



FOLIAR APPLICATION OF SALICYLIC ACID AND SEAWEED EXTRACT MODIFIES LEAFLET ANATOMY OF YOUNG DATE PALM TREES (*PHOENIX DACTYLIFERA* L. CV. KHADRAWI)

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Abstract. The effects of foliar biostimulants on leaflet tissue organisation in date palm remain poorly documented. A field experiment in Salah al-Din, Iraq, evaluated three monthly foliar sprays of salicylic acid (SA; 50, 100 and 150 mg L⁻¹) combined factorially with a seaweed extract (SW; 1, 2 and 3 mL L⁻¹), plus an untreated control, on 19 leaflet anatomical traits of five-year-old *Phoenix dactylifera* L. cv. Khadrawi palms. All SA × SW combinations increased stomatal density on the abaxial (+32.1%) and adaxial (+30.7%) surfaces ($P \leq 0.001$) without reducing stomatal length, raising the stomatal pore area index by 32.7–44.4%. Treated palms also had thicker adaxial and abaxial cuticles (+55.6%), a thicker adaxial epidermis (+13.9%), wider vascular bundles (+27.5%) and thicker phloem fibre caps (+9.6%). SA concentration governed the stomatal and epidermal responses: 150 mg L⁻¹ raised total stomatal density to 580 mm⁻², above 50 and 100 mg L⁻¹ ($P = 0.014$), and gave the thickest adaxial epidermis ($P = 0.047$). SW concentration mainly affected adaxial cuticle thickness ($P = 0.008$), and vascular bundle size depended on the SA × SW combination. Principal component analysis (56.3% of the variation on two axes) and a composite anatomical index ranked SA 150 mg L⁻¹ + SW 1 mL L⁻¹ (T7) first and SA 150 mg L⁻¹ + SW 3 mL L⁻¹ (T9) second. Foliar SA at 150 mg L⁻¹ with seaweed extract therefore promotes a denser stomatal apparatus, a reinforced dermal system and larger vascular bundles, traits linked to greater gas-exchange capacity and resilience.

Keywords: *Phoenix dactylifera*; biostimulant; stomatal density; cuticle; vascular bundle; leaf anatomy; principal component analysis.

1. Introduction

Date palm (*Phoenix dactylifera* L., Arecaceae) is one of the oldest cultivated fruit trees. Its cultivation in Mesopotamia dates back to at least 3000 BCE, and world date production reached roughly 9.5 million tonnes in 2020 (Al-Karmadi and Okoh, 2024). In Iraq the crop is propagated mainly through offshoots, and the young

palms derived from them need several years of vigorous vegetative growth before reaching full productivity. Treatments that strengthen the photosynthetic and protective tissues of their leaves during this juvenile phase are therefore of direct practical value, in line with the wider need for sustainable, locally adapted practices in Iraqi agriculture (Khalaf et al., 2027).

The date palm leaflet is a unifacial, amphistomatic organ. It bears tetracytic stomata on both surfaces, a uniseriate epidermis covered by a cuticle, a colourless hypodermis, a compact chlorenchymatous mesophyll and collateral vascular bundles surrounded by a sheath and fibre caps (Zein El Din et al., 2020). This architecture is highly plastic: osmotic stress, for example, thickens the cuticle and guard-cell walls and makes the mesophyll more compact in date palm plantlets (Zein El Din et al., 2020). Leaflet anatomy is therefore a sensitive and integrative readout of how a treatment alters leaf development. Stomatal density and size set the anatomical ceiling for stomatal conductance and, through it, for CO₂ uptake and water-use efficiency (Bertolino et al., 2019).

Plant biostimulants are substances or microorganisms that, applied in small quantities, improve nutrient use efficiency, tolerance to abiotic stress or crop quality, independently of their nutrient content (du Jardin, 2015). Seaweed extracts, especially those prepared from the brown alga *Ascophyllum nodosum*, are among the most widely used. They contain cytokinins, auxins and gibberellin-like substances, glycine betaine, polysaccharides such as alginates, laminarin and fucoidan, phlorotannins and trace minerals (Shukla et al., 2019). Their effects are attributed mostly to the interacting organic fraction rather than to any single compound; ashing an extract abolishes its activity (Ali et al., 2021). Foliar seaweed extract increased leaf number and chlorophyll content of date palm cv. Saeqi under salinity (Odeh et al., 2026). In *Moringa oleifera*, an algal extract was among the most effective foliar treatments for enlarging the midvein vascular bundle, xylem, phloem and lamina thickness (Abou-shlell et al., 2017).

Salicylic acid (2-hydroxybenzoic acid) is a phenolic phytohormone best known for its role in systemic acquired resistance. It also regulates cell division and cell expansion in a strongly dose-dependent manner: low exogenous concentrations often promote growth, whereas high concentrations inhibit it (Li et al., 2022). In date palm, foliar SA improved growth, relative water content and the K⁺/Na⁺ ratio of salinity-stressed offshoots and restored their indole-3-acetic acid level (Shareef et al., 2022). It also reduced oxidative damage in UV-B-stressed seedlings (Swaïd and Shareef, 2025) and in salinity-stressed palms of cv. Barhi (Suhim et al., 2023). At the anatomical level, the evidence is scarce and contradictory. SA at 0.1–1 mM reduced the thickness of all leaf tissue components in barley (Uzunova and Popova, 2000). In contrast, it increased stomatal frequency on both leaf surfaces of field-grown maize (Abdulameer et al., 2017) and enlarged leaf cells in strawberry (Akin and Aras, 2026).

