RESEARCH ARTICLE

Silicon: its manifold roles in plants

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Abstract

The title of this essay declares that silicon does have roles in plants and all participants in this conference know that that is so. This knowledge, however, is not shared by the general community of plant biologists, who largely ignore the element. This baffling contrast is based on two sets of experience. First, higher plants can grow to maturity in nutrient solutions formulated without silicon. That has led to the conventional wisdom that silicon is not an essential element, or nutrient, and thus can be disregarded. Second, the world's plants do not grow in the benign environment of solution culture in plant biological research establishments. They grow in the field, under conditions that are often anything but benign. It is there, in the real world with its manifold stressful features, that the silicon status of plants can make a huge difference in their performance. The stresses that silicon alleviates range all the way from biotic, including diseases and pests, to abiotic such as gravity and metal toxicities. Silicon performs its functions in two ways: by the polymerization of silicic acid leading to the formation of solid amorphous, hydrated silica, and by being instrumental in the formation of organic defence compounds through alteration of gene expression. The silicon nutrition of plants is not only scientifically intriguing but also important in a world where more food will have to be wrung from a finite area of land, for that will put crops under stress.

Silicon in plants: two views

Silicon, as all of us here know, is an important element in plant nutrition. 'All of us here', however, does not include the larger community of plant biologists, the great majority of whom entirely ignore the element. What goes on here? Why this discrepancy between our interest in the element and its disregard on the part of so many of our plant biological colleagues?

Let us look at these conflicting attitudes separately. First then, ours. The previous three Silicon in Agriculture conferences (Datnoff *et al.,* 2001; Silicon in Agriculture Organizing Committee, 2002; Korndörfer, 2005) provided ample evidence that in many areas large numbers of crops, rice, *Oryza sativa*, prominently among them, benefit from silicon applications. Silicon particularly improves disease resistance (Datnoff *et al.*, 2007) but other stresses, both biotic and abiotic, are also mitigated by silicon applications.

But it is not just experience from the field that has convinced us that silicon is significant in plant biology. No, basic laboratory research leads to the same conclusion. Let me give a few examples from our laboratory.

To be effective in a plant, an element has first to be absorbed by it. We have carried out experiments on silicon transport in wheat (Rafi & Epstein, 1999). In a long-term experiment on the absorption of silicon by wheat, *Triticum aestivum*, four plants were grown in 100-L volumes of nutrient solution with an initial silicon concentration of just above 0.5 mM. At first the plants, still small seedlings, did not make much of a dent in the concentration of silicon, because of the large supply of it in the solution. But as they grew they depleted the silicon and by Day 84 silicon could no longer be detected in the solution. We also grew a parallel set of four plants in a solution to which silicon had not been added. When these plants, by now mature, were then transferred to fresh solutions at the same silicon

concentration as the initial one they rapidly depleted the solution of silicon, and the rate of its uptake differed little whether the plants were preloaded with silicon or grown previously in a minus-silicon solution. The internal silicon concentrations reached levels hundreds of times higher than the ambient ones.

This and similar experiments suggested an active transport of silicon. So subsequently, we did short-term experiments on the kinetics of silicon absorption by wheat (Rains *et al.*, 2006). The concentration isotherm showed simple Michaelis–Menten kinetics.

At an external concentration of 1 mM (28 ppm Si), the rate of absorption was close to the theoretical maximal rate asymptotically approached, V_{max}. The Michaelis constant, K_m, the concentration giving half the maximal rate of absorption, and a measure of the affinity of the transport protein for the element, was 0.086 mM, or 2.4 ppm.

Another feature of silicon absorption is its selectivity. Phosphate, even at concentrations way in excess of the silicon concentration, entirely fails to change the rate of silicon uptake. Only germanium, something of a silicon analogue or doppelgänger, competes with silicon in the process of absorption, and does so with virtually equal affinity for the transporter (Rains *et al.*, 2006). This and much other research clearly indicates the operation of silicon membrane transporters; see the review by Currie and Perry (2007). Furthermore, Ma and his collaborators have characterised several silicon transport genes in rice; see Yamaji *et al.* (2008) and references there.

