



Evaluation of Salicylic Acid's Cytotoxic and Antimicrobial Effects: Insights from *Allium cepa* Meristematic Cells, Protein Electrophoresis, and IRAP Marker

Amal Mohamed AlGarawi¹ · Jumanah Ali Al-Farraj¹ · Latifah Abdullrahman AL-Humaid¹ · Nura Alsakabi¹

Received: 1 September 2025 / Accepted: 23 January 2026 / Published online: 13 February 2026
© The Author(s) 2026

Abstract

Salicylic acid is a phenolic plant hormone widely studied for its role in enhancing tolerance to biotic and abiotic stresses, yet its concurrent cytological, antibacterial, and genetic impacts across different biological systems remain insufficiently characterized. In this study, we adopt a dual bioassay strategy (plant+bacteria) to provide an integrated assessment of salicylic acid's bioactivity and potential toxicity, thereby linking its role in stress physiology to both plant cell division and bacterial growth inhibition within a single experimental framework. The study's purpose is to evaluate the cytological, antibacterial, and genetic effects of salicylic acid on *Staphylococcus epidermidis* and *Allium cepa* root tip meristematic cells. By combining plant cytology with bacterial inhibition assays and molecular profiling (protein electrophoresis and IRAP markers) in the same study, we aim to connect visible cytological alterations with underlying genetic and proteomic variation, and to explore whether the doses that affect plant meristematic cells are also relevant for antibacterial activity. *A. cepa* root tip cells were treated with different concentrations of salicylic acid (1, 5, and 10 mM) to determine the mitotic index (MI), chromosomal aberrations, antibacterial activity, protein electrophoresis, and the inter-retrotransposon-amplified polymorphism (IRAP) markers. In *A. cepa* root tip cells, salicylic acid dramatically decreased MI; this decrease was more pronounced at higher doses and for longer exposure times. The highest percentage of chromosomal aberrations, 81.25% at 10 mM after 24 h, was seen in sticky chromosomes, lagging chromosomes, star anaphase, and bridges. According to antibacterial testing, salicylic acid inhibited *S. epidermidis*; the biggest inhibitory zone (2.367 cm) was produced at 2% concentration. Genetic fingerprinting with IRAP markers yielded 126 amplicons with 59.5% polymorphism, whereas protein analysis identified 20 peptide bands. By lowering the mitotic index and causing chromosomal aberrations in *A. cepa* root tip cells, salicylic acid has an impact on cell division. By using protein and IRAP marker analysis, it shows the considerable genetic diversity in onion root samples and exhibits antibacterial activity against *S. epidermidis*. Future studies should investigate the molecular mechanisms involved and assess the long-term effects of salicylic acid exposure to guide its use in stress management and sustainable crop production.

Keywords Salicylic acid · *Allium cepa* · Cytotoxicity · MI · Meristematic cells · *Staphylococcus epidermidis* · Protein analysis · IRAP markers

1 Introduction

Plant hormone salicylic acid was first identified as the primary component of willow bark extract, which gave rise to its name (Ding and Ding 2020). Salicylic acid was utilized as an anti-inflammatory back in antiquity (Mutlu 2019). Salicylic acid is a phenolic group compound that is found widely in plants. It plays a direct role in plant growth and heat generation, and inducing flowering in buds (Arif et al. 2020). It also affects transpiration by influencing stomata

✉ Amal Mohamed AlGarawi
Aalgarawi@ksu.edu.sa

¹ Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

movement and improves chlorophyll and carotene pigments in photosynthesis (Desoky et al. 2021). It helps plants become more resilient to many stresses, including drought and salt stress, by allowing them to survive in the shadow of these conditions (Li et al. 2022; Al-Tamimi et al. 2022; Leotta et al. 2023; Movahedi et al. 2023). In terms of helping the plant resist stresses, the roles of the hormones salicylic acid (SA) and abscisic acid (ABA) can occasionally be similar. They can also work together to help the plant resistance to abiotic stresses, which it will resist more effectively and remain healthy (Koo et al. 2020).

Crystal clear powder known as salicylic acid is used as a preservative in food and cosmetics. It is also used in skin-care products (Zamora Carballo 2022). Due to its ability to exfoliate the stratum corneum, which makes it a good peeling agent, salicylic acid has some properties that help treat a variety of skin disorders (Calvisi 2021a). Because it disrupts cellular connections rather than breaking or decomposing keratin filaments between cells, salicylic acid is beneficial for acne patients (Kim et al. 2018). When applied topically to the skin, salicylic acid absorbs quickly and can be found in the urine in about a day. Salicylic acid is used on skin that is irritated or red. Although cutaneous absorption rarely causes systemic toxicity, elevated concentrations could be a serious risk (Calvisi 2021b).

Apart from its exfoliating properties, it acts as a topical antibacterial agent. Many drugs, such as aspirin, which relieves pain and fever, are derived from salicylic acid and its derivatives (Elshafie et al. 2023). Although salicylic acid is safe in moderation and in permissible dosages, an overdose of it or its derivatives can cause symptoms that manifest in three to eight hours, depending on the amount consumed (Farkouh et al. 2022). Twelve to twenty-four hours after ingestion, salicylate levels above 100 mg/dL are extremely toxic and can cause cardiac arrest (Runde and Nappe 2021).

The mitotic index, representing the proportion of cells undergoing mitosis in a given cell population, serves as an essential indicator of cell proliferation and overall plant growth. Research indicates that salicylic acid's impact on the mitotic index is concentration-dependent. Low concentrations of salicylic acid have been observed to promote plant growth under unfavourable conditions, whereas high concentrations tend to inhibit growth (Pasternak et al. 2019). For instance, in *Arabidopsis thaliana*, exogenous application of salicylic acid proportionally reduced primary root elongation and specifically induced root waving in a dose-dependent manner (Pasternak et al. 2019). In studies involving *A. cepa* (onion) root meristems, salicylic acid treatment resulted in a decrease in the mitotic index, suggesting an inhibitory effect on cell division (Çavuşoğlu et al. 2022). Similarly, in barley (*Hordeum vulgare*) apical meristems,

salicylic acid application led to a reduction in the mitotic index and an increase in chromosomal abnormalities (Keya et al. 2023). These findings highlight salicylic acid's potential to modulate cell division, with effects varying based on concentration and plant species.

Salicylic acid induces chromosomal abnormalities by generating oxidative stress, disrupting spindle formation, and interfering with chromatin condensation (Jeong et al. 2023). It causes sticky chromosomes, C-metaphase, star-shaped metaphase, anaphase bridges, and lagging chromosomes by affecting microtubule dynamics and cell cycle checkpoints (Porubsky et al. 2022). These abnormalities reflect genetic instability, leading to potential DNA damage and loss of genetic material.

Despite extensive studies on salicylic acid's physiological and stress-related roles, there is a clear lack of integrated research simultaneously investigating its cytogenetic effects on meristematic cells, its impact on protein expression patterns using SDS-PAGE, and its influence on genomic stability using IRAP molecular markers. Moreover, the cross-kingdom biological effects of salicylic acid on both plant cells and pathogenic bacteria remain insufficiently explored.

The salicylic acid provides an adaptive response to cytotoxicity stress when cells are exposed to pesticides, it examined the effect of salicylic acid at a concentration of 0.01 mM for 6 and 12 h on the roots of the *Vicia faba* L bean plant (Dawood et al. 2022). Salicylic acid was applied to the growing tops of onion roots (*Allium cepa*) at a concentration of (10–4 mol/L) for 24 h in a study carried out in Russia to determine the impact of microbiological components on abnormalities rates and cytotoxicity (Sabeen et al. 2020). The impact of jasmine and salicylic acids on cell division in the root tops of *A. cepa* onion plants was investigated by Naheed et al., (Naheed et al. 2022). The following concentrations (1.25, 2.5, 5, 10, 20 mM) were used during the following times (6, 12, 24 h); and the division rate significantly decreases, particularly when the roots are treated in high concentrations. The salicylic acid was also applied to the roots of the *Nigella sativa* L plant for six and twelve hours to study how the plant's adaptation to cadmium toxicity affected the cells. It was observed that these low concentrations of salicylic acid protected the cells from abiotic stress and reduced the percentage of abnormality (El-Ghamery and Mousa 2018).

