

Physiological Resilience, Ionic Homeostasis and Stress-Adaptive Responses Underlying Salinity Tolerance in Citrus Cultivars

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Research Article

Keywords: salinity tolerance, ionic regulation, chlorophyll stability, physiological resilience, secondary metabolites, citrus cultivars

Posted Date: September 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10111681/v1>

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Abstract

Salt stress severely restricts citrus productivity in arid and semi-arid regions by disturbing ionic balance, water relations, and physiological functioning. The present study investigated salinity-induced changes in growth, ionic composition, physiological traits, and stress-responsive metabolites in four commercially important citrus cultivars, namely sweet orange (Blood Red), grapefruit (Star Ruby), kinnow mandarin, and lemon (Baramasi). Plants were exposed to five salinity levels ranging from 0.12 to 7.0 dS m⁻¹ under controlled pot conditions using a completely randomized design with five replications. Increasing salinity progressively reduced plant growth, chlorophyll stability, biomass accumulation, tissue hydration, and leaf health, while membrane injury increased markedly under severe stress. Salinity also disrupted ionic homeostasis through enhanced accumulation of Na⁺ and SO₄²⁻ ions coupled with depletion of Ca²⁺, Mg²⁺, and K⁺ in leaves and roots. Accumulation of anthocyanins and flavonoids increased under saline conditions, suggesting activation of stress-responsive biochemical protection mechanisms. Marked cultivar-specific differences were observed in salinity tolerance. Sweet orange and kinnow maintained lower Na⁺ accumulation, better K⁺/Na⁺ balance, higher chlorophyll stability, improved relative water content, and greater biomass retention under stress conditions. In contrast, grapefruit and lemon exhibited greater ionic imbalance, membrane injury, and physiological disruption. The findings indicate that maintenance of ionic homeostasis, chlorophyll stability, and adaptive biochemical modulation plays an important role in salinity tolerance in citrus cultivars. Among the cultivars evaluated, sweet orange and kinnow exhibited comparatively stronger adaptive resilience under progressive salinity stress.

1. Introduction

Citrus is one of the most important fruit crops cultivated in tropical and subtropical regions, but its productivity is increasingly threatened by soil salinity, particularly in arid and semi-arid areas where poor-quality irrigation water and inadequate drainage promote salt accumulation in the root zone. In many citrus-growing regions, increasing dependence on saline groundwater due to freshwater scarcity has further intensified the problem. Citrus species are generally considered sensitive to salinity, and prolonged exposure to saline conditions adversely affects plant growth, nutrient balance, photosynthetic efficiency, and overall plant vigor (Levy and Syvertsen 2004). Salinity affects plants through a combination of osmotic stress, ionic toxicity, and nutritional imbalance. Elevated salt concentration in the rhizosphere restricts water uptake and creates physiological drought-like conditions within plant tissues. At the same time, excessive accumulation of Na⁺ ions disturbs membrane permeability, enzyme activity, and metabolic functioning, ultimately leading to chlorosis, leaf scorching, premature senescence, and growth suppression under saline environments (Maas 1993). Salinity also interferes with uptake and transport of essential nutrients such as K⁺, Ca²⁺, and Mg²⁺, resulting in ionic imbalance and physiological instability. Since these nutrients are directly associated with osmotic regulation, membrane stability, chlorophyll synthesis, and photosynthetic metabolism, their depletion under saline conditions can severely impair plant growth and biomass accumulation (Tozlu et al. 2000; Grattan and Grieve 1998).

In addition to ionic imbalance, salinity stress promotes excessive generation of reactive oxygen species (ROS), which may damage cellular membranes, chloroplast structures, and metabolic systems. To minimize oxidative injury, plants activate several protective mechanisms involving accumulation of stress-responsive metabolites such as anthocyanins and flavonoids that help maintain cellular stability under stress conditions (Agati et al. 2012). Physiological traits including chlorophyll stability index, relative water content, and membrane injury are therefore widely used to assess salinity tolerance because they reflect the ability of plants to maintain photosynthetic efficiency, tissue hydration, and membrane integrity under adverse environments.

Differences in salinity tolerance among citrus cultivars are often associated with their capacity to regulate ion transport, maintain nutrient homeostasis, sustain water relations, and activate protective metabolic responses under stress conditions (Murkute et al. 2005). However, integrated information linking ionic regulation, physiological stability, and stress-responsive biochemical modulation in commercially important citrus cultivars under progressive salinity stress remains limited. Therefore, the present investigation was undertaken to evaluate the effect of salinity stress on growth behavior, ionic composition, physiological characteristics, and biochemical responses of sweet orange (Blood Red), grapefruit (Star Ruby), kinnow mandarin, and lemon (Baramasi) under controlled conditions, with the objective of identifying cultivars possessing better adaptive tolerance to saline environments.

2. Materials and Methods

2.1 Experimental site and plant material

The experiment was conducted during 2023-2025 at the Experimental Orchard, Department of Horticulture, CCS Haryana Agricultural University, Hisar, Haryana, India (29°10' N, 75°46' E; 215 m above mean sea level). The region is characterized by a semi-arid subtropical climate with hot summers and mild winters. Uniform and healthy seedlings of sweet orange (*Citrus sinensis* L. Osbeck cv. Blood Red), grapefruit (*Citrus paradisi* Macf. cv. Star Ruby), kinnow mandarin (*Citrus reticulata* Blanco cv. Kinnow), and lemon (*Citrus limon* Burm. f. cv.

Baramasi) were transplanted into plastic pots (10 kg capacity) containing homogenized sandy loam soil. The initial electrical conductivity (EC) of the control soil was 0.12 dS m⁻¹.

2.2 Salinity treatments and experimental design

Five salinity treatments comprising control (0.12 dS m⁻¹), 2.5, 4.0, 5.5, and 7.0 dS m⁻¹ were imposed using previously analyzed saline groundwater diluted with canal water to obtain the desired EC levels. During the initial phase of the experiment (2023–24), emphasis was placed on stabilization and maintenance of uniform salinity conditions within the pot system, as irrigation with normal water frequently altered soil EC levels. Salinity was therefore monitored periodically using a digital conductivity meter and adjusted whenever required to maintain stable stress conditions throughout the experimental period. Preliminary observations regarding plant survival and symptom development under different salinity levels were also recorded during this phase. After successful stabilization of salinity treatments, detailed growth, physiological, ionic, and biochemical observations were conducted during 2024–25. The experiment was arranged in a completely randomized design (CRD) with five replications, where each replication consisted of one plant per pot. Uniform agronomic and irrigation practices were maintained throughout the experimental period.

2.3 Growth and foliar injury measurements

Plant height was measured from the soil surface to the apical meristem using a meter scale and expressed in centimeters. Stem diameter was recorded 5 cm above the soil surface using a digital Vernier caliper and expressed in millimeters. Healthy, affected, and dead leaves were counted periodically during the experimental period. Leaves showing chlorosis, necrosis, tip burn, or severe foliar scorching were categorized as affected leaves. At harvest, plants were carefully separated into leaves, stems, and roots. Fresh biomass was recorded immediately after harvest, after which samples were oven dried at 48°C until constant weight to determine dry biomass. Total dry biomass was calculated using the following equation:

Total dry biomass (g plant⁻¹) = Dry leaf weight + Dry stem weight + Dry root weight

Root/shoot ratio was calculated as:

Root/shoot ratio = Dry root weight / (Dry leaf weight + Dry stem weight)

Biomass reduction under salinity stress was estimated using the following equation:

Biomass reduction (%) = [(Control biomass – Stress biomass) / Control biomass] × 100

Leaf moisture retention percentage was determined as:

Moisture retention (%) = [(Fresh leaf weight – Dry leaf weight) / Fresh leaf weight] × 100

2.4 Physiological measurements

2.4.1 Chlorophyll content and chlorophyll stability index (CSI)

Chlorophyll content was measured non-destructively using a SPAD chlorophyll meter (SPAD-502, Konica Minolta) on fully expanded mature leaves. Three readings were recorded from different positions on each leaf, and the mean value was expressed as SPAD units.

