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Article

Effect of Foliar Spraying with Silicon and Gibberellic Acid (GA_3) on Some Anatomical Traits of Date Palm (*Phoenix dactylifera L.*) Leaves cv. Barhi

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Abstract: Date palm (*Phoenix dactylifera L.*) leaflets are the main photosynthetic and gas-exchange organs of the tree, yet the anatomical basis of their response to foliar biostimulants remains poorly documented. This study evaluated the effects of foliar silicon (Si; 0, 1 and 2 mL L−1), gibberellic acid (GA3; 0, 50 and 100 mg L−1) and their factorial combinations on the leaflet anatomy of the Iraqi cultivar Barhi, alongside an unsprayed absolute control. Transverse sections were prepared by paraffin embedding and safranin–fast green double staining, and both epidermises were obtained by peeling and nail-varnish impressions for light-microscopic measurement. All treatments modified quantitative leaflet anatomy without altering its isobilateral, amphistomatic organisation, and responses depended on both dose and combination. Si 1 mL L−1 + GA3 100 mg L−1 produced the thickest mesophyll (716 µm; +23.7% over the absolute control), the widest vascular bundles (76 µm; +38.2%), the largest adaxial stomata (17 × 15 µm) and the thickest abaxial epidermis (23 µm). Si 2 mL L−1 + GA3 50 mg L−1 gave the thickest adaxial and abaxial cuticle (5 and 4 µm) and the highest adaxial stomatal frequency (257 mm−2; +25.4%), whereas Si 2 mL L−1 alone gave the highest abaxial stomatal frequency (266 mm−2; +18.2%). GA3 100 mg L−1 alone more than doubled bundle-sheath thickness (+123.1%). Averaged over GA3 levels, Si at 1 mL L−1 increased mesophyll thickness by 10.0% and vascular-bundle diameter by 30.9% relative to Si 0, and Si progressively raised adaxial stomatal frequency. In contrast, combining the two highest concentrations (Si 2 + GA3 100) reduced mesophyll and adaxial epidermis thickness to their minimum values, indicating antagonism at supra-optimal doses. Moderate Si–GA3 combinations therefore remodel the Barhi leaflet towards greater photosynthetic, conductive and protective capacity, and Si 1 mL L−1 + GA3 100 mg L−1 is recommended to enhance mesophyll and vascular development.

Keywords: Arecaceae; beneficial elements; gibberellin; leaflet anatomy; mesophyll; silicon; stomatal frequency; vascular bundle

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1. Introduction

Date palm (Phoenix dactylifera L., Arecaceae) is the keystone fruit tree of arid and semi-arid regions, underpinning food security, rural livelihoods and cultural heritage across the Middle East and North Africa [1], [2]. Sequencing of its genome has opened new avenues for understanding how the species tolerates extreme heat, drought and salinity [3]. Iraq has historically been one of the principal centres of date palm cultivation, and improving the productivity of such strategic crops through sustainable, resource-efficient practices has become a national priority under mounting climatic and resource pressures [4]. Barhi is among its most valued cultivars, prized for fruits that are consumed fresh at the crisp, sweet khalal stage. Consequently, Barhi has been the subject of

numerous studies aimed at improving its growth, yield and fruit quality through pre-harvest applications [5], [6].

The pinnate leaf (frond) is the principal photosynthetic and gas-exchange organ of the palm. Each leaflet consists of two cutinised epidermises, a homogeneous mesophyll and parallel vascular bundles enclosed in bundle sheaths and sclerenchymatous fibre caps, an architecture that reflects the adaptation of the species to high irradiance and water deficit [7]. Leaflet anatomy is highly plastic: the thickness of the epidermis and mesophyll and the dimensions of vascular bundles in date palm change markedly in response to drought, salinity and heat [7]. Because leaflets, and especially their abaxial surfaces, are the main target of foliar sprays, foliar application offers a practical route for reshaping these tissues and improving their function [7]. Moreover, the method by which inputs are delivered strongly influences seedling responses, with foliar application emerging as an efficient route for young plants [8].

Silicon (Si) is the second most abundant element in the Earth's crust and is widely recognised as a beneficial element for plants. It is absorbed as monosilicic acid and deposited as amorphous silica in epidermal cell walls and beneath the cuticle, forming the so-called cuticle–silica double layer [9], [10], [11]. These deposits reinforce cell walls and improve tolerance to biotic and abiotic stresses [12], [13], [14], while in growing tissues Si can increase cell-wall extensibility and promote cell elongation [15]. Nonetheless, plant responses to Si vary widely with species-specific accumulation capacity and growing conditions [16]. In date palm, Si sprays have improved fruit traits and the biochemical status of trees [17], whereas in 'Nabtet-Ali' seedlings foliar Si increased leaf Si content without measurable effects on vegetative growth [18], underlining the need to examine its effects at the tissue level.

