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Effects of Si in nutrient solution on leaf cuticles

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ABSTRACT

The benefits of silicon for different plant species have been described in many studies in both soilless and traditional soil culture systems. The aim of this work was to quantify the effect of Si on leaf cuticles under different fertigation regimes and the relationship of this effect to water, potassium and nitrate absorption, vegetative growth and plant protection. Cucumber, melon and pepper plants were transplanted into coconut fiber containers with Si in the nutrient solution at 0.6 mM (+Si) and without Si (-Si) under optimal fertigation (OF) and moderate deficit fertigation (DF) conditions. Absorption of water, nitrate and potassium, vegetative growth, leaf firmness, loss of water through cuticle transpiration, cuticle thickness, number of trichomes and Si content in the epidermis and trichomes were measured. Trichome numbers, cuticle thickness and Si content were examined using light and SEM microscopes equipped for X-ray microanalysis. Resistance to two pathogens, *Botrytis cinerea* and *Erysiphe cichoracearum*, was also measured. The results show a loss of growth in the three cv. under DF that was alleviated when Si was supplied in the nutrient solution. +Si significantly improved water absorption and decreased leaf loss, which may explain the improvement shown in the growth parameters. +Si showed clear and significant growth increases in both the epidermis and the cuticle, which could justify both the observed greater resistance to diseases and the lower rates of water loss from the leaves. The three cv. showed high concentrations of Si in the trichomes, even under -Si treatments with a mean Si concentration lower than that in the +Si treatments.

1. Introduction

The benefits of silicon for different plant species grown in hydroponics and in traditional soil have been described in many studies (Samuels et al., 1993; Datnoff et al., 2001; Hernandez-Apaolaza, 2014; Savvas and Ntatsi, 2015; Mantovani et al., 2018). These benefits are especially evident when plants grow in adverse environments under both biotic and abiotic stresses (Cooke and Leishman, 2011). While the mechanisms by which plants use silicon in their defense are far from fully understood, the beneficial effects of silicon are considered to act both actively and passively through cuticle thickening (Van Bockhaven et al., 2013), although the exact mechanism(s) by which silicon modulates plant physiology through the potentiation of host defense mechanisms still needs further investigation at the genomic, metabolomic, and proteomic levels (Debona et al., 2017). When silicon precipitates in its different forms in the epidermis of plants, it influences their physical functions, such as transpiration and mechanical resistance (Cooke and Leishman, 2011; Ma and Takahashi, 2002; Romero-Aranda et al., 2006; Tafolla-Arellano et al., 2018).

Silicon allows improved growth under saline conditions without negative repercussions for the plants, since the silicon also decreases sodium absorption when sodium is in excess (Ahmad and Zaheer, 1992; Ma et al., 2001; Saqib et al., 2008).

Improved plant development has also been reported in herbaceous (Pozo et al., 2015) and woody (Gallegos-Cedillo et al., 2018) plants, as well as improved water (Ma et al., 2001), K (Mali and Aery, 2008a) and nitrogen (Mali and Aery, 2008b) absorption both in hydroponics and in soil.

On the other hand, it is well known that the presence of Si in the leaves increases resistance to hydric stress and salinity, reducing the membrane permeability and transpiration through the cuticle, and consequently improves the hydric balance of the plant (Romero-Aranda et al., 2006). The negative effects of an excess of nitrogen fertilization, which allows fast growth and consequently weaker tissues that are susceptible to attack by plagues and diseases, can be mitigated the rigidity conferred by silicon and the reduction in N absorption (Guntzer et al., 2012).

The physical strength of the leaf resulting from Si accumulation in

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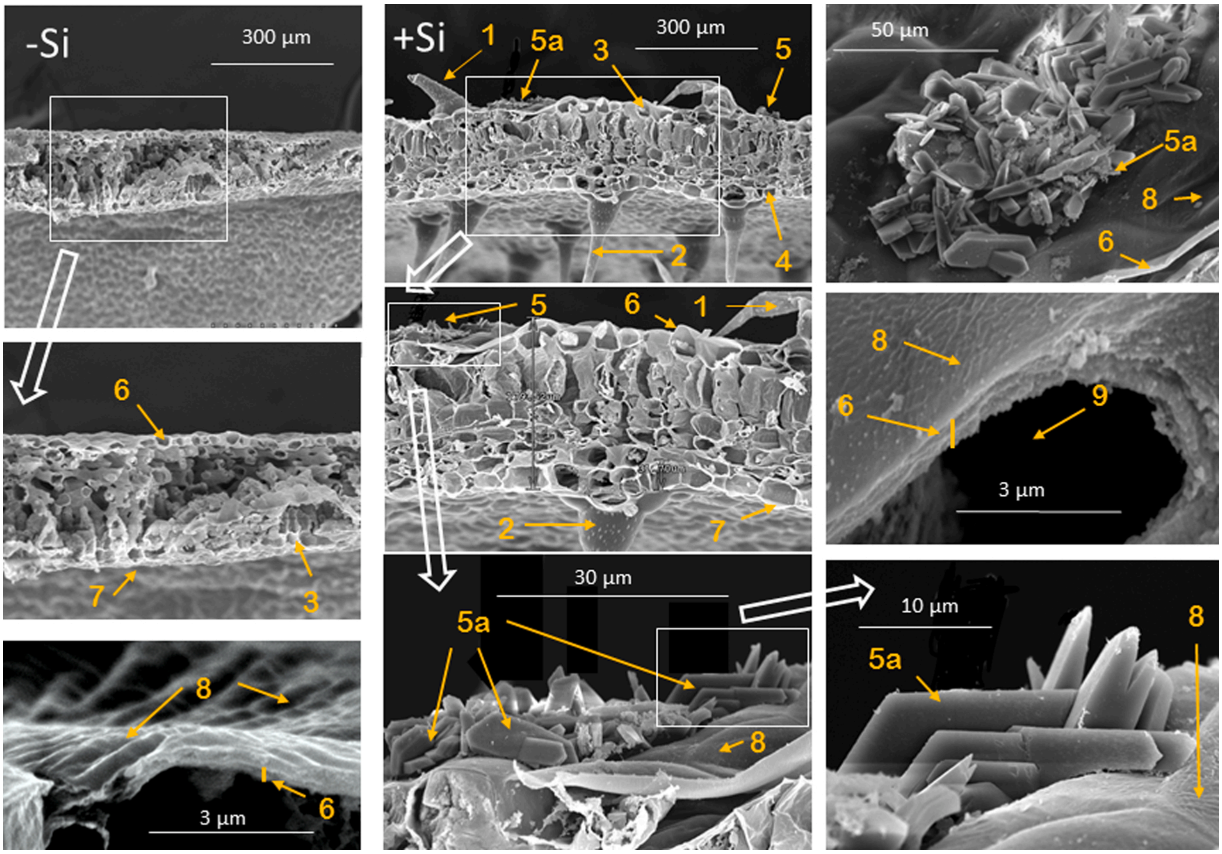