Chlorophyll stability index (CSI) was calculated using the following equation:

CSI (%) = (SPAD value under stress / SPAD value under control) × 100

2.4.2 Relative water content (RWC) and relative stress injury (RSI)

Relative water content (RWC) was determined following the method of Barrs and Weatherley (1962). Fresh leaf samples were weighed immediately to obtain fresh weight (FW), hydrated in distilled water for 6 h to obtain turgid weight (TW), and then oven dried at 80°C for 48 h to determine dry weight (DW). RWC was calculated as:

RWC (%) = [(FW – DW) / (TW – DW)] × 100

Relative stress injury (RSI) was estimated according to Sullivan and Ross (1979) using electrolyte leakage analysis. Leaf samples (200 mg) were incubated in deionized water and initial electrical conductivity (EC₁) was recorded. Samples were subsequently boiled for 1 h and final conductivity (EC₂) was measured. RSI was calculated using the following equation:

$$\text{RSI (\%)} = (\text{EC}_1 / \text{EC}_2) \times 100$$

2.5 Ionic analysis

Leaf and root samples were washed thoroughly with distilled water, oven dried, and ground into fine powder prior to analysis. Sodium (Na^+) and potassium (K^+) concentrations were determined using a flame photometer, whereas calcium (Ca^{2+}) and magnesium (Mg^{2+}) were estimated by EDTA titration. Sulfate (SO_4^{2-}) concentration was determined spectrophotometrically. Ionic concentrations were expressed as mg L^{-1} .

To better understand salinity-induced ionic imbalance, K^+/Na^+ ratio was also calculated in both leaf and root tissues as an indicator of ionic selectivity and salinity tolerance.

2.6 Biochemical analysis

2.6.1 Anthocyanin content

Anthocyanin content was estimated spectrophotometrically and expressed as $\text{mg } 100 \text{ g}^{-1}$ fresh weight.

2.6.2 Total flavonoid content

Total flavonoid content was determined using a colorimetric assay and expressed as $\text{mg quercetin equivalent (QE) g}^{-1}$ fresh weight.

2.7 Multivariate analysis

Heatmap and principal component analysis (PCA) were performed using standardized mean data of ionic, physiological, and biochemical parameters to evaluate trait association patterns and cultivar-specific salinity responses under different salinity levels. PCA was used to identify the major variables contributing toward salinity tolerance and to visualize relationships among cultivars and stress-responsive traits. Heatmap clustering analysis was performed to assess coordinated responses among ionic balance, physiological stability, and biochemical adjustment parameters under saline conditions.

2.8 Statistical analysis

All experimental data were analyzed using analysis of variance (ANOVA) under a completely randomized design (CRD) with five replications using SPSS software (version 26; IBM SPSS Statistics). The effects of salinity levels, cultivars, and their interaction (salinity $\times$ cultivar) were evaluated using two-way ANOVA. Treatment means were separated using the least significant difference (LSD) test at $p \leq 0.05$. Data are presented as mean $\pm$ standard error (SE).

3.1 Growth and foliar injury responses

3.1.1 Effect of salinity stress on plant height of citrus cultivars

Plant height declined progressively with increasing salinity in all citrus cultivars, although the extent of reduction differed among genotypes. Growth suppression became more apparent beyond 4.0 dS m^{-1} salinity, indicating stronger inhibition of vegetative development under severe stress conditions. At 7.0 dS m^{-1} salinity, lemon exhibited the highest reduction in plant height (11.2%), followed by grapefruit (10.3%) and kinnow (9.8%), whereas sweet orange showed comparatively lower reduction (8.3%) (Fig. 1). The comparatively smaller decline observed in sweet orange and kinnow suggests better capacity to sustain growth under saline environments. Reduction in plant height under saline conditions may be associated with osmotic stress, ionic toxicity, and restricted uptake of essential nutrients, which collectively interfere with cell elongation and assimilate partitioning. Excessive accumulation of toxic ions can further disturb metabolic activity and reduce energy availability for active growth processes. Similar genotype-dependent variation in vegetative growth responses under salinity stress has also been reported earlier in citrus cultivars and rootstocks (Parida and Das 2005; Acosta-Motos et al. 2017).

3.1.2 Effect of salinity stress on stem diameter of citrus cultivars

Stem diameter was significantly affected by increasing salinity in all citrus cultivars. The reduction remained relatively small under lower salinity levels but became more pronounced under severe stress conditions. At 7.0 dS m^{-1} salinity, stem diameter declined by 10.32% in lemon, followed by grapefruit (8.73%), kinnow (7.75%), and sweet orange (7.34%) (Fig. 1). The comparatively lower reduction observed in sweet orange and kinnow indicates better maintenance of structural growth under saline environments. Suppression of stem thickening under salinity stress may result from disturbed water relations, reduced nutrient availability, and restricted cellular expansion caused by osmotic and ionic imbalance. Excess salt accumulation can impair secondary growth and reduce biomass allocation toward actively

developing tissues. In contrast, the relatively stable stem growth maintained by sweet orange and kinnow suggests comparatively stronger physiological adjustment and lower cellular injury under stress conditions. Similar reductions in stem growth under saline environments have also been reported in citrus and other horticultural crops (Munns and Tester 2008; Deinlein et al. 2014).

3.1.3 Effect of salinity stress on leaf injury and foliage health of citrus cultivars

Salinity stress had a pronounced effect on foliage health in all citrus cultivars, leading to progressive increase in affected and dead leaves together with reduction in healthy foliage. Visual injury symptoms such as chlorosis, necrosis, marginal scorching, and premature leaf senescence became increasingly severe at higher salinity levels. Lemon was the most affected cultivar under severe salinity stress, recording 4.0 dead leaves plant⁻¹ and 5.4 affected leaves plant⁻¹, whereas sweet orange maintained the highest number of healthy leaves (85.2 leaves plant⁻¹) under the same conditions (Fig. 1). Kinnow also retained comparatively healthier foliage than grapefruit and lemon.

The severe decline in healthy leaves observed in lemon, where healthy foliage decreased to 27.2 leaves plant⁻¹ at 7.0 dS m⁻¹ salinity, clearly reflects greater sensitivity of this cultivar to salt-induced injury. Salinity-mediated foliar damage may be associated with excessive accumulation of Na⁺ in leaf tissues, disruption of chlorophyll synthesis, membrane destabilization, and enhanced oxidative injury. Toxic ion accumulation can further accelerate chlorosis and tissue necrosis by disturbing normal cellular metabolism (Munns 2002). In contrast, the comparatively lower foliar injury observed in sweet orange and kinnow suggests more efficient regulation of ion transport and restricted translocation of toxic salts toward aerial tissues. Similar salinity-induced foliar injury and leaf senescence have also been reported previously in citrus species exposed to elevated salinity stress (Cámara-Zapata et al. 2004; Simpson et al. 2015).

3.1.4 Effect of salinity stress on biomass accumulation and tissue hydration in citrus cultivars

Increasing salinity markedly reduced fresh and dry biomass accumulation in all citrus cultivars, indicating strong inhibition of overall plant growth under saline conditions (Table 1). The reduction became particularly severe at higher salinity levels, especially under 5.5 and 7.0 dS m⁻¹. Lemon exhibited the greatest decline in biomass accumulation under severe salinity stress, where total dry biomass reduction reached 77.8%, followed by grapefruit (64.8%). In contrast, sweet orange and kinnow showed comparatively lower reductions of 59.8% and 43.8%, respectively, indicating relatively greater biomass stability under saline environments. (Fig. 1). Reduction of biomass production under salinity stress may be linked with reduced photosynthetic activity, impaired nutrient uptake, osmotic imbalance, and disruption of metabolic functioning caused by toxic ion accumulation. Reduced carbon assimilation and restricted water uptake under saline conditions further limit dry matter accumulation in actively growing tissues. Similar reductions in biomass production due to salinity stress have also been reported earlier in citrus and other fruit crops grown under saline environments (Ashraf and Harris 2004; Acosta-Motos et al. 2017).

Moisture retention percentage also declined progressively with increasing salinity in all cultivars, although the reduction remained more pronounced in grapefruit and lemon. Lower tissue hydration under saline conditions may be attributed to osmotic stress-induced restriction of water uptake and loss of cellular turgidity. In contrast, sweet orange and kinnow maintained comparatively better tissue hydration, suggesting improved osmotic adjustment and internal water regulation under stress conditions. Salinity also influenced biomass partitioning, as reflected by changes in root/shoot ratio among cultivars. Relatively greater allocation of biomass toward roots under severe salinity may represent an adaptive response aimed at improving water acquisition and survival under stressful environments. The pronounced reduction in both root and shoot biomass observed in lemon further indicates greater susceptibility of this cultivar to salinity-induced ionic and metabolic disturbances. Excessive Na⁺ accumulation under saline conditions can impair membrane stability, disturb nutrient balance, and reduce photosynthetic efficiency, ultimately restricting biomass production and growth performance.

