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Article

Foliar Amino Acids and Silicon Reallocate Leaf Tissues and Reshape Stomatal Architecture in Sour Orange (Citrus Aurantium L.) Seedlings

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Abstract: Sour orange (Citrus aurantium L.) remains the dominant citrus rootstock in Iraq, but its seedlings need a long nursery phase before budding. We tested whether foliar amino acids (AA; 0, 50 and 75 mg L−1) and silicon (Si; 0, 1 and 2 mL L−1), applied alone or combined, remodel the leaf anatomy of one-year-old potted seedlings. The 3 × 3 factorial was arranged in a randomized complete block design with three replicates. Seedlings were sprayed three times at monthly intervals (March–May 2025), and leaves formed during the spray period were sampled in March 2026. Leaves were hypostomatic in all treatments. Control leaves had the smallest stomata (75 μm2), the lowest stomatal density (221 mm−2), the thinnest cuticle (2 μm) and the thinnest lamina (265 μm). Every treatment raised the stomatal pore area index (SPI) above the control, by 32–185%, but the two inputs did so by different routes. Si alone mainly increased stomatal density (up to +22%), whereas AA mainly enlarged the stomata (+62–70% in stomatal area, averaged over Si levels). AA50 + Si1 produced the largest stomata (20 × 12 μm) and the highest SPI (0.091; 2.9-fold the control). Neither input alone thickened the mesophyll (214–225 μm vs. 221 μm in the control), but all four AA × Si combinations did (230–237 μm), indicating a synergistic effect. The single high doses (AA0 + Si2 and AA75 + Si0) instead produced the thickest cuticle (5 μm) and epidermis, and mesophyll thickness was inversely related to the summed thickness of the protective layers ($r = -0.69$, $P = 0.042$). Foliar AA 50 mg L−1 + Si 1 mL L−1 is a promising programme for producing vigorous sour orange rootstock seedlings.

Keywords: Biostimulants; citrus rootstock; leaf anatomy; mesophyll; protein hydrolysate; silicon; stomatal density; stomatal pore area index.

Introduction

Sour orange (*Citrus aurantium* L.) has long been the most widely used citrus rootstock in the world. It is valued for good yield, high fruit quality, cold tolerance and adaptation to heavy and calcareous soils [1]. Its weakness is high susceptibility to decline strains of citrus tristeza virus, which has driven many regions to adopt other rootstocks [2]. In Iraq, however, sour orange remains the dominant rootstock, and most commercial citrus trees are budded onto seedlings raised in local

nurseries. The nursery phase is slow. A seedling must reach a stem diameter suitable for budding, and a well-built leaf canopy is what drives that growth. Any treatment that improves leaf structure and function at this stage can shorten the nursery cycle and lower its cost.

Biostimulants are substances or microorganisms applied to plants to improve nutrient-use efficiency, stress tolerance or crop quality, independently of their nutrient content [3]. Amino acids and protein hydrolysates form one of the main biostimulant classes. Applied to leaves, they enter through polar pores in the cuticle and act both as a readily usable nitrogen source and as signals that stimulate chlorophyll synthesis, photosynthetic electron transport and antioxidant defence [4], [5]. In citrus nurseries, foliar amino acids have improved the vegetative growth of sour orange and other rootstock seedlings [6], [7]. These studies measured plant height, stem diameter, leaf number and leaf pigments; none examined how the leaf is built inside.

Silicon (Si) is the second most abundant element in the Earth's crust. It is not classed as essential for higher plants, but it is now widely accepted as a beneficial element [8], [9]. Plants take up Si as monosilicic acid, which polymerises as amorphous silica in cell walls and beneath the cuticle [10], [11]. The resulting cuticle–silica double layer strengthens the epidermis and reduces cuticular water loss [12], [13]. Silica deposited on guard cells also makes stomata more sensitive to closing signals [14]. Citrus is a low-to-moderate Si accumulator, yet Si-treated citrus leaves form silica granules in the epidermis and a cuticle–silica double layer [15]. Foliar Si has also raised mesophyll silica and leaf toughness in citrus [16]. In other fruit and woody species, Si has altered epidermis thickness, mesophyll thickness and stomatal density [17], [18].

Leaf anatomy sets the limits of leaf function. Stomatal size and density together determine the maximum stomatal conductance a leaf can reach [19]. Smaller stomata respond faster, but larger stomata provide more pore area per unit of epidermis [20], [21]. The two traits are usually inversely related, so a change in one is often balanced by the other [22]. Mesophyll thickness controls light absorption and the internal path of CO_2 to the chloroplasts. This matters especially in hypostomatic leaves such as those of citrus, where CO_2 must diffuse across the full leaf depth [23], [24]. The epidermis and cuticle, in turn, form the main barrier against water loss and pathogen entry [25]. A biostimulant that shifts these traits changes the balance between carbon gain and protection.

Amino acids and Si therefore act on different parts of leaf construction. Amino acids supply nitrogen and metabolic signals to growing tissues, whereas Si mainly reinforces surfaces and cell walls. Their combined effect might be additive, synergistic or antagonistic. A few studies point to synergy for mineral nutrition [26], but their joint effect on leaf anatomy has not been tested in any citrus species.