This leads to another question. In what form is silicon transported upward toward the shoot? We grew wheat to maturity in minus-silicon nutrient solution, and for the experiment proper transferred the plants to a solution containing 0.02 mM silicic acid enriched to 98.7 atom percentage in [29]Si (Casey *et al.*, 2003). (The normal abundance of this stable silicon isotope is a mere 4.67%.) We then detoppped the plants and collected xylem exudate. As in the earlier experiments, chemical analysis showed silicon values hugely in excess of the 0.02 mM external one. When we determined the [29]Si nuclear magnetic resonance (NMR) spectra of the exudate, we found only two aqueous silicate species, viz. mono- and some disilicic acid. We did not detect any organosilicate complexes. This does not, of course, preclude the possibility of such complexes forming as the silicon moves up into the shoot. Indeed, that occurs, as we will see.

How does all this bear on the significance of silicon? Every one of the features of silicon transport that I have discussed is similar to the corresponding ones for other elements such as potassium. Absorption to the extent of very high accumulation ratios, absorption rates following Michaelis–Menten kinetics, selectivity except for the case

of one look-alike element, and finally, upward movement in the xylem as the purely inorganic solute – in all these features plants handle silicon just like potassium and other nutrient elements (Epstein & Bloom, 2005).

When we look at all that kind of evidence for potassium we do not for a moment think that its transport mechanisms came about through some evolutionary caprice. Rather, they were generated through Darwinian natural selection because they fulfilled physiological and biochemical functions. That then surely must apply to silicon transport as well. Together with the field experience I mentioned earlier, this and much other basic research validates the conviction that silicon is an important element in plant nutrition.

Let us now turn to the other view, the one held by the majority of plant biologists, to whom silicon is a plant nutritional nonentity. Why do they not care? The answer to that question is simply this: in the middle of the 19th century, plant physiologists developed the technique of solution culture and promptly found that they did not need to include silicon in the formulation of nutrient solutions. That led to the conclusion that silicon is 'superfluous for the purposes of nutrition and growth', according to the foremost plant physiologist of the mid-19th century, Julius Sachs. Omission of silicon from the formulation of nutrient solutions has been routine ever since.

We must remember that at the time that this mindset about silicon took shape there did not exist any of the field experience and of the physiological evidence that I have mentioned. But even now, with all that evidence at hand, the conventional view persists that silicon performs no essential functions in plants, and therefore can be disregarded. That is not even a reasoned conclusion; rather, plant scientists are entirely oblivious of the element even when the context calls for attention to it (McKersie & Leshem, 1994; Pessarakli, 1999; Sanita di Toppi & Pawlik-Skowaronska, 2003; Jander & Howe, 2008).

That indifference to the element is no longer tenable. The plant physiologist's solution-cultured plants are experimental artifacts: there are no minus-silicon plants in nature and in agriculture. It is possible, indeed likely, that experiments performed with plants grown in conventional, minus-silicon solutions may give misleading results. I will discuss the kinds of experiments where that is most likely to happen.

That said I will now turn devil's advocate and ask: can a case be made for the view that silicon is of dubious significance in plant biology? There is, first, the fact that most plants, except the diatoms and, among vascular plants, the Equisetaceae, can indeed be grown in minus-silicon nutrient solutions. They are not entirely normal

plants (Epstein & Bloom, 2005), but nevertheless, even rice, which accumulates more silicon than any other crop (Ma & Tamai, 2002), can be grown to maturity in nutrient solutions to which silicon has not been added; in our laboratory, we do this routinely with wheat.

Another puzzle is this. The range of the silicon contents of plant species, and even subspecies, is wider than is the case for any other element. Is it likely that plants require for indispensable primary metabolism an element that in some plants is present at a fraction of one percent of that in other plants (Hodson et al., 2005)? By way of contrast, the concentration of potassium in numerous plant genotypes varies by no more than a factor of about 10 (Barker & Pilbeam, 2007). The only other elements that vary widely in their amounts in plant tissues are sodium and chlorine, but that is because of the occurrence of these elements in halophytes, plants adapted to saline habitats.

