DOI: 10.1007/s10535-016-0633-3 BIOLOGIA PLANTARUM 61 (1): 187-191, 2017

BRIEF COMMUNICATION

Silicon modifies both a local response and a systemic response to mechanical stress in tobacco leaves

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Abstract

Both lignin and silicon (Si) are major players in the resistance of plants to mechanical stress (MS). Focusing on the phenolic metabolism, here we studied the short-term effects of a local MS on tobacco (*Nicotiana rustica* L. cv. Basmas) plants with Si (+Si, 1 mM Na_2SiO_3) and without Si (–Si) treatments in order to see how Si may modify local and systemic responses. One week after starting the Si treatment, a half of the plants were exposed to a mechanical pressure applying 980 Pa for 24 h on the upper side of the 3rd leaf of each plant (+MS). The rest of the plants remained unstressed (–MS). Plants were harvested 24 h and 72 h after starting the MS and the leaves directly exposed to the mechanical stress (DMS) and those indirectly exposed to the mechanical stress (IMS) from below and above the DMS leaf were analyzed for phenolic metabolism along with the corresponding leaves from –MS plants. In the DMS leaf, the activities of polyphenol oxidase, phenylalanine ammonia lyase, and cytosolic and covalently-bound peroxidases increased by the MS, while decreased by Si. In accordance with this in the DMS leaf, the content of soluble and cell wall-bound phenolics and lignin were enhanced by the MS but decreased by Si. Interestingly, Si influenced the pattern of response to the MS depending on whether the leaves were directly treated by the MS or not. Silicon treatment augmented MS-induced lignin accumulation in the DMS leaf while rather inhibited lignin formation in the IMS leaves. These data show that Si modified MS-mediated changes in the phenolic metabolism differently in local and systemic leaves.

Additional key words: cell wall-bound phenolics, lignin, *Nicotiana rustica*, peroxidase, phenylalanine ammonia lyase, polyphenol oxidase.

Plant growth and development are affected not only by the usual environmental factors but also by those of mechanical nature. Our knowledge on the effect of mechanical stress (MS) is much more limited than that for other stress factors. It is well known that biotic and abiotic stresses induce defense responses in plants including activation of secondary metabolite pathways (Dixon and Paiva 1995). Activation of the phenylpropanoid pathway as the main metabolic route for the synthesis of natural secondary metabolites, such as phenolics, flavonoids, lignin, *etc.*, has been reported under low temperature, drought, and exposure to high photosynthetically active or UV-radiation (Dixon and Paiva 1995, Weaver and Herrmann 1997, Moura *et al.* 2010).

Besides defenses based on organic compounds, Si plays an important role in plant tolerance to abiotic and biotic stresses (Currie and Perry 2007, Hajiboland 2012). In response to pathogen attack, plants supplied with Si produce phenolics and phytoalexins (Van Bockhaven *et al.* 2012), have higher activities of peroxidases, polyphenol oxidases, and other enzymes of the phenylpropanoid pathway (Chérif *et al.* 1994, Shetty *et al.* 2011). The influence of Si on plant structural resistance has been studied in Si-accumulators and only under long-term growth under field conditions (Guntzer *et al.* 2012). However, the effect of Si on the enhancement of structural support through modification of phenolic metabolism under MS has not been studied so far.

Submitted 8 February 2016, *last revision* 23 March 2016, *accepted* 7 April 2016.
Abbreviations: CW - cell wall; DMS - direct mechanical stress; IMS - indirect mechanical stress; MS - mechanical stress; PAL - phenylalanine ammonia lyase; POD - peroxidase; PPO - polyphenoloxidase.
Acknowledgements: The Research Council and the Center of Excellence for Biodiversity, the University of Tabriz, are greatly appreciated for financial supports.
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In this work, using the phenolic metabolism as indicator of plant response to stress, we studied the short term effect of MS on the leaves of plants grown in the absence or presence of Si. In order to investigate the local *vs*. systemic response to MS and the influence of Si on this response, the phenolic metabolism was investigated in a time-dependent manner using both the leaves receiving the direct pressure stress treatment (DMS) and the indirectly stressed leaves (IMS) located below and above the DMS leaf.

