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Salicylic acid modulates yield and bioactive compound accumulation in chili pepper depending on fruit ripening stage

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Abstract

Salicylic acid (SA) is an endogenous signaling molecule involved in the regulation of plant growth, development, stress responses, and secondary metabolism. Although the effects of exogenous SA and fruit ripening on pepper growth and biochemical composition have been investigated separately, the extent to which SA application modulates yield and bioactive compound accumulation across distinct fruit maturity stages remains insufficiently characterized. This study investigated the effects of foliar SA application on yield, physical fruit quality, biochemical properties, and antioxidant capacity at three maturity stages of chili pepper. A field experiment evaluated three SA treatments (0, 1, and 2 mM) across three maturity stages (green, brown, and red). Fruit maturity significantly affected most evaluated traits, whereas SA application significantly affected yield and selected biochemical and antioxidant traits; significant maturity × SA interactions occurred for several biochemical and antioxidant traits and fruit L*. The 1 mM SA treatment yielded the highest fruit production (1239.86 g m^{-2}), and the brown stage had the highest observed mean fruit weight (19.95 g). Mean fruit weight under 1 mM SA was 19.51 g versus 18.60 g in the control, corresponding to a 4.89% increase relative to the control. The red stage had the highest observed mean values for dry matter, Vitamin C, TPC, TFC, DPPH, and FRAP. Overall, 1 mM SA had the highest mean Vitamin C (236.89 mg 100 g^{-1}), TPC (300.92 mg GAE 100 g^{-1}), DPPH (10.06 µmol TE g^{-1}), and FRAP (6.10 µmol TE g^{-1}) among SA treatments. The highest TPC (406.62 mg GAE 100 g^{-1}) was recorded in red-stage fruits from plants treated with 1 mM SA. Fruit maturity was the predominant factor associated with variation in bioactive compound accumulation and antioxidant capacity, while SA modulated these responses in a dose- and maturity-dependent manner. The 1 mM SA treatment showed the most consistent responses for fruit yield and selected biochemical and antioxidant traits, although effects were not uniform across all parameters. These findings highlight the importance of fruit developmental stage when evaluating the physiological and biochemical effects of exogenous SA in chili pepper.

Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable species originating from Central and South America and having a cultural history spanning thousands of years. Archaeobotanical findings indicate that remains of wild seeds belonging to C. annuum date back to approximately 5000 BC. Following the introduction of pepper to Europe by the Spanish, its spread to Africa and Asia facilitated the rapid adoption of the species in different geographical regions, and it became an important component of Asian culinary cultures, particularly from the late 16th century onward [1–2]. According to FAOSTAT data, global production of green peppers and chili peppers reached approximately 44.8 million tons in 2024 [3]. China, with a production exceeding 18 million tons, is the leading producer, while Mexico, Türkiye, India, and Indonesia are also among the prominent countries in global production [4].

The widespread cultivation of pepper across different geographical regions has also contributed to the diversification of its uses and consumer expectations. Increasing awareness of healthy nutrition and environmental sustainability has supported interest in foods with high nutritional and functional properties

[5], while the growing prevalence of cultural and gastronomic diversity has increased demand for peppers with different taste, aroma, pungency, and color characteristics [6]. Accordingly, color, aroma, texture, pungency, and nutritional composition have emerged as important components of quality and product value in fresh and processed peppers [7–8]. In addition to being consumed fresh and dried, the conversion of pepper into sauces, oleoresins, and other processed products has expanded its applications in the food industry and its potential for added value [9]. Within this diversity, chili pepper stands out for its characteristic pungency, diverse color characteristics, and secondary metabolites exhibiting biological activity. The growing global culture of spicy food consumption and advances in technological production have supported the utilization of chili pepper in fresh, dried, and processed forms [10]. Capsaicinoids, which confer the characteristic pungency to the fruit, have attracted interest in the food, pharmaceutical, and cosmetic industries due to their chemesthetic sensation and biological activities [11]. Studies aimed at characterizing and standardizing capsaicinoids using advanced analytical methods have further demonstrated the importance of chili peppers as sources of functional compounds [12]. These characteristics increase the need for cultivation practices that consider not only yield but also fruit quality and the accumulation of biologically active compounds in chili pepper.

Fruit quality in chili pepper can vary considerably depending on genotype, growing conditions, and the developmental and ripening stage of the fruit. Fruit color is an important attribute influencing consumer preferences and is also associated with changes in phytochemical composition during fruit ripening. Recent analyses have further demonstrated considerable variation in phytochemical composition among pepper species, while ascorbic acid content was positively correlated with fruit diameter, fruit weight, and pericarp thickness [13]. The bioactive compounds present in chili pepper have attracted considerable interest because of their reported anticancer, anti-obesity, and antioxidant properties, highlighting the functional importance of the fruit [14]. However, the levels of these bioactive compounds are not constant throughout fruit development and ripening, but may vary with genotype and harvest time. Changes in total phenolic and flavonoid contents and capsaicin levels have been reported throughout fruit development and ripening [15–17]. Therefore, different maturity stages, such as green, brown, and red, reflect not only changes in color but also changes in the physiological and biochemical status of the fruit [18–19]. Indeed, harvest time and developmental stage have been reported to affect the levels of biologically active compounds in pepper fruits [20–22]. Therefore, the simultaneous evaluation of yield, quality, and biochemical components in chili peppers harvested at different maturity stages may contribute to a more comprehensive understanding of changes in fruit characteristics.

However, variability in environmental conditions remains an important factor affecting yield and fruit quality in pepper production. As a warm-season vegetable species, pepper is increasingly exposed to climate change-induced abiotic stresses, particularly under open-field cultivation, posing an important risk to sustainable production. Stress factors such as increasing temperatures, irregular precipitation regimes, and water scarcity may adversely affect physiological processes and photosynthetic performance, resulting in reductions in yield and fruit quality [23–25]. Furthermore, modeling studies predict that changes in temperature and precipitation regimes may alter suitable ecologies and potential production areas for pepper cultivation in the future [26]. Nevertheless, such geographical shifts are not expected to

completely eliminate the physiological and production pressures caused by high temperature, drought, and other abiotic stresses in existing production systems. In controlled environment agriculture, precise light manipulation and thermal regulation are pivotal for governing plant canopy architecture, photosynthetic performance, and whole-plant resource distribution during early development [27]. Although baseline growth and transplant production duration can be accurately estimated using thermal and light-driven mathematical models in nursery settings [28], transferring these plants to open-field systems exposes them to unpredictable microclimatic extremes. Such environmental fluctuations disrupt photoreceptor signaling, redox homeostasis, and carbon assimilation, thereby accentuating the need for exogenous biostimulants like SA to fortify physiological resilience and optimize fruit biochemical quality.

