

Root convex hull area reflects coordinated root–shoot adaptation to salt stress in spring wheat

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Abstract

Background

Root architecture determines the capacity of crops for spatial exploration under stress conditions; however, existing studies on salt tolerance screening have mostly been confined to single traits such as root length or biomass, overlooking the overall spatial configuration of the root system and its intrinsic linkage with aboveground physiological functions. On this basis, the present study aimed to determine whether root convex hull area can characterize root–shoot synergistic adaptability under salt stress, and whether this synergy involves a physiological mechanism of resource conservation through cortical tissue remodeling.

Methods

Using a paper-based root phenotyping platform, we screened 28 spring wheat varieties originating from the arid regions of northwest China under 200 mM NaCl stress, and compared the differences between large-convex hull area and small-convex hull area varieties in root architecture, root cortical anatomy, stomatal traits, leaf water status, photosystem II efficiency, canopy temperature, and transpiration rate under salt stress.

Results

The results showed that large-convex hull area varieties maintained total root length, maximum depth, and convex hull area under salt stress, whereas small-convex hull area varieties exhibited significant reductions in all these parameters. Meanwhile, compared with small-convex hull area varieties, large-convex hull area varieties possessed greater cortical lacunar tissue area and cortex/stele ratio, as well as higher stomatal density, leaf relative water content, F_v/F_m , and transpiration rate, but lower canopy temperature and smaller stomatal aperture. Convex hull area was positively correlated with leaf water status, photochemical efficiency, cortical lacunar area, and stomatal density, while negatively correlated with canopy temperature and stomatal aperture, indicating that root spatial maintenance, moderate cortical senescence, and stomatal regulation together constitute a functionally coordinated response module under salt stress.

Conclusion

In summary, convex hull area is not merely a descriptive indicator of root morphology, but rather a functional trait that reflects the synergistic integration of belowground exploration capacity and aboveground physiological resilience. This study proposes that convex hull area can serve as a candidate high-throughput phenotypic indicator for salt tolerance screening in wheat at the seedling

stage; nevertheless, its predictive capacity for field yield performance still requires further validation under soil conditions, across the full growth cycle, and under interactions with multiple environmental factors.

Introduction

Salinity is one of the major abiotic stresses limiting global agricultural productivity. Salt stress affects crop growth through a combination of osmotic stress, ion toxicity, nutrient imbalance, and oxidative damage, thereby restricting biomass accumulation, yield formation, and crop quality [1, 2]. According to the latest global assessment by the Food and Agriculture Organization of the United Nations, salt-affected soils cover approximately 1.381 billion ha, accounting for 10.7% of the global land area, while about 10% of both irrigated and rainfed croplands are affected by salinity [3]. Salt-induced land degradation in irrigated areas has been estimated to cause annual crop-production losses of approximately US\$27.3 billion [4]. Moreover, an additional one billion hectares of land are considered at risk because of climate change, increasing aridity, water scarcity, and inappropriate land and water management [3]. Therefore, identifying reliable traits that reflect crop adaptation to salinity is a key priority for salt-tolerance evaluation and breeding.

The root system is the primary organ through which plants perceive rhizospheric salinity changes and absorb water and mineral elements, and its growth and spatial distribution directly influence the plant's adaptability to stress environments. Root architecture is determined by multiple interrelated traits, including root number, root length, branching, root angle, width, depth, and spatial distribution; thus, the use of single indicators such as root length or root number alone may not adequately reflect the plant's capacity to utilize growth space [5, 6]. Root convex hull area is defined as the minimal convex polygon area encompassing all boundary points of a two-dimensional root projection, which can reflect the spatial extension range of the root envelope in both lateral and longitudinal dimensions, and can be combined with traits such as total root length, maximum width, and maximum depth to characterize root architecture [7]. With the development of paper-based culture, flatbed scanning, and image analysis tools such as RootNav and RhizoVision Explorer, complex root phenotypes like convex hull area can now be rapidly and standardly extracted, providing technical conditions for large-scale germplasm screening [8, 9]. However, existing studies on salt tolerance in wheat have mostly focused on aboveground growth, biomass, ion accumulation, and individual root traits such as root length, root number, and root surface area, with relatively insufficient attention paid to root spatial distribution characteristics [10]. Consequently, whether convex hull area can serve as a comprehensive indicator reflecting the capacity for root spatial maintenance under salt stress remains to be systematically investigated.

In addition to external architecture, internal anatomical structures of the root system may also participate in resource allocation and water and ion transport under salt stress. The root cortex, located between the epidermis and the stele, serves as an important pathway for the radial transport of water and ions. Its cell layer number, tissue area, and degree of aerenchyma formation can influence root respiratory consumption, nutrient demand, and radial transport resistance. In Triticeae crops such as

wheat and barley, root cortical senescence can occur in mature root segments, manifesting as the gradual loss of viability in some cortical cells and the formation of lacunae. Previous studies have shown that root cortical senescence can reduce root respiration and the maintenance costs of tissue nitrogen and phosphorus, but excessive loss of cortical tissue may also limit the radial transport of water and nutrients [11]. Modeling studies further suggest that resources saved or reallocated through the reduction of cortical tissue may support new root growth and more extensive soil exploration [12]. Therefore, under salt stress, changes in cortical cell layer number, cortical lacunar tissue, and the cortex/stele ratio may reflect a trade-off between reducing maintenance costs and preserving transport functions in the root system.

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops globally and a vital source of dietary energy and protein for humans. Compared with rice and maize, wheat generally exhibits higher salt tolerance, yet it remains a moderately salt-tolerant crop, with significant genotypic differences in growth maintenance, ion homeostasis, and photosynthetic performance under salt stress [13, 14]. Differences in root architecture may further regulate stomatal traits, transpirational cooling, leaf water status, and photosystem II activity by influencing plant water supply. Previous drought studies have shown that cotton cultivars with smaller lateral root angles and deeper root distributions are able to access more deep-soil water, maintaining higher stomatal conductance and transpiration rates under drought stress, and reducing canopy temperature through transpirational cooling [15]. In addition, substantial variation exists among wheat genotypes in stomatal conductance, evaporative cooling, and photosystem II operating efficiency, and the maintenance of stomatal conductance contributes to lowering leaf temperature and protecting photosynthetic processes. However, drought stress primarily manifests as soil water deficit, whereas salt stress is accompanied by Na^+ and Cl^- accumulation and ionic imbalance in addition to osmotic stress, potentially leading to different synergistic relationships between root architecture and aboveground physiological processes. Although leaf water status, gas exchange, and chlorophyll fluorescence have been employed in salt tolerance evaluation in wheat [16], whether root convex hull area exhibits coordinated responses with stomatal morphology, leaf water status, photosystem II activity, and root cortical tissue remodeling under salt stress remains to be systematically investigated.

Based on this, the present study employed a paper-based root phenotyping platform to screen 28 spring wheat varieties originating from the arid and semi-arid ecological regions of northwest China under salt stress, and selected typical large-convex hull area and small-convex hull area varieties for in-depth analysis according to their convex hull area under salt stress. Through systematic measurements of root architecture, root cortical anatomical structure, stomatal traits, leaf relative water content, F_v/F_m , leaf temperature, and transpiration rate, this study aimed to: (1) clarify the differential responses of root architecture to salt stress between large-convex hull area and small-convex hull area spring wheat varieties, and evaluate the feasibility of convex hull area as a screening indicator for salt tolerance at the seedling stage; (2) elucidate the relationships among root spatial maintenance capacity, stomatal regulation, leaf water status, and photosystem II stability; and (3) delineate the potential link between root cortical tissue remodeling and convex hull area maintenance. We hypothesized that large-convex

hull area varieties could better maintain root spatial expansion under salt stress, and through coordinated adjustments in root cortical structure, stomatal traits, and leaf water status, alleviate the inhibition of shoot growth and physiological functions imposed by salt stress.

Materials and Methods

Plant materials

In this study, 28 spring wheat varieties that have been approved at the national or provincial level in recent years were used as experimental materials. These varieties originated from arid and semi-arid ecological regions of northwestern China, including Ningxia, Inner Mongolia, Gansu, Xinjiang, and Qinghai. All plant materials were provided by the Wheat Research Group of the Department of Plant Breeding and Genetics, Gansu Agricultural University. Detailed information on the varieties is presented in Table S1.

