Karlsruher Institut für Technologie

Jasmonate Signaling and Beyond: A Multi-Layered Perspective on Rice Salt Stress Adaptation

Zur Erlangung des akademischen Grades eines

DOKTORS DER NATURWISSENSCHAFTEN

(Dr. rer. nat.)

von der KIT-Fakultät für Chemie und Biowissenschaften

des Karlsruher Instituts für Technologie (KIT)

genehmigte

DISSERTATION

von

M.Sc. Nitin Dattatray Pawar

1. Referent: **Prof. Dr. Peter Nick**

2. Referent: **Prof. Dr. Holger Puchta**

Tag der mündlichen Prüfung: **24. April 2026**

This dissertation is licensed under a Creative Commons Attribution–NonCommercial–ShareAlike 4.0 International License (CC BY-NC-SA 4.0).
https://creativecommons.org/licenses/by-nc-sa/4.0/

Die vorliegende Dissertation wurde am **Joseph Gottlieb Kölreuter Institut für Pflanzenwissenschaften (JKIP)** des **Karlsruher Instituts für Technologie (KIT)**, Lehrstuhl I für molekulare Zellbiologie, im Zeitraum von **September 2021** bis **März 2026** angefertigt.

Eidesstattliche Erklärung

Hiermit erkläre ich, Nitin Dattatray Pawar, dass ich die vorliegende Dissertation, abgesehen von der Benutzung der angegebenen Hilfsmittel, selbstständig verfasst habe.

Alle Stellen, die gemäß Wortlaut oder Inhalt aus anderen Arbeiten entnommen sind, wurden durch Angabe der Quelle als Entlehnungen kenntlich gemacht.

Diese Dissertation liegt in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vor.

Zudem erkläre ich, dass ich mich beim Anfertigen dieser Arbeit an die Regeln zur Sicherung guter wissenschaftlicher Praxis des Karlsruher Instituts für Technologie (KIT) gehalten habe, einschließlich der Abgabe und Archivierung der Primärdaten, und dass die elektronische Version mit der schriftlichen übereinstimmt.

Karlsruhe, den 11. März 2026

Nitin Dattatray Pawar

Table of Contents

Acknowledgment

I would like to express my sincere gratitude to my supervisor, **Prof. Dr. Peter Nick**, for his continuous guidance, encouragement, and valuable scientific discussions throughout my doctoral research. His support, critical insights, and mentorship greatly contributed to the successful completion of this work.

I am also very thankful to **Dr. Michael Riemann** for his support, constructive feedback, and for providing an inspiring scientific environment at the Karlsruhe Institute of Technology.

My sincere thanks go to all members of the Plant Cell Biology group at KIT for their support and the pleasant working atmosphere during my PhD. I am especially grateful to **Eman**, who was in the final stage of her PhD when I joined the lab, for her guidance and support during my initial phase in the laboratory. I would also like to thank **Christian Metzger** for his support when I first arrived at the institute and for helping me during the early stages of my work and integration into the laboratory environment.

I am grateful to my colleagues and lab members for creating a friendly and supportive working environment. I would like to thank **Manuel Amann, Nathalie Hering, Wenjing Shi, Jathish Ponnu, Islam, Sascha Wetters, Gabriele Jürges, Sabine Purper, and Noemi** for contributing to the positive atmosphere in the laboratory. I also appreciate the collegial and supportive environment provided by the other members of the laboratory.

I would like to acknowledge **Dr. Thierry Heitz** and the team at the **Institut de Biologie Moléculaire des Plantes (IBMP), CNRS, Université de Strasbourg, France**, where I had the opportunity to spend three months as a visiting researcher. This research stay provided valuable scientific insights and greatly enriched my doctoral training.

I also thank the technical staff and the team of the **Botanical Garden at the Karlsruhe Institute of Technology** for their assistance in maintaining the plant material used in this study.

I gratefully acknowledge the **financial support provided by the Government of Maharashtra, India**, which enabled me to pursue my doctoral studies. I also sincerely thank **Oliver Klass** and the **International Office at Karlsruhe Institute of Technology** for their support and for providing the fellowship during the final six months of my doctoral studies.

Finally, I would like to express my deepest appreciation to my parents for their continuous encouragement, support, and belief in my academic journey.

Abbreviations

ABA	Abscisic acid
AOX	Alternative oxidase
ATP	Adenosine triphosphate
Ca^{2+}	Calcium ion
cDNA	Complementary DNA
CRISPR–Cas9	Clustered Regularly Interspaced Short Palindromic Repeats–CRISPR associated protein 9
cpm2	*Coleoptile photomorphogenesis 2*
EC	Electrical conductivity
Ece	Electrical conductivity of saturated soil extract
FAO	Food and Agriculture Organization
FL478	Salt-tolerant rice genotype
GBM	Ganges–Brahmaputra–Meghna delta
HAK	High-affinity potassium transporter
HKT	High-affinity potassium transporter
HK	Histidine kinase
HPLC	High-performance liquid chromatography
JA	Jasmonic acid
JA-Ile	Jasmonoyl-isoleucine
JAR1	Jasmonate resistant 1
JAZ	Jasmonate ZIM-domain protein

K^+	Potassium ion
MAPK	Mitogen-activated protein kinase
MCA	Mid1-complementing activity protein
miRNA	MicroRNA
MSL	Mechanosensitive-like channel
NaCl	Sodium chloride
Na^+	Sodium ion
NHX	Na^+/H^+ antiporter
NSCC	Non-selective cation channel
OPDA	12-oxo-phytodienoic acid
OXI1	Oxidative signal-inducible kinase 1
PCR	Polymerase chain reaction
PIP	Plasma membrane intrinsic protein
ptSOD	Plastidic superoxide dismutase
qPCR	Quantitative polymerase chain reaction
RC222	Salt-sensitive rice genotype
RD22	Responsive to dehydration 22
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SOS	Salt overly sensitive pathway
SOS1	Salt overly sensitive Na^+/H^+ antiporter
TIP	Tonoplast intrinsic protein
WT	Wild type
aoc	Allene oxide cyclase mutant
Hebiba	Jasmonate-deficient rice mutant

Zusammenfassung

Salinität ist ein bedeutender abiotischer Stressfaktor, der die landwirtschaftliche Produktion weltweit beeinflusst. Eine zunehmende Versalzung der Böden bedroht die globale Pflanzenproduktion. Diese ungünstigen Bedingungen zwingen Pflanzen dazu, komplexe Anpassungsmechanismen zu aktivieren. Da Pflanzen sessile Organismen sind, hängt ihr Überleben unter salinen Bedingungen von ihrer Fähigkeit ab, physiologische Veränderungen mit hormonellen Signalwegen zu koordinieren. Verschiedene Pflanzenhormone spielen dabei spezifische Rollen in den pflanzlichen Reaktionen auf biotische und abiotische Stressfaktoren. Unter ihnen nehmen Jasmonsäure (JA) und ihre Derivate, zusammenfassend als Jasmonate bezeichnet, eine zentrale Rolle bei der Regulation pflanzlicher Reaktionen auf Umweltstress ein. Jasmonate vermitteln vielfältige Prozesse, darunter die Aktivierung von Abwehrreaktionen, die Regulation des Wachstums sowie die Anpassung an Stressbedingungen. Dennoch ist die genaue Rolle der Jasmonat-Signalübertragung bei der Entwicklung der Salztoleranz in Reis noch nicht vollständig verstanden.

Um die Rolle von Jasmonaten während der Anpassung an Salzstress zu klären, wurden in dieser Studie zwei komplementäre experimentelle Ansätze verwendet. Der erste Ansatz konzentrierte sich auf hormonelle Defizienz durch die Analyse einer jasmonatdefizienten Allene-Oxid-Cyclase (*aoc*)-Mutante im Vergleich zu ihrem Wildtyp-Hintergrund Kitaake. Der zweite Ansatz untersuchte natürliche Variation in der Salztoleranz durch den Vergleich des toleranten Reisgenotyps FL478 mit dem salzsensitiven Genotyp RC222. Durch die Kombination dieser beiden Systeme war es möglich, jasmonatassoziierte Mechanismen auf morphologischer, physiologischer und molekularer Ebene zu untersuchen.

Die aoc-Mutante zeigte unter salinen Bedingungen eine verbesserte Stresstoleranz im Vergleich zum Wildtyp. Die Mutante behielt eine stärkere Wurzelentwicklung bei, zeigte geringere Blattschäden und akkumulierte während der Salzbehandlung eine höhere Biomasse. Auf physiologischer Ebene wurden die photosynthetische Leistung und die stomatäre Leitfähigkeit in der Mutante weniger stark durch Salinität beeinflusst als in Kitaake. Darüber hinaus wurde das ionische Gleichgewicht in der aoc-Mutante in verschiedenen Pflanzengeweben besser aufrechterhalten, da Natrium und Kalium im Vergleich zum Wildtyp effektiver reguliert wurden.

Molekulare Analysen zeigten unterschiedliche Muster der Genregulation im Zusammenhang mit der Jasmonat-Signalübertragung. Mitglieder der JAZ-Genfamilie wirken als Repressoren innerhalb des Jasmonat-Signalwegs. Diese Gene wurden durch Salinität im Wildtyp stark induziert, zeigten jedoch eine deutlich schwächere Induktion in der jasmonatdefizienten Mutante. Darüber hinaus zeigten Gene, die am Ionentransport und am Schutz vor oxidativem Stress beteiligt sind, darunter **NHX1** sowie plastid-lokalisierte Superoxiddismutase-Gene (**ptSOD1** und **ptSOD2**), genotypspezifische Expressionsmuster während des Salzstresses.

Ähnliche Muster wurden auch beim Vergleich natürlicher Reisgenotypen beobachtet. Der salztolerante Genotyp FL478 zeigte ein verbessertes Wachstum, eine bessere Biomasseerhaltung und eine effektivere Regulation des Ionengleichgewichts als der sensitive Genotyp RC222. Tolerante Pflanzen zeigten während der Salinitätsexposition eine stärker kontrollierte Jasmonatreaktion, wie durch Hormonanalysen während des Salzstresses gezeigt wurde. Die Ergebnisse deuten darauf hin, dass eine reduzierte oder moderierte Jasmonat-Signalübertragung mit einer verbesserten Salztoleranz in Reissämlingen verbunden ist.

Die Ergebnisse zeigen, dass Jasmonate mehrere zentrale Prozesse während des Salzstresses beeinflussen, darunter die Regulation des Wachstums, die Aufrechterhaltung der Ion Homöostase sowie den Schutz vor oxidativen Schäden. Diese Daten liefern ein klareres Verständnis der hormonellen Mechanismen, die der Anpassung an Salinität zugrunde liegen, und könnten zukünftige Strategien zur Verbesserung der Salztoleranz im Reisanbau unterstützen.

Abstract

Salinity is a major abiotic stress affecting agricultural production worldwide. Increasing soil salinity threatens global crop productivity. These unfavourable conditions force plants to activate complex adaptive functions. Because plants are sessile organisms, their survival under saline conditions depends on their ability to coordinate physiological alterations with hormonal signalling pathways. Different plant hormones play particular roles in plant responses to both biotic and abiotic stresses. Among them, jasmonic acid (JA) and its derivatives, collectively known as jasmonates, play central roles in regulating plant responses to environmental stress. Jasmonates mediate diverse processes, including defense activation, growth regulation, and stress adaptation. However, the precise role of jasmonate signalling in the development of salinity tolerance in rice remains insufficiently understood.

To clarify the role of jasmonates during salt stress adaptation, this study used two complementary experimental approaches. The first approach focused on hormonal deficiency by analysing a jasmonate-deficient allene oxide cyclase (*aoc*) mutant in comparison with its wild-type background Kitaake. The second approach examined natural variation in salinity tolerance by comparing the tolerant rice genotype FL478 with the salt-sensitive genotype RC222. By combining these two systems, it was possible to investigate jasmonate-associated mechanisms at morphological, physiological, and molecular levels.

The *aoc* mutant exhibited improved stress tolerance compared with the wild type under saline conditions. The mutant maintained stronger root development, showed less leaf damage, and accumulated higher biomass during salt exposure. At the physiological level, photosynthetic performance and stomatal conductance were less affected by salinity in the mutant than in Kitaake. In addition, ionic balance was better maintained in the aoc mutant across different plant tissues by more effective regulation of sodium and potassium compared with the wild type.

Molecular analyses showed distinct patterns of gene regulation associated with jasmonate signalling. Members of the JAZ gene family act as repressors within the jasmonate signalling pathway. These genes were strongly induced by salinity in wild-type plants but showed substantially weaker induction in the jasmonate-deficient mutant. Furthermore, genes involved in ion transport and oxidative stress protection, including NHX1 and plastid-localized superoxide dismutase genes (ptSOD1 and ptSOD2), showed genotype-specific expression responses during salt stress.

Similar patterns were observed when natural rice genotypes were compared. The salt-tolerant genotype FL478 showed improved growth, better biomass retention, and more effective regulation of ion balance than the sensitive genotype RC222. Tolerant plants manifested a more controlled jasmonate response during salinity exposure, as shown by hormone analyses during salt stress. Reduced or moderated jasmonate signalling is associated with improved salinity tolerance in rice seedlings, as suggested by the results.

The results show that jasmonates influence multiple crucial processes during salt stress, including growth regulation, maintenance of ion homeostasis, and protection against oxidative damage. These data deliver a clearer understanding of the hormonal mechanisms underlying salinity adaptation and may support future strategies intended to improve salt tolerance in rice cultivation.

1. Introduction

All life on Earth is directly or indirectly linked to plants. Plants are the backbone of life on the planet and the main source of food for humans. Since the beginning of human civilization, staple plants like rice have played an important role in food security. Rice is not just the main source of calories for millions of people worldwide. It also shapes economic stability and cultural identity as a key crop across Asia, Africa, and Latin America. As the global population continues to rise and is projected to reach approximately 9.7 billion by 2050, global food demand will increase substantially. Rice production must increase by 25–40% to meet the demand of a rising population amid climate change threats and resource limitations (UN DESA, 2022; FAO, 2024). Increasing agricultural crop production under climatic unpredictability is challenging, as soil degradation reduces the land available for cultivation. Among the diverse biotic and abiotic stresses threatening rice production, salinity is among the most pervasive and destructive. Salinity affects irrigated land, with soil salinity exceeding 20% in some areas and around 7% of all cultivated land. Soil degradation caused by salinity is increasing due to seawater intrusion, rising groundwater tables, land-use changes, and climate-induced hydrological instability (Rengasamy, 2010; FAO, 2021). The region, which is highly vulnerable and densely populated for farming, includes the coastal regions of India, with the Ganges-Brahmaputra-Meghna (GBM) and the Mekong Delta in Vietnam, the Yellow River in China, and the Nile Delta in Northern Egypt. This soil degradation and rising salinity are driven by multiple interacting factors, particularly groundwater salinization, sea-level rise, and seawater intrusion into coastal farmland. The cumulative effects of these factors threaten the regions, which contribute significantly to global rice production (FAO, 2023; Fig. 1; Table 1).

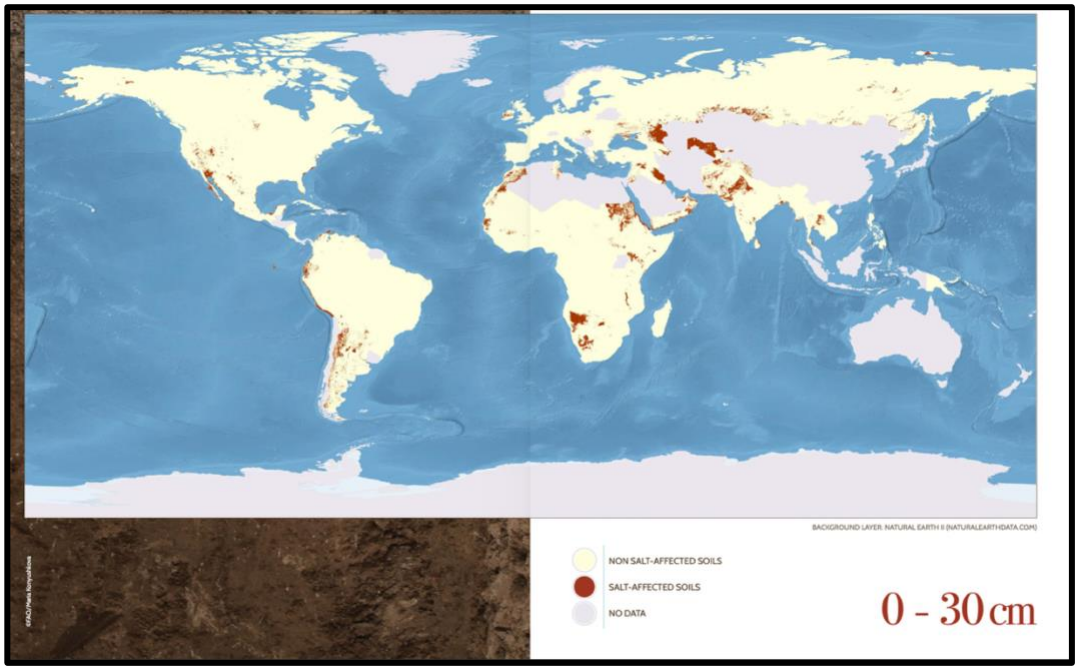


Fig. 1. Global distribution of salt-affected soils in the topsoil layer (0–30 cm). Salt-affected soils are widely distributed throughout continents, including major agricultural and rice-growing regions. Adapted from FAO GSASmap v1.0.

ECe (dS m^{-1})	Salinity intensity	Effect on crop growth	ESP (%)	Sodicity hazard
<0.75	None	None	<15	None
0.75–2	Slight	None	15–30	Slight
2–4	Moderate	Yields of sensitive crops may be restricted	30–50	Moderate
4–8	Strong	Yields of many crops are limited	50–70	High
8–15	Very strong	Only tolerant crops yield satisfactorily	>70	Extreme
>15	Extreme	Only a few very tolerant crops yield satisfactorily	—	—

Table 1. The types of soil salinity and sodicity and their effects on crop growth.

Being a glycophyte plant, rice is highly intolerant of saline conditions. Soil electrical conductivity (EC) of 4–8 dS m^{-1} affects different life stages of plants, including germination, vegetative growth, tillering, panicle formation, spikelet fertility, and grain filling (Munns & Tester, 2008). Salinity stress has two phases: an osmotic phase and an ion-toxicity phase, distinguished in time (Fig. 2).

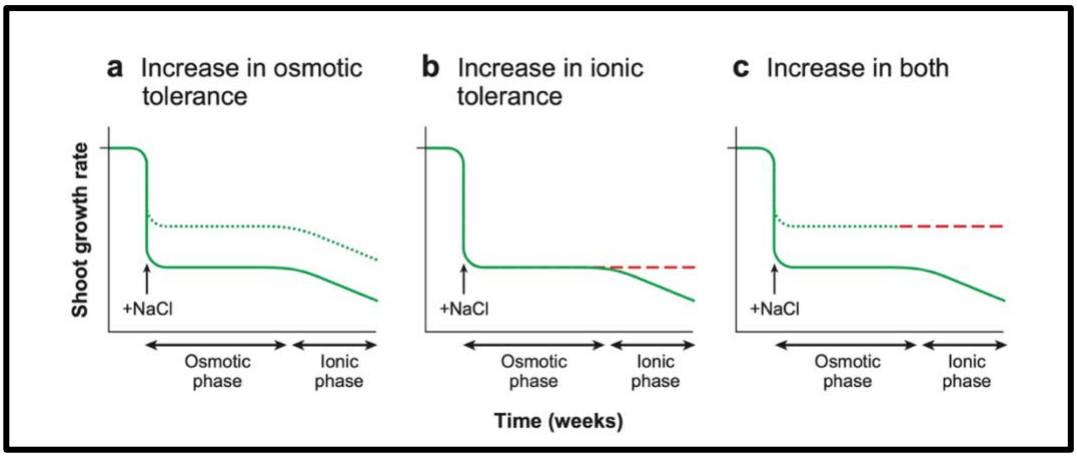


Fig. 2. Two-phase model of salinity stress showing an early osmotic phase and a later ionic toxicity phase affecting shoot growth over time. Panels illustrate improved osmotic tolerance (a), improved ionic tolerance (b), or improvement in both components (c). Adapted from Munns and Tester (2008).

The additive effect of two phases results in reactive oxygen levels exceeding the cell's ability to cope, leading to oxidative damage (Fig. 3).

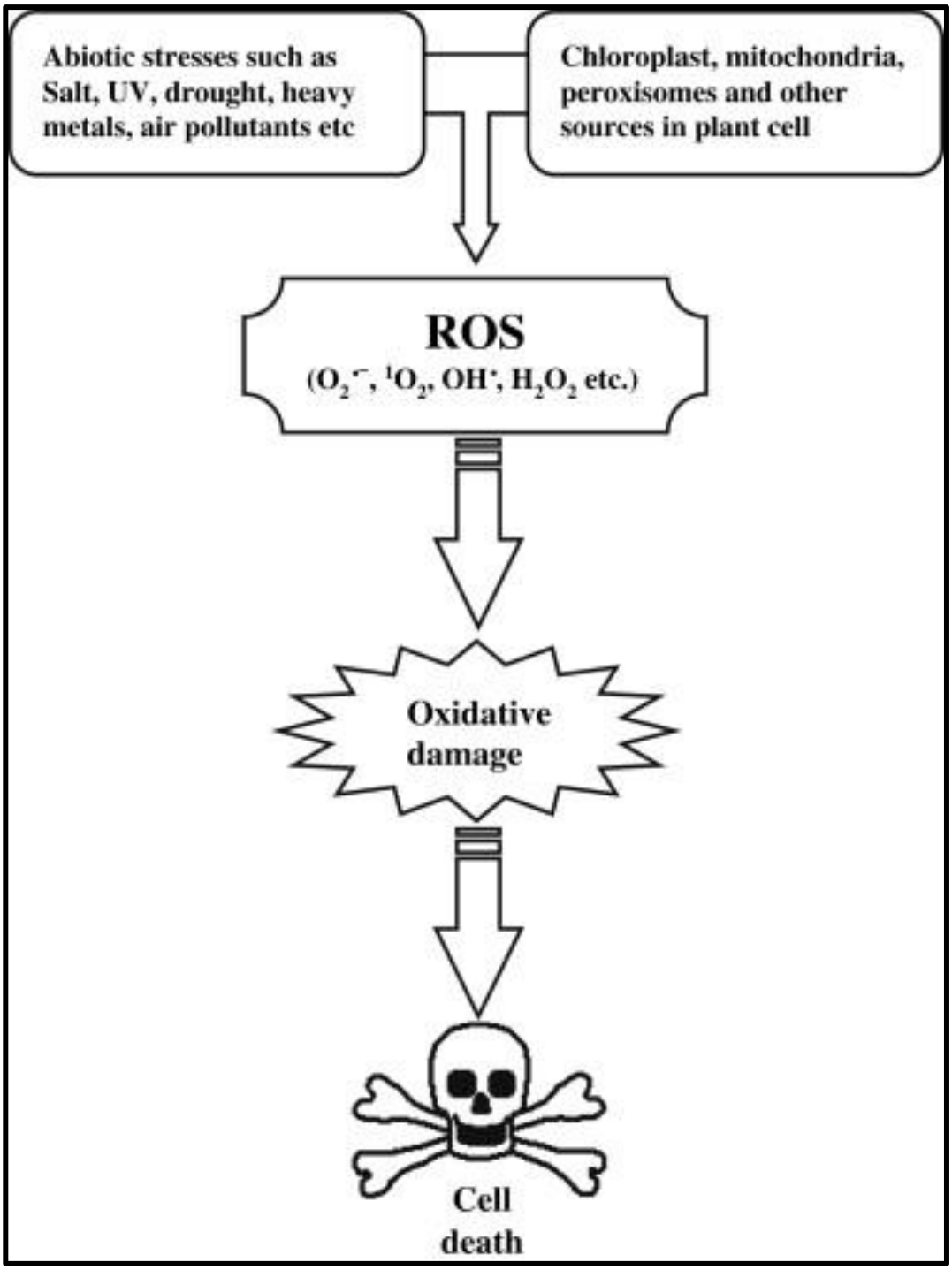


Fig. 3. Abiotic stress–induced production of reactive oxygen species (ROS) and resulting oxidative damage in plant cells. Salinity stress enhances ROS generation, leading to lipid peroxidation, protein oxidation, DNA damage, and ultimately cell death. Adapted from Gill and Tuteja (2010).

Cumulative advances in genomics, transcriptomics, ionomics, and genome editing (such as CRISPR–Cas9) over the last two decades have increased our understanding of how rice adapts to unfavourable saline conditions (Ahmad *et al*., 2022). Although our understanding of plant orchestration in saline conditions is increasing, the role of hormones remains elusive.

Specifically, the role of jasmonic acid (JA) remains poorly characterized. JA plays a vital role in defense responses, growth inhibition, senescence, and developmental processes (Wasternack & Song, 2017). Jasmonic acid (JA) plays an important role in early stress signaling under saline conditions and contributes to the regulation of reactive oxygen species (ROS). However, excessive or prolonged accumulation of JA can have detrimental effects, including growth inhibition, accelerated senescence, and increased salt damage in rice (Riemann *et al*., 2015; Delgado *et al*., 2022). Notably, Hazman *et al*. (2015) found that JA-deficient mutants, such as *aoc*, showed greater adaptability in early growth, delayed senescence, reduced shoot Na^+ accumulation, and increased survival rates to saline conditions than the JA-producing line. In addition, recent studies have shown that not only transcriptional regulators but also post-transcriptional regulators, such as microRNAs (miRNAs), interact with the hormonal pathway to modulate stress responses. Specifically, the communication between JA and miRNAs under salinity conditions remains elusive (Mondal *et al*., 2018). Simultaneously conducting the study on JA-dependent responses and on naturally salt-tolerant and sensitive rice varieties such as FL478 and RC222 may elucidate whether JA suppression replicates or enhances natural tolerance mechanisms. The thesis adopts an integrative approach, combining insights from physiology, ionomics, transcriptomics, miRNA profiling, and comparative genomics to clarify the role of jasmonic acid in rice adaptation to salt stress. Using a jasmonate-deficient line with aoc knockout by CRISPR, wild-type, tolerant, and sensitive genotypes. The study seeks to elucidate the mechanisms by which hormonal and post-transcriptional networks contribute to rice resilience to salinity.

1.1 Global Importance of Rice

Enhanced salt concentration in the soil affects all the developmental stages of rice from germination of the seed to grain filling, leading to a decrease in yield and quality (Flowers and Yeo, 1995; Munns and Tester, 2008; Table 2).

Rice growth stage	Major effects of salinity stress	Typical physiological consequence	Key references
Germination & seedling establishment	Delayed germination, reduced emergence, poor seedling vigor	Weak stand establishment	Zeng & Shannon, 2000; Zeng *et al*., 2001
Vegetative stage (tillering)	Reduced leaf expansion, decreased photosynthesis, inhibited tillering	Reduced biomass accumulation	Flowers & Yeo, 1995; Munns & Tester, 2008
Reproductive stage (panicle initiation–flowering)	Spikelet sterility, impaired pollen viability, disturbed fertilization	Severe yield loss	Zeng *et al*., 2002; Hakata *et al*., 2012
Grain filling	Reduced assimilate transport, increased grain chalkiness, lower 1000-grain weight	Reduced grain quality and yield	Hakata *et al*., 2012; Munns & Tester, 2008

Table 2. Growth-stage–specific sensitivity of rice to salinity stress. Sources: Flowers and Yeo (1995); Zeng and Shannon (2000); Zeng *et al*. (2001, 2002); Munns and Tester (2008); Hakata *et al.* (2012).

In the initial stage of plant development, seed germination and seedling emergence are inhibited and delayed by excessive salt concentration in the soil, respectively, which is reflected in the uneven plant stands (Zeng and Shannon, 2000). Salinity negatively affects cellular growth and photosynthetic efficiency during the vegetative stage, hindering plant growth with reduced dry matter accumulation and fewer tiller formation (Maas and Hoffman, 1977). Among the most sensitive stages of plant development is the reproductive stage, at which salinity disturbance compromises panicle development, and pollen and fertilization, resulting in spikelet sterility (Zeng *et al*., 2002; Moradi and Ismail, 2007). Negative impacts on carbon fixation during grain development result in smaller kernels with increased chalkiness and reduced grain quality (Hakata *et al*., 2012). Even under moderate saline stress, rice plants experience a 50–80% yield reduction, and severe salinity conditions result in complete crop loss (Zeng *et al*., 2001; Munns and Tester, 2008). As salinity impacts all growth stages of the rice plant, recovery from this stress is limited (Munns, 2002), which makes salinity a major obstacle to rice production worldwide and to global food security (Ismail and Horie, 2017).

1.2 Socio-Economic Implications of Salinity Stress

The risk of rising soil salinity endangers rice cultivation as well as wider biological and agricultural systems linked to traditional land use, resulting in notable socio-economic impacts (FAO, 2017; IPCC, 2019)**.** Specifically, the smallfarm holders in coastal and deltaic areas are threatened by increasing salinity in the farmland where agriculture relies on single-cultivation practices with limited capacity to support extensive irrigation, drainage, and soil management infrastructure **(**World Bank, 2020; Ali and Landis, 2025**).** These regions are associated with monoculture practices and are highly vulnerable to factors such as salinity, which can reduce crop yields or lead to complete crop washout **(**Ismail *et al*., 2014; Russ *et al.,* 2020**)**. It has a huge impact on household income and economic stability at the farm and community levels **(**FAO, 2017; World Bank, 2020**)**. Small landholders are forced to leave farming due to a repeated cycle of failed or lost crops caused by soil infertility. This leads to mass migration from rural to urban areas. These factors are altering the region's economy, which relies on rice production and related value chains (World Bank, 2020; FAO, 2021). The combined impacts of salinity show that it is not just a limitation on plant growth but also a major socio-economic challenge.

1.3 Why Salinity Is “a Tough Enemy”

Salinity is among the most destructive abiotic stresses for the model cereal crop (Munns and Tester, 2008; Ismail and Horie, 2017). It is described as a tough enemy of agricultural production due to its persistent, cumulative, and largely irreversible nature (Rengasamy, 2010; FAO, 2017). The major difference between biotic stresses and salinity is that salinity builds gradually over time; it is invisible until crop performance diminishes (Munns, 2002; Qadir *et al.,* 2014). The salt build-up in the soil profile is scarce in many salt-prone regions because it requires a large amount of high-quality water to remove the salt, which is not available (Rengasamy, 2010; Qadir *et al*., 2014). The excessive salt accumulation leads to sodic conditions (Rengasamy and Olsson, 1991). High soil salt accumulation causes dispersion of clay particles (Shainberg and Letey, 1984). This results in lower soil porosity, infiltration, and aeration, which affects the plant's root structure and its ability to absorb water (Qadir and Schubert, 2002). Under stress, plants suffer physiological damage before they exhibit visible symptoms (Munns and Gilliham, 2015). Recovery from the saline condition once it is stabilized is gradual, not cost-effective, and cannot be fully restored, especially with a limited water supply in a farming system (Qadir *et al.,* 2014; FAO, 2017). In contrast, biotic stress, such as

pests or diseases, can often be managed through short-term interventions or chemical treatments (Strange and Scott, 2005). To adapt to this situation and improve productivity, we need an effective solution that combines improved irrigation and drainage practices with sustainable soil and water management strategies and a salt-adaptive rice line (Ismail and Horie, 2017; FAO, 2021).

1.4 Classification of Plants Based on Salt Tolerance

1.4.1 Glycophytes: Salt-Sensitive Plants

A group of plants known as glycophytes comprises numerous terrestrial species such as wheat, maize, rice, barley (which exhibits moderate tolerance), soybean, and most horticultural crops **(**Flowers and Colmer, 2008; Munns and Tester, 2008**)**. These plant species evolved in less salt-contaminated soil **(**Flowers *et al*., 2015**)**. Therefore, they possess weaker physiological and molecular mechanisms for adapting to saline conditions (Munns and Gilliham, 2015; Zhu, 2016).

1.4.1.1 Characteristics of Glycophytes

Glycophytic plants are inherently low in their ability to tolerate external sodium chloride (NaCl) concentrations above approximately 40–80 mM. This is reflected in their growth inhibition and reduced productivity under saline conditions (Munns and Tester, 2008; Flowers and Colmer, 2008). Glycophytic plants are unable to restrict the entry of sodium (Na^+) after interaction with high-salinity soil, leading to excessive ion entry into the plant (Zhu, 2003; Horie *et al*., 2012). In addition, these sensitive plants have limited capacity reduce cytosolic sodium concentration through vacuolar sequestration, which restricts the capacity to compartmentalize Na^+ and Cl^- away from sensitive cytosolic processes (Apse and Blumwald, 2007; Munns and Gilliham, 2015). Changes in ionic homeostasis lead to increase reactive oxygen species (ROS), resulting in oxidative stress with prolonged salt exposure. These changes accelerate membrane impairment, chlorophyll degradation, and premature senescence (Mittler, 2002; Gill and Tuteja, 2010).

1.4.1.2 Rice as a Typical Glycophyte

In high salt soil, the most vulnerable developmental stages of the rice plant are seedling establishment, tillering, and reproductive development, which can lead to irreversible damage to growth and yield formation (Zeng *et al*., 2001; Moradi and Ismail, 2007). If the salt concentration increases beyond 8–12 dS m^{-1}, it causes severe effects on plant development,

such as growth inhibition, spikelet sterility, and near-complete yield loss (Maas and Hoffman, 1977; Zeng and Shannon, 2000). Because rice has a limited strategy for responding to salinity stress, it can serve as a valuable model plant for understanding the physiological and molecular pathways of glycophytes, which can help generate salinity-adapted crop lines using genetic, molecular, and agronomic strategies to improve salinity adaptation in crop plants (Ismail and Horie, 2017; Munns *et al*., 2016).

1.4.2 Halophytes: Naturally Salt-Tolerant Plants

Halophytes are inherently tolerant of salinity, as they evolved to thrive in harsh environments with high salt concentrations in soil and water, including saline soils, salt marshes, coastal zones, and salt deserts (Flowers and Colmer, 2008; Cheeseman, 2015). They also show optimal growth at moderate to high salt levels compared to salt-sensitive glycophyte plants (Flowers *et al*., 2010; Munns and Tester, 2008). The most notable halophyte species include *Salicornia, Atriplex, Suaeda, Halogeton, Bolboschoenus,* and mangrove species such as *Avicennia and Rhizophora* (Parida and Das, 2005; Flowers and Colmer, 2015). These species are adapted to these harsh conditions, as evidenced by their morphological, physiological, and molecular adaptations that confer exceptional salinity tolerance (Flowers *et al*., 2015; Shabala *et al*., 2014).

1.4.2.1 Key Adaptations of Halophytes

Halophytes are adapted to a saline environment, mainly through their efficient orchestration of sodium (Na^+) movement through specialized root barriers and tightly controlled ion transport systems (Flowers and Colmer, 2008; Munns and Tester, 2008; Shabala *et a*l., 2014; Horie *et al*., 2012). Once sodium enters the cell, it is necessary to prevent toxic buildup in the cytosol, where most vital pathways are located. To reduce toxic buildup, it is stored in vacuoles via tonoplast Na^+/H^+ antiporters (NHXs), protecting the cytoplasm from toxicity (Apse and Blumwald, 2007; Bassil and Blumwald, 2014). Another way is that some halophytes have salt-secreting glands or bladders that actively remove excess ions from leaves, boosting salt tolerance (Flowers *et al*., 2010; Yuan *et al*., 2016). Some species of halophyte exhibit morphological succulence via enlarged vacuoles and increased water storage, which enables dilution of intracellular salts and reduce osmotic stress (Flowers and Colmer, 2008; Cheeseman, 2015), while other species biochemically enhance antioxidant defense systems to reduce oxidative stress caused by unfavourable conditions, marked by elevated activities of enzymes that scavenge reactive oxygen species (ROS) (Mittler, 2002; Bose *et al.,* 2014). Combining these adaptive traits, many halophytes are able to thrive in a harsh environment of salinity ranging from 300 to 500 mM

NaCl (Flowers and Colmer, 2008; Parida and Das, 2005). Specifically, increased activation of salt-sensing pathways, such as the salt overly sensitive (SOS) pathway, and efficient ion regulation in the root contribute to this tolerance. Conversely, glycophytes lack efficient mechanisms to prevent toxic cytosolic build-up and structural adaptations. These adaptive traits are vital for generating a rice line that can adapt to a saline environment (Zhu, 2003; Ismail and Horie, 2017).

1.4.3 Intermediate or Moderately Salt-Tolerant Plants

Plant species that are neither halophytes nor glycophytes show moderate salt adaptation (Flowers & Colmer, 2008; Munns & Tester, 2008). These species thrive and maintain productivity at moderate salinity levels. Unlike glycophyte crop species, which show severe growth reduction at moderate salinity (Roy *et al*., 2014). The mechanism behind adaptation to salinity, such as partial ion exclusion via the roots, reduces the accumulation of harmful ions by compartmentalizing them in the vacuole, and an adaptive antioxidant defense system helps diminish the damage caused by salt stress (Tester & Davenport, 2003; Zhu, 2003). The best examples for this species are barley (*Hordeum vulgare*), among the most salt-tolerant cereals (Maas & Hoffman, 1977; Munns *et al*., 2006), quinoa (*Chenopodium quinoa*), a crop with exceptional salinity tolerance despite not being a true halophyte (Adolf *et al*., 2013; Ruiz *et al*., 2016) and pearl millet (*Pennisetum glaucum*), which shows resilience under saline and marginal soil conditions (Ashraf & McNeilly, 2004; Yadav *et al*., 2012). Such species retain certain adaptive halophytic traits, enabling them to balance growth and stress tolerance in saline environments (Flowers *et al.,* 2010).

1.4.3.1 Key Adaptive Features of Intermediate Species

The moderately adaptive plant species are able to protect vital enzyme function and cellular pathways by retaining strong potassium (K^+) levels under saline conditions (Shabala & Cuin, 2008). Plants of this group exhibit the ability to exclude sodium (Na^+) and balance ion movement to reduce toxic sodium buildup in the cytoplasm. To overcome the effects of osmotic stress, these plants increase production of electrically neutral compounds called compatible osmolytes. The main compounds, such as proline, glycine betaine, and soluble sugars, which help stabilize cellular structures and maintain water balance without interfering with normal cellular metabolism, even at peak concentrations (Ashraf & Foolad, 2007). Understanding molecular and physiological pathways in moderate tolerance species will help to unravel the gradual adaptation of these plants to salinity and uncover traits that are both effective and

relevant to crop improvement. Acting as a bridge between halophytes and glycophytes, these species offer practical traits that can realistically be transferred to major crops, including rice, to improve their ability to cope with salinity (Munns *et al*., 2006; Negrão *et al*., 2017; Table 3).

Plant category	Typical salinity tolerance	Key physiological traits	Examples	Key references
Glycophytes (salt-sensitive)	< 40–80 mM NaCl	Poor Na^+ exclusion, limited vacuolar sequestration, strong growth inhibition, high ROS accumulation	rice, wheat, maize, soybean	Flowers & Colmer, 2008; Munns & Tester, 2008
Intermediate / moderately tolerant plants	Moderate salinity	Partial Na^+ exclusion, better K^+ retention, moderate vacuolar compartmentation, osmolyte accumulation	Barley, quinoa, pearl millet	Maas & Hoffman, 1977; Munns *et al*., 2006; Adolf *et al*., 2013
Halophytes (salt-tolerant)	300–500 mM NaCl or higher	Efficient Na^+ sequestration in vacuole, salt glands/bladders, succulence, strong antioxidant systems	*Salicornia, Atriplex, Suaeda*, mangroves	Flowers & Colmer, 2008; Parida & Das, 2005

Table 3. Comparative classification of glycophytes, moderately salt-tolerant plants, and halophytes based on salinity tolerance ranges, key physiological traits, and representative examples

1.5. Physiological Basis for Categorizing Salt Tolerance

The categorization of plant species into glycophytes, moderately tolerant species, and halophytes is primarily determined by how plants interact with and respond to salinity stress, not at a single level but across multiple levels. These interactions include sensing the salt from the soil around roots to control the orchestration of the ion movement via the physiological pathway. At the structural level, organs such as the root and leaf exhibit architectural changes. Hormonal levels interact with other hormonal pathways for stress perception and regulation. At the end, at the molecular level, stress-responsive genes and pathways are induced (Munns & Tester, 2008). Overall, these parameters provide an integrated view of a plant's ability to detect, cope with, and adapt to increased soil salinity (Zhu, 2001; Deinlein *et al*., 2014; Fig. 4).

