

The role of silicon on in vitro plant culture

Kathleen Terhaag

Botanic Department, Biological Sciences Sector, Federal University of Paraná in Curitiba, Paraná, Brazil,
kathleenterhaag@gmail.com

Abstract. Silicon is found in soil and is one of the most dominant mineral nutrients in plants. This mineral helps intolerance to biotic and abiotic stresses, besides increasing photosynthetic activity and nutrient uptake by plants. The vitromorphogenic potential of plant cells and tissues from several species also showed a high increase through the supplementation of silicon sources to the culture media. For this reason, the Si exogenous application to culture media can bring new perspectives to the in vitro cultivation of plants that aim to develop organogenesis, embryogenesis, and other clonal mass propagation techniques. In this regard, this review aims to summarize the role and possibilities of the application of silicon on in vitro cell and tissue cultures.

Keywords. Silicon, micropropagation, culture media, supplementation

1. Introduction

Silicon (Si) is an important inorganic constituent for higher plants. With a few exceptions – mainly certain highly weathered tropical soils – soils contain high rates of Si. In most of them, Si is second only to oxygen (O) as a constituent: the mean values of O are 49% and of Si 31% [1]. For plants, the direct source of Si in the soil is extracted by the roots in the form of orthosilicic acid (H_4SiO_4) which, according to Epstein (1994)[2] is present in concentrations ranging from 0.1 mM to 0.6 mM, something about two orders of magnitude higher than phosphorus (P) concentrations in soil solutions, with an average – but highly variable – the value of 0.0016 mM. Therefore, plants growing in the soil are directly exposed to Si. There is a great amount of botanical, agronomic, horticultural, and phytopathological knowledge of Si that, together with physiological experiments, proves that this mineral cannot be ignored as a plant biological nullity, despite not being considered an essential nutrient for plant growth and development [3]. It is important to note that Si plants content varies greatly in different species and between genotypes of the same species. Another factor that influences the content of this mineral in plants is the availability of Si in the soil, in addition to the plant itself, which can change the chemical form and, consequently, the availability of Si in the substrate. Furthermore, most of the absorbed Si is transferred from the roots to the shoots, and within the shoots, its distribution throughout the plant is quite uneven [4]. Although the Si absorption and

transport process are not fundamentally different from other elements, the uncharged molecule H_4SiO_4 is not redistributed when deposited in the form of crystals, and its translocation within the plant is especially affected by transpiration flux when compared to other elements. Only in the marine diatom *Cylindrotheca fusiformis* a cDNA a plant biological nullity encoding a Si transporter was identified, a finding that represents the first identification of a Si transporter specific to any organism [5]. The concentration of Si in the substrate is affected by many factors, such as silica condensation, temperature, pH, other polymer's presence, different ions, and small molecules. The distance of the Si – O bond and the angle of the Si – O – Si bond play an essential role in the polymerization of different silica species. Environmental reactions and HO groups may differ in several of these species due to composition, solubility, hardness, density, and viscosity. Aminoacids and peptides are effective in the formation of polysilicon species through interactions with different Si species [6]. From that, there is a great amount of botanical, agronomic, horticultural, and phytopathological knowledge of Si that, along with physiological experiments, proves that this mineral cannot be ignored as a plant biological nullity, despite not being considered an essential nutrient for plant growth and development [3]. In this context, this brief review summarizes the role of Si as a mineral supplement in different in vitro plant cultures and highlights its potential for new and future research involving this method of plant cell and tissue growth.