Four-week-old tobacco (*Nicotiana rustica* L. cv. Basmas) plants were transferred to a hydroponic culture containing Hoagland nutrient solution and precultured for further one week. Thereafter, 1 mM Si (as Na_2SiO_3; *Sigma,* St. Louis, USA) was applied (+Si) or not (–Si). One week after the Si addition, mechanical stress (MS) was applied on the 3rd fully expanded leaf (the DMS leaf). For induction of MS, a 40-g stone (2×2 cm) providing a mechanical pressure of 10 g cm^{-2} (980 Pa) (Wang *et al.* 2006) was gently placed on the upper side of the leaves and left for 24 h. A double layer of filter paper was placed between the stone and the leaf surface and the leaf back was sustained. The plants were harvested 24 and 72 h after starting the MS, and in addition of the DMS leaves, the upper and lower indirectly stressed leaves (IMS leaves) were analyzed. In analogy with the +MS plants, the leaves of similar position were analyzed in the –MS plants. Extractions and assays of polyphenol oxidase (PPO, EC 1.14.18.1), phenylalanine ammonia lyase (PAL, EC 4.3.1.5), peroxidase (POD, EC 1.11.1.7), and phenolic compounds in the soluble and cell wall (CW)-bound fractions, and extraction and assay of lignin were performed according to the methods described previously (Hajiboland *et al.* 2015). After autoclave-induced digestion, Si content was determined by a colorimetric method using ammonium molybdate (Elliott and Snyder 1991). The experimental design was a complete randomized block with two factors and four independent replications. Comparisons of means were performed by Tukey's test ($\alpha = 0.05$).

In the treated leaf (DMS), activity of PPO increased by the MS in both the –Si plants and the +Si plants. The mechanical stress-mediated induction of PPO activity appeared already 24 h after the stress treatment and increased further at the subsequent time interval. Silicon treatment decreased PPO activity in both the –MS treatment and the +MS treatment. Consequently, the extent of the MS effect on the induction of PPO activity was considerably lower in the +Si plants compared with the –Si plants. In the leaves of upper and lower positions (IMS), in contrast, MS-induced PPO activity occurred more prominently in the +Si plants. In the IMS leaves of the –Si plants, PPO activity even declined by the MS 24 h after the treatment (Table 1).

A similar trend was observed for PAL activity. In the DMS leaves from the –Si plants, PAL activity increased much more in response to the MS treatment, while the response of the IMS leaves was considerably higher in the +Si ones (Table 1). In the DMS leaf, the MS-induced increase of soluble POD activity was significantly higher in the –Si plants than in the +Si plants at both time intervals. In the IMS leaves 24 h after the MS treatment, soluble POD activity rather decreased under the MS in the –Si plants while increased in the +Si ones. The induction of soluble POD activity in the IMS leaves 72 h after the MS treatment was much greater in the +Si plants compared to the –Si ones (Table 1).

Ionically CW-bound POD responded differently to the MS than the soluble POD. In the DMS leaf, ionically CW-bound POD activity decreased by the MS in both the –Si plants and the +Si plants and at both reported time intervals. In the IMS leaves, the –Si plants responded consistently to the MS by the induction of ionically CW-bound POD. In the +Si plants, in contrast, ionically CW-bound POD activity decreased 24 h after the MS treatment. The observed increase in activity of ionically CW-bound POD in the +Si plants at 72 h was lower compared to the –Si plants and was not statistically significant (Table 1).

In the DMS leaf, an induction of about 8-fold was observed in activity of covalently CW-bound POD 72 h after the treatment only in the –Si plants. In the upper leaves, MS-responsiveness as a reduction (24 h) or an increase (72 h) in activity of the covalently CW-bound POD was only observed in the +Si plants. In the lower leaf, an induction of covalently bound POD was observed only 72 h after the treatment without any difference between the +Si and –Si plants (Table 1).

