SAŽETAK

UTJECAJ SILICIJA NA BILJNE SEKUNDARNE METABOLITE, TE NJEGOV ODNOS SA SIGNALNIM STRESNIM PODRAŽAJIMA

Silicij (Si) se ne smatra esencijalnim elementom za rast i razvoj viših biljaka. Ipak, njegovi se učinci na razne fiziološke, signalne, transkripcijske i biosintetske procese dosljedno očituju uslijed stresa, premda ortosilicijeva kiselina, glavni raspoloživi oblik Si, nema izražen kapacitet za izravno sudjelovanje u molekularno-biokemijskim interakcijama. Stoga iz nesrazmjera između silicijeve kemijske inertnosti i širine pripisanih mu učinaka, kao i iz okolnosti da se ti učinci očituju samo uslijed stresa, dok u odsutnosti stresa izostaju, proizlazi potreba za drugačijim tumačenjem načina na koji Si te učinke zapravo ostvaruje. U suštini, osnova njegova djelovanja proizlazi iz odlike usvojene ortosilicijeve kiseline da se taloži na staničnoj stijenci, čime dolazi do njezine impregnacije i stabilizacije stanica i tkiva. Silicij s te razine djeluje posredno i profilaktički, ograničavajući negativne učinke stresa i ublažavajući prateće poremećaje pa se nizvodni signalni, transkripcijski i biosintetski procesi aktiviraju i odvijaju drugačijim tijekom, što rezultira i drugačijim odgovorom na stres. No moguće je da ova paradigma, iako valjana, ne obuhvaća puni opseg silicijeva djelovanja. U ispitivanju te mogućnosti, sekundarni metabolizam predstavlja pogodan analitički okvir, osobito zato što su njegovi produkti krajnji izraz procesa uključenih u odgovor na stres. Stoga se ovdje, u dva različito koncipirana dijela, promatrajući njegove učinke na derivate fenilpropanoidnog, izoprenoidnog i lipoksigenaznog puta pod specifičnim okolnostima, nastoji razjasniti način silicijeva djelovanja i preispitati doseg prevladavajuće paradigme. U prvom dijelu rada, na maslini (Olea europaea L.), vrsti slabe sposobnosti usvajanja Si, folijarnom primjenom Si uspostavlja se osnova za njegovo profilaktičko djelovanje i istražuje odnos između njegovih učinaka, intenziteta stresa i kapaciteta genotipa da se s njime nosi. Istraživanje je provedeno na dva metabolički različita kultivara masline tijekom ljeta tj. tijekom izraženijeg abiotskog opterećenja te jeseni, tijekom razdoblja njegova popuštanja. Pod ljetnim uvjetima svaki je kultivar razvio vlastiti fenolni profil, a Si ih je dodatno oblikovao. Zabilježen je porast oleuropeina (105-252 %) i verbaskozida (62–126 %), ovisan o genotipu. Na jesen je učinak Si bio ograničen u metabolički konzervativnijem kultivaru, dok je u plastičnijem kultivaru doveo do daljnjeg porasta oleuropeina (37%) i verbaskozida (26%). Rezultati pokazuju da Si ne preusmjerava sekundarni metabolizam neovisno o kontekstu, već se opseg i postojanost njegovih učinaka oblikuju odnosom između intenziteta okolišnog opterećenja, odnosno stresa, te genotipski uvjetovane metaboličke podloge i plastičnosti. Drugi dio rada istražuje obuhvaća li profilaktička paradigma puni opseg silicijeva djelovanja. Ovdje se polazi od autorske pretpostavke prema kojoj Si, impregnacijom stanične stijenke, mijenja operativni kontekst izvanstaničnog sučelja, odnosno sučelja u kojem se rani učinci stresa prvo manifestiraju, pa percipiraju, te preko signalnih kaskada prevode u odgovor. Stoga se istražuje da li Si, uz to što amortizira negativne učinke stresa, ujedno utječe i na to kako biljka na staničnoj razini taj stres percipira, interpretira i prevodi u smislen odgovor. U tu se svrhu obrambeni odgovor ne inducira stresom, već signalima koji ga oponašaju, kako bi se silicijevi učinci mogli promatrati izvan okvira njegova profilaktičkog djelovanja. Uzgajane u strogo kontroliranim uvjetima, mlade biljke ječma (Hordeum vulgare L.), izložene su hlapljivim signalima metil-jasmonatu (MeJA) i metil-salicilatu (MeSA), pomoću kojih se inducirao obrambeni odgovor karakterističan za napad štetnika ili patogena. Nakon izlaganja, uzorkovan je sastav smjese emitiranih obrambenih hlapljivih spojeva. Silicij se pokazao sposobnim značajno izmijeniti profil smjese i u odsustvu stvarnog stresa. Nakon primjene MeJA signala povećane su emisije acetofenona, (E)-2-nonenala i benzilnog alkohola, dok su emisije linaloola i (Z)-3-heksenil-acetata značajno smanjene. Nakon primjene MeSA signala, omjer ovih i drugih spojeva smanjen je pod utjecajem Si. K tome, rezultati olfaktornih testova privlačnosti emitirane smjese ukazali su na značajno češći odabir silicijem opskrbljenih biljaka od strane generalističkog predatora biljnih štetnika. Rezultati pokazuju da Si ne djeluje samo tako što amortizira negativne učinke stresa, već može utjecati i na način na koji biljka oblikuje svoj obrambeni odgovor.

