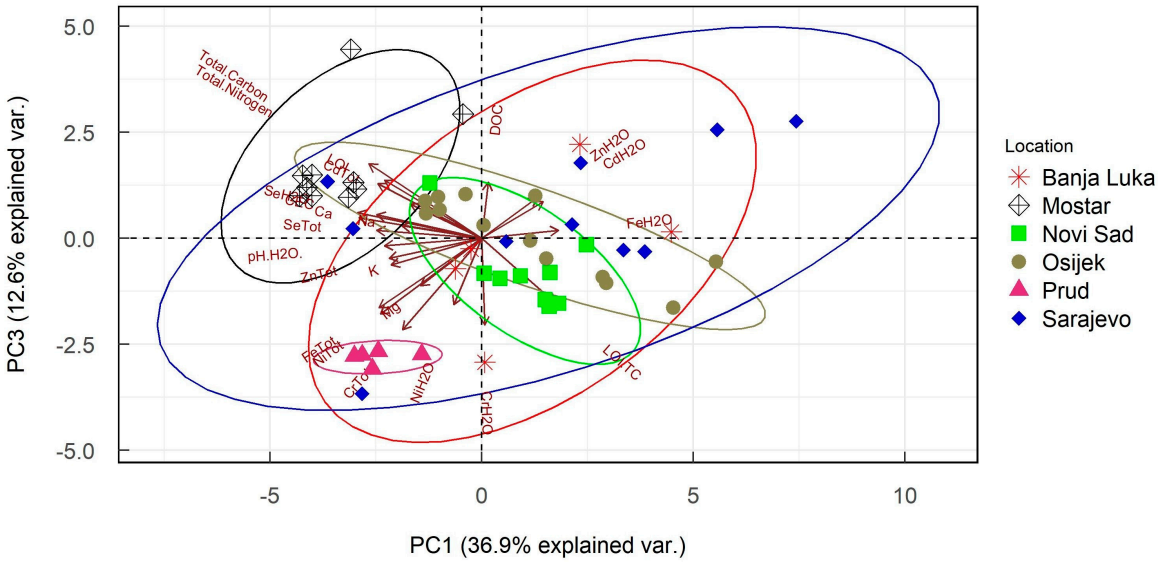

Figure 4. Biplot of principal components 1 and 3 from PCA analysis of variation among six locations with different soil properties. Arrows represent the eigenvalues of each of the 24 selected soil parameters.

Generally, soils from Banja Luka, Sarajevo, and Osijek showed considerable variability in their properties, which can be seen from Figures 1 and 2, where ellipses are scattered across all quadrants. In Figure 3, soils from Prud, Novi Sad, and Mostar are more strongly grouped. Samples from Novi Sad also grouped near the origin of PC1, while soils from Prud and Mostar were mostly on the positive side of PC2. Soils from the Osijek sampling location formed two distinct clusters in PC2, with contrasting properties correlated to this component, while the samples from Banja Luka and Sarajevo showed high diversity and appeared scattered across the assessed PCs. Prud, Mostar, and Novi Sad maintained distinctness and tight grouping in PC3 (Figure 4), similar to PC2. Between PC1 and PC3, distinct clusters of samples from Osijek were not visible, while the samples from Banja Luka and Sarajevo showed similar scattering patterns as in PC2. In all three biplots, samples in the top-left quadrant showed the highest Se_{Tot} values. However, it can be seen in Figure 2 that the samples from this quadrant are also accompanied by the higher values of total Ni, Cd, Fe, and Zn. In both biplots, it was shown that only soils from the Mostar region were consistently grouped in quadrants correlated with parameters assessing Se, while soils from other regions showed a grouping influenced by other soil properties.

3.3. Regression Analysis of the Se_{H_2O} Concentration

Based on the complex relationships between Se_{H_2O} and the other analyzed soil properties and element concentrations (Figure 1), along with the substantial variability among analyzed soils (Figures 3 and 4), three predictive models for Se_{H_2O} were fitted and calibrated. The first PLS model included all analyzed quantitative properties except Se_{Tot} (Figure 5A), while the second also included Se_{Tot} (Figure 5B). The third model included latent variables from model 2 (Figure 5B) along with a random location effect in a mixed model (Figure 5C). Uncalibrated models 1 and 2 explained 89.78% and 93.82%, respectively, of the variance in Se_{H_2O} (not shown), while the calibrated models explained 77.60%, 86.06%, and 90.20% (Figure 5). As expected, the model without Se_{Tot} (Figure 5A) had a higher error

of prediction, compared to the model with it (Figure 5B). The error of prediction was the lowest in the model 3, including the location random effect (Figure 5C).

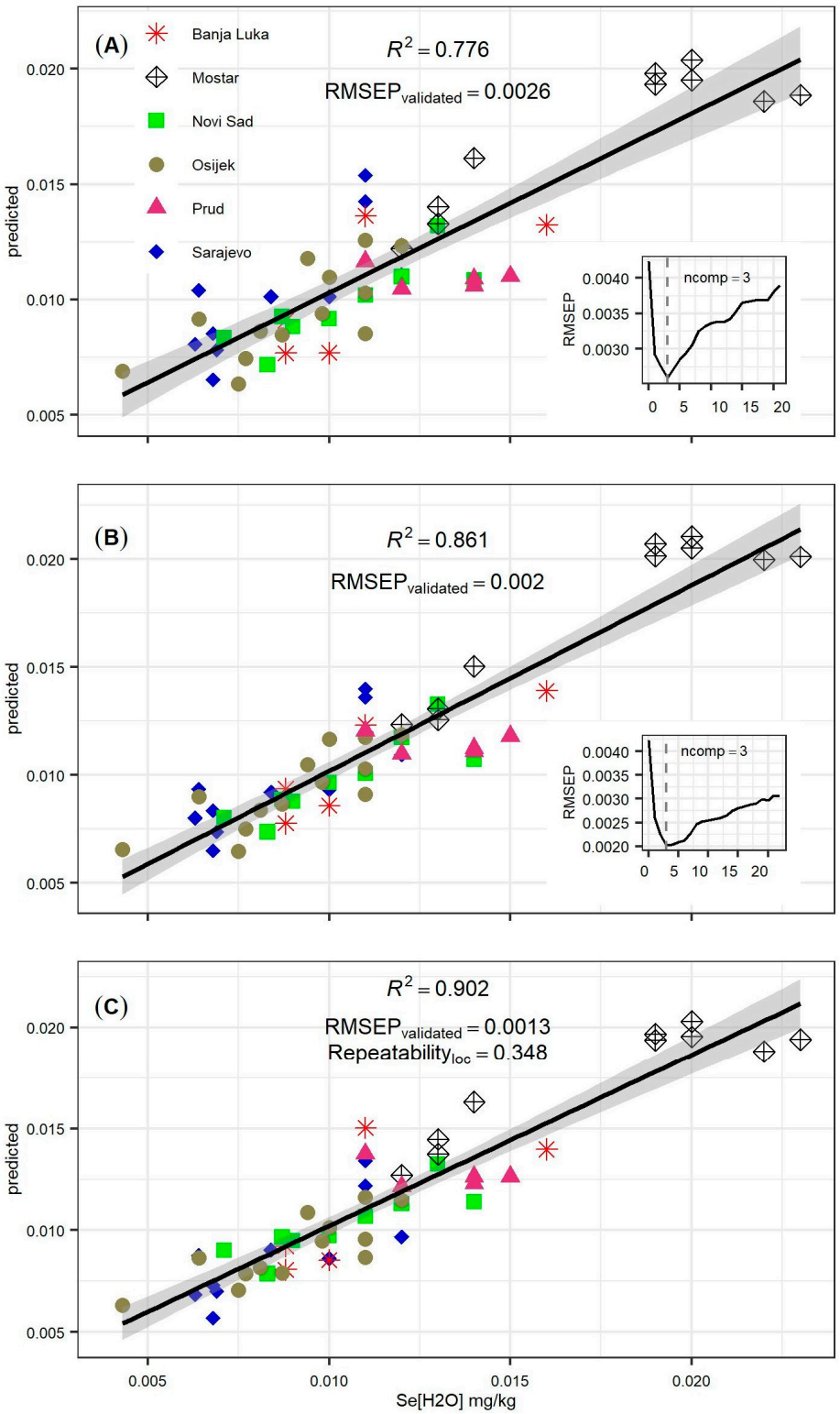

Figure 5. Partial least squares (PLS) regression model for Se_{H_2O}, including soil properties and concentrations of other elements (**A**), Se_{Tot} (**B**), and PLS scores from (**B**) along with location main effect in a mixed model (**C**). Respective validation errors, the selected number of components, and repeatability of the location random effect are shown within plots.

The loading weights of the first component in models 1 and 2 (Figure 6) mostly resembled the correlations between Se_{H_2O} and other soil properties and concentrations of other elements, with the strongest correlations translated to absolute weights > 0.25 (Se_{Tot}, TC, TN, LOI, CEC). The loading weights corresponded to the calculated calibrated model coefficients (Supplementary Figure S1) and variability of a specific variable.

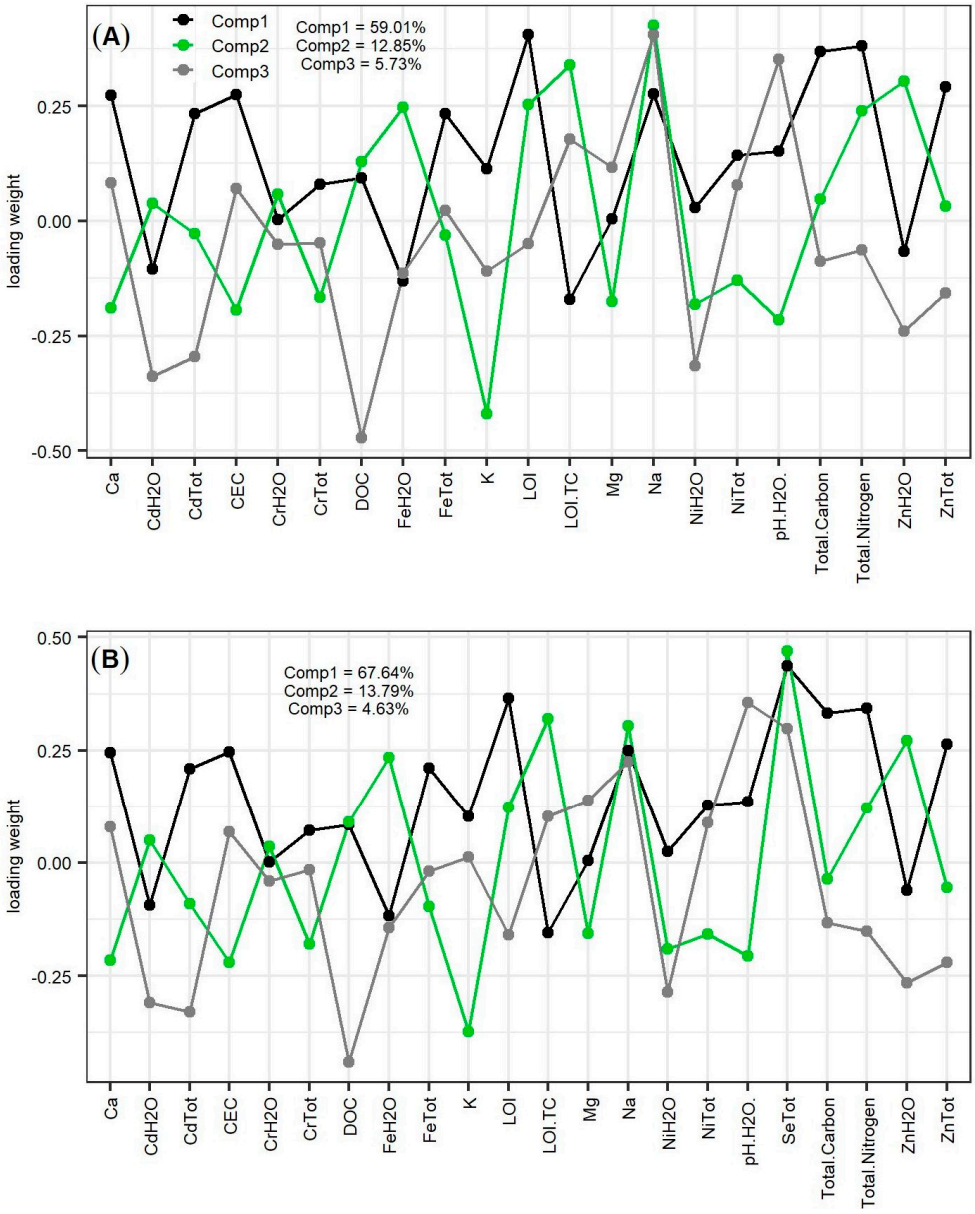

Figure 6. Loading weights from the calibrated PLS model (3 components, Figure 5) for Se_{H_2O}, including soil properties and concentrations of other elements (**A**) and Se_{Tot} (**B**).

4. Discussion

4.1. Relationship between Se Concentrations and Soil Physicochemical Properties: A Principal Component Analysis Approach

PC analysis was conducted to assess soil variability at different locations. PCA showed that the soils in the areas of Banja Luka, Sarajevo, and Osijek were the most variable, while on the other hand, soils from the Mostar, Novi Sad, and Prud showed more specific physical and chemical properties (Figures 1–3). Soils from Banja Luka, Sarajevo, and Osijek were not grouped around similar chemical and physical properties. Instead, soils from Sarajevo and Banja Luka showed a scattering pattern across all quadrants in all three biplots (Figures 1–3),

and soils from the Osijek area could be divided to two groups, mostly defined by PC2 (Figures 2 and 3). According to the Soil Atlas of Europe [53], the Osijek agricultural area is mostly represented by Fluvisols and Luvisols in the north and Mollic Gleysols in the south, while the Banja Luka and Sarajevo agricultural regions lie on diverse soils, such as Pellic Vertisols, Stagnic Luvisols, Dystric Cambisols, Chromic Cambisols, and Fluvisols, corroborating the chemical diversity among samples. Soils around Mostar, Novi Sad, and Prud showed more specific characteristics. The Mostar area had the highest values for numerous observed parameters, as well as the Prud area, while the probed sites of Novi Sad area showed the characteristics of poor soils (low LOI, total C, and total N). All sample sites, except Mostar, had the Se levels below the upper deficiency bound (Table 1), with soils containing less than 0.5 μg g^{-1} of total Se being considered deficient [26]. Physical and chemical properties differed between locations (Table 1). The area of Mostar showed elevated values for the soil properties of LOI, CEC, pH, total C, and total N, and higher concentrations of Ca, Na, Zn_{Tot}, Cd_{Tot} and Se_{Tot} and accordingly, Se_{H_2O} (Figures 1 and 2). There is a possibility that the origins of present elements are similar due to the same primary parent rock [54]. A study by Alloway (2013) reported that soils containing Ag, As, Au, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Se, Th, Tl, U, V, W, and Zn, and also showing a high content of organic matter and clay, usually originate from black shales, including bituminous and oil shales. This corroborates the findings from our research that Se was more effectively incorporated into soil organic matter at higher values of pH (Figure 1) and is also confirmed in the literature [55]. Several studies have highlighted that the capacity of plants to absorb and store Se is influenced not only by the overall Se concentration in the soil, but also by the bioavailability of Se in the soil [14]. As the pH of soil decreases, the surfaces of clay minerals, organic matter, and metal oxyhydroxides become more positively charged, which is conducive to sorption and the retention of Se oxyanions in soil [3]. Situated along rivers, Mostar, Novi Sad, and Prud share a common feature. While the Neretva River runs alongside Mostar, Novi Sad is located near the Danube River, and the Prud area is situated between the Bosna and Sava Rivers. It is possible that the high levels of pH, Ca, CEC, Zn_{Tot} Se_{Tot} and Cd_{Tot} found in soils from Mostar and Prud are a result of the periodic river floods that affect these areas. This is in accordance with investigation of Pavlović et al. (2016) where it was shown that the riparian soil acts as an important sink for different elements, creating favorable conditions for the seeds of plant species that require a bare soil surface for germination, thus retaining high levels of organic matter, as seen in the Mostar area (the highest LOI). On the other hand, Novi Sad region has a moderately warm humid climate with warm summers [56]. A continental climate is prevalent in Sarajevo [57], Banja Luka [58], and Osijek [59]. Soils in arid and semiarid regions can have a high Se content, whereas those in humid and irrigated regions tend to have lower Se levels [25]. The soil samples from Osijek, Novi Sad, and Sarajevo, which experience cooler and more humid weather conditions in comparison to Mediterranean areas, showed lower levels of Se. The concentration of Se in soils is mainly influenced by climate variables, specifically AI, precipitation, and evapotranspiration, as they play a crucial role in regulating soil leaching processes [10].

4.2. Se_{H_2O} and Se_{Tot} Concentrations and Correlation between Different Soil Properties and Se_{H_2O}

Se_{H_2O} analysis was performed as it is considered to be a more effective method for assessing Se deficiency than measuring total Se concentration in soil. This is due to the fact that Se concentration in aqueous soil solutions can serve as a more reliable indicator [60]. The water-soluble fraction of soil Se is a function of soil physicochemical properties and varies with biological reactions [60]. Several studies analyzed soil Se availability referring to water-soluble Se, as plant-available, in spite of the effect of exchangeable Se [28]. The proportions of water-soluble Se in the total soil fractions of analyzed soils were very low (Sarajevo, 3.5%; Banja Luka, 3.26%; Novi Sad, 4.12%; Mostar, 2.72%; Osijek, 3.9%; and Prud, 3.1%). The highest concentrations of Se_{Tot} were recorded in soils from the Mostar region, where the water-soluble fraction accounted for the lowest percentage of the total (2.72%).

This may be attributed to the highest content of soil organic matter (SOM), which can bind with Se and decrease its availability. It is known that the availability of Se in soils, similar to sulfur, generally increases with a higher pH value [55]. This trend can be explained by the increased negativity of the surface charges at alkaline pH levels, which causes electrostatic repulsion between the surface and the negatively charged Se oxyanions [61]. Soil organic matter is another important component that retains Se in the soils. The proportion of SOM-bound Se can be affected by the soil type or the specific composition and content of SOM [35]. This was also corroborated by a significant weak to medium positive correlation between soil pH reaction and Se_{H_2O} (Figure 1). It was shown that alkalinity and salinity can induce the precipitation of some elements and also effect adsorption by affecting the CEC [62]. Furthermore, in our study, Se_{H_2O} and CEC showed a significant moderate positive correlation (0.53) (Figure 1). Calcium and Na also showed medium to strong correlations with Se_{H_2O}, possibly because they are cations participating in the CEC of alkaline soils. Another perspective given by the recent finding of Xu et al. (2020), indicates that the content of Se and Zn increases in calcareous and ferric soils [63]. The results of Imran et al. (2020) suggest that soils on shale parent rock show the highest CEC and the highest Se content, which is in accordance with our results [54].

Our study revealed a strong positive correlation between Se_{H_2O} and LOI, indicating that the water-soluble fraction of Se increases with a higher content of soil organic matter. This finding is consistent with previous research, which has demonstrated that water-soluble and exchangeable Se tends to increase with advanced weathering, as the argillaceous clay, iron oxides, and organic compounds provide more exchange sites for Se [54]. In our study there was a strong positive correlation detected between Se_{H_2O} and Zn_{Tot} which may be explained by the influence of Se on the bioavailability of Zn in soil. Selenium can affect Zn uptake by plants through processes such as adsorption, oxidation, complexation, and precipitation [1]. Zinc in soil typically occurs in the II oxidation state and its activity is influenced by negatively charged adsorptive surfaces such as SOM, clay, and iron, and manganese hydrous oxides. Furthermore, Zn mobilization can occur through the reductive dissolution of Fe oxides [63]. The weak significant positive correlation detected between Ni_{Tot} and Se_{H_2O} was probably caused by soils with reducing conditions that affect both Se and Ni soil dynamics [64]. Our results show positive moderate to strong correlation between Fe_{Tot} and Se_{H_2O}, possibly caused by formation of the organoselenium compounds adsorbed on poorly crystalline iron oxides [54], whereby the OM-bound Se fraction is up to 40–50% [35]. Furthermore, it was found that Fe-oxide-type minerals affect the adsorption of Se [65]. Moreover, amorphous iron is considered to be the most active iron/aluminum oxide and one of the scarce positively charged colloidal minerals present in soil. When in the form of oxygen-containing anions, Se can create a stable inner complex with amorphous iron and co-precipitate with iron hydroxides [14]. Our findings indicate a moderate positive correlation between Se_{H_2O} and Cd_{Tot}. This correlation may be attributed to the reduction of Se in the acidic microenvironments of soil or in the rhizosphere, resulting in the formation of selenide that can bind to Cd and form Cd–Se complexes, which can subsequently reduce Cd uptake by root cells [66]. Selenite, selenate, and their products in soil might also thermodynamically react with Cd to form Cd–Se complexes that become unavailable to the plant root [67], marking another health benefit of Se in soil detoxication. Our study showed that agricultural soils in Southeast Europe have low total Se contents, and that Se dynamics are complex and dependent on various soil properties and types. The total Se content in soils seems to be mainly influenced by the soil parent materials and its availability in soil water solutions. As a result, the overall availability of Se appears to be critically low. However, understanding the correlations and factors that affect the distribution and availability of this essential trace element is crucial for advancing the field of geomedicine [17]. Moreover, recent results indicate that the availability of Se might even worsen due to the climate change, especially in Southeast Europe [10].

4.3. Model for Prediction of Se_{H_2O}

Predicting soil water-extractable Se represents a tentative topic, especially regarding its health benefits in the human diet. In spite of the known high predictive ability of weather patterns for soil Se concentrations [68], our study focused on the predictive ability of the soil geochemical properties with confounding climatological effects. Our study aligns with the findings of Liu et al. (2021), who utilized a predictive model to evaluate Se availability. Their research identified SOM and pH as the key factors impacting model predictability [14]. Our results indicate that the modelling of Se_{H_2O} from soil geochemical parameters in a penalized model could be worthwhile, given the range of soils represented in our study; slightly acidic to slightly alkaline pH, with moderate SOM content (LOI/TC/TN/DOC), with or without information about Se_{Tot} (Figure 5A,B). The addition of Se_{Tot} is expected to increase the model's predictive accuracy due to the established correlation between the extractable and total Se [8], which was also confirmed in our study (Figures 5B and 6B). The study highlighted the significant influence of soil organic matter (SOM) (LOI and TC) on Se extractability in phosphate, which was consistent with our findings in the study of water extracts (shown in Figure 6A,B). Additionally, our study showed that Fe_{Tot} and Ca were crucial in predicting Se concentrations, which was also supported by a study conducted by Xu et al. [63]. This could be caused by the ability of selenite to form inner-sphere complexes, including bidentate and monodentate inner-sphere complexes on the Fe oxide surface [13]. However, Williams Araújo do Nascimento et al. [69] showed that this correlation is valid only in clusters of soils in Fe-rich regions with a specific climate, unlike the stable correlation between Se and SOM across different soils and conditions [70]. Similar findings were also reported by Liu et al. [14], further addressing the importance of aluminum in predicting soil Se content, along with CEC, which was also confirmed in our study. With a comparable sample size to other studies, our study showed that a penalized and mixed model approach might improve predictions of Se_{H_2O}, at least in the constrained soils represented by our study. Due to the high importance of Se to human health and its established beneficial effects in plants, more research determining the key parameters for the building of efficient predictive models is needed. The use of predictive models for mapping Se bioavailability and concentration has become increasingly common, as it plays a crucial role in enhancing, progressing, and verifying these maps [37,71]. However, our study represents a promising step in the precise modelling of Se availability, thus facilitating the assessment of the need for measures such as agronomic biofortification.