Experimental design

This experiment was conducted from December 2, 2024 to April 13, 2025 in an artificial climate chamber at the State Key Laboratory of Aridland Crop Science, Gansu Agricultural University (36°03'N, 103°44'E). Spring wheat seeds were first surface-sterilized sequentially with 75% ethanol and 5% sodium hypochlorite for the specified duration, followed by rinsing five to eight times with distilled water. The sterilized seeds were then placed in Petri dishes lined with filter paper (1.5 cm deep and 15 cm in diameter), with 60 healthy seeds of uniform size and free from mechanical damage evenly arranged per dish, and germinated for 3 d in darkness at 25/22°C (day/night). After germination, seedlings with uniform vigor and balanced root and shoot growth were selected from each dish and transferred to a paper-based phenotyping platform.

For each variety, 30 paper pouches were prepared, with one seedling planted per pouch and positioned at the center of the filter paper to ensure close root–paper contact. One-quarter-strength Hoagland nutrient solution was then added in a timely manner to support normal seedling growth. A total of 840 paper pouches were used in the experiment. Twenty-four hours after transplanting, 15 of the 30 pouches for each variety were randomly assigned to salt stress treatment, while the remaining 15 served as the control and continued to receive one-quarter-strength Hoagland nutrient solution.

When plants reached the two-leaf stage, a 7-day salt stress treatment was imposed with two treatments: normal condition (NC, 0 mM NaCl) and salt stress (SS, 200 mM NaCl), each with five replicates. The pH of the nutrient solution was adjusted to 6.0 ± 0.1 , and the solution was replaced every 3 d. Environmental conditions were maintained as follows: day/night temperatures of approximately $28 \pm 2/25 \pm 2^\circ\text{C}$, a photoperiod of 14/10 h (light/dark), relative humidity of 40–50%, and a light intensity of $600 \mu\text{mol m}^{-2} \text{s}^{-1}$. During the experimental period, aeration was supplied twice daily for 30 min each time.

Determination of the plant height and leaf area

Sample collection was conducted on the seventh day of salt-stress treatment. For each treatment, three representative wheat plants were randomly selected for the determination of morphological traits. Plant height was defined as the length from the coleoptile node to the apex of the main stem and was measured using a ruler, while leaf area was calculated using the length–width coefficient method.

Determination of the leaf physiology

Sample collection was conducted on the seventh day of salt-stress treatment. For each treatment, three representative wheat plants were randomly selected for the determination of the leaf physiology traits.

Canopy temperature was measured using a hand-held infrared thermometer (AGRI-THERM II, Model 6110, USA). The sensor probe was positioned 5 cm away from the upper third of the main stem functional leaf, which was perpendicular to the direction of leaf unfolding. Temperature measurements were recorded as soon as the probe was stable.

Three representative plants were randomly selected between 9:00 and 11:00. The relative water content of the leaves was determined by the swelling drying weighing method. The flag leaves were cut and immediately weighed on an analytical balance to obtain the fresh weight (g). After recording, the fresh samples of the cut leaves were placed in deionized water and left in a 4°C refrigerator for 8–12 hours to determine the expanded weight (g). Subsequently, the samples were placed in an oven and heated to 105°C for 30 minutes, then cooled to 80°C and dried until a constant temperature was reached. The dry weight of the leaf samples was weighed to determine the dry weight (g). The calculation formula: Relative water content = (Fresh weight - Dry weight) / (Expanded weight - Dry weight).

To assess the physiological status of plant leaves, flag leaves were selected for measurements of the maximum quantum yield of photosystem II photochemistry (F_v/F_m), chlorophyll content, and canopy temperature. (F_v/F_m was measured between 00:00 and 02:00 using a portable chlorophyll fluorescence monitoring system (FMS-2, UK).

Gas-exchange parameters of wheat flag leaves were measured using an LI-6400 portable photosynthesis system. Before measurement, the chamber conditions were set to a CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$ and a photosynthetic photon flux density of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Transpiration rate was subsequently recorded.

Determination of the leaf stomatal

On the seventh day of salt-stress treatment, leaf stomatal characteristics were measured between 10:30 and 12:00. Briefly, stomatal traits were assessed using a combined nail polish and transparent tape method, with measurements conducted on the second fully expanded leaf from the top. Nail polish was

evenly applied to the abaxial leaf surface and allowed to air-dry for 2–5 min. Transparent tape was then placed over the dried nail polish layer to obtain an impression, which was subsequently mounted for observation. Stomatal images were captured using an optical microscope (Leica 2500, Leica Microsystems, Germany). For each image, five fields of view were randomly selected, and within each field, five stomata were randomly chosen for analysis. ImageJ software was used to determine stomatal length (μm), stomatal width (μm), and stomatal aperture (μm). The area of each field of view was recorded, and stomatal number per image was counted and divided by the corresponding area to calculate the mean stomatal density (μm^2) for each field.

Determination of the RSA

The imaging–germination platform consisted of plant germination pouches, a pouch holder, germination pouch cabinet, a small photographic lightbox, a high-resolution scanner, and a laptop computer. The paper-based system employed germination pouches made of high-quality Whatman No.1 filter paper combined with standard plant germination bags (180 mm $\times$ 125 mm). The pouch holder was a rectangular structure (20 cm high, 15 cm wide, and 30 cm long) assembled from ten transparent acrylic panels, capable of accommodating nine germination pouches simultaneously, thereby facilitating uniform placement and management of seedlings.

Similarly, the germination pouch cabinet was a rectangular structure (200 cm high, 100 cm long, and 50 cm wide) equipped with four internal shelves, each measuring 100 cm $\times$ 50 cm. An adjustable 220 V LED light strip was installed above each shelf to provide supplemental illumination and ensure precise control of the photoperiod during cultivation. The small photographic lightbox measured approximately 60 cm $\times$ 60 cm $\times$ 60 cm and was internally lined with non-reflective black or dark background materials (e.g., black foam board or black fabric) to minimize reflections and shadows during image acquisition. Evenly distributed LED light strips inside the lightbox provided uniform illumination, ensuring consistent lighting conditions across the imaging area. This enclosed imaging environment provided stable and standardized background and lighting conditions, thereby improving image clarity and consistency.

Root samples were collected on the seventh day of salt-stress treatment. For each treatment group, three representative wheat plants were randomly selected. Whole root systems were imaged using a high-resolution scanner (GP1600AF, COMET, China) at a resolution of 1200 dpi, enabling rapid and clear scanning. The entire imaging system was controlled by a laptop computer (LAPTOP-5MNUD00G, HONOR, China) to ensure stable and uniform lighting conditions during scanning, thereby optimizing image quality. The acquired digital images were saved in TIFF format. Following image acquisition, preprocessing was conducted using Photoshop (Ps), including image scaling, cropping, background adjustment, and horizontal flipping, to enhance image clarity and facilitate subsequent batch analysis. The preprocessed images were then imported into RootNav and RhizoVision Explorer software for quantitative extraction of root traits, including convex hull, lateral root angle, total root length, average length - all roots, lateral root count, average length - lateral roots, maximum depth, maximum width and

width/depth ratio. To ensure standardized data management, all images were systematically renamed according to predefined sample identification codes.

Determination of root anatomical structure

Samples were collected on the seventh day of salt-stress treatment. From each plant, three root segments of approximately 0.5 cm in length were excised from the 6–9 cm regions from the root tip, respectively, with three biological replicates established for each treatment. The collected root segments were first preserved in 75% ethanol for subsequent anatomical analysis of root cortical tissues. Phenotypic analysis of cortical tissue development was performed using acridine orange staining. The stained root segments were then embedded in Tissue-Tek CRYO-OCT compound (Thermo Fisher Scientific, USA), rapidly frozen in liquid nitrogen, and stored at -20°C . Transverse root sections with a thickness of $8\text{ }\mu\text{m}$ were prepared using a cryostat microtome (Leica CM1950, Germany) and immediately mounted onto microscope slides for imaging. Finally, cross-sectional images of the roots were acquired using an inverted fluorescence microscope (ECLIPSE Ti2-U, Nikon, Japan) and a laser confocal scanning microscope (FV-1000, Olympus, Japan). Image-based phenotypic analysis was conducted using ImageJ software (<http://rsbweb.nih.gov/ij>). The extracted anatomical traits included number of cortical layers, cortical lacunar tissue, cortical lacunar/cortex and cortex / columella.