Content of soluble phenolics increased by the MS treatment in both tested time intervals. Accumulation of soluble phenolics was consistently higher in the –Si plants compared to the +Si ones not only in the DMS but also in the IMS leaves. Cell wall-bound phenolics increased by the MS treatment in the DMS leaf. However, Si influenced this parameter differently depending on the time interval. At 24 h after the MS treatment, the Si-treated plants had a slightly higher CW-bound phenolics while the opposite was observed after 72 h. In the IMS, the MS-treatment resulted in a slight or significant reduction of CW-bound phenolics in the +Si plants at both time intervals. However, in the IMS leaves of the –Si plants, similar to the DMS leaf, CW-bound phenolics increased upon the MS treatment. Lignin concentration of the DMS leaf increased by the MS and this effect was higher in the +Si (20 %) compared with the –Si (12 %) plants. In the upper and lower leaves, lignin content decreased by the MS in both the +Si plants and the –Si plants 24 h after the MS treatment. At the subsequent time interval, however, lignin content increased in response to the MS in the –Si plants while diminished in the +Si ones (Table 1).

Silicon content in all analyzed fractions was in the range of 70 - 150 µg kg^{-1}(d.m.) depending on plant fraction, thus, expectedly higher in the +Si compared with –Si plants (Table 1).

Severe mechanical stress damages plants and may have a considerable impact on its ecological adaptation and survival. The phenylpropanoid pathway is activated under stresses such as freezing and wounding (Dixon and Paiva 1995, Weaver and Herrmann 1997, Moura *et al.* 2010). These stresses are associated mainly with damaged cell structure implying the irreplaceable role of phenolics and lignin in plant resistance to mechanical injuries. A coherent response of different components of the phenylpropanoid pathway to the MS observed in this work highlights the important role of CW-bound phenolics and lignin in the structural defense against mechanical perturbations. Higher lignin content was detectable as early as 24 h after the MS treatment in this work. In *A. thaliana* in response to injury caused by

Table 1. Effect of a mechanical stress (MS) on the activities of polyphenol oxidase [PPO, mmol(caffeic acid) mg^{-1}(protein) min^{-1}], phenylalanine ammonia lyase [PAL, pmol(cinnamic acid) mg^{-1}(protein) min^{-1}], three cell fractions of peroxidases (PODs) including soluble POD [µmol(tetraguaiacol) mg^{-1}(protein) min^{-1}], ionically cell wall (CW)-bound POD [µmol(syringaldazine) mg^{-1}(protein) min^{-1}] and covalently CW-bound POD [µmol(syringaldazine) mg^{-1}(wall f.m.) min^{-1}], content of phenolic compounds in the soluble [mg(eq. gallic acid) g^{-1}(f.m.)] and cell wall (CW)-bound fractions [mg(eq. gallic acid) g^{-1}(f.m.)], and lignin [µg mg^{-1}(wall d.m.)] at two time intervals, and concentration of Si [mg kg^{-1}(d.m.)] in a leaf directly exposed to the MS (the DMS leaf) and in its upper and lower leaves (IMS) in tobacco plants grown in a hydroponic medium in the absence (–Si) or presence (+Si) of 1 mM Na_2SiO_3. Data are means ± SDs from four independent replications. Data of each column within each parameter indicated by the same letter are not statistically different ($P > 0.05$).