The increasing likelihood of simultaneous exposure to multiple stress factors, such as high temperature and drought, under climate change makes the development of stress tolerance and the maintenance of photosynthetic efficiency important objectives for climate-adaptive crop production [29]. In this context, biostimulants and plant growth regulators that support plants' capacity to adapt to environmental stresses are considered potential tools for maintaining yield and product quality under changing environmental conditions [30–31]. Among these compounds, SA, an endogenous phenolic signaling molecule, plays a role in the regulation of plant growth and development as well as responses to environmental stresses. The regulatory effects of SA on antioxidant defense and photosynthetic processes may influence plant responses to environmental stresses and contribute to changes in secondary metabolism [32–33]. Exogenous SA application has been shown to modulate physiological and photosynthetic responses in C. annuum under drought and rehydration conditions, with responses varying among application concentrations [34]. The regulatory effects of SA on antioxidant defense systems and the phenylpropanoid pathway may also contribute to changes in secondary metabolism. However, responses to exogenous SA applications may vary depending on the application dose and environmental conditions; low or appropriate concentrations may support physiological processes, whereas high concentrations may adversely affect the photosynthetic system [35]. Therefore, the application dose of SA is considered an important factor determining the direction and magnitude of plant responses.

Although the individual effects of SA on pepper growth and the general dynamics of bioactive compounds during fruit ripening have been independently reported [36–38], the interactive effects of SA application doses and fruit maturity stages on the integrated responses of biochemical quality and antioxidant-related traits remain inadequately elucidated. Moreover, previous work on preharvest SA in pepper has largely examined responses at a particular fruit stage or has focused on subsequent postharvest quality, ripening, senescence, or storage responses, rather than comparing multiple fruit maturity stages within the same experimental framework. Thus, it remains unclear whether the magnitude and direction of SA-associated changes in functional quality traits are consistent across successive stages of fruit ripening. In particular, the extent to which preharvest SA application modulates vitamin C, total phenolic and flavonoid contents, and antioxidant capacity across distinct fruit maturity stages has not been comprehensively characterized. A maturity-dependent assessment is therefore relevant because ripening itself is accompanied by substantial changes in the biochemical and antioxidant characteristics of pepper fruit. Resolving these interactions is essential to determine whether SA can differentially modulate functional

quality attributes depending on fruit developmental stage and thereby identify maturity-specific treatment responses. Accordingly, the aim of this study was to evaluate the effects of foliar-applied SA at different application doses across three distinct maturity stages on yield, physical fruit quality, and the integrated responses of biochemical quality and antioxidant potential, including vitamin C, total phenolic and flavonoid contents, DPPH, and FRAP, in chili pepper. We hypothesized that the effects of SA application would be modulated by fruit maturity stage, leading to stage-dependent responses in yield, fruit quality, bioactive compound accumulation, and antioxidant potential.

Materials and Methods

Experimental site and field management

The field experiment was conducted in 2025 at the Research and Application Area of the Department of Horticulture, Faculty of Agriculture, Ordu University, Türkiye, located at 40°58'00.9"N, 37°56'15.9"E. The soil was tilled to a depth of 25–30 cm before transplanting. Before transplanting, soil samples were collected from the 0–30 cm soil layer to determine the initial soil properties. The soil contained 1.23 kg da^{-1} available P_2O_5 (very low), 32.34 kg da^{-1} available K_2O (high), 0.34% $CaCO_3$ (slightly calcareous), and 3.62% organic matter (good). The total soluble salt content was 0.0648% (non-saline), soil pH was 6.48 (neutral), and the saturation percentage was 109.5%, indicating a heavy clay soil.

Chili pepper plants (*Capsicum annuum* L. cv. 'Atom F1'; Anamas Tohumculuk, Antalya, Türkiye) were transplanted on 22 May at a spacing of 70 cm between rows and 30 cm within rows. Each one-row plot consisted of 20 plants. Thus, each experimental plot covered 4.2 m^2. One border row was maintained between blocks and along the field margins to minimize potential border effects.

Fertilization was applied according to the crop development stage. The total fertilizer application was equivalent to 10 kg da^{-1} N, 8 kg da^{-1} P_2O_5, and 12 kg da^{-1} K_2O. All phosphorus was applied at transplanting. One-third of the total nitrogen was applied at transplanting, while the remaining two-thirds was supplied in five equal applications beginning at flowering. Potassium was supplied in eight equal applications beginning at flowering. Drip irrigation was used throughout the growing period. Weed control and plant-protection practices were carried out as required during the growing period [39]. During the growing period (22 May–15 September 2025), the average temperature, total rainfall, and mean relative humidity at the experimental site were 19.8 °C, 284.5 mm, and 76.2%, respectively.

Experimental design and salicylic acid application

The experiment was established as a 3 × 3 factorial arrangement in a randomized complete block design with three blocks. The factors were fruit maturity stage (green, brown, and red) and foliar salicylic acid (SA) concentration (0, 1.0, and 2.0 mM), resulting in nine treatment combinations per block and 27 experimental plots in total. Treatment combinations were randomly assigned to plots within each block.

Salicylic acid (≥99% purity; Sigma-Aldrich, St. Louis, MO, USA) was prepared as a 3.0 mM stock solution by dissolving 2.0715 g SA in deionized water and adjusting the final volume to 5 L. The 1.0 and 2.0 mM

working solutions were prepared by serial dilution. Tween-20 was added to all spray solutions at 0.05% (v/v). The treatments were applied once, 30 days after transplanting, when the plants had developed a fully established canopy. Foliar spraying was performed in the early morning (08:00–10:00 h) using a hand-held pressure sprayer. The solutions were applied uniformly to both adaxial and abaxial leaf surfaces until visible runoff. Applications were performed under windless conditions, at an ambient temperature of approximately 24 °C and relative humidity of approximately 64%. The control treatment received deionized water containing the same concentration of Tween-20 (0.05%, v/v) as the SA treatments and was applied under the same conditions.