This study tested the hypothesis that foliar amino acids and Si, alone and combined, remodel the leaf anatomy of sour orange seedlings. Specifically, we asked: (i) whether the treatments change stomatal distribution, size and density; (ii) whether they alter the thickness of the cuticle, epidermis and mesophyll; and (iii) whether moderate combined doses outperform either input applied alone at its highest dose.

Materials and methods

Site and plant material

The experiment was carried out in a citrus orchard in Al-Alam sub-district, Tikrit District, Salah al-Din Governorate, Iraq (34°42′ N, 43°41′ E; approximately 94 m above sea level). Uniform one-year-old sour orange (*Citrus aurantium* L.) seedlings raised from seed were grown in plastic pots 20 cm in diameter, filled with a 1:1 (v/v) mixture of peat moss and loamy soil. All seedlings received the same irrigation and cultural practices throughout the trial.

Treatments and experimental design

The trial was a 3 × 3 factorial in a randomized complete block design (RCBD) with three replicates. Each seedling was one experimental unit, giving 27 seedlings (nine treatment combinations × three replicates). Factor A was amino acids (AA) at 0, 50 and 75 mg L^{-1}, supplied as Jisa 1, a liquid bio-organic fertilizer containing 16% free amino acids, 48% organic matter, 9% organic N, 0.35% Zn and

0.31% Mn (China). Factor B was silicon (Si) at 0, 1 and 2 mL L^{-1}, applied as a commercial liquid silicon fertilizer obtained from the local market. Control seedlings were sprayed with water only. Treatment codes are given in Table 1.

Table 1. Treatment combinations and the labels used in the text.

Code	Amino acids (mg L^{-1})	Silicon (mL L^{-1})	Label used in text
A0B0	0	0	Control
A0B1	0	1	AA0 + Si1
A0B2	0	2	AA0 + Si2
A1B0	50	0	AA50 + Si0
A1B1	50	1	AA50 + Si1
A1B2	50	2	AA50 + Si2
A2B0	75	0	AA75 + Si0
A2B1	75	1	AA75 + Si1
A2B2	75	2	AA75 + Si2

Sprays were applied three times at monthly intervals, on 1 March, 1 April and 1 May 2025. The whole canopy was wetted to run-off in the early morning with a 1 L hand sprayer, and no surfactant was added. Leaf samples were collected on 1 March 2026.

Leaf sampling

Fully expanded, mature leaves that had formed during the spray period were taken from the middle of the shoot of each seedling. From each leaf, a segment was cut from the middle of the lamina that included the midrib and part of the blade towards the margin.

Preparation of transverse sections

Permanent transverse sections were prepared by the paraffin method [27], [28], as adapted by Al-Hamdany et al. [29]. Fresh leaf segments, 2–5 mm long, were softened in 1% NaOH for 24–48 h and rinsed in distilled water. They were then fixed for 18–24 h in formalin–acetic acid–alcohol (FAA; 50 mL ethanol, 5 mL glacial acetic acid, 10 mL formaldehyde 37–40% and 35 mL distilled water) and stored in 70% ethanol.

Samples were dehydrated through an ethanol series (80, 90 and 95%, 2 h each) and absolute ethanol (2 h). They were cleared in ethanol–xylene mixtures (3:1, 1:1 and 1:3, v/v) and in pure xylene, 2 h each. Infiltration was carried out with molten paraffin at 55–60 °C, with five to six changes over 4–5 days, the last one overnight. Samples were embedded in paraffin blocks and cut at 10–15 μm on a rotary microtome (Bright, UK). Ribbons were floated on a water bath at 40–45 °C and mounted on slides coated with glycerin–albumin adhesive.

Sections were dewaxed in xylene, rehydrated through a descending ethanol series and stained with 0.2% safranin in 50% ethanol for 2–24 h. They were then dehydrated, counterstained with 1% fast green in absolute ethanol for 3–5 s, cleared in xylene–clove oil (1:1) and xylene, and mounted permanently in Canada balsam.

Epidermal preparations

Adaxial and abaxial epidermal layers were removed from fresh mid-lamina tissue by the stripping method, using fine-tipped forceps, and mounted in glycerin jelly under a cover slip. Clear nail-varnish impressions of both leaf surfaces were also made to confirm stomatal distribution and the outline of ordinary epidermal cells [29]. Terminology followed Dilcher [30].

Measurements

All slides were examined and photographed under an Olympus compound light microscope fitted with a digital camera. Dimensions were measured with a calibrated ocular micrometer at 40× objective magnification. For each treatment, five readings were taken for every trait from the replicate seedlings, and their mean was used.

On epidermal preparations, we recorded stomatal length and width (guard-cell pair), the length and width of ordinary epidermal cells, and the presence or absence of stomata on each surface. Stomatal density (SD, stomata mm^{-2}) was calculated as the number of stomata per field divided by the field area [31]. On transverse sections, we recorded the thickness of the adaxial and abaxial cuticle, the adaxial and abaxial epidermis, and the mesophyll.

Derived anatomical indices

Four indices were derived from the measured traits. Stomatal area (SA, μm^2) was approximated as an ellipse (Eq. 1), and the stomatal pore area index (SPI, dimensionless), a proxy for the anatomical maximum of stomatal conductance, was calculated after Sack et al. [32] (Eq. 2):

$$SA = (\pi/4) \times L_s \times W_s \quad (1)$$

$$SPI = SD \times L_s^2 \times 10^{-6} \quad (2)$$

where L_s and W_s are stomatal length and width (μm). The fraction of abaxial epidermis occupied by stomata was calculated as f_s (%) = $SD \times SA \times 10^{-4}$. Total lamina thickness (LT, μm) was the sum of both cuticles, both epidermal layers and the mesophyll, and mesophyll share (%) was mesophyll thickness divided by LT × 100.