Parameter	Treatments		DMS leaf		IMS (upper) leaf		IMS (lower) leaf	
			24 h	72 h	24 h	72 h	24 h	72 h
PPO	–Si	–MS	11.3 ± 0.59^{b}	10.5 ± 0.98^{b}	20.2 ± 0.63^{b}	6.4 ± 0.89^{c}	9.7 ± 0.80^{a}	7.9 ± 0.75^{c}
	–Si	+MS	22. 3 ± 1.25^{a}	33.2 ± 0.89^{a}	19.2 ± 1.16^{b}	8.1 ± 0.53^{b}	5.8 ± 0.81^{b}	9.7 ± 0.82^{b}
	+Si	–MS	8.1 ± 0.17^{c}	5.7 ± 0.98^{c}	18.1 ± 1.07^{b}	4.0 ± 0.54^{d}	5.0 ± 0.71^{b}	6.9 ± 0.30^{c}
	+Si	+MS	12.1 ± 0.14^{b}	11.7 ± 1.96^{b}	35.0 ± 2.76^{a}	9.8 ± 0.45^{a}	8.2 ± 1.07^{a}	16.3 ± 0.75^{a}
PAL	–Si	–MS	28 ± 2^{a}	32 ± 2^{b}	54 ± 6^{a}	43 ± 6^{ab}	28 ± 3^{a}	37 ± 4^{b}
	–Si	+MS	33 ± 6^{a}	43 ± 4^{a}	48 ± 2^{a}	39 ± 3^{b}	25 ± 5^{a}	40 ± 4^{b}
	+Si	–MS	24 ± 7^{a}	29 ± 5^{b}	30 ± 4^{b}	36 ± 1^{b}	14 ± 5^{b}	35 ± 6^{b}
	+Si	+MS	23 ± 3^{a}	31 ± 4^{b}	48 ± 6^{a}	50 ± 2^{a}	24 ± 2^{a}	61 ± 3^{a}
Soluble POD	–Si	–MS	10.0 ± 1.8^{b}	6.6 ± 1.9^{c}	10.4 ± 1.5^{a}	6.5 ± 2.2^{b}	10.5 ± 3.9^{a}	8.9 ± 1.5^{b}
	–Si	+MS	16.6 ± 0.9^{a}	42.9 ± 1.2^{a}	6.6 ± 1.2^{b}	9.8 ± 0.5^{ab}	5.9 ± 0.3^{b}	21.2 ± 6.2^{a}
	+Si	–MS	7.3 ± 1.2^{c}	4.9 ± 0.7^{c}	5.4 ± 0.9^{b}	1.5 ± 0.4^{c}	7.9 ± 0.6^{ab}	6.2 ± 1.2^{b}
	+Si	+MS	8.6 ± 0.9^{bc}	17.9 ± 3.7^{b}	11.4 ± 1.4^{a}	11.8 ± 2.2^{a}	10.3 ± 1.2^{a}	26.6 ± 2.1^{a}
Ionically bound POD	–Si	–MS	6.10 ± 0.56^{a}	4.73 ± 0.22^{c}	0.58 ± 0.08^{b}	2.31 ± 0.22^{b}	0.83 ± 0.59^{c}	7.45 ± 1.27^{b}
	–Si	+MS	1.12 ± 0.18^{b}	1.69 ± 0.16^{d}	0.86 ± 0.02^{b}	3.02 ± 0.77^{b}	1.37 ± 0.14^{c}	14.17 ± 1.14^{a}
	+Si	–MS	6.34 ± 1.67^{a}	14.76 ± 1.41^{a}	6.01 ± 1.24^{a}	5.29 ± 0.96^{a}	11.24 ± 1.37^{a}	17.35 ± 5.26^{a}
	+Si	+MS	4.79 ± 0.51^{a}	10.64 ± 1.02^{b}	0.85 ± 0.04^{b}	6.48 ± 1.05^{a}	4.60 ± 0.22^{b}	19.97 ± 2.19^{a}
Covalently bound POD	–Si	–MS	1.46 ± 0.42^{a}	0.88 ± 0.02^{b}	1.11 ± 0.19^{b}	0.72 ± 0.04^{b}	1.62 ± 0.36^{a}	1.57 ± 0.14^{ab}