Silicon: its role is defence

In view of all this, we have to face up squarely to the dilemma that weighty arguments can be made both for and against the view that silicon plays a significant role in plants. What is the solution of this conundrum? What role has evolution assigned to this element? The answer is defence – defence against environmental onslaughts, both biotic and abiotic. Under the artificially benign conditions of the plant physiological greenhouse or controlled environment facility silicon often cannot be shown to make much difference. Out in the real world, however, the world of the field, plants are the targets of a host of assaults: insects and other herbivores, bacteria, fungi, wind, cold, heat, salinity, mineral and water shortages or excesses – it is in defence against such onslaughts that silicon comes into play. When considering the role of silicon in plants we must therefore make a rather sharp distinction between plants under fairly benign and definitely adverse conditions.

Defence: physical

Let us then briefly discuss what organisms can do to defend themselves against external threats, physical ones to begin with. The rooted plant lacks the locomotive and behavioural means that animals have to defend themselves. The plant cannot run away, it cannot claw or bite or growl. It has only two means of defence: physical and chemical. The physical features include structures such as thorns, spines, trichomes, raphides, rough, tough epidermal cells, and hard shells and pods. Many plants armour themselves with solid hydrated amorphous silica, or opal, incorporated in cell walls. There is ample evidence for the protection that silicon often provides plant species

against insect pests. Reinforcement of the cell wall by deposition of solid silica in them is one of the ways in which this protection is effected. Currie and Perry (2007) have discussed the biomineralization of silicon.

We have studied this deposition of silicon in the cell wall of wheat leaves and awns (Rafi et al., 1997). The joint use of scanning electron micrography and X-ray microanalysis of the same trichomes showed the localization of silica in the trichomes; none could be detected in the trichomes of plants grown in minus-silicon solutions. It is the silica in trichomes that lends leaves and awns the roughness and the toughness that impede the penetration of herbivores and pathogens through the cell walls. It acts as a physical barrier. That, then, is one of the means by which silicon defends plants subject to attack.

Defence: chemical

The other way plants can play defence is chemistry, and they do so in a very big way. Plants synthesise a huge number of secondary metabolites – compounds that are not part and parcel of indispensable primary 'housekeeping' metabolism but rather are instrumental in attracting or repelling the numerous heterotrophs that share their environment. Hartmann (2008) has traced the early development of ideas concerning biochemical defences that have evolved in the plant kingdom. The early studies were made in the 19th century but then largely forgotten until the mid-20th century (Hartmann, 2008). By now, however, it is clear that secondary metabolites are hugely important in the defence of plants against, mainly, biological attacks, especially by insects, but many other adversities as well. The sheer number of these multifarious chemical structures is immense; by one estimate (Hartmann, 2008) there are more than 200 000 of them.

Secondary metabolites influence the interactions between plants and the organisms that inhabit their environment: insects and other animals, microbes and fungi. Their chemistry is exceedingly diverse, but those that afford defence against biotic stresses are terpenoids, alkaloids and various phenolic compounds (Buchanan et al., 2000). They are obnoxious, repellent or downright toxic to biotic attackers of plants. Secondary metabolites are not, however, the only chemical defence agents in plants. A host of biochemical entities that are part of essential primary metabolism also participate; phytohormones including salicylic acid, jasmonic acid and ethylene loom large. Organic acids are particularly important in the defence against metal toxicities (Delhaize et al., 1993).

How does silicon participate in this chemical warfare? It often promotes the biosynthesis of defence compounds. That much is incontrovertible by now, and I will give

some of the evidence for it. What we do not have good knowledge of, as yet, are the connecting links between silicon and the synthesis of defence metabolites (Datnoff *et al.*, 2007). It is recognised in any event that responses may be specific to the biotic attacker or may be in the nature of systemic acquired resistance.

When plants are under attack by external agents signalling cascades are induced that result in proteins entering the nucleus where they regulate the transcription of target genes. Silicon in the form of solid silica, or plant opal, cannot influence plant biochemistry or molecular biology; only silicon in solution can have such effects (Samuels *et al.*, 1991; Cherif *et al.*, 1992). These investigators produced clear-cut evidence that cucumber was afforded protection against fungal diseases by silicon in solution in the plants, not by polymerised or solid silicon. Soluble silicon, silicic acid, cannot affect metabolism by its mere presence. The conclusion emerges that the sequence of events must begin with silicon becoming the ligand of an organic metabolite. There is indeed evidence of an affinity of many types of organic compounds and complexes for silicon (Kinrade *et al.*, 1999; Balec *et al.*, 2005); that was noted in the previous Silicon in Agriculture conferences and most recently reviewed by Datnoff *et al.* (2007).