Statistical analysis

Microsoft Excel 2019 was used for data recording, organization, calculation of functions, and computation of D values. SPSS 27.0 (IBM, Armonk, NY, USA) was employed for analysis of variance, principal component analysis, correlation analysis, and data standardization. Microsoft Excel 2019 was also used to calculate membership function values and D values. Origin 2022b (OriginLab, Northampton, MA, USA) was applied for data visualization and hierarchical cluster analysis. Data are presented as means $\pm$ standard error (mean $\pm$ SE). One-way analysis of variance followed by the least significant difference (LSD) test was used to determine significant differences at the 95% or 99% confidence levels. Adobe Illustrator 2020 was used for figure assembly.

Results

The small- and large-convex hull area

Through Euclidean distance smoothing and systematic cluster analysis, spring wheat varieties with large-convex hull area and small-convex hull area varieties were selected. The data showed that the 28 spring wheat varieties were divided into five categories, with a Euclidean distance of 0.5 between groups. Class I comprised 6 varieties (21%) with the largest convex hull area; Class II contained 5 varieties (18%) with a relatively large convex hull area; Class III included 10 varieties (36%) with a moderate convex hull area; Class IV consisted of 6 varieties (21%) with a relatively large convex hull area; and Class V was composed of 1 variety, accounting for 4% of the total, with the smallest convex hull area

(Fig. S1). Based on the experimental results, three varieties with large-convex hull areas—Ruichun 1, Ningchun 32, and Ningchun 57—and three varieties with small-convex hull areas—Dingxi 40, Dingxi 48, and Ningchun 15—were identified (Fig. S1). These six varieties were selected for further analysis.

Phenotypic variation in the root related traits

Significant genotypic differences were observed in root traits among spring wheat varieties with different convex hull areas under salt stress (Figs. 1–2). Data showed that under salt stress, the convex hull area of large-convex hull area varieties ranged from 111,861.59–376,043.8, while that of small-convex hull area varieties ranged from 100,853.93–794,903.61. Compared with the control treatment, the convex hull area of small-convex hull area varieties decreased significantly by 51.60% under salt stress ($P < 0.05$), whereas no significant reduction was found in large-convex hull area varieties (Fig. 2A). Moreover, under salt stress, the convex hull area of small-convex hull area varieties was reduced by 28.23% compared with large-convex hull area varieties. Similarly, relative to the control treatment, total root length, average length of all roots, and maximum depth of small-convex hull area varieties decreased significantly by 44.12%, 28.56%, and 10.48%, respectively, under salt stress ($P < 0.05$; Figs. 2C, D, I), while no significant reductions were observed in large-convex hull area varieties. Under salt stress, total root length and maximum width of small-convex hull area varieties were significantly lower than those of large-convex hull area varieties by 37.25% and 40.72%, respectively ($P < 0.05$; Figs. 2C, G).

Phenotypic variation in the stomatal related traits

Under control conditions, genotypic differences in stomatal traits were observed among varieties with different convex hull areas (Fig. 3). However, under salt stress, stomatal width, length, and aperture were inhibited in both large- and small-convex hull area varieties, whereas stomatal density increased. Specifically, compared with the control treatment, stomatal width, length, and aperture in large- and small-convex hull area varieties decreased by 28.34% and 20.92%, 34.58% and 4.12%, and 37.05% and 3.53%, respectively, under salt stress (Figs. 3A-C). Under salt stress, stomatal width, length, and aperture in large-convex hull area varieties decreased by 5.94%, 53.45%, and 62.12%, respectively, compared with small-convex hull area varieties (Figs. 3A-C), while stomatal density significantly increased by 55.96% (Fig. 3D).

Phenotypic variation in the above-ground phenotypic characteristics

Under salt stress, genotypic differences in shoot phenotype and leaf water status were observed among varieties with different convex hull areas (Fig. 4). Specifically, compared with the control treatment, plant height, leaf area, leaf relative water content, F_v/F_m , and transpiration rate in large- and small-convex hull area varieties decreased significantly by 12.15% and 25.05%, 26.49% and 55.21%, 6.30% and 17.14%, 9.64% and 17.99%, and 18.08% and 27.62%, respectively, under salt stress ($P < 0.05$; Figs. 4A-D, F), while canopy temperature increased significantly by 6.88% and 14.76% ($P < 0.05$; Fig. 4E). Under salt stress,

leaf area, leaf relative water content, F_v/F_m , and transpiration rate in large-convex hull area varieties increased significantly by 10.30%, 59.07%, 13.37%, 10.18%, and 14.69%, respectively, compared with small-convex hull area varieties ($P < 0.05$; Figs. 4A-D, F), while canopy temperature decreased significantly by 7.10% ($P < 0.05$; Fig. 4E).

Phenotypic variation in the root cortical tissue

Salt stress reduced cortical cell layer number and the cortex/stele ratio in wheat roots, while increasing cortical lacunar tissue area and the lacunar tissue/cortex ratio, with genotypic differences observed (Fig. 5). Specifically, compared with the control treatment, cortical cell layer number and the cortex/stele ratio in large- and small-convex hull area varieties decreased significantly by 32.43% and 40.00%, and 36.81% and 45.94%, respectively, under salt stress ($P < 0.05$; Figs. 5A, D), while cortical lacunar tissue area and the lacunar tissue/cortex ratio increased significantly by 65.95% and 40.17%, and 129.17% and 126.47%, respectively ($P < 0.05$; Figs. 5B-C). Under salt stress, cortical lacunar tissue area and the lacunar tissue/cortex ratio in large-convex hull area varieties increased significantly by 54.36% and 42.86%, respectively, compared with small-convex hull area varieties ($P < 0.05$; Figs. 5B-C).

Relationships between the root system and key traits in field trials

Stomatal traits, leaf water status, and cortical tissue characteristics were all correlated with root convex hull area. Specifically, stomatal length and stomatal density showed significant positive correlations with root convex hull area (Figs. 6A, C), while stomatal aperture showed a significant negative correlation (Fig. 6B). Leaf relative water content, F_v/F_m , cortical lacunar tissue area, cortical lacunar/cortex ratio, and cortex/stele ratio all increased with increasing root convex hull area (Figs. 6D, E, G, H, I), whereas canopy temperature decreased with increasing root convex hull area (Fig. 6F).

Discussion

Root convex hull area is an important trait for distinguishing salt tolerance in spring wheat

The root system is the primary organ through which plants perceive rhizospheric salinity changes and absorb water and mineral elements, and its growth and spatial distribution directly influence plant adaptability to salt stress [17]. Salt stress first exerts osmotic effects by reducing external water potential, rapidly inhibiting cell elongation and root growth; with the accumulation of Na^+ and Cl^- in tissues, ionic toxicity and nutrient imbalance further restrict root axis elongation, lateral root formation, and root spatial expansion [18]. In this study, we found that after 200 mM NaCl treatment, convex hull area, total root length, average root length, and maximum depth of small-convex hull area varieties decreased significantly, whereas most root traits of large-convex hull area varieties did not undergo

significant changes. This indicates that the differences among spring wheat varieties are not only reflected in root size but also in the capacity to maintain root elongation and spatial expansion under salt stress. Similarly, Rahnema et al. [19] found that salt gradients imposed significantly different degrees of inhibition on root growth among durum wheat genotypes, with some genotypes showing marked decreases in seminal root axis length while others maintained relatively stable root growth, suggesting that root architectural responses to salt stress have a distinct genetic basis. Robin et al. [20] also reported that salt stress significantly inhibited the growth of new root axes and root hairs in wheat, reducing estimated root surface area by 36%–66%, yet the response magnitudes varied among different varieties and different root positions.

Root architecture possesses substantial developmental plasticity and genetic variation, with traits such as root length, root angle, maximum width, and maximum depth collectively determining the distribution range of roots within the growth medium and their resource acquisition capacity [5, 6]. Convex hull area is the minimal convex polygon area encompassing the complete two-dimensional root projection, primarily reflecting the lateral extension and longitudinal reach of the root envelope, and can be combined with traits including total root length, maximum width, maximum depth, and width/depth ratio to characterize root architecture [7]. Therefore, compared with single traits such as root length or root number, convex hull area provides a more comprehensive evaluation of root responses to salt stress from the perspective of spatial occupancy range. In the present study, large-convex hull area varieties maintained relatively stable convex hull area, total root length, and maximum depth under salt stress, indicating their stronger capacity for root spatial maintenance, which may be beneficial for preserving a larger contact range for water and nutrient uptake and alleviating the adverse effects of inhibited root growth on aboveground physiological functions.