	–Si	+MS	1.13 ± 0.40^{a}	4.55 ± 0.67^{a}	1.03 ± 0.25^{b}	0.68 ± 0.02^{b}	1.52 ± 0.12^{a}	1.92 ± 0.60^{ab}
	+Si	–MS	1.42 ± 0.48^{a}	0.46 ± 0.03^{b}	1.65 ± 0.25^{a}	0.58 ± 0.02^{b}	1.18 ± 0.16^{a}	1.33 ± 0.30^{b}
	+Si	+MS	1.40 ± 1.13^{a}	0.42 ± 0.07^{b}	0.54 ± 0.07^{c}	1.55 ± 0.29^{a}	1.12 ± 0.70^{a}	2.29 ± 0.28^{a}
Soluble phenolics	–Si	–MS	2.51 ± 0.25^{b}	3.22 ± 0.22^{b}	4.11 ± 0.06^{b}	5.35 ± 0.25^{ab}	3.29 ± 0.30^{a}	3.15 ± 0.21^{b}
	–Si	+MS	3.12 ± 0.25^{a}	6.04 ± 0.74^{a}	5.76 ± 1.03^{a}	5.77 ± 1.19^{a}	3.72 ± 0.24^{a}	5.02 ± 0.16^{a}
	+Si	–MS	1.07 ± 0.08^{d}	2.04 ± 0.34^{c}	2.00 ± 0.08^{c}	2.29 ± 0.06^{c}	1.67 ± 0.18^{c}	2.63 ± 0.17^{c}
	+Si	+MS	1.98 ± 0.16^{c}	4.10 ± 0.50^{b}	3.00 ± 0.21^{c}	4.05 ± 0.40^{b}	2.42 ± 0.02^{b}	5.09 ± 0.25^{a}
Cell wall-bound phenolics	–Si	–MS	2.33 ± 0.15^{a}	2.67 ± 0.32^{b}	3.31 ± 0.11^{b}	2.51 ± 0.23^{b}	2.18 ± 0.30^{a}	2.39 ± 0.59^{a}
	–Si	+MS	2.57 ± 0.11^{a}	5.86 ± 0.38^{a}	3.73 ± 0.13^{a}	5.30 ± 1.08^{a}	2.75 ± 0.51^{a}	4.73 ± 1.51^{a}
	+Si	–MS	2.22 ± 0.28^{a}	1.76 ± 0.10^{c}	3.17 ± 0.18^{b}	1.86 ± 0.16^{b}	1.09 ± 0.11^{b}	1.63 ± 0.15^{a}
	+Si	+MS	3.03 ± 0.70^{a}	2.22 ± 0.21^{bc}	2.17 ± 0.04^{c}	1.59 ± 0.06^{b}	0.87 ± 0.08^{b}	1.16 ± 0.05^{a}
Lignin	–Si	–MS	289 ± 15^{bc}	269 ± 16^{b}	257 ± 18^{a}	231 ± 26^{b}	347 ± 34^{a}	292 ± 48^{a}
	–Si	+MS	324 ± 18^{a}	302 ± 17^{a}	216 ± 22^{b}	280 ± 26^{a}	286 ± 26^{bc}	334 ± 45^{a}
	+Si	–MS	255 ± 17^{c}	197 ± 11^{d}	96 ± 19^{c}	196 ± 19^{bc}	321 ± 4^{ab}	273 ± 26^{a}
	+Si	+MS	307 ± 15^{ab}	233 ± 8^{c}	75 ± 14^{c}	174 ± 10^{c}	241 ± 25^{c}	232 ± 16^{a}
Si content	–Si		64 ± 6^{b}	64 ± 6^{b}	84 ± 10^{b}	65 ± 5^{b}	64 ± 6^{b}	64 ± 6^{b}
	+Si		124 ± 28^{a}	140 ± 19^{a}	100 ± 13^{a}	131 ± 35^{a}	124 ± 28^{a}	140 ± 19^{a}