5. Conclusions

Most soils worldwide suffer from Se insufficiency, including all locations examined in this study, except Mostar. This study provides valuable information for Se biofortification in Southeast Europe, where physicochemical properties vary greatly by location. Water-extractable Se, which is available to plants, showed positive correlations with LOI, CEC, total C, total N, Ca, Na, Fe_{Tot} Zn_{Tot} Cd_{Tot} and Se_{Tot}. Soils from Mostar, under a Mediterranean climate, had the highest Se content, while soils from other locations with continental, semiarid climates had lower Se contents. The effectiveness of penalized models in predicting water-extractable Se from available geochemical information highlights the dependence of Se concentrations on soil geochemistry, climate conditions, and specific physicochemical properties. Thus, Se biofortification is a necessary step in maintaining a healthy human population in the region. In fact, this predictive model holds great potential for other regions characterized by soil Se deficiency. By making use of basic soil analyses such as SOM, CEC, total N, total C, Ca, and Na, the model's predictive capacity can be significantly enhanced, allowing the acquisition of essential information pertaining to soil Se levels. Ultimately, this approach has the potential to facilitate optimal implementation of Se biofortification, ensuring that an adequate dosage of Se is delivered to crops, and consequently, to people.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13030824/s1, Figure S1: Coefficients of calibrated PLS models A and B from Figure 6 in the main text; Table S1: Communalities (correlations between original variables and PCs) from principal component analysis.

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Selenium Biofortification of Soybean Seeds Influences Physiological Responses of Seedlings to Osmotic Stress

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Abstract: Climate change poses a serious threat to agricultural production. Water deficit in agricultural soils is one of the consequences of climate change that has a negative impact on crop growth and yield. Selenium (Se) is known to be involved in plant defense against biotic and abiotic stress through metabolic, structural, and physiological activity in higher plants. The aim of this study was to investigate the physiological response of Se-biofortified soybean (*Glycine max* (L.) Merrill) seedlings under osmotic stress. For this research, we used biofortified soybean grain obtained after foliar Se biofortification in 2020. The experiment was conducted in a growth chamber with two cultivars (Lucija and Sonja) grown on filter paper in three replicates. The experiment was carried out with two watering treatments: distilled water (PEG-0) and 2.5% polyethylene glycol 6000 (PEG-2.5) on Se-biofortified seeds (Se) and nonbiofortified seeds (wSe). Contents of lipid peroxidation product (LP), free proline (PRO), total phenolic content (TP), ferric reducing antioxidant power (FRAP), and ascorbic acid (AA) were analyzed in 7-days-old seedlings. Significant differences were detected in the Se content of soybean grains between the two cultivars. A milder reaction to PEG-2.5 was observed in cultivar Lucija in both Se and wSe treatments, which might represent the mitigating effects of Se on osmotic stress in this cultivar. Contrarily, in cultivar Sonja, Se adversely affected all analyzed traits in the PEG-2.5 treatment. Ultimately, Se is a pro-oxidant in Sonja, whereas it represents an anti-oxidant in Lucija. In conclusion, different soybean cultivars show contrasting physiological reactions to both osmotic stress and Se. However, the activation of antioxidant pathways in Sonja can also be interpreted as added value in soybean seedlings as a functional food.

Keywords: biofortification; selenium; physiological response; osmotic stress; water deficit; crop improvement; stress tolerance; climate changes

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1. Introduction

Agricultural land is affected with a varying severity of drought, which has become a worldwide problem. Water deficit, extreme temperatures, and low atmospheric humidity lead to drought, which is one of the most limiting factors for better plant performance and higher agricultural yields [1,2]. Drought is by far the most important environmental stress in agriculture, and it is assumed that by 2050, it will contribute to the salinization of more than 50% of arable land in the world [3]. Generally, abiotic stresses are the greatest restriction for crop production worldwide and account for yield reductions of as much as 50% [4,5]. Levels of soil fertility, moisture supply, and other environmental factors influence seed size and seed weight in all crop species [6]. Soybean (*Glycine max* L.) is an

important grain legume with unique chemical composition, making it one of the most valuable agronomic crops [7]. Soybean is one of the most commonly consumed legumes worldwide, with 200 million metric tons produced per year [8] and yields highly affected by water supply [9]. The responses of plants to drought stress are highly complex, involving deleterious and/or adaptive changes [2]. The typical response of plants to low soil fertility and/or chronic osmotic stress is the reduction in quantity of seeds produced rather than in their quality [6].

Drought stress leads to the accumulation of reactive oxygen species (ROS) and increased lipid peroxidation [10]. Oxidative stress caused by a variety of active oxygen species formed under drought stress damages many cellular constituents such as carbohydrates, lipids, nucleic acids, and proteins, which ultimately reduce plant growth, respiration, and photosynthesis [11]. At the cellular level, osmotic stress results in dehydration, which provokes alterations in membrane lipid composition and properties [12]. Lipid peroxidation is a complex process associated with the oxidative deterioration of lipids and the production of various breakdown products [13]. The occurrence of lipid peroxidation in biological membranes causes the impairment of membrane-bound receptors and enzymes, and the increased nonspecific permeability to ions as Ca^{2+} [14]. Low levels of lipid peroxidation during drought conditions are connected to drought tolerance [12]. The ability of higher plants to scavenge the toxic oxygen species appears to be a very important determinant of their tolerance to environmental stresses [15]. Oxidative damage in plants is alleviated by the concerted action of both enzymatic and nonenzymatic antioxidant systems [16]. At the cellular level, drought signals promote the production of stress-protectant metabolites such as proline [17]. Proline plays four major roles during stress, i.e., as a osmotic regulator—osmoprotectant, metal chelator, antioxidative defense molecule, and a signaling molecule [18]. Proline contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions [18]. Proline biosynthesis from glutamate (Glu) appears to be the predominant pathway under stress conditions [19]. As proline acts as the molecular chaperon, it is able to maintain the protein integrity and enhance the activities of different enzymes [18]. It has been found that proline acts as a storage compound and source of nitrogen that enhances growth after stress [20]. Another important group of compounds with antioxidant properties found in plants are polyphenols, which neutralize free radicals by increasing the activities and expressions of antioxidant enzymes and inhibiting the activities of ROS-producing enzymes [21]. Phenolic compounds are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron and chelating metal ions in aqueous solutions. Additionally, they have multiple biological properties such as antitumor, antimutagenic, and antibacterial properties, and these activities might be related to their antioxidant activity [22]. Ascorbic acid (AA), also known as vitamin C, is one of the most abundant water-soluble antioxidant compounds present in plant tissues, also serving as an electron donor in numerous reactions [23]. Ascorbate has been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion, and other developmental processes [24]. It is expected that drought stress would trigger an increase in the biosynthesis of a major antioxidant compound such as AA, and plants with increased AA levels might have improved tolerance to such stresses [23]. Drought combined with ascorbic acid improves the plant responses to stress, reducing the production of harmful substances [24]. Ascorbic acid, the reduced form of vitamin C, is an essential component of the human diet, and small amounts can prevent the deficiency disease, scurvy, while the accumulation of high levels of ascorbate in plasma and tissue may protect against oxidative damage and limit inflammation [25].

Se is an essential element in animal cells and in the human body, but its importance for plants is still the subject of research [26]. The toxicity or benefits of Se are highly dependent on the amount of Se applied [27]. Low levels of Se can stimulate the antioxidant machinery in plants, but it acts as a prooxidant at high levels [28]. Some of the positive effects of Se on plants are: promoting plant growth, alleviating UV-induced oxidative

damage, improving the recovery of chlorophyll from light stress, increasing the antioxidative capacity of senescing plants, and regulating the water status of plants exposed to drought [29]. Additionally, Se can promote the growth and development of plants and increase the tolerance and antioxidant capacity of plants to environmental stresses [28,30] thus helping to attain higher grain yields [4]. Se has been demonstrated to improve plant growth by strengthening the stress tolerance mechanisms such as antioxidant and secondary metabolite metabolism [31]. Despite its manifold positive effects, Se is not considered to be required by higher plants [32], whereas it is an essential trace element for many organisms, including humans [33]. Globally, the human diet is lacking in Se with especially low intakes in vegetarians [34]. Variations in food Se contents greatly depend on soil Se concentrations, consequently also affecting meat and dairy production [35]. It is of great importance to increase Se in the human diet; therefore, Se biofortification in plants is a method for Se-enriched food production [36]. Agronomic biofortification consists of applying fertilizers of mineral elements lacking in the diet in order to increase their concentrations in crops through soil or foliar application [37,38]. The most efficient method of Se biofortification is foliar application with selenate (SeO_4^{2-}) [38]. Different studies have shown that Se could help in the detoxication of ROS and, thus, the enhancement of plant tolerance to oxidative stress [4], and also improve drought resistance by mitigating oxidative stress [39,40]. Hyper-accumulator species have the ability to accumulate Se in the range between 100 and 1000 mg Se kg^{-1} dw (dry weight) without showing toxicity symptoms in contrast to nonaccumulator species of food crops, grasses, and vegetables that hardly accumulate 100 mg Se kg^{-1} before showing symptoms of toxicity [31]. Toxic levels of Se in plants have not been sufficiently investigated; however, it was shown that tolerance varies according to plant species and genotype [41]. Discovering ways to ameliorate the effects of water deficiency on plants will ease competition for freshwater resources, even as the world's population grows [17]. It was thus hypothesized that the Se biofortification of soybean could affect the metabolic pathways of bioactive compound synthesis and the antioxidant activity of the seedlings grown from the biofortified soybean seeds.

The aim of this study was to investigate the physiological responses of seedlings of the two Se-biofortified soybean cultivars to osmotic stress induced by PEG treatment.

2. Material and Methods

2.1. Plant Growth Conditions and Treatments

Seeds of two soybean (*Glycine max* (L.) Merrill) genotypes Sonja and Lucija were obtained from Agricultural institute Osijek, Croatia. Foliar Se biofortification was carried out in growing season 2020 under field conditions with 30 g Se ha^{-1} in the form of sodium selenate (Na_2SeO_4) according to the wheat foliar application of 10, 30, 100, or 300 g ha^{-1} [42]. The foliar application of Se was performed on 22nd of July during the R1 growth stage. The soil was eutric cambisol anthropogenic soil under a temperate continental climate. Soybean grains were harvested in September, dried to 13% moisture, and stored. Biofortified grains were inoculated with Apron (Syngenta, Basel Switzerland) seed treatment fungicide and air-dried 24 h before setting up an experiment. Seeds of each genotype were sown between filter paper that had been previously soaked with 55 mL of distilled water (dH_2O, PEG-0) or 55 mL of 2.5% polyethylene glycol 6000 solution (PEG-2.5). Then, 25 seeds were planted per repetition in single filter paper, folded, and rolled into tubes. Three identical replicates of each treatment were placed in the climate growth chamber at the Faculty of Agrobiotehnical Sciences Osijek, Croatia with the following conditions: 25 °C, 12/12 h day/night regime, and 50% humidity under fluorescent lighting of approximately 200 µmol $m^{-2}s^{-1}$ at 20 cm. Soybean seedlings were harvested 7 days after sowing (VE phase) and stored in a −80 °C freezer.

2.2. Se Content

The Se concentration in soybean grain was determined after digesting in concentrated ultra-pure HNO_3 and H_2O_2 (3:1 ratio) by stepwise heating up to 250 °C using a Milestone

Ultra clave for 1 h and 15 min. Extraction was carried out according to the modified methodology of Matusiewicz et al. [43]. Se concentration was determined using a Perkin Elmer Sciex Elan Inductively Coupled Plasma–Mass Spectrometer (ICP-MS). The standard reference material (SRM) SRM 2709 was used [44].

2.3. Bioactive Compounds (Proline, Total Phenolics, Lipid Peroxidation, and Vitamin C)

2.3.1. Proline

The content of free proline (PRO) in the tissue of hypocotyl was determined according to Bates et al. (1973) [45]. The tissue was homogenized in liquid nitrogen and weighed (about 0.2 g) into plastic tubes. Proline was extracted from the tissue with 10 mL of sulfosalicylic acid (3%). The tissue was separated from the supernatant by centrifugation at 3500 rcf at 4 °C for 15 min. To 2 mL of the supernatant, 2 mL of acidic ninhydrin reagent (2.5%) and 2 mL of glacial acetic acid were added. The mixture thus prepared was stirred on a vortex shaker and heated for 1 h in a water bath at 95–98 °C. After heating, the mixture was quenched in ice water and 4 mL of toluene was added to each sample. The samples were stirred for 20 s and left at room temperature until the upper toluene layer with proline separated from the lower, aqueous layer. The standard curve was made using the basic standard of a solution of L-proline with a concentration of 20 μg PRO mL^{-1} in the concentration range of 0–20 μg PRO mL^{-1}. The proline concentration in the toluene fraction was determined by measuring the absorbance at 520 nm, and it was calculated from a standard curve with known proline concentrations, which were treated in the same way as the samples were. The final results are expressed as μmol proline g^{-1} fresh matter.

2.3.2. Total Phenolics (TP)

The content of total phenols (TP) in hypocotyls of soybean was determined by the spectrophotometric method with Folin–Ciocalteu reagent according to Singleton and Rossi (1965) [46]. The phenols were extracted with 2.5 mL of ethanol (95%) at −20 °C for 48 h from about 0.1 g of tissue macerated in liquid nitrogen. After extraction, the homogenates were centrifuged at 10,000 rcf at 4 °C for 10 min. To a certain volume of supernatant (depending on the expected values of phenol concentration), about 1.5 mL of distilled water (total volume of supernatant and water is 1.6 mL), 100 μL of Folin–Ciocalteu reagent, and 300 μL of Na_2CO_3 (saturated solution) were added. A total of 2 mL of the reaction mixture was stirred on a vortex shaker and incubated in a water bath at 37 °C for 60 min. The phenol content of the incubated and cooled mixture was determined by measuring the absorbance on a spectrophotometer at a wavelength of 765 nm. The phenol concentration was calculated from a standard curve with known gallic acid (GA) concentrations, in the range of 0.05 to 0.5 mg GA mL^{-1}. The final phenol content was expressed as mg GA g^{-1} of fresh matter. Samples of the standard solution, as well as the sample solution, were prepared in triplicate.

2.3.3. Lipid Peroxidation

Lipid peroxidation was conducted according to Heath and Packer (1968) [47]. The hypocotyl tissue was comminuted with liquid nitrogen to a fine powder. About 0.2 g of chopped plant tissue was extracted with 1 mL of trichloroacetic acid (0.1%). After centrifugation at 6000 rcf at 4 °C for 5 min, 1 mL of thiobarbituric acid (0.5%) in trichloroacetic acid (20%) was added to 0.5 mL of supernatant. The mixture was heated in a water bath at 95 °C for 30 min and then cooled. After cooling, the supernatant was isolated by centrifugation at 18,000 rcf for 15 min at 4 °C. The absorbance of the sample supernatant was measured spectrophotometrically at wavelengths of 532 and 600 nm. Thiobarbituric acid (0.5%) in trichloroacetic acid (20%) was used as a blank. The concentration of lipid peroxidation products (TBARS) was calculated using the molar extinction coefficient (ε = 155 mM^{-1} cm^{-1}) and expressed in thiobarbituric acid equivalents (TBA) in units of nmol TBA g^{-1} fresh matter.

2.3.4. Total Vitamin C Content (Ascorbic Acid)

Vitamin C concentration was determined according to the Roe and Kuether protocol (1943) [48] with some modifications. Solutions of TCA 13.3%—trichloroacetic acid, H_2SO_4 65%—sulfuric acid, DNPH 2%—2,4 dinitrophenylhydrazine (2 g of DNPH, 230 mg of thiourea, 270 g of $CuSO_4$, and 100 mL of 5 M of H_2SO_4), and ascorbic acid (stock solution)—the basic standard for making calibration curves (0.1 mL^{-1}) were used. The soybean tissue was macerated with liquid nitrogen, which was wound in plastic tubes from 15 mL, weighing 0.2 g of crushed tissue. Then, 250 µL of soybean juice was pipetted into 15 mL threaded plastic tubes, and the mass of pipetted juice was weighed. Next, 10 mL of distilled water was added to the leaf mass tubes, and 5 mL of distilled water was added to the tubes where the soybean juice was present. After extraction, the samples were centrifuged for 15 min at 4000 rcf at 4 °C. Subsequently, 150 µL was pipetted in two test tubes (2 mL) for the sample and follow-up. For the purpose of the tubes, 175 µL of distilled water, 100 µL of 13.3% TCA, and only a 75 µL sample of DNPH were added, and the prepared extracts were incubated at 37 °C for 3 h. After incubation of the sample and the next probe, 1000 µL of H_2SO_4 was added and DNPH (75 µL) was added in the next probe before that. Standards were prepared in the same way as the sample was, so DNPH was added before incubation. From stock, ascorbic acid solutions were prepared for dilution at concentrations of 0, 25, 50, 75, 100, 125, 150, 175, 200, 250, and 275 g mL^{-1}. All samples were vortexed on a Varian Cary 50 UV-Vis spectrophotometer and measured for absorbance at 520 nm in a 1 cm glass cuvette.

2.4. Analysis of Antioxidant Activity (FRAP)

The ferric reducing antioxidant power (FRAP) method was determined according to Benzie and Straint (1996) with modifications [49]. Freeze-dried material tissue of soybean hypocotyls was weighed (0.2 g) into a plastic tube, and 10 mL of 96% ethanol was added to the sample, closed and shaken for 20 min on the shaker, and then centrifuged for 15 min at 6000 g under 4 °C. The supernatant was transfused with Pasteur-pipettes, put into a 10 mL volumetric flask, and filled with methanol up to the mark. The contents of the volumetric flask were transferred into the scintillation vessel and frozen immediately. A dissolved pellet with 4 mL of n-hexane was centrifuged, and the supernatant was separated in a 10 mL volumetric flask and frozen. A water bath was set to 37 °C. FRAP reagent containing 200 mL of acetate buffer, 20 mL of TPTZ solution, and 20 mL of $FeCl_3$ solution was used. Mixed solutions were placed into the water bath at 37 °C. A spectrophotometer was tuned to a wavelength of 593 nm, and the zero was prepared with 1 mL of FRAP reagent and 100 µL of methanol. The preparation of the standard was as follows: pour 100 µL of standard solution + 1 mL of FRAP solution into the cuvette, mix thoroughly using vortex shaker, and incubate for 4 min at 37 °C in water bath. The dry cuvette was set on the spectrophotometer and measured.

2.5. Statistical Analysis

Statistical analysis was conducted with the R programming language (4.0.4 version). Three-way type III analysis of variance (ANOVA) was carried out with main effects cultivar, Se-biofortification, and PEG-2.5 treatment, and all assumed interactions. Differences between treatment means were considered significant at the $p < 0.05$ probability level in Fisher's LSD test (Table 1). Another one-way ANOVA was carried out for the convenient display of all cultivar-Se-treatment combinations in bar graphs. The differences between means were compared using Fisher's least significant difference at a $p = 0.05$ probability level.

Table 1. Mean values ± standard deviations of Se contents in soybean grains after foliar biofortification with 30 g Se ha^{-1} in 2020. Different letters represent significance of differences at $\alpha = 0.05$ level.

Treatment	Cultivar	μg Se g^{-1}
wSe	Lucija	64.02 ± 36.04 b
	Sonja	101.42 ± 65.87 b
Se	Lucija	2091.67 ± 97.29 a
	Sonja	2315.33 ± 331.8 a
LSD 0.05	333.09	

3. Results

3.1. Se Content in Soybean Grain and Analysis of Variance (Three-Way ANOVA)

Se biofortification resulted in significant increases by factors of 32.67 and 22.83 in grain Se contents in cultivars Lucija and Sonja, respectively. Significant differences were detected between cultivars (Table 1) after foliar Se biofortification in 2020.

Analysis of variance showed significant effects of the cultivar on all analyzed traits except PRO (Table 2). Main effect Se did not significantly affect any of the analyzed physiological traits, while highly significant effects were observed for main effect PEG-2.5. The interaction between Cultivar and Se showed significant effects on all traits except for TP, while the interaction between Cultivar and PEG-2.5 was significant only for PRO and AA. The interaction between Se and PEG-2.5 significantly affected only LP and TP. The three-way interaction did not significantly affect any of the analyzed traits.

Table 2. Results of ANOVA for lipid peroxidation product (LP), proline content (PRO), total phenols (TP), total antioxidant activity (FRAP), and ascorbic acid (AA). Significance of effects is denoted with * ($\alpha = 0.05$), ** ($\alpha = 0.01$), and *** ($\alpha = 0.001$), while *p*-values are given for factors lacking significance.

	LP	PRO	TP	FRAP	AA
Cultivar	**	0.345	*	**	***
Se	0.485	0.783	0.356	0.064	0.061
PEG-2.5	***	***	***	***	***
Cultivar * Se	**	**	0.061	*	*
Cultivar * PEG-2.5	0.492	*	0.086	0.923	*
Se * PEG-2.5	*	0.813	*	0.788	0.103
Cultivar * Se * PEG-2.5	0.937	0.103	0.225	0.625	0.399

Mean values for main effects PEG-2.5 and Se are shown in Table 3. In alignment with the results of ANOVA (Table 2), significant increases in LP and TP were observed in combination with PEG-2.5 and Se-biofortified cultivars over both assessed cultivars.

Table 3. Mean values ± standard error of mean for lipid peroxidation product (LP), proline content (PRO), total phenolic content (TP), total antioxidant activity (FRAP), and ascorbic acid (AA) over main effects PEG and Se. Different letters represent significance of differences at $\alpha = 0.05$ level.