Combined applications of SA and seaweed extract are attractive because the two act through different but complementary routes: SA through hormonal and redox signalling, seaweed extract through a supply of growth regulators, osmolytes and signalling oligosaccharides. In French marigold, SA at 200 mg L⁻¹ gave the largest gains in growth, chlorophyll and leaf nutrient content, while seaweed extract raised carotenoid and essential oil content (El-Ziat et al., 2024). To our knowledge, no study has examined how foliar SA and seaweed extract, alone or in combination, modify the internal structure of date palm leaflets.

The present study therefore aimed to (i) quantify the effects of three SA concentrations combined with three seaweed extract concentrations on the stomatal, dermal and vascular traits of leaflets of young date palms cv. Khadrawi; (ii) separate the contributions of each factor; and (iii) identify, through multivariate analysis, the combination that produces the most favourable leaflet anatomy.

2. Materials and Methods

2.1. Plant material and experimental site

The experiment was conducted in 2025–2026 in an established private date palm orchard in Al-Alam District, Salah al-Din Governorate, Iraq. Twenty-eight young, fruit-bearing palms (offshoot-derived) of cv. Khadrawi, five years old and as uniform as possible in vigour and size, were selected within the orchard. All palms received the orchard's routine irrigation, fertilisation and pest management throughout the experiment.

2.2. Treatments and experimental design

The experiment comprised ten treatments: an untreated control, sprayed with water only, and the nine combinations of three salicylic acid (SA) concentrations (50, 100 and 150 mg L⁻¹) with three concentrations (1, 2 and 3 mL L⁻¹) of a commercial liquid seaweed extract (Magic Seaweed; seaweed extract 25%) (Table 1).

The nine SA × SW combinations were arranged in a randomised complete block design (RCBD) with three replicates (blocks). Each block contained nine palms, one per treatment, so that each palm was one experimental unit (27 palms). One additional palm, sprayed with water only, served as the untreated control, giving 28 palms in total. Treatments were applied as foliar sprays three times at monthly intervals, on 10 May, 10 June and 10 July 2025.

Table 1. Treatment codes and concentrations.

Code	Salicylic acid (mg L ⁻¹)	Seaweed extract (mL L ⁻¹)
Control	0	0
T1	50	1
T2	50	2
T3	50	3
T4	100	1
T5	100	2
T6	100	3
T7	150	1
T8	150	2
T9	150	3

2.3. Sampling

Leaflet samples were collected on 10 August 2026, about 13 months after the last spray. Fully expanded leaflets were taken from the middle part of mature fronds of each palm, and samples were cut from the central portion of the leaflet lamina.

2.4. Preparation of transverse sections

Permanent transverse sections were prepared by the paraffin method (Johansen, 1940; Sass, 1958). Leaflet segments of 2–5 mm were softened in 1% NaOH for 24–48 h, rinsed in distilled water and fixed in FAA (formalin–acetic acid–alcohol: 50 mL ethanol, 5 mL glacial acetic acid, 10 mL formaldehyde 37–40% and 35 mL distilled water) for 18–24 h. The fixed material was washed and stored in 70% ethanol.

Samples were dehydrated through an ethanol series (80%, 90%, 95% and absolute ethanol; 2 h each), cleared in ethanol–xylene mixtures (3:1, 1:1, 1:3) and pure xylene, and infiltrated with paraffin wax (melting point 55–60 °C) in an oven over 4–5 days. The material was embedded in paraffin blocks and sectioned at 10–15 µm with a rotary microtome. Ribbons were floated on a water bath at 40–45 °C, mounted on glycerin–albumin-coated slides and dried on a hot plate at 40–45 °C for 12–24 h.

Sections were dewaxed in xylene, rehydrated through a descending ethanol series and double-stained with 0.2% safranin O in 50% ethanol for 2–24 h and 1% fast green FCF in absolute ethanol for 3–5 s. They were then dehydrated, cleared in xylene–clove oil (1:1) and xylene, and mounted permanently in Canada balsam (Sass, 1958).

2.5. Epidermal preparations

The adaxial and abaxial epidermises were obtained from fresh leaflet segments by the stripping method. Strips were mounted in glycerin jelly and examined directly. Epidermal imprints made with transparent nail varnish were used in parallel to confirm stomatal outlines and ordinary epidermal cell shape (Al-Hamdany et al., 2024).

2.6. Microscopy and measurements

Preparations were examined under an Olympus compound light microscope and photographed with a mounted digital camera. Linear dimensions were measured with a graduated ocular micrometer. Each trait was measured five times (five stomata, five ordinary epidermal cells per surface, or five sections), and the mean was used as the value of the treatment.

The following traits were recorded (in µm unless stated otherwise):

- **Stomatal traits:** stomatal (guard-cell pair) length and width on both surfaces, and stomatal density (stomata mm⁻², calculated from the number of stomata per field of view divided by the calibrated field area) (Stace, 1965).
- **Stomatal pore area index (SPI):** stomatal density × (stomatal length)², both expressed in mm, multiplied by 100. SPI is a dimensionless proxy for the anatomical capacity for gas exchange (Sack et al., 2003).

- **Epidermal traits:** length and width of ordinary epidermal cells on both surfaces; epidermis thickness on both surfaces; cuticle thickness on both surfaces. Terminology followed Dilcher (1974).
- **Vascular traits:** vascular bundle diameter; bundle-sheath thickness; number of bundle-sheath cells; diameter of conducting elements; thickness of the phloem fibre (sclerenchyma) cap.

2.7. Statistical analysis

The nine SA × SW combinations formed a 3 × 3 factorial, and the untreated control was added as an external reference. The factorial part was analysed by two-way analysis of variance on treatment means (five measurements per mean). The SA × SW interaction mean square (4 df) served as the error term (Gomez and Gomez, 1984), and Tukey's one-degree-of-freedom test for non-additivity (Tukey, 1949) was used to detect SA × SW interaction. Marginal means of each factor were compared using the least significant difference (LSD) at $P \leq 0.05$.

