Uniwersytet Rolniczy im. H. Kołłątaja w Krakowie
Wydział Biotechnologii i Ogrodnictwa

Marlena Grzanka
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Rola wanadu w procesie biofortyfikacji w jod kukurydzy cukrowej i sałaty

Autoreferat rozprawy doktorskiej

Praca wykonana pod kierunkiem,
Prof. dr hab. inż. Sylwestera Smolenia
Zakład Żywienia Roślin, Katedra Biologii Roślin i Biotechnologii

Kraków, czerwiec 2022 rok

Pracę doktorską realizowałam w Zakładzie Żywienia Roślin w Katedrze Biologii Roślin i Biotechnologii (ZŻR KBRiB) w latach 2017 – 2022 w ramach studiów doktoranckich prowadzonych w Studium Doktoranckim Uniwersytetu Rolniczego im. Hugona Kołłątaja w Krakowie. Niniejsza praca została wykonana między innymi w ramach projektu badawczego Grant Opus: UMO-2017/25/B/NZ9/00312): 2018-2021 (badania z sałatą) oraz w ramach badań własnych UR z uprawą kukurydzy finansowanych w subwencji Ministerstwa Edukacji i Nauki dla Uniwersytetu Rolniczego im. Hugona Kołłątaja w Krakowie na rok 2018-2021.

W trakcie studiów doktoranckich brałam udział w badaniach naukowych prowadzonych przez ZŻR KBRiB m.in. w projekcie badawczym „Molekularne i fizjologiczne mechanizmy wpływu wanadu na pobieranie jodanów i jodosalicylanów przez sałatę oraz jej przeciwnowotworowe działanie testowane na ludzkich liniach komórkowych". Uczestniczyłam również w projekcie "iFarm- wertykalny system produkcji roślinnej zapewniający stałość wewnętrznych warunków atmosferycznych oraz w pełni kontrolowalne warunki wzrostu roślin" – nie był on jednak tematyką pracy doktorskiej.

Składam serdeczne podziękowania
Prof. dr hab. inż. Sylwestrowi Smoleniowi
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za pomoc w prowadzeniu doświadczenia polowego w rodzinnym gospodarstwie ogrodniczym

Spis treści

Wykaz publikacji stanowiących rozprawę doktorską

Publikacja 1:

Grzanka, M., Smoleń S., Kováčik P. 2020. Effect of vanadium on the uptake and distribution of organic and inorganic forms of iodine in sweetcorn plants during early-stage development. Agronomy 10(11). doi: 10.3390/agronomy10111666.

Punktacja MNiSW$_{2020}$: 100 pkt

IF$_{2020}$: 3,417

Publikacja 2:

Grzanka M., Smoleń S., Skoczylas Ł., Grzanka D. 2021. Biofortification of sweetcorn with iodine: Interaction of organic and inorganic forms of iodine combined with vanadium. Agronomy 11(9), 1720. doi: 10.3390/agronomy11091720.

Punktacja MNiSW$_{2021}$: 100 pkt

IF$_{2021}$: 3,417

Publikacja 3:

Grzanka M., Smoleń S., Skoczylas Ł., Grzanka D. 2022. Synthesis of organic iodine compounds in sweetcorn under the influence of exogenous foliar application of iodine and vanadium. Molecules 27, 1822. https://doi.org/ 10.3390/molecules27061822.

Punktacja MNiSW$_{2022}$:140 pkt

IF$_{2022}$: 4,412

Publikacja 4:

Smoleń S., Kowalska I., Halka M., Ledwożyw-Smoleń I., **Grzanka M**., Skoczylas Ł., Czernicka M., Pitala J. 2020. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10(1), 1-21 doi: 10.3390/agronomy10010001.

Punktacja MNiSW$_{2020}$: 100 pkt

IF$_{2020}$: 3,417

Publikacja 5:

Smoleń S., Czernicka M., Kowalska I., Kęska K., Halka M., Grzebelus D., **Grzanka M**., Skoczylas Ł., Pitala J., Koronowicz A., Kováčik P. 2021 New aspects of uptake and metabolism of non-organic and organic iodine compounds—the role of vanadium and plant-derived thyroid hormone analogs in lettuce. Frontiers in Plant Science 12, 608. doi: 10.3389/fpls.2021.653168.

Punktacja MNiSW$_{2021}$: 100 pkt

IF$_{2021}$: 5,753

Sumaryczny IF pięciu wyżej wyszczególnionych publikacji = 20,416 , a sumaryczna liczba punktów = 540.

Dorobek naukowy

Wykaz publikacji naukowych niebędących tematem rozprawy doktorskiej

1. Smoleń S., Kowalska I., Skoczylas Ł., Liszka-Skoczylas M., **Grzanka M.**, Halka M., Sady W. The effect of salicylic acid on biofortification with iodine and selenium and the quality of potato cultivated in the NFT system. Scientia Horticulturae 2018, 240, 530–543, doi:10.1016/j.scienta.2018.06.060.

   Punktacja MNiSW$_{2018}$: 35 pkt (przed reformą szkolnictwa wyższego)
   IF$_{2018}$: 2,769

2. Smoleń S., Kowalska I., Kováčik P., Sady W., **Grzanka M.**, Kutman U.B. Changes in the chemical composition of six lettuce cultivars (*Lactuca sativa* L.) in response to biofortification with iodine and selenium combined with salicylic acid application. Agronomy 2019, 9, doi:10.3390/agronomy9100660.

   Punktacja MNiSW$_{2019}$: 100 pkt
   IF$_{2019}$: 2,603

3. Rakoczy-Lelek R., Smoleń S., **Grzanka M.**, Ambroziak K., Pitala J., Skoczylas Ł., Liszka-Skoczylas M., Kardasz H. Effectiveness of foliar biofortification of carrot with iodine and selenium in a field condition. Frontiers in Plant Science 2021, 12, 1–16, doi:10.3389/fpls.2021.656283.

   Punktacja MNiSW$_{2021}$: 100 pkt.
   IF$_{2021}$: 5,753

Sumaryczny IF trzech wyżej wyszczególnionych publikacji = 11,125, a sumaryczna liczba punktów = 235.

Sumaryczny IF z ośmiu publikacji stanowiących całkowity dorobek naukowy przed doktoratem = 31,541, a sumaryczna liczba punktów = 775.

Doniesienia Konferencyjne

1. Smoleń S., Sularz O., Koronowicz A., Czernicka M., Kowalska I., Kęska K., Halka M., **Grzanka M**., Grzebelus D., Skoczylas Ł., Pitala J., Tabaszewska M., Liszka-Skoczylas M., Kováčik P. Anticancer potential of lettuce biofortified with iodine in human gastrointestinal cancer cell lines as well as aspects of plant derived thyroid hormone analogs in lettuce. Kick-off meeting COST Action CA 19116 Trace metal metabolism in plants, 24-27.08.2021, Ceskie Budejovice, Czechy.

2. Smoleń S., Kowalska I., Halka M., Ledwożyw-Smoleń I., Czernicka M., Skoczylas Ł., **Grzanka M**., Kęska K. Uptake of iodine in the form of KIO_3 and iodosalicylates depending on the vanadium application – selected aspects of iodine metabolism in lettuce plants cultivated in the NFT hydroponic system. Plant Biology CS 2019, 26-30.08.2019, Ceskie Budejovice, Czechy.

3. **Grzanka M**., Smoleń S. Wpływ wanadu na pobieranie i dystrybucję jodu w roślinach kukurydzy cukrowej we wczesnym stadium ich rozwoju – doświadczenie wazonowe z aplikacją KI, KIO_3 , kwasu 5-jodosalicylowego i 2-jodobenzoesowego. Nowoczesne metody przygotowania prób i analizy wielopierwiastkowej – zastosowanie technik sprzężonych z ICP-MS. 24 września 2019r. Kraków.

4. **Grzanka M**., Smoleń S., Kowalska I., Halka M., Skoczylas Ł., Ledwożyw-Smoleń I., Pitala J. Iodine biofortification of lettuce with the use of iodosalicylates in pot experiments. Plant Nutrition, Growth & Environment Interaction IV: February 24-25, 2020, Vienna, Austria.

5. **Grzanka M**., Smoleń S., Halka M., Sady W. Wpływ wanadu na pobieranie i dystrybucję jodu w roślinach kukurydzy cukrowej we wczesnym stadium ich rozwoju – doświadczenie wazonowe z aplikacją KI i NH_4VO_3. Ogólnopolska Konferencja Naukowa „Współczesne trendy w uprawie i żywieniu roślin ogrodniczych". Poznań (Puszczykowo), Polska, 12 – 14.06.2018.

6. Smoleń S., Kowalska I., **Grzanka M**., Halka M., Sady W., Skoczylas Ł., Liszka-Skoczylas M. Pobieranie i dystrybucja jodu i selenu w roślinach ziemniaka uprawianego w systemie hydroponicznym CKP przy łącznym ich wprowadzeniu do pożywki. Konferencja Naukowa „Współczesne trendy w uprawie i żywieniu roślin ogrodniczych". Poznań (Puszczykowo), Polska, 12 – 14.06.2018.

Wykaz stosowanych skrótów i terminów

T4 - tyroksyna

T3 – trójjodotyronina

vHPO - wanadozależna haloperoksydaza

3,5-diISA - kwas 3,5-dijodosalicowy

4-IBeA - kwas 4-jodobenzoesowy

5ISA - kwas 5-jodosalicylowy

NFT (CKP) - Nutrient Film Technique (Cienkowarstwowe Kultury Przepływowe)

2IBeA - kwas 2-jodobenzoesowy

2,3,5-triIBeA – kwas 2,3,5-trijodobenzoesowy

SA – kwas salicylowy

BeA – kwas benzoesowy

PDTHA - Plant-Derived Thyroid Hormone Analogs - Analogi Hormonów Tarczycy Pochodzenia Roślinnego -

1. Streszczenie

Problem niedoboru jodu w diecie wciąż jest powszechny, jak również idące z nim konsekwencje zdrowotne jakie ludzie ponoszą z tego powodu na całym świecie. Jod jest niezbędny do prawidłowego funkcjonowania tarczycy, stanowi substrat w syntezie tyroksyny i trójjodotyroniny. Jodowanie soli kuchennej nie spowodowało wyeliminowania niedoborów jodu w diecie ludzi. Biofortyfikacja roślin uprawnych wymaga, aby badania rolnicze zostały połączone z zapotrzebowaniem sfery zdrowia i żywienia człowieka. Konieczne jest tu multi-dyscyplinarne podejście naukowców z tychże dziedzin. Celem biofortyfikacji roślin uprawnych w jod jest z ustalenie istotnych progów toksyczności do tego pierwiastka dla roślin jak i optymalizacji tego zabiegu pod kątem bezpieczeństwa dla konsumentów, w dużej mierze spełniając swoją rolę w uzupełnieniu niedoboru tego pierwiastka w diecie. Prowadzenie badań nad biofortyfikacją roślin warzywnych i rolniczych w jod również powinno mieć na celu poszerzenie wiedzy na temat biochemicznych, fizjologicznych i molekularnych aspektów funkcji pierwiastków śladowych na rośliny.

Celem badań było określenie interakcji jodu i wanadu w roślinach kukurydzy cukrowej (*Zea mays* L. subsp. *mays* Grupa Saccharata) i sałaty masłowej (*Lactuca sativa* L. var. *capitata*). Celem dysertacji było zbadanie wpływu na akumulacje i dystrybucję w zależności od zastosowanej formy tego pierwiastka, nieorganicznej w postaci KI (jodku potasu) i KIO_3 (jodanu potasu) oraz organicznej: 5ISA (kwasu 5-jodosalicylowego), 3,5-diISA (kwasu 3,5 – dijodosalicylowego) i 2IBeA (kwasu 2-jodobenzoesowego) w połączeniu z wanadem w formie NH_4VO_3. Zakres badań obejmował, wyznaczenie dawki jodu optymalnej dla roślin oraz bezpiecznej dla konsumenta, oraz dawki wanadu bezpiecznej dla rośliny i efektywnie wspomagającej pobieranie i transport jodu do jadalnych części rośliny (liście – sałata, ziarno – kukurydza). W algach morskich wanad reguluje komórkowe pobieranie/ transport jodu (I) oraz jego ulatnianie się jako I_2 z komórek. Proces ten katalizowany jest przez enzym wanadozależną haloperoksydazę (vHPO). Przed realizacją badań obejmujących tą dysertację nie było żadnych publikacji opisujących wyniki badań na temat zależności jodu i wanadu w roślinach wyższych, podobnie badań wyjaśniających metabolizm jodosalicylanów, jodobenzoesanów oraz ewentualnej syntezy roślinnych analogów hormonów tarczycy.

W wyniku przeprowadzonych badań na kukurydzy, z aplikacją doglebową oraz aplikacją dolistną nie stwierdzono fitotoksycznego efektu zastosowanych dawek jodu w doglebowej aplikacji w fazach BBCH 15 -BBCH 75 w dawce 10μM) oraz w dolistnej aplikacji w doświadczeniu polowym (w dawce 10 μM oraz 100μM). Określono najbardziej korzystny, pod względem wzbogacenia w jod ziaren kukurydzy, sposób aplikacji, częstotliwość i dawkę badanych związków jodu. Zbadano wpływ wanadu na akumulacje jodu w poszczególnych częściach kukurydzy, która była zmienna w zależności od zastosowanej formy jodu i dawki wanadu. Zbadano aktywność vHPO w liściach i korzeniach kukurydzy oraz sałaty masłowej. Zidentyfikowano geny, których wzrost ekspresji/aktywność powiązano z funkcją vHPO w liściach i korzeniach sałaty jak również przedstawiono potencjalne szlaki metaboliczne związków jodu, pobierania, oraz transportu w sałacie uprawianej w systemie hydroponicznym. Stwierdzono naturalną syntezę/obecność 5-ISA, 3,5-diISA, w roślinach sałaty i kukurydzy/ Zbadano udział tych jododsalicylanów w szlaku biosyntezy T3 oraz innych związków PDTHA po ich egzogennej aplikacji w roślinach sałaty.

Uzyskane wyniki poszerzyły wiedzę na temat efektywnej możliwości wzbogacenia generatywnych organów kukurydzy (ziarniaki kukurydzy) w jod oraz możliwość zastosowaniu organicznych związków jodu (jodosalicylanów i jodobenzoesanów) w procesie biofortyfikacją kukurydzy. Realizacja badań poszerzyła wiedzę w zakresie metabolizmu zastosowanych form jodu w roślinach.

2. Summary

Problem of iodine deficiency in the diet is still common, as well as the health consequences of it for people around the world. Iodine is essential for the proper functioning of the thyroid gland and it is a substrate in the synthesis of thyroxine and triiodothyronine. The iodination of table salt did not eliminate iodine deficiency in the human diet. Crop biofortification requires that agricultural research be combined with the health and human nutrition sectors, a multi-disciplinary approach by scientists in these fields is needed. The purpose of biofortification of crops with iodine is to determine significant toxicity thresholds for plants and to optimize this treatment in terms of safety for consumers, largely fulfilling its role of supplementing the deficiency of this element in the diet. Carrying out the researches on the biofortification of vegetable and agricultural plants into iodine must also combine research aimed at broadening the knowledge of the biochemical, physiological and molecular aspects of the functions of trace elements, iodine and vanadium.

The aim of the research was to determine the interaction of iodine and vanadium in sweet corn (*Zea mays* L. subsp. Mays Group Saccharata) and butterhead lettuce (*Lactuca sativa* L. var. Capitata) plants. Investigation of the effect on the accumulation and distribution depending on the form of iodine used, inorganic KI (potassium iodide) and KIO_3 (potassium iodate) and organic 5ISA (5-iodosalicylic acid), 3,5-diISA (3,5-diiodosalicylic acid) and 2IBeA (2-iodobenzoic acid) in combination with vanadium in the form of NH_4VO_3 (ammonium metavanadate). Determining the dose of iodine optimal for plants and safe for the consumer, and the dose of vanadium safe for the plant and effectively enhancing the uptake and transport of iodine to the edible parts of the plant (leaves - lettuce, grain - corn). In marine algae, vanadium regulates the cellular uptake of iodine (I) and its volatilization as I_2, a process catalysed by the enzyme vanadium-dependent haloperoxidase (vHPO). Currently, there are no studies describing the correlation of iodine and vanadium in higher plants, no studies explaining the metabolism of iodosalicylates, iodobenzoates and the possible synthesis of plant thyroid hormone analogues in higher plants. As a result of the tests carried out on sweet corn, with soil application and foliar application, no phytotoxic effect of the applied doses of iodine was found (in the soil application in BBCH 15 and BBCH 75 at a dose of 10 μM) and in the foliar application in the field experiment (at a dose of 10 μM and 100 μM). The most advantageous iodine compound in terms of iodine enrichment in corn grains, application

method, frequency and dose was determined. The effect of vanadium on the accumulation of iodine in individual parts of sweet corn plants was investigated, which was variable depending on the form of iodine used and the dose of vanadium. The activity of vHPO in leaves and roots of sweet corn plants and butterhead lettuce was tested. Genes whose expression increase / activity has been linked to the function of vHPO in lettuce leaves and roots were identified, and the potential pathways of iodine compounds, uptake, and transport in lettuce grown in a hydroponic system were presented. Natural synthesis of 5-ISA, 3,5-diISA was found in control lettuce objects, the participation of these iododsalicylates in the biosynthetic pathway of T3 and other PDTHA compounds after their exogenous application was investigated. The obtained results broadened the knowledge about the effective possibility of enriching the generative organs of plants (corn grains) with iodine and the possibility of using organic iodine compounds (including 2IBeA) in the biofortification process. The research has expanded the knowledge of the metabolism of the applied forms of iodine in plants.

3. Przegląd Literatury

Zjawiska „ukrytego głodu" oraz endemicznego deficytu jodu spowodowane niedoborem jodu w żywności stanowią problem około dwóch miliardów ludzi na całym świecie (Biban i Lichiardopol 2017). Niewystarczające dostarczenie jodu w diecie wciąż stanowi istotny problem globalny, w głównej mierze państw o niskim rozwoju gospodarczym ale i państw wysokorozwiniętych gospodarczo jak Anglia, Niemcy, Australia i Włochy (Andersson i in. 2012, Lyons 2018). Głównym źródłem makro- i mikroelementów, związków fenolowych i witamin niezbędnych do prawidłowego funkcjonowania organizmu człowieka i zwierząt są zboża, warzywa i owoce. Powszechne i szeroko dostępne (w krajach wysokorozwiniętych) suplementy diety nie zastąpią właściwie zbilansowanej diety opartej o produkty pochodzenia roślinnego i zwierzęcego (Mao i in. 2014). Pomimo wprowadzenia jodowania soli kuchennej, problem niedoboru jodu wciąż istnieje, a zgodnie z nowymi zaleceniami WHO powinno się ograniczać spożycie soli, która może powodować problemy z nadciśnieniem i chorobami układu krążenia (Tayie i Jourdan 2010, Weng i in. 2013). Biofortyfikacja roślin uprawnych o dużym znaczeniu globalnym (zboża, ryż, kukurydza) w jod (jak i inne mikroelementy) metodami agrotechnicznymi, stanowi opłacalną i skuteczną metodę uzupełnienia niedoborów pierwiastków deficytowych w diecie (Cakmak i in. 2017, Gonzali i in. 2017, Zou i in. 2019).

3.1. Wpływ i znaczenie jodu i wanadu na funkcjonowanie organizmu człowieka

Jod jest niezbędnym pierwiastkiem w diecie człowieka, odpowiada za właściwe funkcjonowanie hormonu tarczycy (Zimmermann 2016). Jod stanowi substrat konieczny do syntezy tyroksyny-T4 i trójjodotyroniny-T3 (Choudhry i Nasrullah 2018, Lachowicz i in. 2019). Dzienna dawka jodu dla dorosłego człowieka wynosi 150 μg , dla dzieci w wieku 5-12 lat 90-120 μg, poniżej 5 lat 90 μg. Kobiety w ciąży i kobiety karmiące mają największe zapotrzebowanie na jod, mieści się ono w zakresie 220-290 μg (Leung i in. 2011, Zimmermann 2016, Zimmermann i Andersson 2021). W początkowym okresie ciąży wytwarzanie hormonów tarczycy wzrasta o około 50%, związane jest to ze wzrostem stężenia globuliny odpowiedzialnej za wiązanie tyroksyny w surowicy. Wzrost ten wiąże się ze zmianami hormonalnymi, wzrostem poziomu estrogenów jak i aktywizacją receptorów tyreotropowych

(TSH) (Leung i in. 2011). Enzymy, dejodynazy jodotyroninowe typu I, II, III są selenobiałkami uczestniczącymi w metabolizmie hormonów tarczycy. Dejodynaza typu I i II odpowiada za transformacje tyroksyny (T4) do bioaktywnej trijodotyroniny (T3), z kolei w łożysku funkcjonuje dejodynaza typu III, która katalizuje proces rozkładu tyroksyny (T4) do biologicznie nieaktywnej odwrotnej trijodotyroniny (rT3), w konsekwencji zwiększając podaż na hormon tarczycy, a tym samym zwiększając zapotrzebowanie na jod (Beckett i Arthur 2005, Leung i in. 2011, Kryczyk i Zagrodzki 2013). Właściwy rozwój płodu wiąże się z prawidłową gospodarką hormonów tarczycowych przenikających od matki przez łożysko. Hipotyroksynemia u matki i w konsekwencji u płodu skutkuje nieodwracalnymi zaburzeniami neurologicznymi płodu, uszkodzeniem mózgu i w konsekwencji upośledzeniem umysłowym (kretynizm) (Zimmermann 2016, Biban i Lichiardopol 2017). W niektórych przypadkach niewystarczająca dzienna dawka jodu u kobiet w ciąży może prowadzić do niepłodności lub poronienia (Bath 2019). U dorosłych niewystarczająca podaż jodu w diecie jest konsekwencją przerostu tarczycy czyli powstawania tzw. wola endemicznego oraz zwiększenia ryzyka zachorowania na raka żołądka (Gonzali i in. 2017, Zimmermann i Andersson 2021).

Dzienne zapotrzebowanie na wanad dla człowieka nie jest do końca określone przez WHO, można je umieścić w zakresie 26 μg wanadu/kg masy ciała (Costigan i in. 2001), z kolei inne źródła określają zakres mieszczący się w przedziale 10 to 160 μg (Srivastava i Mehdi 2005). Dziennie dostarczamy z pożywieniem od 20 do 60 μg wanadu, który można znaleźć w takich produktach jak świeże ryby, owoce morza, wino, piwo, szpinak, pietruszka, grzyby, pieprz, proso i przetwory mleczne (Badmaev i in. 1999, Panchal i in. 2017), a bezpieczna dawka wanadu nie zagrażająca życiu i zdrowiu to 1,8 mg/dzień (Ivancsits i in. 2002, Tripathi i in. 2018). Drogi dostania się do organizmu człowieka wanadu, to układ pokarmowy, skóra oraz poprzez inhalację - układ oddechowy (Gruzewska i in. 2014). Stężenia wanadu w tkankach i narządach człowieka, w ng/g masy, przedstawiają się następująco: nerka 3.0; tłuszcz i mięśnie 0.55; wątroba 7.5; serce 1.1; tarczycy 3.1; płuca 2.1 (Treviño i in. 2019).

Wanad to metal występujący na kilku stopniach utlenienia od -1 do 5+ (Badmaev i in. 1999, Panichev i in. 2006, Panchal i in. 2017). Poziom toksyczności związków wanadu wzrasta wraz ze wzrostem wartościowości, pięciowartościowe związki wanadu są najbardziej toksyczne dla organizmu człowieka i zwierząt. Bezpiecznymi formami wanadu jest siarczan

wanadylu i sole metawanadanu, z kolei pięciotlenek wanadu jest najbardziej toksyczny dla człowieka (Srivastava i Mehdi 2005, Roberts i in. 2016, Smoleń i in. 2020). Wspomniane związki wanadu, metawanadan i siarczan wanadylu mają istotne znaczenie w ekspresji genów enzymu uczestniczącego w metabolizmie glukozy i lipidów wykazując działanie przeciwcukrzycowe określane jako insulino-naśladowcze (Badmaev i in. 1999, Srivastava i Mehdi 2005, Kordowiak i Holko 2009). Poza wspomnianą rolą przeciwcukrzycową, wanad wykazuje działanie przeciwnowotworowe, obniża poziom cholesterolu, ciśnienie krwi (Kordowiak i Holko 2009) oraz kurczliwość mięśni gładkich (Panchal i in. 2017). Do prozdrowotnych właściwości wanadu zaliczamy jego udział w metabolizmie kości i zębów, reguluje działanie fosfotransferazy, cykladazy adenylowej i kinazy białkowe oraz Na+/K+ ATP-azy dzięki, której możliwy jest błonowy gradient sodu, umożliwiający wychwyt jodu (Uthus i Nielsen 1990, Nielsen i Human 2018). Wanad bierze udział w regulacji aktywności hormonów tarczycy poprzez regulowanie aktywności peroksydazy tarczycowej przy nagłych skokach jodu. Badania jakie były prowadzone na szczurach z suplementacją wanadem przy wzroście poziomu jodu nie powodowały wzrostu poziomów glukozy w przeciwieństwie do osobników niesuplementowanych wanadem (Nielsen i Human 2018).

3.2. Współdziałanie jodu i wanadu w glonach morskich

Wody morskie i organizmy w nich żyjące są największym rezerwuarem jodu na ziemi. Najlepszymi i najbardziej efektownymi akumulatorami jodu są gatunki alg brunatnych, u których jod stanowi 1% zawartości suchej masy *Laminaria digitata* (Leblanc i in. 2006, Fuge i Johnson 2015). Pobieranie jodu przez glony morskie polega między innymi na pozakomórkowym utlenianiem jodku za pośrednictwem wanadozależnej haloperoksydazy (vHPO). Główną częścią istnienia obiegu geochemicznego jodu jest jego ulatnianie się ze środowiska wodnego gdzie istotną rolę odgrywają mikroalgi i makroalgi (ulatnianie biologiczne) jak i poprzez fotochemiczne szlaki (Carpenter 2003, Fuge i Johnson 2015, Carpenter i in. 2021). W wodach morskich jod w głównej mierze jest w formie anionów jodkowych (I^-) i jodanowych (IO_3^-), w mniejszym stopniu w formie organicznej (Fuge i Johnson 2015). Mikroalgi i makroalgi wychwytują i akumulują jod dzięki katalizowaniu tej reakcji przez enzym wanadozależną haloperoksydazę (jodoperoksydazę) (vHPIO) (Leblanc i in. 2006, La Barre i in. 2010). Grupę prostetyczną haloperoksydazy zależnej od wanadu zajmuje metal jakim jest wanad, vHPO utlenia halogenki (Cl, Br, I) i uczestniczy w syntezie

organohalogenków (Leblanc i in. 2006, Verhaeghe i in. 2008, Saiz-Lopez i in. 2016). Specyficzna dla jodu wanadozalezna jodoperoksydaza poprawia wiązanie jodu poprzez katalizowanie utleniania I^- do bardziej lipofilowych związków (kwas jodowy(I)) HIO, a następnie molekularny I_2. Cząsteczki te mają zdolność do łatwego dyfundowania przez błony komórkowe do cytozolu. Proces redukcji HIO lub I_2 do I^- w apoplaście nie jest jeszcze określony przez naukowców (Tymon i in. 2017, Fournier i in. 2014, La Barre i in. 2010, Leblanc i in. 2006). Ulatnianie się jodu w postaci jodoorganicznej jodometanu CH_3I oraz I_2 zarówno z wód morskich i organizmów w nich żyjących (alg) oraz z gleby i roślin lądowych takich jak np. ryż (Yoshida i Muramatsu 1995), stanowi część obiegu tego pierwiastka w przyrodzie. W atmosferze lotne organiczne związki jodu, I_2 są rozkładane w wyniku dysocjacji fotochemicznej generując nowe utlenione związki, które ostatecznie biorą udział w tworzeniu cząstek aerozolu i tworzeniu jąder kondensacji chmur (Leblanc i in. 2006, Verhaeghe i in. 2008).

3.3. Znaczenie jodu i wanadu dla roślin lądowych

Jod i wanad są określane jako „beneficial elements" dla roślin uprawnych, a ich stymulujące działanie można uzyskać po aplikacji niskich stężeń, natomiast wysokie stężenia mogą wywoływać efekt fitotoksyczności (Welch 1973, Medrano-Macías i in. 2016, Smoleń i in. 2020). Oddziaływanie wanadu aplikowanego egzogennie na rośliny zależy od formy chemicznej - stopnia utlenienia, dawki oraz fazy rozwojowej rośliny (Welch 1973). Wanad jest pierwiastkiem o słabej reutylizacji do nadziemnych części rośliny – najwyższy stopień akumulacji tego pierwiastka następuje w korzeniach (Saco i in. 2013). Związane jest to z procesem biotransformacji wanadu podczas pobierania przez korzenie, która polega na redukcji pięciowartościowego wanadu, łatwo utleniającego ketony, aldehydy, katechole, sulfhydryle i olefiny znajdujące się w ścianie komórkowej nawet przy pH 7. W efekcie wanad jest zatrzymywany przez tkanki korzeniowe i wanad (V) zostaje zredukowany do czterowartościowej formy wanadu (IV) (Roychoudhury 2020). Dotychczasowe badania na roślinach z zróżnicowaną dawką wanadu określiły jako toksyczną wartość graniczną powyżej 2 ppm, wywołującą stres oksydacyjny, a w konsekwencji zahamowanie wzrostu, chlorozę i nekrozę liści oraz zaburzenia w pobieraniu niezbędnych składników mineralnych (Imtiaz i in. 2018, Aihemaiti i in. 2019, Roychoudhury 2020). Jak wąska granica jest między

fitotoksycznością, a stymulacją udowodniły badania García-Jiménez w 2018 roku. W uprawie papryki w systemie hydroponicznym z dodatkiem wanadu do pożywki w stężeniu 5 μmol V ·dm^{-3} odnotowano istotnie zwiększenie wzrostu części nadziemnej roślin. Wanad stymulował rozwój pąków kwiatowych i przyspieszył kwitnienie papryki. Zawartość aminokwasów i cukrów w korzeniach i liściach papryki było istotnie wyższe po aplikacji wanadu w dawce 5 μmol V·dm^{-3}, z kolei przy dawce 15 μmol V·dm^{-3} wykazano, że wanad spowolnił wzrost roślin, obserwowano obniżenie średnicy łodygi, liczbę i powierzchnię liści na roślinie oraz świeżą i suchą masę organów nadziemnych i korzeni (García-Jiménez i in. 2018). Badania dla włośnicy zielonej (*Setaria viridis*), gatunku charakterystycznego dla rejonów wydobycia wanadu, a więc tolerancyjnego na wyższe stężenia, wartości progowe były odpowiednio 36,8 i 16,3 mg V·dm^{-3} w roztworze glebowym z kolei 40 i 55,8 mg V·dm^{-3} spowodowało zmniejszenie długości korzeni oraz wysokości łodygi o połowę (Aihemaiti i in. 2019). Sucha masa korzeni mięty wzrosła po aplikacji wanadu w dawce 10, 20 i 40 mg V·dm^{-3} (Akoumianaki Ioannidou i in. 2015), z kolei zawartość sacharozy w burakach cukrowych wzrosła o około 30% po traktowaniu ich 10 mmol V·dm^{-3} (Singh i Wort 1969). W przypadku ciecierzycy (Imtiaz i in. 2018) i chińskiej zielonej gorczycy (*B. campestris ssp*. Chinensis var. Parachinensis) oraz pomidora (Vachirapatama i in. 2011) wanad powodował zahamowanie rozwoju systemu korzeniowego oraz części nadziemnej roślin.

Jod w środowisku glebowym występuje w formach nieorganicznych I^-, IO_3^- oraz w postaci związków organicznych. Dostępność jodu dla roślin jest uzależniona od warunków fizykochemicznych. Wzrost zawartości materii organicznej w glebie powoduje immobilizację jodu, podobnie jak niskie pH, które się wiąże ze wzrostem zawartości jonów Fe i Al powodujących wiązanie jodu do form niedostępnych dla korzeni roślin (Fuge i Johnson 2015). Gleby lżejsze oraz o odczynie zbliżonym do obojętnego zawierają więcej jodu dostępnego w roztworze glebowym dla korzeni roślin wyższych. Odczyn gleby i warunki glebowe wpływają na występowanie odpowiednich form jodu, przy niskim pH i warunkach redoks dominuje I^-, a w przeważającej liczbie procesów oksydacyjnych (utleniających) i pH wysokim (zasadowym do obojętnego) występuję forma IO_3^- (White i Broadley 2009). Transportu jodu w roślinie w znacznej mierze odbywa się przez ksylem, potwierdzając to niższą zawartością (śladowe ilości) jodu w ziarnie w porównaniu do organów wegetatywnych, liści (Mackowiak i Grossl 1999). Badania nad wzbogaceniem organów generatywnych w jod udowodniły iż

transport floemowy jodu jest możliwy, zostało to potwierdzone w roślinach pomidora (Landini i in. 2011, Halka i in. 2019), ryżu i kukurydzy (Cakmak i in. 2017, Zou i in. 2019) oraz truskawki (Li i in. 2017). Niemniej jednak transport jodu w ksylemie jest znacznie wydajniejszy (Hurtevent i in. 2013). Względną ruchliwość jodu w łyku udowodniono w badaniach Zou i in. (2019), uwzględniając istotność fazy rozwojowej rośliny podczas aplikacji jodu. Oprysk dolistny jodanem potasu w fazie wypełniania ziarna pszenicy, spowodował stworzenie dostępnej puli jodu w tkankach liścia powodując transport floemowy do ziarna kukurydzy. W fazie tej następuje intensywny transfer fotoasymilatów do nasion, uruchomienie transportu floemowego (Zou i in. 2019). Na tej podstawie niniejszych badań określono gradient akumulacji jodu w roślinach, który kształtuje się następująco: korzenie> liście> łodyga> owoce>nasiona (Caffagni i in. 2012, Weng i in. 2013, Cakmak i in. 2017, Budke i in. 2020).

Podobnie jak w przypadku wanadu efekt fitotoksyczny bądź stymulacyjny jodu zależy od formy aplikowanego jodu, jego stężenia oraz sposobu aplikacji (doglebowo, dolistnie, hydroponika do pożywki). Zastosowane nieorganiczne związki jodu 10 i 100 μM KI i 100 μM KIO_3 w uprawie ryżu (Mackowiak i Grossl 1999) czy 2,34 mM KIO_3 i 3,01 mM KI w pożywce kukurydzy, pomidora i ziemniaka (Caffagni i in. 2011) jak i jodoorganiczne 3,5-diISA (kwas 3,5-dijodosalicowy), 4-IBeA (kwas 4-jodobenzoesowy) w dawce 25 μM I w pożywce w uprawie pomidora (Halka i in. 2018) oraz 5ISA (kwas 5-jodosalicylowym) w dawce 40 μM I w systemie hydroponicznym NFT w uprawie sałaty (Smoleń i in. 2017) spowodowały negatywny efekt na rozwój roślin.

Biofortyfikacja – biostymulacja sałaty jodem (KI, KIO_3), stymulowała biosyntezę i akumulację prozdrowotnych substancji bioaktywnych, witaminę C i związków fenolowych w badaniach Blasco i in. (2008). Indukcja substancji antyoksydacyjnych stanowi mechanizm odpowiedzi adaptacyjnej podczas biotycznych i abiotycznych czynników stresowych (Kiferle i in. 2021, Medrano-Macías i in. 2016). Zastosowanie jodu w niskich stężeniach spowodowało wzrost plonowania rzepaku i soi (Mao i in. 2014), biomasy szpinaku wodnego (Weng i in. 2008) (Medrano-Macías i in. 2016), szpinaku (Dai i in. 2004) (Gonzali i in. 2017) i truskawki (Li i in. 2017).

4. Hipoteza badawcza

Wanad może wspomagać proces pobierania jodu przez kukurydzę cukrową i sałatę, co zwiększy skuteczność biofortyfikacji części użytkowych tych roślin w jod.

5. Cele badawcze

Celem badań było określenie wpływu wanadu na pobieranie i dystrybucję jodu w roślinach kukurydzy cukrowej (*Zea mays* L. subsp. *mays* Grupa Saccharata) i sałaty masłowej (*Lactuca sativa* L. var. *capitata*) w zależności od zastosowania mineralnych i organicznych związków jodu. Ponadto celem badań było określenie wpływu wanadu (metawanadanu amonu), organicznych i nieorganicznych związków jodu oraz ich łącznej aplikacji (wanad wraz z jodem) na wzrost nadziemnych i podziemnych części rośliny jak i ich skład chemiczny.

6. Materiał i metody

Szczegółowe opisy metod badawczych wykorzystanych do realizacji celów badań opisanego autoreferatu zostały przedstawione w rozdziałach poszczególnych publikacji pt. „Materials and Methods". Ze względu na złożoność metod prowadzenia badań z uprawą kukurydzy cukrowej, przedstawionych w publikacjach nr 1-3 (w porównaniu do doświadczeń z uprawą sałatą – publikacje nr 4 i 5), w tym miejscu przedstawia się ogólny zarys realizacji doświadczeń z kukurydzą cukrową.

Doświadczenia polowe i wazonowe z uprawą kukurydzy cukrowej prowadzone były metodą losowych bloków w układzie zrandomizowanym w czterech powtórzeniach dla każdego obiektu badań. W doświadczeniach wazonowych (publikacja 1 i 2) na jedną kombinacje przypadało 12 roślin. W doświadczeniu, w którym kukurydzę uprawiano do fazy 5 liści właściwych BBCH 15, podłoże stanowił substrat torfowy i piasek wymieszany w stosunku 1:1 (publikacja nr 1). Z kolei w kolejnym wazonowym doświadczeniu z uprawą kukurydzy do fazy dojrzałości mlecznej kolb BBCH 75 podłoże stanowiła gleba mineralna (publikacja nr 2). W obu eksperymentach wazonowych (publikacja nr 1 i 2) aplikację metawanadanu amonu (NH_4VO_3) oraz związków jodu (KI, KIO_3, 5ISA, 2IBeA) prowadzono doglebowo. Dawka wanadu w doświadczeniu opisanym w publikacji nr 1 wynosiła 0,1 μM V, a w doświadczeniu zaprezentowanym w publikacji nr 2 zastosowano dwie dawki wanadu 0,1 i 1,0 μM V. W obydwu doświadczeniach wazonowych aplikowana dawka jodu wynosiła 10 μM I. W trzech doświadczeniach z polową uprawą kukurydzy na jedną kombinację przypadało 60 roślin tj. 4 powtórzenia po 15 roślin. Pierwszy rok badań polowych, Eksperyment nr 1 (2018 rok) jak i drugi rok badań polowych Eksperyment nr 2 (2019 rok) obejmował 15 kombinacji doświadczalnych z dwoma dawkami wanadu 0,1 i 1,0 μM V. W obu tych eksperymentach wykonano aplikacje dolistną w identycznym interwale czasowym, który stanowił 2 tygodnie od fazy BBCH 32 do BBCH 62 (4 aplikacje dolistne). Czynnikiem różnicującym oba eksperymenty była zastosowana dawka związków jodu w Eksperymencie nr 1 (2018 rok) wynosiła 10 μM I, a w Eksperymencie nr 2 wynosiła 100 μM I. W Eksperymencie nr 3 będącym częścią badań opisanych w publikacji nr 3 dolistnie aplikowano wanad w dawce 0,1 μM V i związki jodu w dawce 100 μM I w interwale czasowym wynoszącym 3 dni od fazy rozwojowej BBCH 61 (4 aplikacje dolistne).

Plan badań

Doświadczenia z uprawą kukurydzy cukrowej ‘Złota karłowa’

Wazonowa uprawa kukurydzy we wczesnym stadium rozwoju – aplikacja **DOGLEBOWA jodu i wanadu.** Dawka **jodu 10µM;** dawka **wanadu 0,1µM** .
Publikacja nr 1

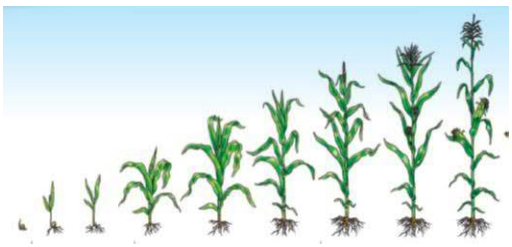
BBCH 00 15

Wazonowa uprawa kukurydzy do fazy dojrzałości zbiorczej kolb. Aplikacja **DOGLEBOWA jodu i wanadu;** dawka **jodu 10 µM;** dawka **wanadu 0,1 µM** oraz **1,0 µM.**
Publikacja nr 2

Polowa uprawa kukurydzy do fazy dojrzałości zbiorczej kolb. **DOLISTNA** aplikacja; dawka **jodu 10 µM,** dawka **wanadu 0,1µM** i **1,0 µM** .
Aplikacja **BBCH 32-61**
Eksperyment nr 1 - Publikacja nr 3

Polowa uprawa kukurydzy do fazy dojrzałości zbiorczej kolb. **DOLISTNA** aplikacja; dawka jodu **100 µM,** dawka **wanadu 0,1 µM** i **1,0 µM.**
Aplikacja **BBCH 32-61**
Eksperyment nr 2 - Publikacja nr 3

Polowa uprawa kukurydzy do fazy dojrzałości zbiorczej kolb. **DOLISTNA** aplikacja; dawka jodu **100 µM** i dawka wanadu **0,1µM.**
Aplikacja **BBCH 61-69**
Eksperyment nr 3 - Publikacja nr 3



BBCH 00 75

Doświadczenie z uprawą sałaty ‘Melodion’

Uprawa sałaty w systemie hydroponicznym CKP
Praca badawcza z uprawą sałaty realizowana była w ramach grantu NCN UMO-2017/25/B/NZ9/00312 pt. „Molekularne i fizjologiczne mechanizmy wpływu wanadu na pobieranie jodanów i jodosalicylanów przez sałatę oraz jej przeciwnowotworowe działanie testowane na ludzkich liniach komórkowych”.
Publikacja nr 4 i 5

7. Streszczenie załączonych publikacji

7.1. Publikacja nr 1

Grzanka, M., Smoleń S., Kováčik P. 2020. Effect of vanadium on the uptake and distribution of organic and inorganic forms of iodine in sweetcorn plants during early-stage development. Agronomy 10(11). doi: 10.3390/agronomy10111666.

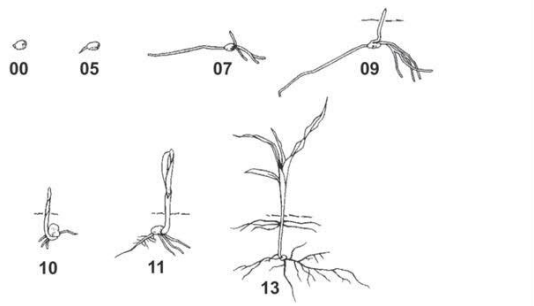
Punktacja $MNiSW_{2020}$: 100 pkt

IF_{2020}: 3,417

Celem badań w kontekście omawianej pracy doktorskiej było określenie wpływu wanadu (aplikowanego w formie metawanadanu amonu) na pobieranie i dystrybucję jodu w roślinach kukurydzy cukrowej (*Zea mays* L. subsp. *mays* Grupa Saccharata) 'Złota Karłowa' po zastosowaniu organicznych (kwas 5-jodosalicylowy /5ISA/ oraz kwas 2 jodobenzoesowy /2IBeA/) i nieorganicznych (jodek potasu/ KI/ oraz jodan potasu /KIO_3/) związków jodu. Układ badawczy składał się z 10 kombinacji, z doglebową aplikacją związków jodu. Od 7 dni po przesadzeniu roślin (BBCH 12) do zbioru roślin (BBCH 15) wykonano 4 aplikacje doglebowe (po 100 ml każda roślina) w odstępach 3 dniowych. Doświadczenie zostało powtórzone 2 krotnie.

Układ doświadczalny:

1. Kontrola (woda destylowana)
2. NH_4VO_3 (0,1µM V)
3. KI (10µM I)
4. KI+ NH_4VO_3 (10µM I +0,1µM V)
5. KIO_3 (10µM I)
6. KIO_3 + NH_4VO_3 (10µM I + 0,1µM V)
7. 5-ISA (10µM I)
8. 5-ISA + NH_4VO_3 (10µM I + 0,1µM V)
9. 2-IBeA (10µM I)
10. 2-IBeA + NH_4VO_3 (10µM I + 0,1µM V)



Metodyka obejmowała:

1. Pomiar parametrów biometrycznych, długość i masa korzeni, wysokość i masa części nadziemnej kukurydzy, oznaczenie zawartości suchej masy.

2. Pomiar aktywności wanadozależnej haloperoksydazy w liściach i korzeniach.
3. Oznaczenie zawartości jodu i wanadu oraz makro i mikroelementów (Ca, K, Mg, P, S, B, Cu, Fe, Zn, Mo) w liściach, pędach i korzeniach.

Odnotowano istotne różnice w morfologii roślin kukurydzy cukrowej po zastosowaniu związków jodu i wanadu. Parametry biometryczne to jest masa i długość korzeni oraz masa części nadziemnej kukurydzy były istotnie wyższe w kombinacjach z aplikacją związków jodu, wanadu ze sobą w stosunku do kontroli. Analiza aktywności wanadozaleznej haloperoksydazy (vHPO) wykazała jej wyższą aktywność w korzeniach niż w organach nadziemnych (liściach), z kolei poziom zróżnicowania aktywności vHPO między obiektami był najwyższy w liściach. Największą zawartość jodu w liściach uzyskano po zastosowaniu organicznej formy jodu z wanadem (2IBeA+V), a w korzeniach w połączeniu nieorganicznej formy jodu z wanadem (KIO_3+V). Otrzymane wyniki oznaczenia akumulacji jodu w liściach, pędach i korzeniach po zastosowaniu nawożenia jod + wanad dają przesłanki do stwierdzenia, że wanad zadziałał stymulująco na akumulację jodu w roślinach kukurydzy cukrowej. Niemal dwukrotnie wyższy poziom akumulacji wanadu w korzeniach niż w liściach, świadczy o słabej reutylizacji tego pierwiastka. W przypadku stanu odżywienia mineralnego roślin kukurydzy, doglebowa aplikacja jodu i wanadu wpłynęła na zmniejszenie akumulacji większości makro i mikroelementów w porównaniu do obiektu kontrolnego ale bez negatywnego wpływu na masę roślin.

7.2. Publikacja nr 2

Grzanka M., Smoleń S., Skoczylas Ł., Grzanka D. 2021. Biofortification of sweetcorn with iodine: Interaction of organic and inorganic forms of iodine combined with vanadium. Agronomy 11(9), 1720. doi: 10.3390/agronomy11091720.

Punktacja MNiSW$_{2021}$: 100 pkt
IF$_{2021}$: 3,417

W publikacji opisano badania wazonowe realizowane w tunelu foliowym na roślinach kukurydzy cukrowej (*Zea mays* L. subsp. *mays* Grupa Saccharata) 'Złota Karłowa'. Rośliny były uprawiane do fazy dojrzałości mlecznej ziarniaków (BBCH 75). Doświadczanie było wykonane w sezonie wiosenno-letnim. Eksperyment był powtórzony dwukrotnie w latach 2018 i 2019. Rośliny uprawiano w doniczkach o pojemności 7 dm^3 w glebie mineralnej. W każdej doniczce znajdowała się jedna roślina, a związki jodu i wanadu aplikowano doglebowo. Doglebową aplikację poprzez fertygację tych pierwiastków wykonywano co 7 dni od fazy rozwojowej BBCH 16-17 do fazy BBCH 75. Układ doświadczenia, ilość kombinacji, zastosowane dawki i użyte związki oraz ilość aplikacji przedstawiono w poniższej tabeli.

Tabela 1. Obiekty badań w doświadczeniu wazonowym opisane w publikacji nr 2.

Lp.	Kombinacja	Dawka jodu	Dawka* wanadu w postaci metawanadanu amonu	Ilość aplikacji I i/lub V od fazy BBCH 16-17
1	Kontrola	-**	-**	-
2	V_1		0.1 μM V	7 aplikacji
3	V_2		1.0 μM V	7 aplikacji
4	KI	10 μM I		7 aplikacji
5	KI+ V1	10 μM I	0.1 μM V	7 aplikacji
6	KI + V2	10 μM I	1.0 μM V	7 aplikacji
7	KIO_3	10 μM I		7 aplikacji
8	KIO_3 + V1	10 μM I	0.1 μM V	7 aplikacji
9	KIO_3 + V2	10 μM I	1.0 μM V	7 aplikacji
10	5ISA	10 μM I		7 aplikacji
11	5ISA + V1	10 μM I	0.1 μM V	7 aplikacji
12	5ISA + V2	10 μM I	1.0 μM V	7 aplikacji
13	2IBeA	10 μM I		7 aplikacji
14	2IBeA + V1	10 μM I	0.1 μM V	7 aplikacji
15	2IBeA + V2	10 μM I	1.0 μM V	7 aplikacji

W tych badaniach przeprowadzono następujące pomiary biometryczne i analizy:

1. Masa części nadziemnej roślin, wysokość roślin, masa i liczba kolb kukurydzy, całkowity uzyskany plon kolb.
2. W świeżym ziarnie kukurydzy (faza mleczna) oznaczono zawartość kwasu askorbinowego i zawartość cukrów.
3. Oznaczenie zawartości jodu i wanadu oraz makro i mikroelementów (Ca, K, Mg, P, S, B, Cu, Fe, Zn, Mo) wykonano w korzeniach, liściach i ziarniakach.

Zastosowane związki jodu i wanadu nie miały istotnego wpływu na plonowanie i wyszczególnione powyżej parametry biometryczne roślin kukurydzy cukrowej. Nie został stwierdzony fitotoksyczny efekt badanych związków jodu i wanadu na rośliny, jak również nie wykazano pozytywnego wpływu wanadu (metawanadanu amonu) oraz organicznych (2IBeA i 5ISA) i nieorganicznych (KI i KIO_3) związków na wzrost i rozwój roślin oraz na plonowanie kolb. Ziarniaki kukurydzy najskuteczniej były wzbogacone w jod po aplikacji związków jodoorganicznch 5ISA oraz 2IBeA (niezależnie od nawożenia wanadem). Wanad w dawce 0.1 $\mu mol \cdot dm^{-3}$ w połączeniu z nieorganicznymi formami jodu KI, KIO_3 powodował zwiększenie akumulacji jodu w liściach, korzeniach i ziarnie w porównaniu do kombinacji bez dodatku wanadu. Aplikacji 5-ISA i wanadu spowodowała wzrost akumulacji wanadu w korzeniach jak i antagonistyczny wpływ wanadu w połączeniu z 5-ISA na akumulacje jodu w korzeniach, liściach i ziarnie kukurydzy cukrowej. Łączna aplikacja wanadu w obu dawkach z 2IBeA stymulowała transport i akumulacje jodu w ziarniakach kukurydzy.

7.3. Publikacja nr 3

Grzanka, M., Smoleń S., Skoczylas Ł., Grzanka D. 2022. Synthesis of organic iodine compounds in sweetcorn under the influence of exogenous foliar application of iodine and vanadium. Molecules 27, 1822. https://doi.org/ 10.3390/molecules27061822.

Punktacja $MNiSW_{2022}$:140 pkt
IF_{2022}: 4,412

Niniejsza publikacja opisuje wyniki trzech niezależnych eksperymentów z polową uprawą kukurydzy cukrowej (*Zea mays* L. subsp. *mays* Grupa Saccharata) 'Złota Karłowa'. Badania były przeprowadzone w 2018, 2019 i 2020 roku. Związki jodu (KI, KIO_3, 5ISA oraz 2IBeA) oraz wanadu (NH_4VO_3) we wszystkich trzech eksperymentach były aplikowane dolistnie. W eksperymencie nr 1 i nr 2 wanad był zastosowany w dwóch dawkach. Dane metodyczne dotyczące aplikacji jodu i wanadu w poszczególnych trzech eksperymentach przedstawione zostały w Tabeli 2.

Tabela 2. Podstawowe informacje na temat różnic w metodologii trzech doświadczeń polowych z dolistną aplikacją jodu i wanadu w uprawie kukurydzy cukrowej.

Eksperyment/ Rok	**Prowadzone doświadczenia**						
	Dawka I	**Dawka V**	**Liczba aplikacji**	**Fazy BBCH, w których wykonano dolistną aplikację**	**Częstotliwość wykonanych aplikacji**	**Faza zbioru (BBCH)**	**Liczba kombinacji**
Eksperyment 1 / 2018	10 μM I	0.1 μM i 1.0 μM V	4	32 – 61	Co 2 tygodnie	75	15
Eksperyment 2 / 2019	100 μM I	0.1 μM i 1.0 μM V	4	32 – 61	Co 2 tygodnie	75	15
Eksperyment 3 / 2019_2020	100 μM I	0.1 μM V	4	61 – 69	Co 3 dni	75	10

We wszystkich trzech eksperymentach przeprowadzono pomiary biometryczne długości kolb, liczbę ziaren w rzędzie oraz masę kolb. Przeprowadzono analizę zawartości jodu i wanadu w liściach i ziarniakach kukurydzy oraz zawartość witaminy C i cukrów rozpuszczalnych w ziarniakach. Ponadto ze względu na wysokie koszty analiz tylko w kolbach z eksperymentu nr 3 oznaczono zawartość jodosalicylanów (5ISA i 3,5-diISA) i jodobenzoesanów (2IBeA, 2,3,5-triIBeA) w liściach i ziarniakach kukurydzy oraz jodki i jodany tylko w ziarniakach.

Przeprowadzone trzy doświadczenia pozwoliły na określenie najefektywniejszej dawki w aplikacji dolistnej pod względem wzbogacenia ziaren kukurydzy w jod - 100 μM I, poziom wzbogacenia ziarna i liści przy dawce 10 μM I był istotnie niższy. Najlepsze efekty wzbogacenia ziaren kukurydzy w jod przy zastosowaniu dokarmiania dolistnego uzyskano wykonując zabiegi co trzy dni w fazie BBCH od 61 do 69.

W żadnym z trzech eksperymentów nie obserwowano jakichkolwiek objawów fitotoksyczności dolistnego stosowania związków jodu i wanadu na roślinach kukurydzy. W eksperymencie nr 2 i 3, w których zastosowano jod w dawce 100 μM, najwyższy całkowity poziom akumulacji tego pierwiastka w ziarnie otrzymano po dolistnym zastosowaniu organicznego związku jodu 2IBeA. Również najwyższy poziom akumulacji jodków (I^-) w ziarnie odnotowano po aplikacji 2IBeA. Zawartość jodanów (IO_3^-) w ziarnie roślin z tejże kombinacji był na poziomie porównywalnym z ziarnem z roślin dokarmianych dolistnie KIO_3. Taki efekt mógł być spowodowany metabolizmem 2IBeA do form jonów nieorganicznych (I^- i IO_3^-). Szlaki metaboliczne takich reakcji w roślinach nie zostały dotychczas opisane w literaturze.

Stymulujący wpływ wanadu na akumulacje jodu w liściach (ale nie w ziarnie) został zaobserwowany w połączeniu aplikacji tego pierwiastka z KI oraz KIO_3. Zastosowanie dolistnie roztworów zawierających KI, KIO_3 i 5ISA z metawanadanem amonu powodowało tendencję do obniżenia zawartości 2,3,5-triIBeA w liściach i ziarniakach.

Uzyskane wyniki wzbogacania ziarna kukurydzy wskazały na możliwość opracowania skutecznych agrotechnicznych metod dolistnej biofortyfikacji ziaren kukurydzy w jod dla praktyki rolniczej i ogrodniczej.

7.4. Publikacja nr 4

Smoleń, S., Kowalska I., Halka M., Ledwożyw-Smoleń I., **Grzanka M.**, Skoczylas Ł., Czernicka M., Pitala J. 2020. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10(1), 1-21 doi: 10.3390/agronomy10010001.

Punktacja $MNiSW_{2020}$: 100 pkt

IF_{2020}: 3,417

W publikacji zostały opisane cztery niezależne doświadczenia na sałacie (*Lactuca sativa* L. var. *capitata* 'Melodion' cv.) uprawianej w systemie hydroponicznym CKP. Badania miały na celu określenie wpływu wanadu w formie metawanadanu amonu (NH_4VO_3) na pobieranie, akumulacje i metabolizm jodu. Układ doświadczeń, zastosowane dawki i związki jodu zamieszczono w Tabeli 3.

Tabela 3. Obiekty badań w czterech doświadczeniach na sałacie w systemie hydroponicznym opisanych w publikacji nr 4.

Numer eksperymentu	Zastosowana forma oraz dawka jodu (μM I) w pożywce	Zastosowana dawka wanadu - /NH_4VO_3/ (μM V) w pożywce
Nr 1	Bez jodu - Kontrola (śladowe ilości 0.0204 μM I)	1. 0 (kontrola) 2. 0,05 3. 0,1 4. 0,4
Nr 2	KIO_3 10 μM (10 μM I)	1. 0 (kontrola) 2. 0,05 3. 0,1 4. 0,4
Nr 3	5ISA 10 μM (10 μM I)	1. 0 (kontrola) 2. 0,05 3. 0,1 4. 0,4
Nr 4	3,5-diISA 10 μM (20 μM I)	1. 0 (kontrola) 2. 0,05 3. 0,1 4. 0,4

We wszystkich czterech eksperymentach przeprowadzono ocenę plonowania oraz analizy chemiczne główki (liści) i korzeni sałaty. Oznaczono między innymi aktywność wanadozależnej haloperoksydazy (vHPO), oznaczono zawartość jodu i wanadu w liściach i korzeniach oraz oznaczono zawartość kwasu salicylowego (SA), kwasu benzoesowego (BeA), jodosalicylanów, jodobenzoesanów i trijodotyroniny (T3) w roślinach.

W obiekcie kontrolnym, w którym w pożywce były śladowe ilości jodu, stwierdzono stymulujący wpływ wrastających dawek wanadu na akumulacje jodu w korzeniach sałaty. Stymulujący efekt wanadu został stwierdzony również w korzeniach w eksperymencie nr 2 z zastosowaniem KIO_3 (najefektywniejsza dawka V 0,10μM) oraz w eksperymencie nr 4 z wprowadzeniem do pożywki 3,5-diISA z wanadem w dawce 0,10μM. Ponadto obserwowano, wzrost zawartości jodu w liściach roślin z kombinacji 3,5-diISA + 0,10 μM V oraz 3,5-diISA + 0,40 μM V vs 3,5-diISA. Wanad w żadnej z zastosowanych dawek nie miał wpływu na wielkość, biomasę roślin sałaty. Aplikowane jodosalicylany 5ISA oraz 3,5-diISA były tak efektywnie pobrane przez rośliny sałaty, że powodowały istotny (7-krotny i 9- krotny) spadek biomasy w porównaniu z kontrolą i nawożeniem roślin KIO_3.

Aktywność vHPO wzrastała w korzeniach sałaty w obecności nieorganicznego związku jodu (IO_3^-) w pożywce. Mechanizmy regulujące procesy dystrybucji, pobierania oraz metabolizmu jakim podlegały zastosowane w dwóch eksperymentach jodosalicylany nie były powiązane z poziomem aktywności enzymu vHPO. Przedstawione wyniki badań w niniejszej pracy pozwoliły udowodnić, że 5ISA oraz 3,5-diISA są związkami –endogennymi - fizjologicznie obecnymi w roślinach sałaty. Z kolei egzogenne ich zastosowanie może zwiększyć syntezę związków jodoorganicznych przez rośliny. Nawożenie roślin 5ISA i 3,5-diISA ukierunkowało metabolizm jodu w roślinach na syntezę T3 w liściach. W obecności śladowych ilości jodu w obiekcie kontrolnym, a także dla jodu stosowanego jako KIO_3, procesy przekształcenia jodosalicylanów do T3 były bardziej wydajne w korzeniach niż w liściach. Zastosowanie egzogennego KIO_3 aktywowało inne szlaki metaboliczne w roślinach sałaty, w tym prawdopodobnie metylację jodu, w stosunku do aplikacji egzogennych 5ISA i 3,5-diISA.

7.5. Publikacja nr 5

Smoleń S., Czernicka M., Kowalska I., Kęska K., Halka M., Grzebelus D., **Grzanka M.**, Skoczylas Ł., Pitala J., Koronowicz A., Kováčik P. 2021 New aspects of uptake and metabolism of non-organic and organic iodine compounds—the role of vanadium and plant-derived thyroid hormone analogs in lettuce. Frontiers in Plant Science 12, 608. doi: 10.3389/fpls.2021.653168.

Punktacja $MNiSW_{2021}$: 100 pkt

IF_{2021}: 5,753

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Niniejsza publikacja opisuje dwuletnie badania z trzema niezależnymi eksperymentami z uprawą sałaty *Lactuca sativa* L. var. *capitata* cv. "Melodion" (tabela 4 i 5). Przeprowadzono dwa niezależne doświadczenia wazonowe, z uprawą sałaty w substracie torfowym i glebie mineralnej. Trzeci eksperyment polegał na uprawie sałaty w systemie hydroponicznym CKP. W tych trzech doświadczeniach aplikowano trzy różne związki jodu: KIO_3 (jodan potasu), 5ISA (kwas 5-jodosalicylowy) i 3,5-diISA (kwas 3,5-dijodosalicylowy), a ponadto SA (kwas salicylowy) oraz NH_4VO_3 (metawanadan amonu).

Tabela 4. Podstawowe informacje na temat metodologii badań w trzech eksperymentach w uprawie sałaty.

Eksperyment	**Prowadzone doświadczenia**					
	Typ doświadczenia	**Typ uprawy**	**Liczba aplikacji**	**Częstotliwość aplikacji**	**Faza zbioru (BBCH)**	**Liczba kombinacji**
Eksperyment 1	hydroponika	Bezglebowa upraw	Ciągłe dostarczanie testowanych związków z pożywką	w sposób ciągły	49	10
Eksperyment 2	wazonowe	Substrat torfowy	8 aplikacji doglebowych	Co 7 dni	49	10
Eksperyment 3	wazonowe	Gleba mineralna	8 aplikacji doglebowych	Co 7 dni	49	10

Tabela 5. Obiekty badań w trzech doświadczeniach opisanych w publikacji nr 5.