2. Biotic and abiotic stresses mediated by Si

The literature is full of reports that Si promotes plant growth. In many cases, the growth stimulus was due to the protection that Si provided to plants against the harmful effects of biotic and abiotic stresses [2]. Plants exposed to these stresses show an increase in the concentration of reactive oxygen species (ROS), such as the superoxide radical (O_2^-), hydroxyl radical (OH^\cdot), and hydrogen peroxide (H_2O_2), which damage biomolecules such as nucleic acids, photosynthetic pigments, and proteins. A decrease in the ROS production and an increase in the plant's antioxidant system from Si supplementation is responsible for greater tolerance of plants to stress [7]. In this sense, an interesting finding is that Si can protect plants due to its interaction with toxic metal ions (such as aluminum, manganese, chromium, cadmium, and lead, among others), reducing its toxicity in plants. Tripathi et al. [8] demonstrated that the application of Si decreased the O_2^- concentrations, H_2O_2 , and malondialdehyde (MDA) in maize leaves grown under arsenic stress by increasing the activity of peroxide ascorbate (PX), dehydroascorbate reductase (DHAR), and glutathione reductase (GSH). Increases in PX, catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) activities in cotton roots and leaves were also related to Si-mediated reductions in cultivars stressed with zinc (Zn) [9]. As an action form, Si seems to decrease these metals uptake due to its action in increasing soil pH, by managing changes in soil metal speciation through the formation of silicate complexes, by compartmentalizing these metals in roots and shoots – to immobilize the molecules and prevent the translocation of metals by the plant – or, in some specific cases, the interaction of Si promotes a homogeneous distribution of these metals throughout the plant, preventing accumulation and consequent necrosis [10–13]. In addition, Si seems to regulate genes expression responsible for alleviating the toxicity of these metals (as the PCS1, SAP1, and SAP14 genes) which encode members of the stress-associated protein (SAP) family, and the AP2 transcription factor genes Erf020, Hsf31, and NAC6 were up-regulated in the roots of cadmium-stressed rice plants, but were down-regulated in the presence of Si, indicating that post-stress addition of Si was able to reverse cadmium stress [14]. A rice leaf proteome study, also under cadmium stress, showed that 50 proteins, including those associated with photosynthesis, redox homeostasis, protein regulation and synthesis, pathogen response, and chaperone activity were up-regulated by Si, while a POX III class and proteins such as thaumatin were found to be regulated by Si independently of cadmium [15]. According to Rizwan et al. [16], Si has also been shown to decrease water stress and

salinity in plants through different mechanisms, which include reducing oxidative stress, modifying gene expression and regulating compatible solutes, increasing absorption and translocation of mineral nutrients – such as phosphorus, potassium, calcium, and magnesium –, modification of gas exchange attributes, osmotic adjustment, and phytohormone synthesis. For example, in the study conducted by Gao et al. [17], photosynthesis, stomatal conductance, transpiration rate, water use efficiency, the number of stomata, and stomatal size in okra plants exposed to salt stress were increased by the application foliar of Si. Matoh et al. [18] reported such a case for rice, which was grown *in vitro* containing solution with additions of NaCl, seawater, or polyethylene glycol in high concentrations. In this experiment, Si reduced NaCl translocation to shoots and increased dry mass production of stressed plants compared to controls. For wheat [19] and barley [20], similar findings were reported, showing that there was the repression of NaCl transporters in plants grown in saline solutions supplemented with Si, with concomitant improvement in growth. Regarding salinity, it is known that Si reduces Na^+ uptake by stimulating the H^+ -ATPase activity of the root plasma membrane [21] and reduces Na^+ translocation promoting its binding to cell walls, thus reducing Na^+ in the leaf apoplast [22]. The interaction between plants and their environment, and in this case, diseases and pests, occurs primarily through the surface of plants, both below and above the ground. The cell wall and its cuticle is the main defense against these violations. In this sense, the granted Si defense to plants refers to the deposition of crystals on the plant cell wall, creating a physical barrier against pathogens and phytophagous insects. However, Si is involved in very complex defense mechanisms but is not fully understood. Some studies show that Si triggers a cascade of biochemical defense mechanisms in infected plants [23], which showed that Si induced resistance to fungal attack in cucumber roots and to the precursors of fungi toxic aglycones appearance that possibly acts as phytoalexins. Currently, Si is related to a type of acquired systemic resistance (SAR), which is characterized by the salicylic acid and proteins related to pathogenesis accumulation. This response is typically induced by pathogens, but its effect can be mimicked by activators, such as Si. Other defense mechanisms mobilized by Si include the accumulation of lignin and, generally, phenolic compounds, in addition to peroxidases and chitinases [24]. Yet, according to Debona et al. [7], studies have revealed that in many plant species nourished with Si, the phenylpropanoid and terpenoid pathways are greatly potentiated, and the transcription of genes involved in host defense and higher activities of defense enzymes are faster and stronger. Unfortunately, the recognition of Si as a fertilizer component that could be used in an integrated pest and disease management strategy is still very recent.