The overall effect of treatment relative to the untreated control was tested with a one-sample t-test comparing the nine treatment means with the control value (8 df). Because the control was represented by a single palm outside the blocks, this comparison should be interpreted with caution. Pearson correlation coefficients were computed between traits across the ten treatments.

Thirteen primary traits were standardised (z-scores) and submitted to principal component analysis (PCA). A composite anatomical index was calculated for each treatment as the mean z-score of the same 13 traits. Analyses were performed in Python 3 with the statsmodels, SciPy and scikit-learn libraries (Seabold and Perktold, 2010).

3. Results

3.1. General leaflet anatomy

In transverse section (Plate 1), the leaflet of cv. Khadrawi showed the typical unifacial organisation of the date palm. It had a uniseriate epidermis on both surfaces, covered by a continuous cuticle, and a compact chlorenchymatous mesophyll. Collateral vascular bundles were arranged in a single row, each enclosed by a parenchymatous bundle sheath and capped by sclerenchymatous phloem fibres. Stomata occurred on both surfaces (amphistomatic) and lay in rows parallel to the leaflet axis (Plate 2). This general pattern was retained in all treatments; the differences between treatments were quantitative.

3.2. Stomatal traits

All SA × SW combinations increased stomatal density on both surfaces relative to the control (Table 2). Abaxial density rose from 193 to a treated mean of 255 stomata mm^{-2} (+32.1%, $P = 0.001$), and adaxial density from 189 to 247 stomata mm^{-2} (+30.7%, $P < 0.001$). The highest values were recorded in T8 on the abaxial surface (301 mm^{-2} , +56.0%) and in T9 on the adaxial surface (302 mm^{-2} , +59.8%). Densities on the two surfaces were similar; the adaxial/abaxial ratio stayed close to 1 (0.81–1.12) and was not altered by treatment ($P = 0.935$).

Stomatal length did not differ from the control on either surface ($P \geq 0.276$), whereas stomatal width increased on both the abaxial (+20.0%, $P < 0.001$) and adaxial (+11.1%, $P = 0.017$) surfaces. Because density rose while length was maintained, the stomatal pore area index increased by 44.4% on the abaxial

surface ($P = 0.006$) and by 32.7% on the adaxial surface ($P = 0.029$). T7 almost doubled the index on both surfaces (+96.9% and +94.4%).

Table 2. Stomatal traits of leaflets of young date palms cv. Khadrawi sprayed with salicylic acid (SA) and seaweed extract (SW). Length and width in μm ; density in stomata mm^{-2} ; SPI = stomatal pore area index (dimensionless). Values are means of five measurements.

Treatment	Abaxial length	Abaxial width	Adaxial length	Adaxial width	Abaxial density	Adaxial density	SPI abaxial	SPI adaxial
Control	14	10	13	9	193	189	3.78	3.19
T1 (SA50 + SW1)	13	11	12	9	252	215	4.26	3.10
T2 (SA50 + SW2)	15	13	13	10	285	230	6.41	3.89
T3 (SA50 + SW3)	13	12	11	10	201	226	3.40	2.74
T4 (SA100 + SW1)	15	12	12	9	236	247	5.31	3.56
T5 (SA100 + SW2)	14	11	13	9	215	235	4.21	3.97
T6 (SA100 + SW3)	17	13	15	10	227	209	6.56	4.70
T7 (SA150 + SW1)	16	12	15	11	291	276	7.45	6.21
T8 (SA150 + SW2)	13	11	12	10	301	283	5.09	4.08
T9 (SA150 + SW3)	15	13	14	12	287	302	6.46	5.92
Mean of T1–T9	14.6	12.0	13.0	10.0	255.0	247.0	5.46	4.24
Change vs control (%)	+4.0	+20.0	0.0	+11.1	+32.1	+30.7	+44.4	+32.7
P (treated vs control)	0.276	<0.001	1.000	0.017	0.001	<0.001	0.006	0.029

3.3. Epidermis and cuticle

Treatment thickened the protective dermal layers (Table 3). Cuticle thickness increased on both the adaxial surface (3.0 to 4.7 μm , +55.6%, $P = 0.010$) and the abaxial surface (2.0 to 3.1 μm , +55.6%, $P = 0.007$). The thickest cuticles occurred in T6 (adaxial, 7 μm) and T5 (abaxial, 5 μm). The adaxial epidermis was 13.9% thicker in treated leaflets ($P = 0.008$) and reached its maximum in T7 (27

μm , +35.0%). The abaxial epidermis increased markedly only in the SA 150 mg L^{-1} treatments T7 (+27.8%) and T9 (+22.2%), with smaller changes elsewhere (−11.1% to +11.1%), so its mean treatment effect was not significant ($P = 0.179$).

Ordinary epidermal cells were larger in treated leaflets. Abaxial cells were 21.4% longer ($P = 0.001$) and 15.2% wider ($P = 0.046$), and adaxial cells were 14.4% wider ($P = 0.026$). Adaxial cell length was unchanged ($P = 0.313$).

Table 3. Cuticle, epidermis and ordinary epidermal cell dimensions (μm) of leaflets of young date palms cv. Khadrawi sprayed with salicylic acid and seaweed extract. Values are means of five measurements.

