

Multi-Level Screening Identifies Salt-Tolerant Chrysanthemum Genotypes Through Physiological, Molecular, and Root Anatomical Responses

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Abstract

Salinity is a major abiotic stress limiting the productivity and ornamental value of chrysanthemum. In the present study, 10 chrysanthemum (*Chrysanthemum morifolium*) genotypes were evaluated for their salt-stress responses using an integrated framework encompassing biochemical, molecular, and anatomical analyses. Significant genotype-dependent variations were observed in antioxidant enzyme activities, lipid peroxidation, and physiological attributes under 100 mM NaCl stress. Bidhan Lalima and Bidhan Sabita exhibited superior salt tolerance, whereas Bidhan Jayanti and Silk Brocate were highly susceptible. Salt stress induced a marked increase in oxidative damage, with MDA content rising by ~1.5-2.5 fold, ranging from ~10.1 to 26.1 nmol g⁻¹ FW across genotypes. Tolerant genotypes such as Bidhan Lalima and Bidhan Sabita maintained lower MDA levels (~13.0-20.6 nmol g⁻¹ FW at 200 mM) compared to highly susceptible genotypes like Bidhan Jayanti and Silk Brocate (~20.5-28.5 nmol g⁻¹ FW). Antioxidant enzyme activities showed genotype-specific responses, with guaiacol peroxidase increasing up to ~2-4 fold and SOD activity ranging from ~0.20 to 0.64 Units mg⁻¹ protein under stress. Defense-related enzymes such as chitinase and β -glucanase also showed significant induction, reaching up to ~9.1 and ~2.5 Units mg⁻¹ min⁻¹, respectively. Gene expression profiling revealed differential regulation of key salt-responsive genes *CmSOD*, *CmWRKY10*, *CmNCED3A*, *CmHSFA4*, and *CmHSP70* with tolerant genotypes showing enhanced expression of antioxidant- and ABA-related pathways. Root anatomical analysis highlighted distinct structural adaptations, including larger vascular areas and modified cortex and endodermal layers in tolerant genotypes, supporting improved water and nutrient transport under saline conditions. Collectively, this multi-tiered approach enabled reliable discrimination of salt-tolerant and susceptible genotypes and provided mechanistic insights into salt adaptation in chrysanthemum. The findings offer a robust foundation for breeding programs focused on developing salt-resilient cultivars for sustainable floriculture in salinity-affected regions.

Categories: Plant genetic resources, Functional genomics, Trait genetics & breeding of crops

Keywords: chrysanthemum, salt stress tolerance, antioxidant enzymes, gene expression, root anatomy, reactive oxygen species (ros)

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Introduction

Soil salinity is a major environmental constraint that adversely affects global agricultural productivity. Currently, salt-affected soils occupy nearly 17 million km² worldwide, of which about 2 million km² are considered suitable for saline agriculture [1]. In India, approximately 6.7 million hectares of land are affected by salinity, posing a serious challenge to agricultural and horticultural productivity, particularly in arid and semi-arid regions [2,3]. The expansion of salinized land is further accelerated by climate change, unsustainable irrigation, and improper fertilization practices. When soil salinity surpasses 1%, plant growth and survival become increasingly difficult. Salinity influences multiple physiological processes, impairing germination, growth, differentiation, and overall development. In ornamental crops, salt stress also diminishes aesthetic quality, limiting their commercial value [4,5]. In floriculture, flower number reductions due to salt stress can negatively affect crop sales, making flower responses under saline irrigation an important consideration for ornamental plant evaluation [6]. Previous studies in ornamental crops such as rose and marigold have reported significant reductions in growth, flowering, and pigment content under saline conditions [7,8].

Plants respond to salt stress through coordinated morphological, physiological, biochemical, and molecular adjustments. These responses include osmotic regulation, ion homeostasis, antioxidant activation, signal transduction, and transcriptional reprogramming mediated by a suite of salt-responsive genes [4]. While these mechanisms are well-documented in several crops, most studies have focused on individual levels of response (e.g., physiological or biochemical) rather than adopting an integrative approach. Since salt tolerance is a complex, genetically regulated trait, the identification of tolerant genotypes is essential for trait improvement.

Increasing population pressure and rapid urbanization have reduced the availability of arable land, emphasizing the need for sustainable strategies to utilize saline soils. Cultivation of salt-tolerant ornamentals offers a practical approach to landscape development in saline regions while avoiding competition with food crops. Consequently, developing high-value salt-tolerant ornamental varieties and evaluating underexplored species has gained considerable attention. This is particularly relevant in peri-urban landscapes and coastal regions where salinity stress is increasingly prevalent.

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the world's most economically important ornamental crops due to its long flowering period, diverse forms, and wide adaptability [5,6,9]. However, its growth and floral quality are highly sensitive to abiotic stresses, particularly salinity and drought. Although several studies have examined physiological responses to salinity in chrysanthemum, comprehensive studies integrating biochemical, molecular, and anatomical responses remain limited. With nearly 30,000 cultivars reported globally [7] and more than 500 in India [8,10], chrysanthemum offers considerable genetic diversity for stress-tolerance breeding. Identifying salt-tolerant genotypes is therefore crucial for selecting elite parents and broadening the genetic base for cultivar improvement [11,12].

At the molecular level, salt-tolerant plants activate intricate defense pathways that collectively shape their physiological and morphological responses [13,14]. However, the relationship between gene expression patterns and biochemical responses under salinity stress is still not well established in chrysanthemum. Given the variability in salt-induced damage across cultivars, rapid and integrative screening methods are essential for selecting resilient chrysanthemum genotypes suited for saline soils. Such approaches will support breeding programs aimed at sustaining commercial chrysanthemum cultivation in salt-affected regions. Even with existing studies, there is a lack of systematic evaluation of multiple chrysanthemum genotypes under graded salinity levels using an integrated framework combining biochemical, molecular, and anatomical analyses.

Despite the economic importance of chrysanthemum, systematic studies integrating biochemical, molecular, and anatomical responses under varying salinity levels remain limited. Furthermore, comparative evaluation of multiple commercially important genotypes under graded salt stress is lacking, restricting the identification of reliable tolerance indicators.

The present study was undertaken to investigate salt stress responses in ten chrysanthemum genotypes exposed to different NaCl concentrations through the assessment of physiological, biochemical, molecular, and anatomical traits. Antioxidant and defense-related biochemical responses were characterized along with the expression profiling of key

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salt-responsive genes, while anatomical adaptations associated with tolerance were also examined. This integrated approach is expected to enhance the understanding of genotype-specific mechanisms underlying salt tolerance and aid in the identification of resilient cultivars suitable for cultivation under saline conditions.

Materials And Methods

Study area and plant materials

The study was carried out at ICAR-Directorate of Floricultural Research under controlled conditions. Ten *Chrysanthemum morifolium* genotypes, namely Silk Brocate, Vanity Pink, Bidhan Jayanti, Bidhan Shweta, Bidhan Lalima, PAUD 11, Bidhan Rajat, Pusa Anmol, Pusa Arunodaya, and Bidhan Sabita, were used as plant material. Rooted cuttings obtained from the institute were established in cocopeat for uniform root development before initiation of the salt-stress treatments.

Salt-stress treatments

After successful rooting, plants were gently washed and transferred to Hoagland's nutrient solution containing different concentrations of NaCl. Four salt levels (50, 100, 150, and 200 mM NaCl) were imposed, with plants grown in Hoagland's solution without NaCl serving as the control. All plants were maintained in a controlled polyhouse to simulate natural growing conditions.

Five plants per genotype were subjected to each salinity level. Salt-stress responses were monitored for 5 days, after which leaf samples were collected. Samples were immediately frozen in liquid nitrogen and stored at -80°C for subsequent biochemical and molecular analyses.

Physio-biochemical analysis

Determination of Malondialdehyde Content

Lipid peroxidation was assessed by estimating malondialdehyde (MDA) following the method of Heath and Packer [15]. Leaf tissue (100 mg) from each replicate was homogenized in 200 μL extraction buffer composed of 20% (w/v) trichloroacetic acid (TCA) and 0.5% (w/v) 2-thiobarbituric acid (TBA). The volume was adjusted to 1 mL and mixed thoroughly. The mixture was incubated at 95°C for 30 minutes and then rapidly cooled on ice. Samples were centrifuged at 5,000 rpm for 10 minutes at 4°C , and the absorbance of the supernatant was recorded at 532 and 600 nm. MDA concentration was calculated using the following standard formula and expressed as nanomoles per gram of fresh weight.

$$\text{MDA Equivalent (nmol/gm FW)} = 1000 \times \text{Abs. } 532\text{nm} - \text{Abs. } 600\text{nm} / 155$$

Preparation of Enzyme Extracts

Enzyme extracts were prepared by homogenizing 100 mg of leaf tissue in 500 μL of sodium phosphate buffer (pH 7.2). The volume was adjusted to 1 mL and vortexed thoroughly. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C , and the resulting supernatant was collected and stored at -80°C for subsequent biochemical assays.