Lp.	Kombinacja	Dawka jodu	Dawka wanadu	Dawka SA
1	Kontrola	-	-	
2	SA	-	-	10 μM
3	KIO_3	10 μM	-	
4	KIO_3 + SA	10 μM	-	10 μM
5	5ISA	10 μM	-	
6	3,5-diISA	10 μM	-	
7	KIO_3 + V	10 μM	0.1 μM V	
8	KIO_3 + SA + V	10 μM	0.1 μM V	10 μM
9	5ISA + V	10 μM	0.1 μM V	
10	3,5-diISA + V	10 μM	0.1 μM V	

We wszystkich trzech eksperymentach przeprowadzono ocenę plonowania główek (liści) sałaty, a w eksperymencie nr 1 z hydroponiczną uprawą również masy korzeni. We wszystkich eksperymentach oznaczono aktywność wanadozależnej haloperoksydazy, zawartość jodu i wanadu w liściach oraz korzeniach, ale tylko w eksperymencie nr 1. W eksperymencie nr 1 wykonano analizę ekspresji genów w liściach i korzeniach metodą Real-time qPCR jak również oznaczano zawartość jodków, jodanów, kwasów organicznych (SA, BeA) i metabolitów jodu (5ISA, 2IBeA, 3,5-diISA, 4IBeA 2,3,5-triIBeA) w soku z parcia korzeniowego oraz w liściach i korzeniach sałaty.

Badania zaprezentowane w tejże pracy opisują przebieg przemian metabolicznych organicznych związków jodu – 5ISA oraz 3,5- diISA w roślinach. Zastosowane jodosalicylany ulegały wewnątrz roślinnej degradacji do jonów I^- lub służyły jako prekursory syntezy trijodotyroniny (T3) i tyroksyny (T4), które są klasyfikowane jako PDTHA (analogi hormonów tarczycy pochodzenia roślinnego). Analiza genetyczna wykazała wyższy poziom ekspresji genu *per64-like* w korzeniach niż w liściach, była skorelowane z wyższą koncentracja wanadu w korzeniach jak i aktywnością vHPO enzymem biorącym udział w procesie pobierania jodu. W korzeniach sałaty po egzogennej aplikacji jodosalicylanów stwierdzono nadekspresje genu *msams5*, który można połączyć z funkcją enzymów HMT/HTMT. Ekspresja genu *samdmt*, była zawiązana z genem kodującym enzym powodujący ulatnianie się (metylacją) kwasu etylosalicylowego – wyższy poziom ekspresji tego genu odnotowano w liściach niż korzeniach. We wszystkich doświadczeniach najwyższą zawartość jodu w liściach sałaty stwierdzono po aplikacji 5ISA.

Aplikacja jodosalicylanów w eksperymencie wazonowym poprzez fertygację wykazała wyższą efektywność wzbogacenia sałaty w jod niż zastosowanie KIO_3. Okazała się

ona bezpieczna dla roślin jak i konsumentów, wniosek na podstawie obliczeń teoretycznych. Sałata uprawiana w substracie torfowym charakteryzowała się najniższą zawartością jodu. Wanad dodany do pożywki spowodował wzrost akumulacji jodu w liściach sałaty w kombinacji 3,5-diISA+V vs. 3,5-diISA w eksperymencie nr 2 i 3 oraz w korzeniach sałaty 3,5-diISA+V vs. 3,5-diISA oraz 5ISA +V vs. 5ISA w eksperymencie nr 1. Wanad akumulowany był głównie w korzeniach sałaty i był w małym stopniu transportowany do liści.

8. Podsumowanie i wnioski

Przeprowadzone badanie w ramach rozprawy doktorskiej pozwoliły, na wyciągniecie następujących wniosków:

1. Stwierdzono zróżnicowany wpływ organicznych i nieorganicznych związków jodu zastosowanych pojedynczo jak i w połączeniu z wanadem na wzrost kukurydzy cukrowej we wczesnych fazach rozwojowych. Wykazano pozytywny wpływ aplikacji jodu i wanadu na rozwój systemu korzeniowego roślin kukurydzy cukrowej.
2. Wszystkie prowadzone badania (w których przebadano skład chemiczny systemu korzeniowego kukurydzy i sałaty) publikacja 1, 2, 4, 5 potwierdziły słabą reutylizacje wanadu z korzeni do nadziemnych części roślin: liści i ziarna kukurydzy oraz liści (główek) sałaty. Najwyższy poziom akumulacji wanadu był w korzeniach zarówno w przypadku kukurydzy jak i sałaty. Było to również skorelowane z wyższą aktywnością enzymu vHPO, w korzeniach niż w liściach: w kukurydzy we wczesnej fazie rozwoju (publikacja 1) jak i w korzeniach sałaty uprawianej w systemie hydroponicznym (publikacja 4 i 5).
3. Efektywniejsze wzbogacenie w jod ziarniaków kukurydzy uzyskano po aplikacji jodoorganicznego związku 2IBeA (Publikacja 2 i 3). Efekt ten był obserwowany w przypadku nawożenia doglebowego w doświadczeniu wazonowym oraz przy dolistnej aplikacji w doświadczeniu polowym. Dolistna aplikacja 2IBeA w dawce 100 μM (w fazie rozwojowej kukurydzy BBCH od 61 do 69), z krótszym interwałem czasowym pomiędzy kolejnymi aplikacjami, była najbardziej skuteczną metodą biofortyfikacji ziarna kukurydzy w jod. Stanowi to podstawę do stworzenia efektywnego programu biofortyfikowania kukurydzy w jodu w praktyce rolniczej i ogrodniczej.
4. Zastosowane jodosalicylany (5ISA, 3,5-diISA) w uprawie hydroponicznej sałaty okazały się zacznie efektywniejsze w procesie wzbogacenia liści w jod niż KIO_3. Jednakże 5ISA : 3,5-diISA spowodowały efekt osłabienia wzrostu roślin sałaty oraz przekroczenie wskaźnika bezpieczeństwa akumulacji jodu dla konsumenta (HQ > 1,0)

przy zastosowanej w hydroponice dawce (10 μM I w formie 5ISA i 3,5-diISA). Tego negatywnego efektu nie obserwowano w przypadku aplikacji 5ISA i 3,5-diISA w dawce 10 μM I pod rośliny sałaty w uprawie na glebie mineralnej i substracie torfowym w doświadczeniu wazonowym.

5. W przypadku sałaty i kukurydzy, dawka wanadu 0,1 μM zwiększyła poziom akumulacji jodu w liściach kukurydzy i sałaty. Wskazuje to na synergistyczny wpływ wanadu na akumulację jodu w roślinach sałaty i kukurydzy.
6. Stwierdzono, że 5-ISA, 3,5-diISA i T3 są naturalnie syntetyzowane w roślinach sałaty, a ich zawartość ulega zwiększeniu po egzogennym zastosowaniu 5-ISA, 3,5-diISA. Wykazano, że jodosalicylany mogą uczestniczyć w szlaku biosyntezy T3 – oraz innych związków z grupy PDTHA.
7. Odnotowano pozytywny wpływ wanadu na akumulację/syntezę odpowiednio egzogennego/endogennego kwasu 5-jodosalicylowego (5ISA) i kwasu 2-jodobenzoesowego (2IBeA). Wanad powodował natomiast obniżenie zawartości kwasu 2,3,5-trijodobenzoesowego (2,3,5-triIBeA – inhibitor auksyny) w liściach i ziarnach kukurydzy.

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10. Wydruki publikacji wchodzących w skład rozprawy doktorskiej

agronomy

MDPI

Article

Effect of Vanadium on the Uptake and Distribution of Organic and Inorganic Forms of Iodine in Sweetcorn Plants during Early-Stage Development

Marlena Grzanka [1,*], Sylwester Smoleń [1] and Peter Kováčik [2]

[1] Unit of Plant Nutrition, Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 31-425 Kraków, Poland; sylwester.smolen@urk.edu.pl

[2] Department of Agrochemistry and Plant Nutrition, Slovak University of Agriculture in Nitra—Slovenska Posnohospodarska Univerzita v Nitre, 94976 Nitra-Chrenová, Slovakia; peter.kovacik@uniag.sk

* Correspondence: marlena.grzanka@student.urk.edu.pl

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Abstract: Iodine and vanadium are elements that are closely related to organisms in water environments. Iodine and vanadium are known as "beneficial elements" that stimulate the growth and development of higher plants. Iodine is an essential element for the synthesis of the thyroid hormones triiodothyronine and thyroxine in the human body, with vanadium also known to be involved in the synthesis of thyroid hormones. The cooperation of both elements in the human body and in algae presents a question regarding the impact of vanadium interaction on iodine uptake in higher plants. The absorption of iodine from seawater in algae is known to be more efficient in the presence of vanadium, with key role in this process played by the iodoperoxidase enzyme, with vanadium acting as a cofactor. The study of the nature of the absorption of iodine by higher plants, and in particular by crops such as corn, remains insufficiently studied. The aim of this study was to investigate the effect of vanadium on iodine uptake via vanadium-dependent iodoperoxidase (vHPO) activity in sweetcorn plants (*Zea mays* L. subsp. Mays Saccharata Group) "Złota Karłowa". The experiment was carried out with organic and inorganic iodine compounds, namely potassium iodide (KI), potassium iodate (KIO_3), 5-iodosalicylic acid (5-ISA), and 2-iodobenzoic acid (2-IBeA), each used in a dose of 10 μM. These compounds were applied with and without vanadium in the form of ammonium methavanadate (NH_4VO_3) at a dose of 0.1 μM. A double control was used, the first without iodine and vanadium and the second with vanadium but without iodine. Root length, root mass, and above-ground weight were significantly higher after iodine and vanadium compared to controls. Plants were collected at the five true leaf stage. vHPO activity level was much higher in the roots than in the leaves, but greater variation in the leaves was observed between treatments in terms of vHPO activity. Vanadium was shown to accumulate in the roots. The use of a relatively low dose of vanadium may have caused changes in the accumulation of this element in the aerial parts of the plant, leaves, and shoots. Fertilization with iodine and vanadium compounds decreased the accumulation of most minerals, macroelements, and microelements compared to controls. The obtained results of iodine accumulation in individual parts after applying iodine and vanadium fertilization testify to the stimulating effect of vanadium on iodine uptake and accumulation.

Keywords: iodine; vanadium; vanadium-dependent haloperoxidases; beneficial elements; stimulating effect; biofortification

1. Introduction

Around two billion people in the World suffer from health issues related to iodine deficiency in their diets [1,2]. The problem of insufficient amount of iodine in the diet concerns areas with a high degree of economic development and less economically developed countries. The recommended daily iodine intake through the diet ranges between 90–250 μg. Iodine is responsible for proper functioning of the thyroid gland by synthesizing thyroxine (T4) and triiodothyronine (T3). Proper iodine concentrations in pregnant women protect against complications in the prenatal period (mental retardation), miscarriage, and infertility [3]. Multilateral organizations, such as WHO (World Health Organization), UNICEF (United Nations Children's Fund), ICCIDDs (International Council for Control of Iodine Deficiency Disorders) have been looking for effective and practical methods to introduce iodine into the human diet for over twenty years [4]. Table salt iodination has so far been the only cost-effective, common solution to supply this essential element to many households [4]. The process of biofortification of plants with iodine (as well as other elements, vitamins, and nutraceuticals) has become an alternative, effective, and cost-effective solution. Agro technical methods of enriching crops with iodine have become the subject of many scientific studies. Previous research on the effectiveness of enriching crop plants with iodine was conducted on several vegetable species, including lettuce [5,6], spinach [7,8], tomato [9], cucumber [10], carrot, celery [11], and potato [12]. Research was also carried out on the efficiency of rice grain enrichment [13] of wheat, corn [14], and buckwheat [15]. In this experiment, an inorganic form of iodine KI and KIO_3 was used for effective iodine biofortification. Halka et al. [16] used an organic form of iodine 5-iodosalicylic acid, 3,5-diiodosalicylic acid, 2-iodobenzoic acid, 4-iodobenzoic acid, and 2,3,5-triiodobenzoic acid to enrich a tomato plant with iodine. Sularz et al. [17] used 5-iodosalicylic acid and 3,5-diiodosalicylic acid for the biofortificaation of the lettuce. To achieve the most effective method of biofortification researchers conduct their experiments by applying iodine compounds to hydroponic culture systems [12], soil, [14] or by foliar spraying of plants [14,18].

The largest reservoir of iodine on Earth is seawater and the organisms living in this environment [19]. Brown algae species are the most efficient iodine accumulators, with an average content of 1% dry matter in *Laminaria digitata* [20]. Iodine uptake by seaweed *L. digitata* involves extracellular iodide oxidation via vanadium-dependent haloperoxidase (vHPO), among other enzymes [21]. In seaweed organisms, one function of iodine is its participation in antioxidant mechanisms that protect capsid and thallus surfaces against oxidative stress [22]. vHPO enzymes play central roles in both iodine uptake from seawater and in the synthesis of volatile hydrogen halides in marine algae [23,24].

Vanadium occurs in several oxidation levels from −1 to +5 [25]. The result of rock leaching and natural volcanic eruption causes distribution of vanadium in the soil and air. It is released into the atmosphere during coal combustion processes and production of petroleum and is considered to be a fertilizer pollution used in agriculture [25,26]. In higher plants, vanadium still arouses the interest of scientists and researchers. The uptake and distribution of vanadium to above-ground plant parts occurs to a small extent. Roots are characterized by the highest degree of accumulation of vanadium [27,28], with vanadium compounds inhibiting plant proton pumps in plasma membranes [21,29], which act as osmotic regulators in cells, i.e., by regulating intracellular pH, response to stress conditions, and mineral deficiencies [30]. The application of high doses of vanadium may cause inhibition of macronutrient uptake and accumulation, resulting in deficiencies. Critically low Ca concentrations were observed in vanadium-treated beans [27], with excess vanadium potentially inhibiting phosphorus uptake, transport, and accumulation in aerial plant parts. Doses above 20 mg $V \cdot kg^{-1}$ soil resulted in these phenomena [31]. Studies conducted on mint by [32] did not show a radical decrease in Ca content in plants after vanadium fertilization.

Daily vanadium intake for humans ranges from 10 to 160 μg [33–35], and is found in mushrooms, parsley, pepper [35–37], seafood, fresh fish [38], beer and wine, spinach, and fennel seeds [39]. Organic and inorganic vanadium compounds help to maintain glucose homeostasis (balance) in type 1 and type 2 diabetes mellitus due to gene expression of enzymes involved in glucose and lipid metabolism [34,39,40]. Vanadium also participates in bone and tooth metabolism and acts as an enzyme cofactor [41], further regulating the action of (Na, K)-ATPase,

phosphotransferase, adenyl cycadadase, and protein kinase [41,42]. An important aspect is the dose and type of vanadium compound used [39]. Vanadium, like iodine, is involved in the metabolism of thyroid hormones [41,43].

Vanadium and iodine are known as beneficial elements for humans and animals, but also for higher plants. The benefits of iodine were supported by many studies on its functions and transformations in plants [24,44,45]. Classification of vanadium as a beneficial element for higher plants was based on research proving its participation in photosynthesis and the metabolism of nitrogen compounds [27,31]. Experiments carried out with legumes confirmed the effectiveness of vanadium and the possibility of replacing molybdenum with vanadium in nitrogen transformations and atmospheric nitrogen fixation [31] as part of the nitrogenase enzyme [46]. Vanadium is mainly found in algae, where it is part of the vanadium-dependent peroxidase enzyme responsible for iodine uptake and accumulation in marine algae tissues [20].

Previous studies conducted on enriching crop plants with iodine (biofortification) showed that vegetable leaves are characterized by higher accumulation and efficiency of iodine biofortification compared to the generative parts of plants [47]. Iodine is transported mainly by xylem [48,49]. This transport route could be an obstacle to the effective enrichment of this element in cereal grains. Mineral elements collected and accumulated in grain are transported by phloem [13], with research results showing that iodine transport through phloem is also possible [14,35]. Based on the many researches and searching for the most effective way of iodine biofortification we based on combination of iodine and vanadium as in *L. digitata*. Vanadium is as a metal ion which readily converts among oxidations states, has the potential to support catalytic processes through oxidation/reduction [50,51]. Mechanisms of iodine uptake and volatilization from cells by *L. digitata* mediated by vanadium-dependent iodoperoxidase [20].

The impact of vanadium on iodine uptake by higher plants, including crops, is not yet determined; therefore, the effect of simultaneous fertilization with iodine and vanadium on sweetcorn plants at an early developmental stage should be determined. The diet of several billion people in the world is based on cereals, including corn. Research carried out regarding iodine and vanadium uptake by sweetcorn plants may help in the development of the iodine enrichment (biofortification) process of this plant.

The aim of this study was to determine the effect of vanadium on the uptake and distribution of iodine in sweetcorn plants at an early vegetative stage of their growth. The hypothesis of this research was that vanadium would stimulate iodine uptake in sweetcorn plants, with the key role of this process played by the iodoperoxidase enzyme with vanadium acting as a cofactor. Next, vanadium and iodine fertilization could modify the uptake of macro- and microelements by plants.

2. Materials and Methods

2.1. Plant Material and Cultivation

The experiment was carried out with sweetcorn (*Zea mays* L. subsp. Mays Saccharata Group) "Złota Karłowa" and conducted by the Faculty of Biotechnology and Horticulture, University of Agriculture in Kraków. Seeds were sown into multipallets (propagation trays: 330 × 520 × 40 mm) with cells (32 × 32 × 40 mm in size) filled with sphagnum peat moss substrate (Hartmann) mixed with sand (1:1). Seedlings at the one true leaf phase were transplanted into 1.5 dm^3 pots and filled with peat substrate. Plants were cultivated in 4 replications of 3 plants (12 plants per combination of one plant per pot; see Figure 1). The total number of plants in the experiment was 120. The plants were grown in a phytotron. During cultivation, the plants were illuminated with a 600 W high-pressure sodium lamp (photosynthetic photon flux density /PPFD/) reaching the plants was approximately 200 μmol m^{-2} s^{-1}), maintaining a photoperiod of 10 h of light: 14 h of darkness. The air temperature was 25 °C during the day and 20 °C at night. The experiment was conducted twice. Organization of the pots in the phytotron was randomized.

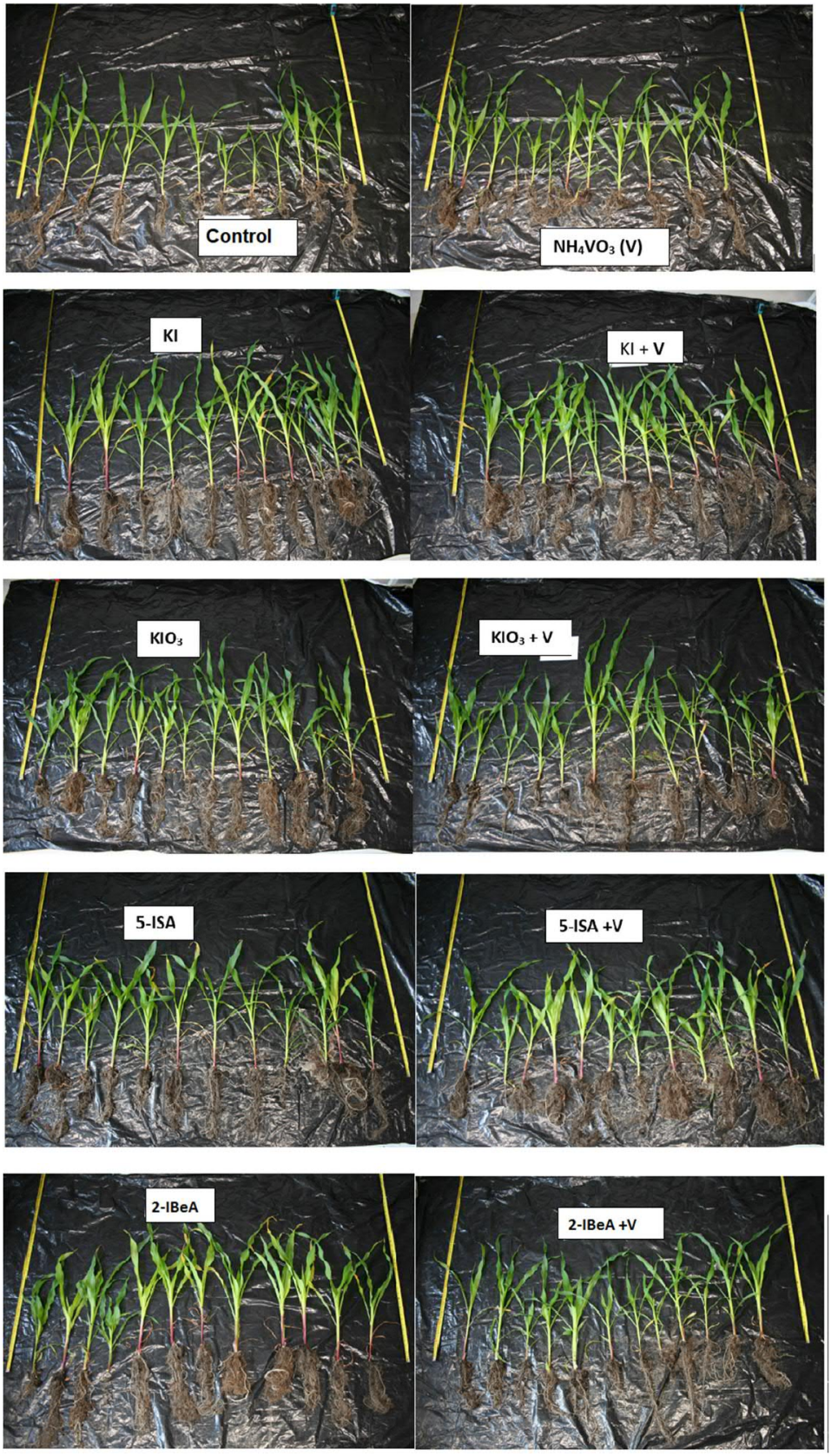


Figure 1. Sweet corn plants after harvest in BBCH 15 the developmental phase.

The study plan included ten combinations of plant treatment with various iodine compounds, including potassium iodide (KI), potassium iodate (KIO_3), 5-iodosalicylic acid (5-ISA), and 2-iodobenzoic acid (2-IBeA) and vanadium in the form of ammonium methanadate (NH_4VO_3) (Table 1). Chosen dose of vanadium and iodine, 10 μM iodine and 0.1 μM vanadium was based on preliminary research, which was done to prepare a two-cycle experiment in phytotron. The main aim was to adjust a safe dose of vanadium and iodine for plants.

Table 1. Doses of applied compounds (μM).

	Dose of Applied Compounds (μM)	
Treatments	**Iodine**	**Vanadium**
Control	-	-
V	-	0.1 μM
KI	10 μM	-
KI+V	10 μM	0.1 μM
KIO_3	10 μM	-
KIO_3+V	10 μM	0.1 μM
5-ISA	10 μM	-
5-ISA+V	10 μM	0.1 μM
2-IBeA	10 μM	-
2-IBeA+V	10 μM	0.1 μM

Compounds of iodine and vanadium were applied four times by fertigation. The fertigation process started on day 7 post-transplantation of the plants into the pots. The time between applications was 3 days (two true leaf stage). A single application dose for one pot (one plant) involved adding 100 mL of each tested solution. The plants were harvested 4 weeks after sowing (five true leaf stage—in BBCH 15 the developmental phase). The experiment was conducted twice, with biometric measurements made during the harvest. Root length and weight as well as height were determined alongside the mass of aerial plant parts. Roots and aerial parts (leaves and stems) were washed in tap water and then distilled water. The second part of preparation for chemical analysis involved chopping these parts up into fragments of about 1–2 cm.

Calculation of iodine or vanadium uptake by plants was performed according to the formula (average of iodine or vanadium concentration $mg \cdot kg^{-1}$ d.w. × total dry weight content in each plant parts this is leaves, roots or stems separately: 1000 = results in μg of I or V·part of $plant^{-1}$).

2.2. Analysis of Fresh Plant Material Sample

The analysis of the total activity of vanadium-dependent haloperoxidases enzymes (vHPO) was determined based on the procedure described by Smoleń et al. [52].

2.3. Analysis of Dry Samples of Roots, Stems, and Leaves

Fresh samples of roots, shoots, and leaves were dried at 70 °C (48 h) in a laboratory dryer with forced air circulation. Dried samples of leaves, roots, and stems were ground in a laboratory mill and stored in a plastic bag until the analysis of iodine, vanadium, macroelement, and microelement contents was carried out.

To determine iodine content, the PN-EN 15111-2008 method was used with the modifications described by Smoleń et al. [53].

The concentrations of V, P, K, Mg, Ca, S, B, Cu, Fe, Mn, Mo, and Zn were determined using the ICP-OES spectrophotometer after microwave digestion in 65% super pure HNO_3. Plant samples of 0.5 g of dry material were placed in 55 mL TFM modified polytetrafluoroethylene (PTFE) vessels and digested in 10 mL of 65% HNO_3 using a CEM MARS-5 Xpress microwave digestion system [54].

2.4. Data Analysis

All data were statistically verified using the one-way analysis of variance (ANOVA) module of the Statistica 12 PL program at a significance level of $\alpha = 0.05$. The significance of differences between the means was estimated using Tukey's test at the assumed significance level of $\alpha = 0.05$.

3. Results

3.1. Plant Growth

Tested treatments of iodine and vanadium fertilization compared to the control (without iodine and vanadium) statistically increased root length and weight, except for root weight after application of V and KIO_3 (Table 2). In the aerial parts of the plants, height was significantly greater than the control after fertilization with KI, KI+V, and KIO_3, and significantly less than the control after application of 2-IBeA+V. Limiting plant growth after 2-IBeA+V demonstrated no negative effect on aerial plant parts, which was similar to what was observed in the control. Significantly higher aerial plant parts were noted after the application of KI, KI+V, KIO_3, KIO_3+V, 5-ISA, 5-ISA+V, and 2-IBeA compared to the control. The application of KIO_3 resulted in a statistically increased shoot/root ratio compared to the application of 5ISA, 5ISA+V, and 2IBeA. Fertilization vanadium and iodine did not significantly affect shoot/root ratio compared to the control.

Table 2. Root length and weight and of the aerial part's height and weight of sweetcorn plants at an early stage of development.

Treatment	Root Length (cm)	Plant Height (Aerial Part of the Plant) (cm)	Root Weight (g)	Plant Weight (Aerial Part of the Plant) (g)	Shoot/Root Ratio
Control	12.08 ± 1.39 ^{a}	58.17 ± 0.64 ^{b,c}	5.11 ± 0.71 ^{a}	26.82 ± 2.21 ^{a}	5.1 ± 0.61 ^{a,b,c}
V	16.83 ± 1.75 ^{b}	58.17 ± 1.37 ^{b,c}	7.17 ± 1.15 ^{a,b}	34.43 ± 4.42 ^{a,b}	5.2 ± 0.88 ^{a,b,c}
KI	22.50 ± 2.10 ^{c,d}	61.50 ± 1.95 ^{d}	14.37 ± 3.21 ^{c,d}	54.06 ± 5.98 ^{c,d}	5.1 ± 1.20 ^{a,b,c}
KI+V	16.46 ± 2.53 ^{b}	65.50 ± 1.00 ^{e}	14.52 ± 3.43 ^{c,d}	62.27 ± 5.56 ^{d}	6.0 ± 1.38 ^{b,c}
KIO_3	17.58 ± 2.94 ^{b}	62.33 ± 1.36 ^{d}	10.07 ± 2.33 ^{a,b,c}	50.65 ± 6.51 ^{c,d}	7.0 ± 1.42 ^{c}
KIO_3+V	20.83 ± 1.71 ^{c,d}	57.92 ± 2.29 ^{b}	8.52 ± 1.03 ^{a,b,c}	43.48 ± 5.69 ^{b,c}	5.0 ± 0.50 ^{a,b,c}
5-ISA	20.33 ± 1.92 ^{c}	60.96 ± 2.19 ^{c,d}	12.61 ± 1.50 ^{b,c,d}	40.14 ± 2.71 ^{b,c}	3.2 ± 0.32 ^{a,b}
5-ISA+V	26.83 ± 0.37 ^{e}	59.50 ± 1.98 ^{b,c,d}	18.95 ± 2.13 ^{d,e}	49.95 ± 6.04 ^{c,d}	2.7 ± 0.23 ^{a}
2-IBeA	23.00 ± 1.49 ^{d}	57.38 ± 3.30 ^{b}	23.02 ± 5.36 ^{e}	44.45 ± 8.08 ^{b,c}	2.5 ± 0.36 ^{a}
2-IBeA+V	21.33 ± 2.18 ^{c,d}	49.00 ± 3.43 ^{a}	9.24 ± 1.48 ^{a,b,c}	30.69 ± 3.48 ^{a,b}	4.0 ± 0.94 ^{a,b,c}

Identical letters in superscript indicate the means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$($n = 8$). The developmental phase of maize: BBCH 15.

Plants after application of 5-ISA+V demonstrated the most developed root system, with roots twice as long as those observed in the control. Roots of plants treated with 5-ISA+V resulted in a mass three times greater than the control (Table 2). However, the largest root mass, more than four times greater than the control, was found after the application of 2-IBeA. Nevertheless, the greatest mass and highest aerial plant parts were obtained after fertilization with KI+V maize (Table 2).

3.2. Uptake and Accumulation of Iodine in Maize Plants

Fertigation of organic and inorganic iodine compounds significantly increased the iodine contents of roots, shoots, and leaves of maize plants compared to the control (Figure 2A–C). Vanadium fertilization combined with organic and inorganic iodine compounds showed a statistically significant increase in the iodine contents of the roots, shoots, and leaves in comparison with KI, KIO_3, 5-ISA, and 2-IBeA application without vanadium (Figure 2A–C). Fertilization of vanadium (without iodine) compared to the control showed a significant increase in corn root, shoot, and leaf iodine contents.

Regardless of the type of iodine compound fertilization, the highest iodine content was observed in the roots, followed by the shoots and, lastly, the leaves (Figure 2A–C). The highest contents of

iodine were found after KIO_3+V fertilization in roots and shoots, and in leaves after the 2-IBeA+V application.

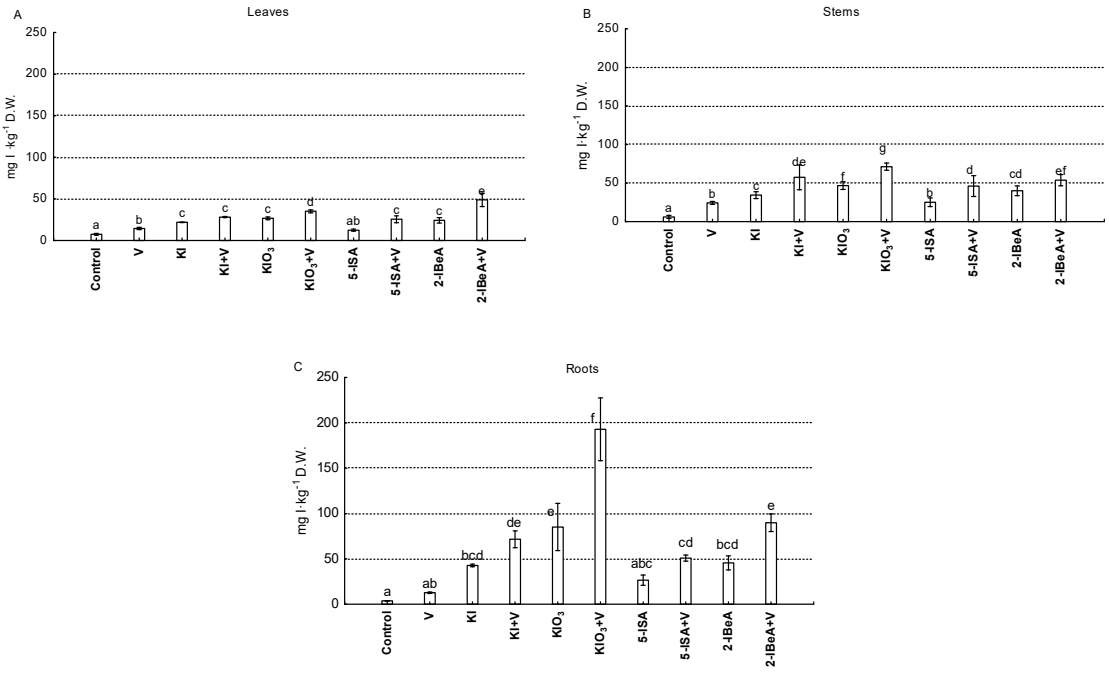
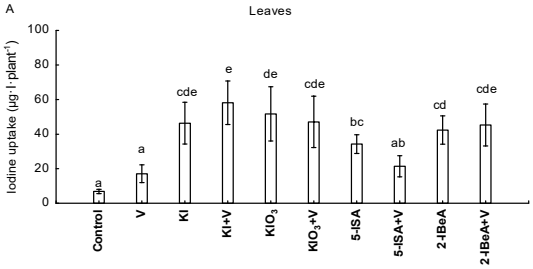


Figure 2. Iodine contents in leaves (**A**), shoots (**B**), and roots (**C**) of maize. Identical letters in superscript indicate means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$ ($n = 8$). The developmental phase of maize: BBCH 15.

Iodine uptake by roots, shoots, and leaves (Figure 3A–C) was different from the results determined by iodine content (Figure 2), probably because the iodine uptake was calculated based on yield, whereas iodine contents in the roots, shoots, and leaves was calculated using dry matter content data (Supplementary Materials Table S1). Plant fertilization with iodine and vanadium compounds (compared to without vanadium) caused a significant increase in iodine uptake by the roots, shoots, and leaves of plants fertilized with KI+V versus KI (Figure 3A–C). The results show the use of KIO_3+V, 5-ISA+V, and 2-IBeA+V versus KIO_3 fertilization, with 5-ISA and 2-IBeA demonstrating no effect on iodine uptake by roots, shoots, or leaves.



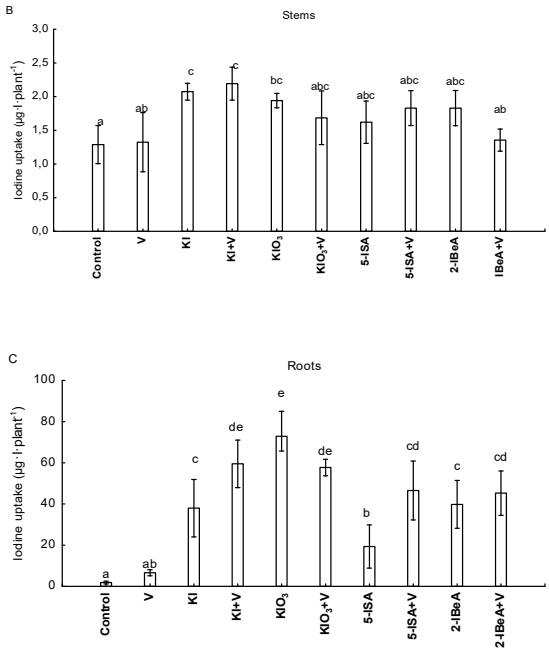
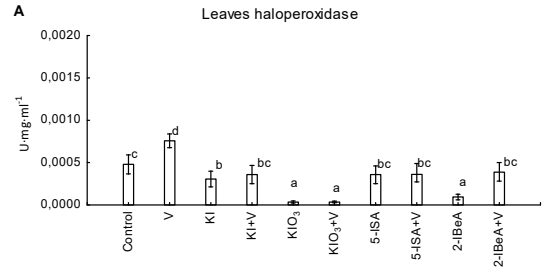


Figure 3. Iodine uptake by leaves (**A**), shoots (**B**), and roots (**C**) of maize. Identical letters in superscript indicate means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$ ($n = 8$). The developmental phase of maize: BBCH 15.

3.3. Activity of vHPO and Content and Uptake of Vanadium by Maize Plants

vHPO activity levels were determined to be higher in the roots than in the leaves of plants (Figure 4A,B). A significant variation between areas in terms of vHPO activity in plants was observed alongside greater variation in the leaves than in the roots of sweetcorn plants.

Vanadium application alone (without iodine) resulted in a significant increase in vHPO activity relative to the control in the leaves. Fertigation of KIO_3, KIO_3+V, and 2-IBeA caused a five-fold reduction in vHPO activity in sweetcorn leaves. Plants fertilized with KI alone (without vanadium) also reduced vHPO activity in the leaves. However, the highest vHPO activity was observed in the roots after KI+V fertilization, with statistically greater vHPO activity found in roots after application of V, 5-ISA, 5-ISA+V, and 2-IBeA+V compared to the control (Figure 4B).



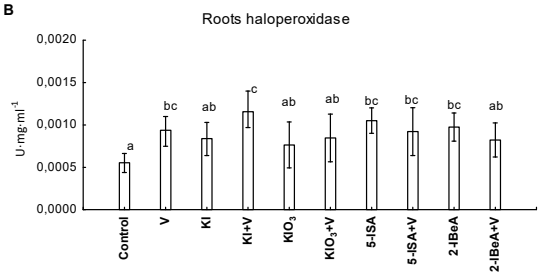


Figure 4. Activity of vanadium-dependent iodoperoxidase (vHPO) in maize leaves (**A**) and roots (**B**). Identical letters in superscript indicate means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$ ($n = 8$). The developmental phase of maize: BBCH 15.

Vanadium contents in the roots, shoots, and leaves were comparable to each analyzed part of the plants (Figure 5A–C). In comparison to the control, vanadium contents increased in maize roots after fertigation of vanadium alone, as well as when combined with all iodine compounds. Comparing vanadium fertilization versus control or the iodine compound applied to the roots, a significant increase in vanadium content in the roots in the two tested treatments was observed, i.e., V versus control and KIO_3+V versus KIO_3. The highest accumulation of vanadium was observed in the roots after fertilization of ammonium metavanadate. It is worth highlighting that the vanadium contents were lower in the shoots and leaves than in the control for all tested treatments except for 2-IBeA+V, with the leaves showing significantly increased V accumulation (Figure 5A–B).

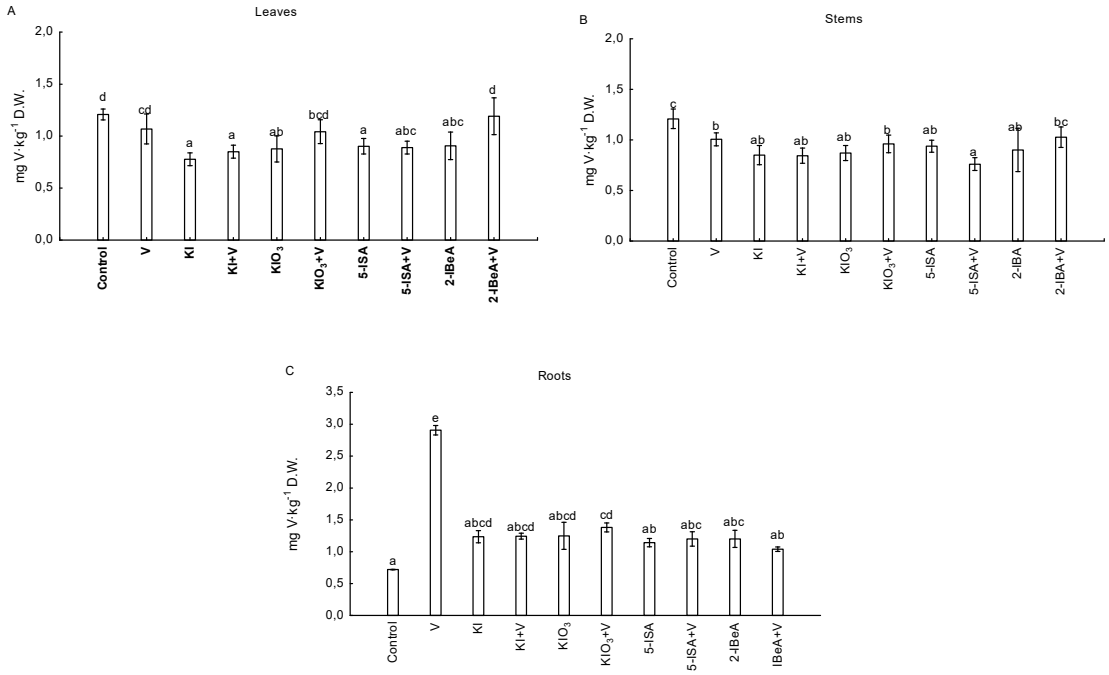


Figure 5. Vanadium contents in leaves (**A**), shoots (**B**), and roots (**C**) in maize. Identical letters in superscript indicate means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$ ($n = 8$). The developmental phase of maize: BBCH 15.

The results presented above show an inefficient effect of NH_4VO_3 fertilization based on low vanadium accumulation in plant (maize) parts, which could be an artifact of too low a dose of

NH_4VO_3. This dose did not cause a spectacular increase in vanadium content in maize, particularly in the leaves and shoots. Therefore, it is likely that the used dose of vanadium was the level of maize physiological vanadium demand. Calculations of V-uptake levels by stems and leaves (Figure 6A–C) showed a close synergistic relationship between vanadium and iodine in maize plants. All iodine compounds used without vanadium applied to the soil resulted in an increase in V-uptake by roots, stems, and leaves compared to the control. Therefore, combined fertilization with iodine and vanadium (compared to KI, KIO_3, 5-ISA, and 2-IBeA without vanadium) did not increase the level of V-uptake by roots, stems, or leaves. The result demonstrating the superior role of iodine over vanadium in the uptake process by corn plants.

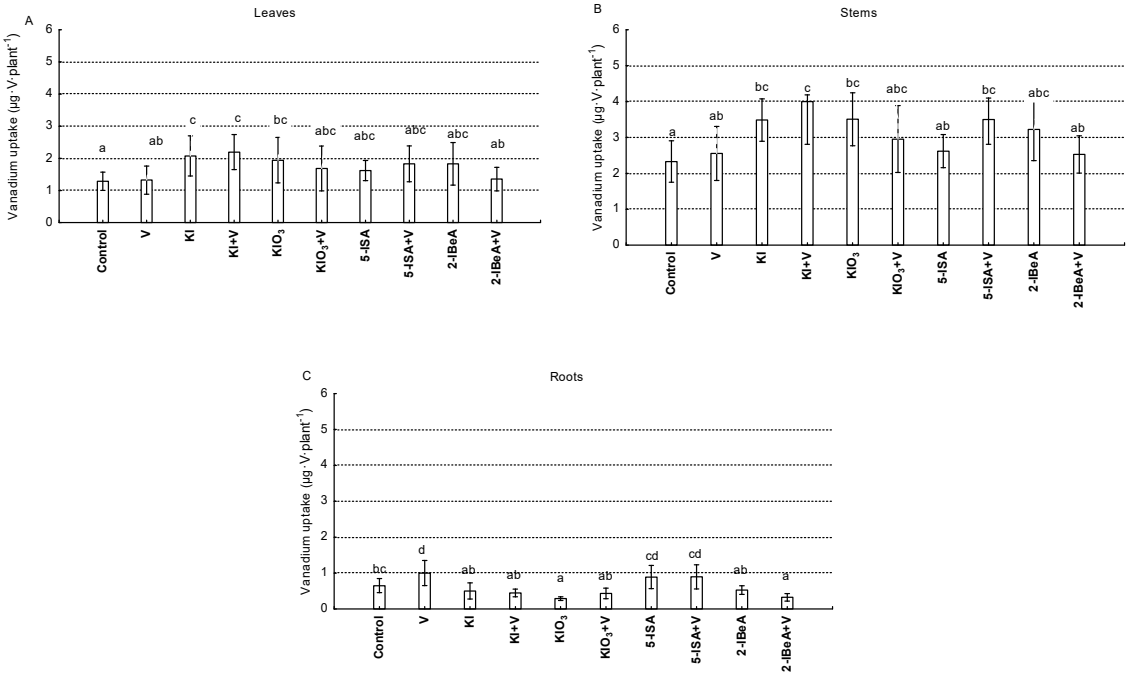


Figure 6. Vanadium uptake by leaves (**A**), shoots (**B**), and roots (**C**) in maize. Identical letters in superscript indicate means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$ ($n = 8$). The developmental phase of maize: BBCH 15.

3.4. Contents of Macroelements and Microelements in Maize Plants

Application of iodine and vanadium treatment showed a significant effect on the contents of all microelements and macroelements in the roots, shoots, and leaves of maize (Tables 3 and 4).

Compared to the control, almost all tested treatments of vanadium and iodine compounds (applied separately and in combination in vanadium) reduced the contents of the following elements: Ca and Mg in stems and leaves; K and S in leaves; P, Cu, Fe, Mn, Zn in roots, stems, and leaves; B in roots and stems; and Mo in roots (Tables 3 and 4); whereras B and Mo contents were increased in leaves. In contrast, Ca content increased in leaves, Zn increased in shoots, and Mn increased in roots, which was significant after fertilization with 2IBeA+V.

Application of KI alone resulted in plants with the lowest contents of all tested treatments, i.e., Ca, Mg, B, and Mn in leaves and Ca in stems. Further, KI+V application resulted in plants with the lowest P and Zn contents in leaves, as well as K, Mg, P, S, and Zn in stems. The exogenous use of 5-ISA+V caused the largest reduction in the contents of K, P, S, B, and Mn in maize roots.

Table 3. Contents of macroelements (Ca, K, Mg, P, and S) in leaves, shoots, and roots of "Złota Karłowa" sweetcorn at an early stage of development.

Leaves (g kg^{-1} d.w)					
Treatment	**Ca**	**K**	**Mg**	**P**	**S**
Control	1.13 ± 0.025 ^{d}	4.68 ± 0.133 ^{f}	0.31 ± 0.003 ^{d}	0.68 ± 0.046 ^{e}	0.24 ± 0.005 ^{a}
V	1.06 ± 0.026 ^{c,d}	4.41 ± 0.143 ^{e}	0.27 ± 0.002 ^{c}	0.58 ± 0.031 ^{d}	0.25 ± 0.002 ^{a,b,c}
KI	0.79 ± 0.010 ^{a}	4.02 ± 0.140 ^{c,d}	0.21 ± 0.004 ^{a}	0.47 ± 0.015 ^{b}	0.25 ± 0.009 ^{a,b}
KI+V	0.92 ± 0.006 ^{b}	3.79 ± 0.087 ^{b,c}	0.22 ± 0.004 ^{a,b}	0.43 ± 0.018 ^{a,b}	0.26 ± 0.006 ^{b,c}
KIO_3	0.90 ± 0.016 ^{b}	4.27 ±0.160 ^{d,e}	0.24 ± 0.008 ^{b}	0.51 ± 0.028 ^{c}	0.25 ± 0.005 ^{a,b,c}
KIO_3+V	1.12 ± 0.035 ^{d}	4.22 ± 0.128 ^{d,e}	0.30 ± 0.004 ^{d}	0.59 ± 0.028 ^{d}	0.26 ± 0.005 ^{b,c}
5-ISA	0.93 ± 0.051 ^{b}	4.13 ± 0.041 ^{d}	0.23 ± 0.006 ^{a,b}	0.52 ± 0.002 ^{c}	0.26 ± 0.015 ^{c}
5-ISA+V	0.92 ± 0.041 ^{b}	3.61 ± 0.028 ^{a,b}	0.24 ± 0.004 ^{b}	0.43 ± 0.002 ^{a}	0.26 ± 0.013 ^{b,c}
2-IBeA	0.98 ± 0.073 ^{b,c}	3.49 ± 0.141 ^{a}	0.22 ± 0.011 ^{a,b}	0.45 ± 0.014 ^{a,b}	0.26 ± 0.023 ^{b,c}
2-IBeA+V	1.23 ± 0.076 ^{e}	3.85 ± 0.190 ^{b,c}	0.26 ± 0.014 ^{c}	0.56 ± 0.027 ^{d}	0.26 ± 0.013 ^{b,c}
Stems (g kg^{-1} d.w)					
Control	0.95 ± 0.031 ^{f}	8.15 ± 0.282 ^{d}	0.41 ± 0.013 ^{e}	0.68 ± 0.008 ^{e}	0.19 ± 0.011 ^{a,b}
V	0.84 ± 0.021 ^{e}	8.33 ± 0.491 ^{d}	0.37 ± 0.006 ^{d}	0.64 ± 0.017 ^{d,e}	0.21 ± 0.013 ^{c}
KI	0.70 ± 0.029 ^{a}	6.61 ± 0.643 ^{a,b,c}	0.29 ± 0.026 ^{a,b}	0.49 ± 0.011 ^{a,b}	0.19 ± 0.01 ^{a}
KI+V	0.71 ± 0.013 ^{a,b}	5.63 ± 0.802 ^{a}	0.26 ± 0.022 ^{a}	0.42 ± 0.044 ^{a}	0.19 ± 0.02 ^{a}
KIO_3	0.75 ± 0.028 ^{c,d}	7.37 ± 0.703 ^{b,c,d}	0.33 ± 0.031 ^{c,d}	0.54 ± 0.01 ^{b,c}	0.20 ± 0.01 ^{a,b,c}
KIO_3+V	0.83 ± 0.041 ^{e}	7.87 ± 0.66 ^{d}	0.37 ± 0.024 ^{d}	0.62 ± 0.007 ^{d,e}	0.19 ± 0.003 ^{a}
5-ISA	0.78 ± 0.017 ^{d}	7.44 ± 0.46 ^{c,d}	0.31 ± 0.013 ^{b,c}	0.58 ± 0.013 ^{c,d}	0.20 ± 0.014 ^{a,b,c}
5-ISA+V	0.73 ± 0.024 ^{a,b,c}	6.25 ± 0.492 ^{a,b}	0.31 ± 0.014 ^{b,c}	0.49 ± 0.01 ^{a,b}	0.19 ± 0.012 ^{a,b,c}
2-IBeA	0.74 ± 0.049 ^{b,c}	5.94 ± 0.596 ^{a}	0.29 ± 0.021 ^{a,b}	0.51 ± 0.004 ^{b,c}	0.19 ± 0.021 ^{a}
2-IBeA+V	0.86 ± 0.010 ^{e}	7.33 ± 0.443 ^{b,c,d}	0.35 ± 0.022 ^{d}	0.67 ± 0.017 ^{e}	0.21 ± 0.01 ^{b,c}
Roots (mg kg^{-1} d.w)					
Control	0.94 ± 0.004 ^{a,b,c}	2.95 ± 0.019 ^{d}	0.20 ± 0.001 ^{b,c}	0.23 ± 0.001 ^{f}	0.48 ± 0.003 ^{c,d}
V	0.87 ± 0.006 ^{a}	2.89 ± 0.031 ^{d}	0.17 ± 0.002 ^{a}	0.18 ± 0.002 ^{d}	0.46 ± 0.003 ^{b,c,d}
KI	1.04 ± 0.002 ^{d}	2.57 ± 0.003 ^{b}	0.22 ± 0.001 ^{c}	0.18 ± 0.001 ^{d}	0.45 ± 0.002 ^{b,c}
KI+V	0.99 ± 0.005 ^{b,c,d}	2.37 ± 0.007 ^{a,b}	0.22 ± 0.001 ^{c}	0.15 ± 0.001 ^{b}	0.38 ± 0.002 ^{a}
KIO_3	1.00 ± 0.033 ^{b,c,d}	2.59 ± 0.084 ^{b,c}	0.20 ± 0.008 ^{c}	0.16 ± 0.005 ^{c}	0.46 ± 0.015 ^{b,c,d}
KIO_3+V	0.97 ± 0.002 ^{b,c,d}	2.82 ± 0.001 ^{c,d}	0.18 ± 0.001 ^{a,b}	0.22 ± 0.001 ^{e}	0.45 ± 0.002 ^{b,c,d}
5-ISA	0.84 ± 0.006 ^{a}	2.91 ± 0.013 ^{d}	0.18 ± 0.001 ^{a,b}	0.20 ± 0.002 ^{d}	0.41 ± 0.003 ^{a,b}
5-ISA+V	0.90 ± 0.004 ^{a,b}	2.14 ± 0.010 ^{a}	0.20 ± 0.001 ^{b,c}	0.14 ± 0.000 ^{a}	0.38 ± 0.002 ^{a}
2-IBeA	1.03 ± 0.051 ^{c,d}	2.41 ± 0.112 ^{b}	0.25 ± 0.013 ^{d}	0.15 ± 0.007 ^{b}	0.50 ± 0.024 ^{d,e}
2-IBeA+V	1.03 ± 0.004 ^{c,d}	3.61 ± 0.026 ^{e}	0.20 ± 0.001 ^{b,c}	0.25 ± 0.001 ^{g}	0.55 ± 0.002 ^{f}

Identical letters in superscript indicate means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$ ($n = 8$). The developmental phase of maize: BBCH 15.

Table 4. Contents of microelements (B, Cu, Fe, Mn, Zn, Mo) in the leaves, shoots, and roots of "Złota Karłowa" sweetcorn at an early stage of development.

Leaves (mg kg^{-1} d.w)						
Treatment	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**Mo**
Control	0.85 ± 0.14 ^{a}	15.77 ± 0.92 ^{f}	134.91 ± 4.91 ^{d}	104.87 ± 0.46 ^{e}	48.60 ± 4.54 ^{d}	0.04 ± 0.03 ^{a,b}
V	1.56 ± 0.41 ^{b}	14.70 ± 0.75 ^{e}	118.64 ± 1.21 ^{c}	92.65 ± 0.37 ^{d}	40.51 ± 2.00 ^{c}	0.86 ± 0.12 ^{a,b}
KI	2.29 ± 0.54 ^{c}	11.53 ± 0.87 ^{a,b}	114.09 ± 3.03 ^{c}	65.83 ± 5.67 ^{a}	35.22 ± 0.94 ^{a,b,c}	1.31 ± 0.40 ^{b}
KI+V	1.80 ± 0.28 ^{b,c}	11.49 ± 0.85 ^{a}	100.55 ± 2.45 ^{a}	74.22 ± 3.73 ^{b,c}	30.53 ± 1.15 ^{a}	1.16 ± 0.27 ^{b}
KIO_3	1.99 ± 0.46 ^{b,c}	12.50 ± 0.79 ^{c}	112.65 ± 2.76 ^{b,c}	80.73 ± 6.08 ^{c}	35.15 ± 1.22 ^{a,b}	1.08 ± 0.30 ^{a,b}
KIO_3+V	1.81 ± 0.42 ^{b,c}	14.20 ± 0.93 ^{d,e}	117.84 ± 3.34 ^{c}	95.77 ± 0.50 ^{d}	38.96 ± 1.51 ^{b,c}	0.56 ± 0.18 ^{a,b}
5-ISA	1.99 ± 0.49 ^{b,c}	12.43 ± 0.80 ^{b,c}	100.93 ± 2.10 ^{a}	77.35 ± 2.37 ^{b,c}	35.07 ± 1.28 ^{a,b}	0.79 ± 0.25 ^{a,b}
5-ISA+V	1.42 ± 0.17 ^{a,b}	11.61 ± 0.91 ^{a,b,c}	103.91 ± 1.82 ^{a,b}	71.44 ± 1.76 ^{a,b}	32.87 ± 0.43 ^{a}	0.59 ± 0.40 ^{a,b}
2-IBeA	1.51 ± 0.28 ^{a,b}	11.37 ± 1.49 ^{a}	98.69 ± 3.95 ^{a}	71.25 ± 2.08 ^{a,b}	33.15 ± 2.09 ^{a}	0.87 ± 0.32 ^{a,b}
2-IBeA+V	1.92 ± 0.39 ^{b,c}	13.49 ± 1.18 ^{d}	110.52 ± 5.28 ^{b,c}	105.93 ± 4.69 ^{e}	46.69 ± 2.80 ^{d}	<0.04 ^{a}
Stems (mg kg^{-1} d.w)						
Control	1.67 ± 0.46 ^{g}	12.88 ± 0.91 ^{g}	101.77 ± 4.09 ^{d}	54.54 ± 6.93 ^{e}	91.1 ± 77.31 ^{g}	<0.04
V	1.46 ± 0.35 ^{d,e}	10.73 ± 0.59 ^{f}	93.19 ± 1.19 ^{c,d}	41.78 ± 2.59 ^{d}	77.89 ± 1.17 ^{f}	<0.04
KI	1.33 ± 0.37 $^{a–d}$	8.29 ± 0.95 ^{b,c}	76.07 ± 6.52 ^{a}	32.78 ± 2.40 ^{b,c}	51.55 ± 3.06 ^{a,b}	<0.04
KI+V	1.37 ± 0.31 ^{c,d,e}	8.24 ± 0.66 ^{b,c}	78.36 ± 5.21 ^{a}	33.52 ± 0.67 ^{b,c}	46.92 ± 3.24 ^{a}	<0.04
KIO_3	1.34 ± 0.33 $^{b–e}$	9.16 ± 0.78 ^{d}	94.52 ± 0.59 ^{c,d}	34.47 ± 1.41 ^{c}	66.45 ± 5.72 ^{e}	<0.04
KIO_3+V	1.52 ± 0.35 ^{e,f}	10.08 ± 0.77 ^{e}	73.41 ± 5.44 ^{a}	34.94 ± 1.94 ^{c}	64.33 ± 3.86 ^{d,e}	<0.04
5-ISA	1.30 ± 0.31 $^{a–d}$	8.72 ± 0.56 ^{c,d}	80.17 ± 2.52 ^{a,b}	31.23 ± 0.25 ^{b}	59.43 ± 3.48 ^{c,d}	<0.04

5-ISA+V	1.18 ± 0.29 ^{a,b,c}	7.71 ± 0.51 ^{a}	71.09 ± 6.99 ^{a}	28.65 ± 0.40 ^{a}	49.65 ± 2.05 ^{a}	<0.04
2-IBeA	1.15 ± 0.28 ^{a,b}	7.89 ± 0.98 ^{a,b}	89.24 ± 2.13 ^{b,c}	31.46 ± 1.90 ^{b}	55.77 ± 5.56 ^{b,c}	<0.04
2-IBeA+V	1.13 ± 0.28 ^{a}	9.97 ± 0.77 ^{e}	94.20 ± 4.54 ^{c,d}	41.45 ± 0.61 ^{d}	94.22 ± 1.44 ^{g}	<0.04
			Roots (mg kg^{-1} d.w)			
Control	1.74 ± 0.03 ^{b}	36.74 ± 0.40 ^{f}	146.66 ± 10.51 ^{e,f}	52.62 ± 0.33 ^{d}	63.91 ± 0.32 ^{d}	1.11 ± 0.04 ^{a,b,c}
V	1.49 ± 0.03 ^{a}	20.42 ± 0.19 ^{e}	116.55 ± 1.20 ^{b,c}	32.39 ± 0.21 ^{b}	96.25 ± 0.93 ^{e}	1.36 ± 0.05 ^{c}
KI	1.66 ± 0.02 ^{a,b}	17.32 ± 0.15 ^{c}	138.62 ± 0.43 ^{d,e,f}	50.45 ± 2.12 ^{d}	61.58 ± 0.33 ^{c,d}	1.18 ± 0.15 ^{b,c}
KI+V	1.59 ± 0.04 ^{a,b}	14.50 ± 0.04 ^{a}	153.51 ± 0.78 ^{f}	35.24 ± 0.34 ^{b}	59.24 ± 0.40 ^{c}	0.76 ± 0.06 ^{a}
KIO_3	1.50 ± 0.04 ^{a}	19.41 ± 0.69 ^{d,e}	129.04 ± 4.64 ^{c,d,e}	41.60 ± 1.42 ^{c}	44.18 ± 1.34 ^{b}	0.95 ± 0.05 ^{a,b,c}
KIO_3+V	1.66 ± 0.07 ^{a,b}	18.33 ± 0.07 ^{c,d}	120.39 ± 0.95 ^{b,c,d}	43.28 ± 0.36 ^{c}	46.95 ± 0.27 ^{b}	0.90 ± 0.04 ^{a,b}
5-ISA	1.63 ± 0.02 ^{a,b}	14.15 ± 0.11 ^{a}	100.09 ± 0.46 ^{a,b}	31.01 ± 0.20 ^{b}	44.78 ± 0.49 ^{b}	0.88 ± 0.06 ^{a,b}
5-ISA+V	1.45 ± 0.09 ^{a}	15.55 ± 0.06 ^{a,b}	108.35 ± 1.00 ^{a,b,c}	23.06 ± 0.22 ^{a}	37.76 ± 0.31 ^{a}	1.03 ± 0.10 ^{a,b,c}
2-IBeA	1.48 ± 0.04 ^{a}	14.61 ± 0.67 ^{a}	122.83 ± 6.42 ^{c,d}	32.19 ± 1.57 ^{b}	35.42 ± 1.37 ^{a}	1.00 ± 0.14 ^{a,b,c}
2-IBeA+V	1.46 ± 0.03 ^{a}	16.71 ± 0.12 ^{b,c}	91.29 ± 3.11 ^{a}	89.66 ± 0.50 ^{e}	43.45 ± 0.19 ^{b}	0.70 ± 0.06 ^{a}

Identical letters in superscript indicate means are not significantly different at $P < 0.05$; different letters indicate statistically significant differences at $P \leq 0.05$ ($n = 8$). The developmental phase of maize: BBCH 15.

4. Discussion

In the present study, the application of 5-ISA+V and KI+V stimulated root system growth and development and, in the case of KI+V, also aerial part mass of maize plants. This may be due to the contribution of vanadium to nitrogen metabolism, whereby vanadium functions as a growth-stimulating factor and is involved in the binding and accumulation of nitrogen in plants [55]. The impact of organic iodine compounds on sweetcorn plants is not well known. After application of 2-IBeA+V, the highest concentrations of iodine in shoots was observed. 2-IBeA can be taken up by plants [16] and can also be precursor of 2,3,5-tri-iodobenzoic acid in plants [52], which is an inhibitor of auxin transport. This may be why decreased plant height and weight after 2-IBeA+V application was observed in this work.

Yang's [56] research on soybeans using different concentrations of vanadium (V) showed that the lowest doses used to stimulate the development of the soybean root system in early development were 0.05 and 0.10 mM V. In our research, vanadium applied with 2-IBeA (2-IBeA+V) showed a different effect on root development compared to the use of 5-ISA+V and KI+V. Research conducted by Halka et al. [57] showed that tomato plant biomass decreased after 2-IBeA treatment.