Treatment	Cuticle adaxial	Cuticle abaxial	Epidermis adaxial	Epidermis abaxial	Epid. cell length abaxial	Epid. cell width abaxial	Epid. cell length adaxial	Epid. cell width adaxial
Control	3	2	20	18	13	11	13	10
T1 (SA50 + SW1)	4	3	22	18	18	13	15	10
T2 (SA50 + SW2)	3	2	21	16	16	11	11	10
T3 (SA50 + SW3)	5	3	25	19	15	11	14	12
T4 (SA100 + SW1)	6	3	21	20	17	15	11	10
T5 (SA100 + SW2)	3	5	20	18	13	9	17	13
T6 (SA100 + SW3)	7	4	21	17	15	13	15	13
T7 (SA150 + SW1)	5	3	27	23	18	15	12	10
T8 (SA150 + SW2)	3	2	23	19	14	12	16	14
T9 (SA150 + SW3)	6	3	25	22	16	15	13	11
Mean of T1–T9	4.7	3.1	22.8	19.1	15.8	12.7	13.8	11.4
Change vs control (%)	+55.6	+55.6	+13.9	+6.2	+21.4	+15.2	+6.0	+14.4
P (treated vs control)	0.010	0.007	0.008	0.179	0.001	0.046	0.313	0.026

3.4. Vascular tissues

Vascular bundle diameter was the most responsive internal trait (Table 4). It increased by 27.5% on average (51 to 65 μm , $P = 0.006$) and reached 84 μm in T7 (+64.7%). The phloem fibre cap thickened by 9.6% ($P = 0.034$), with the highest values in T9 (38 μm) and T4 (37 μm). Bundle-sheath thickness, the

number of bundle-sheath cells and the diameter of conducting elements were not significantly affected ($P \geq 0.169$).

Table 4. Vascular traits of leaflets of young date palms cv. Khadrawi sprayed with salicylic acid and seaweed extract. Dimensions in μm ; sheath cells as number per bundle. Values are means of five measurements.

Treatment	Vascular bundle diameter	Bundle-sheath thickness	Sheath cells (no.)	Conducting element diameter	Phloem fibre cap thickness
Control	51	20	13	4	30
T1 (SA50 + SW1)	48	15	11	3	29
T2 (SA50 + SW2)	53	22	14	6	34
T3 (SA50 + SW3)	64	19	13	4	31
T4 (SA100 + SW1)	72	21	14	5	37
T5 (SA100 + SW2)	69	23	17	3	30
T6 (SA100 + SW3)	61	20	10	4	36
T7 (SA150 + SW1)	84	21	16	5	31
T8 (SA150 + SW2)	75	18	13	7	30
T9 (SA150 + SW3)	59	20	17	5	38
Mean of T1–T9	65.0	19.9	13.9	4.7	32.9
Change vs control (%)	+27.5	–0.6	+6.8	+16.7	+9.6
P (treated vs control)	0.006	0.892	0.312	0.169	0.034

3.5. Main effects of salicylic acid and seaweed extract

Salicylic acid concentration was the dominant factor (Table 5). SA at 150 mg L^{-1} raised total stomatal density to 580 mm^{-2} , against 470 and 456 mm^{-2} at 50 and 100 mg L^{-1} ($P = 0.014$). It also raised adaxial stomatal density ($P = 0.021$), adaxial guard-cell width ($P = 0.049$) and adaxial epidermis thickness ($P = 0.047$), the last significantly only relative to 100 mg L^{-1} . Abaxial stomatal density ($P = 0.071$) and abaxial epidermis thickness ($P = 0.056$) followed the same trend. The

low and intermediate SA concentrations did not differ from each other for any of these traits.

Seaweed extract concentration significantly affected only two dermal traits. Adaxial cuticle thickness was greatest at 3 mL L⁻¹ (6.0 µm) and lowest at 2 mL L⁻¹ (3.0 µm) ($P = 0.008$), and abaxial epidermal cells were longest at 1 mL L⁻¹ ($P = 0.023$). The non-additivity test detected a significant SA × SW interaction for adaxial cuticle thickness ($P = 0.015$) and a strong interaction for vascular bundle diameter ($P < 0.001$). For these two traits, therefore, the response depended on the specific combination rather than on either factor alone. The largest bundle (84 µm) occurred with the highest SA and lowest SW concentration (T7).

Table 5. Marginal means of salicylic acid (SA, mg L⁻¹) and seaweed extract (SW, mL L⁻¹) concentrations for selected leaflet traits, with significance of the main effects and of the SA × SW interaction (Tukey non-additivity test). Within each factor, means followed by the same letter do not differ at $P \leq 0.05$ (LSD).

Trait	SA 50	SA 100	SA 150	P (SA)	SW 1	SW 2	SW 3	P (SW)	LSD 0.05	P (SA × SW)
Total stomatal density (mm ⁻²)	469.7 b	456.3 b	580.0 a	0.014	505.7 a	516.3 a	484.0 a	0.481	68.8	0.339
Adaxial stomatal density (mm ⁻²)	223.7 b	230.3 b	287.0 a	0.021	246.0 a	249.3 a	245.7 a	0.961	39.5	0.597
Abaxial stomatal density (mm ⁻²)	246.0 ab	226.0 b	293.0 a	0.071	259.7 a	267.0 a	238.3 a	0.435	57.5	0.972
Adaxial stomatal width (µm)	9.7 b	9.3 b	11.0 a	0.049	9.7 a	9.7 a	10.7 a	0.160	1.3	0.531
Adaxial epidermis (µm)	22.7 ab	20.7 b	25.0 a	0.047	23.3 a	21.3 a	23.7 a	0.201	3.2	0.561
Abaxial epidermis (µm)	17.7 b	18.3 ab	21.3 a	0.056	20.3 a	17.7 a	19.3 a	0.156	3.0	0.463
Adaxial cuticle (µm)	4.0 b	5.3 a	4.7 ab	0.111	5.0 a	3.0 b	6.0 a	0.008	1.3	0.015
Abaxial epidermal cell length (µm)	16.3 a	15.0 a	16.0 a	0.269	17.7 a	14.3 b	15.3 b	0.023	2.0	0.594
Vascular bundle diameter (µm)	55.0 a	67.3 a	72.7 a	0.228	68.0 a	65.7 a	61.3 a	0.753	24.1	<0.001