Estimation of Protein Content

Protein concentration was determined using the Bradford method [16]. A standard curve was generated using bovine serum albumin ($0-50 \mu\text{g mL}^{-1}$). For each reaction, sample extracts were adjusted to 100 μL with sodium phosphate buffer, followed by the addition of 5 mL of $1\times$ Bradford reagent. After 5 minutes at room temperature, absorbance was measured at 595 nm using a spectrophotometer.

Estimation of Superoxide Dismutase Activity

Superoxide dismutase activity (SOD) activity was measured following Beauchamp and Fridovich [17] and Madamanchi et al. [18], based on inhibition of nitro-blue tetrazolium (NBT) photo-reduction. The reaction mixture consisted of 50 mM sodium phosphate buffer, 100 μL of 100 mM EDTA, 14.3 mM methionine, 671 μL NBT, 25 μL riboflavin, and 10 μL enzyme

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extract. Reactions were exposed to a 400 W light source for 15 minutes, and absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% NBT reduction and expressed as U mg⁻¹ protein min⁻¹.

Estimation of Guaiacol Peroxidase Activity

Peroxidase (POD) activity was estimated according to Kar and Feierabend [19]. The reaction mixture contained distilled water, sodium phosphate buffer (pH 6.0), guaiacol, 10 µL hydrogen peroxide, and 10 µL enzyme extract. The increase in absorbance due to tetraguaiacol formation was recorded at 470 nm for 3 minutes. Activity was calculated using an extinction coefficient of 26.6 mM⁻¹ cm⁻¹ and expressed as U mg⁻¹ protein min⁻¹.

Determination of β-Glucanase Activity

β-Glucanase activity was assessed using a microplate-based assay following Khan and Umar [20]. A glucose standard curve (1 mg mL⁻¹ stock; 10-50 µL) was prepared. Each reaction contained 20 µL laminarin substrate and 10 µL enzyme extract in 96-well PCR plates, incubated at 40°C for 15 minutes. After adding 100 µL DNSA reagent, plates were heated at 95°C for 5 min. Absorbance was recorded at 550 nm, and activity was expressed as µg reducing sugar mg⁻¹ protein min⁻¹.

Determination of Chitinase Activity

Chitinase activity was measured using the microplate assay of Khan and Umar [20]. NAG (1 mg mL⁻¹ stock) standards at 0.5-2.5 µL were prepared. The reaction mixture consisted of 20 µL colloidal chitin substrate and 10 µL enzyme extract. Following incubation at 40°C for 15 min, DNSA reagent was added and incubated at 95°C for 5 min. After cooling, absorbance at 550 nm was recorded, and activity was calculated as µg reducing sugar mg⁻¹ protein min⁻¹.

Statistical Analysis

All biochemical assays were performed in triplicate. Two-way ANOVA was conducted using OPSTAT software considering *genotype and salinity level (NaCl concentration) as the two factors*, with significance set at $P = 0.05$ [21,22].

Principal Component Analysis (PCA) was performed using MetaboAnalyst 6.0 to assess trait variability. Data were autoscaled as per Seisonen et al. [23]. PCA, cluster analysis, and heat map preparation were based on biochemical data under 100 mM salt stress. Heat maps were generated using ClustVis [24] to visualize clustering patterns and gene expression differences between control and salt-stressed plants.

Root Anatomical Studies

Root anatomical traits were examined following Singh et al. [25] and Yin et al. [26]. Small root apex segments were excised using a razor blade, fixed, and stained with safranin for 2 minutes. Excess stain was removed by blotting, followed by washing with 70% ethanol for 1 minute. Sections were mounted in water and observed under a light microscope at 10× magnification. Anatomical features including endodermis thickness, exodermis, pericycle, cortex, epidermis, xylem area, and phloem area were recorded.

Molecular analysis

RNA Isolation and cDNA Synthesis

Total RNA from control and salt-stressed leaves was extracted using the modified SDS-LiCl method [27]. RNA concentration and purity were evaluated using NanoDrop One (Thermo Scientific), and integrity was confirmed on 1% agarose gel electrophoresis. First-strand cDNA synthesis was performed using the cDNA Reverse Transcription Kit (Thermo Fisher Scientific).

Quantitative real-time PCR

Five stress-responsive genes (*CmHSP70*, *CmSOD*, *CmHSFA4*, *CmNCED3A*, and *CmWRKY10*) were analyzed using *CmActin* as the internal reference. Primer sequences are listed in Table 1.

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RT-qPCR was performed on a QIAquant 96 5plex system using 20 µL reactions containing 2× SYBR Green master mix, 10 µM primers, and 50 ng cDNA. Cycling conditions included initial denaturation at 95°C for 30 seconds, followed by 40 cycles of 95°C for 5 seconds and 55°C for 34 seconds. Melt-curve analysis confirmed amplification specificity. Gene expression was quantified by the $2^{-\Delta\Delta C_t}$ method using control samples as calibrators.

Primer	Sequence
CmHSP70 Forward	5'-AAAGAACCAGAACCCGTGAC-3'
CmHSP70 Reverse	5'-ATGTCAAAGCAGACTGTGATCTGTG-3'
CmSOD Forward	5'-GCATGTCAACTGGTCCTCATTACAA-3'
CmSOD Reverse	5'-TCACGAAAGTAAGCCTTGCTATCCC-3'
CmHSFA4-RT Forward	5'-AGTACGAGAAAGTGGGAATG-3'
CmHSFA4-RT Reverse	5'-TTTGATCTGCAAGACTATTAACACT-3'
CmNCED3A-RT Forward	5'-AGTATGGTGGTGAGCCGTTGTATCTAC-3'
CmNCED3A-RT Reverse	5'-GCATTACAATCTGGAGTTCGGACTTC-3'
CmWRKY10 Forward	5'-TGCTCTTTCGCTCCAACCTG-3'
CmWRKY10 Reverse	5'-TTGTTCAACCAAAACCTCGTCA-3'
CmActin Forward	5'-ACAACTGCTGAACGGGAAAT-3'
CmActin Reverse	5'-TCATAGACGGCTGAAAAGG-3'

TABLE 1: Primer sequences of the genes used in the study.