Data analysis

This is a comparative anatomical study, so treatment effects were evaluated from the mean of the five readings of each trait, as is customary in quantitative leaf anatomy. Each treatment was compared with the control as a percentage change, and factor-level (main-effect) means were obtained by averaging over the levels of the other factor. Coordination among traits was tested by Pearson correlation among the nine treatment means, or among the eight treated combinations where stated, using SciPy (version 1.11; [33]), with significance accepted at $P \leq 0.05$.

Results

General leaf anatomy

The leaf of sour orange seedlings was dorsiventral in the control and in all treatments (Fig. 1). A compact, darker palisade layer lay beneath the adaxial epidermis, and below it a loosely arranged spongy parenchyma with large intercellular spaces extended to the abaxial epidermis. Large, spherical secretory (oil) cavities, typical of Rutaceae, were embedded in the mesophyll just below the adaxial epidermis (Fig. 1b, f). Both epidermal layers were single-layered and covered by a continuous cuticle.

In surface view, the ordinary epidermal cells on both surfaces were polygonal, with straight to slightly curved anticlinal walls (Figs. 2 and 3). No stomata were found on the adaxial surface in the control or in any treatment (Fig. 2). Stomata were confined to the abaxial surface, where they were scattered evenly among the ordinary cells (Fig. 3). The leaf was therefore strictly hypostomatic, and neither amino acids nor Si induced adaxial stomata.

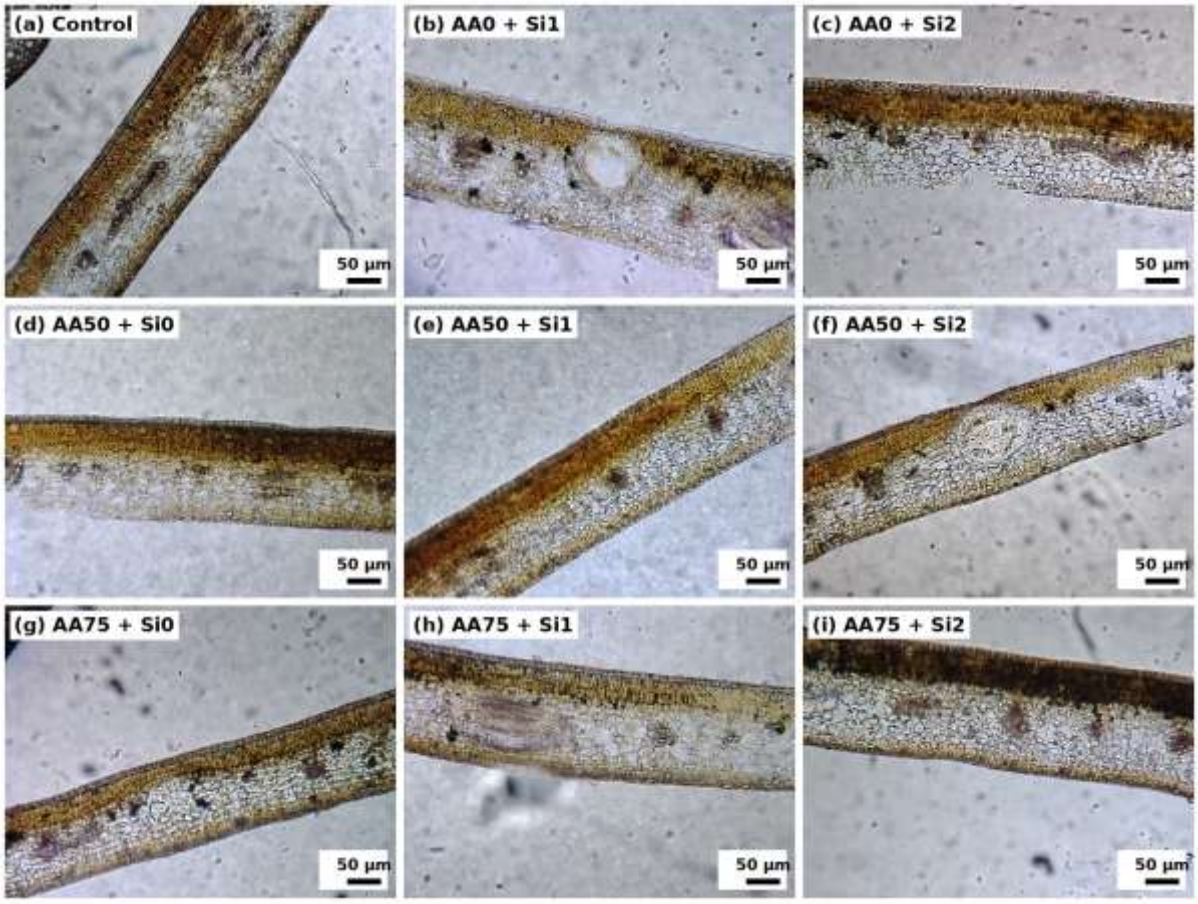


Figure 1. Transverse sections of leaf blades of sour orange seedlings sprayed with amino acids (AA, mg L^{-1}) and silicon (Si, mL L^{-1}); 40× objective, adaxial (palisade) side up. (a) Control; (b) AA0 + Si1; (c) AA0 + Si2; (d) AA50 + Si0; (e) AA50 + Si1; (f) AA50 + Si2; (g) AA75 + Si0; (h) AA75 + Si1; (i) AA75 + Si2. Secretory oil cavities are visible in (b) and (f). Scale bars = 50 µm.