The bacterial population of the skin is naturally diverse. One of the most common of these microorganisms is the common Gram-positive bacterium *S. epidermidis* (Oliveira et al. 2018). *S. epidermidis* is widely used in antimicrobial studies due to its clinical relevance as an opportunistic pathogen, particularly in hospital-acquired infections. It is known for its strong biofilm-forming ability on medical

implants and increasing antibiotic resistance, making it crucial for evaluating new antimicrobial agents (Leja et al. 2019). Additionally, it serves as a comparative model alongside *Staphylococcus aureus* in infection studies (Heilmann et al. 2019). *S. epidermidis* is more than just a passive resident of the skin. It can become infectious once it enters the human host. It can lead to serious consequences and is one of the most common causes of hospital-acquired infections in the United States. The salicylic acid affects the bacterial population of the skin. The composition of the microbial communities in the skin of patients was affected by treatment with 2% salicylic acid (supramolecular salicylic acid), which also resulted in a decrease in *Staphylococcus* from 29% before treatment to 17% after treatment, according to results published in Bilal et al. (Bilal et al. 2023).

Protein electrophoresis is a method that applies an electric field to a gel matrix to separate proteins according to their size, charge, and other characteristics. In molecular biology, the method is frequently employed for diagnostics, genetic diversity research, and protein identification (Alafari and Abd-Elgawad 2021a). It is useful in breeding programs to increase resilience and productivity since it aids in the assessment of genetic variation, stress responses, and developmental changes in plant research (Alafari and Abd-Elgawad 2021b; Alotaibi and Abd-Elgawad 2023).

In molecular genetics, the inter-retrotransposon-amplified polymorphism (IRAP) markers are an essential tool for scientists to evaluate genetic variation among various species. They provide a comprehensive picture of genetic variation by amplifying areas flanked by primers specific to retrotransposons. The IRAP markers are ideal for genetic diversity studies as they detect highly polymorphic and stable retrotransposon insertions (AlGarawi and Abd-Elgawad 2025; Kalendar et al. 2021). They require no prior sequence data, making them cost-effective and widely applicable (Nadeem et al. 2018). The IRAP is especially useful for distinguishing closely related species, assessing evolutionary relationships, and analyzing genetic variation in plant populations (Zanganeh and Sheidai 2022). The IRAP markers, which provide reliability and effectiveness in detecting polymorphisms, have been successfully used in investigations of plant genetic variation (Dongare et al. 2023). Conservation genetics, evolutionary studies, and plant breeding programs are becoming more significant (Kalendar et al. 2021). Particularly for big or complicated genomes, they are economical for genetic connection research and DNA fingerprinting (Cheraghi et al. 2018). The genetic diversity of *A. cepa* root samples treated with salicylic acid was evaluated in this work using IRAP markers, which showed notable genetic variety and polymorphism.

A. cepa is among the best plants for detecting various chemical effects and materials for microbionics, the *Allium*

test allows one to predict potential damage to human DNA by determining how a substance affects genetic material (Pharmawati and Wrsiati 2023). It has been demonstrated that testing onion roots is a sensitive method for regulating genotoxicity in food extracts (Alias et al. 2023). This study provides an integrated evaluation of salicylic acid's biological effects at multiple levels—cytogenetic, biochemical, molecular, and antimicrobial. Using *A. cepa* as a model system, we demonstrate how salicylic acid influences chromosomal behavior, protein expression, and DNA stability, and we show that salicylic acid possesses dose-dependent antibacterial activity. These findings are relevant for understanding the broader biological activity of salicylic acid and for supporting its potential applications in agricultural biotechnology and natural antimicrobial development. The primary objective of this study was to evaluate the effects of different concentrations of salicylic acid on the cytogenetic behavior, protein expression patterns, and DNA integrity in *A. cepa* root meristems, along with an assessment of the antimicrobial potential of salicylic acid against *S. epidermidis*.

1. Does salicylic acid reduce the mitotic index and induce chromosomal abnormalities in *A. cepa* root meristematic cells?
2. Does salicylic acid alter protein expression profiles as detected by SDS-PAGE electrophoresis?
3. Does salicylic acid induce polymorphism in IRAP markers, indicating genomic instability?
4. Does salicylic acid exhibit dose-dependent antibacterial activity against *Staphylococcus epidermidis*?

We hypothesize that exposure to different concentrations of salicylic acid will induce measurable cytogenetic, biochemical, and molecular changes in *Allium cepa* root meristematic cells. Specifically, salicylic acid is expected to (i) reduce the mitotic index and increase chromosomal abnormalities, (ii) alter protein expression profiles as detected by SDS-PAGE electrophoresis, and (iii) induce polymorphism in IRAP markers, indicating changes in genomic stability. Additionally, salicylic acid is hypothesized to exhibit dose-dependent antibacterial activity against *Staphylococcus epidermidis*.

2 Materials and Methods

2.1 Plant Material and Treatment with Salicylic Acid

Medium-sized red onion (*Allium cepa*) bulbs were used for cytological analysis. To promote root development, dry roots were carefully removed, and the bulbs were placed in 250 mL glass beakers filled with tap water and maintained at

room temperature for 24 h until new roots reached approximately 1.5–2 cm in length.

Salicylic acid solutions were prepared at concentrations of 1, 5, and 10 mM by dissolving salicylic acid powder in distilled water using gentle heating and continuous stirring to ensure complete solubility at each concentration. Each treatment group, as well as the control group (kept in distilled water), included three replicates, with five bulbs per replicate. Bulbs were randomly assigned to control and treatment groups using a random number table to avoid allocation bias. In this study, each replicate consisted of an independent set of bulbs treated in separate experimental runs on different days and was therefore considered a biological replicate; within each replicate, measurements taken from multiple roots per bulb were treated as technical replicates. Cytological preparations were coded by a second person so that the observer scoring the mitotic index and chromosomal abnormalities was blinded to the treatment group. The bulbs were exposed to the salicylic acid solutions for 12 and 24 h, after which the roots were rinsed thoroughly with deionized water to remove any residue. This integrated methodological framework was selected and structured to directly address the central research objective by linking each experimental step to a defined aspect of the overarching hypothesis.

2.2 Cytological Analysis Using Light Microscopy

From each replicate, 5–7 root tips (1.5–2 cm) were collected for microscopic analysis. The root tips were fixed in Carnoy's fixative solution (ethanol: acetic acid, 3:1, v/v) for 24 h. Samples were then preserved in 70% ethanol at 4 °C until staining. Prior to staining, root tips were rinsed with distilled water to eliminate ethanol. They were hydrolyzed in 1 N HCl in a water bath at 60 °C for 15 min, then washed with distilled water. Root tips were stained using acetocarmine stain and incubated in the dark at room temperature for 15–20 min.

Each root tip was squashed on a glass slide, covered with a coverslip, and gently pressed to spread the cells. Slides were examined under a light microscope at 400× magnification. All assessments were carried out in three independent replicates to ensure accuracy and reproducibility.

For each treatment and time point, a minimum of 1,000 cells were scored per replicate (totalling 3,000 cells per treatment) to determine:

$$\text{Mitotic Index} = \frac{\text{Number of Cells in Mitosis}}{\text{Total Number of Cells}} \times 100$$

Then calculate the abnormality rate:

$$\text{Total of Abnormality Rate} = \frac{\text{Number of Abnormality Cells}}{\text{Number of Cells in Mitosis}} \times 100$$

2.3 Application of Salicylic Acid Against *S. epidermidis*

Salicylic acid solutions at concentrations of 1, 1.5, 2, and 5 mM were prepared by dissolving salicylic acid powder in distilled water with the addition of 10% of 95% ethanol to improve solubility. These concentrations were selected and expressed as % (w/v) in accordance with common dermatological and antimicrobial formulations of salicylic acid, and because preliminary tests using mM-range solutions did not produce measurable inhibition zones against *S. epidermidis*.

An additional solvent control containing distilled water with 10% of 95% ethanol but no salicylic acid was included in all antibacterial assays to account for any inhibitory effect of ethanol alone. The solvent control showed no inhibitory zones, confirming that ethanol had no antibacterial effect. The bacterium *S. epidermidis* was cultured on Mueller-Hinton Agar (MHA) plates and incubated at 37 °C for 24 h. After incubation, wells were created in the agar using a sterile cork borer, and 100 µL of each salicylic acid solution was added to the wells.

Each concentration and control was tested in three independent replicates. The plates were incubated at 37 °C for 24 h, and the zones of inhibition (in mm) around each well were measured to assess antibacterial activity.