PEG	Se	LP	PRO	TP	FRAP	AA
PEG-0	wSe	36.64 ± 1.86 b	6.88 ± 0.38 b	0.69 ± 0.02 b	3.35 ± 0.22 b	32.19 ± 1.45 c
	Se	33.47 ± 2.95 b	6.89 ± 0.26 b	0.65 ± 0.02 b	4.16 ± 0.49 b	32.69 ± 1.91 bc
PEG-2.5	wSe	44.97 ± 0.94 a	11.26 ± 0.4 a	0.87 ± 0.02 a	6.47 ± 0.42 a	41.04 ± 2.46 ab

Table 3. *Cont.*

PEG	Se	LP	PRO	TP	FRAP	AA
PEG-2.5	Se	50.39 ± 3.09 a	11.46 ± 0.85 a	0.95 ± 0.05 a	7.08 ± 0.59 a	47.62 ± 4.54 a
LSD 0.05	-	7.01	1.54	0.094	1.32	8.39

3.2. Concentration of LP, PRO, TP, FRAP, and AA in Soybean Plant Tissue

A 26% increase in LP content (Figure 1A) was observed in plants under PEG-2.5 treatment. Significant differences between LP contents were detected in PEG-0 plants. The significantly highest level of LP in the PEG-0 was in plants with Se in the cultivar Sonja (39.161 nmol g^{-1}), while the content of LP did not differ significantly between cultivars Lucija (37.325 nmol g^{-1}) and Sonja (35.95 nmol g^{-1}) in wSe. In PEG-0, cultivar Lucija showed a significant reaction of LP to Se with the lowest value of 27.769 nmol g^{-1}. LP content showed significant differences between groups in PEG-2.5 treatment. The significantly highest value of LP of 57.064 nmol g^{-1} was detected in cultivar Sonja in PEG-2.5 treatment with Se seeds. The content of PRO (Figure 1B) in PEG-2.5 plants was 40.38% higher compared to the PEG-0. There were no significant differences detected between plants in PEG-0. Cultivar Sonja with Se in PEG-2.5 showed the significantly highest level of PRO (13.083 µmol g^{-1}). The significantly lowest PRO content in PEG-2.5 with Se was detected in Lucija with 9.84 µmol g^{-1}.

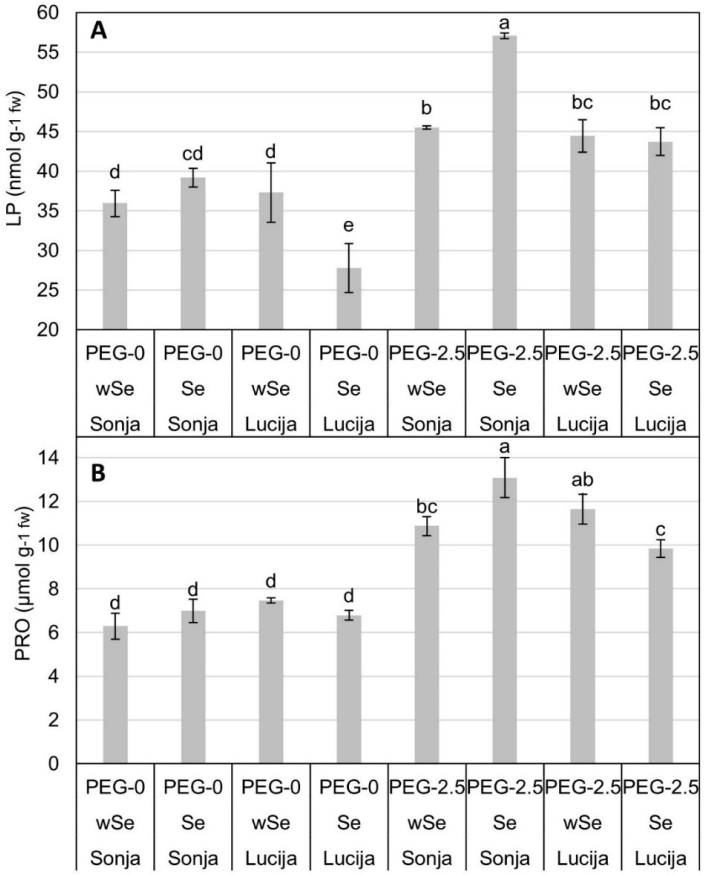

Figure 1. Means and standard errors (SE) of means for PRO (proline) (**A**) and LP (lipid peroxidation product) (**B**). Significance of effects is denoted with different letters at $\alpha = 0.05$ level.

There were no significant differences detected between groups in PEG-0 plants in TP content (Figure 2A). Plants treated with PEG-2.5 had a higher TP content by 27.17% than PEG-0 plants. The only significant difference in PEG-2.5, compared to other groups, was detected in Sonja with Se with a value of 1.049 mg GA g^{-1} fw, while in the other

groups, there were no significant differences. FRAP values (Figure 2B) in PEG-2.5 were higher by 45.29% compared to PEG-0 plants. Significant differences were detected between cultivar-Se groups in PEG-0 treatment. The highest value in the PEG-0 was observed in cultivar Sonja with Se (5.06 Mm g^{-1}), while in cultivar Lucija, no significant differences were detected between wSe and Se treatments, although generally, significantly lower values were observed compared to cultivar Sonja. In PEG-2.5 treatment, for FRAP values, a significant difference was found between cultivar Sonja with Se (8.127 Mm g^{-1}) and all other groups. Plants in PEG-2.5 showed a higher content of AA by 27.41% compared to plants in PEG-0 (Figure 2C). Significant differences in PEG-0 were detected in Lucija with Se, with the lowest concentration of 28.78 mg AA 100 g^{-1}, and Sonja with Se (56.794 mg AA 100 g^{-1}). In PEG-2.5, Sonja with Se showed the highest and significant value of 56.794 mg AA 100 g^{-1}. There were no significant differences detected between the other groups in PEG-2.5.

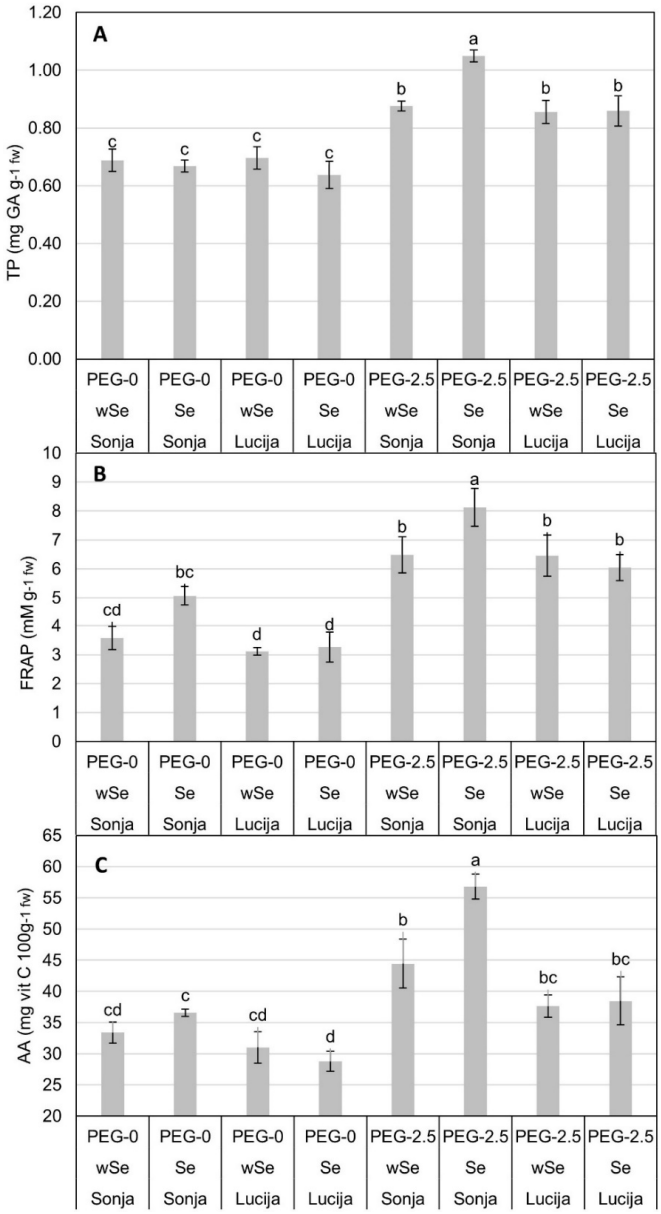

Figure 2. Means and standard errors of mean (SEM) for total phenolic content (**A**), FRAP (total antioxidant activity) (**B**) and AA (ascorbic acid) (**C**). Significance of effects is denoted with different letters at $\alpha = 0.05$ level.

4. Discussion

Significant differences between treatments and cultivars showed contrasting responses of soybean seedlings to Se biofortification in PEG-2.5 treatment (Figure 1A). Similar to our results, PEG-2.5 treatment induced mild stress in soybeans in the study of Basal et al. (2020) [50]. Drought or heat stress can result in the increase in lipid peroxidation [51], while the use of Se at lower doses can stimulate the antioxidant capacity in cucumber (*Cucumis sativus* L.) and reduce lipid peroxidation [26]. In our study, PEG-0 plants with Se in Lucija showed a lower LP by 29.09% compared to Sonja with Se in the same treatment, while LP in plants without Se yielded no significant differences. Our results in Lucija are in accordance with the results in white clover (*Trifolium repens* L.) [39], lettuce (*Lactuca sativa* L.) [52], and in ryegrass (*Lolium perenne* L.) [53] where Se reduced LP. The decrease in LP by exogenous Se may be attributed to its beneficial effects on the antioxidant potential of plants [30]. However, the increase in LP in Sonja with Se (PEG-2.5) was caused by different physiological responses compared to Lucija, which is corroborated by the results of a study conducted on ryegrass where higher doses of Se enhanced the accumulation of the products of LP [53]. Accordingly, Jozwiak and Politycka (2019) [26] conducted research confirming that higher doses of Se can increase LP; therefore, Se at lower concentrations is an antioxidant [54], while high doses can have deleterious effects [53].

Cultivar Sonja in PEG-2.5 treatment with Se showed a 24.79% increase in PRO in seedling tissue compared to Lucija in the same treatment (Figure 1B). Osmolytes, such as proline and glycinebetaine, accumulate under water deficit to help conserve tissue water and protect proteins and cellular membranes from osmotic and oxidative stresses [55]. Proline accumulation is generally regarded as a functional adaption against osmotic stress [56]. The increase in proline accumulation with the increase in severity and duration of drought helps plants maintain tissue water status and avoid drought-induced damage [55]. The increase in TP was detected in cultivar Sonja in PEG-2.5 with Se (Figure 2A). The increase in TP under stress conditions is related to the genetics and growth environment of plants [57]. The antioxidant activity of phenolics is mainly caused by their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers along with their metal chelation potential [58]. Pannico et al. (2020) showed that phenolic compounds increase with Se biofortification in coriander (*Coriandrum sativum* L.), basil (*Ocimum basilicum* L.), and tatsoi (*Brassica rapa* var. *rosularis*) [59]. Phenols have the ability to increase the antioxidant capacity and improve the ability of plants to alleviate oxidative stress [27,60]. Cultivar Sonja showed the highest level of FRAP across both PEG-2.5 and PEG-0 (Figure 2B). Previous investigations have also shown an elevation in the antioxidant activity in Se-treated plants under different abiotic stresses [40]. In a study by Puccinelli et al. (2020), the highest content of antioxidant capacity, total phenol, and rosmarinic acid contents were detected in the Se-treated plants. This could be related to the reaction of plants against the potentially toxic effects of Se in basil [60]. Cultivar Sonja with Se showed the highest content of AA in PEG-2.5 treatment (Figure 2C). AA is an organic acid; under drought stress, respiration is increased and, therefore, these acids act as substrates in the respiration phenomenon [57]. Increasing the AA content in plants can have a triple-positive effect: producing food with a high content of AA for healthy human diets, increasing the postharvest shelf life of products, and increasing the resistance of plants to various kinds of stress [61]. In PEG-0 treatment, decreases in physiological parameters were observed in Se-biofortified plants in cultivar Lucija, while a mild mitigating effect on the osmotic stress in PEG-2.5 treatment with Se was observed through the reduction in proline content. Accordingly, we suggest that biofortified cultivar Lucija might be further examined in field experiments because of its suitability for field crop production. On the other hand, a potentially negative physiological response to Se was observed in cultivar Sonja, where it could act as a prooxidant, presenting a good starting point for the further research of cultivar Sonja for the production of functional Se-enriched food with a high content of phytoactive components.

5. Conclusions

Climate change causes shifts in worldwide climatological scenarios with spring droughts becoming more and more frequent [62]. Accordingly, from our results, it can be seen that the Se biofortification of soybean seeds could help mitigate the effect of water deficit in Lucija. High concentrations of LP, PRO, TP, FRAP, and AA suggest that Sonja with Se had a stronger defense mechanism than Lucija in both PEG-0 and PEG-2.5 treatments. Our results can be interpreted in several ways: that Sonja was more sensitive to Se and had a stronger physiological response, or defense reactions in Sonja are slower and were not captured within the growing setup of our study. There is a possibility that a small difference in concentration is sufficient to cause toxicity in Sonja. Our results also suggest that it might be necessary to determine which Se concentrations are optimal for a particular cultivar when it comes to mitigation of the stress effect caused by water deficit. It is necessary to find an appropriate measure for crop improvement to mitigate the negative effects of climate change, and Se biofortification is certainly one of them [11,39,63,64]. From the conducted research, we conclude that Se biofortification in Lucija might be more appropriate for crop improvement, which should be further tested in field experiments. Sonja should be further investigated in light of functional food production in the form of soybean sprouts because Se directly affected the higher production of TP and FRAP and doubled the concentration of vitamin C (AA). Undoubtedly, both cultivars significantly and considerably increased grain Se, which is of great importance for human health.

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Combining Selenium Biofortification with Vermicompost Growing Media in Lamb's Lettuce (*Valerianella locusta* L. Laterr)

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Abstract: Leafy vegetables are a daily part of the human diet all over the world. At the same time, a worldwide problem of Se malnutrition is present in human populations, mostly due to low soil Se contents. As plants represent the main source of this element in the human diet, with Se being an essential trace element for humans and animals, plant foods containing Se can be used as an efficient means of increasing the Se in the human diet, as well as in animal feed (biofortification). At the same time, the production of growing media relies on limited peat reserves. The use of earthworms facilitates the production of composted organic masses mostly consisting of organic waste, called vermicompost. The aim of this study was to investigate the influence of three different growing media (commercial peat media, vermicompost, and a 1:1 mixture) on Se biofortification's efficacy and yield in lamb's lettuce. The Se biofortification was performed with sodium selenate (Na_2SeO_4). It was shown that biofortification increased the Se contents such that a mass of only 48.9 g of fresh leaves contained enough Se for the recommended daily intake in human nutrition (55 µg Se/day), which represents a significant potential for solving Se malnutrition. Furthermore, the use of a 1:1 vermicompost–commercial substrate mixture showed a similar performance to the peat growing medium, contributing to the preservation of peat reserves.

Keywords: lamb's lettuce; biofortification; selenium; malnutrition; human health; vermicompost; peat

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1. Introduction

Various anthropogenic activities, escalating urbanization, industrialization, and economic growth are leading to the production of huge quantities of solid waste around the globe. The management of this solid waste has now become an ecological and a technical problem for all [1]. Due to the rising costs and uncertain future availability of peat moss, there is a need for alternative components in commercial potting substrates. Nevertheless, peat-based commercial potting substrates have low ion exchange capacities, and there is a concern about the environmental impact of leachates containing high concentrations of chemical fertilizers [2]. The large-scale removal of peat from bogs is also destroying wildlife habitats, and the process of peat regeneration is extremely slow [3]. Combining these two insights with the fact of increasing garbage production and the increasing shortage of resources, one of the possible solutions to these problems might be vermicomposting.

Vermicomposting is an efficient nutrient recycling process that involves harnessing earthworms as versatile natural bioreactors for organic matter decomposition. In other words, earthworms are capable of transforming garbage into "gold" [4]. Vermicomposting is a non-thermophilic biological oxidation process in which organic materials are converted into vermicompost, which is a peat-like material exhibiting high porosity, aeration, drainage and water-holding capacities, and rich microbial activities [4]. The process of vermicomposting is faster than composting because the material passes through the earthworm's gut and presents an excellent soil additive, making it a high value product [5]. It is used as an organic fertilizer, soil amendment, and potting substrate component, with many characteristics matching those of conventional composts [2]. Therefore, many studies have proposed vermicompost as an alternative for maintaining economically viable crop production with minimal environmental pollution [6].

Plants recycle Se within the food chain. Thus, the biofortification of crops with Se, by means of adding Se along with fertilizers, is a useful technique to increase the consumption of Se by animals and humans [7]. In humans, Se absorption from products of plant origin is much easier compared to its absorption from products of animal origin [8]. Dietary Se deficiency has negative effects for human health, and more than 40 types of diseases have been associated with Se deficiency, such as Keshan disease, Kashin-Beck disease, cardiovascular diseases, liver diseases, some type of cancer and cataracts [8]. Similarly to Zn, Se supplementation to coronavirus disease 2019 (COVID-19) infected patients with low Se blood levels could be an option as a natural treatment against the virus [8]. Because Se deficiency in the diet is a common phenomenon in many countries worldwide, plants biofortified with Se are an excellent source of dietary Se that can help alleviate this problem [9]. According to the European Union regulations [10], the recommended daily dose of Se for adults is 55 µg. Se is a very important micronutrient for the proper functioning of humans, animals, and some microorganisms, as a structural component of selenoproteins [11], but its importance for plants is still the subject of research [12]. Some of the positive effects of Se found in plants are: promoting plant growth, alleviating UV-induced oxidative damage, improving the recovery of chlorophyll from light stress, and increasing the antioxidative capacity of senescing plants [12]. The protective role of Se in plants exposed to various environmental stresses, in most cases, has been attributed to the activation of the antioxidative defense system [12,13]. Se is a rare element, with an average concentration in igneous bedrock of only 0.05 mg kg^{-1}, less than any other nutrient element [8]. It exists in four different oxidation states: elemental Se (Se^0), selenide (Se^{-2}), selenite (Se^{+4}) and selenate (Se^{+6}), with other inorganic and organic matrices and soluble forms of selenite and selenate [14]. The plant-available Se in the soil, such as water-soluble and exchangeable Se, consists of mobile fractions that are readily taken up by plants [15].

Fresh-cut or minimally processed fruits and vegetables play an important role in the human diet, as opposed to highly caloric diets rich in lipids and sugars. Consequently, human nutrition has been orienting towards ready-to-eat foods such as pre-cooked and minimally processed vegetables or fruits [16]. With its relatively good storage quality, lamb's lettuce is in increasing demand not only as a leafy salad but also as an ingredient in fresh-cut products and ready-to-eat salad mixtures [17]. Lamb's lettuce has modest heat requirements, such that its production mostly occurs during the colder period of the year, with an optimal growth temperature between 5 and 10 °C [18]. Lamb's lettuce has been reported to have a positive influence on certain diseases, such as diabetes, cardiovascular disorders and cancer [19].

The aim of this study was to investigate the efficiency of Se biofortification with sodium selenate (Na_2SeO_4) in three different growing media: A commercial substrate (CS), vermicompost, and a mixture of these two substrates in a 1:1 ratio.

2. Materials and Methods

2.1. Plant Growth Conditions and Growing Media Characteristics

Three different growing media were used for this experiment: commercial growing substrate (CS), vermicompost, and a CS–vermicompost mixture in a 1:1 ratio. The commercial growing substrate was characterized by the following properties: pH (H_2O) 6.3, electrical conductivity (EC) 0.42 dS/m, specific density 180 kg/m^3, and a total porosity of 85% volume. CS is a mixture of frozen black sphagnum peat and very fine white sphagnum peat, with added water-soluble fertilizer and microelements. CS is a multipurpose substrate for production in containers and nutrients cubes of up to 6 cm for lettuce, cabbage, and celery, etc. The vermicompost used in the experiment was procured from the city municipal waste company UNIKOM d.o.o. Osijek (Osijek, Croatia), produced from municipal waste generated by the maintenance of public green areas (residues from cutting hedges, fallen leaves, grass clippings, and branch cuttings) and processed through the digestive tract of red wigglers (*Eisenia fetida*). The lamb's lettuce cultivation was carried out in a plant growth room (Faculty of Agrobiotehnical Sciences Osijek, Osijek, Croatia) in a walk-in setup. During the experiment, the daytime temperature was set at 24 °C, and the nighttime at 20 °C; the relative humidity was 45%, and the light regime was 16 h day and 8 h night.

2.2. Experimental Design

The experiment was conducted with a completely randomized design. For the biofortification part of the experiment, the seeds of a lamb's lettuce (*Valerianella locusta* L. Laterr), cv. Verte de Cambrai (Franchi sementi, Grassobbio, Italy), with a declared germination rate of 84% and a seed purity of 95% were used. The seeds were sown in styrofoam containers with 60 sowing holes (52 mL each) in a 10 × 6 arrangement. Four containers were prepared per substrate, representing four replicates for each of the growing media (CS, vermicompost, and mixture of both in a ratio of 1:1). Three seeds were sown per growcell. After three weeks, the seedlings were thinned to a single plant per growcell, and then fertilized with a pre-prepared solution of crystalline complex fertilizer N:P:K in a ratio of 20:20:20 + MgO (magnesium oxide) + ME (microelements) (Haifa Group, Haifa, Israel). The biofortification was carried out on the 6th week of the experiment, by adding 15 mL of 40 µM sodium selenate (Na_2SeO_4) solution, which was added per hole to half (30) of the growcells of each container. The plants were harvest 10 days after the Se application. The yield was expressed on a single-plant basis by weighing all of the plants from a single container treatment–substrate combination and dividing this by the number of plants.

2.3. Analysis of the Growing Media

Samples of the vermicompost, commercial substrate and mixtures of the vermicompost and commercial substrate in a 1:1 ratio were analyzed to measure for the following properties: pH, electrical conductivity (EC), organic matter (OM), ash content, the concentration of nitrogen, micronutrients (Zn, Cu, Mo and Ni), toxic elements (Cd, Pb, Cr, Hg and As), and the total Se concentration. The laboratory analyzes and element determination were conducted at the Faculty of Agrobiotehnical Sciences Osijek, Osijek, Croatia.

2.3.1. pH Determination

The electrometric measurement of the reaction (pH value) was performed with pH meters that measured the difference in electrical potential. The electrometric determination of the pH of the vermicompost was performed according to the European standard 13037:2011 [20] in a suspension of 60 mL fresh sample in 300 mL deionized water, i.e., in a volume ratio of 1:5 (sample:water), and after mixing with a shaker for 60 min.

2.3.2. EC

The electrical conductivity was measured according to the European standard EN 13038: 2009 [21] in a suspension of 60 mL fresh sample shaken on a shaker for 60 min in

300 mL deionized water, i.e., in a volume ratio of 1:5 (sample:water). Electrical conductivity is an indicator of the proportion of water-soluble electrolytes in the analyzed sample.

2.3.3. Determination of the Organic Matter and Ash Contents

The total organic matter and ash contents were determined by drying 5 g of the sample at 103 ± 2 °C for at least 4 h, and successively annealing the sample at 450 ± 10 °C for at least 6 h (EN, 2011.b). The sample was annealed in an annealing furnace, with the first weighing of the sample mass after 6 h, and then after each additional hour of annealing to a constant mass, i.e., when the difference between two consecutive weighings was <0.01 g.