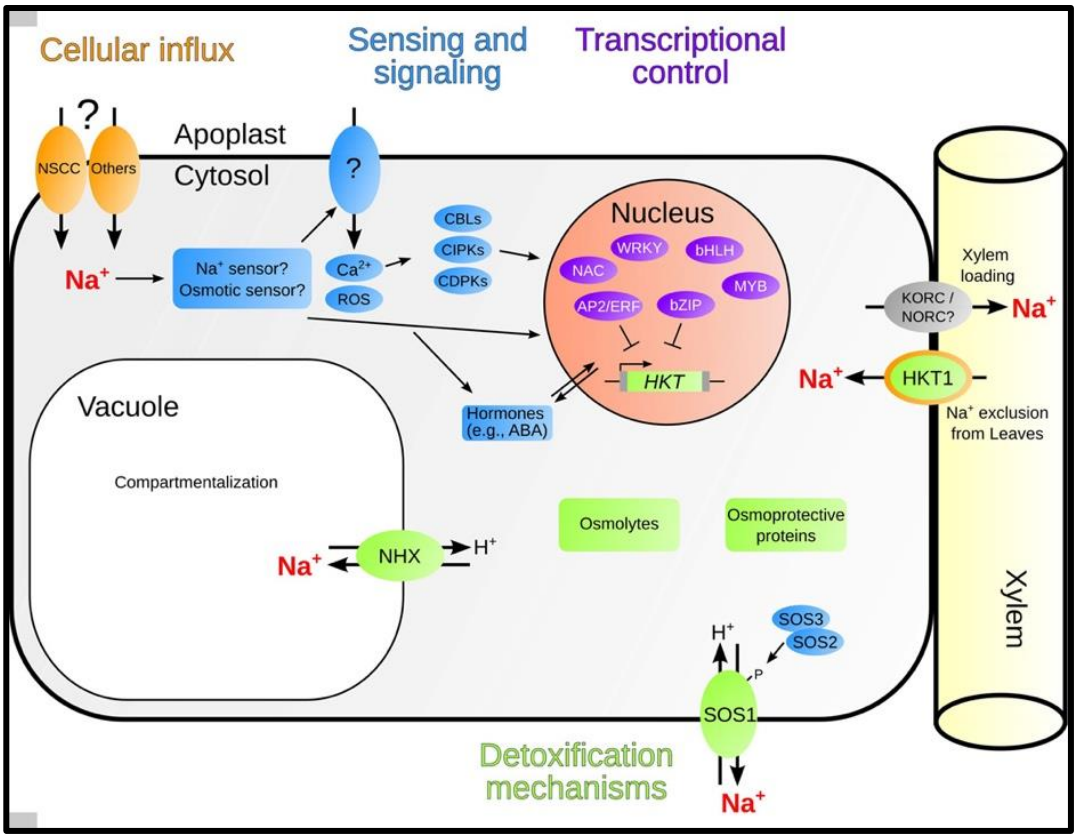


Fig. 4. Integrated physiological and molecular mechanisms underlying plant salt tolerance. Salinity perception triggers Ca^{2+} and reactive oxygen species (ROS) signaling, hormonal responses, transcriptional regulation, ion exclusion at the plasma membrane (SOS1), vacuolar sequestration of Na^+ (NHX), retrieval of Na^+ from the xylem (HKT transporters), osmolyte accumulation, and detoxification processes that collectively determine plant growth and survival under saline conditions. Adapted from Deinlein *et al*. (2014).

1.5.1 Growth and Survival Thresholds under Salinity

The easiest way to categorize plants into different groups is based on how they handle varying levels of salinity (Maas & Hoffman, 1977; Munns, 2002). In the case of sensitive glycophytes, plants are unable to grow at low salinity levels of 40–80 mM, exhibiting reductions in growth and yield. In contrast, the salt-tolerant halophytes grow normally under much higher salinity, often between 200 and 600 mM NaCl, depending on the species (Flowers *et al*., 2010; Glenn *et al*., 1999). Between these groups, moderately tolerant species grow well at low salinity conditions and are viable at moderate salt concentrations (Roy *et al*., 2014).

1.5.2. Ion Homeostasis Capacity

The plant's ability to tolerate salt stress is determined by its regulation of ion movement (Tester & Davenport, 2003). Under saline conditions, it is important to maintain low sodium (Na^+) import to avoid toxic cytoplasmic accumulation and maintain stable Na^+/K^+ ratios. In halophytes, the mechanisms of ion exclusion, sequestration, and redistribution make this possible (Apse & Blumwald, 2007; Shabala & Mackay, 2011). In contrast to salt-sensitive plants, glycophyte plants are unable to prevent sodium influx into the cytoplasm, leading to toxic buildup and altering the Na^+/K^+ ratio. Losing potassium (K^+) disrupts metabolic processes and causes cellular damage (Zhu, 2003; Munns & Tester, 2008). So, the classification of plants tolerant to salinity is based on how they maintain ionic balance under saline conditions (Deinlein *et al*., 2014).

1.5.3 Structural and Anatomical Adaptations

Structural changes are another vital factor in distinguishing salt-tolerant from salt-sensitive plants (Flowers & Colmer, 2008). Halophyte plants have evolved specialized features that help them adapt to saline environments. Features such as specialized structures, called glands or bladders, are located mainly on the leaf epidermis and function to remove excess salts. Succulent tissues accumulate more water to dilute harmful ion concentrations inside cells. To limit transpiration-driven water loss and, indirectly, reduce salt accumulation in shoots, halophytes often develop thick, waxy cuticles (Flowers *et al*., 2010; Dassanayake & Larkin, 2017). In contrast, glycophytes lack these specialized features and therefore exhibit growth inhibition and an inability to adapt to and thrive in saline conditions (Munns, 2002).

1.5.4 Hormonal Response Dynamics

Plant hormones orchestrate many pathways that enable plants to respond to salinity stress (Peleg & Blumwald, 2011). In salt-sensitive glycophytes, saline stress often induces excessive accumulation of stress-responsive hormones, such as abscisic acid (ABA) and jasmonic acid (JA). The balance between them is vital for growth and development; however, stress-induced imbalance leads to strong growth suppression, accelerated senescence, and impaired reproductive development (Fujita *et al*., 2006; Ryu & Cho, 2015). In contrast, halophytes can manage the balance between abscisic acid (ABA) and jasmonic acid (JA) carefully, without causing severe growth inhibition or triggering JA-mediated senescence (Flowers & Colmer, 2008; Peleg & Blumwald, 2011).

1.5.5 Transcriptomic and Epigenetic Resilience

Halophytes maintain stable patterns of gene expression for metabolic and stress-responsive genes, enabling sustained tolerance during prolonged salt exposure (Dassanayake *et al.*, 2011). Chromatin-based regulatory mechanisms, such as DNA methylation, histone modifications, and chromatin remodelling (also called epigenetic mechanisms), frequently support this stability by adjusting gene regulation, so that expression patterns under salt stress closely resemble those under normal conditions (Chinnusamy & Zhu, 2009; Chang *et al.,* 2015). In contrast to halophytes, longer exposure of glycophytes to salt stress causes a broad and poorly coordinated set of changes in gene expression. These transcriptional disturbances interfere with essential metabolic functions and stress signaling networks. As a result, these plants struggle to maintain cellular homeostasis, show impaired stress adaptation, and experience reduced survival under saline conditions (Zhu, 2001; Munns & Tester, 2008). The collective effects of physiological, hormonal, and gene regulatory mechanisms provide a framework for understanding plant salt sensitivity or tolerance.

1.6. Position of Rice Within the Salt-Tolerance Framework (with evidence)

Across the spectrum of plant species with respect to salt adaptation, rice (Oryza sativa L.) is recognised as a glycophyte, meaning it is vulnerable to salt exposure. Even at a low salinity threshold around EC ≈ 3 dS m^{-1} (≈ 30 mM NaCl), yield and growth are strongly affected (Batayeva *et al*., 2018; Neang *et al*., 2020). This variability in salt sensitivity underlies the genetic and genomic diversity of rice accessions (Table 4).

Rice genotype / group	Salt tolerance class	Typical salinity level tested	Key features	References
Pokkali (landrace)	Salt-tolerant	EC 10–12 dS m^{-1}	Strong Na^+ exclusion, good Na^+/K^+ homeostasis, Saltol locus	Diep *et al*., 2024
Nona Bokra (landrace)	Salt-tolerant	EC 10–12 dS m^{-1}	Efficient ion regulation, Saltol-linked tolerance	Diep *et al*., 2024
FL478 (Pokkali × IR29)	Salt-tolerant	EC 12 dS m^{-1}	Improved growth, better ion balance than sensitive lines	Diep *et al*., 2024
NSIC Rc222	Salt-sensitive	EC 12 dS m^{-1}	Strong growth inhibition, poor ion homeostasis	Neang *et al*., 2020
Cultivated rice (general)	Glycophyte	≥ EC 3 dS m^{-1} (~30 mM NaCl)	Yield and growth decline	Batayeva *et al*., 2018; Diep *et al*., 2024

Table 4. Position of rice within the salt-tolerance framework: representative tolerant and sensitive genotypes, salinity thresholds, and key tolerance features. Sources: Batayeva *et al*. (2018); Diep *et al*., (2024)

Some wild and breeding lines of rice exhibit higher heritable salt tolerance than sensitive cultivars (Diep *et al*., 2024). Among the landraces, well-known names are Pokkali and Nona Bokra, with genetically improved salt-tolerant lines such as FL478 exhibiting greater tolerance and performance than salt-sensitive cultivars when exposed to saline conditions **(**Nguyen *et al*., 2022)**.** FL478 (salt tolerant) and NSIC Rc222 (salt sensitive) are used as standard checks for salt tolerance and salt sensitivity, respectively, under controlled salinity screening conditions (e.g., EC 12 dS m^{-1}). The high adaptability to salinity observed in tolerant lines is governed by multiple physiological and molecular mechanisms, such as avoiding toxic sodium accumulation in the cytosol through potassium and sodium transporters linked to the Saltol locus in tolerant cultivars like Pokkali and Nona Bokra. These mechanisms contribute to the maintenance of Na^+/K^+ homeostasis and are supported by stronger stress-response networks such as ROS-related and signaling components (Diep *et al*., 2024). Because of their contrasting responses to salinity, these genotypes provide useful model systems for identifying tolerance-related genes

and networks, understanding how hormonal signaling interacts with stress responses, and determining whether key adaptive traits depend on jasmonic acid (JA). A combined study measuring hormone levels and RNA-seq analysis showed that JA signaling modulates several components of plant responses to salinity, and that JA-deficient lines display altered ion partitioning and overall stress physiology (Ndecky *et al*., 2023). These findings provide a strong idea for the thesis strategy of planning a comparative framework that includes jasmonic acid (JA)–deficient or JA-altered mutants alongside naturally salt-tolerant lines such as FL478 and salt-sensitive benchmark cultivars like *NSIC Rc222*. This approach enables a clearer separation of JA-dependent and JA-independent mechanisms of salinity tolerance, while also allowing key physiological, hormonal, and molecular responses to be evaluated across contrasting genetic backgrounds.

1.7. Sodium Ion–Specific Stresses (Ionic Stress Phase)

1.7.1. Sodium Entry into Plant Cells

Sodium ions influx into the root occurs via several entry pathways. The networks of non-selective cation channels (NSCCs) allow the passive influx of Na^+ and other monovalent cations. which considers a major way to enhance the sodium content inside the plant under saline conditions. Another transport system, such as HKT-type transporters, contributes to Na^+ transport along with K^+ due to imperfect ion selectivity. In addition to passive movement of Na^+ across membranes, apoplastic bypass flow distributes sodium through intercellular spaces and cell walls directly to the vascular system (Yeo *et al*., 1987). The rice root anatomy also plays a vital role because of its leaky nature to Na^+, which makes apoplastic bypass flow a major vulnerability. The flow gives sodium direct access to the xylem, bypassing the cellular barriers from the soil. The rapid increase in Na^+ translocation from roots to shoots leads to toxic buildup in the leaves. Accumulation of salt ions in leaves results in early symptoms such as chlorosis, necrosis, and accelerated senescence. In contrast to rice-sensitive lines, tolerant lines prevent Na^+ translocation by enhancing suberin and lignin deposition in the endodermis and exodermis, thereby strengthening apoplastic barriers and limiting Na^+ entry into the vascular system. These adaptive structural changes, which play a vital role in preventing toxic sodium buildup in leaf tissue, are lacking in sensitive genotypes, making them more vulnerable to ionic toxicity under saline conditions.

1.7.2. Sodium Toxicity and Cellular Dysfunction

1.7.2.1. Na^+/K^+ Imbalance

As a consequence of toxic buildup of Na^+ in the cytoplasm of cells disturbs the potassium (K^+) homeostasis. Increased concentration of Na^+ competes with K^+ for uptake, transport, and binding at enzymatic sites, leading to a reduced cytosolic K^+/Na^+ ratio due to ionic competition. Potassium (K^+) is among the essential ions that play a critical role in enzyme activation, protein synthesis, stomatal regulation, and osmotic balance. When sodium takes the place of potassium inside the cell, the machinery that keeps the cell functioning properly begins to break down, leading to serious cellular dysfunction (Tester & Davenport, 2003). Maintaining a high K^+/Na^+ ratio is therefore central to salinity tolerance, as it helps sustain enzyme activity, metabolic balance, and normal cellular function under salt stress.

1.7.2.2. Membrane Damage and Ion Leakage

An increased concentration of Na^+ in the cell competes with positively charged calcium (Ca^{2+}) associated with membrane phospholipids. Displacement of Ca^{2+} from membrane phospholipids destabilizes cellular membranes and leads to enhanced reactive oxygen species (ROS)-mediated lipid peroxidation. Both processes change membrane permeability, leading to uncontrolled solute leakage, nutrient imbalance due to the leakage of essential macro and micronutrients, disturbance of cellular nutrient homeostasis and loss of membrane integrity (Zhu, 2001; Demidchik *et al*., 2014). As membrane damage progresses, ions move more freely across cellular membranes, further intensifying ion toxicity and reinforcing a cycle of cellular injury.

1.7.2.3 Chlorosis, Necrosis, and Premature Senescence

An increasing concentration of sodium becomes toxic in the leaves. It negatively affects the photosynthetic machinery, reducing leaf photosynthetic capacity. Toxic concentration of sodium disrupts thylakoid membrane integrity and ionic equilibrium, impairing chloroplast function, which leads to compromising the structural organization and functional stability of the photosynthetic apparatus. Disruption of the lipid–protein matrix of the thylakoid changes the proper grana stacking and the spatial arrangement of photosystem complexes. In addition, imbalances in essential ions such as H^+, Mg^{2+}, and Cl^- disturb proton gradients and charge distribution across the thylakoid membrane. As a consequence, disrupting of electron transport through photosystem II, the cytochrome b_6f complex, and photosystem I leads to decreased

photophosphorylation, ATP synthesis, and photosynthetic output. In leaf tissues, Na^+ toxicity also inhibits key enzymes involved in chlorophyll biosynthesis, particularly those of the tetrapyrrole pathway, and accelerates chlorophyll degradation. These effects are mediated in part through jasmonic acid (JA) and ethylene-dependent senescence signaling pathways, which promote stress-induced programmed cell death (Ryu & Cho, 2015). Excessive generation of reactive oxygen species (ROS) is induced due to elevated Na^+, which leads to enhanced chlorophyll breakdown and lipid peroxidation of chloroplast membranes. Collectively, these processes lead to chlorosis, necrosis, reduced photosynthetic efficiency, and irreversible damage to photosynthetic tissues in rice plants exposed to prolonged salinity stress.

1.7.2.4 Reproductive Failure

The reproductive stage is particularly sensitive to salinity stress. Longer exposure to salt stress leads to a higher sodium concentration in the plant, negatively impacting pollen viability and stigma receptivity. It also hinders assimilation and translocation to developing panicles, ultimately restricting fertilization and grain development. Elevated Na^+ levels alter ionic homeostasis and promote ROS production, leading to increased oxidative stress in the reproductive tissue. Elevated oxidative stress overwhelms the cellular antioxidant defense system, resulting in damage to membranes, proteins, and nucleic acids. In addition, salinity-induced hormonal imbalances, particularly involving abscisic acid, auxins, and gibberellins, further interfere with floral organ development, anther dehiscence, and pollen tube growth. The negative effects of salinity on physiological and biochemical processes lead to increased spikelet sterility (Zeng & Shannon, 2000). Because reproductive tissues are highly sensitive to ionic and oxidative stress and possess limited repair capacity, damage incurred during this critical developmental phase is often irreversible and cannot be compensated for later in plant development.

1.7.3 Summary

As a glycophyte, rice struggles to grow under saline conditions. Harsh conditions affect rice growth and productivity due to the limited adaptive capacity of its complex and tightly linked physiological and molecular response networks of physiological and molecular responses, as described in the two-phase model proposed by Munns and Tester., (2008). This amplification of oxidative stress, beyond a plant cell's detoxification capacity through antioxidant systems, leads to damage to the plant. Salinity also disturbs the phytohormone-mediated regulatory

networks of abscisic acid (ABA), jasmonic acid (JA), and ethylene. These hormones play a central role in stress perception and response. Impairment in their network under saline conditions can accelerate growth inhibition, promote premature senescence, and exacerbate tissue damage. Altogether, the outcome of osmotic stress, ionic toxicity, oxidative damage, and hormonal imbalance leads to substantial reductions in biomass accumulation and grain yield in rice.

1.8 The Sensing of Salinity Stress: How Plants Perceive and Respond

Plants are not like moving organisms such as animals. They are sessile in nature and cannot avoid unfavourable environmental conditions. To grow and survive under harsh conditions, organisms depend on how quickly they sense external stress signals and how precisely they interpret their intensity and duration to orchestrate coordinated physiological, molecular, and developmental programs (Zhu, 2002; Knight & Knight, 2001). Salinity is one of the most complex abiotic stressors. The plant senses the increased soil salt concentration through sensing processes operating at the cell wall, plasma membrane, and cytosol (Shabala *et al*., 2015), which trigger physiological and biochemical changes in osmotic potential, membrane tension, ion fluxes, and cellular redox status. This sensing mechanism activates protective responses and longer-term adaptive strategies and subsequently develops longer-term adaptive strategies. Plants detect early changes in osmotic and ionic conditions resulting from reduced soil water potential caused by high salt concentrations and from increased cytosolic sodium ions, which become toxic to cellular processes in the initial phase. These changes are perceived through mechanosensitive channels, ion transporters, and membrane-associated sensors. Following the primary perception, plants engage in the activation of intracellular signaling networks called secondary signaling. These networks depend on Ca^{2+}-mediated signaling, ROS-regulated signaling networks, protein kinase cascades, and phytohormone pathways (Knight *et al*., 1997; Choi *et al*., 2014). The collective effect of these pathways alters cellular metabolism, hormone homeostasis, and gene expression to support stress acclimation, which is reflected in growth inhibition, early senescence, and reduced yield under prolonged salinity. It is therefore important to understand how crops respond to saline conditions and what drives shifts from adaptive regulation to detrimental outcomes under prolonged salinity, with the help of initial sensing and later induced signaling pathways. The following sections of this thesis focus on how salinity perception interfaces with JA signaling to regulate stress adaptation and stress-induced damage in rice.

1.8.1 Initial sense of salt

Plants sense salt within seconds to minutes after exposure to saline conditions. This detection occurs well before the appearance of visible morphological symptoms (Zhu, 2002; Shabala *et al*., 2015). An immediate sensing of changes in water availability is called osmotic sensing, and detecting Na^+-specific disturbances after toxic accumulation in the cytoplasm of the cell leads to changes in cellular ion homeostasis (Munns & Tester, 2008).

1.8.1.2 Changes in Turgor Pressure and Mechanosensing

Cells sense these changes using the special sensor protein known as a mechanosensitive channel, which responds to stretching, pressure, or bending of the cell membrane. As the cell membrane stretches or deforms, it alters the channel's shape, opening it and allowing ions to enter the cell. Key components involved in sensing include the MSL (mechanosensitive-like) and MCA (mid1-complementing activity) families, as well as stretch-activated ion channels (Haswell *et al*., 2011; Shabala *et al.*, 2015). The key ion is specifically Ca^{2+} influx into the cells, which serves as an initial cellular signal initiating downstream stress responses (Knight *et al*., 1997).

1.8.1.3 Aquaporin Regulation

The proteins that play a vital role in water movement across cell membranes are called aquaporins. The activity of these channels is rapidly regulated when a sudden change in external salt or water conditions occurs (osmotic shock). During saline conditions, the water balance is disturbed, leading to altered soil water potential that is sensed by the cell, which responds within minutes by regulating aquaporin activity, thereby regulating aquaporin activity. These aquaporins are located in the plasma membrane (PIPs) and the vacuolar membrane (TIPs). The activity of channels can be reduced through chemical modification and intracellular relocation shortly after exposure to salinity. To maintain intracellular water balance, cells regulate water flux across membranes (Maurel *et al*., 2008).

1.8.1.4. Rapid ROS Signaling

Salinity stress rapidly triggers the production of reactive oxygen species (ROS) in several parts of the cell, including the apoplast, chloroplasts, and mitochondria. This increase in ROS occurs almost immediately after salt exposure and acts as one of the earliest stress signals. Rather than causing damage at this stage, the controlled burst of ROS—often described as a “ROS wave”—spreads the stress signal within individual cells and to neighbouring cells. The ROS wave

functions as an early signaling mechanism that activates downstream defense pathways, enabling adjustments in metabolism and gene expression (Waszczak *et al*., 2028).

1.8.1.5 Calcium Signatures Induced by Salt

Plant cells initially respond to saline stress with conserved responses, such as a transient rise in intracellular calcium (Ca^{2+}) levels. This increase in Ca^{2+} levels occurs within seconds to minutes after salt perception. The altered ion concentration acts as an intracellular signal rather than a random change. The intensity and duration of calcium signaling are determined by the severity of salt stress and the plant's genetic background. The change in the calcium level is called the calcium signature. In this coding communication, the salt overly sensitive (SOS) pathway plays a vital role in restoring ion balance. Beyond ion homeostasis, Ca^{2+} signaling plays an important role in regulating stress-related hormones such as ABA and JA. It influences the levels of both hormones produced and the strength of cell responses to these hormones during salt stress. As a result, calcium signaling helps turn early stress signals into lasting protective responses (Knight *et al*., 1997; Choi *et al*., 2014)

1.8.1.6 Histidine Kinases as Osmotic Stress Sensors

Histidine kinases (HKs) play an important role in helping plants sense osmotic stress caused by high salinity. In *Arabidopsis thaliana*, the protein AtHK1 acts as a true osmosensor, directly detecting changes in cellular water balance, and similar HK proteins have also been identified in rice. These kinases sense osmotic changes through subtle changes in their structure. Once activated, they phosphorylate themselves at specific histidine residues and pass the signal on to downstream response regulators. This signaling cascade activates genes involved in osmotic protection and interacts closely with hormone pathways controlled by abscisic acid (ABA) and jasmonic acid (JA). In rice, HKs contribute to sensing early osmotic changes. This early response provides initial protection but is insufficient during prolonged salt stress.

1.8.1.7 Initial Sensing of Sodium Ion (Na^+) Stress

In addition to osmotic sensing, plants perceive Na^+ as a distinct ionic stress signal.

1.8.1.7.1 Membrane Depolarization and Ion Channel Activation

With prolonged exposure to salt stress, sodium ions (Na^+) accumulate to toxic levels within cells. The accumulation of positively charged Na^+ alters the electrical balance, or membrane potential, across the plasma membrane. Depolarization of the plasma membrane activates membrane-embedded Ca^{2+} channels that are sensitive to voltage changes, as well as annexin-

mediated pathways and other sodium-responsive sensors. The combined effect of ion movement makes the early calcium signal stronger and spreads it farther through the cell, making cells more adaptive and responding more effectively to salt stress (Shabala *et al*., 2005).

1.8.1.7.2 pH and Redox Perturbations

The influx of sodium ions into the cell continues during prolonged exposure to salt stress. Toxic accumulation not only alters ionic balance but also influences internal pH and the cell's redox state, which, in turn, affects enzyme activity, metabolic balance, and stress signaling pathways. The shift in pH and redox balance is sensed at the molecular level by oxidation-sensitive signaling proteins such as OXI1 and MAPKs, and by plasma membrane H^+-ATPases that help regulate proton balance. These components act as mediators, transmitting signals from pH and redox alterations to downstream molecular responses by altering cellular metabolism and gene expression. This allows the cell to cope with unfavourable saline conditions by adjusting its physiological and transcriptional responses to maintain homeostasis and survive (Rentel *et al.*, 2004; Jalmi & Sinha, 2015).

1.8.1.7.3 Plasma Membrane–Cell Wall Interactions

Enhanced cellular sodium (Na^+) levels compete with calcium ions because both carry a positive charge. Calcium (Ca^{2+}) is normally associated with membrane phospholipids; its replacement by sodium ions weakens membrane stability and makes the membrane more vulnerable to damage. In addition, the calcium (Ca^{2+}) acts as a cellular signal to strengthen stress signaling pathways and amplify downstream responses to salinity (Shabala *et al*., 2015).

1.8.1.7.4 Activation of the SOS Pathway

Plants use the salt overly sensitive (SOS) pathway, a central signaling system that senses excessive sodium (Na^+) and activates protective responses to restore ionic balance under saline conditions, limiting sodium toxicity. As salt stress occurs, it triggers calcium (Ca^{2+}) signaling within the cell. The increased Ca^{2+} level, referred to as a calcium signal is detected by the calcium sensor SOS3 (also known as CBL4), which acts as a mediator to activate the protein kinase SOS2 (CIPK24). SOS3 forms a complex with SOS2, and this SOS3–SOS2 complex activates the Na^+/H^+ antiporter SOS1. This transporter is located on the plasma membrane and removes excess sodium from the cell in exchange for protons. The activated mechanism helps cells to reduce the toxic buildup of sodium. In the model cereal crop, the SOS pathway plays

a vital role in sensing salt; however, it is not as robust as in salt-tolerant halophyte plants. As a result, rice is less efficient at removing Na^+ during prolonged salt stress, which contributes to its sensitivity under sustained saline conditions (Zhu, 2002; Munns *et al*., 2016).

1.8.2 Secondary Intracellular Signaling and Salinity Adaptation

Following primary perception, plants activate multilayered intracellular signaling networks that regulate ion transport, gene expression, hormone balance, and antioxidant defenses (Zhu, 2016). These secondary signals orchestrate longer-term physiological and developmental adaptation.

1.8.2.1 Adaptive Responses to Water-Deficit Stress

1.8.2.1.2 Stomatal Closure

The plant's initial response to reduced water uptake caused by osmotic stress is to close the stomata, which reduces transpiration, thereby limiting water loss. First, ABA accumulates in the guard cells, kicking off a series of signals that start the response. These signals lead to calcium (Ca^{2+})–driven changes in the membrane's electrical state, the opening of anion channels such as SLAC1 and QUAC1, and the shutdown of plasma membrane H^+-ATPases. In addition, reactive oxygen species (ROS) and jasmonic acid (JA) signaling help reinforce and fine-tune stomatal closure, allowing the plant to adjust effectively to osmotic stress (Munemasa *et al.,* 2015).

1.8.2.1.3 Role of Abscisic Acid (ABA)

Abscisic acid is a vital plant hormone and plays an important role under unfavourable or stressful conditions. It helps plants to cope with osmotic stress. When plants encounter high salinity or drought, abscisic acid levels rise, especially in guard cells. The accumulation of ABA serves as the first signal that water availability is limited and that plants need to use protective measures. High levels of ABA trigger a spike in intracellular calcium (Ca^{2+}). The calcium buildup acts as a messenger that relays the stress signal, leading to changes in ion transport and cellular activity. These signals trigger stomatal closure, which helps reduce water loss via transpiration. In addition, ABA induces the expression of stress-related genes such as RD22 and LEA. RD22 is a dehydration-responsive gene that helps regulate stress signaling, and LEA

proteins protect cells by stabilizing proteins and membranes during water loss. Salt-sensitive rice plants show high sensitivity to ABA during early development, responding quickly to osmotic stress by initiating a defence response and conserving water. However, although ABA-mediated stomatal closure is beneficial during early stress exposure, prolonged activation under persistent salinity reduces carbon dioxide (CO_2) uptake, ultimately causing diminished photosynthesis, restricted growth, and yield reduction (Lamers *et al.*, 2024).

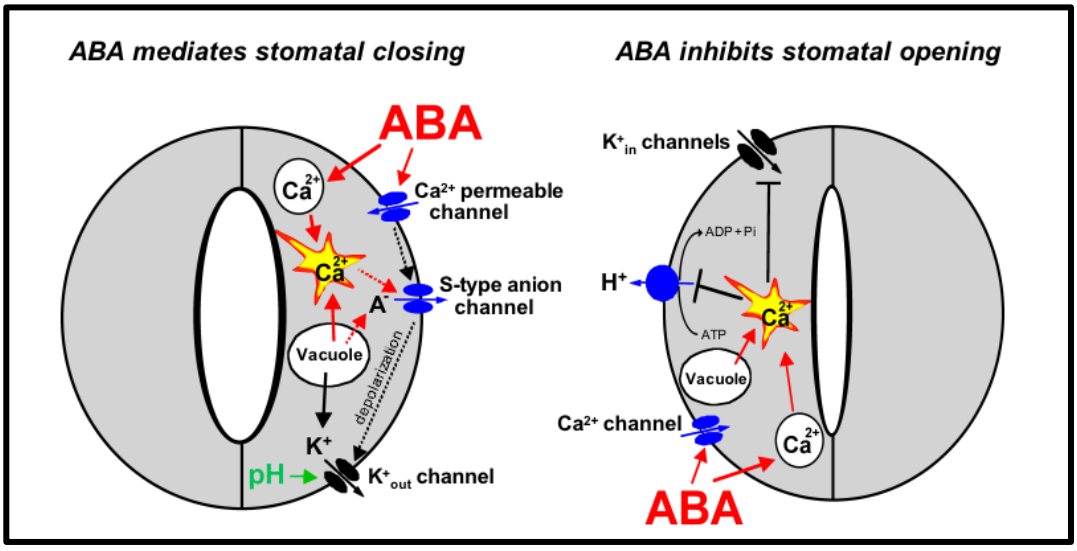


Figure 5. ABA regulation of stomatal movement in guard cells. Abscisic acid (ABA) elevates cytosolic Ca^{2+}, activating anion channels and outward K^+ channels to drive ion efflux, loss of turgor, and stomatal closure. ABA also inhibits H^+-ATPase and inward K^+ channels, preventing K^+ uptake and thereby blocking stomatal opening during osmotic stress.

1.8.2.1.4 Growth Suppression and Architectural Adjustment

Plants face a trade-off between growth and stress adaptation, as sustaining both developmental and adaptive processes is energetically costly. This compromises development, restricting growth by suppressing leaf expansion, tillering, and internode elongation as a strategy to minimise damage and survive under stress conditions such as osmotic and saline stress. This strategy allows the plant to conserve water and energy when resources are limited. To reduce the water loss via transpiring surface, plants control leaf area development and shoot elongation. Coordinated by different hormones, plants make these architectural changes, such as reduced gibberellin biosynthesis, which directly limits cell elongation and overall growth during the

early osmotic phase of salinity stress. Simultaneously, to save energy, the signaling pathways governed by abscisic acid (ABA) and jasmonic acid (JA) prioritized inhibiting growth and increasing stress defense responses. In addition, stress-induced alterations in auxin transport further contribute to modified plant architecture by affecting cell expansion and organ development (Achard *et al*., 2006). The role of jasmonic acid as a growth-inhibitory hormone under stress is particularly evident in studies of JA-deficient mutants. These JA-deficient plants often have larger leaf areas compared to the wild type, which implies that JA plays a key role in restraining growth during stress. Together, these hormonal and developmental adjustments allow plants to balance growth and survival, reallocating resources from expansion to maintenance and stress tolerance (Huot *et al.*, 2014).

1.8.2.1.5 Osmoprotectant Biosynthesis

Under salinity's osmotic stress, plants struggle to absorb water from the soil because of reduced water potential with increased salt concentration. Changes in water potential make it difficult for water to move from a region of higher to a region of lower water potential. To cope with the situation, plants synthesize a range of compatible solutes. These compounds are amino acids (proline), quaternary ammonium compounds (glycine betaine), non-reducing disaccharides (trehalose), and oligosaccharides (raffinose family oligosaccharides), all of which contribute to osmotic adjustment by lowering cellular osmotic potential and stabilizing cellular hydration (Ashraf & Foolad, 2007). These osmoprotectants are neutral in charge and not only help maintain osmotic balance but also preserve the cellular structure under saline conditions. Under saline conditions, denaturation of the macromolecule can be caused by dehydration and ionic stress; however, the osmoprotectants stabilize proteins and membranes to preserve enzymatic function. Among osmolytes, proline plays a dual role in osmotic adjustment by stabilizing proteins and membranes, protecting cell structures, and balancing cellular water potential, as well as in antioxidant defense. Current research provides evidence that jasmonic acid (JA) plays an important role in orchestrating metabolic adjustments that promote stress tolerance. Increased jasmonic acid signaling during salinity and drought stress is directly related to the accumulation of compatible solutes, suggesting that JA coordinates metabolic adjustments during stress adaptation. Despite current research, the precise role of JA in orchestrating the molecular mechanisms and regulatory networks underlying osmoprotectants biosynthesis remains elusive.

1.8.2.2 Adaptive Responses to Ion-Specific Toxicity

1.8.2.2.1 Sodium Efflux and the SOS Pathway

In addition to osmotic stress, the ionic phase of salinity is initiated after the accumulation of toxic sodium ions in the cell's cytoplasm following long-term salt exposure. The ionic phase is more severe than the osmotic phase because the cationic Na^+ disrupts enzymatic activity, membrane integrity, and potassium (K^+) homeostasis, ultimately impairing cellular metabolism and growth. Plants must transport excess Na^+ either out of the cell or into intracellular compartments. To adapt to the situation, plants have evolved highly regulated ion-sensing and signaling mechanisms that play a vital role in Na^+ exclusion and intracellular compartmentalization. The central player in the key adaptive responses is the Salt Overly Sensitive (SOS) signaling pathway, which helps balance ion homeostasis under saline conditions. Toxic accumulation of sodium under salinity stress induces calcium signals. The calcium wave is detected by calcium sensors, triggering the activation of downstream protein kinases that regulate ion transport. The activated signaling network reduces the sodium concentration of the cytosol with the help of the plasma membrane Na^+/H^+ antiporter SOS1 via Na^+ extrusion and Na^+/H^+ exchangers of the NHX family, which are located on the vacuolar membrane, via Na^+ sequestration in the vacuole. The mechanism behind the movement is supported by energy driven by proton gradients established by H^+-ATPases and H^+-pyrophosphatases, which generate the proton motive force required for efficient ion transport (Zhu, 2002; Bassil *et al.,* 2012). Collective activation of SOS1, vacuolar NHX antiporters, and proton pumps reduces the toxic buildup of cytosolic Na^+ concentrations to protect cellular functions during salinity stress (Fig. 6).

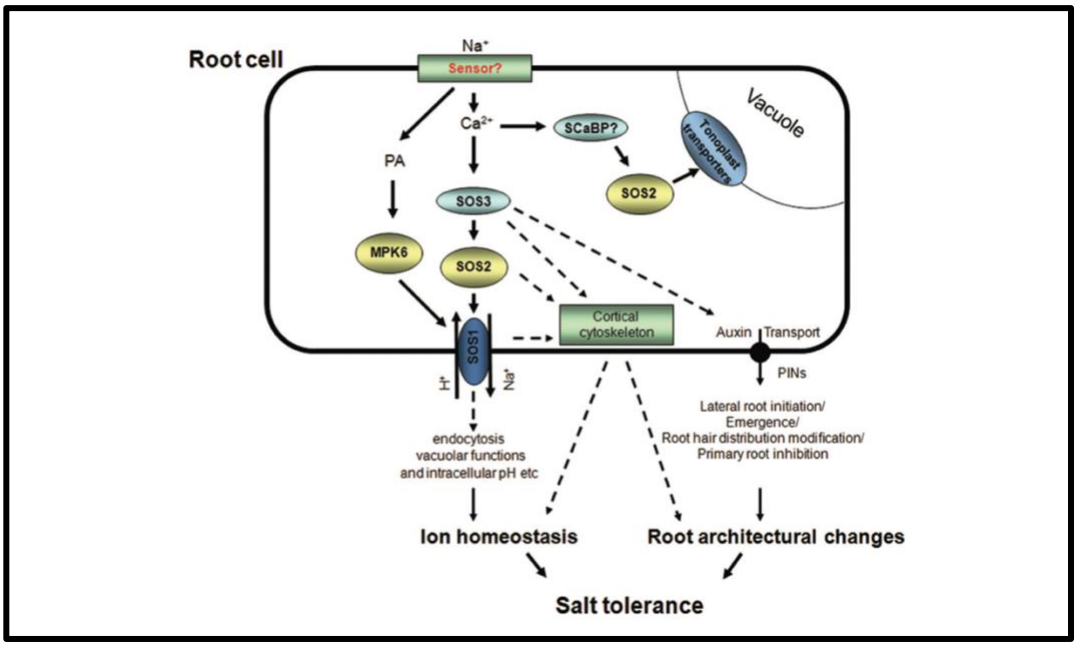


Fig. 6. SOS signaling pathway and ion homeostasis under salinity stress. Salt-induced cytosolic Ca^{2+} signals are sensed by SOS3/CBL proteins, activating the protein kinase SOS2/CIPK, which phosphorylates and activates the plasma membrane Na^+/H^+ antiporter SOS1 to promote Na^+ efflux. The pathway also regulates tonoplast transporters involved in vacuolar Na^+ sequestration and contributes to root architectural changes and ion homeostasis. Adapted from Jia *et al*. (2013); conserved components are present in rice.

1.8.2.2.2 Vacuolar Na^+ Sequestration

Under saline conditions, increased sodium concentration in the cytoplasm has a detrimental effect on cellular mechanisms. To avoid the negative effects of toxic sodium buildup, plants have to implement strategies to reduce sodium accumulation via intracellular or extracellular mechanisms. As mentioned above, sodium efflux is mediated by plasma membrane Na^+/H^+ antiporters such as SOS1. In addition, plants use intracellular compartmentalization to reduce the toxic effects of even high sodium levels within the cell. This mechanism is mediated by NHX family transporters, which are located on the tonoplast and drive Na^+/H^+ exchange. These transporters play a vital role in maintaining osmotic balance and stabilizing Na^+/K^+ homeostasis under saline conditions. In rice plants, OsNHX1 is a vacuolar Na^+/H^+ antiporter that plays a vital role in controlling intracellular Na^+ distribution while preserving metabolic processes. Previous studies have shown that upregulation of the tonoplast transporter NHX1 enhances Na^+ sequestration, maintains ionic balance, and improves salinity tolerance (Fukuda *et al.,* 2004). In this dissertation, delayed expression of the vacuolar Na^+/H^+ antiporter OsNHX1 in the JA-

deficient aoc mutant under saline conditions indicates that jasmonic acid contributes to the regulation of ion homeostasis, particularly vacuolar Na^+ sequestration. Impaired or delayed induction of NHX1 may lead to elevated cytosolic Na^+ accumulation, resulting in ion toxicity and reduced stress adaptation in JA-deficient plants.

1.8.2.2.2.3 Section Transition

Under saline conditions, plants must precisely sense an unfavourable environment to gauge the extent of stress, which involves ion-sensing, signaling, and transport processes that help them interpret these conditions. This information is translated into adaptive and coordinated physiological and molecular responses that minimize ion toxicity and maintain cellular homeostasis. The following sections focus on the framework for the synthesis of the lipid-derived plant hormone jasmonic acid. It orchestrates signaling pathways and functional roles under salinity stress.