Gibberellic acid (GA3) is a key growth regulator that controls cell elongation, cell division and leaf expansion, largely by promoting the degradation of growth-repressing DELLA proteins [19], [20]. Beyond elongation, gibberellins regulate stomatal development [21], [22] and xylem differentiation, expansion and fibre length [23], [24], [25]. In date palm, GA3 is widely used to increase bunch weight and improve the fruit quality of Barhi [5], [6]; moderate concentrations thickened the cuticle and epidermis of Barhi fruits [26], and foliar GA3 altered midrib size and vascular bundle number in date palm seedling leaves [27].

Combined Si and GA3 acted synergistically in date palm seedlings, enhancing growth and heat-stress tolerance through modulation of abscisic acid signalling and antioxidant defences [28]. However, to our knowledge, the anatomical basis of this interaction in the leaves of young palms, and in cv. Barhi in particular, has not been investigated. The present study therefore aimed to (i) evaluate the individual and combined effects of foliar Si and GA3 on cuticle, epidermis and mesophyll thickness in Barhi leaflets; (ii) quantify the responses of stomatal and ordinary epidermal cell dimensions and stomatal frequency on both leaflet surfaces; (iii) assess changes in vascular bundle components; and (iv) identify the combination that best enhances the structural capacity of the leaflet.

2. Materials and Methods

2.1. Experimental site and plant material

The experiment was conducted during the 2025–2026 growing season in Al-Alam district, Salah Al-Din Governorate, Iraq, on one-year-old tissue-culture-derived plants of date palm cv. Barhi, the most widely used planting material for young-palm experiments of this cultivar. Plants were grown in plastic pots 20 cm in diameter filled with a 1:1 (v/v) mixture of peat moss and loamy soil, and were selected for uniform size and vigour and freedom from visible pests and diseases. All plants received identical routine care (irrigation, weeding and pest control) throughout the experiment.

2.2. Treatments and experimental design

A 3 × 3 factorial experiment was laid out in a randomised complete block design (RCBD) with three replicates and one plant per experimental unit. The first factor was silicon at 0, 1 and 2 mL L−1 (coded A1, A2 and A3, respectively), and the second was GA3 at 0, 50 and 100 mg L−1 (coded B1, B2 and B3, respectively). An additional unsprayed

absolute control (A0B0) was included, giving ten treatments in total; the zero level of both factors (A1B1) was sprayed with distilled water only. Silicon was supplied as a commercial liquid potassium silicate fertiliser (K2SiO3; 25% SiO2 and 10% K2O), the most widely marketed foliar silicon source, and solutions were prepared by diluting the required volume in distilled water. GA3 (commercial powder, 90% purity) was dissolved in a few drops of ethanol and brought to volume with distilled water. No surfactant was added to any solution. Plants were sprayed in the early morning to run-off with a 1 L hand-held sprayer, three times at monthly intervals starting on 1 March 2026, and the absolute control plants were left unsprayed. Leaflet samples were collected on 15 July 2026 from the middle portion of fully expanded leaves that had formed during the spray period, so that the measured tissues developed entirely under treatment.

2.3. Preparation of transverse sections

Transverse sections were prepared from fresh leaflet tissue by the paraffin method as follows.

Killing and fixation. Leaflet segments 2–5 mm long were placed in vials containing approximately 20 mL of FAA fixative (50 mL ethanol, 5 mL glacial acetic acid, 10 mL formaldehyde 37–40% and 35 mL distilled water; [29]) for 18–24 h.

Washing and dehydration. Samples were washed twice in 70% ethanol to remove the fixative and then dehydrated through an ascending ethanol series (80, 90 and 95%) for 2 h each, followed by absolute ethanol for 2 h.

Clearing and infiltration. Samples were passed through absolute ethanol–xylene mixtures (3:1, 1:1 and 1:3, v/v) and pure xylene for 2 h each [30]. Half of the xylene was then replaced with molten paraffin wax in an oven at 55–60 °C for 1 h, followed by pure paraffin. Samples were kept in the oven for 4–5 days, the paraffin being renewed every 2 h (five to six changes), with the final change left overnight [31].

Embedding, sectioning and mounting. Samples were oriented and embedded in moulds of molten paraffin (55–60 °C), left for 24 h to solidify, trimmed, mounted on wooden blocks and sectioned at 10–15 μm with a Bright rotary microtome. Ribbons were flattened in a water bath at 40–45 °C, mounted on slides coated with Mayer's glycerine–albumin adhesive, and dried on a hot plate at 40–45 °C for 4–12 h.