Harvesting, yield determination, and fruit sampling

Fruits were harvested once a week from 22 July to 15 September according to the maturity stage assigned to each plot. Fruit number and fruit weight were recorded at each harvest. Fruit yield was calculated as cumulative total fruit yield over the entire harvest period, with all harvests included in the final calculation. Plot-level yield was expressed on an area basis as g m^{-2}.

Fruit physical, dry matter, biochemical, and antioxidant measurements were conducted using samples collected at the third harvest. At this harvest, fruits were collected separately from each of the 27 experimental plots. For physical measurements, 10 fruits were randomly selected from each plot. Fruit weight (g) and length (cm) were measured for each fruit, while fruit diameter and pericarp thickness were measured at the equatorial region using a digital caliper (mm). The measurements obtained from the 10 fruits were averaged for each trait to obtain a single plot-level value.

Physical quality and dry matter measurements

F ruit color was measured at two opposite points along the equatorial region using a chromameter (CR-300; Minolta Co., Ltd., Osaka, Japan). The instrument was calibrated against a standard white tile before measurements. Color was characterized using the CIELAB color system, and lightness (L), chroma (C), and hue angle (h°) were recorded.

Dry matter content (%) was determined gravimetrically. Homogenized fruit samples (approximately 80–100 g) were weighed as the initial fresh weight and dried in a forced-air oven at 70 °C for 48–72 h until two consecutive weighings, performed at 4-h intervals, yielded a constant mass. Dry matter content was calculated as:

Dry Matter (%) = (Dry Weight / Fresh Weight) × 100

Biochemical and antioxidant analyses

For biochemical and antioxidant analyses, 10 fruits were taken from each experimental plot at the third harvest and combined to obtain a representative sample. The fruits were cut longitudinally into approximately 1-cm-wide sections, and 200 g of the prepared fruit material was used for extraction. An equal volume of distilled water was added to the sample (1:1, w/v), followed by homogenization for 5 min with a Waring blender (Waring 8012, Torrington, CT, USA). The homogenate was passed through Whatman

No. 4 filter paper. From the resulting filtrate, 100 mL was transferred to centrifuge tubes and centrifuged at 6000 rpm for 5 min. The supernatant obtained after centrifugation was used for the biochemical and antioxidant determinations. Three technical measurements were performed for each plot-level sample, and their mean was used as the corresponding plot value. Accordingly, the three blocks constituted the independent experimental replicates (n = 3), whereas the repeated measurements within each plot represented technical repetitions.

Total soluble solids (TSS) were measured directly from the fruit extract with a digital refractometer and reported as %. Vitamin C concentration was determined reflectometrically using an RQflex plus 10 system (Merck, Germany) together with ascorbic acid test strips (Cat. No. 116981, Merck, Germany), following Ozturk et al. [40]. For the measurement, 0.5 mL of fruit extract was brought to a final volume of 5 mL with 0.5% oxalic acid. The resulting solution was then analyzed with the reflectometer. Vitamin C concentration was expressed as mg 100 g^{-1} fresh weight.

Total phenolic content (TPC) was quantified by the Folin–Ciocalteu colorimetric procedure described by Çolak et al. [41]. A 400-µL aliquot of the fruit extract was combined with 4.2 mL of distilled water and 100 µL of Folin–Ciocalteu reagent, followed by the addition of 0.3 mL of 2% Na_2CO_3. The reaction mixture was kept for 2 h before its absorbance was recorded at 760 nm using a spectrophotometer. Gallic acid was used for calibration, and TPC was expressed as mg GAE 100 g^{-1} fresh weight.

Total flavonoid content (TFC) was assessed using the colorimetric procedure reported by Çolak et al. [41]. Briefly, 1 mL of diluted fruit extract was brought to 5 mL with distilled water and reacted with 0.3 mL of 5% $NaNO_2$. After addition of 10% $AlCl_3$ and a 6-min reaction period, 1 M NaOH was added, and the mixture was diluted with distilled water to a final volume of 10 mL. Absorbance was measured at 510 nm. Quercetin was used as the calibration standard, and TFC was expressed as mg QE 100 g^{-1} fresh weight.

Ferric reducing antioxidant power (FRAP) was evaluated following Çolak et al. [41] using the assay principle of Benzie and Strain [42]. The working reagent consisted of acetate buffer (0.1 mol L^{-1}, pH 3.6), TPTZ (10 mmol L^{-1}), and ferric chloride (20 mmol L^{-1}). For the assay, 20 µL of fruit extract was added to 2.98 mL of the freshly prepared FRAP reagent. After a 10-min reaction period, absorbance was determined at 593 nm. Results were expressed as µmol TE g^{-1} fresh weight.

DPPH radical-scavenging capacity was determined using the procedure of Blois [43]. In brief, 0.5 mL of ethanolic DPPH solution (0.1 mmol L^{-1}) was combined with the fruit extract and diluted to a final volume of 3 mL. The reaction mixture was stirred and maintained at room temperature for 30 min, after which absorbance was recorded at 517 nm. Trolox was used to construct the calibration curve, and the antioxidant activity was expressed as µmol TE g^{-1} fresh weight.

Statistical analysis

The experimental plot was considered the experimental unit. The 10 fruits sampled within each plot for physical measurements and the composite sample prepared for biochemical and antioxidant analyses were treated as subsamples, and the resulting measurements were averaged to obtain one plot-level

observation. The three blocks represented three independent experimental replications (n = 3) for each fruit maturity × SA combination. Yield data were based on cumulative fruit production across the entire harvest period, whereas physical, biochemical, and antioxidant measurements were based on samples collected at the third harvest.

Data were analyzed using JMP version 13 (SAS Institute Inc., Cary, NC, USA). The assumptions of normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene tests, respectively. A two-way analysis of variance (ANOVA) was performed to evaluate the main effects of fruit maturity stage and SA concentration and their interaction. When significant differences were detected, means were separated using the least significant difference (LSD) test at $P \leq 0.05$. Statistical significance was evaluated at the 5% probability level.

Results and Discussion

Effects of salicylic acid applications and fruit maturity on yield and physical fruit characteristics

The effects of foliar SA applications at different fruit maturity stages on yield and selected physical characteristics of chili pepper fruits are presented in Table 1.