Fig. 1. Scanning electron microscopy images of pepper leaf cross-sections treated with Si in nutrient solution (+Si) (second and third columns) versus a control plant (-Si) (first column). 1 and 2 are trichomes on adaxial and abaxial surfaces. 3 and 4 are adaxial and abaxial epidermal cells, respectively. 5 is the presence of epicuticular crystals and phytoliths on the adaxial surface of leaves, and 5a indicates the same crystal group under different views. 6 and 7 are adaxial and abaxial cuticles, respectively. 8 show the wrinkled epicuticular structure on the adaxial surface and upper epidermal wall. 9 is a space epidermal cell.

the cuticle can explain the mechanism of protection against certain bacterial (Dannon and Wydra, 2004) or fungal diseases well known in rice (Zhang et al., 2013; Schurt et al., 2014) and vegetables (Pozo et al., 2015).

A wide variety of methods are used to apply commercial silicon-based products, including sporadic and continuous applications through fertigation. Although ranges higher than 2 mM can be found, Si in the nutrient solution is usually used at a dose of 0.6–1.5 mM. Authors such as Sonneveld and Straver (1994), recommend 0.5 mM of Si for lettuce and 0.75 for cucurbits such as cucumber and melon, and do not include Si in the nutritive solution for Solanaceae such as tomato and pepper; while Pozo et al. (2015) have found significant benefits and increased leaf and stem cuticle thickness under 0.6 mM in lettuce, tomato, pepper, melon and cucumber. In soilless culture with increasing doses in blueberry cultivation, positive effects have also been found under up to 1.2 mM Gallegos-Cedillo et al. (2018).

The objective of this work was to quantify the effects of Si on the cuticles of cucumber, melon and pepper plants under optimal and deficient fertigation and its relationship with 1) the absorption of water, potassium and nitrate, 2) vegetative development, 3) leaf firmness, and 4) protection against powdery mildew and botrytis.

2. Materials and methods

2.1. Plant growth conditions and fertigation treatments

The experiment was carried out at the University of Almeria (Spain) in an "almeria"-type plastic greenhouse. Seedlings of cucumber (*Cucumis sativus* L. cv. 21 PE 512), melon (*C. melo* L. cv. Nerval) and pepper (*Capsicum annuum* L. cv. Glabriusculum) were transplanted on 15 February 2018 into individual 2.5 L potscontainers. The substrate used was commercial coconut fiber, the physicochemical characteristics of which are described in Pozo et al. (2014). Two treatments were used to apply fertigation: an optimum fertigation volume (FO) and a deficit fertigation volume (FD). FO fertigation management was carried out following the criteria of Urrestarazu, 2015, where each new fertigation application was carried out when the water in the growing unit had reached 10 % of the easily available water following the recommendation of Rodríguez et al. (2014). For each treatment, four repetitions were made in which the drainage from the pot was collected. Fertigation and drainage were averaged daily for 75 days. Volume, pH, electrical conductivity (EC), and concentration of nitrates and potassium were measured and their absorption by plant and day were calculated (Urrestarazu et al., 2015). The nutritional solutions recommended by Sonneveld and Straver (1994) were used, to which 0.60 mM of Si (+Si) of the commercial product as orto potassium silicate FertiSil® (Macasa, Barcelona, Spain) was added; no Si product was added to the control (-Si).

2.2. Vegetative growth parameters

Vegetative development was measured 75 days after transplant and application of treatments. Biomass growth was measured through the dry and fresh weight of the roots, stems and leaves (g plant^{-1}) and leaf area (m^2 plant^{-1}).