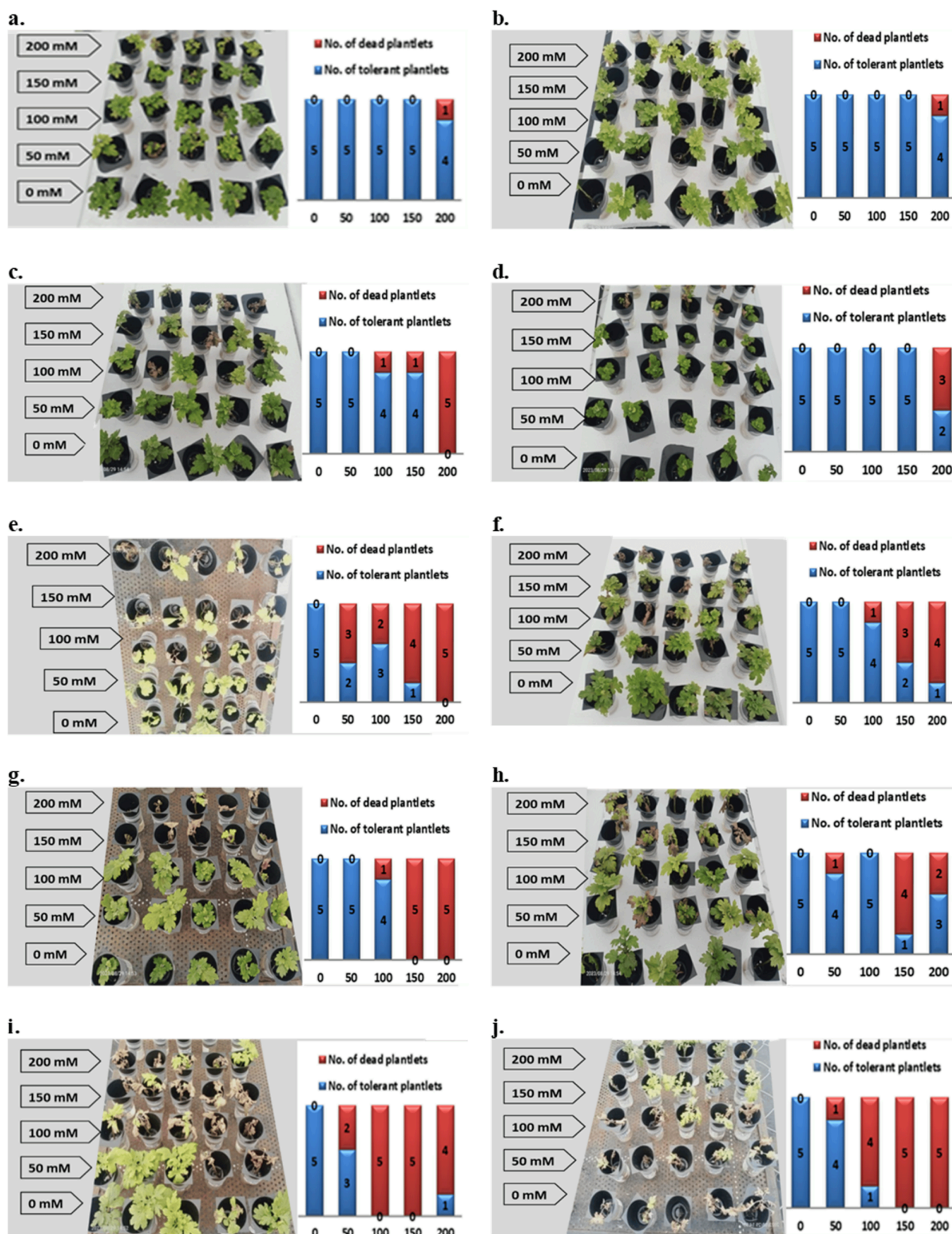
Results And Discussion

Responses of chrysanthemum genotypes to varying salt concentrations

The 10 chrysanthemum genotypes exhibited clear differences in their ability to withstand salt stress over the 5-day exposure period. Bidhan Lalima and Bidhan Sabita showed the highest tolerance, with only minimal mortality even at 200 mM NaCl. Pusa Arunodaya and Pusa Anmol survived up to 150 mM, indicating moderate tolerance. In contrast, Bidhan Jayanti and Silk Brocate were highly susceptible, surviving only up to 50 mM. PAUD-11, Bidhan Rajat, Bidhan Shweta, and Vanity Pink tolerated up to 100 mM (Figure 7).

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FIGURE 1: Phenotypic response of 10 chrysanthemum genotypes to different salinity treatments.

Chrysanthemum genotypes to different salinity treatments (0, 50, 100, 150, and 200 mM NaCl) at the seedling stage, recorded on the 5th day after stress imposition: (a) Bidhan Lalima, (b) Bidhan Sabita, (c) Pusa Arunodaya, (d) Pusa Anmol, (e) Vanity Pink, (f) Bidhan Shweta, (g) PAUD-11, (h) Bidhan Rajat, (i) Bidhan Jayanti, and (j) Silk Brocate.

Based on overall survival and stress responses, the genotypes were grouped as highly tolerant (Bidhan Lalima, Bidhan Sabita), moderately tolerant (Pusa Arunodaya, Pusa Anmol, Bidhan Shweta, PAUD-11, Bidhan Rajat), and highly susceptible (Bidhan Jayanti, Silk Brocate).

Physiological and biochemical responses to salt stress

Two-way analysis of variance (ANOVA) revealed significant effects of genotype (V), salt concentration (T), and their interaction ($V \times T$) on all biochemical parameters measured (Table 2).

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Sample no.	Sample name with salt concentration	Superoxide dismutase (U mg ⁻¹ of protein)	Malondialdehyde content (nmol g ⁻¹ FW)	Guaiacol peroxidase (U mg ⁻¹ of protein)	Chitinase (U mg ⁻¹ Protein min ⁻¹)	β-Glucanase (U mg ⁻¹ Protein min ⁻¹)
1	SILK BROCATÉ -0	0.473 ± 0.003	12.022 ± 0.305	47.191 ± 11.853	3.515 ± 0.334	1.452 ± 0.231
2	SILK BROCATÉ -50	0.487 ± 0.017	24.839 ± 14.051	39.173 ± 10.908	1.984 ± 0.089	0.589 ± 0.055
3	SILK BROCATÉ -100	0.560 ± 0.008	24.946 ± 0.269	87.097 ± 38.871	4.368 ± 0.229	2.326 ± 0.059
4	SILK BROCATÉ -150	0.628 ± 0.010	31.419 ± 0.615	46.302 ± 21.017	3.787 ± 0.274	1.754 ± 0.535
5	SILK BROCATÉ -200	0.616 ± 0.011	28.516 ± 2.099	27.438 ± 8.699	4.604 ± 0.189	2.523 ± 0.192
6	PAUD-11 -0	0.286 ± 0.046	9.140 ± 0.197	29.559 ± 4.585	3.574 ± 0.922	0.351 ± 0.274
7	PAUD-11 -50	0.482 ± 0.034	13.355 ± 0.547	24.520 ± 6.731	1.052 ± 0.409	0.294 ± 0.098
8	PAUD-11 -100	0.320 ± 0.013	15.097 ± 0.274	93.057 ± 40.253	2.371 ± 0.214	0.760 ± 0.103
9	PAUD-11 -150	0.649 ± 0.028	33.398 ± 0.489	51.667 ± 22.728	3.678 ± 0.968	1.835 ± 0.146
10	PAUD-11 -200	0.629 ± 0.072	23.720 ± 0.331	69.511 ± 25.580	3.863 ± 0.406	1.796 ± 0.153
11	PUSA ANMOL -0	0.246 ± 0.027	10.366 ± 0.099	101.516 ± 36.398	3.049 ± 0.407	0.591 ± 0.050
12	PUSA ANMOL-50	0.222 ± 0.013	10.581 ± 0.447	85.846 ± 16.122	2.609 ± 0.768	0.528 ± 0.315
13	PUSA ANMOL -100	0.254 ± 0.018	12.495 ± 0.186	138.935 ± 43.530	3.652 ± 0.811	0.858 ± 0.103
14	PUSA ANMOL -150	0.425 ± 0.058	12.043 ± 0.037	79.818 ± 25.165	2.640 ± 0.446	0.683 ± 0.116
15	PUSA ANMOL -200	0.299 ± 0.016	22.817 ± 0.037	55.467 ± 22.366	2.453 ± 0.468	0.883 ± 0.079
16	BIDHAN RAJAT -0	0.414 ± 0.020	12.452 ± 0.365	81.814 ± 20.821	2.802 ± 0.730	1.114 ± 0.148

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17	BIDHAN RAJAT -50	0.394 ± 0.016	10.430 ± 0.261	157.086 ± 40.584	3.283 ± 0.434	0.810 ± 0.205
18	BIDHAN RAJAT -100	0.412 ± 0.046	11.720 ± 0.355	115.128 ± 33.378	3.034 ± 0.371	1.104 ± 0.181
19	BIDHAN RAJAT -150	0.625 ± 0.015	19.548 ± 0.194	52.360 ± 21.669	1.844 ± 0.229	0.837 ± 0.055
20	BIDHAN RAJAT -200	0.503 ± 0.022	16.516 ± 0.296	73.420 ± 26.070	1.915 ± 0.300	1.070 ± 0.131
21	BIDHAN SABITA -0	0.578 ± 0.010	20.108 ± 0.493	28.242 ± 15.639	1.811 ± 0.651	0.574 ± 0.203
22	BIDHAN SABITA -50	0.551 ± 0.014	15.914 ± 0.788	39.860 ± 17.679	1.624 ± 0.141	0.384 ± 0.096
23	BIDHAN SABITA -100	0.311 ± 0.012	10.989 ± 0.489	227.505 ± 66.524	9.112 ± 0.849	2.318 ± 0.696
24	BIDHAN SABITA -150	0.094 ± 0.010	12.753 ± 0.261	53.949 ± 23.814	3.095 ± 0.402	0.688 ± 0.157
25	BIDHAN SABITA -200	0.503 ± 0.027	22.817 ± 1.049	70.654 ± 34.473	3.683 ± 0.306	1.199 ± 0.164
26	VANITY PINK -0	0.548 ± 0.024	10.645 ± 1.342	84.696 ± 16.245	2.770 ± 0.070	0.614 ± 0.176
27	VANITY PINK - 50	0.604 ± 0.020	17.204 ± 2.310	18.789 ± 5.376	1.554 ± 0.085	0.508 ± 0.009
28	VANITY PINK -100	0.595 ± 0.042	16.065 ± 0.516	10.300 ± 2.696	2.335 ± 1.502	0.760 ± 0.633
29	VANITY PINK - 150	0.586 ± 0.019	20.989 ± 0.458	8.690 ± 1.755	2.957 ± 0.488	1.121 ± 0.447
30	VANITY PINK - 200	0.541 ± 0.047	25.742 ± 1.183	22.214 ± 6.007	3.366 ± 1.023	1.532 ± 0.402
31	BIDHAN SHWETA - 0	0.352 ± 0.010	10.301 ± 0.162	95.806 ± 26.590	2.109 ± 0.141	0.664 ± 0.087
32	BIDHAN SHWETA - 50	0.529 ± 0.017	14.806 ± 0.046	97.024 ± 44.818	1.531 ± 0.134	0.764 ± 0.073
33	BIDHAN SHWETA -100	0.334 ± 0.047	10.280 ± 0.099	50.118 ± 14.310	2.819 ± 0.333	0.805 ± 0.145