Ključne riječi: silicij (Si), maslina (Olea europaea L.); abiotski stres; sekundarni metabolizam; ječam (Hordeum vulgare L.); signalizacija stresa; hlapljivi spojevi; komunikacija bilja.

EXTENDED ABSTRACT

INFLUENCE OF SILICON ON PLANT SECONDARY METABOLITES AND ITS CROSS-TALK WITH STRESS-SIGNALING CUES

Silicon (Si) is not an essential element for growth and development of higher plants. Nevertheless, its beneficial effects are consistently observed when plants are under stress. These effects encompass a wide range of physiological and metabolic processes underpinned and defined by a network of molecular interactions and biochemical reactions. Yet, orthosilicic acid (H_4SiO_4), the main form of Si, lacks the capacity to participate in them, as after uptake it remains mostly non-ionised and weakly reactive at physiological pH. However, driven by transpiration, it polymerises and deposits within the plant cell wall's polysaccharide matrix, constituting the basis for silicon's overarching action under stress. Notably, silicon's beneficial effects are most evident precisely under adverse conditions, whereas in optimal conditions they generally remain latent, limited, or altogether absent. Coupled with its lack of biochemical versatility, this indicates that Si neither participates in biochemical reactions underlying various physiological and metabolic processes nor does it modify them directly on its own. Instead, Si exerts its influence once plants are challenged, mitigating the impact that a given abiotic or biotic stressor might have on them. The prevailing paradigm regarding silicon's operating principle in plants proposes that its deposition within the cell wall stabilises the extracellular domain to the degree that it limits some of the ionic, oxidative, hormonal, and various other disruptions caused by stress. Silicon therefore acts prophylactically, as it restricts stress-induced disturbances at the extracellular level, containing the stressor's impact and dampening its effect on plants. This, in turn, alters the course of downstream signalling, transcriptional, biosynthetic and other metabolic processes, ultimately resulting in an improved response to stress. This paradigm successfully reconciles the apparent discrepancy between silicon's biochemical inertness and the wide range of beneficial effects it exerts under stress. Nevertheless, it remains primarily a working, integrative model that requires further refinement as there are still questions regarding the extent to which it accounts for the full scope of silicon's involvement in shaping how plants respond to stress.