- *Advantages in root convex hull area are closely associated with stomatal regulation and leaf water maintenance*

Stomata are important structures that regulate leaf water loss, CO₂ exchange, and heat dissipation. In this study, we found that under salt stress, stomatal length, width, and aperture decreased in both large- and small-convex hull area varieties, while stomatal density increased. This indicates that salt stress simultaneously induced stomatal morphological shrinkage and a reconfiguration of stomatal number per unit leaf area. This result is largely consistent with the findings of Sehar et al. [21] in wheat, who reported that 100 mM NaCl treatment caused partial stomatal closure and reduced stomatal length and width by 56.7% and 66.6%, respectively. Similar reductions in stomatal size and aperture, along with increased stomatal density, have also been observed in cotton under water stress suggesting that reducing individual stomatal size and adjusting their distribution density may represent a common phenotypic strategy for plants in response to osmotic stress [15]. However, the responses of stomatal density to salt stress are not entirely consistent across studies. These observations indicate that changes in stomatal density are influenced by multiple factors, including genotype, salt concentration, treatment duration, leaf developmental stage, and the measured leaf surface, and thus increased density should not be simply regarded as a universal feature of all salt-tolerant materials. It is particularly important to note that an

increase in stomatal density per unit area does not necessarily imply an increase in the total number of stomata formed. Previous studies in wheat have shown that salt stress can inhibit epidermal cell division and expansion, reducing epidermal cell size; under conditions of restricted leaf area expansion, even if the total stomatal number does not increase, stomatal density per unit area may still rise due to an area-concentration effect [22]. Therefore, the increased stomatal density observed in this study may result from both stomatal developmental regulation and the inhibition of epidermal cell expansion; future work should distinguish these contributions by examining epidermal cell density and stomatal index.

From a functional perspective, the reduction in stomatal size and aperture under salt stress helps limit instantaneous water loss, while the presence of a higher density of smaller stomata may retain a certain number of potential diffusion pathways and enhance the dynamic responsiveness of stomata to environmental changes. Drake et al. [23] found in studies on related plant species that stomatal size is negatively correlated with stomatal opening rate, whereas stomatal density is positively correlated with opening rate, meaning that smaller and more numerous stomata generally exhibit faster opening and closing responses, which helps plants shorten the hysteresis time of stomatal regulation. On this basis, it may be inferred that the high-density–small-stomata combination observed in this study could enable spring wheat to adjust stomatal aperture more rapidly under salt stress, thereby restraining excessive water loss while maintaining, to the extent possible, the necessary CO₂ diffusion and transpirational cooling. However, this interpretation should still be treated with caution, because stomatal density and morphology reflect potential anatomical capacity, whereas actual gas exchange is also influenced by guard cell turgor, leaf water potential, ABA signaling, stomatal distribution, and boundary layer resistance, among other factors. Therefore, further validation through measurements of stomatal conductance, net photosynthetic rate, intercellular CO₂ concentration, and stomatal dynamic responses will be needed to determine whether the observed differences in stomatal structure between different convex hull area varieties truly translate into physiological advantages under salt stress.

Root cortical tissue remodeling may participate in resource allocation under salt stress

In addition to external root architecture, structural changes in the root cortex may also influence salt adaptation in spring wheat. In this study, we found that salt stress reduced cortical cell layer number and the cortex/stele ratio, while increasing cortical lacunar tissue area and the lacunar tissue/cortex ratio, indicating that internal root tissues underwent pronounced remodeling under salt stress. The root cortex is an important pathway for the radial transport of water and ions from the root surface to the stele, and is also one of the primary tissues responsible for root respiration and nutrient consumption. In Triticeae crops such as wheat and barley, root cortical senescence can occur in mature root segments, with some cortical cells gradually losing viability and forming lacunae. Previous studies have shown that root cortical senescence can reduce root respiratory costs and nitrogen and phosphorus maintenance costs, allowing limited assimilates and nutrients to be redistributed to root tips and newly formed roots, thereby promoting further resource exploration by the root system [11, 12].

In the present study, we observed that under salt stress, large-convex hull area varieties exhibited higher cortical lacunar tissue area and lacunar tissue/cortex ratio than small-convex hull area varieties, and both parameters were significantly positively correlated with convex hull area. Functionally, this result shares certain similarities with the adaptive effects of root cortical tissue reduction under drought stress. Guo et al. [24] found that drought stress promoted root cortical senescence in cotton, with drought-tolerant cultivars showing higher degrees of root cortical senescence; root cortical senescence was negatively correlated with root respiration and positively correlated with root length and leaf water potential, and suppressing the expression of genes related to root cortical senescence reduced drought tolerance in cotton. This suggests that moderate reduction of living cortical tissue in mature root segments may enhance the root system's ability to explore limited soil water by lowering root metabolic costs and promoting resource reallocation to newly developing root segments. Similarly, Chimungu et al. [25] found that fewer cortical cell layers in maize roots reduced respiration rate per unit root length by approximately 57%, while promoting deeper root penetration into the soil and improving water uptake, leaf relative water content, and shoot growth. Therefore, the higher degree of cortical lacunarization observed in this study may reduce the proportion of metabolically active tissue in mature root segments, lower the carbon and nutrient maintenance costs of roots, and allocate more resources to root tip elongation and lateral root development, thereby supporting the maintenance of a larger root spatial range under salt stress.

However, cortical tissue reduction may also entail substantial functional costs. Studies in barley have shown that while RCS reduces respiratory and tissue nutrient demands, it also decreases root radial hydraulic conductivity and the radial transport capacity for water and nutrients [11]. In maize, increased root cortical aerenchyma proportion similarly reduces the radial transport rates of phosphorus and calcium from the root surface to the stele, indicating that reduced living cortical tissue may lengthen or partially block certain radial transport pathways [26]. Therefore, a higher degree of cortical lacunarization is not necessarily more advantageous, but may instead reflect a trade-off between reducing metabolic costs and maintaining water and ion transport capacity. For salt stress, this trade-off is particularly critical, because the cortex is involved not only in water transport but also serves as an important radial pathway for Na^+ and other ions entering the stele. Moderate cortical remodeling may reduce tissue maintenance costs and restrict some ion transport, whereas excessive tissue disintegration may compromise root water uptake and nutrient absorption capacity.

External root architecture and internal root structure jointly regulate shoot responses to salt stress

Correlation analyses showed that root convex hull area was positively correlated with leaf relative water content, F_v/F_m , stomatal density, and root cortical lacunar-related parameters, while negatively correlated with stomatal aperture and leaf temperature. These results indicate that spring wheat adaptation to salt stress is not determined by a single trait, but may rely on coordinated regulation among external root architecture, internal anatomical structure, and aboveground physiological processes. First, large-convex hull area varieties maintained greater root width, depth, and total root

length under salt stress, slowing the reduction in root spatial exploration range and potential water-absorbing interfaces. Second, cortical tissue remodeling in mature root segments may reduce the metabolic maintenance costs of some tissues and promote the reallocation of limited carbon and nutrients to newly formed root segments, thereby providing resources for continued root elongation. This inference is consistent with the findings of Schneider et al. [12], who showed that root cortical senescence can improve plant growth under resource-limited conditions by reducing nutrient investment and respiratory consumption in root tissues and reallocating nutrients such as nitrogen and potassium from the cortex. On this basis, plants are able to maintain higher leaf relative water content and regulate water loss through integrated adjustments in stomatal density, stomatal size, and stomatal aperture. Previous studies have found that rice genotypes with narrower root cone angles possess longer roots and a higher proportion of deep roots, while maintaining higher stomatal conductance and lower canopy temperature, indicating that favorable root spatial distribution can support stomatal exchange and transpirational cooling by enhancing water acquisition [27]. Notably, although convex hull area showed a negative correlation with individual stomatal aperture, large-convex hull area varieties still exhibited higher transpiration rates and lower leaf temperatures, suggesting that whole-leaf transpiration capacity is not entirely determined by individual stomatal aperture, but may also be influenced by stomatal density, stomatal size, leaf water status, and root water-supply capacity. Thus, better root water maintenance capacity may allow plants to restrict excessive opening of individual stomata while still maintaining the necessary whole-leaf transpiration and heat dissipation, thereby lowering leaf temperature and alleviating salt-induced damage to photosystem II. Studies on salt stress in wheat have also indicated that leaf water relations, stomatal conductance, transpiration rate, photosynthetic rate, and chlorophyll fluorescence parameters are closely interrelated and can collectively reflect genotypic differences in salt tolerance [16].