1.9 Jasmonic Acid: A Central Regulator of Stress and Development

Jasmonic acid (JA) and its derivatives, such as methyl jasmonate (MeJA), jasmonoyl-L-isoleucine (JA-Ile), hydroxylated and carboxylated jasmonates, glycosylated forms, and the biosynthetic precursor 12-oxo-phytodienoic acid (OPDA), called jasmonate, play a crucial role under biotic and abiotic stress. It is a key lipid-derived phytohormone that plays a vital role in orchestrating plant growth, development, and stress responses (Fig. 7).

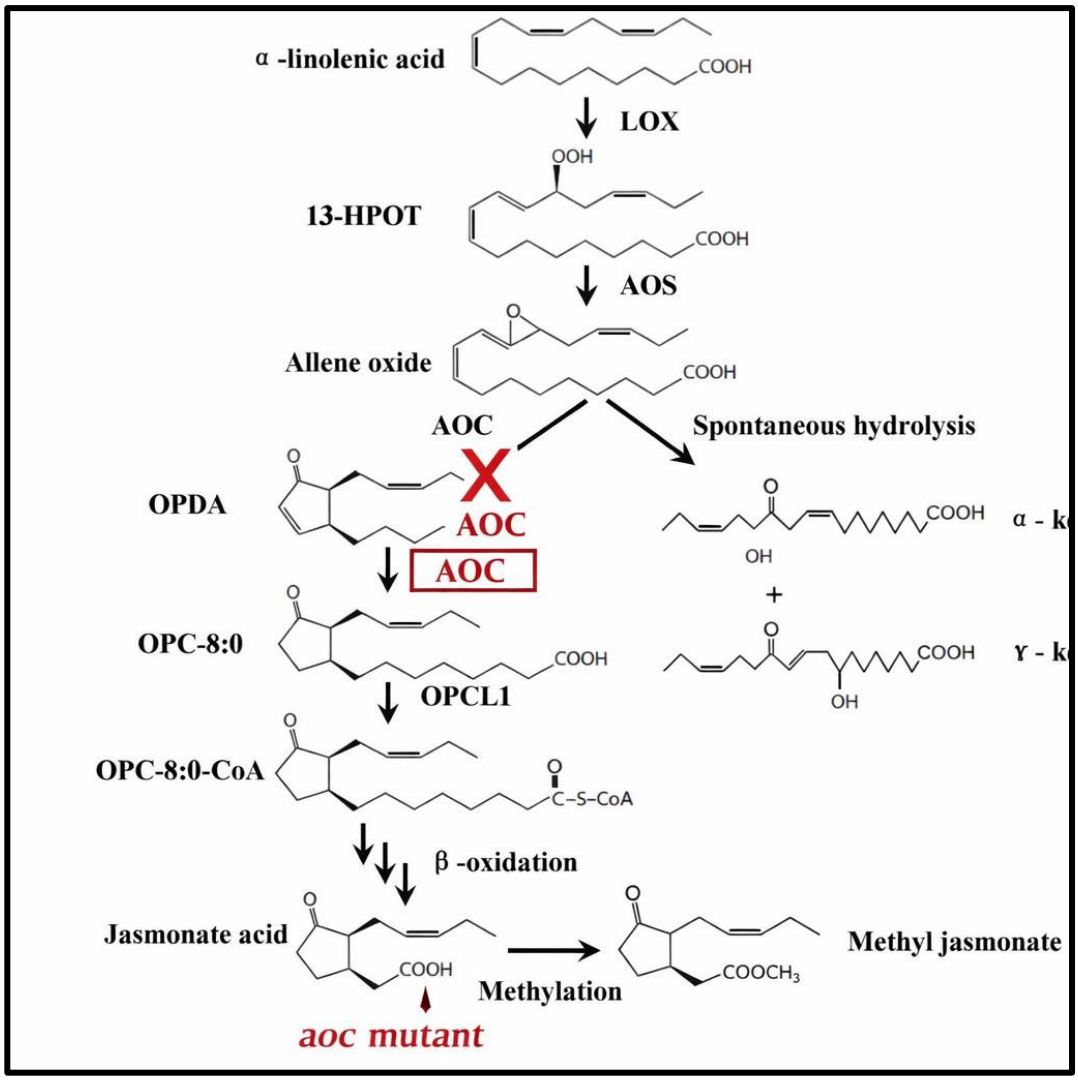


Fig. 7. Jasmonic acid (JA) biosynthesis pathway and position of the AOC mutation. JA is synthesized from α-linolenic acid via the octadecanoid pathway involving 13-lipoxygenase (13-LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) to form OPDA. OPDA is transported to peroxisomes and processed through β-oxidation to produce jasmonic acid and its derivatives. In aoc mutants, disruption of the AOC step blocks OPDA formation and strongly reduces downstream JA biosynthesis.

Initially, jasmonic acid was mainly associated with wound and herbivore defense; however, subsequent research has recognized its role as a key integrator of abiotic stress signaling, including responses to salinity, drought, cold, and oxidative stress (Wasternack & Hause, 2013; Raza *et al.*, 2021). Under saline conditions, JA signaling is involved in several regulatory pathways, which operate during both the early osmotic phase and the later ionic phase. The regulatory pathways, which are active, such as calcium-dependent signaling, the maintenance

of ion homeostasis, and cross communication with other plant hormone signaling, such as abscisic acid (ABA), ethylene (ET), auxin, and gibberellins (GA) (Fujita *et al*., 2006; Kazan, 2015). With multiple hormone pathways interacting, JA plays the crucial role in the growth inhibition, senescence, antioxidant defenses, and stress-responsive gene expression, thereby shaping whole-plant stress adaptation strategies. In salt-sensitive glycophyte species such as rice, JA signaling has positive and negative effects. At first glance, when plants sense the saline condition, JA induces early stress signaling by activating defense-related genes and regulating antioxidant systems and ion homeostasis, resulting in a positive effect (Kazan & Manners, 2008; Riemann *et al*., 2015). However, on the other hand, prolonged or excessive signaling can have negative effects, such as suppressing growth, accelerating leaf aging, and worsening yield penalties during salinity stress (Yang *et al*., 2012; Du *et al*., 2017). Highlighting both positive and negative effects of JA shows the critical importance of tight temporal and spatial control of JA signaling during stress adaptation. Variation among rice genotypes that confer salinity tolerance is based on inherited differences. Salt-adaptive lines show more controlled JA responses, whereas sensitive cultivars display elevated JA accumulation linked to early senescence and growth arrest (Walia *et al*., 2007; Riemann & Takano, 2008). These observations position JA as a critical regulatory node distinguishing adaptive versus maladaptive stress responses under salinity.

1.10 Jasmonic Acid Signaling in Early Salt Sensing and ROS Regulation

Initially, the role of jasmonic acid is linked to wound responses, herbivory, and developmental regulation (Wasternack & Hause, 2013). However, during salinity stress, JA acts as an integrator of multiple early signaling events. As salt disturbs the plasma membrane structure, it activates the phospholipase, releasing α-linolenic acid. The precursor for JA biosynthesis, thereby directly coupling membrane damage to hormone production. Simultaneously, salt stress increased cytosolic calcium via calcium influx. The increased calcium concentration acts on kinases and transcriptional regulators that upregulate genes involved in JA synthesis and signalling. ROS production is increased under stressful conditions, which boosts JA synthesis, and on the other hand, the JA signaling enhances the expression of antioxidant enzymes, developing the feedback loop to balance ROS signaling with detoxification (Fig. 3)**.** JA controls transcriptional reprogramming mediated by the COI1–JAZ–MYC signaling module (Fig. 6), which orchestrates the expression of ion transporters and pumps involved in Na^+ exclusion, vacuolar sequestration, and K^+ retention. As a result, JA plays a vital role in cross-talk between

early physical and chemical stress signals, which are directly linked to homeostasis and gene expression adjustments. JA thus functions as a central integrative hub. Its links membrane-derived signals with Ca^{2+} dynamics, ROS signaling, and transcriptional responses during the early phase of salinity stress (Kazan & Manners, 2008**)**. The concluding research defines the JA not only as a downstream "late defense hormone" but also as a vital element in the initial phase of the salt response system (Wasternack, 2014). In this section, the focus is on connecting the salinity physiology with the JA-focused framework of this thesis and provides a mechanistic context for interpreting JA-deficient mutants (e.g., *aoc*), changing gene expression with downstream regulatory layers such as small RNAs.

1.10.1 Early Salt Sensing: Where JA Enters the Response Cascade

Salt exposure triggers a characteristic sequence of events in roots and leaves **(**Munns & Tester, 2008; Zhu, 2016**):**

- Osmotic shock (seconds to minutes): a rapid reduction in external water potential alters membrane tension and cellular turgor.
- Cytosolic Ca^{2+} signatures (seconds): Ca^{2+} influx serves as a universal alarm signal and activates early kinase networks (Dodd *et al*., 2010**).**
- Initial ROS burst (minutes): apoplastic ROS, largely generated via NADPH oxidases (RBOHs), initiate local and systemic signaling **(**Miller *et al*., 2009; Marino *et al*., 2012**).**
- Hormonal activation (minutes to hours): ABA, JA, and ethylene levels rise and begin to shape downstream transcription and physiology **(**Fujita *et al.,* 2006; Kazan, 2015**).**

ABA is considered the primary hormone in osmotic stress. while JA levels increase after salt exposure within ~5–15 minutes, which is involved in the immediate stress signaling (Riemann *et al.,* 2015; Wasternack & Hause, 2013). JA level enhancement can be explained mechanistically from membrane damage to the formation of substrates for JA biosynthesis via activation of phospholipases and lipoxygenases. Secondly, the high ROS leads to upregulation of the JA pathway gene expression and calcium-controlled kinase activity that influences JA signaling kinetics, including JAZ repressor turnover (Chini *et al*., 2016; Wasternack, 2014). Together, these processes enable JA to respond rapidly to biomechanical and redox disturbances triggered by salinity.

1.10.2 JA as a Regulator of the ROS Burst

As the plant senses the saline soil, it initiates the well-characterized sequence of physiological reactions and signaling events. This was initiated at the primary site of salt perception roots and is subsequently propagated in leaves. The process starts as soon as the soil's salt concentration increases, causing osmotic shock within seconds to minutes. A high salt concentration reduces a plant's ability to absorb water from soil with low water potential. As a result of reduced water uptake, membrane tension and cellular turgor change. Next is the change in calcium level in the cytoplasm, initiated within seconds of salt exposure, called Ca^{2+} signatures. Altered calcium ion levels play a vital role in activating the kinase signaling networks. This is followed by the burst of ROS accumulation. High ROS is generated in the apoplast by NADPH oxidases (RBOHs), which start local and systemic signaling events (Miller *et al*., 2009; Marino *et al*., 2012). Within hours, it leads to hormonal activation, with elevated levels of hormones such as abscisic acid (ABA), jasmonic acid (JA), and ethylene, which orchestrate the downstream processes of transcriptional and physiological regulation (Fujita *et al*., 2006; Kazan, 2015).

1.10.3 Hormonal Crosstalk During Early Stress Perception

JA acts within a tightly interconnected hormonal network in which signaling pathways of multiple phytohormones are integrated, not separately (Fujita *et al*., 2006; Kazan, 2015).

1.10.3.1 Combined JA- and ABA-mediated responses

Early osmotic stress caused by salinity reduces the plant's water-absorption capacity. Therefore, it is important to limit water loss through transpiration. JA and ABA function cooperatively to reduce transpirational water loss by regulating stomatal closure, which leads to a burst of ROS accumulation through RBOH-dependent pathways (Fig. 3, Fig. 6). High ROS accumulation influences the expression of stress-related genes. These changes help to fight the stress condition by controlling water loss and enhancing defense-related transcriptional responses (Munemasa *et al.,* 2011; Riemann *et al*., 2015). However, the timing of signaling is vital; if the pathway continues for a longer time, it can have a negative effect, shifting from initially beneficial responses, which can be observed morphologically, such as growth inhibition and severe developmental penalties.

1.10.3.2 JA–auxin antagonism in roots

Crosstalk between jasmonic acid (JA) and auxin is often antagonistic, with JA exerting inhibitory effects on growth-promoting processes in roots, including root elongation, lateral

root formation, and root hair development (Fig. 6). This negative regulation between the two hormones influences the initial root architectural responses, which ultimately affects water absorption and Na^+ influx (Wasternack & Hause, 2013; Kazan, 2015).

1.10.4 Evidence From JA-Deficient Mutants

It is vital to understand the role of jasmonic acid in saline conditions. One of the best strategies to unravel the precise role of jasmonic acid is to create a line that is unable to produce JA, such as *aoc* and related octadecanoid pathway mutants (Fig.7). In rice JA mutants such as *cpm2* and *hebiba,* it has been previously reported that altered salt stress responses, including reduced sodium accumulation and enhanced reactive oxygen species (ROS) scavenging activity, suggest that JA precursors and related metabolites influence salinity tolerance (Hazman *et al.*, 2015). In a recent publication, Ndecky *et al.* (2023) reported that dynamic changes in JA metabolism and signaling can exert both positive and negative effects on salt stress tolerance. These conclusions are consistent with broader reviews on the network of JA biosynthesis, signaling, and hormonal crosstalk under abiotic stresses, including salinity (Riemann, 2015). These loss-of-function strategies of JA help to unravel how JA influences the mechanism in early stress perception, downstream signaling cascades, and long-term physiological outcomes under saline conditions. One notable observation is a reduced or qualitatively altered early ROS burst, highlighting the role of JA in shaping the strength and timing of early redox signaling following salt exposure. In addition, during the ionic phase initiated by the toxic accumulation of sodium ions in the cytoplasm due to prolonged salinity exposure transporter genes such as SOS1 and NHX1, which reduce toxic sodium concentrations by extruding Na^+ from the cell or sequestering it into the intracellular vacuole, show delayed or reduced induction in JA-deficient plants, which compromises the efficiency of Na^+ detoxification and ion homeostasis. In the jasmonic acid-deficient line, the absence of JA affects ABA response and stomatal behaviour, highlighting crosstalk between the two phytohormones in maintaining transpirational control and water conservation under osmotic stress. With continuous salt exposure, the JA-deficient line accumulates less ROS in the shoots; as a result, it exhibits delayed senescence, pointing to a shift toward prolonged tissue maintenance and reduced stress induced aging (Tang *et al*., 2020; Ndecky *et al*., 2023). Cumulative evidence from previous studies suggests that JA plays both positive and detrimental roles under salinity. At the initial stage of salt stress, jasmonic acid (JA) plays a positive role in early stress signaling, signal amplification, and the activation of adaptive responses. However,

prolonged JA signaling can become detrimental by disturbing cellular redox balance and promoting oxidative stress. Also, the plant pays more focus on defense, which compromises the growth and accelerates the senescence. The severity of saline stress depends on the stress duration, tissue specificity, and genetic background. This dual role of JA under stress conditions forms a central conceptual theme of the present thesis.

1.10.5 Implications for Salinity Tolerance in Rice

The dual behaviour of jasmonic acid (JA) has important implications for salinity tolerance in rice (Kazan, 2015). Physiological, molecular, and genetic studies suggest that JA can act as both a supportive and a limiting factor during salt stress, depending largely on the timing and duration of its activation. In the early phase of saline stress sensing, JA plays a positive role in rapid ROS-based signal transduction. This ROS burst helps stress perception and signal amplification. In addition, JA plays a crucial role in promoting the induction of ion homeostasis networks. These pathways help plants orchestrate the movement of Na^+ through mechanisms of sequestration and exclusion, preventing toxic levels from accumulating. The lipid-derived hormone also crosstalks with ABA, and they influence ABA-mediated responses, particularly those involved in stomatal regulation and water conservation. To adjust gene expression in response to a changing environment, the mobilization of stress-responsive regulators is regulated by JA (Riemann *et al*., 2015; Ndecky *et al*., 2023). However, when JA signaling is sustained over prolonged periods of salinity stress, its effects can become detrimental. A longer presence of JA signaling has a negative effect on growth by inhibiting it, shifting growth-related processes to defensive mechanisms, and compromising overall plant performance. It also accelerates senescence programs, particularly in shoots, and can enhance oxidative stress and ROS accumulation. Combining the outcome of these processes increases the risk of irreversible tissue injury and ultimately reduces yield potential under saline conditions **(**Yang *et al*., 2012; Wasternack & Hause, 2013). Importantly, observations from salt-tolerant rice genotypes, such as FL478, suggest that successful salinity tolerance is not associated with maximal JA activation, but rather with maintaining tighter control over both the amplitude and duration of JA signaling. They avoid excessive JA accumulation at later stress stages while still allowing sufficient early JA signaling to support rapid stress perception and adaptive responses (Walia *et al*., 2005; Kazan, 2015). These patterns strongly support the central hypothesis of this thesis: that fine-tuning or partial suppression of JA signaling, rather than sustained or excessive activation, may represent a viable strategy for improving salinity tolerance in rice. By

maintaining early protective functions of JA while limiting its long-term growth-penalizing effects, plants may achieve a more balanced and resilient response to saline environments. Peethambaran *et al*. (2018) reported that salt-inducible expression of the JA signaling repressor OsJAZ8 improves salinity tolerance at the seedling stage, supporting the idea that transient and moderate JA signaling is beneficial for salt tolerance.

1.11. Integrative Summary: Why Understanding JA Signaling Is Central to This Thesis

When considering how rice plants manage salinity stress, jasmonate signaling plays a central regulatory role. The COI1–JAZ–MYC regulatory module represents a key component of this signaling pathway (Wasternack & Hause, 2013). Once bioactive JA–Ile accumulates, it triggers the rapid degradation of JAZ repressors. This step is crucial because it releases transcription factors that activate changes in gene expression and metabolism required for coping with salt stress (Kazan & Manners, 2008; Howe *et al*., 2018). Several aspects explain why this regulatory framework is central to the present work. First, it clarifies the consequences of JA deficiency. If a plant has an impaired AOC function, the jasmonate pathway becomes disrupted because JA–Ile cannot be produced efficiently. Without this molecule, JAZ repressors remain stable, and downstream stress-responsive genes are not properly activated (Tang *et al*., 2020; Riemann *et al.,* 2015). This pathway, therefore, links hormonal signaling with the physiological mechanisms required for plant survival under salinity. It connects JA signaling with mechanisms of salt tolerance, including ion transport processes involving SOS1, NHX, and HKT transporters, as well as ROS regulation and stress-related developmental responses (Kazan, 2015; Ndecky *et al.,* 2023). These observations suggest that the differences observed between wild-type plants and tolerant genotypes such as FL478 are not necessarily due to fundamentally different signaling components. Instead, they likely reflect differences in the regulation and timing of JA signaling, including signal strength, activation timing, and the tissues in which the response is initiated (Walia *et al.*, 2005; Tang *et al.*, 2020).

1.13 Rationale, Scope and Working Hypotheses of the Thesis

Soil salinization represents one of the major abiotic stresses limiting farming productivity worldwide and currently affects more than 20% of irrigated agricultural land. Under such conditions, plants must activate complex adaptive functions in order to maintain growth and survival. Jasmonic acid is considered to play an important role as part of an elaborate signaling network that interacts with several other salinity-responsive pathways. However, despite recent

studies, the contribution of jasmonates to the regulation of salt stress adaptation in rice remains poorly understood. For this reason, a detailed analysis of jasmonate signaling is required in order to shed light on the mechanisms underlying salt sensitivity and salt tolerance in this crop species.

In the present study, the role of jasmonates during salinity stress was investigated by comparing jasmonate-deficient *aoc* mutant and their wild-type background Kitaake with salt-tolerant (FL478) and salt-sensitive (RC222) rice genotypes in order to obtain a comprehensive view of the role of jasmonates during stress adaptation. Particular emphasis was placed on the question of how JA deficiency affects early salt-stress signaling and the subsequent downstream response pathways. Furthermore, the possible contribution of jasmonates to osmotic adjustment, ion homeostasis and oxidative stress control was examined, together with the potential trade-off between stress tolerance and growth inhibition. Taken together, the present study aims to clarify whether jasmonates primarily function as protective signals, growth-limiting factors, or condition-dependent regulators, thereby providing knowledge that may contribute to future strategies for improving salinity tolerance in rice.

Based on current knowledge, jasmonate deficiency is expected to influence early salt-responsive regulatory processes, including miRNA-mediated post-transcriptional regulation, and may lead to tissue-specific differences between root and shoot responses (Sunkar *et al.,* 2012; Tang *et al.*, 2020). The comparison of aoc mutant (Kitaake background) and kitaake with naturally salt-tolerant genotypes such as FL478 and the salt-sensitive genotype RC222, therefore, provides an opportunity to determine whether natural salinity tolerance depends on JA-related regulatory networks or rather relies on alternative adaptive mechanisms (Walia *et al*., 2005; Reddy *et al.*, 2017).

Salinity stress affects many processes within the plant simultaneously. To overcome the situation and survive under saline conditions with a single mechanism is not possible, which makes adaptation to salt conditions a highly complex trait. This trait orchestrates the coordinated regulation of ion homeostasis, osmotic adjustment, redox balance, hormonal signaling, and developmental plasticity (Munns & Tester, 2008; Reddy *et al*., 2017). For this reason, salt-tolerant and salt-sensitive genotypes constitute important comparative systems for identifying the factors that determine successful or unsuccessful adaptation to saline environments. Contrasting systems in salinity research were used, such as FL478, a well-known salt-tolerant genotype, and RC222, a salt-sensitive genotype (Walia *et al*., 2005; Reddy *et al*., 2017). The FL478 is obtained from the cross between Pokkali and IR29. Its tolerance is mainly

attributed to efficient Na^+ exclusion mediated by OsHKT1;5 (SKC1), improved K^+ retention, and enhanced antioxidant capacity (Ren *et al*., 2005; Kobayashi *et al*., 2017). In addition, tolerant genotypes such as FL478 appear to avoid excessive accumulation of jasmonic acid under salinity conditions, thereby allowing continued growth during continuing early stress signaling (Kazan, 2015; Hazman *et al.,* 2015; Peethambaran *et al*., 2018). In contrast, RC222 exhibits a limited capacity to cope with prolonged salinity stress (Walia *et al.*, 2005; Reddy *et al*., 2017). Under saline conditions, this genotype shows impaired ion homeostasis, reduced osmotic adjustment, and increased oxidative damage compared with tolerant genotypes. Although antioxidant responses and osmolyte accumulation can be detected, these responses are generally insufficient to prevent growth inhibition and stress-induced injury (Ashraf & Foolad, 2007). Increased sensitivity to senescence and diminished recovery after stress additionally indicate weaker regulation of stress-associated hormonal pathways, including jasmonate signaling (Kazan, 2015; Gutjahr *et al*., 2005; Nick, 2006).

2. Materials and Methods

2.1 Overview of Experimental Strategy

Among the abiotic stresses, salinity is the most severe stress in hindering crop productivity around the globe. It has a negative effect on various plant cellular processes, including plant growth, ion homeostasis, photosynthetic efficiency, and hormonal regulation. High soil salinity reduces soil water potential around the root, causing osmotic stress. The plant's ability to maintain the steady water flow is reduced, and cell turgor pressure decreases under osmotic stress. On the other hand, prolonged salt stress leads to toxic ion accumulation and secondary oxidative damage, affecting cellular metabolism and developmental processes. The plant hormones play a vital role in stress signaling, growth modulation, and defense responses. Among them, JA also participates in various plant cellular processes; however, despite recent research progress, the precise role of JA in saline adaptation in rice remains elusive. Specifically, how JA acts during salt stress, either as a negative factor acting as a growth-inhibitory signal, or it has a positive role in adaptive reaction by orchestrating the regulation of ion balance, redox homeostasis, and transcriptional reprogramming. To unravel this mystery, this dissertation study planned to understand jasmonic acid in rice salinity stress responses through integrating phenotypic, physiological, ionomic, molecular, and hormonal analyses. The distinct lines of rice representing contrasting jasmonic acid biosynthesis capacity and salinity tolerance were selected. The lines were wild type (WT) Oryza sativa ssp. japonica cv. Kitaake, a loss-of-function mutant in jasmonic acid biosynthesis (aoc), and two naturally salt-tolerant and sensitive rice lines, FL478 and RC222. The aoc mutant, which is deficient in jasmonic acid biosynthesis due to disruption of the Allene Oxide Cyclase (AOC) gene, was compared directly with its wild-type background to dissect JA-dependent regulatory mechanisms. Along with the salt-tolerant and sensitive genotypes, FL478 and RC222 were included to place jasmonate-related responses within the wider physiological context of naturally evolved salinity tolerance. The structure of the dissertation was planned to integrate early molecular responses with extended growth and physiological outcomes. which covers the initial stress-influence process and a long-term process that helps the plant adapt to an unfavorable condition. At the seedling stage, the salinity stress is given through the hydroponic growth system. The system gives control over nutrient composition, salt concentration, and root-zone exposure. Hydroponic cultivation gives a higher chance to minimize heterogeneity in salt distribution, which is why it was selected over soil-based cultivation. To ensure consistent

stress application across genotypes and facilitate accurate sampling of root tissues for ionomic and molecular analyses. To select the salinity level for the experiment, an initial screening of different salt concentrations was performed. Seedlings were initially exposed to 50 mM, 75 mM, 100 mM, and 150 mM NaCl to evaluate their suitability for inducing measurable salinity stress responses. At 50 mM NaCl, the stress plant showed only mild growth inhibition and limited physiological disruption, and 75 mM NaCl induced moderate stress symptoms; both concentrations were considered insufficient to consistently elicit robust ionic, molecular, and hormonal responses across genotypes. At a higher concentration of 150 mM NaCl, it was a strong stress that showed severe symptoms. Based on the preliminary salt screening, a 100 mM NaCl solution showed promising results and was selected to continue the experimentation. This concentration induced clear and reproducible stress responses without producing excessive lethality or irreversible tissue damage, thereby representing a balanced stress intensity that was neither too mild nor excessively harsh. A salinity level of 100 mM NaCl, therefore, served as an ideal condition to investigate genotype-dependent differences in stress perception, acclimation, and tolerance. This concentration has also been widely employed in rice salinity studies, enabling meaningful comparison of the present findings with prior literature.

The experimental design follows the following objective.

1. Morphological and developmental changes
2. Biomass distribution and water relations
3. Photosynthesis-related performance and gas-exchange behaviour
4. Sodium and potassium partitioning and ionic homeostasis
5. Transcriptional regulation of jasmonate-responsive, ion-transport, and antioxidant genes
6. Hormone profiling of the salt-tolerant and sensitive genotypes FL478 and RC222

Through integrating these datasets, the study intended to determine whether jasmonic acid deficiency enhances salt tolerance mainly through growth regulation, ion homeostasis, redox control, or a combination of these mechanisms, compared with naturally salt-tolerant rice genotypes.

2.2 Plant Material and Genetic Background

For this study, wild-type (WT) rice (Oryza sativa L. ssp. japonica cv. Kitaake) and a loss-of-function mutant in Allene Oxide Cyclase (AOC) generated by CRISPR-Cas9 (Nguyen *et al*., 2020; Ndecky *et al*., 2023) were used. This enzyme is required to generate the precursor 12-oxo-phytodienoic acid (OPDA). Hence, the *aoc* mutant is unable to form jasmonic acid (JA). Since JA depletion leads to male sterility, the homozygous *aoc* mutant seeds had to be isolated from the segregating population of heterozygous AOC/aoc plants. The homozygous mutant seedlings can be easily discriminated from heterozygotes or homozygous wild type by their elongated mesocotyl. In addition, two contrasting rice genotypes were included: the salt-tolerant recombinant inbred line FL478 (derived from Pokkali × IR29), which is widely used as a model genotype for salinity tolerance (Walia *et al*., 2005), and the salt-sensitive rice line RC222, which has been used as a contrasting genotype in salinity response studies (Neang *et al*., 2020). The inclusion of WT, *aoc* mutant, FL478, and RC222 enabled a multi-layered comparative framework. The WT and *aoc* mutant comparison provided mechanistic insight into the role of jasmonic acid biosynthesis in salinity responses, while comparisons with FL478 and RC222 allowed assessment of whether similar or distinct physiological, molecular, and hormonal strategies operate across contrasting genetic backgrounds.

2.3 Seed Sterilization and Germination

The healthy seeds of all four rice genotypes (WT, *aoc* mutant, FL478, and RC222) were selected. The caryopses were dehusked and surface sterilised by first immersed in 70% (v/v) ethanol (VMR, Chemicals) for 1 minute to remove surface-associated contaminants, then rinsed twice with sterile distilled water. Subsequently, the undamaged seeds were treated with 3% (v/v) sodium hypochlorite (ROTH) solution for 20 minutes of incubation and gentle agitation. Followed by 4 times washing steps in ultrapure water to completely remove residual sterilizing agents. The sterilized seeds were sown on Murashige and Skoog (MS) medium (8 g L^{-1}), solidified with 0.5% phytoagar (Duchefa, Netherlands), in Magenta boxes (Sigma-Aldrich) and incubated at 28°C under a 12-hour photoperiod with continuous light (Photosynthetically Available Radiation, PAR, ~475 µmol m^{-2} s^{-1}) and 60% relative humidity for 6 days in growth chamber (Poly Klima). These conditions were selected to promote rapid and uniform germination while minimizing stress unrelated to salinity treatment.

2.4 Hydroponic Growth Conditions

Six-day-old seedlings of uniform size and developmental stage were carefully removed from solid MS and Phytogar media and transferred to the hydroponic solution in the glass cultivation jars. These jars consisted of sterilized floating racks with cutted eppendof tube. Each jar contained 500 mL of nutrient solution and accommodated 20 seedlings, providing approximately 25 mL of nutrient solution per plant. The plant number was picked to ensure adequate nutrient availability to avoid competition and maintain sufficient aeration of the root system. The Hydroponic solution consisted of Murashige and Skoog (MS) basal salts at a reduced concentration of 0.358 g L^{-1} and 1.0 g L^{-1} MES buffer (Roth), and adjusted to pH 5.8. The selected concentration of the compound in the solution is to avoid masking salinity-induced effects and stabilize the pH of the nutrient solution. Under saline conditions, where ion uptake and root exudation can cause significant pH fluctuations. Stable pH conditions are important to sustain membrane transport processes, especially Na^+/H^+ antiport activity and potassium uptake. Seedlings were maintained in hydroponic culture for an additional 3 days before salinity stress was applied.

2.5 Salinity Stress Treatment

Salinity stress(NaCl Roth) was imposed on hydroponically grown seedlings at the age of 11 days of the wild type (WT), *aoc* mutant, FL478, and RC222 at the early vegetative stage. Nutrient solutions were renewed every three days for control and salt stress. Plants were maintained under salinity for twelve days for phenotypic, physiological, and ionomic analyses, while at earlier time points were used for molecular and hormone analyses.

2.6 Measurement of Growth Parameters and Biomass (WT, *aoc* mutant, FL478, and RC222)

Shoot and root lengths were recorded 12 days after salt treatment in all four genotypes (WT, aoc, FL478, and RC222). Shoot length was defined as the distance from the base of the coleoptile to the tip of the youngest leaf, and root length as the distance from the crown to the tip of the seminal root. For each biological replicate, five individual plants per treatment and genotype were measured. Representative morphological images of WT, aoc, FL478, and RC222 plants were selected to illustrate phenotypic differences. Growth stimulation by mutation or genotype and growth inhibition by salt were calculated separately for shoots and roots as percentage change relative to the respective control. For biomass analysis, plants were

separated into seminal root, individual leaves 1–5, and the stem including the youngest leaf, which could not be separated from the stem. Fresh weight was measured immediately after harvest and dissection. Data were collected from five independent plants per genotype and condition.

2.7 Measurement of Growth Parameters and Biomass

Photosynthetic parameters were measured using a portable photosynthesis system (LI-6800, LI-COR, Lincoln, NE, USA). It is equipped with a red/blue LED light source under standardized conditions (photosynthetically active radiation (PAR) 0 to ~2000 µmol m^{-2} s^{-1}, relative humidity 60%, temperature 28 °C) on the youngest fully expanded leaf (leaf 5) at day 12 after the onset of stress (or transfer to salt-free medium, respectively). The Assimilation rate (µmol m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}), stomatal conductance (µmol m^{-2} s^{-1}), and effective quantum yield of photosystem II (Fv′/Fm′) were recorded under control and stress (100 mM NaCl), while PAR was stepwise increased from 0 to 2000 µmol m^{-2} s^{-1}. Simultaneous chlorophyll fluorescence was recorded using the LI-6800 fluorometer module, and ΦPSII (Fv′/Fm′) was automatically calculated after saturating light pulses. Measurements were performed on WT (Kitaake) and aoc mutant plants under both control and salinity stress conditions. Measurements were conducted in the morning within a time window between one and seven hours after dawn. Because plants were measured sequentially, such that the last plant was recorded later than the first, the sequence of measurements was rotated to minimize potential bias from diurnal rhythms. Values for each genotype, therefore represent averages derived from different time points within this window. Data represent mean and standard error from five biological replicates.

.

2. 8 Ion Content Analysis (WT, *aoc* mutant, FL478, and RC222)

Individual plant organs were separated as described above, and harvested tissues were dried to complete removal of water at 48 °C for 48 h (Heraeus, Instruments). The dehydrated material was then ground into a fine powder using a pestle and mortar. For elemental analysis, aliquots of approximately 50 mg of powdered tissue were digested in 2 mL of heated (but not boiling) nitric acid (65% v/v) and 0.5 mL of hydrogen peroxide (30% v/v) within closed Teflon digestion tubes (Gerhardt, UK). The tubes were heated at 110 °C for three hours in a heating block (DigiPrep Jr., S-prep). After digestion, samples were allowed to cool and the volume was adjusted to 20 mL with ultrapure water. Sodium (Na^+) and potassium (K^+) concentrations were determined using an inductively coupled plasma–optical emission spectrometer (ICP–OES; iCAP 7000, Thermo Fisher Scientific). The assay was calibrated using serial dilutions of standard solutions. To normalize variability across replicates, a certified reference material (tomato leaf, NIST 1573a) was included. Results are presented as Na^+ and K^+ concentrations (mg g^{-1} dry weight) and as K^+/Na^+ molar ratios for each sample. Data represent mean and standard error of three biological replicates.

2.9 Gene expression analysis

2.9.1 Total RNA Extraction

Second leaves and roots of seedlings were excised at defined time points (prior to stress, and at 1, 6, and 72 h after addition of 100 mM NaCl). Harvested tissues were immediately frozen in liquid nitrogen and stored at −80 °C until further processing. Total RNA was extracted using the innuPrep Plant RNA Kit (Analytik Jena) according to the manufacturer's instructions. RNA quantity, purity, and integrity were verified spectrophotometrically (NanoDrop) and by agarose gel electrophoresis.

2.9.2 cDNA Synthesis

First-strand cDNA of total mRNAs synthesis was performed using M-MuLV Reverse Transcriptase (New England Biolabs) following the manufacturer's recommendations. For each reaction, 0.5–2 µg of total RNA (or 50–100 ng poly(A)-selected RNA) was used as template. Two reactions were prepared per sample, including one no–reverse transcriptase control to assess genomic DNA contamination. Initially, RNA was combined with 0.4 µL oligo(dT) or random nonamer (N9) primer and 1.0 µL dNTP mix (10 mM each), and nuclease-free water was added to a final volume of 16 µL. The mixture was heated at 65–80 °C for 3–5 min, briefly centrifuged, and immediately placed on ice. Subsequently, 2 µL of 10× RT buffer, 1.25 µL

nuclease-free water, 0.5 µL RNase inhibitor (10 U µL^{-1}), and 0.5 µL M-MuLV Reverse Transcriptase (200 U µL^{-1}) were added to obtain a final reaction volume of 20 µL. For the negative control, reverse transcriptase was replaced with nuclease-free water. The reaction was incubated at 42 °C for 60 min, followed by enzyme inactivation at 90 °C for 10 min. Synthesized cDNA was stored at −20 °C or used directly for downstream applications.

2.9.3 Real-Time PCR

Quantitative real-time PCR (Bio-Rad) was CFX connect Real time system performed as described by Hazman *et al.* (2015) as follows. 95C° for 3 min, and 40 cycles (95C° for 15 s and 63C° for 30s). Data were exported from the Bio-Rad CFX Connect Real-Time PCR Detection System and imported into Opticon Monitor software (Bio-Rad Laboratories, USA) for further analysis. Amplification specificity was confirmed by melt-curve analysis. First, the difference in cycle threshold (Ct) values between each target gene and the endogenous control gene UBQ10 was calculated for both treated and non-treated samples: $\Delta Ct_{\text{treated}} = Ct_{\text{gene}} - Ct_{\text{UBQ10}}$ $\Delta Ct_{\text{non-treated}} = Ct_{\text{gene}} - Ct_{\text{UBQ10}}$. Subsequently, the difference between treated and non-treated samples was calculated as: $\Delta\Delta Ct = \Delta Ct_{\text{treated}} - \Delta Ct_{\text{non-treated}}$ Finally, the relative expression ratio (Qr) of the target gene in treated versus non-treated samples was determined using the following equation: $Qr = 2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). Data represent mean and standard error from three biological replicates, each analyzed in technical triplicates. Primer sequences used to amplify genes of interest are listed in Supplementary Table S1

2.10 Hormone Extraction and Quantification

2.10.1 Endogenous Levels of ABA, OPDA, JA, JA-Ile, 12OH-JA-Ile, 12COOH-JA-Ile, and Salicylic Acid

Second leaves of seedlings from the salt-tolerant genotype FL478 and the salt-sensitive genotype RC222 were harvested at defined time points: T0 (before salt stress), T1 (1 h after addition of 100 mM NaCl), T2 (6 h after addition of 100 mM NaCl), and T3 (24 h after addition of 100 mM NaCl). In addition, at T4 (1 h after salt stress), both second and fourth leaves were harvested. Collected tissues were immediately frozen in liquid nitrogen and stored at −80 °C until further processing. Phytohormones were extracted from frozen leaf tissue following established protocols for jasmonate and related hormone analysis. Briefly,

samples were homogenized in extraction solvent, and internal standards were added to allow accurate quantification. After centrifugation and purification, hormone-containing extracts were subjected to chromatographic separation and mass spectrometric detection. Jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), and related jasmonate derivatives were quantified using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Hormone concentrations were calculated based on calibration curves generated with authentic standards and normalized to tissue fresh weight. Data represent mean and standard error from three biological replicates per genotype and time point.

2.12 Statistical Analysis

All experiments were conducted with either five (for physiological measurements and fresh weight) or three (for ion content, gene expression, and miRNA expression analyses) independent biological replicates. Data are presented as mean ± standard error (SE). Statistical significance between treatments or genotypes was assessed using Student's *t*-test for pairwise comparisons or one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1 Jasmonic acid–deficient aoc mutant versus wild-type Kitaake

3.1.1 Experimental design and sampling strategy

To understand the precise role of jasmonic acid (JA) in rice responses to salinity, we used a loss-of-function mutant in ALLENE OXIDE CYCLASE (*aoc*), which is unable to synthesize JA, and was compared with its corresponding wild-type background, Oryza sativa ssp. japonica cv. Kitaake. Plants were grown for 10 days under regulated conditions (6 days in agar medium and 4 days in hydroponic conditions). Then, they were subjected to either the control medium or to salt stress (100 mM NaCl). Phenotypic, physiological, ionic, and transcriptional responses were analyzed at defined time points to capture both early and late responses to salinity. To understand the early effects of salt on the 2nd and 3rd leaves, we recorded images and conducted molecular analyses. Root and 2nd leaf tissues were sampled prior to stress application and at 1 h, 6 h, and 72 h after salt exposure. For late responses we analysed phenotypic, physiological, ionic resposes after 12 days of salt stress. This experimental design enabled the separation of constitutive genotype effects from salt-induced responses and allowed organ-specific analyses of JA-dependent regulation.