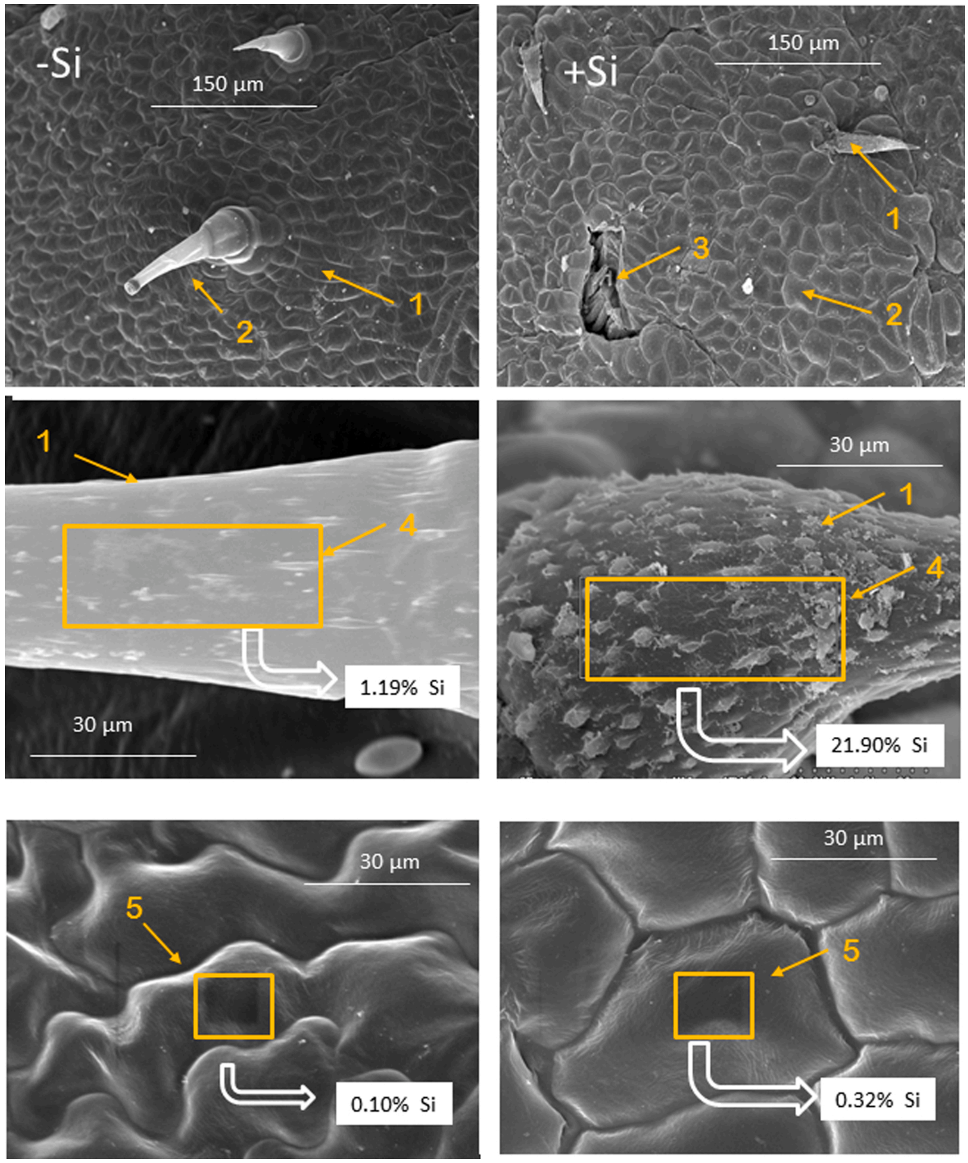

Fig. 2. Scanning electron microscopy images of pepper leaf treated with Si in the nutrient solution (+Si) (second column) versus a control plant (-Si) (first column). 1 and 2 are trichome and epidermal cells on surface leaves. 3 is epicuticular crystals on the adaxial surface of the leaf. 4 and 5 are yellow micro-areas of the trichome or epidermal cells where X-ray microanalysis was performed.

2.3. Test for resistance to deformation and breakage in leaves

The leaf firmness was determined by penetrometry at the end of the experiment by a digital diameter penetrometer (53200-Samar, Tr-Turoni, Forli, Italy) with a 6 mm diameter drill. The results were expressed in Newtons. Resistance to deformation was analyzed by nondestructive measurements of the leaves with a hardness tester (Durofel, Copa Technology, France) equipped with a 0.1 cm^2 tip. A scale of 1 (soft) to 80 (firm) in Shore degrees is used. Four measurements were taken in the middle of each leaf for 10 leaves per replication.

2.4. Measurement of moisture loss in leaves

For the measurement of the loss of moisture from the leaves at the end of the experiment, a sample of four leaves was taken per plant, repetition and treatment. They were sealed by their petiole by

Table 1
Water absorption (L $plant^{-1}day^{-1}$), nitrates and potassium (mmol $plant^{-1}$ day^{-1}) in horticultural crops for a period of 50 days of treatment after transplant. +Si indicates the application of Silicon in the standard nutrient solution (-Si) with an optimal fertigation (OF) and deficit (DF).

	Cucumber			Melon			Pepper		
	Water	K^+	NO_3^-	Water	K^+	NO_3^-	Water	K^+	NO_3^-
OF -Si	0.411b	0.209c	0.210b	0.415b	0.514b	0.367c	0.386b	0.200b	0.212b
+Si	0.455a	0.283b	0.395a	0.449a	0.770a	0.737a	0.431a	0.275a	0.382a
DF-Si	0.340d	0.160d	0.177c	0.347c	0.398c	0.334c	0.317c	0.196b	0.150c
+Si	0.381c	0.309a	0.219ab	0.382b	0.533b	0.391b	0.339bc	0.280a	0.391a

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test.

immersion in paraffin and were weighed on a precision scale to the thousandth of a gram. Then, they were placed in a forced air stove (OMS60, Thermo Scientific®, USA) at 40 °C and weighed again after 10, 30, 60 and 360 min. Leaf water loss data were expressed in mL cm^{-2} $hour^{-1}$.

2.5. Test for protection against gray rot and powdery mildew

The infecting biological materials were *Botrytis cinerea* (gray rot) and *Erysiphe cichoracearum* (powdery mildew).

2.6. Assay to evaluate benefits against gray rot

From each treatment, 4 representative leaves of each crop and treatment were taken. Four discs were extracted from each leaf and taken to a controlled environment for their corresponding inoculation (if applicable), measurement and evaluation of the growth of *B. cinerea* following the procedure described by Wegulo and Vilchez (2007) and (Pozo et al., 2015). The discs extracted from + Si and -Si plants were deposited in Petri dishes boxes. At 3, 7 and 14 days after inoculation, the progression of the disease was observed and recorded as a percentage of the affected surface. For the measurement of the affected surface, the WinDIAS® program (Delta-T Devices Ltd, UK) for interpretation and analysis of images was used.

2.7. Assay to evaluate powdery mildew infection

Four cucumber plants treated with + Si and the control were taken to a controlled environment with a humidity above 85 %, and a spraying of the causal agent of powdery mildew (*Erysiphe cichoracearum*) was performed. After 10 and 15 days, the central leaves of four plants per treatment were sampled. By taking photographs, the affected area (%) was quantified using the ImageJ® (Rueden et al., 2017), image processing program. Calculation of the apparent infection rate as a function of the application the logistic model by Berger (1980), was performed based on the equation:

$$y = 1/(1 + \exp(-[a + rt + rt]))$$

where y = the disease proportion in the range 0 < y < 1, a = logit (*yo*), r = rate, and t = time.