These results are largely consistent with existing findings on the interrelationships among root architecture, stomatal regulation, and canopy temperature. Vinarao et al. [27] further found that rice genotypes with smaller root cone angles had a higher proportion of deep roots and higher stomatal conductance, with canopy temperatures 0.3-1.0°C lower than those of wide-root-cone-angle materials, suggesting that more favorable root spatial distribution supports stomatal exchange and transpirational cooling by improving water acquisition. Similarly, Guo et al. [15] found that cotton cultivars with smaller lateral root angles maintained higher stomatal aperture and transpiration rates under drought conditions, and exhibited lower canopy temperatures.

- *Potential and limitations of convex hull area as a screening tool for salt tolerance in spring wheat at the seedling stage*

This study demonstrates that convex hull area can effectively distinguish spring wheat varieties with different capacities for root spatial maintenance under salt stress, and is significantly correlated with multiple root, stomatal, and leaf physiological indicators, thus showing potential as a screening parameter for salt adaptability at the seedling stage. Compared with single traits such as root length, convex hull area offers greater comprehensiveness; and relative to complex ionomic and physiological

measurements, its image acquisition and batch analysis are more convenient, making it suitable for large-scale preliminary germplasm screening. Ruichun 1, Ningchun 32, and Ningchun 57 exhibited strong root spatial maintenance capacity under salt stress and can serve as candidate materials for subsequent studies on salt adaptation mechanisms and germplasm improvement, while Dingxi 40, Dingxi 48, and Ningchun 15 can be used as control materials for dissecting the genetic basis of contrasting phenotypes.

However, several limitations remain in this study. First, the paper-based platform provides two-dimensional root projections, which cannot fully reflect three-dimensional root distribution in soil, nor does it account for soil mechanical impedance or spatial heterogeneity of salinity. Second, the 200 mM NaCl treatment for 7 days primarily reflects short-term high-salt responses at the seedling stage and cannot directly represent salt tolerance and yield performance over the entire growth cycle. Third, this study did not measure Na^+ and K^+ contents or the K^+/Na^+ ratio, nor did it include functional indicators such as root hydraulic conductivity, root respiration, stomatal conductance, or net photosynthetic rate; therefore, it remains unclear whether the advantages of large-convex hull area varieties primarily arise from ion homeostasis, osmotic adjustment, or water transport. Future studies should conduct independent validation under soil culture and saline field conditions, and combine multiple salt concentrations, different developmental stages, ion accumulation measurements, and root functional assessments to further evaluate the genetic stability of convex hull area and its relationship with final biomass and yield.

Conclusion

In this study, by integrating quantitative root architecture analysis, anatomical histology, and leaf physiological function assessments, we constructed for the first time a root–shoot synergistic adaptation framework centered on root convex hull area under salt stress (Fig. 7). The results showed that large-convex hull area varieties maintained relative stability in total root length, maximum depth, and convex hull area under salt stress, whereas small-convex hull area varieties exhibited comprehensive declines, demonstrating that convex hull area effectively discriminates genotypic differences in root growth plasticity. At the level of internal root structure, this study is the first to extend the phenomenon of root cortical senescence from drought stress to salt stress validation, and found that the degree of cortical lacunarization was positively correlated with the capacity for convex hull area maintenance. Large-convex hull area varieties exhibited higher cortical lacunar tissue area and cortex/stele ratio, suggesting that moderate loss of cortical tissue may support continued root elongation and spatial expansion under salt stress by reducing maintenance costs in mature root segments and promoting resource reallocation to root tips. At the root-shoot coupling level, this study demonstrated that the capacity for convex hull area maintenance systematically modulates stomatal traits and leaf energy metabolism through its influence on root water acquisition. Large-convex hull area varieties, while upregulating stomatal density, maintained smaller stomatal apertures, reflecting an optimized strategy under salt stress in which a higher density of smaller stomata both limits water loss and preserves

diffusion pathways, while relatively sufficient root water supply enables plants to maintain higher transpiration rates and leaf water content, thereby reducing canopy temperature through transpirational cooling and protecting photosystem II activity. In summary, root convex hull area, as a functional phenotype for screening salt tolerance in wheat at the seedling stage, integrates the coordinated responses of external root architecture, internal anatomical structure, and aboveground physiological functions, offering greater comprehensiveness and screening efficiency than single traits. Future studies should evaluate the predictive power of convex hull area for yield under field conditions across multiple salt concentrations and throughout the full growth cycle, and employ genomic approaches to dissect its genetic regulatory network, thereby providing a theoretical basis for molecular design breeding of salt-tolerant wheat.

Declarations

Ethics approval and consent to participate

All plant materials used in this study were provided by the Wheat Research Group of the Department of Plant Breeding and Genetics, Gansu Agricultural University. The use of these plant materials was permitted by the provider. All experimental research on plants complied with relevant institutional, national, and international guidelines and legislation. The plant materials were approved spring wheat varieties, and no wild or endangered plant species were collected in this study. Therefore, no specific collection permits were required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

Congcong Guo and Xinying Li: Data curation, Software, Visualization and Writing – original draft. Chengxin Bai, Xiaoya Gao and Shiji Wang: Conceptualization, Resources and Methodology. Baoyin Zhou and Jiangheng Bai: Data curation, Formal analysis and Software. Hong Fan, Wei He, Yali Sun and Bo Jing: Project administration, Software and Supervision. Cai Zhao and Xiaoyuan Bao: Validation, Visualization, Investigation, and Writing – review & editing All authors: Carefully revised the manuscript and approved the final version.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

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Figures



Figure 1

Representative images of root architecture in large-convex hull area and small-convex hull area spring wheat varieties under salt stress.