3.2 Physiological responses under salinity stress

3.2.1 Chlorophyll content and chlorophyll stability index (CSI)

Salinity stress progressively reduced chlorophyll content in all citrus cultivars, although the magnitude of decline varied considerably among genotypes (Fig. 2). SPAD values decreased from nearly 40.0 under control conditions to 23.2 under severe salinity stress, indicating substantial impairment of the photosynthetic system under elevated salt concentration. Lemon and grapefruit exhibited sharper reductions in chlorophyll content, whereas sweet orange and kinnow maintained relatively higher chlorophyll stability under saline conditions. At 7.0 dS m⁻¹ salinity, lemon recorded the lowest chlorophyll content (23.2), followed by grapefruit (25.3), while sweet orange maintained comparatively higher SPAD values under the same conditions. Reduction in chlorophyll content under saline environments may be due to disruption of chloroplast ultrastructure, inhibition of pigment biosynthesis, and enhanced oxidative damage caused by excessive accumulation of toxic ions (Reetika et al. 2024). Salinity-induced nutritional imbalance, particularly reduced Mg²⁺ availability, may further contribute to chlorophyll degradation and impairment of photosynthetic efficiency. Similar reductions in chlorophyll pigments under salt stress have also been reported in citrus and other perennial fruit crops exposed to saline environments (Acosta-Motos et al. 2017). A

similar declining trend was observed for chlorophyll stability index (CSI), which decreased progressively with increasing salinity levels. Sweet orange maintained the highest CSI value (0.83), followed by kinnow (0.77), whereas grapefruit and lemon showed lower CSI values of 0.75 and 0.71, respectively (Fig. 2). Higher CSI generally reflects better stability of chloroplast membranes and greater protection of photosynthetic machinery under stress conditions. Therefore, the relatively higher CSI maintained by sweet orange and kinnow indicates stronger photosynthetic resilience and improved physiological adjustment under saline environments.

In contrast, lower CSI values observed in lemon and grapefruit suggest greater susceptibility of chloroplast membranes to salinity-induced injury. Excessive accumulation of toxic ions under saline conditions may destabilize membrane integrity and disturb electron transport processes, thereby reducing photosynthetic efficiency and pigment stability. Thus, maintenance of chlorophyll content together with higher CSI appears to be closely associated with better salinity tolerance among citrus cultivars.

3.2.2 Relative water content (RWC) and relative stress injury (RSI)

Relative water content (RWC) declined progressively with increasing salinity in all citrus cultivars, whereas relative stress injury (RSI) increased steadily under saline conditions (Fig. 2). However, the extent of physiological disturbance differed among cultivars. Sweet orange and kinnow exhibited comparatively smaller reductions in RWC, indicating greater ability to maintain tissue hydration and osmotic balance under stress conditions. Under severe salinity (7.0 dS m⁻¹), lemon recorded the lowest RWC (56.2%), followed by grapefruit (60.1%), whereas sweet orange maintained relatively higher tissue hydration (62.2%).

Decline in RWC under saline conditions may be attributed to osmotic stress, restricted water uptake by roots, and disturbance of cellular water balance caused by elevated salt concentration in the rhizosphere. Loss of cellular turgidity under saline environments can adversely affect stomatal functioning, photosynthesis, and overall metabolic activity (Dahro et al. 2023). The comparatively better maintenance of tissue hydration observed in sweet orange and kinnow therefore suggests more effective osmotic adjustment and stronger internal water regulation under stress conditions. Similar genotype-dependent variation in tissue hydration under salinity stress has also been reported in citrus and other horticultural crops (Flowers et al. 2015).

In contrast to RWC, RSI increased progressively with increasing salinity levels, indicating enhanced membrane injury and cellular damage under saline environments. Lemon exhibited the highest RSI value (46.0%) under severe salinity stress, followed by grapefruit (43.1%), whereas sweet orange recorded comparatively lower membrane injury (40.7%). Increased RSI under saline conditions may result from excessive generation of reactive oxygen species, membrane destabilization, and lipid peroxidation induced by toxic ion accumulation. Salinity-mediated oxidative injury can severely impair membrane permeability and disrupt normal cellular functioning (Xiao and Zhou 2023). The comparatively lower RSI observed in sweet orange and kinnow suggests better membrane stability and stronger protective capacity against salinity-induced oxidative damage. Cultivars capable of maintaining lower membrane injury together with better tissue hydration generally exhibit greater physiological resilience under saline conditions. Hence, findings indicate that maintenance of cellular water balance and membrane integrity plays a crucial role in determining salinity tolerance among citrus cultivars.

3.3 Ionic regulation and nutrient imbalance

3.3.1 Effect of salinity on leaf ionic composition of citrus cultivars

Salinity stress significantly altered leaf ionic composition in all citrus cultivars, indicating severe disturbance of nutrient balance under elevated salt conditions (Table 2). Progressive increase in salinity enhanced accumulation of Na⁺ and SO₄²⁻ ions, whereas concentrations of K⁺, Ca²⁺, and Mg²⁺ declined markedly in leaf tissues (Fig. 3). Such ionic imbalance under saline environments is commonly associated with competitive inhibition of nutrient uptake and disruption of membrane transport processes within plant tissues. Similar changes in leaf ionic composition under salinity stress have also been reported in citrus and several other fruit crops (Munns and Tester 2008).

Considerable cultivar-specific variation was observed in the extent of ionic alteration. Grapefruit and lemon accumulated substantially higher Na⁺ concentrations in leaves under severe salinity stress, whereas sweet orange and kinnow maintained relatively lower Na⁺ levels. Increased accumulation of sodium ions in leaf tissues can impair membrane integrity, enzyme functioning, and photosynthetic metabolism, thereby accelerating salinity-induced injury. In contrast, lower Na⁺ accumulation in sweet orange and kinnow suggests stronger ionic regulation and more effective restriction of toxic ion translocation toward aerial tissues.

Leaf SO₄²⁻ concentration also increased progressively with increasing salinity. Grapefruit recorded the highest increase in sulfate accumulation (+42%), whereas kinnow exhibited the lowest increase (+32%). Excessive sulfate accumulation under saline environments may contribute further to osmotic imbalance and ionic toxicity within leaf tissues. The comparatively lower sulfate accumulation observed in sweet orange and kinnow therefore reflects relatively better ion regulation under saline conditions.

In contrast to Na^+ and SO_4^{2-} , concentrations of K^+ , Ca^{2+} , and Mg^{2+} declined progressively with increasing salinity. Lemon and grapefruit showed greater depletion of these essential nutrients, whereas sweet orange and kinnow retained comparatively higher concentrations even under severe stress conditions. Reduction in K^+ concentration under saline environments may impair osmotic adjustment, stomatal regulation, and enzyme activation, while depletion of Ca^{2+} and Mg^{2+} may adversely affect membrane stability and chlorophyll synthesis. Maintenance of relatively higher K^+ , Ca^{2+} , and Mg^{2+} concentrations in sweet orange and kinnow therefore suggests comparatively stronger nutrient retention and physiological stability under saline conditions.

3.3.2 Effect of salinity on root ionic composition of citrus cultivars

Root ionic composition was also markedly affected by increasing salinity levels in all citrus cultivars (Table 3). Progressive accumulation of Na^+ and SO_4^{2-} ions together with reduction in K^+ , Ca^{2+} , and Mg^{2+} concentrations clearly indicates disruption of ionic homeostasis within root tissues under saline environments. Since roots represent the primary site of salt entry into plants, excessive accumulation of toxic ions at the root level may strongly influence nutrient uptake efficiency and overall plant metabolism.

Among the cultivars, lemon exhibited the highest Na^+ accumulation in roots under severe salinity stress, whereas sweet orange maintained comparatively lower root Na^+ concentration. Greater sodium accumulation in root tissues may disturb membrane transport systems, reduce nutrient absorption efficiency, and impair root metabolic activity. Reduced Na^+ accumulation in sweet orange and kinnow therefore suggests more efficient ion exclusion and compartmentalization mechanisms under saline conditions (Deinlein et al. 2014).