Table 1. Effects of SA applications at different fruit maturity stages on selected physical characteristics of chili pepper fruits

Fruit maturity (FM)	SA application	Fruit yield ($g\ m^{-2}$)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (mm)	Pericarp thickness (mm)	Fruit dry matter (%)
Green	Control	1049.73 d	18.33 ef	12.98	15.04	2.22 c	9.44 g
	SA1mM	1215.34 bc	18.47 e	13.02	14.91	2.41 ab	9.57 g
	SA2mM	1262.11 b	18.50 e	13.11	15.45	2.53 a	9.93 f
	Mean	1175.73 A	18.43 C	13.04 B	15.13 C	2.39 A	9.65 C
Brown	Control	1082.23 cd	19.35 cd	13.47	16.31	2.36 b	15.17 d
	SA1mM	1427.49 a	20.47 a	13.53	16.49	2.37 b	15.31 cd
	SA2mM	1042.17 d	20.03 b	13.42	16.37	2.39 b	14.90 e
	Mean	1183.97 A	19.95 A	13.47 A	16.39 A	2.37 A	15.13 B
Red	Control	874.27 e	18.11 f	12.77	15.81	2.21 c	15.44 c
	SA1mM	1076.75 cd	19.59 c	12.80	16.20	1.97 d	15.64 b
	SA2mM	833.85 e	19.10 d	12.93	15.97	1.98 d	16.27 a
	Mean	928.29 B	18.93 B	12.83 C	15.99 B	2.06 B	15.78 A
SA Mean	*Control*	*1002.08 B*	*18.60 C*	*13.07*	*15.72*	*2.26*	*13.35 C*
	SA1mM	*1239.86 A*	*19.51 A*	*13.12*	*15.87*	*2.25*	*13.51 B*
	SA2mM	*1046.04 B*	*19.21 B*	*13.15*	*15.93*	*2.30*	*13.70 A*
	LSD_{FM}:	88.68***	0.18***	0.17***	0.22***	0.08***	0.09***
	LSD_{SA}:	88.68***	0.18***	ns.	ns.	ns.	0.09***
	LSD_{FMxSA}:	153.61***	0.31***	ns.	ns.	0.14***	0.15***

Notes: Values are means of three independent experimental plots (n = 3). Different lowercase letters within a column indicate significant differences among the nine SA × fruit maturity combinations, whereas

uppercase letters indicate significant differences among the corresponding marginal means of fruit maturity stage or SA concentration, according to the LSD test at α = 0.05. ns, not significant (P > 0.05); *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001.

The effects of SA application at different maturity stages on fruit yield and selected physical characteristics of chili pepper are presented in Table 1. The main effect of SA application significantly affected fruit yield, fruit weight, and dry matter content ($p \leq 0.001$), whereas its effects on fruit length, fruit diameter, and pericarp thickness were not significant ($p > 0.05$). Maturity stage significantly affected all examined traits, while significant fruit maturity × SA interactions were observed for fruit yield, fruit weight, pericarp thickness, and dry matter content ($p \leq 0.001$).

Fruit yield was highest under the 1 mM SA treatment (1239.86 g m^{-2}), followed by 2 mM SA (1046.04 g m^{-2}) and the control (1002.08 g m^{-2}). The higher yield observed under 1 mM SA may be associated with physiological effects of SA on plant growth and assimilate allocation reported in previous studies. However, the absence of a further yield increase at 2 mM SA may reflect the biphasic dose–response characteristic of SA, whereby lower concentrations stimulate physiological processes, whereas higher concentrations may elicit weaker or more constrained responses [36,44]. Nevertheless, the yield increases reported at higher SA concentrations in some genotypes [45-46] underscore the context-dependent nature of SA responses and suggest that its effects may be modulated by genotype, concentration, and growing conditions. Across maturity stages, fruit yield was comparable between the green (1175.73 g m^{-2}) and brown stages (1183.97 g m^{-2}), but was lower at the red stage (928.29 g m^{-2}). At the fruit maturity × SA interaction level, the highest observed yield was recorded for the brown stage × 1 mM SA combination (1427.49 g m^{-2}), whereas the lowest was observed under the red stage × 2 mM SA treatment (833.85 g m^{-2}). This interaction indicates that the yield response to SA varied across maturity stages, with the highest observed yield occurring under 1 mM SA at the brown stage.

Fruit weight was also significantly influenced by SA application, with the highest value recorded at 1 mM SA (19.51 g). As fruit maturation progressed, mean fruit weight increased from 18.43 g at the green stage to 19.95 g at the brown stage, before declining slightly to 18.93 g at the red stage. The greatest fruit weight was obtained from the brown stage × 1 mM SA combination (20.47 g). The positive effect of SA on fruit weight may be associated with physiological effects of SA on plant growth and assimilate allocation reported in previous studies, rather than being attributed to a specific cellular mechanism not directly evaluated in the present study. The higher fruit weight observed at the brown stage suggests that the response of fruit growth to SA may vary with developmental stage. These findings are consistent with previous reports demonstrating that SA application can promote fruit development and biomass accumulation [47-48]. Uğur and Saka [49], similarly reported that foliar SA application increased fruit weight in both hot and sweet long peppers, supporting the positive response of fruit weight to SA observed in the present study.

In contrast, neither the main effect of SA nor the fruit maturity × SA interaction significantly affected fruit length or diameter ($p > 0.05$). Variation in these traits was primarily associated with maturity stage, with the greatest fruit length and diameter recorded at the brown stage, reaching 13.47 cm and 16.39 mm,

respectively. Fruit length measured 13.04, 13.47, and 12.83 cm at the green, brown, and red stages, respectively, while corresponding fruit diameters were 15.13, 16.39, and 15.99 mm. These patterns indicate that fruit dimensions were more closely associated with developmental changes during maturation than with the SA concentrations applied in the present study. Although several studies have reported increases in fruit size following SA application [46,50], the magnitude and direction of this response may depend on application rate, timing, and genotype.