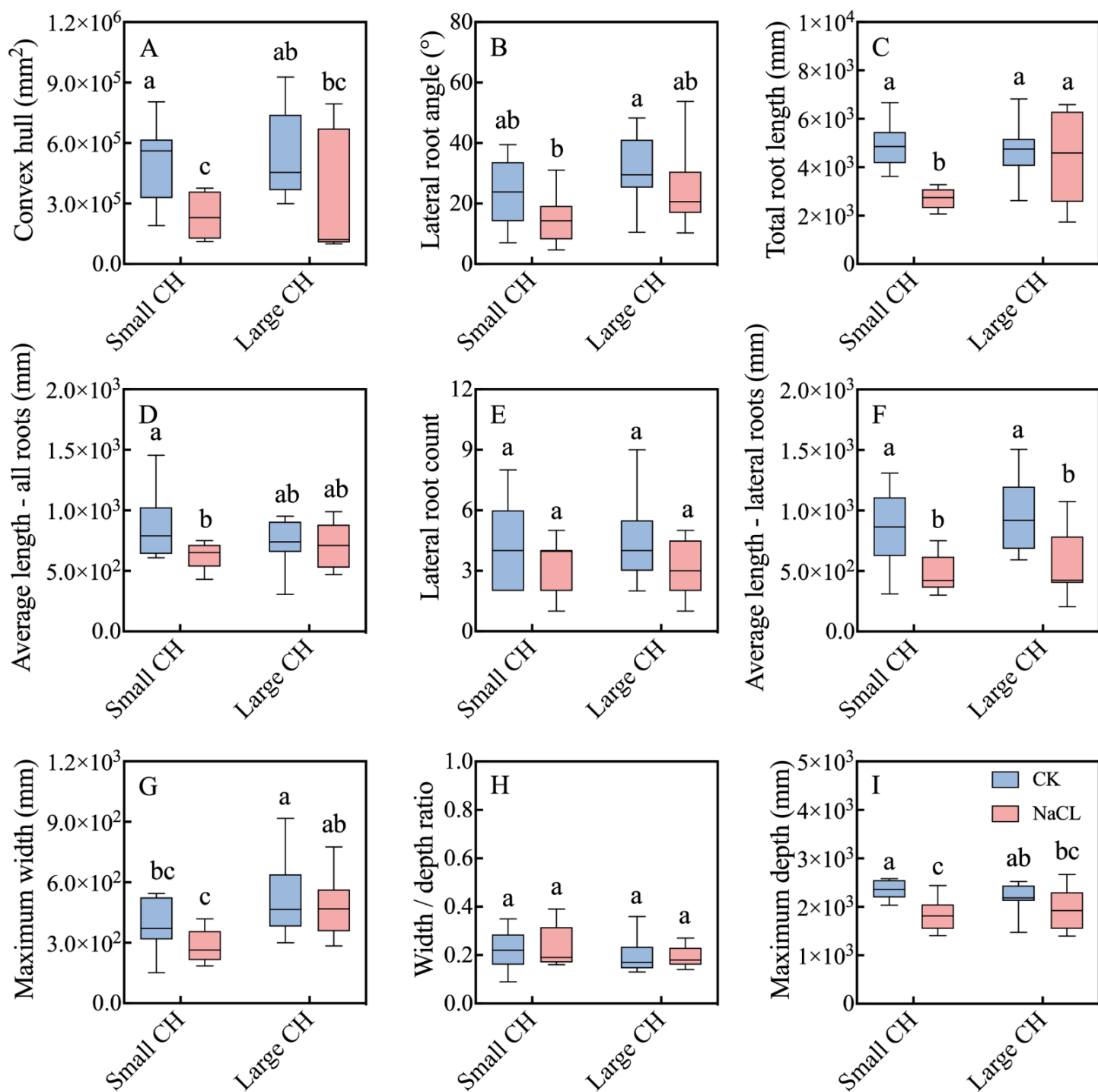


Figure 2

Responses of root traits to salt stress in three large-convex hull area and three small-convex hull area varieties. Statistical analyses of convex hull area (A), lateral root angle (B), total root length (C), average length of all roots (D), lateral root count (E), average length of lateral roots (F), maximum width (G), width/depth ratio (H), and maximum depth (I). CK, control treatment; NaCl, salt stress. Different lowercase letters indicate significant differences among the various salt stress and variety treatments ($P < 0.05$).

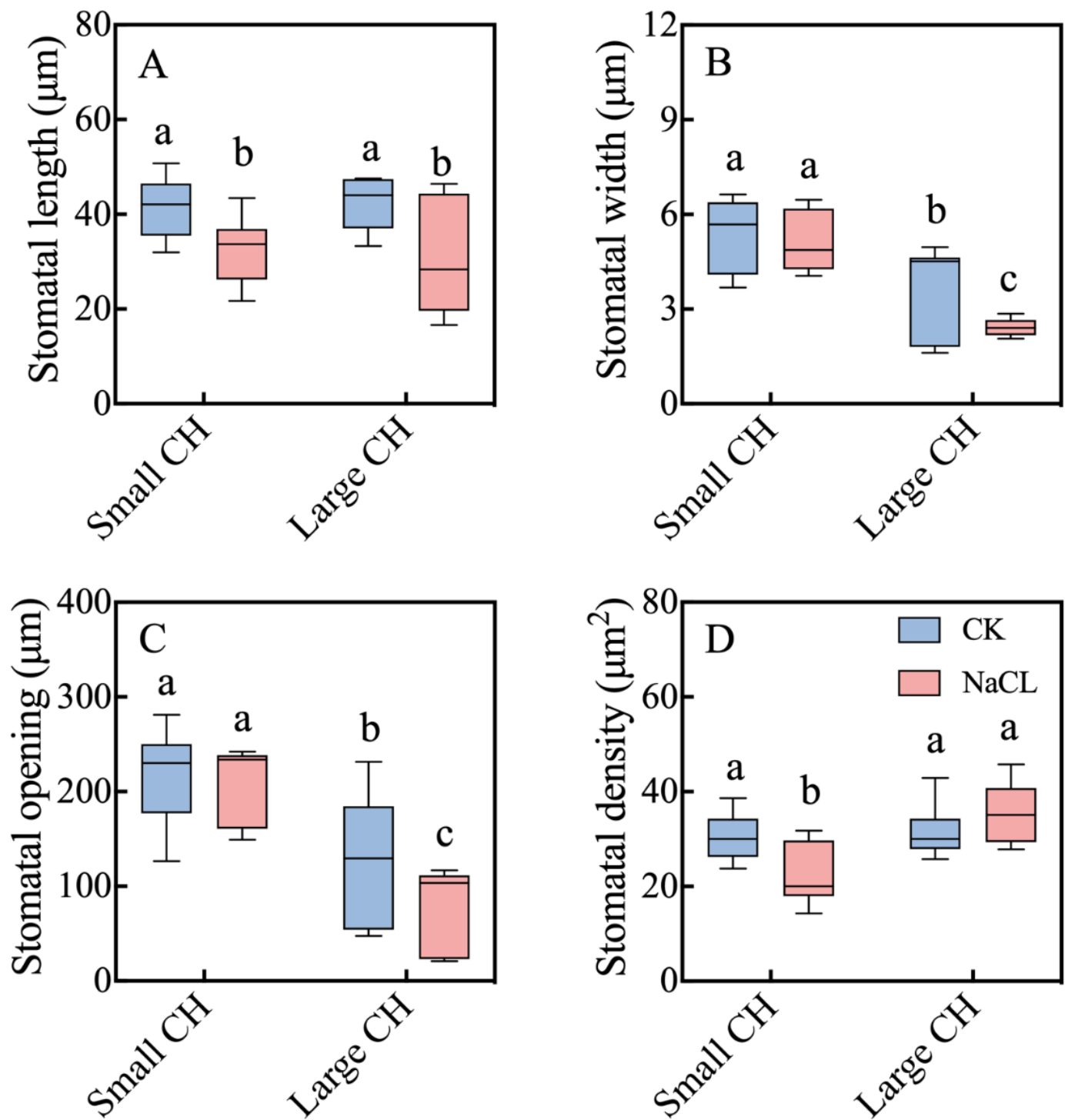


Figure 3

Responses of stomatal traits to salt stress in three large-convex hull area and three small-convex hull area varieties. Statistical analyses of stomatal length (A), stomatal width (B), stomatal opening (C), and stomatal density (D). CK, control treatment; NaCl, salt stress. Different lowercase letters indicate significant differences among the various salt stress and variety treatments ($P < 0.05$).