Transport and uptake of iodine through plant roots can flow actively or passively [58]. Significant differences in both iodine content and uptake were noted between the tested treatments in corn leaves, roots, and shoots. The highest content was recorded in the roots after application of KIO_3+V. Furthermore, it was found that corn roots preferentially uptake IO_3^- over I^-. These observations were different from the preferential I^- uptake over IO_3^- generally described in the literature [47,59]. Smoleń et al. [53] obtained similar results. The application of KIO_3 with humic and fulvic acid caused higher concentrations of iodine in spinach leaves compared to KI complexed with humic and fulvic acid. KIO_3 may be reduced to I_2 and bind aromatic rings from organic matter compounds, causing easier and more effective uptake by plants. Oxidation of I^- to I_2 occurs at different redox potential levels and lower pH levels more suitable to the IO_3^- form.

Distribution of iodine described in the literature in plant parts is as follows: roots > leaves > stem > fruit [2]. In our experiment, the highest concentration of iodine was obtained according to this scheme. The concentration of iodine in combination with KIO_3+V in roots was four times higher compared to the concentration of iodine with this combination found in leaves.

Generally, iodine accumulation in aerial plant parts was lower than in corn roots. However, the level of uptake of iodine was as high as in roots, with iodine uptake by stems being minimal. This was probably because of the much greater leaf mass per plant than root mass (dilution effect of iodine in leaf).

Information regarding the beneficial effects of vanadium on plants and animals exists widely in the literature, with the objection that concentrations of this element are used in trace doses [60]. Vanadium uptake is dependent on pH, with increased vanadium uptake observed at low pH (less

than 4) and uptake in the range of 5 to 8 reduced but stabilized. The use of peat substrate in the current experiment with a pH range of 5.5 to 6.0 represents an acceptable level for uptake enhancement. The application of vanadium (without any iodine compounds) caused higher concentrations in the roots, but transport to stems and leaves was not effective. High pH (alkaline) hindered vanadium uptake in oat roots [60].

Vachirapatama et al. [26] conducted research on Chinese green mustard and tomato. The increasing doses of vanadium applied (i.e., in the range of 1 mg·dm^{-3} to 80 mg·dm^{-3}) caused a gradual decrease in the development of the root system, reducing the mass and length of plants. Similar results were observed in chickpea, with vanadium caused a significant decrease in the mass of roots and aerial parts [61]. Corresponding results were obtained in bean, in which the use of vanadium compounds caused improper development of the main root and development inhibition of the lateral roots [62]. However, Chongkid et al. [63] showed a stimulating effect of vanadium at a dose of 10 mg·dm^{-3} on rice shoot growth, indicating the possibility of a stimulating effect of vanadium on the growth and development of individual species in low doses.

The application of vanadium resulted in higher contents in the stems, leaves, and roots of Chinese mustard and tomato [26]. The results of our research did not show such significant differences in vanadium content between parts of plants (roots, shoots, and leaves), demonstrating different results compared to the works by Vachirapatama et al. [26], Imtiaz [61], and Saco [62]. This may be due to mineral nutrition functioning of plants relative to vanadium. Nevertheless, lower doses of vanadium showed a stimulating effect in the early developmental stage of corn plants.

Almost all of the vanadium treatments and iodine compounds (applied separately and in combination with vanadium) in this research demonstrated a negative impact on mineral nutrition functioning and micronutrient and macronutrient concentrations in sweetcorn compared to the control (without iodine and vanadium). Plant fertilization with KI (in terms of Ca, Mg, B, and Mn contents in leaves and Ca in stems) unfavorably affected the mineral nutrition of plants (and thus the contents of macronutrients and micronutrients), followed by KI+V (in the content of P and Zn in leaves as well as K, Mg, P, S, and Zn in stems) and, to a lower extent, 5-ISA+V (K, P, S, B, and Mn contents in corn roots). Comparison of pairs of treatments with vanadium fertilization compared to treatments without the use of this element (control versus V, as well as all iodine treatments versus all iodine treatments +V) showed that vanadium, depending on the form of iodine used, exerted variable effects on the contents of macro elements and microelements in the roots, shoots, and leaves of corn, causing significant increases, decreases or not affecting individual macro element and microelement concentrations in maize plants. Therefore, it is impossible to clearly determine mineral nutrition process functioning of corn plants depending on fertilization according to vanadium and vanadium plus KI, KIO_3, 5-ISA, or 2-IBeA.

Changes in the contents of mineral elements in parts of plants after iodine application depended highly on the dose and form of iodine used [64]. Iodine can antagonistically or synergistically impact on the uptake of macroelements and microelements [65]. Smoleń and Sady [65] conducted research in iodine-biofortified spinach, showing increased uptake and accumulation of Mg, Na, Ce, and Fe at a dose of 1 mg I dm^{-3}. A higher dose of iodine at 2 mg I·dm^{-3} caused increases in the contents of Na, Fe, Zn, and Al in spinach leaves and decreased contents of P, S, Cu, and Ba.

The dose of vanadium used has a decisive impact on the mineral plant nutrition process. Akoumianaki-Ioannidou et al. [28] noted a decrease in the contents of K, Fe, Zn, and Pb in leaves and K, Fe, Mn, Zn, and Pb in basil roots after vanadium fertilization in the dose range of 5–40 mg V·dm^{-3} substrate. Based on the literature, a positive effect of vanadium is that it allows for better utilization of potassium [66]. The combination of vanadium with the organic form of iodine 2-IBeA showed a positive effect on potassium content in leaves, roots, and stems. Iodine (2IBeA) combined with vanadium increased contents of potassium in roots and stems by around 50% compared to 2-IBeA application.

In the case of soybean plants, the contents of N, P, Mg, Fe, and B in roots and leaves were shaped by vanadium doses (0.6 and 1.2 mM V as $VOSO_4$) and depended on plant phase and leaf location [27]. In soybean plants, vanadium was observed to stimulate increases in K and Mn contents in leaves

while simultaneously reducing its content in the roots. In addition, Kaplan et al. [27] observed an antagonistic effect of vanadium on the content of Ca in roots and Cu, Zn, and Mo in roots and leaves.

5. Conclusions

Ammonium metavanadate fertilization significantly improved the growth of sweetcorn. The applied dose of vanadium and iodine compounds did not demonstrate any toxicity to sweetcorn plants. Iodine and vanadium fertilization increased the iodine contents of plants. The theory of poor vanadium transport from roots to above-ground plant parts was also confirmed. The greatest accumulation of vanadium was observed in the roots. Iodine and vanadium application resulted in higher concentrations of iodine in all applied combinations. The highest iodine content in the leaves was obtained after applying an organic form of iodine alongside vanadium. In the roots, greater accumulation of iodine was achieved by combining it with an inorganic form of vanadium. Based on the concentration of iodine in the roots and higher parts of the plant, transport of the organic form of iodine is more efficient than inorganic forms in sweetcorn. The application of iodine and vanadium significantly changed the mineral nutrition status of maize at an early developmental stage. Extenuation of macronutrient and micronutrient uptake in plants with iodine and vanadium fertilization was observed.

Supplementary materials: The following are available online at www.mdpi.com/2073-4395/10/11/1666/s1, Table S1 Dry matter content in sweet corn plants parts (roots, stems, leaves).

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Biofortification of Sweetcorn with Iodine: Interaction of Organic and Inorganic Forms of Iodine Combined with Vanadium

Marlena Grzanka [1,*], Sylwester Smoleń [1,*], Łukasz Skoczylas [2] and Dominik Grzanka [1]

[1] Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 31-425 Kraków, Poland; dominikgrzanka110@gmail.com
[2] Department of Plant Product Technology and Nutrition Hygiene, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 30-149 Kraków, Poland; lukasz.skoczylas@urk.edu.pl
* Correspondence: marlena.grzanka14@gmail.com (M.G.); sylwester.smolen@urk.edu.pl (S.S.)

Abstract: Around the world, maize cultivation is an essential part of food systems for humans and animals. Effective reactions against the occurrence of diseases related to the deficiency of elements in the human diet are related to the biofortification of plant species of broad importance, including maize. The enrichment of maize with iodine is difficult due to the poor transport of this element to the plant's generative organs. In marine algae, vanadium is part of the structure of the enzyme iodine-dependent peroxidase (vHIPO) that catalyzes the uptake of cellular iodine (I) and its volatilization as I_2. The relationship between iodine and vanadium in higher plants, however, is not well-known. The aim of this research was to determine the effect of vanadium fertilization and the interactions of organic and inorganic iodine compounds with vanadium under soil application. In the pot experiment, NH_4VO_3 was applied to the soil in two doses of 0.1 and 1 μmol·dm^{-3} both separately and in combination, with the following iodine compounds: 5-iodosalicylic acid (5-ISA), 2-iodobenzoic acid (2-IBeA), potassium iodide (KI), and potassium iodate (KIO_3). The iodine compounds were also applied independently to vanadium, while in the control combination, fertilization was performed without I and V. Iodine compounds were applied with doses calculated using the molar mass of this element (i.e., 10 μmol·dm^{-3} I). The highest level of iodine accumulation in grains (regardless of fertilization with V) was obtained after the application of organic compounds 5ISA and 2IBeA. A lower dose of vanadium (0.1 μmol·dm^{-3}) in combination with KI and KIO_3 increased the accumulation of iodine in leaves, roots, and grains compared to the combination without the additional application of vanadium. The combined application of vanadium in both doses with 2-IBeA most effectively stimulated the transport and accumulation of iodine to the maize grain. Under the combined application of 5-ISA and vanadium (10 μmol·dm^{-3}), we observed the stimulating effect of this organic iodine compound on the accumulation of vanadium in the roots as well as the antagonistic effect of vanadium in combination with 5-ISA on the accumulation of iodine in the roots, leaves, and maize grain. Vanadium accumulated mainly in the roots, where the content of this element increased proportionally to its dose. The soil application of 5-ISA increased the total sugar content and vitamin C content in the grain.

Keywords: iodine fortification; organic iodine; inorganic iodine; vanadium; beneficial elements; iodine deficiency; functional food

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1. Introduction

Cereal production is strategically important in many countries around the world. On a global scale, grain production is dominated by cereals which constitute about 50% of all crops [1]. Maize is an essential part of the food systems of humans and livestock. In many countries, especially economically undeveloped ones, wheat, rice and maize account for 60% of dietary calories [2].

Cereals, vegetables, and fruits are the main sources of macro- and microelements, phenolic compounds, and vitamins necessary for human and animal organisms to properly function. However, only a balanced and varied diet can effectively prevent the diseases and ailments related to element and vitamin deficiencies [3–5]. The problem of malnutrition and "hidden hunger" is common in several areas of the world [1,6]. The multidisciplinary approaches of scientists and their cooperation in, e.g., the HarvestPlus program, helped to establish the procedures of biofortification programs [5,7]. The biofortification of major crops via micronutrients using agrotechnical solutions is a cost-effective and sustainable approach to solve the deficiencies of these elements in the human diet [1,3,4].

The WHO recommendations to limit iodized table salt consumption intensified research on the effective enrichment and biofortification of crops with iodine [8]. Iodine is essential for the proper functioning of the thyroid gland and the synthesis of hormones (thyroxin-T4 and triiodothyronine-T3) [9–11]. Deficiency of this micronutrient also negatively affects the metabolic pathway responsible for proper development of the brain, reducing the ability to learn, associate, and form memories, leading to a decrease in the intelligence factor [10–12]. The recommended daily amount of this micronutrient is 200–290 µg for pregnant women, 150 µg for adults, 90 µg for children aged 0–5, and 90–120 µg for children from 5 to 12 years old [10,12,13].

Previous research conducted on the iodine biofortification of rice [14] did not yield the expected results, which was explained by the low mobility of iodine in the phloem and, consequently, the low level of grain enrichment [4]. In a previous study on biofortification, the effect of enriching the grains of maize, wheat, and rice with iodine via foliar application was determined, but for maize, this process was less effective than that for wheat and rice [15].

The possibility of iodine transport (after applying inorganic compounds of this element) to the generative parts of plants was also confirmed not only in cereal crops but also in nectarine and plum fruit [16], tomato [17], and strawberry [18]. Most relevant studies were conducted on the effectiveness of enriching plants with different levels of iodine, as well as the methods of iodine application best suited to obtain a biofortification effect. Soil application was studied in the cultivation of wheat, rice, and maize [15]; lettuce [19]; radish; and kohlrabi [20]. Foliar application was conducted in the cultivation of potatoes, tomatoes, nectarines [16], and lettuce [20] along with application via a nutrient solution in hydroponic cultivation [21]. Determining the optimal methods of enriching plants with iodine depending on the cultivation method and the species of the plant being grown involved, almost exclusively, the use of inorganic iodine compounds KI [22] and KIO_3 [23–25]. Studies testing organic iodine compounds, however, are rare [26,27]. One groundbreaking approach was to test the application of iodosalicylates and iodobenzoates in research on tomato cultivation [26]. These compounds are iodine derivatives of salicylic acid (SA) and benzoic acid (BeA). In plants, benzoic acid (BeA) is a precursor to SA, which is considered a signaling molecule and plant growth regulator [28]. Organic iodine compounds in which iodine is bound to the phenolic nucleus (5-ISA and 2-IBeA) can be absorbed by the roots of crops. In young tomato plants, the authors tested 5-iodosalicylic (5-ISA), 3,5-diiododalicylic (3,5-ISA), 2-iodobenzoic (2-IBeA), and 4-iodobenzoic (4-IBeA) acids [26]. Transitions, including possible secondary iodine metabolites, that can arise in maize plants after the use of low-molecular-weight aromatic iodine compounds and their functions in the plant are not well known. In the lettuce, the authors confirmed the presence of endogenous 3,5-diSA, 5-ISA, 2-IBeA, 4-IBeA, and T3 (triiodothyronine) [21].

The second important aspect is the possible interaction of vanadium on the uptake and accumulation of iodine in plants. Among all plants from individual ecosystems, the most efficient accumulators of iodine are sea algae and brown algae *Laminaria digitata*, which contain 1% dry matter on average [29]. The iodine uptake by *L. digitata* seaweed involves the extracellular oxidation of iodide by vanadium-dependent haloperoxidase (vHPO) [30,31]. In seaweed, one of the functions of iodine is to participate in antioxidant mechanisms that protect the surfaces of the capsid and thallus from oxidative stress [29].

The vHPO enzymes play a central role in both the capture of iodine from sea water and volatile hydrogen halides in the synthesis of marine algae [31,32]. Vanadium-dependent iodo-peroxidases (vHIPO) facilitate iodine uptake by catalyzing the oxidation of I^- to more lipophilic compounds (iodine (I) acid) HIO and subsequently forming molecular I_2, which readily diffuses across the cell membranes into the cytosol. The course of the further reduction of HIO or I_2 to I^- in apoplasts is unknown [29–32]. The presence of an enzyme whose prosthetic group consists of a metal such as vanadium capable of catalyzing the oxidation of halides (Cl^-, Br^-, I^-) in the presence of H_2O_2 to hypohalogenic acids is unknown in higher plants. The first affirmation of vHPO activity in higher plants was observed in lettuce by Smoleń et al. [21].

Vanadium and iodine are well known to be beneficial elements for higher plants. Vanadium's ability to stimulate positive effects on plants at low concentrations was confirmed, as were the toxic effects of higher doses of vanadium on plants. The biological and physiological properties of V also depend on V's oxidation state. Vanadium exists in several oxidation states from −1 to +5 [33]. For peppers cultivated in a hydroponic system with the addition of vanadium into the nutrient solution at a concentration of 5 μmol·dm^{-3}, V significantly increased the growth of the aerial part of the plant, stimulated the development of flower buds, and accelerated the blooming of the pepper. The contents of amino acids and sugars in the roots and leaves of the pepper were significantly higher after the application of 5 μmol·dm^{-3} of vanadium [34]. The dry matter content of mint roots increased significantly after vanadium application at the corresponding doses of 10, 20, and 40 mg V·dm^{-3} [35]. Vanadium caused a decrease in biomass and inhibited the development of the root system in the cultivation of chickpeas, indicating the toxic effect of this element in applicable doses [36]. Application of the vanadium to the nutrient solution at a dose of over 40 mg·dm^{-3} was used to retard the growth of Chinese green mustard (*B. campestris* ssp. *Chinensis var. Parachinensis*) and tomato [37]. Several studies have confirmed that the accumulation of vanadium is much higher in the roots than in the above-ground vegetative and generative parts of the plant [33,36,37].

The aim of this research was to compare the efficiency of maize grain's biofortification into iodine depending on the application of various chemical forms of this element, i.e., aromatic organic compounds and inorganic compounds. Moreover, the aim of this study was to determine the effect of vanadium on the uptake and transport of organic and inorganic iodine compounds by maize.

2. Materials and Methods

2.1. Plant Material and Cultivation

The experiments were performed with sweetcorn (*Zea mays* L. subsp. Mays Saccharata Group) "Złota Karłowa" (a Polish dwarf variety of maize for amateur farmers) by the Faculty of Biotechnology and Horticulture, the University of Agriculture in Kraków (50°05′04.1″ N,19°57′02.1″ E). The experiments using sweetcorn were pot studies. Each pot study was conducted in a foil tunnel in heavy mineral soil. Each experiment was repeated twice. The pot experiments were conducted in the spring/summer season during 2018 and 2019. For the pot experiment in mineral soil, seeds were sown into 7 dm^3 pots filled with heavy mineral soil, and one plant was cultivated per pot. There were 4 replications used with 3 plants per replication (12 plants per treatment). Organization of the pots in the foil tunnel used randomized blocks. The study plan included fifteen treatments (Tables 1 and 2) of plant treatment with various iodine compounds, including KI, KIO_3, 5-ISA, and 2-IBeA, and vanadium in the form of ammonium methavanadate (NH_4VO_3). The dose of iodine was 10 μmol I·dm^{-3} along with two doses of vanadium: 0.1 μmol V·dm^{-3} (V_1) and 1 μmol V·dm^{-3} (V_2). The control plants were not fertilized with iodine or vanadium. The first application of iodine and vanadium compounds started four weeks after sowing (in the plant growth phase, BBCH 16-17). The iodine and vanadium compounds were applied to the soil once a week through manual fertigation. Manual watering with solutions of the compounds studied, at a dose of 200 cm^3·pot^{-1} (one plant^{-1}), was

implemented. We used seven applications of iodine and vanadium compounds through fertigation (Tables 1 and 2). During cultivation, the plants were watered with tap water through a drip irrigation system. Harvesting of the corn cobs was carried out at the full milk maturity stage of the granuloma (BBCH 75). The leaf sheaths were removed from the corn cobs after harvesting. The corn cobs were then counted and weighed successively. After harvest, we measured the heights and weights of the above-ground parts of the plants without corn cobs.

Table 1. Methodological information about application of iodine and vanadium compounds to soil during sweetcorn cultivation in pot experiments.

Year of Experiment	Sowing Date	Date of First Application of Iodine and Vanadium Compounds	Developmental Phase (BBCH) of First Application	Application Cycle	Date Last Application	Date of Harvest	Amount of Applications
2018	8 May 2018	8 June 2018	6–7 leaves	Each 7th day	17 July 2018	24 July 2018	7
2019	9 April 2019	22 May 2019	6–7 leaves	Each 7th day	3 July 2019	10 July 2019	7

Table 2. Design and method of conducting experiment with sweetcorn plants cultivation.

Treatments	Dose * of Iodine Compounds and Dose of Iodine	Dose * of Vanadium as Ammonium Metavanadate	I and/or V Application from BBCH 16-17	Amount of Iodine Applied for One Plant (μmol I·plant^{-1})	Amount of Vanadium Applied for One Plant (μmol V·plant^{-1})
Control	- **	- **	-	- ****	- ****
V_1	-	0.1 μM V	7 times ***	- ****	0.14
V_2	-	1.0 μM V	7 times ***	- ****	1.4
KI	10 μM (10 μM I)	-	7 times ***	14	- ****
KI + V_1	10 μM (10 μM I)	0.1 μM V	7 times ***	14	0.14
KI + V_2	10 μM (10 μM I)	1.0 μM V	7 times ***	14	1.4
KIO_3	10 μM (10 μM I)	-	7 times ***	14	- ****
KIO_3 + V_1	10 μM (10 μM I)	0.1 μM V	7 times ***	14	0.14
KIO_3 + V_2	10 μM (10 μM I)	1.0 μM V	7 times ***	14	1.4
5ISA	10 μM (10 μM I)	-	7 times ***	14	- ****
5ISA + V_1	10 μM (10 μM I)	0.1 μM V	7 times ***	14	0.14
5ISA + V_2	10 μM (10 μM I)	1.0 μM V	7 times ***	14	1.4
2IBeA	10 μM (10 μM I)	-	7 times ***	14	- ****
2IBeA + V_1	10 μM (10 μM I)	0.1 μM V	7 times ***	14	0.14
2IBeA + V_2	10 μM (10 μM I)	1.0 μM V	7 times ***	14	1.4

*—The dose of 200 cm^3 of iodine, vanadium or iodine + vanadium solution pot^{-1} (one plant^{-1}) were applied at a single application. **—Without iodine and vanadium fertilization—the natural content of iodine and vanadium in mineral soil to the experiments. ***—7 times every 7 days. ****—The determined iodine and vanadium content (in TMAH and 1 M HCl extracts, respectively) do not allow for estimating the amount of available iodine and vanadium for plants in the soil solution of mineral soil. It is also not possible to accurately estimate the changes in the content of I and V in the soil solution during the whole period of sweetcorn cultivation using other methods of soil extraction.

2.2. Analysis of Dry and Fresh Samples of Roots, Stems, and Leaves

Fresh samples of roots, leaves, and grain were dried at 70 °C (48 h) in a laboratory dryer with forced air circulation. Dried samples of leaves, roots, and grain were ground in a laboratory mill and stored in a plastic bag until analysis of the iodine, vanadium, microelements, and microelements was carried out. The dry weight content in these samples was determined using the oven-drying method at 105 °C.

To determine iodine content, the PN-EN 15111-2008 method was applied with the modifications described in [38] using ICP-MS/MS (iCAP TQ ICP-MS ThermoFisher Scientific, Bremen, Germany). The concentrations of V, P, K, Mg, Ca, S, Cu, Fe, Mn, Mo, and Zn were determined using an ICP-OES spectrophotometer (Prodigy Spectrometer, Leeman

Labs, New Hampshire, MA, USA) after microwave digestion in 65% super pure HNO_3. Plant samples of 0.5 g dry material were placed in 55 mL TFM-modified polytetrafluoroethylene (PTFE) vessels and digested in 10 mL of 65% HNO_3 using a CEM MARS-5 Xpress (CEM World Headquarters, Matthews, NC, USA) microwave digestion system [39].

The samples of fresh grain (milk stage) were then homogenized and total sugars, as a sum of glucose, fructose, and sucrose, were extracted by boiling 96% ethanol (Destylernia 'Polmos' Sp. z.o.o., Kraków, Poland). The contents of fructose, glucose and sucrose (and their sum as total sugars) were assessed using the capillary electrophoresis technique with the PA 800 Plus system (Beckman Coulter, Brea, CAUSA). Capillaries of 50 µm and a total length of 60 cm (10 cm for detection) were used. A positive power supply of 15 kV was applied, and the temperature was set at 25 °C. The running buffer solution contained 20.0 mmol·dm^{-3} sorbic acid, 0.20 mmol·dm^{-3} CTAB, and 40 mmol·dm^{-3} NaOH, pH 12.2. [40].

The content of L-ascorbic acid in the grains was analyzed via capillary electrophoresis after the homogenization of 20 g samples in 80 cm^3 of 2% oxalate acid (puriss. p.a., Avantor Performance Materials) and further centrifugation for 15 min at 4500 rpm and 5 °C. The supernatants were then filtered through a 0.25 µm cellulose acetate membrane filter and analyzed using a PA 800 Plus capillary electrophoresis system (Beckman Coulter, Indianapolis, IN, USA) with a diode array detector (DAD). We used capillaries of 50 µm i.d. and 365 µm o.d. and a total length of 50 cm (40 cm to detector) and applied a negative power supply of 25 kV. A running buffer solution containing 30 mM NaH_2PO_4 was prepared as proposed in [41] (puriss. p.a., Avantor Performance Materials) with 15 mM $Na_2B_4O_7$ (puriss. p.a., Sigma-Aldrich, Burlington, MA, United States) and 0.2 mM cetyltrimethylammonium bromide (CTAB) (puriss. p.a., Sigma-Aldrich) (pH 8.80).

2.3. Data Analysis

All data were statistically verified using the one-way analysis of variance (ANOVA) module of the Statistica 13.3 PL program at $p < 0.05$. The significance of differences between the means was estimated using Tukey's test at $p < 0.05$.

3. Results

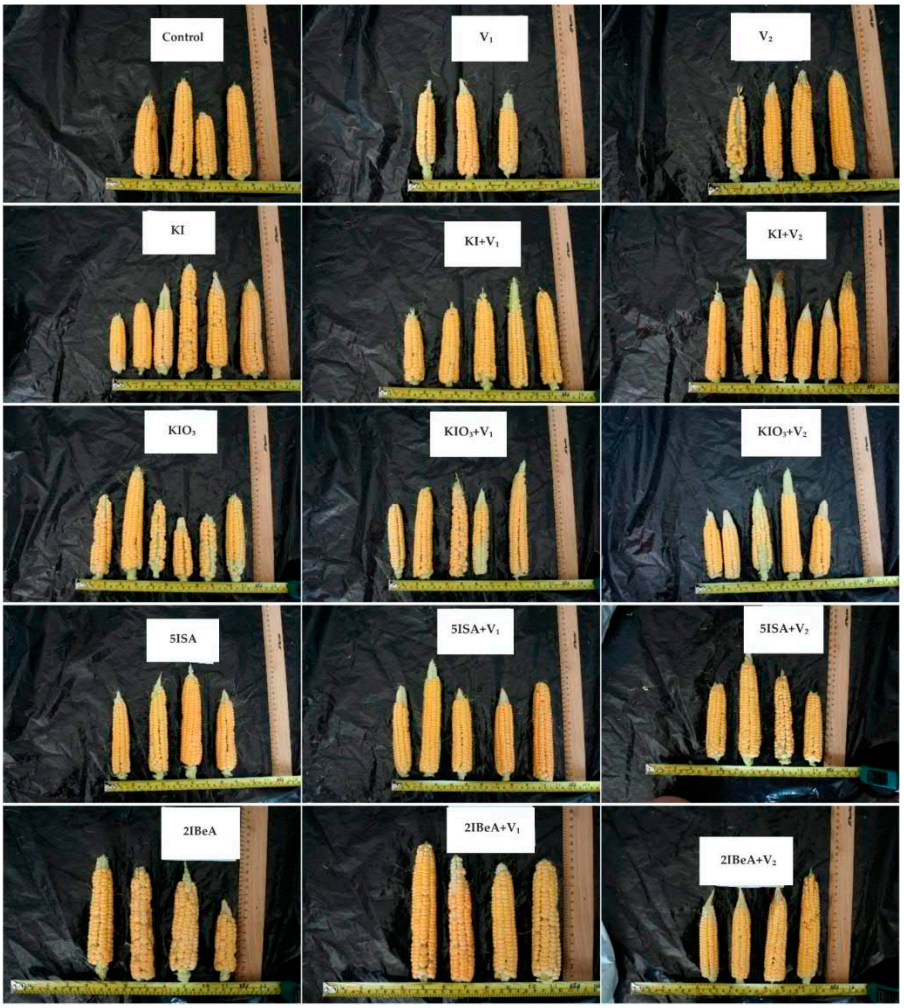
3.1. Plant Biomass and Yield of Corn Cob

The application of iodine and vanadium compounds in each of the applied doses did not have a statistically significant effect compared to the control on the sweetcorn cob yield, the average weight of one cob, and the plant height and weight of the aerial part of a single plant after harvest (Table 3 and Scheme 1).

Table 3. Results of growth of sweetcorn plants and yielding parameters of corn cob.

Treatments	Yield of All Gained Corn Cobs from One Plant (FMW g·Plants^{-1})	Average Weight of One Corn Cob (FMW g)	Weight of Aerial Part of Single Plant after Harvest of Corn Cob (FMW g)	Height of Aerial Part of the Plant at Harvest (cm)
Control	323.0 ± 36.21 a	61.1 ± 6.47 a	295.9 ± 47.82 a	168.4 ± 1.64 a
V_1	278.6 ± 37.76 a	52.9 ± 7.23 a	305.3 ± 29.75 a	177.2 ± 3.53 a
V_2	284.4 ± 35.76 a	56.3 ± 5.99 a	245.0 ± 24.02 a	169.0 ± 5.52 a
KI	355.1 ± 26.00 a	61.6 ± 7.18 a	259.3 ± 20.12 a	167.3 ± 9.03 a
KI + V_1	311.2 ± 29.40 a	57.4 ± 7.35 a	310.2 ± 25.52 a	180.0 ± 6.73 a
KI + V_2	310.1 ± 28.21 a	61.0 ± 8.62 a	264.6 ± 17.71 a	170.3 ± 5.86 a
KIO_3	295.0 ± 32.19 a	58.7 ± 8.57 a	292.3 ± 26.39 a	174.9 ± 8.28 a
KIO_3 + V_1	280.6 ± 39.98 a	54.6 ± 6.82 a	251.7 ± 31.10 a	169.0 ± 8.34 a
KIO_3 + V_2	291.5 ± 14.16 a	58.5 ± 9.11 a	239.0 ± 23.85 a	168.1 ± 6.01 a
5ISA	261.1 ± 21.04 a	53.8 ± 8.88 a	254.8 ± 43.40 a	165.9 ± 10.53 a
5ISA + V_1	291.1 ± 44.03 a	57.7 ± 7.37 a	288.5 ± 23.70 a	176.4 ± 6.17 a
5ISA + V_2	254.3 ± 28.61 a	53.4 ± 6.66 a	228.3 ± 18.05 a	164.0 ± 5.46 a
2IBeA	242.8 ± 43.62 a	58.3 ± 9.90 a	225.3 ± 12.86 a	168.0 ± 7.12 a
2IBeA + V_1	287.8 ± 36.79 a	60.7 ± 6.61 a	294.7 ± 41.53 a	168.8 ± 7.63 a
2IBeA + V_2	239.5 ± 34.41 a	54.9 ± 7.26 a	245.5 ± 16.59 a	175.6 ± 5.09 a

Means followed by different letters for treatments differ significantly $p < 0.05$ ($n = 8$).



Scheme 1. Corn cob at harvest—one from four replications.

3.2. Iodine Accumulation in Sweetcorn Plants

The application of organic and inorganic iodine compounds, both separately and together with vanadium, significantly increased the content of iodine in the roots, leaves, and grains of sweetcorn compared to the control (Figure 1A–C). Based on the effects of the tested iodine compounds (without the application of vanadium), the highest level of iodine enrichment in the grains was obtained with the application of organo-iodine compounds 5-ISA and 2-IBeA (increases of 117% and 110% relative to the control) followed by the inorganic forms KI and KIO_3 (with increases of 70% and 60%, respectively) (Figure 1A). In the leaves, after the application of the organic iodine compounds 5-ISA and 2-IBeA, we observed a lower level of iodine accumulation than that observed after fertilization with KI and KIO_3 (Figure 1B). For roots, the increase in iodine accumulation compared to the control was greatest after the application of 2-IBeA, followed by the application of KI, KIO_3, and 5-ISA (respectively, 124%, 92%, 36%, and 24% higher iodine levels than in the control). In general, the vegetative parts of sweetcorn were characterized by a higher level of iodine accumulation (roots > leaves) than that observed in grains, regardless of the form of iodine applied to the soil (Figure 1A–C).

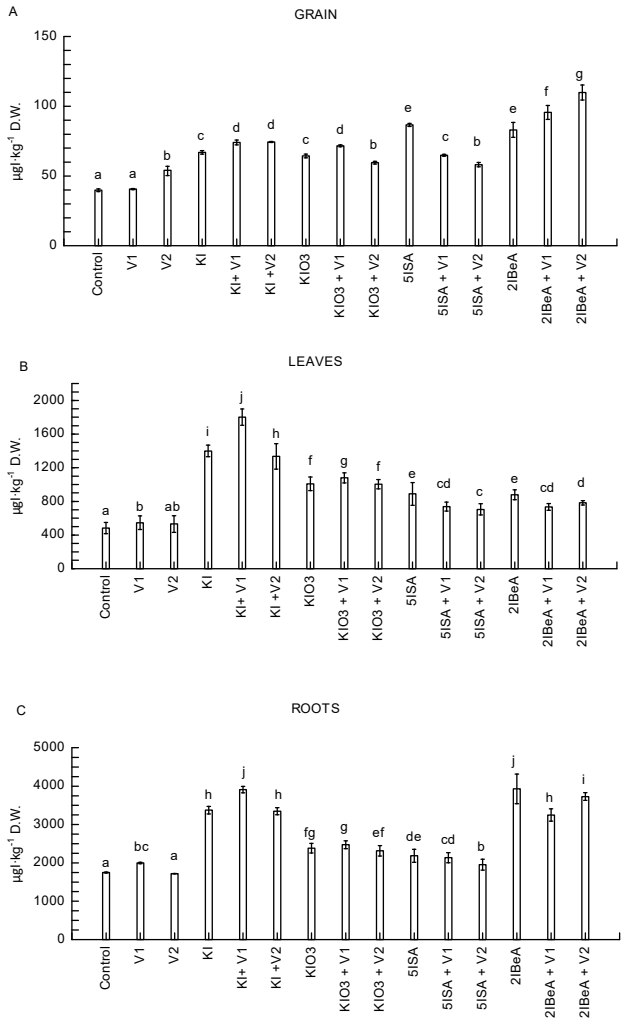


Figure 1. Iodine contents in grain (**A**), leaves (**B**), and roots (**C**) of sweetcorn plants. Means followed by different letters for treatments differ significantly $p < 0.05$ ($n = 8$). Bars indicate standard error.

3.3. The Interaction of Iodine with Vanadium in Individual Parts of the Plant

Under the combined application of KI and ammonium metavanadate at a lower dose (0.1 µmol V·dm^{-3}; KI + V1), a significant increase of the iodine content in roots, leaves, and grain was observed (increases of 15%, 28%, and 10%, respectively, compared to KI). An increase in iodine accumulation was also observed in grains after the combined application of KI with a higher dose of vanadium (1.0 µmol V·dm^{-3}; KI + V2 versus KI; Figure 1A) (an increase of 10% compared to KI). The application of KI + V2 did not increase the accumulation of iodine in the roots and leaves relative to KI (Figure 1B,C). For plants fertilized with KIO_3 + V1, compared to fertilization with KIO_3, we observed increased iodine content in the roots (by 3%), leaves (by 7%), and grains (by 11%). A higher dose of vanadium (KIO_3 + V2) caused a decrease (7%) in the level of iodine accumulation only in grains. The combined application of iodine in the form of the organic compound 5-ISA with vanadium in two doses (0.1 µmol V·dm^{-3} and 1.0 µmol V·dm^{-3}) reduced the level of iodine accumulation in the roots, leaves, and grains (by 2% and 11% in the roots, 17% and 21% in the leaves and 25%, and 32% in the grains, respectively, for doses V1 and V2), compared to the use of only 5-ISA. In the roots, this effect was significant only for the 5-ISA + V2 combination. In roots and leaves, the combined fertilization of 2-IBeA with vanadium in two doses resulted in a significant reduction in iodine content compared to the application of 2-IBeA alone—a reduction of 17% and 5%, respectively, for V1 and V2 in the roots and 16% and 11% for V1 and V2 in the leaves. Meanwhile, in the grain, along with

an increase in the dose of vanadium used together with 2-IBeA, we observed a significant increase in the iodine content—by 14% and 32%, respectively, compared to the application of 2-IBeA alone.

The iodine content in the grain after the application of 2-IBeA + V2 was approximately 180% higher than that in the control and V1 (without iodine fertilization) and 32% higher than that after the application of 2-IBeA and 5-ISA.

3.4. Vanadium Content in Sweetcorn Plants

We found a significant effect of vanadium fertilization on the content of vanadium in the roots, leaves, and grains of sweetcorn when used both separately and together with all applied iodine compounds (Figure 2A–C). In maize grain, we observed significantly higher vanadium content than that in the control for the following combination: KIO_3 + V1 > 5-ISA + V1 > KI + V1 = KIO_3 (Figure 2A).

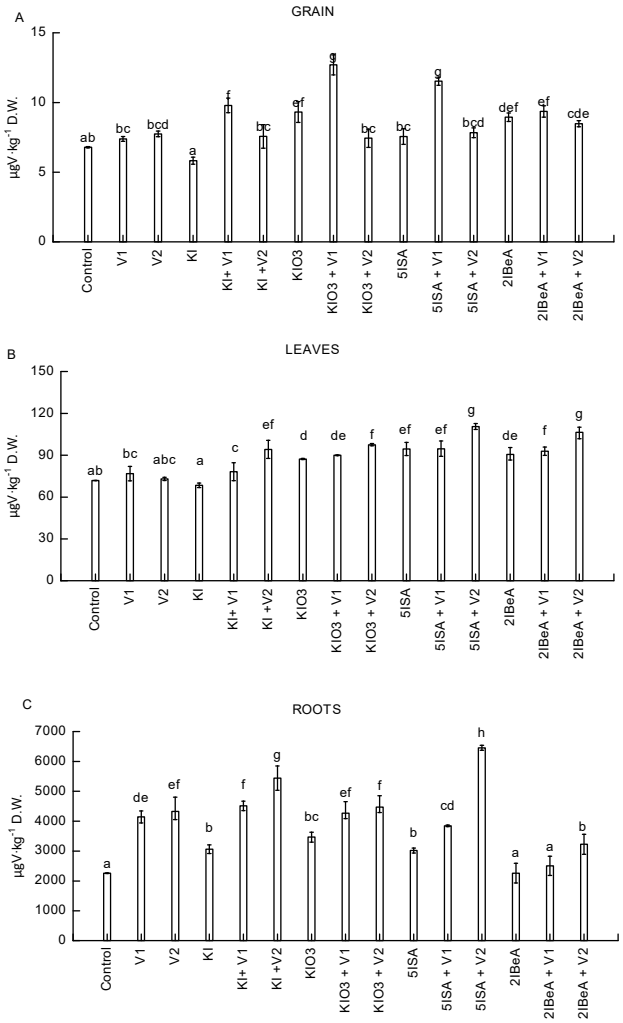


Figure 2. Vanadium contents in grain (**A**), leaves (**B**), and roots (**C**) of maize. Means followed by different letters for treatments differ significantly $p < 0.05$ ($n = 8$). Bars indicate standard error.

The content of vanadium in the leaves was, on average, 60 times lower than that in the roots (Figure 2B,C). In the leaves, all tested combinations with iodine fertilization in the form of KIO_3, 5-ISA, and 2-IBeA (but not KI) without vanadium resulted in a significant increase in the content of vanadium compared to the control (Figure 2B). For the application of individual iodine compounds together with vanadium at a lower dose, the level of vanadium accumulation in leaves was observed in the following order: KI

+ V1 < KIO_3 + V1 < 5-ISA + V1 = 2-IBeA + V1. In total, fertilization with all the tested iodine compounds together with vanadium at a higher dose (V2) significantly increased the accumulation of vanadium in the leaves compared to the application of the iodine compound +V1.

Soil fertilization and ammonium metavanadium were applied separately and together with iodine compounds. We noted that with an increase in the dose of vanadium, there was a significant increase in the level of vanadium accumulation in the roots (Figure 2C) compared to the control (by 83% and 101%). Differences in application-particular iodine percentages without vanadium ranged from 27% for 5-ISA + V2 versus 5ISA to 29% for KIO_3 + V1 versus KIO_3 (Figure 2C). The application of a lower dose of vanadium with 2-IBeA (the 2-IBeA + V1 combination) did not have a statistically significant effect on the content of vanadium in the roots compared to the control object and fertilization with 2-IBeA alone. The highest content of vanadium in the roots was observed when a higher dose of V2 was combined with the application of 5-ISA.

3.5. Content of Total Sugar and Ascorbic Acid in Sweetcorn Grains

The total content of sugars and vitamin C (L-ascorbic acid) in sweetcorn grain significantly depended on the form of iodine and the dose of vanadium applied (Figure 3A,B). Compared to the control, a significant increase in the sugar content of the grain was found only after soil fertilization with 5-ISA and 5-ISA + V1 (by 37% and 36%, respectively; Figure 3A). On the other hand, all the other tested combinations of vanadium and iodine fertilization used with and without vanadium at both doses (except 5-ISA + V2) resulted in a significant reduction of sugar content in maize. This was observed to the greatest extent for the KIO_3 + V2 combination, where a two-fold reduction in sugar content was observed compared to the control. Under the application of KIO_3 and 5ISA, a higher dose of vanadium and fertilization with KIO_3 and 5-ISA, without vanadium, reduced sugar content in the grain was observed. Such relationships were not observed for the fertilization of KI and 2-IBeA under the application of V1 and V2.

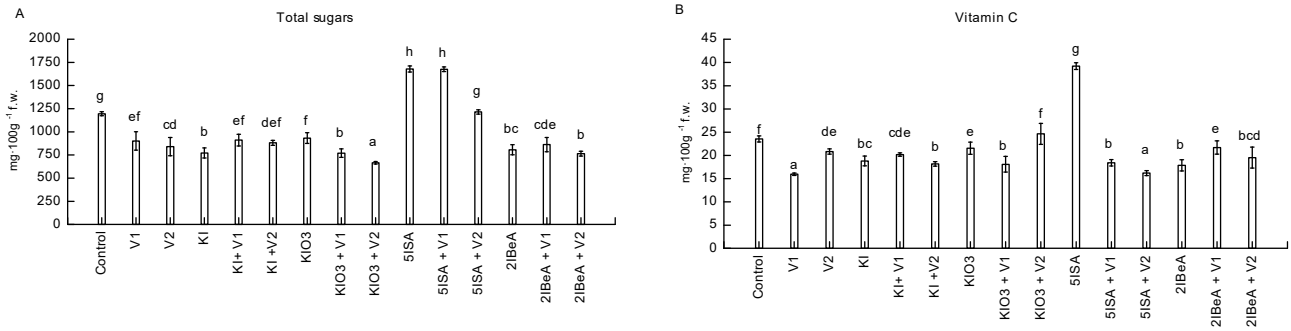


Figure 3. Content of total sugars (**A**) and ascorbic acid (**B**) in sweetcorn grain. Means followed by different letters for treatments differ significantly at $p < 0.05$ ($n = 8$). Bars indicate standard error.

After 5-ISA fertilization, the content of vitamin C was significantly higher than that in the control (Figure 3B). The combined application of 5-ISA with vanadium, at both doses, resulted in a significant reduction of vitamin C content in the grains (by 48% and 61%, respectively, for 5-ISA + V1 and 5-ISA) compared to the use of 5-ISA alone (and compared to the control). The application of vanadium alone, at both doses without the application of iodine, reduced vitamin C content in the grain compared to the control. The fertilization of KI with the additional application of vanadium at both doses did not have a significant effect on the vitamin C content in the grain. On the other hand, when using KIO_3, the additional application of V1 and V2 decreased and increased vitamin C content, respectively. However, when fertilizing with 2-IBeA, the use of vanadium increased the content of vitamin C in grain, which was observed to a greater extent with 2-IBeA + V1.

3.6. Content of Macro- and Microelements and Dry Matter in Maize Plant

The applied iodine and vanadium compounds had various effects on the content of macro- and microelements in the leaves, roots, and grains of sweetcorn (Tables 4 and 5). Generally, all the tested combinations of iodine and vanadium fertilization influenced the observed tendency (statistically significant for some of the combinations) to decrease Ca content in the grain (Table 4) and increase Mn and Fe content in the roots (Table 5). All the tested combinations of iodine and vanadium fertilization caused a significant reduction of Mo content in the grains compared to the control but, at the same time, did not affect the accumulation of Zn in the grains (Table 5).

Table 4. Contents of Ca, K, Mg, P, and S in grain, leaves, and roots of "Złota Karłowa" sweetcorn.

Treatment	Grain (% D.W)				
	Ca	K	Mg	P	S
Control	0.024 ± 0.002 e	0.85 ± 0.07 c	0.14 ± 0.002 b	0.49 ± 0.02 c	0.13 ± 0.006 abc
V1	0.020 ± 0.001b cde	0.85 ± 0.07 c	0.13 ± 0.004 ab	0.48 ± 0.01 bc	0.13 ± 0.002 abc
V2	0.022 ± 0.002 de	0.79 ± 0.08 abc	0.13 ± 0.002 ab	0.47 ± 0.02 bc	0.13 ± 0.003 abc
KI	0.013 ± 0.001 abc	0.77 ± 0.06 abc	0.12 ± 0.004 ab	0.45 ± 0.02 abc	0.13 ± 0.003 abc
KI + V1	0.020 ± 0.004 cde	0.82 ± 0.08 bc	0.12 ± 0.002 ab	0.44 ± 0.02 abc	0.12 ± 0.009 abc
KI + V2	0.019 ± 0.003 bcde	0.83 ± 0.07 bc	0.13 ± 0.005 ab	0.47 ± 0.01 bc	0.13 ± 0.003 abc
KIO_3	0.016 ± 0.002 abcde	0.85 ± 0.07 c	0.13 ± 0.005 ab	0.46 ± 0.02 bc	0.14 ± 0.004 bc
KIO_3 + V1	0.022 ± 0.003 de	0.82 ± 0.07 bc	0.13 ± 0.003 ab	0.46 ± 0.02 abc	0.14 ± 0.007 abc
KIO_3 + V2	0.016 ± 0.001 abcd	0.75 ± 0.04 abc	0.12 ± 0.008 ab	0.45 ± 0.01 abc	0.12 ± 0.005 abc
5ISA	0.016 ± 0.002 abcd	0.70 ± 0.02 a	0.12 ± 0.009 a	0.41 ± 0.02 ab	0.12 ± 0.007 abc
5ISA + V1	0.011 ± 0.002 ab	0.77 ± 0.05 abc	0.13 ± 0.010 ab	0.43 ± 0.01 ab	0.12 ± 0.008 abc
5ISA + V2	0.009 ± 0.001 a	0.75 ± 0.04 abc	0.14 ± 0.003 b	0.41 ± 0.02 ab	0.10 ± 0.008 a
2IBeA	0.012 ± 0.002 ab	0.73 ± 0.05 ab	0.12 ± 0.006 ab	0.41 ± 0.02 ab	0.11 ± 0.007 ab
2IBA + V1	0.017 ± 0.002 bcde	0.78 ± 0.05 abc	0.13 ± 0.008 ab	0.39 ± 0.03 a	0.11 ± 0.012 ab
2IBeA + V2	0.021 ± 0.002 cde	0.81 ± 0.06 bc	0.13 ± 0.006 ab	0.47 ± 0.02 bc	0.15 ± 0.004 c
	Leaves (% D.W)				
Control	2.70 ± 0.28 bcd	1.98 ± 0.15 a	0.49 ± 0.05 abcd	0.34 ± 0.03 ab	0.32 ± 0.03 abcd
V1	2.93 ± 0.09 de	2.14 ± 0.02 a	0.57 ± 0.05 d	0.39 ± 0.03 b	0.39 ± 0.05 d
V2	3.18 ± 0.17 e	2.16 ± 0.11 a	0.51 ± 0.02 cd	0.39 ± 0.04 b	0.36 ± 0.04 bcd
KI	2.54 ± 0.27 abcd	2.22 ± 0.25 a	0.44 ± 0.04 abc	0.37 ± 0.04 b	0.30 ± 0.02 abc
KI + V1	2.74 ± 0.24 cde	2.37 ± 0.15 a	0.51 ± 0.02 cd	0.37 ± 0.02 b	0.37 ± 0.02 cd
KI + V2	2.25 ± 0.20 ab	2.23 ± 0.08 a	0.44 ± 0.03 abc	0.31 ± 0.02 ab	0.28 ± 0.02 ab
KIO_3	2.60 ± 0.19 abcd	2.24 ± 0.16 a	0.48 ± 0.02 abcd	0.27 ± 0.02 a	0.27 ± 0.03 ab
KIO_3 + V1	2.40 ± 0.20 abc	2.36 ± 0.15 a	0.42 ± 0.02 abc	0.34 ± 0.02 ab	0.34 ± 0.02 abcd
KIO_3 + V2	2.63 ± 0.32 abcd	2.32 ± 0.13 a	0.40 ± 0.03 ab	0.32 ± 0.01 ab	0.27 ± 0.0 1a
5ISA	2.30 ± 0.18 abc	2.11 ± 0.11 a	0.39 ± 0.02 a	0.28 ± 0.01 a	0.28 ± 0.02 ab
5ISA + V1	2.51 ± 0.17 abcd	2.24 ± 0.10 a	0.48 ± 0.02 abcd	0.31 ± 0.02 ab	0.32 ± 0.01 abcd
5ISA + V2	2.50 ± 0.13 abcd	2.39 ± 0.12 a	0.46 ± 0.01 abc	0.28 ± 0.03 a	0.26 ± 0.01 a
2IBeA	2.59 ± 0.21 abcd	1.98 ± 0.11 a	0.43 ± 0.02 abc	0.27 ± 0.02 a	0.28 ± 0.02 ab
2IBeA + V1	2.43 ± 0.20 abc	2.39 ± 0.02 a	0.47 ± 0.02 abcd	0.28 ± 0.03 a	0.32 ± 0.02 abcd
2IBeA + V2	2.24 ± 0.22 a	2.34 ± 0.11 a	0.50 ± 0.02 bcd	0.27 ± 0.02 a	0.26 ± 0.01 a
	Roots (% D.W)				
Control	0.86 ± 0.13 ab	1.89 ± 0.22 defg	0.21 ± 0.04 cd	0.17 ± 0.02 ab	0.48 ± 0.06 abc
V1	0.92 ± 0.06 ab	1.79 ± 0.15 bcdef	0.20 ± 0.03 abcd	0.17 ± 0.02 ab	0.50 ± 0.04 abc
V2	0.83 ± 0.07 a	1.82 ± 0.21 cdef	0.21 ± 0.03 bcd	0.16 ± 0.01 ab	0.48 ± 0.05 abc
KI	0.86 ± 0.08 a	1.48 ± 0.10 a	0.19 ± 0.02 ab	0.17 ± 0.03 ab	0.42 ± 0.02 a
KI + V1	0.79 ± 0.10 a	1.61 ± 0.14 abc	0.18 ± 0.03 a	0.18 ± 0.02 ab	0.39 ± 0.04 a
KI + V2	0.96 ± 0.11 ab	1.73 ± 0.17 bcde	0.24 ± 0.05 e	0.16 ± 0.03 ab	0.56 ± 0.07 c
KIO_3	0.95 ± 0.09 ab	1.99 ± 0.22 fg	0.23 ± 0.03 de	0.17 ± 0.02 ab	0.55 ± 0.07 c
KIO_3 + V1	1.05 ± 0.10 b	1.89 ± 0.21 efg	0.21 ± 0.02 abcd	0.16 ± 0.02 ab	0.45 ± 0.05 abc
KIO_3 + V2	0.88 ± 0.04 ab	1.72 ± 0.18 bcde	0.19 ± 0.02 ab	0.17 ± 0.02 ab	0.43 ± 0.03 ab
5ISA	0.89 ± 0.12 ab	1.80 ± 0.20 bcdef	0.19 ± 0.03 ab	0.19 ± 0.02 b	0.47 ± 0.06 abc
5ISA + V1	0.86 ± 0.05 ab	1.59 ± 0.18 ab	0.18 ± 0.02 ab	0.16 ± 0.01 ab	0.46 ± 0.04 abc
5ISA + V2	0.96 ± 0.08 ab	1.68 ± 0.18 abcd	0.18 ± 0.02 a	0.15 ± 0.02 ab	0.39 ± 0.03 a
2IBeA	0.80 ± 0.05 a	2.04 ± 0.30 g	0.19 ± 0.02 abc	0.14 ± 0.02 a	0.43 ± 0.03 ab
2IBeA + V1	0.79 ± 0.02 a	1.68 ± 0.18 abcde	0.18 ± 0.02 ab	0.19 ± 0.03 b	0.39 ± 0.01 a
2IBeA + V2	0.98 ± 0.04 ab	1.76 ± 0.18 bcde	0.20 ± 0.02 abc	0.15 ± 0.01 ab	0.54 ± 0.05 bc

Means followed by different letters for treatments differ significantly at $p < 0.05$ ($n = 8$).

Table 5. Contents of Cu, Fe, Mn, Zn, Mo in the grain, leaves and roots of "Złota Karłowa" sweetcorn.

Treatment	Grain (mg·kg^{-1} D.W)				
	Mo	**Zn**	**Mn**	**Fe**	**Cu**
Control	2.32 ± 0.65 c	53.59 ± 1.59 ab	5.36 ± 0.48 bc	40.13 ± 2.22 cdef	2.42 ± 0.02 abcd
V1	1.09 ± 0.21 ab	46.39 ± 0.50 ab	4.76 ± 0.52 abc	35.61 ± 1.60 abcde	2.47 ± 0.03 abcd
V2	1.25 ± 0.23 ab	51.91 ± 2.04 ab	5.02 ± 0.54 abc	35.00 ± 1.39 abcd	3.09 ± 0.13 e
KI	1.16 ± 0.23 ab	43.82 ± 1.49 a	4.47 ± 0.51 ab	30.58 ± 1.48 a	2.24 ± 0.07 a
KI + V1	1.46 ± 0.40 ab	46.86 ± 2.45 ab	5.16 ± 0.78 bc	32.88 ± 0.99 ab	2.68 ± 0.15 abcde
KI + V2	1.21 ± 0.26 ab	47.83 ± 1.38 ab	5.45 ± 0.86 c	34.05 ± 3.12 abc	2.50 ± 0.03 abcd
KIO_3	1.07 ± 0.15 ab	55.75 ± 4.34 b	5.27 ± 0.62 bc	35.22 ± 1.62 abcd	2.48 ± 0.04 abcd
KIO_3 + V1	1.40 ± 0.32 b	53.21 ± 3.12 ab	4.94 ± 0.35 abc	35.56 ± 1.58 abcd	2.63 ± 0.08 abcd
KIO_3 + V2	1.27 ± 0.20 ab	50.73 ± 2.98 ab	4.51 ± 0.90 ab	33.78 ± 2.56 abc	2.71 ± 0.05 bcde
5ISA	0.61 ± 0.09 a	45.62 ± 4.45 ab	4.17 ± 0.84 a	40.04 ± 2.27 cdef	2.82 ± 0.14 de
5ISA + V1	1.15 ± 0.18 ab	46.44 ± 3.24 ab	4.61 ± 0.84 abc	41.16 ± 1.50 def	2.66 ± 0.07 abcde
5ISA + V2	0.91 ± 0.12 ab	44.19 ± 4.44 a	4.77 ± 1.11 abc	41.88 ± 1.24 ef	2.30 ± 0.04 abc
2IBeA	1.11 ± 0.17 ab	44.49 ± 2.88 a	4.59 ± 0.78 abc	39.63 ± 2.58 cdef	2.27 ± 0.09 ab
2IBeA + V1	0.88 ± 0.07 ab	44.77 ± 4.82 ab	4.95 ± 0.88 abc	38.98 ± 2.52 bcdef	2.73 ± 0.12 cde
2IBeA + V2	1.19 ± 0.18 ab	53.45 ± 1.71 ab	4.95 ± 0.41 abc	42.32 ± 3.46 f	2.47 ± 0.04 abcd
	Leaves (mg·kg^{-1} D.W)				
Control	7.35 ± 1.14 cde	41.07 ± 5.62 a	110.01 ± 17.41 cdef	167.27 ± 11.08 a	5.12 ± 1.00 ab
V1	7.39 ± 1.56 de	44.79 ± 1.64 abc	116.67 ± 7.75 fgh	182.21 ± 2.54 ab	6.46 ± 0.24 b
V2	6.91 ± 1.56 bcde	42.46 ± 3.57 ab	135.11 ± 10.23 i	157.96 ± 7.82 a	5.73 ± 0.65 b
KI	7.75 ± 1.75 e	44.87 ± 5.96 abc	128.19 ± 25.09 ghi	153.04 ± 14.31 a	5.30 ± 1.12 ab
KI + V1	6.33 ± 1.06 abcde	50.26 ± 4.09 bc	130.54 ± 25.00 hi	171.97 ± 7.17 a	4.87 ± 1.02 ab
KI + V2	4.55 ± 0.44 abcd	50.04 ± 8.99 bc	115.22 ± 25.00 efg	172.52 ± 3.96 a	6.01 ± 1.20 b
KIO_3	4.54 ± 0.99 abcd	41.23 ± 3.73 a	97.81 ± 17.01 bcd	161.67 ± 2.83 a	4.56 ± 0.57 ab
KIO_3 + V1	5.59 ± 0.96 abcde	42.92 ± 3.26 ab	97.09 ± 18.50 bc	158.65 ± 7.17 a	5.17 ± 0.86 ab
KIO_3 + V2	3.79 ± 0.24 a	46.70 ± 6.84 abc	98.97 ± 24.04 bcd	170.66 ± 8.59 a	4.88 ± 1.08 ab
5ISA	4.31 ± 0.58 ab	42.69 ± 7.53 ab	100.82 ± 24.53 bcde	163.00 ± 9.51 a	4.63 ± 1.11 ab
5ISA + V1	5.80 ± 1.03 abcde	46.56 ± 3.01 abc	109.87 ± 20.56 cdef	171.81 ± 8.09 a	5.68 ± 0.74 b
5ISA + V2	4.76 ± 0.93 abcd	39.61 ± 2.95 a	91.44 ± 19.44 ab	146.40 ± 5.79 a	5.71 ± 0.69 b
2IBeA	7.27 ± 1.90 cde	52.63 ± 7.46 c	100.07 ± 15.74 bcd	169.44 ± 13.04 a	4.86 ± 0.57 ab
2IBeA + V1	5.49 ± 1.06 abcde	50.51 ± 4.22 bc	112.15 ± 18.15 def	213.36 ± 19.28 b	5.28 ± 0.79 ab
2IBeA + V2	4.52 ± 0.92 abc	43.62 ± 6.12 ab	78.78 ± 12.20 a	166.70 ± 10.49 a	3.47 ± 0.91 a
	Roots (mg·kg^{-1} D.W)				
Control	0.73 ± 0.09 abc	60.81 ± 6.41 abc	55.88 ± 3.37 a	1166.38 ± 82.75 a	8.84 ± 1.03 ab
V1	0.67 ± 0.08 ab	76.49 ± 4.98 d	203.99 ± 28.82 f	2310.88 ± 361.1 fg	8.79 ± 0.56 ab
V2	0.64 ± 0.08 ab	69.43 ± 7.86 abcd	120.29 ± 5.41 abcd	1706.21 ± 201.07 cd	8.53 ± 0.17 ab
KI	0.63 ± 0.10 a	70.15 ± 5.18 abcd	196.50 ± 19.29 ef	2409.72 ± 313.89 g	8.68 ± 0.66 ab
KI + V1	0.67 ± 0.10 ab	58.72 ± 3.04 a	185.26 ± 38.07 def	2285.77 ± 417.23 fg	8.22 ± 0.77 ab
KI +V2	0.81 ± 0.04 abc	72.35 ± 5.76 cd	70.45 ± 2.10 ab	1539.58 ± 50.44 bc	8.63 ± 0.71 ab
KIO_3	1.05 ± 0.02 cde	59.44 ± 4.00 ab	163.31 ± 8.39 cdef	1917.32 ± 55.12 de	7.98 ± 0.25 ab
KIO_3 + V1	1.31 ± 0.23 e	91.91 ± 8.63 e	129.60 ± 27.79 abcdef	2313.99 ± 248.12 fg	8.77 ± 0.48 ab
KIO_3 + V2	1.21 ± 0.07 de	77.54 ± 12.88 d	124.29 ± 26.66 abcde	2158.10 ± 278.91 efg	7.72 ± 0.31 a
5ISA	0.93 ± 0.06 abcd	67.48 ± 6.82 abcd	88.03 ± 4.97 ab	1579.03 ± 51.88 bc	8.13 ± 0.51 ab
5ISA + V1	1.03 ± 0.05 cde	62.11 ± 10.53 abc	109.89 ± 30.24 abc	1470.74 ± 173.41 abc	7.36 ± 0.40 a
5ISA + V2	0.96 ± 0.08 bcd	61.60 ± 11.44 abc	120.48 ± 35.43 abcd	2010.46 ± 447.45 def	9.98 ± 0.99 b
2IBeA	0.80 ± 0.03 abc	71.06 ± 11.95 bcd	100.13 ± 20.84 abc	1743.94 ± 148.61 cd	8.77 ± 0.74 ab
2IBeA + V1	0.96 ± 0.04 abcd	58.85 ± 8.21 a	140.60 ± 61.31 bcdef	1273.04 ± 142.75 ab	7.18 ± 0.24 a
2IBeA + V2	1.24 ± 0.05 de	63.96 ± 10.55 abc	78.66 ± 16.71 ab	1309.44 ± 175.44 ab	7.97 ± 0.28 ab

Means followed by different letters for treatments differ significantly at $p < 0.05$ ($n = 8$).

The 5-ISA application stood out from the other combinations. Compared to the control, the use of 5-ISA significantly reduced the content of Ca, P, K, and Mg in the grain by −62%, −16%, −17%, and −14%, respectively (Table 4). After fertilization with 5-ISA alone, without vanadium, we also noted a reduction in the content of Mn in the grain compared to the control (−22%; Table 5). Moreover, in the leaves, after fertilization with

5-ISA alone, compared to the control, we found a reduction in Mg content (−14% Mg) (Table 4), with the lowest amount related to the lowest Mo content (−41%) (Table 5).

The soil fertilization of plants with vanadium alone at a higher dose (V2) resulted in leaves with the highest content of Ca and Mn (17% and 22% higher than that in the control; see Tables 4 and 5) and grains with the highest content of Cu (28% higher than that in control; see Table 5).

In the roots, fertilization with KI and KI + V1 resulted in the lowest Mg content (−9% and −14% versus the control, respectively), and KI + V2 fertilization yielded the highest value of Mg accumulation (+14% compared to the control) (Table 4). Fertilization with potassium iodide (KI) without vanadium resulted in the lowest K content in the roots (−22% versus the control) (Table 4), the lowest Fe and Cu content in the grain (−24% and –8%, respectively, compared to the control), and the highest Fe in the roots (106% higher than that in the control; see Table 5).

Compared to the control, the applied iodine and vanadium compounds had no significant effect on the content of S in grains, K, Mg, P, and S (Table 4); Fe and Cu (Table 5) in maize leaves; and Ca, P, and S in roots (Table 4).