In an effort to discern whether silicon's involvement in plant stress responses extends beyond its prophylactic role, plant secondary metabolites can provide particularly valuable information, as they represent the integrated outcome of various physiological and metabolic processes through which plants respond to stress. As such, differences in their synthesis, accumulation, or emission between plants grown with or without the addition of silicon can reveal how silicon's influence propagates through the plant’s response network and how it ultimately shapes the way in which plants adapt or respond to stress. Accordingly, through two complementary yet methodologically distinct lines of investigation, this thesis seeks to address key questions surrounding silicon’s mode of action in plants. By examining silicon's effects on different dimensions of plant secondary metabolism, it aims, firstly, to investigate the relationship between silicon and stress, and secondly, to expand the prevailing paradigm concerning the full scope of its involvement in shaping plant stress responses.

The first part of this thesis was conducted on young olive plants (Olea europaea L.). It examined silicon’s influence on the synthesis and accumulation of secondary metabolites as a function of the interplay between the severity of prevailing environmental conditions and the genotype’s inherent adaptive capacity, reflected in its metabolic response strategy. Moreover, given that olive plants posses limited capacity for its natural uptake, within the scope of this investigation silicon was applied foliarly in order to examine whether this approach could successfully establish a functional basis for silicon's prophylactic action.

Silicon's effects were examined in two distinct cultivars, Istarska bjelica and Leccino, across two seasonal contexts differing markedly in the intensity of environmental pressures, first during peak summer and once again in autumn, after those pressures had eased. Analyses were performed on fully developed, directly treated leaves as well as on the juvenile leaves that developed subsequently from plantlets previously treated with Si. Foliar application increased leaf silicon content to a comparable extent in both cultivars, confirming that this approach can be used for silicon's successful assimilation within leaves of species characterised with limited natural capacity for its acquisition from soil. Silicon improved leaf water status and photochemical efficiency, delayed leaf senescence, and yielded

favourable results regarding the chlorophyll and carotenoid indices, but only in physiologically more resilient yet metabolically more conserved cultivar, Istarska bjelica. However, these physiological benefits were clearly evident in summer but not in autumn, when environmental pressures had eased. In contrast to the aforementioned cultivar, Si had little influence on similar traits of the metabolically more versatile cultivar Leccino. Silicon's effects on secondary metabolism were likewise strongly dependent on the intensity of environmental conditions and each genotype's inherent strategy of metabolic adaptation. In summer, concentrations of some of the most important metabolites increased under Si. Silicon increased levels of oleuropein by 105 % in Istarska bjelica and by 252 % in Leccino, while verbascoside levels increased by 62 % in Istarska bjelica and by 126 % in Leccino. As environmental pressures eased in autumn, silicon's influence on the phenolic profile of Istarska bjelica became limited, probably due to the fact that its inherently more conserved metabolism required little further adjustment under the less demanding conditions. Leccino, however, exhibited a more persistent reorganisation of its secondary metabolism. In autumn, its oleuropein and verbascoside levels were 61 % and 26 % higher under Si, while oleuropein increased by a further 37 % relative to the preceding sampling period. To a different degree, the levels of these metabolites were also significantly higher in untreated juvenile leaves that had developed on plantlets previously treated with Si. However, this is more likely due to their translocation from directly treated leaves, where Si enhanced their accumulation, rather than silicon's systemic effect on their local synthesis. All in all, while effective in altering and improving the olive plants' phytochemical profiles, Si did so in a manner that adhered to each genotype's strategy of metabolic adaptation. This clear interdependence between external stress severity and the given genotype's inherent metabolic foundation, aligns with the currently prevailing paradigm of silicon's prophylactic mode of action in plants.