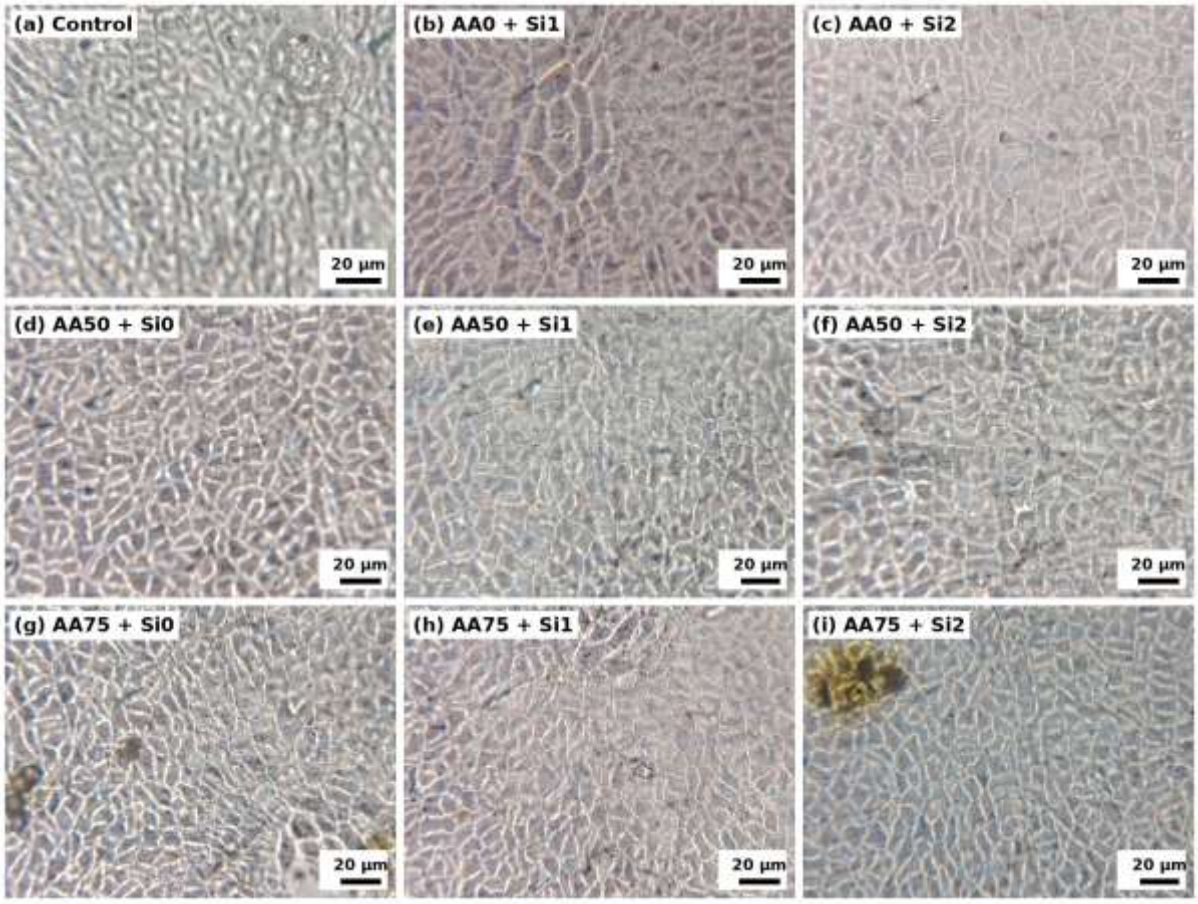


Figure 2. Adaxial (upper) epidermis of sour orange leaves in surface view (40× objective), showing polygonal ordinary epidermal cells and no stomata in any treatment. (a) Control; (b) AA0 + Si1; (c) AA0 + Si2; (d) AA50 + Si0; (e) AA50 + Si1; (f) AA50 + Si2; (g) AA75 + Si0; (h) AA75 + Si1; (i) AA75 + Si2. Scale bars = 20 µm.