Root SO_4^{2-} concentration also increased substantially with increasing salinity levels. Lemon and grapefruit showed greater sulfate accumulation than sweet orange and kinnow, indicating comparatively weaker ionic regulation under stress conditions. Enhanced accumulation of soluble salts within root tissues may aggravate osmotic stress and interfere with nutrient acquisition processes during prolonged salinity exposure.

A progressive decline in root K^+ , Ca^{2+} , and Mg^{2+} concentrations was observed in all cultivars with increasing salinity. The reduction remained more pronounced in lemon and grapefruit, whereas sweet orange and kinnow maintained relatively better nutrient balance under stress conditions. Calcium depletion under saline environments may destabilize membrane structure and increase susceptibility to cellular injury, while reduced Mg^{2+} availability may negatively influence chlorophyll formation and energy metabolism. Better maintenance of these nutrients in sweet orange and kinnow therefore further supports their comparatively greater salinity tolerance.

3.3.3 Sodium and sulfate accumulation under saline environments

Accumulation of Na^+ and SO_4^{2-} ions increased consistently in both leaves and roots with increasing salinity levels, although the magnitude of accumulation varied considerably among cultivars. Lemon and grapefruit accumulated markedly higher concentrations of these ions under severe salinity stress, whereas sweet orange and kinnow maintained relatively lower accumulation within plant tissues. Excessive accumulation of Na^+ under saline conditions can disrupt membrane permeability, enzyme activity, and cellular metabolism, ultimately suppressing plant growth and physiological functioning (Zhang et al. 2021; Isayenkov and Maathuis 2019).

A similar trend was observed for sulfate ions, where grapefruit exhibited maximum increase in leaf SO_4^{2-} concentration, while lemon showed highest sulfate accumulation in roots. Elevated sulfate accumulation may further intensify osmotic imbalance and ionic toxicity under prolonged saline exposure. In contrast, restricted accumulation of Na^+ and SO_4^{2-} in sweet orange and kinnow suggests comparatively stronger ionic regulation and improved stress adaptation under saline environments. It can be easily interpreted from the results that the ability to limit toxic ion accumulation plays a central role in determining salinity tolerance among citrus cultivars.

3.3.4 Potassium homeostasis and K^+/Na^+ balance under salinity stress

Potassium concentration declined progressively in both leaves and roots with increasing salinity levels. However, the extent of reduction varied considerably among cultivars. Grapefruit and lemon showed sharper decline in K^+ concentration, whereas sweet orange and kinnow retained relatively higher potassium levels even under severe salinity stress. Under saline conditions, excessive Na^+ competes with K^+ for uptake and transport processes, thereby disturbing ionic selectivity and cellular homeostasis.

Accumulation of higher K^+ concentration under saline environments is considered essential for osmotic adjustment, stomatal functioning, enzyme activation, and maintenance of cellular turgor (Ahmad et al. 2021). Sweet orange and kinnow also maintained comparatively favorable K^+/Na^+ balance under elevated salinity, indicating stronger ionic selectivity and better cellular regulation under stress conditions. Upholding of optimum K^+/Na^+ ratio has been widely recognized as one of the major physiological determinants associated with salinity tolerance in plants (Shabala and Cuin 2008).

The comparatively lower K^+/Na^+ balance observed in lemon and grapefruit suggests greater susceptibility to ionic toxicity and metabolic disruption under saline environments. On the whole, efficient potassium retention together with restricted sodium accumulation appears to contribute significantly toward improved salinity tolerance in sweet orange and kinnow.

3.3.5 Calcium and magnesium regulation in response to salinity stress

Calcium and magnesium concentrations declined progressively in both leaves and roots with increasing salinity levels (Fig. 3). However, the reduction remained more pronounced in lemon and grapefruit, whereas sweet orange and kinnow retained comparatively higher concentrations of these nutrients under saline conditions. Elevated external Na^+ concentration may interfere with Ca^{2+} uptake because of competition at membrane transport and binding sites within root tissues.

Calcium plays an important role in maintenance of membrane integrity, stabilization of cellular structure, and regulation of membrane permeability. Therefore, depletion of Ca^{2+} under saline environments may enhance membrane destabilization and increase susceptibility to stress-induced injury (Feng et al. 2023). Kinnow maintained relatively higher Ca^{2+} concentration even under severe salinity stress, suggesting better membrane protection and greater physiological stability under saline conditions.

Magnesium depletion also became more evident with increasing salinity levels, particularly in lemon and grapefruit. Since magnesium forms the central component of chlorophyll molecules and is directly involved in photosynthetic metabolism, reduced Mg^{2+} availability may adversely affect chlorophyll stability and biomass production. The comparatively smaller decline in Mg^{2+} concentration observed in sweet orange and kinnow therefore further supports their improved nutrient regulation and physiological adjustment under stress conditions. In view of above, better retention of Ca^{2+} and Mg^{2+} together with improved K^+/Na^+ balance contributed toward stronger ionic homeostasis and greater salinity tolerance in sweet orange and kinnow cultivars.

3.4 Stress-responsive biochemical modulation

3.4.1 Anthocyanin accumulation under salinity stress

Salinity stress significantly influenced anthocyanin accumulation in all citrus cultivars, indicating activation of protective biochemical responses under saline environments (Fig. 4). Anthocyanin content increased progressively with increasing salinity levels, although the magnitude of accumulation differed considerably among cultivars. The increase became more evident under higher salinity levels, particularly at 5.5 and 7.0 dS m^{-1} , suggesting enhanced metabolic adjustment under severe stress conditions.

Among the cultivars studied, sweet orange showed the highest anthocyanin accumulation under severe salinity stress, followed by kinnow, whereas grapefruit and lemon exhibited comparatively lower accumulation. At 7.0 dS m^{-1} salinity, anthocyanin content in sweet orange increased markedly and reached nearly 172 mg 100 g^{-1} , while kinnow also maintained substantially higher accumulation compared with grapefruit and lemon. In contrast, lemon exhibited only limited enhancement in anthocyanin synthesis under severe salinity stress, indicating comparatively weaker biochemical adaptation under saline conditions.

Enhanced anthocyanin biosynthesis under stress environments is generally considered an important adaptive response because these pigments help reduce oxidative injury by scavenging reactive oxygen species and protecting cellular membranes from oxidative damage. Anthocyanins may also contribute toward stabilization of chloroplast structures and protection of photosynthetic machinery under stressful conditions (Singh et al. 2019). Increased accumulation of these compounds under salinity stress has previously been reported in several fruit crops and stress-tolerant genotypes exposed to adverse environmental conditions (Gould 2004).

The comparatively greater anthocyanin accumulation observed in sweet orange and kinnow therefore suggests stronger protective metabolic adjustment and better stress adaptation under saline environments. In contrast, the relatively lower accumulation observed in grapefruit and lemon may reflect comparatively weaker antioxidative protection and greater susceptibility to salinity-induced oxidative damage. The results further indicate that activation of non-enzymatic antioxidant metabolism plays an important role in mitigating cellular injury under elevated salinity stress.

3.4.2 Flavonoid response and protective metabolic adjustment under saline conditions

Flavonoid content also increased progressively with increasing salinity in most citrus cultivars, although the response remained relatively moderate under lower salinity levels (Fig. 4). Differences between control and mild salinity treatments were comparatively small, indicating that lower salt concentrations exerted limited influence on flavonoid biosynthesis. However, under severe salinity stress, flavonoid accumulation increased substantially, particularly in sweet orange and kinnow, suggesting activation of stronger protective metabolic responses under elevated salt stress.

Among the cultivars, kinnow maintained the highest flavonoid accumulation under severe salinity conditions, followed by sweet orange, whereas lemon showed comparatively lower flavonoid content. Increased flavonoid synthesis under saline environments may be associated with their role in antioxidant defense, membrane stabilization, osmotic regulation, and detoxification of reactive oxygen species generated during stress conditions. Flavonoids are also known to participate in protection of membrane lipids and maintenance of cellular redox balance under environmental stress (Rao et al. 2021; Agati et al. 2012).

