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Salicylic Acid Improves Morpho-Physiological Traits and Biomass of Kale under Drought Stress

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Abstract. Drought stress in agricultural land disrupts the physiological processes, growth, and yield of kale (*Brassica oleracea* L. var. *acephala*). This study evaluated the physiological responses, growth, and yield of kale treated with salicylic acid, a potential strategy to enhance plant tolerance to drought stress, in a greenhouse at the Department of Agriculture, Universitas Diponegoro, Semarang. A 4 × 4 factorial experiment was arranged in a Completely Randomized Design (CRD) with three replications. The first factor was drought level (100%, 80%, 60%, and 40% field capacity). The second factor was salicylic acid (SA) concentration 0, 0.75, 1.5, and 2.25 mM). The results indicated that physiological responses (chlorophyll a, chlorophyll b, total chlorophyll, relative water content, and electrolyte leakage) remained largely stable under moderate drought stress (60% FC), whereas plant growth parameters (plant height, leaf number, and leaf area) were reduced by 14.5–20.7% compared with the control (100% FC). At 40% FC, both physiological and growth responses were more severely affected; electrolyte leakage increased markedly, and plant height, leaf area, and dry biomass weight decreased by 24.4%, 47.2%, and 60.5%, respectively, compared with the control ($P < 0.05$). The best treatment was the application of 1.5 mM salicylic acid, which increased the relative water content by 4.13% and decreased the electrolyte leakage by 34.70% compared to untreated plants ($P < 0.05$). This concentration was likely more effective due to optimal stomatal regulation, increased antioxidant enzyme activity, and maintained membrane integrity, indicating that 1.5 mM SA has potential as a biostimulant to improve kale water status and membrane stability. However, field validation across locations and seasons is needed before recommending it for dryland farming.

Keywords: biomass; drought stress; foliar spray; kale; salicylic acid

1. Introduction

Kale (*Brassica oleracea* var. *acephala*) is a horticultural vegetable belonging to the cabbage family (*Brassicaceae*). It is rich in nutrients such as β-carotene, vitamins, sulforaphane, flavonoids, and other antioxidants, making it a highly demanded vegetable among consumers (Satheesh and Fanta, 2020). Kale has significant potential for development in Indonesia due to favourable environmental conditions and high economic value (Thavarajah *et al.*, 2016). However, national kale production declined from 204,000 tons in 2020 to 203,000 tons in 2021 (CSA, 2021). This decline in vegetable production is often associated with seasonal fluctuations, particularly prolonged dry seasons that induce drought stress.

Drought, a major abiotic factor limiting crop productivity worldwide, arises from reduced soil water availability, low rainfall, and poor soil fertility, and is intensified by rising global temperatures and irregular rainfall patterns that alter plant morphology, physiology, and anatomy (Anggraini *et al.*, 2015). Water availability is a key factor in plant cultivation, and optimal plant growth is achieved when soil moisture is maintained at field capacity (FC). Field capacity refers to the amount of water remaining in the soil after gravitational drainage (Darmayati and Sutikto, 2019). Under drought conditions, reduced transpiration lowers turgor pressure and triggers abscisic acid (ABA) synthesis in

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chloroplasts, which inhibits the ATP-dependent proton pump in guard cell plasma membranes. This restricts K^+ influx and osmotic water absorption, driving efflux of K^+ and Ca^{2+} from guard cells and preventing stomatal opening (Yusuf, 2020).

Previous studies on plants within the *Brassicaceae* family have demonstrated varying levels of drought tolerance. For instance, drought stress at 50% and 60% FC was found to maintain growth, leaf number, leaf area, stomatal index, carbohydrate content, biomass, and chlorophyll a and b levels in *Brassica rapa* L. (Mohan *et al.*, 2025). However, reduced crop quality and productivity under drought remain major challenges for farmers. Therefore, it is essential to enhance kale growth and yields under drought stress through physiological regulation, including the exogenous application of phenolic compounds such as salicylic acid.

Salicylic acid (SA) is a phenolic compound, structurally comprising a benzene ring and a hydroxyl group, that functions as a key signaling molecule in plant defense against abiotic stresses such as salinity, drought, and temperature extremes, and contributes to lignin and phytoalexin biosynthesis for pathogen protection (Tarigan *et al.*, 2018). At optimal concentrations, exogenous SA regulates stomatal closure, chlorophyll and protein synthesis, seed germination, and photosynthetic activity, while enhancing antioxidant enzyme activity to improve tolerance to abiotic and biotic stresses (Min *et al.*, 2018). Recent studies have confirmed that SA improves growth and yields under water deficit. Treatment with 0.5 mM SA in mustard greens (*Brassica juncea*) resulted in increased dry weight, leaf area, photosynthetic rate, and proline accumulation (Nazar *et al.*, 2018). Similarly, Kilic (2023) found that 2.0 mM SA enhanced stomatal conductance and antioxidant enzyme activities in kale under severe drought. Comparable benefits were reported in another legume, where salicylic acid application improved the physiological status and production of soybean under drought stress (Rosyida *et al.*, 2026).

However, despite extensive research on salicylic acid in various Brassicaceae crops, there is still limited information on the interactive effects of salicylic acid and drought levels, defined as field capacity, in kale (*Brassica oleracea* var. *acephala*). Most previous studies examined single stress factors or other *Brassica* species, without adequately assessing combined morpho-physiological and yield responses under controlled drought conditions, necessitating a comprehensive assessment of SA across varying drought levels in kale.