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34	BIDHAN SHWETA -150	0.473 ± 0.012	16.344 ± 0.394	91.766 ± 40.735	2.605 ± 0.079	1.037 ± 0.070
35	BIDHAN SHWETA - 200	0.517 ± 0.018	17.484 ± 0.129	71.494 ± 30.400	3.146 ± 0.299	1.132 ± 0.073
36	PUSA ARUNODAYA -0	0.119 ± 0.024	8.710 ± 0.129	66.918 ± 2.127	6.279 ± 0.478	0.676 ± 0.236
37	PUSA ARUNODAYA - 50	0.342 ± 0.015	13.355 ± 5.292	66.804 ± 28.036	3.179 ± 0.784	0.281 ± 0.131
38	PUSA ARUNODAYA -100	0.201 ± 0.039	8.108 ± 0.207	66.492 ± 9.606	4.631 ± 0.225	0.484 ± 0.222
39	PUSA ARUNODAYA - 150	0.431 ± 0.023	11.806 ± 0.065	37.472 ± 12.816	2.376 ± 0.265	0.315 ± 0.162
40	PUSA ARUNODAYA - 200	0.587 ± 0.016	17.140 ± 0.525	49.993 ± 27.923	2.212 ± 0.127	0.687 ± 0.098
41	BIDHAN LALIMA - 0	0.174 ± 0.019	10.086 ± 0.162	32.272 ± 4.781	5.392 ± 1.043	0.764 ± 0.235
42	BIDHAN LALIMA - 50	0.431 ± 0.015	12.172 ± 0.149	16.383 ± 2.894	1.981 ± 0.059	0.347 ± 0.061
43	BIDHAN LALIMA - 100	0.243 ± 0.080	9.665 ± 0.164	22.785 ± 3.588	5.003 ± 0.095	0.495 ± 0.260
44	BIDHAN LALIMA -150	0.501 ± 0.146	16.667 ± 0.074	42.511 ± 2.811	2.255 ± 0.039	0.790 ± 0.080
45	BIDHAN LALIMA -200	0.329 ± 0.013	13.011 ± 0.074	62.672 ± 6.018	3.540 ± 0.254	0.627 ± 0.131
46	BIDHAN JAYANTI -0	0.257 ± 0.026	9.806 ± 0.171	157.040 ± 42.435	5.429 ± 0.255	1.049 ± 0.319
47	BIDHAN JAYANTI -50	0.441 ± 0.020	10.688 ± 0.197	73.190 ± 19.757	2.058 ± 0.677	0.712 ± 0.101
48	BIDHAN JAYANTI -100	0.639 ± 0.016	26.065 ± 2.404	55.556 ± 39.708	4.071 ± 0.338	1.072 ± 0.074

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49	BIDHAN JAYANTI -150	0.625 ± 0.051	15.806 ± 0.065	45.342 ± 16.404	4.228 ± 0.244	1.459 ± 0.171
50	BIDHAN JAYANTI - 200	0.389 ± 0.019	20.502 ± 0.071	59.934 ± 33.074	1.306 ± 0.055	0.537 ± 0.059

TABLE 2: Biochemical analysis of chrysanthemum genotypes under different salt stress (50-200 mM).

The biochemical changes associated with oxidative stress and defense responses are described in Figure 2 (a and b)

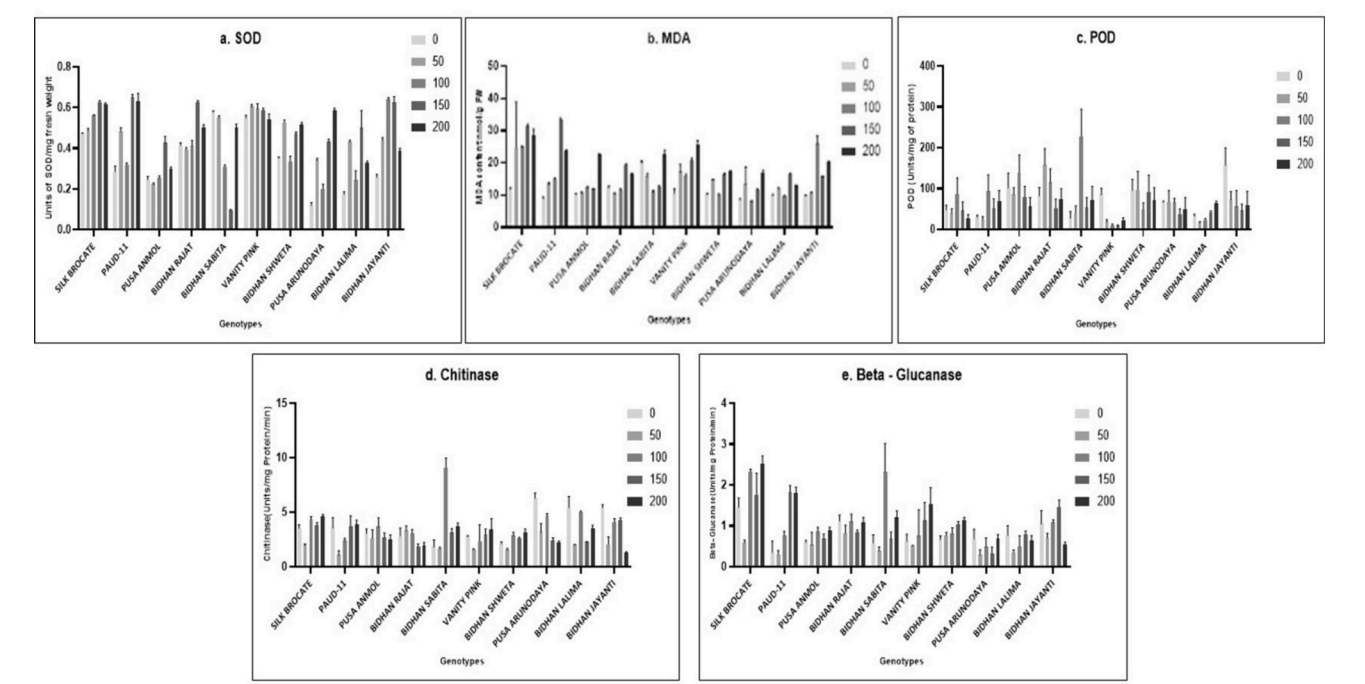


FIGURE 2: Activity of antioxidant enzymes chrysanthemum genotypes at the seedling stage under different salinity conditions.

Activity of antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD), and hydrolytic enzymes chitinase and β -glucanase, along with malondialdehyde (MDA) content in the leaves of ten chrysanthemum genotypes at the seedling stage under different salinity conditions (0, 50, 100, 150, and 200 mM NaCl) on the 5th day after salt stress treatment.

Lipid peroxidation (MDA content)

Salt stress induced a progressive increase in MDA levels across genotypes, with the highest values recorded at 200 mM NaCl. Silk Brocate, PAUD-11, and Vanity Pink exhibited the highest MDA accumulation, indicating greater membrane damage. Conversely, Pusa Arunodaya and Bidhan Lalima showed the lowest MDA levels, suggesting effective reactive oxygen species (ROS) mitigation.

The sharp rise in MDA between 100 and 150 mM NaCl indicates a stress threshold at which oxidative damage accelerates. Significant C.D. values for genotype (0.62), treatment (0.44), and interaction (1.38) substantiate these trends.

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SOD Activity

SOD activity differed significantly among genotypes and salt levels. Bidhan Rajat showed the highest activity, followed by Bidhan Sabita and Bidhan Jayanti, while Pusa Anmol exhibited the lowest values. Salt stress enhanced SOD activity up to 100 mM, after which activity plateaued at 150 and 200 mM NaCl. The interaction effect (C.D. = 0.01) indicates genotype-specific modulation of SOD under stress.

Guaiacol POD Activity

POD activity varied significantly among chrysanthemum genotypes under salinity stress. The highest POD activity was recorded in Bidhan Jayanti, Bidhan Shweta, and Bidhan Rajat, whereas the lowest activity was observed in Vanity Pink and Bidhan Lalima. Significant genotypic variation was detected for POD activity (C.D. = 0.02). In contrast, POD activity showed relatively smaller changes across NaCl treatments, indicating a comparatively moderate response to salinity stress. A significant genotype $\times$ treatment interaction (C.D. = 0.05) was also observed, reflecting differential POD responses among genotypes under varying salt-stress conditions.

Hydrolytic Enzymes: Chitinase and β -Glucanase

Chitinase and β -glucanase activities showed distinct genotypic variation and significant responsiveness to salt stress. Bidhan Sabita, Pusa Arunodaya, and Silk Brocate exhibited the highest chitinase activity, whereas Bidhan Shweta showed the lowest. For β -glucanase, Silk Brocate, Bidhan Sabita, and PAUD-11 were the highest responders, while Pusa Arunodaya and Bidhan Lalima had the lowest activity. The C.D. values (0.38 for chitinase; 0.17 for β -glucanase) indicated strong genotypic effects. Chitinase activity peaked at 100 mM NaCl, whereas β -glucanase activity increased progressively with salinity, reaching its maximum at 200 mM. Significant interaction effects (C.D. 0.84 and 0.37, respectively) show that both enzymes are modulated jointly by genotype and salt intensity.