The comparatively lower flavonoid accumulation observed in lemon under high salinity stress indicates weaker biochemical defense response and greater vulnerability to oxidative injury. In contrast, enhanced accumulation of flavonoids in sweet orange and kinnow suggests more efficient protective metabolic regulation under saline environments. Similar increases in flavonoid biosynthesis under salt stress have also been reported in stress-tolerant horticultural crops where secondary metabolite accumulation contributes toward improved stress resilience and cellular protection (Nakabayashi et al. 2014).

Thus, the present findings demonstrate that salinity-induced enhancement of anthocyanins and flavonoids represents an important adaptive biochemical response in citrus cultivars. Sweet orange and kinnow exhibited comparatively stronger non-enzymatic antioxidant modulation and better protective metabolic adjustment under saline conditions than grapefruit and lemon, thereby reflecting greater physiological resilience under elevated salinity stress.

3.5 Multivariate analysis of salinity responses

3.5.1 Heatmap analysis of ionic, physiological and biochemical responses

Heatmap analysis provided an integrated visualization of cultivar-specific responses under salinity stress by simultaneously evaluating ionic, physiological, and biochemical traits. The clustering pattern clearly differentiated tolerant and sensitive citrus cultivars based on their overall response behavior under saline environments. Sweet orange and kinnow were grouped together with relatively higher K^+/Na^+ balance, chlorophyll stability, tissue hydration, and enhanced accumulation of stress-responsive metabolites, whereas grapefruit and lemon were more closely associated with increased Na^+ and SO_4^{2-} accumulation, membrane injury, and biomass reduction.

The heatmap further demonstrated strong association between excessive accumulation of toxic ions and physiological deterioration under severe salinity stress. Cultivars exhibiting greater Na^+ and sulfate accumulation also showed lower chlorophyll stability index, reduced relative water content, and higher relative stress injury, indicating close interaction between ionic imbalance and physiological disruption under saline conditions. In contrast, sweet orange and kinnow maintained comparatively favorable ionic balance together with better physiological stability and stronger biochemical adjustment. Distinct clustering of anthocyanins, flavonoids, chlorophyll stability, and K^+/Na^+ ratio in sweet orange and kinnow suggests that coordinated regulation of ionic homeostasis and protective metabolic responses contributed significantly toward improved stress adaptation in these cultivars. On the other hand, clustering of membrane injury, biomass reduction, and toxic ion accumulation with grapefruit and lemon reflects comparatively weaker adaptive regulation and greater susceptibility to salinity-induced stress. Figure. 5A showing heatmap analysis effectively summarized the complex interaction among ionic, physiological, and biochemical traits and further confirmed that maintenance of ionic balance together with stronger protective metabolic adjustment plays a central role in salinity tolerance among citrus cultivars.

3.5.2 Principal component analysis (PCA) of salinity tolerance traits

Principal component analysis (PCA) was performed to further examine the relationship among salinity-responsive traits and to identify the major variables contributing toward cultivar differentiation under saline conditions (Fig. 5B). The PCA biplot clearly separated citrus cultivars according to their tolerance behavior and revealed strong association among ionic, physiological, and biochemical parameters under salinity stress (Zou et al. 2016).

Stress-associated traits such as Na^+ accumulation, sulfate concentration, relative stress injury, and biomass reduction were positioned closer to grapefruit and lemon, indicating greater physiological disruption and ionic toxicity in these cultivars under severe salinity stress. In contrast, sweet orange and kinnow were more strongly associated with favorable K^+/Na^+ balance, chlorophyll stability, relative water content, anthocyanin accumulation, and flavonoid content, reflecting comparatively stronger adaptive regulation under saline environments. The PCA pattern further indicated that maintenance of ionic homeostasis was closely linked with physiological stability and biochemical protection under salinity stress. Traits associated with membrane stability and osmotic adjustment showed positive relationship with chlorophyll stability and protective metabolite accumulation, whereas toxic ion accumulation exhibited strong negative association with growth and physiological performance. Similar multivariate relationships among ionic balance, antioxidant metabolism, and stress tolerance have previously been reported in salt-tolerant horticultural crops subjected to saline environments (Petrov et al. 2018). The clear separation of cultivars in PCA space therefore confirms that salinity tolerance in citrus is governed by coordinated interaction

among ionic regulation, water relations, membrane stability, and stress-responsive biochemical mechanisms rather than by a single physiological trait. At the end, the multivariate analysis further strengthened the conclusion that sweet orange and kinnow possess comparatively greater adaptive resilience under saline conditions than grapefruit and lemon.

4. Conclusion

Salinity stress caused significant alterations in growth, physiological functioning, ionic balance, and biochemical responses in all citrus cultivars evaluated in the present investigation. Increasing salinity progressively reduced plant growth, chlorophyll stability, tissue hydration, and biomass accumulation, while accumulation of Na^+ and SO_4^{2-} ions, membrane injury, and stress-responsive metabolites increased under saline conditions. However, the magnitude of these responses differed considerably among cultivars. Sweet orange and kinnow exhibited comparatively greater tolerance to salinity stress through better maintenance of ionic homeostasis, higher K^+/Na^+ balance, improved chlorophyll stability, reduced membrane injury, and stronger accumulation of protective metabolites such as anthocyanins and flavonoids. In contrast, grapefruit and particularly lemon showed greater susceptibility to salinity-induced stress due to excessive toxic ion accumulation, greater physiological disruption, and weaker adaptive biochemical responses. The multivariate analysis further confirmed that salinity tolerance in citrus is governed by coordinated interaction among ionic regulation, membrane stability, water relations, and stress-responsive biochemical adjustment rather than by a single physiological trait. Maintenance of lower Na^+ accumulation together with improved nutrient balance and physiological stability emerged as the major determinants associated with salinity tolerance under saline environments. Collectively, the findings suggest that sweet orange and kinnow possess comparatively stronger adaptive resilience under saline conditions and may therefore be more suitable for cultivation in salt-affected regions. The study also provides useful physiological and ionic insights for future citrus improvement and selection programs aimed at developing salinity-resilient cultivars for sustainable production under increasing soil salinization.

Declarations

Author contributions: SR, RPD and RP conceptualized the research & designed the experiments; RPD contributed experimental materials; SR and MMB executed lab experiments and data collection; RPD analyzed and interpreted data; SR and MMB wrote the manuscript. All authors have read and reviewed the manuscript for final publication.

Funding: This work was funded by host institution only.

Data availability: The data will be shared upon request.

Acknowledgment

The authors sincerely acknowledge CCS Haryana Agricultural University, Hisar, Haryana, India, for providing research facilities and institutional support for conducting the present study.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript

Ethical approval: Not applicable

Consent to participate: Not applicable

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Tables

Table 1: Effect of progressive salinity stress on biomass accumulation, dry matter distribution, moisture retention, and root/shoot dynamics in citrus cultivars under saline conditions

Salinity (dS m ⁻¹)	Cultivar	Fresh leaf wt. (g/plant)	Dry leaf wt. (g/plant)	Dry stem wt. (g/plant)	Dry root wt. (g/plant)	Moisture retention (%)	Total dry biomass (g/plant)	Biomass reduction (%)	Root/shoot ratio	Salinity response
Control	S. Orange	41.9	21.5	9.5	7.6	48.7	38.6	–	0.25	N
2.5		38.5	20.1	8.9	6.7	47.8	35.7	7.5	0.23	SA
4.0		33.6	18.7	8.7	6.7	44.3	34.1	11.7	0.24	MA
5.5		32.2	11.1	6.0	4.7	34.5	21.8	43.5	0.28	SS
7.0		27.8	8.3	3.8	3.4	29.9	15.5	59.8	0.28	MT
Control	Grapefruit	21.5	12.8	5.5	5.0	59.5	23.3	–	0.27	N
2.5		23.7	12.4	5.1	4.7	52.3	22.2	4.7	0.27	SA
4.0		20.1	10.1	4.1	4.6	50.2	18.8	19.3	0.32	MA
5.5		18.5	7.2	3.5	3.2	38.9	13.9	40.3	0.30	SS
7.0		13.8	3.6	2.4	2.2	26.1	8.2	64.8	0.37	S
Control	Kinnow	37.7	14.5	6.7	5.5	38.5	26.7	–	0.26	N
2.5		34.0	14.4	6.1	5.4	42.4	25.9	3.0	0.26	SA
4.0		31.3	14.2	5.2	4.5	45.4	23.9	10.5	0.23	MA
5.5		24.4	10.6	4.5	3.6	43.4	18.7	30.0	0.24	SS
7.0		21.8	9.5	3.3	2.2	43.6	15.0	43.8	0.17	MT
Control	Lemon	16.3	10.7	4.4	3.8	65.6	18.9	–	0.25	N
2.5		17.5	10.1	3.3	3.3	57.7	16.7	11.6	0.25	SA
4.0		14.8	10.0	2.4	3.2	67.6	15.6	17.5	0.26	MA
5.5		12.2	6.4	1.7	1.6	52.5	9.7	48.7	0.20	SS
7.0		8.2	2.2	1.0	1.0	26.8	4.2	77.8	0.31	HS

N = Normal; SA = Slightly affected; MA = Moderately affected; SS = Salt stressed; MT = Moderately tolerant; S = Sensitive; HS = Highly sensitive.

