Stress Resilience and Sugar Transport in *Sorghum bicolor* L. in Response to Salinity

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Karlsruhe, November 2022

Eman Abuslima

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Dedication

I dedicate this thesis to my late parents

"Hoda & Ramadan"

I will never forget how you both were an example of integrity and purity. Everything I am is because of you, and I thank you for always encouraging me to continue on the path I chose in my life.

May God Bless Your Souls

Your Daughter

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Abbreviations:

12-OH-JA: 12-hydroxy-JA

12-OH-JA-Ile: 12-hydroxy-JA-Ile

13-LOX: 13-Lipoxygenase

AAO: Abscisic aldehyde oxidase

ABA: Abscisic acid

ABI5: ABA-insensitiv

ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)

AKT: Potassium channel

AOC: Allen oxide cyclase

AOS: Allen oxide synthase

AQPs: Aquaporins

ASX: Ascorbate peroxidase

BHA: Butylated hydroxyanisole

BSA: Bovine serum albumin

bZIP: basic leucine zipper transcription factor

CAT: Catalase

CAXs: Ca^{2+}/H^{+} exchangers

CC: Companion cells

CDPK: Calcium-dependent protein kinase

CINs: Cytoplasmic invertases

CNGCs: cyclic nucleotide gated NSCCs

COI1: CORONATINE INSENSITIVE 1

CYP707A: ABA 8′-hydroxylase

DA-NSCCs: Depolarization-activated-NSCCs

DPPH: 2,2-dipheny-1 -picrylhydrazyl

Abbreviations

FAO: Food and Agriculture Organization

FER: the malectin-like-domain RK FERONIA

GAPDH: Glyceraldehyde-6-phosphate dehydrogenase

GIPC: Glycosyl Inositol Phospho Ceramides

GR: Glutathion Reductase

H^+-ATPases: PM electrogenic proton pumps

H_2O_2: Hydrogen peroxide

HAK/KUP/KT: High-Affinity K^+/K^+ UPtake/K^+ Transporter

HKT1: High-affinity potassium transporter 1

HXKs: Hexokinases

INV: Invertase

JA: Jasmonic acid

JA-Ile: Jasmonic acid isoleucine

JAR1: JASMONATE RESISTANT1

JAZ: JASMONATE ZIM DOMAIN

JMT: JA-Methyltransferase

LEA: Late embryogenesis-abundant

MAPK: Mitogen activated protein kinase

MCA: Mid1-Complementing Activity

MDA: Malondialdehyde

MED25: Mediator of RNA polymerase II transcription subunit 25

MeJA: Methyl Jasmonate

MES: 2-(N-morpholino)ethanesulfonic acid

MOCA1: Monocation-induced [ca^{2+}] increases 1

MSCCs: Mechanosensitive

MTs: Microtubules

MYC3, MYC4: bHLH zip transcription factor

Abbreviations

NCED1: 9-cis-epoxycarotenoid dioxygenase 1

NHX2: Na^+/H^+ vacuolar antiporter 2

NINJA: Novel of Interactor of JAZ

NSCC: Non-selective cation channel

NSY: Neo xanthin synthase

OPDA: 12-oxophytodienoic acid

OPR: OPDA-Reductase

OSCAs: reduced hyperosmolality-induced $[Ca^{2+}]i$ increase

OST1: Open stomata 1

P5Cs1: D1-pyrroline-5-carboxylate synthase 1

PCD: Programed Cell Death

PEG: Polyethylene glycol

PIPs: Plasma membrane intrinsic proteins

PM: Plasma membrane

POD: Peroxidases

PP2C: Protein phosphatase 2C

PYL: PYR-related

PYR: Pyrabactin resistance

RALF: Rapid alkalinization factor

RBOHD: Respiratory burst oxidase homolog D

RCAR: Regulatory component of ABA receptor

RLKs: Receptor-like kinases

ROS: Reactive oxygen species

SAPK1: Stress-activated protein kinase 1

SCF: Skp1 / Cullin / F box complex

SDR: Short-chain dehydrogenase/reductase

SE: Sieve elements

Abbreviations

SKOR: shaker-like K^+ outward-rectifying channel

SLAC1: Slow anion channel 1

SnRK2: Sucrose nonfermenting-1-related protein kinase 2.

SOD: Superoxide dismutase

SOS1: Salt overly sensitive 1

SPP: Sucrose phosphate phosphatase

SPS: Sucrose phosphate synthase

SUS: Sucrose synthase

TPL: TOPLESS

UBQ: Ubiquitin

V-ATPases: Vacuolar V-type H^+-ATPases

VINs: Vacuolar invertases

VI-NSCCs: Voltage-insensitive- NSCCs

V-PPases: vacuolar V-pyrophosphatases

ZFP: Zeaxanthin epoxidase

Ψp: Turgor pressure

Ψs: Osmotic potential

Ψw: Water potential

Zusammenfassung

Im Jahr 2022 hat die Welt eine multiglobale Krise erlebt, die wir nicht ignorieren können. Die Verschärfung des Klimadilemmas, neue Wellen von Covid-19, ein Konflikt zwischen Nationen, die Inflation und der steigende Bedarf der Bereitstellung von Energie und Nahrungsmitteln heute und 2050, stellen alle vor globale Herausforderungen. Es ist die Zeit, in der Innovation und nachhaltige Landwirtschaft einige der globalen Umweltprobleme lösen könnten, um den Hunger zu verringern, die Ernährungssicherheit zu gewährleisten und organisches Material aus der Landwirtschaft für flüssige Biokraftstoffe zu nutzen. Eine dieser globalen Umweltherausforderungen, die als akute Krise für das Wohlergehen des menschlichen Lebens angesehen wird, ist die Verschlechterung der Bodenstruktur durch die zunehmende Versalzung, die die natürlichen Ökosysteme und die biologische Vielfalt bedroht und die landwirtschaftliche Produktivität verringert.

In diesem Zusammenhang hat sich *Sorghum bicolor* L. als vielversprechende Kulturpflanze erwiesen, die aufgrund hoher Anreicherungen von fermentierbaren Zuckern in den Stängeln als Rohstoff zur Bioenethanol-Produktion genutzt werden kann. Die Fähigkeit Kohlenhydrate in Form von Stärke in den Samen zu speichern, macht sorghum zudem zu einer wertvollen Nahrungsquelle. Darüber hinaus ist sorghum widerstandsfähig und wächst auf Grenzertragsflächen wie beispielsweise an Küsten. Die vielseitige Verwendung von sorghum liefert verschiedene wertvolle Kandidatengenotypen zur Verbesserung der Stresstoleranz, zur Steigerung der Ernteerträge, für die Erzeugung von Biokraftstoffen und Erweiterung ihrer Verbreitung.

In dieser Studie wurde unter standardisierten Bedingungen ein kontrastierendes Paar von sorghum-Genotypen ausgewählt, die unterschiedlich auf Salzstress reagieren: den sogenannten Zuckerhirse-Genotyp „Della“ und den sogenannten Körnerhirse-Genotyp „Razinieh“. Der erste Teil der Arbeit befasst sich mit der Abgrenzung von salzbedingten Schäden und Anpassungen beider Genotypen in einem hydroponischen System. Beide Genotypen wurden vergleichend auf morphologischer, physiologischer und molekularer Ebene untersucht. Zunächst wurde mit Fluoreszenzmikroskopie die Natriumakkumulierung in der Wurzel verfolgt. Des Weiteren wurden die Transkriptlevel von Schlüsselgenen des Natriuminonentransports und der zellulären Homöostase gemessen und Metabolite, Hormone und Parameter für oxidativen Stress quantifiziert. Wir konnten zeigen, dass der salztolerante Genotyp Della im Vergleich zu Razinieh 7-mal weniger Natrium in den Spross

transportiert. Anhand von CoroNa Fluoreszenzfärbungen konnte zudem gezeigt werden, dass die unterschiedlich starke Anreicherung von Natrium im Spross mit der besseren vakuolären Na^+ Sequestrierung in der distalen Zellstreckungszone von Della korreliert. In Blättern von Della zeigte sich eine effizientere Redox-Homöostase mit schnellerer Prolinakkumulation und einer schnelleren und spezifischeren Aktivierung der Enzyme Superoxidedismutase und Ascorbatperoxidase, bei gleichbleibendem effizientem Saccharosetransfer zu den Wurzeln. Dagegen werden in Razinieh die schnelle und hohe Amplitude der Glycinanreicherung in den Blättern in Verbindung mit hohen H_2O_2-Konzentrationen als Indikatoren für eine hohe Photorespirationsrate unter Salzstress angesehen.

Zusammenfassend konnte gezeigt werden, dass der langsamere Natriumionentransport zum Spross in Della die potentielle Schädigung des Photosyntheseapparates verhindert und ihm dadurch mehr Zeit schafft, höhere Mengen an Hexosen in den Blättern zu produzieren, während gleichzeitig mehr Saccharose in die Wurzeln transferiert wird. Diese Situation in Della deutet auf eine länger andauernde Photosynthese und eine verbesserte Redox-Homöostase hin, welche die Salztoleranz dieses Genotyps verbessern. Diese Ergebnisse stimmen mit einem Modell überein welches annimmt, dass ein schnelles systemisches Signal, das in Della von den Wurzeln ausgeht, es ermöglicht antizipative Anpassungsmaßnahmen in den Blättern zu treffen, bevor der Ionenstress eintrifft.

Im zweiten Teil der Arbeit wurde die Zuckerverteilung und die Saccharosemobilisierung unter Salzstress in Della und Razinieh insbesondere im Fahnenblattstadium verglichen. Saccharose wird in ausgereiften Blättern (Quelle) gebildet und über das Phloem für Wachstum und Entwicklung zu Speichergeweben (Senken) transportiert. Dies ist jedoch bei den verschiedenen Genotypen unterschiedlich. Es gibt Genotypen welche nicht-strukturelle Kohlenhydrate in Form von Stärke in den Samen speichern (Körnerhirse) und große Erträge liefern, sowie Genotypen die beträchtliche Mengen an Zucker in ihren Stängeln akkumulieren (Zuckerhirse). Obwohl die Phänotypen von Körner- und Zuckerhirse sich unterscheiden, transportieren beide apoplasmatisch Saccharose unter Verwendung verschiedener Transportertypen, wie beispielsweise sucrose will be eventually exported transporters (SWEETs) und sucrose transporters (SUTs). Es ist daher wahrscheinlich, dass SWEET- und SUT-Proteine in allen sorghum ökotypen eine zentrale Rolle bei der Phleombeladung in den Quellengeweben und der Entladung in Senkengeweben spielen.

Der finale Teil dieser Studie zielte darauf ab, die Unterschiede in der Kohlenhydratekkumulation und -verteilung unter Salzstress zwischen den beiden unterschiedlich salzresistenten Genotypen zu charakterisieren und die Rolle der SbSWEETs und SbSUTs bei der Anreicherung von Saccharose in verschiedenen Pflanzenteilen zu definieren. Um zudem die übergeordnete Signalübertragung zu analysieren, wurde die Regulierung der Promotoren von Saccharosetransportergenen untersucht.

Anhand von Messungen der gesamten löslichen Zucker im Stängel konnte gezeigt werden, dass die Zuckerhirse Della deutlich mehr Zucker akkumuliert als die Körnerhirse Razinieh. Dagegen übertraf unter Salzstress der Kornertrag von Razinieh den Ertrag von Della. Darüber hinaus unterschieden sich beide Genotypen in der Saccharoseverteilung unter Salzstress, was auf unterschiedliche bevorzugte Senkengewebe hinweist. Während in Della Saccharose priorisiert in die Wurzeln transportiert wurde, reicherte sich Saccharose in Razinieh hauptsächlich in den mittleren Internodien an.

Mittels eines Modells basierend auf den Ergebnissen dieser Studie, kann die Be- und Entladung des Phloems mit Saccharose und die Bewegungen zu den verschiedenen Senkengeweben visualisiert werden. Hierbei wird die Rolle der SbSWEET und SbSUT Proteine anhand der Transkriptionslevel der entsprechenden Gene beleuchtet. Die signifikantesten Änderungen und Unterschiede in der Expression von *SbSWEET* und *SbSUT* Genen wurde in den Senkengeweben beider Genotypen beobachtet, was auf den signifikanten Einfluss von Salzstress auf die Saccharoseentladung durch diese Gene deutet. Zudem wurde mittels eines Dual-Luciferase-Reportersystems die Upstream-Signalleitung der *SbSWEET13* und *SbSUT6* Promotoren in Protoplasten untersucht. Es konnte eine erhöhte Reaktivität des *SbSUT6* Promotors auf MeJA in beiden Genotypen nachgewiesen werden, während ABA lediglich in Della den *SbSWEET13* Promotor induzierte. Diese Einblicke über die regulatorischen Eigenschaften von Jasmonaten und ABA, welche die Stresswahrnehmung mit Phytohormonen verknüpfen, geben Anhaltspunkte für die Züchtung Salzstress-toleranter Sorten.

Abstract

In 2022, the world has witnessed a multi-global crisis, which we cannot ignore. These include escalation in the climate change dilemma, new waves of Covid-19, a conflict between nations, the inflation challenge, and providing energy and food for the expected demand now and by 2050. It is the time when innovation and sustainable agriculture could solve some of the global environmental problems to reduce hunger, ensure food security, and utilise agricultural organic material for liquid biofuels. One of such global environmental challenges is the degradation of soil structure by the growing levels of salinization, which threatens the natural ecosystems and biodiversity, decreases agricultural productivity and is considered an acute crisis for the welfare of human life.

In this regard, *Sorghum bicolor* L. has emerged as a promising crop that can be utilised as a bioenergy feedstock and a source for bioethanol production due to its high fermentable sugar accumulation in stems or as a valuable source of food for its capacity to store carbohydrates as starch in the seeds. In addition, sorghum showed an amiable ability to withstand the conditions of marginal lands, such as coastlines. The versatile usage of sorghum provides several valuable candidate genotypes to improve its stress tolerance, boost crop yield, biofuel production and extend its range.

In this study, we have chosen a contrasting pair of sorghum genotypes; that respond differently to salt stress; the sweet sorghum genotype “Della” and the grain sorghum genotype “Razinieh” utilising standardised experimental systems. The first part of the thesis investigated the delineation between salt-driven damages versus adaption events between the two genotypes in a standardised hydroponic system. The two genotypes were compared and studied on the levels of morphology, physiology and molecular biology. First, we followed the sodium accumulation pattern with fluorescent visualisation in the root. This part included examining the transcripts levels of key genes related to sodium ions transport and cellular homeostasis and measuring important marker metabolites, hormones, and oxidative stress parameters. We find that the salt-tolerant genotype Della transferred sodium to the shoots by 7-folds less compared to its susceptible counterpart Razinieh. Based on our results, the difference in sodium accumulation in the shoot is correlated with the superior vacuolar Na^+ sequestration in the distal elongation zone in Della, as indicated by CoroNa fluorescent dye. The leaves of Della showed more efficient redox homeostasis, with swift

proline accumulation and rapid and specific activation of superoxide dismutase and ascorbate peroxidase enzymes while maintaining an efficient sucrose transfer to the roots. Meanwhile, in Razinieh, the rapid and high amplitude in glycine accumulation in leaves accompanied by high H_2O_2 are considered as readouts of high photorespiration rate under salt stress.

In conclusion, the slower rate of sodium ion transport to the shoot in Della lowers the potential damage of the photosynthetic apparatus, giving it more time to produce higher levels of hexoses in the leaves while simultaneously transferring more sucrose to the roots. This situation in Della suggests longer-lasting photosynthesis and improved redox homeostasis, which improves salinity tolerance. This data came with a model hypothesising that a rapid systemic signal generated by Della roots allows gaining time for anticipative adaption measures to occur in the leaves before ionic stress starts.

Further research was performed in the second part of the thesis to compare sugar partitioning and sucrose mobilisation, in particular between sweet sorghum genotype "Della" versus grain sorghum genotype "Razinieh" under salt stress at flag leaf stage. Sucrose is produced in mature leaves (source), loaded into the phloem, and transported to storage tissues (sinks) for growth and development. However, the storage and mobilisation of sucrose differ among the different sorghum genotypes. *Sorghum bicolor* L. includes genotypes that store non-structural carbohydrates as starch in seeds (grain sorghums), hence producing high grain yields, as well as sweet types (sweet sorghums) that accumulate considerable amounts of soluble sugars in their stems. Although the phenotyping of grain and sweet sorghum differs but both are transporting sucrose apoplasmically utilising different types of transporters such as; sucrose will be eventually exported transporters (SWEETs) and sucrose transporters (SUTs) proteins. It is likely that SWEET and SUT proteins play pivotal roles in phloem loading in source leaves and unloading in sink tissues in all sorghum ecotypes.

This part of the study aimed to characterise the differences in carbohydrate accumulation and partitioning under salt stress between a sweet and a grain sorghum genotypes model, which are known to respond differently to salt stress, and to define the roles played by SbSWEETs and SbSUTs in sucrose accumulation in different plant parts. In addition to dissecting upstream signalling controlling the regulation of promoters of candidate sucrose transporters genes.

Abstract

The results we have show that the sweet sorghum genotype “Della” out-performed the grain sorghum genotype “Razinieh” in terms of stem sugars accumulation as indicated by °Brix “total soluble sugars accumulation”. Meanwhile, Razinieh out-performed Della in terms of the high grain yield under salt treatment. In addition, sucrose partitioning differed between the two genotypes under salinity treatment, indicating different preferential sinks. Sucrose mobilisation to the roots in Della under salinity was mostly prioritised, while it was more accumulated in the middle internodes in Razinieh.

We visualized a model comparing sucrose phloem loading and unloading and movement to different sink tissues between the two genotypes, highlighting the predicted roles for SbSWEETs and SbSUTs proteins based on the transcriptional level of corresponding genes. We found that, the most significant alterations in *SbSWEETs* and *SbSUTs* expression were detected in sink tissues of both genotypes, suggesting that the significant effect of salt stress on sucrose unloading to sink tissues is mediated by SbSWEETs and SbSUTs proteins. Second, for dissecting the upstream signalling driving the activation of *pSbSWEET13*, and *pSbSUT6* promoters, we used a dual-luciferase reporter system in sorghum protoplasts suspension. We show an elevated responsiveness of *pSbSUT6* promoter from both genotypes to MeJA, but only *pSbSWEET13* promoter from Della that was induced by ABA. Our findings about the jasmonate and ABA regulatory mechanisms that connect stress sensing and sugar transport have provided insight into the crossover from signalling to stress adaption, that can be addressed for salt tolerance breeding.

1. Introduction

1.1. Environmental threats are affecting agriculture and food availability

Over the previous two decades, natural and artificial drivers have contributed to accelerating climate change. The greenhouse gasses emissions such as Co_2, methane, cause changes in climate by trapping heat which causes global warming and increase the global mean temperature. The subsequent fluctuation in precipitation patterns resulted in rising sea level, changes in evapotranspiration, overwintering of pathogens, increased pest and parasite resistance and decreased plant productivity. However, the high temperature influences precipitation patterns differently depending on the geographical region; it might lead to flooding or drought, Such that the decrease in precipitation affects soil properties, the availability of minerals and increases salinisation (Gelybó et al., 2018).

The resulting drought and soil salinity syndromes are two key environmental issues that are lowering agricultural production around the world. It is expected that, by 2050, drought and salinity occurrences will aggravate to affect almost 50% of total arable land (Chen et al., 2021; Vinocur & Altman, 2005). Parallel to this, the global population is exponentially growing and is predicted to reach 9.3 billion and food requirements are expected to escalate by 85% (FAO 2017).

Understanding the processes of plant salt tolerance is essential for improving agricultural outputs and meeting the nutritional needs of an expanding population and the primary step in this process is identifying salt-stress sensors in plants (Wang et al., 2022). Nevertheless, developing new agricultural practices that rely on crops with low water requirements should be possible to mitigate the adverse effects of prolonged drought and precipitated salt in the soil.

1.2. What is salinity? A challenge to agriculture and the economy

Salinity is a global problem and a critical ecological condition that has a substantial impact on all living forms of the planet. salinity syndrome occurs when soluble salts in soils increase, causing electrical conductivity of the soil to rise to >4 dS/m, which is equivalent to NaCl concentrations above 40 mM and osmotic pressure of approximately 0.2 MPa. Globally, 833 million hectares are

salt-affected soils, which is equivalent to 8.7 % of the planet (FAO, 2021). Globally, soil salinisation destroys about 6% of the cultivated land, with an annual increase of 1–2%, resulting in severe yield losses of staple grain crops, including maize, rice, and wheat (Munns & Tester, 2008). The annual global cost of crop production losses due to salt-induced land degradation in irrigated areas is estimated to reach USD 27.3 billion (Qadir et al., 2019). The direct cost of salinity is the loss of money to farmers due to the low yield. Salinity occurs in arid or semiarid areas; thus, crops are constantly limited by water, but they can also be affected by accumulating salt levels in the soil, particularly when rainfall is less than average (Munns, 2002).

In contrast to other extreme stresses like drought or flooding, the effects of salinity are gradual and hence are concealed. The higher rate of urbanisation, along with the use of primary lands for non-food production (such as the production of fibres and biofuels), is expected to further push agricultural production into marginal areas which are unsuitable for modern crops (Shabala, 2013). Consequently, a qualitative breakthrough in efforts to generate salt-tolerant germplasm is required to meet the challenge of feeding 9.3 billion people by 2050.

1.3. What are the causes of salinity?

Depending on the source, the process of soil salinisation might be primary (natural) or secondary (human-induced). Primary or natural salinisation can be caused by various factors, resulting in salt accumulation in the soil or groundwater over time. I) It can be caused by natural processes such as mineral weathering and soil formation from saline parent rocks. II) sea-level rise because of the increased global warming and melting glaciers and ice sheets, causing seawater to expand thermally. The consequence of rising sea level is that saltwater intrusion affects surface and ground freshwater resources. III) other climatic factors such as low precipitation and the high rates of evapotranspiration accompanied by high-temperature results in salt accumulation on the soil surface (Ullah et al., 2021)

The second factor causing salinisation results from anthropogenic activities changing the soil's hydrologic nature, including; i) irrigation practices that use salt-rich groundwater or have poor drainage systems of natively saline soils in (semi) arid areas. II) recently increasing damming

activities resulted in sediment movement fluctuating in the downstream plains, resulting in seawater intrusion into coastal aquifers (Hzami et al., 2021).

In some case, the deterioration in water resources quantity in the Nile Delta region in Egypt is being alleviated by the frequent reuse of agricultural drainage water, which in turn impact the overall water quality used for irrigation and increases the precipitation of salt and contaminants in soil (Hegazy et al., 2020). Nevertheless, it is anticipated that the construction of the Grand Ethiopian Renaissance Dam (GERD) will cause a further increase in the water budget deficit to one-third (+34%) if filling the reservoir is done within a short term of three-year. This will result in a loss in the cultivated land area by 72% in the next decade and an increase in the dependence on groundwater as a primary source of irrigation (Heggy et al., 2021).

Based on the level of adaption to saline environments, plants can be classified into two broad groups:

Halophytes are a group of plants that grow natively in saline environments and require high salt concentration to reach maximum growth that reaches 200 mM NaCl and represents 1% of the world's flora (Flowers & Colmer, 2008). Many halophytes can thrive in pure or concentrated seawater (mangroves), with NaCl molarities reaching 500mM (Atwell et al., 1999).

Meanwhile, glycophytes are plants that comprise most of the economic crop species. They are unable to reproduce in high saline environments (> 100mM) and eventually die. Some glycophytes have evolved diverse adaptation mechanisms to limit the damaging effects of salt stress, and as a result, they are mainly the focus of research interest. Adaptation usually refers to a level of resistance that is genetically inherited and gained via a long period of selection (Taiz and Zeiger, 2010).

1.4. What are the effects of salinity on plants? Double-phase stress

It is very critical to distinguish between the two-phase growth responses to salinity for screening plants for salt tolerance. The delineation in this time frame between the two phases was established by Munns (1993).

1.4.1. Phase1: osmotic stress component

The presence of salt outside the plant roots causes the initial phase of the growth response. It is sensed by the plants immediately when the salt concentration in the soil solution reaches the approximate threshold value of 40 mM NaCl for most plants or even less for sensitive plants such as rice and Arabidopsis. The increasing salt concentration in soil solution limits the plant's ability to absorb water, resulting in slower growth and is presumed to be controlled by hormonal signals derived from the roots. Such disruption in the plant-water relationship results in an instant but temporary inhibition in cell proliferation rates in the leaves and, to a lesser extent, in the roots (Munns, 2002). Most of the cellular and metabolic events involved in the osmotic phase are shared with the drought-induced, without excessive build-up in Na^+ and Cl^- ions to toxic levels in plant tissues (Munns, 2002, 2005).

1.4.2. Phase 2: ionic stress component

The second phase of growth reduction results from sodium ion toxicity which takes more time (days, weeks or months) to develop. The salt injury occurs when sodium ions enter the transpiration stream and eventually accumulate to excessive levels in transpiring leaves, beyond the cells ability to compartmentalise salts in the vacuole. Ions then rapidly accumulate in the cytoplasm, inhibiting enzyme activity, or accumulate in the cell walls and cause cell dehydration (Flowers & Yeo 1986; Munns, 2002, 2005).

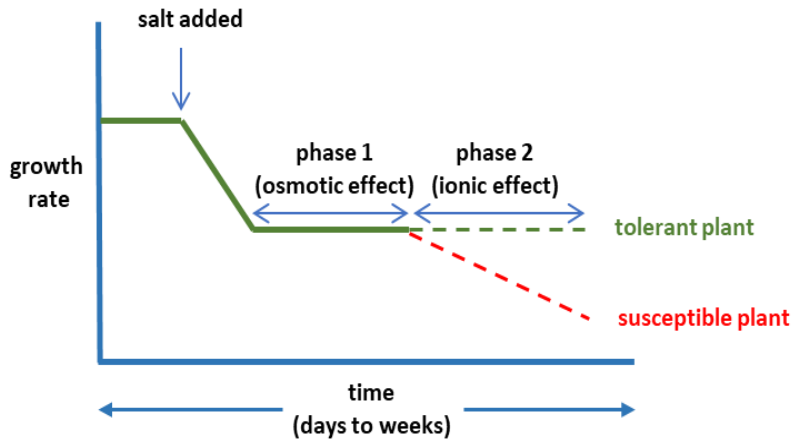

Figure 1.1. Schematic illustration representing the two-phase growth response to salinity (Munns, 2005).

1.5. What are salinity sensing mechanisms?

Plants as sessile organisms, need to adapt in various ways to acclimate to saline environments. These include several signalling transduction pathways that are involved, from salt stress sensing to the regulation of many salt-stress responsive genes to regulate processes such as stomatal closure, ion transport, osmotic balance, and ROS detoxification (Van Zelm et al., 2020; Zhao et al., 2021)

High salinity generates two primary stresses in plants: osmotic and ionic, both of which cause secondary stresses such as oxidative stress (Yang & Guo, 2018; Zhu, 2001). Plants have evolved a range of sensors and receptors (i.e., stress-sensing components) that detect osmotic, ionic, and reactive oxygen species (ROS) signals to activate downstream signal transduction pathways and prevent salt damage (Huang *et al*., 2012; Mukarram *et al*., 2021; Novaković *et al*., 2018). These stress sensors are found on the cell surface, in the endomembrane system, and/or in the cytoplasm (Zhu, 2016).

But what is a sensor?! According to the Oxford definition, a sensor is "a device that detects or measures a physical attribute and records, identifies, or otherwise responds to it". This general term does not identify the sensing process timeline. However, in biology, a cellular or tissue-based sensor should identify either a protein or any other molecule that can respond to environmental changes (stress) and then encode (in the broadest sense of the term) this information into an orchestrated cascade of physical, chemical, and molecular events aimed at optimising the organism performance under different conditions (Shabala et al., 2015). According to several researchers, an absolute stress sensor should have three essential characteristics: (i) the ability to detect stimuli within or outside the cell and to have a unique process for detecting and transducing stress signals; (ii) to be directly affected by the perceived stress either in structural characteristics or activity to trigger signal transduction ultimately; and (iii) its actions must lead to physiological and morphological adaptation of the plant to the stress (Lamers et al., 2020; Vu et al., 2019; Wang et al., 2022).

The possible sensors included in salt stress can be divided into osmotic stress sensors and ionic stress sensors, depending on their mechanisms for the specific temporal and spatial activation needed. This results in the effects of osmotic and ionic components of salt stress are assumed to be separated temporally and spatially. Such that the effect of sodium ions in the soil in limiting

water availability is happening within seconds; meanwhile, the movement of sodium to the shoot hindering photosynthesis is slower. While there is a significant overlap in early and downstream signalling between osmotic and salt stresses, recent observations of both rapid salt-specific signal transduction and sodium-induced growth responses in roots could call this notion into question (Van Zelm et al., 2020).