2.8. Optical and electron microscopy

Four leaves were sampled from the middle part of the plants of each crop and treatment. Each leaf was fixed (Sargent, 1976), for later observation under optical and electron microscopes. Quantification of trichomes was performed using a light microscope (Nikon Eclipse E800, Japan) and a HITACHI S-3500 N LV-SEM (®Hitachi High-Technologies Corporation, Japan) for scanning electron microscopy (SEM) (Fig. 1). The chemical analysis of the sample inside the SEM was performed by Energy Dispersive Spectrosopy (EDS) with INCAx-sight (Oxford, England) using 180 and 320 μm^2 for epidermal cells and trichomes, respectively (Fig. 2).

2.9. Experimental design and statistical analysis

The experiment was carried out following a randomized complete block experimental design (Petersen, 1994). Statistical analysis was performed using ANOVA and Tukey's test with the program STATGRAPHICS PLUS version 5.0.

Table 2
Leaf area (m^2 $plant^{-1}$) and biomass (g $plant^{-1}$) 50 days after transplant in a horticultural crop. +Si indicates the application of Silicon in the standard nutrient solution (-Si) with optimal fertigation (OF) and deficit (DF).

	Cucumber				Melon				Sweet pepper			
	Leaf area											
OF -Si	1.71c				1.83b				1.78c			
+Si	2.32a				2.85a				2.47b			
DF -Si	1.63c				1.77c				1.72d			
+Si	2.22b				2.70a				2.49b			
	Root	Stem	Leaf	Total	Root	Stem	Leaf	Total	Root	Steam	Leaf	Total
	Fresh weight											
OF -Si	16.16c	52.70b	53.84bc	122.70c	58.74b	155.61b	123.44c	337.79c	40.64c	52.15c	47.47b	140.26b
+Si	26.87a	88.36a	162.46a	277.69a	63.92a	237.53a	187.88a	489.33a	70.12a	91.01a	60.06a	221.19a
DF -Si	11.28d	25.57c	35.73c	72.58d	51.83b	137.29c	109.49d	298.61d	38.19c	27.39d	46.91b	112.49c
+Si	22.44b	82.42a	86.76b	191.62b	63.13a	199.28ab	166.15b	428.86b	68.24b	81.30b	67.94a	217.48a
	Dry weight											
OF -Si	0.82c	17.10d	12.04c	29.96c	10.08b	28.27b	25.37b	67.98b	11.56b	15.10b	14.67c	43.77b
+Si	1.19a	22.78a	36.20a	60.17a	15.14a	35.66a	37.10a	82.70a	20.12a	27.48a	16.98b	49.81a
DF -Si	0.70d	8.56c	11.00d	20.26d	7.59c	22.15b	22.37c	53.11c	12.83c	9.47c	14.00c	33.13c
+Si	0.93b	19.39b	21.75b	40.07b	13.91b	37.80a	31.60b	80.22a	19.81b	23.67a	18.60a	45.08b

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test.

Table 3
Thickness (μm) of adaxial (Ad) and abaxial (Ab) epidermis, adaxial cuticle (AdC), leaf lamina (LL) and trichomes (N° mm^{-2}) from scanning electron microscope section after 75 days of Si (+Si) versus a control plant (-Si).

	Cucumber					Melon					Pepper				
	Trichome	Ad	Ab	AdC	LL	Trichome	Ad	Ab	AdC	LL	Trichome	Ad	Ab	AdC	LL
-Si	33b	17.90b	28.96a	0.60b	137.48b	30b	20.83a	29.89b	0.56b	134.30b	16b	17.25b	27.51a	0.58b	128.65b
+Si	39a	21.42a	29.69a	0.64a	177.36a	36a	21.56a	33.39a	0.70a	173.48a	20a	18.68a	27.79a	0.61a	164.83a

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test. n = 4.

3. Results and discussion

3.1. Effect on fertigation parameters

With the application of Si in the nutrient solution (+Si) for the three crops tested and under optimal irrigation (OF) and deficit irrigation (DF), the absorption of water, nitrates and potassium improved by 10, 50 and 70 %, respectively (Table 1). Ma et al. (2001) reported that the presence of Si in the nutrient solution increased water absorption, protecting plants against abiotic stresses (Hernandez-Apaolaza, 2014). This improved absorption has also been described under saline conditions by Shi et al. (2016), when applying Si in the nutrient solution up to 2.5 mM.

With the addition of Si to DF, the absorption of potassium and nitrate in all crops was equal to or better than that in the OF -Si treatments. This suggests that the loss of ionic absorption under moderate water stress is alleviated by the incorporation of Si into the nutritive solution. These results agree with those of Kaya et al. (2006), who reported that silicon addition increased K levels in water-stressed maize leaves. It was reported that increased uptake of K may be attributed to a decrease in plasma membrane permeability and an increase in plasma membrane H+-ATP activity as a result of silicon addition (Liang, 1999).

Likewise, +Si alleviated the decrease in water availability in the DF treatment, recording the same absorption of water as that in OF under -Si. Therefore, the contribution of Si also alleviated the lower water availability of the DF treatment. This result suggests that the improvement in the water balance of the plant is produced not only by the decrease in transpiration due to the potential greater thickness of the cuticle but also by the improvement in water absorption by the roots of cucumber, melon and pepper. In tomato plants, Shi et al. (2016), reported that leaf water content under water stress was preserved when plants were supplied with Si, suggesting that increased water uptake/-transport rather than decreased transpiration was responsible for Si alleviating water stress.

3.2. Effect on vegetative growth parameters

In both OF and DF treatments, the addition of silicon in fertigation registered a clear and significant benefit in all growth parameters in the three crops, with an average increase of more than 20 % (Table 2). The benefit of silicon to the leaf area was similar under OF and DF, but the average Si benefit (more than 10 %) was higher under DF than under OF, suggesting that under stressful situations, the benefit of applying Si in the nutrient solution is greater than that under optimal fertigation conditions. Cucumber cultivation showed an average significant increase twice as high as that found in melon and pepper. This stronger beneficial effect of Si on cucumber can justify why authors such as Sonneveld and Straver (1994), recommend its use in cucurbits such as cucumber and melon and do recommend Si for pepper. However, Pozo et al. (2015), reported similar results to ours in lettuce, tomato, pepper, melon and cucumber plants grown in coconut fiber.

In the -Si treatments, the foliar area of melon and pepper in DF was significantly lower than that of melon and pepper grown with Si in the nutrient solution. The + Si treatments always significantly increased the leaf area with respect to that in the controls.