Table 2: Effect of progressive salinity stress on leaf ionic composition and nutrient homeostasis (Na⁺, K⁺, Ca²⁺, Mg²⁺ and SO₄²⁻ concentrations; mg L⁻¹) in different citrus cultivars under saline conditions.

Cultivar	Ion	Control (0.12 dS m ⁻¹)	2.5 dS m ⁻¹	4.0 dS m ⁻¹	5.5 dS m ⁻¹	7.0 dS m ⁻¹	Change (%)
Sweet orange	Na ⁺	2434	4841	7012	9822	10112	+75
	Ca ²⁺	15377	13789	11453	10141	9032	-41
	Mg ²⁺	5602	4858	4298	3940	3715	-34
	K ⁺	22740	20553	18942	16324	15008	-34
	SO ₄ ²⁻	2558	2923	3287	3590	3955	+35
Grapefruit	Na ⁺	2612	9322	13510	17201	22982	+89
	Ca ²⁺	18930	16467	13002	11345	10387	-45
	Mg ²⁺	5314	4760	4190	3765	3390	-36
	K ⁺	19254	17226	14662	11976	10214	-47
	SO ₄ ²⁻	2387	2875	3345	3715	4148	+42
Kinnow	Na ⁺	2564	6306	8466	10285	12456	+79
	Ca ²⁺	20345	17834	16385	14677	12600	-38
	Mg ²⁺	6016	5665	5123	4789	4385	-27
	K ⁺	25265	23214	21089	18766	16633	-34
	SO ₄ ²⁻	2564	3012	3324	3608	3798	+32
Lemon	Na ⁺	2187	7289	12800	14398	17391	+87
	Ca ²⁺	19890	17623	13321	10899	9644	-51
	Mg ²⁺	6235	5423	4789	4212	3935	-37
	K ⁺	17812	15435	12980	10824	8978	-50
	SO ₄ ²⁻	2188	2619	3018	3488	3645	+40

Table 3: Influence of progressive salinity stress on root ionic distribution and mineral nutrient status (Na⁺, K⁺, Ca²⁺, Mg²⁺ and SO₄²⁻ concentrations; mg L⁻¹) in citrus cultivars under saline environments.

Cultivar	Ion	Control (0.12 dS m ⁻¹)	2.5 dS m ⁻¹	4.0 dS m ⁻¹	5.5 dS m ⁻¹	7.0 dS m ⁻¹	Change (%)
Sweet orange	Na ⁺	5325	8478	14632	23124	26340	+79
	Ca ²⁺	20377	18634	16576	13877	11246	-45
	Mg ²⁺	1698	1567	1421	1246	1034	-39
	K ⁺	11657	10891	9987	9123	8726	-25
	SO ₄ ²⁻	3488	3816	4381	5087	5912	+41
Grapefruit	Na ⁺	3054	7612	16782	31018	50798	+94
	Ca ²⁺	21930	18923	15929	12734	9771	-55
	Mg ²⁺	1474	1341	1209	1078	876	-40
	K ⁺	9756	9032	8312	7243	6143	-37
	SO ₄ ²⁻	2956	3207	3811	4617	5686	+48
Kinnow	Na ⁺	4943	9812	14876	19276	27398	+82
	Ca ²⁺	22345	19972	17326	14724	11527	-48
	Mg ²⁺	1945	1813	1678	1523	1381	-29
	K ⁺	11008	10543	9826	9081	8351	-24
	SO ₄ ²⁻	3582	3918	4417	5086	6106	+41
Lemon	Na ⁺	2973	6789	14127	32786	55879	+96
	Ca ²⁺	22090	19246	15975	12763	9217	-58
	Mg ²⁺	1569	1423	1231	1054	893	-43
	K ⁺	8537	7917	7121	6024	5081	-40
	SO ₄ ²⁻	2380	2812	3367	4117	5112	+53

Figures

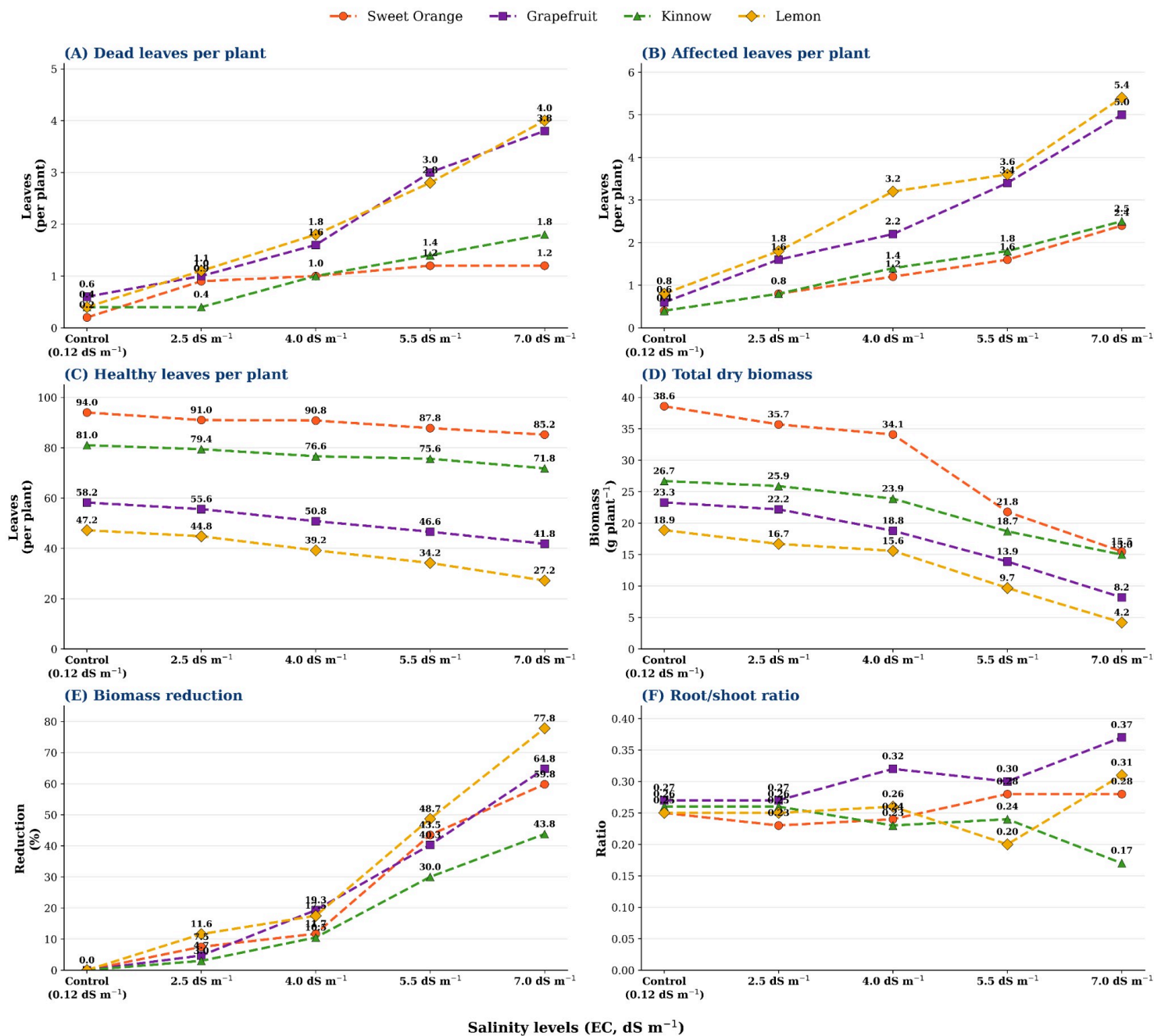


Figure 1

Effect of progressive salinity stress on vegetative growth and foliar injury responses of citrus cultivars under saline conditions. Data represent changes in plant height, stem diameter, healthy leaves, affected leaves, and dead leaves under increasing salinity levels.