Dewaxing and staining. Following Sass [30], slides were dewaxed in xylene (two changes at 50 °C), passed through xylene–absolute ethanol (1:1) for 5 min and a descending ethanol series (96, 80, 70, 50 and 30%) for 5 min each, stained with 0.2% safranin in 50% ethanol for 2–24 h, passed through an ascending ethanol series (30, 50, 70, 80 and 90%) for 5 min each, counterstained with 1% fast green in absolute ethanol for 3–5 s, rinsed in absolute ethanol (5 min), cleared in clove oil–xylene (1:1, 5 min) and xylene (3 min), and permanently mounted in Canada balsam. Slides were dried at 40–45 °C for 24 h, examined under an Olympus compound light microscope and photographed with a digital camera mounted on the microscope.

2.4. Epidermal preparations

Adaxial and abaxial epidermises were obtained from fresh leaflets by peeling, mounted in a drop of glycerine and examined at 40× under the Olympus microscope. Clear nail-varnish impressions of the epidermal surfaces were additionally used to obtain sharp images of stomata and ordinary epidermal cells [32]. Stomatal frequency was expressed as the number of stomata per mm2 of leaflet surface [33], [34].

2.5. Measured traits

In transverse sections, the following traits were measured: adaxial and abaxial cuticle thickness, adaxial and abaxial epidermis thickness, mesophyll thickness, vascular bundle diameter, bundle-sheath thickness and number of sheath cells, diameter of conducting elements, and thickness of the phloem fibre (sclerenchyma) cap. On epidermal preparations, the length and width of stomata and of ordinary epidermal cells and stomatal frequency were recorded on both surfaces. All dimensions were measured in micrometres using a calibrated ocular micrometer, and each value represents the mean of five readings per treatment.

2.6. Data analysis

Each trait value represents the mean of five readings per treatment. To quantify treatment responses, the percentage change of each treatment relative to the absolute control (A0B0) was calculated as [(treatment − control)/control] × 100. Main effects were evaluated by computing the marginal mean of each level of one factor averaged over the three levels of the other factor within the 3 × 3 factorial, the absolute control being excluded from these means. Interaction patterns were identified by comparing each combined treatment with the corresponding single-factor treatments at the same concentrations.

3. Results

3.1. General leaflet anatomy

Transverse sections (Plate 3) showed that the leaflet of cv. Barhi is isobilateral. Both surfaces are covered by a single-layered epidermis overlain by a distinct, safranin-stained cuticle, and each epidermis is immediately underlain by regularly spaced hypodermal sclerenchyma strands that appear as red dots along both surfaces. A homogeneous mesophyll occupies most of the leaflet thickness and encloses a single row of vascular bundles running parallel to the surfaces, interspersed with larger bundles surrounded by a conspicuous bundle sheath and fibre caps. Stomata occurred on both surfaces in longitudinal rows parallel to the veins (Plates 1 and 2), i.e. the leaflet is amphistomatic, in agreement with earlier descriptions of date palm leaves [27], [7]. This general organisation was maintained in all treatments; differences were confined to the quantitative dimensions of the tissues described below. Because the sampled leaves were initiated and expanded entirely during the spray period, these differences reflect developmental responses of the tissues to the treatments rather than modification of already mature leaves.

3.2. Cuticle, epidermis and mesophyll thickness

Adaxial cuticle thickness ranged from 2 to 5 μm (Table 1). The maximum (5 μm; +66.7% over the absolute control) was recorded with GA_3 100 mg L^{-1} alone (A1B3) and with Si 2 + GA_3 50 (A3B2), and the minimum (2 μm) with A2B1 and A3B3. The abaxial cuticle doubled in A3B2 (4 μm vs. 2 μm in the control). Averaged over Si levels, GA_3 at 50 mg L^{-1} raised adaxial cuticle thickness to 4.00 μm compared with 2.67 μm at GA_3 0 (Table 6).

The adaxial epidermis was thickest with GA_3 50 mg L^{-1} alone (A1B2; 31 μm, +24.0%) and thinnest with A3B3 (21 μm, −16.0%). The abaxial epidermis was thickened by most treatments, reaching 23 μm (+27.8%) with Si 1 + GA_3 100 (A2B3), and it increased consistently with GA_3 concentration (17.67, 19.33 and 20.33 μm at 0, 50 and 100 mg L^{-1}) and with Si concentration (18.33, 19.33 and 19.67 μm at 0, 1 and 2 mL L^{-1}).

Mesophyll thickness showed the largest absolute response. It peaked with A2B3 (716 μm, +23.7%), followed by A3B2 (699 μm) and A1B1 (685 μm), and fell to 527 μm (−9.0%) with A3B3. Averaged over GA_3 levels, Si at 1 mL L^{-1} increased mesophyll thickness to 647.3 μm compared with 588.3 μm at Si 0 (+10.0%).

