Ion Response of Sunflower Seedlings to Composite Salt Stress: Postprint

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Date: 2017-11-09T00:00:00+00:00

Abstract

Investigating the ionic response mechanisms of sunflower salt tolerance can provide a scientific basis for rapid screening of salt-tolerant sunflower varieties. This experiment used oil sunflower salt-sensitive variety 'YK18', moderately salt-tolerant variety 'YK06', and salt-tolerant variety 'GF01' as test materials to investigate seed germination and ion accumulation and distribution in germinating seedlings under composite salt (NaCl and Na SO mixed at a 9:1 molar ratio) concentrations of 0 mmol · L ^{1}, 50 mmol · L ^{1}, 100 mmol · L ^{1}, 150 mmol · L ^{1}, 200 mmol · L ^{1}, and 250 mmol · L ^{1}, and utilized ion flux detection technology to dynamically monitor the flow rate and direction of K , Na , Ca^{2} and other ions in plant roots after 24 h of composite salt stress. The results showed that composite salt stress inhibited sunflower seed germination, causing a decrease in germination rate and prolongation of average germination time. After salt stress, sunflower roots exhibited massive K efflux, with the flow rate being 'YK18' > 'YK06' > 'GF01'; as salt stress concentration increased, the Na flow rate in roots shifted from influx to efflux; during influx, 'YK18' showed the highest rate, followed by 'YK06', and 'GF01' the lowest, while during efflux, 'GF01' exhibited the highest flow rate, with its "salt exclusion" phenomenon being particularly evident. After composite salt stress, whole-plant Na accumulation increased, K decreased, and K /Na ratio declined with increasing salt concentration; under low salt concentrations (<150 mmol · L ^{1}), the K /Na ratio in stems of 'GF01' and 'YK06' was lower than that of 'YK18'; under high salt stress (150 mmol · L ^{1}), 'GF01' showed the least whole-plant Na accumulation and the highest leaf K /Na ratio. Additionally, under salt stress, the Ca^{2} absorption rate in sunflower seedling roots accelerated, with 'GF01' being 2 times that of 'YK18'. Thus, oil sunflower plants with different salt tolerances can adapt to salt stress environments by regulating the uptake and efflux of Na , K , and Ca^{2}; salt-tolerant varieties possess stronger K retention capacity and enhance their salt stress tolerance through Na compartmentalization (under low salt stress) and salt exclusion mechanisms (under high salt stress), main-

taining a reasonable K /Na ratio in plant leaves. The findings of this study can provide a theoretical basis for screening and cultivation of salt-tolerant varieties in saline-alkali soils.

Full Text

Ion Response of Sunflower at Sprouting Stage to Mixed Salt Stress

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Abstract

Understanding the ionic response mechanisms underlying sunflower salt tolerance provides a scientific basis for rapid screening of salt-resistant sunflower varieties. This study examined seed germination and ion accumulation patterns in three oil sunflower varieties with contrasting salt tolerance: salt-sensitive 'YK18' , moderately tolerant 'YK06' , and highly tolerant 'GF01' . Seeds were subjected to mixed salt stress (NaCl and Na2SO at a 9:1 molar ratio) at concentrations of 0, 50, 100, 150, 200, and 250 mmol · L [1]. Using scanning ion-selective electrode technique (SIET), we dynamically monitored K , Na , and Ca[2] fluxes in root systems after 24 hours of salt stress. Results demonstrated that mixed salt stress inhibited sunflower seed germination, reducing germination rates and prolonging average germination time. Under salt stress, sunflower roots exhibited substantial K efflux, with efflux rates following the pattern 'YK18' > 'YK06' > 'GF01' . As salt concentration increased, Na flux in roots shifted from influx to efflux. During the influx phase, 'YK18' showed the highest rate, followed by 'YK06' , while 'GF01' had the lowest rate. During the efflux phase, 'GF01' exhibited the highest rate, demonstrating a clear "salt exclusion" phenomenon. Following salt stress, whole-plant Na accumulation increased while K decreased, resulting in declining K /Na ratios with increasing salt concentration. At low salt concentrations (<150 mmol · L [1]), 'GF01' and 'YK06' showed lower K /Na ratios in stems compared to 'YK18' . Under high salt stress (150 mmol · L [1]), 'GF01' accumulated the least Na in whole plants while maintaining the highest K /Na ratio in leaves. Additionally, Ca[2] uptake rates accelerated under salt stress, with 'GF01' showing rates double those of 'YK18' . These findings indicate that sunflower varieties with different salt tolerance