Multivariate Analysis of Physio-Biochemical Traits

To interpret the overall variation among genotypes under salt stress, a PCA was performed on auto-scaled biochemical data. The first two principal components accounted for 81.8% of the total variation, with PC1 explaining 48.4% and PC2 contributing 33.4%. The PCA biplot (Figure 3a) demonstrated clear grouping of variables and genotypes. POD, chitinase, and β -glucanase loaded strongly on the positive region of PC1, clustering with the highly tolerant genotype "Bidhan Sabita." In contrast, SOD and MDA loaded negatively along PC1, aligning closely with the salt-susceptible genotypes "Silk Brocate," "Bidhan Jayanti," and "Vanity Pink." The remaining genotypes were distributed across the quadrants: "Pusa Anmol" and "Pusa Arunodaya" occupied the positive PC2 region, whereas "Bidhan Shweta" and "Bidhan Rajat" were placed in the negative PC2 quadrant. The PCA ordination effectively discriminated tolerant, moderately tolerant, and sensitive genotypes and highlighted the major biochemical contributors to their divergence (Figure 3b).

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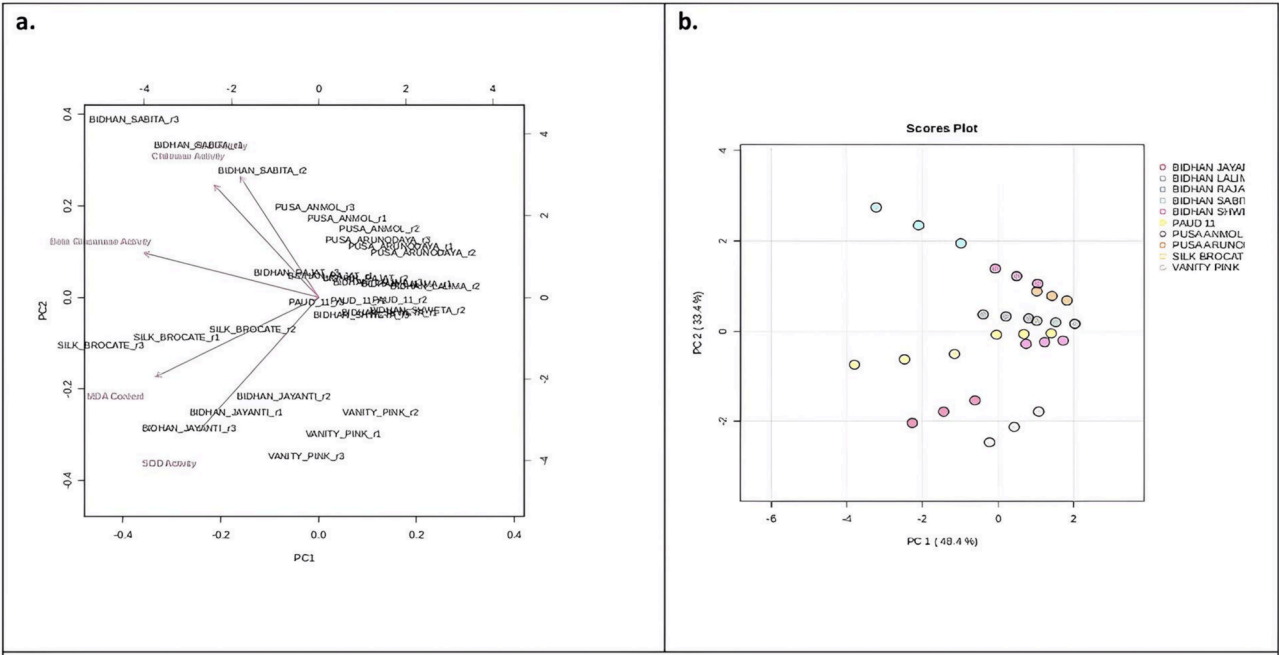


FIGURE 3: Principal component analysis (PCA) of phytochemical traits in chrysanthemum genotypes under 100 mM NaCl-induced salt stress.

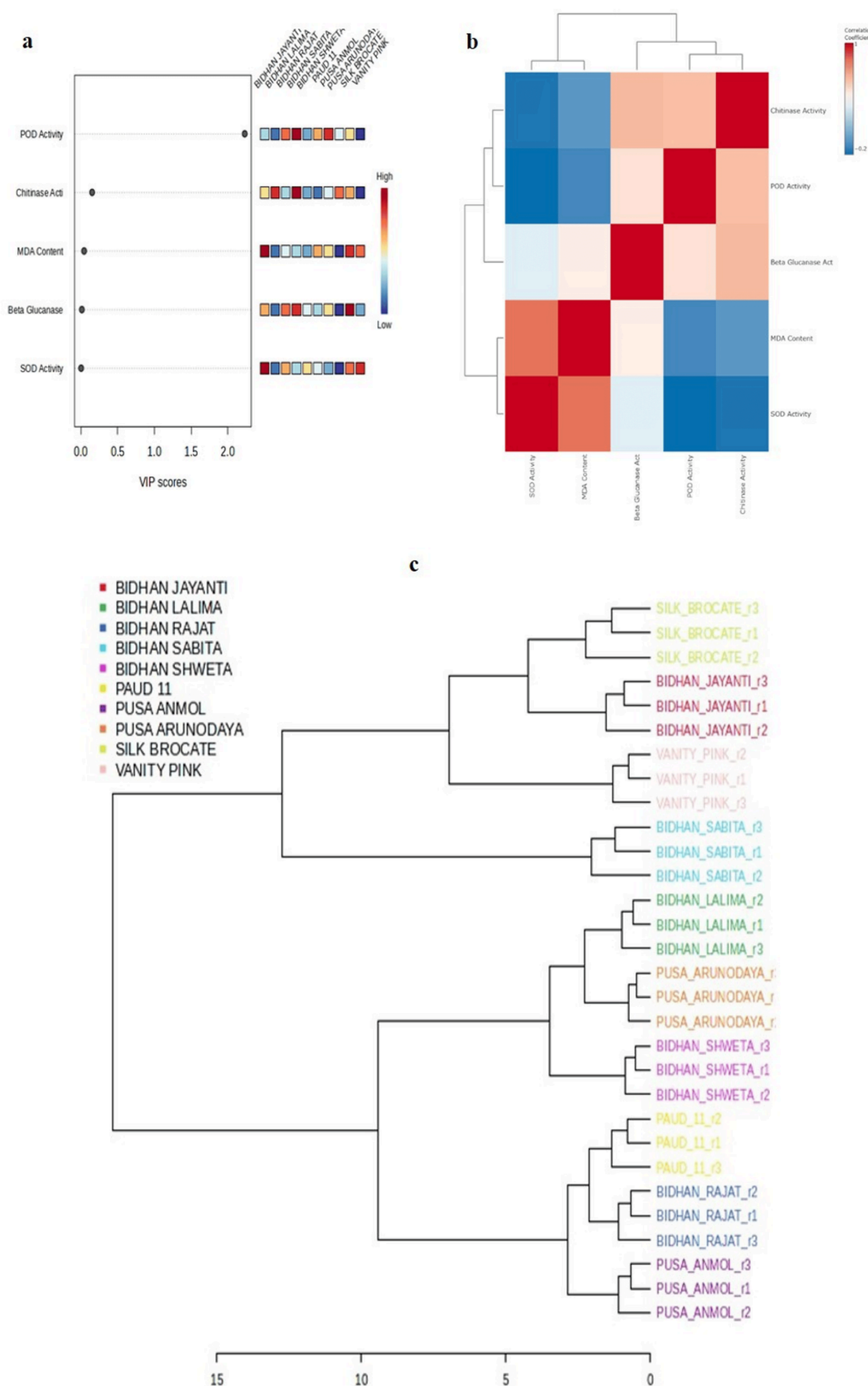
(a) Biplot displaying both PC scores (dots) of genotypes and loadings of the measured variables. (b) PCA score plot illustrating clustering of genotypes based on phytochemical composition.

The Variable Importance in Projection (VIP) values obtained from PLS-DA further identified chitinase as the most influential variable contributing to genotype separation under salt stress (Figure 4a).

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FIGURE 4: Multivariate and correlation analyses of phytochemical data in chrysanthemum genotypes under 100 mM NaCl-induced salt stress.

(a) VIP (Variable Importance in Projection) scores from PLS-DA, indicating the relative importance of individual phytochemicals in discriminating tolerant and susceptible genotypes. (b) Correlation matrix showing pairwise relationships among phytochemicals under salt stress. (c) Hierarchical clustering analysis (HCA) based on phytochemical profiles, illustrating grouping patterns among chrysanthemum genotypes.