2.3.4. Determination of the Organic C Content, Total N and C/N Ratio

The organic carbon content analysis was carried out by wet destruction, in which 50 mg of the dry sample was weighed into destruction cells, poured with 5 mL of 0.27 mol dm^{-3} $K_2Cr_2O_7$ and 7.5 mL of concentrated H_2SO_4, and destroyed for 30 min in a destruction block at 135 °C. After its destruction, the sample was quantitatively transferred to volumetric flasks, made up to 100 mL with deionized water, transferred to centrifuge tubes, centrifuged for 10 min at 2000 G, and filtered. In clear samples and a series of standard glucose solutions, the transmission values at 585 nm were measured with a spectrophotometer, and the organic carbon content (concentration) was expressed in %.

The determination of the nitrogen is based on the preparation of a stock solution of the sample by destroying the sample using mixtures of acids. An NaOH solution was added to the measured volume of the sample stock, and the N was distilled off as ammonia into a sample with an acid of known concentration. The amount of nitrogen in the analyzed sample was calculated from the acid consumption (EN, 2003). The C/N ratio was obtained using the data of the total carbon in the dry matter and the total nitrogen in the fresh matter according to the following formulae:

$$\% \text{ C in fresh matter} = (\% \text{ C in dry matter} \times \% \text{ dry matter}) \div 100 \quad (1)$$

$$\text{C/N} = \% \text{ C in fresh matter} \div \% \text{ N in fresh matter} \quad (2)$$

2.3.5. Determination of the Heavy Metal Concentrations

In order to determine the concentration of heavy metals (Zn, Cu, Ni, Mo, Cr, Cd, Hg, and Pb), a stock solution was prepared by destroying the dry sample by digestion with a mixture of concentrated nitric and hydrochloric acid in a ratio of 1:3 [22]. The heavy metal concentrations were determined by measurement in stock using a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES, Waltham, MA, USA). The concentrations of these elements are expressed in mg/kg dry matter of the sample.

2.4. Plant Material Analysis

Determination of the Total Se and Total Zn Concentrations in the Plant Tissue

The dry plant matter was ground in a special mill without heavy metal residues, and was destroyed by the wet process using the microwave technique. In total, 0.5 g of the dry sample was weighed into a Teflon dish and poured with 9 mL 65% HNO_3 and 2 mL 30% H_2O_2. After the digestion procedure under a controlled pressure and temperature in the microwave, the solution was filtered into metered vessels. Before determining the concentration, the reduction of the Se in the samples was performed. For the Se and Zn reduction procedure, 20 mL of the sample was transferred to clean 125 mL cuvettes with the gradual addition of 20 mL HCl. The solution was then transferred to a 50 mL polypropylene tube and made up to the mark with deionized water. The Se and Zn concentrations in the solutions were measured using the Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) technique.

2.5. Statistical Analysis

The statistical analysis was performed using the R programming language (4.0.4 version) and Microsoft Excel. A two-way type II analysis of variance (ANOVA) was carried out with the main-effects Se treatment and growing media, and assumed two-way interactions. The differences between the treatment means were considered significant at the $p < 0.05$ probability level in Fisher's LSD test. Another one-way ANOVA was carried out for the decomposing of the significant interactions. The differences between the means were compared using Fisher's least significant difference at a $p = 0.05$ probability level.

3. Results

3.1. Physico-Chemical Analysis of the Growing Media

The considerable differences were detected between the properties of the assessed growing media. It is shown in Table 1 that the commercial substrate (CS) had the highest value of organic matter and the highest C:N ratio compared to the other two growing media. CS had the lowest EC, total nitrogen and ash content compared to the other two substrates. The vermicompost had the highest ash content, the highest pH value, EC and total nitrogen content, and compared to the other substrates it contained the lowest amount of organic matter and had the narrowest C:N ratio. The 1:1 mixture showed properties between CS and the vermicompost.

Table 1. Basic properties of the growing media commercial substrate (CS), vermicompost and mixture 1:1.

Growing Media	% Organic Matter	% Ash	pH_{H2O}	EC (mS/m)	Total N (g/kg)	C/N Ratio
CS	39.6	51.4	6.34	42.1	2.2	180:1
Vermicompost	12.6	87.4	9.23	67.9	5.4	23.3:1
Mixture 1:1	22.4	78.6	7.55	59.5	3.1	72.3:1

The concentrations of the trace elements and toxic heavy metals in the growing media are shown in Table 2. CS had the highest determined concentrations of Cd, Se and Pb, and the lowest contents of Zn, Cu, Mo, Ni, Cr, Hg and As compared to the other two analyzed substrates. Vermicompost showed the highest levels of Zn, Cu, Mo, Ni, Cr, Hg and As, and on the other hand, the lowest concentrations of Cd, Se and Pb. The 1:1 mixture's values were between the commercial substrate and the vermicompost.

Table 2. Total concentrations (mg/kg) of the microelements (Zn, Cu, Se, Mo, Ni) and harmful heavy metals (Cd, Pb, Cr, Hg and As) in commercial substrate (CS), vermicompost and mixture 1:1.

Growing Media	Zn	Cu	Se	Cd	Pb	Mo	Ni	Cr	Hg	As
CS	60.2	16.9	0.55	0.64	20.1	0.098	7.1	4.6	<0.01	0.012
Vermicompost	106.0	34.0	0.21	0.49	18.9	0.720	23.0	30.0	0.063	5.570
Mixture 1:1	77.0	23.4	0.32	0.54	19.3	0.411	17.1	19.4	0.041	3.213

3.2. Analysis of Plant Material

The main-effect growing media showed significant effects on the fresh and dry mass per plant and the Se content in the fresh plant material, while the main-effect Se treatment showed significant effects only on the Se content in both the fresh and dry mass (Table 3).

Table 3. Analysis of variance for the fresh and dry weight per plant, and the Se concentrations. * represents significance at $p < 0.05$, *** represents significance at $p < 0.001$. In the case of a lack of significant effects, the *p*-values are given.

	Fresh Weight per Plant	Plant Dry Weight	Se mg/kg FW	Se mg/kg DW
Growing media	***	***	*	0.122
Se treatment	0.6735	0.129	***	***
Growing media: Se treatment	*	*	*	0.121

The yield of fresh mass per plant (g) varied significantly with respect to the treatment and growing media interaction. There were no significant differences detected between the yield in CS and the 1:1 mixture growing media, considering the control and treatment with Se. Significant differences between the Se treatment and the control in the vermicompost were found. A significantly higher yield was detected in the control than in the Se treatment (Figure 1).

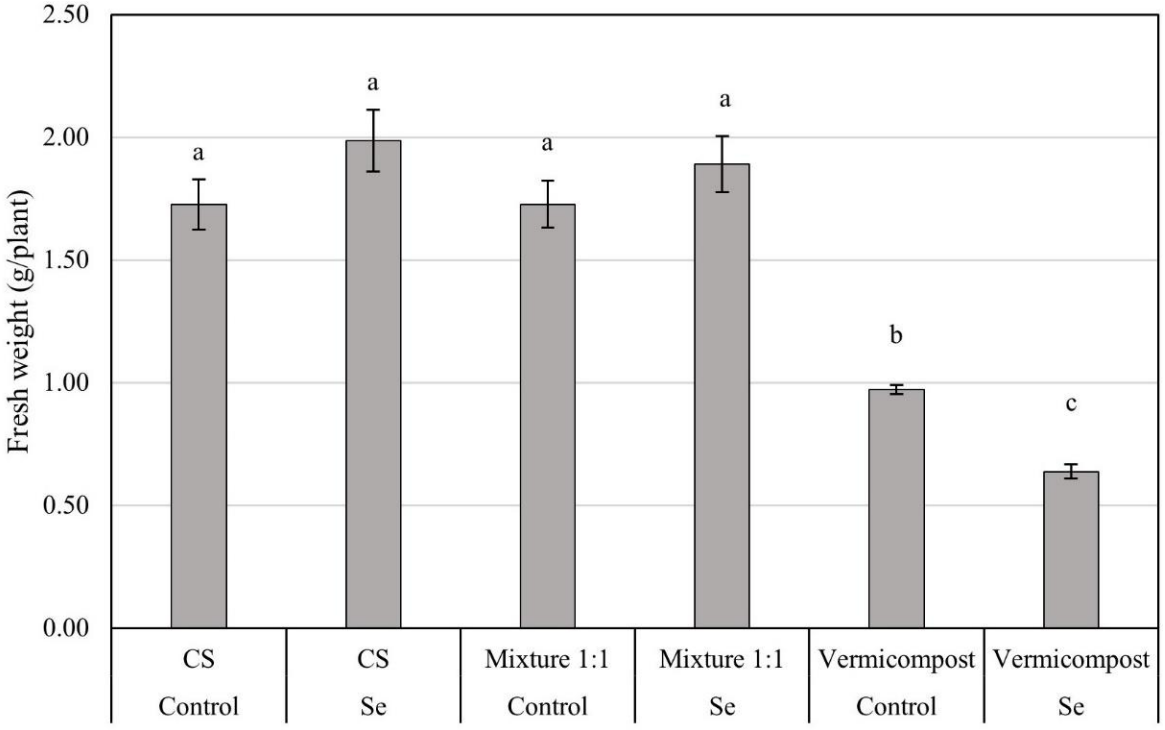

Figure 1. Mean values ± standard error of the fresh weight (FW) per plant (g) over the main-effects Se treatment and growing media commercial substrate (CS), vermicompost and mixture 1:1. The different letters represent the significance of the differences at the $\alpha = 0.05$ level.

Table 4 shows the Se content in the fresh and dry weight of the lamb's lettuce tissue. The Se treatment significantly affected the Se content increase in the dry and fresh plant mass over all three assessed growing media. In the fresh weight of the lamb's lettuce, the Se content increased 172.86-fold compared to the control. The Se content in the dry mass increased by 177.45 times compared to the control. Significant differences were found between the concentration of Se in the fresh weight of the plant tissue with respect to the growing media over both the control and the Se treatment. The 1:1 mixture showed, significantly, the lowest Se contents compared to the other two growing media in the fresh-weight tissue, although no significant differences were found for the Se content in the dry mass of the lamb's lettuce.

Table 4. Mean values ± the standard deviations of the Se contents in the fresh weight (FW) and dry weight (DW) of the lamb's lettuce tissue. The different letters represent the significance of the differences at the $\alpha = 0.05$ level.

	Se FW (mg/kg)	Se DW (mg/kg)
Se Treatment		
Control	0.0065 ± 0.0019^{b}	0.0691 ± 0.0131^{b}
Se	1.1236 ± 0.5914^{a}	12.2615 ± 4.3845^{a}
LSD 0.05	0.2794	2.629
Growing Media		
CS	0.532 ± 0.584^{ab}	6.7207 ± 7.3576^{a}
Vermicompost	0.848 ± 1.01^{a}	7.4656 ± 9.0653^{a}
Mixure 1:1	0.314 ± 0.361^{b}	4.3096 ± 4.6585^{a}
LSD 0.05	0.342	3.221

A significant effect of the Se addition to the growing media on the Zn uptake is shown in Table 5. The fresh weight of the lamb's lettuce in CS had a significantly lower Zn concentration in the treatment with Se. Vermicompost had, significantly, the highest concentration of Zn in the fresh weight with the addition of Se, and in the 1:1 mixture the Zn content was higher in the control compared to the Se treatment in the fresh plant tissue. In the dry mass of the lamb's lettuce in CS, significant differences were detected between the control and treatment with Se. The control showed a higher Zn content compared to the Se treatment. The Se treatment significantly increased the Zn concentration versus the control in the dry weight of the lamb's lettuce tissue in the vermicompost growing media, while no significant differences were found in the Zn concentration in the dry weight of lamb's lettuce grown in 1:1 mixture.

Table 5. Mean values ± standard deviations of the Zn concentration in the fresh-weight (FW) and dry-weight (DW) lamb's lettuce tissue. The different letters represent the significance of the differences at the $\alpha = 0.05$ level.

Treatment	Growing Media	Zn FW (mg/kg)	Zn DW (mg/kg)
Control	CS	14.51 ± 0.910^{a}	170.85 ± 10.65^{a}
Se	CS	11.86 ± 0.064^{b}	149.95 ± 1.05^{b}
Control	Mixture 1:1	9.49 ± 2.462^{cd}	104.55 ± 0.15^{c}
Se	Mixture 1:1	6.83 ± 1.396^{e}	97.67 ± 5.72^{cd}
Control	Vermicompost	9.10 ± 0.635^{d}	88.245 ± 4.68^{d}
Se	Vermicompost	11.70 ± 0.379^{bc}	101.81 ± 6.39^{c}
LSD 0.05		2.226	10.52

4. Discussion

The aim of this study was to investigate the efficiency of Se biofortification in three different growing media: CS (commercial supstrate), vermicompost, and a mixture of the two in a 1:1 ratio. CS had the highest organic matter content, but also the widest C:N ratio (Table 1). The C:N ratio should be between 1:20 and 1:30, which is considered the most favorable C:N ratio because it does not lead to nitrogen depression, so the CS showed the least amount of N. Vermicompost had the optimal C:N ratio of 23.3:1 and the highest amount of N, although it showed the lowest content of organic matter. Electrical conductivity (EC) can serve as a measure of soluble nutrients—both cations and anions [23]—and due to that it would mean that vermicompost is the richest in nutrients, and CS the poorest. The lower EC could result in a lower cations content in the soil solution [23]. The 1:1 mixture had values between the vermicompost and the CS, and thus some properties of both were improved. The reason for the higher nutrient levels in the vermicompost might be the fact that earthworms enhance organic matter degradation [24].

Vermicompost and CS showed values for the trace elements and heavy metal concentrations in reverse order (Table 2). The maximum tolerable levels of the elements in unpolluted soils according to the WHO are: Cd 0.8 mg/kg, Zn 50 mg/kg, Cu 36 mg/kg, Cr 100 mg/kg, Pb 85 mg/kg, and Ni 35 mg/kg [25]. It was found that CS had the highest determined concentrations of Cd, Se and Pb, and the lowest amounts of Zn, Cu, Mo, Ni, Cr, Hg and As compared to the other two substrates. Vermicompost showed the highest concentrations for Zn, Cu, Mo, Ni, Cr, Hg and As, and on the other hand, the lowest concentrations of Cd, Se and Pb. Pb and Cd pollution in acidic soils yields a higher environmental risk, and suggests that efforts to increase the soil pH will effectively decrease both the Cd and Pb accumulation in plants grown in polluted acidic soils [26]. Salinity increases the heavy metal mobilization in soils. The extent of the mobilization depends on the type of heavy metal present, the total amount of heavy metal present, and the type of salt causing the salinization. This means that all of these factors must be explicitly taken into account when assessing the risk of salinization on heavy metal release from the soil [27]. Very low transfers of heavy metals to plant tissues occur at a high pH [28], which is good or bad for certain heavy metals. The term "heavy metals" refers to naturally occurring elements that have a high atomic weight, with a density greater than 4 g/cm^3 [29].

The yield of the lamb's lettuce differed according to the growing media (Figure 1). The highest yield was achieved in CS and the 1:1 mixture, without significant differences between the treatments with Se. With arising ecological issues linked to solid waste management [1] and the exploitation of limited peat reserves presenting a threat for natural habitats [3], this type of mixture might represent a good means of alleviation for these issues. Furthermore, peat substrates show poorer properties compared to vermicomposts [2], and vermicomposts might be per se even better optimized for growing other commercial horticultural plants. However, even for growing lamb's lettuce, a 1:1 mixture can be used as an ecologically safe replacement for a commercial substrate with similar growing performance. Vermicompost showed a significantly lower yield compared to the other two growing media, and significant differences were also found in the treatments with and without Se in the vermicompost. Studies have shown that a high pH (7–10) or a high EC reduced rice [30] and strawberry [31] growth and biomass. On the other hand, the growth of geranium and calendula was better in all vermicompost-based growing media than compost-based growing media, despite the high pH of 7.3 [24]. A high solution pH increased the deficiency of nutrients, particularly Fe and P, and the associated chlorosis and loss of market appeal in leafy vegetables and yield [32]. In our study, high pH values were determined, and the Se treatment (Na_2SeO_4) probably increased the alkalinity even more, and caused a negative effect on the lamb's lettuce yield. The Se biofortification was efficient, and the plants treated with Se had about 170 times higher Se concentrations compared to the control in the dry (177.45 times higher) and fresh (172.86 times higher) weight of the lamb's lettuce (Table 5). A mass of 48.9 g of fresh biofortified lamb's lettuce leaves from our experiment contained enough Se for the recommended daily intake in a human diet (55 µg Se/day). In total, 400 µg Se/day is considered a safe upper limit, and a high dose of Se supplementation showed that intakes up to 3200 µg Se/day gave no obvious Se-related serious toxicities in men [33]. Our results on the efficiency of Se biofortification are in accordance with other research on lettuce [7,9], radish [34], carrot [35] and lentils [36]. Lamb's lettuce grown in vermicompost showed, significantly, the highest Se concentrations in its fresh weight, although there were no significant differences between the growing media and Se concentration in the dry weight of the lamb's lettuce. The yield of the lamb's lettuce was the lowest in vermicompost (Figure 1), such that the Se was more concentrated in the leaf with regard to its fresh weight (Table 5). Several studies have shown that an increase in the soil pH increases the plant's Se uptake [37]. In our study, the Se biofortification was performed with sodium selenite; this could be the reason for the Se accumulation in the lamb's lettuce, because selenate (SeO_4^{2-}) is the predominant Se species in near-neutral pH environments under aerobic conditions, whereas selenite (SeO_3^{2-}) predominates at lower pH and redox potentials [37]. Although there was no significant

difference detected in the concentration of Se in the dry leaf mass of lamb's lettuce, it can be seen that the different substrates produced different concentrations of Se in the leaf. It can be concluded that the rate of Se uptake depends on the concentration and chemical form of the Se in the soil solution, as well as the rhizosphere conditions, such as the pH and the presence of sulfate and phosphate, which alter Se form and compete with Se uptake [38]. In our research, an interesting phenomenon was noticed. In Table 5 it can be seen that Se biofortification had the influence on Zn uptake. CS and 1:1 mixture had a higher Zn content in the control, while contrarily, the Se biofortification decreased the Zn concentration in the fresh weight and dry weight of the lamb's lettuce. The opposite was noted in the vermicompost, in which the addition of Se increased the Zn levels in the fresh and dry weight of the lamb's lettuce leaves, while in the control, the Zn content was lower. It is known that Zn acts synergistically on Se uptake by the roots, and for accumulation in the leaves [39], which is in accordance with our results in vermicompost; therefore, Ei et al. [40] conducted a piece of research in which Se increased more than the Zn accumulation under the combined Se–Zn application. From our research, it can be concluded that, in CS and the 1:1 mixture, Zn and Se had an antagonistic relationship, and in the vermicompost they had synergistic relationship. Zn is an essential element in plants, and is a necessary co-factor of six classes of enzymes in plants, which include oxidoreductases, so these observations suggest that Zn might exert an Se detoxification effect at high Se doses [41]. Our results in CS and the 1:1 mixture are in line with the research form Mangueze et al. [42], which found that Se soil application had an antagonistic effect on Zn in rice grains. It is well-established that the metal transfer from soil-root-shoot/grains is controlled by element speciation, as well as various detoxification/tolerance mechanisms functioning in place inside plants [43].

5. Conclusions

The Se biofortification was successful in all three assessed growing media (CS, vermicompost, and a 1:1 mixture). CS and the 1:1 mixture showed no significant differences in the yield and Se concentration in the fresh and dry lamb's lettuce. On the financial side, 100 kg commercial substrate costs approximately 36 euros. According to our results, the commercial substrate–vermicompost mixture in the 50:50 ratio exerted a similar performance to the commercial substrate in the cultivation of lamb's lettuce. The savings in this scenario would be as much as 50%, or 18 euros per 100 kg, because in our case the vermicompost is produced from green waste from public areas. The use of Vermicompost per se resulted in reduced yields, but the lamb's lettuce grown in the vermicompost showed the highest concentrations of Se, probably due to the high pH value and the use of Se in the form of sodium selenate. In our study, it was observed that Se enhanced the Zn uptake in vermicompost, although Zn was not amended, while in the other two growing media, the Se treatment reduced Zn uptake by lamb's lettuce. Due to its positive performance, we propose vermicompost as an additive to commercial substrates in a 50:50 ratio, thus reducing the use of commercial substrates by 50% due to the increasing lack of peat, while maintaining the growing performance. Due to the extreme importance of Se for the human population, which generates more and more waste and seeks a solution, biofortification and the application of vermicompost are proposed as two potential solutions to address the two uprising issues.

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Naslova rada: Comparative Selenium Biofortification in Lamb's Lettuce and Amaranth in Hydroponic System: Conventional vs. Nanobiotechnological Approaches

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Comparative Selenium Biofortification in Lamb's Lettuce and Amaranth in Hydroponic System: Conventional vs. Nanobiotechnological Approaches

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Abstract

Climate change is a pressing global challenge reshaping ecosystems and agriculture, driving the adoption of alternative food production methods. This is particularly important from the perspective of food composition and food quality. This study investigates the impact of conventional and nanoselenium biofortification on selenium levels, yields and phycological response of baby leaf vegetables in a soilless cultivation system. Selenium biofortification in lamb's lettuce (C3 type) and amaranth (C4 type) plants were performed using sodium selenate (SE200 and SE400) as conventional approach and two different nanoselenium forms, selenium nanoparticles (SeNP) functionalized with polysorbate (PS200 and PS400) and with humic acid (HA200 and HA400). Significant variations in the morphological features of both plant species were observed depending on the substance used for biofortification. Lamb's lettuce exhibits enhanced growth with polysorbate-based SeNP, leading to higher fresh leaf mass and the number of leaves per plant. Amaranth displayed a complex response, with some treatments resulting in suboptimal growth and morphological characteristics. SE400 yielded the highest selenium concentrations in the leaves for both plant species, as expected, with the control group exhibiting the lowest selenium levels. Furthermore, the graph underscores that amaranth assimilates selenium in nanoparticle form (HA200, HA400, PS200, and PS400) with less efficiency than lamb's lettuce, leading to lamb's lettuce demonstrating roughly double the selenium concentrations compared to amaranth. These discoveries bear significant relevance in elucidating daily selenium intake through dietary consumption. The simultaneous application of selenate and SeNP is proposed as a potential solution for optimizing selenium biofortification in different plant species.