Compared to the control, there was a significant decrease (−40%) in dry matter content (Table 6) in the roots after 2-IBeA + V2 fertilization and in the leaves after V1 and V2 fertilization (22.6% and 23.4%; KIO_3 + V1 21, 5%), as well as under all combinations with 5-ISA and 2-IBeA alone and together with vanadium (23% on average). In grain, only 5-ISA + V2 fertilization caused a significant increase in dry matter content (+11.5%) compared to the control.

Table 6. Dry matter content in sweetcorn plants part (roots, leaves, grain).

Treatments	% Dry Weight		
	Roots	Leaves	Grain
Control	12.03 ± 1.14 b	25.95 ± 1.73 b	35.66 ± 2.13 abc
V_1	9.59 ± 0.60 ab	20.06 ± 0.50 a	34.40 ± 1.96 ab
V_2	9.25 ± 0.66 ab	19.88 ± 0.76 a	36.02 ± 1.63 abc
KI	8.79 ± 0.24 ab	22.15 ± 0.84 ab	37.41 ± 1.86 abcd
KI + V_1	10.27 ± 0.71 ab	22.09 ± 0.95 ab	35.97 ± 1.57 abc
KI + V_2	10.17 ± 2.12 ab	22.61 ± 0.61 ab	37.89 ± 2.34 cd
KIO_3	8.80 ± 0.63 ab	22.04 ± 1.57 ab	34.16 ± 1.38 a
KIO_3 + V_1	10.41 ± 0.36 ab	20.37 ± 0.85 a	36.40 ± 1.70 abc
KIO_3 + V_2	8.88 ± 0.39 ab	22.98 ± 2.59 ab	37.81 ± 0.51 cd
5ISA	9.32 ± 0.55 ab	22.26 ± 1.14 ab	37.54 ± 1.10 bcd
5ISA + V_1	10.24 ± 0.84 ab	20.67 ± 1.10 a	37.26 ± 1.26 abcd
5ISA + V_2	8.31 ± 0.49 ab	19.44 ± 1.14 a	39.79 ± 0.95d
2IBeA	9.27 ± 0.35 ab	20.59 ± 0.37 a	36.60 ± 1.43 abcd
2IBeA + V_1	8.47 ± 0.59 ab	20.51 ± 0.68 a	35.87 ± 2.22 abc
2IBeA + V_2	7.28 ± 0.67 a	19.84 ± 0.42 a	35.08 ± 1.79 abc

Means followed by different letters for treatments differ significantly at $p < 0.05$ ($n = 8$).

4. Discussion

The toxic effects of iodine applied in the form of iodide (at doses of 10 μmol·dm^{-3} and 100 μmol·dm^{-3}) and iodate (KIO_3 100 μmol·dm^{-3}) were previously demonstrated in studies on rice biofortification [14]. The authors in [42] applied iodine via fertigation (2.34 mmol·dm^{-3} for KIO_3 and 3.01 mmol·dm^{-3} for KI) for the greenhouse cultivation of tomatoes, potatoes, maize, barley and observed the strongly phytotoxic effect of iodine compounds on plants. The authors observed a decrease in the growth of the biomass of these plants. In studies on the soil and foliar application of KI and KIO_3 at various doses in the cultivation of maize, wheat, and rice, the authors did not observe significant effects of the tested compounds on plant biomass and grain yield [15]. Other scientific reports indicate the bio-stimulating effect of iodine used at low concentrations, such as increases in

biomass in water spinach [27,32] and spinach [9,43] and increases in the yield of soybeans, rapeseed [4], and strawberry [18].

These studies did not show any negative or phytotoxic effects of organic and inorganic iodine compounds applied to the soil and vanadium fertilization on the growth and development of sweetcorn plants. The applied doses of iodine compounds (10 μmol I·dm^{-3}) for all compounds of this element and the doses of vanadium (0.1 and 1 μmol V·dm^{-3}) were found to be safe for sweetcorn plants. The soil application of iodine compounds in combination with vanadium (10 and 0.1 μmol V·dm^{-3}, respectively) in the early developmental stages of sweetcorn (BBCH 15) significantly increased the root mass. The application of KI, KIO_3, and the organic form 5-ISA significantly increased the height of sweetcorn plants [44].

The stimulating effect of vanadium at low doses was confirmed in the cultivation of peppers [34]. A 5 μmol V·dm^{-3} dose of vanadium increased the plant height, stem diameter, number of leaves and flower buds, volume of roots, and weight of fresh and dry pepper biomass. Higher concentrations of vanadium (i.e., 10 and 15 μmol·dm^{-3} V) had a negative effect on pepper plants [34]. Smoleń et al. [21] conducted an experiment on hydroponic lettuce cultivation using organic (5-ISA and 3,5-diISA) and inorganic (KIO_3) iodine compounds at the same dose of 10 μmol I·dm^{-3} and in combination with four different doses of vanadium (0.05, 0.1, 0.2, and 0.4 μmol V·dm^{-3}). The addition of organic iodine compounds to the medium (5-ISA and 3.5-diISA) reduced the size of the lettuce head by 7.5 times compared to the control object, and the object with KIO_3 applied at the same dose. The specificity of hydroponic cultivation is also important, where the iodine compounds are added to the growing medium and supplied throughout the period of growing lettuce. This previous study used seven soil applications for the cultivation of maize.

The soil application of iodine compounds and the combined application of iodine and vanadium in two doses significantly increased the content of iodine in all tested parts of the plant, i.e., roots, leaves, and sweetcorn grain. The strongest enrichment of sweetcorn grain with iodine was achieved by applying the organic compounds 5-ISA and 2-IBeA (analyzing only the effectiveness of the various forms of iodine applied individually without vanadium). The effectiveness of the forms of iodine used and their accumulation varied depending on the part of the plant. In the case of roots, the application of 2-IBeA was found to be the most effective. The distribution according to the efficiency of accumulation was 2-IBeA > KI > KIO_3 > 5-ISA in the roots, KI > KIO_3 > 5-ISA = 2-IBeA in the leaves, and 5-ISA = 2-IBeA > KI = KIO_3 in the grain. In the cultivation of tomato, the dominance of the application of organic forms on the accumulation of iodine in individual parts of the tomato plant was also demonstrated as follows: 4-IBeA > 3,5-diISA > 5-ISA > KI > 2-IBeA > KIO_3 > 2,3,5-triIBeA in leaves and petioles, 2-IBeA > 5-ISA > 2,3,5-triIBeA > 4-IBeA > 3,5-diISA > KI > KIO_3 in roots, and 2-IBeA > KI > 4-IBeA > 5-ISA > 3,5-diISA > KIO_3 > 2,3,5-triIBeA in tomato fruits [17]. In the cultivation of water spinach, the greatest accumulation of iodine was observed after applying the organic compound of iodine as follows: CH_2ICOO^- > I^- > IO_3^- [27]. Research on the effectiveness of iodine enrichment in crops has been mainly carried out using inorganic iodine KI and KIO_3. Many studies on crops have demonstrated more efficient iodine intake in the form of KI than KIO_3 [14,18,42,45–47], the latter of which must be reduced to I^- before uptake [9,32]. On the other hand, greater iodine accumulation with the addition of KIO_3 than KI was found in the cultivation of spinach [24].

Previous research on plants with the most effective degree of iodine accumulation showed leafy vegetables to be the best biofortification targets [43,48,49]. Cereals, including maize, are the dietary foundation of people in both developing and developed countries. The development of effective methods for enriching cereal plants with iodine will facilitate a wide range of methods to combat "hidden hunger", diseases caused by iodine deficiency in the diet [50,51]. The biofortification of rice into iodine did not result in any notable increases of iodine content in the grain, likely due to the specificity of iodine transport in the plant which takes place largely through the xylem. The efficiency of enriching the generative parts of the plant with iodine (rice grains) is limited. The iodine content in the

vegetative parts was several times higher than that in the grain [14]. Landini et al. [22] demonstrated the possibility of iodine transport through the phloem in tomatoes, which was confirmed by the significantly higher content of iodine in the fruit (generative parts of the plant) compared to the control. Similar results indicating effective iodine transport to the generative parts were found in the cultivation of tomato [17,42], strawberry [18], rice, and maize [9,15,52].

Vanadium-dependent haloperoxidases (vHPO) are crucial enzymes in the metabolism of brown algae halides (Cl^-, Br^-, I^-); vanadium-dependent iodoperoxidases play an important role in iodine accumulation [31,32] and antioxidant defence [29]. VHPO enzymes play a central role in both the capture of iodine from seawater and the synthesis of volatile hydrogen halides in marine algae [29,31]. Most studies have focused on *Laminaria digitata*, the brown North Atlantic algae considered to be a biogeochemical pump of iodine and bromine from the ocean to the atmosphere and the strongest accumulator of iodine [29,31]. The interactions of iodine and vanadium in higher plants, however, are not yet well known. The present research showed that vanadium in a lower applied dose (0.1 μmol V·dm^{-3}) in combination with KI increased the content of iodine in the roots, leaves, and grain. The combined application of KIO_3 with vanadium at a lower dose resulted in more effective transport to the higher parts of the plant, which led to higher iodine content in the leaves and grains compared to the application of KIO_3 alone. Under the combined application of 5-ISA and vanadium (1 μmol V·dm^{-3}), we observed a stimulating effect of this organic form of iodine on the accumulation of vanadium in the roots, as well as an antagonistic effect of vanadium in combination with 5-ISA on the accumulation of iodine in sweetcorn. On the other hand, under the combined application of vanadium at both doses (0.1 and 1 μmol V·dm^{-3}), 2-IBeA led to the significantly more effective transport of iodine to, and accumulation in, the grains. The most effective application was observed for 2-IBeA + V2. Vanadium application at a dose of 0.10 and 0.20 μmol V·dm^{-3} V together with the organic form of 3,5-diISA iodine increased the iodine content in lettuce leaves [21].

The ratios of iodine in the roots to leaves and in the roots to the grains for the combinations with organic iodine (2-IbeA + V2, where the iodine content was highest in the grain) and inorganic iodine (KI + V1, where the iodine content was highest in the roots and equally high in the grain) were as follows: for 2-IbeA + V2 30:1 (root: grain), 7:1 (leaf: grain), and 9:1 (root: leaf) for KI + V1; 53:1 (root: grain), 25:1 (leaf: grain), and 2:1 (root: leaf). The difference in the iodine content in the roots after the application of iodine compounds applied individually and in combination with vanadium was several dozen times lower (depending on the combination used) than the iodine content in the kernels of sweetcorn. The iodine accumulation gradient described in the literature is as follows: roots > leaves > stem > fruit > seeds [15,16,53,54]. In the present study, analogous dependencies were obtained for the level of iodine accumulation in the individual parts of the sweetcorn plants.

The reports in the literature on the accumulation of vanadium in various parts of the plant indicate that the roots are characterized by the highest degree of vanadium accumulation and low mobility to the above-ground parts of the plant. The poor reutilization of vanadium into the above-ground parts of plants is well known [35,55,56]. This dependence was also observed for maize. In the roots and leaves of sweetcorn, the content of vanadium increased with an increase in the dose. The concentration of vanadium in the roots was, on average, 50 times higher than that in the leaves. The combined application of 5-ISA with vanadium at a dose of 0.1 μmol·dm^{-3} stimulated the accumulation of vanadium in the grains. The same high content of vanadium in the grain observed in 5-ISA + V1 was also observed in KIO_3 + V1. The ratio of vanadium in roots, leaves, and grains for 5-ISA + V2 (the combinations with the highest content of vanadium in the roots and leaves, reflecting low vanadium mobility) were 875:1 (root: grain), 14:1 (leaf: grain), and 57:1 (root: leaf). In maize leaves and roots, the level of vanadium accumulation increased with an increase in dose. This confirms the results obtained in the cultivation of beans [55], pepper [34], and chickpea [36].

The dry matter content in maize leaves was statistically significantly lower in the objects with the application of vanadium (0.1 and 1 μmol V·dm^{-3}). In the roots, the application of 2-IbeA + V2 in the soil led to statistically significantly lower dry matter content than that in the control. On the other hand, the application of 5-ISA + V2 in the grain caused an increase in dry matter content compared to the control. In the cultivation of pepper, the dry matter content in leaves, roots, and shoots was significantly higher than that in the control at a dose of 5 μmol·dm^{-3} of vanadium [34] and in the cultivation of beans, for which an increase in the dry matter content was observed with an increase in the dose of vanadium compared to the control [35].

Fertilization with 5-ISA yielded the highest content of sugars and vitamin C in grain, and additional fertilization with vanadium using this iodine compound resulted in a reduction in vitamin C content. For KIO_3 used with vanadium, a reduction in sugar content was observed compared to the application of KIO_3 only. In the cultivation of pepper, vanadium stimulated an increase of sugar content in the plant leaves [34]. In sugar beet, the sucrose content increased by 28% in plants treated with 10 mmol V·dm^{-3} [57]. The application of KI in the cultivation of lettuce significantly increased the content of fructose at all tested doses, as well as glucose and sucrose (at doses of 20 and 40 μmol·dm^{-3}, respectively). KIO_3 had a negative effect on the content of fructose, glucose, and sucrose in lettuce leaves.

In many previous studies on the influence of iodine and vanadium (in various forms), different dependencies were observed in terms of the influence of these elements on the functioning of mineral nutrition, i.e., on the macro- and microelements of the plant nutrition process [8,35,58]. With high probability, this result is related to the plant species and the iodine [8] or vanadium doses used [34]. There is no unequivocal relationship between iodine and vanadium on the mineral nutrition of maize plants, which was observed to be specific for each of the iodine compounds used. The combination with the application 5ISA was different from other combinations. Compared to the control, the application of 5ISA significantly reduced the content of Ca, P, K, Mg, and Mn in the grain and Mg and Mo in the leaves. Despite this result, the nutritional intake of the plants with these ingredients was so high that there were few negative consequences on the growth and development of plants or the yield of corn cobs. The literature data show that iodine may have an antagonistic or synergistic effect on the uptake of macro- and microelements [35]. Smoleń and Sady [24] showed increased uptake and accumulation of Mg, Na, Cu, and Fe in spinach cultivation at a dose of 1 mg I·dm^{-3}. A higher dose of iodine, 2 mg I·dm^{-3}, increased the content of Na, Fe, Zn, and Al in spinach leaves and decreased the content of P, S, Cu, and Ba. The fertilization of KI plants had a negative effect on the content of Ca, Mg, B, and Mn in the leaves of maize plants in sweet maize cultivation at an early stage of development (BBCH 15) [44]. This result was caused by the dilution effect, as KI fertilization enabled the maize plants to grow heavier and longer roots, as well as heavier and taller above-ground parts.

5. Conclusions

The effective biofortification of corn grain into iodine was possible despite the poor mobility of this element into the generative organs of plants, including the grains of cereal plants, as described in the literature. The organic iodine compounds 5-ISA and 2-IBeA produced the strongest enrichment of iodine in sweetcorn grains. The synergistic effect of vanadium on the accumulation of iodine in corn grain was demonstrated after the application of the organic compound 2-IBeA, while the application of vanadium together with 5-ISA presented an antagonist effect on the accumulation of iodine in the grains. Organic 5-ISA and inorganic KIO_3, in combination with a lower dose of vanadium, significantly increased the accumulation of this element in the grain. This study confirmed the poor transport of vanadium in plants, as this element was mainly accumulated in the roots of sweetcorn. The application of 5-ISA (without vanadium) significantly influenced the quality parameters of sweetcorn grain, increasing the content of vitamin C and sugars.

Sweetcorn species can thus be considered for iodine biofortification programs. Such programs will allow researchers to incorporate the principles of soil fertilization with iodine in agrotechnical methods for combating iodine deficiency in the diets of people in many regions around the world.

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Synthesis of Organic Iodine Compounds in Sweetcorn under the Influence of Exogenous Foliar Application of Iodine and Vanadium

Marlena Grzanka [1,*], Sylwester Smoleń [1], Łukasz Skoczylas [2] and Dominik Grzanka [1]

[1] Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Krakow, Poland; sylwester.smolen@urk.edu.pl (S.S.); dominikgrzanka110@gmail.com (D.G.)
[2] Department of Plant Product Technology and Nutrition Hygiene, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 30-149 Krakow, Poland; lukasz.skoczylas@urk.edu.pl
* Correspondence: marlena.grzanka14@gmail.com or marlena.grzanka@student.urk.edu.pl

Abstract: A human's diet should be diverse and rich in vitamins, macro- and microelements essential for the proper functioning of the human body. Globally, a high percentage of the human population suffers from malnutrition, deficiencies of nutrients and vitamins also known as the problem of hidden hunger. This problem it is not only common in poor countries, but also occurs in developed countries. Iodine is a nutrient crucial for the proper functioning of the human and animal body. For plants, it is referred to as a beneficial element or even a microelement. The design of the biofortification experiment was determined on the basis of the interaction of iodine and vanadium (synergistic interaction in marine algae), where vanadium-dependent iodoperoxidase catalyzes apoplastic oxidation of iodine, resulting in high efficiency of iodine uptake and accumulation in brown algae (Laminaria digitate). Three independent experiments (Exp.) were carried out with the foliar application of vanadium (V) and iodine (I) compounds. The main differences between the experiments with the adapted proper corn biofortification method were the different application stage between the individual experiments, the application intervals and the dose of the iodine–vanadium compound. In each experiment, the accumulation of iodine and vanadium in the grain was several times lower than in the leaves. The combination iodine and vanadium significantly increased the accumulation of iodine in the grain in the case of applying V with inorganic iodine compounds, and a decrease in the accumulation of I after applying V with organic iodine compound —especially in Exp. No. 3. In grain, the highest content of I^-, IO_3^- was in combination with the application of 2-iodobenzoic acid (products of its metabolism). In most of the tested combinations, vanadium stimulated the accumulation/synthesis of exogenous/endogenous 5-iodosalicylic acid (5ISA) and 2-iodobenzoic acid (2IBeA), respectively, and decreased the content of 2,3,5-triiodobenzoic acid (2,3,5-triIBeA) in leaves and grains. The tested compounds I and V and the combinations of their application had a diversified effect on the vitamin C content in the grains. Vanadium in the lower dose of 0.1 µM significantly increased the sugar content in the grain.

Keywords: iodine; vanadium; biofortification; 5-iodosalicylic acid; 3,5-diiodosalicylic; 2-iodobenzoic acid; 2,3,5-triiodobenzoic acid; foliar nutrition

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1. Introduction

Iodine is an essential element necessary for the proper development and functioning of the human and animal organism. Its major role is related to the function it plays in the proper functioning of the thyroid gland [1]. Iodine is a substrate necessary for the synthesis of thyroid hormones, thyroxine-T4 and triiodothyronine-T3 [1–3]. Pregnant women are particularly at risk of deficiency of this element, and at the same time the most needy of it. Iodine deficiency during pregnancy is associated with health consequences for the baby,

such as impaired fetal brain development, leading to wider and irreversible changes such as cretinism disease. In some cases, insufficient daily dose of iodine in pregnant women can lead to infertility or miscarriage [4,5]. Another health consequence of iodine deficiency is the so-called hypertrophy of the thyroid gland, endemic goiter and increased probability of developing thyroid and stomach cancer [6,7]. The daily dose of iodine is 200–250 µg for pregnant women, 150 µg for adults, 90–120 µg for children from 5–12 years of age, under 5 years old 90 µg, respectively [1,6–8].

Around 2 billion people in the world suffer from iodine deficiency in the diet (hidden hunger of this microelement), and about 50 million are diagnosed with an affliction caused by this microelement deficiency [5,9]. The problem with insufficient iodine intake in the diet and the consequences associated with it occur in economically developed countries such as England, Germany, Australia and Italy [10,11]. Even though iodization has shown a great effect, iodine deficiency often persists. Moreover, WHO restrictions on limiting the consumption of table salt (iodization of table salt as one of the main programs to challenge the deficiency of this element in the diet), justified by the possibility of increasing hypertension (especially in pregnant women), initiated many studies and programs on alternative methods of implementation iodine in the human diet; one of them is the biofortification of crops [12,13]. Agrotechnical methods, soil fertilization, foliar application or genetic methods (plant breeding) also constitute cost-effective biofortification strategies [9,14–16]. A balanced diet is the basis for supplementing all the macro-/microelements and vitamins needed for proper functioning and the core of this balanced diet are vegetables, fruits and grain products [17,18].

Researches on the methods of effective enrichment of plants with iodine takes into account the type of application (foliar application, soil application, fertigation in field and soilless crops, hydroponics), the type of compound applied—inorganic compounds (such as KI, KIO_3) and organic compounds (2IBeA, 5ISA, 3,5-di-ISA, 4IBeA)—and plant type in terms of iodine enrichment into edible parts (fruit, grain, leaf, root) [19–22]. The research objectives and research hypotheses as well as the selection of these factors are different in individual research works. The highest and most effective degree of iodine enrichment is achieved by leafy vegetables [23–25]. Corn, wheat and rice can also be effectively biofortified with iodine, the achievement of which was shown by Cakmak et al. 2017 [22].

Information and current knowledge about the largest reservoir of iodine on Earth, which is seawater and the organisms that live in it, was a base for further study on the possibility of implementing that mechanism on terrestrial plants and combining it with sweet corn biofortification. Iodine volatilization from both the marine and terrestrial environments is a major component of the iodine biogeochemical cycle. There are two pathways of iodine volatilization in the marine environment—photochemical and the dominant biological one from micro- and macro-algae [26–28]. The capture and accumulation of iodine by algae is mediated by the enzyme vanadium-dependent haloperoxidase (vHPO) [29]. Vanadium-dependent haloperoxidase oxidizes halides and participates in the synthesis of organohalogen [28,30]. Vanadium-dependent haloperoxidase, whose prosthetic group is occupied by vanadium (vHIPO), improves iodine binding by catalyzing the oxidation of I^- to more lipophilic compounds (iodine(I) acid) HIO and subsequently molecular I_2. These molecules easily diffuse across cell membranes into the cytosol. The process of further reduction of HIO or I_2 to I^- in the apoplast is not yet well known, same as the presence of vanadium-dependent iodoperoxidase in higher plants [29,31,32]. Smoleń et al. 2020 conducted research on vanadium-dependent iodine peroxidase activity in lettuce plants [20]. The level of vHPO activity in corn plants at an early stage of their development after soil application of ammonium metavanadate together with organic (5ISA, 2IBeA) and inorganic iodine compounds (KI, KIO_3) was also tested by Grzanka et al. [33].

Sweet corn grain is rich in nutritional value, it contains large amounts of protein and most vitamins and microelements. The valuable components of sweet corn grains also include such microelements as selenium, chromium, zinc, copper, nickel and iron. It is

reasonable to add such an important micronutrient as iodine to this list, since maize for dry grain is one of the three most important cereals worldwide for more than 200 million people [34].

The aim of the research was to obtain an effective level of iodine in corn grain and to evaluate a better method of iodine foliar biofortification with the combined use of inorganic (KI, KIO_3) and organic iodine compounds (5ISA, 2IBeA) with vanadium. Moreover, the aim was to determine the effectiveness of iodine enrichment of sweet corn grains by foliar fertilization at various stages of plant development and expand knowledge about the content of iodosalicylates and 3,5-diiodosalicylic acid, iodobenzoates, 2,3,5-triiodobenzoic acid and iodide and iodate after foliar application of organic and inorganic iodine compounds to create a basis for iodine synthesis/transformation in maize plants to answer the question of whether a land plant like corn will be able to create an iodine uptake mechanism stimulated by the application of exogenous vanadium.

2. Results

2.1. Biometrical Parameters and Yield of Corn Cob

In all three experiments, the foliar application of iodine and vanadium compounds in each of the doses used did not have a statistically significant effect on cob yield, average weight of one cob, corn cob length or maximum diameter of the corn cob, as compared to the control (Tables 1–3). However, in each of experiments, the addition of vanadium showed the same trend of increasing yield when combined with KIO_3 with both doses of vanadium. In experiment No. 1 and No. 2, the higher dose of vanadium showed a trend to obtain lower yield in combination with 2IBeA compared to only the 2IBeA application, and a higher yield with the 5ISA + V application compared only with the 5ISA application. According to the analysis, only the use of vanadium in combination No. 1 and No. 2 with a higher dose of vanadium (V2) gave a trend of lower yield compared to the control, in No. 3 the same effect was shown at the lower dose of vanadium (V1). For a separated application of iodine compounds alone in all the performed experiments a similar yielding tendency could not be determined.

Table 1. Results of yield of corn cob, corn cob weight per one plant, corn cob length, number of corn kernels in one row of cob and diameter of one corn cob in experiment No. 1.

Treatment	Yield of Corn Cob ($t \cdot ha^{-1}$)	Corn Cob Weight per One Plant (g)	Corn Cob Length (mm)	Number of Corn in One Rows of Cob	Diameter of One Corn Cob (cm)
Control	10.86 ± 0.91 a	60.73 ± 5.0 a	114.84 ± 3.8 a	9.49 ± 0.3 a	2.94 ± 0.1 a
V1	12.21 ± 1.55 a	64.03 ± 2.9 a	121.18 ± 3.2 a	8.96 ± 0.2 a	2.99 ± 0.1 a
V2	9.78 ± 1.24 a	60.08 ± 6.0 a	107.39 ± 6.0 a	8.49 ± 0.1 a	2.90 ± 0.1 a
KI	7.95 ± 1.29 a	61.25 ± 8.2 a	114.42 ± 9.6 a	9.44 ± 0.1 a	2.95 ± 0.1 a
KI + V1	9.60 ± 2.20 a	57.85 ± 7.5 a	117.48 ± 7.8 a	9.69 ± 0.6 a	2.97 ± 0.1 a
KI + V2	8.39 ± 1.52 a	61.39 ± 7.6 a	120.49 ± 4.4 a	11.72 ± 0.5 a	3.06 ± 0.1 a
KIO_3	10.50 ± 0.79 a	51.94 ± 22.3 a	119.15 ± 4.7 a	9.91 ± 0.4 a	3.03 ± 0.1 a
KIO_3 + V1	10.55 ± 0.12 a	55.30 ± 4.1 a	113.98 ± 5.8 a	9.27 ± 0.2 a	3.03 ± 0.1 a
KIO_3 + V2	12.00 ± 1.85 a	79.52 ± 24.2 a	113.52 ± 7.1 a	11.70 ± 1.4 a	3.05 ± 0.1 a
5ISA	9.55 ± 1.31 a	58.38 ± 2.2 a	114.60 ± 6.9 a	10.77 ± 0.4 a	2.99 ± 0.1 a
5ISA + V1	9.82 ± 0.91 a	61.63 ± 5.6 a	118.59 ± 8.9 a	10.43 ± 0.2 a	3.00 ± 0.1 a
5ISA + V2	9.92 ± 1.70 a	53.50 ± 3.0 a	109.03 ± 3.9 a	10.01 ± 0.3 a	2.89 ± 0.1 a
2IBeA	11.99 ± 1.54 a	58.78 ± 0.9 a	121.76 ± 5.3 a	8.98 ± 0.1 a	3.04 ± 0.1 a
2IBeA + V1	10.10 ± 1.76 a	54.98 ± 2.2 a	114.34 ± 8.5 a	9.43 ± 0.2 a	3.00 ± 0.1 a
2IBeA + V2	10.15 ± 1.75 a	57.68 ± 4.7 a	120.23 ± 11.5 a	9.51 ± 0.3 a	3.06 ± 0.1 a

Means followed by different letters for treatments differ significantly $p < 0.05$ ($n = 8$); ± standard error.

Table 2. Results of yield of corn cob, corn cob weight per one plant, corn cob length, number of corn kernels in one row of cob and diameter of one corn cob in experiment No. 2.

Treatment	Yield of Corn Cob ($t \cdot ha^{-1}$)	Corn Cob Weight per One Plant (g)	Corn Cob Length (mm)	Number of Corn in One Rows of Cob	Diameter of One Corn Cob (cm)
Control	11.7 ± 1.29 a	75.73 ± 3.49 a	142.78 ± 3.98 a	7.89 ± 0.11 a	3.19 ± 0.08 a
V1	11.77 ± 3.55 a	71.61 ± 14.10 a	141.09 ± 4.97 a	7.96 ± 0.20 a	3.19 ± 0.08 a
V2	11.48 ± 1.19 a	80.90 ± 4.80 a	147.46 ± 3.94 a	7.81 ± 0.19 a	3.36 ± 0.07 a
KI	12.52 ± 1.99 a	111.31 ± 39.08 a	134.47 ± 6.72 a	7.96 ± 0.04 a	2.93 ± 0.19 a
KI + V1	10.61 ± 1.23 a	65.56 ± 3.90 a	134.87 ± 1.24 a	8.11 ± 0.11 a	2.99 ± 0.06 a
KI + V2	10.58 ± 1.55 a	74.67 ± 11.21 a	134.63 ± 8.52 a	7.81 ± 0.19 a	3.11 ± 0.16 a
KIO_3	10.41 ± 1.59 a	68.62 ± 4.41 a	139.64 ± 5.20 a	7.94 ± 0.06 a	3.13 ± 0.07 a
KIO_3 + V1	12.79 ± 2.25 a	68.36 ± 2.79 a	137.33 ± 4.03 a	7.95 ± 0.05 a	3.13 ± 0.04 a
KIO_3 + V2	12.97 ± 0.83 a	65.42 ± 4.60 a	130.06 ± 7.43 a	7.93 ± 0.08 a	3.03 ± 0.08 a
5ISA	11.70 ± 0.98 a	79.76 ± 7.82 a	141.53 ± 3.49 a	7.88 ± 0.13 a	3.16 ± 0.11 a
5ISA + V1	11.18 ± 1.41 a	68.18 ± 11.23 a	135.14 ± 4.14 a	7.95 ± 0.04 a	3.13 ± 0.14 a
5ISA + V2	11.88 ± 1.22 a	66.77 ± 8.03 a	139.89 ± 3.30 a	7.75 ± 0.25 a	2.95 ± 0.08 a
2IBeA	10.80 ± 1.90 a	69.69 ± 10.75 a	137.60 ± 5.45 a	8.14 ± 0.09 a	3.15 ± 0.14 a
2IBeA + V1	10.67 ± 2.32 a	61.69 ± 8.95 a	136.85 ± 5.94 a	8.17 ± 0.17 a	2.99 ± 0.10 a
2IBeA + V2	9.92 ± 2.45 a	58.83 ± 2.64 a	133.58 ± 6.40 a	7.76 ± 0.14 a	2.92 ± 0.05 a

Means followed by different letters for treatments differ significantly $p < 0.05$ ($n = 8$); ± standard error.

Table 3. Results of yield of corn cob, corn cob weight per one plant, corn cob length, number of corn kernels in one row of cob and diameter of one corn cob in experiment No. 3.

Treatments	Yield of Corn Cob ($t \cdot ha^{-1}$)	Corn Cob weight per One Plant (g)	Corn Cob Length (mm)	Number of Corn in One Rows of Cob	Diameter of One Corn Cob (cm)
Control	10.6 ± 0.48 a	69.07 ± 4.03 a	144.04 ± 3.64 a	8.21 ± 0.14 a	3.04 ± 0.07 a
V1	8.9 ± 0.61 a	66.65 ± 5.92 a	136.15 ± 3.73 a	8.09 ± 0.06 a	2.94 ± 0.06 a
KI	9.61 ± 0.45 a	61.10 ± 3.40 a	133.48 ± 3.44 a	8.06 ± 0.06 a	2.97 ± 0.06 a
KI + V1	8.26 ± 0.93 a	63.85 ± 3.52 a	136.30 ± 4.13 a	8.18 ± 0.14 a	3.09 ± 0.05 a
KIO_3	10.53 ± 0.92 a	63.10 ± 4.73 a	139.29 ± 4.08 a	8.13 ± 0.08 a	3.04 ± 0.06 a
KIO_3 + V1	11.36 ± 0.91 a	68.94 ± 5.46 a	143.10 ± 4.63 a	7.99 ± 0.10 a	3.08 ± 0.07 a
5ISA	9.6 ± 0.99 a	64.45 ± 3.18 a	141.01 ± 4.56 a	8.10 ± 0.07 a	3.04± 0.06 a
5ISA + V1	10.57 ± 0.81 a	67.21 ± 5.20 a	140.76 ± 4.08 a	8.19 ± 0.21 a	3.09± 0.06 a
2IBeA	9.82 ± 0.63 a	64.70 ± 4.64 a	138.13 ± 3.97 a	8.10 ± 0.15 a	2.98± 0.06 a
2IBeA + V1	11.72 ± 1.02 a	70.87 ± 7.54 a	144.10 ± 3.99 a	8.28 ± 0.22 a	3.09 ± 0.08 a

Means followed by different letters for treatments differ significantly $p < 0.05$ ($n = 8$); ± standard error.

2.2. Iodine Accumulation in Sweet Corn Plants

In all three experiments, foliar application of organic and inorganic iodine compounds used separately and together with vanadium significantly increased the iodine content in sweetcorn leaves and grains compared to the control (Figure 1A–F).

Analyzing the tested iodine compounds (excluding the combined application of iodine with vanadium), the highest iodine content in sweet corn grains in experiment No. 1 was obtained after the application of the inorganic compound KI (4.8 times higher than in the control), in experiment No. 2 and 3, after 2IBeA application, the contents were 52 and 20 times higher, respectively, than in the control (Figure 1A–C). The level of iodine accumulation in grains after the application of 2IBeA in experiment No. 2 was 1.5 times higher than the level of iodine accumulation after application of KI in experiment No. 1. The iodine concentration in grain after the application of 2IBeA in experiment No. 1 was 1.7 times lower than in experiment No. 2 and 2.9 times lower than in experiment No. 3. In all three experiments, the highest level of iodine accumulation in grains was found in experiment No. 3 after 2IBeA application (Figure 1A–C).

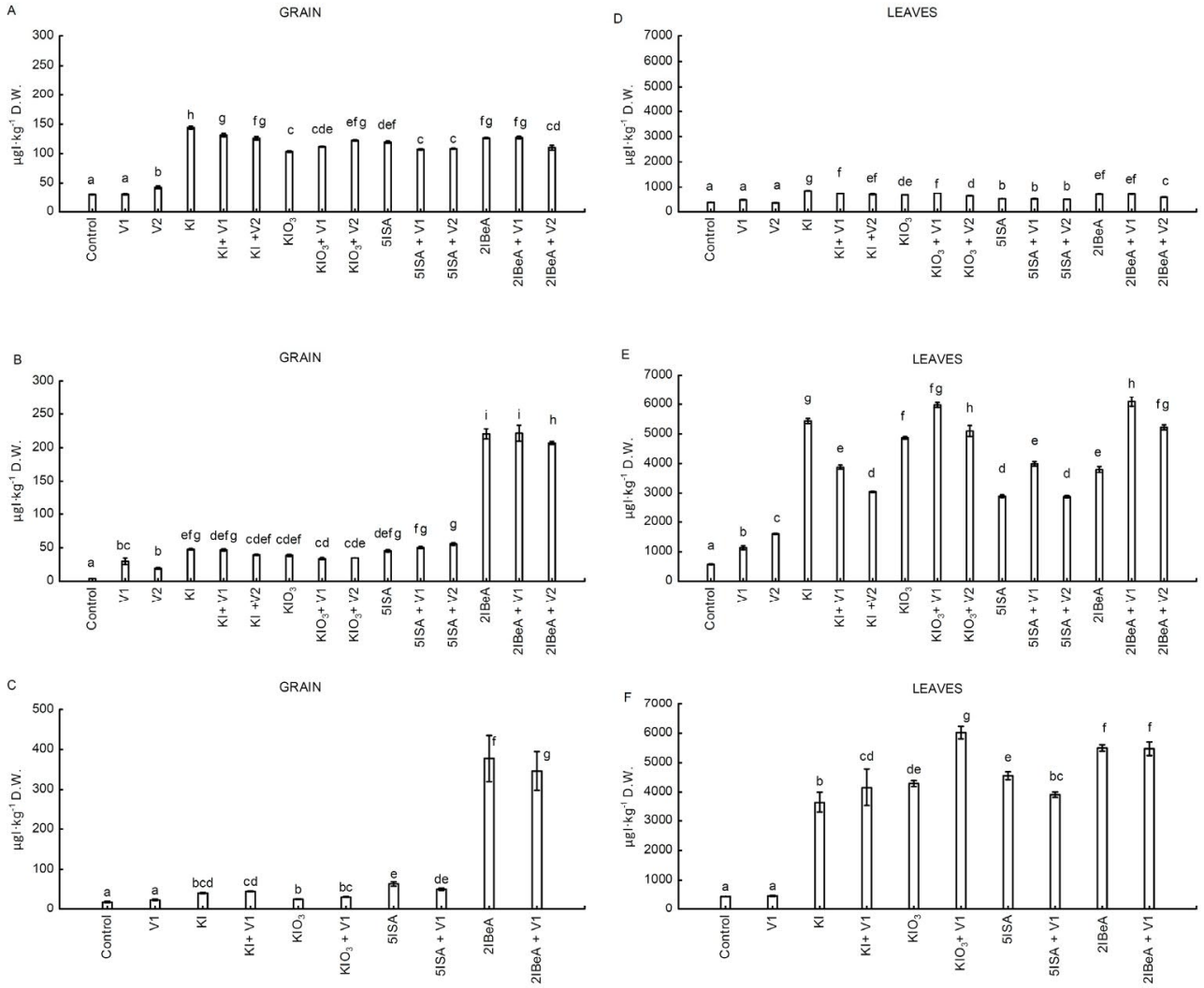

Figure 1. Iodine contents in grain in experiment No. 1 (**A**), No. 2 (**B**) and No. 3 (**C**) and in leaves in experiment No. 1 (**D**), No. 2 (**E**) and No. 3 (**F**) of sweetcorn plants. Means followed by different letters for treatments differ significantly $p < 0.05$ ($n = 8$). Bars indicate standard error.

The vegetative parts of sweet corn (leaves) were distinguished by a higher level of iodine accumulation than grains, regardless of the form of iodine applied (Figure 1D–F). The greatest differences between the iodine content in leaves and grains were observed in experiment No. 2 (on average 25 times higher iodine content in leaves than in grain), and the lowest in experiment No. 1 (an average of 6 times higher iodine content in leaves than in grains). Analyzing the tested iodine compounds (excluding combinations with the application of iodine with vanadium), the highest iodine content in sweet corn leaves in experiment No. 1 was obtained after the application of the inorganic compound KI (2.3 times more than in the control), and in experiment No. 2 it was also KI (9.5 times more than in the control). In the experiment No. 3, the highest degree of iodine accumulation in leaves was determined after application of 2IBeA (12.4 times more than in the control) (Figure 1D–F). The lowest degree of accumulation in leaves compared to the other applied iodine compounds in experiments No. 1 and No. 2 was obtained after 5ISA application. In turn, in experiment No. 3, the application of KI (excluding combinations with the application of iodine with vanadium) resulted in the lowest degree of iodine enrichment of the leaves in relation to other iodine compounds, both organic and inorganic.

2.2.1. The Interaction of Iodine with Vanadium in Individual Parts of the Plant

In experiment No. 1, application of vanadium (in doses of 0.1 μM and 1.0 μM V) together with iodine significantly influenced the iodine content in corn grains only when used in conjunction with KIO_3 (KIO_3 + V1, KIO_3 + V2 versus KIO_3). There was an 8% and 18% increase in iodine accumulation in grains compared to KIO_3, respectively (Figure 1A). The combination of KI, 5ISA and 2IBeA with vanadium (in both doses excluding 2IBeA + V1) reduced the iodine content in corn grains in experiment No. 1 (Figure 1A). In experiment No. 2, no statistically significant differences were found in the accumulation of iodine in grains in combinations with and without the addition of vanadium after the application of KI, KIO_3 and 5ISA. In this experiment, the higher dose of vanadium (1.0 μM V) in combination with 2IBeA resulted in a 7% reduction of iodine content compared to the combination with 2IBeA application without vanadium (Figure 1B). Using of vanadium at a dose of 0.1 μM V in experiment No. 3 in combination with inorganic iodine compounds increased the accumulation of iodine by 9% for KI + V1 in relation to KI and by 19% for KIO_3 + V1 in relation to KIO_3. In the case of combined application of vanadium with organic vanadium compounds, a decrease in iodine content in the grain was observed, by 21% for 5ISA + V1 versus 5ISA, and by 9% for 2IBeA + V1 versus 2IBeA, respectively (Figure 1C).

In the leaves in experiment No.1, the combined foliar application of iodine and vanadium in the form of ammonium metavanadate had no statistically significant effect on the iodine accumulation for the combination with 5ISA versus 5ISA + V1, 5ISA + V2. The lower dose of 0.1 μM V vanadium used with 2IBeA also had no statistically significant effect on the accumulation of iodine in the leaves. On the other hand, after using a higher dose of vanadium with 2IBeA (2IBeA + V2), a 20% reduction of iodine accumulation in leaves was observed compared to 2IBeA applied alone. The stimulating effect of a lower dose of vanadium was found in the combination KIO_3 + V1 vs. KIO_3 (increase by 11%). For KIO_3 + V2 there was a 4.5% decrease in the iodine content in the leaves in relation to KIO_3. In the objects KI + V1 and KI + V2, the iodine content in the leaves was lower by 15% and 18% compared to the application of KI alone (Figure 1D).

In experiment No. 2 no beneficial effect of vanadium on iodine accumulation after the use of KI + V1 and KI + V2 compared to the only application of KI (decrease in iodine content in leaves by 29% and 44%, respectively) (Figure 1E) was shown. In the other tested compounds, the lower dose of metavanadate had a stimulating effect by increasing the iodine content in the leaves: by 22% for KIO_3 + V1 vs. KIO_3, by 37% for 5ISA + V1 vs. 5ISA as well as by 60% 2IBeA + V1 vs. 2IBeA (Figure 1E).

In experiment No. 3, where only one lower dose 0.1 μM V of ammonium metavanadate was used, a statistically significant increase in the iodine content in the leaves was found after the combined application of KI + V1 vs. KI by 13%, by 40% after the use of KIO_3 + V1 vs. KIO_3 and by 7% as a result of foliar application of 2IBeA + V1 vs. 2IBeA. Only the combined application of 5ISA + V1 caused a 14% decrease in the accumulation of iodine in the leaves versus 5ISA (Figure 1F). The level of iodine accumulation in the leaves in experiments No. 2 and No. 3 were several thousand micrograms higher than in experiment No. 1.

2.2.2. The Content of Iodide, Iodates Ion and Organo-Iodine Compounds in Sweet Corn Grain and Leaves

All tested iodine compounds increased the content of iodide (I^-) ions in corn grains in experiment No. 3. The highest, 4.7-fold increase in comparison to the control, was observed after the application of 2IBeA without vanadium. The content of iodide (I^-) ions decreased 1.5 times in combination with the addition of vanadium 2IBeA + V1 compared to the solo application of 2IBeA (Figure 2A). The addition of vanadium in the case of KI application caused a statistically significant increase in the content of I^- ions compared to the application of KI without vanadium. In turn, the level of IO_3^- accumulation in corn grains was the highest in the application of KIO_3 and 2IBeA without vanadium. The

addition of vanadium to these compounds resulted in a 2.4-fold reduction in the content of IO_3^- for combination KIO_3 + V1 vs. KIO_3 and 2.5-fold for 2IBeA + V1 vs. 2IBeA. In the combination of 5ISA + V1 a statistically significant increase (1.4-fold) in the level of IO_3^- content in the grain was shown compared to the combination without vanadium (5ISA) (Figure 2B).

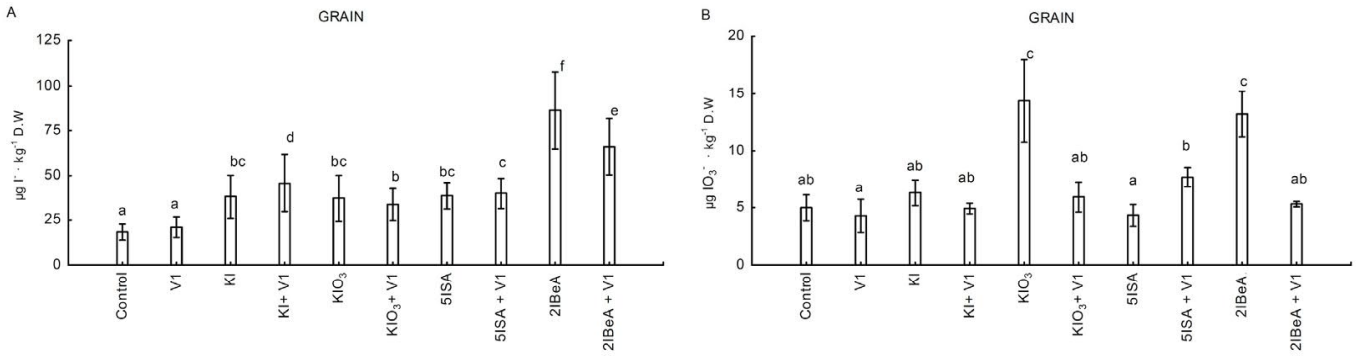

Figure 2. Content of iodide [I^-] (**A**) iodates [IO_3^-] ion (**B**) in sweet corn grain in experiment No. 3. Results only from 1 year of study (2020). Means followed by different letters for treatments differ significantly at $p < 0.05$ ($n = 8$). Bars indicate standard error.

The highest 5ISA content in grain and leaves was found after the foliar application of 5ISA + V1 compared to the control and other combinations (Table 4). It should be noted that the content of 5ISA in grain and leaves after the application of this compound without vanadium was lower than in the combination of 5ISA + V1. The lowest 5ISA content in grain was obtained in the control and the facility with the 2IBeA application. The highest 3,5-diISA content in sweet corn grain was recorded in the grain of the control plants. It was 3.6 times higher than in the combination with the lowest 3,5-diISA content, i.e., after the application of KIO_3. On the other hand, the tendency of the 3,5-diISA content in the leaves was different—the leaves of the control plants contained the least 3,5-diISA, and the most leaves after the application of KIO_3 + V1. In the tested combinations KIO_3 vs. KIO_3 + V1, KI vs. KI + V1 and 5ISA vs. 5ISA + V1, an increasing 3,5-diISA content was noted, which was a significant effect of vanadium addition. The 2IBeA content in leaves and grains was the highest after foliar application of this compound. However, a synergistic effect of vanadium on the accumulation of 2IBeA in grain and an antagonistic effect of vanadium on the accumulation of 2IBeA in the leaves was noted. The higher accumulation of 2IBeA in the grain was found in the case of the combined application of 2IBeA + V1 versus 2IBeA. In the case of the KI and 5ISA application, their combined use with vanadium compared to the application without vanadium resulted in a reduction in the 2IBeA content in the grain, and for 5ISA + V1 versus 5ISA also in the leaves. The content of 2,3,5-triIBeA in corn grain and leaves was several hundred times lower than 5ISA, 3,5-diISA and 2IBeA. The highest content of 2,3,5-triIBeA in grain was recorded after application of 2IBeA, and in leaves after application of V1. In grain and leaves simultaneous foliar application of KI + V1 versus KI; and KIO_3 + V1 versus KIO_3 and 5ISA + V1 versus 5ISA but only in leaves; as well as 2IBeA + V1 versus 2IBeA only in grain, it caused a decrease in 2,3,5-triIBeA content.

Table 4. Content of iodosalicylates [5-iodosalicylic acid (5ISA) and 3,5-diiodosalicylic acid (3,5-diISA)] and iodobenzoates [2-iodobenzoic acid (2IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA)]—all in $\mu g \cdot kg^{-1}$ D.W in sweet corn plants grains and leaves.

Treatment	GRAIN $\mu g\ kg^{-1}$ D.W			
	5ISA	**3,5-diISA**	**2IBeA**	**2,3,5-triIBeA**
Control	1.65 ± 0.02 a	62.62 ± 0.26 h	2.93 ± 0.29 a	0.200 ± 0.007 ab
V1	52.00 ± 1.38 e	20.36 ± 0.40 b	37.75 ± 0.84 e	0.102 ± 0.004 ab
KI	25.92 ± 1.40 d	24.37 ± 0.11 c	20.85 ± 0.31 d	0.284 ± 0.121 bc
KI +V1	15.73 ± 0.18 bc	28.81 ± 0.34 d	1.80 ± 0.04 a	0.031 ± 0.003 a
KIO_3	7.92 ± 0.20 ab	17.00 ± 0.46 a	3.95 ± 0.18 a	0.046 ± 0.015 a
KIO_3 + V1	23.33 ± 0.49 cd	35.16 ± 0.24 f	2.63 ± 0.63 a	0.021 ± 0.001 a
5ISA	60.08 ± 1.38 ef	32.65 ± 0.56 e	13.88 ± 0.37 c	0.159 ± 0.014 ab
5ISA + V1	67.04 ± 5.55 f	38.16 ± 0.24 g	8.81 ± 0.12 b	0.088 ± 0.001 a
2IBeA	2.30 ± 0.10 a	34.42 ± 0.07 ef	365.99 ± 0.83 f	0.465 ± 0.001 c
2IBeA + V1	11.79 ± 0.36 b	39.51 ± 0.73 g	393.61 ± 0.51 g	0.084 ± 0.004 a
	LEAVES $\mu g\ kg^{-1}$ D.W			
	5ISA	**3,5-diISA**	**2IBeA**	**2,3,5-triIBeA**
Control	29.28 ± 0.33 a	15.75 ± 0.09 a	68.61 ± 0.78 d	0.302 ± 0.003 b
V1	59.63 ± 1.50 cd	36.85 ± 0.10 b	30.03 ± 1.33 a	0.6234 ± 0.036 f
KI	48.13 ± 0.23 b	41.08 ± 0.70 c	29.62 ± 0.62 a	0.507 ± 0.048 de
KI + V1	55.75 ± 1.57 c	86.44 ± 0.46 g	29.92 ± 1.06 a	0.099 ± 0.006 a
KIO_3	60.94 ± 0.34 d	36.32 ± 0.46 b	38.53 ± 0.64 ab	0.618 ± 0.009 f
KIO_3 + V1	70.14 ± 1.02 e	53.57 ± 1.86 de	52.41 ± 0.11 c	0.397 ± 0.021 c
5ISA	586.37 ± 0.65 f	51.83 ± 0.25 d	43.38 ± 0.60 bc	0.537 ± 0.033 e
5ISA + V1	1082.80± 0.83 g	60.96 ± 0.19 f	26.84 ± 0.20 a	0.461 ± 0.015 cd
2IBeA	55.91 ± 0.67 c	55.76 ± 0.001 e	2552.32 ± 3.86 f	0.250 ± 0.022 b
2IBeA + V1	55.83 ± 1.30 c	42.75 ± 0.09 c	1363.85 ± 6.98 e	0.234 ± 0.011 b

Means followed by the same letters separately for each part of plants are not significantly different for $p < 0.05$; ±, standard error ($n = 4$). Results only from 2020 year of experiment No. 3.

2.3. Vanadium Content in Sweet Corn Plants

The vanadium fertilization applied separately as well with all iodine compounds showed a significant effect on the vanadium content on the leaves and grain of sweet corn (Figure 3A–F). In experiments No. 1 and No. 2, with the increase in the dose of used vanadium, the content of this element in sweet corn leaves and corn grains increased (Figure 3A,B,D,E). However, only when vanadium was applied in combination with 5ISA were there no statistically significant differences in terms of V content in the grain between the combinations 5ISA + V1 and 5ISA + V2 in both experiment No. 1 and No. 2 (Figure 3A,B). The vanadium content in the leaves was on average about 30 times higher than in the seeds in experiment No. 1. and about 100 times higher than in grains in experiment No. 2. In experiment No.1, the highest content of vanadium in the grain was recorded in the combination KIO_3 + V2, while in experiment No.2, this occurred in the combination of 2IBeA + V2. The application of vanadium in a higher dose in experiment No. 1 resulted in significantly lower accumulation of vanadium in leaves than the combined application of vanadium with iodine in the combinations KI + V2 vs. V2 by 48% and 5ISA + V2 vs. V2 by 31% (Figure 3D). In experiment No. 2, the leaves of plants from all combinations of iodine + V2 were characterized by a higher content of vanadium than after the application of only vanadium in a higher dose (V2; Figure 3E). In experiment No. 2, for individual combinations, the application of iodine with vanadium at a higher dose (iodine + V2) versus V2 alone, an increased accumulation of vanadium in the leaves was noted by: 50% for KI + V2 vs. V2; 43% for KIO_3 + V2 vs. V2; 35% for 2IBeA + V2 vs. V2 as well as 21% for 5ISA + V2 vs. V2 (Figure 3E).

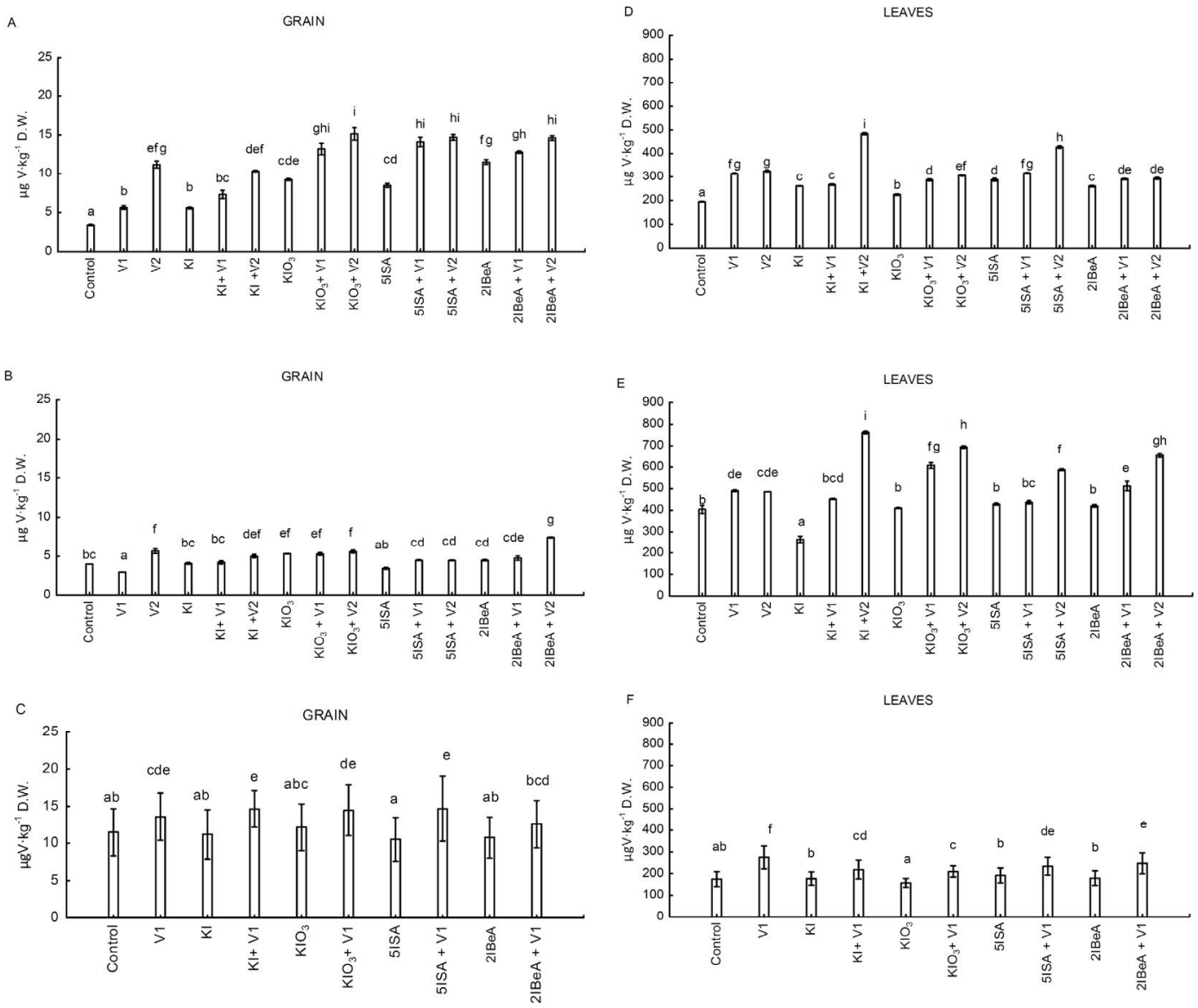

Figure 3. Vanadium contents in grain in experiment No.1 (**A**), No.2 (**B**) and No.3 (**C**) and in leaves in Experiment No.1 (**D**), No.2 (**E**) and No.3 (**F**) of sweetcorn plants. Means followed by different letters for treatments differ significantly at $p < 0.05$ ($n = 8$). Bars indicate standard error.

In the experiment No. 3, only the lower dose of ammonium metavanadate V1 = 0.1 µM was applied. In this experiment, there was also a tendency to increase the vanadium content in sweetcorn leaves and grains after the application of vanadium with iodine compounds versus the application of these iodine compounds without vanadium (Figure 3C,F). In the experiment No. 3, the content of vanadium in leaves was on average 20 times higher than in grains (Figure 3C,F). The highest content of vanadium in grains was obtained after application of 5ISA + V1, and in leaves after application of only vanadium without iodine.

2.4. Total Sugars and Vitamin C Content in Sweet Corn Grains

In all carried out experiments (three separate experiments), the content of total sugars (glucose + fructose + sucrose) and vitamin C (L-ascorbic acid) in sweet corn grains significantly depended on applied iodine compound and dose of vanadium (Figure 4A–F see also Tables S1 and S2). In experiment No. 1, the highest total sugar content was obtained in the control (Figure 4A), and in experiments No. 2 and 3, after the application of a lower dose of vanadium V1 (Figure 4B,C). In experiment No. 1 and No. 2, all applied iodine

compounds had a significantly lower total sugar content compared to the control treatment. The most significant decrease of total sugars content was noted after 2IBeA application (Figure 4A,B). In experiment No. 3, we did not note the same impact of 2IBeA application on total sugar content. Using 2IBeA did not show statistical differences compared to the control (Figure 4C). In experiment No. 2 and 3, the application of 5ISA without vanadium resulted in a significant increase in the content of sugars in the grains.

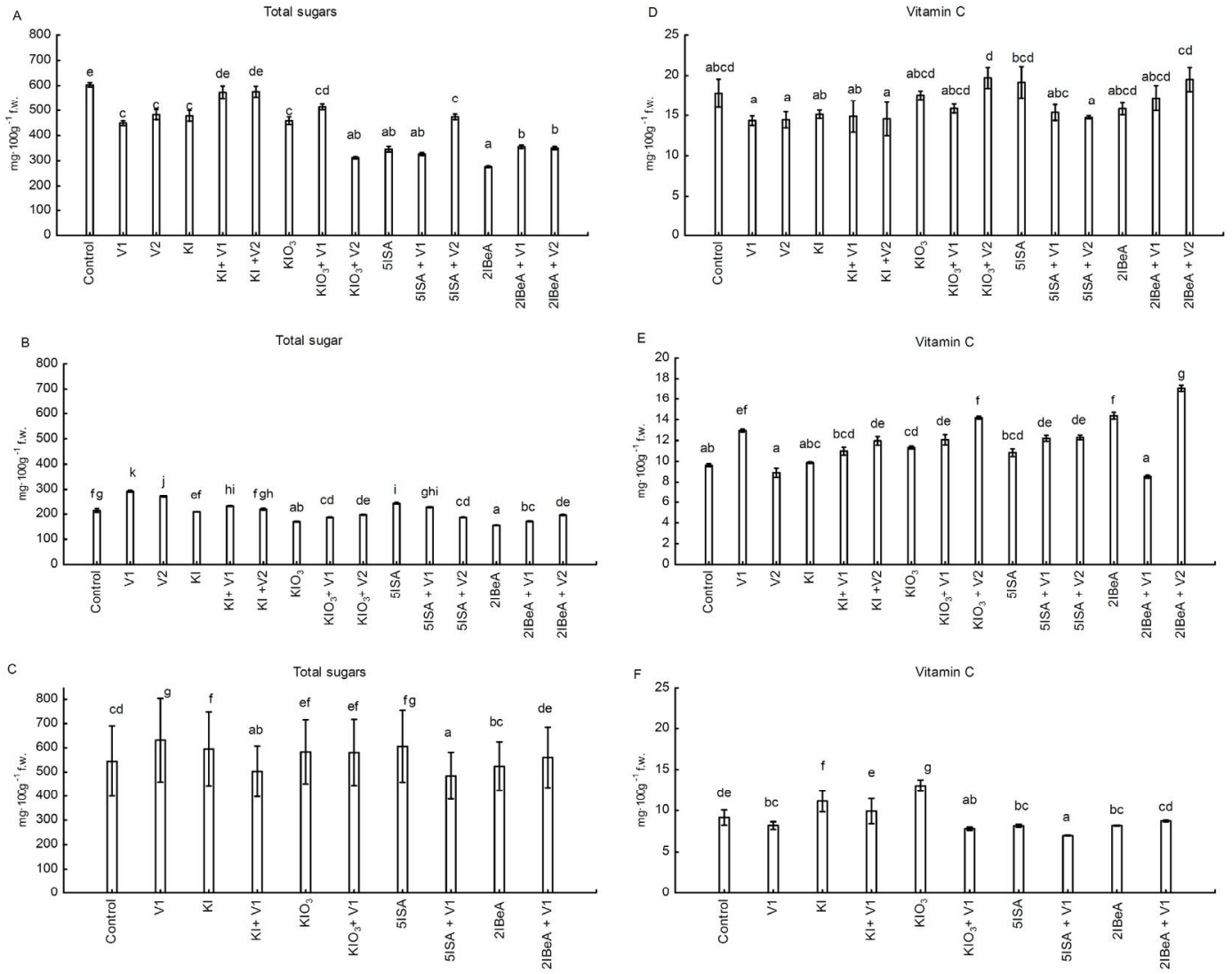

Figure 4. Content of total sugars in grain from experiment No.1 (**A**), No.2 (**B**) and No.3 (**C**), as well as ascorbic acid content in in grain from experiment No.1 (**D**), No.2 (**E**) and No.3 (**F**). Means followed by different letters for treatments differ significantly at $p < 0.05$ ($n = 8$). Bars indicate standard error.