For DF and -Si, the total fresh and dry weight of the three crops tested was also significantly lower, by an average of 20 %. In DF treatments in which Si was applied, this reduction would disappear in pepper and decrease to an average of 14 % for cucumber and melon.

For all vegetative growth parameters evaluated, the DF and + Si treatments obtained results that were the same as or better than those in the OF and -Si treatments. Therefore, the presence of Si in the nutrient solution alleviated hydric stress. Our results suggest an important mitigation of losses from moderate water stress, as has been reported in wheat (Meunier et al., 2017), cucumber (Adaita and Besford, 1986; Hattori et al., 2008), and pepper (Lobato et al., 2009).

3.3. Effect on trichomes

The number of trichomes in cucumber and melon was similar (≈ 30–40 per mm^{-2}) and was lower in pepper (Table 3). This density is similar to the reported number of glandular trichomes in the trichome plants *Ocimum campechianum* and *Ruellia nudiflora* (Martínez-Natarén et al., 2018). The number of trichomes in the three species was increased by 25 % when Si was used in the nutrient solution. An increase in trichome number in soybean plants has been reported when applying 0.8 mM of Si (Tibbitts, 2016), and a significant improvement in trichome number was found when applying 1.5 mM of Si (Liang, 1999). Incorporating Si up to 1 mM did not increase the number of trichomes on cucumber fruits, but the trichomes under + Si had a coarse outer appearance compared to those under -Si, where the trichomes were smooth (Samuels et al., 1993).

3.4. Effect on foliar anatomy

The total thickness of the leaf was similar among the three plants tested. The + Si treatment thickened leaves very significantly, by 30 %, an increase that had already been shown in the highest dry and fresh leaf weight. In the case of cucumber and pepper, the thickness of the epidermal layer of the leaf bundle increased significantly, by 20 and 9%, respectively, while in melon, the abaxial epidermis increased by 10 %, compared to those in the -Si treatments.

3.5. Effect on the cuticle

Cuticle thickness was very similar in all three plants and was within the mean values described by Tafolla-Arellano et al. (2018) and Tafolla-Arellano et al. (2013). With the addition of Si to the nutrient solution, the cuticle recorded an increase of 20, 9 and 5% in cucumber, pepper and melon, respectively. With the same concentration of Si in the nutrient solution, Pozo et al. (2015) found similar increases in tomato, cucumber, pepper, melon and lettuce plants. Therefore, the benefits of growth may be partly due to this thickening of the cuticle (Van Bockhaven et al., 2013).

3.6. Effect on the microanalytic concentration of Si in the cuticle

Hernandez-Apaolaza (2014) have been reported different Si translocation rates to the leaves by the xylem in higher plants. However, specific via to deposit Si on the leaves need further investigation (Savvas and Ntatsi, 2015). Fig. 3 shows the Si content in the cuticle and trichomes of the tested horticultural plants. With the exception of the melon trichomes, the + Si recorded significant increases in Si content in

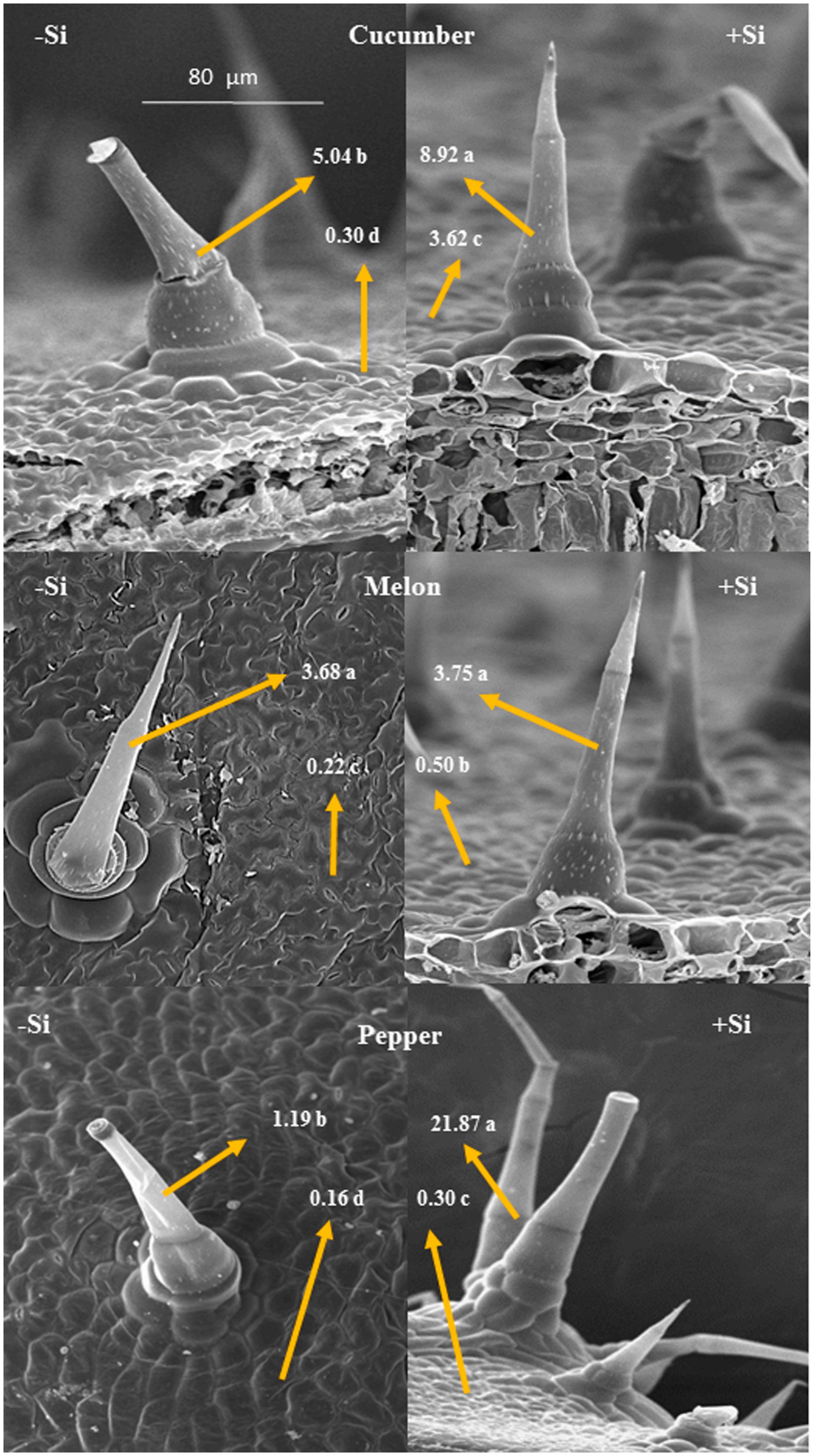

Fig. 3. Si concentration (%) in the different cultivars measured by scanning electron microscopy. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test. $n = 4$.