Pericarp thickness was not significantly affected by the main effect of SA, whereas both maturity stage and the fruit maturity × SA interaction exerted highly significant effects ($p \leq 0.001$). Pericarp thickness was comparable between the green (2.39 mm) and brown stages (2.37 mm), but declined to 2.06 mm at the red stage. At the interaction level, the highest observed pericarp thickness was recorded under the green stage × 2 mM SA treatment (2.53 mm), whereas the lowest value occurred under the red stage × 2 mM SA combination (1.98 mm). This contrasting response indicates that the effect of SA on pericarp thickness varied substantially depending on fruit maturity stage. Rather than attributing this response directly to changes in cell-wall development or cellular water relations, the observed variation may reflect maturity-dependent differences in tissue development and the physiological response to SA. At advanced stages of ripening, however, tissue softening and changes in cell-wall structure associated with ripening may have contributed to the observed reduction in pericarp thickness. The lower value observed with the higher SA concentration at the red stage further suggests that the physiological response to SA may shift across developmental stages [51].

Fruit dry matter content was significantly influenced by both SA application and maturity stage ($p \leq 0.001$). Dry matter content increased across the SA concentrations, reaching its highest observed mean under the 2 mM SA treatment (13.70%). Across maturity stages, dry matter content was 9.65%, 15.13%, and 15.78% at the green, brown, and red stages, respectively, with the highest observed value at the fruit maturity × SA interaction level recorded for the red stage × 2 mM SA combination (16.27%). The increase in dry matter content during ripening may be associated with the progressive accumulation of sugars and other soluble constituents in the fruit [52-53]. The contribution of SA to this response may be related to its reported effects on plant growth and assimilate accumulation, although these processes were not directly measured in the present study. Notably, the highest dry matter content observed under 2 mM SA at the red stage suggests that the effect of SA on dry matter accumulation may become more pronounced at advanced maturity. This finding is consistent with previous reports indicating that SA application can enhance dry matter accumulation and quality-related attributes in pepper fruits [46,54].

Overall, SA application exerted significant effects on fruit yield, fruit weight, and dry matter accumulation, whereas fruit length and diameter were primarily associated with maturity stage. The significant fruit maturity × SA interactions observed for fruit yield, fruit weight, pericarp thickness, and dry matter content indicate that SA responses should be interpreted in relation to fruit developmental stage rather than solely from the main effect of SA application.

Effects of salicylic acid applications and fruit maturity on fruit color characteristics

The responses of fruit color characteristics to different maturity stages and SA applications are presented in Table 2.

Table 2. Effects of different maturity stages and SA applications on color characteristics of chili pepper fruits

Fruit maturity (FM)	SA application	Fruit L*	Fruit chroma (C*)	Fruit hue angle (°)
Green	Control	39.67 c	23.74	99.30
	SA1mM	37.47 d	22.21	100.88
	SA2mM	37.89 de	23.77	100.18
	Mean	38.34 B	23.24 B	100.12 A
Brown	Control	35.99 e	10.59	93.08
	SA1mM	37.42 de	12.16	97.29
	SA2mM	37.26 de	11.33	93.31
	Mean	36.89 C	11.36 C	94.56 B
Red	Control	45.43 a	37.69	41.40
	SA1mM	45.31 ab	37.90	40.87
	SA2mM	43.68 b	38.05	41.45
	Mean	44.81 A	37.88 A	41.24 C
SA Mean	*Control*	*40.36*	*24.01*	*77.93*
	SA1mM	*40.07*	*24.09*	*79.68*
	SA2mM	*39.61*	*24.39*	*78.31*
	LSD_{FM}:	0.97***	1.22***	1.14***
	LSD_{SA}:	ns.	ns.	ns.
	LSD_{FMxSA}:	1.68*	ns.	ns.

Notes: Values are means of three independent experimental plots (n = 3). Different lowercase letters within a column indicate significant differences among the nine SA × fruit maturity combinations, whereas uppercase letters indicate significant differences among the corresponding marginal means of fruit maturity stage or SA concentration, according to the LSD test at α = 0.05. ns, not significant (P > 0.05); *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001.

The effect of fruit maturity stage on L*, C*, and hue values in chili pepper fruits was statistically significant (Table 2). The L* value was highest at the red maturity stage (44.81) and lowest at the brown stage

(36.89), whereas C* showed the highest observed mean at the red stage (37.88) and the lowest at the brown stage (11.36). Hue decreased progressively from 100.12 at the green stage to 94.56 at the brown stage and subsequently to 41.24 at the red stage. The three maturity stages differed significantly from each other for all three color parameters. In contrast, the main effects of SA application on L*, C*, and hue were not statistically significant, with SA treatment means ranging from 39.61–40.36, 24.01–24.39, and 77.93–79.68, respectively. However, the fruit maturity × SA interaction significantly affected L* ($p \leq 0.05$), whereas the interaction effects on C* and hue were not significant.

For L*, the significant fruit maturity × SA interaction indicated that the response pattern varied among maturity stages. At the green stage, L* values were 39.67, 37.47, and 37.89 for the control, 1 mM SA, and 2 mM SA treatments, respectively. At the brown stage, the corresponding values were 35.99, 37.42, and 37.26, while at the red stage they were 45.43, 45.31, and 43.68, respectively. Thus, although the overall SA main effect was not significant, the L* response to SA varied depending on fruit maturity stage. The highest observed L* value was recorded in red-stage fruits under the control treatment (45.43), whereas the lowest was observed in brown-stage fruits under the control treatment (35.99).

The changes observed in L*, C*, and hue throughout fruit maturation may reflect pigment turnover and the progressive development of fruit coloration during ripening. Chlorophyll degradation, chloroplast-to-chromoplast transition, and carotenoid accumulation are recognized processes associated with the transition from green to red in pepper fruits [55-56]. However, because pigment concentrations were not directly determined in the present study, these mechanisms should be considered as possible explanations rather than direct evidence from the current experiment. Consistent with this mechanism, Lim et al. [57] reported pronounced changes in C* and hue values as ripening progressed in bell pepper, while Kim et al. [58] demonstrated that L*, C*, and hue values vary according to harvest stage and cultivar. Gómez-Ladrón de Guevara et al. [59] further established that CIELAB color parameters can serve as useful indicators of pepper maturity. Berry et al. [60] reported that capsanthin and its esterified derivatives are major pigments in ripe Capsicum annuum fruit and that free capsanthin is more strongly associated with colour intensity than total carotenoid content. In the present study, the marked increase in C* and pronounced decline in hue at the red maturity stage similarly reflected the ripening-associated transition from green to red coloration. The absence of significant effects of SA application on C* and hue suggests that these color attributes were governed predominantly by maturity stage rather than by the applied SA concentrations.