In experiment No. 1, the highest content of vitamin C in grains was found after foliar application of KIO_3 + V2 and the lowest after foliar application of V1, V2 and KI + V2 (Figure 4D). In experiment No. 2, the content of vitamin C was the highest after 2IBeA + V2 foliar application and the lowest after 2IBeA + V1 application (Figure 4E). In this experiment, along with the increasing dose of vanadium applied with iodine, the value of ascorbic acid in grains increased after the application of KI, KIO_3 and 5ISA. The aforementioned relations for the increasing content of vitamin C in grains are as follows: KI + V1 vs. KI increase by 11% for KI + V2 vs. KI increase by 21%; KIO_3 + V1 vs. KIO_3 increase by 6%, KIO_3 + V2 vs. KIO_3 increase by 25%; 5ISA + V1 vs. 5ISA increased by 12.9% and 5ISA + V2 vs. 5ISA increased by 13%. In experiment No. 3, the application of 2IBeA + V1 to 2IBeA had no effect

on the vitamin C content in the grains. In the case of the other applied iodine compounds, the addition of ammonium metavanadate resulted in a statistically significant decrease in the ascorbic acid content in the grains (Figure 4F). In this experiment, the highest vitamin C content in grains was found after the application of KIO_3 and then KI.

2.5. Percentage of Recommended Daily Allowance (RDA) for Iodine (RDA-I) and Hazard Quotient (HQ) in Sweet Corn Grain

Based on chemical analyzes of sweetcorn grain in the milk stage, the percent RDA for I (RDA-I) was calculated in 100 g F.MW. sweet corn grains and as well the hazard ratio (HQ) (Table 5).

Table 5. Percentage of the recommended daily allowance (RDA) for iodine (RDA-I) in a 100 g portion of fresh grains of sweet corn and the hazard quotient (HQ) for intake of I through the consumption of 100 g of fresh grains of sweet corn by adults (70 kg body weight), depending on the foliar application of iodine and vanadium in experiments No. 1, 2 and 3.

	Experiment No. 1		**Experiment No. 2**	
Treatment	**Percent RDA-I in 100 g of Fresh Sweet Corn Grains (%)**	**HQ-Iodine for 100 g of Fresh Sweet Corn Grains**	**Percent RDA-I in 100 g of Fresh Sweet Corn Grains (%)**	**HQ-Iodine for 100 g of Fresh Sweet Corn Grains HQ**
Control	1.039 ± 0.226 a	0.00043 ± 0.00001 a	0.092 ± 0.005 a	0.000063 ± 0.000004 a
V1	0.862 ± 0.121 a	0.00043 ± 0.00002 a	0.979 ± 0.043 c	0.001035 ± 0.000016 d
V2	1.134 ± 0.124 a	0.00063 ± 0.00004 b	0.400 ± 0.034 ab	0.000696 ± 0.000014 c
KI	3.794 ± 0.121 d	0.00212 ± 0.00004 h	0.806 ± 0.035 bc	0.000704 ± 0.000015 c
KI + V1	3.591 ± 0.122 cd	0.00193 ± 0.00005 g	0.867 ± 0.039 c	0.000709 ± 0.000008 c
KI + V2	3.362 ± 0.106 bcd	0.00185 ± 0.00004 fg	0.815 ± 0.028 bc	0.000579 ± 0.000014 b
KIO_3	2.897 ± 0.086 b	0.00152 ± 0.00002 c	0.792 ± 0.032 bc	0.000549 ± 0.000011 b
KIO_3 + V1	2.997 ± 0.136 bc	0.00165 ± 0.00001 cde	0.705 ± 0.047 bc	0.000518 ± 0.000008 b
KIO_3 + V2	3.389 ± 0.099 bcd	0.0018 ± 0.00002 efg	0.660 ± 0.009 bc	0.000515 ± 0.000004 b
5ISA	3.309 ± 0.127 bcd	0.00176 ± 0.00003 def	0.842 ± 0.007 bc	0.000685 ± 0.000014 c
5ISA + V1	2.969 ± 0.048 bc	0.00158 ± 0.00002 c	1.004 ± 0.035 c	0.000715 ± 0.00001 c
5ISA + V2	3.162 ± 0.069 bcd	0.00160 ± 0.00001 c	1.058 ± 0.050 c	0.000767 ± 0.000019 c
2IBeA	3.422 ± 0.123 bcd	0.00186 ± 0.00002 fg	4.618 ± 0.092 e	0.00325 ± 0.000041 f
2IBeA + V1	3.378 ± 0.130 bcd	0.00187 ± 0.00003 fg	4.292 ± 0.298 e	0.003389 ± 0.000048 g
2IBeA + V2	2.938 ± 0.199 bc	0.00162 ± 0.00006 cd	3.751 ± 0.111 d	0.003079 ± 0.000014 e

	Experiment No. 3	
Treatment	**Percent RDA-I in 100 g of Fresh Sweet Corn Grains (%)**	**HQ-Iodine for 100 g of Fresh Sweet Corn Grains**
Control	0.387 ± 0.027 a	0.00028 ± 0.00002 a
V1	0.453 ± 0.022 ab	0.00036 ± 0.00003 b
KI	0.707 ± 0.026 c	0.00061 ± 0.00001 d
KI + V1	0.934 ± 0.021 d	0.00067 ± 0.00001 e
KIO_3	0.467 ± 0.016 ab	0.00038 ± 0.00001 b
KIO_3 + V1	0.604 ± 0.034 bc	0.00046 ± 0.00002 c
5ISA	1.145 ± 0.111 e	0.00095 ± 0.00009 g
5ISA + V1	0.987 ± 0.032 de	0.00075 ± 0.00004 f
2IBeA	6.826 ± 0.860 f	0.00535 ± 0.00078 i
2IBeA + V1	6.843 ± 0.653 f	0.00527 ± 0.00075 h

Means followed by the same letters are not significantly different for $p < 0.05$; ±, standard error ($n = 8$). The hazard to consumer exists when the value of HQ exceeds 1.0.

Iodine consumption was calculated on the basis of adult consumption of 100 g of sweet corn grains (average body weight 70 kg). Consumption of 100g of sweet corn grains from plants enriched with I and I + V would significantly increase the RDA-I in relation to the control in all three experiments. The consumption of iodine-biofortified corn grains (applied separately and together with V) would allow the human body to be provided with between 2.89% (KIO_3 application) to 3.79% (KI application) of RDA-I in experiment No. 1,

from 0.66% (application KIO_3 + V2) to 4.62% (application 2IBeA) of RDA-I in experiment No. 2 and from 0.47% (KIO_3 application) to 6.84% (2IBeA + V1 application) of RDA-I in experiment No. 3 (Table 5). The harmfulness of iodine to the human body would occur if the HQ-I index was $\geq$ 1.0. In all three experiments, the calculated HQ-I coefficients were significantly lower than 1.0. The highest value of HQ-I occurred after the use of KI (0.00212 HQ-I) in experiment No. 1, 2IBeA (0.00325 HQ-I) in experiment No. 2 and 2IBeA + V1 (0.00527 HQ-I) in experiment No.3.

3. Discussion

Corn is a very important element in the diet of people all over the world, regardless of the level of economic development of the countries, and it is an important energy crop [35]. Corn and food products derived from it are of great importance for people intolerant to gluten and with celiac disease. Around 1% of the world's population suffers from this type of disorder [36,37]. The implementation of iodine enrichment of crops (corn, cereals, vegetables) in agricultural practice would reduce problems with iodine deficiency occurring all over the world [5,7,10]. The last two decades have provided a lot of research on agrotechnical methods of enriching crops with iodine [6,11]. Many of them proved the effectiveness of the iodine compounds used, which were mainly KI and KIO_3 [22,38]. There had been available results of studies on the biofortification of crops into iodine with the use of organic compounds of this element, including 5-iodosalicylic acid, 3,5-diiodosalicylic acid, 2-iodobenzoic acid, 4-iodobenzoic acid or iodoacetic acid in tomato cultivation [19], lettuce [39,40] and spinach [41]. It is also known that applying iodine in the fertilization of plants in combination with organic stabilizers. In the research of Rangel et al. 2020, the effect of enriching lettuce with iodine was obtained after using the chitosan-I complex (Cs-KIO_3, Ch-KI) [42].

The studies determined the interaction of two trace elements, iodine and vanadium, in corn plants. This interaction—the participation of vanadium in iodine uptake—is described for several species of sea algae [29,31,43]. All of the tested combinations of iodine and iodine with vanadium in the three carried out experiments significantly influenced the degree of iodine accumulation in leaves and grains of sweet corn. Effective enrichment of sweet corn grains with iodine was demonstrated after foliar application of both organic (5ISA, 2IBeA) and inorganic (KI, KIO_3) iodine compounds. The highest effect of enriching sweet corn grain with iodine was obtained after the application of the organic iodine compound 2IBeA in two experiments, No. 2 and No. 3 (analyzing only the effectiveness of iodine compounds applied individually, without vanadium). On the other hand, in experiment No. 1, the inorganic KI compound turned out to be the most effective, and the second in line was the organic 2IBeA. The conducted experiments allowed three aspects determining the effectiveness of enrichment of grains with iodine to be documented, without leading to any phytotoxic symptoms of iodine and vanadium on plants. These include the issue of increasing the dose of iodine from 10 µM I (experiment No. 1) to 100 µM I (experiment No. 2 and 3), compressing the application period between subsequent foliar applications treatments, and carrying out foliar application I and V in various phases of plant development. This is the performance of foliar application I and V at the beginning of flowering (experiment No. 3) doubled the level of iodine accumulation in grains compared to the application of these elements in the earlier stage of plant development (experiment No. 2). The grains of sweet corn treated only with 2IBeA in experiment No. 3 contained about 2.6 times more iodine than in the combination of KI from experiment No. 1 and 1.8 times more than after the application of 2IBeA in experiment No. 2. Satisfactory effects of enriching tomato fruits (generative organs) with iodine after 2IBeA application were obtained by Halka et al. 2019 [44]. In the experiment with tomato in the seedling phase after soil application of iodine compounds, both the roots, leaves and shoots after applying KI had the highest content of iodine, followed by plants treated with 2IBeA and 4-IBeA (4-iodobenzoic acid) [19].

From the point of view of an effective implementation program of foliar iodine biofortification, the 100 μM iodine dose used in experiment No. 3 was safe for plants and did not cause phytotoxic symptoms on sweet corn plants. Phytotoxic symptoms were demonstrated in studies with the application of inorganic forms of iodine in rice plants in doses of 10 and 100 μM KI and 100 μM KIO_3 [45] or while fertigation 2.34 mM KIO_3 and 3.01 mM KI of corn, barley, potato and tomato [46]. In the experiment with the use of organo-iodine compounds 3,5-diISA (3,5-diiodosalicic acid) and 4-IBeA at a dose of 25 μM I in tomato plants due to fertigation in the seedling phase, after the applied organo-iodine compounds a negative effect on the growth and development of above-ground young tomato plants parts was observed [19]. 5ISA in a dose of 40 μM I led to a significant decrease in the biomass of roots and leaves of lettuce grown in the NFT hydroponic system [39].

The used compounds in all experiments with both a lower dose (No. 1) and a higher dose of iodine (No. 2 and No. 3) did not have a statistically significant effect on the sweet corn yield; similar results after soil and foliar application of KI and KIO_3 were obtained Cakmak et al. [22]. In the pot tests on sweet corn, no toxic effect was found and no statistically significant effect on the yield and development of young corn plants at a lower iodine dose of 10 μM I and analogously doses of vanadium 0.1 μM and 1 μM V [43]. The iodine content in the leaves in all experiments was on average 14 times higher than in the grains. The higher dose of iodine compounds used significantly increased the content of iodine in the leaves by about 6 times in experiment No. 2 and No. 3 compared to No. 1. Vegetative parts of plants (leaves, shoots) accumulate higher amounts of this element than the generative parts. Similar results were obtained in studies on rice [22,45], sweet corn [43], green beans [47] and plum trees [21].

The transport of iodine from the leaves to the corn grains after foliar application would have to be via the phloem. The phloem transport of iodine has been confirmed in many studies on the effectiveness of the accumulation of this element in the generative parts [21,38,48]. Nevertheless, xylem transport of iodine is much more efficient. Hurtevent et al. (2013) pointed to the fact of the relative mobility of iodine in the phloem which was proven in the research by Zou et al. 2019 [49,50]. Foliar iodine application (inorganic KIO_3) in the wheat grain filling stage created an available pool of iodine in the leaf tissues, causing phloem transport to the grain. In this phase, there is an intense transfer of photo assimilates to the seeds, and the activation of the phloem transport [50]. The presented research in experiment No. 3 also confirmed the described effects of effective phloem transport as a consequence of more effective accumulation of iodine in grains.

The combined application of iodine with other micronutrients such as Zn, Fe, Se has been described in studies on wheat and rice [9,50]. Foliar application of I, Zn, Se and Fe combined (in most locations where it was performed) resulted in less iodine accumulation than the same dose of iodine applied individually [50]. In the case of vanadium, the interaction of vanadium with iodine, which could be the stimulation or antagonism of this element, is not known in higher plants yet. In a group, brown algae, enzymes as vanadium-dependent haloperoxidases (vHPO) play a key role in the uptake and accumulation of iodine (as well halides metabolism of Cl and Br) [29,31]. They are mainly responsible for the capture of iodine from the water, the synthesis of hydrogen halides in marine algae and antioxidant defense [32,51]. The studies on the interaction of iodine and vanadium in higher plants was conducted, among others, on lettuce by Smoleń et al. [20,40] and in sweet corn by Grzanka at al. [33,43].

In all experiments, in sweet corn leaves the stimulating effect of vanadium on iodine accumulation was preeminent. In experiments No. 1, No. 2 and No. 3, the application of KIO_3 + V1 was statistically significantly more effective in terms of iodine accumulation than the application individually of KIO_3. For 2IBeA in experiment No. 1 and No. 3, iodine accumulation in 2IBeA + V1 was at a similar level of significance with 2IBeA; in experiment No. 2 both doses (0.1 μM and 1 μM) of vanadium significantly increased I accumulation compared to the individually application of 2IBeA. In experiment No. 1, combined application 5ISA with vanadium did not show any impact on the accumulation

of iodine. In experiment No. 2, after using the combination of 5ISA + V1, the iodine content in the leaves was significantly higher than after application of only 5ISA. In turn, in experiment No. 3, only the combination of 5-iodosalicylic acid with vanadium resulted in a decrease in the accumulation of iodine in the leaves (and in grain) compared to the individually applied of 5ISA.

A similar effect was obtained in a pot experiment with soil application of 5ISA with two doses of vanadium (0.1 μM and 1 μM) in sweet corn cultivation [43]. The combined application of iodine and vanadium in a hydroponic system with organic and inorganic iodine compounds gave a variable effect. Lettuce grown in the hydroponic system in the combination of 5ISA + V and for 3,5-diISA + V resulted in a significantly higher level of iodine accumulation in the roots than the individual application of organic compounds 5ISA and 3,5-diISA. In the lettuce leaves the stimulating effect for both compounds used in hydroponics and as well in peat substrate was not observed. On the other hand, in the mineral soil, lettuce leaves had a statistically significantly higher iodine content in/after treatment with 3,5-diISA + V vs. 3,5-diISA [40].

The combined application of 2IBeA with vanadium resulted in a statistically significant decrease in the accumulation of iodides and iodates compared to the individual application of 2IBeA. On the other hand, the combination of KI + V1 increased the accumulation of iodides in the grain of sweet corn compared to the solo application of KI. The combined application of KIO_3, 2IBeA, 5ISA with vanadium resulted in a significant increase in the synthesis/accumulation of 5ISA and 3,5-diISA iodosalicylates in grain. In the leaves, this relationship is consistent with the combined application of the vanadium from KI, KIO_3 and 5ISA. The combined application of vanadium with all iodine compounds, both organic and inorganic, significantly reduced the accumulation of 2,3,5-triIBeA in leaves and grains of sweet corn. The literature indicated that this organo-iodine compound is an auxin inhibitor [52]. The results of the research based on a one-year experiment with a lower dose of vanadium (No. 3 year 2020) confirm the stimulating effect of vanadium (in a dose of 0.1 μM) on the accumulation of iodosalicylates (5ISA, 3,5-diISA) in the leaves and grains of sweet corn in combination with organic and inorganic iodine compounds. Research on tomato [44] and lettuce [20] also confirm the occurrence of iodosalicylates and iodobenzoates in control plants. After foliar application of iodosalicylates and iodobenzoates, their accumulation in sweet corn leaves and grain increased. Similar results were found in studies on tomato and lettuce [20,44].

Transport of vanadium from the roots to the higher parts of plants, mainly to the generative organs, is limited. The highest degree of accumulation of this element is in the roots [53,54]. It is related to the process of vanadium biotransformation during the uptake of vanadium by the roots. This biotransformation consists in the reduction of pentavalent vanadium, which easily oxidize ketones, aldehydes, catechols, sulfhydryls and olefins placed in the cell wall even at pH 7. As a result, vanadium is retained by root tissues and vanadium (V) is reduced to the quadrivalent form of vanadium (IV) [55].

In the conducted experiments No. 1 and No. 2 (with two applied doses of vanadium 0.1 and 1 μM), an increase in the applied dose of vanadium increased the accumulation of this element in leaves and corn grains. The effect of increased accumulation of vanadium with the increasing dose of this element was confirmed in the cultivation of pepper [56], chickpea [57] and beans [54]. In experiments No. 1 and No. 2, the application of KI and 5ISA stimulated the accumulation of vanadium by increasing its content in the leaves compared to the application of only V2 (vanadium in a higher dose) without iodine. At the same time, V2 significantly reduced iodine accumulation in combination with 5ISA and KI. A similar effect was obtained in studies on sweet corn with the application of iodine and vanadium compounds to the soil [43]. In the early development stages of sweet corn, the accumulation of iodine in the roots was highest in combination with the application of solo ammonium metavanadate at a dose of 0.1 μM [33]. Vanadium is determined as a beneficial element to higher plants. The stimulating effect for plants ranges from 1–10 μg L^{-1}; higher doses have a phytotoxic effect on plants [55,58,59]. Vanadium furtherance the

nitrogen fixation process in soils with deficient in molybdenum, low doses stimulate the synthesis of chlorophyll [59,60]. In the carried-out experiments, vanadium doses of 0.1 μM and 1 μM did not cause any phytotoxic effect on plants. Vanadium did not affect the sweet corn yield in a statistically significant way.

The content of total sugars and vitamin C was mainly formed by the weather conditions in the year of the experiment, and it was characterized by a huge diverseness between the studied combinations. In experiment No. 1 and No. 2, the grains had the lowest total sugar content after the foliar application of 2IBeA. The highest total sugars content was in the control for experiment No. 1, and for No. 2 and No. 3 with the application V1 (0.1 μM). In the experiments No. 1 and No. 2, the addition of vanadium to KIO_3 increased the content of vitamin C in the grains. In No. 3, the exclusive application of KIO_3 significantly increased the content of ascorbic acid. In the experience of lettuce the total sugar content and vitamin C was mainly determined by type of applied iodine compounds (organic or inorganic iodine), as well largely the dose determined the variability in sugar and vitamin C content. After the application of 5ISA in a dose of 8.0 μM was obtained the lowest level of vitamin C. The highest total sugar content was recorded in plants grown in a nutrient solution containing KIO_3 + SA. 5ISA in the dose of 40 μM resulted in a higher total sugar content in the lettuce compared to the lower doses (1.6 μM and 8 μM) [39]. The application of KI and KIO_3 increased the content of vitamin C in Opuntia ficus-indica var. Copena V1 almost two times [61] as well in water spinach [41].

An important issue for the implementation of biofortified plants is regular monitoring of iodine intake status to detect excessive intakes. Some data emphasize that healthy adults who are iodine sufficient are curiously tolerant to iodine intake even 1000 μg·day^{-1}. Appropriate iodine intake is the most important for people in chronic iodine deficiency because swift increase of iodine may cause thyroiditis or hyperthyroidism [10]. Upper levels of biofortification of the edible parts plants has to be an I of HQ < 1.0, which indicates a safe level for the consumer. In the presented study, this indicator HQ was not exceeded value 1.0 in all the conducted experiments. In the third experiment after the application of organic iodine compound 2IBeA and 2IBeA + V1, the I-RDA is the highest, so if the average daily intake per adult is 200 g, we can provide approximately 14% of the recommended daily iodine intake.

4. Materials and Methods

4.1. Plants Material, Treadment and Meteorological Data

In 2018–2020, three independent experiments were carried out with the field cultivation of sweet corn (*Zea mays* L. subsp. Mays Saccharata) cv. "Złota Karłowa" on the horticultural farm in southern Poland (50° 16′53.7″ N 19° 47′43.5″ E). The effectiveness of iodine biofortification of corn grains after foliar application of inorganic and organic iodine compounds applied separately and in combination with vanadium in the form of ammonium meatavanadate was investigated. In experiment No. 1 and No. 2 (Table 6), two doses of vanadium was tested, 0.1 μM V and 1.0 μM V, in combination with iodine and was applied at the same plant growth phases 32–61 BBCH. The difference between experiment No. 1 and No. 2 consisted of the fact that in experiment No. 1 (in 2018), iodine was applied at a dose of 10 μM I, and in experiment No. 2 (in 2019), it was applied at a dose of 100 μM I. In experiment No. 3 (in 2019 and 2020), only one lower dose of vanadium 0.1 μM V was used as foliar treatment in combination with the tested iodine compounds at a dose of 100 μM I (Table 7). In experiments No. 1 and No. 2, the application of iodine and vanadium compounds started from the stage of stem development (shoot elongation), i.e., in the 2 nodes phase—BBCH 32. The period between successive applications was on average 14 days and ended in the 61 BBCH phase (i.e., 14 days before harvesting the corn cobs). In experiment No. 3, the application of iodine and vanadium compounds in the form of a foliar spray was started in the phase of visible stamens in the spikelets of the middle part, when the cob emerged from the leaf sheath, i.e., from the BBCH 61 phase. The period between successive applications was 3 days. In all three experiments, four

treatments of foliar application of iodine and vanadium compounds were performed. In each experiment, the following iodine compounds were applied: KI, KIO_3, 5-iodosalicylic acid (5ISA) and 2-iodobenzoic acid (2IBA). The detailed scheme of the three experiments and the tested combinations are presented in Tables 1 and 2.

Table 6. Design and method of conducting experiments with sweet corn in field experiment; experiments No. 1 (2018 year) and No. 2 (2019 year).

Treatment	Experiment No. 1					
	Dose of I Compounds	**Dose of V as Ammonium Metavanadate**	**Amount of Application**	**BBCH Phases during Foliar Application**	**Intervals between Applications**	**Harvest Phase (BBCH)**
Control	-	-	-	32–61 *	2 weeks	75
V1	-	0.1 μM V	4 times	32–61 *	2 weeks	75
V2	-	1.0 μM V	4 times	32–61 *	2 weeks	75
KI	10 μM I	-	4 times	32–61 *	2 weeks	75
KI + V1	10 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
KI + V2	10 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75
KIO_3	10 μM I	-	4 times	32–61 *	2 weeks	75
KIO_3 + V1	10 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
KIO_3 + V2	10 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75
5ISA	10 μM I	-	4 times	32–61 *	2 weeks	75
5ISA + V1	10 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
5ISA + V2	10 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75
2IBeA	10 μM I	-	4 times	32–61 *	2 weeks	75
2IBeA + V1	10 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
2IBeA + V2	10 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75
Treatment	**Experiment No. 2**					
	Dose of I Compounds	**Dose of V as Ammonium Metavanadate**	**Amount of Application**	**BBCH Phases during Foliar Application**	**Intervals between Applications**	**Harvest Phase (BBCH)**
Control		-	-	32–61 *	2 weeks	75
V1		0.1 μM V	4 times	32–61 *	2 weeks	75
V2		1.0 μM V	4 times	32–61 *	2 weeks	75
KI	100 μM I	-	4 times	32–61 *	2 weeks	75
KI + V1	100 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
KI + V2	100 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75
KIO_3	100 μM I	-	4 times	32–61 *	2 weeks	75
KIO_3 + V1	100 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
KIO_3 + V2	100 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75
5ISA	100 μM I	-	4 times	32–61 *	2 weeks	75
5ISA + V1	100 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
5ISA + V2	100 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75
2IBeA	100 μM I	-	4 times	32–61 *	2 weeks	75
2IBeA + V1	100 μM I	0.1 μM V	4 times	32–61 *	2 weeks	75
2IBeA + V2	100 μM I	1.0 μM V	4 times	32–61 *	2 weeks	75

* BBCH 32 [Principal growth stage: Stem elongation- phase 2 nodes detectable]. BBCH 61 [(M) stamens in middle of tassel visible, (F) tip of ear emerging from leaf sheath].

Table 7. Design and method of conducting experiments with sweet corn in field experiment No. 3 in 2019 and 2020 years.

Treatment	Experiment No. 3					
	Dose of I Compounds and Dose of I	Dose of V as Ammonium Metavanadate	Amount of Application	BBCH Phases during Foliar Application	Intervals between Applications	Harvest Phase (BBCH)
Control		-	-	61–69 **	3 days	75
V1		0.1 μM V	4 times	61–69 **	3 days	75
KI	100 μM	-	4 times	61–69 **	3 days	75
KI + V1	100 μM	0.1 μM V	4 times	61–69 **	3 days	75
KIO_3	100 μM	-	4 times	61–69 **	3 days	75
KIO_3 + V1	100 μM	0.1 μM V	4 times	61–69 **	3 days	75
5ISA	100 μM	-	4 times	61–69 **	3 days	75
5ISA + V1	100 μM	0.1 μM V	4 times	61–69 **	3 days	75
2IBeA	100 μM	-	4 times	61–69 **	3 days	75
2IBeA + V1	100 μM	0.1 μM V	4 times	61–69 **	3 days	75

** BBCH 61 [(M) Stamens in middle of tassel visible, (F) tip of ear emerging from leaf sheath]. BBCH 69 [End of flowering: stigmata completely dry].

In each experiment, there were 60 plants for one combination, i.e., 4 replications, 15 plants for one replication—the experiments were carried out in a randomized system, separately for each experiment. Treatments were randomized over the entire experiment area of each experiment. The distance between the rows was 67.5 cm, and the distance between the plants in the row was 15 cm. In each year of the study, no fodder corn was cultivated within a few kilometers from the experimental field. The same pre-sowing fertilization was used in all experiments: 130 kg $N \cdot ha^{-1}$, 80 kg $P_2O_5 \cdot ha^{-1}$, 170 kg $K_2O \cdot ha^{-1}$, 40 kg $MgO \cdot ha^{-1}$. Chemical protection of plants against weeds, diseases and pests was also followed in accordance with the plant protection program in force in Poland. In all experiments, corn cobs were harvested at full milk maturity of the granuloma (BBCH 75). After harvesting, the casings were removed from the corn cobs, sequentially counted and weighed to evaluate the yield size. All the corn cobs from each plant/each replicate were collected for chemical analysis, and then manually obtained grains (half of the grains from each corn cob). Simultaneously with the collection of the corn cob, one leaf from five plants from each replicate was collected for chemical analysis—the leaves located directly under the corn cob were collected.

The average daily temperature in the period from April to September in 2018 differed from the average daily temperature in 2019 and 2020 (in which it was similar) (Table 8). In 2018, the hottest month was July, while in 2019 the hottest month was June, and in 2020 the hottest month was August. April was the warmest in 2018, and in 2020 the average monthly temperature was halved. The largest difference in the average daily temperature between 2018 and 2019 was in May, and between 2019 and 2020 in June. The sum of rainfall in 2019 in the period from April to September was the highest compared to 2018 and 2020. Compared to 2020, it was higher by 64.8 mm, and compared to 2018 by 93.3 mm. The sum of rainfall in 2018 and 2020 was evenly distributed in individual months from April to September. In turn, in 2019, May and August were the months with the highest rainfall, and June and July with the lowest rainfall, respectively 14.5 mm and 22.0 mm.

Table 8. Meteorogical data 2018–2020.

Month	Mean Daily Air Temperature (°C)		
	Year 2018	**Year 2019**	**Year 2020**
April	13.3	8.49	6.08
May	16.9	11.44	10.25
June	18.9	20.33	17.09
July	20.6	18.54	18.19
August	20.5	17.27	19.42
September	14.7	13.69	14.47
Mean	17.48	14.96	14.25
	Sum of Rainfall (mm)		
April	29.4	59.1	23
May	59.4	131.7	76.2
June	72.1	14.5	71.8
July	81.7	22.0	55.7
August	52.5	128.7	82.9
September	46.0	78.4	60.0
Sum	341.10	434.4	369.6

4.2. Analysis of Fresh Samples of Leaves and Grains

The samples of fresh grain (milk stage) were then homogenized, and total sugars, as a sum of glucose, fructose, and sucrose, were extracted with boiling 96% ethanol (Destylernia 'Polmos' Sp. z o.o., Kraków, Poland). The content of fructose, glucose and sucrose and their sum as total sugars was assessed by using the capillary electrophoresis technique with the PA 800 Plus system (Beckman Coulter, United States). Capillaries of ø 50 mm and total length of 60 cm (10 cm for detection) were used. A positive power supply of 15 kV was applied, and the temperature was set at 25 °C. The running buffer solution comprised 20.0 mmol/L sorbic acid, 0.20 mmol/L CTAB, and 40 mmol/L NaOH, pH 12.2. [62].

The content of L-ascorbic acid in fresh grains was analyzed by capillary electrophoresis after the homogenisation of 20 g samples in 80 cm^3 of 2% oxalate acid (puriss. p.a., Avantor Performance Materials) and further centrifugation for 15 min at 4500 rpm, 5 °C. The supernatants were filtered through a 0.25 µm cellulose acetate membrane filter and analyzed using a PA 800 Plus capillary electrophoresis system (Beckman Coulter, Indianapolis, IN, USA) with diode array detector (DAD) detection. Capillaries of 50 µm i.d. and 365 µm o.d. and those of a total length of 50 cm (40 cm to detector) were used. A negative power supply of 25 kV was applied. The running buffer solution was prepared as proposed by [63], containing 30 mM NaH_2PO_4 (puriss. p.a., Avantor Performance Materials), 15 mM $Na_2B_4O_7$ (puriss. p.a., Sigma-Aldrich, Darmstadt, Germany) and 0.2 mM cetyltrimethylammonium bromide (CTAB) (puriss. p.a., Sigma-Aldrich) (pH 8.80).

4.3. Analysis of Dry Samples of Leaves and Grains

Fresh samples of leaves and grain were dried at 70 °C (48 h) in a laboratory dryer with forced air circulation. Dried samples of leaves and grain were ground in a laboratory mill and stored in a plastic bag until the analyses of iodine and vanadium contents were carried out. The dry weight content in these samples was determined using the oven-drying method at 105 °C.

To determine iodine content, the PN-EN 15111-2008 method was used with the modifications described by [64] using ICP-MS/MS (iCAP TQ ICP-MS ThermoFisher Scientific, Bremen, Germany). The concentrations of V were determined using the ICP-OES spectrophotometer (Prodigy Spectrometer, Leeman Labs, New Hampshire, MA, USA) after microwave digestion in 65% super pure HNO_3. Plant samples of 0.5 g of dry material were placed in 55 mL TFM modified polytetrafluoroethylene (PTFE) vessels and digested in 10 mL of 65% HNO_3 using a CEM MARS-5 Xpress (CEM World Headquarters, Matthews, NC, USA) microwave digestion system [65].

Speciation of I, i.e., iodides and iodates, was analyzed in the dried samples of grains (only from experient No. 3 in 2020) using high-performance liquid chromatography (HPLC)–ICP-MS/MS. The content of these two I ions was measured using a modified extraction procedure described by [66], whereby 0.05 g of air-dried, ground plant samples were mixed with an extraction solution containing 4 cm^3 of 25% TMAH (Sigma-Aldrich Co., LLC) and 10 cm^3 0.1 M NaOH (Chempur, Piekary Śląskie, Poland), in 1 dm^3 of demineralized water. The samples were placed in 7-mL polypropylene tubes, whereupon 5 mL of the extraction mixture was added. Once mixed, the samples were incubated for 1 h at 50 °C in an ultrasonic bath and then cooled to approximately 20 °C, mixed thoroughly, and centrifuged for 15 min at 4500 revolutions/min. The supernatants were filtered through a 0.22 µm syringe filter. The content of I ions in filtered samples was analyzed using HPLC—ICP-MS/MS. For I^- and IO_3^- speciation forms, HPLC (Thermo Scientific Ultimate 3000; Thermo Fisher Scientific, Bremen, Germany) was coupled to ICP-MS/MS (iCAP TQ). This method employed a strong anion exchange column (Thermo Scientific; Dionex IonPac AS11 [4 × 250 mm]) and a pre-column (Thermo Scientific; Dionex IonPac AG11 [4 × 50 mm]). The column temperature was set to 30 °C. Demineralized water, 50 mM NaOH, and 0.5% TMAH were used as eluents. To separate both I ions, a mobile phase was used, containing 2.5 mM NaOH and 0.125% TMAH with an isocratic flow. The flow rate was 1.5 mL/min, with an injection volume of 10 µL, and total analysis time of 7 min. The 127I.16O isotope of I was determined, using the S-TQ-O2 mode. Standards were prepared through the dissolution of KI and KIO_3 (Sigma-Aldrich Co., LLC) in demineralized water.

Grains only from experient No. 3 in 2020 were analyzed for iodosalicylates and iodobenzoates [5-iodosalicylic acid (5ISA) and 3,5-diiodosalicylic acid (3,5-diISA); 2-iodobenzoic acid (2IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA)], using the liquid chromatography LC–MS/MS system after extraction with 75% ethanol [20]. Measurements were made using the HPLC Ultimate 3000 system (Thermo Scientific) and an LC-MS/MS: 4500 Qtrap, Sciex spectrometer. Chromatographic separation was carried out on a Luna 3 µm phenyl-hexyl 100 Å (150 × 3 mm, internal diameter 3 µm) column (Phenomenex, Torrance, CA, United States). Electrospray ionization in negative ion mode was used. MS/MS was performed for quantitative analysis. The LC-MS/MS system was controlled using Analyst 1.7 with HotFix 3 software, which was also used for data processing [20].

The percentage of the RDA for I (RDA-I) and Se (RDA-Se) in 100-g portions of the sweet corn grain was calculated based on chemical analyses of the corn grain as well as the hazard quotient (HQ). The intake of I and Se was calculated based on the consumption of 100 g of fresh sweet corn grain by adults (average of 70 kg body weight). All of the calculations were based on the methods described by Smoleń (2019) [67].

4.4. Statistical Analysis

The statistical analysis was performed by using Statistica 13.1 PL programme. All of the data of plant analysis were examined using analysis of variance (ANOVA). Statistically significant differences were assessed by the post-hoc Duncan's test. p values less than 0.05 were considered as statistically significant.

5. Conclusions

Compared to the control, there was a tendency to increase the yield of maize cobs after the application of KIO_3 with vanadium. The most effective level of enrichment of sweet corn grain was achieved in experiment No. 3. The three experiments carried out allowed for the determination of the safest, and at the same time, most effective dose of iodine (100 µM) for sweet corn grain, optimal application time corn development phase BBCH 61–69 and application interval. The highest accumulation of iodine in the grain after foliar application of 2IBeA and the clearly visible positive effect of vanadium on the accumulation of iodine in the leaves with inorganic compounds KI and KIO_3 were observed in experiment No. 3. Experiments confirmed the low mobility of vanadium in plants; the content was the highest in the vegetative plant parts compared to the generative (grain). An interesting

aspect was that after the application of the organic iodine compound, 2IBeA was the largest accumulation of I^- in the grain, and after the application of this foliar organic compound, the synthesis/accumulation of IO_3^- was at the same statistical level as after KIO_3. This may be the consequence of all the transformation of these organic iodine compounds all the way from leaves to grains of the corn; details of the transformation/reduction are not well known.

All tested iodine and vanadium compounds (in the form of ammonium metavanadate) and their combinations had a different effect on the vitamin C content in the grain. The achieved results of corn grain enrichment showed an effective perspective for the biofortification of the generative parts of plants. The testes iodine and vanadium compounds did not have any phytotoxic effect on sweet corn plants as well did not any statistical impact into the yielding.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27061822/s1, Table S1: Content of total sugars, ascorbic acid in grain and % dry weight from Experiment No.1 at 2018 and No.2 at 2019, Table S2: Content of total sugars, ascorbic acid in grain from and % dry weight Experiment No.3 at 2019/2020.

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SelectedAspects of Iodate and Iodosalicylate Metabolism in Lettuce Including the Activity of Vanadium Dependent Haloperoxidases as Affected by Exogenous Vanadium

Sylwester Smoleń [1,*], Iwona Kowalska [1], Mariya Halka [1], Iwona Ledwożyw-Smoleń [1], Marlena Grzanka [1], Łukasz Skoczylas [2], Małgorzata Czernicka [1] and Joanna Pitala [3]

1 Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Krakow, Poland; iwona.kowalska@urk.edu.pl (I.K.); maryskagalka@gmail.com (M.H.); iwona.ledwozyw-smolen@urk.edu.pl (I.L.-S.); marlena.grzanka14@gmail.com (M.G.); malgorzata.czernicka@urk.edu.pl (M.C.)

2 Department of Fruit, Vegetable and Mushroom Processing, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 30-149 Krakow, Poland; lukasz.skoczylas@urk.edu.pl

3 Laboratory of Mass Spectrometry, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Krakow, Poland; joanna.pitala@urk.edu.pl

* Correspondence: sylwester.smolen@urk.edu.pl; Tel.: +48-12-662-52-39

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Abstract: In marine algae, vanadium (V) regulates the cellular uptake of iodine (I) and its volatilization as I_2, the processes catalyzed by vanadium-dependent haloperoxidases (vHPO). Relationships between I and vanadium V in higher plants, including crop plants, have not yet been described. Little is known about the possibility of the synthesis of plant-derived thyroid hormone analogs (PDTHA) in crop plants. The activity of vHPO in crop plants as well as the uptake and metabolism of iodosalicylates in lettuce have not yet been studied. This studyaimed to determine the effect of V on the uptake and accumulation of various forms of I, the metabolism of iodosalicylates and iodobenzoates and, finally, on the accumulation of T3 (triiodothyronine—as example of PDTHA) in plants. Lettuce (*Lactuca sativa* L. var. *capitata* 'Melodion' cv.) cultivation in a hydroponic NutrientFilm Technique (NFT) system was conducted with the introduction of 0 (control), 0.05, 0.1, 0.2, and 0.4 μM V doses of ammonium metavanadate (NH_4VO_3) in four independent experiments. No iodine treatment was applied in Experiment No. 1, while iodine compounds were applied at a dose of 10 μM (based on our own previous research) as KIO_3, 5-iodosalicylic acid (5-ISA) and 3,5-diiodosalicylic acid (3,5-diISA) in Experiment Nos. 2, 3 and 4, respectively. When lettuce was grown at trace amount of I in the nutrient solution, increasing doses of V contributed to the increase of (a) I content in roots, (b) I uptake by whole lettuce plants (leaves + roots), and (c) vHPO activity in leaves (for doses 0.05–0.20 μM V). Vanadium was mainly found in roots where the content of this element increased proportionally to its dose. The content of V in leaves was not modified by V introduced into the nutrient solution. We found that5-ISA, 3,5-diISA and T3 were naturally synthesized in lettuce and its content increased when 5-ISA, 3,5-diISA were applied. Quantitative changes in the accumulation of organic metabolites (iodosalicylates and iodobenzoates) accumulation were observed, along with increased T3 synthesis, with its content in leaves exceeding the level of individual iodosalicylates and iodobenzoates. The content of T3 was not affected by V fertilization. It was concluded that iodosalicylates may participate in the biosynthesis pathway of T3—and probably of other PDTHA compounds.

Keywords: beneficial elements; iodobenzoates; iodosalicylates; plant-derived thyroid hormone analogs; T3; thyroid hormone; triiodothyronine; vanadium-dependent haloperoxidases

1. Introduction

Vanadium [1–3] and iodine [4,5] are included into the group of trace elements that are beneficial for human and animal organisms. In the human body, vanadium regulates the functioning of the following enzymes: Na/K ATPase, phosphotransferases, adenylate cyclase, and protein kinases [6,7]. Vanado-dependent enzymes exhibiting insulin-mimetic properties have also been described. Vanadium compounds may participate in the regulation of blood pressure, lipid profiles, and, along with iodine, the proper functioning of the thyroid gland [3,8].

Excessive intake of vanadium is toxic for humans [9] and farm animals [10] with the effect dependent on the dose, duration of the exposure, and chemical form of V. Vanadium pentoxide (V_2O_5) is the most toxic for human organisms, while the safest forms are vanadyl sulfate ($VOSO_4$) and metavanadate salts [11]. To date, the recommended daily allowance (RDA) for vanadium has not been determined [12]. The report of WHO only suggests that the tolerable upper intake level for adults is approximately 26 μg vanadium/kg body weight/day [6,7]. The lowest doses reported to cause adverse effects in humans were about 200 μg vanadium/kg/day, which is up to 1000 times higher than the approximate daily intake of that element, i.e., 0.2–0.3 μg/kg/day [13].

The role and importance of iodine as a trace element for humans and animal organisms is better recognized. Depending on the age, sex, and physiological condition, the iodine RDA for humans is between 90 and 200 μg I·day^{-1}[14]. Iodine is crucial for the synthesis of thyroid hormones: thyroxine (T4) and triiodothyronine (T3). Its deficiency causes a broad spectrum of disorders and diseases, from endemic goiter even to mental disability. Prenatal iodine deficiency leads to neurological fetal defects, including severe mental disability (cretinism) [15,16].

Vanadium and iodine are included into the group of beneficial elements for higher plants, i.e., that in low concentrations, they may stimulate plant growth and development. The effect of exogenous vanadium on plants strongly depends on its dose, chemical form (mainly oxidation state) and growth conditions, particularly with respect to the conditions of root system development [4,5,17]. Low concentrations of V (below 0.04 mg V·dm^{-3}) were revealed to improve chlorophyll biosynthesis and the uptake of P, K, Ca, and Mg by plants [18]. An increase of K and Mn accumulation after V application was noted in soybean leaves [19]. In the studies conducted by Sentíes-Herrera et al. [20], foliar application of 10 and 20 μM V (as NH_4VO_3) contributed to increased growth of sugar cane plants that were characterized by greater stem diameter and improved anatomic structure related to sugar accumulation. A positive effect of vanadium was also reflected by higher chlorophyll content in leaves as well as increased plant height, weight, number of leaves, and flowers of tomato plants [21].

The effect of iodine on plants is also strongly dependent on its dose, oxidation state, and cultivation conditions. In general, iodides (I^-) are more easily taken up than iodates (IO_3^-) but exhibit higher toxicity to plants [4]. Blasco et al. [22] observed that, in soilless cultivation of lettuce, application of I^- in doses exceeding 80 μM I reduced plant biomass, while, for iodates, such an effect was not noted even at the dose of 240 μM I.

Plant preference towards the source of iodine varies depending on plant species. The order of iodine uptake by barley and pea is as follows—$I^- > CH_2ICOOH^- > IO_3^- > IO_4^-$ [23], while for water spinach—$CH_2ICOOH^- > I^- > IO_3^-$ [24]. There is scarce information on the possibility of taking up organoiodine compounds with iodine bound to aromatic ring as in the case of iodosalicylates. Our previous research [25] revealed that 5-iodosalicylic acid can be absorbed by lettuce, effectively increasing iodine content in plant tissues. To date, no studies that have documented the effect of other iodosalicylates, such as 3,5-diiodosalicylic acid on lettuce plants, have been presented. The influence of that compound on young tomato plants was, however, tested [26].

Functioning and relations between iodine and vanadium in higher plants have not yet been recognized. Medrano-Macias et al. [4] informed about a possible interaction between these elements in terrestrial plants. However, plant response to inorganic or organic iodine as related to vanadium bioaccessibility is yet to be described. Adversely, in marine algae, that issue is widely studied.

In marine algae, enzymes belonging to the family of vanadium-dependent haloperoxidases (vHPO; vanadium-dependent bromo-, chloro- and mainly iodoperoxidases) participate in the process of iodine uptake from seawater into plant tissues [27]. Vanadium-dependent iodoperoxidase (vHIPO), present in the cell wall of *Laminaria digitata*, catalyzes the process of I^- oxidation into hypoiodous (I) acid (HIO), which is further transformed into molecular I_2. These compounds (HIO and I_2) are more lipophillic than I^-, which facilitates its penetration through the cell membrane into the cytosol. The course of the processes of further reduction of HIO or I_2into I^- in the apoplast is not known. vHIPO enzymes also participate in the process of volatilization of I_2 from cells to seawater. Hypoiodous acid (HIO) is also synthesized in the cell wall throughout the process, which is triggered in marine algae as a response to oxidative stress [27]. The activity of vHIPO enzymes was detected in various algae species, such as *Pelvetia canaliculata* [28], *Gracilaria fisheri* [29], and *Laminaria digitata* [30]. Nevertheless, the functioning of this enzyme in crop plants has not yet been sufficiently studied.

To date, plant fertilization with vanadium in hydroponic cultivation has not yet been commonly applied. That element is only slightly mentioned in the respective handbooks [31]. Our studies allow for filling the information gap on vanadium influence on crop plants, mainly with respect to iodine biofortification.

The aim of this study was to compare the effect of different doses of vanadium on iodine uptake (effectiveness of biofortification) by lettuce plants grown in the presence of KIO_3 and iodosalicylates: 5-iodosalicylic acid (5-ISA) or 3,5-diiodosalicylic acid (3,5-diISA) as source iodine compounds. The study was also directed at evaluating the activity of vHPO enzymes in lettuce leaves and roots under the influence of vanadium applied solely or together with iodine into the nutrient solution. It was also aimed at analyzing the chemical composition of plants affected by the application of vanadium as well as inorganic and organic iodine compounds.

2. Materials and Methods

2.1. Plant Material and Treatments

The hydroponic cultivation of lettuce *Lactuca sativa* L. var. *capitata* 'Melodion' cv. was conducted in an NFT (nutrient film technique) system located in a greenhouse of the Faculty of Biotechnology and Horticulture, University of Agriculture in Kraków (50°05′04.1″ N, 19°57′02.1″ E).

Four independent experiments (Experiment no. 1, 2, 3 and 4) were carried out according to the same procedure of lettuce cultivation (Table 1). Seeds were sown into 112-cell propagation trays (330 × 520 × 40) mm with (32 × 32 × 40) mm sized one cell filled with peat substrate mixed with sand (1:1 *v*/*v*). Seedlings of 4–5 true leaves were transplanted into the NFT system. Substrate was thoroughly rinsed from seedling root system with the use of tap water. Seedlings were placed into holes (spaced 25 cm apart) of styrofoam slabs filling NFT beds—a "dry hydroponic" method of cultivation without substrate. After transplanting, plants were watered for one minute every 5 min during the day between 5:00 a.m. and 7:00 p.m. and during the night between 1:00 a.m. and 2.00 a.m. The nutrient solution used for the cultivation contained the following amounts of macro- and microelements ($mg \cdot dm^{-3}$): N 150, P 50, K 200, Mg 40, Ca 120, Fe 2, Mn 0.55, Zn 0.33, B 0.33, Cu 0.15, and Mo 0.05. At the beginning of lettuce cultivation in the NFT system, the EC (electrical conductivity) of nutrient solution of all treatments in all the experiments was 1.75 $mS \cdot cm^{-1}$ and the pH of all nutrient solutions was adjusted to 5.70 with the use of 38% nitric acid in accordance with the recommendations of the Research Institute of Horticulture in Poland [32]. Measurements of pH and EC were carried out with the use of a pH-meter CP-505 and conducto-meter CC-505 (both by Elmetron Sp.j., Zabrze, Poland), respectively. The results of pH measurement were used to regulate the pH nutrient solution with the use of 38% nitric acid while readings of EC were stable during the whole cultivation period.

Table 1. Design, schedule and method of conducting experiments with lettuce cultivation in the NutrientFilm Technique (NFT) hydroponic system.

Experimental Factor	Experiment No. 1	Experiment No. 2	Experiment No. 3	Experiment No. 4
Dose of vanadium as ammonium metavanadate (μM V nutrient solution).	0 (control *) 0.05 0.1 0.2 0.4	0 (control *) 0.05 0.1 0.2 0.4	0 (control *) 0.05 0.1 0.2 0.4	0 (control *) 0.05 0.1 0.2 0.4
Concentration and chemical form of iodine in nutrient solution (the same for each treatments in separateexperiment) **	Control * (0.0204 μM I)	KIO_3 (potassium iodate) 10 μM (10 μM I)	5-ISA (5-iodosalicylic acid) 10 μM (10 μM I)	3,5-diISA (3,5-diiodo-salicylic acid) 10 μM (20 μM I)
Growing season	Autumn	Autumn	Autumn-winter	Autumn-winter
Seed sowing	28 August 2018	28 August 2018	22 October 2018	22 October 2018
Planting seedlings to NFT gutter	20 September 2018	20 September 2018	16 November 2018	16 November 2018
Iodine and vanadium application in rosette stage of plants.	05 October 2018	05 October 2018	07 December 2018	07 December 2018
Harvest of the lettuce heads (number of days after sowing/after planting seedlings to NFT gutter)	05 November 2018 (69/46 days)	05 November 2018 (69/46 days)	08 January 2019 (78/53 days)	08 January 2019 (78/53 days)
Parameters				
Temperature for heating strategy (day/night)	16 °C/10 °C	16 °C/10 °C	16 °C/10 °C	16 °C/10 °C
Temperature for ventilation strategy (day/night)	22 °C/15 °C	22 °C/15 °C	22 °C/15 °C	22 °C/15 °C
Hours of natural light supplementation with 600-W high-pressure sodium lamps	5.00–8.00 and 16.00–19.00	5.00–8.00 and 16.00–19.00	5.00–8.00 and 16.00–19.00	5.00–8.00 and 16.00–19.00

* control—trace concentration of iodine and vanadium in nutrient solution: 0.0204 μM I and 0.009 μM V of nutrient solution (I and V from tap water and fertilizers). ** Dose of iodine compounds for each vanadium treatment in Experiments Nos. 2–4.

Mineral nutrients were introduced into the solution with the use of the following fertilizers: calcium nitrate, monopotassium phosphate, potassium nitrate, potassium sulphate, magnesium nitrate (all produced/ distributed by Yara, Poland), and potassium chloride (ICL Speciality Fertilizer, City, Poland). Micronutrients were introduced in the form of multi-element fertilizer 'Mikro plus' (Intermag, Olkusz, Poland). The distinguishing factor of Experiment Nos. 1, 2, 3, and 4 was the chemical form of iodine applied with increasing doses of vanadium (Table 1). In each experiment, five doses of vanadium applied in the form of ammonium metavanadate (NH_4VO_3) were introduced into the nutrient solution: 0 (control) 0.05, 0.1, 0.2, and 0.4 μM V. For increasing V doses, the same concentration of iodine was applied. In Experiment No. 1, control iodine treatment was used, i.e., a trace amount of iodine in the nutrient solution. In subsequent experiments, the following iodine compounds were used: KIO_3 in Experiment No. 2; 5-iodosalicylic acid (5-ISA) in Experiment No. 3; and 3,5-diiodosalicylic acid (3,5-diISA) in Experiment No. 4.

Inorganic (KIO_3) and organic forms of iodine, i.e., 5-ISA and 3,5-diISA were applied in a dose of 10 μM calculated per molar mass of a whole compound. The dose was chosen based on own previously published [25,26] and unpublished results, including patent application P.410806; see Acknowledgments). According to that, the iodine dose was 10 μM I for KIO_3 and 5-ISA as well as 20 μM I for 3,5-diISA (Table 1). Iodine content in the control nutrient solution (**Experiment No. 1**) assayed by an ICP-MS QQQ (TQ ICP-MS ThermoFisher Scientific, Bremen, Germany) technique was 2.6 μg I·dm^{-3} (0.0204 μM I)—trace amount delivered with tap water and mineral fertilizers. In each experiment, iodine and vanadium were first introduced at the stage of rosette with 4–5 true leaves. Prior to the introduction, respective amounts of 5-ISA and 3,5-diISA were dissolved in a small amount of water containing a few drops of 1M NaOH in order to increase its solubility.

The compartment in a greenhouse was equipped with 10 individual NFT sets with 650-dm^3nutrient solution containers, facilitating lettuce cultivation in recirculating hydroponics. Each NFT set consisted of three 5.5 m long beds. Each experiment was conducted separately in randomized block design with four repetitions within one NFT set. Plants were cultivated in four replications of 15 plants (60 plants per combination). In a single cultivation period, two experiments were performed, each with five levels of vanadium application into the nutrient solution (ten combinations in total, each combination in an individual NFT set). Experiment Nos. 1 and 2 were carried out in the autumn season and Experiment Nos. 3 and 4 in the autumn–winter season of 2018. Cultivation in both terms (autumn and autumn–winter season) was done with the same microclimate conditions regulated with the use of a greenhouse climate computer control system (Table 1). Natural light was supplemented between 5:00 a.m. and 8:00 a.m. as well as 4:00 p.m. and 7:00 p.m. with the use of 600-W high-pressure sodium lamps, which allowed for obtaining a 14h day/10h night photoperiod. During the harvest, the biomass of lettuce roots and heads were evaluated. Chemical analyses were performed using the collected plant material i.e., five heads and all the roots from plants from each replication.

2.2. Activity of Vanadium-Dependent Haloperoxidases (vHPO)

An analysis of a total activity of vHPO enzymes in the fresh samples of lettuce roots and leaves was conducted. No information has been found in the literature on measuring the activity of vHPO enzymes in higher plants. Therefore, the analysis was performed based on the methods used for marine algae [28,29,33,34]. The adapted analytical method allowed us to measure the total activity of vanadium-dependent haloperoxidase enzymes (vHPO) in root and leaf tissues of lettuce.

Roots and leaves of lettuce were washed in tap and distilled water, dried with the use of laboratory paper and cut into small fragments. The samples of 2.5 g were weighted into 30 mL Falcon tubes and homogenized with 5 mL ice-cold 20 mM Tris HCl buffer (pH 8.5) containing 1% PVP-40 [29]. Homogenized plant material was transferred into centrifuge tubes and centrifuged for 15 min at 4500 rpm, 2 °C. The supernatant was collected, transferred to fresh centrifuge tubes, and further centrifuged for 10 min at 12,000 rpm, 2 °C. An amount of 2 mL of supernatant was collected from the Eppendorf tubes for the enzymatic activity analysis.

The enzymatic activity of vHPO was assayed spectrophotometrically with the use of a Hitachi U2900 Spectrophotometer (Hitachi High Technologies Corporation, Tokyo, Japan). The total volume of reaction mixture was 3 mL and contained (according to the order of mixing): (a) 1.3 mL of Tris-HCl pH 8.5; (b) 0.1 mL of 1 mM KI; (c) 0.25 mL of 1 mM NH_4VO_3; (d) 0.5 mL of extract (or double distilled H_2O for blank); and (e) 0.1 mL of 100 µM bromothymol blue (TB). The mixture was vortexed for a few seconds, transferred into the cuvette and then 0.75 mL of 10 mM H_2O_2 was added to initiate the reaction. All reagents, i.e., KI, NH_4VO_3, TB, and H_2O_2 were dissolved in Tris-HCl pH 8.5. The absorbance was read at 0 and 20 min time at 620 nm [33]. The activity of vanadium-dependent haloperoxidase was calculated based on the increase of absorbance after 20 min and expressed as $U \cdot mg^{-1} \cdot min^{-1}$ protein. The protein content was assayed according to the Lowry method with bovine serum albumin as a standard [35].

2.3. Analysis of Dry Samples of Roots and Leaves

Each lettuce head was cut in half (the leaves from each growth stage was collected), mixed within the replication, frozen at −20 °C and lyophilized with the use of a Christ Alpha 1-4 lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). Lettuce roots were prepared accordingly. Samples of lyophilized leaves and roots were ground in a laboratory grinder FRITSCH Pulverisette 14 (FRITSCH GmbH, Weimar, Germany) and stored in tightly closed polyethylene bags (at room temperature) until the analysis.

Iodinedetermination. In order to evaluate the content of iodine in root and leaf samples, the alkaline extraction of samples with tetramethylammonium hydroxide(TMAH) was conducted according to the procedure described in previous work [36]: 0.5 g air-dried leaf or root samples, 10 mL double-distilled

water and 1 mL of 25% TMAH (Sigma-Aldrich, St. Louis, MO, USA) were put into 30mLFalcon tubes. After mixing, samples were incubated for 3 h at 90 °C. After incubation, samples were cooled to a temperature of approximately 20 °C and filled to 30 mL with double-distilled water. After mixing, samples were centrifuged for 15 min at 4500 rpm. The measurements of iodine content using an ICP-MS triple quadruple spectrometer (TQ ICP-MS Thermo Fisher Scientific) were conducted in the supernatant without decanting [37].

Vanadium determination. An analysis of vanadium content was conducted with the use of ICP-OES technique (using an ICP-OES Prodigy Spectrometer, Leeman Labs, New Hampshire, MA, USA) after microwave digestion in 65% super pure HNO_3[38]. The 0.5 g plant samples were placed in 55 mL TFM (TFM—Modified Poly-Tetra-Fluoro-Ethylene (PTFE)) vessels and were digested in a 10 mL 65% super pure HNO_3 (Merck Whitehouse, Station, NJ, USA) in a CEM MARS-5 Xpress (CEM World Headquarters, Matthews, NC, USA) microwave digestion system. The following procedure was applied: 15 min time needed to achieve a temperature of 200 °C and 20 min maintaining this temperature. After cooling, the samples were quantitatively transferred to 25 mL graduated flasks with redistilled water.

Determination of salicylic acid (SA), benzoic acid (BeA), iodosalicylates, iodobenzoates and triiodothyronine (T3). The content of SA, 5-ISA, 3,5-diISA, 2-iodobenzoic acid (2-IBeA), 4-iodobenzoic acid (4-IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA), and T3 was analyzed in the root and leaf samples. The following extraction procedure was applied: 50 mg of plant material was placed in 7 mL polypropylene tubes, 5 mL of 76% ethanol containing 50 ng·mL^{-1} of deutered salicylic acid (SA-d4, Sigma-Aldrich). Samples were vortexed and subjected to 1-h ultrasound-assisted extraction at 50 °C. Samples were then centrifuged (5 min, 4500 RPM) and supernatants filtered with the use of nylon 0.22 µm syringe filters (FilterBio NY Syringe Filter, Phenomenex, Torrance, CA, USA). The measurements of SA, BeA, 5-ISA, 3,5-diISA, 2-IBeA, 4-IBeA, and 2,3,5-triIBeA were determined by an LC-MS/MS technique (Ultimate 3000, Thermo Scientific, QTrap 4500, Sciex) according to the previously described procedure [39]. Chromatographic separation was carried out on a Luna 3 µm Phenyl-Hexyl 100 Å (150 mm × 3 mm, i.d. 3 µm) column (Phenomenex, Phenomenex, Torrance, CA, USA) with the following mobile phases: A—water with formic acid 0.3% (at the beginning 60%); B—acetonitrile with formic acid 0.3% (40%). After 2 min, the proportions of the mobile phase were increased linearly up to obtain 98% phase B at 8 min and held for 4 min. The starting proportions were restored over a 3 min period after the 15-min analysis. The injection volume was 10 µL. The mobile phase was directed to MS ion source between 1 and 14 min of the separation. For detection, electrospray ionization (ESI) in negative ion mode was used. Tandem mass spectrometry MS/MS was used for quantitative studies. 136.8/93.1, 120.9/76.9, 262.9/126.7, 388.8/126.7, 246.9/126.6, 246.9/144.7, 498.7/454.4, 671.8/350.2 and 141/96.8 transitions were monitored for SA, BeA, 5-ISA, 3,5-diISA, 2-IBeA, 4-IBeA, 2,3,5-triIBeA, T3 sodium salt and for SA-d4, respectively. The LC-MS/MS system was controlled using Analyst 1.7 with HotFix 3 software, which was also used for data processing.

2.4. Statistical Analysis

All data were statistically verified using the ANOVA module of the Statistica 12.0 PL program (https://www.tibco.com/products/data-science, StatSoft Inc., Tulsa, OK 74104, USA) at significance level of $p < 0.05$. In the case of significant effects, homogenous groups were distinguished on the basis of a Tukey test. The obtained results were statistically verified separately for each of the four experiments.

3. Results

3.1. Plant Biomass

The weights of lettuce heads from Experiment Nos. 3 and 4 were approximately 7.5 times lower than those from Experiment Nos. 1 and 2 (Figure 1 and Table 2). This indicates that 5-ISA and 3,5-diISA

applied in a 10 µM dose similarly inhibited the growth and development of plants as compared to the same concentration of KIO_3.

Increasing doses of vanadium in the nutrient solution had no significant effect on head and root biomass in lettuce grown in the control as well KIO_3 or 5-ISA combinations (Experiment Nos. 1, 2, and 3, Table 2). Only the application of the highest doses of vanadium 0.40 µM V with 3,5-diISA caused a 27.1% reduction of lettuce head biomass, yet the effect was not statistically significant and also not observed in roots (Experiment 4, Table 2). Application into the nutrient solution of 0.10 and 0.20 µM V with 3,5-diISA significantly improved root biomass of lettuce, respectively, by 56.8% and 43.7%.

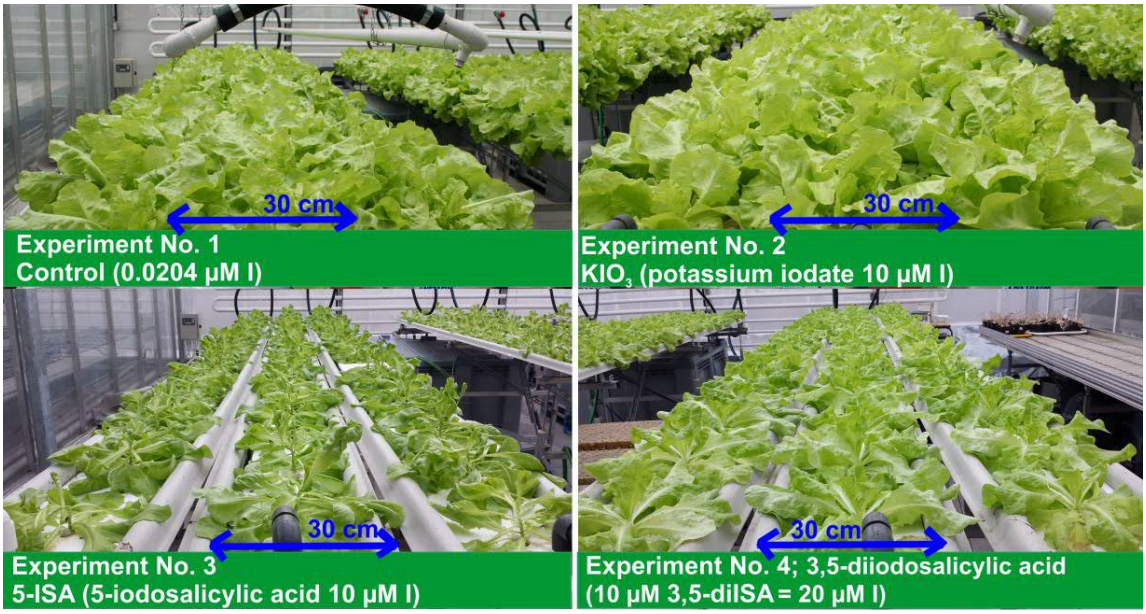

Figure 1. Lettuce plants prior harvest from Experiment Nos. 1, 2, 3 and 4.