3.6. Trait associations

Across the ten treatments, stomatal densities on the two surfaces were positively correlated ($r = 0.771$, $P = 0.009$), as were stomatal lengths ($r = 0.862$, $P = 0.001$). Adaxial stomatal density correlated positively with both abaxial ($r = 0.733$, $P = 0.016$) and adaxial ($r = 0.652$, $P = 0.041$) epidermis thickness, so a denser stomatal apparatus developed together with a thicker epidermis. Adaxial cuticle thickness correlated with phloem fibre cap thickness ($r = 0.727$, $P = 0.017$), indicating a coordinated reinforcement of protective and supporting tissues.

Conducting element diameter correlated positively with abaxial stomatal density ($r = 0.717$, $P = 0.020$).

3.7. Multivariate overview

The percentage changes relative to the control are summarised in Figure 1. The first two principal components explained 56.3% of the total variation (PC1 34.2%, PC2 22.1%; Figure 2). PC1 was loaded positively by adaxial stomatal density (0.411), abaxial epidermis thickness (0.378), vascular bundle diameter (0.326), adaxial epidermis thickness (0.325) and abaxial stomatal density (0.314). It therefore represents a general gradient of stomatal and dermal-vascular development. PC2 contrasted abaxial stomatal length (0.435), abaxial cuticle thickness (0.395), adaxial cuticle thickness (0.340) and adaxial stomatal length (0.335) against conducting element diameter (-0.307) and abaxial stomatal density (-0.271).

Along PC1, the control (-2.73) and T1 (-2.87) occupied the negative end, whereas the SA 150 mg L⁻¹ treatments T7 (3.82) and T9 (3.22) occupied the positive end. T6 separated on PC2 (3.54) through its thicker cuticles and longer stomata, and T8 lay at the opposite end (-3.40) through its high stomatal density and wider conducting elements. The composite anatomical index ranked the treatments T7 (0.95) > T9 (0.82) > T4 (0.26) > T6 (0.15) > T8 (-0.03) > T5 (-0.05) > T2 (-0.12) > T3 (-0.37) > control (-0.76) > T1 (-0.86).

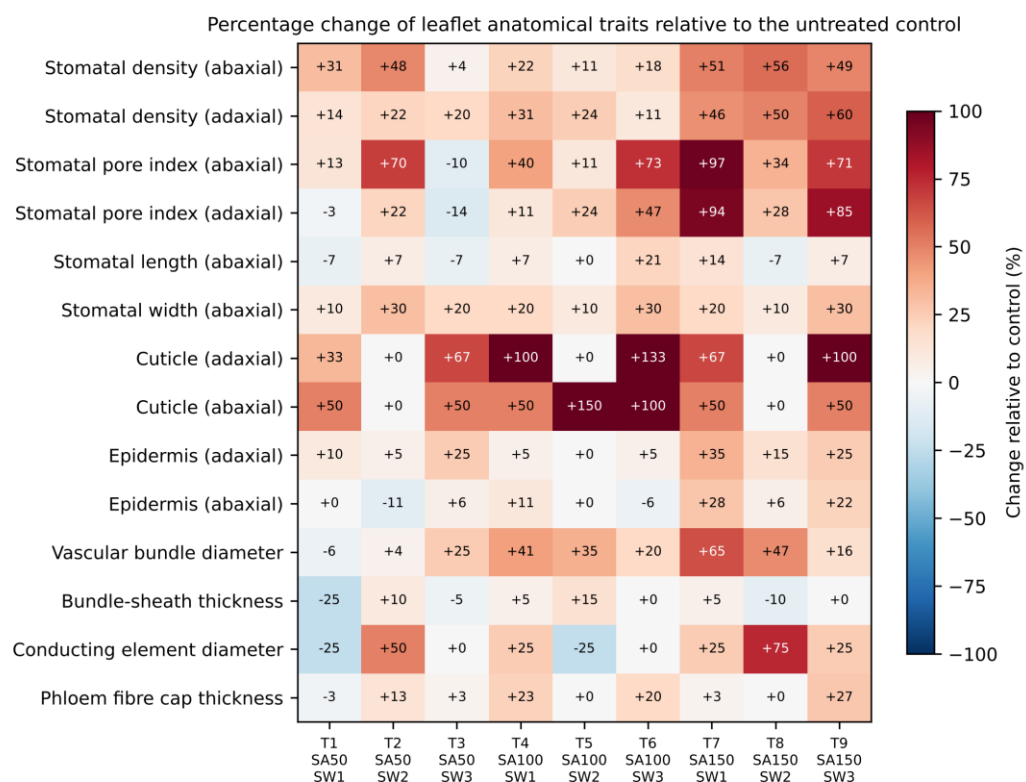


Figure 1. Percentage change of 14 leaflet anatomical traits of date palm cv. Khadrawi in each salicylic acid (SA, mg L⁻¹) × seaweed extract (SW, mL L⁻¹) treatment relative to the untreated control. Red indicates an increase and blue a decrease.