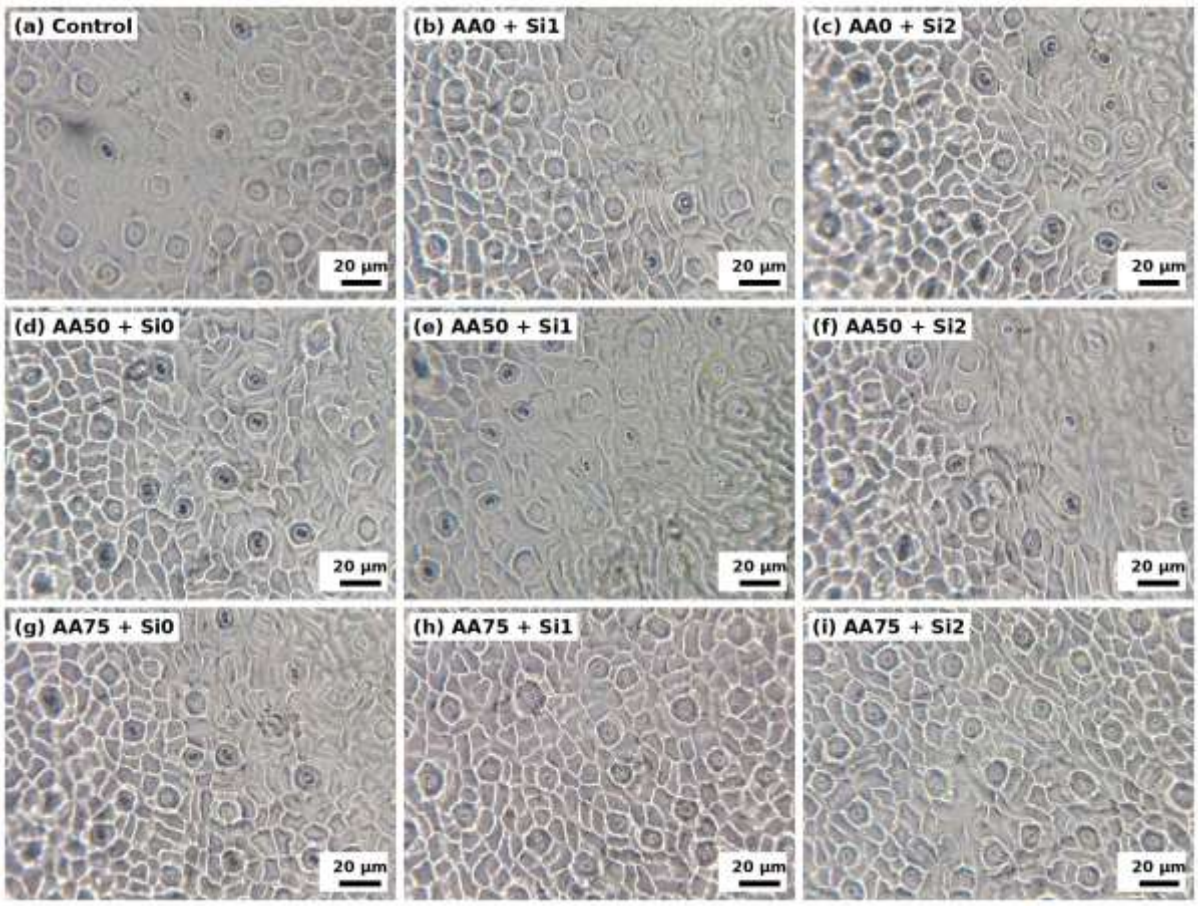


Figure 3. Abaxial (lower) epidermis of sour orange leaves in surface view (40× objective), showing stomata scattered among the ordinary epidermal cells. (a) Control; (b) AA0 + Si1; (c) AA0 + Si2; (d) AA50 + Si0; (e) AA50 + Si1; (f) AA50 + Si2; (g) AA75 + Si0; (h) AA75 + Si1; (i) AA75 + Si2. Scale bars = 20 µm.

Stomatal size, density and pore area

Control leaves had the smallest stomata in the trial (12 × 8 µm; 75 µm²) and the lowest stomatal density (221 stomata mm⁻²) (Table 2). Every treatment increased both traits, stomatal area by 8–150% and density by 3–22%. As a result, the stomatal pore area index (SPI) rose from 0.032 in the control to 0.042–0.091 in the treated seedlings (+32 to +185%), and the fraction of epidermis occupied by stomata from 1.7% to 2.2–4.3%.

The two inputs raised stomatal capacity by different routes. Si applied alone mainly increased the number of stomata: density rose by 22% under AA0 + Si1 (269 mm⁻²) and by 19% under AA0 + Si2 (262 mm⁻²), whereas stomatal area increased by only 8% and 41%. Amino acids applied alone mainly enlarged the stomata: stomatal area rose by 88% under AA50 + Si0 and by 130% under AA75 + Si0, with smaller gains in density (16% and 10%). Averaged over Si levels, AA50 and AA75 increased stomatal area by 70% and 62% relative to AA0 (149 and 142 vs. 88 µm²); averaged over AA levels, Si hardly changed stomatal area (124–130 µm²).

AA50 + Si1 combined the largest stomata (20 × 12 µm; 189 µm²; +150%) with the smallest gain in density (227 mm⁻²; +3%), and had the highest SPI (0.091), 2.9 times that of the control. Among the eight treated combinations, stomatal area and density were inversely related ($r = -0.70$, $P = 0.051$; Fig. 4). The control lay well below this line, its stomata being both small and sparse, so the relationship disappeared when the control was included ($r = -0.22$, $P = 0.57$).

The stomatal length-to-width ratio was 1.50 in the control and 1.50–1.80 at AA0 and AA50, but fell to 1.30–1.33 at AA75 whatever the Si dose; stomata at the highest amino acid dose were therefore noticeably rounder. Ordinary epidermal cells ranged from 8 × 10 µm (AA50 + Si0) to 13 × 19 µm (AA50

+ Si2), compared with 11 × 12 μm in the control, and their area was not related to stomatal density (r = −0.43, P = 0.25).