1. Introduction

Selenium is an essential micronutrient that is crucial for the proper functioning of humans, animals, and certain microorganisms, primarily serving as a structural component of selenoproteins [1]. As a structural component of vital enzymes and proteins, it plays a significant role in various aspects of human well-being, such as immune responses and the prevention of cancer[2]. Selenium is involved in a range of metabolic processes, including thyroid hormone metabolism, antioxidant defense, and immune function and acts as an antioxidant, potentially exerting a positive influence on plant metabolism and delaying plant senescence by reducing ethylene production[3]. It is covalently integrated into amino acids, primarily selenocysteine (SeCys) and selenomethionine (SeMet), serving as a cofactor for antioxidant enzymes like glutathione peroxidase when present in these chemical forms [4]. An estimated 0.5 to 1 billion individuals are affected by selenium deficiency, primarily due to selenium intake falling below the recommended dietary allowance (RDA) of 50–70 µg Se per day [5]. Biofortification of cereals with selenium has been already suggested as approach for reducing the health problems. Selenium concentrations in soils worldwide are reported to be very low [6], leading to its deficiency in both human and animal diets [7]. To address this deficiency or issue, biofortification has proven to be a valuable tool in agronomic practices, where selenium is most commonly

applied in the form of sodium selenate (Na_2SeO_4) to the soil or directly through foliar application to the plant [8,9,10–13]. Through this process, selenium is conveyed to the consumable plant parts via accessing the metabolic pathway of its analog sulphur[14]. Biofortification programs involving lettuce showed that the use of selenate at low concentrations could be more advantageous as it promotes shoot biomass growth, selenium translocation, and selenium levels in the shoot biomass [15]. Lately, the advent of state-of-the-art technologies has indicated the utilization of selenium in the form of selenium nanoparticles (SeNP) as a substitute for conventional selenium fertilizers to augment the Se-organic compound levels in crops [16–18]. Nanoselenium has garnered increased attention, owing to its superior bioavailability and reduced toxicity compared to inorganic and organic forms [19]. There are various approaches for the synthesis of SeNP, such as physical, chemical, and biological available in the literature [20]. Recent study determined that foliar supplementation with SeNP played a significant role in mitigating the detrimental effects of salt stress on various aspects of *Phaseolus vulgaris* plant growth, including physiology, biochemistry, and green yield. Thus, foliar feeding with SeNP can be suggested as a noteworthy strategic approach for enhancing the growth and productivity of *Phaseolus vulgaris* plants under soil salinity stress in open field conditions [21]. Similar study on *Hordeum vulgare* seedsrecommended the use of SeNP as a growth and development stimulant for agricultural seeds under stressful conditions, with improvements in morphofunctional characteristics [22]. It has been also demonstrated that Fusarium oxysporum is capable of producing selenium-based nanoparticles through a safe and cost-effective aerobic green approach[23]. Given that the global human population is projected to reach approximately 9 billion by the year 2050, it becomes evident that food security is one of the key issues of the new millennium and, arguably, the most pressing challenge for the agricultural sector where climate change exacerbates their challenges[24,25]. Natural resources alter the agronomic approach towards novel production methods to reduce the consumption of natural resources. In greenhouse production, hydroponic systems offer a viable alternative to soil, enabling crop cultivation in diverse environments where traditional agriculture is unfeasible[26]. The floating system is a soilless cultivation technique where plants are grown on alveolar or crack-style polystyrene sheets floating in containers containing a nutrient solution[27]. The floating system represents one of the simplest cultivation systems [28]. The cultivation of plant crops in these systems is garnering greater interest, particularly in the production of "baby leaf" vegetables and this growing demand is fueled by consumers seeking prepackaged salads, which require top-quality ingredients and stringent hygiene standards [29]. Another advantage of soilless hydroponic systems is that it eliminates the need for soil fumigation and reduces soil-borne diseases, which can increase the safety of packaged vegetables by minimizing chemical residue problems [30]. In hydroponic cultivation, various research studies are conducted concerning the solution temperature [31] and various recipes for nutrient solutions [25,32]. Leafy greens are essential in the economies of many countries, especially in the Mediterranean and Northern European regions, where they're commonly used in ready-made salads [33]. Soilless cultivation is advised for salad production due to better control over mineral nutrients, resulting in higher-quality freshly harvested produce [34]. Precisely managing nutrient solutions, including factors like concentration, chemical forms, temperature, and pH, is vital for successful leafy vegetable production. Soilless cultivation offers a valuable agricultural practice and improved yields for leafy greens [32,35,36]. Due to climate change, the problem of world hunger, and changes in crop profiles in European and other countries around the world, it is becoming desirable to look for new plants with a high nutritional potential that can be combined with health benefits [37]. Lamb's lettuce is gaining prominence in Europe as a convenient ready-to-eat leafy salad, often featured in "ready to eat salad mixtures" [38]. In response to high-calorie diets, new food processing technologies are emerging, especially in pre-packaged salad production [39,40]. Furthermore, studies such as those by Hawrylak-Nowak et al. (2018) and Puccinelli et al. (2021) confirm the success of selenium biofortification in lamb's lettuce [1,3]. In our recent research, we efficiently conducted selenium biofortification of lamb's lettuce using a substrate-based cultivation method[41]. Lamb's lettuce is a highly nutritious functional food, rich in carotenoids, phenolic compounds,

folic acid, sterols, and fatty acids. Its cultivation, including the use of hydroponic techniques, is widespread [35,42–44]. Another noteworthy and valuable "baby leaf" is Amaranth, an important nutritional crop. This unique plant is consumed for its leaves as a vegetable and for its seeds as a cereal [45]. Amaranth stems and leaves offer affordable and plentiful dietary fiber, protein with essential amino acids, vitamins, carotenoids, minerals, and various antioxidants and phytochemicals, like betacyanin, anthocyanin, carotenoids, and ascorbic acid [4647]. It has a bility to adjust to environmental variations is one of the reason for its successful introduction and rapid distribution [4849]. Amaranth leaves are very tasty and have nutritional quality as high as grains. They can be eaten either fresh in salads, or cooked in the same way as spinach [49]. In the study by Mala et al. (2017), successful biofortification of amaranth was achieved [50]. In the research conducted by Munandar et al. (2019), the biofortification of iodine concentration in Amaranth leaves, grown through hydroponic techniques, also achieved successful outcomes [51]. Lately, there have been several studies classifying amaranth as functional food due to its higher concentrations of phytoactive compounds that offer benefits to human health [37,46,47,49,51]. Given the pressing issues of climate change, a growing global population, and the ever-increasing need to produce high quality food while ensuring human health, this study aimed to investigate the effectiveness of conventional versus nanoselenium biofortification in a soilless cultivation system by means of multielemental analysis, plant yielad, morphological, physiological and biochemical parameters of baby leaf vegetables (specifically lamb's lettuce and amaranth).

2. Material and Methods

2.1. Study Area

The research was conducted at the Faculty of Agrobiotechnical Sciences Osijek in Livana, Eastern Croatia (45°32' north latitude, 18°44' east longitude), within a greenhouse. The greenhouse has a total area of 120 m^2 and is covered with a high-quality ethylene-vinyl-acetate film that provides UV stability. It has a working height of 2.60 m and features automated side ventilation openings on both sides, operated by an electric motor connected to a temperature and humidity sensor through a control unit. The greenhouse is classified as a heated protected area and is equipped with an independent oil-fired heating unit that uses heating oil as fuel. Throughout the experiment, the greenhouse temperature fluctuated between 19.5 and 21°C during the daytime and between 14.5 and 16°C during the nighttime.

2.2. Experimental design

The pools had dimensions of 80 x 120 cm and a depth of 30 cm, with a nutrient solution depth of 20 cm, resulting in a total volume of 200 liters of nutrient solution per pool. An air pump was used in conjunction with the pools. The nutrient solution for the floating hydroponic system was prepared following the Hoagland's recipe, with the concentration of all essential elements reduced by 50% as recommended for leafy vegetable cultivation (Supl. Table 1). In the experiment, seeds of lamb’s lettuce (*Valerianella locusta*) from the Dutch producer RIJK ZWAAN were used, with the STYLUS RZ variety. Amaranth (*Amaranthus caudatus*) was purchased from REIN SAAT (Austria). Salts from Gram-mol d.o.o. (Croatia), TTT d.o.o. (Croatia), or Haifa Chemicals (Haifa, Israel) were used to prepare the base nutrient solution. The vegetation experiment was designed using a completely randomized design with 4 biological replicates. Each biological replicate consisted of 20 plants, resulting in a multifactorial experiment. Three seeds of lamb's lettuce and amaranth were planted in each sowing spot, and after germination, thinning was performed to retain two plants per spot. This resulted in a total of 80 plants per container in 2 containers, amounting to 160 plants per experimental variant. Polystyrene containers were pre-filled with a 1:1 volume ratio mixture of vermiculite and commercial substrate. Amaranth sprouted 3-4 days after sowing, with a total germination period of 6-7 days. Lamb's lettuce started to sprout 5-6 days after sowing, and germination continued until the 10th day after sowing. The containers with plants were transferred to the floating hydroponic system on

the 16th day after sowing. Throughout the experiment, the greenhouse maintained a temperature range of 19.5-22 °C during the day and 14.5-16 °C at night.

2.3. Synthesis and characterization of SeNPs

2.3.1. Chemicals and Reagents

Sodium selenite (Na_2SeO_3), polysorbate 20 (PS) and humic acid (HA) were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany), while L-(+)-ascorbic acid was obtained from Alfa Aesar (Kandel, Germany). All chemicals were utilized without additional purification, while all glassware was subjected to cleaning with 10% (v/v) HNO_3 (Merck Suprapur, Darmstadt, Germany), followed by rinsing with ultrapure water (UPW) prior to usage.

2.3.2. Synthesis of SeNPs

PS-functionalized SeNPs (PS-SeNPs) were prepared following the slightly modified procedure outlined by Vahdati et al.[52] Briefly, 1.2 mM of 90 mL sodium selenite (Na_2SeO_3) was introduced into the round-bottom flask. Then, 10 μL of the PS solution was added after each 2 mL of 56.7 mM L-ascorbic acid to the final volume of 100 mL. The mixture was stirred at 400 rpm for 20 minutes when adopted clear orange color. The synthesized PS-SeNPs underwent centrifugation for 20 minutes at 12 000 g twice. The pellet was subsequently re-suspended in UPW and kept at a temperature of 4 °C.

The synthesis of HA-stabilized SeNPs (HA-SeNPs) was carried out using already published procedures with some alterations.[52–54] First, 10 mL of 1 mM of sodium selenite (Na_2SeO_3) was added to a flask along with 10 mL of humic acid (0.001 %). Subsequently, 10 mL of 0.01 M L-ascorbic acid (0.01 M) was added dropwise under constant stirring. The reaction was allowed to proceed for 20 minutes, after which the mixture was centrifuged for 15 minutes at 15 000 g. The resulting pellet was re-suspended in UPW and stored at 4 °C

2.3.3. Characterization of SeNPs

Dynamic light scattering (DLS) and electrophoretic light scattering (ELS) methods were employed in order to determine size and surface charge of SeNPs. Hydrodynamic diameter (d_H) was obtained by DLS measurements as an average result from six consecutive measurements, while zeta (ζ) potential values were determined using the ELS method, as an average result from three measurements. The size-volume distribution function was employed to present the outcomes of the DLS analysis whereas for the calculation of ζ potential the Henry equation, employing the Smoluchowski approximation, was used. The data analysis utilized ZS Xplorer Zetasizer software from Malvern Instruments, Malvern, UK. Both DLS and ELS experiments were performed using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) equipped with a 532nm laser, and scattered light intensity was measured at an angle of 173°. Additionally, visualization of SeNPs was carried out through transmission electron microscopy (TEM). TEM images were captured using a JEOL JEM 1010 transmission electron microscope (JEOL, Tokyo, Japan) operating at 80 kV. SeNPs were resuspended in ultrapure water (UPW) at a final concentration of 10 mg Se/L and deposited onto Formvar®-coated carbon grids in a dropwise manner. The ImageJ processing software (LOCI, University of Wisconsin, Madison, WI) was utilized for the analysis of TEM images to determine the primary diameter (d_{TEM}) of particles. A minimum of 30 particles per type of SeNPs were included in the analysis.

2.4. Chemical Analysis of Plant Material

2.4.1. Preparation of Plant Material Samples

Root and leaf samples were dried in a drying oven (Kambic®) at 105 °C for 1 hour to halt enzymatic activity in the plant tissue. Subsequently, drying was continued at 70 °C until a constant weight was achieved. After drying, the samples were ground using a mill (Retsch GM200 - heavy metal free technology) and assigned a laboratory number.

2.4.2. Determination of Nitrogen Concentration

The determination of nitrogen concentration was carried out after the destruction of plant material on a digestion block (Kjeldahl - Büchii B-324) using a mixture of acids consisting of 96% concentrated sulfuric acid (LabExpert) and 4% perchloric acid (LabExpert) with the addition of 30% hydrogen peroxide (Fisher). Samples of 1 gram of dry plant material were transferred to a digestion flask and covered with 5 ml of the mixture of sulfuric and perchloric acids, along with 2 ml of hydrogen peroxide. After the plant material absorbed the acid mixture, a total of 10 ml of hydrogen peroxide was gradually added using a pipette. The samples were heated on the digestion block at a temperature up to 360 °C until the acid mixture solution became completely clear. The cooled solution was cautiously diluted by adding approximately 50 ml of deionized water and then filtered through filter paper into 100 ml measuring flasks. Nitrogen distillation was performed by displacing ammonia from the sample solution (with a 40% sodium hydroxide solution) into a receiver containing a known volume of 0.01 mol dm-3 sulfuric acid. The neutralization of part of the sulfuric acid with distilled ammonia in the receiver resulted in ammonium sulfate. The amount of ammonium sulfate formed was equivalent to the amount of nitrogen present in the plant material sample. The excess sulfuric acid remaining in the receiver was determined by titration with 0.02 mol dm-3 sodium hydroxide solution. The nitrogen concentration was expressed as a percentage of nitrogen in the dry matter of the analyzed plants [55].

2.4.3. Determination of Ca, Mg, K, P, S, Cu, Fe, and Mn Concentration

The determination of Ca, Mg, K, P, S, Cu, Fe, Mn, and Se concentrations in the plant material sample was based on the use of a sample basic solution obtained through acid digestion. The plant material samples were digested using a wet procedure in a microwave oven (CEM MARS 6). In a Teflon vessel, 0.5 g of dry plant material sample was weighed using an analytical balance (Kern®) and covered with 6 ml of 65% HNO3 (Fisher) and 2 ml of 30% H2O2 (Fisher). After the digestion process, the cooled solution was quantitatively transferred to 50 ml centrifuge tubes (Sarstedt®) and filled to the mark with distilled water. The concentrations of macro and microelements were directly measured from the diluted sample digestate using the ICP-OES technique (PerkinElmer Optima 2100 DV) and expressed in mg kg-1 of plant material (Supl. Table 2 and Table 3).

2.4.4. Determination of Selenium Concentration

After microwave digestion, the concentrations of selenium in the digested plant material solution were determined using the ICP-MS technique (Agilent 7500 Series). Two reference materials were used: BCR129 (Hay Powder, European Commission) and 1567b (Wheat Flour, Standard Reference Material, National Institute of Standards and Technology), which were prepared in the same manner as the plant samples. The concentration of Se in the dried leaf samples was also used to calculate the total Se content in fresh leaf matter by calculating the percentage of dry matter in the leaf and its water content. The total selenium content in fresh leaves was obtained using the following formula:

Total selenium content in fresh matter = Se mg kg^{-1} dry matter x (1 - (H_2O %/100))

The total selenium content in fresh leaves was calculated to assess the success of biofortification in optimizing the system and accurately representing selenium intake in the human body through the consumption of 200 g of fresh arugula or spinach leaves.

2.5. Physiological and Biochemical Parameters Determination in Lamb's lettuce and Amaranth Leaves

2.5.1. Photosynthetic Pigment Concentration

The concentration of chlorophyll is determined using the method described by Lichtenthaler (1987) [56]. It involves extracting pigments from finely ground plant tissue (0.1 g) by adding 1 ml of 80% acetone. After extraction, the homogenates are centrifuged for 15 minutes at 22,000 x g and at a temperature of +4 °C. The supernatant is decanted into test tubes, and the extraction process is repeated several times until the precipitate becomes colorless, with the obtained extracts being pooled. In the pooled diluted extracts, photosynthetic pigments are determined by spectrophotometric measurement of absorbance at three wavelengths: 470 nm, 645 nm, and 662 nm. The results are expressed in mg of chlorophyll or carotenoids per gram of fresh weight (mg/g FW).

2.5.2. Total Phenolic Content

The total phenolic content in the ethanol extracts of arugula and spinach leaves is determined spectrophotometrically using the Folin-Ciocalteu method [57]. This method is based on the reaction of Folin-Ciocalteu reagent (a complex of phosphomolybdic-phosphotungstic acid) with a reducing agent (phenolic compound), resulting in the formation of a blue color. To perform the analysis, 20 µl of ethanol extract, 1.58 ml of distilled water, and 100 µl of Folin-Ciocalteu reagent are added to a reaction mixture and mixed on a rotary shaker. After a waiting period of at least 30 seconds but not exceeding 8 minutes, 300 µl of saturated Na_2CO_3 solution is added to the samples, followed by thorough vortexing. The samples are then incubated in a water bath at 37 °C for 1 hour. The absorbance of the prepared samples is measured at 765 nm, and the content of total phenols in the ethanol extracts is calculated using a calibration curve with gallic acid as the standard.

2.5.3. Ascorbic Acid (AA) Concentration

The concentration of ascorbic acid is determined using a spectrophotometric method described by Benderitter et al. (1998) [58]. Ascorbic acid is measured in freshly prepared aqueous extracts of plant tissue. To perform the analysis, finely ground tissue (0.2 g) is mixed with 1 ml of distilled water. After homogenization using a vibrating mixer, the homogenates are centrifuged for 5 minutes at 6,000 g at a temperature of +4 °C. To the resulting supernatant (300 µl), 100 µl of 13% trichloroacetic acid (TCA) solution, 25 µl of distilled water, and 75 µl of 2,4-dinitrophenylhydrazine (DNPH) reagent are added. The reaction mixture is then incubated for 60 minutes in a water bath at +37 °C. For each sample, a blank sample is prepared in the same manner, except that the DNPH reagent is added only after the incubation period, to prevent any reaction from occurring. After incubation, 500 µl of 65% sulfuric acid (H_2SO_4) is added to the reaction mixture, followed by thorough mixing on a vibrating mixer. In the blank sample, the DNPH reagent is added along with the H_2SO_4. The absorbance of the resulting solution is measured at a wavelength of 520 nm. The concentration of ascorbic acid is determined using a calibration curve constructed with increasing concentrations of ascorbic acid as standards. The results are expressed in milligrams of ascorbic acid per 100 grams of fresh weight (mg 100 g^−1 fresh weight).

2.5.4. Total Antioxidant Activity

The total antioxidant activity of ethanol extracts of arugula and spinach leaves is determined using the method described by Brand-Williams et al. (1995)[59]. This method is based on the reduction of DPPH· (2,2-diphenyl-1-picrylhydrazyl) radicals. In closed microtubes, 50 µl of ethanol extract is added and then brought to a volume of 1 ml with a DPPH solution. A blank sample is prepared in the same manner, but pure ethanol is added instead of the sample. The reaction takes place in tightly closed microtubes with

gentle mixing at 20 °C for 15 minutes. The absorbance of the prepared samples is measured at 515 nm, and the total antioxidant activity of the ethanol extracts is calculated using a calibration curve constructed with Trolox as the standard. The results are expressed in Trolox equivalents.

2.5.5. Lipid Peroxidation Products (LPO)

The amount of LPO products is determined by the method described by Verma and Dubey (2003) through the measurement of TBARS (thiobarbituric acid reactive substances), primarily MDA (malondialdehyde)[60]. The tissue sample is extracted with 1 ml of a 0.1% solution of trichloroacetic acid (TCA). After 10 minutes of incubation on ice, the homogenates are centrifuged for 5 minutes at 6,000 g at a temperature of +4 °C. To the obtained supernatant (0.5 ml), 1 ml of a 0.5% solution of thiobarbituric acid in a 20% TCA solution is added. The reaction mixture is mixed on a vibrational shaker and then incubated for 30 minutes in a water bath at +95 °C, causing the breakdown of lipid peroxides and the formation of products (mostly MDA) that react with thiobarbituric acid. After the incubation period, the reaction is stopped by holding it on ice for 10 minutes, followed by centrifugation of the reaction mixture for 10 minutes at 22,000 g at a temperature of +4 °C. The absorbance of the resulting supernatant is measured at wavelengths of 532 nm and 600 nm, with the absorbance at 600 nm subtracted from the absorbance at 532 nm to correct for non-specific reactions. The amount of TBARS, namely MDA, is determined using a standard curve equation, with 1,1,3,3-tetramethoxypropane used as the standard. The results are expressed as nmol per gram of fresh weight (nmol g-1 fresh weight).

2.6. Statistical analysis

Each plant species was analyzed separately. Means over technical replicates (biological replicates) were used as input for the analysis of variance (ANOVA). Analysis of variance followed the factorial design of experiments according to general formula $y_{ij} = T_i + P_j + TP_{ij} + \varepsilon$ where T_i is a Se treatment i, P_j is a plant part j and TP_{ij} is an interaction term. Contrarily, analysis of biochemical traits was limited to leaves and thus ANOVA followed reduced model $y_{ij} = T_i + \varepsilon$. Pearson's product-moment correlations were calculated between biochemical traits and Se concentrations in different plant parts.

Further, principal component analysis (PCA) was conducted with means of biological replicates of morphological traits and Se concentrations in different plant parts. Analysis was focused on first two components explaining the largest amount of variance. All analyses were carried out in R [61].

3. Results

3.1. Physico-chemical characteristics of SeNPs

The HA- and PS-SeNPs were synthesized through a reduction method, employing L-ascorbic acid as a reducing agent and HA as well as PS for surface stabilization. The general principle of nanoparticles synthesis comprises three phases: reduction, nucleation, and growths. In the nucleation phase, selenium ions (Se^{4+}) are reduced to the elemental form Se (0), which then forms nuclei. These nuclei serve as the foundation for further NP growth. Surface agents, in our case HA and PS, then bind to and stabilize the SeNPs through electrostatic interactions or sterically, respectively[62]. TEM, DLS and ELS techniques were used to obtain d_{TEM}, d_{H} and ζ potential values of SeNPs, respectively, as presented in Table 1. Negative ζ potential for both types of SeNPs suggests their good colloidal stability whereas one population observed by DLS refers to monodispersed systems[63]. TEM micrographs (Figure 1) showed that both SeNPs were spherical in size, while their primary size were found smaller than hydrodynamic diameters as DLS monitor the behavior of NPs within the medium, encompassing not only NP size but also the presence of a hydration shell on their surface[64].