1.5.1. Sensing the osmotic stress component

When plants encounter salt stress, they first sense the rapidly generated osmotic stress by high salinity and respond immediately. Osmotic stress causes cells to lose their water potential and become dehydrated, which disturbs essential metabolic activities in the cell. Plant cell osmotic receptors detect and transmit osmotic stress signals, triggering downstream gene expression and controlling the biosynthesis of osmotic protective metabolites to prevent cell dehydration (Wang et al., 2022). In the following, we are reviewing different types of osmosensors in plants:

1.5.1.1 Aquaporins

Plants are modifying their water status regularly in response to changing environmental conditions, and aquaporins (AQPs) play a key role in this process. Aquaporins are a type of channel protein that transports water, small molecules, and rarely ions across membranes (Chaumont & Tyerman, 2014; Murata et al., 2000). The reduction in plant hydraulic conductivity under water-related stress has been reported to be a typical response mediated by AQPs, which have been hypothesised to function as osmosensors (MacRobbie, 2006). For example, exposure of Arabidopsis plants to salt (100 mM NaCl) resulted in a significant decrease (70%) in hydraulic conductivity (*Lpr*) combined with overall transcriptional downregulation of aquaporins and in the abundance of AtPIP1 proteins after 30 minutes from exposure to salt (Boursiac et al., 2005).

Hill et al. (2004) proposed that aquaporins serve as osmotic and turgor pressure gradient detectors, communicating information to signalling chains. Aquaporins are composed of tetramers, and each monomer's membrane is in an hourglass form with a narrow channel in the centre filled with water and flanked by an inner and outer atrium. This makes osmotic solutes to exclude from the atrial, causing negative pressure, and promoting channel protein deformation. The presence of an osmotic gradient produces an unequal strain and deformation in the two parts of the molecule, which is the

driving force for water flow down the central channel. Similar asymmetric deformations will result from a pressure gradient across the membrane. As a result, each monomer may sense pressure or osmotic gradients via its protein structure. The work on aquaporins NOD26 in root cortical cells in Cara plant shows changes in water permeability in response to pressure or osmotic conditions (Vandeleur et al., 2005; Wan et al., 2004; Ye et al., 2004, 2005). As a result, aquaporins are considered one of the osmotic gradient sensor candidates that trigger the quick hypoosmotic transient (for review see Ismail et al., 2020).

1.5.1.2 Mechanosensitive Channels (MSCs)

Mechanical stimulation does not only allow cells to survive, but it also helps them to build their own architecture and perform biological activities according to the needed context. For example, a mechanical stimulus such as osmotic stress can modify the turgor pressure of plant cells which causes dehydration and deformation. This helps to understand why cells and organisms developed mechanosensitive channels (MSC_S). Mechanosensitive channels may operate as mechanosensors, sensing cell deformation and responding to osmotic stress-induced changes resulting in Ca^{2+} influx (for review see Ismail et al., 2020).

MSCs are interesting proteins because they can function as both sensors and effectors. They are embedded in the plasma membrane, positioned between the cell wall and the cytoskeleton. Thus, they can translate mechanical stimuli like membrane tension and curvature into electrical or biochemical signals, allowing adaptive response by regulating a diverse range of cellular functions. MSC_S are directly triggered by mechanical stimuli and transform mechanical force into electrical trans-membrane potential change in milliseconds. As a result, MSC_S are the fastest transducers known in biological systems for review see (Peyronnet et al., 2014).

1.5.1.3. Protein Kinases

Stress sensing and Intracellular signal transduction achieved through phosphorylation of signalling proteins by protein kinases is a critical step in plant responses to different stress conditions (for review see Chen et al., 2021; Zhao et al., 2021). Protein kinases modify the activity of their substrates by catalysing the reversible transfer of γ-phosphate from adenosine triphosphate (ATP) to a particular amino acid (Ser, Thr, or Tyr) on their target proteins, thus changing their activity causes downstream signal transmission (Stone and Walker, 1995). Under drought and salt stress,

several protein kinases have been identified as osmotic sensors, including Histidin kinase 1 (HK1) from Arabidopsis. When AtHK1 was overexpressed in the yeast double mutant sln1 sho1, which lacks two osmosensors, it could survive in high salinity media and had high osmotic stress tolerance by transmitting the stress signal to a downstream MAPK cascade, suggesting that HK1 has a role in sensing and transducing osmotic stress signals thus, modifying stress responses (Urao et al., 1999).

Regulation of osmotically driven stomatal movements in response to environmental stimuli mostly are mediated by protein kinases such as SnRK2s. Grondin et al. (2015) demonstrated that open stomata 1 (OST1; also known as SnRK2.6) in Arabidopsis can phosphorylate PIP2;1 at Ser121 in an ABA-dependent way to regulate the water transport activity and increase guard cell water permeability**.** During ABA signalling, SnRK2s can directly phosphorylate and activate downstream targets like transcription factors such as; Abscisic acid responsive element-binding factor (ABF)-type (Fujita et al., 2013) and the anion channel Slow anion channel 1 (SLAC1) (Geiger et al., 2009).

1.5.1.4. Hormonal regulation – ABA and JA play the music

1.5.1.4.1 ABA biosynthesis and mode of action

Plants as sessile organisms, communicate under stressful conditions by chemical signals to achieve defence along with development. Phytohormones are small molecules and chemical signals that regulate plant growth and development and respond to sudden environmental stimuli, either in situ or remotely distanced from their site of synthesis (Colebrook et al., 2014; Davies, 2016). Thus, plants can alter the signal transduction and production of "stress hormones" under stressful conditions to stimulate protective measurements. For example, ABA mediates stomatal closure as an immediate consequence of the reduction in water potential around the roots (Christmann et al., 2013), and the ability to cope with salinity stress correlates with the swiftness and amplitude of ABA (Ismail *et al.*, 2014b). Not only ABA, but other endogenous hormones such as; (jasmonates) JAs, IAA, Brassinosteroids (BRs), Gibberllins (Gas), and Ethylene (ET), are suggested to "cross-talk" by performing a signalling network between roots and shoots under stress conditions (Jackson, 1997). When plant roots are subjected to osmotic stress, ABA concentration in both root and leaf tissues increases significantly and swiftly (within a few minutes).

ABA biosynthesis is first committed through the cleavage of β-carotene (C40) in plastids to zeaxanthin (see Figure 1.2). Then, zeaxanthin is catalysed into violaxanthin by zeaxanthin epoxidase (ZEP). After that neo-xanthin synthase (NXY) is catalysing the conversion of violaxanthin to neo xanthin and 9-*cis*-violaxanthin. Then, the oxidative cleavage of neo xanthin and 9-*cis*-violaxanthin, catalysed by the enzyme "9-*cis*-epoxy carotenoid dioxygenase" (NCED), which produces a C15 intermediate product called xanthoxin. The product xanthoxin is exported to the cytosol where xanthoxin is converted to ABA in two enzymatic reactions. First, xanthoxin is converted to an ABA aldehyde by an enzyme called short-chain alcohol dehydrogenase/reductase (SDR). The final step is the oxidation of the abscisic aldehyde to ABA, catalysed by the abscisic aldehyde oxidase (AAO) (for reviews see Asad et al., 2019; Dar et al., 2017; Sah et al., 2016).

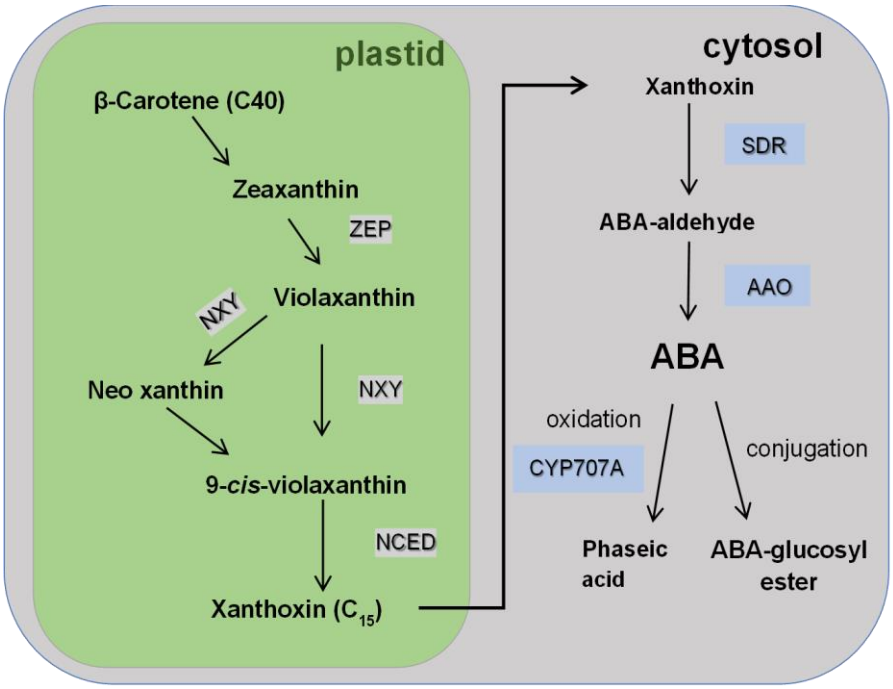

Figure 1.2. ABA biosynthsis and catabolism. Abbreviations: ZFP, zeaxanthin epoxidase; NSY, neo xanthin synthase; NCED, 9-cis-epoxycarotenoid dioxygenase; AAO: abscisic aldehyde oxidase; SDR, short-chain dehydrogenase/reductase; CYP707A, ABA 8′-hydroxylase (Figure modified from (Asad et al., 2019)).

Then ABA signalling process starts with the binding of ABA to the PYR/PYL/RCAR receptor, which inhibits the activity of the protein phosphatases 2C (PP2C), releasing a phosphorylation cascade of Ser/Thr kinases (SnRK2) (See **Figure 1.3**). Then, SnRK2s drive the activation of

various anion efflux channels leading to a decrease in turgor pressure and stomatal closure (Osakabe et al., 2014). Not only this, but it also culminates in activating bZIP transcription factors that will activate genes improving osmotolerance, such as LEA proteins or proline (for reviews see Banerjee & Roychoudhury, 2017; Joshi-Saha et al., 2011; Sah et al., 2016; Zarattini & Forlani, 2017).

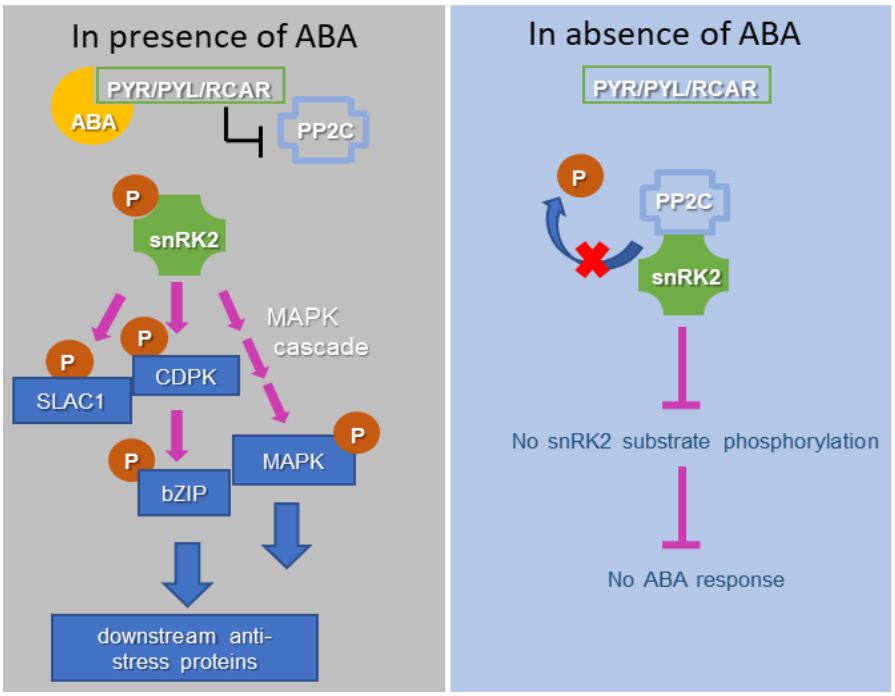

Figure 1.3. The schematic representation of the main ABA signalling pathway in plants with and without ABA presence. Abbreviations: ABA, Abscisic acid; MAPK, mitogen activated protein kinase; PP2C, protein phosphatase 2C; PYR, pyrabactin resistance; PYL, PYR-related; RCAR, regulatory component of ABA receptor; SnRK2, sucrose nonfermenting-1-related protein kinase 2.; SLAC1, slow anion channel 1; CDPK, Calcium-dependent protein kinase; bZIP, basic leucine zipper transcription factor (Figure modified from (Sah et al., 2016)

The origin of the ABA signal is still questioned, even though the involvement of ABA in salinity-induced stomatal closure is without dispute. It was long believed that ABA is produced in osmotically stressed roots and then quickly transferred to the shoot with the transpiration stream (Jiang & Hartung, 2008; Wilkinson & Davies, 2002). However, recent research suggested that stress-induced stomatal closure might not necessitate ABA transport from root to shoot. The NCEDs genes, which encode the first step of ABA biosynthesis, are primarily expressed in the vascular parenchyma of leaves (Endo et al., 2008) and are quickly upregulated by osmotic stress.

1.5.1.4.2. JA biosynthesis and mode of action

Jasmonates (JAs), are collectively referred to as Jasmonic acid (JA) and its related compounds, including the volatile fragment-methyl jasmonate (MeJA). They are widely distributed lipid-derived compounds that operate as a master switch to control plant response to several biotic and abiotic stresses such as drought and salt stress, and pathogen infection, and insect attack (Riemann et al., 2015; Wasternack & Hause, 2013; Zander et al., 2020).

JA biosynthesis is initiated first in the plastids i) by the formation of 13-hydroperoxylinolenic acid (13-HPOT) from triunsaturated fatty acids (18:3) through lipoxygenases (13-LOXs) (See **Figure 1.4**) (Bell et al., 1995; Wasternack & Hause, 2013). Subsequently, the generated substrates(13-HPOT) will be consumed in two-step enzymatic reactions inside the plastids by allene oxide synthase (AOS) and allene oxide cyclases (AOCs) to produce 12-oxo-phytodienoic acid (*cis*-OPDA). ii) consequently, *cis*-OPDA is transported into the peroxisome and is reduced from cyclopentenones to cyclopentanones by the OPDA reductase (OPR). The following steps involve three rounds of the ß-oxidation of OPC8 to produce (+)-7-*iso*-JA. After being transported into the cytosol, JA undergoes different modifications or conjugations to yield at least 12 different derivatives, such as jasmonic acid isoleucine (JA-Ile), 12-hydroxy-JA-Ile (OH-JA-Ile), 12-hydroxy-JA (OH-JA), 12-*O*-glucoside (12-*O*-Glc-JA), 12-HSO_4-JA and JA-methyl ester (MeJA). These metabolic byproducts of the jasmonate pathway all exhibit variable degrees of biological activity (Koo, 2018; Wasternack & Hause, 2013).

The biologically active JA conjugate with the amino acid Ile, produced from the inactive JA by JAR1, is necessary for JA signalling. It is anticipated to diffuse into the nucleus, where it can bind to COI1 –JAZ receptor complexes to activate the jasmonate signalling pathway (Staswick & Tiryaki, 2004).

The degradation of a transcriptional repressor is as part of JA signalling (See **Figure 1.5**). The jasmonate ZIM/TIFY-domain (JAZ/TIFY) proteins, also known as JAZ proteins, are the repressor in JA signalling. JAZ proteins are degraded in a SCF (for SKP1 -CUL1- F-box)-type ubiquitin ligase SCF^{COI1}-dependent manner via the 26S proteasome in response to increased JA levels as a result of stimulation by various stress factors. This results in the swift activation of JA responses, such as the expression of JA-responsive genes (Chini et al., 2007; Pauwels & Goossens, 2011; Thines et al., 2007).

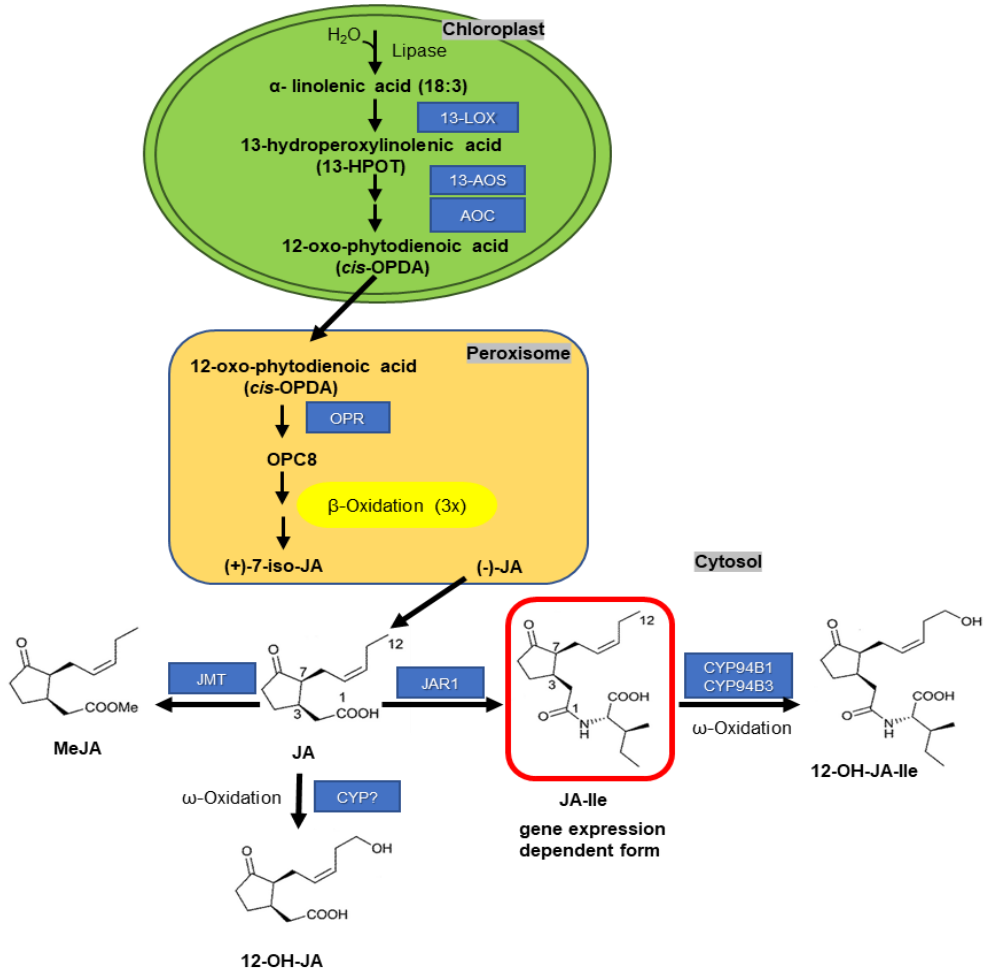

Figure 1.4. Biosynthesis and enzymatic modifications of jasmonic acid. Abbreviations: 13-LOX, 13-Lipoxygenase; AOS, Allen oxide synthase; AOC, Allen oxide cyclase; OPR, OPDA-Reductase; JA, Jasmonic acid; JA-Ile, jasmonic acid isoleucine; MeJA, Methyl jasmonate; JAR1, JASMONATE RESISTANT1; JMT, JA-Methyltransferase; 12-OH-JA-Ile: 12-hydroxy-JA-Ile; 12-OH-JA, 12-hydroxy-JA. (Figure modified from (Dhakarey et al., 2016; Jimenez-Aleman et al., 2019; Peethambaran, 2017).

On the contrary, with fewer JA-Ile molecules - the resting state - MYC2 is linked to the G-box in the promoter region of the JA-responsive genes, and then JAZ repressors bind to MYC2 to inhibit transcription (**Figure 1.5**). In addition, the NINJA adaptor protein binds the Corepressor TPL to the JAZ proteins. Again, as soon as the action of JA-Ile dislocates JAZ repressors, transcription factors such as MYC2, MYC3 and MYC4 are librated, triggering early JA responses.

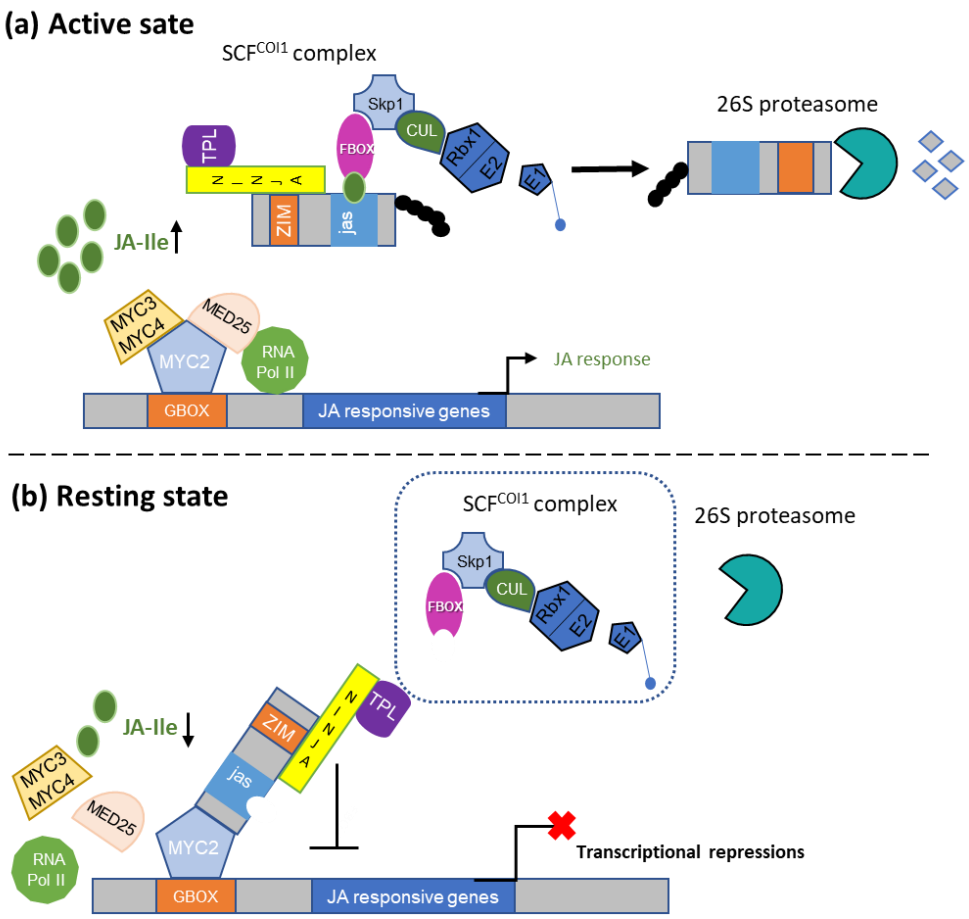

Figure 1.5. Jasmonate biosynthesis and signalling. a) The active state with an abundance of JA-Ile, b) The resting state when JA-Ile is scarce. Abbreviations: JA-Ile, jasmonic acid isoleucine; JAZ: JASMONATE ZIM DOMAIN, NINJA, Novel of Interactor of JAZ; TPL, TOPLESS; SCF, Skp1 / Cullin / F box complex; COI1, CORONATINE INSENSITIVE 1; MYC3, MYC4: bHLH zip transcription factor; MED25, Mediator of RNA polymerase II transcription subunit 25, (Figure modified from (Peethambaran, 2017).

Nevertheless, the accumulation of ABA is controlled by jasmonic acid at several levels, especially under salinity (reviewed in Ismail *et al.*, 2014b). However, the fact that rice mutants lacking the ability to accumulate JA, are still able to accumulate almost the same levels of ABA as the wild type, when they are challenged by osmotic stress demonstrates that JA-independent pathways exist as well (Tang *et al.*, 2020). While JA is undoubtedly not the only upstream regulator of ABA accumulation (at least with respect to osmotic stress), the decision between adaption and programmed cell death in response to salinity depends on the temporal signature of JA signalling. A rapid, but transient signature is followed by efficient accumulation of ABA triggering protective events that lead to cellular adaptation, while a sluggish, but persistent signature of JA signalling

culminates in cell death (reviewed in Ismail *et al.*, 2014b). As proof of concept for this signature model, rice was engineered with a tailored version of the JA signalling protein JAZ8 that is dominant-negative with respect to proteolysis, enforcing a transient JA signature. In fact, these transgenic plants exhibited elevated salinity tolerance (Peethambaran *et al.*, 2018).

1.5.2. Sensing the ionic stress component

1.5.2.1. First, sodium import and sensing

It has been hypothesised that plants detect osmotic changes rather than sodium ions; meanwhile, sodium-specific responses occur much later as a result of sodium (or chloride) toxicity on the leaves (Munns & Tester, 2008). However, Choi *et al*. (2014) identified rapid salt-specific calcium waves in Arabidopsis roots. Furthermore, the sodium-specific effect on root growth direction (halotropism) suggests the incidence of root-based sodium sensors (Galvan-Ampudia et al., 2013). Usually, spatial sodium sensing can be intercellularly, extracellularly, or at the plasma membrane via ion transporters (Lamers et al., 2020). Recently, (Jiang et al., 2019) identified monovalent cation sensors "monocation-induced [Ca^{2+}] increases 1 (MOCA1)" which function in extracellular Na^{+} sensing. MOCA1 produces glycosyl inositol phosphoryl-ceramide (GIPC) sphingolipids at the plasma membrane, which sense and bind Na^{+} under high concentration leading to Ca^{+2} inward channels gating and increases cyt Ca^{+2} concentration, which consequently upregulates the Ca^{+2} -related downstream processes, for review see (Ismail et al., 2020).

Increased salinity can cause Na^{+} to displace pectin-bound Ca^{2+} in vitro and interrupt pectin cross-linking (Feng et al., 2018; Munarin et al., 2012). Interestingly, FERONIA (FER), a receptor-like kinase (RLK), is predicted to sense the decreased cross-linking of pectin directly or indirectly perceive the changes in the cell wall structure through leucine-rich repeat extensins and the peptides rapid alkalinisation factor (RALF) 22/23 (Zhao et al., 2020). As a result, *fer* mutants show a significant reduction in the late-stage of Ca^{2+} spikes compared to wildtype, as well as increased cell swelling and eventually bursting close to the root tip. Consequently, exogenous calcium or borate supplementation can improve the *fer* phenotype by enhancing pectin cross-linking (Feng et al., 2018). The previous findings help to understand that cell wall reinforcement is activated and maintained throughout growth recovery owing to FER-dependent late-induction of Ca^{2+}. Although

downstream signalling of FER receptors happens several hours after salt treatment, but it is needed to stimulate critical responses to salt stress, which will likely most result in salt adaptation and tolerance (Ismail et al., 2020; Van Zelm et al., 2020).

1.5.2.2. Ca^{2+} Ion spike

Calcium ions (Ca^{2+}), the most common universal second messenger, are required in a wide range of biological processes in all living forms, from bacteria to plants to specialised neurons. Despite their universality, Ca^{2+} signals are distinguished by their distinctive patterns generated by a substantial Ca^{2+} buffering capacity (Dodd et al., 2010). Ca^{2+}-modulated proteins and/or adaptors can detect these Ca^{2+} signals, which are later amplified by being released from membrane-enclosed organelles, particularly vacuoles. On the other hand, uncontrolled high calcium levels can trigger degradative processes or apoptosis. As a result, complex mechanisms such as Ca^{+2} -chelation, export, and compartmentalisation in different intracellular organelles such as the ER, chloroplast, mitochondria, and the vacuole tightly regulate intracellular Ca^{2+} levels as well as the spatial distribution of this signal (Bouché et al., 2005; Clapham, 1995). The usual Ca^{2+} level in the cytoplasm is 100-200 nM, while it is 1 -2 mM in the cell wall and organelles. Ion influx or efflux from the extracellular space (cell wall or apoplast in plants) or intracellular compartments (such as vacuole, chloroplast, or mitochondria) can generate such cytoplasmic Ca^{2+} (cyt Ca^{2+}) signals. In particular, the vacuolar Ca^{2+} release into the cytosol has been established in response to various signalling pathways, including biotic and abiotic stressors and hormone signals (Hirschi, 2001). For example, in terms of plant–water relations, Ca^{2+} signals have fundamental dual functions, which are at the same time paradoxical. Under non-stressful conditions, submicromolar Ca^{2+} concentrations can phosphorylate AQP SoPIP2;1 at Ser274 by a PM-associated protein kinase, resulting in fluxing water (Baral et al., 2015; Johansson et al., 1996); which makes resting cells have nanomolar Ca^{2+} concentrations in the cytosol (100–200 nM) (Clapham, 2007). Activating AQPs, on the other hand, can cause cell turgor loss and plasmolysis in response to water stress. As a result, plants first aim is to prevent, or at the very least reduce water loss, and Ca^{2+} signals appeared to have in/directly role in regulating AQP gating under osmotic stress conditions.