Table 1. Effect of foliar silicon and gibberellic acid on cuticle, epidermis and mesophyll thickness (μm) of date palm cv. Barhi leaflets.

Treatment	Cuticle		Epidermis		Mesophyll
	Adaxial	Abaxial	Adaxial	Abaxial	
A0B0 — Absolute control (unsprayed)	3	2	25	18	579
A1B1 — Si 0 + GA_3 0 (water spray)	3	2	28	16	685
A1B2 — Si 0 + GA_3 50	4	2	**31**	19	532
A1B3 — Si 0 + GA_3 100	**5**	3	29	20	548
A2B1 — Si 1 + GA_3 0	2	2	24	18	601
A2B2 — Si 1 + GA_3 50	3	3	27	17	625

Treatment	Cuticle		Epidermis		Mesophyll
	Adaxial	Abaxial	Adaxial	Abaxial	
A2B3 — Si 1 + GA_3 100	4	3	23	**23**	**716**
A3B1 — Si 2 + GA_3 0	3	2	29	19	659
A3B2 — Si 2 + GA_3 50	**5**	**4**	25	22	699
A3B3 — Si 2 + GA_3 100	2	2	21	18	527

Values are means of five readings. Si in mL L^{-1}; GA_3 in mg L^{-1}. Bold values indicate the maximum in each column.

The thicker cuticle in Si-containing treatments is consistent with the deposition of amorphous silica beneath the cuticle and in the outer walls of epidermal cells, forming the cuticle–silica double layer [9], [10]. Electron microscopy has shown dense silica layers beneath the cuticle and thicker epidermal cell walls in Si-treated leaves [12]. The effect of GA3 on the cuticle and epidermis can be attributed to its stimulation of cell expansion and wall loosening [19]. Our observation that the moderate GA3 concentration (50 mg L−1) was more effective than the higher one in thickening the adaxial epidermis and cuticle mirrors findings in Barhi fruits, in which an intermediate GA3 concentration produced the thickest cuticle and epidermis, with a decline at the highest dose [26]. Doaigey et al. [27] likewise observed slightly wider epidermal cell lumens in date palm seedling leaves sprayed with high GA3.

The thicker mesophyll, particularly under Si 1 + GA3 100, probably reflects two complementary mechanisms: gibberellin-driven cell expansion through DELLA degradation [20], and Si-enhanced cell-wall extensibility in growing tissues, which promotes cell elongation without affecting cell number [15]. A thicker mesophyll implies a larger volume of chloroplast-bearing tissue and hence greater photosynthetic capacity per unit leaflet area. Conversely, the thinner mesophyll and adaxial epidermis under the highest combined dose (A3B3) suggests that the optimum was exceeded, a typical dose-dependent response for growth regulators and beneficial elements whose effects vary with dose and plant status [16]. The water-sprayed treatment (A1B1) also exceeded the absolute control in mesophyll thickness, which may reflect leaflet wetting by the spray itself or natural plant-to-plant variation; A1B1 was therefore used as the reference level when calculating main effects within the factorial.

3.3. Ordinary epidermal cells

Ordinary epidermal cells responded in opposite directions on the two surfaces (Table 2). On the abaxial surface, mean cell length decreased progressively with Si concentration (22.33, 21.00 and 17.00 μm at 0, 1 and 2 mL L^{-1}); the shortest cells were recorded with A3B3 (15 μm, −25.0%) and the longest with A1B2 (25 μm, +25.0%). On the adaxial surface, by contrast, cell length and width increased with Si: mean width rose from 10.33 to 14.00 μm at 2 mL L^{-1}, reaching 15 μm (+66.7%) with A3B2, which also shared the longest adaxial cells (19 μm) with A2B2.

Table 2. Effect of foliar silicon and gibberellic acid on the dimensions (μm) of ordinary epidermal cells of date palm cv. Barhi leaflets.

Treatment	Abaxial epidermis		Adaxial epidermis	
	Length	Width	Length	Width
A0B0 — Absolute control (unsprayed)	20	11	16	9
A1B1 — Si 0 + GA_3 0 (water spray)	23	12	13	11
A1B2 — Si 0 + GA_3 50	**25**	10	14	10
A1B3 — Si 0 + GA_3 100	19	**13**	15	10
A2B1 — Si 1 + GA_3 0	21	11	17	11
A2B2 — Si 1 + GA_3 50	23	11	**19**	10
A2B3 — Si 1 + GA_3 100	19	9	12	10
A3B1 — Si 2 + GA_3 0	16	10	15	13

Treatment	Abaxial epidermis		Adaxial epidermis	
	Length	Width	Length	Width
A3B2 — Si 2 + GA_3 50	20	12	**19**	**15**
A3B3 — Si 2 + GA_3 100	15	10	17	14

Values are means of five readings. Bold values indicate the maximum in each column.