perforation of a leaf with the tip of a pipette, the expression of genes encoding enzymes of the lignin biosynthetic pathway occurred during two distinct phases, at 2.5 h (the early phase) and 48 -72 h (the late phase) after injury (Soltani *et al.* 2006). In a woody species *Chamaecyparis obtusa*, however, the content of lignin measured in the CW of necrotic tissue was significantly higher at 28 and 56 d after injury (Kusumoto 2005). Up-regulation of lignin biosynthesis in the cells surrounding the wound site forming a "barrier zone" has been considered as a strategy to avoid pathogen infection and water loss (Moura *et al.* 2010).

The activation of the phenylpropanoid pathway in the IMS leaves soon after 24 h of the MS treatment in this work imply the involvement of a phloem-mobile molecule for communication between the treated and non-treated leaves. In contrast to other factors involved in the systemic regulation of shoot and root growth, such as nutritional status, our knowledge on the phloem-mobile signaling molecules after MS is much more limited and mostly restricted to evidence found in studies on the response of wounded tissue following insect feeding (Łukaszuk and Ciereszko 2012). The exact nature of a signaling molecule mediating communication between treated and non-treated leaves after MS needs to be characterized, and a question should be addressed if that is different from the wound-induced signaling molecule.

Reduction of activities of PAL, PPO, and soluble and covalently bound POD, as well as content of CW-bound phenolics and lignin in the +Si plants was a distinct evidence for down-regulation of the phenylpropanoid pathway by Si. This effect was not affected by the MS treatment and down-regulation of this pathway was observed by Si in the MS plants, too. Post-transcriptional and/or allosteric regulation of enzymes (Van Bockhaven *et al.* 2012) and/or a Si effect *via* hormone signaling as was observed in the leaves challenged with pathogens (Fauteux *et al.* 2006, Cabot *et al.* 2013) were probably involved in the Si-mediated modifications in the phenylpropanoid pathway of the leaves. Lower lignin content in the Si-treated plants may reflect the role of Si enhancing the mechanical strength of CW, allowing a trade-off between lignin and Si accumulation that results in down-regulation of lignin synthesis. Such a trade-off between Si and lignin may be of advantage because Si as a structural material is a cheaper source for cell structural resistance than lignin. Cell wall lignification is a highly energy-consuming process, whereas Si deposition requires less energy than lignin synthesis; the ratio of the energy requirement for lignin to that for Si is 20:1 (Raven 1983). In monocot Si-accumulator species, Si plays an important role in leaf erectness and shade or lodging avoidance, and Si accumulating ability is an ecological advantage for these species (Currie and Perry 2007, Hajiboland 2012). In tobacco as a non-accumulator species, however, our results confirm also that Si may play a significant role and substitutes partially lignin in its function for production of structural support.

The MS-induced changes of enzyme activities and content of phenolics and lignin considerably differed between the DMS and IMS leaves and Si treatments. In the IMS leaves, in contrast to the DMS leaf, an increase in activities of PAL, PPO, and the soluble fraction of POD by the MS was more prominently expressed in the +Si compared with –Si plants. In some cases, the direction of the change by the MS was different between the +Si and –Si plants. This was particularly observed for ionically-bound POD, CW-bound phenolics, and lignin. These data suggest that Si supplementation changed the pattern of phenolics metabolism and fractionation in leaves depending on whether they were directly treated with the MS or not. Silicon treatment enhanced MS-induced lignin accumulation in the treated leaf while in the leaves not directly challenged with the MS it rather inhibited lignin biosynthesis. Considering phenolics metabolism as the down-stream target of MS signaling, it is likely that Si affected it differently because of a different signaling pathway evoked by the MS in the local and systemic leaves. Silicon is increasingly being associated with modulation of primary signal transduction (Van Bockhaven *et al.* 2012, Detmann *et al.* 2013).

The Si-induced enhancement of lignin biosynthesis in the leaves directly challenged by the MS supports the role of Si in providing structural strength also of leaves in a non-accumulator species. However, regarding a high carbon and energy demand of lignin biosynthesis and an earlier cessation of leaf expansion following lignification of the CW, its down-regulation by Si in the IMS leaves may also be considered an ecological advantage for Si-supplemented plants. The lower lignin biosynthesis in the IMS leaves maintained the whole plant growth, thus, favored plant energy balance and provided a possibility for a higher carbon allocation for dry matter production instead of structural hardiness.

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