Interestingly, the propagation of long-range calcium waves was caused by high salt, but not by osmotic stress treatment. As already described, GIPC produced by MOCA1 can be bound by monovalent cations and initiate a Ca^{2+} influx; however, the involved Ca^{2+} channel remains to be

identified (Jiang et al., 2019). To mediate the dissipation of Ca^{+2} signal, calcineurin B–like proteins (CBLs) bind calcium and promote protein phosphorylation through their interaction with CBL-interacting protein kinases (CIPKs). Various CBL-CIPKs have been discovered to coordinate such a set of cellular responses to sodium by decoding the produced Ca^{2+} signals (Manishankar et al., 2018). The most well-studied CBL-CIPK pathway is the salt overlay sensitive (SOS) pathway. Calcium is first detected by SOS3/CBL4 (Jiping Liu & Zhu, 1998), and then the latter attaches to SOS2/CIPK24 (Halfter et al., 2000; Jiping Liu et al., 2000). Then, the H^+/cation antiporter SOS1/NHX7 (Halfter et al., 2000), which can transport sodium out of the cell, is phosphorylated by the SOS2-SOS3 complex.

In summary, Ca^{2+} is a crucial signalling molecule at the cellular level throughout the plant. Ca^{2+} signals are complex, as they regulate not only Na^+ but also other critical secondary messengers like H^+ and ROS, as well as hormones. Furthermore, Ca^{2+}-signals are self-regulated to ensure action specificity and prevent Ca^{2+}-induced cell death.

1.5.2.3. Proton (H^+) influx

Protons (H^+) play an important role in cell signalling, either directly or in coordination with phytohormones or Ca^{2+} signalling (Gao et al., 2004) reviewed in (Ismail et al., 2014; 2020). The proton is a multifunctional cellular component that plays a role in plant growth and development (Falhof et al., 2016; Palmgren, 2001). Furthermore, H^+ entry occurs rapidly (within seconds) as a downstream of Ca^{2+} signals, most likely through PM non-selective cation/anion channels and is expected to function as a second messenger under stress (Gao et al., 2004; Geilfus & Mühling, 2013; Maathuis, 2014; Monshausen et al., 2009). For example, cytoplasmic alkalinisation can transmit methyl-JA (MeJA) and ABA signalling during stomatal closure in Arabidopsis (Suhita *et al*., 2004), and is also involved in plant responses to salinity and drought stresses (Kurkdjian & Guern, 1989).

As a result, plants have evolved various techniques for utilising H^+ protons promptly while also avoiding its adverse effects. Plasma membranes are armed with several proton pumps that generate proton-derived pH gradients and, as a result, energise membranes with the necessary driving force for ion and metabolite transport (Geilfus & Mühling, 2013). Especially, proton pumps (P-type H^+-ATPases) that extrude H^+ out of the cytosol in concert with the vacuolar V-type H^+-ATPases (V-

ATPases) and V-pyrophosphatases (V-PPases) (Hedrich, 2012; Kriegel *et al*., 2015). Usually, PM H^+-ATPases (12 members of the Arabidopsis PM H^+-ATPases, AHAs) acidify the apoplast in non-stressed conditions, supporting cell development (Wolf et al., 2012). However, when plant cells are disrupted under stressful situations, the opposite occurs. For example, the proton influx will happen simultaneously with calcium, and the apoplastic alkalinisation that results have been used extensively as a significant marker for the rapid activation of calcium influx channels either by elicitors (Felix et al., 1999) or by abiotic stresses such as salinity stress (Geilfus & Mühling, 2013; Ismail et al., 2012; 2014). Comparing two Vitis cell lines with different salt tolerance showed that efficient adaptation in *V. rupestris* was associated with a faster and more long-lasting apoplastic alkalinisation than the salt-sensitive *V. riparia* (Ismail et al., 2014). It's also worth noting that removing protons from the apoplast liberates anionic binding sites for sodium ions. Simultaneously, the high steady-state level of apoplastic superoxide as a significant second signal will be enhanced (Ismail et al., 2014). In summary, proton influx can improve early sodium and calcium signals even though it does not function as an independent signal.

1.5.2.4. Reactive oxygen species (ROS)

ROS are unavoidable byproducts of aerobic plant metabolism that can become hazardous if no measures are adopted. ROS are generated regularly in plant compartments such as mitochondria, chloroplasts, and peroxisomes. They were once assumed to be damaging byproducts of aerobic metabolism, but they are now recognised as key participants in a cell-sophisticated signalling network (Miller et al., 2010). Plants have evolved essential ROS-scavenging techniques and the ability to generate ROS as essential cellular second messengers. Recent findings revealed an essential role of ROS signalling in a variety of signal transduction pathways mediated by temporal-spatial coordination of ROS and other signals that lead to the production of stress-specific molecules, compounds, and hormones reviewed in (Baxter et al., 2014; Gilroy et al., 2014; Kimura et al., 2017).

The accumulation of ROS in the apoplast under stress, are triggered by the within-second stimulus-induced increases of cyt Ca^{2+} (Baxter et al., 2014; Knight et al., 1997; Monshausen et al., 2009). The PM NADPH oxidases, also known as respiratory burst oxidase homologues (RBOHs), are important players in the ROS generation network in plants (Suzuki et al., 2011; Torres & Dangl, 2005). First, superoxide (O^{2-}) is formed at the apoplast by the function of RBOH proteins, and by

the action of superoxide dismutase (SOD), it dismutases to H_2O_2 spontaneously or catalytically (Lin et al., 2009; Wi et al., 2012). Later, the membrane-permeable H_2O_2 can be used as a signalling molecule to control cellular metabolism during growth, development, and response to environmental stimuli (Sagi et al., 2004; Xia et al., 2009).

Recent studies revealed different regulatory mechanisms of RBOHD and RBOHF as well in regulating stomatal closure under abiotic stress. In Arabidopsis, Ca^{2+} binding and phosphorylation work together to activate RBOHD and RBOHF ROS-producing activities (Kimura et al., 2012; Ogasawara et al., 2008). The activation of RBOHD involves conformational changes in EF-hand motifs caused by Ca^{2+} binding, which requires a Ca^{2+} influx in the cytosol (Ogasawara et al., 2008). During ABA-dependent stomatal closure, OPEN STOMATA 1 (OST1) phosphorylates the RBOHF at Ser174 and Ser13 (Sirichandra et al., 2009). Taken together, these observations suggest that changes in regulatory mechanisms may account for some of the varied activities of RBOH signalling in plants.

1.6. What happens after the recognition of the stress? "Adaptive mechanisms to salinity"

Plants have adapted many response mechanisms to cope with abiotic stress, such as stress avoidance, tolerance, escape, and recovery processes (Chen et al., 2021; Fang & Xiong, 2015). After sensing stress conditions, plant cells initiate these reactions in response to stressful circumstances to restore cellular and organismal equilibrium. These mechanisms also mitigate long-term stress consequences (Mickelbart et al., 2015).

1.6.1. Stomatal closure

Stomatal aperture reduction is the most striking and first observed trait in plant reaction to salinity. Stomatal conductance is promptly impacted by the osmotic action of the salt outside the roots causes, initially and briefly due to disturbed water relations and then shortly after due to the local synthesis of ABA (Fricke et al., 2006).

Most researches show that environmental signals cause stomatal movement depending on coordinated changes to protect cell turgor (ionic fluxes and sugar), cytoskeleton organisation, membrane transport, and gene expression. Numerous research findings suggest that specific

stimulus signalling pathways may be insufficient for regulating stomatal opening (for reviews see Hetherington, 2001; Schroeder et al., 2001).

For example, AQPs play an essential role in stomatal behaviour for controlling the water status of the plant. For instance, stomatal closure under water stress requires increasing the water activity (water efflux) of the guard cell via AQPs; however, a direct connection is still lacking (Maurel et al., 2016). Grondin et al. (2015) reported that PIP2;1 might be phosphorylated at Ser121 in an ABA-dependent manner by open stomata 1 (OST1; also known as Snf1-related protein kinase 2.6; SnRK2.6). However, the stomatal regulation in the transgenic Arabidopsis mutants *pip2;1-1* and *pip2;1-2* was comparable to the wild type. In addition, Wang et al. (2015) found stomatal closure happens before the considerable increase in foliar ABA in *Vitis vinifera* during drought stress. These findings imply that stomatal closure is initiated by passive hydraulic cues but is maintained by ABA, although more concrete data is still required (Ismail et al., 2020).

1.6.2. Two levels of plant tolerance mechanisms to sodium ions

Since salinity is a widespread condition that has accompanied plant evolution from its beginnings, plants have evolved mechanisms to cope and survive with the challenges of salt stress, depending on the species, even on saline soils. One type of mechanism targets the distribution of sodium ions, such as: (i) reduced net rate of Na^+ uptake by roots (Jiang et al., 2019); (ii) extrusion of Na^+ to the apoplast; (iii) reduced transition of Na^+ into the transpirational stream, and (iii) sequestering Na^+ in the vacuole, to retain it in specific tissues while maintaining turgescence.

The second type of mechanism targets cellular adaptations, for instance, by synthesis of compatible osmolytes that will mitigate the gradient in water potential to the rhizosphere or by deploying enzymatic and non-enzymatic antioxidants that will help to restore redox homeostasis perturbed by invading sodium ions (Hoque et al., 2008; Nahar et al., 2016; Wu et al., 2019). Under certain circumstances, cells under the challenge of salinity can turn to programmed cell death rather than investing resources for local adaptation. While this response is deleterious to the cell itself, it may benefit the plant as an entity because resources can be allocated to the meristematic tissues that allow for regeneration once the stress episode is over (Li et al., 2007). In some cases, the dead organs can be shed, thus removing the ions accumulated in them.

1.6.2.1. Avoiding salt stress by controlling sodium distribution

The extracellular and intracellular ion balance of plant cells is disturbed by high salinity, and Na^+ build-up in the cytoplasm hinders K^+ uptake and has a detrimental impact on the entire metabolic processes. The resultant increase in cyt Ca^{2+} has the potential to cause cell death and, if fine-tuned, might also be employed as a signal to start the adaption process.

1.6.2.1.1. Sodium ion uptake

To understand the first type of adaptive response, it is necessary to comprehend the molecular mechanisms responsible for sodium transport (for review, see Keisham et al., 2018). Under normal physiological conditions, plants typically maintain a high cytosolic K^+/Na^+ ratio and a negative electrical membrane potential difference (-140 mV) across the plasma membrane. Under salt stress conditions, the rise in soil Na^+ concentration generates an electrochemical gradient that encourages passive Na^+ transfer from the soil into the cytosol (Blumwald et al., 2000). The accumulation of Na^+ ions around the roots facilitates Na^+ efflux into root cells by non-selective cation channels (NSCC) and high-affinity potassium transporters (HKTs) of subfamily (II) along the generated electrochemical gradient (Byrt et al., 2017).

The voltage-insensitive NSCCs (VI-NSCCs) class is considered the main route for sodium ions influx into the roots and therefore has been studied with regard to salt tolerance (Demidchik & Maathuis, 2007; Kronzucker & Britto, 2011). Several reports have demonstrated the permeability of Na^+ through VI-NSCCs in root cells (Demidchik et al., 2002; Maathuis & Sanders, 2001; Tyerman et al., 1997; White & Lemtiri-chlieh, 1995), as well as that Na^+ influx into intact tissues via $NSCC_S$ is partially hindered by Ca^{2+} and that it is sensitive to its blockers, such as quinine, but still needs further validation (Essah et al., 2003; Wang et al., 2015). In Arabidopsis roots, AtCNGC3 has been reported to be involved in Na^+ influx. A null mutation in AtCNGC3 has been shown to diminish the net absorption of Na^+ (40–80 mM) during the early stage of exposure to NaCl. However, exposure to NaCl (80–120 mM) causes both the wild-type (WT) and mutant seedlings to accumulate equivalent Na^+ concentrations (Gobert et al., 2006). These findings show that AtCNGC3 was involved in Na^+ absorption during the early phases of salt stress. Numerous studies have suggested that cyclic nucleotides can affect unidirectional Na^+ flux or net fluxes, supporting the idea that CNGCs are involved in Na^+ transport in plants (Essah et al., 2003; Maathuis, 2006).

1.6.2.1.2. Sodium ion exclusion

It is widely acknowledged that cytosolic Na^+ exclusion is essential for defending plants against salinity stress. Na^+ efflux from plant cells is an active mechanism when there are high external Na^+ concentrations (Blumwald et al., 2000). Thus, the ion exchange activity of Na^+ influx and efflux controls net Na^+ build-up in plant cells. To date, Na^+ export from the cytosol to the apoplast is described to be achieved by plasma membrane Na^+/H^+ antiporter "SOS1", which is mainly expressed in plant root apex (Shi et al., 2002). When cyt Ca^+ increases, it binds to calcineurin B-like protein (CBL4), also known as SOS3 and promotes its association with the protein kinase (CIPK24), also known as SOS2. This SOS3/SOS2 complex phosphorylates the Na^+/H^+ antiporter SOS1 located on the plasma membrane to initiate the Na^+ exclusion from the cytosol (Harper et al., 2004; Munns & Tester, 2008; Plasencia et al., 2021). According to Cuin et al. (2011), the salt-tolerant wheat variety "Kharchia" exhibited the highest root Na^+ exclusion ability of the eight studied wheat varieties. The transgenic plants ability to tolerate salt has been improved by overexpressing SOS1 (Yang et al., 2009; Yue et al., 2012). Meanwhile, the loss of SOS1 function caused a hyper-salt-sensitive phenotype in the halophyt *Thellungiella salsuginea* (Oh et al., 2009). This finding further confirmed the vital role of the SOS1 Na^+/H^+ antiporter in Na^+ exclusion and overall plant salinity tolerance.

1.6.2.1.3. Sodium ion compartmentalisation

Plants also can reduce Na^+ toxicity in the cytosol by storing Na^+ in the vacuole because the vacuole occupies the largest portion of the cell volume while most metabolism occurs in the cytoplasm. Na^+/H^+ antiporters mediate the vacuolar Na^+ sequestration, a common and significant process in plant salt tolerance (Apse et al., 1999; Mansour et al., 2003; Rahnama et al., 2011). Vacuolar Na^+ sequestration is a prerequisite to preventing cytoplasmic Na^+ elevation, maintaining the cytosolic K^+/Na^+ ratio, and regulating vacuolar osmotic potential in plants under salt stress (Maathuis & Amtmann, 1999). To date, NHXs Na^+/H^+ antiporters are the most well-known transporters for vacuolar Na^+ sequestration. Many species, including Arabidopsis (Apse et al., 1999), tomato (Zhang & Blumwald, 2001), rice (Chen et al., 2007), and tobacco (Gouiaa et al., 2012), exhibit improved salt tolerance when NHX1 antiporter is overexpressed. Transgenic rice cells demonstrated considerably faster growth rates and total Na^+ contents than the wild type (WT) when OsNHX1 was overexpressed, which also improved salinity survival in the cells (Fukuda et

al., 2004). These findings demonstrate unequivocally that vacuolar Na^+ sequestration is a critical factor in determining the total salt tolerance of plants.

Then, the tonoplast Na^+ and K^+-permeable channels called FV (fast-activating) and SV (slow-activating) channels regulate Na^+ leakage from vacuole to cytosol. In the salt-stressed halophyte quinoa, negative inhibition of FV and SV channel activity has been demonstrated to reduce such leakage (Bonales-Alatorre et al., 2013), indicating that effective control of Na^+ leakage from the vacuole to cytosol may be a key mechanism in plant overall salt stress.

1.6.2.1.4. Sodium ion retrieval from xylem

controling Na^+ transfer to shoots is considered an essential trait in salt-tolerant plants and involves a membrane passage at the endodermis. In addition, the subfamily I of HKT transporters plays a vital role in this process as it resides at the plasma membrane of root xylem parenchyma cells and, by retrieving Na^+ from xylem vessels, it can prevent sodium from reaching the shoot (for reviews see Almeida *et al.*, 2013; Keisham *et al.*, 2018). A knockout study in tomato has confirmed that HKT transporters, such as HKT1;1 and HKT1;2, are crucial for maintaining Na^+/K^+ homeostasis, Na^+ exclusion, and Na^+ partitioning from root to shoot (for review see Romero-Aranda et al., 2021). Mutations in AtHKT1;1 and OsHKT1;1 under salt stress cause the Na^+ concentration of phloem sap to decrease, whereas xylem sap receives an increase in Na^+ uptake. The role of OsHKT1;5 was suggested to restrict Na^+ transport to young rice leaves via phloem parenchyma-based Na^+ exclusion in dispersed vascular bundles of basal nodes, whereas AtHKT1;1 was discovered to be involved in Na^+ recirculation to roots via the phloem (Joshi et al., 2022; Shohan et al., 2019).

1.6.2.2. Tolerating salinity “cellular adaption”

1.6.2.2.1. Enzymatic & non-enzymatic antioxidants

During stress, high-energy electrons are transported to molecular oxygen (O_2), forming reactive oxygen species (ROS) (Mittler et al., 2002). Then, ROS accumulation can cause an oxidative burst, while at low concentrations, they act as signalling molecules. Thus, the delicate balance in the temporal and spatial accumulation of ROS is crucial and maintained by an efficient functioning of

the antioxidants system comprising enzymatic and non-enzymatic components (for review see Hossain & Dietz, 2016; Jiahao Liu et al., 2021). Usually, chloroplasts are the major sites for ROS production under abiotic stress compared to mitochondria and peroxisomes (Gill & Tuteja, 2010). During stress conditions such as drought and salinity, when CO_2 fixation is limited, two primary processes are involved in forming ROS during photosynthesis as electron sinks to alleviate the over-reduction of the photosynthetic electron chain. First, the direct photoreduction of O_2 to the superoxide (Mehler reaction) radical by PSI when the electron acceptor $NADP^+$ is limited. Second, the photorespiratory cycle includes oxygenation of Rubisco in the chloroplast and generating high levels of H_2O_2 in peroxisomes as a result of glycolate oxidation (for review see Miller et al., 2010). In response to ROS build-up, different scavenging pathways are activated to eliminate the accessory ROS such as water–water cycle, including SOD in chloroplasts, the ascorbate–glutathione cycle in chloroplasts, cytosol, mitochondria, apoplast and peroxisomes, glutathione peroxidase and CAT in peroxisomes (Mittler et al., 2002).

First, the superoxide radicals produced at PSI are scavenged to hydrogen peroxide by a membrane-bound copper/zinc superoxide (Cu/ZnSOD) in the presence of PSI, and then the H_2O_2 is converted to water by a membrane-bound thylakoid ascorbate peroxidase (tAPX) in what is called water-water cycle. Such rapid dismutation of O_2^- by SOD is a frontline defence against oxidative stress, for i) reducing the chances of converting O_2^- to $^{\cdot}OH$, radicals through metal catalysed Haber-Weiss reaction (Mittler et al., 2002), and ii) the generated H_2O_2 relieves the chances of $^1O_2$ formation at PSII (Miller et al., 2010), thus protecting photosystems I&II and relieving the chances of cytotoxicity of $^{\cdot}OH$ and $^1O_2$ which are very destructive and can cause enhanced levels of lipid peroxidation and cellular death (for reviews see Ahanger et al., 2017; Miller et al., 2010; Mushtaq et al., 2020). Ascorbate peroxidases (APXs) are also involved in the scavenging of H_2O_2 in the ascorbate–glutathione cycle or different cell compartments utilising ascorbate as the electron donor. APX, on the other hand, has a higher affinity for H_2O_2 than CAT and POD, suggesting that it may play a more critical role in the management of ROS stress or be responsible for fine-tuning ROS signalling (Ahmad et al., 2010a).

Second, the photorespiratory oxygenation of Rubisco in the chloroplast is another major sink of electrons to relieve partial oxidation of PSII acceptors under limited availability of CO_2. The oxidation of glycolate in peroxisomes produces most of the H_2O_2 released under stress conditions

(Apel & Hirt, 2004). Then, CATs localised in peroxisomes are the principle antioxidative enzymes to scavenge the H_2O_2 released during photorespiration (Apel & Hirt, 2004; Miller et al., 2010).

In addition, GR is a key enzyme in the ascorbate glutathione cycle which catalyse the reduction of H_2O_2 via ascorbate oxidation. GR catalyses the rate-limiting step in this cycle by reducing glutathione disulphide (GSSG) to reduced glutathione (GSH) to regenerate ascorbate and balances cellular GSH/GSSG ratio (Ahmad et al., 2010b; Noctor et al., 2002). Consequently, GSH can scavenge ROS such as O_2^- and ·OH to prevent accumulative oxidative damage. Concurrently the ratio of $NADP^+$/NADPH increases, thereby ensuring the availability of $NADP^+$ to accept electrons from the over-reduced photosynthetic electron transport chain (Mushtaq et al., 2020). While the Asc–GSH cycle is the primary H_2O_2 in different cell compartments with CAT in peroxisomes (Noctor and Foyer 2016), class III peroxidases (PODs) are the main H_2O_2-scavenging enzymes in the extracellular space (Hiraga et al., 2001). PODs scavenge H_2O_2 by catalysing the oxidation of phenolic substrates using H_2O_2 as an electron acceptor (Sakihama et al., 2002).

On the other hand, the non-enzymatic, such as secondary metabolites that are produced through phenylpropanoid pathway comprising phenols and flavenoids are known as hydrogen donors to scavenge ROS, depending on the number and position of the free OH groups (Aryal et al., 2019; Chen et al., 2021; Gill & Tuteja, 2010).

In summary, launching such a network of antioxidant molecules that can reduce the free radicals by donating electrons or hydrogen atoms is essential to maintaining the normal redox potential status of the cell (Hossain & Dietz, 2016; Kiani et al., 2021; Sharma et al., 2019).

1.6.2.2.2. Osmoprotectants

Different Studies showed that plant tolerance to drought and salinity has occurred through osmotic regulations by synthesising different types of osmotically active compounds or osmoprotectants (for reviews see Pandey et al., 2022; Singh et al., 2015).

Compatible solutes or osmoprotectants are small organic compounds with a low molecular weight that are electrically neutral, readily soluble, and non-toxic at molar concentrations and can assist plant growth and survival in highly osmotic environments (Ahn et al., 2011; Lang, 2007). They can mitigate the damaging risk caused by the overproduced ROS, prevent membrane injury by stabilising proteins and membranes and lower the osmotic potential of membranes to avoid cell

dehydration (Wani et al., 2013). In addition, osmoprotectants can accumulate inside the cell and keep the osmotic difference between the cytosol and the surroundings in balance (Nahar et al., 2016; Tiwari et al., 2010; Yamaguchi et al., 2007).

For example, proline also is a common osmoprotectant that defends plant cells under osmotic stress (Hare & Cress, 1997; Kavi Kishor et al., 2005). Proline has also been shown to operate as a molecular chaperone, preserving the integrity of proteins and boosting enzyme activity. Proline is thought to have a role in antioxidant defence by scavenging ROS and quenching singlet oxygen ((Matysik et al., 2002). Under stressful conditions, proline build-up in the cytoplasm accelerates hydraulic conductivity (Ashraf & Foolad, 2007). Increased proline production during stress can also help chloroplasts maintain a low NADPH:$NADP^+$ ratio, support electron flow between photosynthetic excitation centres, stabilise the redox balance and prevent photoinhibition and damage to the photosynthetic machinery (Hare & Cress, 1997). In halophytes, the build-up of proline is a typical adaptive response to salt stress. The members of the Aizoaceae family accumulate substantial amounts of proline, demonstrating its function in osmoprotection (Deuschle et al., 2001; Lokhande et al., 2011). There is evidence of a similar osmotic adjustment, mediated by proline accumulation, in other halophyte species from different families (Slama et al., 2015).

1.7. Sugars: not only osmoprotectants

As autotrophic organisms, plants need water and light energy to fix carbon dioxide and trigger the photosynthetic process in the chloroplast (Baker et al., 2012; Gangola & Ramadoss, 2018; Tarkowski & Van den Ende, 2015). This mechanism aids in the maintenance of two large pools of metabolites that, depending on the needs of the plant cell, can be transformed into one another via reversible enzymatic processes. These two pools are; the triose phosphates pool, which are made up of 3-phosphoglycerate (3-PGA) and dihydroxyacetone phosphate (DHAP), and the hexose phosphates pool which contains; glucose 1-phosphate, glucose 6-phosphate, fructose 1-phosphate, and ADP-glucose (Granot et al., 2013; Griffiths et al., 2016). The triose phosphates serve as essential carbon carriers from chloroplast to cytosol, where they are transformed into hexose phosphates which are utilised in sugar production and metabolism (Griffiths et al., 2016).

Many different structural and non-structural sugars can be formed by a wide variety of sugars in plants. Long-chain compounds from the structural sugars, such as cellulose and hemicellulose, give plants their biomass and structural integrity (Hartmann & Trumbore, 2016). At the same time, the non-structural sugars, also called soluble sugars, include; monosaccharides (triose, tetrose, pentose, and hexose), disaccharides (sucrose, maltose, and trehalose), oligosaccharides (raffinose and stachyose), and polysaccharides (starch and fructan). Soluble sugars are not only a versatile metabolites complex that controls various processes but also serves as an energy source for growth and development, signalling molecules, as well as osmoprotectants under stress environments and as a component of the antioxidative system (Hennion et al., 2019; Salmon et al., 2020).

1.7.1 Sugar metabolism

Sugar synthesis requires sunlight and carbon dioxide and occurs in specialised plant-cell compartments, which are chloroplasts. The synthesised triose-phosphate is either utilised in starch synthesis or transported immediately to the cytosol to synthesise sucrose. Sucrose is the main transport and storage molecule in most plants since it is a non-reducing sugar with particular chemical activity. One molecule of sucrose is formed of one glucose and one fructose molecule, and they are connected by α (1→ 2) glycosidic bond (Chibbar et al., 2016). In the cytosol, sucrose is synthesised from triose phosphates (product of photosynthesis), in two catalytic processes, first by sucrose-phosphate synthase (SPS) and the other by sucrose-phosphate phosphatase (SPP) (Ruan, 2014). Alternatively, in darkness after starch degradation, the resultant hexose sugars will be exported to cytosol. Then sucrose can also be synthesised through a reversible reaction between NDP-glucose (nucleotide diphosphate like uridine diphosphate) and fructose catalysed by sucrose synthase (SUS) enzyme (Nguyen et al., 2016). It is noteworthy that SUS controls both sucrose synthesising and degradation and is present in soluble and membrane-bound forms in the plant cell.

Sucrose is the primary type of sugar that is carried over long distances in plants (Sauer, 2007; Wind et al., 2010). Sucrose can be loaded into the phloem symplastically. i.e. via plasmodesmata or apoplastically i.e. by specific membrane transporters. In the apoplastic mode of transport, SWEETS (Sugars will eventually be exported transporters) are first utilised to shuttle sucrose from

mesophyll cells into the apoplast; subsequently, sucrose is taken up by a class of membrane-localised sucrose/H^+ symporters (SUTs) to import sucrose into phloem companion cells (Chen et al., 2012; Gautam et al., 2022).

Sucrose accumulation attracts water, increasing turgidity and a mass assimilation flow towards sink tissues. Either apoplasmic or symplasmic sucrose unloading from phloem to sink cells is followed by sucrose breakdown by cytoplasmic invertases (CINs) or sucrose synthase (SUS) in the cytosol (Barratt et al., 2009; Bieniawska et al., 2007), or transported to the vacuole and hydrolysed by vacuolar invertases (VINs) (Vu et al., 2020). Later the produced hexoses from sucrose cleavage are consumed in the glycolysis process or sugar polymers synthesis, such as; cellulose, fructan, and starch (Wind et al., 2010).

1.7.2. Sugars play several roles in abiotic stress tolerance

Sugars are chemically active molecules that play a key role in biological and physio-chemical processes, including photosynthesis, respiration, seed germination, flowering, and senescence. Therefore, altering the concentration or content of sugars in plants may enhance their ability to respond to or adapt to abiotic stress (Ahmad et al., 2020; Slama et al., 2015). In the following, we discuss the different functions of sugars for maintaining tolerance to abiotic stresses.

1.7.2.1. Scavenging ROS

Under oxidative stress, plants utilise some antioxidative based systems to scavenge ROS to maintain growth (Foyer & Shigeoka, 2011; Gangola et al., 2013; Gill & Tuteja, 2010). Sugars also are considered antioxidant molecules. In vitro, disaccharide molecules such as sucrose, trehalose, maltose, and lactose significantly affect quenching free radicals depending on the number of •OH residues (Morelli et al., 2003). Meanwhile, monosaccharides were less reactive toward hydroxyl radicals and more susceptible to their destruction than disaccharides (Morelli et al., 2003).