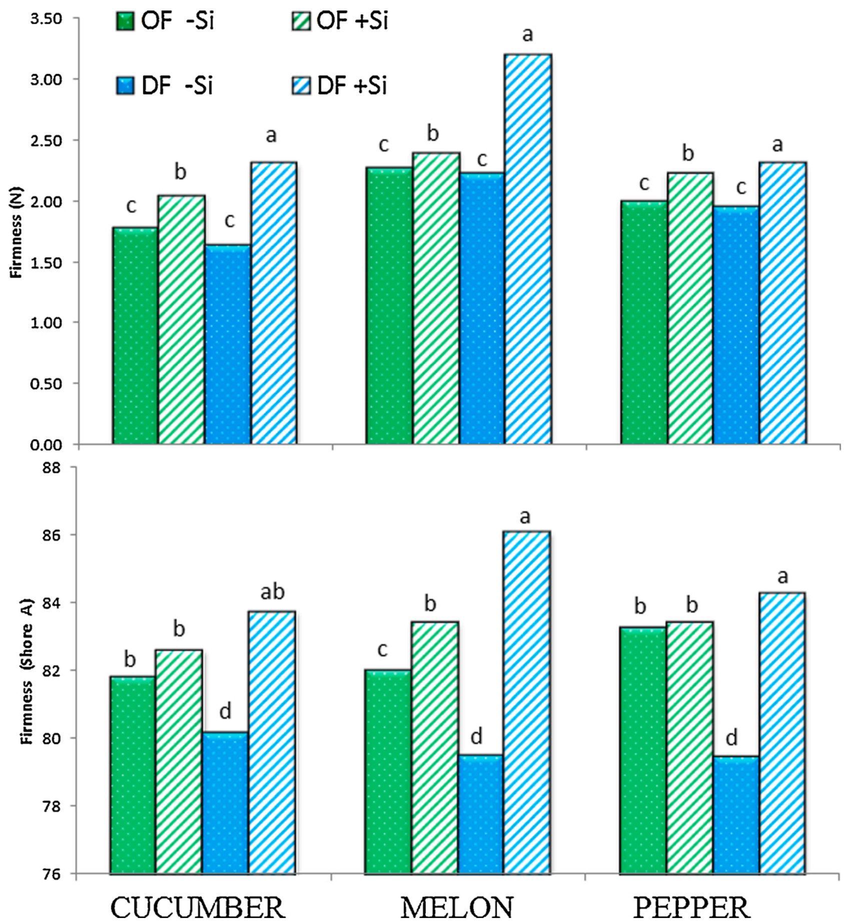

Fig. 4. Resistance to deformation (Shore) and firmness (N) of the leaves with the application of Si in the nutrient solution in pepper, melon and cucumber crops. +Si indicates the application of silicon in the standard nutrient solution (-Si) under optimal (OF) and deficit (DF) fertigation. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test. n = 4.

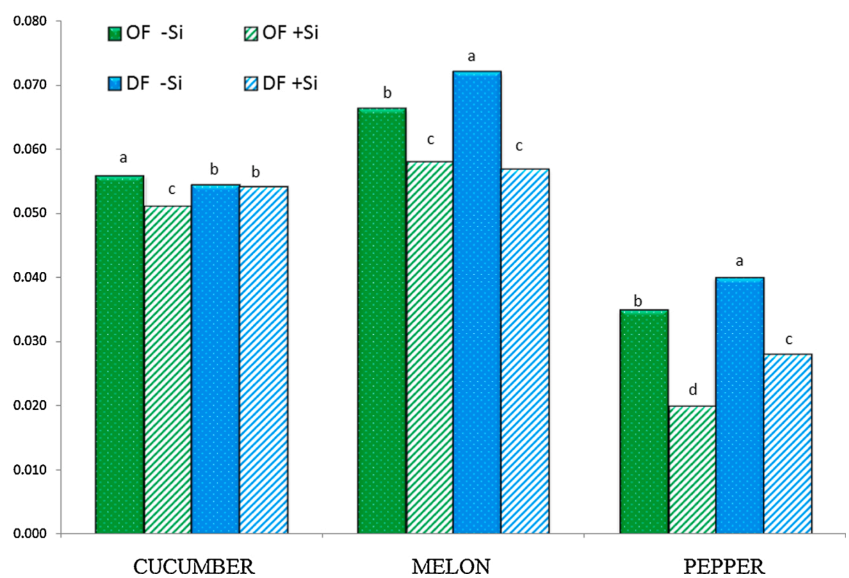

Fig. 5. The leaf desiccation rate (ml cm^{-2} $hour^{-1}$) with the application of Si in the nutrient solution in pepper, melon and cucumber crops. +Si indicates the application of silicon in the standard nutrient solution (-Si) under optimal (OF) and deficit (DF) fertigation. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test. n = 4.

both trichomes and the cuticle compared to those in the -Si treatments, and phytoliths and oxalate crystals were only observed in the + Si treatments. In the case of cucumber and pepper, the trichomes had a significantly higher Si concentration (>75 %) than those under the -Si treatments. Many studies have reported a higher presence of Si in the external layers of the leaves when Si is applied in the nutrient solution