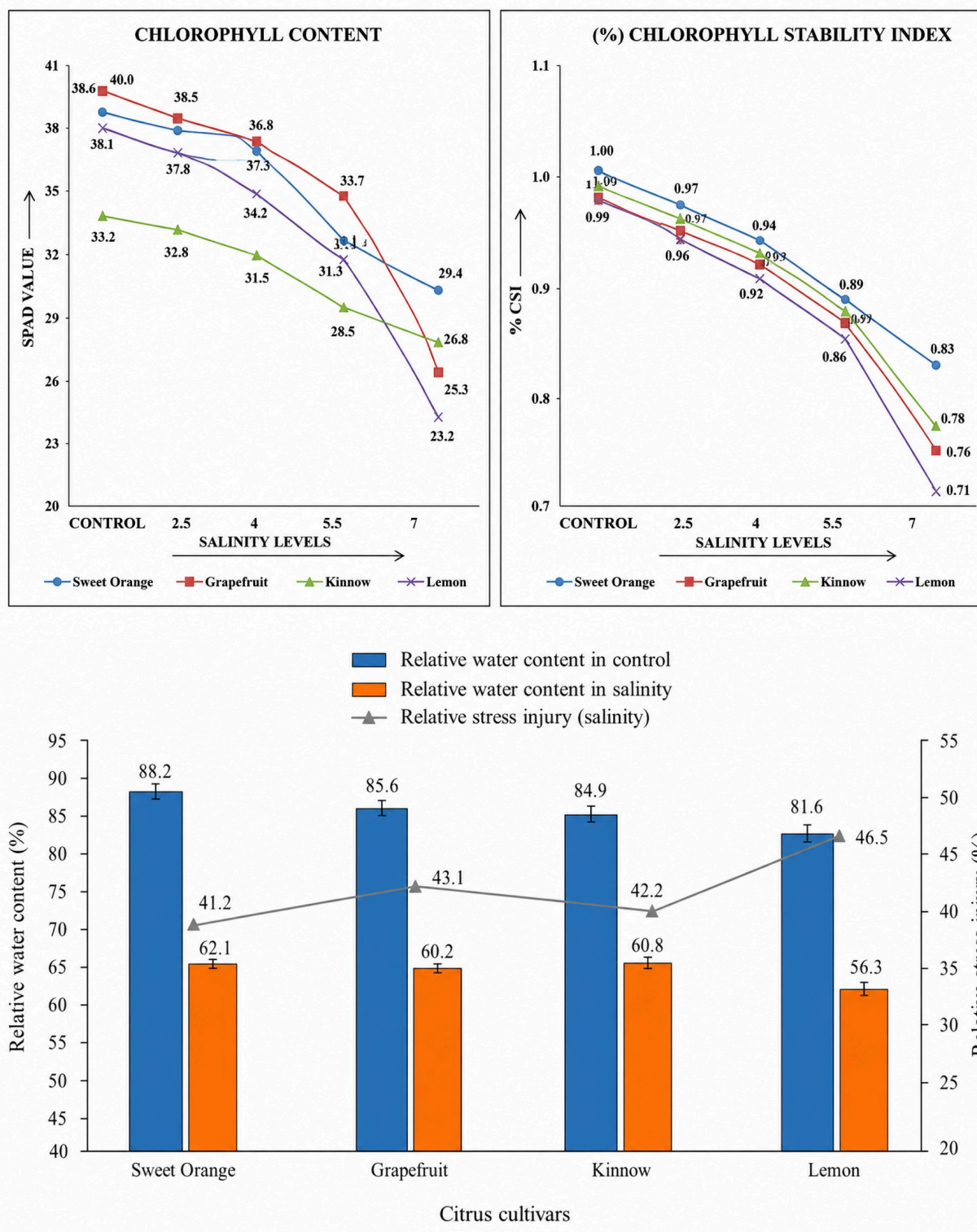


Figure 2

Effect of salinity stress on chlorophyll content, chlorophyll stability index (CSI), relative water content (RWC), and relative stress injury (RSI) in different citrus cultivars under saline environments.

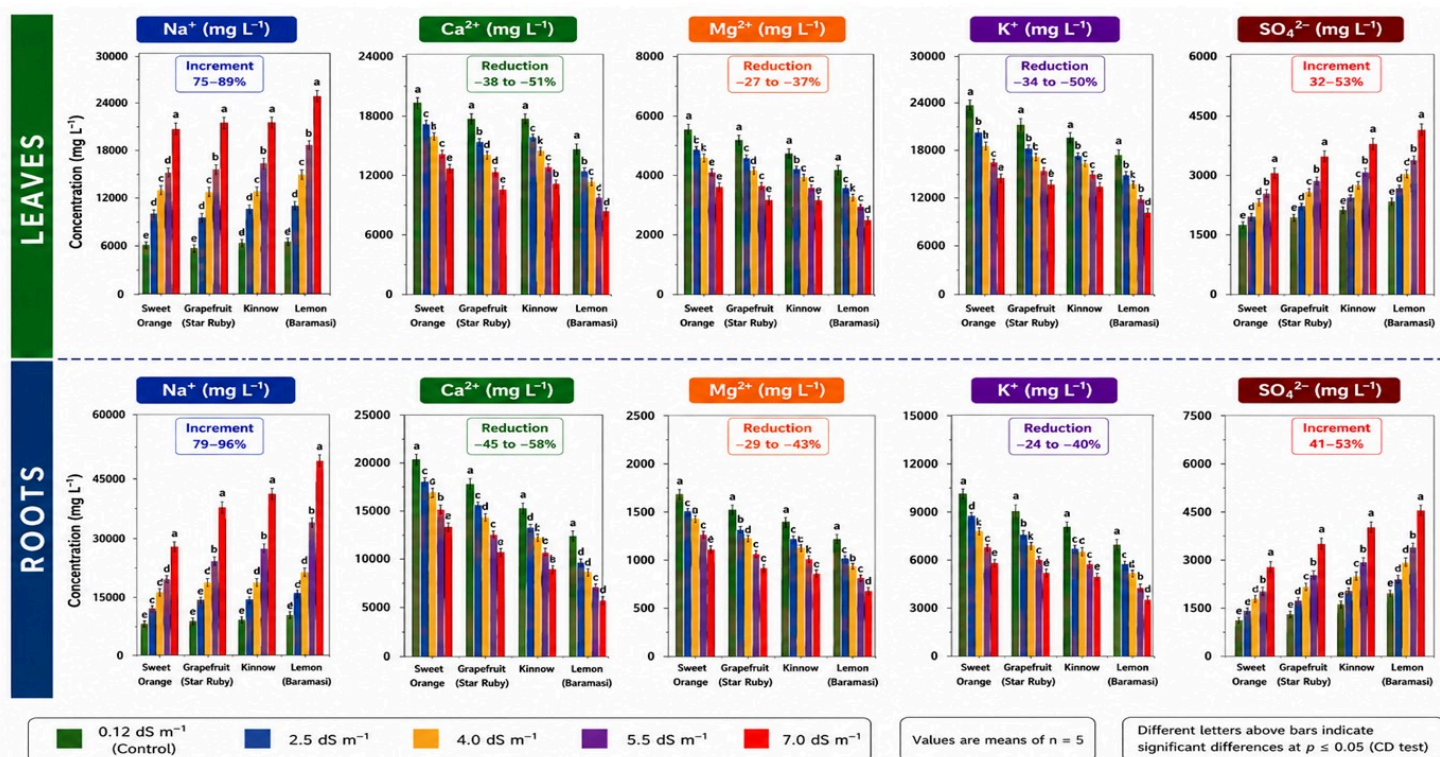


Figure 3

Influence of progressive salinity stress on leaf and root ionic composition of citrus cultivars showing variation in Na⁺, K⁺, Ca²⁺, Mg²⁺, and SO₄²⁻ concentrations under saline conditions