2.4 Protein Extraction and Analysis

We isolated soluble proteins from onion plant's roots on germination's. Roots were weighed, homogenized twice with 5 ml of 0.5 M NaCl, and centrifuged at 10,000 rpm for 10 min. The clear supernatant, including salt-soluble proteins, was collected in a new Falcon tube.

Total protein concentration in each extract was determined by the Bradford assay using bovine serum albumin as a standard, and equal amounts of protein (per lane) were loaded onto the gels to allow comparison of band patterns and intensities. Protein profiling by SDS-PAGE was employed to detect salicylic acid-induced changes in root protein expression that could be associated with the cytogenetic alterations observed in *A. cepa* meristematic cells and to provide a biochemical layer complementing the cytological and IRAP-based genetic analyses. Using a discontinuous buffer system consisting of 4% stacking gel and 12% separating gels, SDS-polyacrylamide gel electrophoresis was used for electrophoretic separations of salt-soluble proteins (Alafari and Abd-Elgawad 2021b). Tris/glycine (1 M, pH 8.3) was used as an electrode buffer for the electrophoresis, which was conducted at 15 °C for for around an hour with a steady current of 10 mA/gel from the stacking gel and 15 mA/gel from the separating gel. After the electrophoretic assembly was removed, gels were stained for approximately

2 h with 0.05% Coomassie Brilliant Blue R250. They were then destained with a standard methanol–acetic acid solution until clear band patterns were obtained, and gels were photographed for subsequent analysis.

2.5 Extraction and Purification of Genomic Deoxyribonucleic Acid (DNA)

The DNA was extracted from four samples onion root by DNeasy Plant Mini Kit (Qiagen Santa Clarita, CA), this was performed following the manufacturer's instruction. The DNA quality and quantity were detected using both NANO-DROP 2000 (Thermo Scientific, USA) and a 1% agarose gel (ethidium bromide staining).

2.6 The Inter-Retrotransposon Amplified Polymorphism (IRAP) Analysis

a) The Polymerase Chain Reaction for Inter-Retrotransposon Amplified Polymorphism (IRAP-PCR) Reactions Eleven primers IRAP were used in the detection of polymorphism (Table 1). Genomic DNA was isolated from *A. cepa* root tips using a standard CTAB protocol, and DNA quality and concentration were assessed by spectrophotometry (A260/A280) and 1% agarose gel electrophoresis before PCR. The eleven IRAP primers were synthesized by a commercial supplier and selected based on their reported polymorphism and reliability in *Allium* and related species. For each primer, a no-template control (NTC) containing all PCR components except DNA was included in every run to monitor potential contamination and nonspecific amplification. To assess reproducibility, a subset of samples was amplified in at least two independent PCR runs per primer, and only clear bands that appeared consistently in replicate runs were scored; IRAP profiles were then converted to

a binary matrix (1 = presence, 0 = absence) for polymorphism and genetic similarity analyses. The amplification reaction was carried out in a 20 μ L reaction volume containing 10 μ L Master Mix (sigma), 2.5 μ L primer (10 pmol), 2.5 μ L template DNA (10 ng), and 5 μ L dH₂O, according to (Badr et al. 2020).

b) Thermocycling Profile Polymerase Chain Reaction (PCR) The PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94 °C. Each cycle consisted of a denaturation step at 94 °C for 45 s, an annealing step at 47 °C for 50 s, and an elongation step at 72 °C for 1 min. The primer extension segment was extended to 7 min at 72 °C in the final cycle.

c) Detection of the PCR Products The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 μ g/mL) in 1X TBE buffer at 95 volts. The PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

2.7 Data Analysis

The DNA banding patterns generated from the IRAP reaction were analyzed by the Gel Analyzer 3 program, where bands were scored as (1) for the presence or (0) for absence. For each primer, the percentage of polymorphism was calculated by dividing the number of polymorphic bands by the total number of scored bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated among the different genotypes, and these coefficients were used in an unweighted pair group method with arithmetic mean (UPGMA) clustering to construct a phylogenetic tree

Table 1 List of IRAP primers, sequences, annealing temperatures, and banding characteristics used in the molecular analysis of *A. cepa* root DNA

Primer Name	Primer sequence (5'→3')	Annealing temperatures	TB	MB	PB	% P	F
IRAP-01	TTAGACCCGGAACCGCCGTG	47 °C	13	2	11	85	0.58
IRAP-02	ATCCTTCGCGTAGATCAAGCGCCA	48 °C	9	5	4	44	0.78
IRAP-03	GAAGTACCGATTACTTCCGTGTA	46 °C	13	1	12	92	0.46
IRAP-04	ATGTGACAGTCGACTAACCAC	49 °C	10	6	4	40	0.75
IRAP-05	TGGCGCTTGATCTACGCGAAGGA	45 °C	12	6	6	50	0.71
IRAP-06	AACTTGATCCAGATCATCTCC	47 °C	10	5	5	50	0.75
IRAP-07	CCATGGCGAGCAGATGTGCT	47 °C	14	4	10	71	0.64
IRAP-08	ATGCCGTATTCTCAGCATCC	48 °C	12	4	8	67	0.71
IRAP-09	CAGGCAAGAATGAGCGTCTC	46 °C	9	6	3	33	0.86
IRAP-10	ATGGTTGTCGAAACTCCAGC	47 °C	15	6	9	60	0.68
IRAP-11	CTTGCTGAAAAGTGTGTGAGAGG	47 °C	9	6	3	33	0.78
Total			126	51	75	-	-
Average			11.5	4.7	6.8	56.8	0.70

TB, Total bands; MB, monomorphic bands; PB, polymorphic bands; %P, percentage polymorphism; F, band frequency

(dendrogram) in PAST software Version 1.91, according to the Euclidean similarity index (Hammer and Harper 2024). The PIC value for each primer was calculated according to <http://irscope.shinyapps.io/iMEC/> (Amiryousefi et al. 2018).

Statistical analyses were performed using Python (version 3.12.4). Data are presented as mean \pm standard error of the mean (SE). Differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test to assess pairwise differences. Groups that do not share a common letter are considered significantly different at $p < 0.05$, whereas groups sharing at least one letter are not significantly different. The significance threshold was set at $\alpha = 0.05$ for all tests.

3 Results

3.1 Cytological Effects of Salicylic Acid on *A. cepa* Root Meristem Cells

The cytological analysis of *A. cepa* root tip meristematic cells treated with salicylic acid revealed concentration- and time-dependent changes in mitotic activity and chromosomal integrity. Tables 2 and 3 summarize the mitotic index (MI) and chromosomal abnormality rates at 12 and 24 h.

A significant reduction in the MI was observed with increasing salicylic acid concentrations. Although treatment duration had a limited effect, a notable decrease in MI occurred, particularly at higher concentrations. For instance, at 1 mM salicylic acid, the MI dropped from 12% at 12 h to 8.66% at 24 h, indicating a delayed but significant inhibitory effect on cell division (Table 2).

Chromosomal abnormalities increased proportionally with both salicylic acid concentration and exposure time. The highest abnormality rate (81.25%) was recorded at 10 mM salicylic acid after 24 h, while the lowest (50%) occurred at 1 mM salicylic acid after 12 h (Table 3).

Microscopic examination revealed various chromosomal abnormalities (Figs. 1a–m). Common aberrations included sticky chromosomes observed in multiple mitotic stages. Prophase showed hollow nuclei, despiralization, and chromatin budding. Metaphase exhibited C-metaphase figures and star-shaped chromosomes. Anaphase displayed lagging chromosomes, star-shaped figures, and chromatin bridges, suggesting genetic instability and possible DNA fragmentation. Multiple bridges were often observed, which may lead to micronucleus formation. Telophase exhibited fewer abnormalities.

3.2 Antibacterial Activity of Salicylic Acid

The antibacterial effect of four salicylic acid concentrations (1, 1.5, 2, and 5 mM) was tested against *S. epidermidis*. Distilled water, used as a negative control, showed no inhibition (0%). The solvent control (distilled water with 10% of 95% ethanol) showed no detectable inhibition zones against *S. epidermidis*, confirming that the observed antibacterial activity was attributable solely to salicylic acid. The 2 mM salicylic acid concentration produced the largest average inhibition zone (2.367 cm), while the smallest zone was observed with 1.5 mM salicylic acid (2.167 cm). The inhibition zones at 1- and 5-mM salicylic acid were 2.250 cm and 2.267 cm, respectively (Figs. 2 and 3, and Tables 4 and 5).