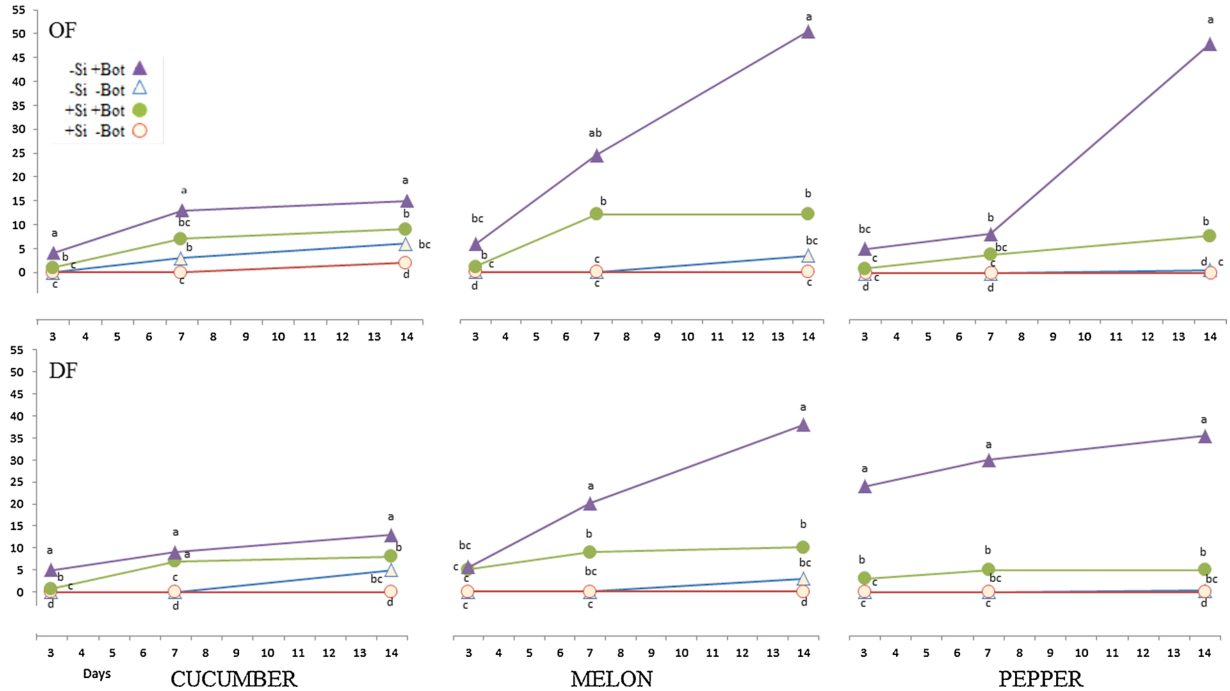

Fig. 6. Severity of gray rot disease over time on leaf discs expressed as the percentage of the infected area. +Si + Bot, −Si + Bot, +Si − Bot, and − Si − Bot refer to discs from plant growth in the nutrient solutions with silicon (Si) and with *Botrytis cinerea* inoculum, without Si and with *B. cinerea* inoculum, with Si and without *B. cinerea* inoculum, and without Si and without *B. cinerea* inoculum, respectively. Different letters indicate significant differences T $P \leq 0.05$ at same time after inoculation according to Tukey's test. OF and DF indicate optimun and deficit fertigation, respectively.

(Ma and Takahashi, 2002). Although the concentration of Si was much lower when Si was not applied in the nutrient solution, a higher proportion of Si was found in all cases in the trichome than in the cuticles, suggesting that Si is preferably mobilized to the trichomes themselves for formation. Meunier et al. (2017), described the formation of the highest number of trichomes with the presence of Si in the nutrient solution. However, the metabolic mechanism of Si translocation to the trichomes requires further study.

3.7. Effect on leaf firmness

Fig. 4 shows the effect on the mechanical resistance of the leaves depending on the type of fertigation and the application of Si. It is well known that Si contributes to a higher mechanical resistance in the leaves (Tafolla-Arellano et al., 2018, 2013; Kaufman et al., 1979; Sahebi et al., 2015). Our results show that the highest mechanical resistance, deformation resistance and resistance to leaf rupture always occurred with Si application and under DF. With the exception of the OF treatment in pepper, the presence of Si reduced deformation by 15 % and reduced penetration by 25 %. This greater resistance to deformation led to less deformation from wind (Debona et al., 2017; Tafolla-Arellano et al., 2018; Martínez-Natarén et al., 2018), and therefore can partly explain the better vegetative growth, probably because the leaves remained intact during a longer period of exposure to light.

3.8. Effect on leaf drying

With the exception of the DF treatment in cucumber, the application of Si led to a lower loss of moisture through the cuticle (Fig. 5). The reduction in water loss was directly proportional to the cuticle thickness of the three cv. tested. This decrease in water loss from the leaves was also recorded by Tibbitts (2016). This result also agrees with the well-known decrease in transpiration that occurs when Si is supplied (Ma, 1990; Tafolla-Arellano et al., 2018).

The results suggest that the addition of Si to the nutrient solution

Table 4
Apparent rate of infection and % affectation of the leaves by Oidio (*Erysiphe cichoracearum)* on a cucumber crop. +Si indicates the application of Silicon in the standard nutrient solution (-Si) with an optimal fertigation (OF) and deficit (DF).

	% Rate of infection	Nº plants affected within 15 days	Apparent rate of infection
OF -Si	7.37a	5.3b	0.0294a
+Si	2.13b	5.5b	0.0119bc
DF -Si	8.79a	7.1a	0.0156b
+Si	3.71b	6.4b	0.0088c

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test. n = 4.

produces 1) a decrease in water loss from the leaves and therefore lower desiccation, justified in part by an increase in the thickness of the cuticle and epidermis; and 2) a greater absorption of water, which improves the hydric balance and explains in part the improvement in the vegetative development. These results are in agreement with the results reported in tomato by Romero-Aranda et al. (2006) and Haghighi and Pessarakli (2013), and in potato by Shi et al. (2016).