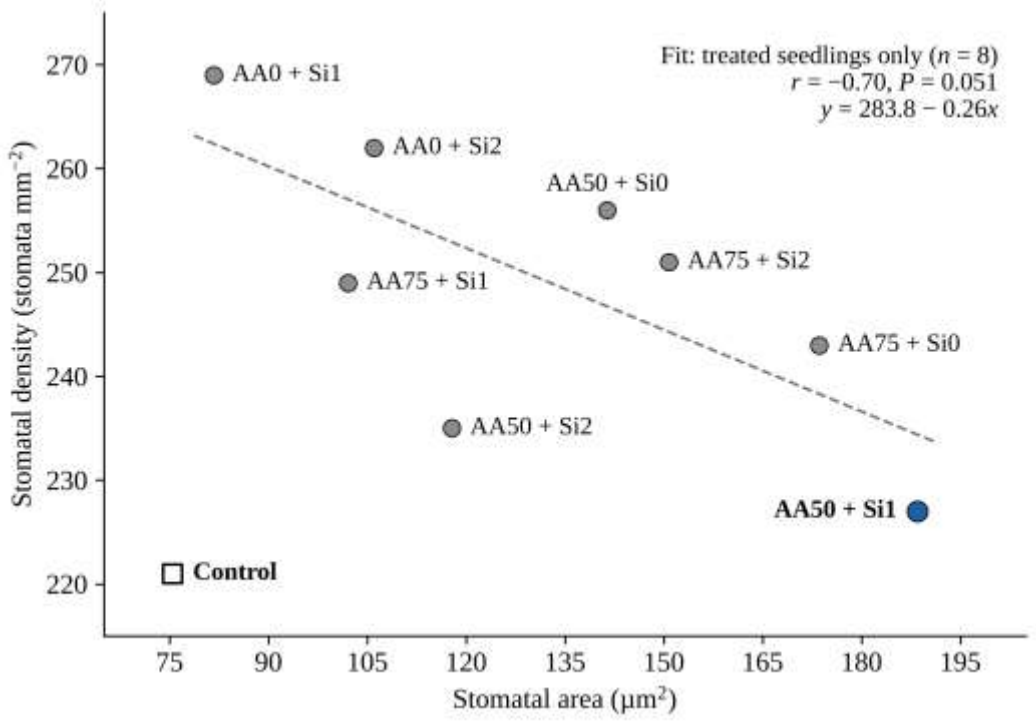


Figure 4. Relationship between stomatal area and stomatal density on the abaxial leaf surface of sour orange seedlings sprayed with amino acids (AA, mg L^{-1}) and silicon (Si, mL L^{-1}). Each point is a treatment mean; the open square is the control. The dashed line is the least-squares fit for the eight treated combinations. AA50 + Si1 (blue) combined the largest stomata with a density close to that of the control.

Cuticle, epidermis and mesophyll

Control leaves had the thinnest lamina (265 μm) (Table 3). All treatments increased lamina thickness, but only slightly (268–283 μm; +1 to +7%). Mesophyll made up 80–85% of the lamina. Neither input applied alone thickened the mesophyll: it was 221 μm under both AA-alone treatments, as in the control, 225 μm under AA0 + Si1, and only 214 μm (−3%) under AA0 + Si2. In contrast, all four AA × Si combinations thickened it, to 237 μm (AA50 + Si1), 236 μm (AA50 + Si2 and AA75 + Si1) and 230 μm (AA75 + Si2), i.e. by 4–7%.

Control leaves also had the thinnest cuticle (2 μm on both surfaces). Every treatment thickened the adaxial cuticle (3–5 μm), most of all the two single high doses, AA0 + Si2 and AA75 + Si0 (5 μm; +150%). The same two treatments had the thickest adaxial (25 μm; +19%) and abaxial (21 μm; +11%) epidermis. Conversely, the combinations AA50 + Si1 and AA75 + Si2 had a thinner epidermis than the control, particularly on the abaxial side (15 and 16 μm vs. 19 μm). The adaxial epidermis was always thicker than the abaxial one (ratio 1.11–1.33).

Across all nine treatments, the combined thickness of the protective layers (both cuticles and both epidermal layers) was inversely related to mesophyll thickness (r = −0.69, P = 0.042; Fig. 5). Treatments that built more mesophyll invested less in surface layers, and vice versa.