The second part of this thesis investigated whether the prevailing paradigm on silicon's mode of action accounts for the full scope of its involvement in shaping how plants respond to stress, or whether it exerts an additional, yet uncharacterised function. Here, it was hypothesized that, rather than just acting prophylactically by dampening the severity of stress-induced impairments, silicon's deposition within the cell wall also profoundly preconditions the extracellular interface to the extent that it changes the operational context in which early stress-induced disruptions are perceived, transmitted, and ultimately translated into a metabolic response. However, under actual stress, these two functions cannot be distinguished from one another, since any difference between plants with and without silicon may simply reflect the lower degree of damage mitigated by silicon's prophylactic action. In order to bypass this limitation and to assess silicon's potential additional function, plants had to be brought into a defence-activated state while remaining free from any form of stress and the damage it would otherwise impose. Therefore, rather than using an actual stressor to trigger the plants' defence response, two different volatile signaling cues were employed to mimic it, ensuring the plants remained free from any confounding disruptions or damages that could otherwise be mitigated by Si. Should Si lead to significant metabolic changes even under such conditions, it would provide compelling evidence that its influence extends beyond mere prophylactic protection. Young barley plants (Hordeum vulgare L., cv. Fairytale) were therefore first supplied with Si and then exposed to either methyl jasmonate (MeJA) or methyl salicylate (MeSA),

stress-associated volatile signals that induce metabolic rearrangement and activate various defence pathways related to leaf tissue damage, herbivory, or pathogen attack. After exposure, silicon's influence on these pathways was monitored through the lens of subsequently emitted volatile organic compounds, namely, secondary metabolites released in response to biotic stress which serve as crucial airborne signals in plant defense and communication. Silicon supplementation did not increase total volatile emissions after exposure to either signal, despite promoting biomass accumulation. Instead, it selectively reconfigured the composition of the subsequently emitted volatile blends in a signal-specific manner. Following exposure to methyl jasmonate, silicon supplementation led to a reduced emission of the most dominant compounds in the blend, namely linalool and (Z)-3-hexenyl acetate, while increasing the emission of acetophenone, (E)-2-nonenal, and benzyl alcohol. Following exposure to methyl salicylate, the emissions of linalool, (Z)-3-hexenyl acetate, β-myrcene, methyl salicylate, and benzaldehyde all reduced under silicon's influence, while none of the other captured and analyzed

volatiles showed a significant increase. Evidently, under silicon's influence, these shifts in blend composition were substantial enough to give rise to completely different volatile profiles, a metabolic rearrangement that was substantiated by multivariate non-metric multidimensional scaling (NMDS) analyses. Furthermore, since induced volatile compounds serve as an indirect line of plant defence, essentially carrying airborne semiochemical information in order to, among other functions, attract natural enemies of herbivores, silicon's influence on the induced volatile bouquet had to be validated not only analytically, but through its biological functionality as well. This step was crucial to confirm that the observed chemical alterations under Si were not inconsequential, but functionally relevant components of the plant's defense. Ultimately, olfactory behavioural assays demonstrated that these silicon-induced shifts translated into a true ecological advantage, rendering the volatile profiles characteristically more attractive to the sevenspot ladybug (Coccinella septempunctata Linnaeus), a generalist predator of various phytophagous insects. However, this behavioural preference was only limited to the blends emitted by Si-supplied plants that had been exposed to MeJA. Given that the young barley plants within this line of investigation were brought into a defence-activated state via an exposure to a volatile signal rather than being subjected to genuine stress, silicon had no actual impairment, disruption, or damage to attenuate. Consequently, the metabolic and behavioural changes observed under silicon's influence cannot be explained solely by its well-documented prophylactic mode of action in plants. Silicon, therefore, alongside its established protective role, may also shape the way in which plants process stress-related information, and the way they translate it into a response. Due to its recognized biochemical inertness, this is hypothesized to occur without silicon's direct participation in the corresponding metabolic pathways, operating instead indirectly from the level of the extracellular interface. While this beneficial element clearly protects plants during actual stress, the fact that it can modulate defence responses induced by a stress-mimicking signal alone indicates that its role extends beyond mere damage control. Consequently, the currently prevailing paradigm is not challenged but further extended. Silicon not only limits the detrimental consequences of stress but also contributes to the formation of a functionally more meaningful and coordinated defence response.

Keywords: olive (Olea europaea L.); silicon (Si); foliar application; abiotic stress; secondary metabolites; phenolic compounds; barley (Hordeum vulgare L.); stress signalling; methyl jasmonate; methyl salicylate; volatile organic compounds; semiochemical communication