There are distinct three ways antioxidants use to scavenge ROS: electron transfer, hydrogen atom transfer, and radical addition reaction (Hernandez-Marin & Martínez, 2012). The second mechanism is assumed to be the primary ROS scavenging mechanism for sugars, during this mechanism, hydrogen is preferably utilised from C–H rather than O–H because of lower bond

energy (Matros et al., 2015). It is verified that the sugars are partially breaking down during the scavenging response (Peshev et al., 2013). The reaction between sugars and ROS results in free hexoses and carbon-centred radicals. Then the resulting free sugars can also react with hydroxyl radicals (Peshev et al., 2013).

1.7.2.2. Sugars as osmoprotectants

As clarified previously, several classes of osmoprotectants have been found in plants, and sugars, including; sucrose, trehalose, and fructans are significant classes of these compounds (Slama et al., 2015). In order to preserve the hydrophilic interactions in plant cells, which are essential to stabilise native macromolecules and membrane structure during dehydration, the hydroxyl groups of the sugars can substitute for water molecules (Pukacka et al., 2009). In addition, osmoprotective carbohydrates has also been linked to the maintenance of ion partitioning and homeostasis in the plant cell, aiding in the preservation of appropriate cell functioning and improving the tolerance to salt stress (for review see Gangola & Ramadoss, 2018). In terms of concentration needed, trehalose is the most promising osmoprotective sugar and, in some cases, can be replaced by sucrose and other sugars in plants (Nahar et al., 2016). Hence it was necessary to target essential genes in sugar biosynthetic pathways to increase tolerance to abiotic stress (Gangola & Ramadoss, 2018).

1.7.2.3. Sugars as signalling molecules

Besides being osmoprotectant or structural and energy molecules, sugars can also serve as sensors/endogenous stimuli for imitating phytohormonal actions under stress conditions (for reviews see Khanna et al., 2022; Rodriguez et al., 2019). In order to sustain the growth, nutrition, and stress signalling pathways in plants, several sugars, including sucrose, trehalose-6-phosphate, and fructose, operate as signalling components (Martínez-Noël & Tognetti, 2018).

For example, abiotic stress results in energy deprivation in plant cells, which activates SnRKs [sucrose nonfermenting1 (SNF1)-related protein kinases], which is correlated with various signalling pathways and the activity of transcription factors that control the biosynthesis of stress-related compounds in plants (Valluru & Van den Ende, 2011). SnRKs are positive regulators of ABA-related transcription factors (Saddhe et al., 2017). Stress-induced abscisic acid pathway includes auto-phosphorylation and activation of the SnRK2. Following its activation, a number of downstream targets, including MYC2, WRKY, and amylase phosphorylation, also become active.

Galactinol synthase and raffinose synthase genes, which code for galactinol and raffinose synthesis, are regulated by WRKY transcript regulation. Additionally, they reduce oxidative damage by removing ROS (Bhattacharya & Kundu, 2020). This interaction is crucial to operating as a regulatory centre for sugar and phytohormone signalling systems in plants under stress (Sakr et al., 2018).

1.7.3. Carbohydrates partitioning

Assimilated sugars have three possible destinations: i) they can be used as precursors for other metabolic pathways, ii) reserved as an energy source iii) or they can be transported to sink organs. Distributing assimilating sugars from the source (photosynthetic leaves) to sink organs, including roots, young leaves, storage organs, flowers, seeds, and fruits, is known as "carbohydrates partitioning" (for reviews see Gautam et al., 2022; Kaur et al., 2021; Saddhe et al., 2021).

The primary form of transferred sugar is sucrose, between 80% and 90% of all solutes transported by the phloem conduit (Stein & Granot, 2019). Although the precise cause of it being the most popular means of transportation is uncertain, its unreactive structure can provide a compelling argument (Kühn et al., 1999). However, several species can also transport other sugars, including sorbitol, mannitol, and polyols from the raffinose family (Rennie & Turgeon, 2009).

There are two major routes in sucrose loading into the phloem. I) the apoplastic pathway, which is energy-dependent, utilising proton gradient induced by plasma membrane-localized H^+-ATPase transporters. II) the second route is symplastic loading, in which sucrose is transported from mesophyll cells to sieve elements via SE-CC but through plasmodesmata (Brodribb & Holbrook, 2005; Sauer, 2007). In addition, a third technique that includes sucrose being loaded symplastically into the specialized companion cells is reported. This method is known as "polymer entrapment" because sucrose is transformed into polymer forms such as raffinose and stachyose, which cannot diffuse back to the mesophyll cells (Schulz, 2007).

An adequate carbon supply is necessary to maintain the homeostasis of the source and sink relationship, which is further influenced by plant age, development stage, source or sink tissue type, and environmental conditions (Nardozza et al., 2013). As a result, the intricate molecular network involved in sugar storage and transport is dynamic, which is essential for sudden

environmental changes. Hence, the spatial distribution of sugars can be affected by local and remote signals to maintain homeostasis between "source and sink" during stressful situations (Lemoine et al., 2013; Rosa et al., 2009).

Then, clarifying the different factors modifying carbohydrates partitioning and their genetic control under physiological and stressful circumstances is crucial. Various sugar transporters that integrate signal transduction are involved in sensing biotic and abiotic stress responses and adaptation, allowing plants to control the partitioning of carbohydrates (Kong et al., 2019; Pommerrenig et al., 2018).

1.7.4. Sucrose transporters in plants

Sucrose transporters are known to play a central role in regulating cellular, tissue, and long-distance distribution of sugars during plant growth and development and even under stress conditions. Consequently, it is intriguing to comprehend their function, regulation, and evolution. These include two prominent families of sucrose transporters which are; sucrose will eventually be exported transporter (SWEETs), and sucrose transporters (SUTs) (Chen et al., 2010, 2015). Members of the SWEET family play a significant role in phloem loading and vacuolar sugar export (Chen et al., 2012). On the other hand, the different regulation of SUTs enables plants to respond to environmental factors such as temperature, light regime, photoperiod, pathogen attack, and other challenges (for reviews see Khanna et al., 2022; Saddhe et al., 2021). In the following, we discuss detailed information about the functional role and regulation of SWEETs and SUTs transporters under abiotic stresses.

1.7.4.1. SWEET transporter family

The SWEET transporters are a new family of sugar efflux/bidirectional transporters in plants that are essential for maintaining pollen, nectar, and seed development (Chen et al., 2010). SWEETs are among the essential transporters that stimulate sugar flux around the plasma membrane and are found in both eukaryotes and prokaryotes. Bioinformatics studies have also discovered what are known as semi-SWEETs in bacteria with three transmembranes. As a result, 3-transmembrane or semi-SWEET duplication caused the emergence of eukaryotic SWEETs following evolution.

SWEETs maintain the source-sink connection in plants and play a crucial role in the long-distance transport of sugars (Chen et al., 2012). In plants, SWEETs are encoded by a multigene family and have seven transmembrane domains (Chen et al., 2010; Yuan & Wang, 2013). Using phylogenetic analysis, SWEET transporters are divided into four clades (clades I–IV). Hexoses are transported preferentially by SWEETs in clades I and II, whereas vacuolar transporters belong to clade IV. OsSWEET11, OsSWEET12, OsSWEET13, OsSWEET14, and OsSWEET15 in rice are examples of Clade III SWEETs known to be sucrose transporters (Chen et al., 2015; Yang et al., 2006; Yang et al., 2018). It has been discovered as well that SWEET transporters have a role in pathogen susceptibility. For instance, the pathogen *Xanthomonas oryzae*, which causes rice blight, has effectors that target SWEETs to generate more sugars in the host as an asset for pathogen survival (Asai & Kobayashi, 2016; Chen et al., 2010).

1.7.4.1.1. Regulation of SWEET transporters under abiotic stress

Understanding the function of the plant SWEET family in controlling sugar transport under abiotic stress tolerance has advanced considerably during the last decade (Chen et al., 2015; Jeena et al., 2019). Abiotic stresses alter sugar homeostasis by disrupting metabolic and photosynthetic activities. In a typical situation, plants finely regulate the activity of photosynthesis, the production and distribution of sugar, from the source to the sink organs (Chen et al., 2012). However, under the conditions of water defeciency, the transcripts level of AtSWEET15 was significantly upregulated in Arabidopsis, suggesting a role in sucrose apoplastic unloading (Durand et al., 2016). AtSWEET15 also is induced under osmotic stresses, including salinity and drought in ABA-dependent pathway (Seo et al., 2011).

Further research showed other examples, such as the regulation in the tonoplast content of glucose and fructose under cold and low nitrogen supply conditions, mainly by AtSWEET16 and AtSWEET 17 in Arabidopsis leaves and roots (Guo et al., 2014; Klemens et al., 2014). Meanwhile, freezing tolerance was more evident in the *Atsweet11 Atsweet12* double mutants than in the wild-type or single mutant (Hir et al., 2015). Furthermore, under osmitic stress conditions, the transcripts level of clade III SWEET members (AtSWEET11- and 15) were up-regulated in source leaves, resulting in an enhanced efflux of sugars to the apoplast area, ready for phloem loading (Durand et al., 2016). Overall, SWEET gene family functions in various physiological activities, including stress responses.

1.7.4.2. Sucrose transporters (SUTs)

Sucrose transporters (SUTs) act as a bridge for sucrose import across the plasma membranes of cells. SUTs are energy-dependant transmembrane proteins that co-transport protons and sucrose in the same direction in 1:1 stoichiometric ratio (Lalonde et al., 2004). It is known that SUTs play a role in the phloem loading in source photosynthetic leaves in maize (Slewinski et al., 2009). Henceforth, studying SUTs in sorghum is interesting because of the potential significance of their functions in the apoplasmic loading of sucrose into source leaves via phloem and the apoplasmic unloading of sucrose into stem storage sinks (Milne et al., 2013).

Yeast complementation test identified a proton sucrose symporter from spinach leaves (Riesmeier et al., 1992). Later, several SUT1 orthologues in wheat, barley, and maize have also been characterized (Aoki et al., 2002). The rice genome only has five SUT members, while *Arabidopsis thaliana* has nine sucrose transporter genes (also known as SUCs) (Kühn & Grof, 2010). SUTs play various roles in sugar transportation from source to sink; first, phloem loading in source tissue, then sucrose uptake in sink cells, and finally, vacuole transit for storage (Slewinski et al., 2010). Hence, SUTs can control biomass partitioning, plant development, pollen germination, and fruit size.

1.7.4.2.1. Regulation of SUT transporters under abiotic stress

Several studies have been done to functionally confirm sucrose transporters before using them as potential candidate genes to increase plant tolerance to abiotic stress (Julius et al., 2017). In *Arabianopsis thaliana* the expression of *AtSUC9* is induced under different treatments such as salt, osmotic stress, and cold (Jia et al., 2015). In addition, in *Atsuc9* mutant, the expression of ABA-inducible genes is suppressed and showed a low level of endogenous ABA under stressful conditions. On the other hand, *Atsuc4* mutant lines had higher levels of sucrose, fructose and glucose in the shoots than the roots, under salt stress and causing an imbalance in sugar distribution (Gong et al., 2013). The expression of *AtSUC2* and *AtSUC4* also was modified under different treatments, including exogenous ABA, salt, osmotic stress, and low temperature (Gong et al., 2015). The high expression of *AtSUC2* under salt stress has improved sucrose transport and phloem loading in Arabidopsis leaves; however, lower expression was seen during osmotic stress (Gong et al., 2015).

In addition, the overexpression of *VvSUC27* in tobacco improved ROS scavenging and the expression of ABA biosynthesis-related genes, improving tolerance to abiotic stress (Cai et al., 2017). Meanwhile, Arabidopsis plants overexpressing *VvSUC11* and *VvSUC12* showed improved drought tolerance in addition to different phenotyping with more lateral branches and thicker leaves, and it was demonstrated that these transporters enhanced sucrose loading from source leaves (Cai et al., 2020).

1.8. *Sorghum bicolor* L. as a model crop

By 2050, the population of the world is forecast to exceed nine billion. To meet such anticipated pressure, securing food and energy sources is obliged (Palmgren et al., 2015). These demands an enhanced crop performance even under insufficient arable land. Hence, breeding programs should include essential measures such as minimising crop loss, improved abiotic and biotic stress tolerance, and efficient assimilates delivery into storage organs to maximise crop yield.

For this context, sorghum (*Sorghum bicolor* L.) has gained attention and is considered a promising candidate for a better understanding molecular and physiological mechanisms of salt stress tolerance in cereals. Sorghum is a moderately salt-tolerant crop (Almodares et al., 2014). Unlike cereal crops such as wheat, maise, and barely, sorghum has minimum requirements to grow in both marginal and coastal lands (Boursier et al., 1987; Wang et al., 2020). Nevertheless, regarding the economic value, *Sorghum bicolor* L. is the fifth most vital cereal crop and glycophyte used as a food source for human and animals and as a potential source of biofuel production (Bihmidine et al., 2015; Paterson, 2008).

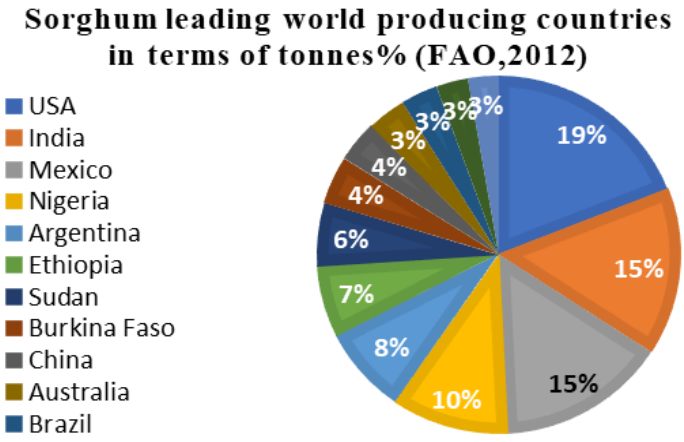

Figure 1.7. Global production of *Sorghum bicolor* L. in terms of tonnes%. (Figure modified from (FAO, 2012)).

The traditional uses of sorghum have expanded its position in bioeconomy. As a C4 crop, sorghum grows relatively faster due to its ability to minimise resource losses maintained by low photorespiration rate, which is accompanied by a significant mass transfer of sucrose from the leaves towards the stem (Bihmidine et al., 2015; Kanbar, Shakeri, et al., 2021). Sorghum is one of the plants with the highest potential to produce bioethanol compared to other cereal crops (Irving, 2015). However, the growing criticism is mainly directed at using agricultural land for bioenergy production (Thompson, 2012). Sorghum would let to get over the "no food for fuel" crisis in virtue of its pronounced stress-resilience, diverse uses, and capacity to adapt to marginal soils.

There are two main types of *Sorghum bicolor L.*; grain sorghum which has significant economic value mainly in Africa and China, for accumulating carbohydrates as starch in the seed (Dicko et al., 2006; Jacob et al., 2021; Qingshan & Dahlberg, 2001). The other is sweet sorghum which is utilised as a valuable feedstock and for producing bioethanol because it has been found to store significant amounts of soluble sugars (mainly sucrose) in the stem (Calviño & Messing, 2012; Slewinski, 2012). Both sweet and grain sorghums are categorised as *Sorghum bicolor* L. and genetically related. Despite the phenotypic distinctions of sweet and grain sorghum genotypes, they are not discernible along racial subtypes by molecular markers, according to population genetic studies (Bihmidine et al., 2015; Morris et al., 2013). Nevertheless, grain versus sweet sorghums constitutes an excellent comparative paradigm for studying the genes and mechanisms governing carbohydrate partitioning in cereals due to the various terminal sink tissues and storage forms for carbohydrate deposition (Calviño et al., 2009; Felderhoff et al., 2012; Murray et al., 2008; Ritter et al., 2008; Shakoor et al., 2014). Whether these traits, such as carbohydrate partitioning, are affected under salinity in the mature stage of growth in grain and sweet sorghum still needs comprehensive research.

1.9. The scope of the study

The main ideas of this research are 2-folds; i) fisrt, to find which adaptive measures are utilised by salt-tolerant sorghum genotype under salt stress, & ii) second, to study how carbohydrate partitioning is affected in sweet and grain sorghum genotypes under salinity stress. To achieve these goals, we designed experimental research in two different life stages of sorghum to be able to define two main working models explaining; i) the delineation in physiological and molecular

events between salt adaption versus salt susceptibility, ii) the predicted activity in sucrose transporters in two sorghum genotypes models under salt stress.

Therefore, this approach was designed by two prominent hypothesis-driven research;

A) Understanding how two sorghum genotypes models will handle adaptive responses to salt stress

The main idea of this part is based on a comparative approach to determine whether one or many of the numerous events elicited by salt stress are an expression of damage or are an expression of adaption?! To address this question, we used hydroponic cultures under the same conditions to investigate biologically similar systems that are contrasting in their response to stress. We employed a pair of sorghum genotypes, Della; sweet sorghum, versus Razinieh; grain sorghum which in our previous study showed a difference in response to Pi depletion with superiority to Razinieh (Kanbar et al., 2021). However, the current study shows that Della is salt tolerant according to ion uptake pattern, along with biochemical and molecular analysis. Based on qRT-PCR and Na^+ visualizing approaches in the roots, it is evident that the less Na^+ uptake in the tolerant genotype and the better K^+ retention in shoot compared to the susceptible is orchestrated at both transcriptional and functional levels. In parallel, the different temporal patterns of endogenous hormones, amino acids, and antioxidants activity are revealed in time-controlled and early coordinated responses to acquire adaption to salinity.

B) Comparing the partitioning of sugars from source to sink organs between "sweet" and "grain" sorghum genotypes models under salt stress

We investigated the flag leaf stage in this study to compare the high biomass sweet sorghum genotype "Della" of thick stems with amounts of soluble sugars as the main stored form of carbohydrate versus the grain sorghum genotype "Razinieh" which produces heavier panicles.

Della was developed from a cross of Dale and ATx622 and selected by pedigree breeding methodology after the 6th generation and determined to be a pure line in 1990 (Harrison & Miller, 1993). Meanwhile, Razinieh is a Syrian landrace developed by bulk breeding method to enhance crop productivity (Kanbar et al., 2021). These two genotypes were selected for this study for the following reasons: I) They have been proven to be contrasting in their response to salt stress,

according to findings in the work package **(A)**. II) They have been used in other reports (Kanbar et al., 2021; McKinley et al., 2016), providing sufficient background data. III) At the phenotypic level, they are highly divergent, suggesting that they may have different mechanisms for controlling carbohydrate partitioning.

To see if candidate *SbSUTs* and *SbSWEETs* genes might be associated with differential sugar accumulation in roots, stems and leaves of the two genotypes. We investigated the expression pattern of candidate sugar transporter genes and endogenous sugar content in different source and sink tissues. Based on our findings, a working model is proposed highlighting the movement of sucrose from source to sink and the potential functions of several sucrose transporter proteins.

In summary, the output of this work may be used to identify essential key genes in sorghum induced by abiotic stress. They could serve as markers in breeding programs for improving tolerance to salt and/or osmotic stress (marker-assisted breeding), taking into account the critical role of sucrose transporters in contributing to biomass partitioning and controlling crop stalk and panicle yield production.

2. Methodology

2.1. Two contrasting sorghum genotypes to salt stress under standardised hydroponic system

2.1.1. Plant materials, growth, and stress application

In this study, we used seeds from two varieties of *Sorghum bicolor* (L.) Moench. Razinieh, grain sorghum; a Syrian landrace improved by bulk breeding (Kanbar et al., 2020), and Della, sweet sorghum variety developed from the cross of Dale and ATx622 in Virginia Polytechnic Institute (McKinley et al., 2016). The caryopses were sown in magenta boxes (Duchefa, The Netherlands) containing a solid growth medium of 0.5% phytoagar medium mixed with 8% MS medium (Duchefa, The Netherlands). Seedlings were grown for ten days in a culture room at 25 °C with a 12 h photoperiod of 120 µmol $m^{-2}s^{-1}$ light intensity and at 22 °C for 12 h in darkness. Seedlings with uniform emergence and size were selected and transferred to hydroponic cultures; of custom-made sterilised floating racks in a glass jar containing 500 mL milli-pore water enriched with half-strength (2.15 g/ L) MS basal salt mixture as standard culture solution for three days for adaption. Later for salt treatment, seedlings were divided into two groups of treatments; i) control treatment with the same standard culture solution, and ii) salt treatment with a solution containing half-strength MS + 100 mM NaCl. The leaves and roots of control and stressed plants were harvested, frozen in liquid nitrogen, and stored at –80 °C to be used for biochemical and molecular analysis.

2.1.2. Phenotypic and biochemical analysis

To determine biomass, shoots and roots were excised by a sterilised razor blade upon harvesting and then oven-dried at 48°C for two days to reach a constant dry weight. Leaf area was quantified from digital images using quantitative image analysis (ImageJ, https://imagej.nih.gov/ij/). Relative Water Content (RWC) was determined as described in Tang et al. (2020), and chlorophyll was extracted and quantified according to Metzner et al. (1965). Malondialdehyde (MDA) as a readout for lipid peroxidation was measured using the thiobarbituric acid (TBA) method (Heath & Packer, 1968), and hydrogen peroxide (H_2O_2) content was measured using potassium buffer pH 7 and 1 M KI according to Shi et al. (2005). Data represent means values from three independent biological replicates.

2.1.3. Determination of sodium and potassium ions content

Samples from each treatment (˜50 mg) were oven-dried (at 48°C for two days) and crushed to a fine powder (TissueLyser, Qiagen), then placed in 50 mL digestion tubes (Gerhardt, UK). Subsequently, 0.5 mL ultrapure water, 2 mL HNO_3 (conc.) and 0.5 mL H_2O_2 (30% v/v) were added to the samples prior to incubation in a heating block (DigiPrep jr, S-prep) system at 110°C for 2-3 h. After cooling, 0.5 mL of H_2O_2 were added twice to rinse the walls of the digestion tube. The final volume of each sample was adjusted to 20 mL with 1% v/v HNO_3. The digest is used for measuring sodium and potassium contents by inductively coupled plasma optical emission spectrometry (ICP-OES, 715ES, Varian, radial mode) in the Laboratory for Environmental and Raw Materials Analysis (LERA) at KIT. Two reference materials (grass - 14th needle/leaf interlaboratory test; tomato leaves - NIST 1573a) were used in the digestion process in order to evaluate the quality of the process. The accuracy of both materials was 90%, 102% in tomato leaves and 99%, and 96% in grass leaf in measuring Na^+ and K^+ respectively. Samples from each treatment was collected from 3 biological replicates. Blank samples were subjected to digestion and measurement in the same way, excluding the plant sample.

2.1.4. Quantification of non-enzymatic antioxidant activities

Sample preparation**.** Methanolic extracts were prepared from sorghum seedling shoots. Freeze-dried sorghum shoots were ground to a fine powder (TissueLyser, Qiagen), and a specified amount powder of each sample (~100 mg) was macerated with 20 mL of HPLC grade methanol, shaken at 150 rpm (IKA®KS 260 basic shaker) in darkness for two h and stored overnight at -20°C. The mixture was filtered using filter paper (Whatman, No. 2). The supernatant was collected, and the methanol evaporated completely by *Büchi® rotary evaporator R-205* basic at 250 mbars (25 kPa) at 45°C. Subsequently, the dried residue was dissolved in HPLC grade methanol again to adjust a concentration of ~10 mg/mL, which was then aliquoted. This preparation, termed in the following as crude extract, was used for the determination of synthetic free radicals scavenging activity, as well as quantification of total phenolics and flavonoids contents. The extraction and the measurement were carried out from three biological replicates (each biological replicate comprised shoots collected from 5 individual plants).

2.1.4.1. Determination of DPPH free radical-scavenging activity

In order to measure radical scavenging capacity in sorghum shoots, we measured the activity of the extracts against the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) as described by (Alara et al., 2018) with minor modifications. Briefly, serial dilutions (100 – 700 µg/mL of the crude extract) - were adjusted to 2 mL of HPLC grade methanol 99.8%, and then complemented with 2 mL of freshly prepared DPPH (40 ppm) in methanol. After incubation of 30 min in the dark, the absorbance at 517 nm was recorded. The DPPH scavenging capacity was determined using the following formula: $(A_{con}–A_s)/A_{con}\times100$; where A_{con} is the absorbance of the methanolic DPPH blank, and A_s the absorbance of the sample in the methanolic DPPH solution. Then, The IC_{50} values (amount of crude extract required to inhibit half/50% of the DPPH radical activity) were estimated using a linear regression model of the percentage of inhibition over sample crude extract in µg per reaction medium. Butylated hydroxyanisole (BHA), a very efficient antioxidant, was used as positive control.

2.1.4.2. Determination of ABTS activity

The scavenging of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) as readout for antioxidant activity was measured as described by Rufino et al. (2007). In brief, the ABTS radical cation ($ABTS^+$) was generated by mixing a 7 mM ABTS stock solution with 140 mM potassium persulfate and leaving the mixture in the dark at 20°C for 16 h. The working solution was prepared by diluting 1 mL of this $ABTS^+$ solution to 100 mL methanol to obtain an absorbance of 0.70 ± 0.05 at 734 nm. Again, serial dilutions (100 – 700 µg/mL of the crude extract) were adjusted to 2 mL using HPLC grade methanol, then transferred into a tube, mixed with 3 mL of the $ABTS^+$ solution, and incubated in the dark for 30 min at 20°C. The quenching of A_{734} by the sorghum shoot extracts was compared to the A_{734} of the unmixed $ABTS^+$ working solution and plotted as inhibition percentage over the amounts of the crude extract to determine the IC_{50} values, i.e. (the amount of the crude extract in µg required to inhibit 50% of the ABTS radical formation) using a model of linear regression. Again, BHA was as positive control.

2.1.4.3. Determination of total polyphenols and flavonoids.

The total phenolics content was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965) from 100 µl of the methanolic crude extract (~10 mg/mL). The absorbance was measured spectrophotometrically at 765 nm. The total phenolic content was determined as ferulic acid equivalents (mg FA/g DW). Additionally, 250 µl of the methanolic extract (~10 mg/mL) were also used to measure total flavonoids content in each sample by the aluminum chloride colorimetric assay (Zhishen et al., 1999). The absorbance was measured at 510 nm and total flavonoids content of sorghum shoots methanolic extract was expressed as quercetin equivalents (mg QA/g DW).

2.1.5. Quantification of enzymatic antioxidant activity

Specific activities of several antioxidant enzymes were determined in biological triplicates following quantification of total protein content. Fresh leaf samples (~300 mg fw) were grounded by mortar and pestle in 5 mL potassium phosphate buffer (0.1 M, pH 7.5). The liquid extracts were centrifuged at 12000×g for 20 min, at 4°C with Hermel Z 383 K centrifuge, and the supernatants were collected. 100 µl from each supernatant was adjusted to 1 mL with the same buffer, and protein content was determined according to Bradford (1976). Absorbance was recorded spectrophotometrically at 595 nm and calibrated using bovine serum albumin as a standard. The remaining supernatant was used for the following assays of antioxidant enzyme activities: Superoxide Dismutase (SOD) activity was assayed by monitoring the inhibition of the photochemical reduction of Nitroblue Tetrazolium (NBT) at 560 nm (Beauchamp & Fridovich, 1971), Ascorbate Peroxidase (APX) was assayed following Nakano and Asada (1981), general Peroxidase activity (POX) according to Malik and Singh (Malik & Singh, 1980), catalase (CAT) activity following the method of Aebi (Aebi, 1974), and Glutathione Reductase (GR) according to Venisse et al. (2001).

2.1.6. RNA extraction, cDNA synthesis and quantitative real-time PCR

Total RNA was isolated from the 2nd leaves and roots of control and salt-stressed plants (after 0, 1, 6, 24 and 72 h of stress treatment) using the InnuPrep plant RNA kit (Analytika Jena RNA kit)

according to the manufacturer's instructions. cDNA synthesis and quantitative real-time PCR were conducted as described in (Hazman et al., 2015). *Ubiquitin* (*SbUBQ*) was chosen as internal standard. The relative expression between the different treatments were compared using the $2^{-\Delta Ct}$ method (Livak & Schmittgen, 2001). Each data point represents the mean and standard error from three independent biological replicates (each biological replicate in three technical replicates). The primer sequences for the targeted genes are provided in **Supplementary Table S2.1**.