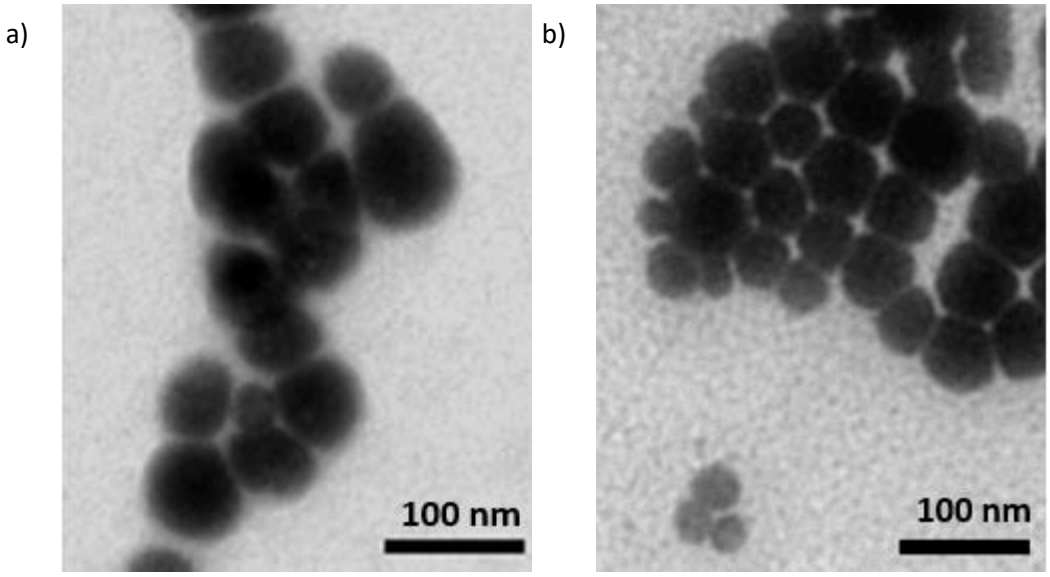

Figure 1. TEM micrographs of a) HA-SeNPs and b) PS-SeNPs

Table 1. Physico-chemical characteristics of selenium nanoparticles functionalized with humic acid (HA-SeNPs), and polysorbate 20 (PS-SeNPs). The primary diameter (*d*, nm) was determined by TEM, hydrodynamic diameters (d_H, nm) in ultrapure water (UPW) was obtained by DLS and ζ potential (mV) was measured using the ELS method. All measurements were done at 25 °C and SeNPs concentration of 100 mg Se/L.

SeNPs type	d_{TEM}/nm	d_H/nm	ζ/mV
HA-SeNPs	52.4 ± 7.6	64.1 ± 2.3	-31.2 ± 0.6
PS-SeNPs	39.6 ± 8.1	53.7 ± 0.4	-29.1 ± 2.4

3.2. Plant morphological properties and after selenium biofortification

Selenium was applied in three different forms, as sodium selenate (Na_2SeO_4) at concentration of 200 µmol Se/m^3 (SE200) and 400 µmol Se/m^3 (SE400) and SeNPs stabilized with HA (HA200 = 200 µmol Se/m^3, HA400 = 400 µmol Se/m^3) or PS (PS200 = 200 µmol Se/m^3 and PS400 = 400 µmol Se/m^3).

Significant variations in the morphological features of lamb's lettuce plants under different selenium biofortification treatments were observed. In the absence of selenium supplementation (control group), lamb's lettuce plants exhibited statistically significant differences in their morphological characteristics, as depicted in Figure 2. Specifically, the control group exhibited the least statistically significant values for all assessed morphological traits, except for Fresh mass per plant (1.47 g) and Root length (13 cm). Among the various selenium treatments, SE200 (administered at 200 µmol Se/m3 sodium selenate) yielded the lowest selenium concentrations in lamb's lettuce plants while simultaneously displaying the highest fresh root mass (1.34 g). Interestingly, HA400 exhibited a superior fresh plant mass yield, while both humic acid treatments (HA200 and HA400) resulted in identical dry matter content within the lamb's lettuce plants (rosettes). Notably, the most pronounced differences and distinctive morphological characteristics in lamb's

lettuce plants were observed in selenium treatments involving nanoparticles synthesized with polysorbate (PS200 and PS400). These treatments featured the longest lamb's lettuce roots and the highest number of leaves per plant, ultimately resulting in superior fresh and dry matter yields in the edible portion of the plant (rosettes).

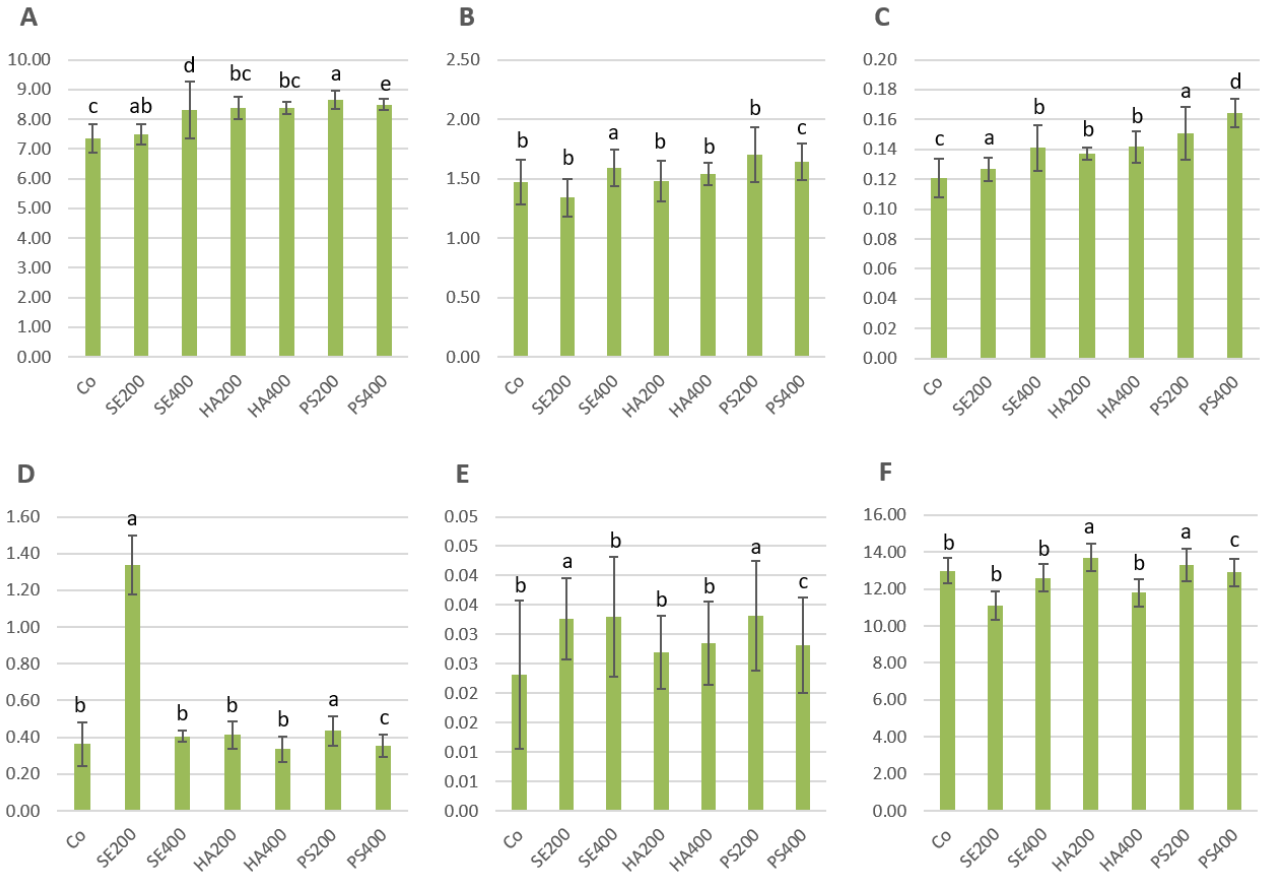

Figure 2. Mean values ± standard deviations of morphology structure in lamb's lettuce plants after six different treatments of selenium biofortification. Leaves per plant (**A**), FW (fresh weight) per plant (**B**), DW (dry weight) per plant (**C**), root FW per plant (**D**), root DW per plant (**E**), root length (**F**) Different letters represent significance of differences at $\alpha = 0.05$ level.

Our investigation of amaranth plants revealed statistically significant variations among the selenium biofortification treatments (Figure 3). Notably, the treatment involving polysorbate (PS400) exhibited the most modest differences in amaranth plants bearing approximately half the number of leaves compared to other selenium-treated groups. Moreover, the PS400 treatment resulted in fresh and dry leaf masses approximately 3.2 to 5.5 times smaller than those in the other treatments. Morphological attributes in the PS400 treatment also exhibited diminished values, encompassing root characteristics. Of particular interest, the PS200 treatment displayed the highest values for the observed morphological characteristics (excluding fresh leaf mass and fresh root mass). Contrastingly, the PS200 treatment outperformed almost all other treatments, while PS400 displayed the lowest values for the morphological attributes of amaranth. The control group (lacking selenium supplementation), it was evident that these plants exhibited reduced fresh mass in comparison to the other selenium-treated groups (with exceptions being HA400 and PS400). The lowest dry mass was also observed, except, once more, in the PS400 treatment. Intriguingly, the SE400

treatment yielded the highest fresh leaf mass, representing the consumable portion, despite having the fewest leaves (excluding the PS200 treatment). For amaranth plants treated with sodium selenate (SE200 and SE400), statistically significant differences were identified for all observed morphological attributes, with the exception of root length and stem length. In the HA200 and HA400 treatments, similar values were recorded, with no statistically significant differences detected among the observed morphological properties, except for root length.

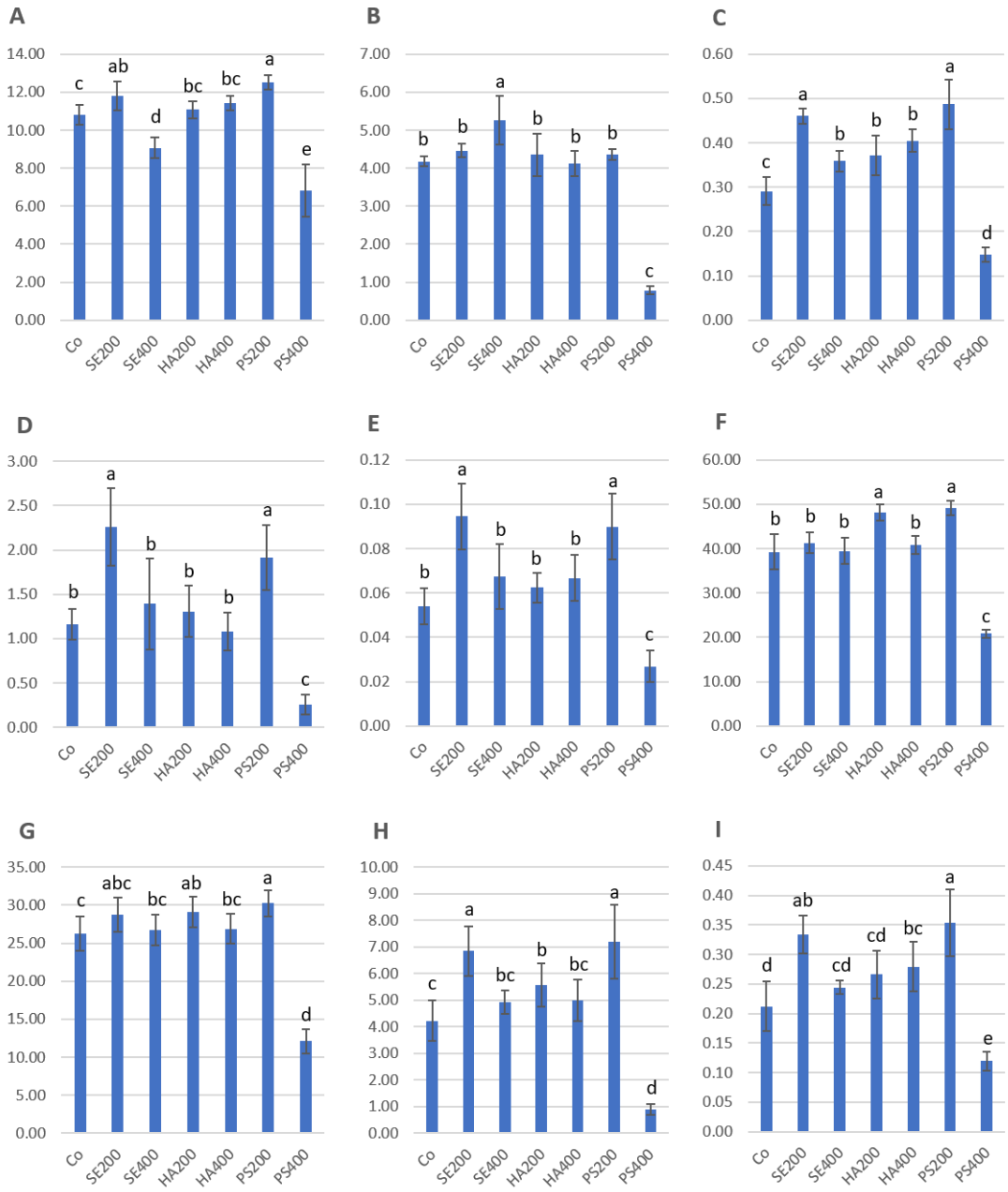

Figure 3. Mean values ± standard deviations of morphology structure in amaranth plants after six different treatments of selenium biofortification. Leaves per plant (**A**), leaves per plant FW (**B**), leaves per plant DW (**C**), root FW per plant (**D**), root DW per plant (**E**), root length (**F**), stem length (**G**), stem FW per plant (**H**) and stem DW per plant (**I**) Different letters represent significance of differences at $\alpha = 0.05$ level.

3.3. Selenium content in plants following biofortification

Figure 4 presents selenium concentrations within the edible portions of lamb's lettuce and amaranth plants. The graph unmistakably demonstrates that SE400 produced the highest selenium concentrations in the leaves for both plant species, while, as anticipated, the control group displayed the lowest selenium levels. Additionally, the graph highlights that amaranth assimilates selenium in nanoparticle form (HA200, HA400, PS200, and PS400) with less efficiency than lamb's lettuce, resulting in lamb's lettuce exhibiting approximately double the selenium concentrations compared to amaranth. These findings hold significant relevance in determining daily selenium intake through dietary consumption. This means, for example, that lamb's lettuce treated with PS400, in a 100 g package, would have 65.9 µg/kg of selenium, and amaranth treated with SE400 would have 85.4 µg/kg per package (100 g) which is for both the recommended daily intake [5].

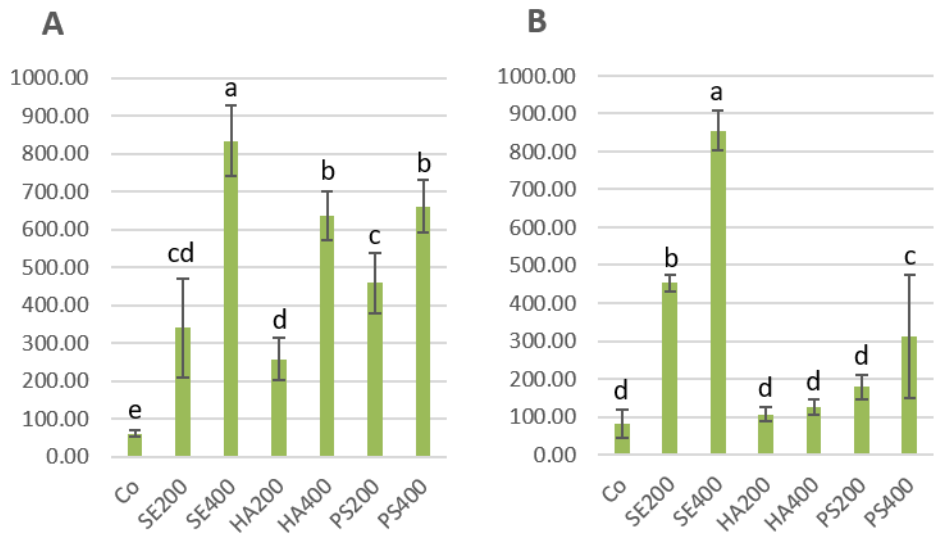

Figure 4. Selenium concentrations (µg/kg) in the edible part of the plant (leaf) per treatment in lamb's lettuce (**A**) and amaranth (**B**)

3.4. Physiological and Biochemical Parameters in plants fortified with different selenium forms

In Table 2, the composition of pigments (Chla a, Chl b, Chl a+b, Car, Chl a/Chl b, Car/Chl a + Chl b) in lamb's lettuce plants is presented. The highest significant values for parsley plants were observed for Chla under the HA200 treatment, while the lowest significant values were recorded for HA400. Furthermore, for Chl b, lamb's lettuce exhibited the highest significant value under the nanoselenium PS400 treatment and the lowest in the control plants. No significant differences were observed for Chl a+b in lamb's lettuce plants, while for Car, the highest values were recorded in plants under the HA200 treatment, and the lowest significant value was associated with HA400.

The Chl a/Chl b ratio revealed the most significant differences in control plants, with the highest value, while the lowest values were observed in treatments HA400, PS200, and PS400. Finally, the Car/Chla+Chlb ratio was significant in both control plants and the HA200 treatment, with the smallest ratio observed in the PS400 treatment in lamb's lettuce plants.

Table 2. Mean values ± standard deviations of pigments content in lamb's lettuce plants after six different treatments of selenium biofortification. The concentrations of pigments are indicated in mg/g of fresh weight. Different letters represent significance of differences at α = 0.05 level.

Treatment	Chl a	Chl b	Chl a+b	Car	Chla/Chlb	Car/Chla + Chl b
Co	0.84±0.03ab	0.27±0.01b	1.11±0.04a	0.24±0.01ab	3.08±0.02a	0.22±0.003a
SE200	0.87±0.04ab	0.30±0.01ab	1.17±0.04a	0.24±0.01ab	2.92±0.10bc	0.21±0.007b
SE400	0.85±0.05ab	0.28±0.02ab	1.14±0.08a	0.24±0.01ab	3.01±0.05ab	0.21±0.001ab
HA200	0.92±0.07a	0.31±0.02ab	1.23±0.09a	0.27±0.01b	3.00±0.04ab	0.22±0.002a
HA400	0.78±0.21b	0.28±0.06b	1.06±0.26a	0.22±0.05a	2.82±0.11c	0.20±0.003bc
PS200	0.87±0.05ab	0.30±0.01ab	1.17±0.06a	0.25±0.01ab	2.86±0.12c	0.21±0.006abc
PS400	0.91±0.04ab	0.32±0.01a	1.23±0.06a	0.25±0.01ab	2.84±0.10c	0.20±0.006c

Figure 5 illustrates the concentrations of total phenols (PHE), ascorbic acid (AA), and lipid peroxidation (LP) in lamb's lettuce plants. The most significant differences in phenol content were observed in control plants and treatments with HA200 and PS400, showing the lowest values, while the highest value was recorded in the SE200 treatment. The content of AA was highest in the control, with the order from the highest to the lowest for the treatments as follows: SE200, SE400, HA200, HA400, PS200, and PS400. Ultimately, based on the presented Graph 5, it can be observed that the concentration of lipid peroxidation did not show significant differences in lamb's lettuce plants.

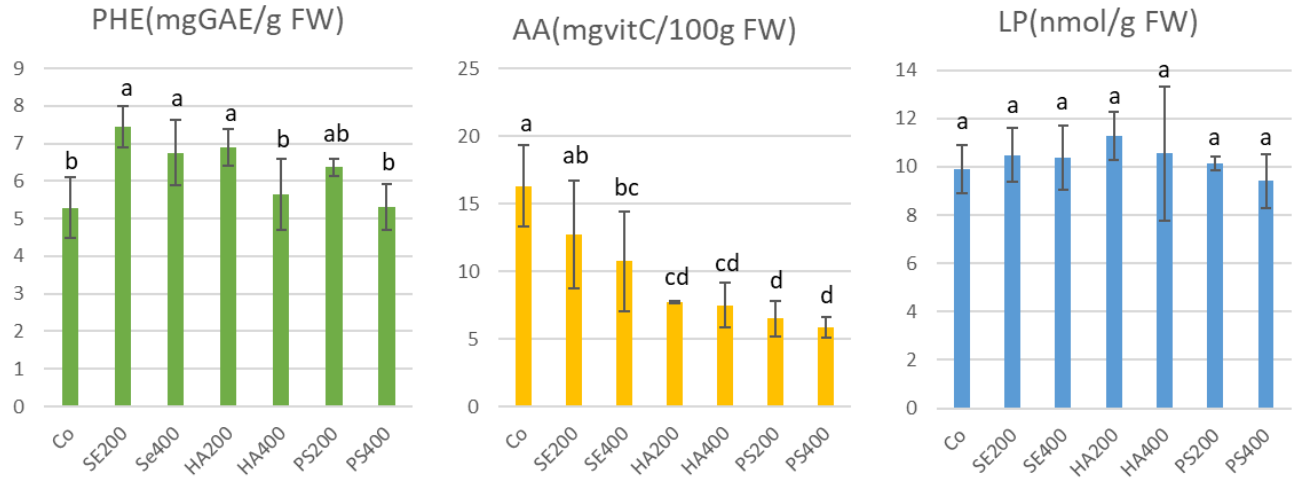

Figure 5. Means and standard deviation of total phenolic content (PHE), ascorbic acid (AA) and lipid peroxidation (LP) in lamb's lettuce plants. Significance of effects is denoted with different letters at α= 0.05 level.

Table 3 presents the composition of pigments (Chla a, Chl b, Chl a+b, Car, Chl a/Chl b, Car/Chl a + Chl b) in amaranth plants. The highest significant values for amaranth plants were observed for Chla under the SE400 treatment, while the lowest significant values were recorded in the control plants. Other treatments for Chla ranged in concentrations from 80 mg/g fresh weight to 90 mg/g fresh weight. Furthermore, for Chl b, the treatment with SE400 exhibited the highest significant value in amaranth, and the lowest was observed in the control plants. The most significant concentration for Chl a+b, with the highest value, was found in the SE400 treatment, while the lowest was in the control plants of amaranth. For Car, the treatment with PS200 had the highest value, and the lowest values were in the control and treatments with HA 200

and HA400. The Chl a/Chl b ratio revealed the most significant differences in the control plants, with the highest value, while the lowest values were associated with the SE400 treatment. Finally, the smallest value for the Car/Chla+Chlb ratio was observed in the SE200 and SE400 treatments, while the highest values were in the PS200 treatment and the control.

Table 3. Mean values ± standard deviations of pigments content in amaranth plants after six different treatments of selenium biofortification. The concentrations of pigments are indicated in mg/g of fresh weight. Different letters represent significance of differences at α = 0.05 level.

Treatment	Chl a	Chl b	Chl a+b	Car	Chla/Chlb	Car/Chla + Chl b
Co	0.77±0.04c	0.18±0.01d	0.95±0.05c	0.22±0.02d	4.36±0.02a	0.23±0.01ab
SE200	0.88±0.01b	0.21±0.01b	1.09±0.02±b	0.23±0.01bcd	4.13±0.33abc	0.21±0.01de
SE400	0.93±0.02a	0.23±0.01a	1.16±0.02a	0.24±0.01abc	3.99±0.32c	0.21±0.01e
HA200	0.80±0.02c	0.20±0.01bc	1.00±0.02c	0.22±0.01cd	4.00±0.03bc	0.22±0.01bcd
HA400	0.81±0.03c	0.19±0.01cd	1.00±0.04c	0.22±0.01bcd	4.26±0.05ab	0.22±0.01abc
PS200	0.89±0.04ab	0.21±0.01b	1.10±0.05b	0.26±0.01a	4.14±0.12abc	0.23±0.01a
PS400	0.90±0.02ab	0.22±0.01b	1.11±0.02ab	0.24±0.01ab	4.18±0.09abc	0.22±0.01cd

Figure 6 shows the concentrations of total phenols (PHE), ascorbic acid (AA), and lipid peroxidation (LP) in amaranth plants. The most significant differences in phenol content were observed in the PS200 treatment, where the highest value was recorded, and the lowest value was in the SE200 treatment. The AA content was highest in PS200, while the lowest value was in SE200. The content of lipid peroxidation varied significantly with selenium treatments, with a notable difference identified in PS200, having the highest value for lipid peroxidation, and the lowest value measured in the SE200 treatment. The HA200 treatment showed a high value for lipid peroxidation with a significant difference compared to other treatments, except in the control plants. Significant differences in amaranth plants for lipid peroxidation were also observed in the SE400, HA400, and PS400 treatments.