Table 2. Effects of KIO_3, 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA)andvanadium application on lettuce biomass.

Exp. No.	Treatments	Leaf Biomass (Lettuce Head, g)	Root Biomass from One Plant (g)	Whole Plant Biomass (Roots + Leaves, g)
1	Control	238.5 ± 6.06a	22.6 ± 0.72a	261.1 ± 6.60a
	0.05 µM V	253.1 ± 12.78a	25.1 ± 1.60a	278.3 ± 13.94a
	0.10 µM V	267.6 ± 5.21a	27.1 ± 1.14a	294.8 ± 4.62a
	0.20 µM V	269.7 ± 3.51a	27.4 ± 1.05a	297.0 ± 3.04a
	0.40 µM V	247.4 ± 18.76a	24.4 ± 1.59a	271.8 ± 19.68a
2	Cont. + KIO_3	254.4 ± 3.37a	23.8 ± 0.63a	278.1 ± 3.43a
	0.05 µM V + KIO_3	233.8 ± 3.74a	23.6 ± 0.86a	257.3 ± 3.71a
	0.10 µM V + KIO_3	246.7 ± 14.74a	25.5 ± 1.26a	272.2 ± 15.03a
	0.20 µM V + KIO_3	252.5 ± 8.37a	24.8 ± 1.58a	277.3 ± 9.59a
	0.40 µM V + KIO_3	245.6 ± 13.26a	21.4 ± 1.79a	267.0 ± 14.94a
3	Cont. + 5-ISA	29.50 ± 1.14a	4.50 ± 0.46a	34.0 ± 1.58a
	0.05 µM V + 5-ISA	29.25 ± 1.90a	4.88 ± 0.47a	34.1 ± 2.37a
	0.10 µM V + 5-ISA	28.50 ± 1.85a	4.38 ± 0.55a	32.9 ± 2.11a
	0.20 µM V + 5-ISA	27.33 ± 1.85a	4.25 ± 0.60a	31.6 ± 2.16a
	0.40 µM V + 5-ISA	32.38 ± 0.80a	4.13 ± 0.24a	36.5 ± 0.79a
4	Cont. + 3,5-diISA	40.94 ± 3.36ab	3.59 ± 0.30ab	44.5 ± 3.64ab
	0.05 µM V + 3,5-diISA	39.38 ± 1.14ab	4.69 ± 0.18bc	44.1 ± 1.16ab
	0.10 µM V + 3,5-diISA	43.13 ± 4.29ab	5.63 ± 0.26c	48.8 ± 4.51b
	0.20 µM V + 3,5-diISA	49.22 ± 2.98b	5.16 ± 0.47c	54.4 ± 3.20b
	0.40 µM V + 3,5-diISA	29.84 ± 2.82a	3.13 ± 0.26a	33.0 ± 3.05a

Means in the column followed by different letters separately for each experiment differ significantly at $p < 0.05$ ($n = 4$).

3.2. Iodine Uptake and Accumulation by Lettuce Plants

A various effect of vanadium on iodine content in leaves and roots of lettuce was noted depending on the chemical form of iodine present in the nutrient solution (Table 3).

Each dose of vanadium significantly increased iodine content in roots of plants grown in the control and KIO_3 combinations (Experiment Nos. 1 and 2, Table 3); for the trace amount of iodine in the nutrient solution, iodine level in roots increased proportionally to the applied level of vanadium (Experiment No. 1). Iodine content in leaves of plants from Experiment No. 1 was higher when lower doses of V were applied i.e., 0.05 and 0.10 µM V.

In comparison to the control, application of all the doses of vanadium caused a significant decrease in iodine content in the leaves of plants fertilized with KIO_3 (Experiment No. 2, Table 3) as well as in the leaves and roots of lettuce from 5-ISA combinations (Experiment No. 3). For 3,5-diISA, only its highest dose, i.e., 0.40 µM V reduced iodine content in roots (Experiment No.4). At the same time, no clear effect of vanadium doses on iodine content was noted in leaves of lettuce from that experiment.

To sum up, only for trace amounts of iodine in the nutrient solution in Experiment No. 1 did additional application of increasing doses of vanadium increase iodine uptake for a single head(all leaves from plants), roots, and/or whole plants of lettuce (Table 4). When iodine was applied in the form of KIO_3, 5-ISA, and 3,5-diISA, the application of the highest dose of vanadium decreased iodine uptake by a whole lettuce plant. Additionally, the application of 0.10 and 0.20 µM V + 3,5-diISA increased iodine uptake by lettuce heads, roots, and whole plants (head+roots). In all experiments, the two lowest vanadium doses, i.e.,0.05 and 0.10 µM V, decreased the vanadium uptake by the roots as compared to respective control combinations.

Table 3. Effect of KIO_3, 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA) and vanadium application on the content of iodine in leaves and roots of lettuce plants.

Exp. No.	Treatments	Content of Iodine (mg I·kg^{-1} D.W.)	
		in Leaves	in Roots
1	Control	0.21 ± 0.006a	2.91 ± 0.440a
	0.05 µM V	0.51 ± 0.009d	10.46 ± 0.149b
	0.10 µM V	0.55 ± 0.005e	12.89 ± 0.252c
	0.20 µM V	0.29 ± 0.003b	15.21 ± 0.156d
	0.40 µM V	0.32 ± 0.002c	17.43 ± 0.345e
2	Cont. + KIO_3	10.56 ± 0.184d	33.51 ± 0.565a
	0.05 µM V + KIO_3	8.01 ± 0.044b	45.77 ± 1.256c
	0.10 µM V + KIO_3	9.14 ± 0.140c	52.88 ± 1.117d
	0.20 µM V + KIO_3	8.65 ± 0.285bc	35.41 ± 0.163b
	0.40 µM V + KIO_3	6.99 ± 0.146a	46.80 ± 1.019c
3	Cont. + 5-ISA	286.77 ± 3.462b	1 111.30 ± 20.512a
	0.05 µM V + 5-ISA	243.50 ± 0.583a	1 029.87 ± 4.949b
	0.10 µM V + 5-ISA	247.68 ± 7.197a	941.42 ± 11.650a
	0.20 µM V + 5-ISA	233.31 ± 1.982a	919.30 ± 18.200a
	0.40 µM V + 5-ISA	238.82 ± 1.480a	943.14 ± 7.584a
4	Cont. + 3,5-diISA	9.68 ± 0.084b	646.79 ± 54.627bc
	0.05 µM V + 3,5-diISA	8.82 ± 0.036a	678.97 ± 34.856bc
	0.10 µM V + 3,5-diISA	12.11 ± 0.127d	764.66 ± 18.047c
	0.20 µM V + 3,5-diISA	9.50 ± 0.329ab	603.05 ± 19.232ab
	0.40 µM V + 3,5-diISA	11.23 ± 0.135c	466.95 ± 36.763a

Means in the column followed by different letters separately for each experiment differ significantly at $p < 0.05$ ($n = 4$).

Table 4. Iodine and vanadium uptake by single lettuce head (all leaves from plants), roots as well as whole plant (head + roots).

Exp. No.	Treatments	Iodine Uptake			Vanadium Uptake		
		by Single Head (μg I·head^{-1})	by Roots (μg I roots·plant $^{-1}$)	by Whole Plant (Head + Roots) (μg I plant $^{-1}$)	by Single Head (μg V·head^{-1})	by Roots (μg V roots·plant $^{-1}$)	by Whole Plant (Head + Roots) (μg V plant $^{-1}$)
1	Control	1.85 ± 0.05a	1.92 ± 0.1a	3.76 ± 0.1a	8.54 ± 0.2a	1.95 ± 0.1a	10.49 ± 0.2a
	0.05 μM V	4.99 ± 0.09c	8.28 ± 0.1b	13.27 ± 0.1b	9.44 ± 0.1bc	4.26 ± 0.1b	13.70 ± 0.1b
	0.10 μM V	5.60 ± 0.04d	12.61 ± 0.2c	18.20 ± 0.2d	10.09 ± 0.2c	9.34 ± 0.1c	19.43 ± 0.1c
	0.20 μM V	3.03 ± 0.03b	13.95 ± 0.1d	16.98 ± 0.1c	9.93 ± 0.2c	10.98 ± 0.7d	20.91 ± 0.6c
	0.40 μM V	3.15 ± 0.02b	14.37 ± 0.3d	17.52 ± 0.2cd	8.76 ± 0.2ab	19.10 ± 0.1e	27.86 ± 0.1d
2	Cont. + KIO_3	106.0 ± 1.8c	24.5 ± 0.4a	130.5 ± 2.1d	9.51 ± 0.1bc	1.19 ± 0. 1a	10.7 ± 0.1a
	0.05 μM V + KIO_3	75.4 ± 0.4a	33.7 ± 0.9b	109.2 ± 0.8ab	8.43 ± 0.1a	3.86 ± 0.1b	12.3 ± 0.1a
	0.10 μM V + KIO_3	84.5 ± 1.3b	36.9 ± 0.8c	121.4 ± 1.9c	8.57 ± 0.2a	6.71 ± 0.1c	15.3 ± 0.3b
	0.20 μM V + KIO_3	85.5 ± 2.8b	31.3 ± 0.1b	116.8 ± 2.7bc	8.81 ± 0.1ab	10.36 ± 0.1d	19.2 ± 0.2c
	0.40 μM V + KIO_3	70.0 ± 1.5a	31.8 ± 0.7b	101.7 ± 1.3a	9.59 ± 0.1c	16.14 ± 1.2e	25.7 ± 1.2d
3	Cont. + 5-ISA	502.8 ± 6.0b	218.0 ± 4.0b	720.9 ± 8.8ab	2.73 ± 0.1a	0.11 ± 0.1a	2.83 ± 0.1a
	0.05 μM V + 5-ISA	469.4 ± 1.1a	256.3 ± 1.2c	725.7 ± 2.2b	3.03 ± 0.1ab	0.48 ± 0.1b	3.51 ± 0.1a
	0.10 μM V + 5-ISA	511.6 ± 14.8b	221.7 ± 2.7b	733.4 ± 14.5b	3.58 ± 0.1b	1.20 ± 0.1c	4.78 ± 0.1b
	0.20 μM V + 5-ISA	457.8 ± 3.8a	229.0 ± 4.5b	686.8 ± 2.5a	3.44 ± 0.1b	4.03 ± 0.1d	7.47 ± 0.1c
	0.40 μM V + 5-ISA	531.8 ± 3.3b	171.5 ± 1.5a	703.3 ± 2.7a	3.67 ± 0.1b	6.19 ± 0.1e	9.86 ± 0.3d
4	Cont. + 3,5-diISA	28.1 ± 0.2b	164.8 ± 13.9b	192.8 ± 13.8b	4.68 ± 0.1bc	0.33 ± 0.1a	5.01 ± 0.1a
	0.05 μM V + 3,5-diISA	23.9 ± 0.1a	226.2 ± 11.6c	250.1 ± 11.7c	4.13 ± 0.2b	1.63 ± 0.1b	5.76 ± 0.2a
	0.10 μM V + 3,5-diISA	37.1 ± 0.4d	322.9 ± 7.6d	360.3 ± 8.0d	4.83 ± 0.2c	3.17 ± 0.1c	8.00 ± 0.2b
	0.20 μM V + 3,5-diISA	31.7 ± 1.1c	223.6 ± 7.1c	256.8 ± 6.9c	6.06 ± 0.1d	6.76 ± 0.1d	12.82 ± 0.1c
	0.40 μM V + 3,5-diISA	22.0 ± 0.2a	107.4 ± 8.5a	127.4 ± 8.3a	3.40 ± 0.1a	8.63 ± 0.1e	12.03 ± 0.2c

Means in the column followed by different letters separately for each experiment differ significantly at $p < 0.05$ ($n = 8$).

3.3. Vanadium Uptake and Accumulation by Lettuce.

In each experiment, the level of vanadium in lettuce leaves was not affected by exogenous application of that element into the nutrient solution (Table 5). However, a gradual and significant increase of vanadium content in roots caused by increasing vanadium level in the nutrient solution was noted in all four experiments. The average content of vanadium in leaves was lower than in roots—approximately by 91.0%, 91.4%, 86.4%, and 88.1%, respectively, for Experiment Nos. 1, 2, 3, and 4.

Table 5. Effect of KIO_3, 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA) and vanadium application on the content of vanadium in leaves and roots of lettuce plants.

Exp. No.	Treatments	Content of Vanadium (mg V·kg^{-1} D.W.)	
		in Leaves	in Roots
1	Control	0.95 ± 0.024ab	2.96 ± 0.027a
	0.05 μM V	0.96 ± 0.011ab	5.38 ± 0.035b
	0.10 μM V	0.99 ± 0.014b	9.55 ± 0.146c
	0.20 μM V	0.95 ± 0.018ab	11.98 ± 0.802d
	0.40 μM V	0.90 ± 0.021a	23.18 ± 0.111e
2	Cont. + KIO_3	0.95 ± 0.015a	1.64 ± 0.014a
	0.05 μM V + KIO_3	0.89 ± 0.019a	5.24 ± 0.115b
	0.10 μM V + KIO_3	0.93 ± 0.024a	9.45 ± 0.169c
	0.20 μM V + KIO_3	0.89 ± 0.018a	13.51 ± 0.115d
	0.40 μM V + KIO_3	0.96 ± 0.009a	23.76 ± 1.846e
3	Cont. + 5-ISA	1.55 ± 0.018a	0.56 ± 0.009a
	0.05 μM V + 5-ISA	1.57 ± 0.077a	1.94 ± 0.033b
	0.10 μM V + 5-ISA	1.73 ± 0.029a	5.11 ± 0.072c
	0.20 μM V + 5-ISA	1.75 ± 0.039a	16.19 ± 0.169d
	0.40 μM V + 5-ISA	1.25 ± 0.132a	34.05 ± 0.581e
4	Cont. + 3,5-diISA	1.62 ± 0.027 a	1.29 ± 0.081a
	0.05 μM V + 3,5-diISA	1.53 ± 0.079 a	4.90 ± 0.051b
	0.10 μM V + 3,5-diISA	1.58 ± 0.083 a	7.50 ± 0.059c
	0.20 μM V + 3,5-diISA	1.82 ± 0.022 a	18.23 ± 0.053d
	0.40 μM V + 3,5-diISA	1.73 ± 0.045 a	37.50 ± 0.582e

Means in the column followed by different letters separately for each experiment differ significantly at $p < 0.05$ ($n = 4$).

It can therefore be stated that increasing doses of ammonium metavanadate in the nutrient solution proportionally increased vanadium uptake by the roots from a single plant as well as by whole plants (Table 4). The level of vanadium translocation into leaves was not directly affected by vanadium dose.

3.4. Effect of Iodine and Vanadium on vHPO Activity in Lettuce Plants

Depending on the chemical form of applied iodine, various effects of vanadium application on vHPO activity in lettuce leaves and roots were noted (Table 6). A significant increase of vHPO activity in lettuce leaves caused by increasing doses of vanadium was noted only in plants grown in the presence of trace amounts of iodine in the nutrient solution (Experiment No.1). Vanadium doses of 0.05, 0.1, and 0.2 µM V applied to plants fertilized with KIO_3 contributed to an approximately four-time reduction of vHPO activity in leaves, while for the highest dose (0.4 µM V), vHPO activity was comparable to the control (Experiment No. 2). Only the highest dose of vanadium, i.e., 0.40 µM V,decreased vHPO activity in the leaves of lettuce grown with 3,5-diISA as an iodine source (Experiment no. 4). No changes in the activity of vHPO in roots were observed in all the conducted experiments (Table 6).

Table 6. Effect of KIO_3, 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA) and vanadium application on the activity of vanadium-dependent haloperoxidase(vHPO).

Exp. No.	Treatments	vHPO Activity (U·µg^{-1} protein)	
		in Leaves	in Roots
1	Control	0.298 ± 0.021a	3.110 ± 0.036a
	0.05 µM V	0.505 ± 0.040ab	5.163 ± 0.012a
	0.10 µM V	0.728 ± 0.010bc	5.413 ± 0.007a
	0.20 µM V	1.243 ± 0.011d	3.690 ± 0.006a
	0.40 µM V	0.903 ± 0.040c	3.548 ± 0.014a
2	Cont. + KIO_3	1.213 ± 0.030bc	3.000 ± 0.043ab
	0.05 µM V + KIO_3	0.310 ± 0.010a	2.840 ± 0.027ab
	0.10 µM V + KIO_3	0.465 ± 0.022ab	1.645 ± 0.009a
	0.20 µM V + KIO_3	0.238 ± 0.020a	2.883 ± 0.030ab
	0.40 µM V + KIO_3	1.908 ± 0.010c	5.105 ± 0.010b
3	Cont. + 5-ISA	1.585 ± 0.040b	0.750 ± 0.041a
	0.05 µM V + 5-ISA	0.910 ± 0.020ab	0.484 ± 0.078a
	0.10 µM V + 5-ISA	1.385 ± 0.052b	0.593 ± 0.051a
	0.20 µM V + 5-ISA	1.088 ± 0.01b	0.590 ± 0.015a
	0.40 µM V + 5-ISA	0.568 ± 0.020a	0.530 ± 0.052a
4	Cont. + 3,5-diISA	1.066 ± 0.027b	0.397 ± 0.061a
	0.05 µM V + 3,5-diISA	0.901 ± 0.008b	0.313 ± 0.060a
	0.10 µM V + 3,5-diISA	0.587 ± 0.005ab	0.342 ± 0.063a
	0.20 µM V + 3,5-diISA	0.499 ± 0.014ab	0.347 ± 0.059a
	0.40 µM V + 3,5-diISA	0.263 ± 0.005a	0.421 ± 0.080a

Means in the column followed by different letters separately for each experiment differ significantly at $p < 0.05$ ($n = 8$).

3.5. The Content of BeA, SA, Iodosalicylates, Iodobenzoates, and T3 in Lettuce Plants

Only in plants fertilized with 5-ISA did the tested vanadium doses increase the level of 5-ISA in lettuce rootssignificantly, at the same time decreasing its content in leaves as compared to the control, i.e., 5-ISA applied without vanadium (Experiment No. 3; Table 7.). Vanadium applied in doses of 0.1, 0.2 and 0.4 µM V decreased the level of SA and increased the content of 5-ISA in the leaves of plants grown in the presence of 3,5-diISA (Experiment No. 4).

Irrespective of vanadium dose, the content of 5-ISA, 3,5-diISA, 2-IBeA, 4-IBeA, and 2,3,5-triIBeA in the leaves of plants grown in the presence of trace amount of iodine (Experiment No. 1) was a few times higher than in plants treated with KIO_3 (Experiment No. 2). Moreover, the content of T3 in the roots of plants from the experiments with trace iodine amount and KIO_3 (No. 1 and 2) was approximately

20 times higher than in the leaves. The content of T3 in the leaves and roots from 5-ISA and 3,5-diISA combinations remained at a similar level. At the same time, the content of T3 in leaves from these plants was from 6 to 9 times higher than in lettuce grown in the presence of trace iodine amounts and KIO_3 in the nutrient solution (Experiment Nos. 1 and 2). The highest amount of SA, 2-IBeA, 4-IBeA, and 2,3,5-triIBeA in leaves was noted in plants non-fertilized with iodine (Experiment 1, Table 7).

The content of BeA in the leaves of lettuce not fertilized with iodine (Experiment No. 1) was similar to that of SA, while, in plants fertilized with KIO_3 (Experiment No.2), it was two times higher.

The application of 5-ISA and 3,5-diISA resulted in the significant increase in the content of 5-ISA and 3,5-diISA in lettuce leaves and roots (as compared to plants grown in the presence of trace amounts of iodine and KIO_3), which suggests a possible translocation of these compounds from roots to leaves. It was also found that the application of 5-ISA (Experiment No. 3) increased the content of SA and 3,5-diISA only in roots (Table 7).

Table 7. Benzoic acid (BeA), salicylic acid (SA), 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA), 2-iodobenzoic acid (2-IBeA), 4-iodobenzoic acid (4-IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA), and triiodothyronine (T3) in leaves and roots of lettuce plants.

Exp. No.	Part of Plants	Treatments	(mg·kg^{-1} D.W.)							
			BeA	**SA**	**5-ISA**	**3,5-diISA**	**2-IBeA**	**4-IBeA**	**2,3,5-TIBA**	**T3**
1	Leaves	Control	2.18 ± 0.83a	2.86 ± 0.07b	2.92 ± 0.01b	4.78 ± 0.28b	2.12 ± 0.05b	2.04 ± 0.06b	2.13 ± 0.08b	1.34 ± 0.10a
		0.05 μM V	3.49 ± 0.64a	2.79 ± 0.01b	2.96 ± 0.04b	5.01 ± 0.09b	2.06 ± 0.03b	1.98 ± 0.03b	2.13 ± 0.01b	1.01 ± 0.08a
		0.10 μM V	2.38 ± 0.43a	2.82 ± 0.04b	3.11 ± 0.06b	5.26 ± 0.13b	2.14 ± 0.03b	2.12 ± 0.05b	2.21 ± 0.05b	1.14 ± 0.07a
		0.20 μM V	4.19 ± 0.45a	3.33 ± 0.03c	4.09 ± 0.03c	6.91 ± 0.05c	2.75 ± 0.03c	2.92 ± 0.06c	2.96 ± 0.07c	1.45 ± 0.07a
		0.40 μM V	2.45 ± 0.15a	1.83 ± 0.05a	1.93 ± 0.06a	3.32 ± 0.09a	1.36 ± 0.03a	1.73 ± 0.04a	1.49 ± 0.03a	1.26 ± 0.39a
	Roots	Control	4.79 ± 1.60a	1.51 ± 0.02e	0.02 ± 0.001b	0.03 ± 0.002a	0.033 ± 0.003a	0.039 ± 0.001bc	0.022 ± 0.001a	33.02 ± 0.56d
		0.05 μM V	2.67 ± 0.95a	1.21 ± 0.01d	0.02 ± 0.002ab	0.02 ± 0.003a	0.026 ± 0.005a	0.042 ± 0.002c	0.025 ± 0.003a	24.38 ± 0.85bc
		0.10 μM V	3.32 ± 0.51a	1.04 ± 0.01c	0.02 ± 0.001ab	0.04 ± 0.006a	0.033 ± 0.002a	0.048 ± 0.002c	0.137 ± 0.107a	26.14 ± 0.49c
		0.20 μM V	4.35 ± 1.29a	0.78 ± 0.01a	0.02 ± 0.001a	0.03 ± 0.001a	0.028 ± 0.001a	0.029 ± 0.004b	0.026 ± 0.001a	20.19 ± 1.23a
		0.40 μM V	3.91 ± 0.55a	0.87 ± 0.02b	0.02 ± 0.001a	0.02 ± 0.001a	0.035 ± 0.002a	0.014 ± 0.002a	0.023 ± 0.001a	21.59 ± 0.61ab
2	Leaves	Cont. + KIO_3	3.52 ± 0.33ab	1.04 ± 0.01c	0.45 ± 0.005a	0.80 ± 0.016b	0.38 ± 0.019a	0.46 ± 0.010a	0.39 ± 0.005a	1.35 ± 0.08a
		0.05 μM V + KIO_3	2.49 ± 0.39a	0.93 ± 0.01b	0.46 ± 0.008a	0.81 ± 0.011b	0.37 ± 0.006a	0.49 ± 0.037a	0.38 ± 0.006a	1.26 ± 0.20a
		0.10 μM V + KIO_3	2.25 ± 0.49a	0.92 ± 0.01b	0.45 ± 0.007a	0.81 ± 0.014b	0.43 ± 0.058a	0.46 ± 0.004a	0.40 ± 0.019a	1.57 ± 0.20a
		0.20 μM V + KIO_3	3.87 ± 1.33ab	1.15 ± 0.01d	0.46 ± 0.004a	0.72 ± 0.027a	0.39 ± 0.007a	0.51 ± 0.012a	0.39 ± 0.007a	1.44 ± 0.05a
		0.40 μM V + KIO_3	5.77 ± 0.46b	0.52 ± 0.01a	0.45 ± 0.005a	0.81 ± 0.005b	0.40 ± 0.012a	0.49 ± 0.008a	0.41 ± 0.005a	1.82 ± 0.30a
	Roots	Cont. + KIO_3	2.45 ± 0.64a	0.73 ± 0.01a	0.025 ± 0.001c	0.13 ± 0.005b	0.028 ± 0.002a	0.023 ± 0.002a	0.021 ± 0.001a	24.43 ± 0.94a
		0.05 μM V + KIO_3	2.18 ± 0.22a	0.83 ± 0.02b	0.013 ± 0.001a	0.03 ± 0.002a	0.034 ± 0.002a	0.018 ± 0.003a	0.022 ± 0.001a	22.35 ± 1.15a
		0.10 μM V + KIO_3	1.94 ± 0.72a	0.76 ± 0.02ab	0.016 ± 0.001ab	0.03 ± 0.002a	0.031 ± 0.003a	0.020 ± 0.004a	0.021 ± 0.002a	26.33 ± 1.38a
		0.20 μM V + KIO_3	1.37 ± 0.33a	1.37 ± 0.02c	0.019 ± 0.001b	0.03 ± 0.002a	0.027 ± 0.004a	0.027 ± 0.005a	0.022 ± 0.003a	26.30 ± 1.73a
		0.40 μM V + KIO_3	2.33 ± 0.38a	0.73 ± 0.01a	0.031 ± 0.001d	0.41 ± 0.004c	0.075 ± 0.011a	0.028 ± 0.002a	0.039 ± 0.000a	23.75 ± 0.89a
3	Leaves	Cont. + 5-ISA	0.81 ± 0.17a	1.14 ± 0.33a	2.38 ± 0.07c	0.20 ± 0.009a	0.09 ± 0.002a	0.12 ± 0.005a	0.10 ± 0.003a	7.50 ± 0.49a
		0.05 μM V + 5-ISA	0.94 ± 0.26a	1.16 ± 0.01a	1.95 ± 0.06b	0.26 ± 0.012b	0.09 ± 0.005a	0.12 ± 0.006a	0.10 ± 0.005a	8.30 ± 0.53a
		0.10 μM V + 5-ISA	0.72 ± 0.25a	1.24 ± 0.04a	1.90 ± 0.04b	0.23 ± 0.021ab	0.09 ± 0.003a	0.10 ± 0.001a	0.10 ± 0.001a	8.38 ± 0.15a
		0.20 μM V + 5-ISA	1.20 ± 0.36a	1.18 ± 0.05a	1.24 ± 0.02a	0.18 ± 0.009a	0.09 ± 0.009a	0.10 ± 0.010a	0.10 ± 0.002a	8.07 ± 0.46a
		0.40 μM V + 5-ISA	0.99 ± 0.26a	1.40 ± 0.05a	1.92 ± 0.06b	0.23 ± 0012ab	0.09 ± 0.003a	0.10 ± 0.003a	0.10 ± 0.001a	7.13 ± 0.36a
	Roots	Cont. + 5-ISA	0.86 ± 0.13a	16.47 ± 0.61c	14.24 ± 0.24a	30.3 ± 0.26a	0.07 ± 0.005b	0.03 ± 0.004ab	0.03 ± 0.004a	9.70 ± 0.18b
		0.05 μM V + 5-ISA	2.54 ± 0.55b	8.80 ± 0.13a	28.18 ± 0.33e	31.0 ± 0.60a	0.06 ± 0.001a	0.03 ± 0.003ab	0.02 ± 0.002a	6.47 ± 0.07a
		0.10 μM V + 5-ISA	2.30 ± 0.36ab	10.37 ± 0.40b	16.34 ± 0.73b	42.8 ± 2.08c	0.07 ± 0.002ab	0.05 ± 0.003b	0.02 ± 0.001a	6.48 ± 0.73a
		0.20 μM V + 5-ISA	1.84 ± 0.09ab	11.12 ± 0.15b	21.34 ± 0.27d	36.1 ± 0.18b	0.07 ± 0.001ab	0.03 ± 0.002a	0.03 ± 0.007a	7.72 ± 1.08ab
		0.40 μM V + 5-ISA	1.52 ± 0.45ab	8.11 ± 0.14a	18.79 ± 0.30c	35.6 ± 0.21b	0.07 ± 0.003ab	0.03 ± 0.005a	0.03 ± 0.002a	6.10 ± 0.46a

Table 7. *Cont.*

Exp. No.	Part of Plants	Treatments	(mg·kg^{-1} D.W.)							
			BeA	**SA**	**5-ISA**	**3,5-diISA**	**2-IBeA**	**4-IBeA**	**2,3,5-TIBA**	**T3**
4	Leaves	Cont. + 3,5-diISA	1.22 ± 0.37a	0.64 ± 0.03c	0.24 ± 0.004a	10.46 ± 0.41c	0.08 ± 0.007a	0.09 ± 0.004a	0.09 ± 0.003a	10.52 ± 0.24a
		0.05 µM V + 3,5-diISA	0.83 ± 0.19a	0.69 ± 0.01c	0.26 ± 0.002ab	6.28 ± 0.06a	0.09 ± 0.007a	0.08 ± 0.005a	0.10 ± 0.005ab	11.12 ± 0.27ab
		0.10 µM V + 3,5-diISA	0.98 ± 0.15a	0.53 ± 0.01b	0.31 ± 0.005b	7.62 ± 0.26b	0.09 ± 0.004a	0.09 ± 0.008a	0.11 ± 0.005ab	13.36 ± 0.73c
		0.20 µM V + 3,5-diISA	1.80 ± 0.63a	0.41 ± 0.02a	0.27 ± 0.004c	8.07 ± 0.04b	0.10 ± 0.003a	0.09 ± 0.002a	0.11 ± 0.005ab	14.44 ± 0.37c
		0.40 µM V + 3,5-diISA	1.64 ± 0.36a	0.55 ± 0.01b	0.35 ± 0.011d	11.38 ± 0.28c	0.10 ± 0.010a	0.11 ± 0.011a	0.12 ± 0.007b	12.96 ± 0.52bc
	Roots	Cont. + 3,5-diISA	1.33 ± 0.43a	5.91 ± 0.08c	3.34 ± 0.04a	701.8 ± 2.31a	0.03 ± 0.001b	0.013 ± 0.002b	0.003 ± 0.0006ab	8.20 ± 0.93a
		0.05 µM V + 3,5-diISA	2.54 ± 0.41a	6.68 ± 0.03d	4.99 ± 0.02c	712.4 ± 4.58a	0.02 ± 0.001a	0.011 ± 0.001ab	0.005 ± 0.0001b	9.41 ± 0.89a
		0.10 µM V + 3,5-diISA	1.54 ± 0.41a	6.79 ± 0.06d	3.07 ± 0.04a	694.4 ± 11.33a	0.01 ± 0.001a	0.009 ± 0.001ab	0.003 ± 0.0004ab	10.61 ± 0.25a
		0.20 µM V + 3,5-diISA	1.88 ± 0.40a	5.54 ± 0.02b	3.20 ± 0.02a	716.7 ± 5.02a	0.01 ± 0.001a	0.007 ± 0.0008ab	0.001 ± 0.0003a	9.69 ± 0.79a
		0.40 µM V + 3,5-diISA	1.80 ± 0.77a	5.24 ± 0.06a	3.72 ± 0.13b	681.9 ± 14.15a	0.04 ± 0.001c	0.005 ± 0.0006a	0.002 ± 0.0005ab	8.89 ± 0.38a

Means in the column followed by different letters separately for each experiment differ significantly at $p < 0.05$ ($n = 8$).

4. Discussion

4.1. Biomass Production and Vanadium Accumulation in Lettuce Plants

In the present study, the application of vanadium into the nutrient solution had no effect on the biomass of lettuce roots and leaves. Even the application of the highest dose of vanadium (0.4 μM V) did not cause any visible symptoms of its toxicity to plants. This clearly suggests that the applied doses were on the safe level for lettuce plants. Welch and Huffman [17] did not note any significant effect of 1 μM V (as NH_4VO_3) on the yield of lettuce and tomato as compared to the control combination (characterized by a trace amount of vanadium, i.e., <0.07 μM V).

The studies by Vachirapatama et al. [40] revealed the possibility of increasing root-to-leaf transfer of vanadium when very high, potentially lethal doses of that element are applied to plants. In the plants of Chinese green mustard, the high content of vanadium in the nutrient solution between 0.39 and 1.57 mM V caused a proportional increase in vanadium content in respective plant organs according to the order: roots > steams > leaves. Importantly, roots contained a few hundred times more V than leaves and stems [40]. Similar relations were found in the plants of sweet basil fertilized with NH_4VO_3 in doses of 0.1, 0.1, 0.39, and 0.79 mM V [41]. It was also revealed that fertilization with 1 μM V (as NH_4VO_3) proportionally increased the content of vanadium in the leaves and roots of lettuce and tomato [17].

In the present studies, increasing vanadium doses caused a gradual increase in its content only in the roots (Figure 2). The obtained results are in agreement with observations made on various crops, including tomato and Chinese green mustard [40], soybeans [19], rice [42] and lettuce [43]. In these studies, vanadium accumulated in roots rather than in above-ground parts of the plant. It needs to be mentioned that the applied doses between 0.05 and 0.40 μM V, apart from being safe for plants, may have been too low to observe an efficient root-to-leaf distribution of that element. Vanadium doses exceeding 0.79 mM V impaired the growth and development of tomato and Chinese green mustard plants [40]. A toxic dose of vanadium (as $VOSO_4$) for soybean plants was 1.2 mM V[19]. In the case of rice plants, vanadium toxicity was revealed for the 0.39 mM V dose [42]. When excessive vanadium concentrations were applied, plastid degradation occurred in maize and horse bean plants [43]. Gil et al. [44] observed a 15% decrease in lettuce biomass, even for the lowest dose of 0.002 mM V (as NH_4VO_3) and the application of 0.02 mM V reduced plant biomass by approximately 64%. Moreover, root darkening, a decrease in the number of secondary roots, and a loss of leaf turgidity was observed.

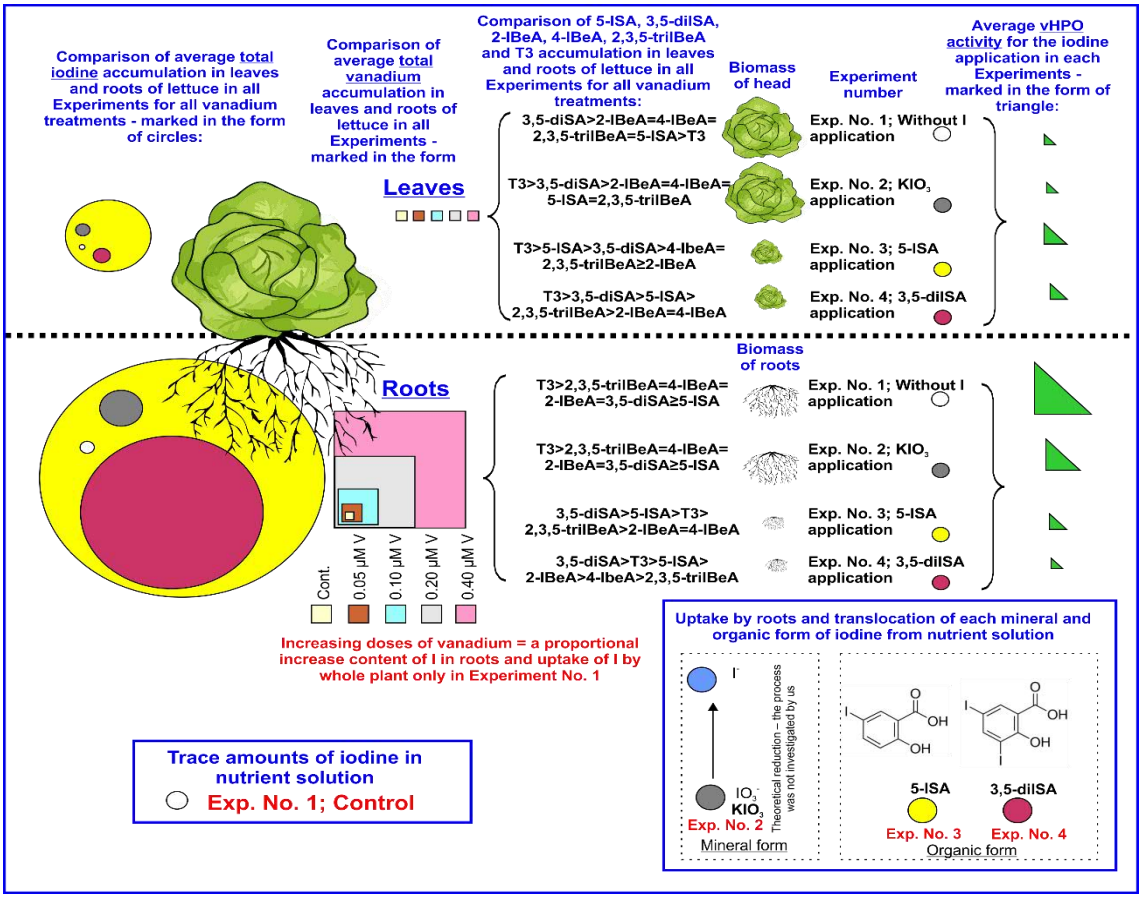

Figure 2. Summary of the study.

4.2. Iodine Accumulation vs. Vanadium Application and vHPO Activity

The enzyme vanadium-dependent haloperoxidase (vHPO) that is present in marine alga *Laminaria digitata* participates in the process of iodine uptake/release from cells with hypoiodous acid (HIO) as an intermediate [27]. An indirect role of vHPO in the volatilization of elemental iodine I_2 by marine algae is related, among others, to plant response to oxidative stress. Furthermore, the release of volatile methyl iodide also occurs in marine algae [4] and higher plants such as*Arabidopsis thaliana*, rice [45,46], or lettuce [47].

In the analyzed literature, no information can be found on the interaction between vanadium application and the level of iodine accumulation in crop plants. The effect of vanadium on the activity of vHPO in crop plants also has not been studied. Basically, the description of the structure, activity, and role of vHPO rely on the results obtained for enzymes isolated from various species of marine algae. Considering the structure and functioning of vHPO, it is classified into the group of histidine phosphatase/peroxidase super family [48]. Studies conducted by Colin et al. [49] revealed that in vitro activity of vHPO isolated from *Laminaria digitata* increased when KI was applied in the range of 0–10 mM I and dropped for KI applied in doses >20 mM I.

The trial was undertaken to determine the enzymatic activity of vHPO in lettuce with no previous analytical protocol and based on the assumption that lettuce extracts may exhibit enzymatic activity typical for vHPO. The assayed activity of vHPO is a total activity of vanadium-dependent peroxidases in plant extract and is a derivative of interaction between iodine and vanadium or other halogens. The results of the study indirectly indicate the functioning of various mechanisms regulating the activity of vHPO in lettuce, depending on the application of vanadium and different iodine compounds (KIO_3, 5-ISA and 3,5-diSA).

A significant increase in vHPO activity after the application of vanadium was noted only in the leaves of plants non-fertilized with iodine(Experiment No. 1) and was positively correlated with iodine content in roots (r^2 = 0.78*) and leaves (r^2 = 0.79*).These results suggest that vanadium fertilization may improve via increasing vHPO activity, the process of iodine uptake, and accumulation in lettuce roots and leaves only when plants are cultivated in the presence of a trace amount of that element in the nutrient solution (Experiment No. 1). Application of 3,5-diISA reduced vHPO activity in lettuce roots to levels lower than those noted for 5-ISA. At the same time, application of the highest dose of V together with 3,5-diISA decreased the activity of vHPO in leaves the most. That observation was accompanied by a slight increase in T3 content in leaves, but no effect on leaf accumulation of SA, BeA, 3,5-diSA, 5-ISA, 2-IBeA, 4-IBeA and 2,3,5-triIBeA was observed.

The reduction in the vHPO activity in roots of plants treated with 5-ISA and 3,5-diISA suggests that these iodosalicylates are taken up by the roots by different mechanisms than those related to iodide uptake with the participation of vHPO as described in marine alga. It is worth mentioning that iodates (IO_3^-) undergo reduction to I^- in the root zone most probably by a specific reductase [50] or, alternatively, nitrate reductase [4].

4.3. SA and Iodosalicylate Metabolism

In the plant organisms, benzoic acid (BeA) is a precursor of SA, the latter being considered as a signaling molecule and plant growth regulator [51]. On the other hand, 2,3,5-triiodobenzoic acid (2,3,5-triIBeA) plays a role of auxin inhibitor [52]. Van de Wouwer et al. [53] revealed that *p*-iodobenzoic acid (synonym: 4-IBeA) and its derivatives inhibit the process of lignification by reducing the activity of cinnamate 4-hydroxylase—a key enzyme in the phenylopropanoid pathway leading to the synthesis of lignin polymers. Crisan [54] revealed that exogenous 3-iodobenzoic acid (3-IBeA—its content was not determined in our study) stimulated root elongation and the formation of adventitious roots.

Previous studies on tomato plants showed that plant preference towards the uptake and accumulation of 5-ISA and 3,5-diISA in leaves and roots depended on the growth stage of plant: stage of 5–6 true leaves [26], intensive vegetative growth, and fruiting [39]. In the present studies, iodine applied as 5-ISA was taken up and distributed more easily than 3,5-diISA.

It was revealed that 3,5-diSA, 5-ISA, 2-IBeA, 4-IBeA, and T3 are present and synthesized in lettuce plants and, to our knowledge, this is a first report of that matter. In the case of **PDTHA** (Plant Derived Thyroid Hormone Analogs), its synthesis in plant tissues has been previously hypothesized by Lima et al. [55]. The exact pathway of PDTHA synthesis is yet to be described. It is worth mentioning that the first report of PDTHA was presented by Fowden [56], who revealed that, after fertilization with iodine, the plants of astra and *Salicornia* sp. contained (and therefore synthesized): 3,5-diiodothyrosyne, 3,5-diiodothyronine and 3,5,3′-triiodothyronine. Bean and barley plants grown in the presence of iodine contained only 3,5-diiodothyrosyne. A specific enzymatic system is required for the production of PDTHA in plants. Fenical [57] informed that the enzymatic process of halogenation, i.e., the incorporation of iodine (or other halogens), was described for mushrooms. Furthermore, the presence of 3-iodothyrosyne, 2,5-diiodothyrosyne, and 3,5,3′-triiodothyronine has been confirmed in *Rhodophyta* algae [57]. To our knowledge, the process of synthesis and metabolism of iodosalicylates and T3 in higher plants has not yet been described. Based on quantitative relations between analyzed iodosalicylates and iodobenzoates in the roots and leaves of lettuce from all four experiments, a hypothetical overview of that process can be proposed (Figures 2 and 3).

It needs to be underlined that the synthesis and metabolism of the analyzed compounds in lettuce plants varied depending on the form of applied iodine rather than the dose of vanadium. In all four experiments, the highest content of 2,3,5-triIBeA, 5-ISA, 2-IBeA and 4-IBeA was measured in the leaves of control plants, i.e., not fertilized with iodine (Experiment No. 1). On the other hand, the content of 3,5-diSA in the leaves of the control plants was slightly lower than after the application of 3,5-diSA (Experiment No. 4). It seems that the basic iodine metabolites in the plants grown in the presence of trace amounts of iodine were iodosalicylates, iodobenzoates, and T3. However, the obtained results do

not provide the background for the possible physiological function of these organoiodine compounds in lettuce plants.

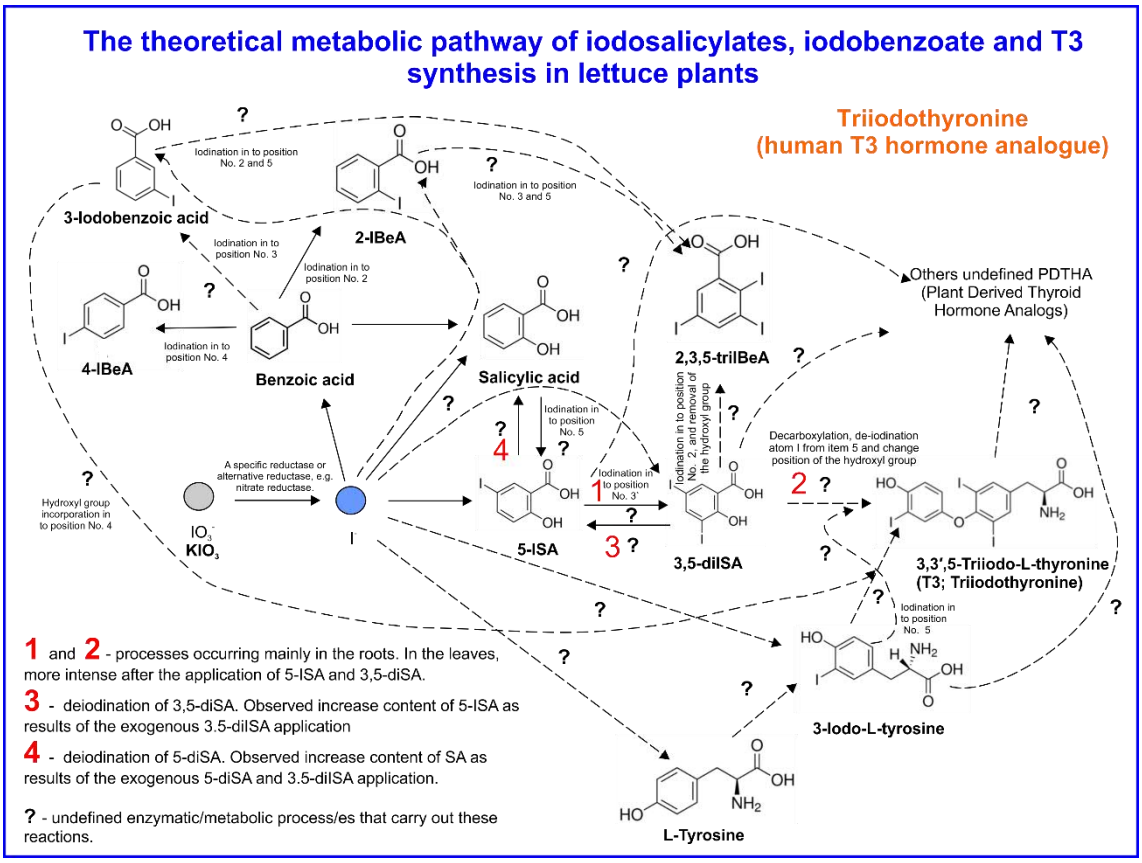

Figure 3. Theoretical metabolic pathway of iodosalicylates and iodobenzoates and T3 synthesis in lettuce plants.

The application of KIO_3 into the nutrient solution decreased the level of iodosalicylates and iodobenzoates in lettuce leaves. Most likely, in the case of increased accumulation of inorganic iodine, different pathways of its metabolism were activated (i.e., including iodine volatilization through methylation) than those engaged in the synthesis of iodosalicylates and iodobenzoates. Based on these results, it can be concluded that, after the application of iodosalicylates, endogenous iodobenzoates (2-IBeA, 4-IBeA and 2,3,5-triIBeA) as well as 3,5-diISA and 5-ISA are degraded, conversed into other compounds or volatilized in methyl forms. One of the formed compounds includes T3 as its level was higher in lettuce leaves fertilized with 5-ISA and 3,5-diISA than in the control and KIO_3 plants.

Taking the above into consideration, this supports the proposed description of possible synthesis of T3 and the metabolism of exogenous iodosalicylates in lettuce plants (Figure 3). Biosynthesis of T3 is independent of applied vanadium dose as well as of vHPO activity and most likely occurs in roots. The transport of T3 from roots to leaves is strongly limited or alternatively; T3 is conversed in leaves into other compounds from the PDTHA group.

Plant fertilization with KIO_3 did not modify the level of T3 in leaves and roots of lettuce, which suggests that the content of T3 in lettuce leaves remains stable for a trace and increased concentration of inorganic iodine in the root zone and plant tissues. In the conditions of increased concentrations of 5-ISA and 3,5-diSA in plants, an efficient distribution of both iodosalicylates into the leaves was observed. This was followed by a substantial increase in the T3 level in leaves and a decrease of T3 content in roots as compared to plants from the control and KIO_3 experiments.Therefore, it can be concluded that 5-ISA was converted into 3,5-diISA in both leaves and roots and the latter compound

may have been utilized for the synthesis of T3 through, for instance, its joining with 3-iodo-L-tyrosine or other metabolic pathways that are presented in Figure 3.

A slight decrease in the biosynthesis of BeA was also observed in the roots of plants grown in the presence of KIO_3 as compared to the control plants (Experiment No. 2 versus Experiment No. 1). Plant fertilization with KIO_3 also contributed to a simultaneous decrease of the content of (a) SA in roots and leaves; and(b) 5-ISA, 3,5-diSA, 2-IBeA, 4-IBeA, and 2,3,5-triIBeA in leaves. These results indicate that different pathways of iodine metabolism were activated by KIO_3applicationthat was directed, among others, on iodine methylation. In the case of plant fertilization with 5-ISA and 3,5-diISA, iodine metabolism was directed to the synthesis of T3 or other organic iodine compounds including those classified as PDTHA. This may have been a direct cause of obtaining lower biomass after plant treatment with iodosalicylates. In addition, the content of 5-ISA, 3,5-diSA, and T3 in the leaves of plants treated with iodosalicylates was higher than the physiological level noted in the control plants. This could have been another factor that modified the functioning of phytohormones related to the growth and development of lettuce plants fertilized with iodosalicylates.

Furthermore, the obtained results indirectly indicate that the exogenous 5-ISA and 3,5-diSA weakened the synthesis of 2-IBeA, 4-IBeA, and 2,3,5-triIBeA in lettuce plants. A significantly higher content of SA as well as a decreased content of BeA in the roots of plants fertilized with 5-ISA and 3,5-diISA suggest that some share of iodosalicylates, apart from T3/PTDHA synthesis, could have undergone the process of de-iodination into SA (Figure 3).

5. Conclusions

Within the range of applied doses, vanadium had no influence on lettuce growth. Application of 5-ISA and 3,5-diISA resulted in a higher total level of iodine accumulation and uptake by plants (leaves+roots) as compared to KIO_3. At the same time, the content of iodine reached the toxicity value as the plants were characterized by approximately nine- and seven-times lower biomass as compared to the control and KIO_3 plants.

The enzymatic system of vHPO was engaged in the uptake of inorganic iodine forms by lettuce plants, particularly for trace amounts of I^- in the root environment as well as those formed after the reduction of IO_3^-. Iodosalicylates: 5-ISA and 3,5-diISA underwent various mechanisms regulating the uptake, distribution and metabolism processes in plants that were independent of vHPO functioning.

The values of 5-ISA and 3,5-diISA analysis in the control plants indicate that these compounds are physiologically present in lettuce. It seems that exogenous application of iodosalicylates may increase the plant ability to synthesize organoiodine compounds containing iodine bound into the aromatic group.

After plant fertilization with 5-ISA and 3,5-diISA, iodine metabolism in plants was directed into the synthesis of T3 and, most likely, other organoiodine compounds, including PDTHA. The application of exogenous KIO_3 activated other metabolic pathways in lettuce plants, probably including iodine methylation.

In the presence of trace amounts of iodine as well as for iodine applied as KIO_3, the processes of iodosalicylates conversion into T3 were more efficient in roots than in leaves. In the case of exogenous application of iodosalicylates, the increase of T3 biosynthesis in leaves was related to improved transport of 3,5-diISA rather than 5-ISA. Increased uptake of iodosalicylates also reduced the biosynthesis of BeA, which is a precursor of SA. On the other hand, some share of taken up 5-ISA and 3,5-diISA could have been degraded or conversed into SA through elimination of one or two molecules of iodine. The hypothetical activation of that metabolic pathway only in the roots of plants fertilized with iodosalicylates could have been caused by a significant increase of T3 content in leaves. An increase in SA level in lettuce from the combinations with 5-ISA and 3,5-diISA could have been an effect of increased synthesis of SA as a response to the stress effect exerted by 5-ISA and 3,5-diISA.

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New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce

Sylwester Smoleń[1†], Małgorzata Czernicka[1†], Iwona Kowalska[1†], Kinga Kęska[1†], Maria Halka[1†], Dariusz Grzebelus[1†], Marlena Grzanka[1†], Łukasz Skoczylas[2†], Joanna Pitala[3†], Aneta Koronowicz[4†] and Peter Kováčik[5†]*

[1] Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Kraków, Poland, [2] Department of Plant Product Technology and Nutrition Hygiene, Faculty of Food Technology, University of Agriculture in Krakow, Kraków, Poland, [3] Laboratory of Mass Spectrometry, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Kraków, Poland, [4] Department of Human Nutrition and Dietetics, Faculty of Food Technology, University of Agriculture in Krakow, Kraków, Poland, [5] Department of Agrochemistry and Plant Nutrition, Slovak University of Agriculture in Nitra, Nitra, Slovakia

The process of uptake and translocation of non-organic iodine (I) ions, I^- and IO_3^-, has been relatively well-described in literature. The situation is different for low-molecular-weight organic aromatic I compounds, as data on their uptake or metabolic pathway is only fragmentary. The aim of this study was to determine the process of uptake, transport, and metabolism of I applied to lettuce plants by fertigation as KIO_3, KIO_3 + salicylic acid (KIO_3+SA), and iodosalicylates, 5-iodosalicylic acid (5-ISA) and 3,5-diiodosalicylic acid (3,5-diISA), depending on whether additional fertilization with vanadium (V) was used. Each I compound was applied at a dose of 10 μM, SA at a dose of 10 μM, and V at a dose of 0.1 μM. Three independent 2-year-long experiments were carried out with lettuce; two with pot systems using a peat substrate and mineral soil and one with hydroponic lettuce. The effectiveness of I uptake and translocation from the roots to leaves was as follows: 5-ISA > 3,5-diISA > KIO_3. Iodosalicylates, 5-ISA and 3,5-diISA, were naturally synthesized in plants, similarly to other organic iodine metabolites, i.e., iodotyrosine, as well as plant-derived thyroid hormone analogs (PDTHA), triiodothyronine (T3) and thyroxine (T4). T3 and T4 were synthesized in roots with the participation of endogenous and exogenous 5-ISA and 3,5-diISA and then transported to leaves. The level of plant enrichment in I was safe for consumers. Several genes were shown to perform physiological functions, i.e., *per64-like*, *samdmt*, *msams5*, and *cipk6.*

Keywords: 5-iodosalicylic acid, 3,5-diiodosalicylic acid, vHPO, CBL-interacting serine/threonine-protein kinase 6, plant-derived thyroid hormone analogs

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***Correspondence:**
Sylwester Smoleń
sylwester.smolen@urk.edu.pl

†ORCID:
Sylwester Smoleń
orcid.org/0000-0001-8093-3801
Małgorzata Czernicka
orcid.org/0000-0001-5768-5758
Iwona Kowalska
orcid.org/0000-0002-0012-6143
Kinga Kęska
orcid.org/0000-0002-4329-1499
Maria Halka
orcid.org/0000-0002-3502-8930
Dariusz Grzebelus
orcid.org/0000-0001-6999-913X
Marlena Grzanka
orcid.org/0000-0002-3810-6906
Łukasz Skoczylas
orcid.org/0000-0002-8349-4477
Joanna Pitala
orcid.org/0000-0001-5906-4402
Aneta Koronowicz
orcid.org/0000-0002-1653-2867
Peter Kováčik
orcid.org/0000-0002-0185-3342

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INTRODUCTION

Iodine in Plants

Iodine (I) is a beneficial element for plants and studies have determine the effectiveness of iodide (I^-) or iodate (IO_3^-) uptake by plants through roots or leaves (upon foliar application) and their potential translocation (Medrano-Macías et al., 2016; Antonyak et al., 2018). The effectiveness of I ion accumulation has been determined for a number of species of plants. The majority of the studies have been performed in the context of I biofortification of plants. They were conducted with the aim to prepare an I deficiency prevention program, other than through the consumption of kitchen salt based on plant enrichment with I (White and Broadley, 2009). Among the papers published within the last 15 years, there has been research on species such as kohlrabi (Golob et al., 2020), strawberry (Budke et al., 2020), lettuce (Blasco et al., 2008; Dávila-Rangel et al., 2020), basil (Incrocci et al., 2019; Kiferle et al., 2019), green bean, lettuce (Dobosy et al., 2020), cabbage, cowpea (Ojok et al., 2019), broccoli raab, curly kale, mizuna, and red mustard (Gonnella et al., 2019). There was also research devoted to the I uptake mechanism (Kato et al., 2013; Humphrey et al., 2019).

Another subject of research was the impact of I on oxyreduction, e.g., in lettuce (Blasco et al., 2008; Dávila-Rangel et al., 2020), the process of photosynthesis in kohlrabi (Golob et al., 2020) and basil (Kiferle et al., 2019), nitrate (V) content in four *Brassica* genotypes (Gonnella et al., 2019), and changes in the content of macro- and microelements in green bean and lettuce (Dobosy et al., 2020). Furthermore, there was also research devoted to the efficacy of I^- and/or IO_3^- uptake by plants, depending on the additional application of other elements, such as selenium in kohlrabi (Golob et al., 2020), zinc, selenium, and iron in wheat (Zou et al., 2019), and zinc and selenium in wheat and rice (Cakmak et al., 2020). The impact of I on the induction of plant resistance to diseases was also analyzed (Ajiwe et al., 2019). There are also works focused on the process of methylation, i.e., volatilization to the atmosphere, of volatile I forms (Leblanc et al., 2006; Itoh et al., 2009).

There were also studies that tackled a unique subject of the plants' ability to take up I applied in the form of organic compounds, e.g., iodoacetate anion (Weng et al., 2008) or organic I complexes. For instance, Dávila-Rangel et al. (2020) showed that the application of the chitosan-I complex enhanced I uptake by lettuce. The ability of tomato plants to take up organic I compounds was also determined, in which I was bound with the aromatic ring (Halka et al., 2019). The conclusion for lettuce was that the effectiveness of I biofortification with 5-ISA was higher than upon application with KIO_3 (Smoleń et al., 2017). Smoleń et al. (2020) showed the enrichment of lettuce with I using KIO_3, 5-ISA, and 3,5-diISA; however, they did not study the molecular mechanisms associated with the process of uptake and metabolism of these three I compounds.

Previous literature on physiology and/or biochemistry of plants has not considered the issue of activity and function of plant-derived thyroid hormone analogs (PDTHA), which are compounds that contain I; hence, the scarcity of scientific data on the subject. Fowden (1959) demonstrated that radioactive I binds to I organic compounds, e.g., T3 in bean, barley, aster, and Salicornia plants. Lima et al. (2012) presented general information on the potential presence of PDTHA compounds in plants. Based on their model research, Pessoa et al. (2010) concluded that in *Arabidopsis thaliana* exogenous T4 may be bound by the transthyretin-like protein. Plants were observed to produce transthyretins, i.e., proteins that may potentially act as T3 and/or T4 transporters (Eneqvist et al., 2003). However, in the available literature, it is unclear whether T3/T4 receptors might be present in plants, and descriptions of the potential genes that might encode such proteins is lacking. Obtaining information on the issue seems crucial for the determination of functionality and mechanism of PDTHA activity in plants.

Vanadium vs. Iodine

Vanadium is classified as a beneficial element for plants (Welch and Huffman, 1973; Mengel and Kirkby, 1996) and is also beneficial for animals and humans (Anke et al., 2002). In the human body, V regulates the activity of a number of enzymes (WHO, 2001; Anke et al., 2002; Gruzewska et al., 2014). It also improves thyroid function (Afkhami et al., 2009). There is no recommended dietary allowance (RDA) established for humans (Trumbo et al., 2001). The official information on the effect of V on humans was issued by WHO (World Health Organization) several decades ago and only contains rough suggestions concerning V doses for humans (10 $\mu g\ V \cdot 24\ h^{-1}$).

A positive impact of V on the growth and development of plants was observed at low doses < 0.04 mg $V \cdot dm^{-3}$ in the nutrient solution (Kaplan et al., 1990; Pilbeam and Drihem, 2007). Such doses have been observed to have a synergistic effect on the uptake of selected macroelements by plants or to increase the foliar content of photosynthetically active pigments (Kaplan et al., 1990; Pilbeam and Drihem, 2007). Higher photosynthetic activity following V application results in the accumulation of sugars in sweetcorn (Sentíes-Herrera et al., 2018), leading to larger biomass growth in the aboveground parts of the plants (Basiouny, 1984).

In hydroponic systems, the availability of V for roots is higher than that in the soil. Therefore, the V tolerance/harmfulness for plants (Gil et al., 1995; Chongkid et al., 2007; Vachirapatama et al., 2011) is much lower than that in the soil (Zhang et al., 2012; Akoumianaki-Ioannidou et al., 2016; Imtiaz et al., 2018). Next to the dose, the plants' response to V also depends on I oxidation and is a generic property of plants (Kaplan et al., 1990; Gil et al., 1995; Vachirapatama et al., 2011). The low root absorbability of V is due to its poor mobility in the soil (Cappuyns and Swennen, 2014). The process of V sorption is related to the fact that VO_2^+ very easily reacts with humic acids in soil organic matter (SOM), making V not easily available to plants (Pilbeam and Drihem, 2007). Vanadium has not been commonly included in the process of preparing nutrient solutions for hydroponic systems (Jones, 2016). The conducted research made it possible to determine the plant's response to the interaction between the simultaneous fertilization of plants with I and V.