3. The role of Si in plant tissue culture development

The growth and morphogenesis of in vitro cultures of plant cells, tissues, and organs are directly influenced by the composition of the culture medium, which is often modified to stimulate the growth and development of specific plant materials. In general, the plant tissue culture medium is composed of inorganic nutrients, organic supplements, a carbon source, growth regulators, and a solidifying agent. The optimization of mineral nutrients is a fundamental step in the process of preparing the culture medium, aiming at the growth and morphogenesis of plant cells, tissues, and organs cultivated through in vitro techniques [25,26]. As previously seen, the exogenous application of Si improves the growth and yield of several plant species, especially when subjected to different types of biotic and abiotic stresses [27]. Although the availability of Si in hydroponic culture systems and the substrate is still quite restricted, it is known that the supplementation of this mineral in nutritious culture media or soilless substrate has significantly improved the growth characteristics, yield, and quality of several crops [28]. The Si inclusion in the culture medium also demonstrated the morphogenetic potential improvement in plant cells, tissues, and organs cultivated in vitro. Studies have shown that the Si inclusion in the tissue culture medium increases callus growth, stem regeneration, and root induction and stimulates somatic embryogenesis, in addition to improving the morphological, anatomical, and physiological characteristics of shoots [29–33]. Beyond that, Si treatment prolongs the longevity of calluses and organs with the potential for plant regeneration [26]. Concerning somatic embryogenesis, some studies show that Si promotes viable embryo development in its most diverse forms. For example, Na₂SiO₃ induced somatic embryos formation in callus generated from root explants of *Phragmites australis* [34]. Abiri et al. [35] showed the efficiency of potassium metasilicate in callus proliferation and somatic embryo formation from root explants of rice cultivar. In palm leaf explants, K₂SiO₃ and Na₂SiO₃ demonstrated the highest rates of somatic embryo formation – and germination of these embryos – from in vitro cultured embryogenic calluses, compared to treatments in which there was no addition of somatic sources [36]. In vitro tissue culture of woody plants often includes many disorders, such as tissue browning, which results from the phenolic oxidative activity, in addition to hyperhidrosis. In this sense, it has been shown that Si can completely prevent this tissue oxidation in guava (*Psidium guajava*) by sealing the nodal explants cut ends using a silicone mixture, without causing any subsequent damage to the rest of the propagation in vitro. [37]. Fadl [36] demonstrated that the levels of oxidation and hyperhidrosis decreased with the

greater addition of K₂SiO₃ and Na₂SiO₃ to the culture medium of embryogenic palm callus. The addition of Si to the culture medium caused less hyperhidrosis in *Ornithogalum dubium* [38] and *Cotoneaster wilsonii* [39]. Si treatment also improved the survival rate of grape callus at low temperatures due to the prevention of tissue oxidation [40]. Si has not been included in any commercial tissue culture medium formulation despite its demonstrated efficacy. These studies indicate that the effect of Si on the morphogenetic potential of in vitro plant cultures depends on the species used, the genotype, and the concentration of Si in the culture medium. Furthermore, it is important to note that the absorption and transport of Si depend on the plant species being used, as well as the external concentration of Si and the active or passive capacity of Si absorption by each different plant species [6,41]. Despite knowing that Si is a mineral that can be efficiently used as a supplement to the culture medium of several plant species, further studies are needed to better understand the biochemical and molecular mechanism of Si in other mass propagation methods such as organogenesis and somatic embryogenesis. In conclusion, Despite knowing that more studies are needed to better understand the biochemical and molecular mechanism of Si in other methods of mass propagation, such as organogenesis and somatic embryogenesis, it is necessary to admit that Si is a mineral that can be efficiently used. as a supplement to the culture medium of several plant species which aim at mass propagation.