3.9. Effect of Si on plant protection

It is well known that the leaves Si depositions protect plants of multiple abiotic and biotic stresses (Datnoff et al., 2001; Ma and Takahashi, 2002; Ma and Yamaji, 2006; Tafolla-Arellano et al., 2018). Fig. 6 shows the protective effect of the addition of Si to the nutrient solution against the pathogen *Botrytis cinerea*. From 7 days after inoculation, significant differences appeared in the progression of gray rot in the inoculated discs of the three horticultural plants tested compared to that in the noninoculated plants. The trends in OF and DF were analogous. These results were very similar to those found by Pozo et al. (2015), in

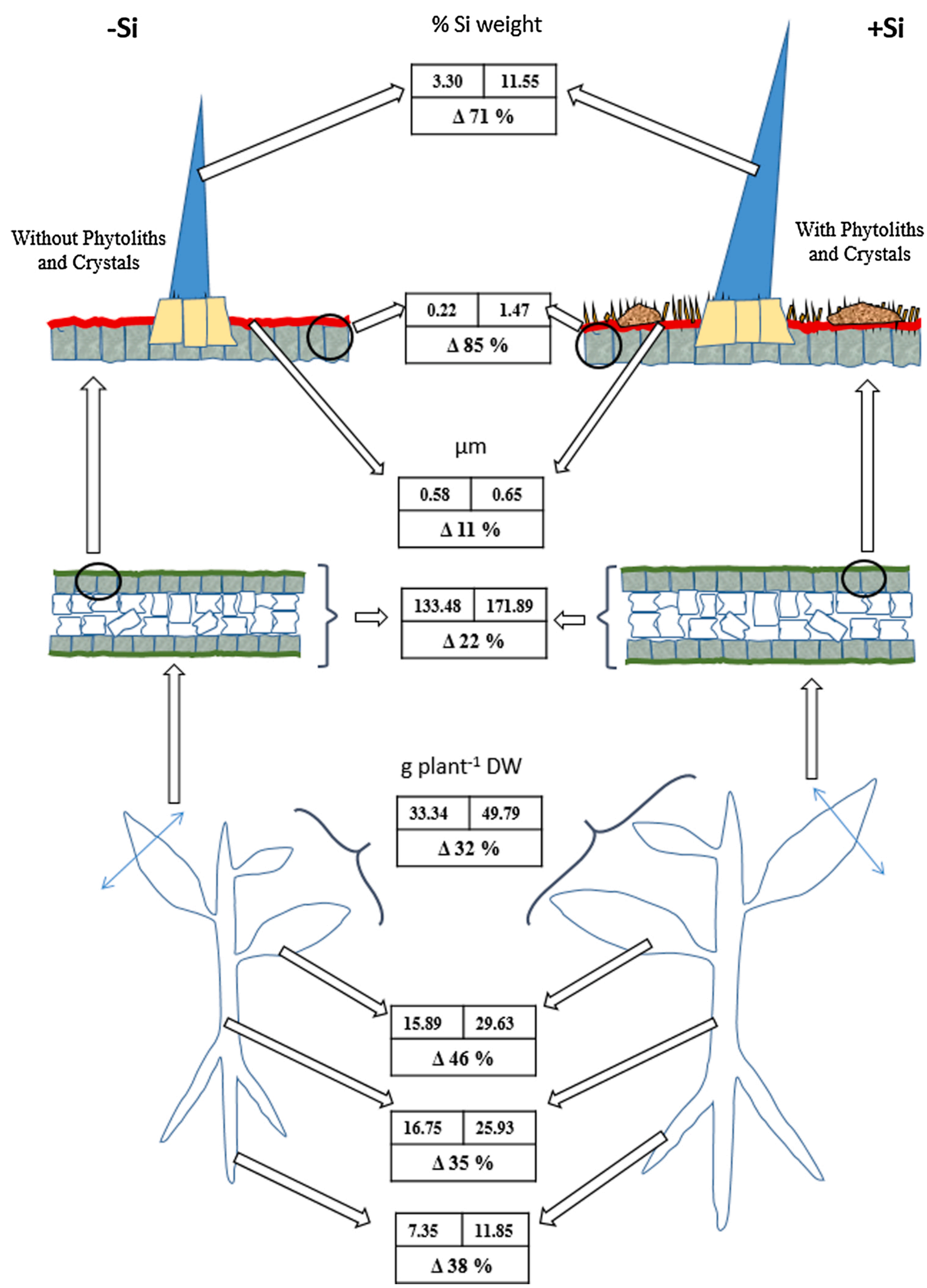

Fig. 7. Averages of the effects on growth and Si concentration in the cuticle and trichomes in vegetable plants treated with Si in nutrient solution (+Si) versus the effects of a control (-Si).

the same horticultural plants and with the same dose of Si in the nutrient solution. The high cuticle thickness described above in the tested horticultural plants exerted greater protection against the pathogenic agent *B. cinera.*

In both OF and DF, the presence of Si in the nutrient solution in the cucumber crop exerted a significant protective effect against affectation in the leaves and in the apparent affectation rate (Table 4). As with the protection against the pathogenic agent *B. cinerea*, the increased cuticle thickness of the cucumber crop under + Si produced a protective effect against powdery mildew. This effect of silicon in soilless culture had also been reported by Adaita and Besford (1986), in cucumber protection against powdery mildew.

In general, our results suggest better benefit under stress condition, this is in accordance with reported by Hernandez-Apaolaza (2014): "its beneficial effects are usually expressed more clearly when plants are subjected to various abiotic and biotic stresses".

4. Conclusion

The addition of Si to the nutrient solution improves plant growth. We suggest that, in addition to the metabolic implications, this improvement may be for two reasons: 1) The water balance of the cucumber, melon and pepper plants is improved by the Si in the nutrient solution both a) by the improvements in water absorption by the roots and in transport towards the leaves, and b) by the lower water loss through the cuticle due to its improved thickness; and 2) The improved turgidity (point 1) and the rigidity conferred by the thicker cuticle and the phytoliths and trichomes allow the leaf to spend a longer time being exposed to light. The application of Si alleviated the loss of growth caused by moderate deficit irrigation. The Si transported by the xylem clearly preferentially accumulates in the trichomes, which implies active transport towards these structures; the number of trichomes also increases when Si is applied through fertigation (Fig. 7). The thicker cuticle and consequently the more rigid leaves increase plant resistance to diseases that need to cross an epidermal barrier.

CRediT authorship contribution statement

Francisca Ferrón-Carrillo: Methodology, Formal analysis, Resources. **Miguel Urrestarazu:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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