levels adapt to saline environments by regulating Na , K , and Ca^2 absorption and efflux. Salt-tolerant varieties possess stronger K retention capacity and enhance salt tolerance through Na compartmentalization (under low salt stress) and salt exclusion mechanisms (under high salt stress), thereby maintaining appropriate K /Na ratios in leaves. This study provides theoretical guidance for screening and cultivating salt-tolerant varieties in saline-alkali soils.

Keywords: Oil sunflower; Sprouting stage; Mixed salt stress; Ion flux; Salt tolerance

Introduction

Soil salinization has become a global environmental issue, with increasingly limited arable land worldwide. Enhancing crop salt tolerance represents an effective approach for utilizing saline-alkali soils. Oil sunflower (*Helianthus annuus* L.) offers advantages including short growth periods, strong stress resistance, and stable yields, enabling normal growth and reproduction in moderately saline soils. Cultivating oil sunflower not only provides economic benefits but also gradually improves the physicochemical properties of saline soils, making it an ideal crop for such environments. Therefore, investigating the mechanisms of sunflower salt tolerance is crucial for variety selection and cultivation management.

Numerous studies have examined sunflower growth and physiological characteristics under salt stress, significantly contributing to our understanding of salt tolerance mechanisms and evaluation indicators. Research has shown that salt stress inhibits seed germination across sunflower varieties, with inhibitory effects intensifying as salt concentration increases. Salt stress also affects leaf area, plant height, membrane permeability, and reactive oxygen species scavenging capacity. Some researchers have identified biomass under salt stress as a key indicator for evaluating sunflower salt tolerance in the Hetao Irrigation District. The detrimental effects of salt on crops—including ion toxicity, osmotic stress, and nutritional imbalance—are directly related to plant regulation of inorganic ion absorption, transport, and distribution. Investigating salt tolerance mechanisms from the perspective of ion uptake and allocation provides more direct insights into plant salt tolerance evaluation.

"Na exclusion" and "Na compartmentalization" represent primary physiological characteristics of plant salt tolerance. Many studies suggest that salt tolerance mechanisms in oil sunflower are similar to those in cotton (*Gossypium* spp.), with restricting Na transport to shoots being a major factor contributing to sunflower salt tolerance. Zheng et al. found that compartmentalizing Na primarily in stems and roots maintains appropriate K/Na ratios in leaves, and that plants exhibit strong selectivity for K during ion uptake and transport. Other researchers reported that under seawater stress, K /Na ratios in sunflower seedlings consistently remained highest in leaves and lowest in roots, indicating that roots and stems intercept Na while roots selectively absorb and transport

K to shoots under low seawater stress. These studies collectively demonstrate that Na compartmentalization is a crucial feature of sunflower salt tolerance, though reports of "Na exclusion" in sunflower under salt stress remain scarce.

Ion flux detection technology, particularly the non-invasive scanning ion-selective electrode technique (SIET), enables measurement of ion movement rates and directions in the rhizosphere without damaging plant tissues. Compared to static information such as ion concentrations and contents, ion flux detection more intuitively reveals physiological mechanisms underlying plant responses to stress. This study employed mixed salt stress to simulate field soil salinity, investigating seed germination in oil sunflower varieties with different salt tolerance levels and examining Na and K absorption and accumulation using SIET. Our objective was to further elucidate the salt tolerance mechanisms of oil sunflower from an ion uptake perspective, providing theoretical guidance for screening salt-alkali tolerant varieties, promoting their adoption, and informing production practices in saline regions.

Materials and Methods

1.1 Experimental Materials Three oil sunflower varieties were used: highly salt-tolerant 'GF01', moderately salt-tolerant 'YK06', and salt-sensitive 'YK18', all provided by Inner Mongolia Agricultural University.