This study aimed to determine the physiological responses, growth, and yield of kale under varying drought stress levels and salicylic acid (SA) concentrations, providing a scientific basis for using SA to mitigate drought stress and support robust kale production amid increasing water scarcity.

2. Materials and Methods

2.1 Experimental design

This experiment was conducted from December 26, 2024, to February 20, 2025, in a greenhouse at the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia, with a temperature ranging 30.05-42.13 °C, relative humidity 60-80%, and light intensity 5,136.65-30,302.08 lux inside (9,603.19-52,179.65 lux outside).

The experiment was arranged in a Completely Randomized Design (CRD) with a 4 × 4 factorial arrangement and three replications per treatment combination. A total of 48 experimental units (4 drought levels × 4 SA concentrations × 3 replications) were used, with treatment combinations randomly assigned within each replication block using a random number generator to ensure unbiased allocation. The first factor was drought stress level expressed as field capacity (FC): 100% FC (D0), 80% FC (D1), 60% FC (D2), and 40% FC (D3). The second

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factor was salicylic acid (SA) concentration: 0 mM (S0), 0.75 mM (S1), 1.5 mM (S2), and 2.25 mM (S3). SA powder (98% purity) was dissolved in propylene glycol and diluted with distilled water to prepare liquid solutions for foliar spray application. In total, 16 treatment combinations were randomly assigned to experimental units.

2.2 Plant materials and growth conditions

Kale seeds obtained from a commercial market were sown in seedling trays and, at 14 days after sowing, uniform seedlings with well-developed true leaves were transplanted in the early morning (to minimize stress) into pots (30 cm diameter) containing soil (6 kg pot^{-1}), manure (43.2 g pot^{-1}), and husk charcoal (12 g pot^{-1}). Basal fertilization used urea (200 kg ha^{-1}; 2.4 g plant^{-1}), triple superphosphate (150 kg ha^{-1}; 1.8 g plant^{-1}), and potassium chloride (100 kg ha^{-1}; 1.2 g plant^{-1}), after which plants were maintained under greenhouse conditions per the experimental treatments.

2.3 Drought stress treatment

Field capacity (FC) of the soil was determined using a gravimetric method (Mustamu *et al.*, 2025). Soil samples were saturated with water and allowed to drain freely for 24–48 h until gravitational drainage ceased. The soil was then weighed to determine the mass at field capacity, and the oven-dry mass was determined after drying at 105 °C until a constant weight was reached. Field capacity was calculated as:

$$Field\ capacity\ (\%) = \frac{W\ FC - W\ dry}{W\ dry} x\ 100\%$$

where FC is field capacity (%), W FC is the soil mass at field capacity (g), and W dry is the oven-dry soil mass (g).

Drought treatments were imposed by maintaining soil moisture at 100% FC (D0), 80% FC (D1), 60% FC (D2), and 40% FC (D3). Soil moisture was controlled using a gravimetric pot-weighing method. The target pot mass for each drought level was calculated as:

$$W\ target\ (g) = \left(W\ dry + \frac{FC\ (\%)}{100} x\ W\ dry\right) x\ f$$

where W target is the target pot mass (g), W dry is the oven-dry soil mass (g), FC is field capacity (%), and f is the drought fraction (1.00, 0.80, 0.60, and 0.40).

The irrigation volume applied was determined as:

$$V\ water = W\ target - W\ current$$

where V water is the volume of water added and W current is the pot mass before irrigation (g). Pots were weighed regularly and irrigated carefully to maintain the designated soil moisture levels, avoid surface runoff, and ensure uniform wetting.

2.4 Preparation and Foliar Application of Salicylic Acid

SA solutions were prepared following Li *et al.* (2019) with slight modifications. Due to its limited solubility in water, SA powder (98%) was dissolved in a small volume of propylene glycol, then diluted with distilled water to a final volume of 1 L for each of the four concentrations (0, 0.75, 1.5, and 2.25 mM; S0–S3). Solutions were applied as a foliar spray (~20 mL plant^{-1} per spraying) each morning at 14, 21, 28, 35, 42, and 49 days after planting, for a total of ~120 mL plant^{-1} during the experimental period.

2.5 Measured Parameters

Plant stress-tolerance responses were evaluated using morphological, physiological, and biomass-related traits. Morphological parameters included plant height, leaf number, leaf area, and root length, which were measured using standard agronomic procedures. Leaf chlorophyll content was determined using the 80% acetone extraction method described by Arnon (1949), and absorbance was measured at 645 and 663 nm using a spectrophotometer. Leaf relative water content (RWC) was measured following the method of Barrs and Weatherley (1962) using fresh, turgid, and dry leaf weights. Electrolyte leakage was determined according to Dionisio-Sese and Tobita (1998) by measuring the electrical

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conductivity of leaf leachates before and after heat treatment. Fresh biomass was measured immediately after harvest using an analytical balance, and dry biomass after oven-drying samples at 70 °C to constant weight; these data were used to calculate the stress sensitivity index (SSI) following the method of Fischer and Maurer (1978). Based on the SSI value, treatments were classified as tolerant/resistant when SSI < 1, at the tolerance threshold when SSI = 1, and sensitive/vulnerable when SSI > 1.

2.6 Statistical analysis

All data were analyzed by factorial analysis of variance (ANOVA) to evaluate the effects of drought stress, salicylic acid, and their interaction. Significant treatment means were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Relationships among morphological, physiological, and biomass traits were explored using principal component analysis (PCA) and hierarchical cluster analysis (HCA), with results presented as a dendrogram and a heat map, respectively. ANOVA and DMRT were performed using SAS software (version 9.4), whereas PCA, dendrogram, and heat map analyses were conducted using R software (version 4.3.1).