Fig. 7. Overview of experimental setup and representative phenotypes of wild-type (WT) and *aoc* mutant rice seedlings under control and salinity stress conditions. A) Three-day-old dark-grown WT (Kitaake) seedlings arranged for imaging. (B) Three-day-old dark-grown *aoc* mutant seedlings arranged for imaging. (C) Magenta box cultivation system containing Murashige and Skoog (MS) medium solidified with phytoagar, used for initial seedling growth prior to transfer to hydroponic culture. (D) Representative phenotype of *aoc* mutant plants grown under control conditions (left) and after exposure to 100 mM NaCl in hydroponic culture (right). (E) Representative phenotype of WT (Kitaake) plants grown under control conditions (left) and after exposure to 100 mM NaCl in hydroponic culture (right).

3.1.2 Salinity-Induced Phenotypic Responses in WT and JA-Deficient Seedlings

3.1.2.1 JA-Deficient *aoc* Mutant Exhibits Reduced Sensitivity to Salinity Stress

The first practical step in plant stress is to examine the phenotype to make an integrative assessment of plant performance. It also generates the initial step as it relates to the next experimental and practical investigation. To examine the role of jasmonic acid in rice responses to salinity, wild-type (WT) and jasmonic acid–deficient *aoc* seedlings were grown under hydroponic conditions and exposed to either control medium or 100 mM NaCl. First, the phenotype was analyzed after 72 hours of salt exposure on the 2nd and 3rd leaves (Fig. 8), which clearly shows strongly necrotic regions of both 2nd and 3rd leaves in WT compared to the *aoc* mutant. Then again, phenotypes were analyzed 12 days after the onset of salt treatment. The stress effect showed clear morphological differences between the two genotypes under both control and saline conditions. For salt stress, we could observe that WT plants exhibited pronounced growth inhibition with leaf chlorosis and visible tissue damage (Fig. 9A). In contrast, an *aoc* mutant had less necrotic leaves with a greener appearance and vigorous overall growth habit (Fig. 9B). Without salt exposure, the *aoc* mutant plants already appeared larger than WT plants. Quantitative analysis supported these observations. A clear significant difference was observed for the shoot length between *aoc* mutant (50.4 cm) and WT plants (41.0 cm) in control conditions. In a saline condition, shoot length decreased in both genotypes but remained higher in *aoc* mutant seedlings (38.8 cm) compared with WT plants (32,0 cm) (Fig. 9C). When relative changes were calculated, shoot length in the *aoc* mutant was approximately 20% higher than WT under both control and salinity conditions (Fig. 9E). In saline conditions, the WT plants show more necrotic leaves, like leaf 2nd and 3rd, while the 4th leaf showed some necrotic regions with rolling compared to the *aoc* mutant (Fig. 9A), which is showing more necrosis on the 2nd leaf, while the 3rd leaf was half necrotic and the 4th leaf was greener with less salt stress symptoms (Fig. 9B). Together, these results show that the *aoc*

mutant displays a less salt-sensitive phenotype and sustains superior growth under salinity stress. Importantly, this improved growth performance is already evident under non-stress conditions, indicating that jasmonic acid deficiency affects both basal growth capacity and salinity-induced growth responses.

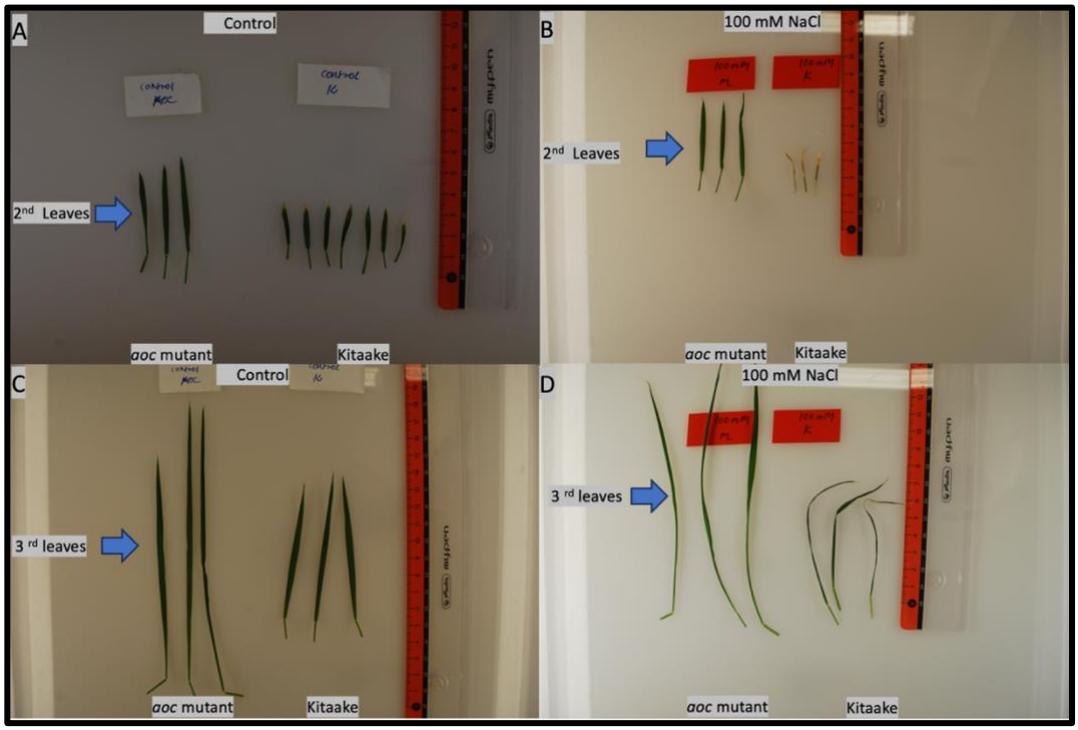


Fig. 8. Jasmonic acid deficiency reduces salt-induced leaf necrosis in rice seedlings. Wild-type (WT; cv. Kitaake) and jasmonic acid–deficient *aoc* mutant seedlings were grown under hydroponic conditions and exposed to either control medium or 100 mM NaCl. Representative images of the 2nd and 3rd leaves were recorded after 72 h of treatment. (A) 2nd leaves under control conditions. (B) 3rd leaves under control conditions. (C) 2nd leaves after 100 mM NaCl treatment. (D) 3rd leaves after 100 mM NaCl treatment. Under salt stress, WT leaves show pronounced chlorosis and necrotic regions, whereas *aoc* mutant leaves display markedly reduced necrosis, indicating enhanced tolerance to salinity. Scale bar shown in each panel.

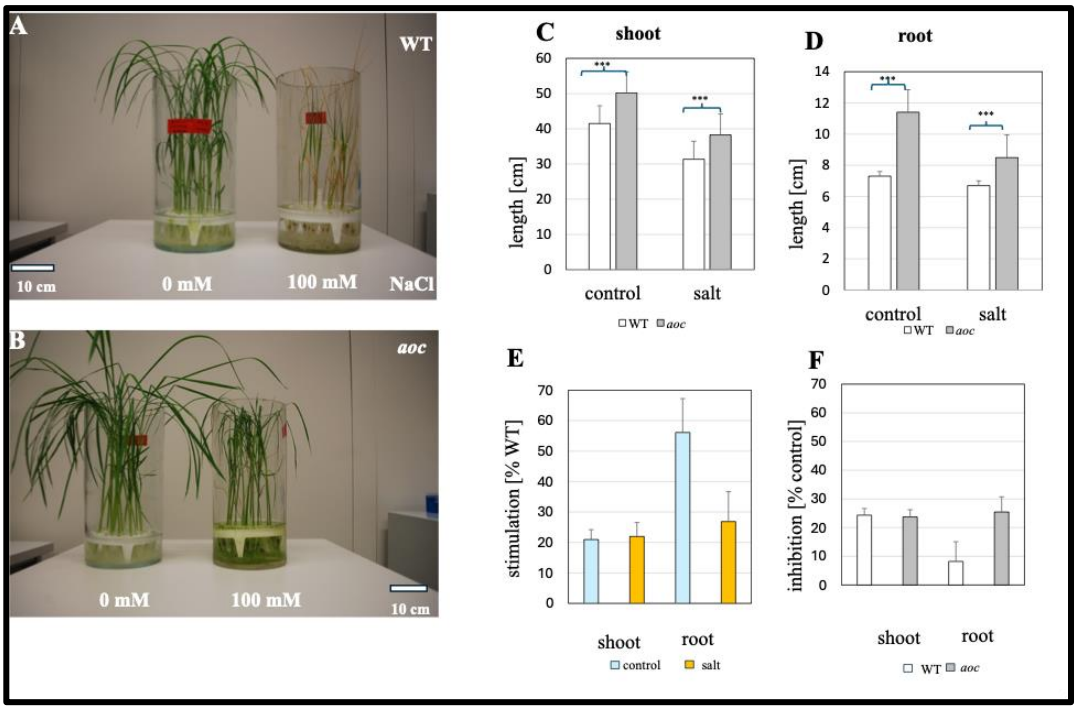


Fig. 9. Jasmonic acid deficiency promotes enhanced growth and reduced salt sensitivity in rice seedlings. Wild-type (WT; cv. Kitaake) and jasmonic acid–deficient *aoc* mutant plants were grown hydroponically and analyzed 12 days after the onset of salt treatment. (A) WT plants under control (0 mM NaCl) and salt (100 mM NaCl) conditions. (B) *aoc* mutant plants under control and salt conditions. Under salinity, WT plants exhibit strong growth inhibition, leaf chlorosis, and visible tissue damage, whereas *aoc* plants maintain greener leaves and a more vigorous growth habit. (C) Shoot length and (D) root length of WT and *aoc* plants under control and salt conditions. (E) Relative stimulation of shoot and root length in *aoc* compared to WT under control and salt conditions. (F) Relative inhibition of shoot and root growth by salinity in WT and *aoc* plants. Data represent means ± SE. Scale bar = 10 cm.

3.1.2 Effect of Salinity Stress on Root Elongation

Roots are the primary site of salt perception and ion uptake and therefore provide a sensitive indicator of plant growth performance under salinity stress. To understand the effect of salinity on genotypic growth, we examined the length of seminal roots after 12 days of salinity stress (Fig. 9). Results of the *aoc* mutants' roots were shown to be longer (11.0 cm) than those of WT plants (6.9 cm) in control conditions (Fig. 9D). Salt exposure resulted in a significant reduction in root elongation in both genotypes. However, roots of *aoc* seedlings remained significantly longer under salt stress (8.4 cm) compared with WT plants (6.8 cm) (Fig. 9D). Relative to the control condition, WT showed a 10% reduction in root growth, while the *aoc* mutant exhibited a stronger reduction of about 25% relative to its controls (Fig. 9F). Still, the absolute root length under salt stress remained higher in the *aoc* mutant than in the wild type. These data indicate that jasmonic acid deficiency promotes increased root elongation under both control and saline conditions and contributes to the maintenance of longer roots during salt stress, even though salinity negatively affects root growth in both genotypes.

3.1.3 Organ-Specific Biomass Accumulation

3.1.3.1 Enhanced Fresh Biomass Accumulation in the *aoc* Mutant

As we observed the superior growth of an *aoc* mutant, we wanted to check whether it links to altered biomass allocation, and examined the fresh weight of individual plant organs after 12 days of growth under control conditions and exposure to 100 mM NaCl. The relative stimulation of growth in the *aoc* mutant was compared with WT across several organs, as shown in Fig. 10. The jasmonic acid–deficient *aoc* mutant showed higher fresh biomass across all analyzed organs than the WT. The difference in biomass was already noticed at control conditions and became pronounced under saline stress. This implied that mutant tissues retained growth more efficiently than WT tissues during salt exposure. The stimulation of change throughout various tissues varied and showed organ- and developmental-stage dependence. Without salt exposure, moderate increases in fresh weight were observed for most leaves, the stem-containing fraction, and roots. In saline conditions, younger leaves like 3rd and 4th, and the leaf 6th/stem fraction showed strong stimulation. In case older leaves (leaf 2nd) recorded minor changes between genotypes. A higher biomass enhancement was observed in the roots of the *aoc* mutant in saline conditions. The difference in the *aoc* mutant root was approximately 70–90% higher than WT. These data indicate that JA deficiency enhances fresh biomass accumulation across the plant but preferentially promotes growth in actively developing tissues.

3.1.3.2 Root Biomass Increase Is Associated with Enhanced Cellular Biomass Accumulation

An increase in fresh weight may reflect either enhanced cell proliferation (increased cell number or cellular biomass) or increased cell expansion and water content. To distinguish between these possibilities, fresh weight and dry weight were analyzed in parallel (Suppl. Fig. S1). In roots, the increase in fresh weight observed in the *aoc* mutant was accompanied by a proportional increase in dry weight under both control and saline conditions. The similar scaling of fresh and dry weight indicates enhanced root biomass in the mutant, which signifies increased cellular biomass, consistent with elevated cell proliferation and/or increased synthesis of structural cellular components. This finding indicates that JA deficiency supports maintenance of root growth capacity under salinity by sustaining biomass production rather than merely increasing tissue hydration.

3.1.3.3 Leaf Biomass Increase Is Predominantly Driven by Cell Expansion

In contrast to roots, a qualitatively different relationship between fresh and dry weight was observed in younger leaves (Suppl. Fig. S1, red arrows). Although the fresh weight of leaves 3

and 4 showed a strong increase in the *aoc* mutant under salinity, the corresponding increase in dry weight was comparatively small. The recorded difference between the fresh and dry weight indicates the role of cell expansion and water accumulation rather than increased cell number in the elevated fresh weight of *aoc* mutants. The cell expansion linked to improved turgor maintenance, altered cell wall properties, or changes in osmotic adjustment. Therefore, JA deficiency promotes shoot growth under salinity primarily through mechanisms that enhance cellular expansion rather than through stimulation of cell proliferation.

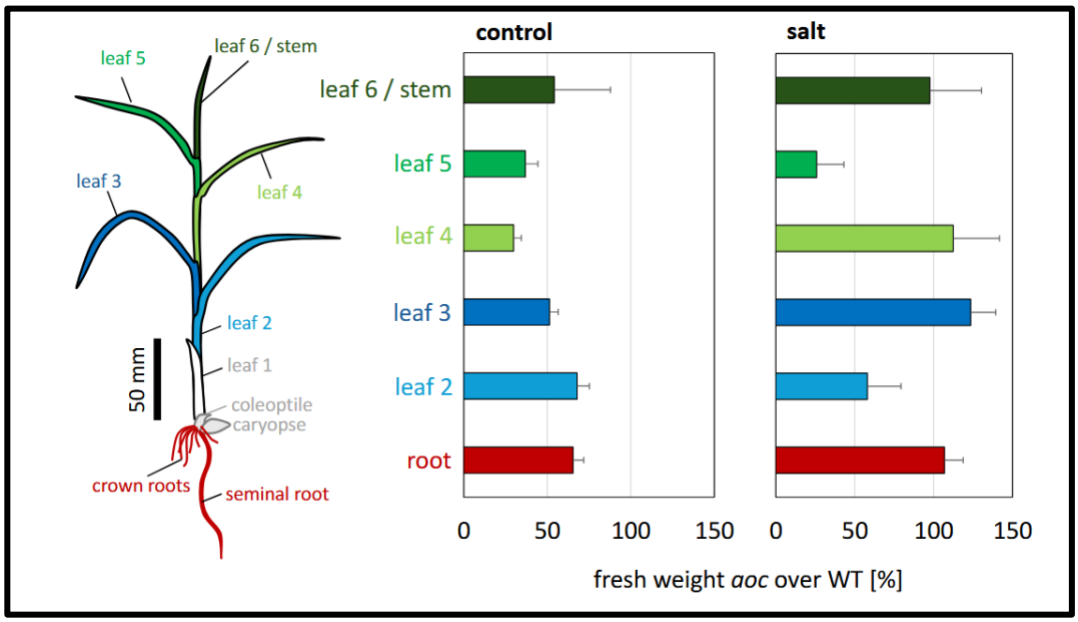


Fig. 10. Organ-specific stimulation of fresh biomass accumulation in the jasmonic acid–deficient *aoc* mutant. Wild-type (WT; cv. Kitaake) and *aoc* mutant seedlings were grown hydroponically for 12 days under control conditions or in the presence of 100 mM NaCl. Individual organs (roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and leaf 6th/stem fraction) were harvested separately as illustrated in the schematic diagram. Fresh weight of each organ was determined and expressed as relative change of *aoc* compared with WT (%, WT = 100). Under control conditions, the *aoc* mutant shows moderately increased fresh biomass in most organs. Under saline conditions, biomass stimulation is strongly enhanced, particularly in younger leaves (leaf 3rd and leaf 4th), the leaf 6th/stem fraction, and roots, whereas older leaves (leaf 2nd) display only minor differences between genotypes. Roots exhibit the strongest stimulation, reaching approximately 70–90% higher fresh weight in *aoc* compared with WT. Data represent means ± SE.

3.1.4. Photosynthetic Performance under Salinity

3.1.4.1 JA Deficiency Attenuates Salinity-Induced Decline in Carbon Assimilation

To understand the role of Jasmonate in the photosynthetic machinery during salt exposure, we measured net CO_2 assimilation rates on the 5th leaf of wild-type (WT) and *aoc* mutant seedlings under control and salt-stress conditions (Fig. 11A). Measurements were conducted over a range of photosynthetically available radiation (PAR; 0 – 2000 µmol m^{-2} s^{-1}). The assimilation rate was increased at low PAR (0 – 500 µmol m^{-2} s^{-1}) in both genotypes in the control environment. Then recorded the gradual approach to saturation at higher irradiances. The higher assimilation

rate at saturating PAR (>1000 µmol m^{-2} s^{-1}) was higher in WT than in *aoc* mutant. It was recorded that the mutant saturated at approximately 30% lower values. Non-saline conditions resulted in lower photosynthetic capacity in the *aoc* mutant than in WT. Salt exposure has been shown to reduce carbon assimilation in both genotypes across the entire PAR range. In WT, the assimilation rate was close to zero from lower to higher irradiance, pointing towards the complete inhibition of CO_2 fixation. Meanwhile, the *aoc* mutant showed measurable carbon fixation across the PAR range. At 500 – 2000 µmol m^{-2} s^{-1}, the moderate range reduced carbon fixation to 25–35% of control values. These observed values of carbon fixation in WT and *aoc* mutant in saline conditions suggest JA plays a role in reducing the salinity-induced inhibition of carbon assimilation, especially at light conditions that normally support high photosynthetic activity.

3.1.4.2 The *aoc* Mutant Maintains Higher Transpiration Rates across the PAR Range under Salinity

The next after assimilation rate, transpiration rate was measured as an indicator of salt stress and gas-exchange performance because saline conditions affect plant water balance and stomatal function. In control conditions, at PAR between 0 and 500 µmol m^{-2} s^{-1}, transpiration increased rapidly and gradually approached saturation at higher irradiance in both genotypes (Fig. 11B). However, in the *aoc* mutant, the transpiration rate was consistently higher, approximately 40 – 60%, at saturating light than in WT. After salt application, the transpiration collapsed to near-zero values across all PAR levels in WT. In contrast, the *aoc* mutant maintained detectable transpiration from low to high PAR. In the *aoc* mutant, transcription rates observed were approximately 20 – 30% of those observed under control conditions at moderate and high irradiance.

3.1.4.3 Stomatal Conductance Is Sustained in the *aoc* Mutant across Light Intensities

To determine whether the differences observed in transpiration were associated with changes in stomatal behavior, stomatal conductance was analyzed. Across the PAR, stomatal conductance showed the same trend as transpiration (Fig. 11C). Stomatal conductance increased steeply at low PAR and gradually approached saturation at higher irradiance in both genotypes. The *aoc* mutant maintains higher stomatal conductance from 500 to 2000 µmol m^{-2} s^{-1} light intensities than WT plants, notably at moderate to high light intensities. WT plants showed low to near-zero stomatal conductance from moderate to high light intensities after salt

exposure. In contrast, the *aoc* mutant plant showed a countable stomatal conductance across the entire light range. At moderate to high light intensities, the *aoc* mutant maintained approximately 20 – 30% of its normal (control) stomatal conductance or transpiration capacity. Results indicate that JA deficiency mitigates salinity-induced stomatal closure, thereby supporting continued gas exchange under light conditions that normally favor high photosynthetic rates.

3.1.4.4 Photochemical Efficiency of Photosystem II Is Similar across PAR in WT and *aoc* mutant

Altered stomatal conductance and transpiration can influence photosynthetic performance. Then we examined whether differences in gas exchange are associated with changes in photochemical efficiency. To that end, we determined the effective quantum yield of Photosystem II (Fv/Fm) across the PAR range (Fig. 11D). Both WT and *aoc* mutant plants showed high Fv/Fm values at low PAR. These values declined with increasing irradiance. These trends showed that normal light-dependent down-regulation of PSII efficiency. In saline conditions, the trend across the entire PAR range observed was towards the lower end in both genotypes. No consistent genotype-dependent differences in Fv/Fm were observed in either control or saline conditions, indicating that PSII photochemical efficiency is not substantially affected by JA deficiency.

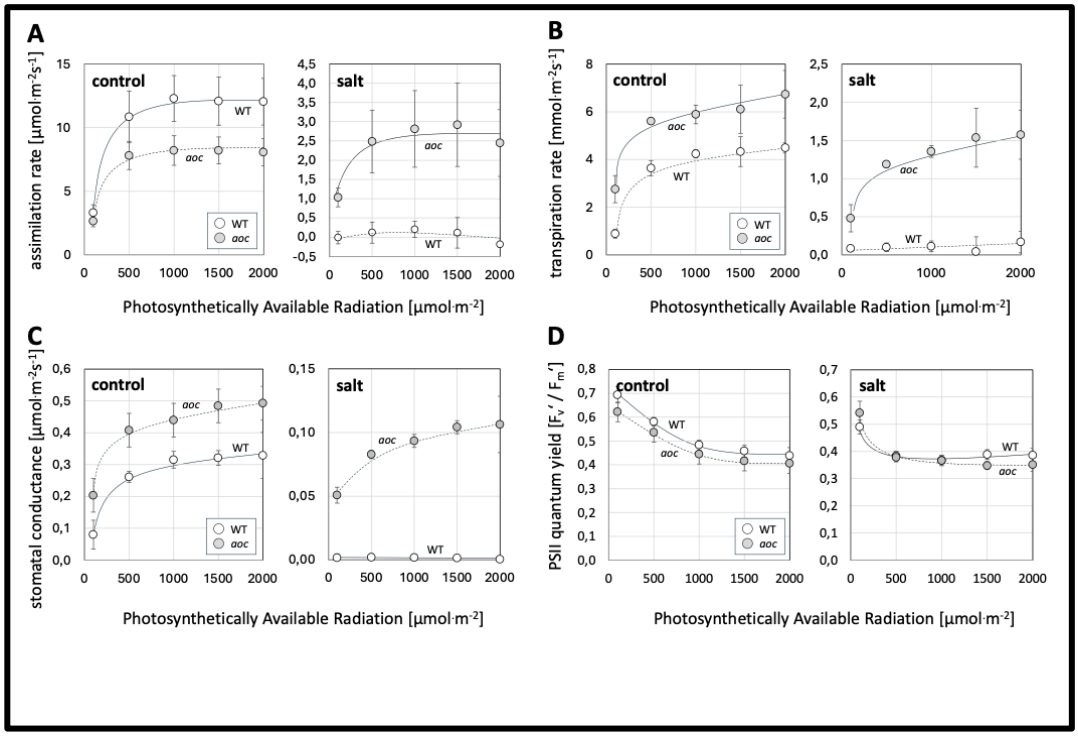


Fig. 11. Photosynthetic performance of wild-type and mutant rice seedlings under control and salinity conditions. Net CO_2 assimilation rate (A), transpiration rate (B), stomatal conductance (C), and effective quantum yield of Photosystem II (Fv/Fm) (D) were measured on the 5th leaf of WT (cv. Kitaake) and jasmonic acid–deficient *aoc* seedlings under control conditions and after exposure to 100 mM NaCl across a range of photosynthetically available radiation (PAR; 0–2000 µmol m^{-2} s^{-1}). Data represent means ± SD.

3.1.5 Sodium and Potassium Partitioning

3.1. 5.1 Organ-Specific Sodium Accumulation in WT and *aoc* mutant Plants

Sodium (Na^+) content was quantified in individual organs after 12 days of growth under control conditions or exposure to 100 mM NaCl (Fig. 12A–D) to examine whether the physiological differences between WT and the *aoc* mutant were linked with ion homeostasis. Na^+ concentrations have been recorded at low levels in all plant tissues in both genotypes compared to saline conditions **(**WT: root ~0.50, leaf 2nd ~0.40, leaf 3rd ~0.15, leaf 4th ~0.15, leaf 5th ~0.05, leaf 6th/stem ~0.25 mg g^{-1} dry weight; *aoc* mutant: root ~0.40, leaf 2nd ~0.30, leaf 3rd ~0.12, leaf 4th ~0.05, leaf 5th ~0.10, leaf 6th/stem ~0.35 mg g^{-1} dry weight**)** (Fig. 12A–B). However, *aoc* mutant displayed significantly lower Na^+ levels than WT across most organs, with the exception of leaf 5th in the same condition. Strong Na^+ content enhancement was recorded for both genotypes after salt exposure compared to the control **(**WT: root ~35, leaf 2nd ~80, leaf 3rd ~45, leaf 4th ~65, leaf 5th ~50, leaf 6th/stem ~70 mg g^{-1} dry weight; aoc: root ~40, leaf 2nd ~50, leaf 3rd ~40, leaf 4th ~45, leaf 5th ~25, leaf 6th/stem ~55 mg g^{-1} dry weight**)** (Fig. 12C, D). Promoted Na^+ accumulation measured in the leaves 2nd, 4th, and the leaf 6th/stem fraction in WT plants. In contrast, the aerial tissue of the *aoc* mutant showed significantly reduced sodium accumulation. The approximately 25–30% less Na^+ was recorded in the *aoc mutant*, specifically 2nd, 4th, and the leaf 6th/stem fraction, compared to WT. Root tissue of both genotypes did not differ significantly in sodium accumulation, still *aoc* mutant had higher levels than WT **(**~40 mg g^{-1} compared to ~35 mg g^{-1}**).**

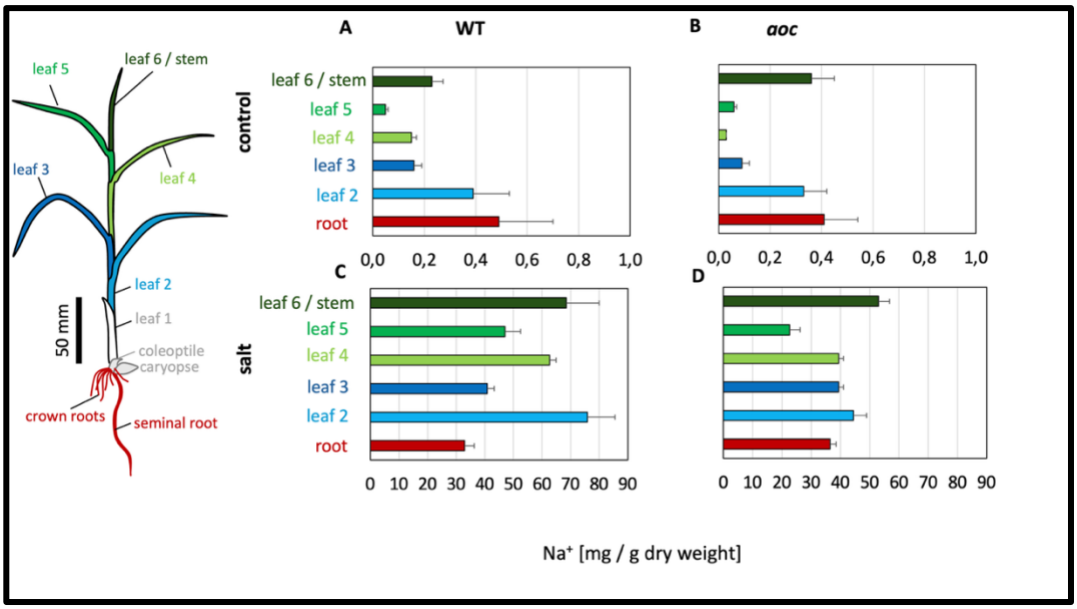


Fig. (6)12. Organ-specific sodium (Na^+) accumulation in wild-type and aoc mutant rice seedlings. Wild-type (WT; cv. Kitaake) and jasmonic acid–deficient aoc seedlings were grown hydroponically for 12 days under control conditions or in the presence of 100 mM NaCl. (A–B) Na^+ concentrations in individual organs of WT (A) and aoc(B) seedlings under control conditions. (C–D) Na^+ concentrations in individual organs of WT (C) and aoc (D) seedlings after salt treatment. Na^+ content was determined in roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and the leaf 6th/stem fraction, as indicated in the schematic. Data are presented as Na^+ concentration (mg g^{-1} dry weight). Values represent means ± SE.

3.1. 5.2 Potassium Accumulation Is Differentially Affected by Genotype and Salinity

To assess potassium (K^+) status and determine whether it differs in parallel with Na^+ accumulation under saline conditions, K^+ content was analyzed in the same organs (Suppl. Fig. S2 A–D). The K^+ levels were recorded as higher in the *aoc mutant* than in WT plants across all organs in non-saline conditions (WT: root ~38 mg g^{-1} dry weight, leaf 2nd ~35 mg g^{-1}, leaf 3rd ~38 mg g^{-1}, leaf 4th ~40 mg g^{-1}, leaf 5th ~40 mg g^{-1}, leaf 6th/stem ~75 mg g^{-1}; *aoc* mutant : root ~42 mg g^{-1}, leaf 2nd ~50 mg g^{-1}, leaf 3rd ~40 mg g^{-1}, leaf 4th ~42 mg g^{-1}, leaf 5th ~45 mg g^{-1}, leaf 6th/stem ~110 mg g^{-1}) (Suppl. Fig. S2A, B). After salt application, a strong reduction in K^+ content in both genotypes (WT: root ~8 mg g^{-1} dry weight, leaf 2nd ~15 mg g^{-1}, leaf 3rd ~38 mg g^{-1}, leaf 4th ~38 mg g^{-1}, leaf 5th ~20 mg g^{-1}, leaf 6th/stem ~15 mg g^{-1}; *aoc* mutant: root ~10 mg g^{-1}, leaf 2nd ~25 mg g^{-1}, leaf 3rd ~20 mg g^{-1}, leaf 4th ~15 mg g^{-1}, leaf 5th ~25 mg g^{-1}, leaf 6th/stem ~15 mg g^{-1}) (Suppl. Fig. S2C, D). In the youngest shoot tissues (leaf 6th/stem), a pronounced decline was observed, with K^+ levels decreasing by approximately four- to five-fold compared with control conditions (WT ~75 → ~15 mg g^{-1}; *aoc* mutant~110 → ~15 mg g^{-1}). The higher basal K^+ levels observed in the *aoc* mutant were no longer evident with salt exposure. In shoot tissue, leaves 3rd and 4th of the *aoc* mutant showed K^+ levels were significantly lower than those

in WT plants (leaf 3rd: ~20 vs ~38 mg g^{-1}; leaf 4th: ~15 vs ~38 mg g^{-1}). Thus, although JA deficiency is associated with elevated basal K^+ levels under control conditions, this difference is not maintained under salt stress.

3.1. 5.3 K^+/Na^+ Ratio Reveals Organ-Specific Ionic Balance

To integrate the observed changes in Na^+ and K^+ accumulation and evaluate their combined impact on ionic balance, the molar K^+/Na^+ ratio was calculated for each organ (Fig. 13A–D). Because salinity tolerance depends strongly on the balance between K^+ and Na^+, the molar K^+/Na^+ ratio was calculated for each organ (Fig. 13). Under control conditions, the *aoc* mutant exhibited significantly higher K^+/Na^+ ratios than WT across all organs (WT: root ~70, leaf 2nd ~90, leaf 3rd ~90, leaf 4th ~80, leaf 5th ~110, leaf 6th/stem ~300; *aoc* mutant: root ~100, leaf 2nd ~160, leaf 3rd ~120, leaf 4th ~140, leaf 5th ~170, leaf 6th/stem ~350). This reflects the combination of lower Na^+ levels (Fig. 12) and higher K^+ levels (Suppl. Fig. S2) in the mutant. Salinity stress caused a dramatic reduction of the K^+/Na^+ ratio in both genotypes, decreasing by almost three orders of magnitude (WT: root ~0.12, leaf 2nd ~0.18, leaf 3rd ~0.85, leaf 4th ~0.55, leaf 5th ~0.40, leaf 6th/stem ~0.22; *aoc* mutant: root ~0.10, leaf 2nd ~0.35, leaf 3rd ~0.45, leaf 4th ~0.28, leaf 5th ~0.90, leaf 6th/stem ~0.25) (Fig. 13C–D). However, organ-specific differences between genotypes remained. In leaves 2nd and 5th, K^+/Na^+ ratios tended to be higher in the *aoc* mutant (leaf 2nd ~0.35 vs ~0.18; leaf 5th ~0.90 vs ~0.40), whereas in leaves 3rd and 4th, ratios were significantly higher in WT plants (leaf 3rd ~0.85 vs ~0.45; leaf 4th ~0.55 vs ~0.28). These patterns indicate complex, organ-specific regulation of ionic balance under salinity.

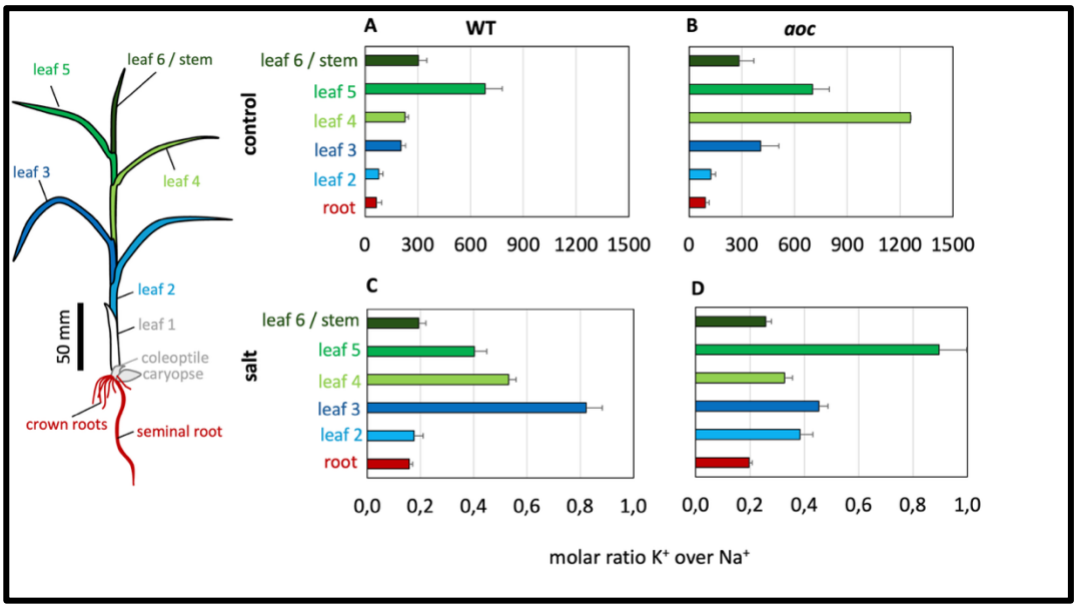


Fig. 13. Organ-specific molar K^+/Na^+ ratios in wild-type and aoc mutant rice seedlings. Wild-type (WT; cv. Kitaake) and jasmonic acid–deficient ^{aoc} seedlings were grown hydroponically for 12 days under control conditions or in the presence of 100 mM NaCl. (A–B) Molar K^+/Na^+ ratios in individual organs of WT (A) and aoc (B) seedlings under control conditions. (C–D) Molar K^+/Na^+ ratios in individual organs of WT (C) and aoc (D) seedlings after salt treatment. Ratios were calculated from Na^+ and K^+ concentrations measured in roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and the leaf 6th/stem fraction, as indicated in the schematic. Values represent means ± SE.

3.1. 5.4 Integration of Ion Partitioning Patterns

The observed data indicated that the *aoc* mutant maintains ion homeostasis more effectively than the WT by differentially orchestrating Na^+ and K^+ partitioning. Absence of JA is connected to lower sodium accumulation in the newly emerging tissue without disturbing the root. Finally, the *aoc* mutant showed the capacity to restrict Na^+ accumulation in aerial tissues with maintained potassium level, which likely contributes to its reduced salt-sensitive phenotype.

3.1.6 Salinity Induces Gene Expression in a Jasmonate-Dependent Manner

After ion data measurement, which indicated that the *aoc* mutant alters the partitioning of Na^+ and K^+ and balances ion homeostasis under saline conditions. It was important to analyze Jasmonate network components to determine their relationship to physiological differences. We analysed the transcript levels of different genes, such as JAR1, JAZ genes, transport genes, and antioxidant genes.

3.1.6.1 Salinity Differentially Modulates JAR1 Transcript Levels in WT and *aoc* mutant

To understand the connection between the jasmonate network and saline conditions, we analysed the transcript level of JAR1 in the older leaf (2^{nd} leaf) in WT and *aoc* mutant under control and salt stress. The genes encode the protein that plays a vital role in forming the bioactive form of the jasmonate JA-Ile (Fig. 14A). Stable transcript levels were observed throughout the time course in the WT under non-saline conditions. In contrast, the JAR1 transcript exhibited a pronounced transient increase in the *aoc* mutant. At the early time point, the transcript amplitude was fourfold higher in the *aoc* mutant than in WT and returned to basal levels by 6 h. After salt application, the JAR1 transcript is modulated, and a slight but significant transient decrease was observed in WT plants, whereas a slight but significant transient increase was observed in the *aoc* mutant. These results show that both genotype and salinity influence JAR1 expression and suggest compensatory transcriptional regulation of JAR1 in response to the absence of endogenous jasmonate production.

3.1. 6.2 JAZ Genes Are Strongly Induced by Salinity in WT but Not in the *aoc* Mutant

In light of the differential regulation of JAR1 under salinity, we next analyzed whether downstream JA signaling components show comparable genotype-dependent responses to salt stress. Transcript levels of four JAZ genes (JAZ8, JAZ9, JAZ11, and JAZ13) were monitored in parallel to assess salinity-responsive JA signaling (Fig. 14B–E). Under control conditions, transcript levels of all four JAZ genes were very low in both WT and *aoc* mutant plants. Following salinity treatment, WT plants showed strong induction of all tested JAZ transcripts. In contrast, expression of these genes in the *aoc* mutant remained close to background levels throughout the time course. This striking difference demonstrates that salinity-induced expression of JAZ genes strictly depends on the presence of jasmonates.

3.6.3 Distinct Temporal Induction Patterns among JAZ Family Members

To further resolve the JA signaling response to salinity, we compared the temporal induction patterns of individual JAZ family members. Although all four JAZ genes were induced by salinity in WT plants, their temporal response patterns differed. JAZ9 and JAZ11 displayed gradual and moderate increases in transcript abundance starting at the first time point (1 h) and continuing over the time course (Fig. 14C–D). In contrast, JAZ13 showed a much steeper increase, with strong accumulation detectable already at 1 h and continuing to rise thereafter, identifying JAZ13 as the most strongly salt-responsive JAZ gene among those tested (Fig. 14E). JAZ8 exhibited a biphasic response pattern: a small but significant early and transient increase was observed shortly after salt application, followed by a later and more pronounced accumulation at extended time points (Fig. 14B). These data reveal gene-specific kinetics of JAZ induction during salinity stress.

3.1.6.4 Dependence of Salinity-Induced JAZ Expression on Jasmonates

While individual JAZ genes exhibit distinct temporal induction patterns under salinity in WT plants, we next asked whether this salt-responsive expression strictly depends on endogenous jasmonate biosynthesis. The near-complete absence of JAZ transcript accumulation in the *aoc* mutant under salinity indicates that induction of JAZ8, JAZ9, JAZ11, and JAZ13 requires endogenous jasmonate production. This finding suggests that salinity-triggered activation of these JAZ genes is mediated primarily through jasmonate-dependent signaling rather than through parallel jasmonate-independent pathways.