3. Acknowledgement

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported this work.

4. References

- [1] Bache BW. G. Sposito 1989. The Chemistry of Soils. xiii 277 pp. Price £25.00 (hard covers). New York, Oxford: Oxford University Press. ISBN 0 19 504615 3. Geol Mag 1990;127:190–191. <https://doi.org/10.1017/S0016756800014059>.
- [2] Epstein E. The anomaly of silicon in plant biology. Proc Natl Acad Sci U S A 1994;91:11–7. <https://doi.org/10.1073/pnas.91.1.11>.
- [3] Abreu RAA, Assis FA, Souza BHS, Nascimento AM, Latini AO, Pio LAS. Effects of silicon application on the biochemistry of passion fruit and performance of Dione juno juno (Lepidoptera: Nymphalidae). Arthropod Plant Interact 2021;15:417–29. <https://doi.org/10.1007/s11829-021-09827-3>.

- [4] Epstein E. SILICON. *Annu Rev Plant Physiol Plant Mol Biol* 1999;50:641–64. <https://doi.org/10.1146/annurev.arplant.50.1.641>.
- [5] Hildebrand M, Volcani BE, Gassmann W, Schroeder JI. A gene family of silicon transporters. *Nature* 1997;385:688–9. <https://doi.org/10.1038/385688b0>.
- [6] Sahebi M, Hanafi MM, Siti Nor Akmar A, Rafii MY, Azizi P, Tengoua FF, et al. Importance of Silicon and Mechanisms of Biosilica Formation in Plants. *Biomed Res Int* 2015;2015:396010. <https://doi.org/10.1155/2015/396010>.
- [7] Debona D, Rodrigues FA, Datnoff LE. Silicon's Role in Abiotic and Biotic Plant Stresses. *Annu Rev Phytopathol* 2017;55:85–107. <https://doi.org/10.1146/annurev-phyto-080516-035312>.
- [8] Tripathi DK, Singh S, Singh VP, Prasad SM, Chauhan DK, Dubey NK. Silicon Nanoparticles More Efficiently Alleviate Arsenate Toxicity than Silicon in Maize Cultivar and Hybrid Differing in Arsenate Tolerance. *Front Environ Sci* 2016;4:46. <https://doi.org/10.3389/fenvs.2016.00046>.
- [9] Anwaar SA, Ali S, Ali S, Ishaque W, Farid M, Farooq MA, et al. Silicon (Si) alleviates cotton (*Gossypium hirsutum* L.) from zinc (Zn) toxicity stress by limiting Zn uptake and oxidative damage. *Environ Sci Pollut Res Int* 2015;22:3441–50. <https://doi.org/10.1007/s11356-014-3938-9>.
- [10] Adrees M, Ali S, Rizwan M, Zia-Ur-Rehman M, Ibrahim M, Abbas F, et al. Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: A review. *Ecotoxicol Environ Saf* 2015;119:186–97. <https://doi.org/10.1016/j.ecoenv.2015.05.011>.