1.2.1 Germination Test Under Mixed Salt Stress NaCl and Na SO were mixed at a 9:1 molar ratio to create five treatment concentrations (S1–S5): 50, 100, 150, 200, and 250 mmol·L ^{1}, with distilled water as the control (CK). Each treatment had three replicates. The germination test procedure was as follows: Uniform, plump sunflower seeds were surface-sterilized with 0.1% HgCl , then placed in 12.0 cm diameter petri dishes lined with two filter papers as a germination bed. Fifty seeds were evenly distributed in each dish, and different salt solutions were added to moisten the filter paper and seeds. Dishes were covered and incubated at 25°C in a constant-temperature illuminated incubator. Filter papers were replaced daily to maintain constant salt concentration. Germination counts were recorded daily (fungal contamination was promptly removed to prevent effects on germination), with germination defined as radicle emergence reaching half the seed length. Germination parameters were calculated as follows:

Germination rate (%) = (Final germinated seed number / Total seeds tested) × 100
Germination potential (%) = (Seeds germinated by day 3 / Total seeds tested) × 100
Germination index = Σ(G /D)
Average germination time = Σ(G × D)/G

where G represents the number of germinated seeds on day t, D is the corresponding germination day, and G is the germination rate.

1.2.2 Hydroponic Sunflower Cultivation Uniform, plump seeds were surface-sterilized with 0.1% HgCl for 8 minutes, rinsed thoroughly with sterile water to remove residual HgCl , and placed in 12.0 cm petri dishes lined with two filter papers moistened with deionized water. After cotyledon emergence and radicle growth to 3–5 cm, seedlings were transferred to half-strength Hoagland nutrient solution for hydroponic culture. When plants developed one pair of true leaves, different concentrations of mixed salt (NaCl + Na SO) were added. After 24 hours of salt stress, ion fluxes (K , Na , Ca^2) at the root surface and K , Na contents in leaves, stems, and roots were measured.

1.2.3 Inorganic Ion Flux Detection Ca^2 , Na , and K fluxes were measured using ion flux detection technology (Portable Plant Microscopic Dynamic Ion Flux Detection System AI-IFDD-I, Beijing Research Center for Information Technology in Agriculture). Glass microelectrodes with tip diameters of 4–5 µm were silanized and backfilled with approximately 10–15 mm of corresponding ion filling solution (Ca^2 : 100 mmol · L^{-1} CaCl ; Na : 250 mmol · L^{-1} NaCl; K : 100 mmol · L^{-1} KCl). Selective ion exchangers were then front-loaded into the electrode tips (K : Potassium ionophore I-cocktail A; Na : Sodium ionophore II-cocktail A; Ca^2 : Calcium ionophore I-cocktail A; all from Sigma-Aldrich, St. Louis, MO 63103, USA). The ion exchanger columns measured approximately 20–40 µm for Ca^2 and Na , and about 180 µm for K . Fabricated electrodes were mounted on chlorinated Ag/AgCl electrode holders with the silver wire immersed in the backfill solution to ensure electrolyte connection to the preamplifier. A solid electrode served as the reference. Microelectrodes were calibrated before use; K and Na Nernst slopes around 58 and Ca^2 around 29 were considered acceptable. Calibration solutions were: Ca^2 –0.05 and 0.5 mmol · L^{-1} CaCl ; K –0.1 and 1 mmol · L^{-1} KCl; Na –0.5 and 5 mmol · L^{-1} NaCl. The test solution for all three ions contained 0.1 mmol · L^{-1} KCl, 0.1 mmol · L^{-1} CaCl , 0.1 mmol · L^{-1} MgCl , 0.5 mmol · L^{-1} NaCl, 0.2 mmol · L^{-1} Na SO , and 0.3 mmol · L^{-1} MES at pH 6.0.

For measurements, sunflower seedling roots were placed in test solution and secured with small glass blocks. The measurement region was the root meristematic zone approximately 10 mm from the root tip, as cells in this zone are sensitive and can rapidly and accurately reflect ion uptake under salt stress. The electrode tip was positioned 10 µm from the root surface as the origin and moved reciprocally between two points 30 µm apart perpendicular to the root surface to obtain voltage differences [Figure 1: see original paper]. Each sample was measured stably for over 10 minutes with six replicates, and results were averaged.