2.1.7. Metabolite analysis

Extraction of total soluble metabolites. Total metabolites were extracted as described earlier (Gemmer et al., 2020) with few modifications. In brief, plant tissue samples were lyophilised, weighed and then were pulverised using a Retsch-ball mill (MM 400, Retsch, Germany) for 1 minute at 25 Hz. Subsequently, each sample was resuspended in a 900 µl methanol: chloroform: water solution (3:2:4, v/v) containing 4 µg/mL of $_{13}$C-sorbitol as an internal quantitative standard. The mixture was shaken for 30 s with intermittent cooling on ice. It was then centrifuged for 14,000 g for 5 minutes at 4°C. The upper phase was collected and filtered through a 0.22 µm syringe filter (Carl Roth GmbH, Germany). This filtered extract was then used for analysis of soluble sugars and total free amino acids. Data represent means and standard errors from four independent biological replicates.

2.1.7.1. Determination of soluble sugars by GC-MS

Aliquots of 10 µl of the filtered extract were dried in a vacuum concentrator (Christ, Germany) without heating for 45 minutes. The tissue samples along with the respective reference standards for glucose, fructose, and sucrose (Sigma, USA) were derivatised (PAL, Chromtech evolution). Thirty microliters of methoxamine hydrochloride (20 mg/mL in pyridine) were added to these samples, and the mixture shaken for 60 min at 45°C. Subsequently, 45 µl of BSTFA (N,O-Bis(Trimethylsilyl)trifluoroacetamide) were added and the samples were shaken again for 120 min at 45°C. The derivatised samples were injected at 250°C in a splitless mode with a helium gas flow set to 1 $mL \cdot min^{-1}$. All the samples along with the respective sugar standards were analysed by GC-MS (GC/MS/MS Agilent 7890A / 5975C / Chromtech Evolution 3, Agilent, Santa Clara, USA).

The temperature program was set to 60°C followed by a linear ramp of 10°C·min^{-1} to 180°C and holding at this temperature for 8 minutes. This was followed up by another linear ramp of 10°C·min^{-1} to 325°C and holding at this temperature for 3 minutes. Throughout the entire run, the transfer line was set to 290°C, the source to 230°C, and the quadrupole to 150°C. The raw data were processed by the Mass Hunter Qualitative Analysis software (Agilent, B.07.00) and the identification of the chromatographic peaks was validated using the mass spectra library NIST 14 (National Institute of Standards and Technology), along with the data from the sugar standards. For sugar calibration and quantification, reference standards for all three sugars were measured in triplicates for 8 different concentrations. The standard curve was then used to calibrate the sugar concentrations in the respective plant samples. These standards were also used for in-batch and inter-batch correction of the data analysis. Peak areas were normalised with the respective sugar standards and the dry weight of the samples used for extraction.

2.1.7.2. Determination of total free amino acids using HPLC-FLD

For amino-acid analysis, 1 µl of sample filtrate was derivatised with o-phthalaldehyde (OPA) and 9- fluorenylmethyl chloroformate (FMOC) as described in the instruction manual of the producer (1260 Infinity II amino-acid solution, Agilent, Santa Clara, CA, USA). The chromatographic separation of the total free amino acids was performed by HPLC (Agilent 1260 Infinity II) comprising a quaternary pump, an autosampler, a degasser, and a fluorescence detector. Online automated OPA/FMOC based derivatisation for the amino-acid standards as well as the plant tissue samples was performed using the autosampler of the Agilent 1260 Infinity II system. Amino acids were separated on a Poroshell HPH-C18 column (4.6 × 100 mm, 2.7 µm) using the binary gradient mobile phase with the same pre-set specifications as in 1260 Infinity II amino acid solution system. Before every injection, the column was equilibrated for 2 min, and 0.5 µl of the derivatised sample were injected at a column temperature of 40°C. The reference standards for the 21 amino acids were measured in triplicates for 5 different concentrations for each batch of the HPLC run. The obtained standard curve was then used to calibrate the amino-acid concentrations in the respective plant samples. These standards were also used for in-batch and inter-batch correction of the data analysis. Peak areas were normalised for the respective amino acid standards and the dry weight of the samples used for extraction.

2.1.8. Estimation of endogenous hormones

The endogenous levels of jasmonic acid (JA), its bioactive isoleucine conjugate (JA-Ile), its precursor 12-oxophytodienoic acid (OPDA), and of abscisic acid (ABA) were quantified at 0, 1, 6, and 12 h of the stress treatment. The levels of these endogenous hormones were measured simultaneously using a standardised method based on ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) according to (Balcke et al., 2012) using [$_2H_5$]OPDA, [$_2H_6$]JA, [$_2H_2$]JA-Ile, and [$_2H_6$] ABA as internal standards. Data represent mean and standard errors from three independent biological replicates.

2.1.9. Imaging of vacuolar and cytosolic Na^+ distribution in different root zones

The fluorescent CoroNa green acetoxymethyl ester (cat. No. C36676, Invitrogen) has strong affinity to Na^+ cations and was chosen to evaluate Na^+ distribution and accumulation in vacuole and cytosol in different root zones under salt treatment. We followed the protocol established by Wu et al. (2018). In brief, four-day-old sorghum seedlings (grown on solid agar medium in darkness) were transferred to standardised hydroponic culture containing 100 mM NaCl for 24 h. Two segments of 10 mm length were excised from seminal sorghum roots—one in the maturation zone (30–40 mm from the apex), the other in the apex (the first 10 mm). Root segments were simultaneously stained with 20 μM CoroNa Green-AM and 20 μM FM4-64 (Molecular Probes) for 2 hours in darkness. The samples were then rinsed with 5 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer, pH 6.3, adjusted with KOH, to remove the unbound dye and then analysed under an AxioObserver Z1 inverted microscope (Zeiss, Jena, Germany) equipped with a spinning disc device (Yokogawa CSU-X1 Spinning Disc Unit, Yokogawa Electric Corporation, Tokyo, Japan), and a cooled digital CCD camera (AxioCam MRm; Zeiss). Images were recorded using the 488 nm (CoroNa) and the 509 nm (FM4-64) emission lines of the Ar-Kr laser and a Plan-Apochromat 25x/1.44 DIC oil objective operated via the Zen 2012 (Blue edition, Zeiss) software. Mean fluorescence intensity was quantified (ImageJ, https://imagej.nih.gov/ij/) for each cell in the cytoplasm and in the vacuole in relative units from images that had been recorded with constant exposure time and laser power. Readings from 53–98 individual cells for each genotype from 3 independent biological replicates were averaged and reported.

2.1.10. Imaging of Casparian strips

Sorghum seedlings were cultivated precisely as described in section **2.1.1.** Root samples were collected from both control and salt-treated seedlings after 6 days of salt treatment. Root samples were cut at 15 mm from the root tip, then washed gently with de-ionised water and fixed immediately. The roots were incubated for 30 min in fixative (4 % w/v paraformaldehyde and 3% v/v glutaraldehyde in 0.025 M sodium phosphate buffer) followed by three washings with 0.025 M sodium phosphate buffer alone. Subsequently, the samples were dehydrated through a rising ethanol series and eventually embedded in paraffin wax (Paraffin 52-54°C, Carl Roth GmbH; Germany). Cross sections of 15 µm thickness were cut by a microtome (Jung, Heidelberg). Finally, sections were stained for 1 h with 0.1% (w/v) berberine hemisulphate and for an additional hour with 0.5% (w/v) aniline blue (Brundrett *et al.*, 1988). Stained sections were viewed with a Zeiss Axioskop FS Fluorescence Microscope using blue light (filters: excitation 450–490 nm, dichroic mirror 510 nm, emission LP 520). Each treatment was conducted in three independent biological replicates.

2.2. Sugar partitioning from source to sink organs under salt stress at flag leaf stage

2.2.1. Plant growth conditions

Plants were grown in the greenhouse at Botanical Garden of Karlsruhe Institute of Technology (Karlsruhe, Germany) during the summers of 2019 and 2020. Surface-sterilised seeds of the sorghum genotypes Della and Razinieh were sown and germinated in 5 L plastic pots containing Floraton 3 (Floragard Vertriebs GmbH, www.floragard.de) soil for 6 weeks. Later, seedlings were transferred into 10 L pots containing peat-based substrate (Tonsubstrat; Klasmann-Deilmann; http://www.klasmann-deilmann.com) until the end of the experiment, under glasshouse conditions with temperatures maintained at 25 ± 15°C during the day, and 22 ± 2°C during the night. The mean relative humidity for crop growing periods was about 20-50 % during the daylight and ranging from 40-80% during the night. Plants were exposed to 12-h photoperiod supplemented with lighting intensity of about 1000 µmol/m2/s PAR by Light bulbs (400 W / 220 E40 55,000 lm) (SON-T AGRO, Philips) fixed at 3 m height. Seedlings were thinned to one per pot at 1-week post-germination. Irrigation was performed to maintain 80 % of field capacity from cultivation

day until the end of the experiment. Salt application (100 mM NaCl) started pre-flowering at the emergence of the flag leaf; the final and uppermost leaf develops on the stem stalk (flag leaf stage) by applying 400 mL of a 100 mM NaCl solution over a period of 2 months until plants reach the mature grain stage. In parallel, a mock control was run, where the plants were treated in the same way by de-ionised water. A continuous flow of fresh air was maintained during the experiment.

2.2.2. Phenotyping

First, we examined the effects of salt stress on sorghum plants development. Morpho-physiological parameters were recorded from the entire plant, the internodes, and each adjacent leaf blade at 0 day (the start of salt treatment) and then at 10, 20, and 30 days after the treatment. The entire plant, the internodes, and each adjacent leaf blade were collected for recording plant height (cm), internodes number and weights, leaves number, leaf blade area (cm^2), sugar concentration (°Brix), and juice yield as; volume (mL/internode), and weight (g/internode). All data were recorded from three biological replicates. To quantify the juice yield and to measure the sugar concentration of the juice directly after harvest, a conventional cane crusher (VEVOR Juicer 110LBS/H, India) was used for crushing the canes. Sugar concentration as °Brix was recorded with a manual refractometer (Model PAL, Atago Co. Ltd., Tokyo, Japan) for each internode.

2.2.3. Determination of sugar accumulation and ions content

Samples were harvested from representative parts selected along the entire plant: roots, middle internodes with adjacent middle leaves, and flag internodes with adjacent flag leaves after 0 day (control plants utilised as ground level), then from both control and treated plants after 10, 20, and 30 days of salinity treatment. Then washed several times gently with de-ionised water and were divided into two groups to be processed differently for the following two experiments.

2.2.3.1. Extraction and measurement of sugars content. After washing the harvested samples, they were immediately frozen in liquid nitrogen and kept in –80 °C to be analysed for sugars content as described in sections **2.1.7.** & **2.1.7.1.**

2.2.3.2. Measuring sodium and potassium ions content. The selected samples were incubated at 80℃ in a drying oven for three days. Then the dry tissues were homogenised into a fine powder

(TissueLyser, Qiagen). Then ~50 mg from each sample was used for measuring ions content by (ICP-OES) as mentioned previously in section **2.1.3.**

2.2.4. RNA extraction, cDNA synthesis and quantitative real-time PCR

For gene expression studies, root tissues along with the middle and flag Internodes with adjacent leaves were employed after excision from the plant. The internodes and the leaves were quickly sectioned at their midpoint in addition to removing the mid-rib from leaves with a sharp blade; then immediately, the middle parts were frozen in liquid nitrogen and stored at -80°C until processing. Samples were collected after 0, 1 and 5 days from salt treatment. The isolation of total RNA and cDNA synthesis were performed as described in the section **2.1.6.**, using the geometric mean of *ubiquitin* (*SbUBQ*) and glyceraldehyde-6-phosphate dehydrogenase (*SbGAPDH*) transcripts as internal standards. Transcript levels between the different samples were compared using the $2^{-\Delta Ct}$ method (Livak & Schmittgen, 2001). Three biological replicates were utilised for each treatment. Three technical replicates were conducted from each biological replication. The details of the oligonucleotide primers to amplify the genes of interest are provided in **Supplemenatry Table S2.1**.

2.2.5. Cloning and analysing the promoters of *pSbSWEET13, pSbSUT2,* and *pSbSUT6*

Genomic DNA from the leaves of Della and Razinieh was extracted using the CTAB (cetyltrimethylammonium bromide) protocol (Lodhi et al., 1994) and then used as a template. Upstream promoter sequences of *SWEET13*, *SUT2*, and *SUT6* genes were amplified from genomic DNA of both Della and Razinieh, using Q5® High-Fidelity DNA polymerase (NEB, Germany) based on oligonucleotide primers derived from the sorghum reference genome (BTx623) and given in **Supplementary Table S2.2.** The promoter fragments Amplicons were obtained using 36 cycles of 10 s denaturation at 98 °C, 30 s annealing at 65 °C, 120 s elongation at 72 °C. After elution from the gel and purification (Invisorb® Fragment CleanUp STRATEC), 2ul (=100 ng) amplicons were ligated into the pGEM®-T Easy Vector (*Promega* GmbH, Mannheim) and then transformed into E-coli DH5α for DNA sequencing (GATC Biotech, Cologne, Germany). Later, the six promoter regions were also ligated into a GATEWAY version of luciferase vector pLuc

(Supplementary Figure S2.1b), using GATEWAY BP and LR recombination reactions (Invitrogen Corporation, Paisley, UK), respectively and verified by DNA sequencing (GATC Biotech, Cologne, Germany). Putative regulatory elements were analysed with the PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/), and compared with the reference genome with Multiple Sequence Alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo/).

2.2.6. Sorghum protoplast isolation for transient transfection and a dual-luciferase reporter

Sorghum protoplasts were isolated according to (Meng *et al.*, 2020) with some modifications. First, the caryopses of the sorghum genotype Della were surface sterilised and sowed as in section **2.1.1.** Shoot tissues of uniform seedlings were collected and cut into two sections; stems and leaves, with a sharp razor blade. Then, a bundle of stems or leaves tissues from 30 plants were cut together into 3 mm strips. The strips were static incubated in 10 mL mannitol solution (500 mM), pH 5.7, in darkness for 30 minutes. Then, the mannitol solution is replaced with 10 mL enzyme solution (500 mM mannitol, 0.6% cellulose, 0.375 macerozyme, 0.1% pectolyase, 0.1% BSA, and 0.1% polyvinylpyrrolidone K30) and incubated for 4:30 h in the dark at 26°C and agitated at 40 rpm. An equal volume of W5 solution (154 mM NaCl, 125 mM CaCl2, 5 mM KCl, and 2 mM MES, pH 5.7) was added, mixed, and shaken for 1 h at 80 rpm. The mixed solution containing protoplasts was then filtered through a 70 nm nylon mesh (Corning® cell strainer, REF 431751) into a 50 mL tube and centrifuged (Universal 320R von Andreas Hettich GmbH & Co. KG, Tuttlingen) at 1500 rpm for 3 min to collect the protoplasts. The isolated protoplasts were suspended in 300 µL of MMG suspension solution (0.4M mannitol, 15 mM $MgCl_2$, 4 mM MES, pH 5.7) and examined under microscope (**Supplementary Figure S2.3)** . Every solution used was sterilised by a 0.22 µm filter (Rotilabo®-SYRINGE FILTERS, PVDF, sterile, Carl Roth GmbH & Co. KG, Karlsruhe) before use.

Protoplast transfection. PEG-transfection was carried out as described in (Meng et al., 2020; Yoo et al., 2007) with minor changes. Mix 300 µL protoplast suspension (1-2 x 10^6 cells/mL) with 75 µl (20 µg/mL) of plasmid DNA and 75 µl (20 µg/mL) of plasmid pRLUC in a two mL microcentrifuge tube. The co-expression with pRLUC with constitutive promotor (Cauliflower Mosaic Virus (CaMV) 35S for internal standardisation (Horstmann et al., 2004) (**Supplementary**

Figure S2.2). Then, 300 µL 40% PEG solution (40% PEG 4000, 0.1M $CaCl_2$, 0.4M mannitol, pH 5.7) was added immediately and gently mixed by shaking up and down. The final solution was incubated for 20 min at 26°C. Then 650 µL W5 was added to dilute the PEG. The protoplasts were collected by centrifugation (Universal 320R von Andreas Hettich GmbH & Co. KG, Tuttlingen) at 500× g for 5 min and suspended in 650 µL of incubation buffer (0.5M mannitol, 4 mM KCl, 4 mM MES, pH 5.7). The incubation solution with protoplasts was stored at 26°C for 48h.

Protoplast's treatment. After expression for 48 h, the protoplasts were subjected to different treatments before assaying the luciferase activity. Promoters activation was measured in response to 25 µM ABA, 50 µM MeJA, 200 mM NaCl and 25% PEG 6000. After 1 h from each treatment, the cells were harvested, centrifuged (microcentrifuge, VWR Microstar 17 von VWR International GmbH, Darmstadt) with 8000 g for 1 min, and 650 µl of the supernatant was discarded. The remaining protoplasts were then lysed by adding 100 µl of 2× passive lysis buffer (PLB, Promega, Madison, Wl) to the cells on ice, and vortexed for 30 seconds, followed by shaking for 10 minutes at 500 rpm. After centrifugation of the lysates for 1 min at 10.000 g, luciferase activities were measured with the dual-luciferase reporter assay system (PJK, Kleinblittersdorf, Germany) according to (Duan et al., 2016). In brief, for each lysate supernatant (20ml), 50 µl of each Beetle Juice and Renilla Glow Juice were added. The emitted inflorescence was measured with a lumat LB9507 Luminometer (Berthold Technologies, Bad Wildbad, Germany). All transfection experiments were performed in triplicate, and the relative luciferase activity was calculated as the ratio between the firefly and Renilla (control) luciferase activity.

3. Results

3.1. Chapter 1: Identifying adaptive responses in sorghum under salt stress

3.1.1. Sodium translocation to the shoot in Della is less as compared to Razinieh

The degree of adaptation or susceptibility to salt stress might depend on differences in Na^+ uptake and distribution between different plant parts. For this reason, we followed Na^+ content in roots and shoots of Della and Razinieh under salt stress from day 0 to day 12 after the start of stress treatment. This time course revealed three distinct stages of Na^+ uptake into the roots (**Figure 3.1a**):

(i) During the first day, sodium uptake to the roots occurred rapidly, and the roots accumulated sodium content to around three times the initial value. Both genotypes behaved equally during this "initial phase" of stress challenge. (ii) Over the course of the next five days, which is revealed as a "decision phase", sodium content remained stable, showing that sodium uptake from the medium and transfer to the shoot was in a dynamic equilibrium. (iii) Later, the roots underwent a "manifestation phase," during which the two genotypes were diverged. While Della could keep the plateau, Razinieh was losing control and further increased Na^+ content at days 9 and 12 by 26% and 47%, as compared to Della under salt stress.

In contrast with sodium uptake to the shoot, the two genotypes showed two different patterns from the very beginning (**Figure 3.1b**). Here, Na^+ content increased at a constant rate during the first three days of salt stress. The rate of uptake in Razinieh was around twice that seen in Della. Again, between days 3 and 6, there was a"decision phase". In this phase, Della kept a plateau, while Razinieh increased further, albeit more slowly (by around 13%) as compared to the initial increase. Following day 6, a second wave of sodium increase ensued. Even the values in Della barely increased and stopped at day 9, while in Razinieh the increase of sodium in the shoot proceeded in a manner that seemed unrestrained. Taking advantage of the fact that sodium content in the root was in a steady state during the "decision phase" (between days 1 and 6, **Figure 3.1a**), we estimated the coefficient for transfer from root to shoot by a simple mathematical model based on the condition that, in steady state, the influx into the root equals the efflux from the root into the

shoot, in order to deduce the time constants (**Supplementary Figure S3.1**). In Razinieh, compared to Della, the transfer coefficient was seven times higher.

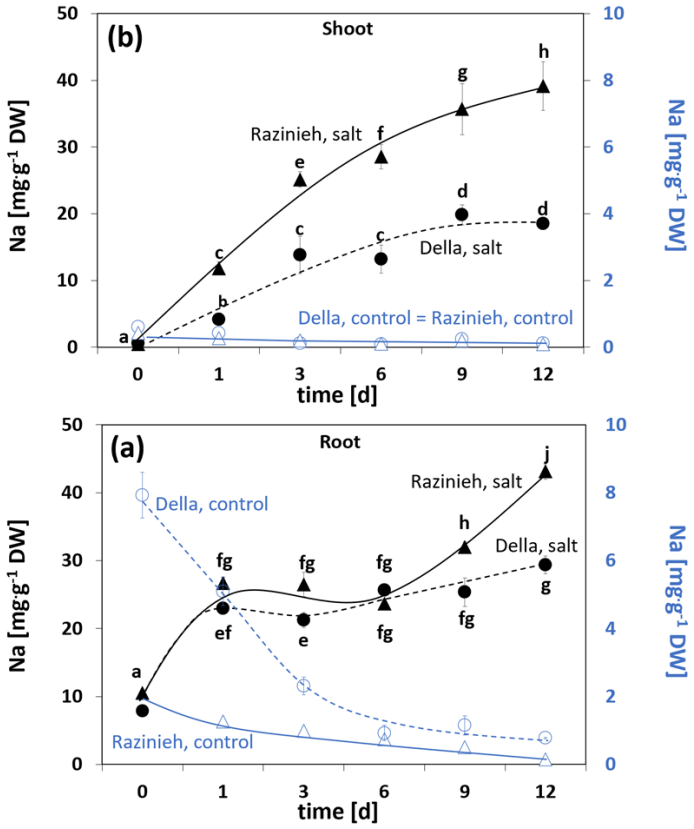

Figure 3.1. Content of sodium ions in (a) roots and (b) shoots of sorghum genotypes Della and Razinieh. Thirteen days old Della (dashed line), and Razinieh (solid line) seedlings were stressed in aqueous NaCl (100 mM) solution and collected after 0,1, 3, 6, 9, and 12 days. Values represent the mean of at least three independent biological replicates ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05). Published data in Abuslima et al., Frontiers in Plant Science (2022).

We also measured the potassium content in the same plants since sodium stress frequently disturbs potassium homeostasis. Here, we observed a mild perturbation in the shoots, while the roots were more severely affected (**Supplementary Figure S3.2**). Concomitantly with the increase of sodium content, potassium was significantly (P<0.05) depleted from roots of both genotypes (**Supplementary Figure S3.2b**). Consequently, the ratio of potassium over sodium was declining. Under control conditions, the ratio of K over Na (on a mg base) was 6.5± 0.17 for Della, but only 4.17±0.12 for Razinieh. After the first day of salt stress, this value had already decreased to <2 in both genotypes (**Supplementary Figure S3.2d**). In contrast, in the shoots of both genotypes,

potassium content in the controls increased mildly but significantly with progressive development (**Supplementary Figure S3.2a**). Such potassium increase in Della was mostly maintained, whereas in Razinieh, the potassium content remained the same as seen in the initial level at day 0. However, the ratio of potassium over sodium was drastically declined again in shoots of both genotypes (**Supplementary Figure S3.2c**). While the initial values were 75.9 ±15.6 for Della and 90.13±6.3 for Razinieh, these values had droped to about 10 in Della and to 2.9 for Razinieh after the first day of salt stress. Additionally, during the following days, the values for Razinieh were significantly less than those seen for Della.

In summary, sodium uptake into the roots is comparable between the two genotypes up to day 6, but the transfer of sodium to the shoot is 7-fold lower in Della as compared to Razinieh. After day 6, a second wave of sodium increase can be observed in the root. This is partially mitigated in Della, while it proceeds unrestrained in Razinieh.

3.1.2. Leaves of Della are more resilient to salt stress

From the time course of sodium uptake, the "decision phase" from day 1 to 6 of salt exposure turned out to be crucial, because, after this phase, the response patterns of the two genotypes became qualitatively different. This stimulated the question, how the physiology would change during this "decision phase". To test this, sorghum seedlings (13 days of age) were treated with 100 mM NaCl and collected after 1, 3, and 6 days from stress treatment. As readout, phenotypic traits, including coverage of green area, chlorophyll content, and Relative Water Content (RWC) were monitored for the second leaf (**Figure 3.2**).

All these data indicated that Razinieh is susceptible to salt stress. After 6 days of salt exposure, the leaves of Razinieh showed wilting and necrosis (**Figure 3.2a**) while, the leaves of Della did not differ from the controls. This was also reflected in the relative coverage of the green leaf area, scored for the second leaf (**Figure 3.2b**). In Razinieh, this parameter showed a clear reduction already from day 3 by around 1/3 of the control value, while the value remained stable in Della. The stress susceptibility of Razinieh was also demonstrated by a decrease in chlorophyll content compared to the control, albeit the effect was partially masked by the fact that chlorophyll content increased with proceeding development in Razinieh (**Figure 3.2c**). As a result, the value seen in Razinieh under salt stress at day 3 was significantly lower than in the control but still higher than the value in Della. This indicates that the non-necrotic area of the Razinieh leaf partially

compensated the loss of area by an increase in pigment concentration. The decrease in green area and chlorophyll content was paralleled by a significant decline of RWC (**Figure 3.2d**). This decline was not seen in Della but was already detectable in Razinieh at day 3 and even had amplified to around ¼ of the control value at day 6.

The negative impact on photosynthetic pigments (**Figure 3.2c**), water status (**Figure 3.2d**), and the loss of ion balance (**Supplementary Figure S3.2c**) should impair seedling growth. In fact, root and shoot dry weight decreased significantly (P<0.05) in Razinieh but not in Della (**Supplementary Figure S3.3a, b**).

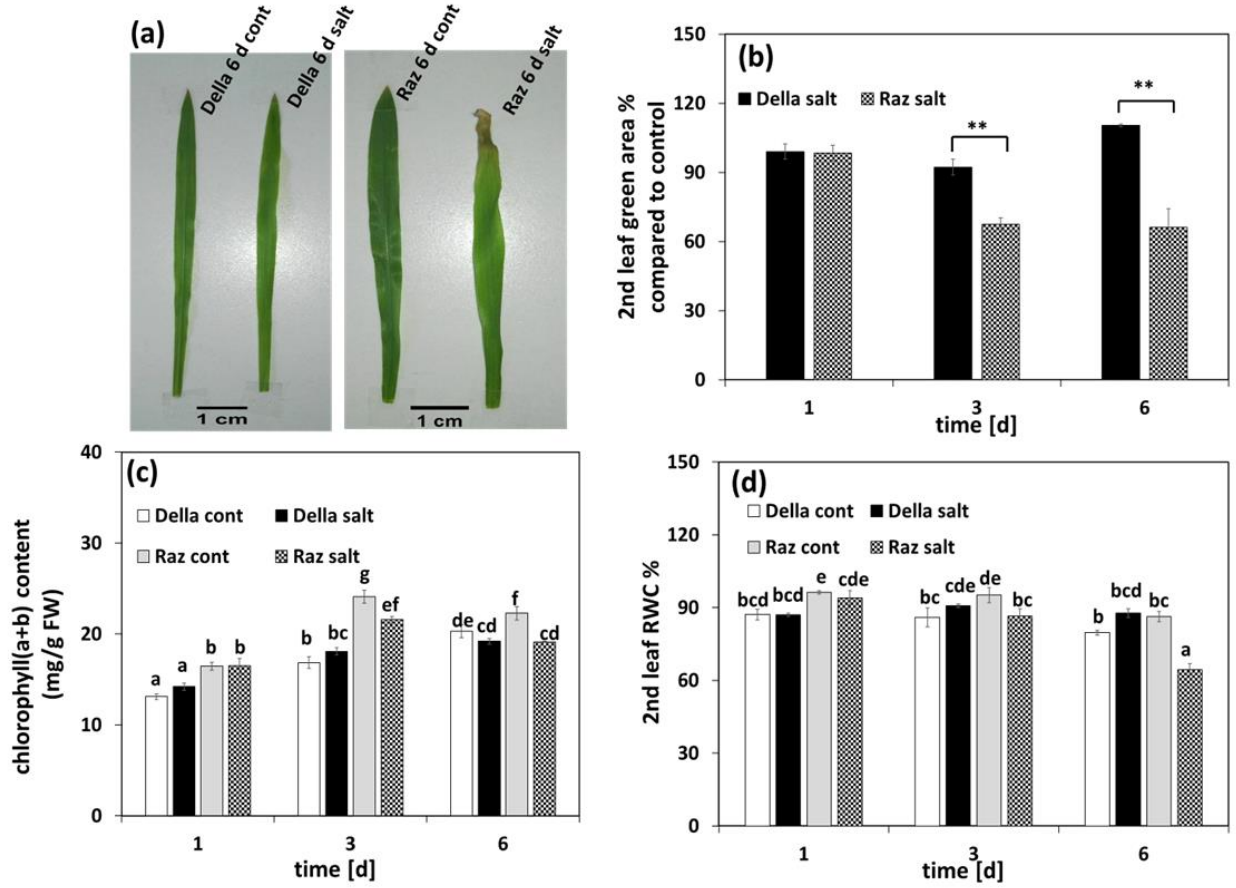

Figure 3.2. Phenotyping traits of thirteen-days-old sorghum seedlings subjected to 100 mM NaCl in aqueous solution and sampled after 1, 3, and 6 days after treatment (a) The second leaf of Della versus Razinieh after 6 days from salt treatment (b) The percentage of second leaf green area under stress compared to each corresponding control (c) chlorophyll (a+b) content of second leaves (d) RWC% of second leaves. Values represent the mean of at least three independent replicates ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05). Asterisks indicate a statistically significant difference between genotypes, as determined by Student's *t*-test (**p<0.01). Published data in Abuslima et al., Frontiers in Plant Science (2022).