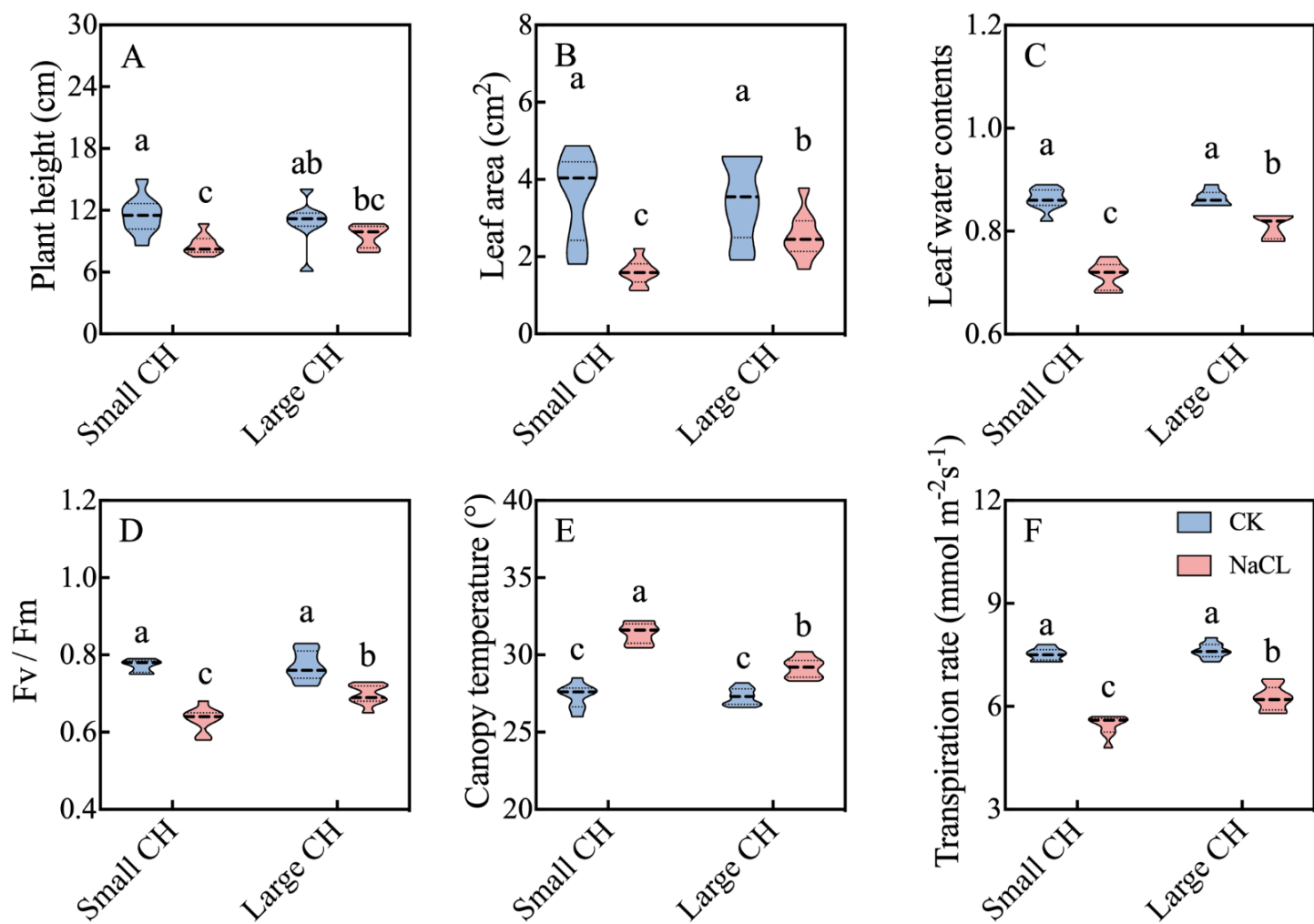


Figure 4

Responses of shoot phenotypes to salt stress in three large-convex hull area and three small-convex hull area varieties. Statistical analyses of plant height (A), leaf area (B), leaf relative water content (C), F_v/F_m (D), canopy temperature (E), and transpiration rate (F). CK, control treatment; NaCl, salt stress. Different lowercase letters indicate significant differences among the various salt stress and variety treatments ($P < 0.05$).

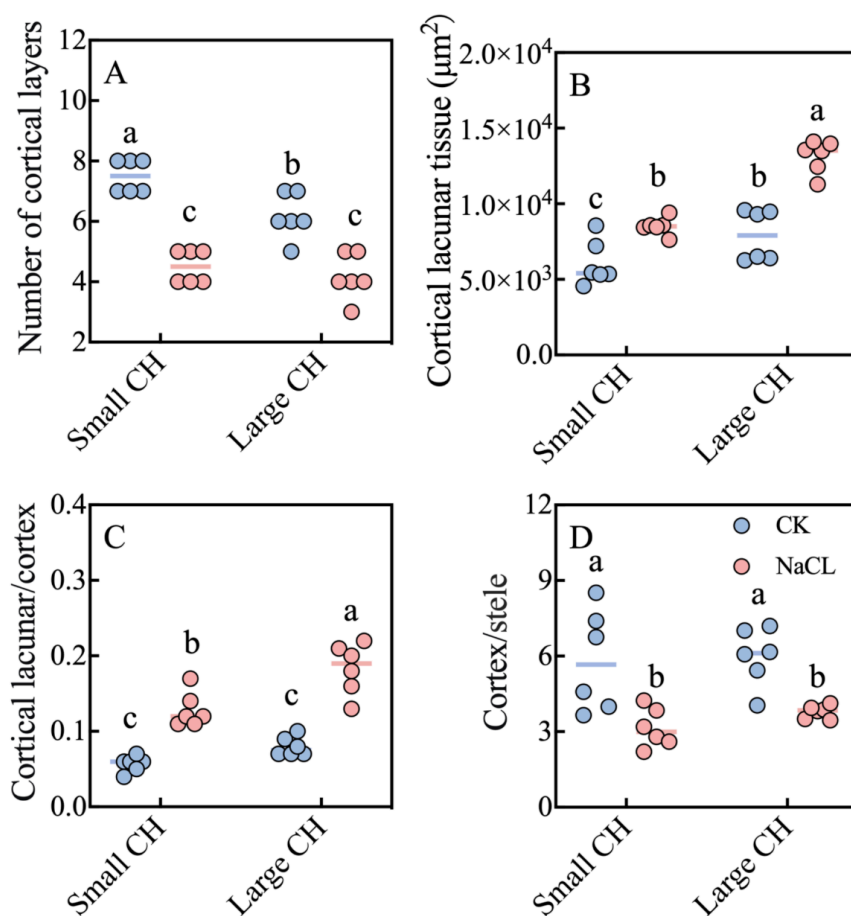
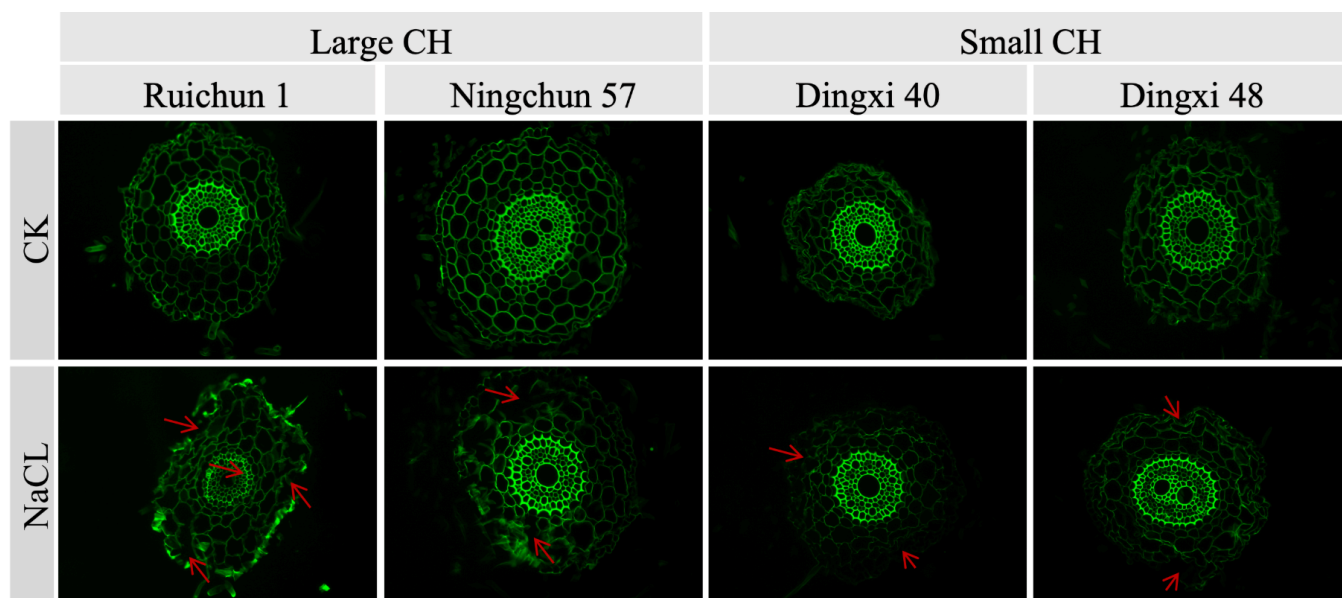


Figure 5

Responses of root cortical tissues to salt stress in two large-convex hull area and two small-convex hull area varieties. On the 7th day after salt stress treatment, cross-sections were taken from the primary roots of large-convex hull area varieties (Ruichun 1 and Ningchun 57) and small-convex hull area varieties (Dingxi 40 and Dingxi 48) at 6-9 cm from the root tip, and stained with acridine orange for fluorescence microscopy. Red arrows indicate cortical lacunar tissue regions (A). Statistical analyses of

number of cortical layers (B), cortical lacunar tissue area (C), cortical lacunar/cortex ratio (D), and cortex/stele ratio (E). CK, control treatment; NaCl, salt stress. Different lowercase letters indicate significant differences among the various salt stress and variety treatments ($P < 0.05$).