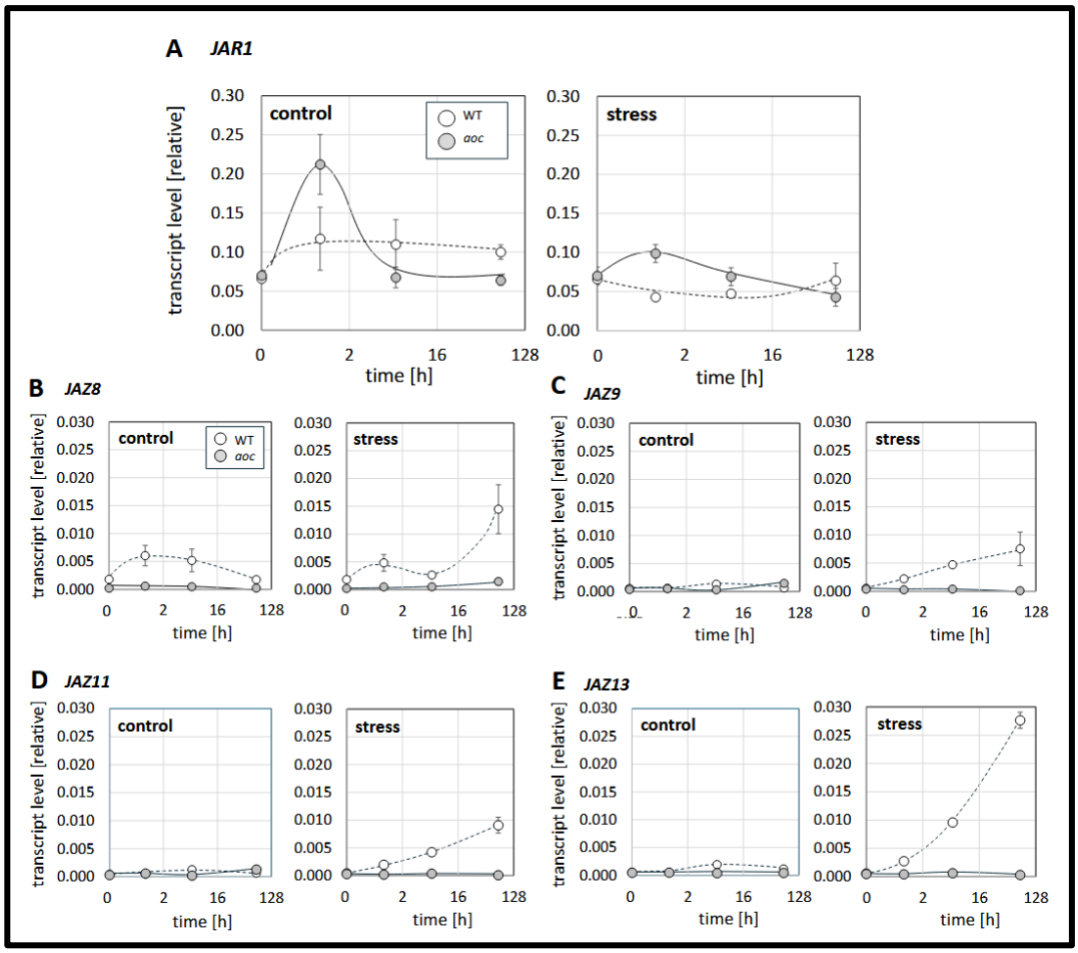


Fig. 14. Salinity-induced regulation of JAR1 and JAZ gene expression in wild-type and *aoc* mutant rice**.** Relative transcript levels of JAR1 (A), JAZ8 (B), JAZ9 (C), JAZ11 (D), and JAZ13 (E) were determined by RT-qPCR in the 2nd leaf of wild-type (WT; cv. Kitaake) and jasmonic acid–deficient *aoc* mutant seedlings under control conditions and after salt treatment. Samples were collected at the indicated time points after treatment onset. Transcript levels are shown relative to reference genes. Data represent means ± SE (n = 3 biological replicates, each with three technical replicates).

3.1.7 Salinity-Dependent Regulation of Ion Transporter Genes in Leaf 2nd

Given that the *aoc* mutant displays altered jasmonate signaling and improved Na^+/K^+ partitioning under salinity, we next investigated whether these physiological differences are associated with genotype-specific regulation of ion transporter genes in leaf 2nd.

3.1.7.1 NHX1 Is Induced Early by Salinity in the *aoc* Mutant but Late in WT

Because reduced sodium accumulation in leaf 2nd represents a major difference between WT and *aoc* plants (Fig. 15), transcript levels of the vacuolar sodium/proton antiporter NHX1 were monitored over time in leaf 2nd under control and salinity conditions (Fig. 15A). Under control conditions, steady-state NHX1 transcript levels were low and comparable between WT

and *aoc* mutant plants throughout the time course. Following salt treatment, NHX1 expression in the *aoc* mutant increased rapidly and significantly, with induction already detectable at 1 h. In contrast, WT plants did not show any early NHX1 induction. At the late time point (72 h), the response pattern was reversed. Here, WT plants displayed a strong increase in NHX1 transcript levels, exceeding those observed in the *aoc* mutant by approximately five-fold. These results indicate distinct temporal regulation of NHX1 between genotypes, with early induction in the *aoc* mutant and delayed induction in WT plants.

3.1.7.2 HAK12 Shows Transient Induction in the *aoc* Mutant but Not in WT

To determine whether additional transporters involved in sodium handling show similar genotype-specific regulation, transcript levels of HAK12, proposed to contribute to sodium exclusion from shoots, were next examined (Fig. 15B). In WT plants, HAK12 transcript levels remained relatively constant under control conditions and gradually decreased under salinity. In contrast, the *aoc* mutant displayed a transient but significant increase in HAK12 expression at 1 h under both control and saline conditions. Importantly, the amplitude of this transient induction was approximately doubled under salinity compared with control conditions. Thus, early HAK12 induction represents a mutant-specific response that is enhanced by salinity.

3.1.7.3 HAK16 Exhibits Strong Late Induction under Salinity in WT but Not in *aoc* mutant

We next analyzed expression of HAK16, which is implicated in potassium uptake and translocation to the shoot (Fig. 15C). Under control conditions, HAK16 transcript levels were similar between WT and *aoc* mutant plants. Upon salinity treatment, WT plants exhibited a strong late induction of HAK16, particularly at 72 h. In contrast, this late induction was largely absent in the *aoc* mutant. This pattern closely resembles the late induction observed for NHX1 in WT plants.

3.7.4 HAK17 Responds to Salinity Predominantly in WT

To further assess potassium transport-related responses, transcript levels of HAK17, proposed to mediate potassium uptake under high sodium conditions, were examined (Fig. 15D). In WT plants, HAK17 displayed a transient induction under control conditions and a pronounced late induction under salinity. In contrast, the *aoc* mutant showed no substantial changes in HAK17 expression across the time course under either condition.

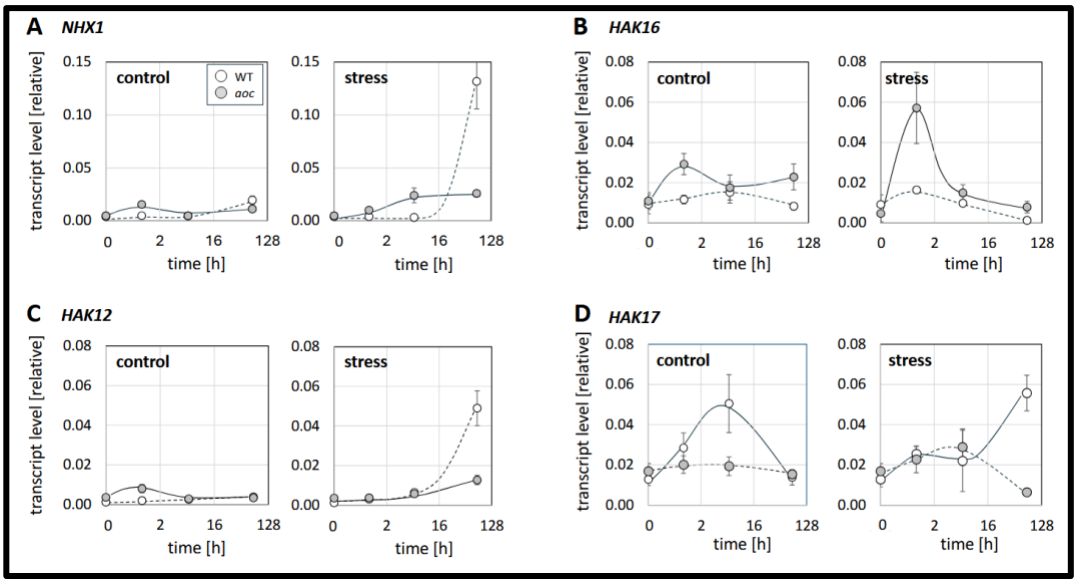


Fig. 15. Salinity-dependent regulation of NHX1 and HAK transporter gene expression in leaf 2nd of wild-type and *aoc* mutant rice**.** Relative transcript levels of NHX1 (A), HAK16 (B), HAK12 (C), and HAK17 (D) were determined by RT-qPCR in the 2nd leaf of wild-type (WT; cv. Kitaake) and jasmonic acid–deficient *aoc* seedlings under control conditions and after salt treatment. Samples were collected at the indicated time points after treatment onset. Transcript levels are shown relative to reference genes. Data represent means ± SE (n = 3 biological replicates, each with three technical replicates).

3.1.7.5 Root Ion Transporter Gene Expression under Salinity

The reduced sodium accumulation in leaf 2nd of the *aoc* mutant might be linked towards more pronounced sequestration of sodium (Fig. 12) and potassium (Suppl. Fig. S2) in the root. Therefore, we analyzed transcript levels of the same panel of ion transporter genes in root tissue (Suppl. Fig. S3). The vacuolar sodium–proton antiporter NHX1 (Suppl. Fig. S3A) remained uninduced and showed comparable transcript levels between WT and aoc mutant plants under both control and salinity conditions. In contrast, the expression pattern of HAK16, a transporter involved in potassium uptake into roots and its translocation to the shoot, differed clearly between the two genotypes (Suppl. Fig. S3B). Already under control conditions, basal transcript levels were higher in the *aoc* mutant and increased further from the first time point (1 h) after the onset of salinity. However, this response lagged behind the rapid and strong induction observed in leaves (Suppl. Fig. S3B) (Fig. 7B). For HAK12 (Suppl. Fig. S3C), although transcript levels increased under salinity, induction was weaker in the *aoc* mutant than in WT plants. A similar pattern was observed for HAK17 (Suppl. Fig. S3D). Overall, transcriptional responses of ion transporters in roots were limited and delayed compared with those observed in shoot tissues. This indicates that the major differences in sodium exclusion and potassium

partitioning between WT and *aoc* mutant plants are predominantly established at the shoot level rather than in the root.

3.1.7.6 Integration of Transporter Expression with Sodium Accumulation Patterns

The strong late induction of NHX1 together with elevated expression of HAK16 and HAK17 in WT leaves coincides with the pronounced accumulation of sodium in leaf 2nd observed at late time points (Fig. 12). The temporal characteristics of these responses suggest that they represent secondary or damage-associated transcriptional responses to elevated cellular sodium levels. Consistently, these late inductions are largely absent in the *aoc mutant*, which accumulates substantially less sodium in leaf 2nd. Instead, the mutant exhibits early induction of NHX1 and HAK12 in leaves, which may contribute to limiting sodium accumulation before severe ionic stress develops. In roots, transcriptional responses of NHX1, HAK16, HAK12, and HAK17 were comparatively weak and delayed (Suppl. Fig. S3). This indicates that the major differences in sodium exclusion and potassium partitioning between WT and *aoc* mutant plants are predominantly established at the shoot level rather than in the root.

3.1. 8 Plastidic Superoxide Dismutase Genes Are More Readily Induced in the *aoc* Mutant

Because altered ion accumulation and transporter regulation in leaf 2 are expected to influence cellular redox homeostasis, we next investigated whether antioxidant defense genes exhibit genotype-dependent regulation under salinity.

3.1.8.1 Induction of ptSOD1 and ptSOD2 in Response to Experimental Perturbation

Sodium accumulation and salinity stress can interfere with electron transport processes in chloroplasts, leading to enhanced production of reactive oxygen species (ROS), particularly superoxide. Superoxide is detoxified by superoxide dismutases (SODs), which convert it into hydrogen peroxide, subsequently removed by catalases and peroxidases. To assess whether JA deficiency affects antioxidant gene expression, steady-state transcript levels of two plastid-localized SOD genes, ptSOD1 and ptSOD2, were monitored over time in WT and *aoc* mutant plants under control and salinity conditions (Fig. 16). Under both control and salinity conditions, transcript levels of ptSOD1 and ptSOD2 showed transient increases in the *aoc* mutant, reaching peak levels approximately 6 h after the start of the treatment. In contrast, WT plants displayed only weak or no induction of these genes over the same time course. At their peak, transcript levels of both ptSOD genes in the *aoc* mutant were approximately four-fold higher than in WT plants, indicating a markedly stronger transcriptional response in the mutant.

3.1. 8.2 Induction Is Not Strictly Salinity-Specific but Is Enhanced in the *aoc Mutant*

Notably, similar transient induction of ptSOD1 and ptSOD2 was also observed in the *aoc* mutant under control conditions. This indicates that the upregulation of plastidic SOD genes is not exclusively triggered by salinity stress but can also be elicited by experimental perturbation associated with transfer to the hydroponic system. Despite this lack of strict salinity specificity, the magnitude of induction was consistently higher in the *aoc* mutant than in WT plants under both control and saline conditions.

3.1.8.3 Genotype-Dependent Sensitivity of Antioxidant Gene Regulation

Together, these data demonstrate that plastidic SOD genes respond sensitively to environmental or experimental perturbations and that this response is more pronounced in the jasmonic acid–deficient *aoc* mutant. This suggests that JA deficiency is associated with an enhanced transcriptional responsiveness of plastidic antioxidant genes.

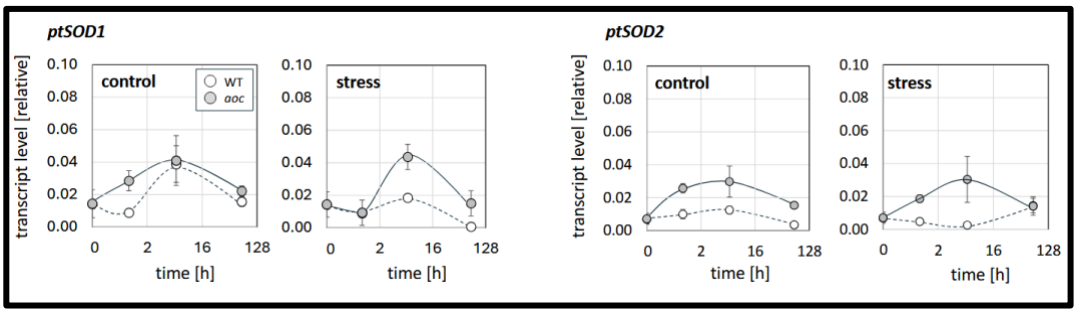


Fig. (16). Salinity-dependent regulation of plastidic superoxide dismutase genes in wild-type and *aoc* mutant rice**.** Relative transcript levels of ptSOD1 and ptSOD2 were determined by RT-qPCR in leaf tissue of wild-type (WT; cv. Kitaake) and jasmonic acid–deficient *aoc* seedlings under control conditions and after salt treatment. Samples were collected at the indicated time points after treatment onset. Transcript levels are shown relative to reference genes. Data represent means ± SE (n = 3 biological replicates, each with three technical replicates).

3. **RESULT**

3.2. Salt-tolerant FL478 versus salt-sensitive RC222

3.2.1 Experimental design and sampling strategy

The same experimental arrangements that were described in Section 3.1 were used. The salinity effects on the tolerant genotype FL478 and the salt-sensitive genotype RC222 were analyzed under control conditions and following exposure to 100 mM NaCl. Both genotypes were compared at phenotypic, physiological, ionic, hormonal, and transcriptional parameters. To allow their study, the adaptive and sensitive mechanism was studied using identical growth conditions, treatment regimes, and sampling strategies. Samples were collected from the second leaf and roots before salt application (T0) and at defined time points after stress onset (T1 = 1 h, T2 = 6 h, and T3 = 72 h) for transcriptomic analysis. Hormonal responses were quantified in the second leaf tissue at early stages (1 h, 6 h, and 48 h), while at 72 h, both the second and fourth leaves were analyzed to examine spatial differences during later phases of salinity exposure. To understand the long-term effect on plant growth and development, we analyzed phenotypic, physiological, and ionic measurements after prolonged salt treatment. All results are presented in parallel for FL478 and RC222 to facilitate direct genotype-specific comparisons and to relate early molecular and hormonal responses to longer-term physiological and ionic outcomes under salinity stress.

3.2.2 Salinity-Induced Phenotypic Responses in FL478 and RC222 Seedlings

3.2.2.1 Differential Sensitivity of FL478 and RC222 to Salinity Stress

To understand plant responses to unfavourable conditions, it is important to analyse the phenotypic marker. These morphological markers provide a link between the plant performance and establish a framework for subsequent physiological and molecular analyses. To understand genotypic salinity responses, seedlings of the salt-tolerant rice line FL478 and the salt-sensitive genotype RC222 were grown hydroponically under control conditions or exposed to 100 mM NaCl. After 72 hours of salt exposure, the first phenotypic observation was recorded for the 2nd and 3rd leaves (Fig. 17). The salt-tolerant line FL478 showed less chlorosis and fewer necrotic areas on the 2nd and 3rd leaves, in contrast to the salt-sensitive line RC222, which exhibited pronounced tissue damage on the same leaves. These observations indicated an earlier onset of salt-induced injury in RC222, while FL478 maintains greater leaf integrity during the initial

phase of stress. Longer saline stress exposure makes both genotypes more vulnerable. Plants were visually assessed for growth performance and leaf damage after 12 days of salt application (Fig. 18A). FL478 plants showed more vigorous shoot growth than RC222 under control conditions (Fig. 18B). After salt application, the growth of both lines was inhibited. On one hand, RC222 plants showed a stronger reduction in shoot growth accompanied by extensive leaf chlorosis, rolling, and visible tissue damage (Fig. 18 A). In contrast, FL478 plants retained greener leaves and a more robust growth habit under saline conditions (Fig. 12B). Interestingly, the one notable observation recorded that FL478 plants appeared larger than RC222, indicating intrinsic differences in basal growth capacity between the genotypes in non saline condition. These visual differences were supported by quantitative measurements of shoot length (Fig. 18C). Under control conditions, FL478 plants developed significantly longer shoots (40.8 cm) than RC222 plants (34.8 cm). Exposure to 100 mM NaCl reduced shoot elongation in both genotypes; however, FL478 maintained longer shoots (22.4 cm) compared with RC222 (19.5 cm) under salinity. When relative changes were calculated, shoot growth in RC222 was reduced by approximately 15–20% compared with FL478 under both control and salt conditions (Fig. 18E), reflecting consistently lower shoot performance in the salt-sensitive genotype. FL478 exhibited tolerance to the saline condition, as it reported necrosis only on older leaves, such as the 2nd leaf. In the case of the sensitive genotype RC222, it showed more necrosis on leaves 2 and 3, with rolling and bleaching also apparent in younger leaves (Fig. 18B). The visual observation showed a clear difference between genotypes in handling the saline stress. The RC222 was more susceptible to salt-induced shoot injury. In contrast, Fl78 exhibited shoot growth and leaf integrity more effectively under stress. In conclusion, the combined results showed that FL478 exhibited a more effective defense against salt stress than RC222, characterized by superior shoot growth and reduced leaf damage under salinity stress. Importantly, these differences were already evident under non-stress conditions, suggesting that genotypic variation in basal growth capacity contributes to differential salinity tolerance.

Fig. 17. Differential salt-induced leaf necrosis in FL478 and RC222 rice seedlings**.** FL478 and RC222 seedlings were grown under hydroponic conditions and exposed to either control medium or 100 mM NaCl. Representative images of the 2nd and 3rd leaves were recorded after 72 h of treatment. (A) Leaves under control conditions. (B) Leaves after 100 mM NaCl treatment. Under salt stress, RC222 leaves exhibit pronounced chlorosis and extensive necrotic regions in both the 2nd and 3rd leaves, whereas FL478 leaves show reduced tissue damage and retain a greener appearance, indicating enhanced tolerance to salinity. Scale bar shown in each panel.

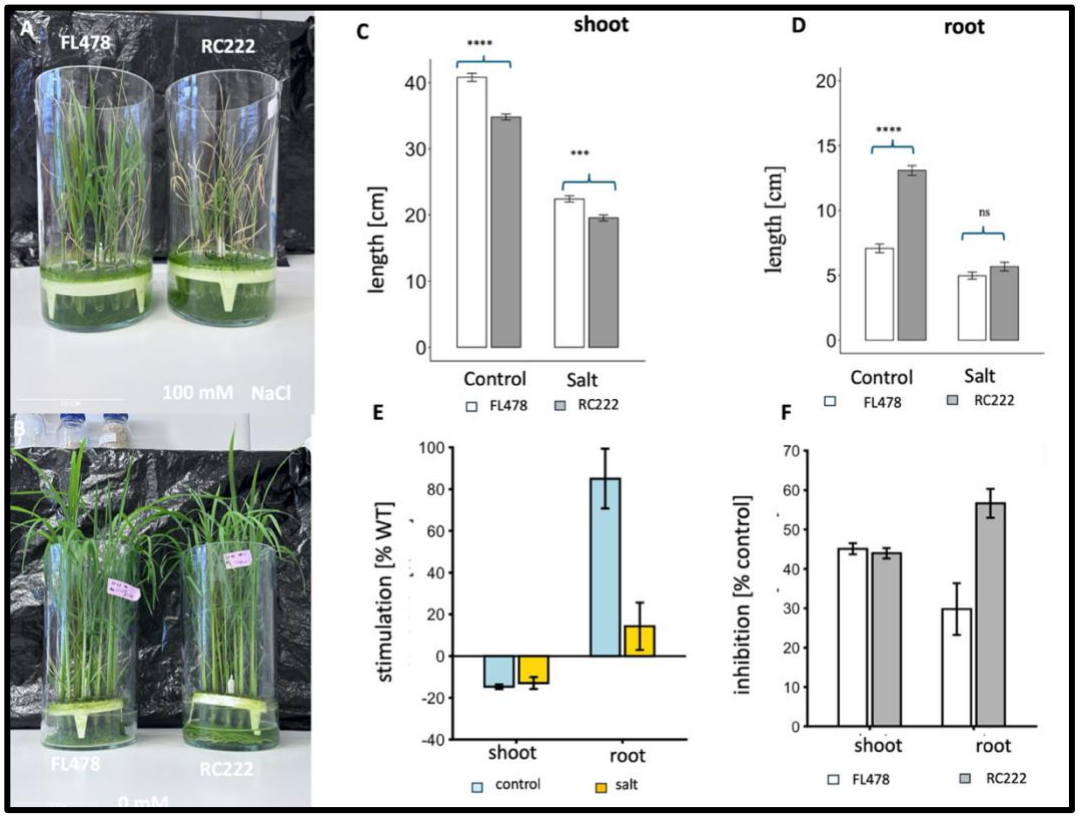


Fig. 18. Genotype-dependent growth responses of FL478 and RC222 rice seedlings to salinity. FL478 and RC222 plants were grown hydroponically and analyzed 12 days after the onset of salt treatment. (A) Representative images of FL478 and RC222 plants exposed to 100 mM NaCl. (B) Representative images of plants grown under control conditions. Under salinity, RC222 plants exhibit pronounced growth inhibition, leaf chlorosis, and visible tissue damage, whereas FL478 plants maintain greener leaves and a more vigorous growth phenotype. (C) Shoot length and (D) root length of FL478 and RC222 under control and salt conditions. (E) Relative stimulation of shoot and root length in RC222 compared to FL478 under control and salt conditions. (F) Relative inhibition of shoot and root growth by salinity in FL478 and RC222. Data represent means ± SE. Scale bar = 10 cm.

3.2.3 Effect of Salinity Stress on Root Elongation

Water-absorbing plant organ roots are the first sensory tissue to sense the salt in the soil. As it plays a vital role in ion uptake, it provides a sensitive indicator of plant growth performance under salinity stress. The seminal root length was analyzed after 12 days of salt exposure to assess genotypic differences in root growth (Fig. 18D). RC222 developed significantly longer roots (13.1 cm) than FL478 (7.08 cm) under non-saline conditions. It showed enhanced basal root elongation in RC222 than in FL478. As expected, after salt exposure, both genotypes showed a marked reduction in root length. Nevertheless, RC222 roots remained longer under salinity (5.68 cm) than those of FL478 (4.97 cm). Root growth in RC222 was more strongly reduced by saline conditions (approximately 55–60%) than in FL478 (approximately 30%) by Relative inhibition analysis (Fig. 18F). Despite the stronger negative force of salt in hindering root growth. RC222 showed higher root length than FL478 in saline conditions. The observed results showed that RC222 possesses an intrinsically stronger root elongation capacity, which is partially maintained under salinity stress, whereas FL478 displays greater stability in relative root growth reduction.

3.2.4 Organ-Specific Biomass Allocation in FL478 Compared with RC222

3.2.4.1 Enhanced Fresh Biomass Accumulation in FL478 under Salt Stress

To determine whether the salt-tolerant genotype FL478 showed changes in biomass allocation compared with RC222, we analyzed the fresh weight of individual plant organs after 12 days of hydroponic growth under control conditions and 100 mM NaCl. Fresh weight values were recorded for both genotypes and were expressed relative to RC222 (WT = 100), as shown in Fig. (19). FL478 exhibited fresh biomass levels comparable to or moderately higher than RC222 across most analysed organs, including leaves, the stem fraction, and roots under non-

saline conditions. In contrast, exposure to salt stress resulted in a pronounced organ-specific redistribution of biomass. Strong increases in fresh weight were observed in younger leaves (leaf 3rd and leaf 4th), while the leaf 6th/stem fraction showed only moderate differences between the genotypes, whereas older leaves (leaf 2nd) showed smaller relative changes between genotypes. Root fresh weight was reduced by salinity in both genotypes; however, FL478 maintained a higher relative biomass than RC222. These results indicate that FL478 preserves shoot growth more efficiently than RC222 under saline conditions, with preferential biomass allocation to actively developing aerial tissues.

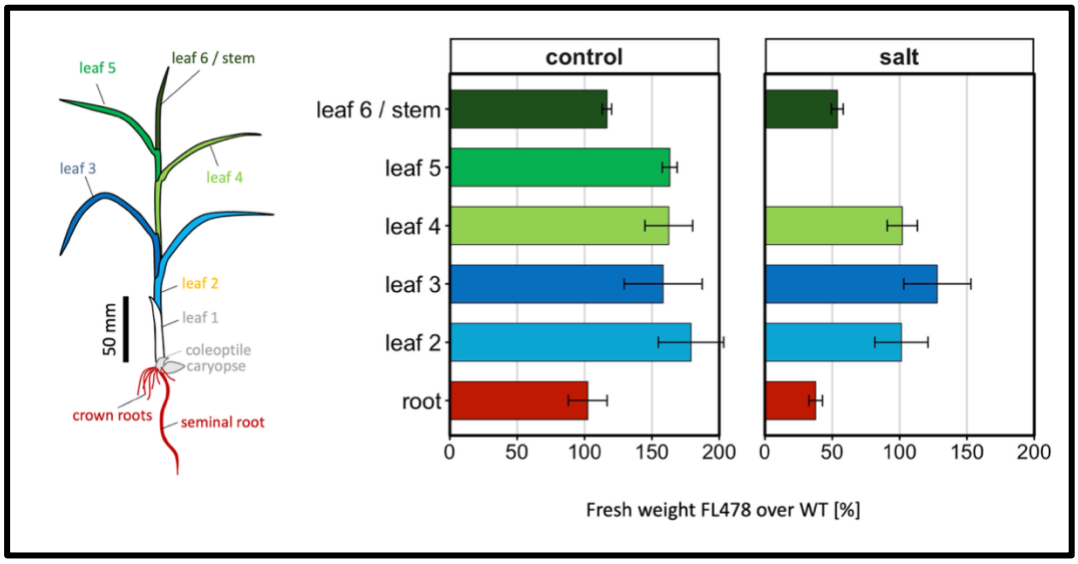


Fig. 19. Fresh weight of individual organs (root, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and leaf 6th/stem fraction) of FL478 seedlings expressed as percentage relative to RC222 (set to 100%). Seedlings were grown hydroponically under control conditions or in the presence of 100 mM NaCl. Bars represent mean fresh weight values, and error bars indicate ± SE. Under control conditions, FL478 shows comparable or moderately increased biomass across most organs. Under salt stress, biomass distribution is altered in an organ-specific manner, with pronounced effects observed in younger leaves, the leaf 6th/stem fraction, and roots.

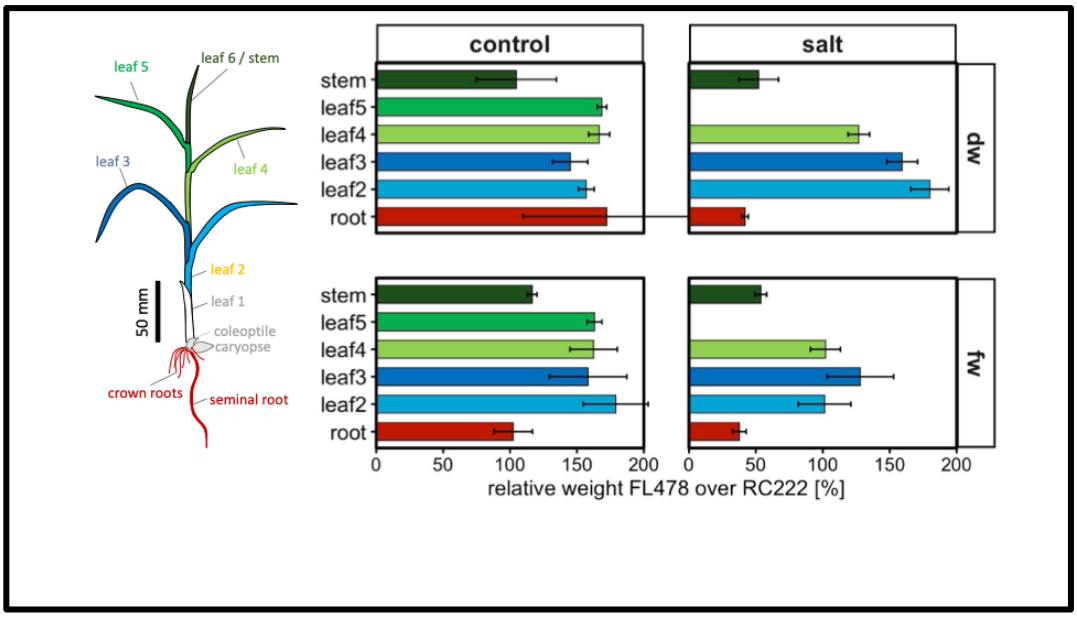
3.2.4.2 Root Biomass Responses Are Reflected by Coordinated Changes in Fresh and Dry Weight

Fresh weight (FW) and dry weight (DW) were analyzed in parallel to determine how changes in fresh biomass were associated with increased cellular biomass or altered tissue hydration (Supplementary Fig. 4). Reduction in the fresh weight of the root was accompanied by proportional decreases in dry weight under saline conditions. It showed that changes in root biomass primarily reflect alterations in cellular biomass accumulation rather than changes in

water content alone. The coordinated scaling of FW and DW suggests that salt stress restricts root biomass production in both genotypes, although FL478 retains a higher relative biomass than RC222.

3.2.4.3 Salt-Induced Leaf Biomass Changes in FL478 Are Partially Driven by Tissue Hydration.

Younger leaves exhibited a distinct relationship between fresh and dry weight than the roots under saline conditions (Supplementary Fig. 4). FL478 showed stronger increases in fresh weight than RC222 in leaves 3rd and 4th, the corresponding increases in dry weight were comparatively smaller. The observed difference between FW and DW indicated that high leaf biomass in FL478 was influenced by enhanced tissue water content and cell enlargement rather than by a corresponding increase in cellular biomass in saline conditions. The observed result indicated that FL478 maintains shoot growth through organ-specific strategies, combining sustained cellular biomass production in roots with enhanced expansion and water accumulation in developing leaves in saline conditions.



Supplementary Fig. 4. Differential organ-specific fresh and dry biomass accumulation in FL478 under control and salt stress. Fresh weight (FW) and dry weight (DW) of individual organs were measured in RC222 (wild type) and FL478 seedlings grown hydroponically for 12 days under control or salt stress conditions. Roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and the stem fraction were harvested separately. Data are shown as relative change of FL478 compared with RC222 (%, WT = 100). Bars represent means ± SE.

3.2.5 Salinity-Induced Hormonal Responses in FL478 and RC222

3.2.5.1 Salinity-Induced Changes in Jasmonate Levels

In the leaf tissues (2^{n}d and 4th) of both genotypes, endogenous hormone levels were measured at early (1 h and 6 h) and late (48 h and 72 h) time points following exposure to 100 mM NaCl. Jasmonic acid (JA) and its bioactive conjugate jasmonoyl-isoleucine (JA-Ile) were measured in these tissues, and spatial differences between leaves and genotypes were evaluated at later stages of stress. In the second leaf, the level of jasmonic acid (JA) remained below 15 pmol g^{-1} FW in FL478 and 30 pmol g^{-1} FW in RC222 (Fig. 20A), while the bioactive JA-Ile levels were minimal and did not exceed 20 pmol g^{-1} FW in either genotype under control conditions (Fig. 20B). JA levels increased after 1 hour of saline exposure in both genotypes (Fig. 20A). At 1 h, JA rapidly accumulated to approximately 40–60 pmol g^{-1} FW, and the trend remained the same at 6 h in RC222. In contrast, a moderate increase was recorded, with JA concentrations not exceeding 30 pmol g^{-1} FW during the early response phase in FL478. A comparable trend was observed for JA-Ile (Fig. 20B). RC222 showed a pronounced induction of JA-Ile, reaching 120–180 pmol g^{-1} FW at 1 h, whereas JA-Ile levels in FL478 remained below 30 pmol g^{-1} FW.

3.2.5.2 Accumulation of Jasmonate Catabolites during Salinity Stress

As jasmonate turnover plays a key role in regulating the duration and intensity of stress signaling, jasmonate catabolites such as 12-hydroxy-JA-Ile, 12-hydroxy-JA, and 12-carboxy-JA-Ile were quantified to assess the dynamics of jasmonate inactivation. Catabolite levels were recorded as low in both genotypes under non-saline conditions. RC222 showed a delayed accumulation of jasmonate catabolites after salt exposure. At 1 h, 12OH-JA-Ile levels remained below 10 pmol g^{-1} FW, despite high JA-Ile concentrations (Fig. 14 C). At later time points, RC222 accumulated substantial amounts of catabolites, with 12OH-JA and 12COOH-JA-Ile reaching 200–500 pmol g^{-1} FW, particularly in the second leaf (Fig. 20D–E). In FL478, accumulation of jasmonate catabolites was measurable at earlier time points. 12OH-JA-Ile levels reached 10–20 pmol g^{-1} FW at early stages and remained lower than those observed in RC222 throughout the experiment (Fig. 1420). At 72 h, catabolite levels in FL478 were lower and more evenly distributed between the second and fourth leaves (Fig. 20C; Fig. 21E).

3.2.5.3 OPDA, ABA, and Salicylic Acid Levels under Salinity

Levels of the jasmonate precursor OPDA were higher in RC222 than in FL478 under both control and salt conditions (Fig. 21F). Under salinity, OPDA accumulated mainly in the second

leaf of RC222, whereas OPDA levels in FL478 remained low throughout the experiment. Abscisic acid (ABA) levels increased in response to salinity in both genotypes (Fig. 15G). RC222 showed a pronounced ABA accumulation at later time points, reaching 500–800 pmol g^{-1} FW, particularly in the second leaf. In FL478, ABA levels increased moderately and remained below 300 pmol g^{-1} FW. Salicylic acid levels showed only minor changes in response to salinity and were comparable between FL478 and RC222 at all analyzed time points and leaf positions (Fig. 21H).

3.2.5.4 Summary of Hormonal Responses

Jasmonates and ABA levels were recorded as higher in both genotypes after saline exposure. In the salt-sensitive genotype, RC222 exhibited more pronounced and consistent accumulation of JA, JA-Ile, OPDA, and ABA. In contrast, FL478 showed the least hormone accumulation and the least persistence over time. Spatial analysis revealed higher hormone levels in older leaves of RC222, while FL478 displayed lower and more uniform distribution between leaves.

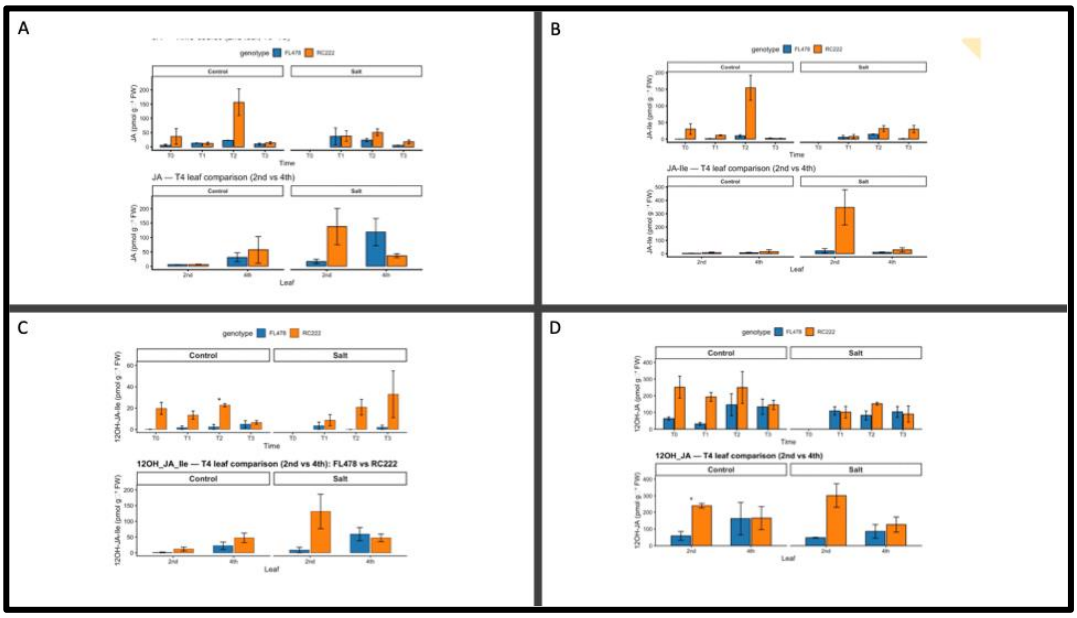


Fig. 20. Salinity-induced changes in jasmonates and jasmonate catabolites in FL478 and RC222. Endogenous levels of jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), and major jasmonate catabolites were quantified in leaves of FL478 and RC222 under control conditions and after exposure to 100 mM NaCl. **(**A**)** JA levels measured in the second leaf at different time points under control and salt conditions. **(**B**)** JA-Ile levels measured in the second leaf at corresponding time points. **(**C**)** Levels of 12-hydroxy-JA-Ile (12OH-JA-Ile) measured over time in the second leaf. **(**D**)** Levels of 12-hydroxy-JA (12OH-JA) measured over time in the second leaf. **(**E**)** Levels of 12-carboxy-JA-Ile (12COOH-JA-Ile) measured over time in the second leaf. Lower panels show leaf-position-dependent hormone accumulation at later stages. JA (A), JA-Ile (B), 12OH-JA-Ile (C), 12OH-JA (D), and 12COOH-JA-Ile (E) levels were compared between the second and fourth leaves under control and salt conditions at T4. Data represent mean ± SE. Hormone concentrations are expressed as pmol g^{-1} fresh weight (FW).