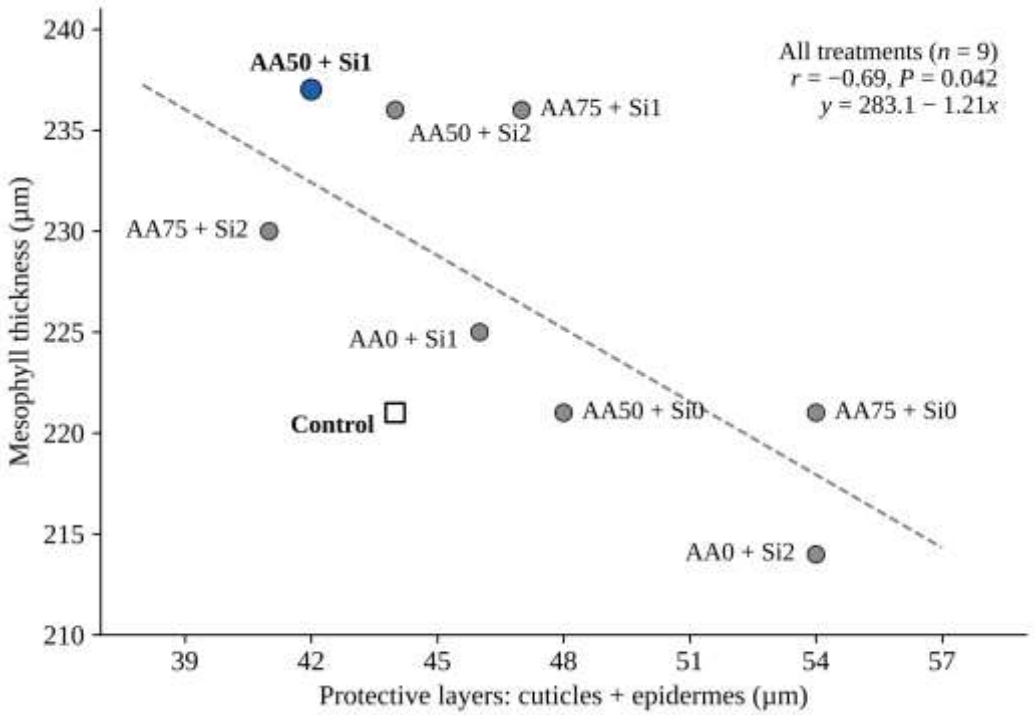


Figure 5. Relationship between the combined thickness of the protective layers (adaxial and abaxial cuticle and epidermis) and mesophyll thickness in sour orange seedlings sprayed with amino acids (AA, mg L^{-1}) and silicon (Si, mL L^{-1}). Each point is a treatment mean; the open square is the control. The dashed line is the least-squares fit for all nine treatments.

Discussion

Foliar amino acids and Si did not change the basic plan of the sour orange leaf, but they changed how it was built. Compared with the control, every treatment produced a leaf with greater stomatal capacity and a thicker cuticle. Beyond this common response, the two inputs acted by different routes: Si mainly increased the number of stomata, amino acids mainly increased their size, and only their combination thickened the mesophyll.

A conserved hypostomatic plan

No treatment induced adaxial stomata. Hypostomaty is a fixed trait of *Citrus* leaves, and in such leaves all CO_2 must reach the palisade cells by diffusing upwards from the abaxial surface through the whole mesophyll [23]. The treatments therefore did not alter where stomata develop; they acted on how many formed and how large they grew on the abaxial surface. This agrees with the view that stomatal patterning is under strong genetic control, whereas density and size are the plastic traits that respond to environmental and internal signals [34], [35].

Both biostimulants raised stomatal capacity

Control leaves sat at the low end of every stomatal trait: the smallest stomata, the lowest density and therefore the lowest stomatal pore area index. All eight treatments raised SPI, by 32–185%. Because SPI is a robust anatomical proxy for maximum stomatal and leaf hydraulic conductance [19], [32], the treated seedlings were built for a higher potential rate of CO_2 uptake than the controls. The control seedlings, grown in pots without supplementary foliar inputs, may have been limited in the nitrogen and structural resources needed to build stomata; both biostimulants appear to have relieved this limitation. Stomatal development is known to respond to the internal nutrient and hormonal status of the leaf [34], and foliar amino acids and Si both improve nutrient status and photosynthetic metabolism [3], [12]. The adaxial cuticle was also thicker than in the control in every treatment, which is consistent with improved carbon supply for cuticle synthesis and, where Si was applied, with silica deposition beneath the cuticle [11].

Silicon alone favours many small stomata

Si applied without amino acids raised stomatal density the most (+19 to +22%) while keeping stomata relatively small. Smaller stomata have shorter diffusion paths for ions and water across the guard cells, so they open and close faster [20], [21]. A leaf built from many small stomata can track fluctuating light and humidity closely and limit unnecessary water loss. Si may reinforce this behaviour at the level of the guard cell itself. Silica deposited on guard cells increased stomatal sensitivity to abscisic acid and lowered stomatal conductance in tall fescue [14], and Si-treated citrus leaves accumulate silica granules in their epidermal cells [15]. Effects of Si on stomatal density depend on species and conditions: Si raised density in micropropagated banana [17] but lowered it in young cacao under water deficit [18]. In sour orange seedlings, the Si-alone phenotype is consistent with a water-conserving, fast-responding stomatal design.

Amino acids favour fewer, larger stomata and more pore area

Amino acids raised stomatal capacity mainly through size. Averaged over Si levels, AA50 and AA75 enlarged stomata by 70% and 62%, whereas Si had almost no main effect on stomatal area. AA50 + Si1 took this to the extreme, with stomata 2.5 times the area of the control and an SPI 2.9 times higher, while density stayed close to the control value. Among the treated seedlings, stomatal size and density were inversely related ($r = -0.70$, $P = 0.051$). Although marginal with eight treatment means, this agrees with the size–density scaling found across thousands of species [19], [22]. The control lay below this line. Together, these patterns suggest a two-step response: the biostimulants first raised the total investment in stomata, and the inputs then decided whether that investment went into more stomata (Si) or larger ones (AA).