3.1.3. Leaves of Della are endowed with more effective redox homeostasis

Sodium ions can enter through the outer membrane of mitochondria and plastids and perturb electron transport, leading to the accumulation of Reactive Oxygen Species (ROS). The ability for ROS scavenging is, thus, a crucial parameter for salt tolerance. To address this, oxidative stress markers were evaluated in both genotypes, such as lipid peroxidation and hydrogen peroxide content, and the activity of enzymatic and non-enzymatic antioxidative systems as a readout of redox balance under salt stress. Malon Dialdehyde (MDA) is a stable end product of lipid peroxidation and can be used to monitor the peroxidation of membrane lipids.

The levels of MDA under salt stress were moderately but significantly higher in both Della and Razinieh by day 3 compared to the controls; however, at day 6 there was a rapid increase (by a factor of 3) in Razinieh. Nevertheless, the increase in MDA value in Della was 40 % less than the value seen in Razinieh **(Figure 3.3a)**.

Oxidative damage markers such as MDA are more persistent than the reactive oxygen species causing them. The level of MDA, thus, represents the integral over time of the stress level. However, the increase of steady-state values for H_2O_2 in response to salt stress was also more pronounced in Razinieh than in Della (**Figure 3.2b**). At day 6, the H_2O_2 level had increased by 41 % in Razinieh and only by 23% in Della compared to the corresponding controls. In summary, these data show that salt stress perturbs the redox balance, whereby Della performs better than Razinieh, which progressively fails to constrain the accumulation of reactive oxygen species.

The ability to mitigate the oxidative burst elicited under salinity depends on the efficiency of enzymatic and non-enzymatic antioxidants. While both genotypes responded to salt stress with significant increases in the activity of enzymatic scavengers (**Figure 3.3c**), there were particular differences in the amplitude and even in the quality of the response. The salt-tolerant genotype Della showed a rapid but transient activation of Superoxide Dismutase (SOD) and Ascorbate Peroxidase (ASX), while the susceptible genotype Razinieh did not show this response, except for some very late and minor activation of both enzymes. A variation of this pattern was seen for Peroxidases (POD), where Della produced a rapid and robust increase of activity again, while Razinieh responded with a delay but eventually, at day 6, exhibited a very high POD activity. This late increase in POD activity correlates with the accumulation of phenolics (potential substrates for peroxidases) in Razinieh after day 6, which is not seen with the initial rise of POD activity in

Della (**Supplementary Figure S3.4a**), suggesting a different functional context for these POD activities.

In contrast to SOD, ASX and POD, Glutathion Reductase (GR), and Catalase (CAT) activities followed a contrasting pattern. In both genotypes, the activity of GR, showed a steady increase, which peaked to significantly higher levels in Razinieh. For CAT, there was a transient increase at day 1, but again this amplitude was more distinct in Razinieh. Interestingly, at day 6, when peroxide levels were significantly increasing, catalase activities had dropped to low levels in both genotypes. In conclusion, fast and transient activation of SOD, ASX, and POD are linked with salt tolerance, whereas higher but late activation of GR and POD under stress operate as indicators for stress damage.

To test for non-enzymatic antioxidants, such as phenolics, we assayed scavenging of the synthetic free radicals ABTS and DPPH. Methanolic shoot extracts of salt-stressed plants produced dose-dependent scavenging of these synthetic radicals, but to a different extent (**Supplementary Figure S3.5a, b**). To quantify this difference, the concentrations required to reach 50% inhibition (IC_{50}) were determined, whereby lower values mean higher antioxidant activity (**Figure 3.3d, e**). Both assays showed clearly that the IC_{50} values were significantly lower in Della as compared to Razinieh, for DPPH by 21%, and for ABTS by 26%. The positive control BHA produced IC_{50} values that were around one order of magnitude lower, which confirmed the validity of the assay system. These data show that the rapid activation of POD activity in Della (**Figure 3.3c**) correlated with a higher activity of non-enzymatic antioxidants (**Figure 3.3d, e**). In contrast, in Razinieh, the activation of POD was much later and was not accompanied by the same degree of non-enzymatic antioxidants.

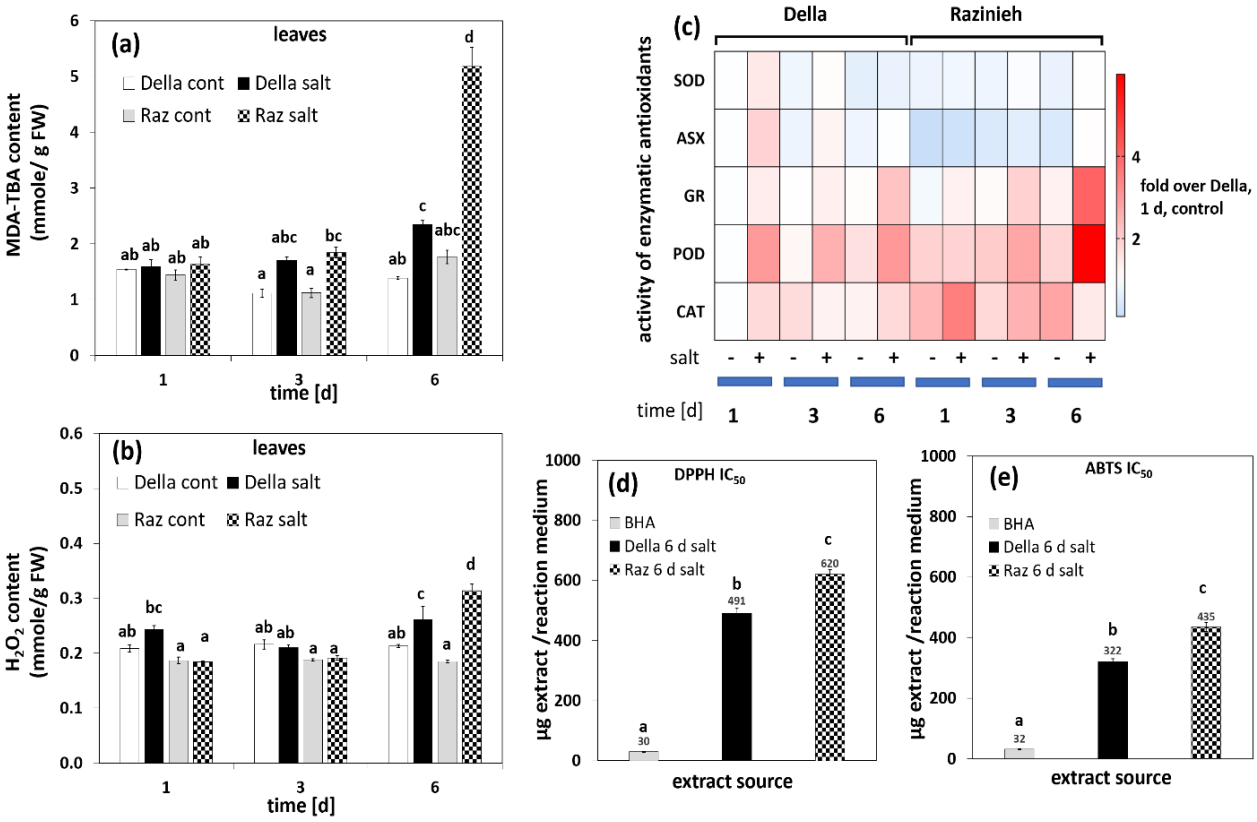

Figure 3.3. (a) The level of estimated malondialdehyde (MDA) in the second leaves of control and salt-stressed Della and Razinieh seedlings. (b) Levels of aqueous peroxide in the second leaves of control and salt-stressed Della and Razinieh seedlings. (c) Heat map profile of enzymatic antioxidants in second leaves of both Della and Razinieh under salinity stress compared to the control of Della at day 1. (d) IC50 values were calculated from DPPH free radical scavenging activity of 6 days stressed shoots depending on regression analysis in Supplementary Fig. S6a. (e) IC50 values were calculated from ABTS free radical scavenging activity of 6 days stressed shoots depending on regression analysis in Supplementary Fig. S6b. Values represent the mean of at least three independent biological replicates ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05). Published data in Abuslima et al., Frontiers in Plant Science (2022).

3.1.4. The earlier production of sugars and proline in leaves correlates with salt stress in Della

To get insight into the more pronounced redox homeostasis of Della leaves, we followed the salinity responses of sugars (**Figure 3.4a**), and amino acids (**Figure 3.4b**) in roots and leaves of both genotypes. This response was typically more evident in the leaves than in the roots.

The most visible change was in the leaves of Della with the fast and robust accumulation of both fructose and glucose, which was considerably slower and less prominent in the leaves of Razinieh (**Figure 3.4a**). As opposed to leaves, sucrose accumulated preferentially in the root, again more steadily in Della than in Razinieh. The resting level of sucrose was similar in roots of both genotypes, but after one day of salt stress, sucrose content in the roots of Della had increased by 125%, and increased in Razinieh by 85%. The situation in the leaves was different. Under both the control and salt treatment, sucrose concentration was twice as high as in Razinieh when compared to Della. Although sucrose seems to be repartitioned from the shoot into the root in both genotypes, the sucrose content in the leaf can be sustained and increased under salt stress, slightly but significantly. For the amino acids, the rapid and robust accumulation of proline in the leaves was the most striking response (**Figure 3.4b**). Although the accumulation was seen in both genotypes, it occurred earlier and to a higher amplitude in Della. Generally, the resting levels for most amino acids were elevated in Razinieh over those seen in Della, and they remained so under salt stress. This was especially evident for glycine in the leaves. Worth to be mentioned is also the induction of glutamine by salt, which was observed in both genotypes, but which was more pronounced in Razinieh. These phenomena were barely detectable in the roots.

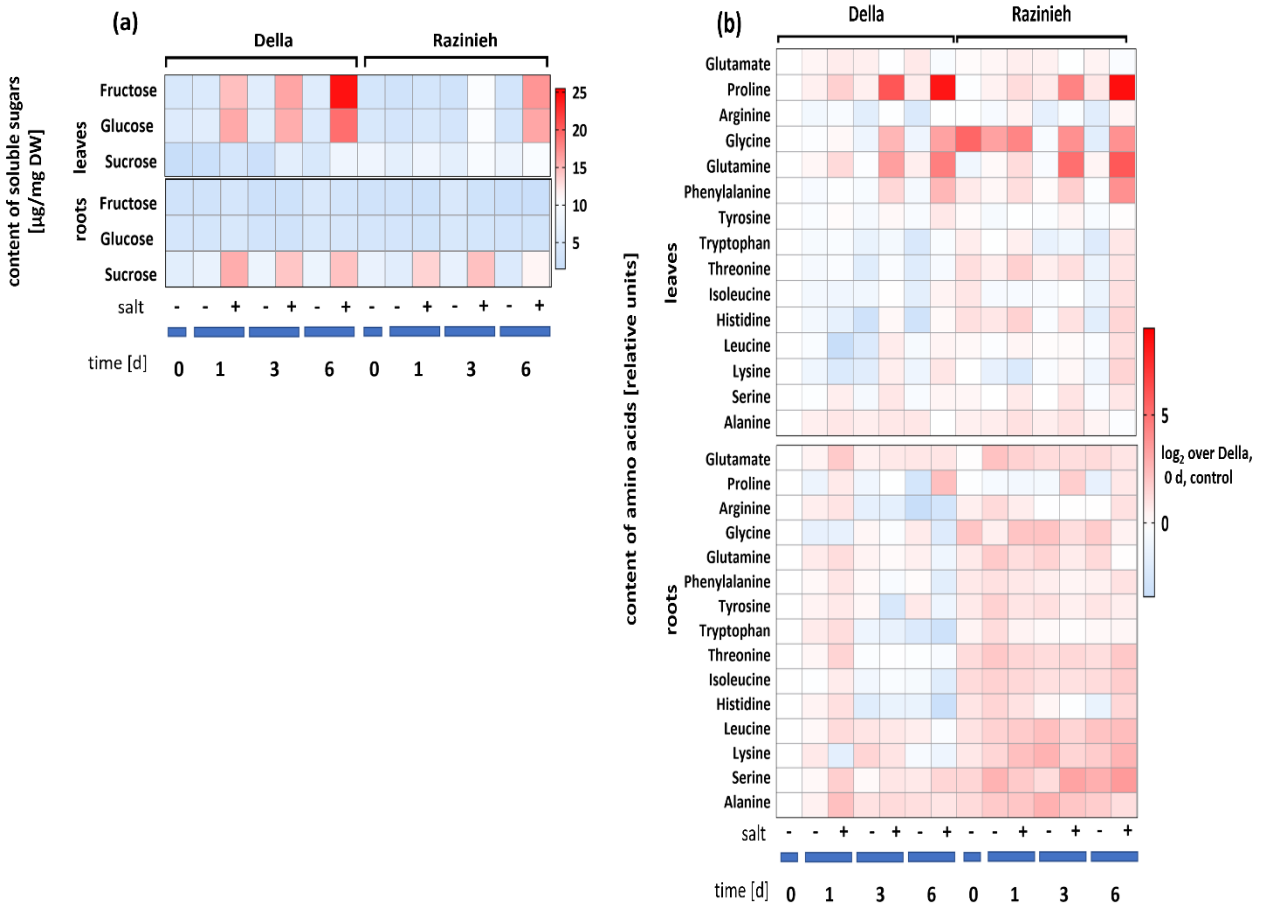

Figure 3.4. Metabolites abundance estimated in roots and 2 leaves in control and salt-stressed Della and Razinieh thirteen days old seedlings stressed in aqueous NaCl (100 mM) solution for 1, 3, 6 days. (a) absolute values of soluble sugars: glucose, fructose, and sucrose. (b) Log2 ratios of mean amino acids abundance compared to the control of Della at day 0. Values represent the mean of at least three independent experiments. Published data in Abuslima et al., Frontiers in Plant Science (2022).

3.1.5. Vacuolar Na^+ sequestration in the root distal elongation zone is superior in Della under salinity

To investigate the mechanisms underlying the reduced transfer of sodium to the shoot in Della (**Figure 3.1b**), we followed the expression of *SbSOS1* transcripts, encoding the plasma membrane localised sodium exporter, and of *SbNHX2* encoding the tonoplast-localised sodium transporter (**Figure 3.5a**). The expression level of *SbSOS1* was unexpectedly about an order of magnitude less in the root than in the leaf **(Supplementary Figure S3.6a, b)**, suggesting that this exporter does not have a significant impact in the root.

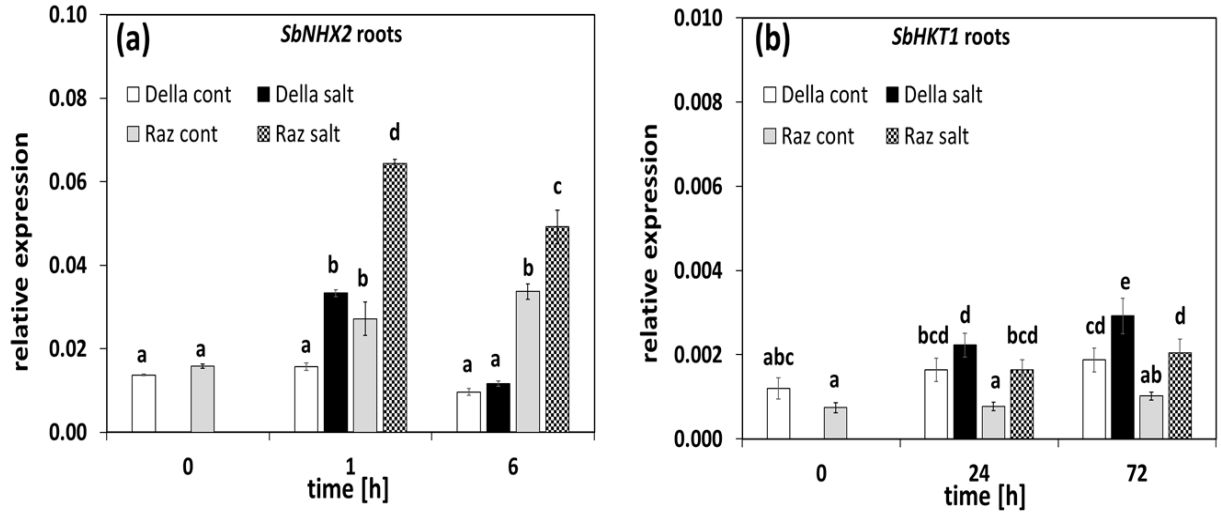

Figure 3.5. The steady-state transcripts level of salt stress-related genes in sorghum roots. Thirteen days old Della (white bars), and Razinieh (gray bars) seedlings were stressed in aqueous NaCl (100 mM) solution to measure steady sate transcripts of (a) *SbNHX2* after 1, and 6 h and (b) *SbHKT1* after 24 h and 72 h after salt treatment. Values represent the mean of at least three independent biological replicates ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05). Published data in Abuslima et al., Frontiers in Plant Science (2022).

This minor role seemed to be even smaller for Razinieh. At the start of the experiment, the ground level of *SbSOS1* in the root was less than half in Razinieh as compared to Della (**Supplementary Figure S3.6b**). Although it increased significantly during further development in Razinieh, it did not reach the level seen in Della at the onset of the experiment. In Della, the ground level decreased strongly, by a factor of four, during the six hours of the control experiment. While salinity did not modulate the expression in Della, it did accelerate the increase of this transcript in Razinieh.

Overall, Razinieh roots acquired the transcript levels seen in Della at the beginning of the experiment only with a temporal delay (1 h under salt stress, 6 h in the absence of salt stress). The situation in the leaf was contrasting (**Supplementary Figure S3.6a**). Here, the ground transcript levels of *SbSOS1* were 2.5 times higher in Razinieh than Della. They subsequently decreased in Razinieh but persisted in Della under control conditions. Under salinity, the transcripts level were temporarily inhibited in both genotypes but later were recovered. In conclusion, the higher levels of *SbSOS1* transcripts in the root of Della were related to lower levels seen in the leaves, but this relation was the opposite for Razinieh. The initial steady-state level of this transcript appeared to be inversely related to the inducibility by salt.

For the Na^+/H^+ vacuolar antiporter *SbNHX2*, the initial levels were comparable in roots of both genotypes. Under salt treatment, the transcript level was rapidly induced in Razinieh to twice as high the steady-state level in Della after 1 h (**Figure 3.5a**). In contrast, salt stress did not significantly induce *SbNHX2* transcripts in leaves of Razinieh, and in Della, there was even a significant reduction (**Supplementary Figure S3.7c**). Similarly to *SbSOS1*, the levels of *SbNHX2* transcripts were much higher in the leaf than those seen in the root, independently of the genotype.

To test whether other transporters might withhold sodium from entering the shoot, we measured the expression of high-affinity K^+ (potassium) transporters in roots. This type of transporter mediates the retrieval of sodium from the xylem into the xylem parenchyma. The resting level of *SbHKT1* was significantly higher in Della compared to Razinieh (**Figure 3.5b**). Although *SbHKT1* transcripts were elevated in both genotypes in response to salt stress, the levels in Razinieh in response to salt stress were only just approached those observed in Della prior to induction. Thus, the decreased sodium transfer from the root to the shoot may be a result of the active expression of *SbHKT1* transporter**.**

Further, we investigated the actual distribution of sodium between the cytosol and vacuole in different root zones using a double-staining with the fluorescent sodium dye CoroNa Green and FM4-64 (labelling the plasma membrane) and in order to comprehend these complex patterns of the two sodium transporter genes. The double-staining facilitated the discrimination between sodium in the cytoplasm, which was adjacent to the plasma membrane, and sodium sequestered in the vacuole, which was separated from the plasma membrane by the cytoplasm. In addition, delineating the plasma membrane also allowed to quantify signal intensity separately for cytoplasm and vacuole. Della generally showed superior Na^+ sequestration to the vacuole than

Razinieh. However, the pattern was dependent on the progression of cell differentiation along the different root zones (**Figures 3.6-8**). In the meristematic zone, the sodium signal in the vacuole was 2.5-fold higher as compared to the cytoplasm in Della (mean intensity of 37.9±1.5 versus 10.9 ±0.7, P<0.01). On the contrary, the Na^+ signals between vacuole and cytoplasm for Razinieh were more or less similar (18.5±0.9 versus 21.1±0.8; P<0.05), indicating a homogenous distribution of sodium (**Figure 3.6**).

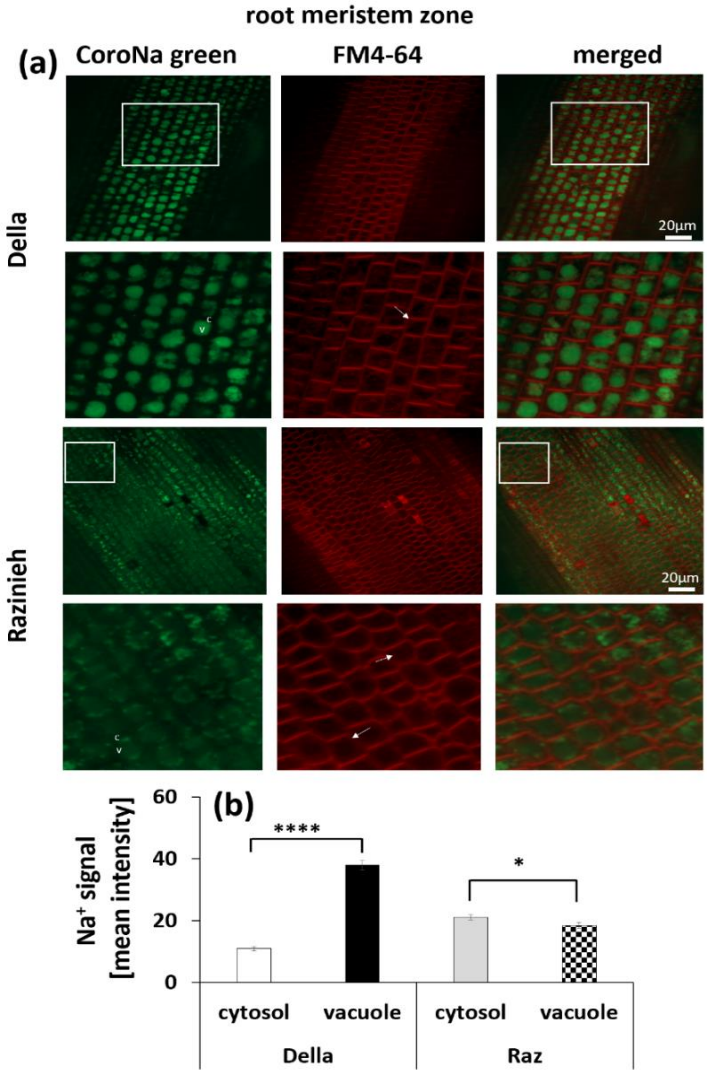

Figure 3.6. Na^+ signal intensity in the root meristem zone of 4-day-old sorghum seedlings grown in darkness treated with 100 mM NaCl for 24 h (a) Representative images of root meristem zone cells of Della and Razinieh genotypes stained with CoroNa Green dye, and FM4- 64 dye (b) Averaged values for Na^+ signal intensity in the cytosol and vacuole in the root meristem zone. Mean ±SE [n=77 (Della) and 57 (Razinieh)]. Asterisks indicate a statistically significant difference between treatment and corresponding control, as determined by Student's t-test (****P< 0.0001). Published data in Abuslima et al., Frontiers in Plant Science (2022).

The most distinct qualitative difference between the two genotypes was seen in the distal elongation zone of the root (**Figure 3.7**). Here, Della was able to efficiently sequester Na^+ in the vacuole with five times signal intensity higher than the cytoplasm (49.0±1.5 versus 9.9±0.4, P<0.01). In Razinieh, on the other hand, the sodium signal intensity in the vacuole was less than in the cytoplasm (20.8±0.5 versus 38.0±1.5; P<0.05), indicating that additional sodium was not sequestered in the vacuole but rather was kept in the cytoplasm.

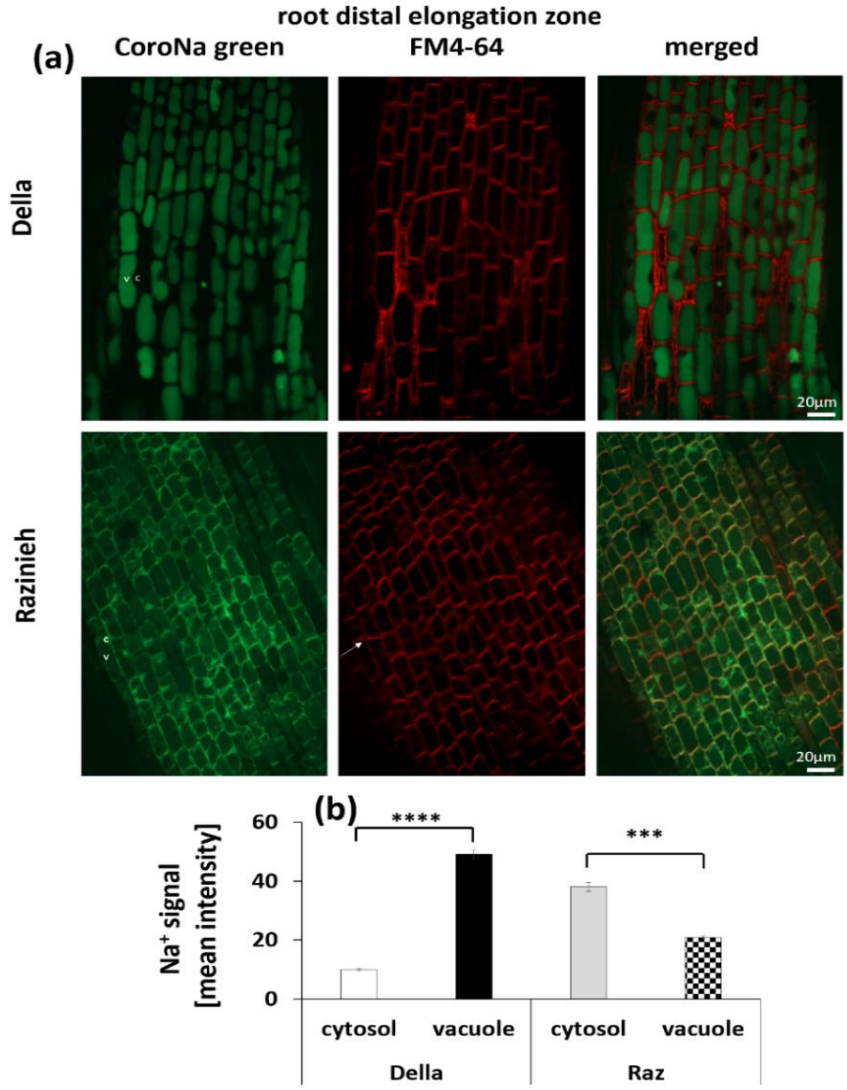

Figure 3.7. Na^+ signal intensity in the cytosol and vacuole in the root distal elongation zone of 4-day-old sorghum seedlings grown in darkness were treated with 100 mM NaCl for 24 h (a) Representative images of root distal elongation zone of Della and Razinieh genotypes, cells stained with CoroNa Green dye, and FM4- 64 dye. (b) Averaged values for Na^+ signal intensity in the cytosol and vacuole in the root distal elongation zone. Mean ±SE [n=98 (Della) and 53 (Razinieh)]. Asterisks indicate a statistically significant difference between treatment and corresponding control, as determined by Student's t-test (***p<0.001, ****P< 0.0001). Published data in Abuslima et al., Frontiers in Plant Science (2022).

The pattern in the differentiation zone (where the rhizodermis had already developed root hairs) was again different (**Figure 3.8**). Here, the vacuoles of both genotypes demonstrated higher signal than in the cytoplasm. In Della, the vacuolar signal was 20.5±0.4 versus 9.5±0.5 in the cytoplasm (P<0.01), for Razinieh a vacuolar signal was 37.4±1.4 versus a cytoplasmic signal of 27.3±1.3 was recorded (P<0.05) (**Figure 3.8**). Overall, in the differentiation zone, the sodium signal for Razinieh roots was nearly two times higher than in Della.