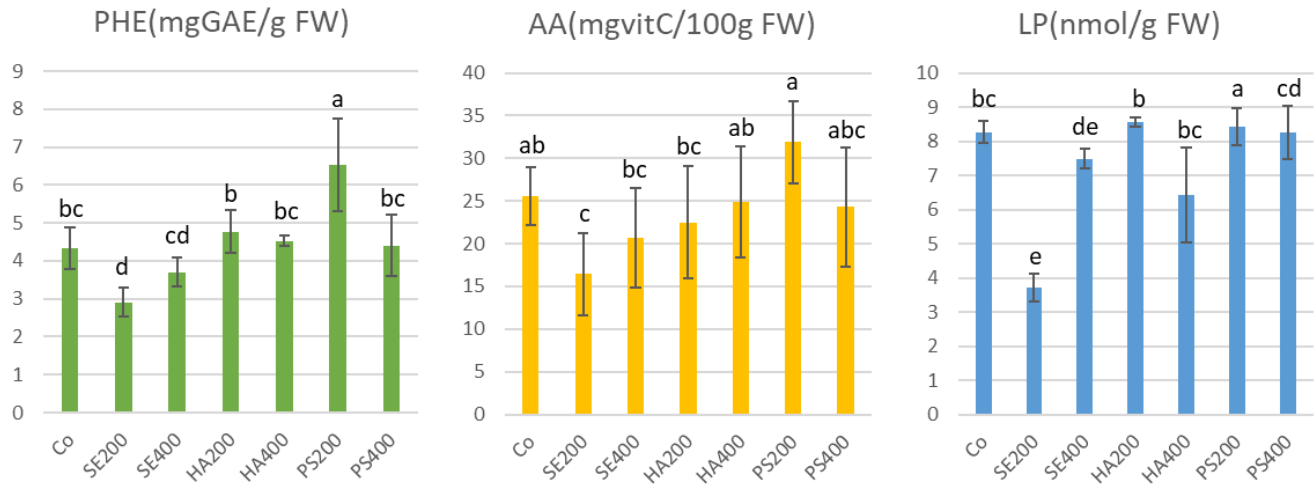

Figure 6. Means and standard deviation total phenolic content (PHE), ascorbic acid (AA) and lipid peroxidation (LP) in amaranth plants. Significance of effects is denoted with different letters at α= 0.05 level.

3.5. PC (principal component) analysis

Principal component analysis of explained 67.6% of total variance in morphological traits and Se concentrations in different plant parts of Lamb's lettuce (Figure 7). PC1 was positively correlated to dry mass per plant, number of leaves per plant and Se concentrations in leaves and roots. PC2 showed negative correlations with fresh root mass per plant and dry root mass per plant, and positive with root length. Accordingly, the positioning of control and SE200 treatment at negative side of PC1 indicated lower dry mass per plant and lower concentrations of Se in leaves and roots. Contrarily, all other treatments grouped at the positive side of PC1. All treatments except control and SE200 grouped around the origin of PC2, indicating close-to average responses in most of the traits. However, control and SE200 grouping at positive and negative sides, respectively, indicated differences in fresh root mass per plant, dry root mass per plant and root length between the groups.

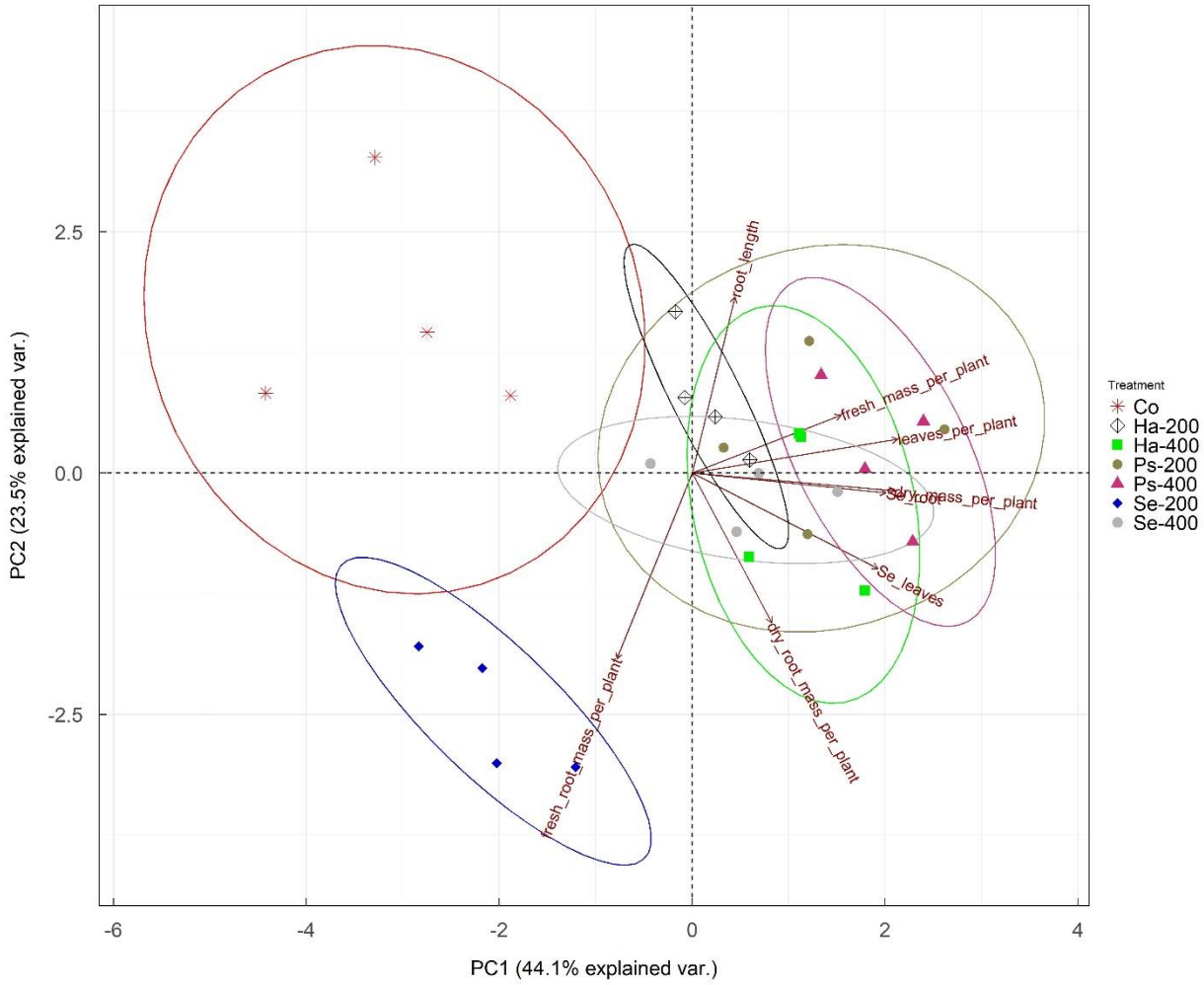

Figure 7. Principal component analysis of treatment effects on morphological traits and Se concentrations in different plant parts of Lamb's lettuce and eigenvectors

Principal component analysis in Amaranth (Figure 8) explained 85.8% of total variation in the dataset in first two PCs. Loading analysis indicated negative correlations between all morphological traits and PC1, and correlations with Se concentrations in different plant parts close to zero. Contrarily, PC2 was correlated positively with Se concentrations in leaves and stem. Grouping of different treatments along PC1 indicated above-average performance of plants in PS400 treatment in all morphological traits, with all other treatments grouped close to the PCs origin and its negative side. Grouping in PC2 indicated highest concentrations of Se in SE400 treatment with close to average performance in morphological traits. In the SE200 treatment, plants showed below-average performance in morphological traits with higher than average Se concentrations. All other treatmetns grouped at the negative side of PC2 with close-to average or below average performances.

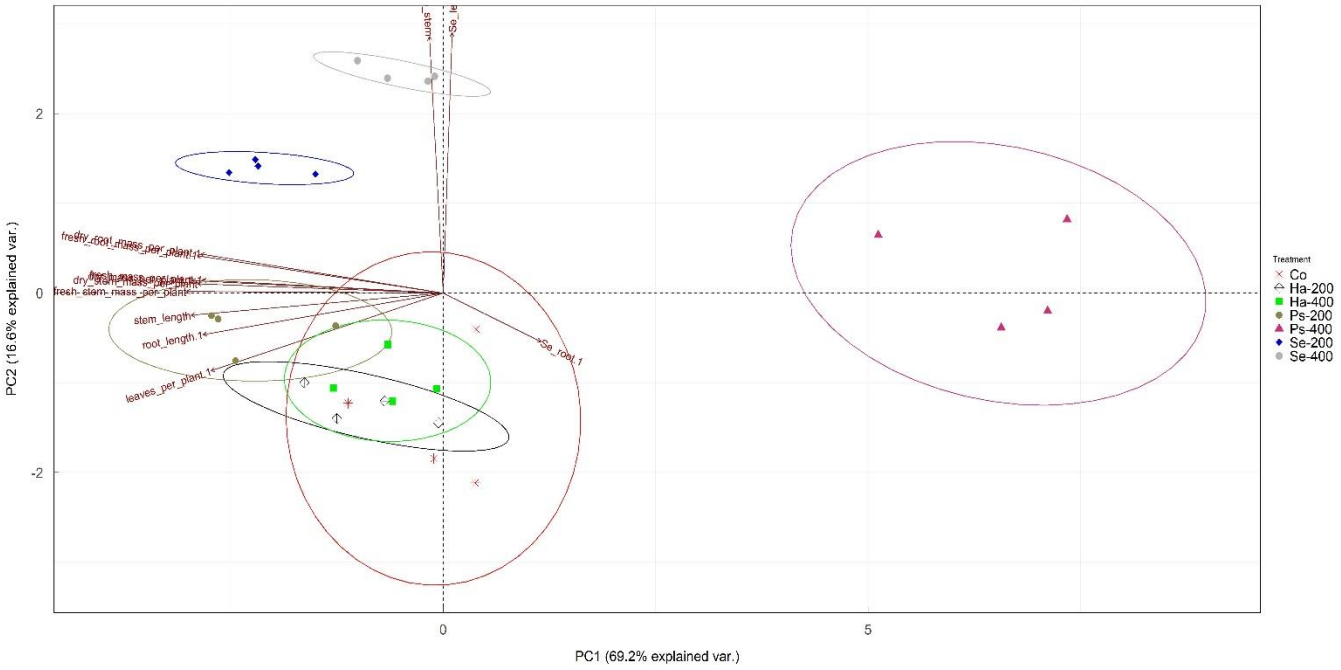

Figure 8. Principal component analysis of treatment effects on morphological traits and Se concentrations in different plant parts of Amaranth and eigenvectors

Discussion

The increased number of conducted studies on the suitability of floating hydroponics for growing baby leaf vegetables can confirm our results in terms of lamb's lettuce (Table 1) yield [35,44] [3]. From the conducted research, similar to ours, it has been determined that the addition of selenium to the hydroponic growth

solution stimulates plant growth in lamb's lettuce plants [44]. In a study conducted by Ashotovich Nagdalian et al. (2023), it was found that nanoselenium enhances seed germination and various barley attributes, suggesting the use of nanoselenium as a plant growth promoter [22]. As in our research, where we observed an increase in fresh plant mass compared to control plants treated with nanoselenium in both plant species (Table 1 and Table 2). This might be attributed to alterations in the cellular redox status, leading to heightened synthesis of non-enzymatic antioxidants like lycopene and carotenoids. Additionally, it could be linked to an increased activity of key enzymes involved in the antioxidant pathway, such as glutathione peroxidase, with selenium serving as a cofactor, and the possibility of obtaining ionic selenium from nanoparticles [17]. In Figure 1, we can observe a particular distinction among plant species, wherein amaranth exhibited a lower uptake of selenium in the form of nanoparticles. The precise mechanism by which these nanoparticles are absorbed remains incompletely understood. One of the widely accepted hypotheses suggests that absorption occurs through both intra- and extracellular pathways within plant tissues until reaching the xylem. The specific process by which nanoparticles traverse the Casparian strip remains unclear, but it is possible that it occurs through the meristematic zone. While the cell wall serves as a physical barrier, it contains pores with diameters ranging from 5 to 20 nm, allowing nanoparticles smaller than this range to penetrate freely [17]. There is a possibility that the nanoselenium particles for this plant species (amaranth) were too large. In the study conducted by Bai et al. (2021), it was found that nano-particle uptake in C3 species was greater than in C4 plants, with maize and amaranth serving as examples of C4 plant types. This is one of the possible explanations for why amaranth plants did not uptake selenium in the form of nanoparticles in our research [65]. In accordance with univariate analysis of variance, the multivariate analysis, specifically the Principal Component Analysis (PCA) in this case (Figure 2 and Figure 3), has demonstrated that the PS200 treatment in lamb's lettuce yielded the most favorable results for almost all observed traits, whereas in amaranth, all treatments were relatively similar, with the exception of the nanoparticles in the PS400 treatment. Our findings are consistent with other studies that investigated the effects of selenates, selenites, and nanoselenium on morphological characteristics, as seen in the research on Faba bean (Vicia faba L.) conducted by Sindireva et al. (2023), St John's wort (Hypericum perforatum L.) in the study by Rezaei Nazari et al. (2022), and sweet wormwood (Artemisia annua L.) in the research by Logvinenko et al. (2022) [66–68]. In our study, an intriguing phenomenon was observed, where selenium nanoparticles with polysorbate in the PS400 treatment led to less favorable morphological characteristics in amaranth plants, which appeared wilted. One possible reason for this outcome could be the accumulation of nanoparticles in the Casparian strip or band, as suggested by Li and Wu (2022). In the research by Cervantes-Avils and colleagues (2021), it was noted that, after five days in soybean root exudates, the size of nano-$Cu(OH)_2$ increased nearly twofold (from 518 nm to 938 nm), and nano-MoO_3 increased in size as well (from 372 nm to 690 nm) [69]. Another contributing factor could be that the root epidermis acts as a secondary barrier for efficient root uptake of nanoparticles. When nanoparticles interact with the root epidermis, the primary routes for root uptake of nanoparticles are the apoplastic and symplastic pathways, as highlighted by Su et al. (2019) [70]. Within the apoplastic pathway, limitations in cell wall porosity, plasmodesmata diameter, and width are significant factors that restrict the efficiency of root nanoparticle uptake[71]. In research by Lin and Xing (2008) ZnO nanoparticles exhibited varying zeta potentials and aggregate sizes when comparing their presence in the rhizosphere and bulk nutrient solutions. These differences suggest that root exudates have the potential to influence the properties and behavior of ZnO nanoparticles [72]. Our experiments yield compelling evidence that aligns with the findings of El-Badri et al.'s research in 2022. It demonstrates that the morpho-physiochemical response of rapeseed to bioSeNPs surpasses that of both the control group and Se (IV) treatments, especially under normal and salt stress conditions. Furthermore, the investigation underscores the significant effectiveness of bioSeNPs in enhancing seed germination, seedling growth, elevating photosynthesis capacity, boosting secondary metabolism, and strengthening the defense system capabilities [73]. Consistent with our data presented in the

correlation plots (Figure 4 and Figure 5), it can be inferred that our selenium treatments, in various forms, had a limited impact on physiological parameters. This suggests that substantial alterations were not induced in the plants, except in the case of lamb's lettuce, where a moderately negative correlation was observed between selenium content in the roots and pigment composition. Conversely, amaranth plants displayed a moderate positive correlation with pigments but a strong negative correlation with the pigment ratio Car/Chl.a/Chl.b, in relation to selenium concentrations in both leaves and roots. Selenium did not exert a significant influence on other physiological parameters, including phenols, ascorbic acid, and lipid peroxidation. These findings are in agreement with the study conducted by Neysanian et al. (2020), who showed improved growth, productivity and fruit quality in tomato plants using selenate and selenium nanoparticles, thus confirming our results through their analyzes of ascorbate, non-protein thiols, proline and soluble phenols [74]. Results by Puccinelli et al. (2021) also provided evidence that the simultaneous addition of selenium and iodine at doses of 13 M and I 5 M, respectively, increased the content of the two microelements in lettuce leaves without any negative interactions in the plants [3]. From the data provided above, it is evident that selenium in the form of nanoparticles has the potential to enhance morphological characteristics, such as plant yield and the number of leaves per plant, while not eliciting physiological responses. This is a significant parameter in modern plant cultivation practices, with the potential for broader commercial utilization, particularly in the context of selenium nanoparticles.

Conclusion

In summary, our research demonstrates that the use of selenium nanoparticles synthesized with polysorbate (treatments PS200 and PS400) results in the highest values for parameters such as the number of plants per lamb's lettuce leaf and the highest fresh leaf mass yield. Following this, the treatments in descending order of yield in lamb's lettuce are SE400>HA400>HA200>SE200>Co. For amaranth plants, the order is as follows: SE400>SE200>PS200>HA200>Co>HA400>PS400. Importantly, no physiological changes were observed in either plant species, indicating the potential application of nanoparticles with humic acid and polysorbate in a commercial sense, as part of biofortification program and growth promotors, as mentioned previously by various authors [15,21,22]. Given that lamb's lettuce (a C3 plant type) responded more positively to the addition of nanoselenium, while amaranth (a C4 plant type) exhibited a relatively weaker response. We propose further exploration of nanoparticles in comparison between these two plant types: C3 and C4, in order to elucidate the mechanisms of uptake and the efficiency of biofortification with nanoselenium. As a potential solution, considering the advantageous properties of both conventional selenium and selenium nanoparticles, we suggest simultaneous application. For instance, selenate could elevate selenium levels in the edible part of the plant, while nanoselenium could stimulate growth and serve as a growth promoter, especially in certain species. For amaranth plants, we recommend biofortification with selenate or other nanoparticles, considering the unique characteristics of C4 plant types.

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Naslov izvornog znanstvenog rada broj 1: Agronomic Biofortification of Significant Cereal Crops with Selenium — A Review

Proširenі sažetak:

Selen (Se), nemetal u tragovima, esencijalan je element velike važnosti za zdravlje ljudi i životinja, prisutan u različitim oksidacijskim stanjima u okolišu. Nedostatak selena povezan je s brojnim bolestima, a biofortifikacijsko povećanje moguće je rješenje za globalnu pothranjenost selenom. Žitarice poput riže, pšenice, kukuruza i ječma značajni su izvori selena u ljudskoj prehrani jer su najčešće konzumirane namirnice. U radu je istraživana agronomska biofrtifikaciju selenom, fokusirajući se na učinkovite metode za povećanje sadržaja selena u žitaricama (pšenica, riža, kukuruz i ječam). Iako je selen bitan za niže biljke, njegova važnost u višim biljkama još uvijek je predmet istraživanja. Fiziološki koristan učinak selena na biljke uključuje povećanje antioksidativnih metabolita poboljšavajući otpornost na reaktivne kisikove vrste u uvjetima stresa. Nedostatak selena u prehrani rezultira zdravstvenim rizicima pa čak i bolestima poput Keshanove bolesti, Kashin–Beckove bolesti, karcinoma, kardiovaskularnih problema, bolesti jetre i katarakte. S ciljem ublažavanja nedostatka selena, istražuju se različiti pristupi biofortifikaciji, uključujući agronomske i genetske strategije. Žitarice, kao glavni izvor hrane, prikladni su usjevi za biofortifikaciju. Agronomska biofrtifikacija uključuje povećanje koncentracije selena u usjevima dodatkom selena u tlo ili folijarnom primjenom. Istraživanje procjenjuje učinkovitost različitih oblika selena, uključujući selenat i selenit. Rezultati naglašavahju prednost folijarne primjene nad primjenom na tlu ili u tlo, pri čemu se selenat pokazao učinkovitijim zbog brze apsorpcije i translokacije. Zbog složenosti metodologije biofortifikacije selenom u postojećoj literaturi provedena je meta - analiza kako bi identificirali najučinkovitiji način biofortifikacije. Meta – analizom utvrđeno je da je folijarna primjena učinkovitija od primjene na tlu, te da je selenat preferirani oblik za agronomsku biofortifikaciju selenom. Zanimljiva spoznaja je bila da je kod riže efikasnija biofrtifikacija sa selenitom. Kroz istraživanje literaure, sugerira se mogući razvoj računalnog modela za predviđanje učinkovitosti primijenjenog selena s obzirom na vrstu usjeva i tla, oblik selena te metodu primjene. Dodatno, prepoznata je potreba za istraživanjem divljih biljnih vrsta s visokim koncentracijama selena, posebice segetalnih korova koji su često karakteristični za određene okoline, ponekad imaju ljekovita svojstva i mogu biti ugroženi zbog intenzivne poljoprivredne prakse. Rezultati istraživanja doprinose smanjenju nedostatka selena i

poboljšanje zdravlja i dobrobiti populacija poboljšanjem nutricionističke kvalitete prehrambenih usjeva.