1.2.4 K and Na Content in Plants Under Mixed Salt Stress Ion content in plant tissues was determined using flame photometry following Hou et al. Fresh sunflower seedling leaves, stems, and roots (5.00 g each) were collected, killed at 110.5°C for 30 minutes, dried to constant weight at 70–80°C, ground, and sieved through a 420 µm mesh. One gram of dry sample was mixed with

30 mL deionized water, shaken, heated in a boiling water bath for 2 hours, cooled, and brought to 50 mL volume for measurement. Three replicates were performed and results averaged.

Results

2.1 Effects of Mixed Salt Stress on Sunflower Seed Germination Under control conditions, all three varieties germinated normally . Salt stress reduced germination rate, germination index, and germination potential while extending average germination time. At low concentration (50 mmol · L ^{1}), germination rates and indices decreased with increasing salt concentration. The salt-sensitive variety 'YK18' showed significantly reduced germination rates compared to 'GF01' . When salt concentration increased to 150 mmol · L ^{1}, 'YK18' germination rate decreased by 22.13%, while 'GF01' decreased by only 17.58%. At 200 mmol · L ^{1}, 'YK18' germination rate fell below 50%, and at 250 mmol · L ^{1}, both 'YK06' and 'YK18' germination rates dropped below 30%, while 'GF01' maintained 48.16% germination. Under identical salt treatments, 'YK18' showed the longest average germination time and 'GF01' the shortest, with significant differences among varieties ($P < 0.05$).

2.2 Effects of Mixed Salt Stress on K Flux at Sunflower Root Surface Under normal growth conditions, oil sunflower roots absorbed K , with the salt-tolerant variety 'GF01' showing the highest absorption rate, followed by moderately tolerant 'YK06' , and salt-sensitive 'YK18' the lowest. After 24 hours of mixed salt stress, K efflux occurred at the root surface, with efflux rates increasing with salt concentration [Figure 2: see original paper]. The salt-tolerant variety 'GF01' exhibited the lowest K efflux rate, while 'YK18' showed the highest, with significant inter-varietal differences. At 150 mmol · L ^{1} salt treatment, 'GF01' K efflux rate was approximately 112 pmol · cm ^{2} · s ^{1}, representing 2.00-fold and 1.43-fold increases compared to 50 and 100 mmol · L ^{1} concentrations, respectively. The salt-sensitive variety 'YK18' showed K efflux rates at 150 mmol · L ^{1} that were 3.19-fold and 1.73-fold higher than at 50 and 100 mmol · L ^{1}, respectively, with a greater magnitude of increase than 'GF01' . At 200 mmol · L ^{1}, 'GF01' and 'YK06' showed similar K efflux rates, both significantly lower than 'YK18' ($P < 0.05$). Using 150 mmol · L ^{1} treatment as an example, K efflux rates in all three varieties showed minor fluctuations but remained generally stable, reflecting the immediate physiological status of different varieties [Figure 3: see original paper].

2.3 Effects of Mixed Salt Stress on Na Uptake at Sunflower Root Surface Under normal growth conditions, all varieties showed minimal Na uptake. With increasing mixed salt concentration, a pattern of initial influx followed by efflux emerged [Figure 4: see original paper]. At low salt concentrations (50–100 mmol · L ^{1}), Na influx occurred in root systems. When salt concentration reached 150 mmol · L ^{1}, all three varieties shifted to Na efflux,

with maximum efflux rates reaching 7,294 pmol · cm ^{2} · s ^{1} at 200 mmol · L ^{1} [Figure 5: see original paper]. Significant inter-varietal differences were observed: during the influx phase, 'GF01' showed the lowest rate (897 pmol · cm ^{2} · s ^{1} at 50 mmol · L ^{1}, representing 39% of 'YK06' and 19.9% of 'YK18'). At 100 mmol · L ^{1}, all three varieties showed increased Na influx rates, but 'GF01' remained the lowest. During the efflux phase, 'GF01' exhibited the highest rate: at 200 mmol · L ^{1}, its average Na efflux rate was 6,512 pmol · cm ^{2} · s ^{1}, 3.64-fold higher than 'YK06' and 5.78-fold higher than 'YK18' .