Effects of salicylic acid applications and fruit maturity on biochemical and antioxidant properties

The effects of different maturity stages and SA applications on selected biochemical and antioxidant properties of chili pepper fruits are presented in Table 3.

Table 3. Effects of different maturity stages and SA applications on selected biochemical and antioxidant properties of chili pepper fruits

Fruit maturity (FM)	SA application	Fruit TSS (%)	Vitamin C (mg 100 g^{-1})	DPPH (mmol TE g^{-1})	FRAP (mmol TE g^{-1})	TPC (mg GAE 100 g^{-1})	TFC (mg QE 100 g^{-1})
Green	Control	2.47	104.67 e	5.37 h	3.19 ef	91.85 g	36.88 g
	SA1mM	2.53	140.33 d	6.04 g	3.62 e	137.32 f	46.64 f
	SA2mM	2.67	102.00 e	4.87 i	2.80 f	91.99 g	33.56 g
	Mean	2.56 C	115.67 C	5.43 C	3.20 C	107.06 C	39.03 C
Brown	Control	4.07	250.33 c	6.61 f	4.88 d	280.06 e	57.45 e
	SA1mM	4.23	254.00 c	9.49 d	5.07 d	358.81 c	69.85 d
	SA2mM	4.17	269.00 b	8.37 e	5.95 c	387.99 b	83.51 c
	Mean	4.16 B	250.78 B	8.16 B	5.30 B	342.29 B	70.27 B
Red	Control	5.13	322.33 a	14.25 b	6.78 b	325.55 d	99.85 a
	SA1mM	5.03	316.33 a	14.64 a	9.61 a	406.62 a	98.23 a
	SA2mM	5.10	271.33 b	13.15 c	6.80 b	356.41 c	91.44 b
	Mean	5.09 A	303.33 A	14.01 A	7.73 A	362.86 A	96.51 A
SA Mean	*Control*	*3.89*	*225.78 B*	*8.74 B*	*4.95 B*	*232.49 C*	*64.73 B*
	SA1mM	*3.93*	*236.89 A*	*10.06 A*	*6.10 A*	*300.92 A*	*71.57 A*
	SA2mM	*3.98*	*214.11 C*	*8.80 B*	*5.19 B*	*278.80 B*	*69.50 A*
	LSD_{FM}:	0.85***	6.27***	0.16***	0.42***	5.82***	2.16***
	LSD_{SA}:	ns.	6.27***	0.16***	0.42***	5.82***	2.16***
	LSD_{FMxSA}:	ns.	10.86***	0.27***	0.72***	10.09***	3.74***

Notes: Values are means of three independent experimental plots (n = 3). Different lowercase letters within a column indicate significant differences among the nine SA × fruit maturity combinations, whereas uppercase letters indicate significant differences among the corresponding marginal means of fruit maturity stage or SA concentration, according to the LSD test at α = 0.05. ns, not significant (P > 0.05); *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001.

Fruit maturity significantly affected all biochemical and antioxidant traits evaluated in the present study (Table 3). Soluble solids content (TSS) increased progressively with fruit maturation, from 2.56% at the green stage to 4.16% at the brown stage and 5.09% at the red stage. In contrast, neither the main effect of SA application nor the fruit maturity × SA interaction significantly affected TSS (p > 0.05). Thus, variation in TSS was primarily associated with fruit maturity rather than with the applied SA concentrations and may

reflect the accumulation of soluble sugars and other soluble constituents during ripening, consistent with previous observations in pepper fruits [61].

Fruit maturity also significantly affected vitamin C, DPPH, FRAP, total phenolic content (TPC), and total flavonoid content (TFC) ($p \leq 0.001$). The highest maturity-stage means for all five traits were observed at the red stage, with values of 303.33 mg 100 g^{-1} for vitamin C, 14.01 µmol TE g^{-1} for DPPH, 7.73 µmol TE g^{-1} for FRAP, 362.86 mg GAE 100 g^{-1} for TPC, and 96.51 mg QE 100 g^{-1} for TFC. Papathanasiou et al. [62] similarly reported that total phenolic content, ascorbic acid content, and antioxidant capacity varied across developmental and maturation stages in three C. annuum cultivars, with the highest antioxidant activity observed at the red maturity stage. Hamed et al. [63] also reported substantial variation in total phenolic and flavonoid contents, ascorbic acid, and DPPH and ABTS antioxidant activities between mature green and red/yellow C. annuum fruits, further supporting the importance of maturity stage in determining the bioactive composition and antioxidant potential of pepper fruits. Similarly, Ghasemnezhad et al. [61] and Bhandari et al. [53] reported that fruit ripening substantially alters biochemical attributes such as vitamin C, phenolic compounds, and antioxidant capacity in peppers. However, the magnitude and direction of these changes may vary depending on the compound and genotype [61]. Ornelas-Paz et al. [64] likewise reported that vitamin C content varied with ripening stage in different pepper types, with red and yellow fruits reaching higher levels than green fruits. Moreover, Medina-Juárez et al. [65] found that higher DPPH radical-scavenging activity among C. annuum cultivars was associated with higher levels of several phenolic compounds and ascorbic acid, suggesting that these bioactive constituents may contribute to the antioxidant potential of pepper fruits. This pattern is consistent with the higher vitamin C and antioxidant values observed at the red maturity stage in the present study.

The effects of SA application on biochemical properties varied with fruit maturity stage. Based on the marginal means, 1 mM SA resulted in the highest mean values for vitamin C (236.89 mg 100 g^{-1}), DPPH (10.06 µmol TE g^{-1}), FRAP (6.10 µmol TE g^{-1}), and TPC (300.92 mg GAE 100 g^{-1}). For TFC, both 1 mM SA (71.57 mg QE 100 g^{-1}) and 2 mM SA (69.50 mg QE 100 g^{-1}) were statistically higher than the control (64.73 mg QE 100 g^{-1}), whereas the two SA concentrations did not differ significantly from each other. These results indicate that the response to SA varied among the evaluated biochemical traits and was not uniformly concentration-dependent. Similar dose- and stress-dependent responses have been reported in pepper, where SA applications affected phenolic compounds, flavonoids, vitamin C, and antioxidant-related properties to varying degrees [66-67]. The present findings are also consistent with Dobón-Suárez et al. [36], who reported that preharvest SA applications at 0.5 and 1 mM increased total phenolic content in green pepper fruits, while subsequent work indicated that SA treatment may influence the maintenance and accumulation of phenolic compounds during harvest and storage [37]. These results further indicate that increasing SA concentration from 1 to 2 mM did not consistently result in additional increases in biochemical or antioxidant traits.