In marine algae, V functions in I uptake into cells. This functionality of V is attributed to its presence at the active

site of iodoperoxidase and other haloperoxidases (vHPO), i.e., V-dependent bromoperoxidase or chloroperoxidase (Leblanc et al., 2006). The structure, regulatory activity, and functionality of vHPO was described, owing to research on a number of marine alga species (Almeida et al., 2000; Colin et al., 2003; Kongkiattikajorn and Pongdam, 2006; Leblanc et al., 2006). Haloperoxidases (HPOs) are responsible for the oxidation of halogens that was conducted in the presence of H_2O_2 (Colin et al., 2005).

The process of cell I uptake by marine algae, catalyzed by vHPO, consisted of the oxidation of I^- to HIO, which was further transformed to molecular I_2. HIO and I_2 are produced within the cell wall. Being more lipophilic than I^-, they easily penetrate through the wall to the cytosol (Leblanc et al., 2006). Medrano-Macías et al. (2016) reported that there is likely a relationship between I and V in terrestrial plants. However, the function of vHPO in domesticated plants is not known. Smoleń et al. (2020) identified vHPO-like enzyme activity in lettuce and a relationship between its activity and I root uptake in the setting of trace I content in the rhizosphere. Thus far, no gene in the genome of lettuce has been assigned the function of vHPO.

The response of plants to I and V application on physiological and biochemical properties has not been diagnosed. The issue has been, to a limited extent, described in preliminary research by Smoleń et al. (2020). This situation is different for marine algae, as these plants actively take up I, accumulate it in their tissues, and carry out methylation (Leblanc et al., 2006; Keng et al., 2020). The methylation process (I volatilization) has also been described for selected terrestrial plant species (Attieh et al., 2000; Nagatoshi and Nakamura, 2007; Itoh et al., 2009). Among the studies conducted were biotechnological studies on deactivation of the process in *A. thaliana* (Landini et al., 2012). Iodovolatilization is carried out with the participation of vHPO. In contrast, the methylation of iodic hydrocarbons (CH_xI_x) is carried out with the participation of S-adenosyl-l-methionine (SAM)-dependent halide methyltransferase (HMT) or SAM-dependent halide/thiol methyltransferase (HTMT). These enzymes use iodide as a substrate (Medrano-Macías et al., 2016; Gonzali et al., 2017). No gene encoding HMT or HTMT has been identified in the genome of lettuce.

Salicylic Acid (SA) and SA-Derivatives vs. Iodine

A volatile ester of methyl salicylic acid (MeSA) can be volatilized in roots and leaves. MeSA is produced in the process of esterification of salicylic acid, during which CH_3 is joined to the SA carboxylic group (Taiz and Zeiger, 2010; Zhang et al., 2013). In tomatoes, MeSA is synthesized by an enzyme named salicylic acid carboxyl methyltransferase (SAMT) (Tieman et al., 2010). This volatile ester participates in SAR (systemic acquired resistance) in plants (Gao et al., 2014). SA is classified as a plant phytohormone (Gust and Nürnberger, 2012) or as a phytohormone-like compound (Hayat et al., 2010). Literature lacks information on whether endogenous and exogenous iodosalicylates, such as 5-ISA and 3,5-diISA, in plants may undergo further catabolic reactions. No enzymes related to the catabolism of 5-ISA and 3,5-diISA have been identified to date in plants. Additionally, there is no data indicating whether SAMT can participate in the methylation of 5-ISA, 3,5-diISA, or the SA produced as a result of the potential degradation of these iodosalicylates. Moreover, genes encoding proteins with a SAMT-like function in lettuce have not been found.

The aim of this study was to determine the process of uptake and metabolism of I applied to plants as KIO_3 and iodosalicylates. Additionally, the study aimed to determine the effect of V on these processes. Another objective was to document the selected molecular processes in the metabolism of non-organic and organic I compounds in roots and leaves of lettuce, considering aspects related to the synthesis of PDTHA.

A novelty in this study, when compared with previously published ones, was research on the plants' ability to take up non-organic and organic I compounds (iodosalicylates) through the roots, as well as whether and to what extent these compounds can be metabolized and transported within the plants' roots-leaves system. Additionally, selected genes were examined and assigned a potential putative role for encoding enzyme proteins demonstrating functions typical of vHPO, SAMT, and HMT/HTMT.

MATERIALS AND METHODS

Plant Material and Treatments

Lactuca sativa L. var. *capitata* cv. "Melodion" was planted in two pot studies and one hydroponic study. This research was performed within the camp of the University of Agriculture in Kraków (50°05′04.1″N 19°57′02.1″E).

A nutrient film technique (NFT) was used for the hydroponic system in a greenhouse setting. The hydroponic experiment was named Experiment 1 (**Table 1**). The pot studies were in turn conducted in a foil tunnel with the plants being farmed in 2 types of substrate, a peat substrate as organic soil (Experiment 2) and loam soil as an example of a heavy mineral soil (Experiment 3). Each of the 3 experiments was repeated twice in the spring season during 2 consecutive years of the study in 2018 and 2019.

The subject of this study was plant fertilization with I (**Table 1**), that is, with potassium iodate (KIO_3), 5-iodosalicylic acid (5-ISA), and 3,5-diiodosalicylic acid (3,5-diISA), as well as with ammonium metavanadate (V). An additional application of salicylic acid (SA) was also used to compare the effects of both iodosalicylic acids. The same configuration of the combinations tested was used in all 3 experiments: (1) Control; (2) SA; (3) KIO_3; (4) KIO_3+SA; (5) 5-ISA; (6) 3,5-diISA; (7) KIO_3 + V; (8) KIO_3 + SA + V; (9) 5-ISA + V; and (10) 3,5-diISA + V. In each experiment, application started at the rosette stage (5–6 true leaves). The following concentrations were used: 10 μM for all I compounds (molar mass equivalents), 10 μM for SA, and 0.1 μM for V. The experiments differed in the frequency of application. In hydroponic systems, the nutrient solution (containing the compounds tested) was applied continually (fixed concentration). A different application strategy was used in the pot systems. The I and V compounds and SA were applied to the soil once a week through manual fertigation (manual

watering with solutions of the compounds studied, at a dose of 100 mL·pot^{-1} (one plant^{-1}). In total, in Experiments 2 and 3, plants were fertilized with I, V, and SA 8 times. This research strategy was planned purposefully. Our aim was to avoid the risk of accumulation of I concentrations that would be toxic to plants in either the peat substrate or mineral soil. When designing the study, we had no information on whether or to what extent iodosalicylates applied to the soil would be taken up by the plants. Additionally, the aim was to measure the effectiveness of biofortification of lettuce using different I compounds with or without V, depending on the method of cultivation and substrate.

In a hydroponic system, it was possible to obtain the entire root system for chemical analyses without damaging it, among other things. This allowed model research on the uptake and transport of different forms of I in the roots-leaves system. In the pot system, it was impossible to isolate roots from the soil because the root system outgrew the volume of soil in the pots. Therefore, in Experiments 2 and 3, roots were not subjected to chemical analyses.

In each year of the study, seeds were sown in early March (13 March 2018 and 4 March 2019). They were sown in multi-pallets filled with substrate, that is, with peat substrate and sand 1:1 (V/V). The multi-pallets had 112 cells (14 rows × 8 cells), each sized 3.2 × 3.2 × 4 cm. Young plants in the phase of 4–5 true leaves were replanted to the NFT hydroponic system (in Experiment 1) or to pots (in Experiments 2 and 3; 10 April 2018 and 2 April 2019). The plants were potted together with the entire root ball in either peat substrate or heavy mineral soil. The volume of substrate in pots was 1.5 dm^3. The chemical properties of the peat substrate and heavy mineral soil before cultivation are presented in **Table 2**; a detailed description of methods used for chemical analysis of soil is reported in the **Supplementary Material**. No fertilization was performed before or during lettuce cultivation in either of the two pot experiments. This was because the nutrient content, pH, and EC (electrical conductivity) were optimal for growing this species in the peat substrate or heavy mineral soil I (Sady, 2000; **Table 2**).

In the NFT hydroponic system, target replanting was preceded by thorough rinsing of the substrate between the seedlings'

TABLE 1 | Design and method of conducting experiments with lettuce cultivation in the hydroponics NFT Experiment No. 1 as well as in pot experiments: Experiment Nos. 2 and 3.

Treatments	Hydroponics NFT Experiment No. 1					
	Dose of I compounds and dose of I	Dose of V as ammonium metavanadate	Dose of SA	I, V, and/or SA application from the rosette phase	Amount of I applied for one plant (μ mol I·plant^{-1})	Amount of V applied for one plant (μ mol V·plant^{-1})
Control	–*	–*	–	–	13.2	0.09
SA	–*	–*	10 μM	Permanent	13.2	0.09
KIO_3	10 μM (10 μM I)	–	–	Permanent	100	0.09
KIO_3+SA	10 μM (10 μM I)	–	10 μM	Permanent	100	0.09
5-ISA	10 μM (10 μM I)	–	–	Permanent	100	0.09
3,5-diISA	10 μM (20 μM I)	–	–	Permanent	200	0.09
KIO_3+V	10 μM (10 μM I)	0.1 μM V	–	Permanent	100	0.98
KIO_3+SA+V	10 μM (10 μM I)	0.1 μM V	10 μM	Permanent	100	0.98
5-ISA+V	10 μM (10 μM I)	0.1 μM V	–	Permanent	100	0.98
3,5-diISA+V	10 μM (20 μM I)	0.1 μM V	–	Permanent	200	0.98
	Pot experiments: Peat substrate Experiment No. 2 and mineral soil Experiment No. 3					
Control	–**	–**	–	–	–****	–****
SA	–**	–**	10 μM	8 times***	–****	–****
KIO_3	10 μM (10 μM I)	–	–	8 times***	2.67	–****
KIO_3+SA	10 μM (10 μM I)	–	10 μM	8 times***	2.67	–****
5-ISA	10 μM (10 μM I)	–	–	8 times***	2.67	–****
3,5-diISA	10 μM (20 μM I)	–	–	8 times***	5.34	–****
KIO_3+V	10 μM (10 μM I)	0.1 μM V	–	8 times***	2.67	0.027
KIO_3+SA+V	10 μM (10 μM I)	0.1 μM V	10 μM	8 times***	2.67	0.027
5-ISA+V	10 μM (10 μM I)	0.1 μM V	–	8 times***	2.67	0.027
3,5-diISA+V	10 μM (20 μM I)	0.1 μM V	–	8 times***	5.34	0.027

Trace concentration of I and V in nutrient solution: **0.0204 μM I and **0.009 μM V** of nutrient solution (I and V from tap water and fertilizers).*

***Without I and V fertilization—the natural content of I and V in peat substrate and mineral soil prior to the Experiments No 2 and 3 was presented in **Table 2**.*

****8 times every 7 days.*

*****The determined I and V content (in TMAH and metricconverterProductID1 M1 M HCl extracts, respectively see **Table 2**) do not allow estimating the amount of available I and V for plants in the soil solution of peat substrate (organic soil) and mineral soil. It is also not possible to accurately estimate the changes in the content of I and V in the soil solution during the whole period of lettuce cultivation using other methods of soil extraction.*

roots with tap water. Seedlings were placed in the openings (spaced 25 cm apart) in Styrofoam slabs filling the NFT beds. No substrate was used in slabs. Once planted, seedlings in the NFT system were watered during the day. Each experiment consisted of four replicates in a randomized block design. In hydroponic Experiment 1, there were 15 plants per replicate and 60 plants per combination (600 plants per experiment). In pot Experiments 2 and 3, there were 7 plants per replicate and 28 plants per combination (a total of 560 plants in both pot experiments).

The type of nutrient solution, its preparation (content of macro- and microelements), regulation of pH and EC, and type of fertilizers in the NFT were the same as in our previous studies with lettuce in the same hydroponic system (Smoleń et al., 2019b). The I used in the base nutrient solutions (control) was iodide I^- (25.52 μg $I \cdot dm^{-3}$) and iodate IO_3^- (0.29 μg $I \cdot dm^{-3}$). The content of I was natural (from water and dissolved fertilizers).

Once planted in spacers in the NFT systems, the plants were watered during the day between 5 am and 7 pm and at night between 1 and 2 am, for 1 min at 5-min intervals. In pot experiments, the plants were watered with tap water using drip irrigation. A single dose of water was about 100 $mL \cdot plant^{-1}$ (pot^{-1}). The frequency of watering was adjusted to weather conditions and sizes of the plants; factors that determined the rate of substrate drying involved controlling watering by the irrigation computer with the option to sum the amount of solar radiation to start irrigation. The adopted watering strategy made it possible to eliminate water leaching from pots. On the days when the compound solutions were applied to the substrate in pot Experiments 2 and 3, the plants were not watered through the drip system.

Plants were harvested at the phase of head development, that is on 15 May 2018 and 7 May 2019 in hydroponic Experiment 1, and on 9 May 2018 and 16 May 2019 in pot Experiments 2 and 3. Then, the heads (lettuce leaves) were weighed. Hydroponic Experiment 1 was the only experiment where lettuce leaf harvesting was immediately followed by pipette collection of a secretion produced as a result of root pressure [white secretion (RootSec) on the surface of the root neck, visible after cutting the heads at collar level (lettuce leaves), as shown in **Supplementary Figure 1**]. The secretion was collected for the determination of the chemical forms of I, transported from roots to the aboveground parts of plants. A total of two samples were collected for each combination, each containing 5 mL of root secretion. Immediately after collection, the secretion was 1:1 mixed with buffer (20 mM Tris HCl buffer, pH 8.5). Then, the samples were frozen at −20°C and stored until analyzed using two mass spectrometry techniques. Iodides (I^-) and iodates (IO_3^-) were analyzed using high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS)/MS, while organic I compounds were analyzed using liquid chromatography-mass spectrometry (LC-MS)/MS. Methodologic details are presented in the following subsections.

In Experiment 1, the collection of root secretions was followed by the measurement of lettuce root biomass, while in pot Experiments 2 and 3 these measurements were abandoned, as the root biomass could not be separated from the soil without damaging the roots. Chemical analyses were performed on roots of all plants from the hydroponic system, and on four randomly selected heads from each biological replicate in all three experiments.

TABLE 2 | Selected chemical properties of the soil prior to the Experiments Nos. 2 and 3.

Physicochemical soil characteristic	Peat substrate—Experiment 2	Mineral soil—Experiment 3
pH_{H_2O}	5.50	6.70
$pH_{(KCl)}$	5.33	6.28
EC ($mS \cdot cm^{-1}$)	1.18	1.46
Eh(mV)	270.1	221.6
Macroelements:		
$N\text{-}NH_4$ ($mg \cdot dm^{-3}$)	158.7	68.2
$N\text{-}NO_3$ ($mg \cdot dm^{-3}$)	103.1	205.9
$N\text{-}NH_4$+ $N\text{-}NO_3$ ($mg \cdot dm^{-3}$)	261.8	274.1
P ($mg \cdot dm^{-3}$)	125.1	94.5
K ($mg \cdot dm^{-3}$)	316.8	122.2
Mg ($mg \cdot dm^{-3}$)	180.0	175.4
Ca ($mg \cdot dm^{-3}$)	2 046.2	1 686.8
S ($mg \cdot dm^{-3}$)	227.8	287.4
Na ($mg \cdot dm^{-3}$)	20.6	55.9
Iodine ($mg\ I \cdot kg^{-1}$)	6.25	5.56
Vanadium ($mg\ V \cdot kg^{-1}$)	0.84	6.57
Al-hydroxides ($mg \cdot kg^{-1}$)	145.8	400.53
Fe-hydroxides ($mg \cdot kg^{-1}$)	624.3	5 118.0
Mn-hydroxides ($mg \cdot kg^{-1}$)	15.4	433.7
BeA ($mg \cdot kg^{-1}$)	1.768	0.864
SA ($mg \cdot kg^{-1}$)	0.156	0.034
5-ISA ($mg \cdot kg^{-1}$)	0.0125	0.0036
3,5-diISA ($mg \cdot kg^{-1}$)	0.008	0.008
2-IBeA ($mg \cdot kg^{-1}$)	0.028	0.013
4-IBeA ($mg \cdot kg^{-1}$)	0.001	0.002
2,3,5-triIBeA ($mg \cdot kg^{-1}$)	0.013	0.026
Exchange hydrolytic acidity ($me \cdot 100\ g^{-1}$)	0.99	22.13
Cation exchange capacity ($me \cdot 100\ g^{-1}$)	21.03	47.62
Total soil sorption capacity ($me \cdot 100\ g^{-1}$)	22.02	69.74
Soil organic matter (%)	100	1.92
Soil texture (according to the ISSS classification).	Peat soil (organic soil)	Loam soil 35 sand 28 % silt 37% clay

Where: benzoic acid (BeA), salicylic acid (SA), 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA), 2-iodobenzoic acid (2-IBeA), 4-iodobenzoic acid (4-IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA).

Activity of Vanadium-Dependent Haloperoxidases

Fresh leaf samples collected in all three experiments and root samples from Experiment 1 were used to measure the

total activity of vHPO enzymes. The analysis was performed with a method adapted for lettuce by Smoleń et al. (2020) based on that for marine algae. The activity of vHPO was calculated based on the increase in absorbance within 20 min (wavelength: 620 nm) and converted into $U \cdot mg^{-1} \cdot min^{-1}$ protein. Protein content in enzyme extracts was measured using the Lowry method (Waterborg, 2002). Bovine serum albumin was used as a standard.

Freeze-Drying of Samples

Fresh root and leaf samples (lettuce) were washed in tap and distilled water. Samples of lettuce heads were vacuum-dried. Each head was halved (leaves were peeled in each growth phase) and mixed thoroughly as part of each replicate. Root and leaf samples were frozen at −20°C. The freeze-drying of frozen samples was performed with the Christ Alpha 1–4 unit (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). Vacuum-dried samples of roots and leaves were ground in a lab mill (FRITSCH Pulverisette 14; FRITSCH GmbH, Weimar, Germany) and stored in sealed polyethylene bags until further chemical analyses (described in the next three sections).

Analysis of Total Iodine and Vanadium in Dry Samples of Roots and Leaves

The analysis of I content in samples of lettuce leaves and roots was performed by inductively coupled plasma mass spectrometry (ICP-MS/MS) with a triple quadruple spectrometer (iCAP TQ ICP-MS Thermo Fisher Scientific, Bremen, Germany), preceded by alkaline extraction of 0.2 g samples by tetramethylammonium hydroxide (TMAH; Smoleń et al., 2019a,c; based on Pn-En 15111, 2008).

Vanadium content in leaf and root samples was measured using inductively coupled plasma optical emission spectrometry (ICP-OES) (Prodigy Spectrometer, Leeman Labs, New Hampshire, MA, United States). The mineralization and measurement procedures were consistent with the method described by Smoleń et al. (2020).

The results of I and V content in the plant samples and biomass measurements were used to calculate I uptake (I-uptake) and V uptake (V-uptake) by plants.

Analysis of Iodides (I^-) and Iodates (IO_3^-) in Roots and Leaves by HPLC-ICP-MS/MS

The content of iodides (I^-) and iodates (IO_3^-) was only determined in root and leaf samples of hydroponic lettuce (Experiment 1); in pot experiments, there was no possibility to collect root samples for analysis. The content of these I ions was measured using a modified extraction procedure described by Smoleń et al. (2016). Briefly, a 0.05 g analytic portion of air-dried, ground plant samples was extracted (in 7 mL polypropylene tubes) using a solution containing 4 mL 25% TMAH (Sigma-Aldrich, St. Louis, MO, United States) and 10 mL 0.1 M NaOH (Chempur, Piekary Śląskie, Poland) dissolved to a final volume of 1 L with demineralized water. Once mixed, the samples were incubated for 1 h at 50°C in an ultrasonic bath, then cooled to approximately 20°C, mixed thoroughly, and centrifuged for 15 min at 4,500 rpm. The supernatants were filtered through a 0.22 μm syringe filter. The content of I ions in filtered samples was analyzed using HPLC-ICP-MS/MS. For I^- and IO_3^- speciation forms, HPLC (Thermo Scientific Ultimate 3000; Thermo Fisher Scientific, Bremen, Germany) was coupled to ICP-MS/MS (iCAP TQ). This method employed a strong anion exchange column [Thermo Fisher Scientific; Dionex IonPac AS11 (4 × 250 mm)] and a precolumn [Thermo Fisher Scientific; Dionex IonPac AG11 (4 × 50 mm)]. The column temperature was set to 30°C. Demineralized water, 50 mM NaOH, and 0.5% TMAH were used as eluents. To separate both I ions, a mobile phase, containing 2.5 mM NaOH and 0.125% TMAH at an isocratic flow, was used. The flow-rate was 1.5 mL/min, with an injection volume of 10 μL and total analysis time of 7 min (**Supplementary Figures 2**, **3**). The HPLC-column effluent was introduced directly into ICP-MS/MS (iCAP TQ ICP-MS). Iodine was determined at 127I.16O isotope, using S-TQ-O2 mode. Standards were prepared through dissolution of KI and KIO_3 (Sigma-Aldrich, St. Louis, MO, United States) in demineralized water.

To ensure correct iodide and I measurements, a "standard addition method" was used; it was applied independently for each leaf and root sample from each of the 10 combinations (**Supplementary Figures 4–6**). The standard addition method has been applied because that the alternative and easier "standard series method," which is described in numerous publications, would not provide correct analytical results. The difficulty to obtain reliable assaying results with the standard series method was due to the different matrix effects of root and leaf extracts obtained from each of the 10 combinations tested in Experiment 1. The effect could be eliminated through the use of the standard addition method, which allowed us to obtain correct analytic results.

Determination of Salicylic Acid, Benzoic Acid, Iodosalicylates, Iodobenzoates, and Plant-Derived Thyroid Hormone Analogs

Roots of plants from the hydroponic system (Experiment 1) and lettuce leaves in all three experiments were tested using LC-MS/MS to measure the content of SA, BeA (benzoic acid), 5-ISA, 3,5-diISA, 2-iodobenzoic acid (2-IBeA), 4-iodobenzoic acid (4-IBeA), 2,3,5-triiodobenzoic acid (2,3,5-triIBeA), iodotyrosine (I-Tyr), sodium salt triiodothyronine (T3-Na), triiodothyronine (T3), and thyroxine (T4) (**Supplementary Figure 7**). The root and leaf content of these compounds was analyzed in extracts prepared with 75% ethanol containing 50 $ng \cdot mL^{-1}$ of deuterated salicylic acid (SA-d4, Sigma-Aldrich). Sample extraction and filtration procedures were the same as described in our previous research (Smoleń et al., 2020).

The compounds were also measured in **RootSec** after lettuce harvesting. The **RootSec** were mixed with 20 mM Tris HCl buffer (pH 8.5). The collection and storage of **RootSec** is described in section "Plant Material and Treatments."

Root secretions stored in the TRIS-HCl buffer were mixed in vortex prior to analysis and centrifuged at 5°C for 15 min at 4,500 rpm. Then, the supernatant was filtered with a 0.22 μm nylon syringe filters (FilterBio NY Syringe Filter, Phenomenex, Torrance, CA, United States) and analyzed using LC-MS/MS according to Smoleń et al. (2020).

Biofortification Target and the Safety of Iodine-Enriched Lettuce Consumers

The results of I measurements in lettuce leaves were used in each of the experiments to calculate the following coefficients: (1) recommended daily allowance of iodine (% RDA-I) and (2) hazard quotient for iodine (HQ-iodine). They were calculated for 100 g of fresh lettuce leaves, considering the daily I requirement of adults of 150 μg. The % RDA-I and HQ-iodine coefficients were calculated using mathematical formulas described in detail by Smoleń et al. (2019a).

Gene Expression Analysis

Plants cultivated in the hydroponic NFT system (Experiment 1) were used as material for gene expression analysis. Leaves and roots for RNA extraction were collected directly before harvest. The leaf and root samples were collected from 8 plants (2 plants from each of the 4 replications), separately for each of the 10 treatments. The third youngest leaf and root samples (portions of 5–10 cm, with tips) were collected for each plant. The samples were immediately frozen in liquid nitrogen and stored at −80°C until isolation of RNA. Total RNA extraction was carried out with a Direct-zol^{TM} RNA MiniPrep Plus RNA isolation kit (Zymo Research, Irvine, CA, United States), according to the manufacturer's instructions. RNA samples were treated with 1 U μl^{-1} RNase-free Dnase I (Ambion Inc., Austin, TX, United States) and 40 U μl^{-1} RiboLock RNase Inhibitor (Thermo Fisher Scientific, Wilmington, DE, United States) to avoid contamination by DNA and RNA degradation. The quality and integrity of RNA samples were verified by electrophoresis in 1% agarose gel in denaturing conditions. The concentration and quality of RNA were evaluated spectrophotometrically using NanoDrop 2000c (Thermo Fisher Scientific, Wilmington, DE, United States) at 230, 260, and 280 nm. cDNA synthesis was conducted in four biological replicates, each comprising two plants. One microgram of RNA from each sample was transcribed into cDNA using the iScript cDNA synthesis kit (BioRad laboratories, Hemel Hempstead, United Kingdom), according to the manufacturer's instructions. The cDNA was frozen at −20°C until it was used as a template in real-time qPCR using the StepOnePlus^{TM} Real-Time PCR System (Applied Biosystems, Foster City, CA, United States), according to the following steps: denaturation at 95°C for 10 min; 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. The melting curves were obtained by melting the amplicons from 60 to 95°C for 15 s; the temperature was increased by 0.3°C per cycle.

For expression analysis, five genes possibly related to selected metabolic pathways for I and/or iodosalicylates in lettuce, i.e., *per12-like*, *per-64-like*, *samdmt*, *cipk6,* and *msams5*, were chosen (**Supplementary Table 1**). Moreover, differential expression patterns of those genes were shown by RNA-seq in leaves and roots of *L. sativa* "Melodion" of control plants and supplemented with SA, KIO_3 and KIO_3+V (data unpublished). Gene-specific primers for real-time qPCR were designed using Primer3Plus[1] based on *L. sativa* var. *capitata* "Melodion" transcript sequences *de novo* assembled from RNAseq, deposited in the NCBI GeneBank (Acc. No MT649253, MT649254, MT663549-MT663551) and Lettuce Genome Resource[2] (**Supplementary Table 1**).

The absence of primer-dimer and hairpin structures was determined using IDT-OligoAnalyzer 3.1[3]. The utility of the designed primers was validated in a reverse-transcriptase polymerase chain reaction (RT-PCR) and confirmed by electrophoresis in 1% agarose gel (**Supplementary Figure 8**). Primer specificity was verified by observing single peaks in all melting curves. The total volume of the reaction mixture was 25 μL. The mixture included 12.5 μL Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Fisher Scientific), 0.7 μM of (5 μM) each primer (forward and reverse), 2 μL of a 5-fold diluted template cDNA, and a total volume of 25 μL made up with nuclease-free DEPC-treated water (diethylpyrocarbonate; Thermo Fisher Scientific, Wilmington, DE, United States). qPCR reactions were conducted in four biological and three technical replicates. No template controls were included. Amplification efficiencies for all primer pairs were evaluated using serial 10-fold dilutions of pooled cDNA. The efficiency of each primer pair was calculated from the slope of the standard curve using the formula $E = 10^{-1/slope}$ and converted into percentage values according to the following formula: $\%E = (E - 1) \times 100\%$.

Actin (*act*) (Smoleń et al., 2016) and protein phosphatase 2A regulatory subunit A3 (*pp2aa3*) (Sgamma et al., 2016) were used as endogenous reference genes. As *Pp2aa3* expression was more stable than that of *act* (**Supplementary Figure 9**), relative quantification of gene expression was calculated using the $2^{-\Delta\ \Delta\ C(T)}$ method (Livak and Schmittgen, 2001) with Ct value normalization to *pp2aa3*. In the case of *per64-like*, *per12-like*, *cipk6*, and *msams5*, the relative gene expression was compared with control samples from roots, whereas in *samdmt*, it was compared to control samples from leaves, since expression of *samdmt* in root control samples was not detectable (**Supplementary Figure 8**).

Statistical Analyses

All data were statistically verified using one-way analysis of variance (ANOVA) in the Statistica 12.0 PL (StatSoft Inc., Tulsa, OK 74104, United States)[4] program at a significance level of $p < 0.05$. In the case of significant effects, homogenous groups were distinguished on the basis of a *post-hoc* Tukey HSD test. The results obtained were verified statistically by one-way ANOVA and *post-hoc* Tukey HSD test, separately for each of the three experiments and separately for leaves and roots of lettuce in Experiment 1.

[1]https://primer3plus.com/cgi-bin/dev/primer3plus.cgi
[2]https://lgr.genomecenter.ucdavis.edu
[3]https://eu.idtdna.com/calc/analyzer
[4]https://www.tibco.com/products/data-science

RESULTS

Plant Biomass

Hydroponic Experiment 1 was the only experiment where the application of 5-ISA and 3,5-diISA (without or with V) caused a reduction in the weight of leaves and whole plants (roots + leaves) compared with the control (**Table 3**). No negative impact of 3,5-diISA and 3,5-diISA+V on the weight of roots was observed. A comparable increase in root weight was observed for the application of 5-ISA and 5-ISA+V, compared with the control. In this experiment, combined fertilization with V and KIO_3, KIO_3+SA, 5-ISA, and 3,5-diISA had no effect on the weight of roots and heads (leaves) of lettuce, compared with the application of these compounds without V.

None of the combinations of SA, V, and I compounds had a significant impact on the weight of lettuce heads in either of the two pot experiments (Experiments 2 and 3).

Gene Expression in Roots and Leaves of Plants Cultivated in a Hydroponic System (Experiment 1)

I compounds, V, and SA applied to the nutrient solution had a statistically significant impact on the expression of the following genes in lettuce roots and leaves: *per64-like*, *per12-like*, *samdmt*, *cipk6*, and *msams5* (**Figures 1A–E**). Basically, the expression of *per64-like* in leaves and *samdmt* in roots was relatively very low compared with roots and leaves, respectively. As for the remaining three genes (*per12-like*, *cipk6*, and *msams5*), their expression in roots was higher than in leaves.

Compared with the control, all combinations with the application of I, I+SA, and V, caused increased expression of all five genes, i.e., *per64-like*, *per12-like*, *samdmt*, *cipk6*, and *msams5*, in roots (**Figures 1A–E**). The highest expression level of *per64-like* in roots was observed after application of KIO_3+SA and 5-ISA. Additionally, exogenous 5-ISA in roots caused the highest expression of *cipk6* and *msams5;* application of 3,5-diISA led to the most pronounced expression of *per12-like*, while plants fertilized with KIO_3 had the highest expression of *samdmt.* Foliar activity of these five genes in lettuce was completely different that in roots compared with the control. The highest foliar expression of individual genes was as follows: *per64-like* following the application of exogenous 3,5-diISA, *per12-like* following the application of 5-ISA+V, *samdmt* following the application of SA, *cipk6* following the application of exogenous 5-ISA, and *msams5* following the application of KIO_3.

Iodine Accumulation and Uptake by Lettuce

Inorganic (IO_3^-) and organic (5-ISA, 3,5-diISA) I accumulated in larger amounts in roots than in leaves (**Figures 2A,B**; hydroponic Experiment 1). Root accumulation of I upon the application of both iodosalicylates was higher than upon using KIO_3 as a fertilizer. Vanadium added to the nutrient solution caused a significant reduction of I content in roots for the combination of KIO_3+SA+V vs. KIO_3+SA. Additionally, V caused a significant increase in I content in roots for the combination 5-ISA+V vs. 5-ISA, and for 3,5-diISA+V vs. 3,5-diISA (**Figure 2B**).

Compared with the control and SA, application of each I compound (without V, with V, and with KIO_3+SA) caused a significant increase in foliar I content (**Figures 2A,C**), RDA-I%, and HQ-iodine, as well as I uptake by a single lettuce head (leaves from one plant) in all three Experiments (**Supplementary Table 2**).

The highest foliar I content, RDA-I%, and HQ-iodine, as well as I uptake by a single lettuce head, was found in plants with applied 5-ISA in all three experiments (**Figures 2A,C** and **Supplementary Table 2**). Adding V to the compound (5-ISA+V vs. 5-ISA) reduced foliar I content in both pot experiments (Experiments 2 and 3) but not in the hydroponic system (Experiment 1). Consequently, RDA-I%, HQ-iodine, and I uptake by

TABLE 3 | Fresh weight of roots, leaves /lettuce head/ and whole plants /roots+leaves/ in hydroponic NFT Experiment No. 1 as well as in lettuce leaves /head/ in pot Experiment Nos. 2 and 3.

Treatments	Hydroponics NFT Experiment No. 1			Peat substrate Experiment No. 2	Mineral soil Experiment No. 3
	FW of leaves/lettuce head/(g)	FW of roots from one plant (g)	FW of whole plants /roots+leaves/(g)	FW of leaves/lettuce head/(g)	FW of leaves/lettuce head/(g)
Control	291.4 ± 35.2b	31.7 ± 4.5a	323.1 ± 39.6b	174.1 ± 12.2a	186.6 ± 22.8a
SA	291.3 ± 29.5b	33.1 ± 3.7ab	324.4 ± 33.2b	169.3 ± 14.4a	168.5 ± 16.1a
KIO_3	296.8 ± 34.1b	34.6 ± 3.8ab	331.4 ± 37.7b	169.8 ± 12.2a	180.5 ± 23.1a
KIO_3+SA	276.5 ± 42.2b	32.6 ± 3.8ab	309.1 ± 45.9b	161.3 ± 14.3a	176.9 ± 23.6a
5-ISA	171.1 ± 35.7a	39.4 ± 6.1bc	210.5 ± 41.8a	167.7 ± 10.5a	194.7 ± 18.5a
3,5-diISA	147.7 ± 37.8a	30.1 ± 5.2a	177.8 ± 42.9a	171.9 ± 13.9a	185.5 ± 20.3a
KIO_3+V	305.9 ± 33.0b	35.2 ± 3.2ab	341.1 ± 36.1b	167.9 ± 10.7a	186.5 ± 19.4a
KIO_3+SA+V	305.6 ± 28.0b	33.1 ± 4.1ab	338.7 ± 32.0b	163.7 ± 13.3a	171.7 ± 21.9a
5-ISA+V	160.7 ± 35.8a	42.9 ± 5.8c	203.6 ± 41.4a	173.7 ± 9.8a	172.5 ± 18.8a
3,5-diISA+V	152.0 ± 41.1a	33.1 ± 6.3ab	185.1 ± 47.3a	168.3 ± 10.5a	180.2 ± 21.6a

Means in the column followed by different letters separately for each experiments differ significantly at $P < 0.05$ ($n = 8$).

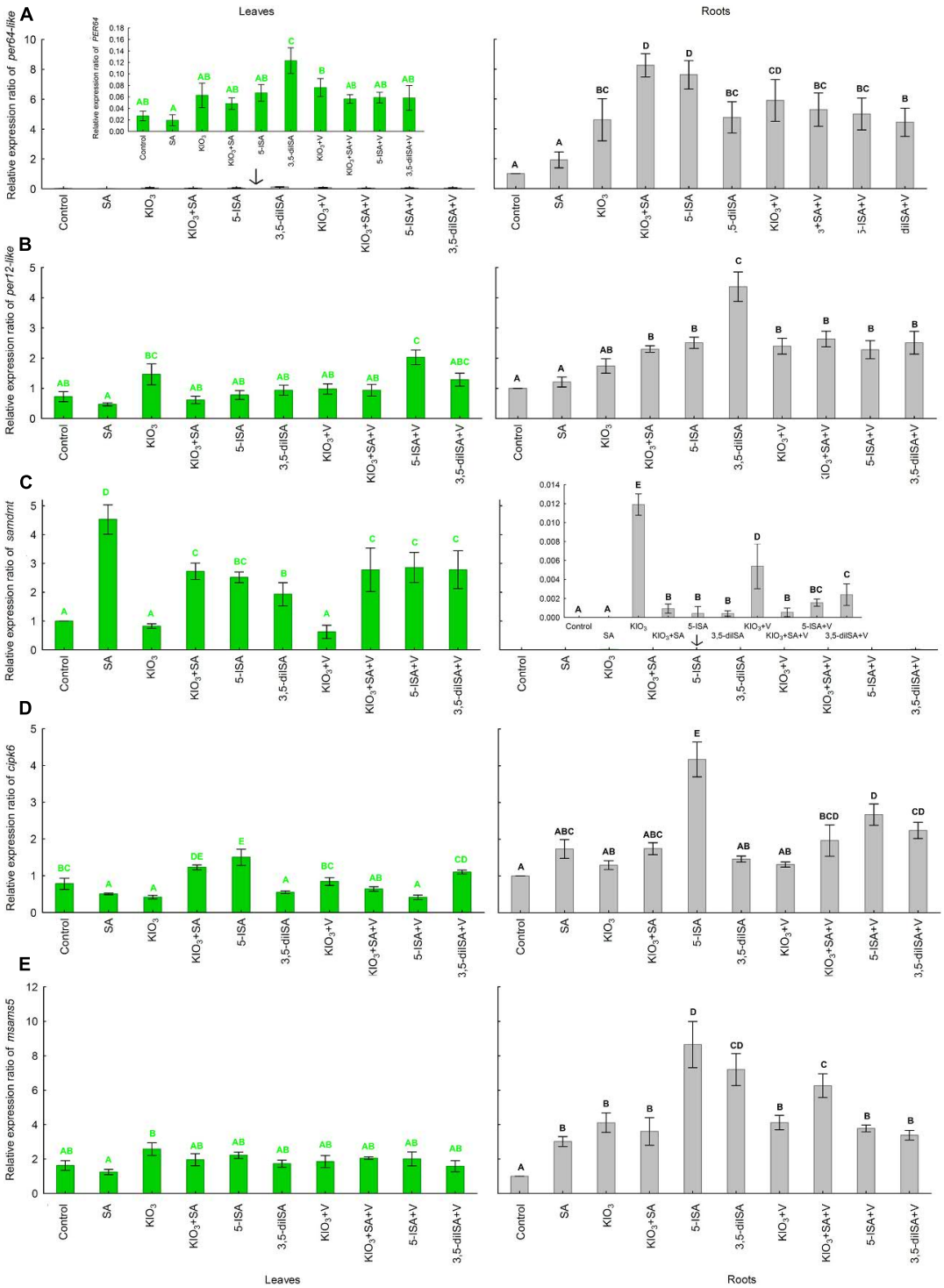

FIGURE 1 | Relative expression of *per64-like* **(A)**, *per12-like* **(B)**, *samdmt* **(C)**, *cipk6* **(D)**, and *msams5* **(E)** genes in leaves and roots of lettuce plants cultivated in hydroponic NFT Experiment No. 1. Means followed by different letters for treatments differ significantly at $P < 0.05$. Bars indicate standard error ($n = 4$).

a single lettuce head was significantly lowered for 5-ISA+V vs. 5-ISA in both pot experiments (**Supplementary Tables 2**, **3**).

Combined fertilization with KIO_3+V had no impact on leaf I content in any of the three experiments, compared with KIO_3 without V. As for the application of 3,5-diISA+V (compared with

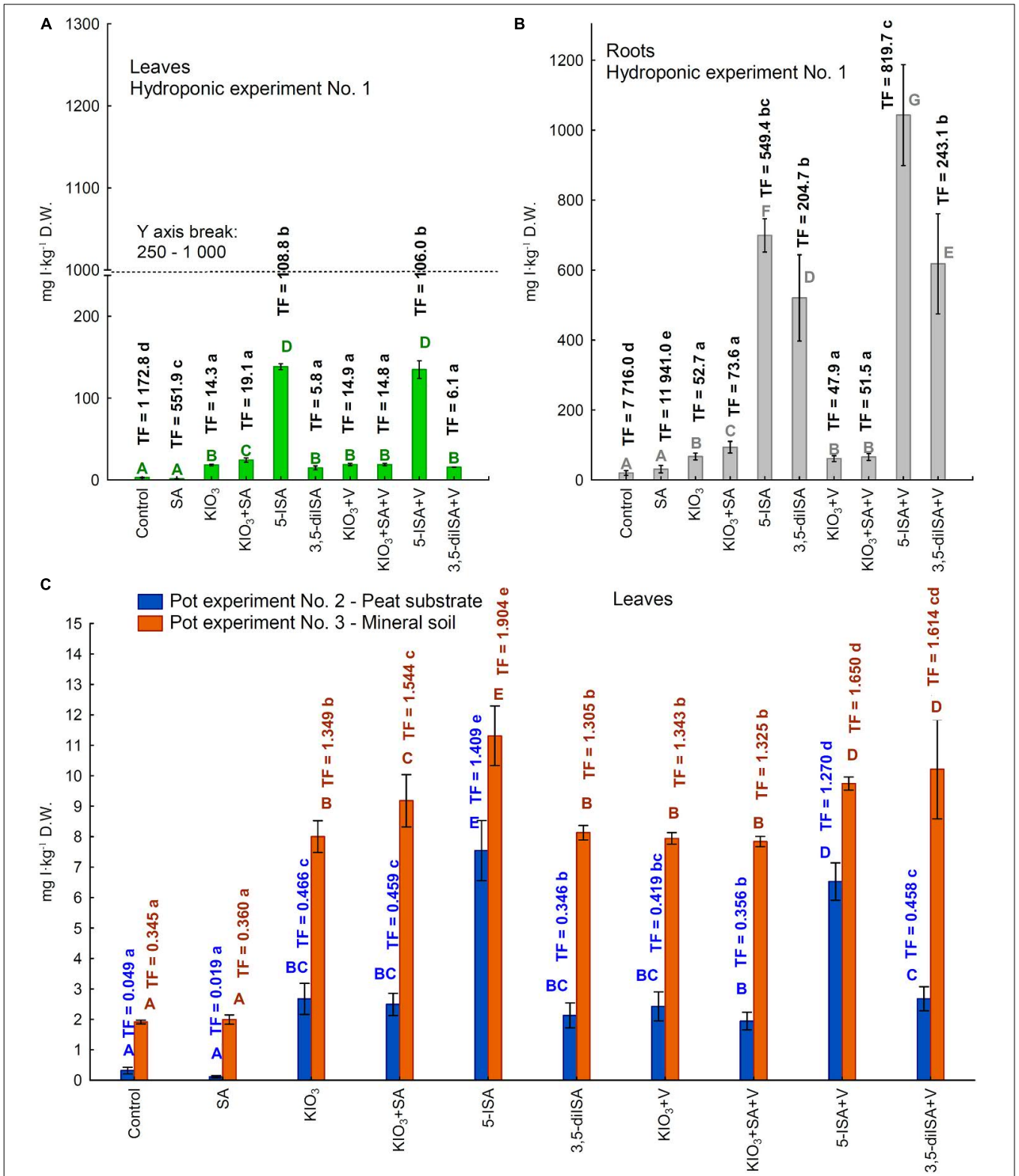

FIGURE 2 | Concentrations of iodine in leaves /heads/ **(A)** and roots of lettuce **(B)** cultivated in hydroponic NFT Experiment No. 1 as well as in leaves of plants cultivated in pot Experiment No. 2 and 3 **(C)**. Transfer factor /TF/ of iodine to roots and leaves /heads/ in the all experiments. Means followed by different letters for treatments, separately for each experiments differ significantly at $P < 0.05$. Color-coded capital letters for iodine content (green and gray for leaves and roots in Experiment 1 and blue and orange for leaves in Experiments 2 and 3); lowercase letters in the same colors for the TF factor in these experiments. Bars indicate standard error ($n = 8$).

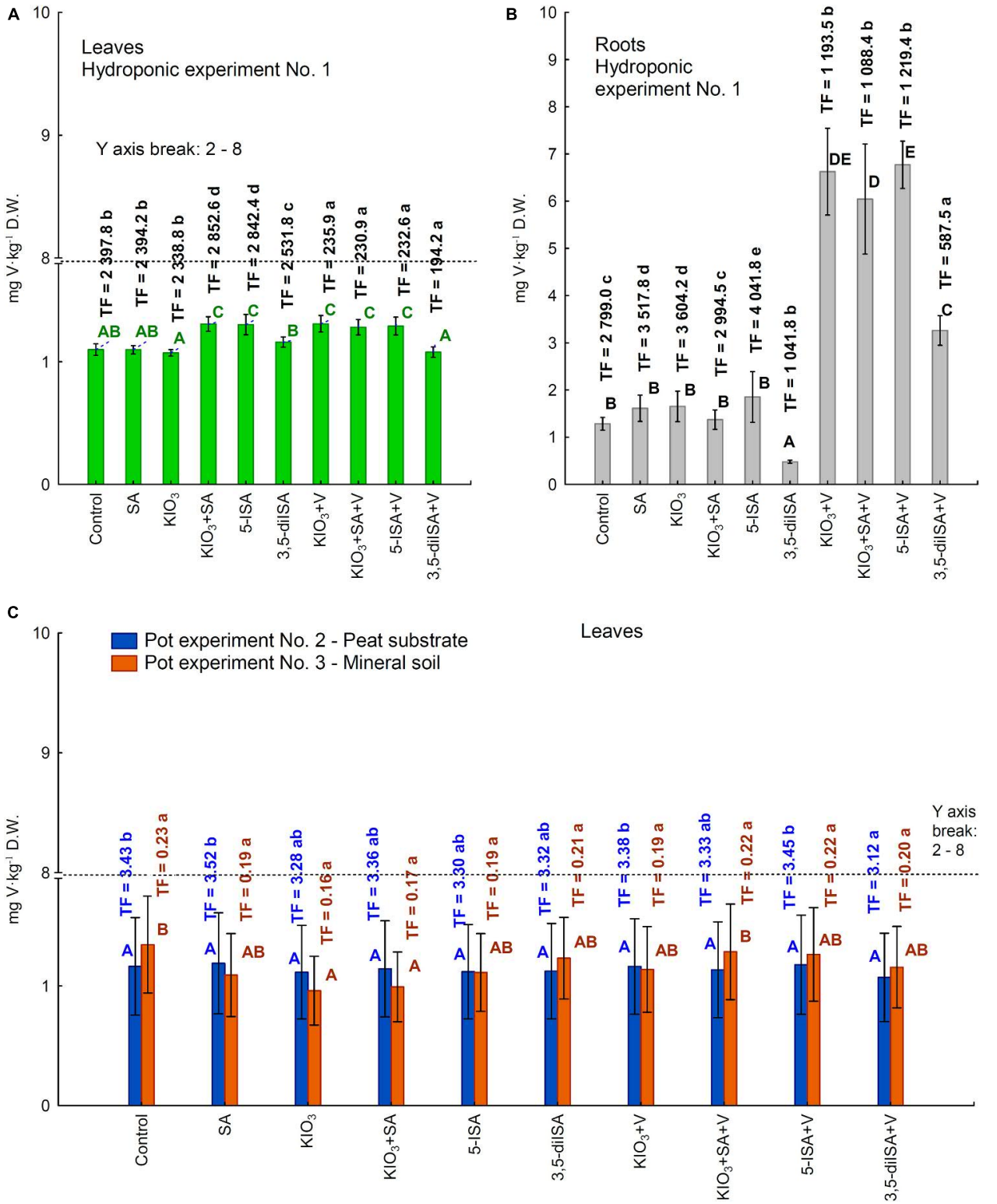

FIGURE 3 | Concentrations of vanadium in leaves /heads/**(A)** and roots of lettuce **(B)** cultivated in hydroponic NFT Experiment No. 1 as well as in leaves of plants cultivated in pot Experiment No. 2 and 3 **(C)**. Transfer factor /TF/ of vanadium to roots and leaves /heads/ in the all experiments. Means followed by different letters for treatments, separately for each experiments differ significantly at $P < 0.05$. Color-coded capital letters for vanadium content (green and gray for leaves and roots in Experiment 1 and blue and orange for leaves in Experiments 2 and 3); lowercase letters in the same colors for the TF factor in these experiments. Bars indicate standard error ($n = 8$).

3,5-diISA without V), only Experiment 3 with a peat substrate showed a significant increase in leaf I content, RDA-I%, HQ-iodine, and I uptake by a single lettuce head (**Supplementary Tables 2**, **3**).

In all three experiments, the calculated TFs for I uptake were significantly modified by application of the I compounds tested, as well as SA and V (**Figures 2A–C**). The TF values reflected I uptake by plants (I content in roots or leaves) depending on its availability in the rhizosphere, i.e., in the nutrient solution in the hydroponic system (Experiment 1) or mineral soil or peat substrate (Experiments 2 and 3).

Vanadium Uptake and Accumulation by Lettuce

In hydroponic Experiment 1, V was accumulated in larger amounts in roots than in leaves (**Figures 3A,B**). Fertilization with V combined with KIO_3, KIO_3+SA, and 5-ISA caused an approximately 4-fold increase in V content in roots compared with the control (**Figure 3B**). Following fertilization with 3,5-diISA without applying ammonium metavanadate, the content of V in roots was lower than in the control (**Figure 3B**); V uptake by roots of a single plant and by whole plants (roots + head) was consequently lower (**Supplementary Table 3**). Application of 3,5-diISA+V resulted in a significant increase in V content in roots and V uptake by roots of a single plant and by whole plants (roots + head) compared with 3,5-diISA without V. However, the increase in root V content and in V uptake by roots and whole plants was less effective for fertilization with 3,5-diISA+V than for KIO_3+V, KIO_3+SA+V, and 5-ISA+V than for 3,5-diISA, KIO_3, KIO_3+SA, and 5-ISA, respectively. This was confirmed by TF values in roots for the respective combinations with and without V fertilization.

Following the application of KIO_3+V, KIO_3+SA+V, and 5-ISA+V, the foliar content of V for hydroponic Experiment 1 was significantly higher than that for the control (**Figure 3A**); however, it was still at the same level as following the application of KIO_3+SA and 5-ISA without ammonium metavanadate. Additionally, in Experiment 1, the combination of KIO_3+V was the only one for which V uptake by a single head in Experiment 1 was significantly higher than that for KIO_3 without ammonium metavanadate (**Supplementary Table 3**).

In the two pot experiments with lettuce, foliar V content (**Figure 3C**) and V uptake by a single head (leaves from one plant) (**Supplementary Table 3**) were significantly lower than in the control but only for the KIO_3 and KIO_3+SA combination in Experiment 3. For the remaining combinations, the content of V and V uptake by a single head was the same as in the control in both pot experiments (**Figure 3C** and **Supplementary Table 3**).

In Experiment 3, the TF for foliar V was about 20-fold lower than in Experiment 2 (**Figure 3C**), which was due to lower V content in the peat substrate than in mineral soil (**Table 2**).

vHPO Activity in Lettuce

The activity of vHPO in roots (Experiment 1) and leaves of lettuce in all three experiments was significantly modified by the application of the compounds studied to the nutrient solution or substrate in the pots (**Figures 4A–C**). In hydroponic Experiment 1, the vHPO activity measured in roots was 2.5- to 4.0-fold higher than that in leaves (**Figures 4A,B**). Foliar activity of vHPO in all three experiments was in the range between 0.08 and 1.43 $U{\cdot}ng^{-1}$ protein.

The root activity of vHPO following the application of KIO_3+SA and 5-ISA (**Figure 4B**) in the hydroponic system was significantly higher than that in the control. These two combinations also produced a significantly higher foliar activity of vHPO than that in the control (**Figure 4A**). For all four combinations with ammonium metavanadate fertilization and 3,5-diISA (without V), the root activity of vHPO was lower than in the control, while foliar activity of vHPO was significantly higher than that in the control (**Figures 4A,B**). Furthermore, 3,5-diISA (vs. 3,5-diISA+V) was the only combination where the activity of vHPO in both roots and leaves was the same. Additional fertilization with V together with KIO_3, KIO_3+SA, and 5-ISA caused a significant increase in vHPO in leaves and a decrease in the roots compared with the application of these compounds without V.

In each of the three experiments, the highest vHPO activity was measured in leaves of plants representing different combinations: 3,5-diISA+V in Experiment 1 (1.27 $U{\cdot}ng^{-1}$ protein), 5-ISA+V in Experiment 2 (0.49 $U{\cdot}ng^{-1}$ protein), and 5-ISA+V in Experiment 3 (1.43 $U{\cdot}ng^{-1}$ protein) (**Figures 4A,C**). The lowest foliar activity of vHPO was identified in the control in Experiment 1 (0.21 $U{\cdot}ng^{-1}$ protein) and for SA in Experiments 2 and 3 (0.08 $U{\cdot}ng^{-1}$ protein in each experiment).

BeA, SA, and Iodine Metabolites in Secretions Collected as a Result of Root Pressure

The contents of I^-, IO_3^-, BeA, SA, 5-ISA, 2-IBeA, 4-IBeA, 2,3,5-triIBeA, I-Tyr, T3-Na, T3, and T4 measured in root secretions collected as a result of root pressure (**RootSec**) from plants in Experiment 1 differed significantly between the combinations studied (**Table 4**).

Compared with the control and SA application in all remaining combinations, I content is RootSec was markedly higher. The highest content of I^- was found after the application of 5-ISA (without V); it was 9.7-fold higher than when KIO_3 was applied to the nutrient solution alone (**Table 4**). Compared with the control, a significant increase of IO_3^- was observed in RootSec following the application of KIO_3, KIO_3+SA, 5-ISA+V, and 3,5-diISA+V, with the highest RootSec content of IO_3^- being observed for fertilization with KIO_3+SA.

3,5-diISA was detected in RootSec exclusively after the application of 3,5-diISA (with or without V). Additionally, the RootSec content of 5-ISA was on average 6.2-fold higher for these two combinations than for the control. However, the highest content of 5-ISA in RootSec (25- to 46-fold higher than in the control) was demonstrated following its application to the nutrient solution. Plants treated with exogenous 5-ISA were characterized by the highest content of T3 and T4 and the

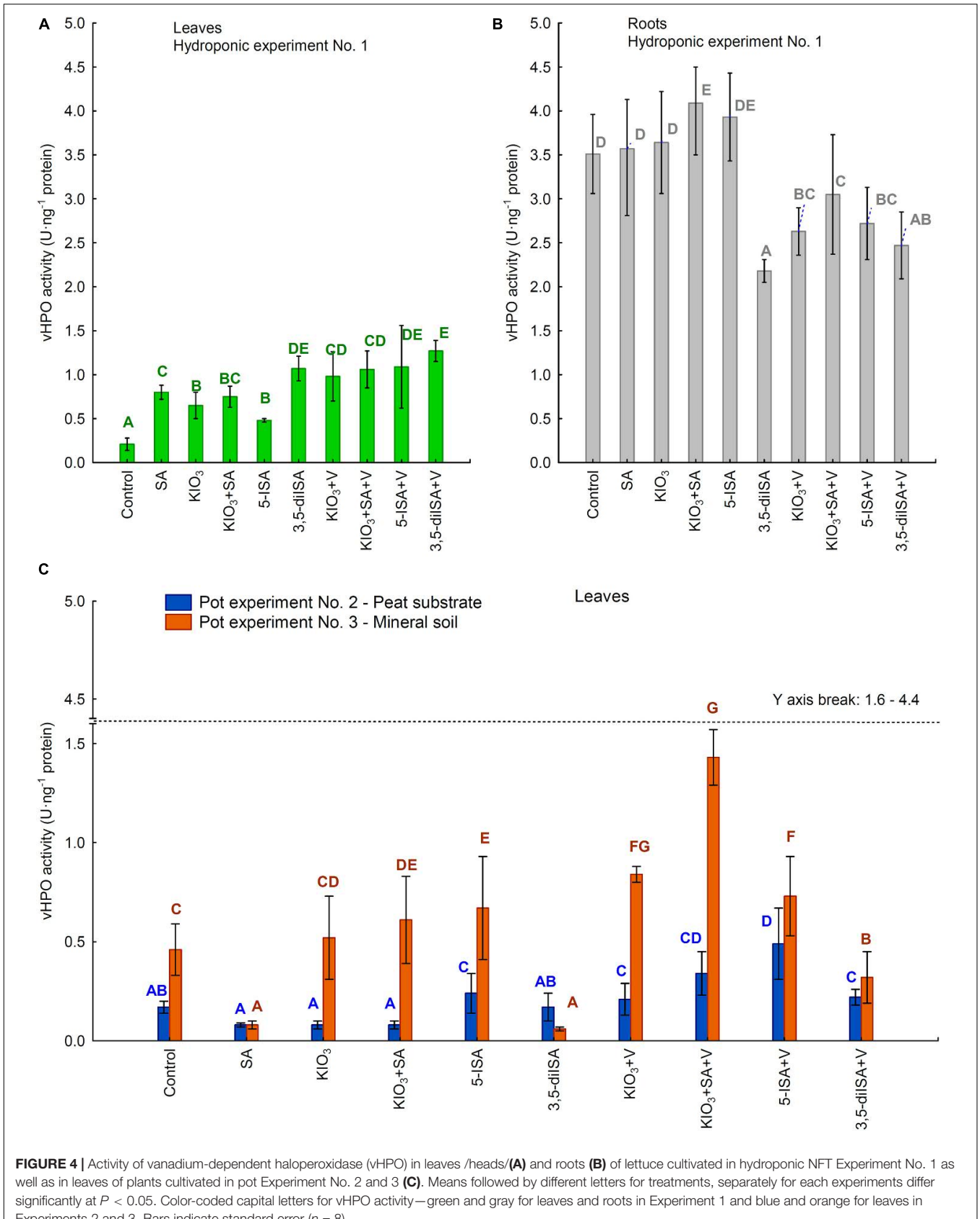

FIGURE 4 | Activity of vanadium-dependent haloperoxidase (vHPO) in leaves /heads/**(A)** and roots **(B)** of lettuce cultivated in hydroponic NFT Experiment No. 1 as well as in leaves of plants cultivated in pot Experiment No. 2 and 3 **(C)**. Means followed by different letters for treatments, separately for each experiments differ significantly at $P < 0.05$. Color-coded capital letters for vHPO activity—green and gray for leaves and roots in Experiment 1 and blue and orange for leaves in Experiments 2 and 3. Bars indicate standard error ($n = 8$).

lowest content of BeA and 2,3,5-triIBeA in RootSec. The RootSec content of T4 was equally high for fertilization using 3,5-diISA.

For all combinations with fertilization using ammonium metavanadate, application of the compound caused a reduction in T3 content in RootSec; the comparison encompassed combinations with KIO_3, KIO_3+SA, 3,5-diISA, 5-ISA without and with V (**Table 4**). The application of 5-ISA+V vs. 5-ISA (without V) had a reduced content of SA, 5-ISA, T3, and T4 and increased 2-IBeA in RootSec. When it comes to fertilization using 3,5-diISA+V vs. 3,5-diISA (without V), it caused a significant increase in BeA, 2-IBeA, and 4-IBeA and a decrease in the content of 5-ISA, T3, and T4 in RootSec.

Application of exogenous SA to the nutrient solution caused a significant increase in the RootSec content of 2,3,5-triIBeA compared with all the remaining combinations (**Table 4**).

Determination of Iodides (I^-) and Iodates (IO_3^-) in Roots and Leaves of Lettuce in a Hydroponic System (Experiment 1)

Roots and leaves had a higher content of iodides (I^-) than iodates (IO_3^-), from 12.5 times in roots for SA treatment to 53,750 times in leaves for 5-ISA+V treatment (**Table 5**). There were trace amounts of IO_3^-

TABLE 4 | Results of the determination of iodides, iodates, organic acids, and iodine metabolites in secretions collected as a result of root pressure (RootSec)—this is in white secretion on the surface of the root neck after cutting the heads (lettuce leaves).

Treatments	($\mu g \cdot dm^{-3}$)*				
	Iodides (I^-)	**Iodates (IO_3^-)**	**BeA**	**SA**	
Control	16.1 ± 0.25a	1.08 ± 0.07ab	68 ± 9.0b	638.6 ± 10.0b	
SA	10.4 ± 0.37a	0.74 ± 0.15ab	98 ± 0.6cd	214.6 ± 18.3a	
KIO_3	184.3 ± 3.69b	2.02 ± 0.05c	116 ± 9.9cde	2 163.0 ± 100.0c	
KIO_3+SA	215.2 ± 19.76bc	2.94 ± 0.71d	88 ± 10.0bcd	118.2 ± 18.9a	
5-ISA	1 789.9 ± 18.29e	0.70 ± 0.11ab	2 ± 1.0a	740.6 ± 4.0b	
3,5-diISA	291.2 ± 23.36c	0.67 ± 0.02ab	128 ± 10.0de	258.4 ± 51.1a	
KIO_3+V	203.5 ± 3.34b	0.28 ± 0.07a	10 ± 1.4a	106.6 ± 5.4a	
KIO_3+SA+V	169.8 ± 15.74b	0.65 ± 0.30ab	74 ± 9.8bc	104.3 ± 1.3a	
5-ISA+V	389.6 ± 70.51d	1.54 ± 0.06bc	132 ± 10.0de	254.0 ± 82.9a	
3,5-diISA+V	171.2 ± 10.99b	2.13 ± 0.32cd	138 ± 10.0e	221.3 ± 49.3a	
	5-ISA	**3,5-diISA**	**2-IBeA**	**4-IBeA**	
Control	8.4 ± 0.82a	<LOQ**	0.05 ± 0.01abc	0.04 ± 0.01a	
SA	8.3 ± 0.57a	<LOQ	0.15 ± 0.01d	0.19 ± 0.01c	
KIO_3	8.7 ± 0.73a	<LOQ	0.07 ± 0.01abc	0.10 ± 0.01abc	
KIO_3+SA	7.2 ± 0.24a	<LOQ	0.26 ± 0.03e	0.08 ± 0.01ab	
5-ISA	386.1 ± 11.45d	<LOQ	0.02 ± 0.01ab	0.06 ± 0.01ab	
3,5-diISA	58.6 ± 0.67b	63.9 ± 6.07a	0.01 ± 0.00a	0.06 ± 0.01ab	
KIO_3+V	7.4 ± 0.36a	<LOQ	0.08 ± 0.01bc	0.11 ± 0.03abc	
KIO_3+SA+V	7.7 ± 1.10a	<LOQ	0.06 ± 0.01abc	0.16 ± 0.02bc	
5-ISA+V	215.7 ± 97.09c	<LOQ	0.09 ± 0.02cd	0.08 ± 0.01ab	
3,5-diISA+V	46.2 ± 1.48b	83.6 ± 3.64b	0.10 ± 0.01cd	0.36 ± 0.05d	
	2,3,5-triIBeA	**I-Tyr iodotyrosine**	**T3-Na**	**T3**	**T4**
Control	0.25 ± 0.01a	0.52 ± 0.08bc	484.1 ± 40.5ab	2.81 ± 0.10d	0.22 ± 0.01ab
SA	2.67 ± 0.46b	0.56 ± 0.02bc	422.4 ± 41.6ab	1.31 ± 0.01b	1.83 ± 0.10cd
KIO_3	0.45 ± 0.10a	0.55 ± 0.06bc	746.7 ± 58.6c	2.84 ± 0.10d	0.01 ± 0.00a
KIO_3+SA	0.17 ± 0.07a	0.71 ± 0.06c	608.9 ± 77.5bc	3.46 ± 0.10e	0.64 ± 0.09b
5-ISA	0.01 ± 0.002a	0.52 ± 0.24bc	342.0 ± 3.4ab	5.00 ± 0.06f	4.78 ± 0.10h
3,5-diISA	0.09 ± 0.01a	0.16 ± 0.01ab	285.2 ± 36.1a	2.10 ± 0.10c	4.04 ± 0.15g
KIO_3+V	0.08 ± 0.02a	2.17 ± 0.10d	270.6 ± 60.5a	0.16 ± 0.01a	2.34 ± 0.21de
KIO_3+SA+V	0.08 ± 0.01a	2.26 ± 0.01d	409.7 ± 1.6ab	1.34 ± 0.10b	0.15 ± 0.01ab
5-ISA+V	0.17 ± 0.02a	0.50 ± 0.08bc	225.3 ± 2.0a	3.83 ± 0.10e	1.63 ± 0.10c
3,5-diISA+V	0.13 ± 0.02a	0.01 ± 0.00a	591.3 ± 109.0bc	1.16 ± 0.01b	3.14 ± 0.10f

**The content of the following compounds was analyzed: iodides (I^-), iodates (IO_3^-), benzoic acid (BeA), salicylic acid (SA), 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA), 2-iodobenzoic acid (2-IBeA), 4-iodobenzoic acid (4-IBeA), 2,3,5-triiodobenzoic acid (2,3,5-trilBeA), iodotyrosine (I-Tyr), sodium salt triiodothyronine (T3-Na), triiodothyronine (T3), and thyroxine (T4).*

***Limit of quantification (LOQ) for 3,5-diISA was 2.748.*

TABLE 5 | Concentrations of iodides (I^-) and iodates (IO_3^-) in roots and leaves of lettuce in hydroponics NFT Experiment No. 1 (speciation of iodine analyzed HPLC-ICP-MS/MS) as well percentage of the iodides and iodates in relation to the total iodine.