2.4 Effects of Mixed Salt Stress on Ca² in Sunflower Under normal growth conditions, plants showed minimal Ca² influx at slow rates. Mixed salt stress significantly accelerated Ca² uptake rates, which correlated positively with salt concentration. Significant inter-varietal differences existed, following the pattern 'GF01' > 'YK06' > 'YK18' [Figure 6: see original paper]. The Ca² uptake rate in salt-tolerant varieties increased more rapidly with salt concentration; at 150 mmol · L ^{1}, 'GF01' Ca² uptake rate was double that of 'YK18' [Figure 7: see original paper].

2.5.1 K Accumulation and Distribution Compared to the control, K accumulation in all tissues decreased significantly under low salt concentration (50 mmol · L ^{1}). At 100 mmol · L ^{1}, total plant K accumulation recovered, but decreased again under high salt concentrations (150 mmol · L ^{1}), with all three varieties showing similar trends [Figure 8: see original paper]. These results align with the rapid increase in K efflux detected at the root surface under 150 mmol · L ^{1} salt stress [Figure 2: see original paper]. Among the three varieties, 'GF01' showed the highest total K accumulation. Under high salt stress (150 mmol · L ^{1}), inter-varietal differences in K distribution became apparent: 'GF01' showed significantly reduced K accumulation in roots and stems but relatively stable levels in leaves. At 200 mmol · L ^{1}, 'GF01' root K decreased sharply to 27.63% of control levels, while leaf K content remained high (4.02 mg · g ^{1}), even exceeding control levels (3.39 mg · g ^{1}) and accounting for 48% of total plant K accumulation. In contrast, 'YK06' and 'YK18' leaf K accumulation accounted for only 28.6% and 31.6% of total plant K , respectively.

2.5.2 Na Accumulation and Distribution Under normal conditions, total plant Na accumulation was low (approximately 0.15 mg · g ^{1}). Mixed salt stress increased Na accumulation in roots, stems, and leaves [Figure 9: see original paper], with varying degrees of increase among varieties and tissues. At low salt concentrations (100 mmol · L ^{1}), Na content in roots and stems exceeded that in leaves across all varieties. Inter-varietal comparisons revealed smaller differences in whole-plant, leaf, and root Na accumulation, but significant differences in stem Na accumulation: the salt-tolerant variety 'GF01' accumulated 1.628 mg · g ^{1}, significantly higher than the other two varieties (1.215 and 1.189 mg · g ^{1}). At high salt concentrations (150 mmol · L ^{1}), inter-varietal differences in stem and root Na accumulation were minimal, but leaf Na accumulation in the

salt-sensitive variety 'YK18' increased rapidly to 2.30 mg · g ^{1}, 1.6-fold higher than 'GF01' and 'YK06' . At 200 mmol · L ^{1}, total Na content in 'YK06' and 'YK18' increased dramatically to 11.01 and 11.04 mg · g ^{1}, respectively, significantly higher than 'GF01' (9.41 mg · g ^{1}). Notably, leaf Na content in moderately tolerant 'YK06' approached 'YK18' levels (approximately 4.10 mg · g ^{1}), 1.75-fold higher than 'GF01' . Additionally, at 200 mmol · L ^{1}, 'GF01' root Na content significantly exceeded leaf Na content, while the other varieties showed similar Na levels in roots and leaves.

2.5.3 Changes in K /Na Ratio Mixed salt stress significantly reduced K /Na ratios in all organs across the three varieties [Figure 10: see original paper]. As salt concentration increased, K /Na ratios gradually declined, but the extent of reduction varied among varieties. In 'GF01' , K /Na ratios showed leaf > root > stem at 0–50 mmol · L ^{1}, shifting to leaf > stem > root at 100 mmol · L ^{1}, maintaining the highest K /Na ratio in leaves under salt stress. In 'YK06' , the pattern was leaf > stem > root at 50–100 mmol · L ^{1}, changing to stem > leaf > root at 150 mmol · L ^{1}, with leaf K /Na ratios declining significantly at 150 mmol · L ^{1}. In 'YK18' , the pattern was leaf > stem > root at 50 mmol · L ^{1}, becoming stem > leaf > root at 100 mmol · L ^{1}, with leaf K /Na ratios declining markedly at 100 mmol · L ^{1}. Thus, leaf K /Na ratio can serve as an indicator of damage severity from mixed salt stress in sunflower.