Hierarchical clustering of chrysanthemum genotypes

Hierarchical clustering analysis (HCA) revealed two major clusters that clearly distinguished salt-tolerant from susceptible genotypes (Figure 4c). Cluster I consisted of two sub-clusters: one containing the highly susceptible genotypes ("Bidhan Jayanti," "Silk Brocate," "Vanity Pink"), and the other comprising the highly tolerant genotype "Bidhan Sabita." Cluster II was further divided into multiple sub-groups. The tolerant genotypes "Bidhan Lalima" and "Pusa Arunodaya" clustered together, while moderately susceptible genotypes such as "Bidhan Rajat" and "PAUD-11" formed another sub-cluster. "Bidhan Shweta" and "Pusa Anmol" appeared as independent sub-clusters. Interestingly, the moderately tolerant genotype "Pusa Anmol" grouped closer to susceptible types, suggesting a divergent physiological response compared with other moderately tolerant genotypes.

Correlation analysis among biochemical parameters

Pearson correlation analysis (Table 3) provided additional insights into the relationships among oxidative stress markers and defense-related enzymes under 100 mM NaCl stress. A strong positive correlation was observed between MDA and β -glucanase activity ($r = 0.613$, $P < 0.01$). MDA showed weak positive associations with chitinase activity ($r = 0.179$) and POD activity ($r = 0.086$). SOD activity exhibited a strong positive correlation with MDA ($r = 0.830$, $P < 0.01$) and a weak positive correlation with β -glucanase activity ($r = 0.376$, $P < 0.05$). In contrast, SOD showed weak negative correlations with POD activity ($r = -0.200$) and chitinase activity ($r = -0.145$). A moderate positive correlation was observed between POD and β -glucanase activity ($r = 0.485$, $P < 0.01$), while chitinase activity was strongly and positively correlated with β -glucanase activity ($r = 0.625$, $P < 0.01$) (Figure 4b).

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Biochemical parameter	SOD activity	MDA content	POD activity	Chitinase activity	β-Glucanase activity
SOD Activity	1.000	0.830**	-0.200	-0.145	0.376*
MDA Content	0.830**	1.000	0.086	0.179	0.613**
POD Activity	-0.200	0.086	1.000	0.352	0.485**
Chitinase Activity	-0.145	0.179	0.352	1.000	0.625**
β-Glucanase Activity	0.376*	0.613**	0.485**	0.625**	1.000

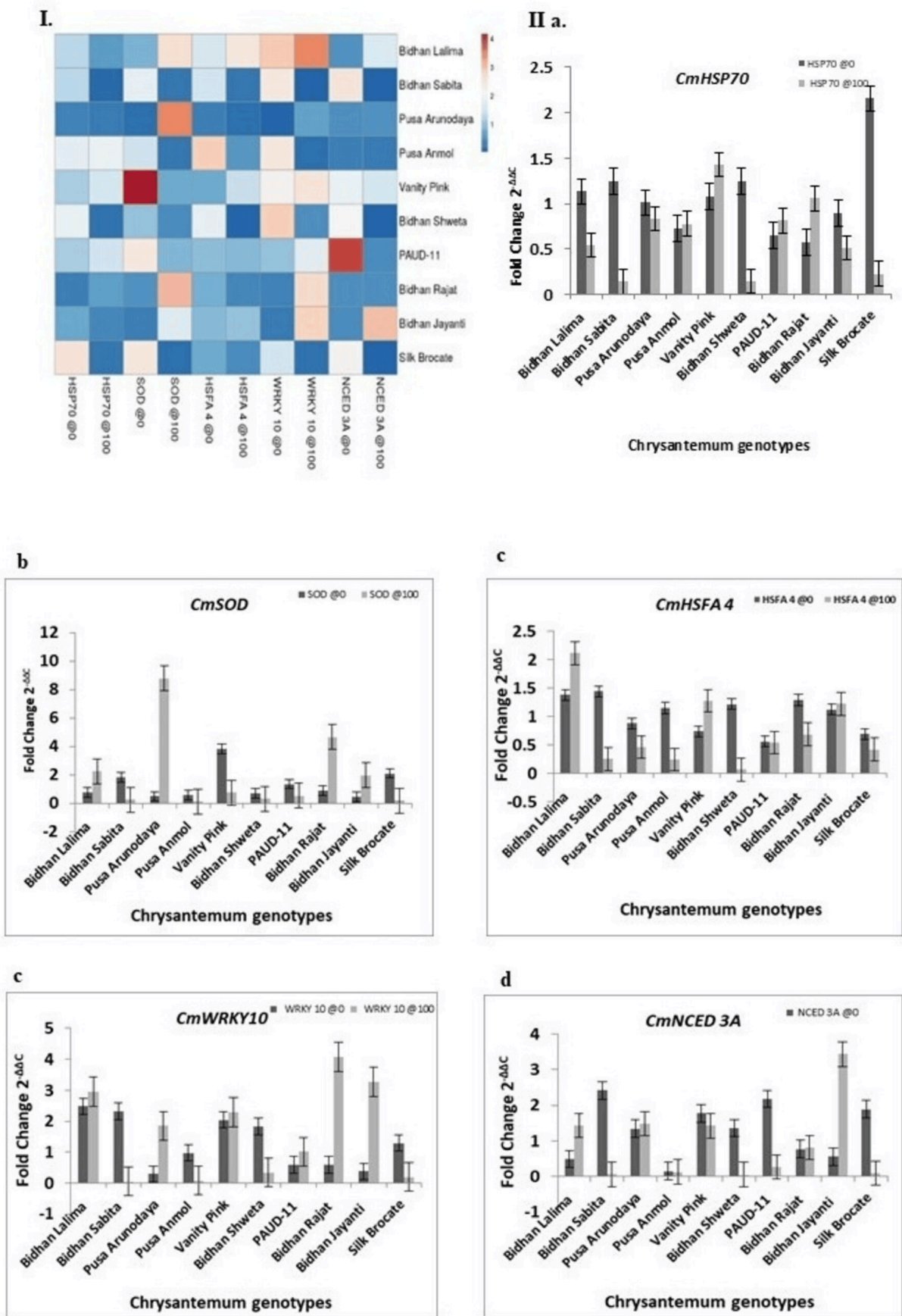
TABLE 3: Pearson's correlation coefficients (r) among biochemical parameters of chrysanthemum genotypes under 100 mM NaCl-induced salt stress.

Note: Values represent Pearson's correlation coefficients (*r*); * and ** denote statistically significant correlations at *P* < 0.05 and *P* < 0.01, respectively. Diagonal values (1.000) represent self-correlations. Pearson's correlation coefficients (*r*) were calculated using *n* = 30 observations (10 genotypes × 3 biological replicates) under 100 mM NaCl stress.

Gene expression changes under salt stress in chrysanthemum genotypes

To complement the physiological and biochemical analyses, the expression profiles of five key salt-responsive genes *CmSOD*, *CmHSP70*, *CmHSFA4*, *CmWRKY10*, and *CmNCED3A* were examined in 10 chrysanthemum genotypes under 100 mM NaCl stress (Appendix Table 4).

All genotypes survived 100 mM salt treatment, allowing comparative assessment of their molecular responses (Figure 5).



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FIGURE 5: Transcriptomic response of ten chrysanthemum genotypes to salt stress (100 mM NaCl).

I. Heatmap representation of gene expression profiles under control (0 mM NaCl) and salt stress conditions at the seedling stage, 5 days post-treatment. II. Relative expression levels of selected stress-responsive genes in leaves: (a) *CmHSP70*, (b) *CmSOD*, (c) *CmHSFA4*, (d) *CmWRKY10*, and (e) *CmNCED3A*, as quantified by RT-qPCR. Data represent means $\pm$ SE of three biological replicates.

Among the highly tolerant genotypes, Bidhan Lalima showed a marked increase in *CmSOD* expression, consistent with its elevated SOD activity under stress. This genotype also exhibited enhanced expression of *CmHSP70*, *CmHSFA4*, *CmWRKY10*, and *CmNCED3A*. In contrast, Bidhan Sabita displayed reduced expression of these genes. Among the moderately tolerant genotypes, Pusa Arunodaya exhibited higher expression of all stress-related genes compared with Pusa Anmol, although their transcript levels remained lower than those of the susceptible genotypes.

In the susceptible group, Bidhan Rajat and Vanity Pink showed generally high expression of all stress-responsive genes. Vanity Pink especially displayed elevated *CmHSP70*, *CmHSFA4*, *CmWRKY10*, and *CmNCED3A* expression, while Bidhan Rajat showed comparatively higher *CmSOD* expression. Notably, Vanity Pink exhibited higher SOD activity than Bidhan Rajat despite its lower *CmSOD* transcript level, indicating possible post-transcriptional regulation. Among highly susceptible genotypes, Bidhan Jayanti showed increased expression of *CmSOD*, *CmHSFA4*, *CmWRKY10*, and *CmNCED3A* compared with Silk Brocate, whereas Silk Brocate exhibited higher *CmHSP70* expression. Across genotypes, *CmSOD* expression was highest in the tolerant genotypes Bidhan Lalima and Pusa Arunodaya, as well as in the susceptible genotype Bidhan Rajat. In this study, *CmHSP70* exhibited high basal expression in tolerant genotypes, whereas its strongest induction under salt stress occurred in the susceptible genotypes Bidhan Rajat and PAUD-11, and the moderately tolerant Pusa Arunodaya.