Table 2 Effect of salicylic acid (SA) treatments on mitotic activity in root tip cells at 12 h and 24 h, expressed as total and dividing cells and mean mitotic index (MI \pm SE)

Treatments Time	Total cells observed		Dividing cells		Mean of Mitotic Index (MI) \pm SE	
	12 h	24 h	12 h	24 h	12 h	24 h
1-Control (0 mM SA)	300	300	43	39	14.33 \pm 0.11a	13.00 \pm 0.10a
2-1 mM SA	300	300	36	26	12.00 \pm 0.10b	8.66 \pm 0.09b
3-5 mM SA	300	300	19	18	6.33 \pm 0.09c	6.00 \pm 0.09c
4-10 mM SA	300	300	17	16	5.66 \pm 0.08c	5.00 \pm 0.08c

Treatments with different letters within the same column differ significantly (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$)

Table 3 Effect of salicylic acid (SA) treatments on abnormality rate (mean \pm SE) and distribution of mitotic phases in root tip cells at 12 h and 24 h

Treatments Time	Prophase %		Metaphase %		Anaphase %		Telophase %		Abnormality Rate (%) \pm SE	
	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
1- Control (0 mM SA)	11.62	20.51	11.62	7.69	13.95	7.69	6.97	7.69	44.18 \pm 0.12a	43.58 \pm 0.09a
2-1 mM SA	22.22	30.76	16.66	11.53	5.55	11.53	5.55	11.53	50.00 \pm 0.27a	65.38 \pm 0.09b
3-5 mM SA	21.05	22.22	21.05	27.77	15.78	16.66	10.52	16.66	68.42 \pm 0.09b	83.33 \pm 0.09c
4-10 mM SA	23.52	18.75	17.64	31.25	23.52	18.75	11.76	12.5	76.47 \pm 0.12b	81.25 \pm 0.10c

Within each column, values with different letters differ significantly (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$)

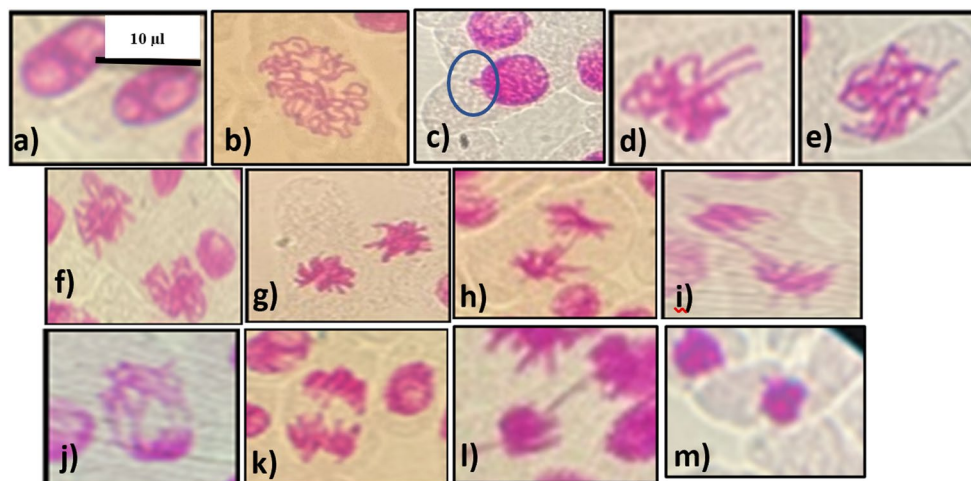


Fig. 1 Mitotic chromosomal abnormalities observed in *A. cepa* root tip cells after salicylic acid (SA) treatment: **a** Binucleolar in Interphase at 5 mM. **b** Despiralization prophase at 10 mM. **c** Nuclear bud in prophase at 1 mM. **d** Sticky metaphase at 5 mM. **e** Disturbed metaphase at 1 mM. **f** Star anaphase at 1 mM. **g** Sticky star anaphase at 10 mM.

h Multiple bridge in anaphase 5 mM. **i** Multipolar with single bridge in anaphase 10 mM. **j** Unoriented chromosome and bridge in anaphase 5 mM. **k** Multipolar and unoriented chromosome with bridge at anaphase 10 mM. **l** Sticky and bridge in anaphase with lagging chromosome 10 mM. **m** Sticky telophase at 5 mM salicylic acid

3.3 Protein Profiling by SDS-PAGE

Protein electrophoresis of *A. cepa* root samples revealed 20 peptide bands ranging in molecular weight from 18 to 124 kDa (Table 6). Sixteen bands were common across treatments and controls. Specific bands disappeared in response to salicylic acid treatment: the 110 kDa band was absent at 1 and 5 mM; the 74 and 60 kDa bands at 10 mM; and the 43 kDa band under various treatments, indicating protein expression changes linked to salicylic acid concentration.

Euclidean similarity indices calculated from protein banding patterns showed the highest similarity (97%) between the control, 1 mM, and 5 mM treatments, while the lowest similarity (91%) was observed between 5 mM and 10 mM treatments. A UPGMA dendrogram (Fig. 4) grouped the control, 1 mM, and 5 mM salicylic acid into one cluster, with 1 mM salicylic acid forming a sub-branch. The 10 mM treatment formed a distinct cluster, suggesting greater variation in protein expression at higher salicylic acid concentrations.

3.4 The IRAP Molecular Marker Analysis

The IRAP analysis using 11 primers yielded 126 bands, of which 75 were polymorphic, indicating 59.5% polymorphism (Fig. 5, and Table 6). Polymorphic bands per primer ranged from 3 (IRAP-09, IRAP-11) to 12 (IRAP-03), with total band numbers ranging from 9 to 15. An average of 6.8 polymorphic bands per primer was recorded.

Genetic similarity based on IRAP polymorphism revealed the highest similarity (82%) between the 1 mM, 5 mM, and 10 mM treatments. The lowest similarity (68%) occurred

between the control and 1 mM salicylic acid. A UPGMA dendrogram (Fig. 6, and Table 7) grouped the control, 1 mM, and 10 mM into one cluster, while 5 mM salicylic acid formed a separate sub-branch, reflecting distinct molecular responses at different concentrations.

4 Discussion

This study evaluated the cytogenetic, protein expression, and molecular marker responses of *A. cepa* root meristematic cells to various concentrations of salicylic acid, and separately assessed the antibacterial activity of salicylic acid itself. The results revealed a clear concentration-dependent cytotoxic and genotoxic effect of salicylic acid, with significant suppression of mitotic activity, increased chromosomal abnormalities, and alterations in protein and genomic profiles.

The *Allium* test remains a robust plant bioassay for evaluating chromosomal aberrations and chemically induced genotoxicity (Bonciu et al. 2018). The findings demonstrate that salicylic acid significantly reduced the mitotic index (MI), particularly at higher concentrations (5 and 10 mM). Although treatment duration (12 vs. 24 h) alone did not drastically affect MI, a notable reduction was observed at 10 mM salicylic acid over 24 h (MI dropped from 12% to 8.66%). These results are consistent with previous reports indicating that salicylic acid inhibits cell division by interfering with mitotic spindle formation and cell cycle progression (Kamal et al. 2021; Pellestor et al. 2022).

Salicylic acid also induced diverse chromosomal abnormalities in a dose-dependent manner. Sticky chromosomes