Discussion

3.1 Seed Germination and Growth of Different Sunflower Varieties Under Mixed Salt Stress Salt stress inhibits seed water imbibition and expansion, causing delayed germination. Studies by Wang et al. and Liu et al. on sunflower under salt stress demonstrated significantly reduced germination and emergence rates with delayed emergence times. At low salt concentrations (40–120 mmol · L ^{1}), sunflower seeds showed delayed emergence without significant differences in emergence or seedling rates, whereas high salt stress (160–240 mmol · L ^{1}) caused both delayed emergence and significantly reduced rates. Sang et al. found that sugar beet (*Beta vulgaris* L.) under Na SO + NaCl mixed salt stress showed declining germination rates significantly different from controls. In this study, sunflower under mixed salt stress (NaCl + Na SO) exhibited reduced germination rates, indices, and potential, with extended average germination time. These results align with previous research, likely because reduced water potential under salt stress impedes seed imbibition. As salt concentration increases, plant cell membrane structure is damaged, causing substantial solute leakage and toxic Na influx that leads to cellular dehydration and affects germination. Additionally, this study found that at 200 mmol · L ^{1}, the salt-tolerant variety maintained nearly 50% germination, while the salt-sensitive 'YK18' barely germinated. The salt-sensitive variety 'YK18' showed the longest average germination time, while 'GF01' showed the shortest, with significant inter-varietal differences ($P < 0.01$), likely determined by genotype.

3.2 K and Na Uptake in Different Sunflower Varieties Under Mixed Salt Stress Inorganic ions are essential for plant growth and development. Research indicates that all plants possess both "salt absorption" and "salt exclusion" mechanisms to varying degrees, which directly or indirectly affect ion absorption, accumulation, and compartmentalization. Under salt stress, plants absorb more Na and Cl while reducing K , Ca^2 , and Mg^2 uptake, disrupting internal ionic balance. K is a crucial nutrient for maintaining normal plant growth, and many scholars believe that plant salt resistance depends partly on K supply or retention capacity. Using SIET for real-time dynamic monitoring, this study found that K flux at the root surface shifted rapidly from slight influx to efflux under mixed salt stress, with efflux rates increasing with salt concentration. Concurrent measurements showed reduced K accumulation in root systems, confirming K loss under salt stress. The salt-tolerant variety 'GF01' exhibited the lowest K efflux rate and highest root K accumulation, confirming superior K retention capacity under salt stress.

Many studies consider controlling Na absorption and distribution as an effective salt tolerance mechanism. Primary pathways include preferential Na accumulation in roots and stems to prevent leaf accumulation, extrusion through plasma membranes, or compartmentalization in vacuoles via tonoplast Na /H antiporters to reduce damage. At low salt concentrations (100 $mmol \cdot L^{1}$), all varieties showed Na influx, with 'GF01' showing the lowest influx rate and whole-plant Na accumulation. This variety intercepted Na in stems to maintain relatively low leaf Na levels, consistent with Hou et al.' s findings on K and Na uptake and distribution in sunflower under salt stress. This compartmentalization likely results from high expression of cytoplasmic Na /H antiporters in stem cells that store Na in vacuoles, reducing transport to shoots. This study also revealed that at higher salt concentrations (150 $mmol \cdot L^{1}$), plants exhibited "salt exclusion," more pronounced in salt-tolerant varieties. 'GF01' showed the highest Na efflux rate and lowest Na accumulation in whole plants and leaves, demonstrating the strongest salt tolerance. The other two varieties showed slower Na efflux and higher Na accumulation, indicating poorer salt tolerance. At high salt concentration (200 $mmol \cdot L^{1}$), 'GF01' root Na content significantly exceeded leaf Na , while other varieties showed similar root and leaf Na levels, consistent with Han et al.' s findings in salt-tolerant maize. Thus, the ability of salt-tolerant varieties to intercept Na in roots and stems constitutes a primary mechanism for strong salt tolerance.