3. Results and Discussion

3.1 Morphological and Biomass Responses of Drought-Stressed Kale under SA treatments

Drought stress exerted a highly significant effect ($p < 0.01$) on plant height, leaf number, leaf area, fresh weight, and dry weight, whereas root length was not significantly affected (Table 1). All five significant traits showed a curvilinear response to FC, peaking at 80% FC and being significantly higher than at 100% FC, according to Duncan's Multiple Range Test. This indicates that full water saturation was not physiologically optimal for kale growth, most likely because a waterlogged rhizosphere restricts oxygen diffusion into the root zone, impairing aerobic respiration, ATP-dependent nutrient uptake, and shoot biomass accumulation (Pan *et al.*, 2021).

Beyond 80% FC, growth declined progressively as water deficit increased. At 60% FC, reductions relative to 80% FC reached 14.5% (height), 12.2% (leaf number), 20.7% (leaf area), 31.2% (fresh weight), and 49.3% (dry weight), while at 40% FC the reductions intensified further to 24.4%, 22.5%, 47.2%, 39.4%, and 60.5%, respectively. Dry weight was consistently the most sensitive parameter (CV = 17.35%), declining roughly twice as much as plant height (CV = 8.81%) at the same stress level, indicating that drought preferentially constrains carbon assimilation over cell elongation. This likely reflects the typical water-deficit sequence, in which reduced leaf turgor first induces ABA-mediated stomatal closure, restricting CO_2 diffusion and net photosynthesis, before limiting meristematic cell division and expansion (Haghpanah *et al.*, 2024; Sinay, 2015; Utami *et al.*, 2020; Anindya *et al.*, 2023). Leaf area was also reduced disproportionately (up to 47.2% at 40% FC) relative to leaf number (22.5%), indicating that kale's dominant adaptive response to water deficit was to shrink individual leaf size rather than reduce leaf number. These results may reflect a classical drought-avoidance strategy that minimizes transpiring surface and conserves water at the cost of photosynthetic area (Busaifi, 2017).

In contrast, root length showed no significant response to drought ($p > 0.05$), though raw means increased slightly with stress intensity, from 30.15 cm at 100% FC to 33.90 cm at 40% FC. This direction, though not statistically confirmed, is consistent with the drought-avoidance strategy of allocating photoassimilates toward root elongation to improve access to deeper soil water at the expense of shoot growth (Nour *et al.*, 2024). The lack of a statistically significant effect in this study, in contrast to reports of drought-enhanced root elongation in other species (Rosawanti, 2016; Mohan *et al.*, 2025), may reflect the fact that drought-induced plasticity

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in *Brassicaceae* is likely more often expressed through changes in root diameter, lateral branching density, and root-to-shoot dry-mass ratio rather than through primary root elongation, none of which were captured by the present measurement.

Table 1. Morphological and biomass parameters of kale (*Brassica oleracea* var. *acephala*) as affected by drought stress levels and salicylic acid concentrations.