- [11] Ding X, Zhang S, Li S, Liao X, Wang R. Silicon Mediated the Detoxification of Cr on Pakchoi (*Brassica Chinensis* L.) in Cr-contaminated Soil. *Procedia Environ Sci* 2013;18:58–67. <https://doi.org/https://doi.org/10.1016/j.proenv.2013.04.009>.
- [12] Li L, Zheng C, Fu Y, Wu D, Yang X, Shen H. Silicate-mediated alleviation of Pb toxicity in banana grown in Pb-contaminated soil. *Biol Trace Elem Res* 2012;145:101–8. <https://doi.org/10.1007/s12011-011-9165-z>.
- [13] Liang X, Wang H, Hu Y, Mao L, Sun L, Dong T, et al. Silicon does not mitigate cell death in cultured tobacco BY-2 cells subjected to salinity without ethylene emission. *Plant Cell Rep* 2015;34:331–43. <https://doi.org/10.1007/s00299-014-1712-6>.
- [14] Farooq MA, Detterbeck A, Clemens S, Dietz K-J. Silicon-induced reversibility of cadmium toxicity in rice. *J Exp Bot* 2016;67:3573–85. <https://doi.org/10.1093/jxb/erw175>.
- [15] Nwugo CC, Huerta AJ. The Effect of Silicon on the Leaf Proteome of Rice (*Oryza sativa* L.) Plants under Cadmium-Stress. *J Proteome Res* 2011;10:518–28. <https://doi.org/10.1021/pr100716h>.
- [16] Rizwan M, Ali S, Ibrahim M, Farid M, Adrees M, Bharwana SA, et al. Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. *Environ Sci Pollut Res* 2015;22:15416–31. <https://doi.org/10.1007/s11356-015-5305-x>.
- [17] Gao X, Zou C, Wang L, Zhang F. Silicon Decreases Transpiration Rate and Conductance from Stomata of Maize Plants. *J Plant Nutr - J PLANT NUTR* 2006;29:1637–47. <https://doi.org/10.1080/01904160600851494>.
- [18] Matoh T, Kairusmee P, Takahashi E. Salt-Induced Damage to Rice Plants and Alleviation Effect of Silicate. *Soil Sci Plant Nutr* 1986;32:295–304. <https://doi.org/10.1080/00380768.1986.10557506>.
- [19] Ahmad R, Zaheer SH, Ismail S. Role of silicon in salt tolerance of wheat (*Triticum aestivum* L.). *Plant Sci* 1992;85:43–50. [https://doi.org/https://doi.org/10.1016/0168-9452\(92\)90092-Z](https://doi.org/https://doi.org/10.1016/0168-9452(92)90092-Z).
- [20] Liang Y. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. *Plant Soil* 1999;209:217. <https://doi.org/10.1023/A:1004526604913>.
- [21] Xu CX, Ma YP, Liu YL. Effects of silicon (Si) on growth, quality and ionic homeostasis of aloe under salt stress. *South African J Bot* 2015;98:26–36. <https://doi.org/https://doi.org/10.1016/j.sajb.2015.01.008>.
- [22] Habibi G, Norouzi F, Hajiboland R. Silicon