Is the initial response to stress the only one to involve silicon? We do not know, but we do know that when stressed, plants often respond by up- and down-regulation of genes, and that silicon affects that response. Fauteux *et al.* (2006) examined the role of silicon in *Arabidopsis* infected by powdery mildew. In plants not so infected silicon made little difference in gene expression. In plants infected by powdery mildew numerous genes were differentially expressed, and silicon promoted that response. Watanabe *et al.* (2004) had found that several rice genes were regulated by silicon but they were few in number. These authors correctly commented that that was because of the benign conditions under which the plants had been grown in their experiments. There is much overlap or cross-talk between responses to pathogens and other biotic and abiotic stimuli (AbuQamar *et al.* (2008) and references there).

This discussion of chemical defence has so far dealt mainly with biotic stresses – pathogens and pest organisms. The role of silicon in mitigating the effects of these assaults has taken pride of place in the research on silicon in plants, as documented by Datnoff *et al.* (2007). Research on relief from abiotic stress has lagged. There is, however, perfectly good evidence that silicon ameliorates the damage to plants caused by such adversities as metal toxicities, salinity, drought or water logging, temperature extremes, and still others; see Epstein (1999), Ma (2005), and Currie and Perry (2007) and references there.

These stresses differ in an important way from biotic ones. Pathogens attack some target points on the plant's surface, and so do insects and nematodes. In general that is not the case for abiotic stresses. They impinge directly on the whole plant, or at least, the whole root system, or the shoot, in its entirety. Drought, for example, affects the plant all the way from the tips of its roots to the uppermost leaves. Similar considerations hold true for most other abiotic stresses. Metal toxicity or salinity targets the entire root system, and a brief cold spell, the shoot. We might therefore surmise that long-distance signalling plays a lesser role in responses to abiotic stresses than is known to be the case for biotic ones, but there is no evidence one way or the other. It is intriguing that silicon provides defence against environmental aggressions that differ so greatly in their targeting of the plants.

There is another difference between the responses of plants to biotic and abiotic stresses. Biotic stresses tend to elicit defence mechanisms in plants, as we have seen. The biotic stress agents, be they pests or microorganisms or viruses, in turn evolve means to overcome these defences; in other words, there results an evolutionary arms race (Dawkins & Krebs, 1979; Holab, 2001). The plants often use silicon in their defence, as discussed here. No such mutual relationship exists between plants and abiotic stressors. The defence mechanisms that have evolved in plants do not elicit countermeasures in toxic metals or salinity or drought. Silicon plays roles in the defence of plants against these stresses too. Thus, whether there is an arms race or purely unilateral defence, silicon is often involved. We are just beginning to elucidate the biochemical and molecular biological mechanisms by which it fulfills activating or potentiating roles.

Conclusion

Silicon plays an astonishingly large number of diverse roles in plants, and does so primarily when the plants are under stressful conditions, whereas under benign conditions its role is often minimal or even nonexistent. It cannot therefore be counted among the indispensable, primary essential nutrients. Rather, its roles may be compared with those of the organic secondary metabolites. It is indeed likely that this 'quasi-essential' element is instrumental in the generation of these defence metabolites. Therefore, in analogy to organic secondary metabolites, silicon may be looked upon as an inorganic secondary nutrient. Seeing the manifold roles that silicon plays in the defence of plants against all manner of adversities we must admit that we are still far from formulating a 'unified field theory' of silicon in agriculture.

This discussion has mainly dealt with pure science. There is, however, another focus of interest: applied science. The planet's population, now 6.8 billion, will grow to 9 billion in about three decades. We will need to grow more food on a finite area of land, the best of which is already cropped. More intensive cropping of good land, or putting marginal land into production, will put crops under stress – the very condition under which the role of silicon often looms large. There is work to be done!

Acknowledgements

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