Treatments	Parameters					
	Plant Height	Leaf Number	Leaf Area	Root Length	Fresh Weight	Dry Weight
Drought Stress (% FC)	(cm)	(leaf)	(cm^2)	(cm)	(g)	(g)
100	46.27 ± 0.79 b	21.75 ± 0.49 ab	4.221 ± 368.28 b	30.15 ± 1.45	253.92 ± 7.80 a	66.51 ± 2.52 a
80	49.96 ± 1.28 a	22.79 ± 0.90 a	6.233 ± 322.61 a	29.63 ± 0.91	236.33 ± 9.69 a	61.26 ± 2.65 a
60	42.70 ± 1.36 c	20.00 ± 0.76 b	4.940 ± 258.68 b	32.56 ± 1.52	174.60 ± 3.63 b	33.71 ± 2.42 b
40	37.75 ± 0.70 d	17.67 ± 0.41 c	3.289 ± 233.30 c	33.90 ± 1.11	153.92 ± 7.94 b	26.25 ± 1.75 c
Significance	**	**	**	ns	**	**
Salicylic Acid (mM)						
0	44.51 ± 1.70	20.75 ± 0.80	4.573 ± 425.63	29.28 ± 0.89	201.58 ± 16.25	46.13 ± 5.79
0.75	43.89 ± 1.80	20.08 ± 0.88	4.492 ± 302.23	30.35 ± 1.16	204.85 ± 13.57	49.04 ± 5.99
1.5	43.42 ± 1.77	20.54 ± 0.92	4.646 ± 479.19	32.92 ± 1.66	196.08 ± 14.25	43.72 ± 4.73
2.25	44.86 ± 1.63	20.83 ± 0.93	4.972 ± 516.90	33.68 ± 1.22	216.25 ± 13.72	48.84 ± 6.08
Significance	ns	ns	ns	ns	ns	ns
% FC × mM						
100-0	45.52 ± 0.61	21.83 ± 1.20	3.408 ± 113.53	27.92 ± 2.46	274.17 ± 1.45	66.42 ± 5.30
100-0.75	48.12 ± 1.40	21.50 ± 1.32	4.533 ± 395.17	28.17 ± 2.35	242.33 ± 8.02	68.42 ± 3.51
100-1.5	46.40 ± 2.80	21.67 ± 1.17	3.570 ± 452.88	34.27 ± 4.50	250.50 ± 24.83	61.45 ± 6.95
100-2.25	45.05 ± 0.99	22.00 ± 0.76	5.374 ± 1.163.89	30.27 ± 1.14	248.67 ± 19.10	69.75 ± 5.55
80-0	50.50 ± 3.30	22.67 ± 2.20	6.278 ± 315.58	28.72 ± 0.66	221.83 ± 14.45	60.92 ± 7.72
80-0.75	49.95 ± 2.83	22.83 ± 1.01	5.166 ± 452.92	28.75 ± 1.75	246.83 ± 23.10	65.67 ± 0.30
80-1.5	46.57 ± 2.52	22.17 ± 1.45	6.995 ± 417.35	30.70 ± 3.36	216.00 ± 5.53	51.75 ± 4.79
80-2.25	52.83 ± 0.75	23.50 ± 3.04	6.492 ± 957.70	30.35 ± 1.33	260.67 ± 25.14	66.69 ± 1.51
60-0	42.97 ± 3.97	19.83 ± 1.48	5.190 ± 608.76	30.08 ± 2.67	168.50 ± 5.39	31.58 ± 1.96
60-0.75	40.53 ± 1.89	19.00 ± 1.80	5.092 ± 458.78	31.67 ± 3.19	178.07 ± 11.07	40.17 ± 6.54
60-1.5	44.75 ± 3.86	21.67 ± 2.17	4.880 ± 289.51	32.03 ± 4.26	181.50 ± 8.61	33.33 ± 6.36
60-2.25	42.55 ± 1.51	19.50 ± 0.76	4.598 ± 839.17	36.45 ± 2.00	170.33 ± 2.68	29.75 ± 3.25
40-0	39.05 ± 1.50	18.67 ± 0.83	3.416 ± 713.86	30.42 ± 1.36	141.83 ± 19.92	25.58 ± 3.69
40-0.75	36.95 ± 0.76	17.00 ± 0.87	3.177 ± 406.04	32.83 ± 1.82	152.17 ± 0.83	21.92 ± 1.30
40-1.5	35.97 ± 1.35	16.67 ± 0.44	3.138 ± 294.94	34.67 ± 2.32	136.33 ± 13.17	28.33 ± 4.36
40-2.25	39.02 ± 1.65	18.33 ± 0.73	3.425 ± 633.22	37.67 ± 1.58	185.33 ± 9.73	29.17 ± 4.15
Significance	ns	ns	ns	ns	ns	ns
Coefficient of Variation	8.81%	12.51%	22.05%	13.87%	12.25%	17.35%

Note: Values are means ± SE (n = 3). Different letters within the same column indicate significant differences ($p < 0.05$; Duncan's Multiple Range Test). **. *. ns indicate significant at $p < 0.01$. $p < 0.05$, or not significant, respectively.

SA application had no significant effect on morphological or biomass parameters ($p > 0.05$), though 1.5 mM SA showed a non-significant tendency to increase leaf area, root length, and dry weight, contrasting with prior reports that SA maintained leaf area and promoted cell division in lettuce under drought (Shehata *et al.*, 2020; Hayat *et al.*, 2010; Andriani *et al.*, 2015; Sayyari *et al.*, 2013). This may reflect a sub-optimal concentration window (0–2.25 mM), since SA efficacy is strongly dose-, species-, and stage-dependent (Yang *et al.*, 2023). In this study, the weak SA effect may reflect reduced uptake caused by high greenhouse temperatures (up to 42 °C), which accelerate SA degradation and alter stomatal/cuticular conductance (Waraich *et al.*, 2012; Min *et al.*, 2018). Moreover, moderate drought (60–40% FC) was likely generating insufficient ROS signal to trigger SA's antioxidant–ABA priming cascade (Hussain *et al.*, 2019;

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Haghpanah *et al.*, 2024), where SA failed to mitigate drought-induced biomass loss despite benefiting well-watered plants (Santos *et al.*, 2024), contrasting with cases where SA raised dry weight via improved photosynthesis (Habibi, 2015).

No significant drought × SA interaction was found (Table 1), suggesting SA acted independently of drought intensity, possibly due to kale's inherent constitutive tolerance (Handayani *et al.*, 2018) and redundancy with already-elevated endogenous SA under stress (Haghpanah *et al.*, 2024), unlike other *Brassicaceae* where SA measurably enhanced drought resistance (Khan *et al.*, 2015; Nazar *et al.*, 2015). Overall, drought was likely the dominant driver of growth variation, possibly acting via a turgor–stomatal–photosynthesis cascade (Haghpanah *et al.*, 2024; Nour *et al.*, 2024), while SA's benefit remains conditional on concentration, uptake, and stress intensity (Yang *et al.*, 2023), and in this study likely manifests more at the physiological/biochemical level than in morphology or biomass, as explored next.

3.2 Physiological responses of kale to drought stress and SA applications

Chlorophyll content, an indicator of photosynthetic capacity, increased progressively from 100% to 60% FC before declining at 40% FC (Table 2). This indicates an adaptive response by the plant, in which water deficiency is low enough to activate photosynthesis to compensate for carbon assimilation (Farooq *et al.*, 2017; Purbajanti *et al.*, 2019). Drought stress increases the production of reactive oxygen species (ROS) in chloroplasts, which disrupts the photosynthetic electron transport chain (Gao *et al.*, 2026; Qiao *et al.*, 2024). In the case of moderate stress (60% FC), ROS may have remained under control by the plant's enzymatic and non-enzymatic antioxidant system, which could explain why the photosynthetic system continued to function (Rosyida & Kristanto, 2022), whereas under severe stress (40% FC) the observed decline in chlorophyll may reflect ROS overwhelming the antioxidant system; however, ROS levels and antioxidant enzyme activity were not directly measured in this study, so this interpretation remains tentative.