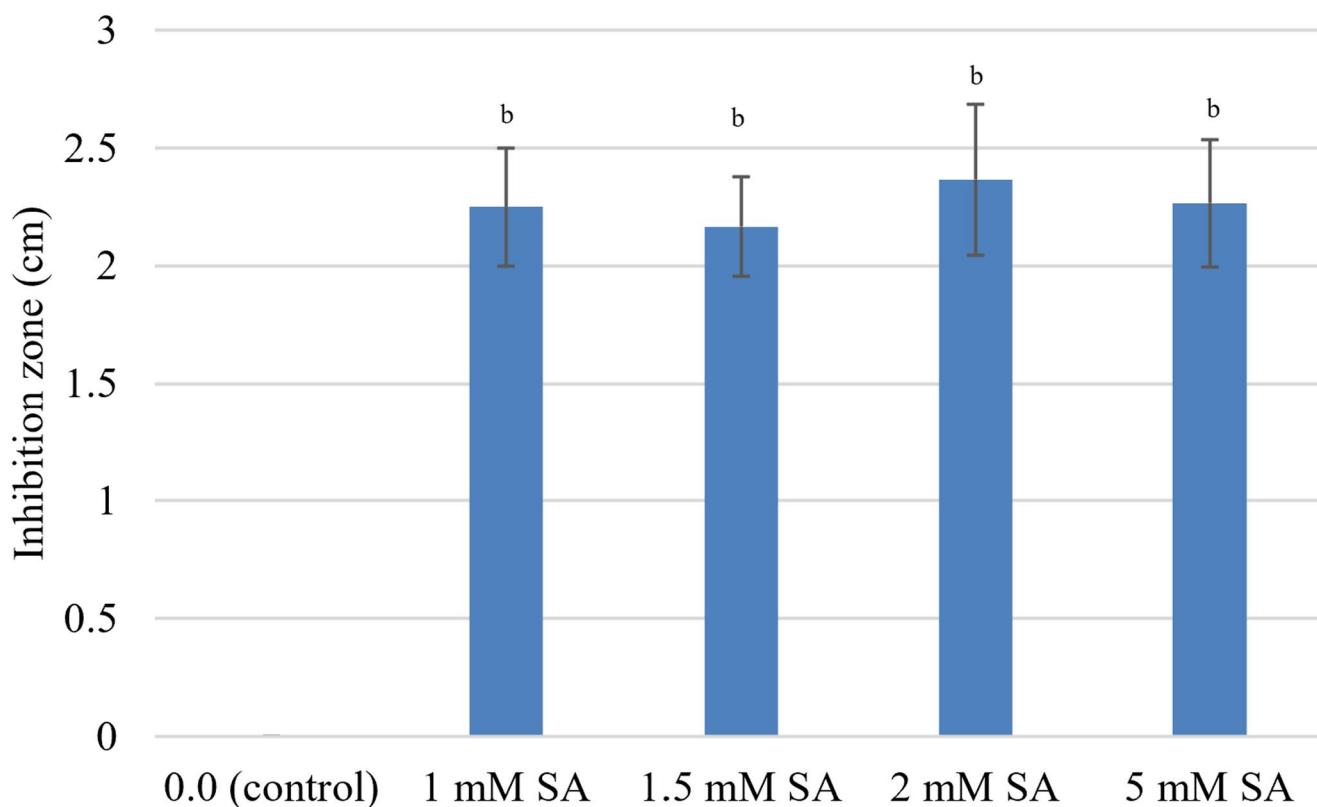


Fig. 2 Effect of salicylic acid (SA) concentration on inhibition zone diameter (mean ± SE). Values with different letters differ significantly within the column (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$)

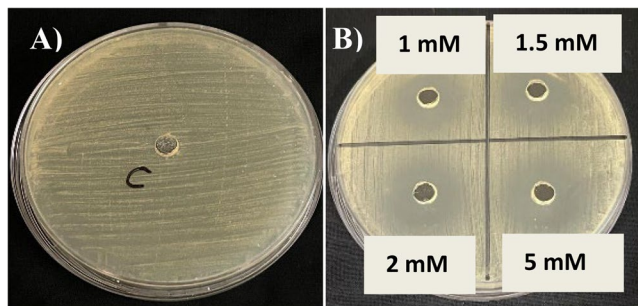


Fig. 3 Effect of salicylic acid on *S. epidermidis* growth on nutrient agar plates. **A** Control (distilled water) and **B** salicylic acid treatments

Table 4 Effect of salicylic acid (SA) concentration on inhibition zone diameter (mean ± SE)

Salicylic acid concentrations	Inhibition Zone ± SE
0.0 (control)	0.000 ± 0.00a
1 mM SA	2.250 ± 0.25b
1.5 mM SA	2.167 ± 0.21b
2 mM SA	2.367 ± 0.32b
5 mM SA	2.267 ± 0.27b

Values with different letters differ significantly within the column (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$)

Table 5 Effect of salicylic acid (SA) concentration on inhibition zone diameter (cm) in replicate plates (mean ± SE)

Salicylic acid concentrations	Plates				Mean Inhibition Zone (cm) ± SE
	1	2	3	4	
0.0 (control)	-	-	-	0.0 cm	0.000 ± 0.00a
1 mM SA	2.3 cm	1.95 cm	2.5 cm	-	2.250 ± 0.16b
1.5 mM SA	2.25 cm	2 cm	2.25 cm	-	2.167 ± 0.08b
2 mM SA	2.4 cm	2.4 cm	2.3 cm	-	2.367 ± 0.03b
5 mM SA	2.65 cm	1.85 cm	2.3 cm	-	2.267 ± 0.23b

Values with different letters differ significantly within the column (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$)

were prevalent at all mitotic stages, while specific aberrations such as lagging chromosomes, chromatin bridges, C-metaphase, star metaphase, and micronuclei were most pronounced during anaphase and metaphase. These anomalies reflect mitotic spindle disturbances, chromatin fragmentation, and genetic instability (Dawood and Latif 2023; Jangra et al. 2022; Pharmawati and Wrsiati 2023). Notably, bridges and lagging chromosomes often co-occurred, suggesting a risk of DNA breakage and micronucleus formation, which could lead to chromosomal loss or rearrangements (Krupina et al. 2021; Pellestor et al. 2022).

Table 6 Electrophoretic protein banding patterns in *A. cepa* roots exposed to different concentrations of salicylic acid

Band MW (kDa)	1– control	2– 1 mM	3– 5 mM	4– 10 mM	Band frequency
124	1	1	1	1	1.0
116	1	1	1	1	1.0
110	1	0	1	0	0.5
102	1	1	1	1	1.0
86	1	1	1	1	1.0
82	1	1	1	1	1.0
78	1	1	1	1	1.0
74	1	1	1	0	0.8
60	1	1	1	0	0.8
54	1	1	1	1	1.0
51	1	1	1	1	1.0
47	1	1	1	1	1.0
44	1	1	1	1	1.0
43	1	0	0	0	0.3
38	1	1	1	1	1.0
35	1	1	1	1	1.0
30	1	1	1	1	1.0
26	1	1	1	1	1.0
21	1	1	1	1	1.0
18	1	1	1	1	1.0

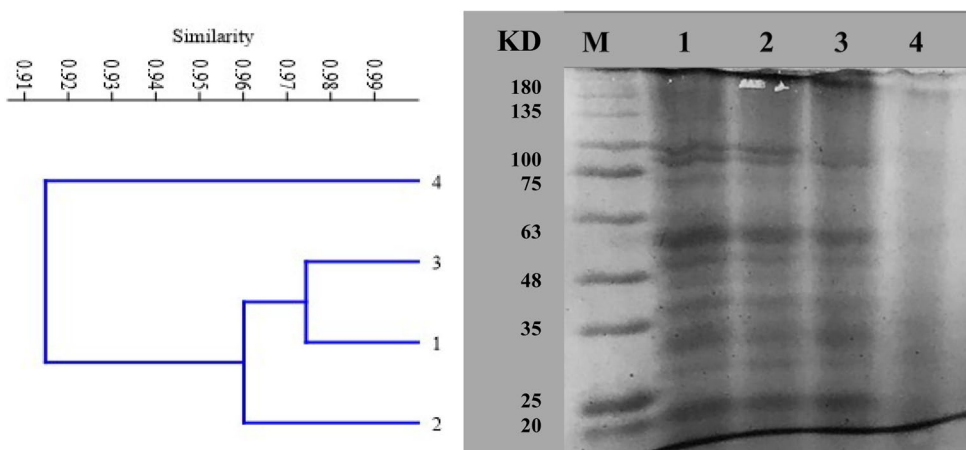
Interestingly, a minor level of chromosomal abnormalities was observed in the control group, likely due to prior exposure of market-sourced onions to agrochemicals or environmental contaminants. Nonetheless, the marked increase in aberrations following salicylic acid treatment confirms its genotoxic potential (Kamal et al. 2021).

Salicylic acid is widely recognized as a central hormone involved in plant defence and developmental regulation (Chen et al. 2021; Zhang YueLin and Li Xin 2019). At the cellular level, it modulates root architecture by suppressing cell division in the root apical meristem (RAM) through downregulation of CYCLIN B1;1 and enlargement of meristematic cells, as observed in *Arabidopsis* and rice mutants (Bagautdinova et al. 2022; Zhou et al. 2021). The findings

of reduced MI and aberrant mitotic structures align with this mode of action and suggest that high salicylic acid concentrations may disrupt cell cycle regulation and reactive oxygen species (ROS) homeostasis in root cells.