A plausible explanation for the AA effect is the dual role of foliar amino acids: as a nitrogen source that supports cell expansion, and as signals that stimulate chlorophyll synthesis and photosynthetic metabolism [3], [5]. A leaf with greater photosynthetic demand benefits from greater stomatal capacity. This anatomical gain offers a mechanism for the faster vegetative growth reported when sour orange and other citrus rootstock seedlings were sprayed with amino acids [6], [7]. Larger stomata are slower to close [20], so this design probably carries a higher water cost under drought; in an irrigated nursery, where water is not limiting, it is an advantage. The rounder stomata at AA75 (length-to-width ratio 1.30–1.33, vs. 1.50 in the control) suggest that the highest amino acid dose altered guard-cell expansion; whether this affects pore aperture should be tested with direct measurements of pore dimensions and gas exchange.

Mesophyll gain required both inputs

The clearest evidence of interaction between the two biostimulants came from the mesophyll. Neither input alone thickened it: amino acids alone left it at the control value (221 μm), Si1 alone added only 4 μm, and Si2 alone reduced it to 214 μm. All four AA × Si combinations, in contrast, thickened it to 230–237 μm. Because neither main effect could produce this gain by itself, the response is best described as synergistic. A thicker mesophyll offers more chloroplast-lined cell surface for CO_2 dissolution and more tissue for light capture per unit leaf area [24]. In a hypostomatic citrus leaf it also lengthens the internal diffusion path [23], and the higher SPI of the combined treatments may compensate for this by supplying more CO_2 at the abaxial surface. Synergy between foliar amino acids and Si has been reported for nutrient accumulation in grassland (Radkowski et al., 2020); the present results extend this concept to leaf anatomy.

The mesophyll gain came largely at the expense of the protective layers. Lamina thickness changed by at most 7%, and across all nine treatments mesophyll thickness was inversely related to the summed thickness of cuticles and epidermes ($r = -0.69$, $P = 0.042$). The leaf therefore appears to work within a nearly fixed thickness budget and to allocate it either to photosynthetic tissue or to surface protection. The single high doses, AA0 + Si2 and AA75 + Si0, allocated it to protection, with a 5 μm adaxial cuticle (2.5 times the control) and the thickest epidermis. For Si, this is the expected response: Si polymerises beneath the cuticle and in epidermal cell walls, forming the cuticle–silica double layer [11], [15], and Si supply thickened both cuticle and epidermis in several horticultural crops, reducing water loss and fungal penetration [12], [36]. The same response under the highest amino acid dose alone is less expected. Biostimulant effects are strongly dose-dependent [37], and some individual amino

acids act mainly by stimulating defence pathways rather than growth [5].A high amino acid load may therefore have been perceived partly as a stress signal, redirecting resources to the surface layers. This interpretation should be tested by profiling cuticular waxes and stress markers.

Implications for nursery practice

The best leaf structure came from moderate doses combined, not from either input at its highest dose. Si appears to secure the structural integrity of the epidermis at low cost, which allows the nitrogen and signalling supplied by amino acids to be invested in the mesophyll and the stomatal apparatus. A growth-oriented programme for sour orange rootstock seedlings could therefore use AA 50 mg L^{-1} + Si 1 mL L^{-1}, which gave the highest stomatal pore area and the thickest mesophyll. Si alone, or a single high dose of either input, gave smaller, denser stomata and thicker protective layers; this phenotype could be useful for hardening seedlings before transplanting to the field, when water stress and pathogen pressure increase.

Limitations and future work

This study has six limitations. It covers one season and one sampling date, taken ten months after the last spray. Anatomical traits were measured without parallel data on gas exchange, leaf water status, or leaf Si and nitrogen contents. The amino acid product also supplied organic N, Zn and Mn, so part of the AA response may reflect these nutrients rather than amino acids alone. Stomatal index could not be calculated because the number of epidermal cells per field was not recorded. Treatment effects were evaluated descriptively from trait means, so the differences reported here should be confirmed in a larger, fully replicated trial. Finally, anatomy was assessed at the light-microscope level only. Future work should link these anatomical shifts to photosynthesis, water-use efficiency and time to budding size, locate silica deposition by SEM–EDX, and repeat the trial over two seasons.

Conclusions

Foliar amino acids and Si remodelled the leaf anatomy of sour orange seedlings without changing its basic hypostomatic plan:

- Every treatment raised the stomatal pore area index (by 32–185%) and the adaxial cuticle thickness above the control.
- Si alone mainly increased stomatal number and, at 2 mL L^{-1}, thickened the cuticle and epidermis, i.e. a protective, water-conserving leaf.
- Amino acids mainly increased stomatal size and pore area.
- Only the AA × Si combinations thickened the mesophyll, indicating a synergistic effect.
- AA 50 mg L^{-1} + Si 1 mL L^{-1} gave the largest stomata, the highest stomatal pore area index (2.9-fold the control) and the thickest mesophyll, i.e. a leaf built for carbon gain.

We recommend AA 50 mg L^{-1} + Si 1 mL L^{-1} for growth-oriented production of sour orange rootstock seedlings, whereas Si-based protection may be more useful when hardening seedlings before transplanting. Linking these anatomical traits to gas exchange, water-use efficiency and time to budding is the next step.

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