SA treatments significantly influenced plant water status and membrane stability under drought stress (Table 2). Leaf RWC increased with SA application up to 1.50 mM, then declined at the highest concentration. Relative to the untreated control (0 mM SA), RWC increased by 2.25%, 4.13%, and 1.54% at 0.75, 1.50, and 2.25 mM SA, respectively, peaking at 1.50 mM SA (85.73 ± 0.29%) before declining by 2.48% at 2.25 mM, indicating that SA's benefit for leaf hydration diminished at higher concentrations.

EL, an indicator of membrane integrity, showed a contrasting trend, decreasing with increasing SA concentration up to 1.50 mM and subsequently increasing at 2.25 mM. Relative to the untreated control, EL decreased by 12.34%, 34.70%, and 18.31% under 0.75, 1.50, and 2.25 mM SA, respectively, reaching its lowest at 1.50 mM SA (36.73 ± 3.15%) before rising 25.10% at 2.25 mM SA, indicating reduced membrane protection at excessive concentrations. These results suggest that severe drought substantially compromised membrane stability, most likely through oxidative stress and lipid peroxidation, thereby increasing solute leakage from leaf cells (Hasanuzzaman *et al.*, 2017; Yousefvand *et al.*, 2022).

This concentration-dependent response is consistent with the hormetic behavior of SA (Sharma *et al.*, 2018), whereby moderate concentrations optimize stress tolerance. In contrast, excessive concentrations may impose additional physiological constraints or trigger metabolic imbalance and oxidative stress that offset the protective benefits of SA (Huang & Jin, 2025). The improvement in RWC due to SA is attributed to SA's role in redox regulation and proline metabolism, which result in membrane stability and the water-retention ability of cells (Sharma *et al.*, 2018).

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Table 2. Physiological parameters of kale (*Brassica oleracea* var. *acephala*) as affected by drought stress levels and salicylic acid concentrations.

Treatments	Parameters				
	Chlorophyll a	Chlorophyll b	Chlorophyll Total	Leaf RWC	Electrolyte Leakage
Drought Stress (% FC)	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(%)	(%)
100	1.22 ± 0.08 b	0.52 ± 0.04 b	1.73 ± 0.11 b	85.66 ± 0.51 a	36.73 ± 2.83 b
80	1.35 ± 0.04 b	0.60 ± 0.02 b	1.95 ± 0.06 b	84.52 ± 0.38 ab	37.31 ± 3.90 b
60	1.52 ± 0.07 a	0.71 ± 0.04 a	2.22 ± 0.12 a	83.17 ± 0.84 bc	44.89 ± 5.26 b
40	1.33 ± 0.07 b	0.60 ± 0.04 b	1.93 ± 0.10 b	82.48 ± 0.84 c	69.30 ± 5.64 a
Significance	*	**	**	**	**
Salicylic Acid (mM)					
0	1.31 ± 0.03	0.57 ± 0.02	1.87 ± 0.05	82.33 ± 1.03 c	56.25 ± 6.61 a
0.75	1.46 ± 0.07	0.67 ± 0.04	2.12 ± 0.11	84.18 ± 0.70 ab	49.31 ± 5.94 a
1.5	1.35 ± 0.08	0.61 ± 0.05	1.97 ± 0.12	85.73 ± 0.29 a	36.73 ± 3.15 b
2.25	1.30 ± 0.08	0.57 ± 0.05	1.87 ± 0.13	83.60 ± 0.42 bc	45.95 ± 6.29 ab
Significance	ns	ns	ns	**	*
D × S					
100-0	1.36 ± 0.03	0.59 ± 0.01	1.95 ± 0.03	85.00 ± 2.03	32.98 ± 6.81
100-0.75	1.41 ± 0.19	0.63 ± 0.09	2.04 ± 0.29	86.19 ± 0.20	31.30 ± 7.68
100-1.5	1.18 ± 0.13	0.48 ± 0.05	1.66 ± 0.18	86.48 ± 0.51	37.03 ± 2.53
100-2.25	0.92 ± 0.02	0.37 ± 0.01	1.29 ± 0.03	84.99 ± 0.53	45.60 ± 1.88
80-0	1.27 ± 0.07	0.55 ± 0.03	1.82 ± 0.09	83.49 ± 0.31	36.34 ± 1.98
80-0.75	1.29 ± 0.14	0.59 ± 0.05	1.87 ± 0.19	84.77 ± 1.21	31.61 ± 3.87
80-1.5	1.48 ± 0.05	0.67 ± 0.03	2.16 ± 0.08	85.71 ± 0.39	43.16 ± 9.37
80-2.25	1.35 ± 0.03	0.58 ± 0.02	1.94 ± 0.04	84.13 ± 0.30	38.14 ± 13.92
60-0	1.26 ± 0.13	0.55 ± 0.07	1.81 ± 0.19	80.69 ± 2.86	50.93 ± 9.79
60-0.75	1.63 ± 0.11	0.77 ± 0.07	2.40 ± 0.18	83.92 ± 1.39	40.87 ± 8.61
60-1.5	1.51 ± 0.18	0.70 ± 0.11	2.20 ± 0.29	84.84 ± 0.63	59.46 ± 12.93
60-2.25	1.68 ± 0.05	0.80 ± 0.04	2.48 ± 0.10	83.24 ± 0.36	28.32 ± 1.53
40-0	1.34 ± 0.04	0.57 ± 0.04	1.91 ± 0.08	80.15 ± 1.66	76.99 ± 5.43
40-0.75	1.50 ± 0.15	0.68 ± 0.09	2.18 ± 0.23	81.83 ± 1.57	43.13 ± 3.69
40-1.5	1.24 ± 0.21	0.61 ± 0.14	1.85 ± 0.34	85.89 ± 0.62	85.34 ± 1.57
40-2.25	1.25 ± 0.09	0.54 ± 0.04	1.79 ± 0.13	82.04 ± 0.96	71.76 ± 12.30
Significance	ns	ns	ns	ns	ns
Coefficient of Variation	15.08%	18.78%	16.03%	2.51%	28.39%