The significant fruit maturity × SA interactions for vitamin C, DPPH, FRAP, TPC, and TFC indicate that the response to SA depended on fruit maturity stage. For vitamin C, the highest value was observed at the red stage under the control treatment (322.33 mg 100 g^{-1}), which did not differ significantly from the red

stage × 1 mM SA treatment (316.33 mg 100 g^{-1}). At the brown stage, the highest vitamin C value was recorded under 2 mM SA (269.00 mg 100 g^{-1}), whereas at the green stage, 1 mM SA resulted in the highest value (140.33 mg 100 g^{-1}). Thus, the effect of SA on vitamin C was not consistent across maturity stages.

A similar maturity-dependent pattern was observed for antioxidant capacity. DPPH values were highest at the red stage, reaching 14.64 µmol TE g^{-1} under 1 mM SA, followed by 14.25 µmol TE g^{-1} under the control and 13.15 µmol TE g^{-1} under 2 mM SA. At the brown stage, 1 mM SA also produced the highest DPPH value (9.49 µmol TE g^{-1}), and 1 mM SA also gave the highest value at the green stage, 6.04 µmol TE g^{-1}. For FRAP, the highest interaction mean was recorded at the red stage under 1 mM SA (9.61 µmol TE g^{-1}), while the lowest was observed at the green stage under 2 mM SA (2.80 µmol TE g^{-1}). At the brown stage, 2 mM SA resulted in the highest FRAP value (5.95 µmol TE g^{-1}). Thus, 1 mM SA produced the highest DPPH values at all three maturity stages, whereas for FRAP the most effective SA concentration differed with fruit maturity (1 mM at the green and red stages, 2 mM at the brown stage).

The positive effect of SA on phenolic compound accumulation was particularly evident at 1 mM. In the present study, TPC was 232.49, 300.92, and 278.80 mg GAE 100 g^{-1} under the control, 1 mM SA, and 2 mM SA treatments, respectively. At the interaction level, TPC was highest at the red stage under 1 mM SA (406.62 mg GAE 100 g^{-1}), followed by the brown stage under 2 mM SA (387.99 mg GAE 100 g^{-1}). At the green stage, 1 mM SA resulted in the highest TPC value (137.32 mg GAE 100 g^{-1}), whereas the control and 2 mM SA treatments did not differ significantly. These results are consistent with previous studies indicating that SA application can promote phenolic compound accumulation in pepper fruits. Dobón-Suárez et al. [36] reported that preharvest applications of 0.5 and 1 mM SA increased total phenolic content in green pepper fruits. Similarly, Dobón-Suárez et al. [37] showed that SA application contributed to the maintenance and accumulation of total phenolic compounds during harvest and subsequent storage. Khazaei and Estaji [67] also reported that SA application enhanced phenolic compound accumulation in pepper, although the magnitude of this response varied depending on application dose and stress conditions.

Total flavonoid content increased progressively across maturity stages, from 39.03 mg QE 100 g^{-1} at the green stage to 70.27 mg QE 100 g^{-1} at the brown stage and 96.51 mg QE 100 g^{-1} at the red stage. The significant fruit maturity × SA interaction further showed that the response to SA differed among maturity stages. At the green stage, 1 mM SA resulted in the highest TFC value (46.64 mg QE 100 g^{-1}), whereas at the brown stage, 2 mM SA produced the highest value (83.51 mg QE 100 g^{-1}). At the red stage, the control (99.85 mg QE 100 g^{-1}) and 1 mM SA (98.23 mg QE 100 g^{-1}) did not differ significantly, while both were higher than 2 mM SA (91.44 mg QE 100 g^{-1}). The overall SA means showed that 1 and 2 mM SA were both higher than the control, but did not differ significantly from each other. This finding indicates that the effect of SA on flavonoid accumulation was dependent on both application dose and fruit maturity stage. Dos Anjos et al. [68] reported that SA application increased total phenolic content in Capsicum frutescens fruits, whereas total flavonoid content did not differ significantly from the control. Mahdavian et al. [69] demonstrated that SA applications could affect metabolites associated with flavonoids and phenolic

compounds such as rutin in pepper. Similarly, Thiruvengadam et al. [70] reported that different phytohormone applications could enhance phenolic and flavonoid accumulation in Chinese cabbage.

With respect to antioxidant capacity, both DPPH and FRAP values were significantly affected by fruit maturity and SA application. For both parameters, 1 mM SA produced the highest overall mean values, while the 1 mM SA treatment at the red stage resulted in the highest values recorded in the study for DPPH (14.64 μmol TE g^{-1}) and FRAP (9.61 μmol TE g^{-1}). These results suggest that SA application at an appropriate dose may support the antioxidant capacity of pepper fruits. A relationship between total phenolic content and antioxidant activity in pepper fruits has previously been reported, with phenolic compounds potentially contributing to radical-scavenging activity [71-72]. Similarly, Medina-Juárez et al. [65] reported that higher DPPH radical-scavenging activity in C. annuum cultivars was associated with higher levels of several phenolic compounds and ascorbic acid. Hamed et al. [63] likewise observed considerable differences in phenolic compounds, flavonoids, ascorbic acid, and antioxidant activity between mature green and red/yellow C. annuum fruits, underscoring the importance of fruit maturity in determining the bioactive composition and antioxidant potential. Taken together, the concurrent increases in phenolic compounds, flavonoids, and vitamin C may have contributed to the higher antioxidant activity observed during ripening, although the present study did not establish causal relationships among these constituents and antioxidant assays.