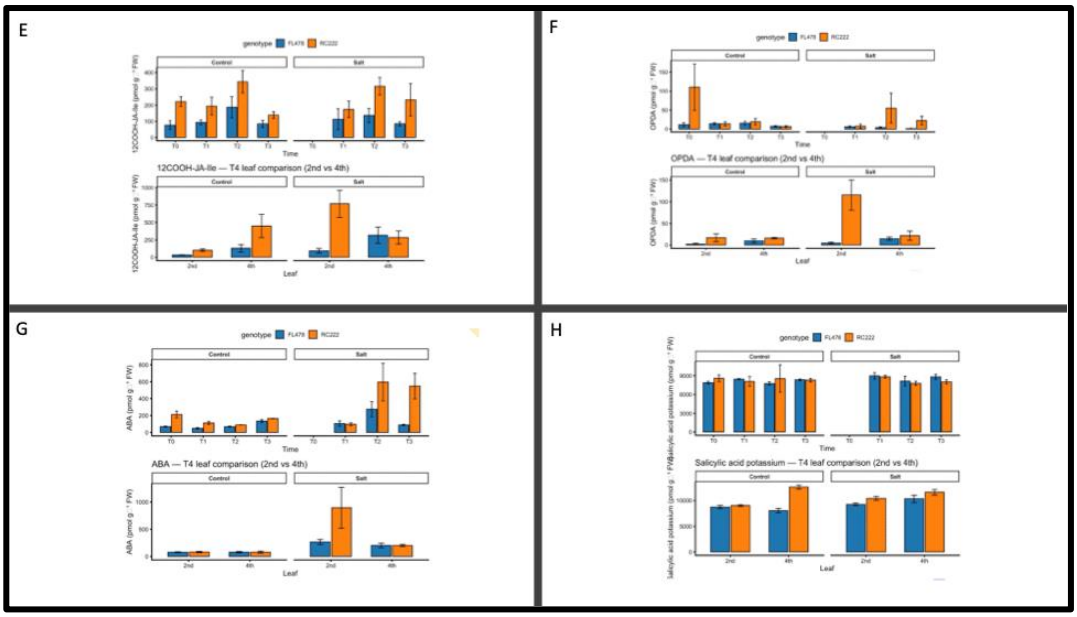


Fig. 21. Effects of salinity on OPDA, ABA, and salicylic acid levels in FL478 and RC222. Endogenous levels of additional stress-related hormones were quantified in leaves of FL478 and RC222 under control conditions and after exposure to 100 mM NaCl. **(F)** OPDA levels measured in the second leaf at different time points under control and salt conditions, including comparison between the second and fourth leaves at T4. **(G)** Abscisic acid (ABA) levels were measured in the second leaf over time and compared between the second and fourth leaves at T4. **(H)** Salicylic acid levels were measured in the second leaf over time and compared between the second and fourth leaves at T4. All hormone concentrations are expressed as pmol g^{-1} fresh weight (FW). Data represent mean ± SE.

3.2.6 Sodium and Potassium Partitioning

3.2.6.1 Organ-Specific Sodium Accumulation in FL478 and RC222 Plants

After 12 days of growth under control conditions or after exposure to 100 mM NaCl, the sodium (Na^+) content was recorded from the individual tissue organs of FL478 and RC222 seedlings (Fig. 22A–D). The ion analysis was vital to assess genotype-dependent differences in ion partitioning. As expected, all analyzed tissues in both genotypes showed low Na^+ concentrations under control conditions (below ~0.25 mg g^{-1} dry weight in all organs) (Fig. 22A, B). However, some variation among them was recorded, but no pronounced genotypic differences in sodium accumulation were observed under non-saline conditions. The scenario changed after salt exposure, resulting in a marked increase in Na^+ accumulation in all organs of both genotypes. The highest Na^+ concentrations were detected in shoot tissues, particularly in leaf 2nd (~60 mg g^{-1} dry weight in FL478 and ~75 mg g^{-1} in RC222), leaf 3rd (~30 mg g^{-1} in FL478 and ~50 mg g^{-1} in RC222), leaf 4th (~20 mg g^{-1} in FL478 and ~55 mg g^{-1} in RC222), and the leaf 6th/stem fraction(~25 mg g^{-1} in FL478 and ~55 mg g^{-1} in RC222). Notably, RC222 accumulated higher Na^+ levels than FL478 in most aerial organs under salt stress (Fig. 22C, D). This difference was most pronounced in leaves 3rd and 4th as well as in the leaf ^{6th}/stem fraction, where Na^+ concentrations in RC222 exceeded those in FL478 by approximately 20–30%. In contrast, root Na^+ accumulation did not differ substantially between the two genotypes under saline conditions (~18–22 mg g^{-1} dry weight in both genotypes) (Fig. 22C, D), indicating that genotypic differences in sodium homeostasis were primarily associated with Na^+ partitioning within the shoot rather than exclusion at the root level.

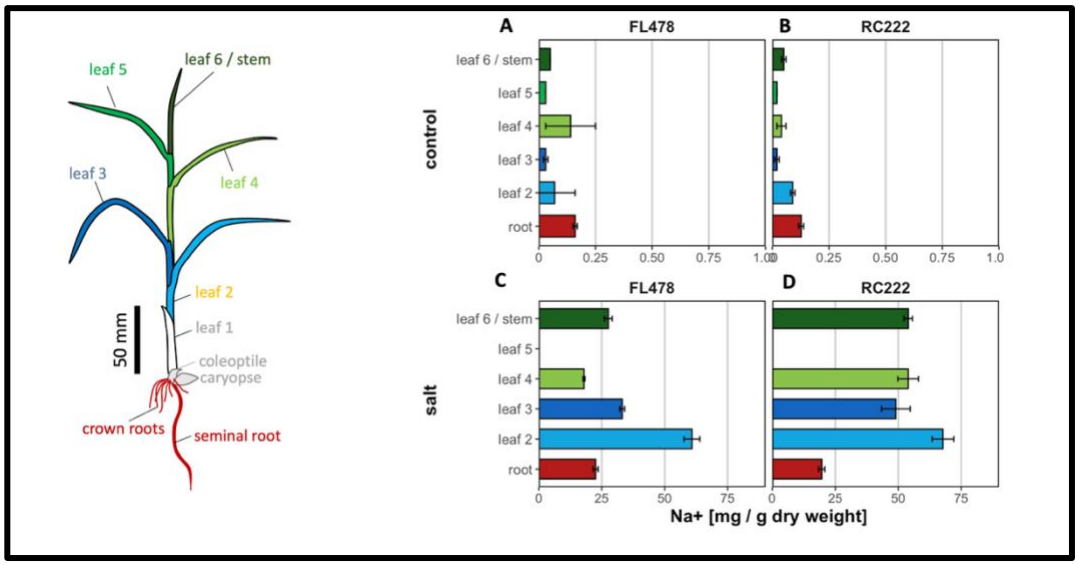


Fig. 22. Organ-specific sodium (Na^+) accumulation in FL478 and RC222 seedlings. (A–B) Na^+ concentrations in individual organs of FL478 (A) and RC222 (B) seedlings under control conditions after 12 days of hydroponic growth. (C–D) Na^+ concentrations in individual organs of FL478 (C) and RC222 (D) seedlings after exposure to 100 mM NaCl. Sodium content was measured in roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and the leaf 6th/stem fraction. Values represent Na^+ concentration expressed as mg g^{-1} dry weight. Bars represent mean values and error bars indicate ± SE.

3.2.6.2 Potassium Accumulation Is Differentially Affected by Genotype and Salinity

As we observed genotypic differences in Na^+ accumulation in Figure 16, we measured the K^+ content to determine the balance between these ions (Suppl. Fig. 5A–D). FL478 and RC222 showed comparable K^+ levels across most tissues(35–60 mg g^{-1} dry weight across organs). However, higher K^+ concentrations were detected in younger shoot organs than in roots in both genotypes under control conditions (leaf 6th/stem ~55–60 mg g^{-1}, leaf 4th ~45–50 mg g^{-1}, leaf 3rd ~38–40 mg g^{-1}, whereas roots contained ~35–45 mg g^{-1} dry weight) (Suppl. Fig. 5A–B). With salinity exposure, K^+ content decreased in both genotypes. The plummeting concentration of potassium was pronounced in the shoot tissues, including leaf 3rd, leaf 4th, and the leaf 6th/stem fraction (decreasing to ~15–25 mg g^{-1} dry weight depending on the organ). The greatest relative decline was observed in the youngest shoot tissues, where K^+ concentrations decreased by approximately three- to five-fold compared with control conditions (leaf 6th/stem decreased from ~55–60 mg g^{-1} to ~18–22 mg g^{-1} dry weight). Under saline conditions, RC222 generally displayed lower K^+ levels than FL478 in several aerial organs, most notably in leaves 3rd and 4th (RC222 leaf 3rd ~35 mg g^{-1} and leaf 4th ~28 mg g^{-1} compared with ~22–25 mg g^{-1} and ~20–22 mg g^{-1} in FL478 under salt treatment) (Suppl. Fig. 5C–D). These results show that salinity-induced potassium depletion is more pronounced in RC222, showing a reduced capacity to maintain K^+ homeostasis during salt stress.

3.2.6.3 K^+/Na^+ Ratio Reveals Organ-Specific Ionic Balance

The molar K^+/Na^+ ratio was calculated for each organ to integrate changes in Na^+ and K^+ accumulation and determine the cumulative effect of these ions on ionic balance (Fig. 23A–D). High K^+/Na^+ ratios across all tissues were recorded for both genotypes under control conditions (~150–700 depending on the organ) (Fig. 23A–B). This was reflected across all tissues in low Na^+ concentrations and adequate potassium availability. Ionic homeostasis was largely preserved despite minor genotypic differences. Severe disruption of ionic homeostasis caused by Salt stress, as evidenced by a marked reduction in the K^+/Na^+ ratio in all organs of both

genotypes (~0.15–0.90 depending on the tissue) (Fig. 23C–D). However, clear organ-specific and genotype-dependent differences were evident. In FL478, higher K^+/Na^+ ratios were recorded in the shoot tissues, specifically leaf 2nd (older tissue) (~0.18) and leaf 5th (younger tissue) (~0.40), than in RC222 (leaf 2nd ~0.38 and leaf 5 ~0.88). However, the more pronounced depletion of the K^+/Na^+ ratio was recorded in leaves 3rd and 4th of RC222 (leaf 3rd ~0.50 and leaf 4th ~0.38 compared with ~0.88 and ~0.55 in FL478), reflecting the combined effects of enhanced Na^+ accumulation and stronger K^+ depletion. Root tissues showed the smallest relative differences in K^+/Na^+ ratios between genotypes ~0.20 in FL478 and ~0.18 in RC222) (Fig. 23C–D), consistent with the comparable Na^+ accumulation observed in roots under salt stress.

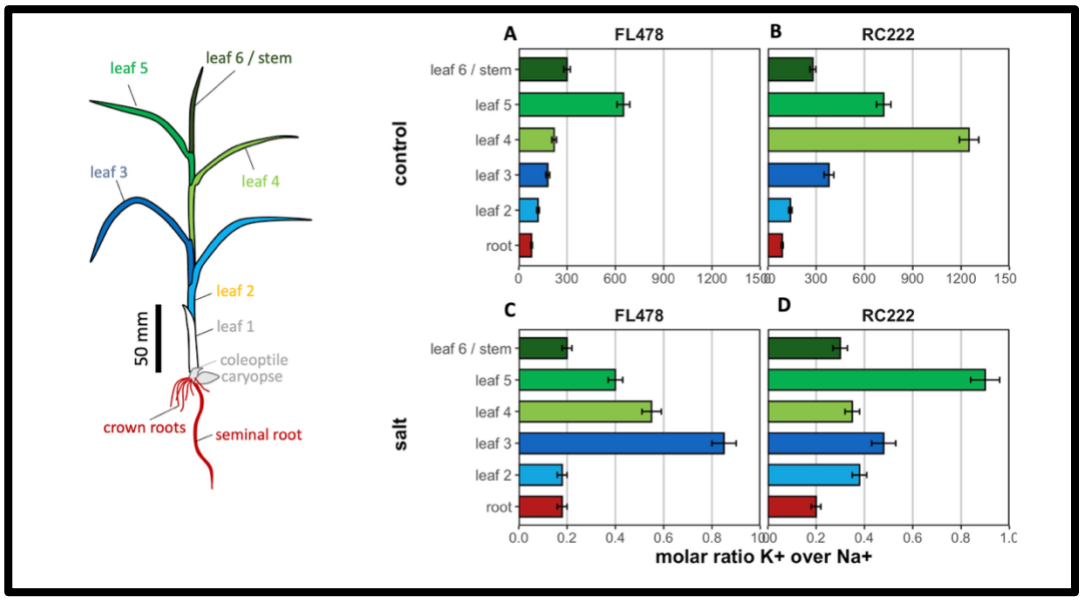


Fig. 23. Organ-specific molar K^+/Na^+ ratios in FL478 and RC222 seedlings under control and salt stress conditions.(A–B) K^+/Na^+ ratios in individual organs of FL478 (A) and RC222 (B) seedlings grown under control conditions.(C–D) K^+/Na^+ ratios in individual organs of FL478 (C) and RC222 (D) seedlings after exposure to 100 mM NaCl. Ratios were calculated from measured Na^+ and K^+ concentrations in roots, leaf 2nd , leaf 3rd , leaf 4th , leaf 5th , and leaf 6th /stem fraction after 12 days of hydroponic growth. Bars represent mean values ± SE.

3.2.6.4 Integration of Ion Partitioning Patterns

We observed that FL478 showed less Na^+ accumulation in aerial tissues with an effective restrictive strategy. Higher K^+ levels were recorded in FL478, resulting in more favorable K^+/Na^+ ratios in several organs. In contrast, RC222 showed reduced ionic homeostasis, with enhanced Na^+ accumulation and greater potassium depletion under saline conditions. Different ion partitioning of sodium and potassium across all organs of both genotypes was recorded. The different ion-orchestrating strategies of the two genotypes likely contributed to the contrasting

salt tolerance phenotypes observed between them. This points out how vital organ-specific ion regulation is in determining overall salinity responses.

3.2.7 Salinity-Dependent Regulation of JAZ Genes in the Second Leaf

To determine whether jasmonate signalling differs between the salt-tolerant genotype FL478 and the salt-sensitive genotype RC222, transcript levels of the jasmonate-responsive genes JAZ9 and JAZ13 were analysed in the second leaf under control conditions and during early and late phases of salinity stress.

3.2.7.1 JAZ9 Is Strongly Induced by Salinity in FL478 but Only Weakly in RC222

We observed that JAZ9 expression was extremely low in both genotypes across the time course under control conditions (Fig. 24A-B). The transcript level of JAZ9 remained low in the absence of salt exposure. However, small fluctuations were observed under control conditions. A clear genotype-dependent difference was recorded under saline conditions. In FL478, transcript levels of JAZ9 increased rapidly and reached a distinct peak at the early time point (around 6 h). After the initial induction, JAZ9 transcript levels gradually declined but remained measurable at later time points. In contrast, transcript levels in RC222 remained low throughout the entire time course and were substantially lower than those observed in FL478. After salt application, RC222 showed a weak and transient increase in JAZ9 expression. Expression analysis showed that salinity triggers a rapid, transient activation of JAZ9 in the tolerant genotype, whereas the sensitive genotype shows only limited transcriptional responsiveness.

3.2.7.2 JAZ13 Exhibits a Strong and Transient Induction in FL478 under Salinity

JAZ13 gene showed the same trend as JAZ9, observed in both genotypes under control conditions (Fig. 24C-D). The expression analysis revealed that neither JAZ genes were not strongly expressed in the absence of stress. A striking induction of JAZ13 was recorded in FL478 in saline conditions. Expression of JAZ13 increased dramatically at early time points and reached a pronounced peak around 6 h after salt exposure. Thereafter, transcript abundance declined again but remained above basal levels at later stages. In contrast, the salt-sensitive genotype RC222 showed only a minor and transient increase in JAZ13 transcript levels. Even at the peak time point, expression remained far below the levels observed in FL478. Together, these results demonstrate that salinity induces a strong and early activation of JAZ signalling in the tolerant genotype, whereas this response is markedly attenuated in the sensitive genotype.

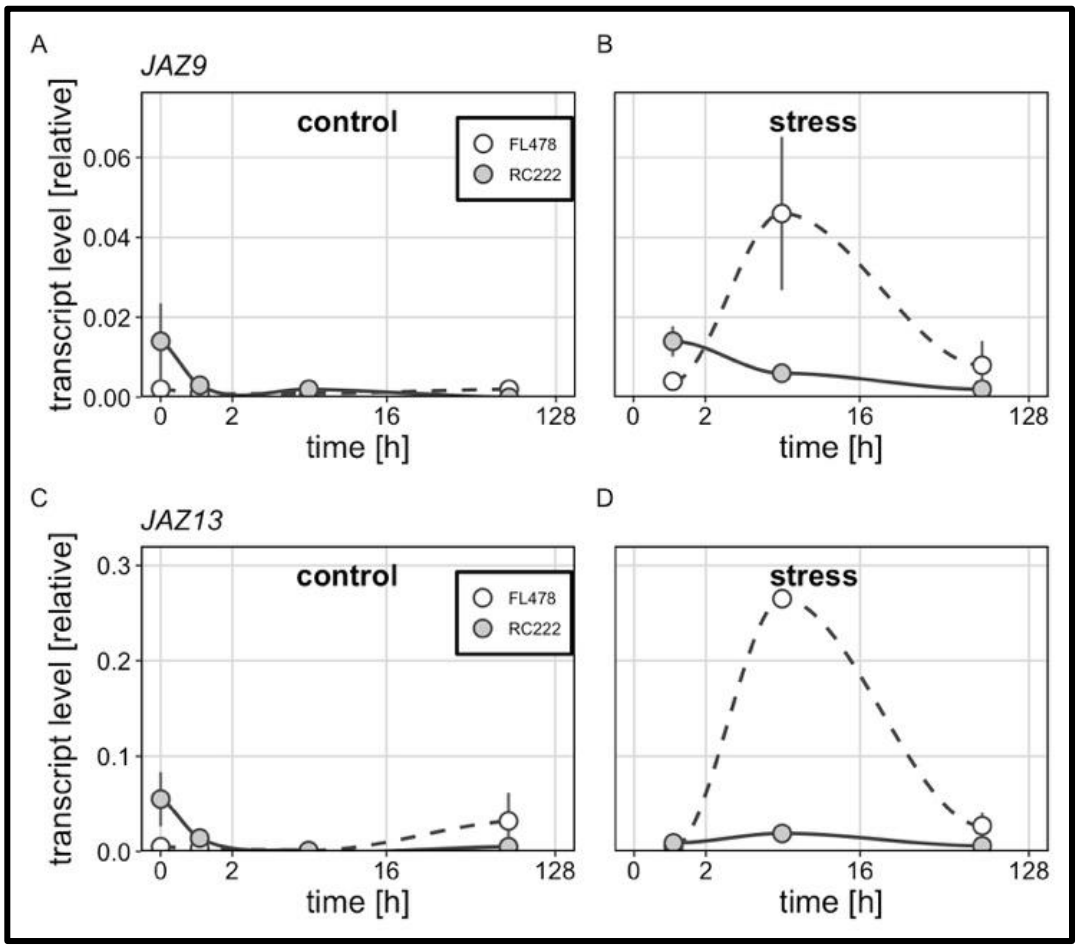


Fig. 24. Time-course expression of JAZ9 and JAZ13 in the second leaf of FL478 and RC222 under control conditions and after salt treatment (100 mM NaCl). Data represent mean ± SE of three biological and three technical replicates.

3.2.8 Salinity-Dependent Regulation of Ion Transporter Genes in the Second Leaf

To investigate whether genotype-dependent salt tolerance is associated with differential regulation of ion transport processes, transcript levels of the potassium transporter HAK4 and the vacuolar sodium/proton antiporter NHX1 were analysed in the second leaf under control conditions and during salinity stress.

3.2.8.1 HAK4 Shows a Moderate and Transient Induction in FL478 under Salinity

The HAK4 transcript levels were low in both genotypes across the time course in control conditions (Fig. 25A). Elevated basal expression of HAK4 was observed in RC222 compared

to FL478 at early time points. However, at later time points, HAK4 expression declined rapidly in both genotypes. A transcriptomic difference in HAK4 at the genotype-dependent level was observed after salt exposure. In the salt-tolerant genotype FL478, HAK4 transcript levels increased transiently and reached a clear peak during the early phase of salt stress (around 6 h). After this early induction, transcript levels gradually declined again at later time points (Fig. 25B). In contrast, RC222 showed only minor changes in HAK4 expression during the stress treatment. Transcript levels remained low and relatively constant throughout the time course. These results show that HAK4 is transiently activated by salinity in the tolerant genotype, whereas the sensitive genotype exhibits only weak transcriptional responsiveness.

3.2.8.2 NHX1 Is Strongly and Rapidly Induced in FL478 but Remains Weakly Expressed in RC222

At early time points, the vacuolar transporter NHX1 was shown to have a detectable transcript level. The transcript level declined over the time course. At later stages, very low levels were recorded in both genotypes without salt (Figure. 25C). As previously reported for other genes, salinity triggered a striking genotype-specific response for transporter genes as well. In response to salt exposure, transcript levels of NHX1 increased strongly and showed a distinct peak at the early time point (around 6 h) in FL478, indicating rapid activation of vacuolar sodium sequestration mechanisms. At later time points, transcript abundance declined again after reaching a peak. In contrast, RC222 showed only weak, nearly constant NHX1 expression during salt treatment, with no pronounced induction observed over the time course (Fig. 25D). Together, these results show that salinity induces an early activation of ion sequestration and potassium transport processes in the tolerant genotype, whereas this response is largely absent in the sensitive genotype.

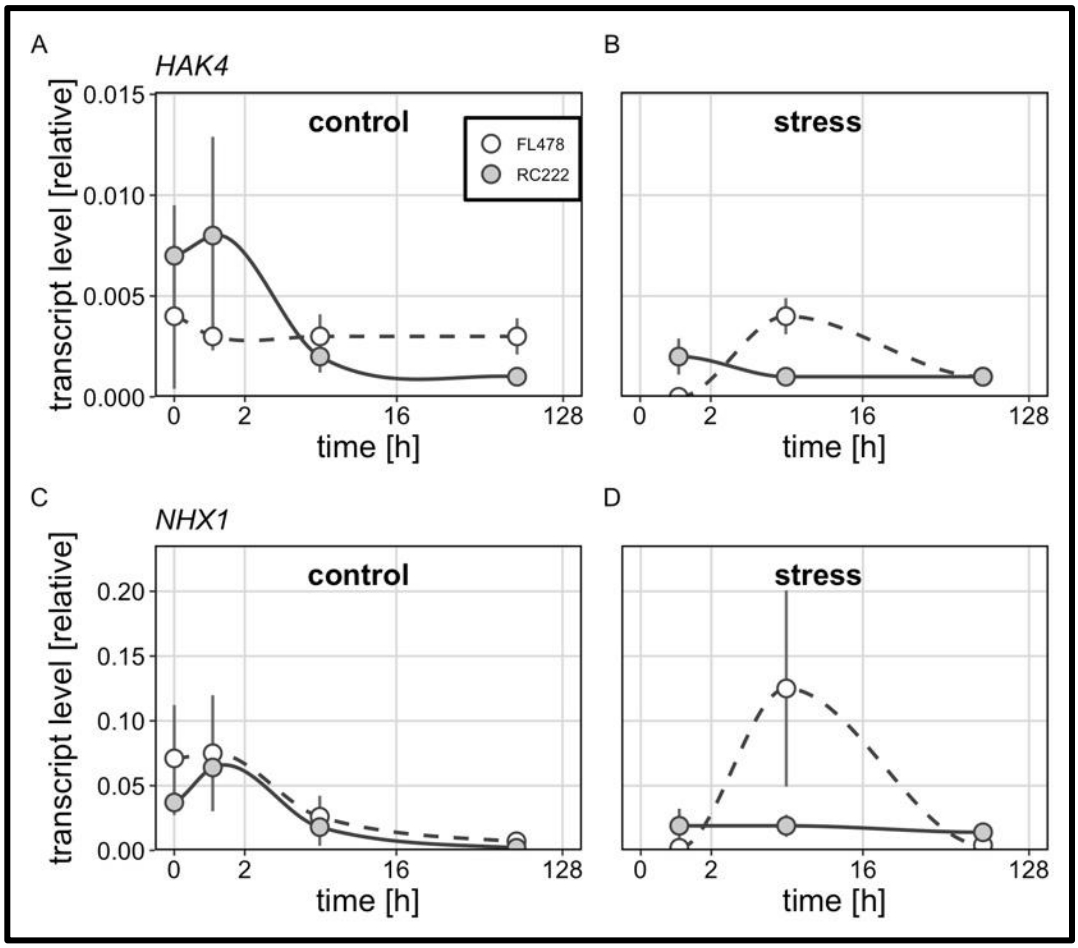


Fig. 25. Time-course expression of HAK4 and NHX1 in the second leaf of FL478 and RC222 under control conditions and after salt treatment (100 mM NaCl). Data represent mean ± SE of three biological and three technical replicates.

3.2.8.3 FE1 Expression Shows Transient Induction under Control Conditions but Only Minor Changes during Salinity

The next transcriptomic analysis was performed to understand the transcriptional responses of genes involved in ion homeostasis and stress adaptation. We analysed FE1 gene transcript levels in the 2nd leaf (older leaves) under control and salinity conditions. In both genotypes, FE1 transcript levels were very low at the beginning of the time course under control conditions (Fig. 26). At the intermediate time point (around 6 h), a clear peak was observed in FL478, followed by a decline towards later stages. In contrast, RC222 maintained consistently low FE1 transcript levels throughout the control time course, with no pronounced induction observed (Fig. 26A). FE1 expression remained low in both genotypes. Only minor changes in transcript abundance were detected over time, and no strong genotype-dependent induction was observed under salinity conditions (Fig. 26B). FE1 expression is transiently activated in the tolerant

genotype under non-stress conditions, whereas salinity does not strongly induce FE1 transcription in either genotype.

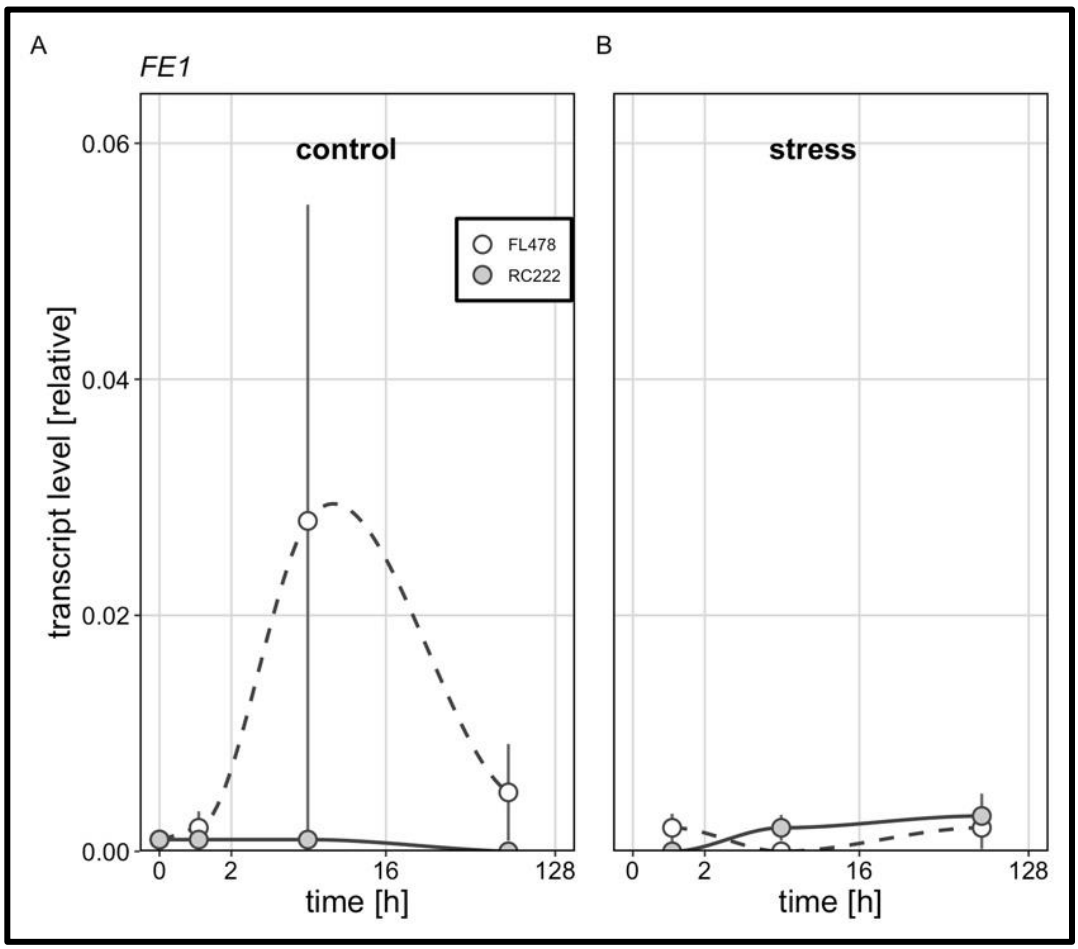


Fig. 26. Time-course expression of FE1 in the second leaf of FL478 and RC222 under control conditions and after salt treatment (100 mM NaCl). Data represent mean ± SE of three biological and three technical replicates

3.2.9 Salinity-Dependent Regulation of JAZ Genes in Roots

To determine whether jasmonate signalling responses differ between shoot and root tissues, transcript levels of JAZ9 and JAZ13 were analysed in roots of FL478 and RC222 under control conditions and following salinity stress (Fig. 27).

3.2.9.1 Root JAZ9 Expression Shows Moderate and Genotype-Specific Changes during Salinity

Expression of the JAZ9 gene was observed in both genotypes (Fig. 27). The clear difference between early and later time points indicates that the level was higher at the beginning and decreased thereafter. At later time points, transcript levels gradually increased again,

particularly in RC222, resulting in slightly higher expression in the sensitive genotype at the end of the time course (Fig. 27A). Following salt exposure, clear genotype-specific differences became apparent. During the early phase of stress, JAZ9 transcript levels in RC222 were already elevated at the early time point and remained higher than those of FL478. In contrast, FL478 showed only a modest, delayed increase in JAZ9 transcript levels in response to salinity(Fig. 27B). At later stages of stress, transcript levels in both genotypes converged to similarly low values. These results indicate that, in contrast to leaves, JAZ9 expression in roots shows only moderate and genotype-specific regulation under salinity stress.

3.2.9.2 JAZ13 Is Preferentially Induced in RC222 Roots during Early Salinity Response

The expression pattern of JAZ13 was analysed to extend these observations (Fig. 27). In both genotypes, the transcript level of JAZ13 was very low throughout the time course. However, at later stages, slight increases were detected (Fig. 27C). After salt exposure, a genotype-dependent expression pattern was recorded. A clear transient induction of JAZ13 was exhibited by RC222. At an early time point, a transcript peak was observed around 6 h, followed by a decline at later time points under saline conditions. On the other hand, during the entire stress treatment, only minimal changes in JAZ13 transcript abundance were recorded in FL478 (Fig. 27D). Transcript analysis indicated that JAZ gene regulation is tissue-specific. Strong salinity-induced activation in leaves of the tolerant genotype contrasts with a preferential induction in roots of the sensitive genotype.

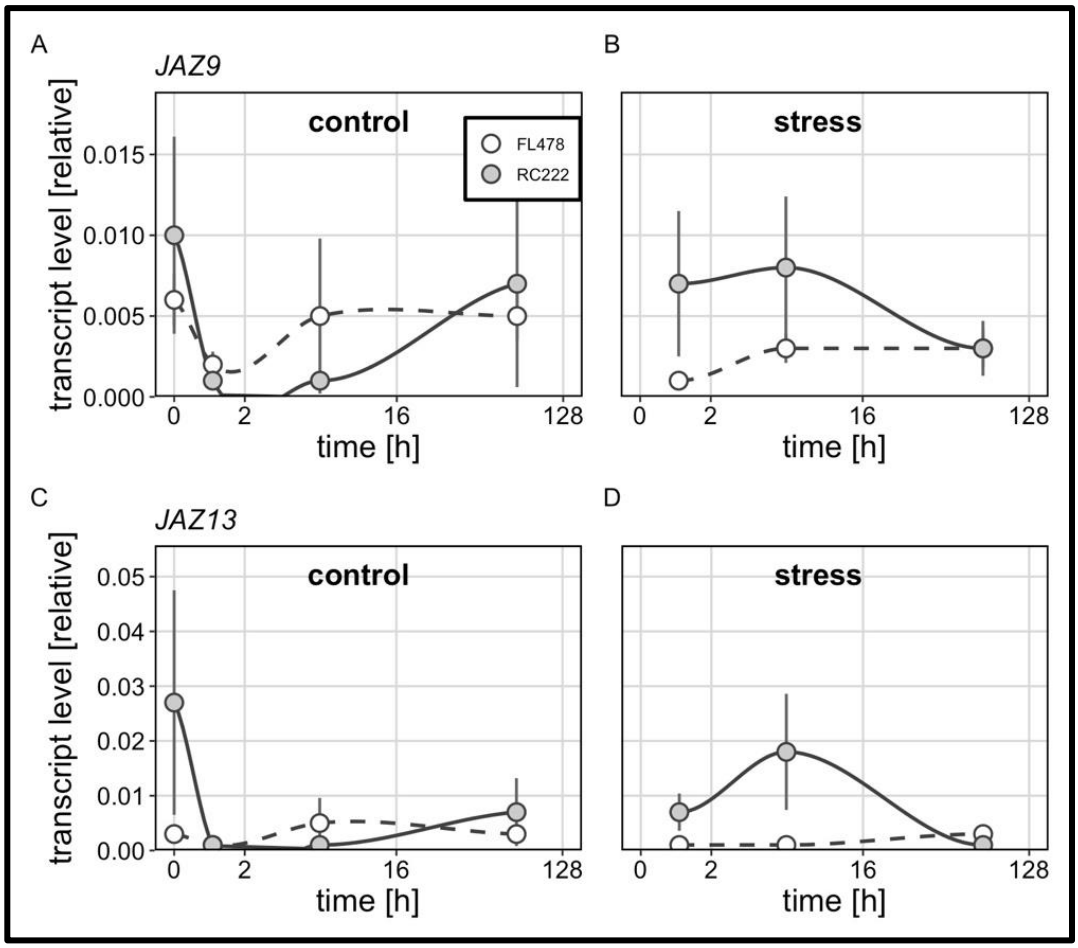


Fig. 27**.** Time-course expression of JAZ9 (A–B) and JAZ13 (C–D) in roots of FL478 and RC222 under control (left) and salt stress (right) conditions. Under control conditions, both genes showed generally low transcript levels with minor late increases. Under salinity, genotype-specific responses were observed: RC222 displayed higher early JAZ9 expression and a clear transient induction of JAZ13 (peak around 6 h), whereas FL478 showed only weak and delayed changes. Overall, JAZ gene expression in roots shows moderate, genotype-dependent regulation under salt stress.

3.2.9.3 Root HAK4 Expression Is Transiently Induced by Salinity in Both Genotypes

Transcript levels of HAK4 were analysed in FL478 and RC222 to check potassium transport processes in roots in response to salinity (Fig. 28). HAK4 transcript levels were low in both genotypes and showed only moderate fluctuations over time under control conditions. FL478 maintained relatively stable transcript levels, whereas RC222 showed a slight increase at early time points and a gradual increase at later time points (Fig. 28A). Both genotypes showed a clear transient induction of HAK4 expression after salt exposure. During the early phase of stress, transcript levels increased at approximately 6 h in both genotypes, reaching peak values before declining at later time points. The amplitude of induction was slightly higher in FL478

than in RC222 (Fig. 28B). These results indicate that salinity triggers a rapid activation of potassium transport processes in the roots of both genotypes.

3.2.9.4 NHX1 Expression in Roots

Vacuolar sodium sequestration in roots contributes to genotype-specific salt tolerance. We analysed NHX1 transcript levels to determine its role (Fig. 28). The transcript of the transporter gene was measurable at early time points but declined rapidly thereafter in both genotypes. In FL478, a transient increase was observed at intermediate time points. In contrast, RC222 showed only minor changes throughout the time course (Fig. 28C). NHX1 transcript levels remained relatively stable in both genotypes under saline conditions (Fig. 28D). During the entire stress treatment, no strong induction was observed, aside from small fluctuations. These results show that, in contrast to leaves, transcriptional regulation of NHX1 in roots is limited and does not show pronounced salinity-induced activation.

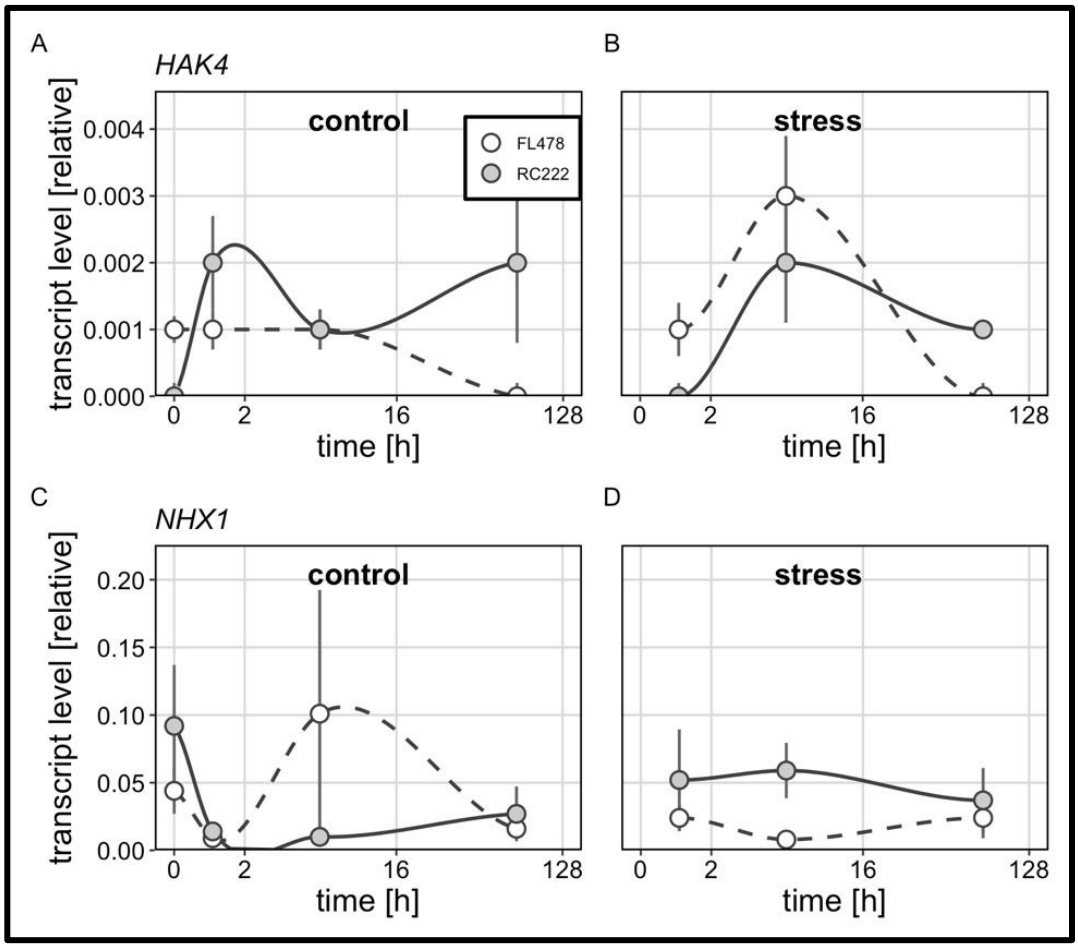


Fig. 28. Time-course expression of potassium transporter genes HAK4 and NHX1 in FL478 and RC222 under control and salt stress**.** Relative transcript levels were measured at 0, 2, 6, and 72 h in the salt-tolerant genotype FL478 (solid line) and the salt-sensitive genotype RC222 (dashed line). (A,B) HAK4 expression under control (A) and salt stress (B). Salt treatment induced a transient early peak, particularly pronounced in RC222, followed by a decline at later time points. (C, D) NHX1 expression under control (C) and salt stress (D). Under salinity, FL478 maintained higher NHX1 transcript levels compared with RC222, indicating stronger activation of ion sequestration mechanisms in the tolerant genotype. Values represent mean ± SE.