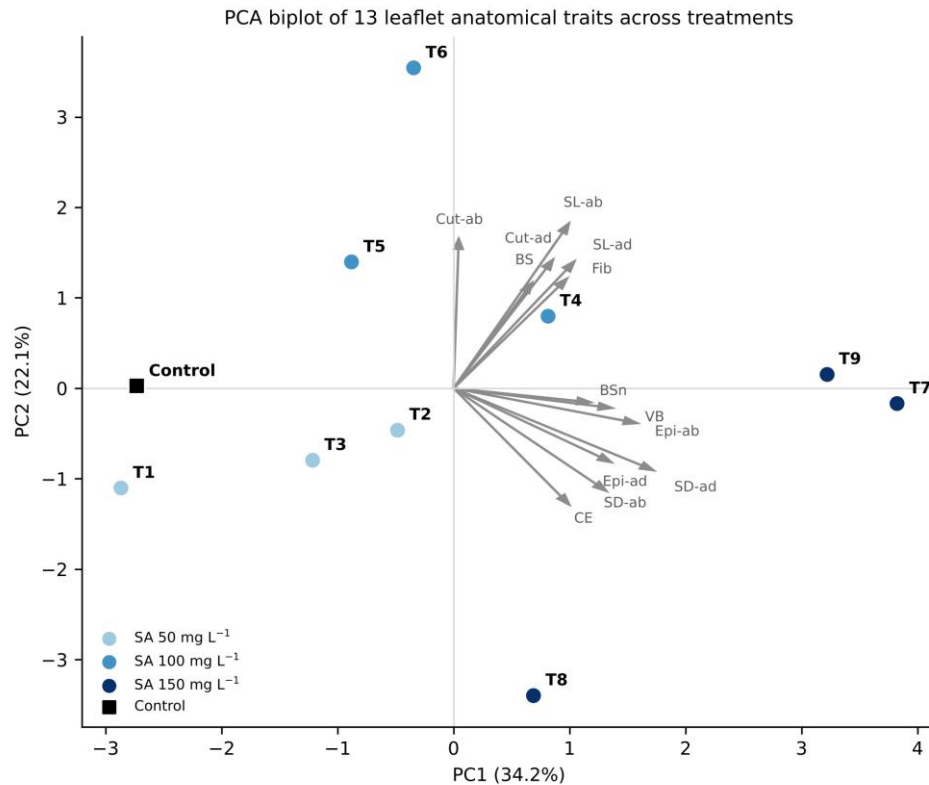


Figure 2. Principal component analysis biplot of 13 leaflet anatomical traits. Points are treatment scores, coloured by SA concentration; arrows are trait loadings. SD = stomatal density; SL = stomatal length; Cut = cuticle; Epi = epidermis; VB = vascular bundle diameter; BS = bundle-sheath thickness; BSn = bundle-sheath cell number; CE = conducting element diameter; Fib = phloem fibre cap; ab = abaxial; ad = adaxial.

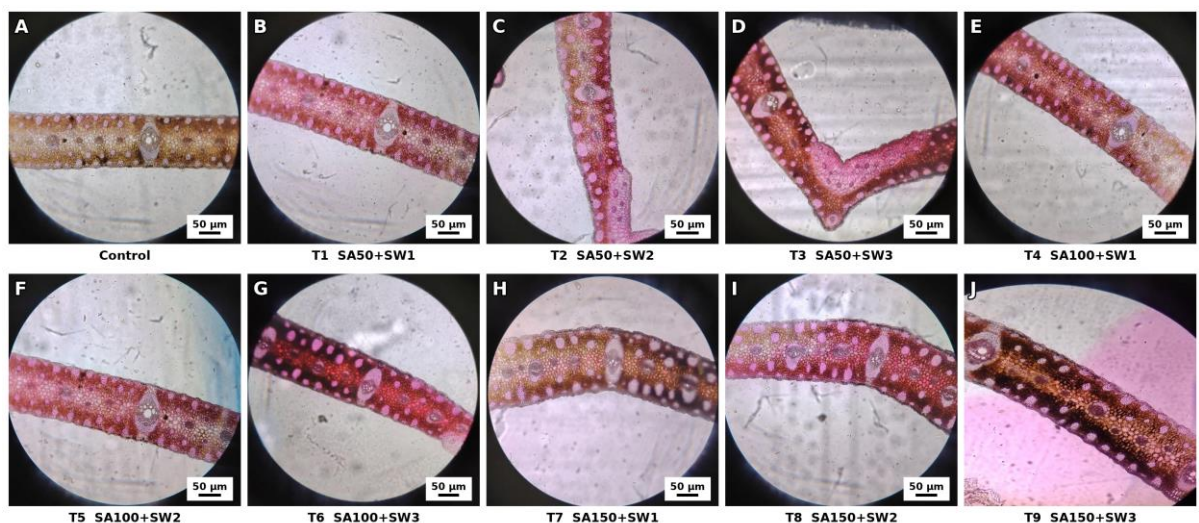


Plate 1. Transverse sections of leaflets of young date palms cv. Khadrawi (safranin-fast green, $\times 40$). (A) Control; (B–D) SA 50 mg L⁻¹ with SW 1, 2 and 3 mL L⁻¹ (T1–T3); (E–G) SA 100 mg L⁻¹ with SW 1, 2 and 3 mL L⁻¹ (T4–T6); (H–J) SA 150 mg L⁻¹ with SW 1, 2 and 3 mL L⁻¹ (T7–T9). Each section shows the uniseriate epidermis on both surfaces, the compact mesophyll, the row of vascular bundles and the sclerenchymatous fibre strands (stained pink). Scale bars = 50 μ m (panel J was photographed at a different enlargement and carries no bar). Measured values are given in Tables 3 and 4.