Due to similar hydrated ionic radii, Na competes with K for transmembrane transport sites, partially replacing K functions and increasing cellular osmotic potential. However, excessive Na inactivates metabolic enzymes, causing toxicity that inhibits growth or causes death. Maintaining K /Na ratios within reasonable ranges is therefore crucial for salt resistance. Ao et al. found that salt stress increased Na content while reducing K content in tomato, decreasing K /Na ratios in roots, stems, and leaves and inhibiting growth. In this study, mixed salt stress reduced K content and increased Na content, significantly decreasing K /Na ratios in all organs, most notably in leaves. Yang et al. re-

ported similar patterns in paper mulberry (*Broussonetia papyrifera*) seedlings, where leaf K /Na ratios were significantly lower than root ratios. Higher leaf K /Na ratios are important for maintaining normal photosynthesis and respiration under salt stress. In this study, 'GF01' maintained significantly higher leaf K /Na ratios than 'YK06' and 'YK18' , correlating with stronger whole-plant Na compartmentalization and better K retention.

3.3 Effects of Mixed Salt Stress on Ca² in Different Varieties Ca² is essential for maintaining normal plant cell physiological function, participating in most cellular metabolic processes and acting as a second messenger to regulate gene expression. Under salt stress, Ca² stabilizes cell walls and membranes, activates or inhibits various ion channels, modulates specific enzyme activities, and induces stress-resistance gene expression, thereby enhancing environmental adaptation. Zhao et al. found that exogenous calcium significantly improved germination rate and index in salt-stressed winter wheat variety 'Shaan 266' , alleviating salt damage. Liu et al. also reported that exogenous Ca(NO) significantly mitigated salt stress inhibition of maize seed germination, with effects increasing with concentration. Thus, Ca² acts as a signaling molecule in cellular signal transduction under salt stress, and enhanced Ca² absorption represents an important resistance mechanism. In this study, Ca² influx in root systems accelerated after 24 hours of mixed salt stress, with rates increasing with salt concentration. The salt-tolerant variety 'GF01' showed the fastest absorption, while the salt-sensitive 'YK18' showed the slowest, suggesting that differential Ca² absorption among varieties may contribute to differences in salt tolerance.

Xue et al. demonstrated that Na can displace membrane-bound Ca² under salt stress, causing massive K efflux and disrupting ionic balance, thereby inhibiting growth. Exogenous calcium application alleviated NaCl stress, promoted enzyme activity, regulated inorganic ion transport, and enhanced salt resistance. Li et al. found that Ca² and calmodulin (CaM) regulated Na uptake and accumulation in winter wheat through the salt overly sensitive (SOS) pathway, promoting K absorption. Wang et al. also reported that Ca² activated both K -specific channels and non-selective cation channels (NSCCs), promoting K uptake with enhancement proportional to Ca² concentration. Combined with our findings on Na and K uptake and accumulation, the salt tolerance mechanism of 'GF01' may involve Ca² directly or indirectly (via CaM) activating the SOS pathway, inducing expression of plasma membrane Na -H antiporters and K transporters, thereby accelerating Na extrusion and K uptake, increasing leaf K /Na ratios, and restoring cellular ionic balance. However, specific pathways and mechanisms require further investigation.

This study employed ion flux detection technology to examine K , Na , and Ca² fluxes in roots of sunflower varieties with different salt tolerance levels under mixed salt stress. Results demonstrate that salt-tolerant varieties possess strong K retention capacity, enhance salt tolerance through Na compartmen-

talization at low salt concentrations, and exhibit distinct "salt excretion" at high salt concentrations in addition to Na compartmentalization. Accelerated Ca^2 absorption under salt stress also represents a salt tolerance mechanism. As a non-invasive technique, ion flux detection provides an efficient and convenient method for studying root ion uptake mechanisms under salt stress and can be reliably applied to resistance identification and physiological mechanism research in plant breeding.

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Note: Figure translations are in progress. See original paper for figures.