- alleviates salt stress in pistachio plants. *Prog Biol Sci* 2014;4:189–202.
- [23] Chérif M, Benhamou N, Menzies JG, Bélanger RR. Silicon induced resistance in cucumber plants against *Pythium ultimum*. *Physiol Mol Plant Pathol* 1992;41:411–25. [https://doi.org/https://doi.org/10.1016/0885-5765\(92\)90053-X](https://doi.org/https://doi.org/10.1016/0885-5765(92)90053-X).
- [24] Schneider S, Ullrich WR. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiol Mol Plant Pathol* 1994;45:291–304. [https://doi.org/https://doi.org/10.1016/S0885-5765\(05\)80060-8](https://doi.org/https://doi.org/10.1016/S0885-5765(05)80060-8).
- [25] Reed BM, Wada S, DeNoma J, Niedz RP. Mineral nutrition influences physiological responses of pear in vitro. *Vitr Cell Dev Biol - Plant* 2013;49:699–709. <https://doi.org/10.1007/s11627-013-9556-2>.
- [26] Sivanesan I, Park SW. The role of silicon in plant tissue culture. *Front Plant Sci* 2014;5:571. <https://doi.org/10.3389/fpls.2014.00571>.
- [27] Ma JF, Yamaji N. Silicon uptake, and accumulation in higher plants. *Trends Plant Sci* 2006;11:392–7. <https://doi.org/10.1016/j.tplants.2006.06.007>.
- [28] Voogt W, Sonneveld C. Chapter 6 Silicon in horticultural crops grown in soilless culture. In: Datnoff LE, Snyder GH, Korndörfer GH, editors. *Silicon Agric.*, vol. 8, Elsevier; 2001, p. 115–31. [https://doi.org/https://doi.org/10.1016/S0928-3420\(01\)80010-0](https://doi.org/https://doi.org/10.1016/S0928-3420(01)80010-0).
- [29] Islam M, Ahmed M, Mahalder D. In Vitro Callus Induction and Plant Regeneration in Seed Explants of Rice (*Oryza Sativa* L.) 2004;1.
- [30] Máthé C, Mosolygó Á, Surányi G, Beke A, Demeter Z, Tóth VR, et al. Genotype and explant-type dependent morphogenesis and silicon response of common reed (*Phragmites australis*) tissue cultures. *Aquat Bot* 2012;97:57–63. <https://doi.org/https://doi.org/10.1016/j.aquabot.2011.11.005>.
- [31] Braga F, Nunes C, Favero A, Pasqual M, Carvalho J, Castro E. Anatomical characteristics of the strawberry seedlings micropropagated using different sources of silicon. *Pesqui Agropecuária Bras* 2009;44:128–32.
- [32] Soares J, Pasqual M, Rodrigues F, Villa F, Araújo A. Silicon sources in the micropropagation of the *Cattleya* group orchid. *Acta Sci Agron* 2011;33:503–7.
- [33] Sivanesan I, Jeong BR. Silicon Promotes Adventitious Shoot Regeneration and Enhances Salinity Tolerance of *Ajuga multiflora* Bunge by Altering Activity of Antioxidant Enzyme. *Sci World J* 2014;2014:521703. <https://doi.org/10.1155/2014/521703>.
- [34] He C, Wang L, Liu J, Liu X, Li X, Ma J, et al. Evidence for ‘silicon’ within the cell walls of suspension-cultured rice cells. *New Phytol* 2013;200:700–9. <https://doi.org/https://doi.org/10.1111/nph.12401>.
- [35] Abiri R, Maziah M, Shaharuddin NA, Yusof ZNB, Atabaki N, Hanafi MM, et al. Enhancing somatic embryogenesis of Malaysian rice cultivar MR219 using adjuvant materials in a high-efficiency protocol. *Int J Environ Sci Technol* 2017;14:1091–108. <https://doi.org/10.1007/s13762-016-1221-y>.
- [36] Fadl R. EFFECT OF SILICON ON SOMATIC EMBRYOGENESIS AND SHOOT REGENERATION OF DRY DATE PALM (*PHOENIX DACTYLIFERA* L.) CV BARTAMUDA. *Egypt J Desert Res* 2014;64:65–82. <https://doi.org/10.21608/ejdr.2014.5810>.
- [37] Youssef MA, El-Helw MR, Taghian AS, El-Aref HM. IMPROVEMENT OF PSIDIUM GUAJAVA L. USING MICROPROPAGATION. *Acta Hort.*, International Society for Horticultural Science (ISHS), Leuven, Belgium; 2010, p. 223–30. <https://doi.org/10.17660/ActaHortic.2010.849.24>.
- [38] Ziv M. Silicon effects on growth acclimatization and stress tolerance of bioreactor cultured *Ornithogalum dubium* plants. *Acta Hort* 2010;865:29–35. <https://doi.org/10.17660/ActaHortic.2010.865.2>.
- [39] Sivanesan I, Song J, Hwang S, Jeong B. Micropropagation of *Cotoneaster wilsonii* Nakai—a rare endemic ornamental plant. *Plant Cell Tissue Organ Cult* 2010;105:55–63. <https://doi.org/10.1007/s11240-010->

9841-2.

- [40] Xiaoyu D, Min T, WeiShuang W. Effects of Silicon on Physiology and Biochemistry of *Dendrobium moniliforme* Plantlets under Cold Stress, 2013.
- [41] Liang Y, Sun W, Zhu Y-G, Christie P. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environ Pollut* 2007;147:422-8.
<https://doi.org/10.1016/j.envpol.2006.06.008>.