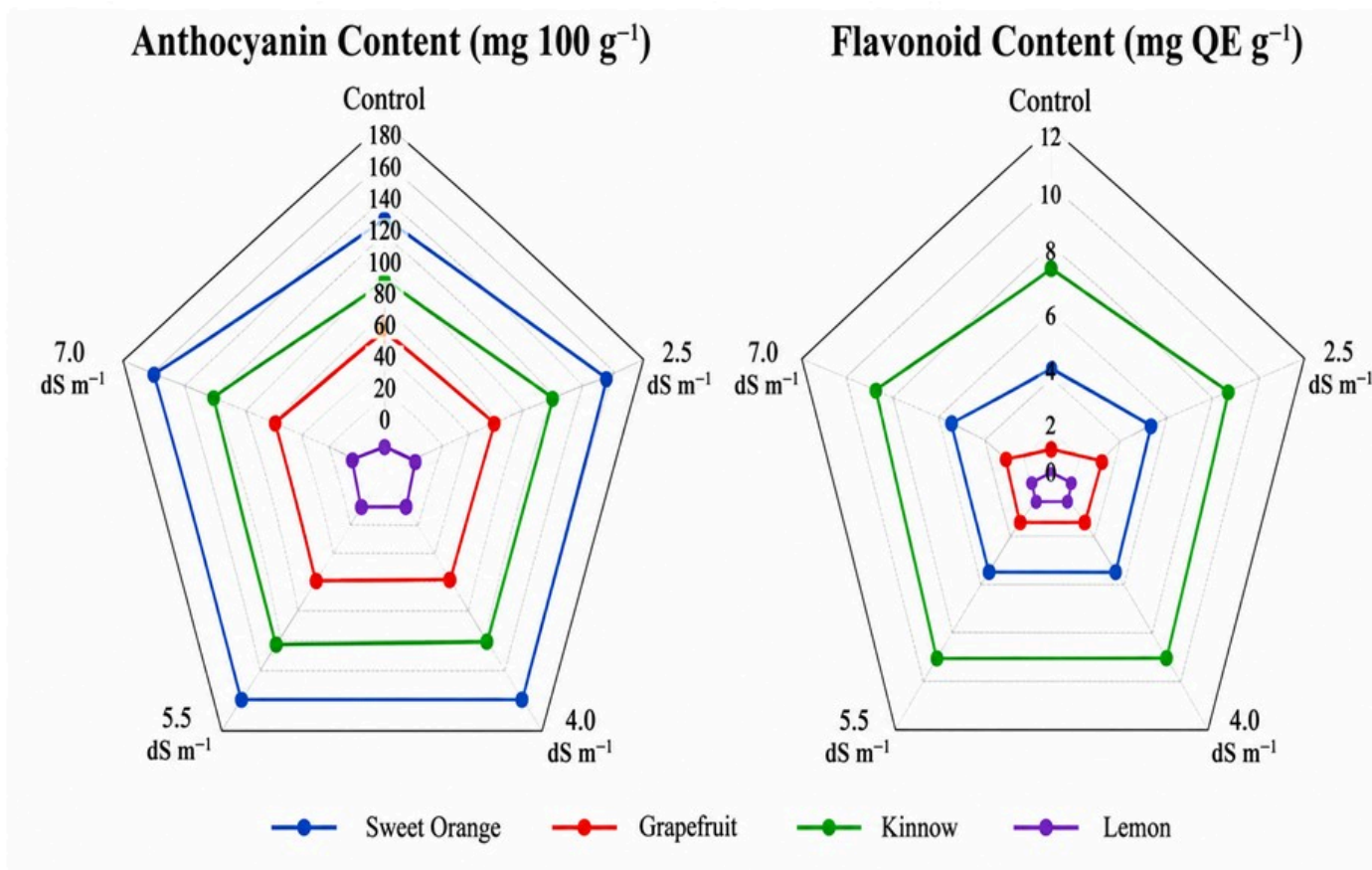


Figure 4

Effect of salinity stress on anthocyanin and flavonoid accumulation in citrus cultivars under progressive saline environments.

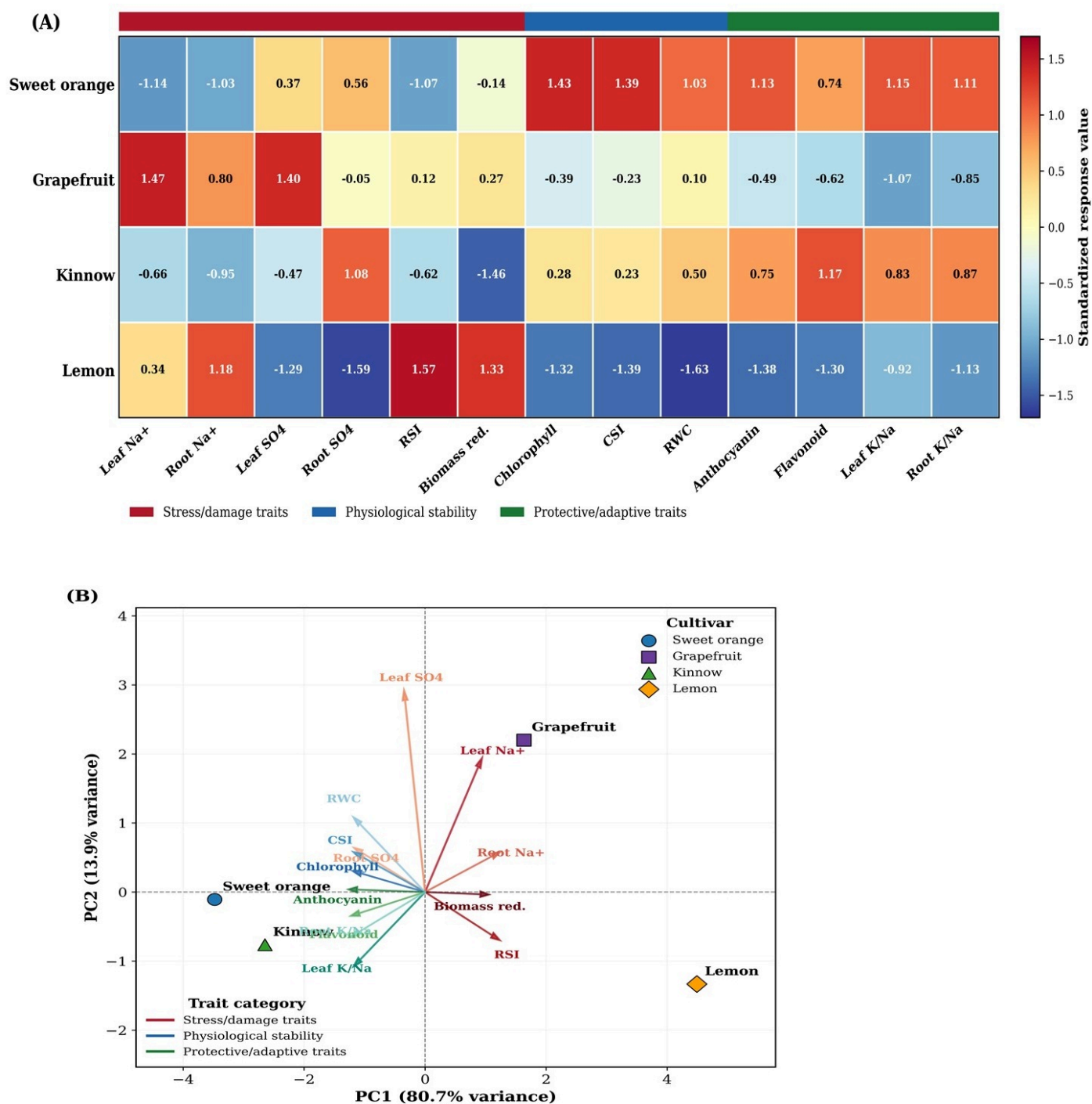


Figure 5

(A): Heatmap illustrating integrated ionic, physiological, and biochemical responses associated with salinity tolerance in different citrus cultivars under saline condition.

(B): Principal component analysis (PCA) showing association among ionic, physiological, and biochemical traits contributing to salinity tolerance and cultivar differentiation under saline environments.