4. Discussion

Jasmonates are lipid-derived phytohormones that regulate plant growth, development, and defence responses against a wide range of environmental stresses. While their role in plant immunity is well established, increasing evidence indicates that jasmonate signalling also participates in abiotic stress responses, including salinity (Creelman and Mullet, 1997; Wasternack, 2007; Browse, 2009; Wasternack and Hause, 2013; Kazan and Manners, 2012). Among the important staple crops, rice is one of the most sensitive to salinity stress. This has a strong effect on early seedling development, when osmotic stress and sodium toxicity severely restrict growth and productivity (Munns and Tester, 2008; Horie *et al*., 2012; Reddy *et al*., 2017). Understanding the hormonal and molecular mechanisms controlling salt tolerance in rice is therefore of major agronomic importance (Roy *et al*., 2014; Munns and Gilliham, 2015; Zhu, 2016). Recent studies have revealed a complex and sometimes contradictory role of jasmonates during salt stress (Kurotani *et al*., 2015; Hazman *et al*., 2015; Peethambaran *et al.*, 2018; Ndecky *et al*., 2023; Valenzuela *et al*., 2016; Li *et al*., 2024). Jasmonate accumulation is rapidly induced after salt exposure. It contributes to hormonal reprogramming and stress signalling (Moons *et al*., 1997; Song *et al*., 2021). Several studies suggest that excessive jasmonate signalling may negatively affect salt tolerance in rice seedlings. Jasmonate-deficient allene oxide cyclase (*aoc*) mutants display improved salt tolerance, reduced sodium accumulation in leaves, and enhanced ROS-scavenging capacity (Hazman *et al.,* 2015). Similarly, attenuation of jasmonate signalling using enhanced JAZ activity has been associated with improved salt tolerance (Peethambaran *et al*., 2018). More recently, transcriptomic and physiological analyses have shown that jasmonate signalling regulates multiple components of the salt stress response (Ndecky *et al*., 2023). This includes processes related to ion transport, oxidative stress responses, and senescence. Taken together, the results indicate that the timing, intensity, and spatial regulation of jasmonate signalling, rather than its simple presence or absence, determine plant performance under salt-induced stress (Chini *et al.,* 2007; Hazman *et al.,* 2015; Peethambaran *et al.,* 2018; Ndecky *et al.,* 2023)**.** To further clarify the role of jasmonate signalling in rice salt stress adaptation, the present study combined two complementary experimental systems: the jasmonate-deficient *aoc* mutant in the Kitaake background and the comparison of the contrasting rice genotypes FL478 (salt tolerant) and RC222 (salt sensitive) **(**Ndecky *et al.,* 2023; Ren *et al*., 2005)**.** Through integrating analyses of growth performance, gas exchange, ion partitioning, hormone dynamics, along with stress-responsive gene expression, this work aimed to dissect the contribution of jasmonate signalling to salt tolerance.

Importantly, the convergence of physiological, hormonal, and transcriptional responses across these independent systems provides strong evidence that modulation of jasmonate signalling represents a central regulatory component of salt stress adaptation in rice seedlings.

4.1 Improved salt tolerance in the jasmonate-deficient *aoc* mutant and the tolerant genotype FL478

The first major outcome of this study is the observation that attenuation of jasmonate signalling is consistently associated with improved salinity tolerance in rice seedlings **(**Ndecky *et al*., 2023). Unexpectedly, the jasmonate-deficient *aoc* mutant displayed a less salt-sensitive phenotype than its wild-type background Kitaake. Visual assessment during the early phase of stress already revealed reduced necrotic areas in the second and third leaves of *aoc* mutant compared with the wild type after 72 h of 100 mM NaCl exposure, indicating that JA deficiency is associated with an early reduction of leaf injury symptoms (Ueda and Kato, 1980; He *et al*., 2002; Hazman *et al*., 2015; Lim *et al*., 2020; Kim *et al*., 2023) (Chapter 3.1; Fig. 8). After 12 days of salt exposure, Kitaake showed strong growth inhibition and chlorosis, whereas *aoc* mutant maintained greener leaves and better growth (Chapter 3.1; Fig. 9). Quantitative measurements confirmed this visual observation, as shoot length in the aoc mutant remained approximately 20% higher than in Kitaake under both control and saline conditions (Chapter 3; Fig. 3C–E; Wasternack and Hause, 2013**).** This result is in line with the concept that rice seedlings, as a salt-sensitive glycophyte, are strongly impaired by salt stress at the level of growth and leaf integrity (Munns and Tester, 2008; Horie *et al*., 2012) and supports previous evidence that impaired JA biosynthesis can improve salt tolerance rather than weaken it (Hazman *et al*., 2015). A similar contrast was observed in the natural genotype system, where FL478 showed less chlorosis and necrosis than RC222 after 72 h of salt exposure (Chapter 3.2; Fig. 17). After 12 days of salt stress, RC222 showed stronger growth inhibition and severe leaf damage, whereas FL478 maintained greener leaves and better growth (Chapter 3.2; Fig. 18). Longer shoots were observed for the FL478 compared to the RC222 under control conditions. which exhibits genotype-specific differences in basal growth potential (Chapter 3.2; Fig. 18C). This constitutive variation suggests that tolerance involves pre-adapted physiological features in addition to stress-responsive mechanisms (Li *et al*., 2017). The overall conclusion from the two experimental systems is that lessened sensitivity to salinity is associated with attenuated jasmonate signalling (Hazman *et al*., 2015; Ndecky *et al*., 2023)**.** The *aoc* mutant provides genetic evidence that reduced JA biosynthesis decreases leaf injury and preserves growth under salinity, while FL478 naturally maintains better leaf integrity than RC222 (Chapter 3.2; Fig.

18). This is consistent with previous reports showing that JA-deficient mutants display greater salt tolerance and reduced damage symptoms, including lower sodium accumulation in leaves (Hazman *et al*., 2015). Therefore, the present phenotypic data strongly suggest that jasmonate signalling contributes to the development of salt-induced injury in rice seedlings and that attenuation of this pathway is associated with improved tolerance. Importantly, this phenotypic foundation provides the basis for the mechanistic analyses discussed in the following sections.

4.2 Organ-specific biomass allocation under salinity reveals distinct growth strategies

The phenotypic differences described above raised the question of whether improved tolerance is reflected in organ-specific growth responses and biomass allocation structures. Fresh and dry biomass of individual organs were analysed under control and salt stress conditions. This analysis showed clear genotype-dependent differences in biomass allocation schemes that provide important insight concerning the mechanisms underlying salt tolerance. Kitaake showed a strong reduction in shoot and root biomass under salinity, whereas the *aoc* mutant maintained higher fresh and dry weight across most organs (Chapter 3.1; Fig. 10; Supplementary Fig. S1). The comparison of fresh weight (FW) and dry weight (DW) revealed organ-specific differences in the nature of growth responses (Poorter *et al*., 2012; Xu *et al*., 2024). In several shoot organs, increases in fresh weight were proportionally larger than increases in dry weight. This indicates that part of the growth advantage of *aoc mutant* may result from improved tissue expansion and water retention rather than increased structural biomass accumulation (Munns and Tester, 2008; Zhu, 2016). In contrast, root fresh and dry weight showed more parallel changes. Notably, this effect was particularly pronounced under salinity, where root fresh biomass in the *aoc* mutant reached approximately 70–90% higher values than in Kitaake (Chapter 3.1; Fig. 10). This suggests that the root growth advantage of *aoc* mutant reflects genuine biomass production rather than increased water content (Uga *et al*., 2013; Horie *et al*., 2012; Ismail and Horie, 2017). Shoots and roots respond differently to jasmonate deficiency under saline conditions, as demonstrated by the organ-specific differences (Hazman *et al*., 2015; Han *et al*., 2023; Wu *et al*., 2025; Ndecky *et al*., 2023). The natural genotype systems exhibited a comparable pattern. Under salt-induced stress, RC222 displayed pronounced reductions in fresh and dry biomass across most organs, whereas FL478 maintained higher biomass accumulation (Chapter 3.2; Fig. 19; Supplementary Fig. 4). As in the mutant system, the difference between fresh and dry weight responses indicated that tolerant plants maintained both structural growth and tissue hydration during salinity (Munns and Tester, 2008;

Xu *et al*., 2024). Maintenance of biomass during salt stress is a key component of tolerance because growth reduction is one of the earliest consequences of osmotic stress (Munns and Tester, 2008; Roy *et al*., 2014). The observation that tolerant plants maintained biomass under prolonged salt exposure suggests reduced cellular damage and improved physiological stability during stress (Zhu, 2016; Hasanuzzaman *et al.,* 2020). The parallel biomass patterns detected in the *aoc mutant* and FL478 support the hypothesis that attenuation of jasmonate signalling aids in improved growth maintenance under salinity (Hazman *et al*., 2015; Peethambaran *et al.*, 2018; Ndecky *et al.,* 2023). Together, the biomass data extend the phenotypic observations and demonstrate that the improved visual appearance of tolerant plants is supported by sustained biomass accumulation at the organ level. These results deliver an important link between hormone signalling and whole-plant growth responses during salt stress. Importantly, the maintenance of biomass suggests improved biological stability and energy supply. This prompted further analysis of photosynthetic performance in the following section.

4.3 Maintenance of photosynthetic performance in the jasmonate-deficient *aoc* mutant

To better understand the physiological basis of the growth differences between Kitaake and the *aoc* mutant, photosynthetic performance and gas-exchange parameters were analysed during salt stress. Under control conditions, Kitaake and the *aoc* mutant showed comparable photosynthetic performance, although small differences in maximum assimilation rates were detectable (Chapter 3.1; Fig. 11A). Salinity impacted Kitaake and the *aoc* mutant differently and revealed clear genotype-dependent differences. CO_2 assimilation and stomatal conductance declined strongly in Kitaake, whereas the *aoc* mutant maintained substantially higher photosynthetic activity under saline conditions (Chapter 3.1; Fig. 11A, C). This result implies that jasmonate deficiency supports the maintenance of photosynthesis during salt stress (Peethambaran *et al*., 2018; Ndecky *et al.,* 2023). The reduction in photosynthesis in Kitaake agrees with the well-known sensitivity of rice photosynthesis to salinity (Munns and Tester, 2008; Zeng *et al*., 2003; Nounjan *et al*., 2018). Salt stress can restrict photosynthesis through both stomatal and non-stomatal limitations. It may result from reduced stomatal aperture, impaired electron conduction, and disrupted enzymatic processes (Flexas *et al*., 2004; Munns and Tester, 2008). Reduced carbon assimilation in Kitaake was observed under ionic stress, indicating both stomatal limitation and metabolic impairment**.** At the same time, the *aoc mutant* maintained higher gas-exchange rates under salinity (Chapter 3.1; Fig. 11A). This suggests improved physiological steadiness in the mutant background. Importantly, photochemical

efficiency of Photosystem II (Fv/Fm) remained similar between genotypes under both control and saline conditions, indicating that improved tolerance in the aoc mutant is primarily associated with stomatal and gas-exchange regulation rather than protection of PSII photochemistry (Chapter 3.1; Fig. 11D) Salt stress needs sustained photosynthesis because ion transport, osmotic adjustment, and restoration processes demand large amounts of energy (Jacoby *et al*., 2011; Munns and Tester, 2008). The higher photosynthetic performance of the *aoc* mutant therefore provides a physiological explanation for its capacity to maintain growth and biomass under salinity (Ambavaram *et al*., 2014). Importantly, the maintenance of gas exchange in the *aoc* mutant occurred despite the reduced shoot sodium accumulation described in the following section. This suggests that improved salt tolerance in the jasmonate-deficient background cannot be explained simply by reduced transpiration (Chapter 3.1; Fig. 11C). Instead, improved regulation of ion transport and cellular stress responses is likely involved. Jasmonates interact with ABA signalling and influence stomatal behaviour (Kazan and Manners, 2012), suggesting that altered hormonal regulation contributes to the improved photosynthetic performance of the *aoc* mutant. Together, the gas-exchange data show that jasmonate deficiency enables rice seedlings to maintain photosynthetic activity during salt stress. This intrinsic physiological advantage likely contributes to the improved development and decreased tissue damage observed in the *aoc* mutant.

4.4 Sodium and potassium partitioning reveal improved ion homeostasis in tolerant plants

To identify mechanisms underlying salt tolerance, it was important to determine the distribution of salt ions under saline conditions. After analysing the growth and physiological responses, we recorded the distribution of sodium and potassium between roots and shoots. Salt ion distribution in the different plant tissues is a key determinant of plant performance under salinity (Tester and Davenport, 2003; Flowers and Colmer, 2008; Kobayashi *et al*., 2017). The increased accumulation of salt ions in photosynthetic tissues, such as leaves, disrupts photosynthesis and accelerates tissue damage (Lutts *et al*., 1996; Dionisio-Sese and Tobita, 2000; Munns *et al.*, 2006; Deng *et al.,* 2025). In the jasmonate-deficient system, sodium concentrations in roots were comparable between Kitaake and the *aoc* mutant, whereas clear differences were observed in shoot tissues. During salt stress, Kitaake accumulated substantially higher Na^+ levels in leaves, while the *aoc mutant* maintained significantly lower sodium concentrations in shoot tissues (Chapter 3; Fig. 12). This observation indicates that the reduced salt sensitivity of *aoc* mutant is not caused by reduced sodium uptake at the root surface but rather by altered long-distance transport of sodium from roots to shoots (Hazman *et al*.,

2015; Horie et al., 2012; Kobayashi *et al*., 2017). A comparable pattern was observed in the natural genotype system. Sodium accumulation in roots was similar between FL478 and RC222, whereas RC222 accumulated markedly higher Na^+ levels in leaves compared with the tolerant genotype FL478 (Chapter 3.2; Fig. 22). The parallel behaviour of the two experimental systems strongly suggests that improved salt tolerance is associated with reduced sodium accumulation in photosynthetic tissues. Shoot sodium exclusion is widely recognised as a major determinant of salt tolerance in rice (Ren *et al.,* 2005; Ismail and Horie, 2017). Sodium ions transported to the shoot disrupt chloroplast function (Oi *et al*., 2019; Mitsuya *et al*., 2003). They interfere with potassium-dependent enzymatic processes and accelerate leaf senescence (Shabala and Cuin, 2008; Zhu, 2016). The reduced Na^+ accumulation observed in the *aoc mutant* and FL478 therefore provides a mechanistic explanation for their improved growth and maintenance of photosynthesis (Jiang *et al*., 2024; Cotsaftis *et al*., 2011). In addition to sodium, potassium levels and K^+/Na^+ ratios differed between genotypes. The tolerant backgrounds maintained more favourable K^+/Na^+ ratios in shoot tissues than Kitaake and RC222 (Chapter 3; Supplementary Fig. 2; Chapter 3.2; Fig. 23). Maintenance of potassium homeostasis is essential for enzyme activation, osmotic adjustment, and membrane stability throughout the course of salt stress (Tian *et al*., 2021; Zhou *et al.,* 2022). A high K^+/Na^+ ratio is therefore widely used as a physiological indicator of salt tolerance (Roy *et al*., 2014; Kakar *et al*., 2019). Importantly, the observation that sodium levels were similar in roots but differed in shoots suggests that tolerance is mainly determined by root-to-shoot ion transport rather than root sodium uptake capacity. The convergence of mutant and natural variation systems therefore indicates that attenuation of jasmonate signalling is associated with improved regulation of sodium partitioning during salt stress.

4.5 Hormone dynamics during salt stress: differential jasmonate and ABA regulation in tolerant and sensitive genotypes

Hormonal reprogramming represents a central component of plant adaptation to salinity. To determine whether differences in salt tolerance between FL478 and RC222 are associated with altered hormone dynamics, endogenous levels of jasmonates (JA, JA-Ile, and OPDA) and ABA were analysed during salt stress. We observed differences in both the magnitude and timing of hormone accumulation. The observed differences were genotype-dependent. Ndecky *et al*. (2023) reported time-resolved hormone profiling revealing rapid, organ-specific changes in jasmonate metabolism during salt exposure in rice plants. Strong and sustained accumulation of jasmonic acid (JA) and its bioactive conjugate, JA-Ile, was observed in RC222 after salt

exposure. In contrast, FL478 showed a more moderate and transient induction. JA-Ile levels were markedly higher in RC222 at early time points, indicating stronger activation of jasmonate signalling in the sensitive genotype (Chapter 3.2; Fig. 20A–B). A comparable trend was observed for OPDA, the precursor of JA, which accumulated more strongly in RC222 than in FL478 (Chapter 3.2; Fig. 21F). OPDA is not only a biosynthetic intermediate but also an independent signalling molecule with specific transcriptional effects (Stintzi *et al.,* 2001; Taki *et al*., 2005). The stronger OPDA accumulation in RC222, therefore, points to enhanced activation of the oxylipin pathway in the sensitive genotype (Kurotani *et al*., 2015). Jasmonate biosynthesis is well reported to be induced by salt stress in rice seedlings (Moons *et al*., 1997). At the same time, increasing evidence suggests that excessive or prolonged jasmonate signalling can negatively affect salt tolerance (Hazman *et al*., 2015; Peethambaran *et al.,* 2018). Jasmonate-deficient rice mutants display greater salt tolerance and reduced shoot sodium accumulation (Hazman *et al*., 2015). Ndecky *et al*. (2023) reported a similar study showing that sustained JA signalling enhances stress-associated transcriptional programs, including senescence and oxidative stress pathways, during prolonged salinity exposure. These studies point to the importance of the amplitude and duration of jasmonate accumulation as determinants of stress outcome (Wasternack, 2007; Browse, 2009). Consistent with this interpretation, jasmonate catabolites accumulated earlier and to lower levels in FL478, whereas RC222 showed delayed but much stronger catabolite accumulation at later stages, indicating slower jasmonate turnover in the sensitive genotype (Chapter 3.2; Fig. 20C–E). ABA levels were also differentially regulated under stress conditions (Sah *et al*., 2016; Vishwakarma *et al*., 2017). Salt stress induced ABA accumulation in both genotypes, but RC222 showed stronger and more prolonged ABA accumulation than FL478 (Chapter 3.2; Fig. 21G). Coordinated regulation of JA and ABA pathways during salinity has been demonstrated in rice leaves, illustrating strong hormonal cross-talk during stress (Ndecky *et al*., 2023). This plant hormone plays a vital role in osmotic stress responses and stomatal sealing (Sah *et al*., 2016; Vishwakarma *et al*., 2017). While early ABA accumulation is essential for adaptation, excessive or sustained ABA signalling can restrict growth and photosynthesis (Jiang *et al*., 2024). The stronger ABA accumulation in RC222 may therefore help cause its stronger growth inhibition during salt stress. Simultaneous induction of JA and ABA was observed in RC222, indicating intensified hormonal cross-regulation in the sensitive background. JA and ABA signalling pathways are known to intersect at multiple regulatory nodes, including shared transcription factors and overlapping stress-responsive gene networks (Kazan, 2015; Yu *et al.,* 2020). The more moderate hormonal response observed in FL478 may therefore reflect a better-

balanced stress signalling network that prevents excessive growth inhibition while still allowing responsive adaptations. Although endogenous jasmonate levels were not directly measured in Kitaake and *aoc mutants* in the present study, previous work showed that JA-deficient *aoc* mutant accumulate less JA and JA-Ile during salt stress (Hazman *et al*., 2015). Consistently, Ndecky *et al*. (2023) demonstrated that attenuation of JA signalling modifies ion partitioning and stress-associated gene expression during salinity. The similarity between the moderated jasmonate response in FL478 and the genetically reduced jasmonate signalling in *aoc mutant* further supports the hypothesis that attenuation of jasmonate signalling plays a role in improved salt tolerance. Taken together, the hormone analyses indicate that salt-sensitive genotypes present stronger and more prolonged accumulation of jasmonates and ABA, whereas tolerant plants display a more controlled hormonal response. Precise regulation of jasmonate and ABA signalling, therefore, appears to be a key determinant of salt stress adaptation in rice seedlings (Ndecky *et al*., 2023).

4.6 Early activation of ion transporter genes indicates improved regulation of Na^+ and K^+ homeostasis

To link physiological differences in ion partitioning with molecular mechanisms, it is vital to check transporter gene expression. We analysed the expression of key ion transporter genes under salt stress across all four genotypes. Particular focus was placed on the vacuolar Na^+/H^+ antiporter NHX1, which mediates sequestration of sodium into vacuoles, and the potassium transporter HAK4, which contributes to the maintenance of K^+ homeostasis under salinity (Ren *et al.,* 2005; Tian *et al.,* 2021). Across both experimental systems, salinity triggered genotype dependent differences in the timing of transporter activation. In the tolerant backgrounds FL478 and the jasmonate-deficient *aoc* mutant, NHX1 expression increased rapidly during the early phase of salt exposure. In contrast, the sensitive genotypes RC222 and Kitaake showed weak or delayed induction (Chapter 3.1; Fig. 15A; Chapter 3.2; Fig. 25CD). In Kitaake, a pronounced increase in NHX1 transcripts became evident only at later stages, coinciding with higher sodium accumulation in shoot tissues under saline conditions (Chapter 3; Fig. 9A). The sensitive genotype RC222 displayed a reduced and delayed transcriptional response compared with the tolerant genotype FL478 in saline conditin (Chapter 3.2; Fig. 25D). Early activation of vacuolar sodium sequestration is widely regarded as a critical protective mechanism because it limits cytosolic Na^+ accumulation during the initial phase of salt stress (Horie *et al*., 2012; Ismail and Horie, 2017). In FL478 and the *aoc* mutant, early NHX1 induction was observed. This transcriptional response aligns with their reduced shoot sodium accumulation (Chapter 3; Fig.

6; Chapter 3.2; Fig. 16). On the other hand, the delayed responses in RC222 and Kitaake likely represent secondary, damage-associated transcriptional activation after ionic stress has already developed. A similar pattern was observed for potassium transport. In the natural genotype system, FL478 exhibited a rapid, transient increase in HAK4 expression following salt exposure, whereas RC222 showed only minor, delayed changes (Chapter 3.2; Fig. 25B). In the mutant system, early activation of HAK transporters was again more prominent in *aoc* mutant than in Kitaake (Chapter 3; Fig. 9). These consistent temporal differences across both systems suggest that early mobilisation of potassium transport processes is a defining feature of tolerant genotypes (Zhou *et al.,* 2022). Maintenance of potassium uptake is essential under salinity because Na^+ competes with K^+ for transport and disrupts potassium-dependent enzymatic and metabolic processes (Shabala and Cuin, 2008). The more favourable K^+/Na^+ ratios observed in FL478 (Chapter 3.2; Fig.23) and *aoc* muatnt (Chapter 3; Supplementary Fig. 2) are therefore mechanistically supported by the earlier activation of HAK transporters (Chapter 3; Fig. 9; Chapter 3.2; Fig. 19). In contrast, stronger potassium depletion and lower K^+/Na^+ ratios in RC222 and Kitaake are consistent with delayed transporter responses. Importantly, the convergence between the mutant and natural variation systems strengthens the mechanistic interpretation. In both comparisons, attenuation of jasmonate signalling either genetically in *aoc* mutant or physiologically in FL478 was associated with rapid activation of ion transport genes, reduced shoot sodium accumulation, and improved ionic balance. Conversely, stronger or prolonged jasmonate signalling in Kitaake and RC222 coincided with delayed transporter activation and enhanced sodium accumulation. Previous studies have shown that salt-tolerant rice cultivars activate ion transport mechanisms earlier than sensitive genotypes (Ren *et al*., 2005; Horie *et al*., 2012). Moreover, jasmonate signalling has been implicated in the regulation of ion transporter networks during salinity (Hazman *et al*., 2015; Ndecky *et al*., 2023). The parallel responses observed across Kitaake, *aoc* muatnt and FL478, RC222 therefore suggest a unifying model in which moderated jasmonate signalling permits early and effective activation of sodium sequestration and potassium uptake mechanisms. Importantly, transcriptional responses of ion transporters were considerably weaker and delayed in roots compared with leaves, indicating that genotype dependent regulation of Na^+ and K^+ partitioning is established predominantly at the shoot level (Chapter 3; Supplementary Fig. S3**).** Taken together, the integrated transcriptional and ionic data from all four genotypes indicate that salt tolerance is not determined solely by transporter capacity, but by the timing of transporter activation. Early responses in FL478 and *aoc* mutant appear to prevent the accumulation of toxic ions. In

contrast, delayed activation in RC222 and Kitaake likely reflects compensatory responses after ionic imbalance has already impaired cellular function.

4.7 Tissue-specific regulation of jasmonate signalling revealed by JAZ9 and JAZ13 expression

To further understand how jasmonate signalling is regulated during salt stress, the expression of the jasmonate-responsive repressors JAZ9 and JAZ13 was analysed in leaves and roots across both experimental systems. JAZ proteins act as key negative regulators of jasmonate signalling and form part of a feedback loop that modulates the strength and duration of JA responses (Kazan and Manners, 2012; Wasternack and Hause, 2013). Changes in JAZ transcript abundance therefore provide important insight into the spatial and temporal regulation of jasmonate signalling during salinity. In the jasmonate-deficient mutant system, JAZ regulation was strongly attenuated in the *aoc* mutant compared with Kitaake. Salt stress induced JAZ transcript accumulation in Kitaake, whereas this induction was strongly reduced or largely absent in *aoc* mutant (Chapter 3.1; Fig. 14A–B–C–E). This observation is consistent with the role of JAZ genes as rapidly induced feedback regulators of jasmonate signalling (Chini *et al*., 2007). The weak JAZ induction in *aoc* mutant therefore confirms the strongly diminished jasmonate signalling capacity of the *aoc* mutant and provides molecular evidence that the JA pathway is functionally impaired. Genotype-dependent regulation of JAZ genes was also evident in the natural variation system. In leaf tissues, the tolerant genotype FL478 showed a rapid and transient induction of JAZ9 and JAZ13, whereas the sensitive genotype RC222 displayed weaker or delayed responses (Chapter 3.2; Fig. 24). Rapid induction of JAZ genes is often interpreted as evidence of an efficient feedback loop that restricts prolonged activation of jasmonate responses (Chini *et al*., 2007; Kazan and Manners, 2012). The stronger and more transient induction observed in FL478 therefore suggests more effective attenuation of jasmonate signalling in photosynthetic tissues. In contrast, root tissues showed an opposite tendency. RC222 displayed stronger or more persistent JAZ transcript changes in roots compared with FL478 (Chapter 3.2; Fig. 27). This contrasting pattern indicates that jasmonate signalling is regulated in a tissue-specific manner. It further suggests that the sensitive genotype experiences more prolonged jasmonate activation in roots. Sustained JA signalling has been associated with growth inhibition and altered root development (Kazan, 2015), which may contribute to the reduced stress performance of RC222. The comparison of both experimental systems reveals a striking convergence. In Kitaake, strong JAZ induction reflects active jasmonate signalling. In the *aoc* mutant, JAZ feedback regulation is strongly reduced due to

genetic impairment of JA biosynthesis. In the natural genotype system, FL478 shows rapid and transient JAZ induction, suggesting efficient feedback control of jasmonate signalling. In contrast, RC222 displays weaker leaf induction but more persistent root responses, indicating prolonged or spatially imbalanced JA signalling. These findings agree with previous studies demonstrating that members of the JAZ gene family are rapidly induced by salinity and play important roles in coordinating stress responses (Toda *et al*., 2013; Peethambaran *et al*., 2018). Overexpression of JAZ genes has been shown to improve salt tolerance in several species, supporting the idea that controlled repression of jasmonate signalling is beneficial during prolonged abiotic stress (Wu *et al*., 2015). Taken together, the results demonstrate that efficient and tissue-specific feedback regulation of jasmonate signalling is closely associated with salt tolerance. Rapid attenuation of JA signalling in leaves, combined with balanced regulation in roots, appears to support improved maintenance of growth and physiological performance during salt stress.

4.8 Reduced oxidative stress is associated with moderated jasmonate signalling

Salt stress is well known to induce oxidative stress through excessive production of reactive oxygen species (ROS) (Mittler, 2002; Kim *et al.,* 2023). These arise as a secondary consequence of ionic imbalance and impaired photosynthesis (Munns and Tester, 2008; Moradi and Ismail, 2007). Accumulation of ROS leads to lipid peroxidation, protein oxidation, and membrane damage (Moradi and Ismail, 2007; Lutts *et al.,* 1996). This ultimately accelerates leaf senescence and growth inhibition (Saddiq *et al.,* 2021). The extent of oxidative stress therefore represents a key determinant of plant performance under salinity (Akram *et al*., 2025). In the jasmonate-deficient system, antioxidant responses differed clearly between Kitaake and the aoc mutant. Salt stress triggered stronger transcriptional activation of antioxidant-related genes in *aoc* mutant (Chapter 3.1; Fig. 16). In particular, plastidic superoxide dismutase (ptSOD) showed stronger induction in the mutant. Superoxide dismutases play a central role in detoxifying superoxide radicals by converting them into hydrogen peroxide, which is subsequently removed by downstream antioxidant enzymes (Gill and Tuteja, 2010). In *aoc mutant*, stronger ptSOD induction suggests an enhanced capacity to detoxify ROS and limit oxidative damage. Interestingly, transient induction of plastidic SOD genes was also observed in the aoc mutant under control conditions, indicating a generally enhanced transcriptional responsiveness of antioxidant pathways to environmental or experimental perturbations (Chapter 3; Fig. 16)**.** A comparable relationship between oxidative stress and salt tolerance was

observed in the natural genotype system. FL478 accumulated less sodium in shoot tissues and maintained better physiological performance than RC222. In leaves, enhanced Na^+ accumulation disrupts the photosynthetic machinery in chloroplasts, leading to increased ROS production (Munns and Tester, 2008; Horie *et al*., 2012). The higher shoot Na^+ levels observed in RC222 therefore indicate increased oxidative stress in the sensitive genotype. In contrast, reduced Na^+ accumulation in FL478 likely limits ROS formation. The comparison of both experimental systems indicates a consistent pattern. The tolerant backgrounds, *aoc* mutant and FL478, combine reduced shoot Na^+ accumulation with improved antioxidant capacity. In contrast, the sensitive genotypes Kitaake and RC222 show higher sodium accumulation and weaker protection against oxidative damage. Previous studies support this interpretation. Improved ROS-scavenging capacity and reduced lipid peroxidation have been reported in jasmonate-deficient rice mutants under salt stress (Hazman *et al*., 2015). Jasmonate signalling has also been shown to regulate oxidative stress pathways and senescence-associated gene expression during prolonged salinity (Ndecky *et al*., 2023). The present findings broaden these observations by demonstrating that both the *aoc mutant* and the tolerant genotype FL478 display traits consistent with improved oxidative stress management. Together, the results suggest a mechanistic link between jasmonate signalling, ion homeostasis, and oxidative stress. Reduced shoot sodium accumulation in aoc mutant and FL478 likely limits ROS production, while enhanced antioxidant responses additionally mitigate oxidative damage. In contrast, stronger jasmonate accumulation and higher sodium levels in Kitaake and RC222 are associated with increased oxidative stress and stronger growth inhibition. Taken together, the data indicate that moderated jasmonate signalling helps to improved oxidative stress management during salt stress. This represents an additional crucial element of the enhanced tolerance observed in the *aoc* mutant and FL478.

4.9 Integrated model of jasmonate-mediated salt tolerance in rice seedlings

Physiological, hormonal, and transcriptional evidence from two independent experimental systems collectively highlights the role of jasmonate signalling in rice salt stress adaptation. In salt conditions, the *aoc* mutant and naturally tolerant genotype FL478 exhibited similar responses, whereas Kitaake and RC222 showed features typical of salt-sensitive plants. The convergence of these responses strongly suggests that jasmonate signalling represents a central regulatory component linking growth, ion homeostasis, and stress responses during salt exposure. At the whole-plant level, both *aoc* mutant and FL478 maintained better growth, greener leaves, and higher biomass accumulation than Kitaake and RC222 (Chapter 3; Fig. 9A,

B; Chapter 3.2; Fig. 18). Maintenance of growth under salinity is particularly important because rice seedlings are highly sensitive to osmotic and ionic stress (Munns and Tester, 2008; Horie *et al*., 2012). The phenotypic similarity between the jasmonate-deficient mutant and the tolerant genotype indicates that moderated jasmonate signalling contributes to improved growth maintenance during salt stress.

Physiological measurements additionally showed that the *aoc* mutant maintained higher photosynthetic performance under salinity than Kitaake (Chapter 3.1; Fig. 11). Sustained carbon assimilation is essential because it provides the energy required for ion transport and stress adaptation (Ambavaram *et al*., 2014). Improved photosynthetic performance in the mutant therefore provides a physiological explanation for its increased growth. Together with the reduced sodium accumulation observed in FL478 and aoc mutant, these findings show that tolerant plants experience less metabolic interference during salt exposure. Pronounced differences in ion measurements were observed in shoot tissues among genotypes, whereas root values were comparable (Chapter 3; Fig. 12; Chapter 3.2; Fig. 22). In the leaves of the aoc mutant and FL478, less Na^+ accumulation with more favourable K^+/Na^+ ratios than in Kitaake and RC222. Shoot Na^+ exclusion is widely recognised as a major determinant of salt tolerance in rice (Ren *et al.,* 2005; Ismail and Horie, 2017). The similar ion partitioning patterns detected in the mutant and tolerant genotype, therefore, represent a central physiological link between jasmonate signalling and salt tolerance.

Hormone analyses revealed that RC222 displayed stronger and more prolonged accumulation of jasmonates and ABA than FL478 (Chapter 3.2; Fig. 20; Fig. 21). Previous studies showed that jasmonate-deficient rice mutants accumulate less JA and exhibit improved salt tolerance (Hazman *et al*., 2015). Excessive jasmonate signalling has been shown to amplify senescence and oxidative stress during prolonged salinity, recently reported by Ndecky *et al*. (2023). The moderated hormonal response observed in FL478, therefore, resembles the genetically reduced jasmonate signalling of *aoc*, further supporting the idea that excessive JA accumulation contributes to salt sensitivity.

At the transcriptional level, tolerant plants displayed earlier activation of ion transporters and antioxidant responses together with more efficient feedback regulation of jasmonate signalling through JAZ genes (Chapter 3.1; Fig. 14; Fig.15;Chapter 3.2; Fig. 24; Fig. 25). In contrast, Kitaake and RC222 exhibited delayed transporter activation, stronger jasmonate accumulation, and increased oxidative stress. Early activation of protective responses is critical because delayed responses often occur after cellular damage has already developed (Horie *et al*., 2012).

Taken together, the results support a model in which salt stress induces jasmonate accumulation that influences ion transport, oxidative stress, and growth regulation. Excessive jasmonate signalling in sensitive genotypes promotes growth inhibition, delayed protective responses, and enhanced oxidative damage. In contrast, attenuation or rapid feedback regulation of jasmonate signalling enables early activation of defensive mechanisms, reduced sodium accumulation in shoots, improved oxidative stress management, and maintained growth. Thus, jasmonate signalling emerges as a central regulatory hub that determines whether rice seedlings enter a protective or damage-dominated response during salt stress.

5. Conclusion and outlook

Salinity represents one of the most severe abiotic stresses limiting rice productivity worldwide. As a glycophytic crop species, rice is particularly sensitive to elevated soil salinity, which disrupts ionic balance, impairs photosynthesis, and promotes oxidative stress. Understanding how plants coordinate hormonal signaling with physiological adaptation under saline conditions is therefore essential for developing strategies to improve salt tolerance in rice. The present thesis investigated the role of jasmonate signaling in rice salt stress responses using two complementary experimental systems. In the first system, the jasmonate-deficient aoc mutant was compared with its wild-type background Kitaake. In the second system, the naturally salt-tolerant genotype FL478 was compared with the salt-sensitive genotype RC222**.** By combining physiological measurements, ion profiling, hormone analysis, and gene expression studies, this work provides a comprehensive view of how jasmonate signaling contributes to the regulation of salinity responses in rice seedlings.

The results demonstrate that reduced jasmonate signaling is associated with improved tolerance to salinity stress. The jasmonate-deficient *aoc* mutant exhibited reduced sensitivity to salt stress compared with wild-type plants. Under saline conditions, the mutant maintained improved root growth, higher biomass accumulation, and more stable photosynthetic performance. In addition, altered sodium and potassium partitioning patterns indicated improved ionic balance in specific plant organs. At the molecular level, several jasmonate-responsive genes were differentially regulated during salinity exposure. Members of the JAZ gene family, which act as repressors of jasmonate signaling, were strongly induced by salt stress in wild-type plants but showed markedly reduced induction in the jasmonate-deficient mutant. Furthermore, genes associated with ion transport and oxidative stress responses, including NHX1 and plastidic superoxide dismutase genes (ptSOD1 and ptSOD2), exhibited genotype-dependent regulation, suggesting that jasmonate signaling influences both ion homeostasis and antioxidant defense mechanisms.

Comparative analysis of natural rice genotypes revealed similar physiological patterns. The salt-tolerant genotype FL478maintained better growth performance and ionic balance under salinity stress than the salt-sensitive genotype RC222. Hormone profiling further indicated distinct jasmonate dynamics during salt exposure, suggesting that moderated jasmonate signaling may represent an adaptive mechanism contributing to improved stress tolerance. Taken together, the results support a model in which jasmonate signaling acts as a central

regulatory component linking stress perception, growth regulation, ion transport, and oxidative stress responses during salinity exposure. While early jasmonate signaling may contribute to stress perception and activation of defense pathways, prolonged or excessive jasmonate accumulation appears to restrict growth and exacerbate stress damage. In contrast, reduced jasmonate levels, as observed in the aoc mutant, and moderated jasmonate signalling in the tolerant genotype FL478**,** allow plants to maintain growth while still activating protective responses. These findings provide new insight into the complex role of jasmonate signaling during salinity stress and highlight the importance of hormonal balance in determining plant stress tolerance. Future research should further investigate how jasmonate signaling interacts with other hormonal pathways, particularly abscisic acid (ABA) and auxin, during salt stress responses. In addition, the regulatory networks linking jasmonate signaling with ion transporter activity and antioxidant systems require deeper investigation at the transcriptional and post-transcriptional levels. Advances in genomic and genome-editing technologies, such as CRISPR–Cas9, may enable targeted modification of key regulators of jasmonate signaling, offering promising opportunities for the development of salt-tolerant rice cultivars.

Overall, the results of this thesis contribute to a better understanding of how hormonal signaling networks regulate plant adaptation to salinity stress and provide a foundation for future strategies aimed at improving stress resilience in rice and other important crop species.

6. References

Adolf, V.I., Jacobsen, S.E. and Shabala, S. (2013). Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). *Environmental and Experimental Botany*, 92, 43–54. https://doi.org/10.1016/j.envexpbot.2012.07.004.

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van der Straeten, D., Peng, J. and Harberd, N.P. (2006). Integration of plant responses to environmentally activated phytohormonal signals. *Science*, 311(5757), 91–94. https://doi.org/10.1126/science.1118642.

Ambavaram, M.M.R., Basu, S., Krishnan, A., Ramegowda, V., Batlang, U., Rahman, L., Baisakh, N. and Pereira, A. (2014). Coordinated regulation of photosynthesis in rice increases yield and tolerance to environmental stress. *Nature Communications*, 5. 5302. https://doi.org/10.1038/ncomms6302.

Apse, M.P. and Blumwald, E. (2007). Na^+ transport in plants. *FEBS Letters*, 581, 2247–2254. https://doi.org/10.1016/j.febslet.2007.04.014.

Ashraf, M. and McNeilly, T. (2004). Salinity tolerance in Brassica oilseeds. *Critical Reviews in Plant Sciences*, 23, 157–174. https://doi.org/10.1080/07352680490433286.

Ashraf, M. and Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, 59, 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006.

Bassil, E. and Blumwald, E. (2014). The ins and outs of intracellular ion homeostasis: NHX-type cation/H^+ transporters. *Current Opinion in Plant Biology*, 22, 1–6. https://doi.org/10.1016/j.pbi.2014.08.002.

Batayeva, D., Labaco, B., Ye, C., Li, X., Usenbekov, B., Rysbekova, A., Dyuskalieva, G., Vergara, G., Reinke, R. and Leung, H. (2018). Genome-wide association study of seedling stage salinity tolerance in temperate japonica rice germplasm. *BMC Genetics*, 19(1), 2. https://doi.org/10.1186/s12863-017-0590-7.