The shorter abaxial pavement cells coinciding with higher abaxial stomatal frequency under Si (Table 4) reflect the well-known reciprocal relationship between pavement cells and guard cells, which arise from shared protodermal lineages; diverting more of these cells into the stomatal pathway results in smaller pavement cells between stomatal files [35]. The wider adaxial cells under Si are consistent with its role in increasing wall extensibility in growing tissues [15] and with the preferential accumulation of silica in epidermal cell walls [12], [11].

3.4. Stomatal dimensions and frequency

All treatments maintained or increased stomatal dimensions on both surfaces relative to the absolute control (Table 3; Plates 1 and 2). On the adaxial surface, Si 1 + GA_3 100 (A2B3) produced the longest (17 μm, +70.0%) and widest (15 μm, +87.5%) stomata, and GA_3 100 mg L^{-1} gave the highest marginal means for adaxial stomatal length and width (15.00 and 12.33 μm). On the abaxial surface, the longest stomata (16 μm, +45.5%) were recorded with A1B3 and A3B1, and the widest (13 μm, +30.0%) with Si 2 alone (A3B1).

Table 3. Effect of foliar silicon and gibberellic acid on stomatal dimensions (μm) of date palm cv. Barhi leaflets.

Treatment	Abaxial stomata		Adaxial stomata	
	Length	Width	Length	Width
A0B0 — Absolute control (unsprayed)	11	10	10	8
A1B1 — Si 0 + GA_3 0 (water spray)	13	10	13	10
A1B2 — Si 0 + GA_3 50	13	9	10	8
A1B3 — Si 0 + GA_3 100	**16**	11	14	12
A2B1 — Si 1 + GA_3 0	13	10	15	12
A2B2 — Si 1 + GA_3 50	15	9	13	11
A2B3 — Si 1 + GA_3 100	13	11	**17**	**15**
A3B1 — Si 2 + GA_3 0	**16**	**13**	11	9
A3B2 — Si 2 + GA_3 50	15	10	15	13
A3B3 — Si 2 + GA_3 100	12	10	14	10

Values are means of five readings. Bold values indicate the maximum in each column.

Abaxial stomatal frequency ranged from 220 to 266 stomata mm^{-2} (Table 4), peaking with A3B1 (+18.2%), whereas A3B3 was close to the control (220 mm^{-2}). On the adaxial surface, every treatment increased stomatal frequency above the absolute control (205 mm^{-2}), with the maximum in A3B2 (257 mm^{-2}, +25.4%) followed by A2B1 (253 mm^{-2}); the marginal mean rose steadily with Si concentration (226.0, 244.7 and 245.7 mm^{-2}). The adaxial-to-abaxial frequency ratio ranged from 0.91 to 1.08, confirming a nearly even distribution of stomata on both leaflet surfaces.

Table 4. Effect of foliar silicon and gibberellic acid on stomatal frequency (stomata mm^{-2}) of date palm cv. Barhi leaflets.

Treatment	Abaxial surface	Adaxial surface
A0B0 — Absolute control (unsprayed)	225	205
A1B1 — Si 0 + GA_3 0 (water spray)	236	216
A1B2 — Si 0 + GA_3 50	228	225
A1B3 — Si 0 + GA_3 100	246	237

Treatment	Abaxial surface	Adaxial surface
A2B1 — Si 1 + GA_3 0	235	253
A2B2 — Si 1 + GA_3 50	249	243
A2B3 — Si 1 + GA_3 100	251	238
A3B1 — Si 2 + GA_3 0	**266**	249
A3B2 — Si 2 + GA_3 50	247	**257**
A3B3 — Si 2 + GA_3 100	220	231

Values are means of five readings; frequency was calculated per mm^2 of leaflet surface, not per microscope field. Bold values indicate the maximum in each column.

Larger stomata under GA3 are expected given its stimulation of cell expansion, including that of guard cells [19], [20]. Gibberellins also promote stomatal formation, acting at an early step of the stomatal lineage [21], and their signals are integrated into the core regulatory network that sets stomatal density and patterning [22]. The increase in stomatal frequency with Si in the present study contrasts with reductions reported in rice and cacao [36] and with the lack of change in maize [37], confirming that Si effects on the epidermis depend on species, dose and growing conditions [16]. Functionally, the combination of higher stomatal density and larger stomata raises the anatomical maximum stomatal conductance and gas-exchange capacity [38], which could support higher photosynthetic rates, while the thicker cuticle–silica layer may limit cuticular water loss under dry conditions [10], [13].