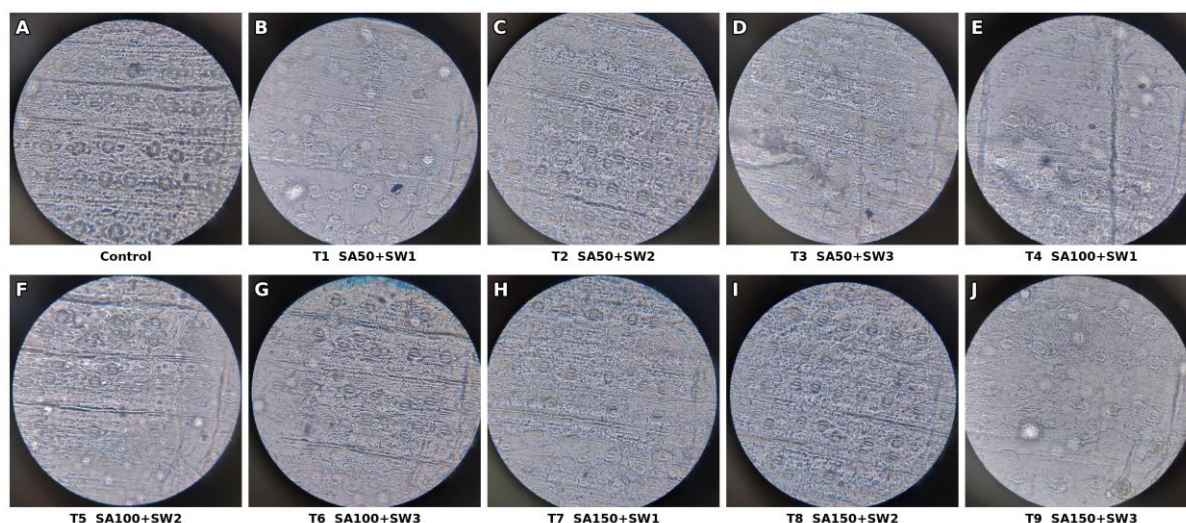


Plate 2. Abaxial (lower) epidermis of leaflets of young date palms cv. Khadrawi showing stomata arranged in longitudinal rows ($\times 40$). (A) Control; (B–D) T1–T3; (E–G) T4–T6; (H–J) T7–T9. Treatment codes as in Table 1. Measured values are given in Table 2.

4. Discussion

This study shows that foliar salicylic acid and seaweed extract do not change the basic anatomical plan of the date palm leaflet but substantially re-tune its quantitative traits. The response was organised around three tissue systems: the stomatal apparatus, the dermal (epidermis–cuticle) system and the vascular bundle. SA concentration was the main driver of stomatal and epidermal development, seaweed extract concentration of cuticle thickness, and the combination of both factors determined vascular bundle size.

4.1. A denser stomatal apparatus without smaller stomata

The most consistent effect was the 31–32% increase in stomatal density on both leaflet surfaces, reaching up to 60% in the SA 150 mg L⁻¹ treatments. Stomatal density is fixed during leaf development by the number of asymmetric divisions in the stomatal lineage and their fate. Cytokinin is an endogenous signal in this process: it interacts with the master regulator *SPEECHLESS* to modulate the number and type of lineage divisions and the final balance between guard cells and pavement cells (Vatén et al., 2018). Seaweed extracts supply cytokinins and up-regulate the plant's own cytokinin biosynthesis genes (Ali et al., 2021; Shukla et al., 2019).

Salicylic acid, in turn, acts on cell division and interacts with auxin transport and gibberellin signalling (Li et al., 2022). In date palm offshoots, foliar SA restored indole-3-acetic acid levels depressed by salinity (Shareef et al., 2022). A hormonal environment favouring division in the young, still-developing leaflet primordia is therefore the most plausible explanation for the higher density. Our result agrees with the increase in stomatal frequency on both surfaces of field-grown maize sprayed with SA (Abdulameer et al., 2017).

Increases in stomatal density are usually accompanied by smaller stomata, a well-known density–size trade-off (Bertolino et al., 2019). Here stomatal length was maintained and guard-cell width even increased. As a result, the stomatal pore area index rose by 33–44% on average and almost doubled in T7. Because SPI scales with the maximum anatomical capacity for stomatal conductance (Sack et

al., 2003), treated leaflets acquired a greater potential for CO₂ uptake, which may support faster growth of young palms. The adaxial/abaxial density ratio was unaffected (0.81–1.12). This shows that the treatments enhanced stomatal development on both surfaces of this amphistomatic leaflet without altering its typical balanced distribution (Zein El Din et al., 2020).

4.2. Why the highest SA concentration was most effective

The effects of SA on growth are biphasic: low doses promote growth and high doses inhibit it, but the threshold differs widely among species and organs (Li et al., 2022). The concentrations used here correspond to approximately 0.36, 0.72 and 1.09 mM. In barley, SA at 0.1–1 mM reduced the thickness of all leaf tissues (Uzunova and Popova, 2000), whereas in date palm the 1.09 mM level gave the strongest positive response.

This contrast is consistent with the structure of the date palm leaflet. Its thick cuticle, continuous hypodermis and coriaceous lamina limit foliar penetration, so the internal concentration reached after spraying is likely to be far lower than the applied one. The route of application is known to matter: in *Senna* seedlings grown in Mosul, the growth response to nano-fertilisers depended strongly on the application method, and spraying combined with soil addition outperformed either route alone (Kahlel et al., 2021). A long-lived monocot palm also probably requires a stronger stimulus than an annual cereal to reach the stimulatory range. The lack of difference between 50 and 100 mg L⁻¹ for stomatal density and epidermis thickness suggests that the effective threshold for this cultivar lies between 100 and 150 mg L⁻¹. This agrees with other studies in which 100–200 mg L⁻¹ SA improved growth or stress tolerance of date palm (Swaid and Shareef, 2025) and other horticultural crops (El-Ziat et al., 2024).