Bose, J., Rodrigo-Moreno, A. and Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of Experimental Botany*, 65, 1241–1257. https://doi.org/10.1093/jxb/ert430.

Browse, J. (2009). Jasmonate passes muster: A receptor and targets for the defense hormone. *Annual Review of Plant Biology*, 60, 183–205. https://doi.org/10.1146/annurev.arplant.043008.092007

Cheeseman, J.M. (2015). The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytologist*, 206, 557–570. https://doi.org/10.1111/nph.13217.

Chini, A., Gimenez-Ibanez, S., Goossens, A. and Solano, R. (2016). Redundancy and specificity in jasmonate signalling. *Current Opinion in Plant Biology*, 33, 147–156. https://doi.org/10.1016/j.pbi.2016.07.005.

Chinnusamy, V. and Zhu, J.K. (2009). Epigenetic regulation of stress responses in plants. *Current Opinion in Plant Biology*, 12, 133–139. https://doi.org/10.1016/j.pbi.2008.12.006.

Choi, W.G., Toyota, M., Kim, S.H., Hilleary, R. and Gilroy, S. (2014). Salt stress-induced Ca^{2+} waves are associated with rapid systemic signaling in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 6497–6502. https://doi.org/10.1073/pnas.1319955111.

Cotsaftis, O., Plett, D., Shirley, N., Tester, M. and Hrmova, M. (2012). A two-staged model of Na^+ exclusion in rice explained by 3D modeling of HKT transporters and alternative splicing. *PLoS ONE*, 7(7), e39865. https://doi.org/10.1371/journal.pone.0039865.

Creelman, R.A. and Mullet, J.E. (1997). Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48, 355–381. https://doi.org/10.1146/annurev.arplant.48.1.355.

Dassanayake, M., Oh, D.H., Haas, J.S., Hernandez, A., Hong, H., Ali, S., Yun, D.J., Bressan, R.A., Zhu, J.K., Bohnert, H.J. and Cheeseman, J.M. (2011). The genome of the extremophile plant *Thellungiella parvula*. *Nature Genetics*, 43, 913–918. https://doi.org/10.1038/ng.889.

Deinlein, U., Stephan, A.B., Horie, T., Luo, W., Xu, G. and Schroeder, J.I. (2014). Plant salt-tolerance mechanisms. *Trends in Plant Science*, 19, 371–379. https://doi.org/10.1016/j.tplants.2014.02.001.

Delgado, C., Mora-Poblete, F., Ahmar, S., Chen, J.T. and Figueroa, C.R. (2021). Jasmonates and plant salt stress: Molecular players, physiological effects, and improving tolerance by using genome-associated tools. *International Journal of Molecular Sciences*, 22(6), 3082. https://doi.org/10.3390/ijms22063082.

Demidchik, V., Straltsova, D., Medvedev, S.S., Pozhvanov, G.A., Sokolik, A. and Yurin, V. (2014). Stress-induced electrolyte leakage: the role of K^+-permeable channels and involvement in programmed cell death and metabolic adjustment. *Journal of Experimental Botany*, 65(5), 1259–1270. https://doi.org/10.1093/jxb/eru004.

Diep, N.P.N., Hau, L.P., Do, T.I., Trang, T.T.T., Tinh, P.A. and Ky, H. (2025). Genetic and physiological characteristics of salt-tolerance in nine rice varieties at seedling stage. *Hue University Journal of Science: Natural Science*, 134(1S-3), 151–161. https://doi.org/10.26459/hueunijns.v134i1S-3.7954.

Dionisio-Sese, M.L. and Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Science*, 135(1), 1–9. https://doi.org/10.1016/S0168-9452(98)00025-9.

Dodd, A.N., Kudla, J. and Sanders, D. (2010). The language of calcium signaling. *Annual Review of Plant Biology*, 61, 593–620. https://doi.org/10.1146/annurev-arplant-070109-104628.

Du, H., Liu, H. and Xiong, L. (2013). Endogenous auxin and jasmonic acid levels are differentially modulated by abiotic stresses in rice. *Frontiers in Plant Science*, 4, 397. https://doi.org/10.3389/fpls.2013.00397.

FAO (2017). *The future of food and agriculture: Trends and challenges*. Rome: Food and Agriculture Organization of the United Nations.

FAO (2021). *The State of the World's Land and Water Resources for Food and Agriculture – Systems at Breaking Point*. Rome: Food and Agriculture Organization of the United Nations.

FAO (2023). *Global Status of Salt-Affected Soils*. Rome: Food and Agriculture Organization of the United Nations.

FAO (2024). *FAOSTAT Statistical Database*. Rome: Food and Agriculture Organization of the United Nations.

Flexas, J., Bota, J., Loreto, F., Cornic, G. and Sharkey, T.D. (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. *Plant Biology*, 6(3), 269–279. https://doi.org/10.1055/s-2004-820867.

Flowers, T.J. and Colmer, T.D. (2008). Salinity tolerance in halophytes. *New Phytologist*, 179, 945–963. https://doi.org/10.1111/j.1469-8137.2008.02531.x.

Flowers, T.J. and Colmer, T.D. (2015). Plant salt tolerance: adaptations in halophytes. *Annals of Botany*, 115, 327–331. https://doi.org/10.1093/aob/mcu267.

Flowers, T.J. and Yeo, A.R. (1995). Breeding for salinity resistance in crop plants: Where next? *Australian Journal of Plant Physiology*, 22, 875–884. https://doi.org/10.1071/PP9950875.

Flowers, T.J., Galal, H.K. and Bromham, L. (2010). Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology*, 37, 604–612. https://doi.org/10.1071/FP09269.

Flowers, T.J., Munns, R. and Colmer, T.D. (2015). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Annals of Botany*, 115(3), 419–431. https://doi.org/10.1093/aob/mcu217.

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006). Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology*, 9(4), 436–442. https://doi.org/10.1016/j.pbi.2006.05.014.

Fukuda, A., Nakamura, A., Hara, N., Toki, S. and Tanaka, Y. (2011). Molecular and functional analyses of rice NHX-type Na^+/H^+ antiporter genes. *Planta*, 233(1), 175–188. https://doi.org/10.1007/s00425-010-1289-4.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016.

Glenn, E.P., Brown, J.J. and Blumwald, E. (1999). Salt tolerance and crop potential of halophytes. *Critical Reviews in Plant Sciences*, 18, 227–255. https://doi.org/10.1080/07352689991309207.

Hakata, M., Kuroda, M., Miyashita, T., Yamaguchi, T., Kojima, M., Sakakibara, H., Mitsui, T. and Yamakawa, H. (2012). Suppression of α-amylase genes improves quality of rice grain ripened under high temperature. *Plant Biotechnology Journal*, 10(9), 1110–1117. https://doi.org/10.1111/j.1467-7652.2012.00741.x.

Haswell, E.S., Phillips, R. and Rees, D.C. (2011). Mechanosensitive channels: what can they do and how do they do it? *Structure*, 19, 1356–1369. https://doi.org/10.1016/j.str.2011.09.005.

Hazman, M., Hause, B., Eiche, E., Nick, P. and Riemann, M. (2015). Increased tolerance to salt stress in OPDA-deficient rice allene oxide cyclase mutants is linked to an increased ROS-scavenging activity. *Journal of Experimental Botany*, 66(11), 3339–3352. https://doi.org/10.1093/jxb/erv142.

He, Y., Zhu, Z., Yang, J., Ni, X. and Zhu, B. (2009). Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environmental and Experimental Botany*, 66(2), 270–278. https://doi.org/10.1016/j.envexpbot.2009.02.007.

Horie, T., Karahara, I. and Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: an overview with the central role of transporters. *Rice*, 5, 11. https://doi.org/10.1186/1939-8433-5-11.

Howe, G.A., Major, I.T. and Koo, A.J. (2018). Modularity in jasmonate signaling for multistress resilience. *Annual Review of Plant Biology*, 69, 387–415. https://doi.org/10.1146/annurev-arplant-042817-040047.

Huot, B., Yao, J., Montgomery, B.L. and He, S.Y. (2014). Growth–defense tradeoffs in plants: A balancing act to optimize fitness. *Molecular Plant*, 7(8), 1267–1287. https://doi.org/10.1093/mp/ssu049.

IPCC (2019). *Climate Change and Land: An IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse Gas Fluxes in Terrestrial Ecosystems*. Geneva: Intergovernmental Panel on Climate Change.

Ismail, A.M. and Horie, T. (2017). Genomics, physiology and breeding approaches for improving salt tolerance in rice. *Annual Review of Plant Biology*, 68, 405–434. https://doi.org/10.1146/annurev-arplant-042916-040936.

Ismail, A.M., Singh, U.S., Singh, S., Dar, M.H. and Mackill, D.J. (2013). The contribution of submergence-tolerant (Sub1) rice varieties to food security in flood-prone rainfed

lowland areas in Asia. *Field Crops Research*, 152, 83–93. https://doi.org/10.1016/j.fcr.2013.01.007.

Jalmi, S.K. and Sinha, A.K. (2015). ROS mediated MAPK signaling in abiotic and biotic stress—striking similarities and differences. *Frontiers in Plant Science*, 6, 769. https://doi.org/10.3389/fpls.2015.00769.

Ji, H., Pardo, J.M., Batelli, G., Van Oosten, M.J., Bressan, R.A. and Li, X. (2013). The Salt Overly Sensitive (SOS) pathway: Established and emerging roles. *Molecular Plant*, 6(2), 275–286. https://doi.org/10.1093/mp/sst017

Kazan, K. (2015). Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends in Plant Science*, 20(4), 219–229. https://doi.org/10.1016/j.tplants.2015.02.001.

Kazan, K. and Manners, J.M. (2008). Jasmonate signaling: Toward an integrated view. *Plant Physiology*, 146(4), 1459–1468. https://doi.org/10.1104/pp.107.115717.

Knight, H., Trewavas, A.J. and Knight, M.R. (1997). Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. *The Plant Journal*, 12, 1067–1078. https://doi.org/10.1046/j.1365-313X.1997.12051067.x.

Kobayashi, N.I., Yamaji, N., Yamamoto, H., Okubo, K., Ueno, H., Costa, A., Tanoi, K., Matsumura, H., Fujii-Kashino, M., Horiuchi, T., Nayef, M.A., Shabala, S., An, G., Ma, J.F. and Horie, T. (2017). OsHKT1;5 mediates Na^+ exclusion in the vasculature to protect leaf blades and reproductive tissues from salt toxicity in rice. *The Plant Journal*, 91(4), 657–670. https://doi.org/10.1111/tpj.13595.

Kurotani, K., Hayashi, K., Hatanaka, S., Toda, Y., Ogawa, D., Ichikawa, H., Ishimaru, Y., Tashita, R., Suzuki, T., Ueda, M., Hattori, T. and Takeda, S. (2015). Elevated levels of CYP94 family gene expression alleviate the jasmonate response and enhance salt tolerance in rice. *Plant and Cell Physiology*, 56(4), 779–789. https://doi.org/10.1093/pcp/pcv006.

Lamers, J., van der Meer, T. and Testerink, C. (2020). How plants sense and respond to stressful environments. *Plant Physiology*, 182(4), 1624–1635. https://doi.org/10.1104/pp.19.01464.

Lim, C.W., Baek, W., Jung, J., Kim, J.H. and Lee, S.C. (2020). Function of jasmonic acid in plant responses to abiotic stresses. *Journal of Plant Biology*, 63, 341–351. https://doi.org/10.1007/s12374-020-09287-5.

Lutts, S., Kinet, J.M. and Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany*, 78(3), 389–398. https://doi.org/10.1006/anbo.1996.0134.

Maas, E.V. and Hoffman, G.J. (1977). Crop salt tolerance – current assessment. *Journal of the Irrigation and Drainage Division*, 103, 115–134.

Manik, S.M., Shi, S., Mao, J., Dong, L., Su, Y., Wang, Q. and Liu, H. (2015). The calcium sensor CBL-CIPK is involved in plant's response to abiotic stresses. *International Journal of Genomics*, 2015, 493191. https://doi.org/10.1155/2015/493191.

Marino, D., Dunand, C., Puppo, A. and Pauly, N. (2012). A burst of plant NADPH oxidases. *Trends in Plant Science*, 17(1), 9–15. https://doi.org/10.1016/j.tplants.2011.10.001.
Maurel, C., Verdoucq, L., Luu, D.T. and Santoni, V. (2008). Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology*, 59, 595–624. https://doi.org/10.1146/annurev.arplant.59.032607.092734.

Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M.A., Shulaev, V., Dangl, J.L. and Mittler, R. (2009). The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling*, 2(84), ra45. https://doi.org/10.1126/scisignal.2000448.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7, 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9.

Mitsuya, S., Kawasaki, M., Taniguchi, M. and Miyake, H. (2003). Light dependency of salinity-induced chloroplast degradation. *Plant Production Science*, 6(3), 219–223. https://doi.org/10.1626/pps.6.219.

Mondal, S. and Borromeo, T.H. (2016). Screening of salinity tolerance of rice at early seedling stage. *Journal of Bioscience and Agriculture Research*, 10(1), 843–847. https://doi.org/10.18801/jbar.100116.102.

Moons, A., Prinsen, E., Bauw, G. and Van Montagu, M. (1997). Antagonistic effects of abscisic acid and jasmonates on salt stress-inducible transcripts in rice roots. *The Plant Cell*, 9(12), 2243–2259. https://doi.org/10.1105/tpc.9.12.2243.

Moradi, F. and Ismail, A.M. (2007). Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salinity stress during seedling and reproductive stages in rice. *Annals of Botany*, 99, 1161–1173. https://doi.org/10.1093/aob/mcm052.

Munemasa, S., Hauser, F., Park, J., Waadt, R., Brandt, B. and Schroeder, J.I. (2015). Mechanisms of abscisic acid-mediated control of stomatal aperture. *Current Opinion in Plant Biology*, 28, 154–162. https://doi.org/10.1016/j.pbi.2015.10.010.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment*, 25, 239–250. https://doi.org/10.1046/j.0016-8025.2001.00808.x.

Munns, R. and Gilliham, M. (2015). Salinity tolerance of crops – what is the cost? *New Phytologist*, 208, 668–673. https://doi.org/10.1111/nph.13519.

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.

Munns, R., James, R.A. and Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, 57, 1025–1043. https://doi.org/10.1093/jxb/erj100.

Nick, P. (2006). Noise yields order – auxin, actin, and polar patterning. *Protoplasma*, 227, 1–11. https://doi.org/10.1007/s00709-005-0132-3.

Ndecky, S., Nguyen, T.H., Eiche, E., Cognat, V., Pflieger, D., Pawar, N., Betting, F., Saha, S., Champion, A., Riemann, M. and Heitz, T. (2023). Jasmonate signaling controls negative and positive effectors of salt stress tolerance in rice. *Journal of Experimental Botany*. https://doi.org/10.1093/jxb/erad086.

Negrão, S., Schmöckel, S.M. and Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, 119, 1–11. https://doi.org/10.1093/aob/mcw191.

Nguyen, T.H., Mai, H.T.T., Moukouanga, D., Lebrun, M., Bellafiore, S. and Champion, A. (2020). CRISPR/Cas9-mediated gene editing of the jasmonate biosynthesis OsAOC gene in rice. In: *Methods in Molecular Biology*, 2085, 199–209. https://doi.org/10.1007/978-1-0716-0142-6_15.

Ouk, R., Oi, T., Sugiura, D. and Taniguchi, M. (2024). Structural changes of mesophyll cells in rice leaf tissue in response to salinity stress based on three-dimensional analysis. *AoB Plants*, 16(2), plae016. https://doi.org/10.1093/aobpla/plae016.

Parida, A.K. and Das, A.B. (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety*, 60, 324–349. https://doi.org/10.1016/j.ecoenv.2004.06.010.

Peethambaran, P.K., Glenz, R., Höninger, S., Islam, S.M.S., Hummel, S., Harter, K., Kolukisaoglu, Ü., Meynard, D., Guiderdoni, E., Nick, P. and Riemann, M. (2018). Salt-inducible expression of OsJAZ8 improves resilience against salt stress. *BMC Plant Biology*, 18, 311. https://doi.org/10.1186/s12870-018-1521-0.

Peleg, Z. and Blumwald, E. (2011). Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology*, 14, 290–295. https://doi.org/10.1016/j.pbi.2011.02.001.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J. and Postma, J.A. (2012). Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, 39, 839–850. https://doi.org/10.1071/FP12049.

Qadir, M. and Schubert, S. (2002). Degradation processes and nutrient constraints in sodic soils. *Land Degradation & Development*, 13, 275–294. https://doi.org/10.1002/ldr.504.

Qadir, M., Quillérou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R.J., Drechsel, P. and Noble, A.D. (2014). Economics of salt-induced land degradation and restoration. *Natural Resources Forum*, 38, 282–295. https://doi.org/10.1111/1477-8947.12054.

Raza, A., Charagh, S., Zahid, Z., Mubarik, M.S., Javed, R., Siddiqui, M.H. and Hasanuzzaman, M. (2021). Jasmonic acid: a key frontier in conferring abiotic stress tolerance in plants. *Plant Cell Reports*, 40(8), 1513–1541. https://doi.org/10.1007/s00299-020-02614-z.

Reddy, I.N.B.L., Kim, B.K., Yoon, I.S., Kim, K.H. and Kwon, T.R. (2017). Salt tolerance in rice: Focus on mechanisms and approaches. *Rice Science*, 24(3), 123–144. https://doi.org/10.1016/j.rsci.2016.09.004.

Ren, Z.H., Gao, J.P., Li, L.G., Cai, X.L., Huang, W., Chao, D.Y., Zhu, M.Z., Wang, Z.Y., Luan, S. and Lin, H.X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genetics*, 37(10), 1141–1146. https://doi.org/10.1038/ng1643.

Rengasamy, P. (2010). Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology*, 37, 613–620.

Rengasamy, P. and Olsson, K.A. (1991). Sodicity and soil structure. *Australian Journal of Soil Research*, 29, 935–952. https://doi.org/10.1071/SR9910935.

Rentel, M.C., Lecourieux, D., Ouaked, F., Usher, S.L., Petersen, L., Okamoto, H., Knight, H., Peck, S.C., Grierson, C.S., Hirt, H. and Knight, M.R. (2004). OXI1 kinase is necessary for oxidative burst-mediated signalling. *Nature*, 427, 858–861. https://doi.org/10.1038/nature02353.

Riemann, M., Dhakarey, R., Hazman, M., Miro, B., Kohli, A. and Nick, P. (2015). Exploring jasmonates in the hormonal network of drought and salinity responses. *Frontiers in Plant Science*, 6, 1077. https://doi.org/10.3389/fpls.2015.01077.

Roy, S.J., Negrão, S. and Tester, M. (2014). Salt resistant crop plants. *Current Opinion in Biotechnology*, 26, 115–124. https://doi.org/10.1016/j.copbio.2013.12.004.

Ryu, H. and Cho, Y.G. (2015). Plant hormones in salt stress tolerance. *Journal of Plant Biology*, 58, 147–155. https://doi.org/10.1007/s12374-015-0103-z.

Saddiq, M.S., Iqbal, S., Afzal, I., Ibrahim, A.H., Bakhtavar, M.A., Hafeez, M.B., Jahanzaib and Maqbool, M.M. (2019). Mitigation of salinity stress in wheat (*Triticum aestivum* L.) seedlings through physiological seed enhancements. *Journal of Plant Nutrition*, 42(10), 1192–1204. https://doi.org/10.1080/01904167.2019.1609509.

Shabala, S. and Cuin, T.A. (2008). Potassium transport and plant salt tolerance. *Physiologia Plantarum*, 133, 651–669. https://doi.org/10.1111/j.1399-3054.2007.01008.x.

Shabala, S. and Mackay, A. (2011). Ion transport in halophytes. *Advances in Botanical Research*, 57, 151–199. https://doi.org/10.1016/B978-0-12-387692-8.00005-9.

Shabala, S., Wu, H. and Bose, J. (2015). Salt stress sensing and early signalling events in plant roots: current knowledge and hypothesis. *Plant Science*, 241, 109–119. https://doi.org/10.1016/j.plantsci.2015.10.003.

Shainberg, I. and Letey, J. (1984). Response of soils to sodic and saline conditions. *Hilgardia*, 52, 1–57. https://doi.org/10.3733/hilg.v52n02p057.

Stintzi, A., Weber, H., Reymond, P., Browse, J. and Farmer, E.E. (2001). Plant defense in the absence of jasmonic acid: The role of cyclopentenones. *Proceedings of the National Academy of Sciences of the United States of America*, 98(22), 12837–12842. https://doi.org/10.1073/pnas.211311098.

Strange, R.N. and Scott, P.R. (2005). Plant disease: a threat to global food security. *Annual Review of Phytopathology*, 43, 83116. https://doi.org/10.1146/annurev.phyto.43.113004.133839.

Tang, G., Ma, J., Hause, B., Nick, P. and Riemann, M. (2020). Jasmonate is required for the response to osmotic stress in rice. *Environmental and Experimental Botany*, 175, 104047. https://doi.org/10.1016/j.envexpbot.2020.104047.

Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Ainai, T., Yagi, K., Sakurai, N., Suzuki, H., Masuda, T., Takamiya, K., Shibata, D., Kobayashi, Y. and Ohta, H. (2005). 12-oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. *Plant Physiology*, 139(3), 1268–1283. https://doi.org/10.1104/pp.105.067058.

Tester, M. and Davenport, R. (2003). Na^+ tolerance and Na^+ transport in higher plants. *Annals of Botany*, 91, 503–527. https://doi.org/10.1093/aob/mcg058.

Ueda, J. and Kato, J. (1980). Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). *Plant Physiology*, 66(2), 246–249. https://doi.org/10.1104/pp.66.2.246.

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N., Inoue, H., Takehisa, H., Motoyama, R., Nagamura, Y., Wu, J., Matsumoto, T., Takai, T., Okuno, K., Yano, M. and Hori, K. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics*, 45, 1097–1102. https://doi.org/10.1038/ng.2725.

UN DESA (2022). *World Population Prospects 2022*. New York: United Nations Department of Economic and Social Affairs.

Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R.K., Kumar, V., Verma, R. and Sharma, S. (2017). Abscisic acid signaling and abiotic stress tolerance in plants: A review on current knowledge and future prospects. *Frontiers in Plant Science*, 8, 161. https://doi.org/10.3389/fpls.2017.00161.

Walia, H., Wilson, C., Condamine, P., Liu, X., Ismail, A.M., Zeng, L., Wanamaker, S.I., Mandal, J., Xu, J., Cui, X. and Close, T.J. (2005). Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiology*, 139(2), 822–835. https://doi.org/10.1104/pp.105.065961.

Wang, Y. and Wu, W.H. (2013). Potassium transport and signaling in higher plants. *Annual Review of Plant Biology*, 64, 451–476. https://doi.org/10.1146/annurev-arplant-050312-120153.

Wasternack, C. (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany*, 100(4), 681–697. https://doi.org/10.1093/aob/mcm079.

Wasternack, C. and Hause, B. (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. *Annals of Botany*, 111(6), 1021–1058. https://doi.org/10.1093/aob/mct067.

Waszczak, C., Carmody, M. and Kangasjärvi, J. (2018). Reactive oxygen species in plant signaling. *Annual Review of Plant Biology*, 69, 209–236. https://doi.org/10.1146/annurev-arplant-042817-040322.

World Bank (2020). *World Development Report 2020: Trading for Development in the Age of Global Value Chains*. Washington, DC: World Bank.

Wu, H., Ye, H., Yao, R., Zhang, T. and Xiong, L. (2015). OsJAZ9 acts as a transcriptional regulator in jasmonate signaling and modulates salt stress tolerance in rice. *Plant Science*, 232, 1–12. https://doi.org/10.1016/j.plantsci.2014.12.010.

Yadav, S., Irfan, M., Ahmad, A. and Hayat, S. (2012). Causes of salinity and plant responses to salinity stress: a review. *Journal of Environmental Biology*, 33, 667–685.

Yeo, A.R. and Flowers, T.J. (1986). Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Australian Journal of Plant Physiology*, 13, 161–173. https://doi.org/10.1071/PP9860161.

Yu, J., Zhang, Y., Di, C., Zhang, Q., Zhang, K., Wang, C., You, Q., Yan, H., Dai, S.Y., Yuan, J.S., Xu, W. and Su, Z. (2016). JAZ7 negatively regulates dark-induced leaf senescence in *Arabidopsis*. *Journal of Experimental Botany*, 67(3), 751–762. https://doi.org/10.1093/jxb/erv487.

Yuan, F., Guo, J., Shabala, S. and Wang, B. (2018). Reproductive physiology of halophytes: current standing. *Frontiers in Plant Science*, 9.

Yusuf, A., Li, M., Zhang, S.Y., Odedishemi-Ajibade, F., Luo, R.F., Wu, Y.X., Zhang, T.T., Ugya, A.Y., Zhang, Y. and Duan, S. (2025). Harnessing plant–microbe interactions: Strategies for enhancing resilience and nutrient acquisition for sustainable agriculture. *Frontiers in Plant Science*, 16, 1503730. https://doi.org/10.3389/fpls.2025.1503730.

Zeng, L. and Shannon, M.C. (2000). Salinity effects on seedling growth and yield components of rice. *Crop Science*, 40(4), 996–1003. https://doi.org/10.2135/cropsci2000.404996x.

Zeng, L., Shannon, M.C. and Lesch, S.M. (2001). Timing of salinity stress affects rice growth and yield components. *Agricultural Water Management*, 48(3), 191–206. https://doi.org/10.1016/S0378-3774(00)00146-3.

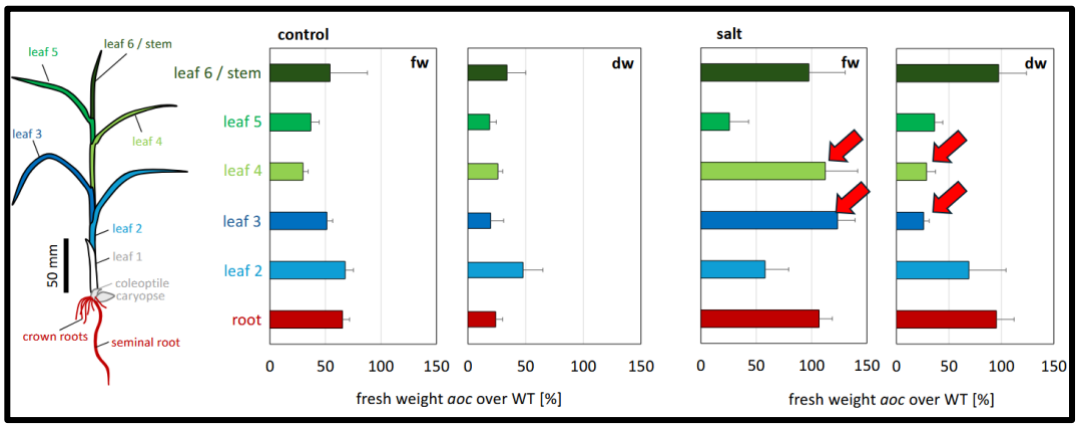
Zeng, L., Shannon, M.C. and Grieve, C.M. (2002). Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. *Euphytica*, 127, 235–245. https://doi.org/10.1023/A:1020262932277.

Zhu, J.K. (2001). Plant salt tolerance. *Trends in Plant Science*, 6, 66–71. https://doi.org/10.1016/S1360-1385(00)01838-0.

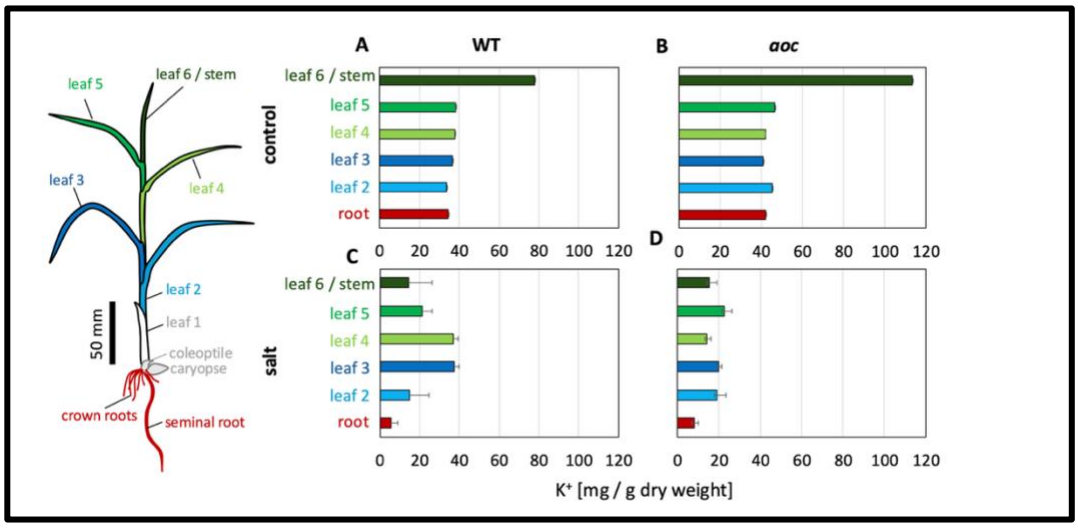
Zhu, J.K. (2003). Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology*, 6, 441–445. https://doi.org/10.1016/S1369-5266(03)00085-2.

Zhu, J.K. (2016). Abiotic stress signaling and responses in plants. *Cell*, 167, 313–324. https://doi.org/10.1016/j.cell.2016.08.029.

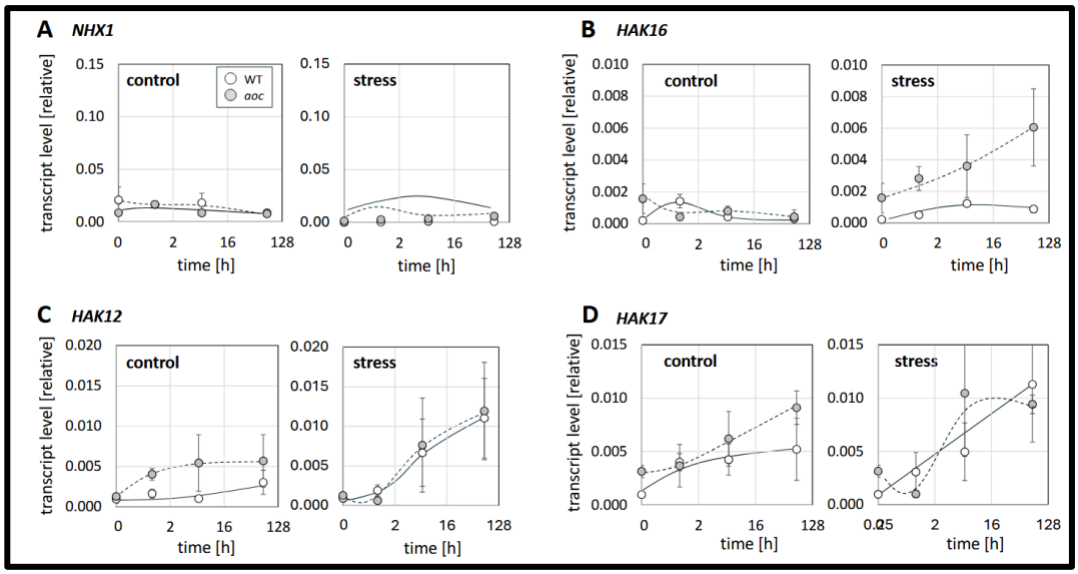
7. Supplementary data



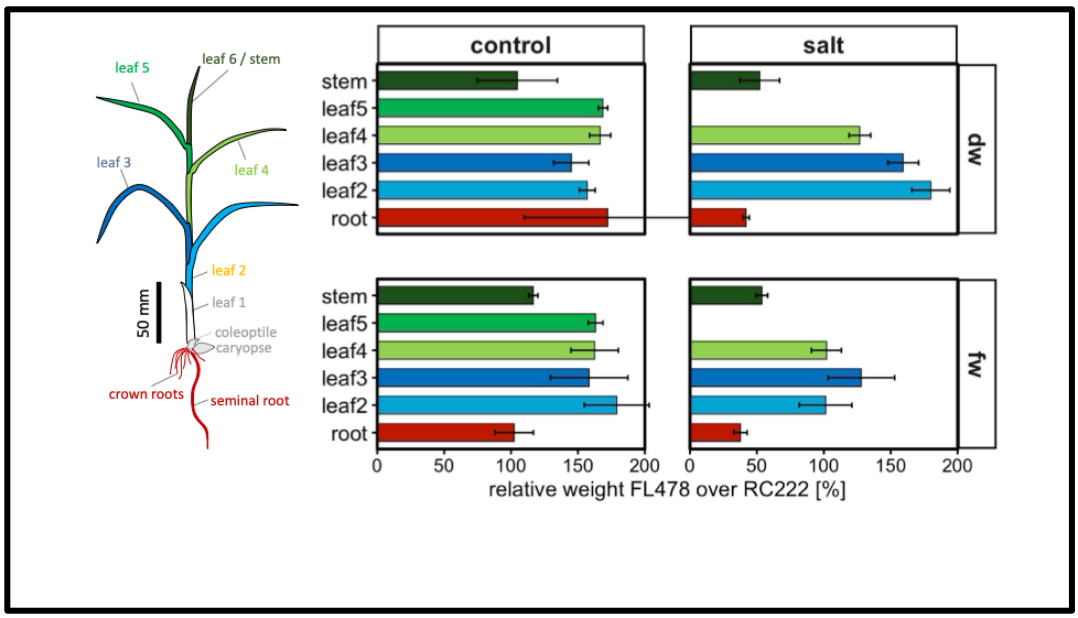
Supplementary Fig. S1. Differential relationship between fresh and dry weight accumulation in roots and leaves of the *aoc* mutant. Fresh weight (FW) and dry weight (DW) of individual organs were measured in WT and *aoc* seedlings grown for 12 days under control conditions or 100 mM NaCl. Data are presented as relative change of *aoc* compared with WT (%, WT = 100). In roots, the increase in fresh weight observed in the *aoc* mutant is accompanied by a proportional increase in dry weight under both control and saline conditions, indicating enhanced cellular biomass accumulation. In contrast, in younger leaves (leaf 3rd and leaf 4th), strong increases in fresh weight under salinity are associated with comparatively small changes in dry weight (red arrows), suggesting that biomass stimulation in leaves is predominantly driven by cell expansion and increased water content rather than increased cell number. Data represent means ± SE.



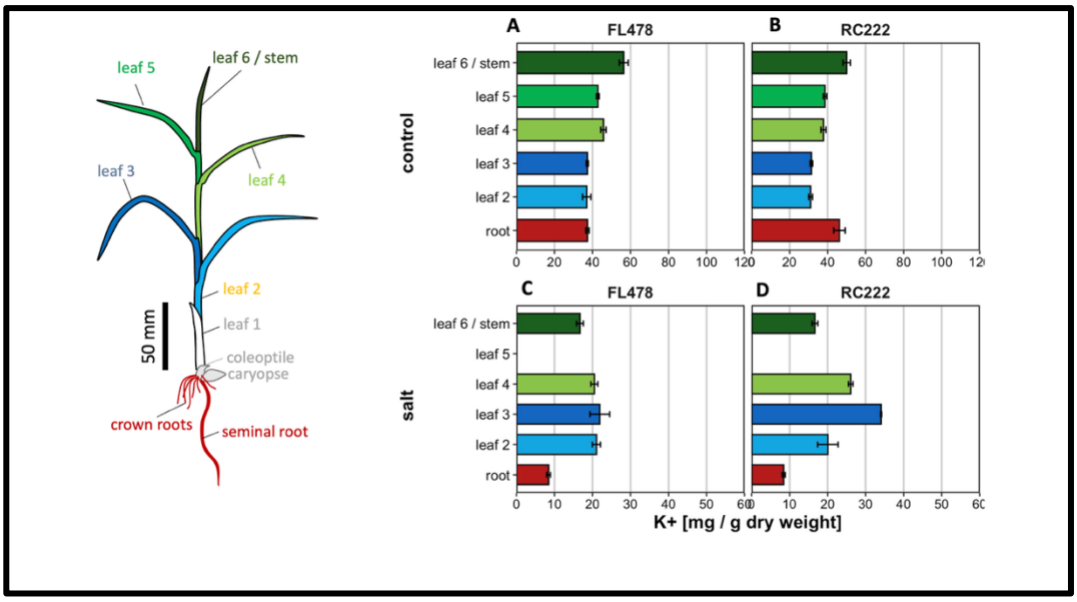
Suppl. Fig. S2. Organ-specific potassium (K^+) accumulation in wild-type and aoc mutant rice seedlings. Wild-type (WT; cv. Kitaake) and jasmonic acid–deficient aoc seedlings were grown hydroponically for 12 days under control conditions or in the presence of 100 mM NaCl. (A–B) K^+ concentrations in individual organs of WT (A) and *aoc*(B) seedlings under control conditions. (C–D) K^+ concentrations in individual organs of WT (C) and *aoc* (D) seedlings after salt treatment. K^+ content was determined in roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and the leaf 6th/stem fraction, as indicated in the schematic. Data are presented as K^+ concentration (mg g^{-1} dry weight). Values represent means ± SE



Supplementary Fig. S3. Salinity-dependent regulation of ion transporter gene expression in roots of wild-type and *aoc* mutant rice. Relative transcript levels of NHX1 (A), HAK16 (B), HAK12 (C), and HAK17 (D) were determined by RT-qPCR in roots of wild-type (WT; cv. Kitaake) and jasmonic acid–deficient *aoc* mutant seedlings under control conditions and after salt treatment. Samples were collected at the indicated time points after treatment onset. Transcript levels are shown relative to reference genes. Data represent means ± SE (n = 3 biological replicates, each with three technical replicates)



Supplementary Fig. 4. Differential organ-specific fresh and dry biomass accumulation in FL478 under control and salt stress. Fresh weight (FW) and dry weight (DW) of individual organs were measured in RC222 (wild type) and FL478 seedlings grown hydroponically for 12 days under control or salt stress conditions. Roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and the stem fraction were harvested separately. Data are shown as relative change of FL478 compared with RC222 (%, WT = 100). Bars represent means ± SE.



Supplementary Fig. 5. Organ-specific potassium (K^+) concentrations in FL478 and RC222 seedlings under control and salt stress conditions. (A–B) K^+ concentrations in individual organs of FL478 (A) and RC222 (B) seedlings grown under control conditions. (C–D) K^+ concentrations in individual organs of FL478 (C) and RC222 (D) seedlings after exposure to 100 mM NaCl. Potassium content was measured in roots, leaf 2nd, leaf 3rd, leaf 4th, leaf 5th, and the leaf 6th/stem fraction after 12 days of hydroponic growth. Values are expressed as mg g^{-1} dry weight. Bars represent mean values ± SE.

No.	Gene Name	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
1	JAZ8	ACGAAAGTGCAAGTGAGGCA	GGTGGACGGGAAGTTCTCAA
2	JAZ9	GGCCGGTCGAGTTGGAA	GGTCAGGCTCGGCGAAAT
3	JAZ13	CAGTACACGTCAGCTTTAATCCCATA	CTTGTGACAGATAGGAATAATCGTGC
4	HAK12	CTTCCACATGTTCCGTTGTG	CCAGCCTTTCCAGTTAGCAG
5	HAK16	CAGCTTCGTGCTATTCACCA	TCTTCTCGAGCTGGCTCTTC
6	HAK17	AACACCACTTGTGCTTGCTG	CCGTAGTACCAGCCGAACAT
7	HAK4	CTCTGTCAGCACGCAAACAT	ACCTTCTCAGTCTTCCAGGC
8	FE1	ATGATGGCATGATTCGACAA	AAGAGGATTGATGGCATTCG
9	FE2	CTTGATGCCCTGGAACCTTA	ATGGGTTGCCGTGTTGTAT
10	JAR1	AGGAGGCATCAAAGTTCCTGG	CTCAGCTCCCAGAAGATCACG
11	NHX1	TGTGCTCCGACAACCTGTAA	TACATGCAGGGTGGCAACTA
12	UBI10	GAGCCTCTGTTCGTCAAGTA	ACTCGATGGTCCATTAAACC

Supplementary Table S1. List of primers used for gene expression (qPCR)