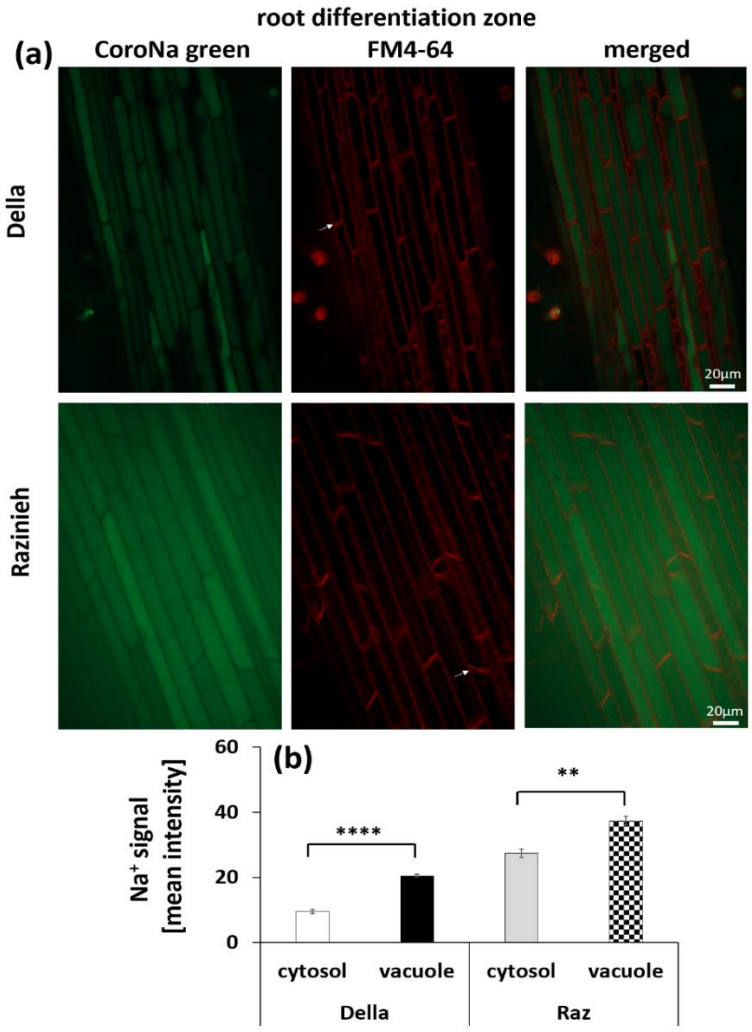

Figure 3.8. Na^+ signal intensity in the cytosol and vacuole in the root differentiation zone of 4-day-old sorghum seedlings grown in darkness were treated with 100 mM NaCl for 24 h. (a) Representative images of root differentiation zone of Della and Razinieh genotypes, cells stained with CoroNa Green dye, and FM4- 64 dye. (b) Averaged pooled values for Na^+ signal intensity in the cytosol and vacuole in the root differentiation zone. Mean ±SE [n=37 (Della) and 47 (Razinieh)]. Asterisks indicate a statistically significant difference between treatment and corresponding control, as determined by Student's t-test (**P<0.01, ****P< 0.0001).

As an additional mechanism, we also evaluated the development of the Casparian Strip in the endodermis- in root differentiation zone-because this hydrophobic barrier would facilitate the passage of ions through the membrane separating it from the central cylinder, including ions entering through apoplastic water flow. The Casparian Strip was visible under control conditions, but it wasn't very pronounced or visibly distinct between Della and Razinieh (**Supplementary Figure S3.8a, c**). However, the entire endodermis as well as the surrounding layer of the central cylinder showed a robust signal in response to salt stress (**Supplementary Figure S3.8a, c**). This response was particularly pronounced in Razinieh, less in Della. While the Casparian Strip in Della appeared to be more localised, the entire radial wall in Razinieh appeared to have been suberinised. In summary, while the Casparian Strip responded to salinity, the pattern was inverse to that seen for sodium transfer to the shoot (**Figure 3.1b**). Razinieh, with its strong expanded Casparian Strip transferred more sodium to the shoot than Della, where the Casparian Strip was less developed. In accordance with the observation that the distal elongation zone (where the Casparian Strip is not yet developed) showed the most significant variation in sodium partitioning between the genotypes, rather than the differentiation zone (where the Casparian Strip is already established).

3.1.6. Expression levels of ABA-related genes are differentially regulated under salt stress

The maintenance of turgescence and the synthesis of osmolytes are crucial for successful adaptation to salinity. Both phenomena are under control of the plant hormone abscisic acid (ABA). To test the possibility of this hypothesis, we measured steady-state transcript levels of selected Abscisic acid-related genes under 100 mM NaCl after 1 h and 6 h of treatment. These included 9-cis-epoxycarotenoid dioxygenase (*SbNCED1*) encoding a rate-limiting enzyme in ABA biosynthesis, and representative members of the downstream responses activated to ABA; (*SbSAPK1*), a member of SnRK2 family, and *SbbZIP-TF-TRAB-1 like* (basic region leucine zipper transcription factor).

The induction of *SbNCED1* expression in Della was relatively more rapid and at higher levels compared to in Razinieh **(Figure 3.9a)**. After 1 h of salt treatment, in roots, the transcripts level were induced up to 9-fold in Della and only by 3.5-fold in Razinieh compared to respective controls. Additionally, in leaves, the induction in Della was again more evident (about 10-fold

within 1 hour of salt treatment) than in Razinieh (about 2-fold). Albeit, the ground level of this transcript was significantly higher in Razinieh compared to Della.

For *SbSAPK1*, the ground transcript level in the roots was twice as high in Della compared to Razinieh but did not increase during subsequent salt stress. In Razinieh, this transcript was induced during salt stress to around the level seen in Della prior to stress (**Figure 3.9a**). In contrast to the leaves, the ground level was higher in Razinieh but eased off a bit during the salt treatment. In Della, there was a transient induction approaching the value seen for Razinieh prior to stress. Thus, the regulation pattern for this transcript was inversed between roots and leaves.

The transcripts for *SbbZIP-TF-TRAB-1* in the roots showed a transient induction under salt treatment, but it was more pronounced in Della as compared to Razinieh **(Figure 3.9a)**. In the leaves, the ground level was significantly higher in Della, but since these transcripts accumulated in Razinieh, the values became comparable in both genotypes after 6 h of salinity.

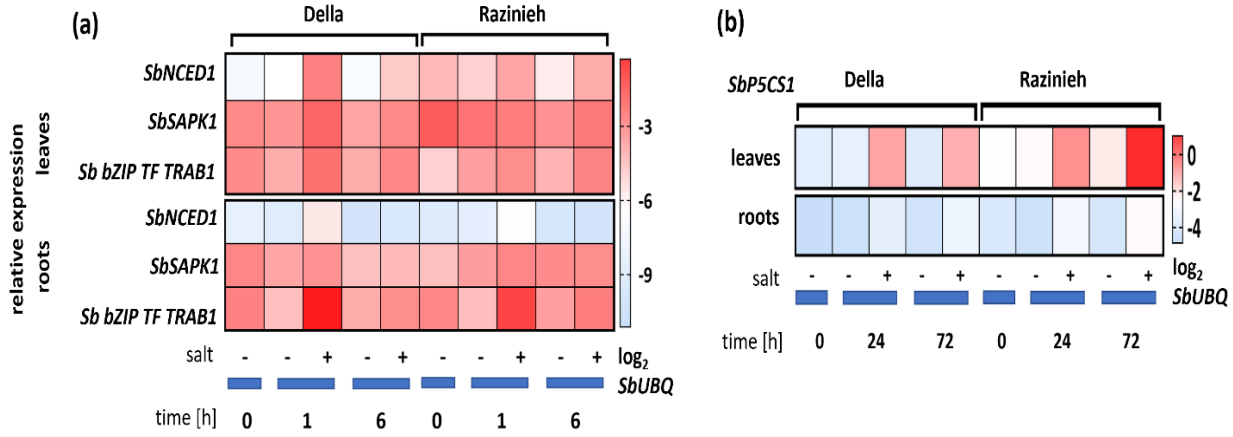

Figure 3.9. Heat maps representing the Log2 fold change of steady-state transcripts level of stress-related genes in response to salinity (a) *SbNCED1*, *SbSAPK1*, *bZIP-TF-TRAB1-like* and (b) *SbP5Cs1*. Thirteen days old Della and Razinieh seedlings were treated with 100 mM NaCl for 1, 6, 24 and 72 h. Values represent the mean of at least three independent biological replicates ±SE. Published data in Abuslima et al., Frontiers in Plant Science (2022).

3.1.7. *SbP5CS1* gene encoding a rate-limiting enzyme in proline synthesis is upregulated more strongly in Razinieh

Accumulation of proline protects against the ionic component of salt stress since proline protects proteins and membranes from structural damage. We had seen (**Figure 3.4b**) that proline was accumulating in leaves of both genotypes in response to salinity (swifter and stronger in Della), while this response was not very pronounced in the roots. To further understand this adaptive response, we followed the expression of *SbP5Cs1* (D^1-pyrroline-5-carboxylate synthase1), encoding the first committed enzyme of the pathway that directs glutamate to proline biosynthesis (**Figure 3.9b**).

To relate transcripts to the resulting metabolite, 24 h and 72 h were selected as time points. In principle, the pattern of the transcript paralleled that of proline. In roots, the induced expression of *SbP5Cs1* was relatively weak under salt treatment, with around 2-fold higher level in Razinieh over Della at both time points. In contrast, the induction was clear and more evident in leaves; the transcripts level were significantly higher in Razinieh than those seen in Della after 72 h of salt stress. This is interesting to note because the actual proline content at this time point was less accumulated in Razinieh as compared to Della (**Figure 3.5b**), despite that both genotypes eventually achieved a similar level after 6 days of treatment (around 190- fold compared to the corresponding controls (**Figure 3.5b**). These data are congruent with the notion that the induction of *SbP5Cs1* in the leaves precedes and predicts the subsequent accumulation of proline. While the accumulation of proline appears to start earlier in Della than in Razinieh, then in the latter, it has to be balanced by inducing more expression of this critical enzyme.

3.1.8. Different patterns of induced jasmonate accumulation under salt stress between the two genotypes

Since most adaptive responses under salt stress are under the control of endogenoues levels of hormones content, we monitored the steady-state content of ABA, OPDA, JA, and JA after 0,1, 6, and 12 hrs after salt treatment **(Figure 3.10).** The most distinct change was a significant but temporary accumulation of JA in the roots of Razinieh, which was not seen in Della. Interestingly, this was not translated into a corresponding accumulation of the conjugate JA-Ile, indicating that

JA was not further converted locally. Also, the precursor, OPDA, did not increase in response to salinity, possibly, because it was swiftly converted to JA. It should be mentioned that the ground level of JA in Razinieh roots was 3-fold higher that in Della. Alebit JA increased by about ~3-fold in Della within an hour of salt treatment; it did not even approach the ground level observed in Razinieh. It was interesting to see that the response in the leaves was the opposite. Here in Della, JA accumulated as a result of salinity, whereas in Razinieh, the response was less evident. Again, unlike the roots, here, JA was effectively conjugated to JA-Ile, whereas an identical increase in JA-Ile followed the change in JA level. Surprisingly, the steady-state levels of ABA did not increase under salt treatment but rather was stabilised in comparison to the variations seen for the jasmonates, with the ground levels in the leaves of Della being around 20% higher than seen in Razinieh.

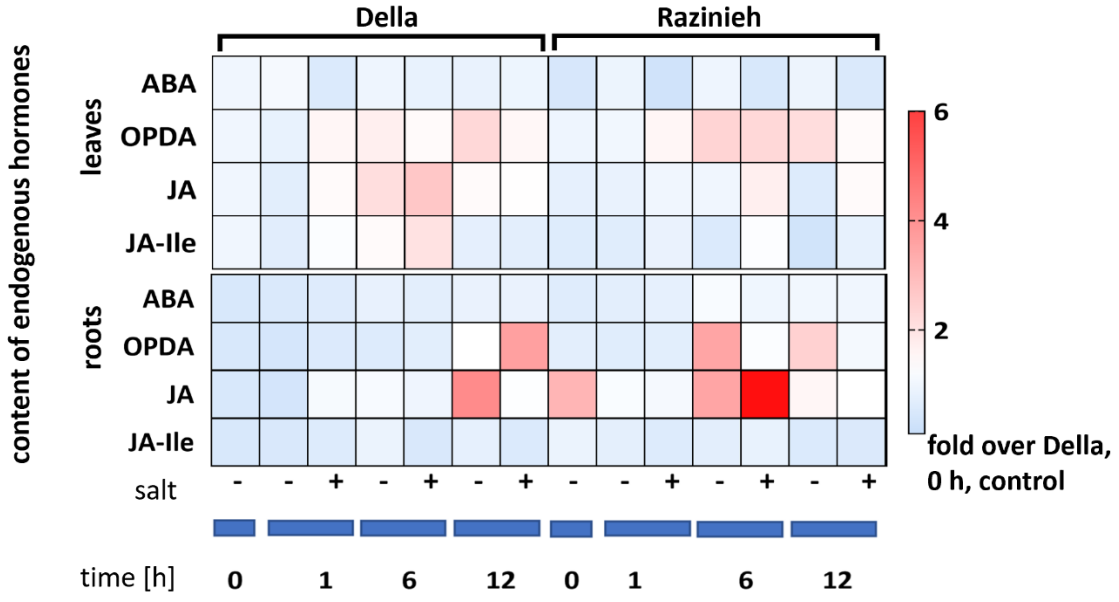

Figure 3.10. Heat maps representing the Log2 fold change of endogenous hormones content compared to the control of Della at day 0 in roots and second leaves of Della and Razinieh seedlings treated with 100 mM NaCl for 1, 6, and 12 h. Values represent the mean of at least three independent biological replicates ±SE. Published data in Abuslima et al., Frontiers in Plant Science (2022).

3.2. Chapter 2: The different patterns in sugar partitioning between sweet and grain sorghum genotypes under salt stress is reflected by differential phenotypic traits

3.2.1. Differential phenotypic traits between Della and Razinieh genotypes

To understand better the main phenotypic distinctions between sweet and grain sorghum genotypes, we started the salt treatment with flag leaf emergence **(Figure 3.11a)** and recorded some morpho-physiological parameters for 30 days after the treatment. In terms of sugar concentration as °Brix, juice volume, and juice weight, we found that "Della" the sweet sorghum genotype, outperformed "Razinieh" the grain genotype (**Figur 3.11b, Supplementary Figure S3.8a, b**). Meanwhile, Raznineh outperformed Della regarding the panicle yield (**Figure 3.11d**).

The characteristics of the stem that relate to its ability to act as a sink for soluble sugars primarily determine cane and juice yields in sorghum. In order to differentiate between the strength of the stem sink of both genotypes, we examined in each internode (from base to top); the sugar concentration (°Brix), internode length, weight, juice content, and area of the adjacent leaf.

In Della, sugar concentration, juice content and internodes fresh weight were significantly higher under both ground level and salt treatment **(Figure 3.11, Supplementary Figure S3.8a-c).** On the other hand, our results show that in Razinieh, the sugar content (in degree Brix) increased only significantly after 10 days of stress treatment (2.9±0.45 to 4.1±1.5, $P < 0.05$). Meanwhile, in Della, the sugar content shows an accumulative pattern under both control and salinity conditions and increased significantly (7.2±0.85 to 9.1±2.65, $P < 0.05$) after 30 days of salt treatment in comparison to control **(Figure 3.11b).** Again in Razinieh, the change in the green leaf area after 10 days of salt treatment was very interesting. It shows a significant decrease from internode 2 to internode 6 by approximately 30 %; meanwhile, at internode 12 green leaf area in Razinieh increased under salt treatment by 200% compared to the control **(Supplementary Figure S3.9e).**

Juice volume was evaluated for both the control and salinity treatments in order to determine whether the increase in soluble sugars content (as indicated by °Brix) was caused by a loss of water from the stem **(Supplementary Figure S3.8a)**. The juice volume was more or less constant in Della from 181.6 ml per plant under control to 167.5 ml per plant after 30 days of salinity

treatment. Meanwhile, a significant decrease was noticed in the juice volume in Razinieh after 10 days of the stress (control = 88.6 ml/plant; salinity stress = 51.5 ml/plant).

The next step was to look at ion accumulation in roots, internodes and leaves in both genotypes after 0, 10, 20, and 30 days of salt treatment. Expectedly, root tissues had accumulated significant sodium amounts under stress treatment. Again Della can withhold sodium in the roots more efficiently than Razinieh **(Figure 3.12a**). Moreover, Razinieh accumulated significantly higher sodium concentrations in the middle internodes by 2-folds compared to the control after 30 days of treatment and in mature grains by 6-fold compared to the control **(Figure 3.12b, f).** This is linked with a concomitant decrease of potassium content in Razinieh, especially in roots and middle leaves under stress treatment compared to the control **(Supplementary Figure S3.10a, d).** Albeit, the expression of salt-related genes; *SbSOS1*, *SbNHX1* and *SbHKT1* in roots of both genotypes have not responded to salt treatment **(Supplementary Figure S3.11).**

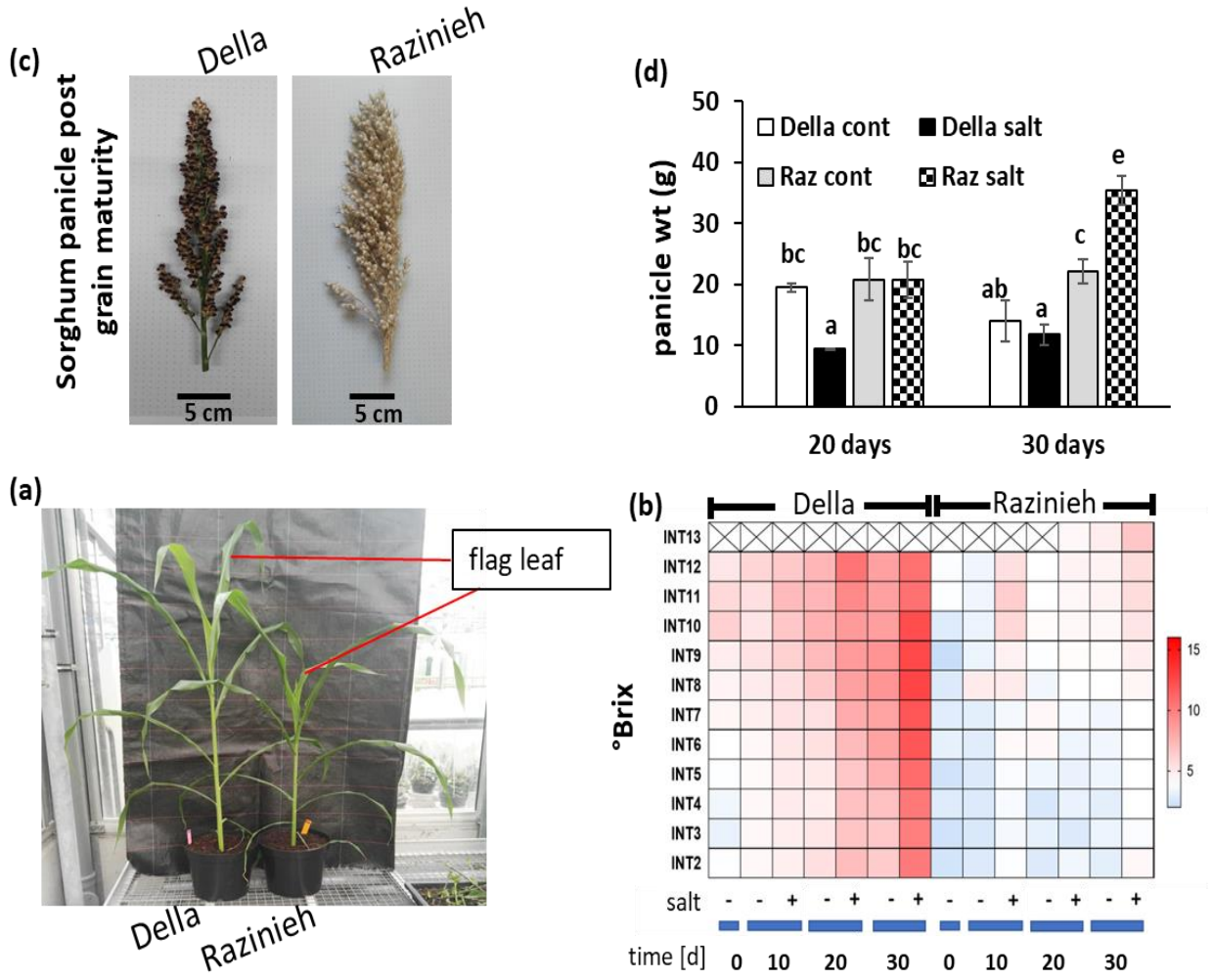

Figure 3.11. Differences between Della and Razinieh plants in phenotypic traits at flag leaf stage: (a) Della and Razinieh plants at flag leaf stage (at 0 day; the start of salt treatment), (b) concentration of total soluble sugars in each internode under control and salt treatment indicated as °Brix, (c) sorghum panicle collected after grain maturity under salt treatment, (d) panicle weight recorded after 20 and 30 days after salt treatment.

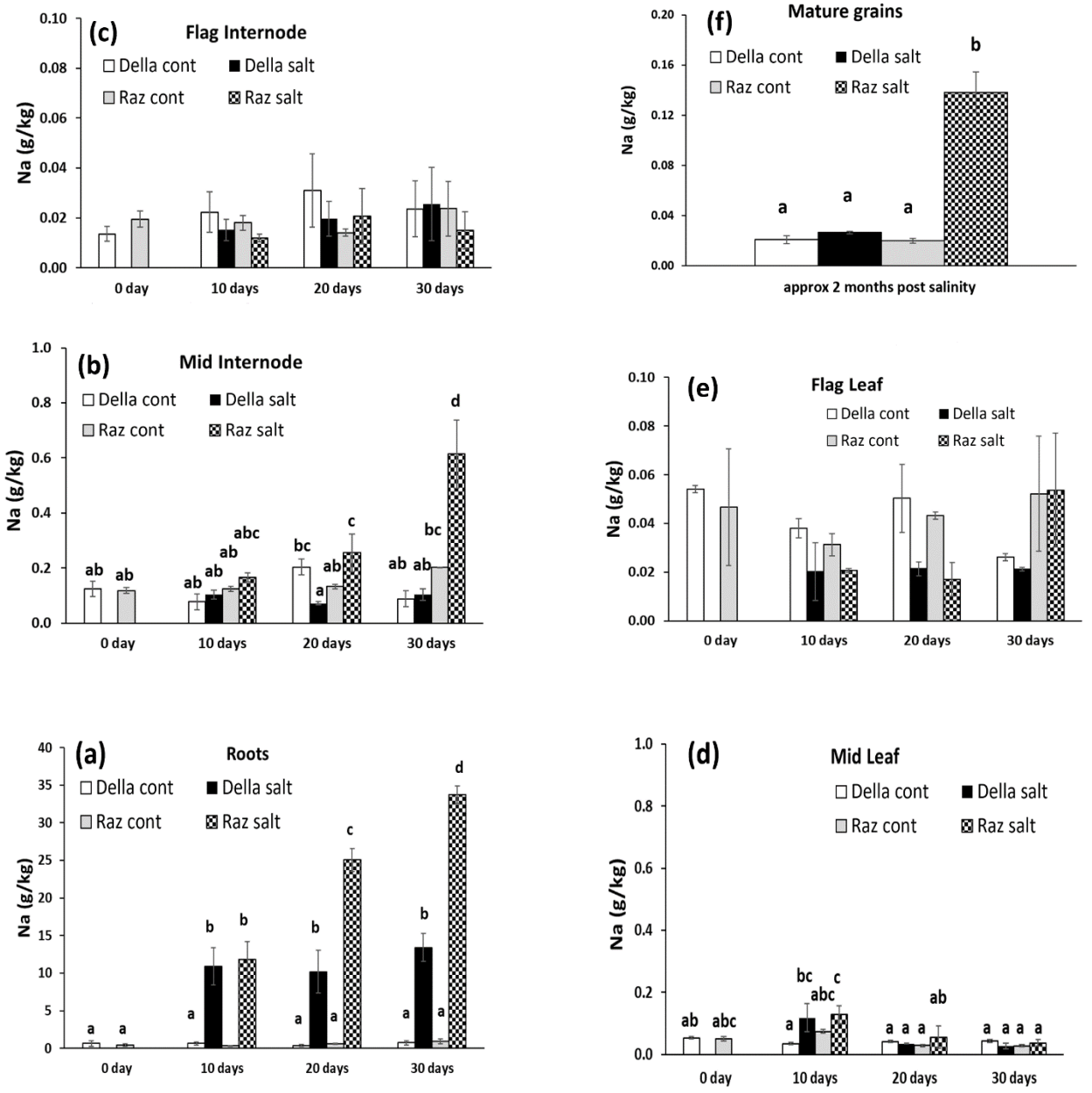

Figure 3.12. Sodium ion concentration in roots, internodes, leaves and grains of a sweet (Della) and a grain (Razinieh) sorghum genotypes at flag leaf stage under control and salt treatment (100mM NaCl) after 0, 10, 20, and 30 days of treatment. Values represent the mean of at least three independent biological replicates ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05).

3.2.2. Alterations in sugar profile in sorghum roots, internodes, and leaves in response to salt stress

The next step was testing sugar partitioning under salt stress. We preferentially examined the effect of salt stress on sucrose, glucose and fructose contents in roots, internodes and leaves of both genotypes **(Figure 3.13).**

Quantifying sugars showed a high and significant increase in sucrose concentration in both genotypes, especially in the roots under salt stress treatment. Sucrose was preferentially accumulated in the roots of Della from **(19.4 to 79.4 mg/g dw)** and in Razinieh from **(14.3 to 24.6 mg/g dw),** respectively, after 10 days of the stress treatment compared with untreated plants **(Figure 3.13a).**

Further, we quantified the changes in sugar concentrations in internodes and leaves of both genotypes **(Figure 3.13b-e).** Similar to the root tissues, the amount of sucrose increased sharply but to a lesser extent in the middle internodes under salt stress. However, sucrose accumulation was more evident in Razinieh under both control and salt treatment compared to Della. Sucrose concentration was increased significantly in Della from **4.3 to 9.6 mg/g dw** and in Razinieh from **7.13 to 12.6 mg/g dw** after 10 days of salt treatment compared to respective controls **(Figure 3.13c).**

Similar effects of salt stress on sucrose concentration were observed in the middle leaves of Della after 30 days from the stress. Sucrose concentration was increased from **3.3 to 7.2 mg/g dw** at the expense of a significant decrease in glucose and fructose contents compared to the control **(Figure 3.13b).** Interestingly, in flag internodes, the ground level in sucrose content was ≈ 7- folds higher in Razinieh compared to Della; however, the most prominent change to salt treatment was seen in Della as a significant decrease in fructose content from **10.1 to 5.7 mg/g dw** and in glucose content from **6.8 to 2.2 mg/g dw** compared to control **(Figure 3.13e).**

These results indicated that the differential effects of salt stress on sugars content and transport in sweet versus grain sorghum genotypes eventually resulted in different phenotypic traits under abiotic stress.