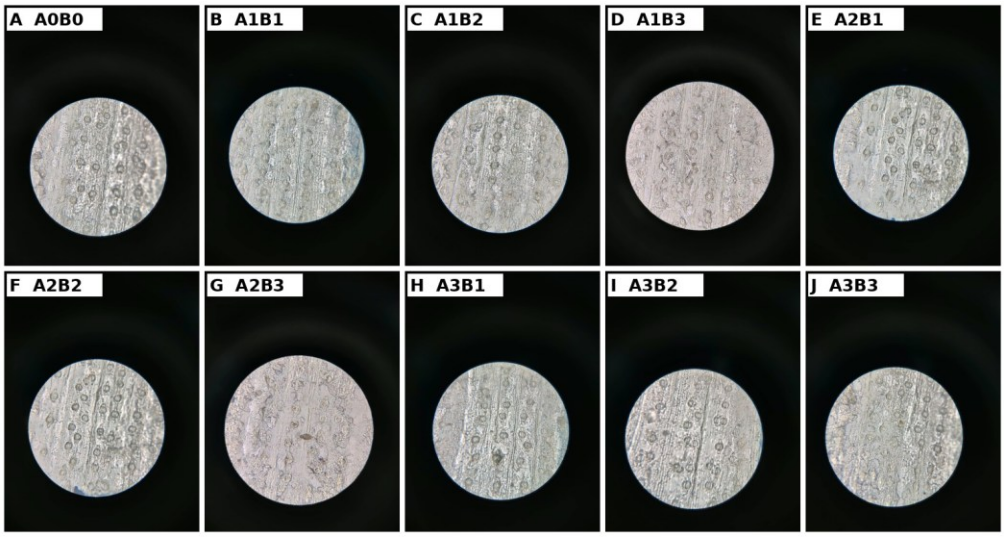


Plate 1. Adaxial epidermis of date palm cv. Barhi leaflets showing stomata under the studied treatments (40×). A, A0B0; B, A1B1; C, A1B2; D, A1B3; E, A2B1; F, A2B2; G, A2B3; H, A3B1; I, A3B2; J, A3B3.

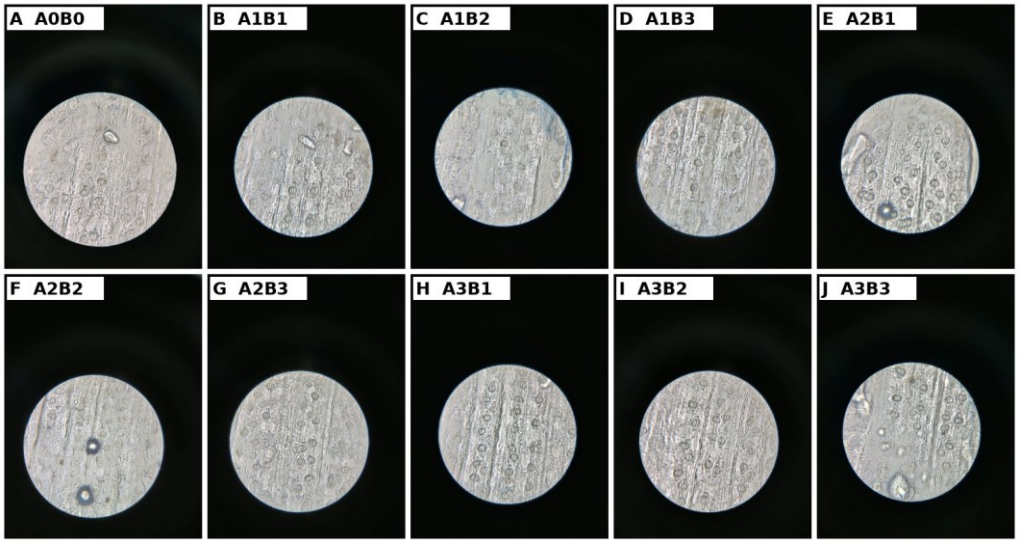


Plate 2. Abaxial epidermis of date palm cv. Barhi leaflets showing stomata under the studied treatments (40×). Panel order as in Plate 1.

3.5. Vascular bundles

Vascular bundle diameter increased in seven of the nine factorial treatments (Table 5), reaching 76 μm (+38.2%) with A2B3, followed by A3B1 (72 μm) and A2B1 (71 μm); the smallest bundles (49 μm) occurred with A1B3. Averaged over GA_3 levels, Si at 1 mL L^{-1} increased bundle diameter to 70.67 μm compared with 54.00 μm at Si 0 (+30.9%). All treatments thickened the bundle sheath (19–29 μm vs. 13 μm in the control), with the maximum in A1B3 (+123.1%), and GA_3 100 mg L^{-1} gave the highest marginal mean (26.33 μm). The number of sheath cells ranged from 10 to 15, being highest in A2B1 and A3B2. The diameter of conducting elements increased at the higher Si concentration (marginal mean 7.33 μm) and peaked in A3B3 (9 μm, +28.6%). Phloem fibre caps were thicker in all treatments (37–63 μm vs. 33 μm), with the highest values in A1B1 (63 μm) and A1B2 (54 μm); their marginal mean declined with increasing GA_3 (52.67, 46.67 and 38.00 μm).