4.3. Reinforcement of the dermal system

Treated leaflets had cuticles about 56% thicker on both surfaces, a thicker adaxial epidermis and larger epidermal cells. The cuticle is the main barrier against non-stomatal water loss, UV radiation and pathogen entry. Plant secondary metabolites add a chemical layer to this protection; for example, terpenes extracted from *Eucalyptus camaldulensis* showed biological activity against the soil-borne fungi *Macrophomina* spp. (Saadallah et al., 2023). Cuticular wax biosynthesis is controlled by stress-responsive transcription factors and adjusts to environmental signals (Shaheenuzzaman et al., 2021; Zhao et al., 2025). In date palm, osmotic stress likewise thickens the cuticle and the guard-cell walls (Zein El Din et al., 2020).

The thicker cuticle induced here can therefore be interpreted as a primed, acclimation-like phenotype. SA is a central node of stress signalling, and its moderate rise is known to activate protective metabolism, including antioxidant enzymes, in date palm (Suhim et al., 2023; Swaid and Shareef, 2025). Seaweed polysaccharides and their oligomers trigger defence signalling through the SA, jasmonate and ethylene pathways (Ali et al., 2021), and the extracts contain phenolics and betaines that support stress-related metabolism (Shukla et al., 2019). This may explain why seaweed concentration was the factor that significantly influenced adaxial cuticle thickness. The significant SA × SW interaction for this trait, and the low value at the intermediate seaweed rate, indicate that the two signals are integrated rather than simply additive.

The thicker cuticle is functionally important in the context of the higher stomatal density. More stomata increase the potential for transpiration. A reinforced cuticle and epidermis reduce residual, non-stomatal water loss and help young palms maintain a favourable water balance under the high evaporative demand typical of Iraqi summers. The positive correlation between epidermis thickness and stomatal density ($r = 0.65\text{--}0.73$) shows that these two components developed together.

The enlargement of ordinary epidermal cells (abaxial cells 21% longer and 15% wider) reflects increased cell expansion. SA enhanced leaf cell expansion in strawberry (Akin and Aras, 2026), and seaweed extracts up-regulate auxin and gibberellin biosynthesis genes (Ali et al., 2021), both of which drive cell-wall loosening and expansion.

4.4. Enlargement of the vascular bundle

Vascular bundle diameter increased by 27.5% on average and by 65% in T7. Differentiation of xylem and phloem is driven primarily by polar auxin flow, modulated by cytokinin. Both hormones are supplied or stimulated by seaweed extracts (Ali et al., 2021; Shukla et al., 2019), and SA helps maintain auxin status in date palm (Shareef et al., 2022). A comparable enlargement of the midvein vascular bundle, xylem and phloem was produced by foliar algal extract in *Moringa oleifera* (Abou-shlell et al., 2017).

Unlike the stomatal response, vascular bundle size depended strongly on the specific SA $\times$ SW combination (non-additivity $P < 0.001$). The largest bundles occurred with the highest SA and lowest seaweed rate (T7), followed by T8 and T4. This suggests that an optimal hormonal balance, rather than the maximum supply of exogenous growth regulators, favours vascular development.

Larger bundles increase the capacity for water delivery to the mesophyll and for export of assimilates. This hydraulic–stomatal coordination, documented across many species (Sack et al., 2003), is reflected in our data by the positive correlation between conducting element diameter and abaxial stomatal density ($r = 0.717$). The thicker phloem fibre cap, correlated with adaxial cuticle thickness ($r = 0.727$), points to a coordinated reinforcement of the mechanical and protective tissues of the leaflet.

4.5. Integrated performance and practical implications

The multivariate analysis integrated the 13 traits into a single picture. The control and the lowest combination (T1) occupied one end of PC1, while the SA 150 mg L⁻¹ combinations, especially T7 and T9, occupied the other, characterised by high stomatal density, thick epidermis and large vascular bundles. The composite anatomical index identified SA 150 mg L⁻¹ + seaweed extract 1 mL L⁻¹ (T7) as the most favourable combination. T7 combined the highest SPI, the thickest adaxial epidermis and the largest vascular bundle. Because T7 used the lowest seaweed concentration, it also represents the most economical effective combination.

Several limitations should be considered. Statistical inference was based on treatment means, with the interaction used as the error term. This approach is conservative for main effects and tests the interaction only through Tukey's one-degree-of-freedom component, so an analysis of the individual replicate measurements would give greater power. The untreated control was a single palm outside the blocks, so comparisons with it should be confirmed with a control replicated in every block. Leaflets were sampled about 13 months after the last

spray, so the differences reflect persistent rather than immediate effects of the treatments. The study covered one cultivar and one season and was purely anatomical. Linking the observed changes to gas exchange, chlorophyll fluorescence, leaf water status and tree growth and yield would confirm their physiological significance.

5. Conclusions

Foliar application of salicylic acid combined with seaweed extract modified the leaflet anatomy of young date palms cv. Khadrawi without altering its basic organisation. Treated leaflets developed about 31% more stomata per mm² on both surfaces with no reduction in stomatal size, cuticles about 56% thicker, a thicker adaxial epidermis, larger epidermal cells, vascular bundles about 28% wider and thicker phloem fibre caps. SA concentration was the principal driver of stomatal and epidermal development, with 150 mg L⁻¹ clearly superior to 50 and 100 mg L⁻¹ for stomatal density. Seaweed extract mainly modulated cuticle thickness and, in interaction with SA, vascular bundle size. Spraying SA at 150 mg L⁻¹ with seaweed extract at 1 mL L⁻¹ (T7) produced the most favourable overall leaflet anatomy and is recommended for further evaluation as a practical treatment to improve the leaf structure and resilience of young date palms.

Conflict of interest: The authors declare that they have no conflict of interest.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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