Capsaicinoids may contribute to antioxidant capacity in pepper fruits, as capsaicin has been reported to exhibit antioxidant-related activity [73]. However, capsaicinoids were not quantified in the present study; therefore, their contribution to the DPPH and FRAP responses observed here cannot be directly assessed. Furthermore, the observed physiological responses in this study may be associated with the capacity of SA to modulate antioxidant defense systems and reactive oxygen species (ROS) metabolism in plants. Hayat et al. [74] reported that exogenous SA application at appropriate concentrations can regulate antioxidant defense systems and antioxidant enzyme activities. In a complementary study, Jiménez-Pérez et al. [75] demonstrated that vitamin C, flavonoids, and carotenoids in sweet pepper responded differently to fertilization, irrigation, and microbial biostimulant treatments depending on genotype and ripening stage. Notably, microbial biostimulant application increased specific flavonoids in certain genotype–maturity combinations, while its effects on vitamin C and carotenoids were generally limited. These findings support the view that biochemical responses to agronomic interventions are compound-specific and depend on fruit maturity and genetic background. Similarly, Sobczak-Samburska et al. [38] reported that foliar SA application can support biochemical quality and antioxidant capacity in sweet pepper fruits. Hosseini et al. [66] also showed that SA application under cold-stress conditions can positively affect vitamin C, flavonoid, and antioxidant-related properties in sweet pepper. Collectively, these findings suggest that SA may modulate biochemical and antioxidative responses in pepper under different experimental conditions.

Overall, the maturity-stage means of all biochemical and antioxidant traits evaluated in the present study increased progressively from the green to the red stage. For vitamin C, DPPH, FRAP, TPC, and TFC, the significant fruit maturity × SA interactions indicate that the effects of SA depended on fruit developmental stage. Although 1 mM SA showed the highest overall mean values for vitamin C, DPPH, FRAP, and TPC,

stage-specific responses differed among traits, and 2 mM SA produced the highest values for some traits at the brown stage. Therefore, the present findings support a maturity-dependent modulation of biochemical and antioxidant traits by SA rather than a uniform concentration-dependent response across all maturity stages.

Conclusions

This study demonstrated that foliar salicylic acid (SA) application influenced yield, physical fruit quality, and biochemical and antioxidant characteristics of chili pepper fruits in a dose- and maturity-dependent manner. Among the concentrations evaluated, 1 mM SA produced the most favorable overall response, resulting in the highest fruit yield and fruit weight, as well as higher overall mean values for vitamin C, antioxidant activity, and total phenolic content. However, the magnitude and direction of the SA response varied across maturity stages, highlighting fruit developmental stage as an important determinant of SA effectiveness.

Fruit maturation was the dominant factor shaping biochemical quality and color development. The red maturity stage exhibited the highest stage-mean values for vitamin C, total phenolic and flavonoid contents, as well as DPPH and FRAP activities, whereas dry matter progressively increased from the green to the red stage. The simultaneous increase in these bioactive compounds and antioxidant-related parameters during ripening indicates that advancing maturity substantially alters the functional quality profile of chili pepper fruits. In contrast, fruit color attributes were predominantly associated with maturity stage, with the transition from green to red characterized by a marked increase in chroma and a decline in hue. Fruit weight was highest at the brown maturity stage, indicating that the most suitable harvest stage may differ according to the quality attribute targeted.

Importantly, significant SA × maturity interactions were detected for fruit yield, fruit weight, pericarp thickness, dry matter, vitamin C, DPPH, FRAP, total phenolic content, and total flavonoid content. A significant interaction was also observed for the L color parameter.* These interactions demonstrate that the effects of foliar SA cannot be adequately characterized by its main effect alone and instead depend on the developmental stage at which fruit quality is evaluated. The particularly high DPPH and FRAP activities obtained with 1 mM SA at the red maturity stage further indicate that the combination of an appropriate SA concentration and fruit maturity stage may be associated with higher antioxidant activity. The stage-dependent response of pericarp thickness, which differed in direction between green and red maturity stages, further supports the importance of considering SA treatment and fruit maturity together.

The present study extends previous findings by demonstrating that biochemical and antioxidant responses to preharvest SA application are strongly conditioned by fruit maturity stage, emphasizing the importance of evaluating SA dose and developmental stage as interacting factors rather than as independent determinants of fruit quality. The findings indicate that the effectiveness of preharvest SA application should be interpreted in the context of fruit developmental stage rather than as a uniform dose-dependent response.

Overall, the findings identify 1 mM foliar SA as the most promising treatment among the concentrations tested for improving yield and selected biochemical quality attributes, while also demonstrating that its effects are strongly conditioned by fruit maturity. From a practical perspective, brown-stage harvest may be considered when greater fruit weight is prioritized, whereas red-stage harvest may be preferable when higher vitamin C content, antioxidant activity, and more intense red coloration are desired. Accordingly, the results suggest that combining an appropriate preharvest SA treatment with a maturity-oriented harvest strategy may provide a more targeted approach to improving the yield and functional quality of chili pepper fruits. Further studies involving repeated SA applications at different developmental stages and across pepper cultivars are needed to assess the consistency and practical applicability of these responses.

Abbreviations

ABTS 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

ANOVA Analysis of Variance

CIELAB Commission Internationale de l'Éclairage L*a*b* Colour Space.

da Decare

DAT Days After Transplanting

DPPH 2,2-diphenyl-1-picrylhydrazyl

F1 First filial generation

FAOSTAT Food and Agriculture Organization Corporate Statistical Database

FM Fruit Maturity

FRAP Ferric Reducing Antioxidant Power

GAE Gallic Acid Equivalents

JMP Statistical software developed by SAS Institute

LSD Least Significant Difference

mM Millimolar

mmol mikromol

ns Not Significant

QE Quercetin Equivalents

RCBD Randomized Complete Block Design

RH Relative Humidity

ROS Reactive Oxygen Species

SA Salicylic acid

SAS Statistical Analysis System

TE Trolox Equivalents

TFC Total Flavonoid Content

TPC Total Phenolic Content

TSS Total Soluble Solids

Declarations

Ethics approval and consent to participate

There is no ethics approval and consent to participate in this manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Supplementary Information

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Author Contributions

K.Ö.: research, methodology, conducting field trials, data organization, fruit analyses, biochemical analyses. A.U.: research, methodology, conceptualization, data organization, statistical analyses, project management, consulting, writing.

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Data availability

All datasets generated for this study are included in the article.

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