The antibacterial assay revealed that while all salicylic acid concentrations inhibited the growth of *S. epidermidis*, the size of the inhibition zones did not increase linearly with concentration. The 2- and 5-mM treatments produced the largest zones (~2.36 cm), while 1 and 1.5 mM had slightly smaller, yet comparable effects. These results indicate that salicylic acid has dose-dependent inhibitory activity against *S. epidermidis* under the tested conditions. The antibacterial activity of salicylic acid against *S. epidermidis* in the present study was assessed only by the agar well diffusion (zone of inhibition) method, and no broth-based MIC or MBC assays were performed, which limits the quantitative interpretation of its antimicrobial potency; future studies should therefore include standardized MIC/MBC determinations to corroborate and extend these preliminary findings. These findings agree with previous studies indicating that salicylic acid exhibits antimicrobial activity at moderate concentrations but may plateau or decline at higher levels due to potential saturation effects or acid-induced stress on bacterial membranes (Parvekar et al. 2020). The 1.5 mM salicylic acid concentration, though slightly less effective than 2 mM, still demonstrated substantial activity and may represent a safer alternative for applications requiring lower toxicity.

Protein electrophoresis revealed both monomorphic and polymorphic bands, reflecting salicylic acid-induced changes in protein expression. The 10 mM treatment resulted in the disappearance of several protein bands, including those at 43, 60, 74, and 110 kDa, indicating repression of specific proteins at higher concentrations. The UPGMA dendrogram grouped control, 1 mM, and 5 mM treatments closely, while 10 mM formed a distinct branch, suggesting that salicylic acid at high doses alters the proteomic profile of *A. cepa* roots significantly. This agrees with prior work showing that salicylic acid can modulate stress-responsive proteins and

Fig. 4 Dendrogram showing the genetic similarity of *A. cepa* roots under salicylic acid treatments using UPGMA analysis. 1; control; 2; 1 mM, 3; 5 mM, 4; 10 mM salicylic acid

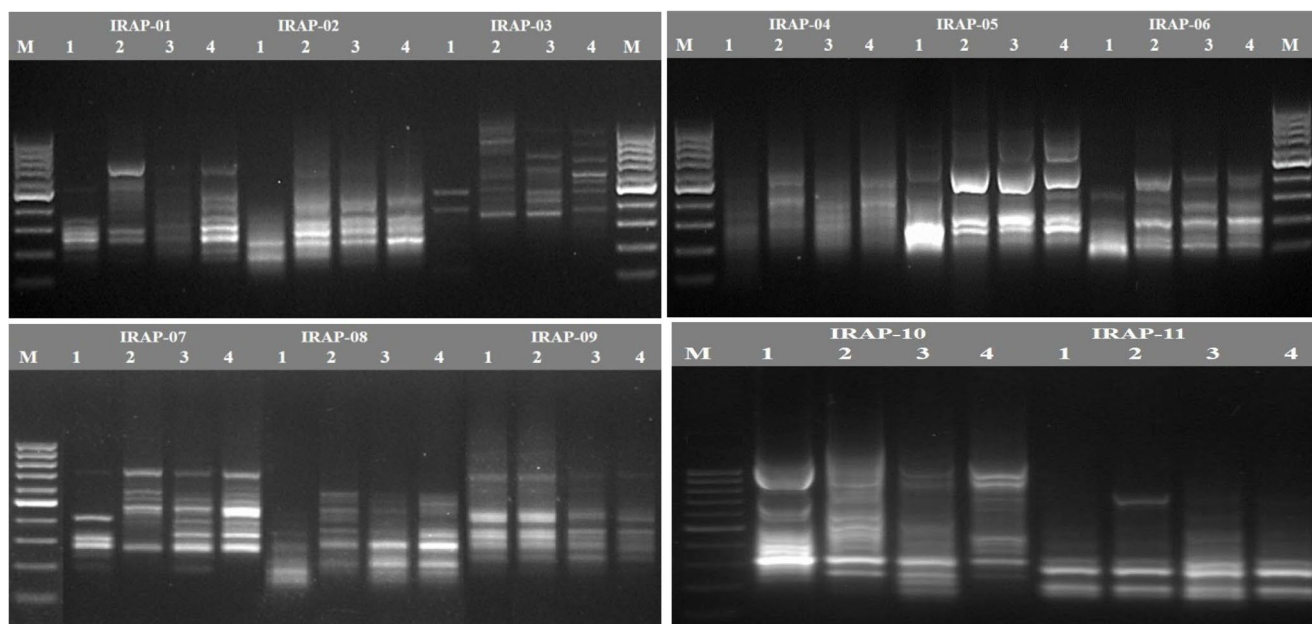


Fig. 5 IRAP profiling of *A. cepa* roots treated with salicylic acid. 1; control; 2; 1 mM, 3; 5 mM, 4; 10 mM salicylic acid

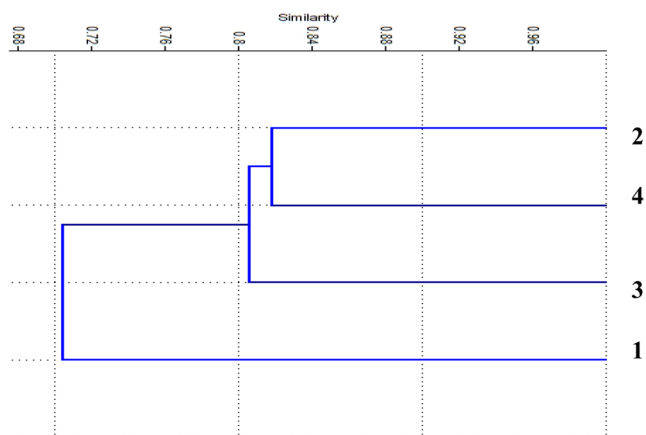


Fig. 6 Dendrogram showing genetic relationships among *A. cepa* roots under salicylic acid stress based on IRAP markers. 1; control; 2; 1 mM, 3; 5 mM, 4; 10 mM salicylic acid

Table 7 Unweighted pair group method with arithmetic mean (UPGMA) dendrogram illustrating the genetic relationships among *A. cepa* root samples treated with different concentrations of salicylic acid based on IRAP marker analysis

	1– control	2– 1 mM	3– 5 mM	4– 10 mM
1– control	100			
2– 1 mM	68	100		
3– 5 mM	74	80	100	
4– 10 mM	69	82	82	100

gene expression involved in growth, defence, and metabolism (Alafari and Abd-Elgawad 2021b; Alotaibi and Abd-Elgawad 2023; Boorboori and Li 2024; Fu et al. 2024).

Inter-Retrotransposon Amplified Polymorphism (IRAP) marker analysis further confirmed salicylic acid-induced genetic variability. Out of 126 bands, 59.5% were polymorphic, with the highest number of polymorphic amplicons detected using IRAP-03. The high polymorphism rate demonstrates the sensitivity of IRAP markers in detecting genomic changes due to chemical stress, consistent with findings in other plant species (Bidyananda et al. 2024; Kalendar et al. 2021). Notably, the lowest genetic similarity (68%) was found between control and 1 mM salicylic acid, while the highest (82%) was shared among 1-, 5-, and 10-mM treatments. The clustering pattern differed from that observed in protein profiling, which grouped 1, 2, and 3 together, while IRAP clustered 1, 2, and 4. This discrepancy highlights the complementary nature of protein-based and DNA-based markers: the former reflects gene expression and environmental influence, while the latter captures stable genomic changes.

The study revealed that higher concentrations of salicylic acid caused mitotic inhibition and chromosomal abnormalities, altered protein expression profiles, and induced DNA polymorphism as detected by molecular markers on *A. cepa* roots. Additionally, salicylic acid exhibited moderate antibacterial activity against *S. epidermidis*, suggesting its potential utility in microbial management. Future studies should explore the underlying molecular mechanisms in greater detail and evaluate the long-term implications of salicylic acid exposure on plant development, reproduction, and agricultural sustainability.