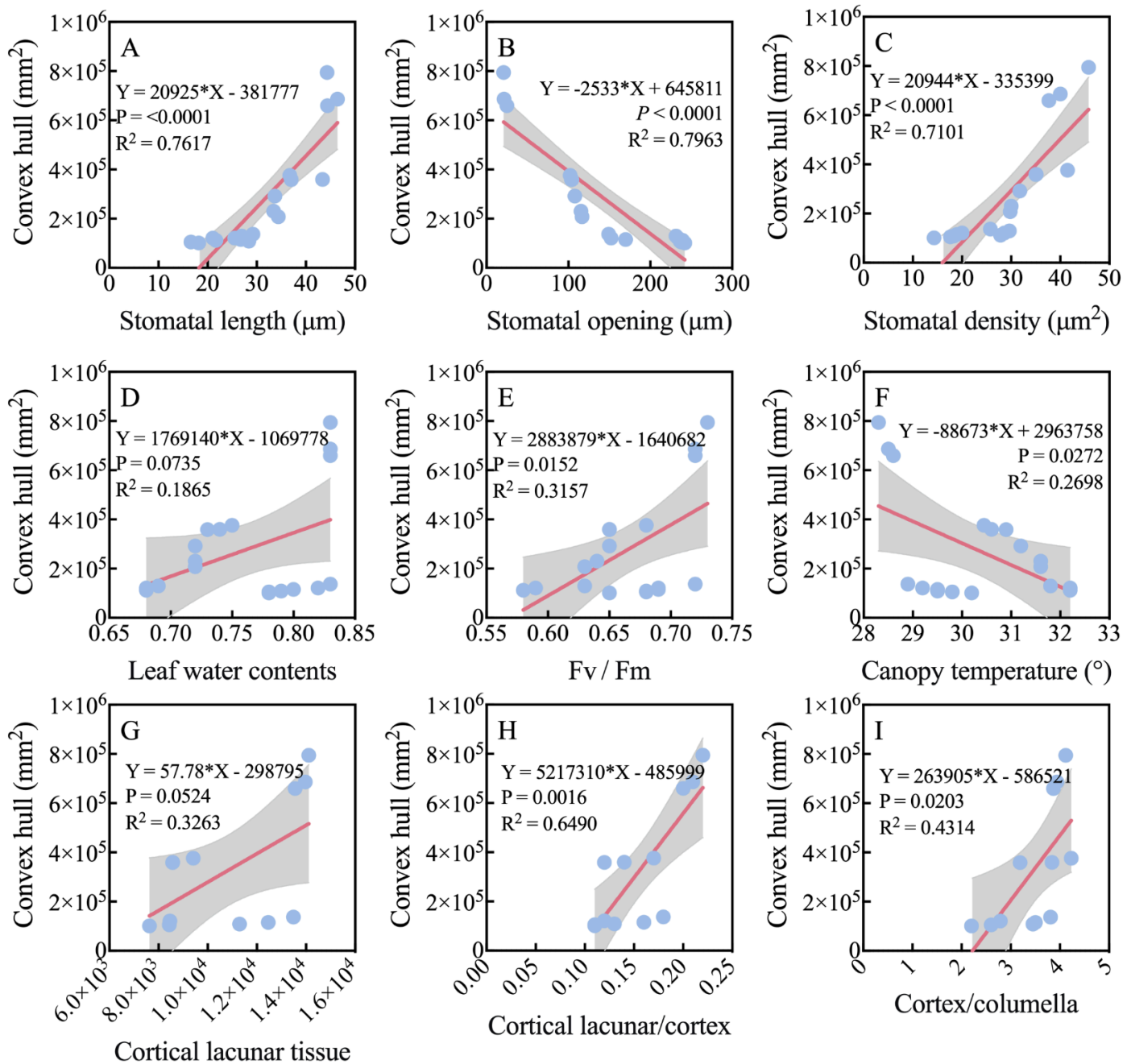


Figure 6

Linear regressions of stomatal traits, leaf water status, and root cortical tissue characteristics with convex hull area under salt stress. Correlations of stomatal length (A), stomatal aperture (B), stomatal density (C), leaf relative water content (D), F_v / F_m (E), canopy temperature (F), cortical lacunar tissue area (G), cortical lacunar/cortex ratio (H), and cortex/stele ratio (I) with root convex hull area.

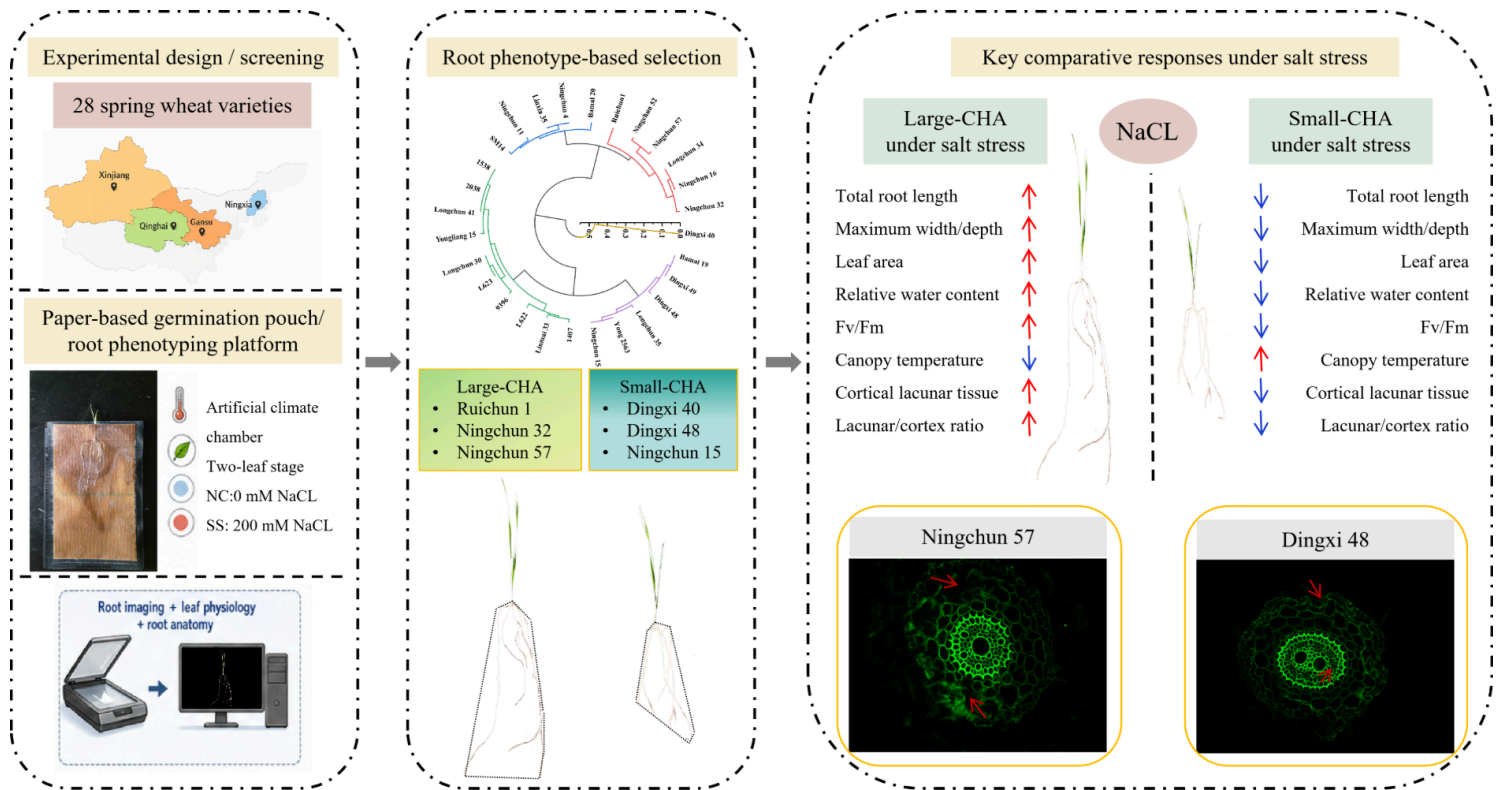


Figure 7

Conceptual model illustrating root convex hull area-mediated root–shoot coordination in spring wheat under salt stress.

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