Root anatomical observations of chrysanthemum genotypes

Among the salt-tolerant genotypes, Pusa Anmol exhibited comparatively larger xylem and phloem areas, which may enhance water and nutrient transport under saline conditions. This genotype also possessed thinner pericycle and endodermis layers, indicating a structurally efficient root system for mitigating salt-induced impacts. In contrast, Pusa Arunodaya recorded the largest cortex area, followed by Bidhan Lalima, Bidhan Sabita, and Pusa Anmol, suggesting that cortex expansion may serve as a supportive or storage-related adaptation under salt stress. Bidhan Sabita showed increased exodermis and epidermis thickness, potentially improving root protection in saline environments (Figure 6).

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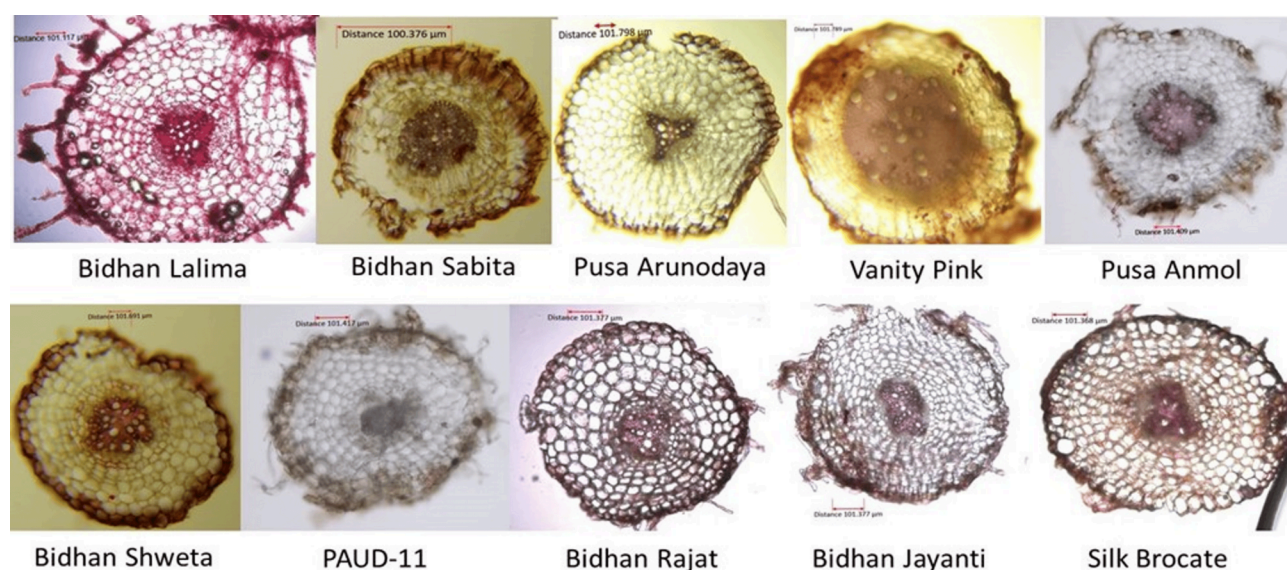


FIGURE 6: Anatomical features of root tissues in salt-tolerant and salt-susceptible chrysanthemum genotypes under salt stress (100 mM NaCl).

Representative transverse sections were observed under a light microscope to compare structural modifications associated with salinity tolerance. Scale bar $\approx$ 100 μ m

Among the salt-susceptible genotypes, Bidhan Shweta and Bidhan Rajat displayed reduced xylem and phloem areas, reflecting weaker vascular adaptation to salinity. PAUD-11 exhibited the smallest xylem and phloem areas, whereas Vanity Pink showed better vascular development, indicating relatively stronger structural adjustment compared with Bidhan Jayanti and Silk Brocate (Figure 6). Thickness of the pericycle and endodermis was moderate in Bidhan Rajat and PAUD-11, while Bidhan Shweta showed the least development of these layers.

Overall, the observed anatomical variations represent genotype-specific root traits that may be associated with differential responses to salt stress in chrysanthemum. Tolerant genotypes showed comparatively more developed vascular structures and protective tissues, whereas susceptible genotypes exhibited relatively limited anatomical adjustments under the same conditions. These observations should be interpreted as potential adaptive responses correlated with salinity tolerance, rather than as definitive mechanistic evidence. Nevertheless, these findings highlight the possible relevance of root anatomical attributes in understanding genotype-dependent variation in salt stress response in chrysanthemum.

Discussion

The 10 chrysanthemum genotypes exhibited clear differences in their ability to withstand salt stress over the 5-day exposure period. The results reflect genotypic differences in early stress perception and acclimation capacity, similar to previous reports in chrysanthemum [28].

Salt stress induced a progressive increase in MDA levels across genotypes, with the highest values recorded at 200 mM NaCl. The highest MDA accumulation, indicating greater membrane damage. Conversely, Pusa Arunodaya and Bidhan Lalima showed the lowest MDA levels, suggesting effective ROS mitigation. The sharp rise in MDA between 100 and 150 mM NaCl indicates a stress threshold at which oxidative damage accelerates. Lower MDA content in tolerant genotypes supports the opinion that delayed membrane injury and superior ROS homeostasis contribute to salt tolerance [29,28]. SOD activity differed significantly among genotypes and salt levels. Tolerant genotypes exhibited higher SOD under stress, consistent with the role of SOD as the first line of ROS detoxification and an indicator of cellular stress resistance [30,31]. These patterns align with earlier findings in chrysanthemum [28]. POD activity varied significantly among

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genotypes. While genotype differences were pronounced (C.D. = 0.02), POD activity across NaCl levels remained relatively stable, indicating a moderate response to salinity. The significant genotype $\times$ treatment interaction (C.D. = 0.05) suggests that POD contributes complementarily to antioxidant defense but is not the primary determinant of salt tolerance. This agrees with reports that POD responses are often genotype-dependent and supportive rather than central to stress adaptation [32].

Chitinase and β -glucanase activities showed distinct genotypic variation and significant responsiveness to salt stress. Given their roles in cell-wall remodeling and stress signaling, elevated hydrolytic enzyme activities may support structural adaptation under salt stress [33,34]. Tolerant genotypes showed strong inducible activity, suggesting a functional link to stress adaptation. Collectively, the biochemical data (Appendix Table 5) demonstrate that salt tolerance in chrysanthemum is strongly genotype-dependent and influenced by coordinated modulation of ROS-scavenging enzymes, membrane integrity, and hydrolytic defense enzymes. Tolerant genotypes consistently showed lower oxidative damage and stronger antioxidant responses under stress, whereas susceptible genotypes exhibited higher MDA and weaker enzymatic defenses. These findings provide biochemical evidence for the observed phenotypic tolerance and identify key candidate genotypes for breeding salt-resilient chrysanthemum.

PCA of the auto-scaled biochemical data revealed distinct patterns of variation among chrysanthemum genotypes under salt stress. The PCA biplot clearly separated tolerant, moderately tolerant, and sensitive genotypes, indicating differential biochemical responses to salinity stress. The distribution of variables in the ordination plot suggested that antioxidant and defense-related enzymes contributed substantially to genotype discrimination (Figure 3b).

Further, Partial Least Squares-Discriminant Analysis (PLS-DA) identified chitinase activity as the most influential variable associated with genotype differentiation, as indicated by the highest Variable Importance in Projection (VIP) score (Figure 4a). This finding suggests that chitinase may serve as an important biochemical marker linked to salinity tolerance in chrysanthemum genotypes. Hierarchical clustering of chrysanthemum genotypes

HCA further supported the multivariate findings by grouping the genotypes into two major clusters corresponding to salt-tolerant and salt-susceptible groups (Figure 4c). The clustering pattern reflected similarities in biochemical response profiles among the genotypes under salinity stress. Together, the PCA and HCA analyses demonstrated consistent discrimination among genotypes and reinforced the role of physio-biochemical traits in determining salinity tolerance in chrysanthemum.

Pearson correlation analysis further highlighted the coordinated interaction among oxidative stress indicators and defense-related enzymes in chrysanthemum genotypes under salinity stress (Table 3). The strong positive association between MDA and β -glucanase activity suggests coordinated activation of oxidative stress responses and β -glucanase-mediated defense mechanisms under salt stress. The weak positive associations of MDA with chitinase and POD activities indicate a limited relationship between lipid peroxidation and these defense enzymes. The strong positive correlation between SOD activity and MDA content indicates that elevated SOD activity reflects an adaptive antioxidant response to increased oxidative stress under saline conditions. However, the persistence of high MDA levels suggests that the antioxidant response is insufficient to completely prevent lipid peroxidation in susceptible genotypes. The weak positive association between SOD and β -glucanase activity, together with the moderate positive correlation between POD and β -glucanase activity and the strong positive correlation between chitinase and β -glucanase activity, further supports the coordinated functioning of antioxidant and defense-related enzymes during salt stress. Overall, these relationships indicate an integrated antioxidant and defense network that contributes to salinity tolerance in chrysanthemum genotypes (Figure 4b).