Ključne riječi: biofortifikacija, selen, meta – analiza, žitarice, selenat, selenit

Naslov izvornog znanstvenog rada broj 2: Modelling Leverage of Different Soil Properties on Selenium Water-Solubility in Soils of Southeast Europe

Prošireni sažetak:

Selen (Se), esencijalan nemetal za ljude, životinje i mikroorganizme, dok je kod biljaka beneficijalan, ima značajnu ulogu u prehrambenom lancu, pri čemu je tlo glavni izvor selena. Biološka dostupnost selena u tlu značajno utječe na njegovu prisutnost u prehrambenom lancu, a različita svojstva tla, uključujući kationski izmjenjivački kapacitet (KIK, eng. –"*cation-exchange capacity"* - CEC) i organsku tvar tla (*eng*. "*soil organic matter"* - SOM), značajno utječu na dostupnost selena. Za potrebe kreiranja modela korišteni su rezultati fizikalno-kemijskih analiza uzoraka tla prikupljenih s lokaliteta u 6 područja u jugoistočnoj Europi, tj. u Hrvatskoj (Osijek), Bosni i Hercegovini (Sarajevo, Banja Luka, Mostar i Prud) i Srbiji (Novi Sad). U uzorcima tla analizirana je koncentracija ukupnog selena (Se ekstrahiran pomoću HNO_3 - SeTot) i vodotopivog selena (Se ekstrahiran vodom - SeH_2O). Među istraženim područjima, samo su tla iz područja Mostara imala koncentracije selena iznad granice deficita (0,5 mg kg^{-1}), povezane s povišenim vrijednostima KIK, organske tvari tla, ukupnog ugljika (C), ukupnog dušika (N), ukupnog cinka i ukupnog kadmija. Istražujući složene odnose između kemijskih svojstava tla i SeH_2O, analiza glavnih komponenata (eng." *principal component analysis*" - PCA) objasnila je 73,7 % varijance u skupu podataka unutar prve tri glavne komponente (PC-a). Na temelju ovih podataka razvijen je model parcijalnih najmanjih kvadrata (eng. "*partial least squares regression*" - PLS) koji predviđa razine SeH_2O u tlu s visokom točnošću od 77 % do 90 %, ovisno o ulaznim podacima. Pri tome su LOI, KIK, ukupni C, ukupni N i SeTot čimbenici s najvećim opterećenjima u modelu. Ovi rezultati ističu da je na ovim poljoprivrednim područjima potrebna biofortifikacija kako bi se nadomjestio nedostatak selan u prehrani ljudi i hranidbi životinja. Razvijeni prediktivni model ima veliki potencijal i za druge regije koje karakterizira nedostatak selena u tlu. Korištenjem osnovnih analiza tla poput sadržaja organske tvari tla, kationskog izmjenjivačkog kapaciteta, sadržaja ukupnog N, ukupnog C, ukupnog Ca i ukupnog Na, točnost modela može se značajno poboljšati. Dakle, upotreba regresijskih modela za predviđanje dostupnosti selena može pomoći u učinkovitijoj i ekonomičnijoj implementaciji mjera biofortifikacije. Razvijeni model na tlima različitih područja omogućuje daljnju optimizaciju validacijom sa ili bez rezultata provedenih biofortifikacija na novim lokalitetima izvan istraživaninjia obuhvaćenih područja. Rezultati

istraživanja mogu biti značajan doprinos u razvoju održivih tehnologija u poljoprivredi za proizvodnju kvalitetnije hrane s dodanom nutritivnom vrijednosti.

Ključne riječi: selen, svojstva tla, nedostatak selena, regresijski model, jugoistočna Europa, biofortifikacija

Naslov izvornog znanstvenog rada broj 3: Selenium Biofortification of Soybean Seeds Influences Physiological Responses of Seedlings to Osmotic Stress

Prošireni sažetak:

Klimatske promjene predstavljaju ozbiljnu prijetnju globalnoj poljoprivrednoj produktivnosti uz sve češći nedostatak vode u poljoprivrednim tlima kao izravnu posljedicu koja štetno utječe na rast i prinos usjeva. Selen (Se) je značajna činitelj obrambenih mehanizama biljaka uslijed biotskog i abiotskog stresa. U radu su istraživani fiziološki odgovori klijanaca soje (*Glycine max* (L.) Merrill) uzgojene pod osmotskim stresom iz sjemena biofortificiranog selenom. Koristeći u prethodnoj vegetaciji biofortificirano zrno soje (dobiveno folijarnim biofortifikacijom natrijevim selenatom), eksperiment uključuje dva kultivara soje (Lucija i Sonja). Kultivari su uzgojeni na filtrirnom papiru u komori za rast s različitim tretmanima suše: kontrola uz zalijevanje deioniziranom vodom (PEG-0) i tretman s 2,5% polietilen glikolom 6000 (PEG-2,5), kako upotrebom selenom biofortificiranih sjemenki (Se) i sjemenki koje nisu biofortificirane (wSe), u tri ponavljanja. U 7-dnevnim klijancima soje utvrđen je sadržaj produkta peroksidacije lipida (LP), slobodnog prolina (PRO), ukupnog fenolnog sadržaja (TP), askorbinske kiseline (AA) i određivanje ukupne antioksidativne moći putem redukcije željeza (FRAP). Sadržaj selena u biofortificiranom zrnu soje pokazao je značajne varijacije među kultivarima, pri čemu je kultivar Lucija pokazao blažu fiziološku reakciju na tretman PEG-2,5 u slučaju biofortificiranog sjemena soje selenom i kontrole (wSe-bez selena), sugerirajući potencijal selena za ublažavanje učinaka osmotskog stresa. Suprotno tome, kod kultivara Sonja, selen je negativno utjecao na analizirana svojstva u tretmanu PEG-2,5, ukazujući na prooksidativno djelovanje selena u kultivaru Sonja, za razliku od kultivara Lucija kod kojeg je djelovao kao antioksidans. Unatoč prooksidativnom utjecaju selena kod kultivara Sonja, aktivacija antioksidacijskih puteva dodatno valorizira klijance kultivara Sonja, posebice ako ih smatramo funkcionalnom hranom.Ovo istraživanje ističe genotipski specifično međudjelovanje osmotskog stresa i selena.

Ključne riječi: biofortifikacija, selen, fiziološki odgovor, soja, osmotski stres, suša, otpornost na stres

Naslov izvornog znanstvenog rada broj 4: Combining Selenium Biofortification with Vermicompost Growing Media in Lamb's Lettuce (*Valerianella locusta* L. Laterr)

Prošireni sažetak:

Listnato povrće je važan dio svakodnevne prehrane ljudi. Istovremeno, nedostatak selena (Se) predstavlja rasprostranjeni globalni problem, prvenstveno uzrokovan niskim razinama selena u tlu. Budući da su biljke značajan izvor selena u ljudskoj prehrani, upotreba namirnica biljnog podrijetla bogatih selenom može biti učinkovita strategija za povećanje unosa selena u prehrani ljudi. Također, s obzirom na ograničene rezerve treseta, značajnog za proizvodnju uzgojnih medija, istraživana je mogućnost upotrebe vermikomposta iz organskog otpada kao alternativu tresetu korištenom za pripremu sjetvenih supstrata. Cilj ovog istraživanja bio je ispitati učinkovitost biofortifikacije matovilca selenom u tri različita uzgojna medija: komercijalni supstrat, vermikompost i mješavinu ova dva medija u omjeru 1:1. Biofortifikacija matovilca selenom bila je uspješna u sva tri ispitana uzgojna medija. Komercijalni supstrat i mješavina 1:1 nisu pokazali značajne razlike u prinosu i koncentraciji selena u svježem i suhom listu matovilca. Utvrđeno je da je biofortifikacija povećala sadržaj selena tako da je masa od samo 48,9 g svježih listova matovilca sadržavala dovoljno selena za preporučeni dnevni unos u ljudskoj prehrani (55 µg selena dan^{-1}), što predstavlja značajan potencijal za rješavanje problema nedostatka selena u prehrani. Nadalje, korištenje smjese vermikomposta i komercijalnog supstrata u omjeru 1:1 pokazalo je slične rezultate kao i korištenje komercijalnog uzgojnog medija, što može doprinijeti očuvanju rezervi treseta. Korištenje vermikomposta kao samostalnog medija za uzgoj rezultiralo je smanjenim prinosima, ali u matovilcu uzgojenom u vermikompostu izmjerene su najveće koncentracije Se. Na temelju pozitivnih rezultata, možemo zaključiti da se vermikompost u 1:1 smjesi s komercijalnim supstratom može uspješno korisiti u uzgoju selenom biofortificiranog matovilca.

Ključne riječi: matovilac, biofortifikacija, selen, pothranjenost, vermikompost, treset

Naslov izvornog znanstvenog rada broj 5: Comparative Selenium Biofortification in Lamb's Lettuce and Amaranth in Hydroponic System: Conventional vs. Nanobiotechnological Approaches

Prošireni sažetak:

Veliki značaj selena u prehrani ljudi i hranidbi životinja rezultirao je stvaranjem različitih metode biofortifikacije selenom uz upotrebu različitih oblika selena ili načina uzgoja. Cilj ovog istraživanja bio je istražiti utjecaj oblika selena na prinos, morfološke karakteristike, fiziološki odgovor "*baby leaf*" povrća (matovilca i amaranta) i uspješnost biofortifikacije u hidroponskom uzgoju. Znanstveni rad istražuje učinke biofortifikacije selena na matovilcu (C3 vrsta) i amarantu (C4 vrsta) koristeći različite tretmane selena, uključujući nanočestice sintetizirane s polisorbatom (PS200 i PS400), huminskom kiselinom (HA200 i HA400) te tretman natrijevim selenatom (SE200 i SE400). Istraživanje otkriva značajne varijacije morfoloških karakteristika obje vrste povrća. Matovilac pokazuje poboljšan rast primjenom nanočestica selena na bazi polisorbata, uz veću masu svježeg lista i veći broj listova po biljci. S druge strane, odgovor amaranta je kompleksniji, jer na neke tretmane rezultira suboptimalnim rastom i promjenama morfoloških karakteristika. Istraživanjem je utvrđeno da uporaba nanočestica selena sintetiziranih s polisorbatom (tretmani PS200 i PS400) rezultira najvećim brojem listova po biljci matovilca i najvećim prinosom svježeg lista. Ostali tretmani u nizu sve manjeg prinosa zauzimaju sljedeći poredak: SE400>HA400>HA200>SE200>kontrola. Istovremeno je kod amaranta utvrđen redoslijed SE400>SE200>PS200>HA200>kontrola>HA400>PS400. Također, nisu primijećene fiziološke promjene niti kod matovilca niti kod amaranta, što znači da se nanočestice s huminskom kiselinom i polisorbatom mogu koristiti za uzgoj selenom obogaćenog matovilca i amaranta. Ipak, matovilac kao C3 biljna vrsta je pozitivnije reagirao na dodatak nanoselena, dok je amarant kao C4 vrsta pokazao relativno slabiji odgovor, te je potrebno daljnje istraživanje nanočestica u usporedbi fizioloških mehanizama i učinkovitosti biofortifikacije nanoselenom ove dvije biljne vrste. Prema rezultatima istraživanja, selenat bi se pri tome mogao učinkovitije koristiti za povećanje razine selena u jestivom dijelu biljke, dok bi se nanoselen mogao koristiti kao stimulans za rast i postizanje većeg prinosa.

Ključne riječi: selen, nanoselen, biofortifikacija, matovilac, amarant, plutajući hidroponski sustav

SAŽETAK

Selen (Se) je esencijalan mikroelement za ljude, životinje, mikroorganizme, dok je za biljke beneficijalan. Nedostatak selena u prehrani predstavlja globalni problem čiji intenzitet često ovisi o koncentracijama i raspoloživosti selena u tlu. Selen ima ključnu ulogu u antioksidacijskim procesima, regulaciji funkcija reproduktivnog i imunološkog sustava u zdravlju ljudi. Kao odgovor na ovaj izazov, biofortifikacija postaje značajna strategija za povećanje sadržaja selena u biljkama i hrani biljnog podrijetla. U okviru istraživanja u ovoj disertaciji provedena je meta-analize agronomske biofortifikacije pšenice, kukuruza, ječma i riže selenom. Rezultati i straživanja sugeriraju da folijarna primjena selena postiže bolje rezultate od primjene u tlu, te da je aplikacija selenata najčešće učinkovitija od aplikacije selenita, izuzevši u biofortifikaciji rižemgdje je efikasniji selenit. Objavljeni znanstveni radovi sugeriraju razvoj modela za predviđanje učinkovitosti primijenjenog selena s obzirom na vrstu usjeva i svojstva tla, oblik selena i metodu primjene. U disertaciji je razvijen model za predviđanje raspoložive vodotopive frakcije selena u tlu. Za potrebe kreiranja modela korišteni su rezultati fizikalno-kemijskih analiza uzoraka tla prikupljenih s lokaliteta u 6 područja u jugoistočnoj Europi, tj. u Hrvatskoj, Bosni i Hercegovini i Srbiji. U uzorcima tla analizirana je koncentracija ukupnog selena ekstrahiranog pomoću HNO_3 i vodotopivog selena ekstrahiranog vodom. Razvijen je prediktivni model parcijalnih najmanjih kvadrata koji na temelju fizikalno-kemijskih svojstava tla s visokom pouzdanošću predviđa sadržaj raspoložive frakcije Se u tlu. Istraživani su također i različiti aspekti biofortifikacije soje, matovilca i amaranta. Fiziološki odgovori klijanaca soje na stres izazvan sušom istraživani su na dva kultivara (Lucija i Sonja) uzgojena iz sjemena u prethodnoj vegetaciji biofortificiranog selenom. Istraživani su fiziološki parametri (askorbinska kiselina, ukupni fenoli, prolin, ukupna antioksidativna aktivnost i lipidna peroksidacija) kao odgovor na osmotski stres izazvan polietilen glikolom (PEG tretmanom) klijanaca soje (Glycine max L. Merr.) čije je sjeme u prethodnoj vegetaciji biofortificirano selenom, te su utvrđene različite reakcije dva kultivara soje (Sonja i Lucija). Utvrđeno je genotipski specifično međudjelovanje osmotskog stresa i selena jer je u kultivaru Sonja selen biofortifikacijom akumuliran u zrnu djelovao kao prooksidans, a u kultivaru Lucija kao antioksidans. Istraživanjem utjecaja različitih supstrata (komercijalni supstrat, vermikompost i njihove mješavine) na učinkovitost biofortifikacije selenom i prinos u uzgoju matovilcu, utvrđena je pogodnost smjese vermikomposta i tresetnog medija za uzgoj i biofortifikaciju matovilca. Također, u istraživanju biofortifikacije natrijevim selenatom (Na_2SeO_4)

i nanočesticama selena u hidroponskom uzgoju dvije vrste "baby leaf" povrća (matovilac i amarant), analizama morfoloških i fizioloških značajki "*baby leaf*" povrća utvrđena je različita učinkovitost ovih pristupa biofortifikaciji. Cilj ovog zadnjeg dijelu istraživanja u okviru disertacije bio je istražiti utjecaj oblika selena na prinos, morfološke karakteristike, fiziološki odgovor "*baby leaf*" povrća (matovilca i amaranta) i uspješnost biofortifikacije u hidroponskom uzgoju. Istraživan je učinak biofortifikacije selenom matovilca i amaranta s različitin tretmanima selenom, uključujući nanočestice sintetizirane s polisorbatom, huminskom kiselinom te tretman natrijevim selenatom. Istraživanje otkriva značajne varijacije morfoloških karakteristika obje vrste povrća. Matovilac pokazuje poboljšan rast primjenom nanočestica selena na bazi polisorbata, uz veću masu svježeg lista i veći broj listova po biljci. S druge strane, odgovor amaranta je kompleksniji, jer neki tretmani rezultiraju suboptimalnim rastom i promjenama morfoloških karakteristika. Najznačajniji zaključci su da je vodotopivi selen u tlima u pozitivnoj korelaciji s organskom tvari, kationskim izmijenjivačkim kapacitetom, koncentracijama ukupnih ugljika, dušika, kalcija, natrija, željeza, cinka, kadmija i ukupnog selena; da proizvodnja selenom biofortificiranog sjeman soje može pomoći ublažiti učinak nedostatka vode kod nekih kultivara, dok kod drugih kultivara može rezultirati visokim koncentracijama prolina, ukupnih fenola, askorbinske kiseline i ukupnom antioksidativnom aktivnosti; da se vermikompost može koristiti u uzgoju selenom biofortificiranog matovilca radi smanjenja upotrebe komercijalnih supstrata; da se biofortifikacijom matovilca selenom može povećati koncentracija selena u listu do te mjere da bi dnevna konzumacija 50-ak grama matovilca bila dovoljna za unos dnevne preporučene količine selena; da upotreba nanočestica selena s polisorbatom poboljšava prinos i broj listova biofortificiranog matovilca u hidroponskom uzgoju.

SUMMARY

Selenium (Se) is an trace element essential for humans, animals, microorganisms, while it is beneficial for plants. The lack of selenium in the diet is a global problem, the intensity of which often depends on the concentration and availability of selenium in the soil. Selenium plays a key role in antioxidant processes, regulation of reproductive and immune system functions in human health. In response to this challenge, biofortification becomes a significant strategy for increasing the selenium content in plants and plant-based foods. As part of the research in this dissertation, a meta-analysis of agronomic biofortification of wheat, corn, barley and rice with selenium was performed. The results and observations suggest that the foliar application of selenium achieves better results than the application in the soil, and that the application of selenate is usually more effective than the application of selenite, except in rice biofortification, where selenite is more effective. Published scientific papers suggest the development of a model to predict the efficiency of applied selenium with respect to crop type and soil properties, selenium form and application method. The dissertation developed a model for predicting the available water-soluble fraction of selenium in the soil. For the purposes of creating the model, the results of physical-chemical analyzes of soil samples collected from localities in 6 areas in South-Eastern Europe, i.e. in Croatia, Bosnia and Herzegovina and Serbia, were used. The concentrations of total selenium extracted with HNO_3 and water-soluble selenium were analyzed in the soil samples. A partial least squares prediction model was developed which, based on the physico-chemical properties of the soil, predicts the content of the available fraction of Se in the soil. Different aspects of biofortification of soybeans, lamb's lettuce and amaranth were also investigated. Physiological responses of soybean seedlings to drought stress were investigated on two cultivars (Lucija and Sonja) grown from seeds in the previous vegetation biofortified with selenium. Physiological parameters (ascorbic acid, total phenols, proline, total antioxidant activity and lipid peroxidation) were investigated in response to osmotic stress induced by polyethylene glycol (PEG treatment) of soybean seedlings whose seeds were biofortified with selenium in the previous vegetation, and different reactions of two soybean cultivars were determined. A genotypic-specific interaction between osmotic stress and selenium was established, because in the cultivar Sonja, selenium accumulated in the grain by biofortification acted as a prooxidant, and in the cultivar Lucija as an antioxidant. By researching the influence of different substrates (comercial supstrate, vermicompost and their mixtures) on the efficiency of biofortification with selenium and the yield

in the cultivation of lamb's lettuce, the suitability of a mixture of vermicompost and comercial medium for the cultivation and biofortification of lamb's lettuce was determined. Also, in the study of biofortification with sodium selenate (Na_2SeO_4) and selenium nanoparticles in the hydroponic cultivation of two types of "*baby leaf*" vegetables (lamb's lettuce and amaranth), analyzes of the morphological and physiological characteristics of "*baby leaf*" vegetables determined the different effectiveness of these approaches to biofortification. The aim of this last part of the research within the dissertation was to investigate the influence of the form of selenium on the yield, morphological characteristics, physiological response of "*baby leaf*" vegetables (lamb's lettuce and amaranth) and the success of biofortification in hydroponic cultivation. The effect of selenium biofortification of lamb's lettuce and amaranth was investigated with different selenium treatments, including nanoparticles synthesized with polysorbate, humic acid and treatment with sodium selenate. The research reveals significant variations in the morphological characteristics of both types of vegetables. Lamb's lettuce shows improved growth with the application of polysorbate selenium nanoparticles, with greater fresh leaf mass and a more leaves per plant. On the other hand, the response of amaranth is more complex, because some treatments result in suboptimal growth and changes in morphological characteristics. The most significant conclusions are that water-soluble selenium in soils is positively correlated with organic matter, cation exchange capacity, concentrations of total carbon, nitrogen, calcium, sodium, iron, zinc, cadmium and total selenium; that the production of selenium biofortified soybean seed can help alleviate the effect of water deficit in some cultivars, while in other cultivars it can result in higher concentrations of proline, total phenolics, ascorbic acid and total antioxidant activity; that vermicompost can be used in the cultivation of selenium biofortified lamb's lettuce to reduce the use of commercial substrates; that the biofortification of lamb's lettuce with selenium can increase the concentration of selenium in the leaf to such an extent that the daily consumption of about 50 grams of lamb's lettuce would be sufficient for the intake of the daily recommended amount of selenium; that the use of selenium nanoparticles with polysorbate improves the yield and number of leaves of biofortified lamb's lettuce in hydroponic cultivation.

ŽIVOTOPIS

Lucija Galić je rođena u Osijeku 16. ožujka 1994. godine. Nakon uspješno završene srednje škole, stupila je u radni odnos na rok od godinu dana u zubotehničkom laboratoriju Zdenko Bobinac u Belišću radi stručnog ispita, koji je uspješno položila 2015. godine. Iste godine, upisuje se na Poljoprivredni fakultet u Osijeku, smjer Hortikultura, gdje se ističe kao studentica s dvije pohvale i dekanovom nagradom na trećoj godini preddiplomskog studija. Na preddiplomskom studiju, diplomira 2018. godine s temom "Biofortifikacija lisnatog povrća kondicioniranjem medija za uzgoj" pod mentorstvom prof. dr. sc. Zdenka Lončarića. Tijekom studija, aktivno sudjeluje na raznim događanjima poput simpozija Slavonika, međunarodnog simpozija agronoma u Vodicama, Festivala znanosti te Agro startup konferencije. Prikazuje stručnost i angažman te je nagrađena od tvrtke Inspecto d.o.o. za iznimna postignuća. Nakon preddiplomskog studija, upisuje diplomski studij "Ishrana bilja i tloznanstvo" i uspješno ga završava 2020. godine s temom "Fenolni spojevi u biljkama" pod mentorstvom prof. dr. sc. Miroslava Lisjaka. Od 2020. godine zaposelna je kao doktorandica (asistent) na projektu "Primjena nanobiotehnologije za suplementaciju hrane sa selenom" s ugovorom na četiri godine te obvezom završetka poslijediplomskog sveučilišnog (doktorskog) studija, te upisuje poslijediplomski sveučilišni (doktorski) studij "Poljoprivredne znanosti" smjer Agrokemija. Njezin rad i istraživački doprinos imaju perspektivu unapređenja poljoprivrednih praksi, posebno u području biofortifikacije i tloznanstva.

CURRICULUM VITAE

Lucija Galić was born in Osijek on March 16, 1994. After successfully completing high school, she entered into a one-year employment contract at the dental laboratory of Zdenko Bobinac in Belišće for the purpose of a professional exam, which she successfully passed in 2015. In the same year, she enrolled at the Faculty of Agriculture in Osijek, specializing in Horticulture, where she distinguished herself as a student with two commendations and the Dean's Award in the third year of the undergraduate studies. During her undergraduate studies, she graduated in 2018 with a thesis on "Biofortification of leafy vegetables through conditioning of the growing medium," under the mentorship of Prof. Dr. Zdenko Lončarić. Throughout her studies, Lucija actively participated in various events such as the Slavonika symposium, an international agronomist symposium in Vodice, the Science Festival, and the Agro Startup Conference. She demonstrated expertise and commitment, earning financial recognition from Inspecto d.o.o. for outstanding achievements. Following her undergraduate studies, she enrolled in the master's program "Plant Nutrition and Soil Science," successfully completing it in 2020 with a thesis on "Phenolic Compounds in Plants" under the mentorship of Prof. Dr. Miroslav Lisjak. In 2020, Lucija entered into employment as a doctoral student (assistant) on the project "Application of nanobiotechnology for selenium food supplementation," with a four-year contract and an obligation to complete the doctoral studies. Simultaneously, she enrolled in the doctoral program "Agricultural Sciences," specializing in Agrochemistry. Her work and research contributions have the potential to enhance agricultural practices, particularly in the fields of biofortification and soil science.