Experiment No. 1. Hydroponic NFT/Part of plant	Treatments	(mg·kg^{-1} D.W.)			Percentage of the sum of iodides and iodates in relation to the total I content* (%)	Percentage of the sum of analyzed organic-I** in relation to the total I content* (%)	Theoretical percentage share of the content of other unanalyzed organic or inorganic I compounds in the total I content (%)
		Iodides (I^-)	Iodates (IO_3^-)	The sum of two forms of I: I^- and IO_3^-			
Leaves	Control	2.34 ± 0.005b	0.0272 ± 0.0008d	2.365 ± 0.005b	57.7 ± 0.80c	3.26 ± 0.11d	39.0
	SA	0.97 ± 0.006a	0.0293 ± 0.0009d	0.998 ± 0.006a	56.3 ± 0.53c	2.39 ± 0.26c	41.4
	KIO_3	15.06 ± 0.042f	0.0146 ± 0.0006b	15.078 ± 0.042f	73.4 ± 0.64f	0.71 ± 0.05b	25.9
	KIO_3+SA	17.49 ± 0.140g	0.0139 ± 0.0001b	17.504 ± 0.140g	56.0 ± 0.33c	0.16 ± 0.02a	43.9
	5-ISA	97.06 ± 0.189i	0.0488 ± 0.0006f	97.113 ± 0.189i	67.6 ± 1.77e	0.25 ± 0.01ab	32.1
	3,5-diISA	8.63 ± 0.044d	0.0002 ± 0.0001a	8.625 ± 0.044d	5.3 ± 0.60a	0.75 ± 0.01b	93.9
	KIO_3+V	14.12 ± 0.041e	0.0215 ± 0.0004c	14.143 ± 0.041e	63.1 ± 0.36d	0.30 ± 0.06ab	36.6
	KIO_3+SA+V	18.92 ± 0.229h	0.0023 ± 0.0001a	18.918 ± 0.229h	84.1 ± 1.20h	0.66 ± 0.01ab	15.3
	5-ISA+V	118.25 ± 0.623j	0.0022 ± 0.0001a	118.252 ± 0.624j	72.4 ± 0.12f	0.31 ± 0.01ab	27.3
	3,5-diISA+V	3.46 ± 0.009c	0.0428 ± 0.0017e	3.507 ± 0.010c	23.6 ± 0.20b	7.01 ± 0.12f	69.4
Roots	Control	7.70 ± 0.0187b	0.8780 ± 0.0007e	8.577 ± 0.019b	22.7 ± 0.32g	2.41 ± 0.04	74.9
	SA	0.16 ± 0.0025a	0.0137 ± 0.0001a	0.169 ± 0.002a	0.3 ± 0.01a	0.70 ± 0.02	99.0
	KIO_3	10.87 ± 0.0131c	0.9914 ± 0.0006ef	11.866 ± 0.013c	12.8 ± 0.12d	0.55 ± 0.05	86.7
	KIO_3+SA	34.99 ± 0.4050e	0.2436 ± 0.0037d	35.235 ± 0.408e	25.6 ± 0.24h	0.40 ± 0.04	74.0
	5-ISA	72.58 ± 0.0764f	1.7800 ± 0.0428g	80.364 ± 0.119f	14.0 ± 0.23e	0.86 ± 0.03	85.1
	3,5-diISA	8.12 ± 0.0183b	0.9589 ± 0.0005f	9.080 ± 0.018b	1.1 ± 0.01ab	9.18 ± 0.10	89.8
	KIO_3+V	13.53 ± 0.0426d	0.0531 ± 0.0004ab	13.583 ± 0.042d	16.4 ± 0.15f	1.32 ± 0.06	82.3
	KIO_3+SA+V	11.27 ± 0.0564c	0.1136 ± 0.0009c	11.382 ± 0.057c	12.5 ± 0.23d	2.10 ± 0.06	85.4
	5-ISA+V	119.26 ± 1.6836g	0.9511 ± 0.0266ef	120.212 ± 1.710g	6.2 ± 0.08c	0.44 ± 0.00	93.4
	3,5-diISA+V	11.59 ± 0.0606c	0.0695 ± 0.0003bc	11.661 ± 0.060c	1.2 ± 0.01b	7.99 ± 0.14	90.8

Total I content determinate after TMAH extraction (see **Figure 1).*

***Organic-I—I in all organic I compounds analyzed in roots and leaves of lettuce from hydroponic Experiment No. 1 (see **Supplementary Table 4**, 5). Sum content of I in form: 5-ISA, 3,5-diISA, I-Tyr, T3-Na, T3, 2-IBeA, 4-IBeA, 2,3,5-triIBeA in relation to the total I content.*

Means in the column followed by different letters separately for each experiments differ significantly at $P < 0.05$.

in leaves and roots in all combinations subjected to analysis. Even in roots and leaves of plants fertilized with KIO_3, the content of IO_3^- was lower than or similar to the control.

The lowest root and leaf content of I^- (lower than in the control) was detected in plants treated with exogenous SA (**Table 5**). The highest content of I^- in roots and leaves was found in plants treated with 5-ISA+V; it was significantly higher than in combinations where 5-ISA was used alone.

A comparison of combinations with I applied to the nutrient solution shows the following quantitative root content of I^- (**Table 5**): 5-ISA+V > 5-ISA > KIO_3+SA > KIO_3+V > 3,5-diISA+V = KIO_3+SA+V = KIO_3 > 3,5-diISA. The quantitative content of I^- in leaves was as follows: 5-ISA+V > 5-ISA > KIO_3+SA+V > KIO_3+SA > KIO_3 > KIO_3+V > 3,5-diISA > 3,5-diISA+V.

An additional application of V together with KIO_3, KIO_3+SA, 5-ISA, and 3,5-diISA in each of these four combinations had a different impact on the I^- content in leaves and roots (**Table 5**) and resulted in an increase or decrease in I^- content in leaves and roots, compared with application of exogenous I compounds without V.

The percentage of the sum of iodides (I^-) and iodates (IO_3^-) in relation to total I content was within the range from 0.3 for SA to 25.6 for KIO_3+SA in roots, and from 5.3 for 3,5-diISA to 84.1 for KIO_3+SA+V in leaves (**Table 5**).

In the supporting information files were included the results of content of BeA, SA, iodine metabolites in roots and leaves (**Supplementary Tables 4**, **5**) as well as content of I and V in soil after lettuce cultivation (**Supplementary Table 6**).

DISCUSSION

The aim of research on I biofortification of plants is to establish biofortification regimens, define the threshold of I toxicity for plants, and optimize I biofortification

of plants to make it safe and adequate to consumers' needs (Lawson et al., 2016). Research on I biofortification must be connected with research targeted at expanding knowledge on biochemical, physiologic, and molecular aspects of the functions of trace elements in plants (White and Broadley, 2009).

Plant Biomass and Iodine Biofortification Efficiency Depending on the Chemical Form of Iodine, Vanadium Application, and Type of Cultivation

The dose of I in the hydroponic system was 37.5-fold higher than that in both pot experiments. In consequence, the I compounds (particularly as 5-ISA and secondarily as 3,5-diISA) were applied at a concentration that was too high, negatively impacting plants (reduced yield) in hydroponic Experiment 1. Smoleń et al. (2017) showed that in the hydroponic system, 5-ISA was toxic to lettuce when applied at a dose of 40.0 μM I; the symptoms were not reported after application of 5-ISA at a dose of 1.6 and 8.0 μM I. The authors did not conduct research on 3,5-diISA. Based on the results of a study by Smoleń et al. (2017) and the reduced lettuce biomass shown in this study (Experiment 1), we presume that the threshold of transition from harmful to toxic I activity in lettuce is somewhere between the dose of 8.0 μM and 10.0 μM of I applied as 5-ISA. Because only one dose of 3,5-diISA was tested, it was impossible to establish an exact threshold of harmfulness/toxicity of exogenous 3,5-diISA on lettuce, as was done for 5-ISA.

Welch and Huffman (1973) did not report a significant impact of 1 μM V (as NH_4VO_3) on the yield of lettuce or tomato compared with the control without V fertilization, for doses ranging from 0.05 to 0.40 μM V. In the three experiments described in the publication, simultaneous fertilization with KIO_3, 5-ISA, and 3,5-diISA plus V at a dose of 0.1 μM V (vs. no V) had no negative impact on the biomass of lettuce.

The highest efficacy of I biofortification on lettuce following the application of 5-ISA (in each of the three experiments) compared with 3,5-diISA and KIO_3 was justified in research by Smoleń et al. (2017). The authors demonstrated that 5-ISA at a dose of 8.0 μM I was enough to achieve a similar effect of I biofortification in lettuce leaves, as in the case of using KIO_3 at a dose of 40.0 μM I. In Experiment 1, the dose of 5-ISA (or, to a lesser extent, 3,5-diISA and KIO_3) was too high in the context of a need to balance I content in the daily diet of consumers. This is indicated by the RDA for I (%) in a 100 g portion of fresh lettuce leaves $>$ 480% and HQ $>$ 0.66; the compound would be harmful to consumers if HQ exceeded 1.0. The two iodosalicylates mentioned above, as well as 2-IBeA, 4-IBeA, 2,3,5-triIBeA, and I-Tyr, and T3 were naturally synthesized in lettuce, which further confirms the results of previous studies on lettuce (Smoleń et al., 2020). Halka et al. (2019) showed that these organic I compounds were present in tomato fruits and willow bark. The problem with defining a "target range" for I biofortification of lettuce with non-organic I compounds (KI and KIO_3), as well as the determination of human demand of I and estimations of lettuce consumption by the general population were addressed in a study by Lawson et al. (2016). Vegetables enriched with KI and KIO_3 have been found to be safe both for humans (Tonacchera et al., 2013) and laboratory rats (Piątkowska et al., 2016). In our studies, exogenous 5-ISA and 3,5-diISA caused a significantly higher accumulation of these compounds in leaves and roots. This information may provoke questions on consumer safety where iodosalicylates are used for I enrichment of plants. There is no direct data on the effect of exogenous iodosalicylates used in vegetable growth on animals or humans. We presume that exogenous iodosalicylates may increase health-promoting effects of domesticated plants. This is because the fertilization of plants using inorganic KI or KIO_3 compounds increases synthesis and accumulation of organic I metabolites in plants, as confirmed in research on lettuce (Smoleń et al., 2020) and tomato (Halka et al., 2019). Even though organic I metabolites, including T3, are present in marine algae in much higher amounts than in lettuce (Fenical, 1975), marine algae are still consumed by a number of people worldwide (González et al., 2019).

In hydroponic Experiment 1 with lettuce, I enrichment of plants was higher than in both pot experiments. This was probably because I taken up directly from the nutrient solution was more readily available to roots and because the dose of I per plant was higher than in Experiments 2 and 3. The effectiveness of I biofortification in plants is much higher in hydroponic and soilless systems than when soil fertilization is used (Blasco et al., 2008). This is due to high I sorption by soil, a phenomenon that is absent in hydroponic nutrient solutions. Iodine sorption in soil is attributable to the mineral fractions and SOM (Kashparov et al., 2005), especially the humified aromatic ring of organic matter but not fresh organic matter (Schlegel et al., 2006). After SOM, the following compounds also participate in I sorption by mineral soils: hydroxides Fe/Al (Yoshida et al., 1992), Cu(I)-Fe (III)-sulfides and Cu(I)-sulfides (Lefèvre et al., 2003), as well as Cu/Cr and Cu/Al (Pless et al., 2007). Additionally, I desorption by soil is very slow, which inhibits I uptake by roots (Dai et al., 2004). The SOM also contains SA and its derivatives (Hue et al., 1986). Molecular I or its non-organic anions in the soil may react with aromatic rings of compounds included in SOM (Yamada et al., 1996). Endogenous iodosalicylates and iodobenzoates were identified in the soil prior to lettuce cultivation. The peat substrate was richer in BeA, SA, 5-ISA, and 2-IBeA than mineral soil. In both pot experiments, lettuce heads grown in the peat substrate accumulated less I than those grown in mineral soil. The sorption of I anions (IO_3^-) by organic soils was higher than in mineral soils (Yamaguchi et al., 2005). This has also been confirmed by the results of analyses of the peat substrate and mineral soil after lettuce cultivation. Post-cultivation I content in the peat substrate was on average 1.5-fold higher than in mineral soil. The highest efficacy of I enrichment of lettuce following the application of 5-ISA in the peat substrate and mineral soil may have resulted from the low degradation or conversion of low-molecular-weight organic aromatic I compounds in soil, which then may be taken up by roots.

Relative Expression of Analyzed Genes vs. Iodine Uptake. Iodine Metabolism in Lettuce

The absence of impact or insignificant increase in V content in leaves following ammonium metavanadate fertilization reported in the three experiments was also confirmed in the literature. When V is applied through a soil fertilizer or nutrient solution, it accumulates in roots and its transport to aboveground parts of the plants is very limited. This was also reported for the cultivation of tomato, Chinese green mustard (Vachirapatama et al., 2011), soybean (Kaplan et al., 1990), rice (Chongkid et al., 2007), and lettuce (Gil et al., 1995). Increased V transfer to the aboveground parts of plants is possible if high concentrations of the element, which are potentially toxic to plants, are used in fertilization (Chongkid et al., 2007; Vachirapatama et al., 2011).

Peroxidases are linked to a number of physiological functions. These include the removal of H_2O_2, oxidation of toxic reductants, biosynthesis and degradation of lignin, and participation in many other biochemical processes (additional descriptions in **Supplementary Data 1**). The ion Ca^{2+} has been described as a cofactor for peroxidase (Pandey et al., 2017). Enzymes from the group of V-dependent haloperoxidases (vHPO) contain the bare metal oxide vanadate, as a prosthetic group (Wever and Hemrika, 2001) (see also **Supplementary Data 1**). In the presence of H_2O_2, they oxidize halides (I, Be, Cl) in the following reaction: $H_2O_2 + X^- + H^+ \rightarrow H_2O + HOX$, where X represents Cl^-, Br^-, or I^- (Wever and Hemrika, 2001; Leblanc et al., 2006). The function of vHPO is well-described for marine algae (Leblanc et al., 2006; Verhaeghe et al., 2008). In marine algae, the vHPO enzyme plays a dual function. It can participate in the process of I uptake into cells and is involved in the process of I excretion from cells to the environment in the form of I_2 (Leblanc et al., 2006).

An additional application of V with different I compounds and SA had no definitive impact on the expression of *per64-like*, *per12-like*, *samdmt*, *cipk6*, or *msams5* in either roots or leaves. *per64-like* was the only gene whose expression decreased in the roots of plants treated with KIO_3+SA+V, 5-ISA+V, and 3,5-diISA+V (in nutrient solution) when compared to the application of the same compounds without V. For these very same combinations (with and without V), the root activity of vHPO was reported to decrease. Therefore, the level of expression of the *per64-like* gene was correlated with the activity of vHPO in roots. The decreased activity of vHPO was accompanied by a lower expression of *per64-like* in plants treated with KIO_3+SA+V, 5-ISA+V, and 3,5-diISA+V. These results are sufficient to assign vHPO-like activity to an enzyme encoded by *per64*-like, rather than the one encoded by *per12-like*. Perhaps peroxidase encoded by *per64-like* may have vHPO-like function (may be a V-dependent enzyme). The results of pairwise alignment of protein sequences of *A. thaliana* PER12 and PER64 with vIPO1 *L. digitata* and vBPO *C. officinalis* and *A. nodosum* showed common regions between them (see also **Supplementary Data 1**). Further *in-silico* research is needed to this end or research directed at isolating PER64-like enzyme to be able to examine its structure and functionality depending on the application of V.

Colin et al. (2005) proved that the *in-vitro* activity of vHPO isolated from *Laminaria digitata* grew intensively within the range 0–10 mM KI and dropped suddenly when KI > 20 mM was used. In the three experiments conducted as part of our study, exogenous KIO_3, 5-ISA, and 3,5-diISA (applied without V) had a different effect on root and foliar activity of vHPO. Before its uptake by roots or immediately thereafter, IO_3^- must be reduced to I^- (Kato et al., 2013). Surprisingly, there was no significant difference in vHPO activity in the roots of plants treated with KIO_3 alone (Experiment 1) compared with the control, despite a simultaneous 2.6-fold increase in the expression of the *per64-like* gene. The results indirectly indicate that other molecular and biochemical mechanisms than vHPO must be involved in the process of root uptake and transport of I^- produced following IO_3^- reduction. These mechanisms are probably responsible for chloride transport. The transport of I^- within root cells and to the xylem is analogous to the translocation of Cl^- ions and takes the form of symport (H^+/anion) or antiport (Na:K/Cl) or is effected through I channels that are permeable to Cl^-/I^- (White and Broadley, 2001; Roberts, 2006; Colmenero-Flores et al., 2007).

The three experiments share the observation that none of the tested I compounds silenced foliar activity of vHPO. 3,5-diISA was the only compound that reduced the activity of vHPO in roots, an observation consistent with Smoleń et al. (2020). This may be because of the specific effect of 3,5-diISA on vHPO, which inhibited the activity of the enzyme (already at a dose of 10 μM; or 20 μM I) but did not suppress the expression of the *per64-like* gene, which has been linked with vHPO-like functions. Notably, lower expression of *per64-like* was reported in the roots of plants treated with 3,5-diISA but only compared with KIO_3+SA and 5-ISA, with a simultaneous increase in gene expression in comparison with the control. 5-ISA increased the foliar activity of vHPO in all three experiments compared with the control, which translated into the highest I accumulation in lettuce leaves. The potential mechanism most likely to stimulate foliar activity of vHPO through 5-ISA and 3,5-diISA is not fully understood. It could be a result of catabolism in both iodosalicylates to I^-, as only in this form could I be taken up intracellularly with the aid of vHPO in a mechanism resembling the one described for marine algae by Leblanc et al. (2006).

The activity of vHPO and expression of *per64-like* in leaves and roots were determined at the final stage of cultivation (shortly before lettuce harvesting), which means that the measurements were made after the plants have been exposed to exogenous V and I compounds for an extended time. While theoretically it may seem that V fertilization should increase the activity of vHPO in plants, due to the higher availability of the enzyme's cofactor, lettuce fertilization with V caused decreased activity of vHPO and expression of the *per64-like* gene. Reduction in vHPO activity following test fertilization with I compounds + V is consistent with the previous findings of Smoleń et al. (2020). The authors showed that fertilization with V without the simultaneous use of I (plant cultivation with trace amounts of I in the nutrient solution) significantly enhanced the activity of vHPO. Given these results, the assignment of a vHPO-like function to the protein encoded by *per64-like* seems to be substantively justified.

Fertilization with V did not cause an increase in foliar V content in any of the experiments conducted as part of this research (except for KIO_3+V vs. KIO_3 in Experiment 1). However, a considerable increase in root V content following V fertilization was observed. The results were consistent with the literature. V fertilization of Chinese greens, at a dose of 0.39, 0.79, and 1.57 mM, caused a proportional increase in V content in the plants, with the following preserved concentration gradient: roots > stems > leaves system (Vachirapatama et al., 2011). A similar V concentration gradient was obtained by Akoumianaki-Ioannidou et al. (2016) for sweet basil fertilized with NH_4VO_3. The rate of transfer of V from roots to leaves can only be increased if it is used at very high doses, which may be harmful to plants. The negative effect of V on plants also depends on its chemical form. Findings for soybean showed that a harmful dose of $VOSO_4$ was 1.2 mM V (Kaplan et al., 1990). A dose of 0.39 mM V was reported to be harmful for rice (Chongkid et al., 2007). The adverse effects of V may include root darkening, decreased number of secondary roots, decreased turgor pressure, loss of leaf firmness, and plastid degradation in plants (Gil et al., 1995). In Experiment 1, we did not observe any negative impact of V on the development of roots, which was demonstrated by the biomass obtained from the roots of one of the plants.

Expression of *cipk6* Gene

The available literature describes the likely biochemical mechanisms of PDTHA activity in plants by comparing their function to thyroid hormones (T3 or T4) in humans. Eneqvist et al. (2003) stated that higher plants can produce a protein homologous to human transthyretin, which is responsible for T3 and T4 transport (TransThy-T3/T4trans). Additionally, the TransThy-T3/T4trans protein from higher plants, including *A. thaliana*, tomato, and potato was more closely related in the phylogenetic tree of the transthyretin protein family to the protein found in *Homo sapiens* than to TransThy-T3/T4trans from bacteria or fungi. In model research, Pessoa et al. (2010) showed that exogenous T4 can be bound by transthyretin-like protein in *A. thaliana*. Furthermore, Power et al. (2000) showed that transthyretin in humans belongs to the group of proteins that includes thyroxine-binding globulin and albumin. The chemical bond is responsible for transporting thyroid hormones in blood. Davis et al. (2000) showed that in humans, T4 can activate signal transduction proteins, such as mitogen-activated protein kinase (MAPK). Additionally, T4 can enhance the activity of several nuclear transactivator proteins, through serine phosphorylation by MAPK. A similar complex mechanism may exist in lettuce. Findings show that the protein encoded by *cipk6* can likely perform the function of a T3 and/or T4 receptor (see the functional annotation of the *cipk6* gene in **Supplementary Data 2**). This assumption is justified by results of chemical analyses of roots and RootSec. The synthesis of T3 and T4 (and possibly other isomers of these PDTHAs) probably occurs, with the participation of iodobenzoates and/or iodosalicylates (5-ISA and 3,5-diISA) as the substrates of PDTHA (**Figures 5**, **6**). The results showed that the process occurs primarily in roots. T3 and T4 are then transported to the aboveground parts of plants (**Figures 5**, **6**). The observation of root synthesis of T3 are consistent with the outcome of our previous study (Smoleń et al., 2020). In the present study, we measured T4 directly in leaves and roots. The key problem with T3 and T4 measurements in plant tissues is that there are no analytical protocols dedicated to the analysis of PDTHA content in plants. However, the content of T4 was measured directly in RootSec. Therefore, it is important that we postulate the need to search for and elaborate on the optimum methods or analytical procedures that would enable measurements of the total T4/T3 and other PDTHAs in the tissue of plants. We believe that the results of our analyses of non-organic and organic I metabolites (particularly T3 and T4) in roots, RootSec, and leaves, provide grounds to assign the function of a T3 and/or T4 receptor in lettuce to a protein encoded by *cipk6*. For *cipk6*, a significant correlation coefficient was reported between its expression vs.: 1) the content of T3 (r = 0.30*), 5-ISA (r = 0.72*), and total content of I (r = 0.84*) directly in roots and 2) the content of T3 (r = *0.60**), T4 (r = *0.58**), 5-ISA (r = 0.89*), and total content of I (r = 0.89*) in RootSec. A statistically significant correlation between the expression of *cipk6* and total I content (r = 0.26*) was found in leaves; however, no correlation was reported between the expression of *cipk6* and the content of T3 or 5-ISA. This led us to the conclusion that the root activity of *cipk6* is closely related to the presence of PDTHA. The effect of PDTHA on *cipk6* gene expression in leaves was smaller. This may be proof that the function of the dominant PDTHA receptor in aboveground parts of plants may be performed by a protein encoded by another gene/group of genes. However, the results of research presented herein are insufficient to thoroughly describe the physiological function of PDTHA in lettuce.

The highest expression of *cipk6* in roots and leaves of plants treated with 5-ISA was unequivocally associated with I uptake and metabolism in these plants. The plants were found to demonstrate the highest I-uptake and total I content, and had increased 5-ISA, 2-IBeA, 3,5-diISA, I^-, and IO_3^- levels in roots and leaves. In addition, RootSec from plants where 5-ISA had been applied were also found to have the highest content of 5-ISA and I metabolites, such as I^-, T3, and T4. The results clearly showed that there is a close interdependence between a larger preference to take up 5-ISA (compared with IO_3^- and 3,5-diISA) and the metabolism of I compounds which, among others, induces the synthesis of PDTHA and increases the expression of *cipk6*, a gene of the T3 and/or T4 receptor.

Expression of the *msams5* Gene

The functions of S-adenosyl-l-methionine (SAM)-dependent halide methyltransferase (HMT) or SAM-dependent halide/thiol methyltransferase (HTMT), enzymes responsible for I volatilization to CH_3I or CH_2I_2, are subject to a complex control mechanism and are typical of a number of marine algae species (Keng et al., 2020) and terrestrial plants (Attieh et al., 2000; Nagatoshi and Nakamura, 2007; Itoh et al., 2009; Landini et al., 2012). These enzymes are not specific for I as a substrate but can also participate in the methylation of other group-17 elements (halogens) from the periodic table. A different, complicated affinity in the substrate-enzyme system is as follows: $I^- > Br^- > Cl^-$ (Manley, 2002; Murphy, 2003). In *B. oleracea*,

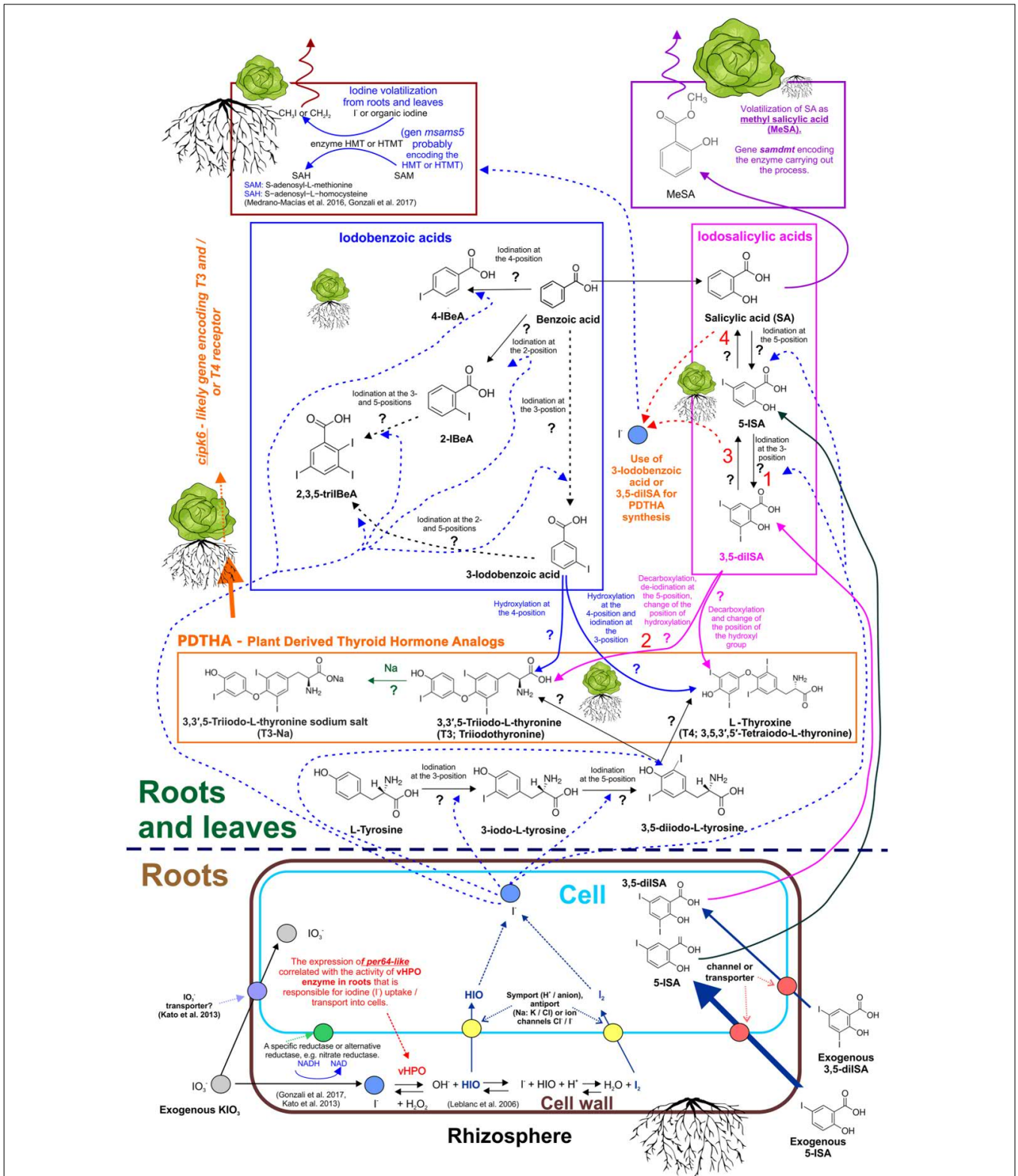

FIGURE 5 | Mechanism of uptake of non-organic and organic iodine compounds. Theoretical metabolic pathway of iodosalicylates, iodobenzoates, and plant-derived thyroid hormone analogs (PDTHA) in lettuce—summary of the study and literature data. 1 and 2—Processes that mainly occur in roots. Foliar activity intensifies after application of 5-ISA and 3,5-diISA. 3—Deiodination of 3,5-diISA. Observed increased content of 5-ISA following exogenous application of 3,5-diISA. 4—Deiodination of 5-diSA. Observed increased content of SA following exogenous application of 5-diSA and 3,5-diISA. **?**—Undefined enzymatic/metabolic processes that carry out these reactions.

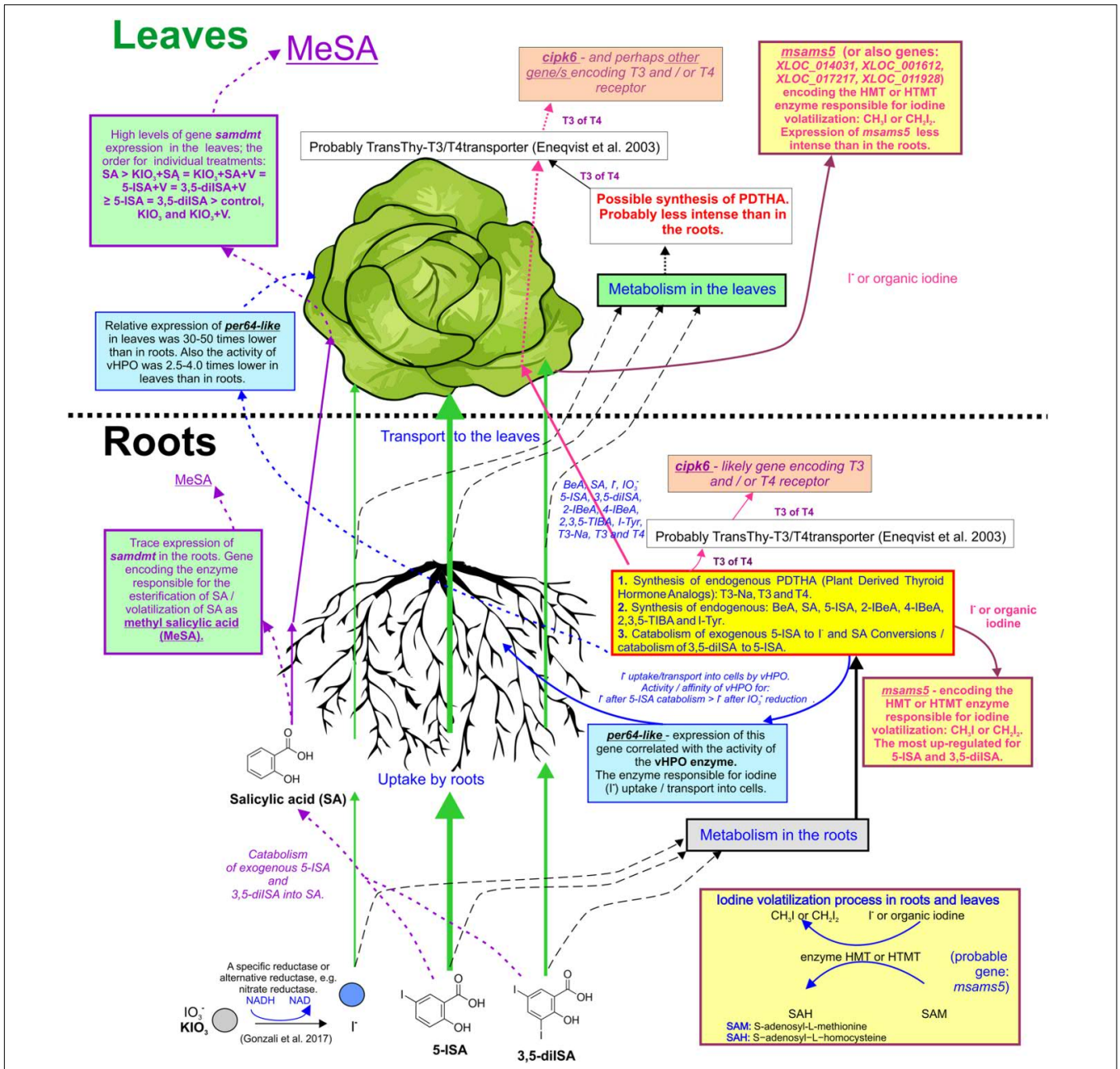

FIGURE 6 | Graphic summary of study outcomes: uptake, transport, and metabolic pathways of iodine compounds and SA. Genes with described activity and function: *Peroxidase 64-like (per64-like)*: the gene's activity has been linked to the activity/functionality of V-dependent haloperoxidase (vHPO) in roots and leaves of lettuce. *Peroxidase 12-like (per12-like)*: no correlation has been found between the gene's expression and vHPO activity in roots. *S-adenosyl-L-methionine-dependent methyltransferase (samdmt)*: gene that possibly encodes an enzyme that conducts the process of esterification/volatilization of methyl salicylic acid (MeSA). *CBL-interacting serine/threonine-protein kinase 6 (cipk6):* gene that likely encodes triiodothyronine (T3) and/or thyroxine (T4) receptors. *S-adenosylmethionine synthase 5 (msams5):* gene that possibly encodes an enzyme responsible for I volatilization in the process of methylation (synthesis of CH_3I or CH_2I_2).

HTMT was also involved in the process of methylation of [SH]- and [SCN]- groups to CH_3SH (Attieh et al., 2000).

The available literature does not name the specific gene(s) responsible for I methylation in lettuce. The results justify the assignment of the potential HMT or HTMT function to an enzyme, S-adenosylmethionine synthase 5, encoded by the *msams5* gene. Characteristics of the *L. sativa* MSAMS5 protein are shown in **Supplementary Data 3**. Significant overexpression (about 8.5-fold higher than the control) of the *msams5* gene in the roots of plants treated with exogenous 5-ISA was detected. These results and, indirectly, the total root content of I and iodine metabolites show that a protein of the

msams5-encoded enzyme may be immediately associated with I methylation by lettuce roots. Iodine volatilization through roots (with the participation of *msams5*-encoded enzyme) was higher following the application of iodosalicylates than when using KIO_3. This indicates that the enzyme protein encoded by *msams5* might have played a dominant role in I methylation in roots but not in leaves. Compared with the control, foliar expression of *msams5* was slightly, yet significantly, increased only following the application of KIO_3. Therefore, foliar expression of the *msams5* gene did not reflect the reported accumulation of I or its organic or non-organic compounds. The process of methylation (volatilization) of gaseous I from leaves probably occurred with the participation of the enzyme(s) encoded by a gene(s) other than *msams5*. On the basis of the transcriptome analysis (Kęska et al., 2019), we were able to identify other genes in lettuce leaves that may potentially be associated with synthesis of enzymes with HMT- or HTMT-like function, i.e., with the synthesis of CH_3I or CH_2I_2. The list includes the following genes described in the genome of lettuce: *S-adenosylmethionine synthase (XLOC_014031)* (Lsat_1_v5_gn_6_117861.1), *lysine-specific demethylase REF6 methyltransferase (XLOC_001612)* (Lsat_1_v5_gn_1_28820.1), *probable methyltransferase At1g27930 (XLOC_017217)* (Lsat_1_v5_gn_8_148061.1), and *histone-lysine N-methyltransferase (XLOC_011928)* (Lsat_1_v5_gn_5_154240.1).

Notably, *in vitro* emission of CH_3I, CH_3Br, and CH_3Cl by rice depended on the stadium of the plants' development (Redeker et al., 2004). Secretion of CH_3I by rice during the day was nearly twice that observed at night (Muramatsu and Yoshida, 1995; Muramatsu et al., 1995). Gonzali et al. (2017) suggested that because the structure of HMT or HTMT and other methyltransferases is homologic, they are probably involved in plant salinity tolerance or play a role in the protection of plants against diseases. The interpretation assumed in the literature is that the process of I methylation (synthesis of CH_3I or CH_2I_2) serves to detoxify plants from excess I content in tissues (Landini et al., 2012; Medrano-Macías et al., 2016; Gonzali et al., 2017). The results justify the presumption that the process of volatilization of gaseous I compounds, i.e., CH_3I or CH_2I_2 (associated with HMT- or HTMT-like enzymatic activity) may also perform a different physiological function that is not yet described in the literature.

The activity of S-adenosylmethionine synthases, including those encoded by *msams5*, requires divalent cations, such as Mg^{2+}, Mn^{2+}, or Ca^{2+}, and monovalent cations, such as K^+ or Na^+ (**Supplementary Data 3**). The V cation (VO^{2+}) may replace divalent cations at the active site of this type of enzyme (Chasteen, 1995). Morrell et al. (1986) showed that biotransformation (oxidation) of V from vanadate (VO_3^-) to vanadyl (VO_2^+) during its uptake by plants is possible. In the three experiments conducted as part of the research, V was applied as VO_3^-. The research results obtained imply that VO_3^- transformation to VO_2^+ was weaker in the presence of exogenous 5-ISA and 3,5-diISA. This is indicated by reduced activity of *msams5*, which encodes an enzyme dependent on VO_2^+ and not on VO_3^-, and decreased V uptake by roots and leaves, in particular for the 3,5-diISA+V combination (in Experiment 1).

Expression of *samdmt* Gene

The process of methylation (volatilization of methyl salicylic acid ester [MeSA]) occurs with the participation of an enzyme called salicylic acid carboxyl methyltransferase (SAMT) (Tieman et al., 2010). The synthesis of MeSA is one of the many processes in the production of SA derivatives in plants. MeSA participates in processes responsible for SAR. MeSA is volatilized from roots and overground parts of plants and can be transported by the phloem (Gao et al., 2014). The process of biosynthesis of MeSA is catalyzed by SA methyltransferases (SAMT/BSMT); the reconversion of MeSA back to SA by methyl esterase (MES, SABP2) is also possible (Park et al., 2009; Gao et al., 2014).

In our study the expression of the *S-adenosyl-L-methionine-dependent methyltransferase* gene (*samdmt)* (**Supplementary Data 4**) in lettuce leaves was clearly associated with application of exogenous SA, SA + KIO_3 (KIO_3+SA, KIO_3+SA+V), and both iodosalicylates, 5-ISA and 3,5-diISA, with or without V. We reported a significant correlation between the foliar activity of *samdmt* and (1) the **RootSec** content of SA transported from roots to leaves ($r = 0.80^*$) and (2) the foliar content of SA and 5-ISA ($r = 0.72^*$ for SA and $r = 0.77^*$ for 5-ISA). Additionally, the results of measurements of all organic I metabolites and SA in roots and leaves showed that exogenous 5-ISA and 3,5-diISA underwent at least a 2-way transformation (**Figures 5**, **6**). Conversely, 5-ISA and 3,5-diISA served as substrates in PDTHA synthesis, while they underwent a catabolic/decomposition reaction: (1) to I^- ions that could be used as substrates in CH_3I or CH_2I_2 synthesis with the participation of the *msams5*-encoded enzyme or (2) to the SA molecule. This is indicated by the elevated SA content in roots and leaves and, consequently, by an increased foliar expression of *samdmt* in plants treated with exogenous 5-ISA or 3,5-diISA. Therefore, the results justify the assignment of a SAMTase-like function (esterification of SA; volatilization of MeSA) to the *samdmt* gene. The process was probably far more intense in leaves than in roots. This was exemplified by the relatively high expression of *samdmt* in leaves and trace functionality of the gene in the roots. For this very reason, no significant correlation was found between the activity of *samdmt* and root content of SA, 5-ISA, and 3,5- diISA.

CONCLUSION

The direction of metabolic conversion of KIO_3, 5-ISA, and 3,5-diISA in plants was documented. Both iodosalicylates were applied exogenously and underwent degradation inside the plants to I ions or served as precursors of synthesis of T3 and T4, classified as PDTHAs. The assignment of the role of encoding protein receptor T3 or T4, mainly in lettuce roots, to *cipk6* was proposed.

There are reasons to believe that the *per64-like,* rather than the *per12-like* gene, may act as a V-dependent haloperoxidase (vHPO), an enzyme that participates in I uptake (expression of

per64-like gene in roots > leaves). The expression of *msams5* was sufficiently specific to link the gene to the functions of HMT/HTMT enzymes. This gene was overexpressed in roots in systems where exogenous iodosalicylates were applied. The expression of *samdmt*, in turn, makes it naturally shortlisted for the role of a gene encoding the enzyme responsible for esterification/volatilization of ethyl salicylic acid (activity: leaves > roots).

V added to the nutrient solution caused a significant reduction and growth of I content in roots, but not in leaves, for the combination of: KIO_3+SA+V vs. KIO_3+SA and 3,5-diISA+V vs. 3,5-diISA, respectively. V was mostly accumulated in roots, with its transfer to leaves being limited. The level of *per64-like* expression was correlated with root activity of vHPO.

Plant enrichment with I through 5-ISA and 3,5-diISA was more effective than that through KIO_3. The results of pot experiments indicated that the I compounds tested, including 5-ISA and 3,5-diISA, in particular, may be used in I enrichment of plants through fertigation without the fear of harming the plants. The level of plant enrichment in I was safe for consumers. This is implied by the fact that the highest HQ-I in pot studies was 0.071. Consumers' safety would be at risk if the HQ exceeded 1.0. In Experiment 1 (hydroponic system), the efficacy of I uptake from the nutrient solution was higher than in the mineral soil or peat substrate. However, in the context of balancing the reference daily allowance of I for humans, the achieved level of I accumulation (especially following application of 5-ISA) was too high [as shown by RDA-I (%) > 480%, HQ > 0.66]. This means that doses < 10 μM of I compounds can be recommended for hydroponic systems, especially where both iodosalicylates are used.

I-enriched lettuce strongly reduces the *in vitro* development of cancerous cells in colon cancer (Koronowicz et al., 2016). It seems appropriate to study the use of lettuce enriched with 5-ISA and 3,5-diISA in nutrigenomics.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in the online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

SS, MC, IK, and JP: methodology. SS, MC, and KK: formal analysis. SS: funding acquisition. SS, IK, MH, MG, ŁS, JP, and AK: investigation of lettuce cultivation and chemical analyses of plant, nutrient solution, and soil samples. MC, KK, and DG: investigation of molecular analyses of lettuce. SS, MC, IK, and KK: resources. SS and MC: supervision. SS, MC, and KK: writing—original draft. SS, MC, IK, KK, MH, MG, DG, ŁS, and PK: writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.653168/full#supplementary-material

Supplementary Data 1 | The functional annotation of *per12-like i per64-like* genes.

Supplementary Data 2 | The functional annotation of *cipk6* gene.

Supplementary Data 3 | The functional annotation of *msams5* gene.

Supplementary Data 4 | The functional annotation of *samdmt* gene.

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GLOSSARY

2,3,5-triIBeA = 2,3,5-triiodobenzoic acid
2-IBeA = 2-iodobenzoic acid
3,5-diISA = 3,5-diiodosalicylic acid
4-IBeA = 4-iodobenzoic acid
5-ISA = 5-iodosalicylic acid
BeA = benzoic acid
HMT = halide methyltransferase
HPLC-ICP-MS)/MS = high-performance liquid chromatography with inductively coupled plasma mass spectrometry
HPOs = haloperoxidases
HTMT = SAM-dependent halide/thiol methyltransferase
ICP-OES = inductively coupled plasma optical emission spectrometry
I-Tyr = iodotyrosine
LC-MS/MS = liquid chromatography-mass spectrometry
MAPK = mitogen-activated protein kinase
MeSA = volatile ester of methyl salicylic acid
NFT = nutrient film technique
PDTHA = plant-derived thyroid hormone analogs
RDA = recommended dietary allowance
RootSec = secretion produced as a result of root pressure
SA = salicylic acid
SAM = S-adenosyl-l-methionine
SAMT = salicylic acid carboxyl methyltransferase
SAR = Systemic Acquired Resistance
SOM = soil organic matter
T3 = triiodothyronine
T3-Na = sodium salt triiodothyronine
T4 = thyroxine
TF = transfer factor
TMAH = tetramethylammonium hydroxide
TransThy-T3/T4trans = transthyretin, responsible for T3 and T4 transport
vHPO = vanadium-dependent haloperoxidases
per64-like = Peroxidase 64-like
per12-like = Peroxidase 12-like.
samdmt = S-adenosyl-L-methionine-dependent methyltransferase
cipk6 = CBL-interacting serine/threonine-protein kinase 6
msams5 = S-adenosylmethionine synthase 5

11. Oświadczenia autorów publikacji wchodzących w skład pracy doktorskiej

Oświadczenia Publikacji nr 1

Grzanka, M., Smoleń S., Kováčik P. 2020. Effect of vanadium on the uptake and distribution of organic and inorganic forms of iodine in sweetcorn plants during early-stage development. Agronomy 10(11). doi: 10.3390/agronomy10111666.

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- współudziałem w opracowaniu metodologii doświadczenia,
- przeprowadzeniem doświadczenia wazonowego,
- przygotowaniem materiału roślinnego do analiz laboratoryjnych,
- wykonywanie analizy laboratoryjnej związanej z oznaczeniem enzymów: wanadozależnej haloperoksydazy, katalazy i peroksydazy,
- wykonaniem analizy statystycznej wyników
- opracowaniem, interpretacją , wizualizacją oraz dyskusją uzyskanych wyników,
- przygotowaniem wstępnej i ostatecznej wersji manuskryptu,
- udzieleniem odpowiedzi na uwagi recenzentów oraz poprawę manuskryptu po recenzji.

Marlena Grzanka

mgr inż. Marlena Grzanka

al. 29 Listopada 54, 31-425 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
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Wydział Biotechnologii i Ogrodnictwa
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- pomocą w sformułowaniu problemu badawczego,
- współopracowaniem metodologii badań,
- pomocą w organizacji oraz nadzorem nad przeprowadzeniem doświadczenia wazonowego,
- nadzorem nad wykonaniem analiz laboratoryjnych,
- pomocą w interpretacji uzyskanych wyników,
- pomocą w przygotowaniu ostatecznej wersji manuskryptu,
- pomocą w udzieleniu odpowiedzi na uwagi recenzentów oraz poprawieniu manuskryptu po recenzji,
- pozyskaniem funduszy na badania i na opublikowanie artykułu.

prof. dr hab. inż. Sylwester Smoleń

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

Slovak University of Agriculture in Nitra - Slovenska posnohospodarska univerzita v Nitre

Department of Agrochemistry and Plant Nutrition
Slovak University of Agriculture in Nitra—Slovenska
Posnohospodarska Univerzita v Nitre, 94976 Nitra - Chrenová,

Agroinštitút Nitra, štátny podnik

Agroinštitút Nitra, štátny podnik
Akademická č. 4, 949 01 Nitra

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Declaration on the participation of co-authors in the publication

I declare that in the publication of Grzanka M., Smolen S., Kováčik P. Effect of vanadium on the uptake and distribution of organic and inorganic forms of iodine in sweetcorn plants during early-stage development. Agronomy 2020, 10, 1666; doi: 10.3390 / agronomy10111666 my participation was related to:

- help in editing the final version of the manuscript,
- raising funds to publish the article.

prof. Ing., CSc. Kováčik Peter

Oświadczenia Publikacji nr 2

Grzanka M., Smoleń S., Skoczylas Ł., Grzanka D. 2021. Biofortification of sweetcorn with iodine: Interaction of organic and inorganic forms of iodine combined with vanadium. Agronomy 11(9), 1720. doi: 10.3390/agronomy11091720.

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dr Łukasz Skoczylas, prof. URK

ul. Balicka 122, 30-149 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
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prof. dr hab. inż. Sylwester Smoleń

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

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- pomoc w przygotowaniu manuskryptu w zakresie opracowania figur i tabel.

Dominik Grzanka

Dominik Grzanka
wolontariusz afiliowany przy WBiO UR w Krakowie
Student VI roku medycyny, Śląski Uniwersytet Medyczny.

al. 29 Listopada 54, 31-425 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
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mgr inż. Marlena Grzanka

al. 29 Listopada 54, 31-425 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka

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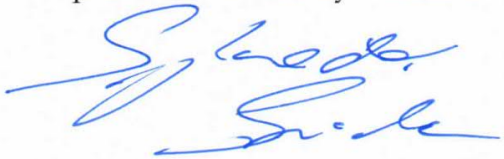

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- pomoc w przeprowadzeniu doświadczeń polowych,
- pomoc w przygotowaniem materiału roślinnego do analiz laboratoryjnych,
- pomoc w przygotowaniu manuskryptu w zakresie opracowania figur i tabel.

Dominik Grzanka

Dominik Grzanka
wolontariusz afiliowany przy WBiO UR w Krakowie
Student VI roku medycyny, Śląski Uniwersytet Medyczny.

al. 29 Listopada 54, 31-425 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam że w publikacji Grzanka, M., Smoleń S., Skoczylas Ł., Grzanka D. 2022. Synthesis of Organic Iodine Compounds in Sweetcorn under the Influence of Exogenous Foliar Application of Iodine and Vanadium. Molecules 27, 1822. https://doi.org/ 10.3390/molecules27061822, mój udział związany był z:

- sformułowaniem problemu badawczego
- współpracowaniem metodologii badań,
- przeprowadzeniem doświadczeń polowych,
- przygotowaniem materiału roślinnego do analiz laboratoryjnych,
- wykonaniem analizy statystycznej wyników,
- opracowaniem, interpretacją , wizualizacją oraz dyskusją uzyskanych wyników,
- przygotowaniem wstępnej i ostatecznej wersji manuskryptu,
- udzieleniem odpowiedzi na uwagi recenzentów oraz poprawą manuskryptu po recenzji.

Marlena Grzanka

mgr inż. Marlena Grzanka

al. 29 Listopada 54, 31-425 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka

Oświadczenia Publikacji nr 4

Smoleń S., Kowalska I., Halka M., Ledwożyw-Smoleń I., **Grzanka M**., Skoczylas Ł., Czernicka M., Pitala J. 2020. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10(1), 1-21 doi: 10.3390/agronomy10010001.

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UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Technologii Żywności
Katedra Technologii Produktów Roślinnych i Higieny Żywienia

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- pomocą w przeprowadzeniu badań,
- pomocą w przygotowaniu ostatecznej wersji manuskryptu,
- pomocą wudzieleniu odpowiedzi na uwagi recenzentów.

dr Łukasz Skoczylas, prof. URK

Skoczylas Łukasz

ul. Balicka 122, 30-149 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- pomocą w przeprowadzeniu badań,
- pomocą w przygotowaniu manuskryptu do druku.

dr inż. Małgorzata Czernicka, prof. URK

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- pomocą w przeprowadzeniu doświadczeń wegetacyjnych z uprawą sałaty,
- wykonaniem pomiarów biometrycznych plonowania,
- pomocą w wykonaniu analizy aktywności enzymatycznej vHPO,
- pomocą w przygotowywaniu materiału roślinnego i pożywek do analiz chemicznych.

mgr inż. Marlena Grzanka

Marlena Grzanka

al. 29 Listopada 54, 31-425 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 2020, 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- pomocą w sformułowaniu problemu badawczego,
- współopracowaniem metodologii badań,
- nadzorem nad przeprowadzeniem doświadczeń z uprawą sałaty,
- pomocą w przygotowaniu ostatecznej wersji manuskryptu,
- pomocą w poprawie publikacji po recenzji.

dr hab. inż. Iwona Kowalska, prof. URK

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- sformułowaniem problemu badawczego,
- opracowaniem metodologii badań,
- organizacją i nadzorem nad przeprowadzeniem doświadczeń z uprawą sałaty,
- nadzorem nad wykonaniem analiz laboratoryjnych,
- analizą statystyczną wyników i pomocą przy ich wizualizacji,
- przygotowaniem manuskryptu,
- interpretacją uzyskanych wyników,
- udzieleniem odpowiedzi na uwagi recenzentów oraz poprawieniem manuskryptu po recenzji,
- pozyskaniem funduszy na badania i opublikowanie artykułu.

prof. dr hab. inż. Sylwester Smoleń

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- pomocą w przeprowadzeniu doświadczeń z uprawą sałaty,
- opracowaniem metod i wykonaniem analiz chemicznych technikami ICP-MS/MS oraz LC-MS/MS,
- pomocą w przygotowaniu manuskryptu w zakresie opisu ww. metod analitycznych.

Joanna Pitala

mgr Joanna Pitala

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- pomocą w przeprowadzeniu doświadczeń wegetacyjnych z uprawą sałaty,
- wykonaniem pomiarów biometrycznych plonowania,
- wykonaniem analizy aktywności enzymatycznej vHPO,
- pomocą w przygotowywaniu materiału roślinnego i pożywek do analiz chemicznych.

Dr inż. Maria Halka

Maria Halka

al. 29 Listopada 54, 31-425 Kraków
Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S., Kowalska I., Halka M., Ledwozyw-Smoleń I., Grzanka M., Skoczylas Ł., Czernicka M., Pitala J. Selected aspects of iodate and iodosalicylate metabolism in lettuce including the activity of vanadium dependent haloperoxidases as affected by exogenous vanadium. Agronomy 10 (1), 1, doi:10.3390/agronomy10010001 mój udział związany był z:

- pomocą w przeprowadzeniu doświadczeń wegetacyjnych z uprawą sałaty,
- pomocą w wykonania analizy statystycznej wyników i ich wizualizacji,
- pomocą w przygotowaniu ostatecznej wersji manuskryptu,
- pomocą w poprawie publikacji po recenzji.

Iwona Ledwożyw-Smoleń

dr inż. Iwona Ledwożyw-Smoleń

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

Oświadczenia Publikacji nr 5

Smoleń S., Czernicka M., Kowalska I., Kęska K., Halka M., Grzebelus D., **Grzanka M.**, Skoczylas Ł., Pitala J., Koronowicz A., Kováčik P. 2021 New aspects of uptake and metabolism of non-organic and organic iodine compounds—the role of vanadium and plant-derived thyroid hormone analogs in lettuce. Frontiers in Plant Science 12, 608. doi: 10.3389/fpls.2021.653168.

Punktacja MNiSW$_{2021}$: 100 pkt

IF$_{2021}$: 5,753

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 mój udział związany był z:

- wykonaniem analiz molekularnych sałaty oraz pozyskaniem odczynników i materiałów do ich przeprowadzenia,
- wykonaniem analizy bioinformatycznej uzyskanych wyników badań molekularnych.
- przygotowaniem manuskryptu i poprawą publikacji po recenzji w zakresie części badań biotechnologicznych opisanych w manuskrypcie.

dr inż. Małgorzata Czernicka, prof. URK

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 mój udział związany był z:

- nadzorem nad wykonaniem analiz molekularnych sałaty,
- rewizją i edycją przygotowanego manuskryptu.

prof. dr hab. inż. Dariusz Grzebelus

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 mój udział związany był z:

- wykonaniem analiz molekularnych sałaty,
- wykonaniem analizy bioinformatycznej uzyskanych wyników badań molekularnych.
- pomocą w przygotowaniu manuskryptu i poprawieniu publikacji po recenzji w zakresie części badań biotechnologicznych opisanych w manuskrypcie.

dr inż. Kinga Kęska

al. 29 Listopada 54, 31-425 Kraków
Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120

ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 mój udział związany był z:

- sformułowaniem problemu badawczego,
- współopracowaniem metodologii badań,
- organizacją i nadzorem nad przeprowadzeniem doświadczeń z uprawą sałaty,
- nadzorem nad wykonaniem analiz laboratoryjnych,
- analizą statystyczną wyników,
- przygotowaniem manuskryptu,
- interpretacją uzyskanych wyników,
- udzieleniem odpowiedzi na uwagi recenzentów oraz poprawieniem manuskryptu po recenzji,
- pozyskaniem funduszy na badania i opublikowanie artykułu.

prof. dr hab. inż. Sylwester Smoleń

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 mój udział związany był z:

- pomocą w sformułowaniu problemu badawczego,
- współopracowaniem metodologii badań,
- pomocą w przeprowadzeniu doświadczeń z uprawą sałaty,
- nadzorem nad wykonaniem analiz laboratoryjnych,
- pomocą w przygotowaniu ostatecznej wersji manuskryptu,
- pomocą w pozyskaniu środków na badania,
- pomocą w poprawie publikacji po recenzji.

dr hab. inż. Iwona Kowalska, prof. URK

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Biotechnologii i Ogrodnictwa
Katedra Biologii Roślin i Biotechnologii

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 mój udział związany był z:

- z pomocą w prowadzeniu doświadczeń wegetacyjnych z uprawą sałaty,
- wykonaniem pomiarów biometrycznych oceny plonowania,
- pomocą w przygotowywaniu materiału roslinnego, pożywek i prób gleby do analiz chemicznych.

mgr inż. Marlena Grzanka

Marlena Grzanka

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,

ePUAP: ESP:/URKRAKOW/skrytka

Slovak University of Agriculture in Nitra - Slovenska posnohospodarska univerzita v Nitre

Department of Agrochemistry and Plant Nutrition
Slovak University of Agriculture in Nitra—Slovenska
Posnohospodarska Univerzita v Nitre, 94976 Nitra - Chrenová,

Agroinštitút Nitra, štátny podnik

Agroinštitút Nitra, štátny podnik
Akademická č. 4, 949 01 Nitra

Oświadczenie o udziale współautorów w publikacji

Oświadczam, że w publikacji Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 mój udział związany był z:

- pomocą w redagowaniu finalnej wersji manuskryptu.

Declaration on the participation of co-authors in the publication

I declare that in the publication of Smoleń S.; Czernicka, M.; Kowalska, I.; Kęska, K.; Halka, M.; Grzebelus, D.; Grzanka, M.; Skoczylas, Ł.; Pitala, J.; Koronowicz, A.; Kováčik, P. New Aspects of Uptake and Metabolism of Non-organic and Organic Iodine Compounds—The Role of Vanadium and Plant-Derived Thyroid Hormone Analogs in Lettuce. Frontiers in Plant Science 2021, 12, 608. https://doi.org/10.3389/fpls.2021.653168 my participation was related to:

- help in editing the final version of the manuscript.

prof. Ing., CSc. Kováčik Peter

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

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- **z pomocą w prowadzeniu doświadczeń wegetacyjnych z uprawą sałaty,**
- **wykonaniem pomiarów biometrycznych oceny plonowania,**
- **wykonaniem analizy aktywności enzymatycznej vHPO.**

Dr inż. Maria Halka

Maria Halka

al. 29 Listopada 54, 31-425 Kraków
Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

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- pomocą w przeprowadzeniu doświadczeń z uprawą sałaty,
- opracowaniem metod i wykonaniem analiz chemicznych technikami ICP-MS/MS, HPLC-ICP-MS/MS oraz LC-MS/MS,
- pomocą w przygotowaniu manuskryptu w zakresie opisu ww. metod analitycznych.

Joanna Pitala

mgr Joanna Pitala

al. 29 Listopada 54, 31-425 Kraków
31-120 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21,
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

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Katedra Żywienia Człowieka i Dietetyki

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Signed by / Podpisano przez:

Aneta Koronowicz

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dr hab. inż. Aneta Koronowicz, prof. URK

ul. Balicka 122, 30-149 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka

UNIWERSYTET ROLNICZY
im. Hugona Kołłątaja w Krakowie

Wydział Technologii Żywności
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- pomocą w przygotowaniu ostatecznej wersji manuskryptu,
- pomocą wudzieleniu odpowiedzi na uwagi recenzentów.

dr Łukasz Skoczylas, prof. URK

Skoczylas Łukasz

ul. Balicka 122, 30-149 Kraków
tel. +48 (12) 662 51 87, e-mail: kbrib@urk.edu.pl

Adres do korespondencji: al. Mickiewicza 21, 31-120 Kraków
ePUAP: ESP:/URKRAKOW/skrytka