5 Conclusions

This study demonstrates the cytotoxic and genotoxic effects of salicylic acid (SA) on *A. cepa* root meristematic cells, particularly at higher concentrations and longer exposure durations. The reduction in mitotic index and the increase in chromosomal abnormalities—including sticky chromosomes, lagging chromosomes, and anaphase bridges—reflect SA's disruptive impact on cell division, likely through interference with spindle apparatus formation and chromosomal segregation. In addition to cytological effects, salicylic acid exhibited antimicrobial activity, though the inhibition zones did not increase linearly with concentration, suggesting that lower doses may still exert effective biological activity while minimizing phytotoxic effects. This highlights SA's potential as a selective and environmentally safe antimicrobial agent. Molecular assessments provided further insights into the genomic and proteomic responses of onion plants under SA stress. Protein profiling showed concentration-dependent changes in expression patterns, while IRAP marker analysis revealed significant genetic polymorphism among treated samples. Interestingly, the differential clustering observed in protein and DNA marker data emphasizes the importance of integrating multi-level molecular analyses to fully understand stress responses. The findings demonstrate that salicylic acid induces concentration-dependent changes in mitotic activity and chromosomal stability in *A. cepa*, affects protein expression profiles, and generates detectable DNA alterations as revealed by molecular markers. Additionally, salicylic acid exhibited measurable antibacterial activity, supporting its potential use as a natural antimicrobial agent. These results contribute to our understanding of salicylic acid's biological effects at both cellular and molecular levels. Future scope: Future research should investigate the molecular and epigenetic mechanisms of salicylic acid-induced genotoxicity, assess gene expression changes, explore effects across different plant species and stages, and evaluate the long-term and reversible impacts. These insights will guide safe and effective use of salicylic acid in plant biotechnology and sustainable agriculture.

Acknowledgements Ongoing Research Funding program, (ORF-2025-931), King Saud University, Riyadh, Saudi Arabia.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no known financial interests or personal relationships that could influence this work.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Al-Tamimi N, Langan P, Bernád V, Walsh J, Mangina E, Negrão S (2022) Capturing crop adaptation to abiotic stress using image-based technologies. *Open Biol* 12:210353. <https://doi.org/10.1098/rsob.210353>
- Alafari HA, Abd-Elgawad ME (2021a) Differential expression gene/protein contribute to heat stress-responsive in *Tetraena propinqua* in Saudi Arabia. *Saudi J Biol Sci* 28:5017–5027. <https://doi.org/10.1016/j.sjbs.2021.05.016>
- Alafari HA, Abd-Elgawad ME (2021b) Heat-Induced protein and superoxide dismutase changes in wild *Tetraena propinqua* ssp. *Migahidii* seedlings. *Pak J Biol Sci* 24:310–318. <https://doi.org/10.3923/pjbs.2021.310.318>
- AlGarawi AM, Abd-Elgawad ME (2025) Genetic diversity of closely related *Calligonum* species collected from Saudi habitats by analyzing the *MatK* and *rpoC1* genes, and *scot* and IRAP markers. *Plant Biotechnol Rep* 19:55–66. <https://doi.org/10.1007/s11816-024-00952-y>
- Alias C, Feretti D, Viola GVC, Zerbini I, Bisceglie F, Pelosi G, Zani C, Buschini A, Carcelli M, Rogolino D, Restivo FM, Degola F (2023) Allium Cepa tests: A plant-based tool for the early evaluation of toxicity and genotoxicity of newly synthesized antifungal molecules. *Mutat Res Genet Toxicol Environ Mutagen* 889:503654. <https://doi.org/10.1016/j.mrgentox.2023.503654>
- Alotaibi MO, Abd-Elgawad ME (2023) Soil structure influences proteins, phenols, and flavonoids of varied medicinal plants in al Jubail, KSA. *Saudi J Biol Sci* 30:103567. <https://doi.org/10.1016/j.sjbs.2023.103567>
- Amiryousefi A, Hyvönen J, Poczar P (2018) iMEC: online marker efficiency calculator. *Appl Plant Sci* 6:e01159. <https://doi.org/10.1002/aps3.1159>
- Arif Y, Sami F, Siddiqui H, Bajguz A, Hayat S (2020) Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. *Environ Exp Bot* 175:104040. <https://doi.org/10.1016/j.envexpbot.2020.104040>
- Badr A, El-Sherif N, Aly S, Ibrahim SD, Ibrahim M (2020) Genetic diversity among selected *Medicago sativa* cultivars using inter-retrotransposon-amplified polymorphism, Chloroplast DNA barcodes and morpho-agronomic trait analyses. *Plants* 9:995. <https://doi.org/10.3390/plants9080995>
- Bagautdinova ZZ, Omelyanchuk N, Tyapkin AV, Kovrizhnykh VV, Lavrekha VV, Zemlyanskaya EV (2022) Salicylic acid in root growth and development. *Int J Mol Sci* 23:2228. <https://doi.org/10.3390/ijms23042228>
- Bidyananda N, Jamir I, Nowakowska K, Varte V, Vendrame WA, Devi RS, Nongdam P (2024) Plant genetic diversity studies: insights from DNA marker analyses. *Int J Plant Biol* 15:607–640. <https://doi.org/10.3390/ijpb15030046>