Table 5. Effect of foliar silicon and gibberellic acid on vascular bundle traits of date palm cv. Barhi leaflets.

Treatment	Bundle diameter (μm)	Sheath thickness (μm)	Sheath cells (no.)	Conducting element diameter (μm)	Phloem fibre cap (μm)
A0B0 — Absolute control (unsprayed)	55	13	13	7	33
A1B1 — Si 0 + GA_3 0 (water spray)	62	20	10	6	**63**
A1B2 — Si 0 + GA_3 50	51	23	13	5	54
A1B3 — Si 0 + GA_3 100	49	**29**	11	5	39
A2B1 — Si 1 + GA_3 0	71	25	**15**	5	45
A2B2 — Si 1 + GA_3 50	65	19	11	7	40
A2B3 — Si 1 + GA_3 100	**76**	23	13	5	37
A3B1 — Si 2 + GA_3 0	72	19	13	8	50
A3B2 — Si 2 + GA_3 50	58	21	**15**	5	46
A3B3 — Si 2 + GA_3 100	66	27	10	**9**	38

Values are means of five readings. Bold values indicate the maximum in each column.

The superiority of Si 1 + GA3 100 for bundle diameter agrees with the established role of gibberellins in xylem formation and expansion. Gibberellin–auxin cross-talk releases ARF6 and ARF8 from DELLA repression to drive the xylem expansion phase [24], and

gibberellins promote PIN1-dependent polar auxin transport within the cambium, biasing stem-cell daughters towards xylem identity [25]. In date palm itself, high GA3 increased midrib size and vascular bundle number in seedling leaves [27]. The striking thickening of the bundle sheath in all treatments is of particular interest in palms, because sheath cells of large bundles in date palm leaves contain silica bodies [27]; this tissue is therefore a likely sink for absorbed Si and a source of mechanical support and protection for the vascular tissue [11].

The decline in phloem fibre cap thickness with increasing GA3 may indicate that gibberellin channels cellular resources towards elongation and expansion rather than secondary wall thickening. Although elevated gibberellin biosynthesis lengthened xylem fibres in transgenic trees [23] and altered lignin biosynthesis in tobacco [39], these effects concern fibre length and wall chemistry rather than the cross-sectional thickness of fibre caps. The wider conducting elements at the higher Si concentration may be linked to the improved water and nutrient status commonly associated with Si supply [14], which would enhance hydraulic efficiency within the leaflet.

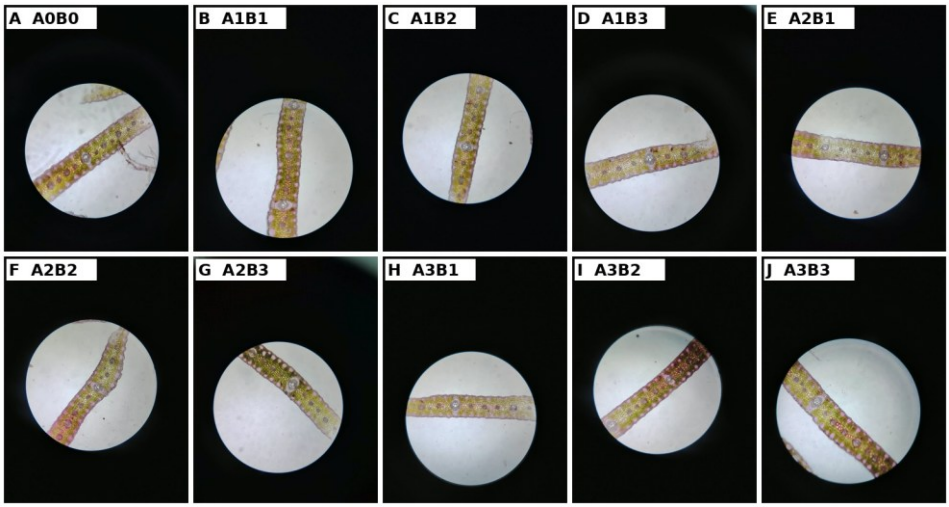


Plate 3. Transverse sections of date palm cv. Barhi leaflets under the studied treatments (40×), showing the cuticle, both epidermises, hypodermal fibre strands (red), mesophyll and vascular bundles. Panel order as in Plate 1.