Note: Values are means ± SE (n = 3). Different letters within the same column indicate significant differences ($p < 0.05$; Duncan's Multiple Range Test). **, *, ns indicate significant at $p < 0.01$, $p < 0.05$, or not significant, respectively.

SA treatment may also upregulate the activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which could help prevent the oxidative damage to membranes, keeping them intact and preventing ion leakage (Silva *et al.*, 2023), supporting Khan *et al.* (2015) who stated that SA improves water status and decreases electrolyte leakage in plants. As antioxidant enzyme activity was not directly measured in this study, this explanation remains a plausible interpretation rather than a confirmed mechanism. However, the lack of significance of SA's effect on chlorophyll content contrasts with previous findings that SA increases chlorophyll biosynthesis by raising Aminolevulinic Acid content (Huang & Jin, 2025). Although SA at 0.75 mM tended to increase chlorophyll under moderate drought, the response was inconsistent, possibly due to suboptimal concentrations, application frequency, or high greenhouse temperatures (up to 42 °C), which may have limited SA uptake (Waraich *et al.*, 2012). Beyond antioxidant regulation, SA may also interact with ABA, influencing

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stomatal behavior and improving water-use efficiency under drought conditions (Chen *et al.*, 2023). In *Brassica napus*, mild drought priming was shown to preferentially raise endogenous SA signaling relative to ABA, which in turn strengthened the glutathione-based redox pathway and reduced ROS accumulation during subsequent, more severe drought (Muchlas *et al.*, 2024).

No significant interaction between drought stress and SA was observed for any physiological parameter, indicating that SA's effects on leaf RWC and EL were independent of drought intensity (Table 2). This is possibly because the drought levels used (60–40% FC) were not sufficient to induce SA-dependent defense mechanisms, or because kale's own defense mechanisms against drought are superior to those induced by external SA (Handayani *et al.*, 2018). In this case, the results differ from those observed in other Brassicaceae species, in which the use of SA increased drought tolerance via improved antioxidant properties (Khan *et al.*, 2015; Hasanuzzaman *et al.*, 2017). This distinct division of roles indicates that SA is primarily responsible for water status and membrane stability in kale, rather than for modulating the drought-stress response itself. These findings consistent with SA study on drought-stressed soybean, where SA at up to 1 mM also failed to counteract growth and yields reductions imposed by 40–60% FC drought (Salsabila *et al.*, 2024), also consistent with reports that salicylic acid modulates physiological status and yield formation in soybean under drought conditions (Rosyida *et al.*, 2026), and where the beneficial effect of biostimulants on RWC was most apparent only when combined with soil amelioration under mild drought (Budiyanto *et al.*, 2024), suggesting that crop species, stress intensity, and soil management all modulate the threshold at which exogenous SA can express a measurable biostimulant effect.

3.3 Multivariate Analysis of Kale Responses to Drought Stress and Salicylic Acid

Cluster dendrogram analysis (Figure 1) revealed distinct functional groups among the morpho-physiological and biomass traits of kale under drought stress and salicylic acid treatment. Leaf area (LA) and fresh weight (FW) each formed separate clusters at high linkage distances, reflecting their high sensitivity to water stress and SA treatment, as well as their importance to plant performance. Two further clusters emerged: leaf number (LN), chlorophyll A/B/total (CHA, CHB, CHT), and stress sensitivity index (SSI) formed one closely associated group, while relative water content (RWC), electrolyte leakage (EL), dry weight (DW), plant height (PH), and root length (RL) formed another. This clustering pattern indicates that exogenous SA enhances drought tolerance in kale by modulating multiple, functionally distinct groups of morpho-physiological and biomass traits.

Heatmap correlation histogram (Figure 2) showing the distribution patterns of morpho-physiological traits and biomass parameters of kale (*Brassica oleracea* var. *acephala*) across the 16 drought × SA treatment combinations (as detailed in Figure 2) for plant height (PH), leaf number (LN), leaf area (LA), root length (RL), chlorophyll A/B/total (CHA, CHB, CHT), relative water content (RWC), electrolyte leakage (EL), fresh and dry biomass weight (FW, DW), and stress sensitivity index (SSI).