The highly tolerant genotype also exhibited enhanced expression of *CmHSP70*, *CmHSFA4*, *CmWRKY10*, and *CmNCED3A*, suggesting activation of multiple stress-responsive pathways. In contrast, Bidhan Sabita displayed reduced expression of these genes, indicating genotypic variation in the mechanisms contributing to tolerance. The evidence suggests that Bidhan Lalima relies strongly on SOD-mediated ROS detoxification and ABA-responsive pathways during salt stress. The

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high number of downregulated genes in leaves under high salinity could be attributed to the efficiency in conserving resources and energy under stress conditions by repressing the transcriptional process of genes mainly associated with oxidative activities and cell wall compartment, which could be constitutively active [35].

Susceptible genotype Vanity Pink exhibited higher SOD despite its lower *CmSOD* transcript level, indicating possible post-transcriptional regulation. Similarly, elevated *CmNCED3A* expression in tolerant genotypes highlights a likely involvement of ABA biosynthesis in modulating tolerance. Previous studies have shown that ABA-responsive genes—including *DREB1A*, *DREB2A*, *NCED3A*, *NCED3B*, and *CuZnSOD* play essential roles in abiotic stress mitigation [36]. The transcription factor *CmWRKY10*, known to enhance drought tolerance through ABA-mediated signaling [37] showed higher expression in tolerant genotypes even under control conditions and was further induced under salt stress.

The heat shock protein *HSP70* is well established as a key mediator of defense against abiotic stresses [38]. In this study, *CmHSP70* exhibited high basal expression in tolerant genotypes, whereas its strongest induction under salt stress occurred in the susceptible genotypes. *CmHSFA4*, implicated in ROS homeostasis and ion transporter regulation [39], showed highest expression in tolerant genotypes reinforcing its strong molecular tolerance signature.

Overall, stress-susceptible genotypes exhibited higher transcript accumulation across most genes compared with tolerant genotypes under stress, likely reflecting greater oxidative and osmotic injury. The diverse expression patterns observed among the genotypes point to distinct regulatory mechanisms underlying salt stress responses in chrysanthemum. These findings highlight the need for further functional characterization of key transcription factors and ABA-responsive genes to elucidate the molecular basis of salt tolerance.

Anatomical examination of root sections revealed distinct structural differences among chrysanthemum genotypes under salt stress. The larger xylem and phloem areas, which may enhance water and nutrient transport under saline conditions. The genotype with thinner pericycle and endodermis layers, indicates a structurally efficient root system for mitigating salt-induced impacts. The large cortex area suggests that cortex expansion may serve as a supportive or storage-related adaptation under salt stress. The increased exodermis and epidermis thickness, potentially improving root protection in saline environments.

Wider xylem vessels and conducting elements are associated with enhanced hydraulic conductivity and efficient water transport under abiotic stress conditions. These anatomical modifications may help maintain transpiration, water uptake, and osmotic balance, thereby contributing to stress adaptation and tolerance. However, excessively large vessels may also increase vulnerability to embolism and hydraulic dysfunction under severe stress. Therefore, improved stress tolerance depends on maintaining a balance between hydraulic efficiency and xylem safety [40,41].

The susceptible genotypes displayed reduced xylem and phloem areas, reflecting weaker vascular adaptation to salinity. Genotypes with better vascular development, indicating relatively stronger structural adjustment compared with Bidhan Jayanti and Silk Brocate (Figure 6). A larger cortex area in suggests a possible tolerance mechanism through cortex expansion. Overall, the anatomical variations highlight genotype-specific root traits associated with salt tolerance in chrysanthemum. Tolerant genotypes displayed more efficient vascular structures and protective tissues, whereas susceptible ones exhibited limited anatomical adjustments. These findings emphasize the relevance of root anatomical attributes in understanding and improving salt tolerance in chrysanthemum.

Conclusions

This study comprehensively evaluated salt-stress tolerance in 10 chrysanthemum genotypes through an integrated assessment of biochemical, molecular, physiological, and anatomical responses. Significant variations in enzymatic activities and lipid peroxidation revealed distinct stress-mitigation strategies among genotypes. Bidhan Lalima and Bidhan Sabita consistently exhibited superior salt tolerance, while Bidhan Jayanti and Silk Brocate emerged as highly susceptible. Two-way ANOVA further confirmed that both genotype and salt concentration significantly influenced stress responses. Molecular analyses demonstrated differential expression of key stress-responsive genes *CmSOD*, *CmWRKY10*,

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CmNCED3A, *CmHSFA4*, and *CmHSP70* indicating diverse regulatory mechanisms governing salt adaptation. Root anatomical observations provided additional insights, with tolerant genotypes showing well-developed vascular tissues and structural modifications conducive to efficient water and nutrient transport under saline conditions.

Overall, this multi-dimensional approach proved effective for reliably discriminating salt-tolerant and susceptible chrysanthemum genotypes. The integration of biochemical, molecular, and anatomical markers offers a robust framework for rapid and accurate screening. The insights generated here provide a valuable foundation for breeding programs aimed at developing salt-resilient chrysanthemum cultivars, contributing to sustainable floriculture practices in saline-prone environments. However, the present study was conducted under controlled conditions and included a limited set of biochemical and molecular parameters. Therefore, further validation under field conditions is necessary before the identified genotypes can be recommended for commercial cultivation in saline environments. Future studies focusing on ion transport mechanisms, hormonal regulation, metabolomic profiling, and large-scale transcriptomic analyses may provide deeper insights into the complex mechanisms underlying salinity tolerance in chrysanthemum.

Appendices

Sample name	CmHSP70	CmSOD	CmHSFA 4	CmNCED 3A	CmWRKY 10
Silk Brocate	0.229251011	0.162667732	0.418993567	0.093104841	0.196826994
Vanity Pink	1.433955248	0.739693755	1.274560627	1.424050196	2.289448321
Bidhan Jayanti	0.51584159	1.958840595	1.222640278	3.434261746	3.271608234
Bidhan Shweta	0.141120551	0.311002913	0.065607293	0.052738487	0.340328529
Bidhan Lalima	0.543367431	2.234574276	2.114036081	1.42899414	2.948538435
PAUD-11	0.812252396	0.530343871	0.543367431	0.271683716	1.00695555
Bidhan Rajat	1.057018041	4.642815658	0.685391402	0.815072332	4.069918768
Pusa Anmol	0.77916458	0.116225618	0.244855074	0.126306431	0.082183923
Pusa Arunodaya	0.835087919	8.78474251	0.460093825	1.479387509	1.846764621
Bidhan Sabita	0.138696184	0.250867937	0.25348987	0.057911754	0.050240687

TABLE 4: Relative expression of genes in chrysanthemum genotypes under salt stress (100 mM).

Multi-Level Screening Identifies Salt-Tolerant Chrysanthemum Genotypes Through Physiological, Molecular, and Root Anatomical Responses

	MDA	SOD	POD	Chitinase	β -glucanase
A (Varieties)					
SILK BROCATÉ	24.35	0.041	0.061	3.65	1.73
PAUD-11	18.94	0.039	0.049	2.91	1.01
PUSA ANMOL	13.66	0.029	0.057	2.88	0.71
BIDHAN RAJAT	14.13	0.055	0.077	2.58	0.99
BIDHAN SABITA	16.52	0.046	0.050	3.87	1.03
VANITY PINK	18.13	0.038	0.029	2.60	0.91
BIDHAN SHWETA	13.84	0.04	0.104	2.44	0.88
PUSA ARUNODAYA	11.82	0.033	0.061	3.74	0.49
BIDHAN LALIMA	12.32	0.036	0.025	3.63	0.61
BIDHAN JAYANTI	16.57	0.046	0.125	3.42	0.97
C.D.	0.62	0.004	0.024	0.38	0.17
SE(m)	0.22	0.002	0.009	0.13	0.06
B (Concentration)					
0mM	11.36	0.038	0.06	3.67	0.79
50mM	14.33	0.037	0.07	2.09	0.52
100mM	14.54	0.048	0.07	4.14	1.10
150mM	19.08	0.039	0.06	2.95	1.05
200mM	20.83	0.041	0.07	3.01	1.20
C.D.	0.44	0.003	N/A	0.27	0.12
SE(m)	0.16	0.001	0.01	0.09	0.04
A X B					
C.D.	1.38	0.01	0.05	0.84	0.37
SE(m)	0.49	0.003	0.02	0.30	0.13

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	0mM	50mM	100mM	150mM	200mM
MDA	11.36	14.33	14.54	19.08	20.83
SOD	0.038	0.037	0.048	0.039	0.041
POD	0.06	0.07	0.07	0.06	0.07
Chitinase	3.67	2.09	4.14	2.95	3.01
β-glucanase	0.79	0.52	1.10	1.05	1.20

TABLE 5: Statistical analysis using two-way ANOVA.
MDA: Malondialdehyde, SOD: Superoxide dismutase, POD: Peroxidase; ANOVA, Analysis of Variance

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

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Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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