3.6. Main effects and Si × GA_3 interaction

Table 6 summarises the marginal means of the principal traits. Silicon was the dominant factor for traits linked to photosynthetic and conductive capacity (mesophyll thickness, vascular bundle diameter and adaxial stomatal frequency), with the best response at 1 mL L^{-1}, whereas GA_3 dominated adaxial stomatal dimensions, abaxial epidermis thickness and bundle-sheath thickness, with the best response at 100 mg L^{-1}.

Table 6. Marginal means of the main effects of silicon and gibberellic acid on key anatomical traits of date palm cv. Barhi leaflets (µm unless stated otherwise).

Trait	Absolute control	Si (mL L^{-1})			GA_3 (mg L^{-1})		
		0	1	2	0	50	100
Adaxial cuticle thickness	3	**4.00**	3.00	3.33	2.67	**4.00**	3.67
Abaxial epidermis thickness	18	18.33	19.33	**19.67**	17.67	19.33	**20.33**
Mesophyll thickness	579	588.33	**647.33**	628.33	**648.33**	618.67	597.00
Adaxial stomatal length	10	12.33	**15.00**	13.33	13.00	12.67	**15.00**
Adaxial stomatal width	8	10.00	**12.67**	10.67	10.33	10.67	**12.33**
Adaxial stomatal frequency (mm^{-2})	205	226.00	244.67	**245.67**	239.33	**241.67**	235.33

Trait	Absolute control	Si (mL L^{-1})			GA_3 (mg L^{-1})		
		0	1	2	0	50	100
Abaxial stomatal frequency (mm^{-2})	225	236.67	**245.00**	244.33	**245.67**	241.33	239.00
Vascular bundle diameter	55	54.00	**70.67**	65.33	**68.33**	58.00	63.67
Bundle-sheath thickness	13	**24.00**	22.33	22.33	21.33	21.00	**26.33**
Phloem fibre cap	33	**52.00**	40.67	44.67	**52.67**	46.67	38.00

Each level mean of one factor is averaged over the three levels of the other factor within the 3 × 3 factorial; the absolute control is not included. Bold values indicate the highest mean for each factor.

4. Discussion

Three interaction patterns emerged. (i) Synergism under Si 1 + GA3 100 (A2B3), which produced the highest values for four key traits (mesophyll thickness, vascular bundle diameter, adaxial stomatal dimensions and abaxial epidermis thickness), exceeding the corresponding single-factor treatments at the same concentrations (A2B1 and A1B3). (ii) A protective pattern under Si 2 + GA3 50 (A3B2), which combined the thickest cuticle on both surfaces, the highest adaxial stomatal frequency and the widest adaxial epidermal cells with a thick mesophyll (699 μm). (iii) Antagonism at the highest combined dose (A3B3), which gave the thinnest mesophyll and adaxial epidermis, the shortest abaxial epidermal cells and the lowest abaxial stomatal frequency. The synergistic pattern agrees with Khan et al. [28], who found that integrated Si and GA3 application to date palm seedlings was more effective than either alone in promoting growth and reducing oxidative stress through regulation of abscisic and salicylic acid signalling. Silicon itself can modulate endogenous gibberellins and other hormones [14], which helps explain why the anatomical response depended on the balance between the two inputs rather than on the absolute dose of either. Antagonism at high doses is probably due to supra-optimal hormonal stimulation coinciding with silica deposition that may limit wall extensibility in maturing tissues, since Si increases wall extensibility in growing zones but stiffens walls in more mature regions [15], [16].

5. Conclusion

Foliar Si and GA_3 induced clear quantitative changes in the leaflet tissues of date palm cv. Barhi without altering its isobilateral, amphistomatic organisation. Si at 1 mL L^{-1} was the most effective level for increasing mesophyll thickness (+10.0%) and vascular bundle diameter (+30.9%) relative to Si 0, and Si progressively increased adaxial stomatal frequency. GA_3 at 100 mg L^{-1} had the strongest effect on adaxial stomatal dimensions and on abaxial epidermis and bundle-sheath thickness, whereas 50 mg L^{-1} was optimal for adaxial cuticle thickness. The combination Si 1 mL L^{-1} + GA_3 100 mg L^{-1} acted synergistically on photosynthetic and conductive tissues, Si 2 mL L^{-1} + GA_3 50 mg L^{-1} gave the best protective surface traits, and combining the two highest concentrations was antagonistic. We therefore recommend Si 1 mL L^{-1} + GA_3 100 mg L^{-1} for enhancing mesophyll and vascular development in Barhi, Si 2 mL L^{-1} + GA_3 50 mg L^{-1} where surface protection is the priority in drier sites, and avoidance of the highest combined dose. Future multi-season studies should link these anatomical changes with gas exchange, leaf Si content, yield and fruit quality.

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