Principal Component Analysis (PCA) (Figure 3) showed that the total variation explained by the first two principal components was 86.06%, with PC1 and PC2 contributing 60.45% and 25.61%, respectively. PC1 was highly correlated with growth and biomass traits: plant height (PH), leaf number (LN), leaf area (LA), root length (RL), relative water content (RWC), fresh weight (FW), and dry weight (DW), whose closely aligned, positive vectors indicate that PC1 reflects plant vigor. Stress-related traits (electrolyte leakage, EL; stress sensitivity index, SSI) loaded on the opposite side of PC1, confirming an inverse relationship between stress intensity and growth

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performance. PC2 was driven by chlorophyll-related traits (CHA, CHB, CHT), which clustered separately from biomass traits, reflecting an independent physiological response of photosynthetic pigments. The contrasting vector directions of chlorophyll and stress-related traits suggest that greater chlorophyll stability corresponds to lower membrane injury, consistent with the link between chlorophyll degradation and electrolyte leakage from oxidative injury (Handayani *et al.*, 2013). In conclusion, the PCA results indicate that applying exogenous salicylic acid increases drought tolerance in kale by enhancing regulation of growth, water status, and photosynthesis, while minimizing stress injury.

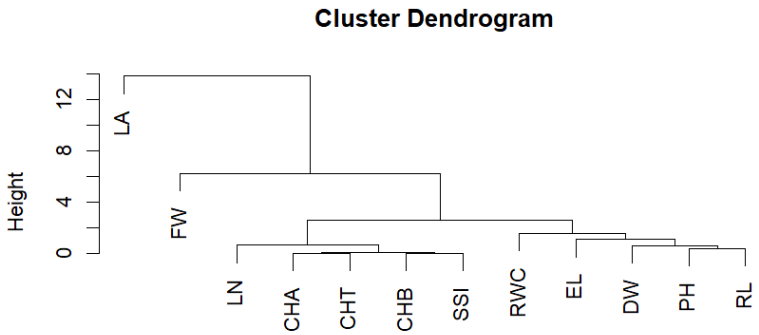

Figure 1. Cluster dendrogram analysis of morpho-physiological traits and biomass parameters of kale. Abbreviations: plant height (PH), Leaf Number (LN), leaf area (LA), root length (RL), chlorophyll A (CHA), chlorophyll B (CHB), total chlorophyll (CHT), relative water content (RWC), electrolyte leakage (EL), fresh weight of biomass (FW), dry weight of biomass (DW), and stress sensitivity index (SSI).

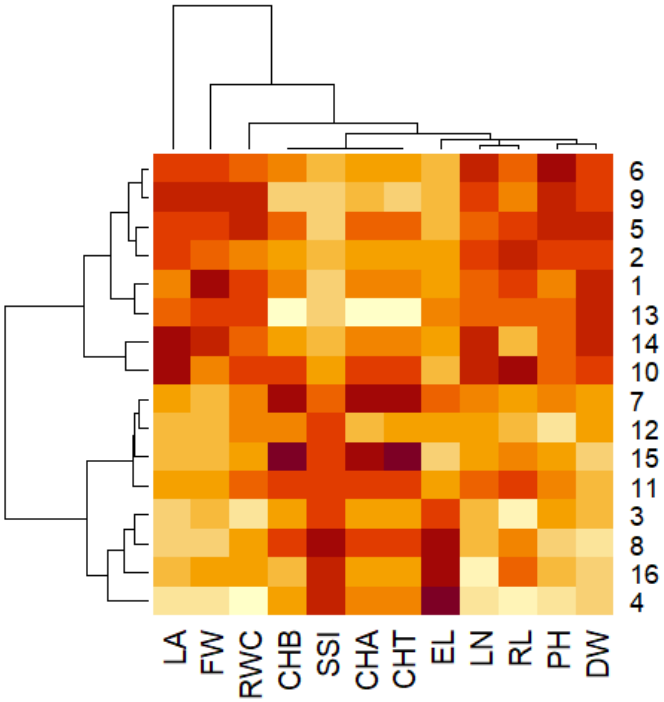

Figure 2. Heat map correlation histogram of morpho-physiological traits and biomass parameters of kale under different salicylic acid concentrations and drought stress levels. The colors represent variable values, with darker colors indicating higher values and lighter colors indicating lower values.

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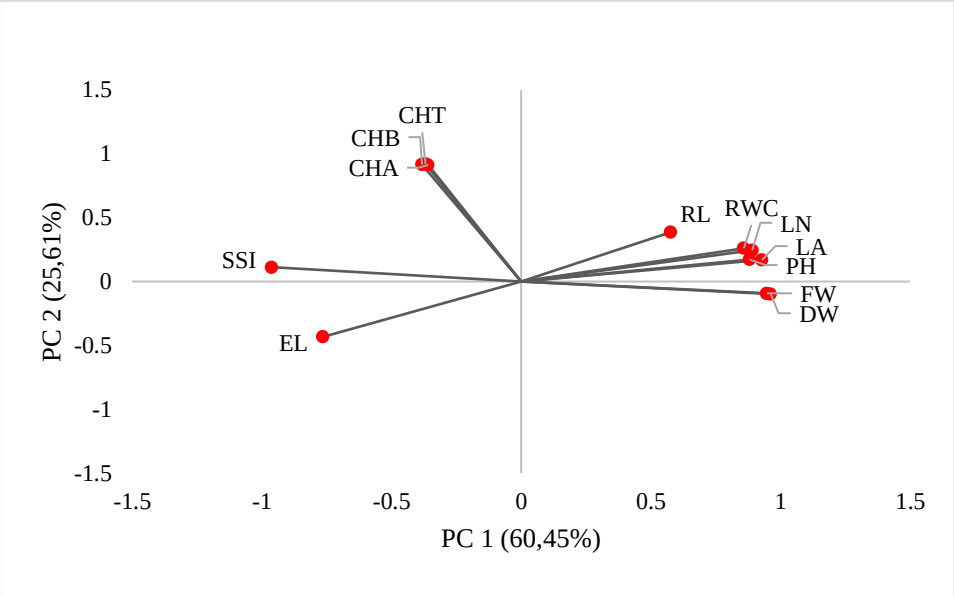

Figure 3. Principal component analysis (PCA) illustrating the relationships among morpho-physiological traits and biomass parameters of kale under salicylic acid application and drought stress.