- Bilal H, Xiao Y, Khan MN, Chen J, Wang Q, Zeng Y, Lin X (2023) Stabilization of acne vulgaris-associated microbial dysbiosis with 2% supramolecular Salicylic acid. *Pharm* 16:87. <https://doi.org/10.3390/ph16010087>
- Bonciu E, Firbas P, Fontanetti CS et al (2018) An evaluation for the standardization of the Allium Cepa test as cytotoxicity and genotoxicity assay. *Caryologia* 71:191–209. <https://doi.org/10.1080/0087114.2018.1503496>
- Boorboori MR, Li J (2024) The effect of salinity stress on tomato defense mechanisms and exogenous application of Salicylic acid, abscisic acid, and melatonin to reduce salinity stress. *Soil Sci Plant Nutr* 1–18. <https://doi.org/10.1080/00380768.2024.2405834>
- Calvisi L (2021a) Efficacy of a combined chemical Peel and topical Salicylic acid-based gel combination in the treatment of active acne. *J Cosmet Dermatol* 20:2–6. <https://doi.org/10.1111/jocd.14281>
- Calvisi L (2021b) Efficacy of a combined chemical Peel and topical Salicylic acid-based gel combination in the treatment of active acne. *J Cosmet Dermatol* 20:2–6. <https://doi.org/10.1111/jocd.14281>
- Çavuşoğlu D, Macar O, Kalefetoğlu Macar T, Çavuşoğlu K, Yalçın E (2022) Mitigative effect of green tea extract against mercury (II) chloride toxicity in Allium Cepa L. model. *Environ Sci Pollut Res* 29:27862–27874. <https://doi.org/10.3390/ph16010087>
- Chen J, Zhang J, Kong M, Freeman A, Chen H, Liu F (2021) More stories to tell: Nonexpressor of Pathogenesis-related Genes1, a Salicylic acid receptor. *Plant Cell Environ* 44:1716–1727. <https://doi.org/10.1111/pce.14003>
- Cheraghi A, Rahmani F, Hassanzadeh-Ghorttapeh A (2018) IRAP and REMAP based genetic diversity among varieties of Lallelantia iberica. *Mol Biol Res Commun* 7:125–132. <https://doi.org/10.22099/mbrc.2018.29924.1327>
- Dawood MF, Latef AAHA (2023) Allium Cepa under stressful conditions. Medicinal plant responses to stressful conditions. CRC, pp 1–20
- Dawood MFA, Zaid A, Latef AAHA (2022) Salicylic acid Spraying-Induced resilience strategies against the damaging impacts of drought and/or salinity stress in two varieties of *Vicia Faba* L. Seedlings. *J Plant Growth Regul* 41:1919–1942. <https://doi.org/10.1007/s00344-021-10381-8>
- Desoky E-SM, Selem E, Abo El-Maati MF, Hassn AA, Belal HE, Rady MM, Al-Harbi MS, Ali EF (2021) Foliar supplementation of clove fruit extract and Salicylic acid maintains the performance and antioxidant defense system of Solanum tuberosum L. under deficient irrigation regimes. *Hortic* 7:435. <https://doi.org/10.3390/horticulturae7110435>
- Ding P, Ding Y (2020) Stories of Salicylic acid: a plant defense hormone. *Trends Plant Sci* 25:549–565. <https://doi.org/10.1016/j.tplants.2020.01.004>
- Dongare MD, Alex S, Soni KB, Sindura KP, Nair DS, Stephen R, Jose E (2023) Cross-species transferability of IRAP retrotransposon markers and polymorphism in black pepper (*Piper nigrum* L). *Genet Resour Crop Evol* 70:2593–2605. <https://doi.org/10.1007/s10722-023-01590-z>
- El-Ghamery A, Mousa M (2018) Salicylic acid triggers adaptation cadmium cytogenetic toxicity in roots of *Nigella sativa* L. *Egypt J Bot* 58:297–310. <https://doi.org/10.21608/ejbo.2018.3049.1159>
- Elshafie HS, Camele I, Mohamed AA (2023) A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *Int J Mol Sci* 24:3266. <https://doi.org/10.3390/ijms24043266>
- Farkouh A, Hemetsberger M, Noe CR, Baumgärtel C (2022) Interpreting the benefit and risk data in between-drug comparisons: illustration of the challenges using the example of mefenamic acid versus ibuprofen. *Pharm* 14:2240. <https://doi.org/10.3390/pharmaceutics14102240>
- Fu S, Wang L, Li C, Zhao Y, Zhang N, Yan L, Li CM, Niu Y (2024) Integrated Transcriptomic, Proteomic, and metabolomic analyses revealed molecular mechanism for salt resistance in soybean (*Glycine max* L.) seedlings. *Int J Mol Sci* 25:13559. <https://doi.org/10.3390/ijms252413559>
- Hammer Ø, Harper DA (2024) Paleontological data analysis. Wiley
- Heilmann C, Ziebuhr W, Becker K (2019) Are coagulase-negative Staphylococci virulent? *Clin Microbiol Infect* 25:1071–1080. <https://doi.org/10.1016/j.cmi.2018.11.012>
- Jangra A, Sharma G, Sihag S, Chhokar V (2022) The dark side of miracle plant-*Aloe vera*: a review. *Mol Biol Rep* 49:5029–5040. <https://doi.org/10.1007/s11033-022-07176-9>
- Jeong H-W, Ryu TH, Lee H-J, Kim K-H, Jeong R-D (2023) DNA damage triggers the activation of immune response to viral pathogens via Salicylic acid in plants. *Plant Pathol J* 39:449. <https://doi.org/10.5423/PPJ.OA.08.2023.0112>
- Kalendar R, Muterko A, Boronnikova S (2021) Retrotransposable elements: DNA fingerprinting and the assessment of genetic diversity. In: Besse P (ed) *Molecular plant taxonomy*. Springer US, New York, NY, pp 263–286. https://doi.org/10.1007/978-1-0716-0997-2_15
- Kamal MI, Zaied KA, Hussein MK, Abd El-Hady AH (2021) Cytogenetic effects of naphthalene acetic acid and benzylaminopurine in meristematic cells of onion roots. *J Agric Chem Biotechnol* 12:11–24. <https://doi.org/10.21608/jacb.2021.148055>
- Keya SS, Mostofa MG, Rahman MM, Das AK, Sultana S, Ghosh PK, Anik TR, Ahsan SM, Rahman MA, Jahan N, Tran L-SP (2023) Salicylic acid application improves photosynthetic performance and biochemical responses to mitigate saline stress in cotton. *J Plant Growth Regul* 42:5881–5894. <https://doi.org/10.1007/s00344-023-10974-5>
- Kim H, Kim JT, Barua S, Yoo S-Y, Hong S-C, Lee KB, Lee J (2018) Seeking better topical delivery technologies of moisturizing agents for enhanced skin moisturization. *Expert Opin Drug Deliv* 15:17–31. <https://doi.org/10.1080/17425247.2017.1306054>
- Koo YM, Heo AY, Choi HW (2020) Salicylic acid as a safe plant protector and growth regulator. *Plant Pathol J* 36:1–10. <https://doi.org/10.5423/PPJ.RW.12.2019.0295>
- Krupina K, Goginashvili A, Cleveland DW (2021) Causes and consequences of micronuclei. *Curr Opin Cell Biol* 70:91–99. <https://doi.org/10.1016/j.ccb.2021.01.004>
- LejaK, Szudera-Kończal K, Myszkka K, Czaczyk K (2019) Antibacterial effect of natural oils – an opportunity to solve the problem of antibiotic resistance on the example of pseudomonas spp. *Post Mikrobiol - Adv Microbiol* 58:177–190. <https://doi.org/10.21307/PM-2019.58.2.177>
- Leotta L, Toscano S, Ferrante A, Romano D, Francini A (2023) New strategies to increase the abiotic stress tolerance in woody ornamental plants in Mediterranean climate. *Plants* 12:2022. <https://doi.org/10.3390/plants12102022>
- Li A, Sun X, Liu L (2022) Action of Salicylic acid on plant growth. *Front Plant Sci* 13:878076. <https://doi.org/10.3389/fpls.2022.878076>
- Movahedi A, Dzinyela R, Aghaei-Dargiri S, Alhassan AR, Yang L, Xu C (2023) Advanced study of drought-responsive protein pathways in plants. *Agron* 13:849. <https://doi.org/10.3390/agronomy13030849>
- Mutlu H (2019) Willow tree extracts as novel plant-based biostimulants. Master's Thesis, Biyoteknoloji Enstitüsü
- Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N, Özkan H, Chung G, Baloch FS (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnol Biotechnol Equip* 32:261–285. <https://doi.org/10.1080/13102818.2017.1400401>
- Naheed R, Akhtar N, Afzal MS, Farhat F, Farooq MU (2022) Evaluation of mitigating effects of Salicylic acid against various levels of salinity in onion (*Allium cepa*). *Adv Life Sci* 9:92–97. <https://doi.org/10.62940/als.v9i1.1305>

- Oliveira WF, Silva PMS, Silva RCS, Silva GMM, Machado G, Coelho L, Correia MTS (2018) Staphylococcus aureus and Staphylococcus epidermidis infections on implants. *J Hosp Infect* 98:111–117. <https://doi.org/10.1016/j.jhin.2017.11.008>
- Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S (2020) The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. *Biomater Investig Dent* 7:105–109. <https://doi.org/10.1080/26415275.2020.1796674>
- Pasternak T, Groot EP, Kazantsev FV, Teale W, Omelyanchuk N, Kovrizhnykh V, Palme K, Mironova VV (2019) Salicylic acid affects root meristem patterning via auxin distribution in a concentration-dependent manner. *Plant Physiol* 180:1725–1739. <https://doi.org/10.1016/j.jhin.2017.11.008>
- Pellestor F, Gaillard JB, Schneider A, Puechberty J, Gatinois V (2022) Chromoanagenesis, the mechanisms of a genomic chaos. *Seminars in cell & developmental biology*. Elsevier, pp 90–99. <https://doi.org/10.1016/j.semcdb.2021.01.004>
- Pharmawati M, Wrsiati LP (2023) Chromosomal and nuclear alteration induced by nickel nitrate in the root tips of *Allium Cepa* var. *Aggregatum*. *Pollut* 9:702–711. <https://doi.org/10.22059/poll.2022.349167.1634>
- Porubsky D, Höps W, Ashraf H, Hsieh P, Rodriguez-Martin B, Yilmaz F, Ebler J, Hallast P, Maggiolini FAM, Harvey WT (2022) Recurrent inversion polymorphisms in humans associate with genetic instability and genomic disorders. *Cell* 185:1986–2005. <https://doi.org/10.1016/j.cell.2022.04.017>
- Runde TJ, Nappe TM (2021) Salicylates Toxicity. StatPearls [Internet] Treasure Island (FL): StatPearls Publishing
- Sabeen M, Mahmood Q, Bhatti ZA, Irshad M, Bilal M, Hayat MT, Irshad U, Akbar TA, Arslan M, Shahid N (2020) Allium Cepa assay based comparative study of selected vegetables and the chromosomal aberrations due to heavy metal accumulation. *Saudi J Biol Sci* 27:1368–1374. <https://doi.org/10.1016/j.sjbs.2019.12.011>
- Zamora Carballo I (2022) Development of a range of products for acne treatment on sensitive skin and preliminary design of its manufacturing process. *Treballs Finals de Grau (TFG) - Enginyeria Química*. <https://hdl.handle.net/2445/188069>
- Zanganeh F, Sheidai M (2022) Population genetic diversity and genetic affinity analyses of sweet orange cultivars (*Citrus sinensis* (L.) Osbeck) by using IRAP molecular markers. *Genet Resour Crop Evol* 69:2437–2446. <https://doi.org/10.1007/s10722-022-01382-x>
- Zhang YueLin ZY, Li Xin LX (2019) Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Curr Opin Plant Biol* 50:29–36. <https://doi.org/10.1016/j.pbi.2019.02.004>
- Zhou D, Shen W, Cui Y, Liu Y, Zheng X, Li Y, Wu M, Fang S, Liu C, Tang M (2021) Apical Spikelet Abortion (ASA) controls apical panicle development in rice by regulating Salicylic acid biosynthesis. *Front Plant Sci* 12:636877. <https://doi.org/10.3389/fpls.2021.636877>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.