The stress sensitivity index (SSI), which relates dry-weight biomass reduction to stress intensity, was used to evaluate and compare drought tolerance among treatments. Treatments were classified as tolerant/resistant when SSI < 1, as being at the tolerance threshold when SSI = 1, and as sensitive/vulnerable when SSI > 1. Based on dry-weight data, kale plants at 80% FC fell into the tolerant/resistant category, whereas those at 60–40% FC were classified as sensitive/vulnerable, indicating that kale can adapt to a range of stress levels but with reduced tolerance under more severe drought.

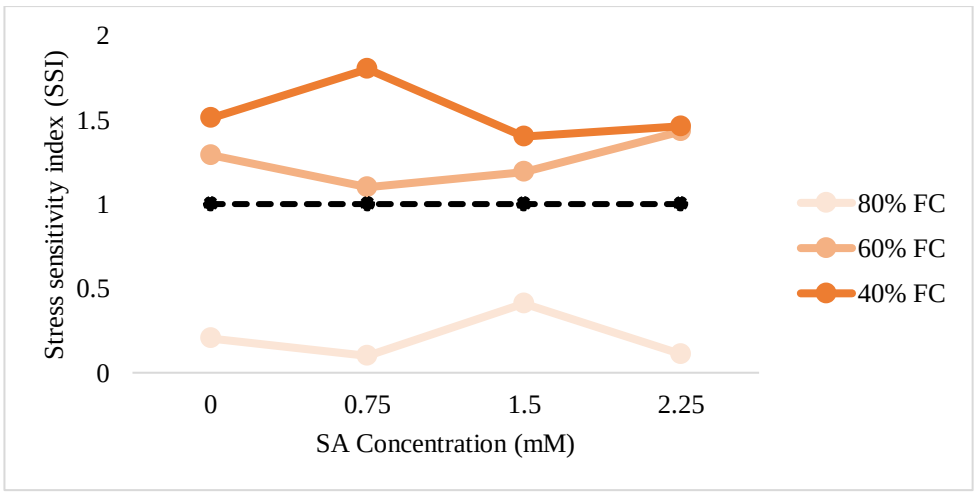

Figure 4. Interaction plot showing the effect of drought stress levels and salicylic acid (SA) concentrations on the Stress Sensitivity Index (SSI) of kale (*Brassica oleracea* var. *acephala*). The dashed horizontal line indicates the tolerance threshold (SSI = 1), where values below 1 represent tolerant or resistant responses and values above 1 indicate sensitivity to drought stress.

As shown in Figure 4, SSI values increased as soil moisture decreased from 80% to 40% FC, showing that drought intensity strongly drove the stress response while SA concentration had only a minor influence within each drought level. Lower

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SSI values at 80% FC compared with 60–40% FC reflect inhibited photosynthesis and water/nutrient uptake under intensifying drought, ultimately reducing biomass accumulation (Anindya *et al.*, 2023).

4. Limitations and Future Directions

This study offers useful insights into the effects of drought stress and salicylic acid on the morpho-physiological attributes of kale, though several limitations remain. It was conducted in a controlled greenhouse (temperature 30–42 °C, humidity 60–80%) rather than field conditions with fluctuating weather, wind exposure, soil variation, and biotic stress. It also used only one kale variety within a single growing season, and no biochemical or molecular analyses (antioxidant enzyme activity, proline content, or gene expression) were performed. Future work should verify these results through multi-location, multi-season field trials across kale varieties, incorporate biochemical and molecular analyses, and optimize SA application method, timing, and concentration (e.g., 0–3.0 mM).

5. Conclusion

Drought at 60% FC supported physiological tolerance (chlorophyll a: 1.52 mg g^{-1}; chlorophyll b: 0.71 mg g^{-1}; total chlorophyll: 2.22 mg g^{-1}; electrolyte leakage: 44.89%) and vegetative growth (plant height: 42.70 cm; leaf number: 20.00; leaf area: 4.940 cm^2), whereas severe drought at 40% FC negatively affected all parameters, with electrolyte leakage reaching 69.30% and dry weight decreasing by 60.5% relative to 100% FC. Application of 1.5 mM salicylic acid increased RWC and decreased electrolyte leakage by 4.13% and 34.70%, respectively, compared to the untreated plants. These results indicate that 1.5 mM SA has potential as a biostimulant for improving kale water status and membrane stability. However, in this study, SA improved physiological traits only, rather than implying an overall improvement in drought tolerance or plant performance. As the findings were obtained under controlled greenhouse conditions in a single growing season, this recommendation should be regarded as preliminary pending field validation across multiple locations, seasons, and kale varieties.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declared that no generative AI was used in the preparation of this manuscript.

Authorship Contribution Statement

RSD contributed to conceptualization, methodology, investigation, data curation, and writing the original draft. AD contributed to the investigation, data curation, formal analysis, and writing the original draft. KRN contributed to conceptualization, methodology, validation, and review. MAR contributed to data interpretation and writing the original draft. FJP and NIM contributed to validation and reviewing. All authors reviewed, revised, and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript in Agro Bali : Agricultural Journal.

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