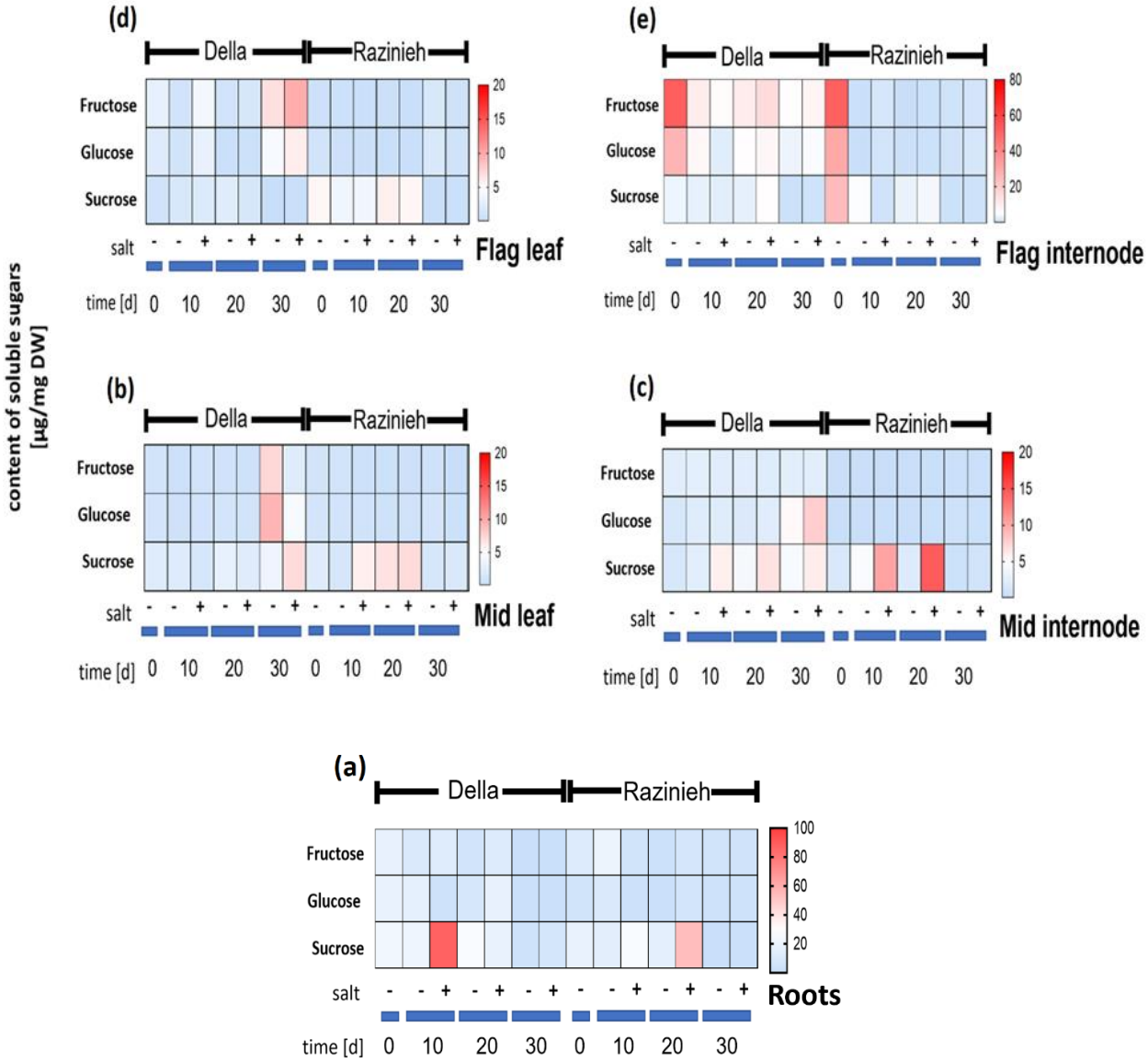

Figure 3.13. Absolute values of soluble sugars: fructose, glucose and sucrose in roots, leaves and internodes of a sweet (Della) and a grain (Razinieh) sorghum genotypes at flag leaf stage under control and salt treatment (100mM NaCl) after 10, 20, and 30 days of treatment. Values represent the mean of at least three independent biological replicates.

3.2.3. Differential expression of *SbSUTs* and *SbSWEETs genes*

Since sucrose is apoplastically transported through phloem in sorghum, we investigated the potential effect of salt stress in sucrose metabolism and phloem loading and unloading by analysing the transcripts of sucrose related genes, including synthesis and cleavage (*SbSPSs SbSUSs*, and *SbCINVs)*, and transport (*SbSUTs*, *SbSWEETs*) in both source and sink tissues. **(Figure 3.14).**

From sucrose synthesis genes, we have studied the expression of both *SbSPS1* and *SbSPS4.* Interestingly, in flag leaf, the transcripts levels of both genes were higher in Della at ground level by 35-fold and 3-fold compared to Razinieh. The most salient difference was in the middle leaf after 1 day to salinity, whereas *SbSPS1* expression showed a contrasting pattern between the two genotypes; with a significant decrease (5-folds) in Della, but it showed a significant increase in Razinieh (4-folds) compared to corresponding controls. However, in the middle internode, *SbSPS1* expression increased in response to salinity after 1 day of treatment in both genotypes but with superiority to Razinieh. In the roots, although the ground transcript levels of both genes were similar, the expression was more responsive to salinity in Razinieh. Meanwhile, no change was noticed in Della.

The expression of both *SbSUS3*, and *SbSUS4* genes, showed a significant increase in Della middle internode by ≈ 1-fold and ≈ 6-fold, respectively after 1 day of salt treatment compared to control. On the other hand, *SbCINV1* transcripts were significantly decreased by 4-folds in Della flag internode after 1 day of salt treatment compared to control. Meanwhile, in flag leaf *SbCINV2* decreased in Della after 1, and 5 days of salt treatment by 17-fold and 5-fold, respectively. No significant change was noticed in Razinieh.

All *SbSUTs and SbSWEETs* were expressed at measurable levels in all tissues examined. In normal conditions, analysis of *SbSUT1* and *SbSUT4* genes showed that these genes are more strongly expressed in all studied tissues, with higher levels of *SbSUT4* observed in Razinieh compared to Della. However, in roots, *SbSUT6* and *SbSWEET6* are the most expressed sucrose transporter genes. In addition, the transcripts level of *SbSUT6* was 3.5-fold higher in Della compared to Razinieh at the ground level. Albeit, the expression of *SbSUT6* and *SbSWEET6* was lower in leaves and internode tissues for both genotypes compared to the roots **(Figure 3.14).**

Under salt treatment, the most remarkable difference with respect to the expression of different sucrose transporters is observed in sink tissues **(Figure 3.14)**. Nevertheless, the response of *SbSUTs* and *SbSWEETs* transcripts to salt stress is complex and varied depending on each individual gene, the organ (roots, internodes), and the genotype **(Figure 3.14)**. As a result, the transcription pattern will be discussed independently for each genotype.

In Della, in flag internodes, *SbSUT2 and SbSUT5* transcripts were decreased significantly by 3-fold and 47-folds, respectively; meanwhile, both *SbSUT1 and SbSUT6* transcripts increased significantly by 3-fold during the first day of salt treatment compared to control **(Figure 3.14)**. Interestingly sharp and significant increase was noticed with *SbSWEET13* expression in middle and flag internodes by 2-fold and 57-fold compared to control, which was found to be related to the significant increase of *SbbZIP-TF-TRAB1* by 1.5-fold and 6-fold, respectively in the same tissues **(Figure 3.14)**.

In Razinieh, on the other hand, *SbSUT2* and *SbSUT5* transcripts observed in the flag internode did not show any change under salt treatment. Albeit, the increase in *SbSUT1* and *SbSUT6* transcripts in flag internodes was relatively high compared to Della. Interestingly the ground level of *SbSWEET13* transcripts in Razinieh was relatively high compared to Della by 5-fold in both middle and flag internodes but did not respond to salt treatment in internodes or leaves tissues. However, in roots, *SbSWEET13* expression increased in Razinieh after 1 d of salt treatment by 20-fold and correlated with the increase of *SbbZIP-TF-TRAB1* by 3-fold compared to control **(Figure 3.14)**.

As a result, the fluctuations in the expression levels of these genes could not directly link to the different phenotyping or the significant differences in sugar storage in roots and internodes of Della and Razinieh.

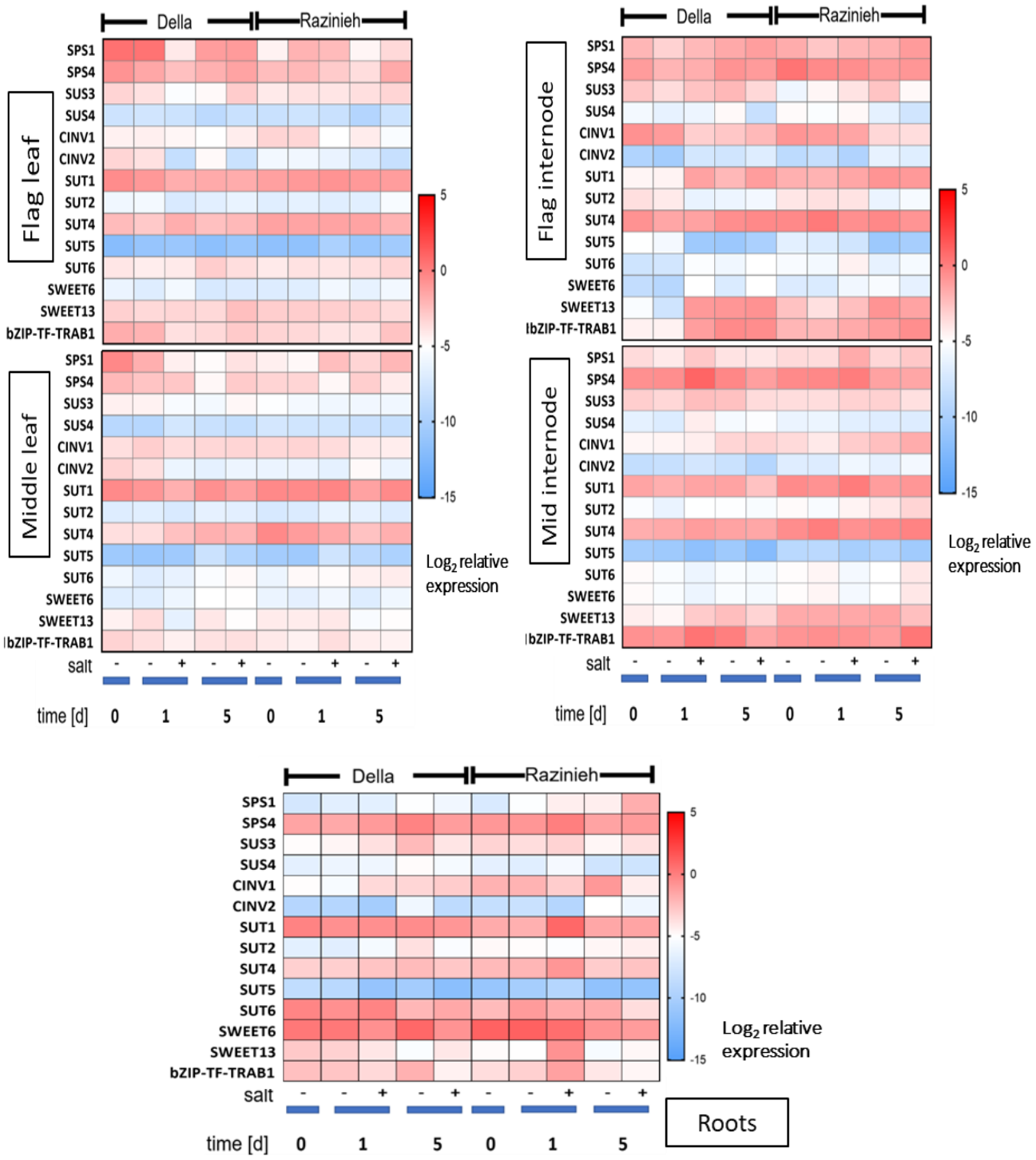

Figure 3.14. Relative expression analysis of selected sucrose metabolism genes in a sweet (Della) and a grain (Razinieh) sorghum genotypes at flag leaf stage under control and salt treatment (100mM NaCl) after 0, 1, and 5 days of treatment. Values represent the mean of at least three independent biological replicates.

3.2.4. *Cis*-element comparison of the promoters *pSWEET13, pSUT2,* and *pSUT6* between Della and Razinieh genotypes

To get insight into the regulatory features of three candidate sucrose transporter genes, we cloned all six promoter regions (*pSWEET13*, *pSUT2*, and *pSUT6* from both Della and Razinieh) 1600~2000 bp upstream of ATG start codon. We found a number of abiotic stress-related *cis*-acting elements distributed across the selected promoter regions, such as; Abscisic acid responsive element (ABRE) with ACGTG; methyl-jasmonate responsive element with CGTCA and TGACG; dehydration responsive element with CCGAC, and bZIP binding sites with ACGTG conserved sequence.

However, these elements have different frequencies within the promoters of different genes **(Figure 3.15)**. For example, *pSUT6* promoter of both alleles has 3 methyl-jasmonate responsive elements (CGTCA) motives **(Figure 3.15c)**; meanwhile, both alleles of *pSUT2* had only 2 methyl-jasmonate responsive motives **(Figure 3.15b)**. In the case of *pSWEET13*, the allele of Della harbours one CGTCA motif, which is not found in the *pSWEET13* allele from Razinieh **(Figure 3.15a)**. On the other hand, ABRE (ACGTG) is more frequently present both alleles of *pSUT2* compared to *pSUT6* and *pSWEET13.* Likewise, dehydration responsive element DRE (CCGAC) was very frequent in both alleles of *pSUT2*; meanwhile, this element was less frequent in *pSWEET13* and absent in both alleles of *pSUT6.* The full alignment with the details of these *cis*-elements are given in **Supplementary Figure S3.12.**

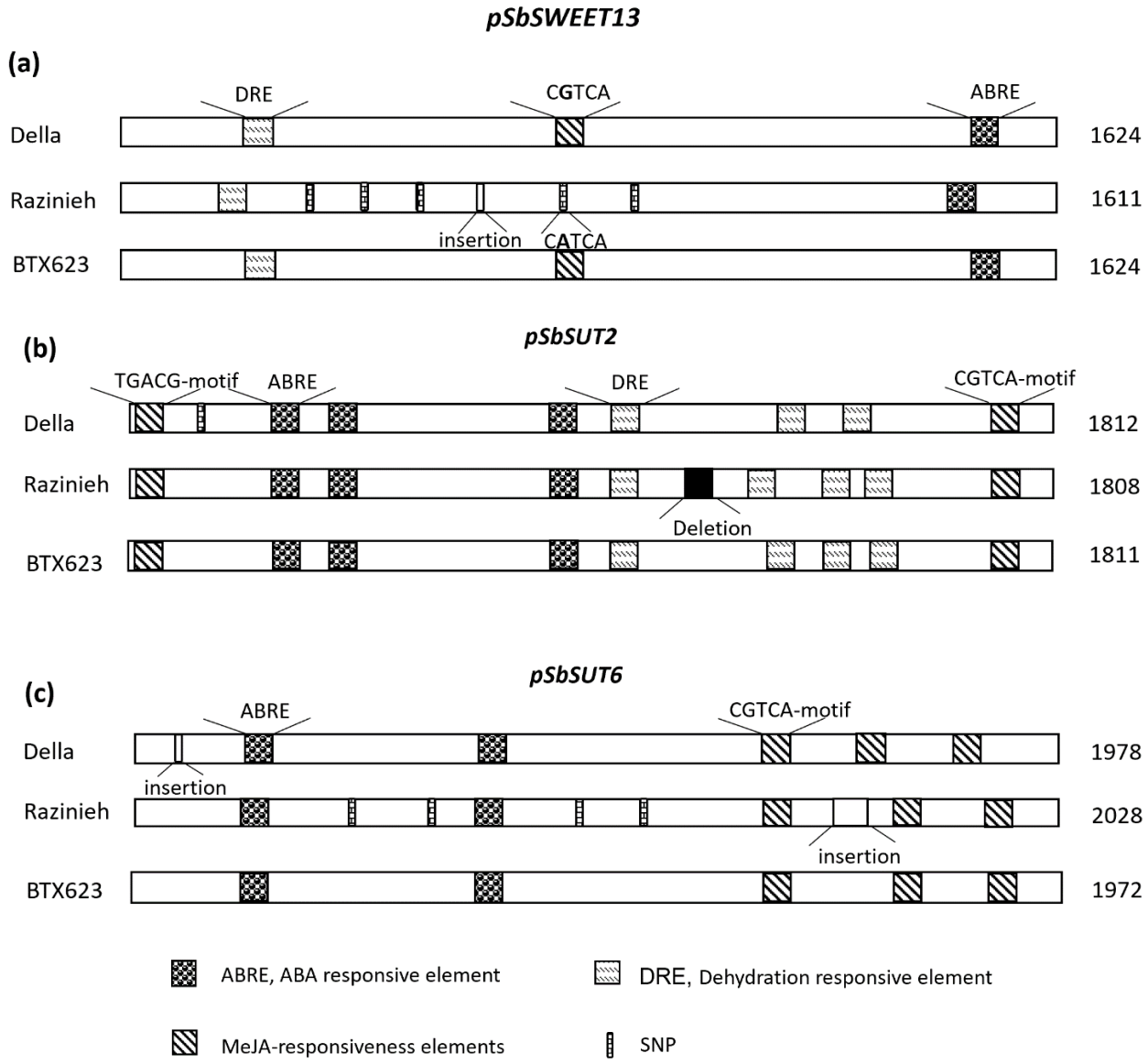

Figure 3.15. Comparison of *pSbSWEET13*, *pSbSUT2*, and *pSbSUT6* promoters of a sweet (Della) and a grain (Razinieh) sorghum genotypes, along with the reference genome BTX623. The distribution of different stress related *cis*-element is represented on sense strand are the following; ABA responsive elemnet (ABRE) with ACGTG, dehydration responsive element (DRE) with CCGAC, Methyl jasmonate (MeJA) responsive elements with TGACG, and CGTCA.

3.2.5. ABA activates promoter activity *of pSbSWEET13*, meanwhile; MeJA activates promoter activity of *pSbSUT6*

The abundance of different stress-related *cis*-acting elements in the studied promoters suggests that the expression of *SbSUTs* and *SbSWEETs* is very sensitive to abiotic stresses. Using the dual-luciferase reporter assay system in sorghum protoplasts suspension, we tested the inducibility of the studied promoters to known early signalling events such as induction by Abscisic acid or methyl jasmonate or osmotic stress to investigate whether the differences in the inducibility of the *pSWEET13, pSUT2,* and *pSUT6* promoters an account of the stress-specific differences in the expression of different sucrose transporter genes.

In the next step, we measured the activation of the six promoters in response to different treatments such as 25 µM ABA, 50 µM MeJA, 25% PEG (6000), and 200 mM NaCl after 1 h against a control treatment "mock treatment" which did not induce any significant activation in the activity of the promoters **(Figure 3.16).**

In the case of *pSWEET13,* the responsiveness to ABA, and PEG was more evident in Della compared to Razinieh. Meanwhile, in response to NaCl, both alleles were more or less equally induced; however, no activity stimulation was found with MeJA treatment in both alleles **(Figure 3.16a)**. In contrast, *pSUT2* promoter was not induced strongly under any of the different treatments. In addition, the activity of *pSUT2* promoter of both alleles was even inhibited by ABA and PEG treatments **(Figure 3.16b)**. Interestingly the activity of *pSUT6* promoter from allele of Razinieh was induced by 1.2 folds with MeJA treatment; however, it was entirely repressed with the other treatments. However, *pSUT6* promoter from allele of Della, shows equal inducibility under MeJA, PEG, and NaCl treatments by around 1-fold **(Figure 3.16c).**

These results indicated that the differential responsiveness of promoters of the selected sucrose transporter genes to different upstream signalling events is suggested to mediate the induction of sucrose transporter genes as observed by quantitative real-time expression analyses.

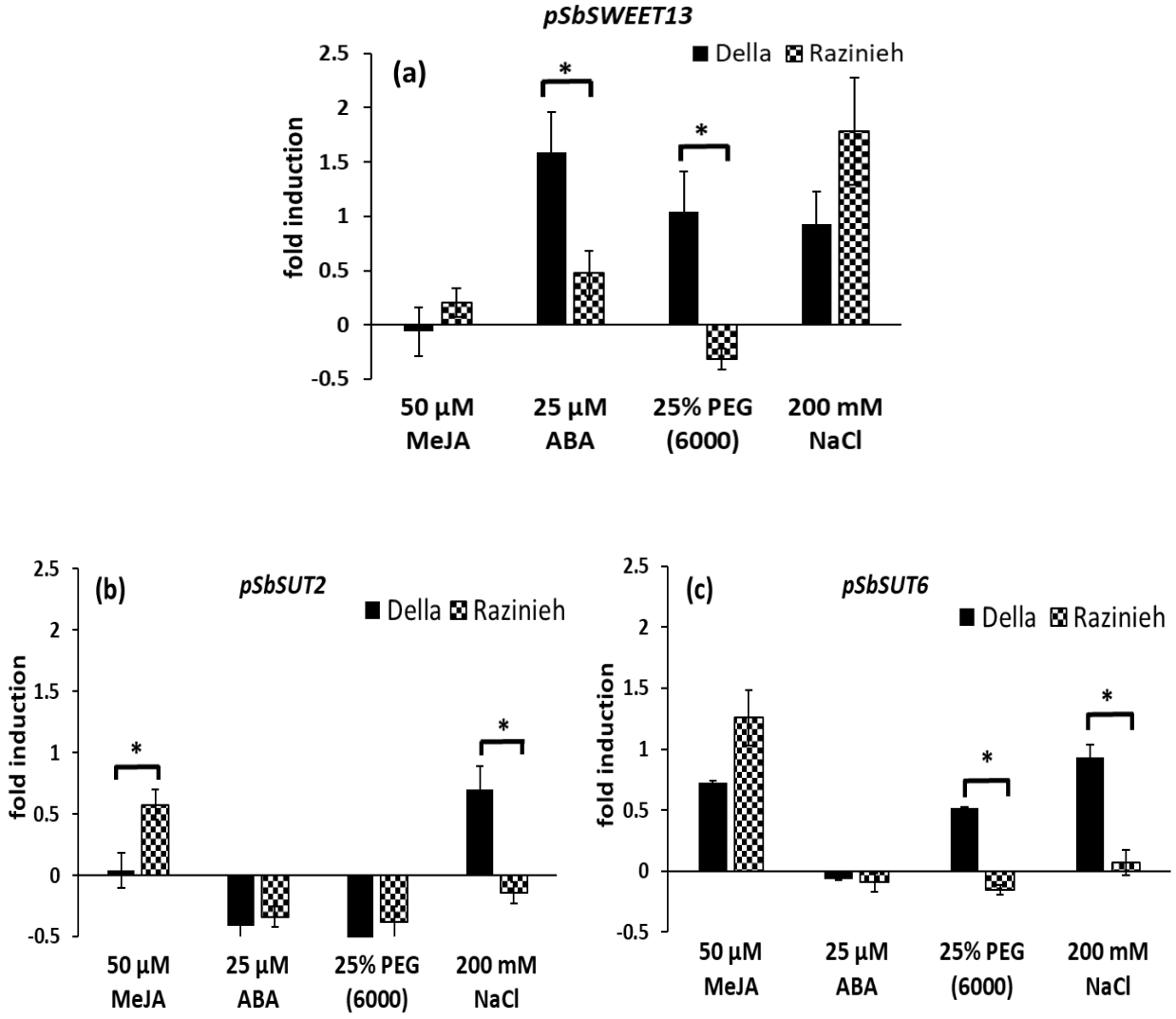

Figure 3.16. Activities of *pSbSW*EET*13*, *pSbSUT2*, and *pSbSUT6* promoters derived from Della and Razinieh sorghum genotypes after 1 h of each treatment; 50 µM MeJA, 25 µM ABA, 25% PEG(6000), and 200 mM NaCl. All plasmids were transferred transiently to Della genotype protoplasts using PEG method. The columns show the normalised induction level relative to the untreated control after 1 h from each treatment. Asterisks indicate a statistically significant difference between genotypes, as determined by Student's *t*-test (*p<0.05)

4. Discussion

4.1. Chapter 1: The salt tolerant sorghum genotype can efficiently withhold sodium translocation to the shoot: a case gives more time to adapt to salt stress

In this study, we examined a contrasting pair of sorghum genotypes to salt stress; Della (sweet sorghum) and Razinieh (grain sorghum). We demonstrated how salt tolerance in Della is related to the restricted transfer of sodium from root to shoot, which is supported by superior vacuolar Na^+ sequestration in the root distal elongation zone, as shown by CoroNa green fluorescent dye. Furthermore, when plants were exposed to salinity, the leaves of Della showed swift proline accumulation with better redox homeostasis; meanwhile, sucrose was transported to the roots more effectively. Other distinct changes also included glycine accumulation, steady-state levels of jasmonic acid and its bioactive conjugate JA-Ile, and a number of transcripts implicated in ABA and proline biosynthesis.

Stress-related events can be of dual nature, and the delineation between stress adaptation and stress damage becomes possible when the observed differences are compared among a contrasting pair of genotypes. Our study included a working model explaining the observed conditions between tolerance and susceptibility to salt stress, providing the notion of the different amplitude and speed of systemic signal. In Della, the salt-tolerant genotype, the vacuolar sodium sequestration in the distal elongation zone and the decreased sodium translocation to the shoots are accompanied by effective sucrose translocation from the shoot to the root. These adaptive measures maintain root growth under salinity and ameliorate ionic stress effect on photosynthesis, allowing compatible osmolytes such as proline to accumulate swiftly. The reduced photorespiration rate revealed by lower glycine levels in Della are an indicator of the ensuing buffering against oxidative stress, which preserves the photosynthetic efficiency. The potential role of jasmonates as local and systemic signals that coordinate the response to salt stress can be deduced from the provided metabolic and phytohormonal indicators of salt adaptation versus salt damage which are shown in the model (**Figure 4.1**).

4.1.1. Della is more efficiently able to retain sodium in the root

To confine the impact of chronic sodium uptake, the challenged plant can either (i), impede the influx of excess ions, (ii), sequester sodium in the vacuole to remove it from the cytosol, (iii), induce necrosis to mobilise the resources of the affected organ and isolate it from the remaining organism, or (iv), sustain growth and development by adjusting metabolism and physiology to ameliorate the elevated levels of sodium ions. These strategies are not mutually exclusive and depend on the respective conditions (stress duration, severity & plant developmental status) and how the challenged plant will allocate resources to these four strategies. Our data indicate that the superior performance of Della is linked with a more efficient exclusion of sodium from shoot and leaves. The evidence is as follows: while comparable levels of sodium accumulate in the roots (**Figure 3.1b**), Della can more efficiently prevent sodium from entering the shoot **(Figure 3.1a)**, with the transfer coefficient being 7-times lower than in Razinieh **(Supplementary Figure S3.1)**.

What are the reasons for this restricted sodium transfer to the shoot? A possible mechanism might be a more efficient extrusion of sodium through the SOS1 exporter. In fact, the resting levels of *SbSOS1* transcripts are elevated (more than twice) in Della roots over those in Razinieh, indicative of a more active extrusion system **(Supplementary figure S3.6b)**. A second mechanism would be sequestration in the vacuole of root cortex cells. This is strongly supported by our CoroNa Green data. The main difference between the genotypes occurs already in the distal elongation zone of the root, where Della is effectively sequestering sodium into the vacuole while Razinieh is unable to do so (**Figure 3.7**). However, the differentiation zone for the two genotypes appears to be quite similar. Therefore, the symplastic pathway of sodium uptake through the root hairs seems unrelated to this condition. Thus, the development of a Casparian strip can also be discounted as a mechanism for the differential transfer of sodium because it seems more evident in Razinieh which is more permissive to sodium translocation to the shoot **(Supplementary figure S3.7).**

This is supported by the apparent induction of *SbNHX2* in roots of Razinieh under salt stress, indicative of a more substantial requirement for this protein, while Della does not exhibit such an induction (**Figure 3.5a**). The resting levels are comparable, however (contrasting with the situation for *SbSOS1*, which is not responsive to salt stress). Overall, sodium retention in the root appears to be substantially regulated by sodium sequestration into the vacuole, despite sodium exclusion maybe having only a minor effect. This is consistent with previous findings about the responses

of barley to salinity (Wu et al., 2019). A third mechanism to confine sodium transfer to the shoot, might be sodium retrieval from the xylem sap through HKT1 transporters (for review see Keisham et al., 2018). In Della, the higher ground level of *SbHKT1* transcripts in the root (**Figure 3.5b**) correlates with the reduced transfer of sodium to the shoot **(Figure 3.1a)**.

The massive increase of cytosolic sodium should lead to membrane depolarisation, rendering the passive import of potassium through AKT1 channels in the root plasma membrane thermodynamically unfavourable (Wu et al., 2018). In addition, membrane depolarisation will also activate K^+ outward rectifying channels such as GORKs (Adams and Shin, 2014; Jayakannan et al., 2013), and the ROS-activated NSCCs (Sun et al., 2009; Wu et al., 2015). In fact, we are able to observe significant decreases in potassium content under salt conditions. This decrease was strong in the roots of both genotypes **(Supplementary Figure S3.2b)**, while in the shoot, potassium levels sustained significantly better in Della **(Supplementary Figure S3.2b)**. The mechanisms behind this superior potassium homeostasis in Della shoots remain to be elucidated, but molecular candidates are known. The activation of K^+ selective KUP/HAK/KT transporters or the stelar potassium outward rectifiers (Maathuis, 2006) might be employed in regulating potassium efflux to the xylem under K^+ reduction (Han et al., 2016). In rice, knockout of OsHAK1 causes salt sensitivity, while overexpression of this transporter enables the plants to withstand salt stress (Chen et al., 2015).

4.1.2. Sustaining sucrose allocation to the roots in Della correlates with adaption to salt

Sugars, primarily sucrose, glucose, and fructose, are the primary products of photosynthesis and are particularly sensitive to abiotic stress (for review, see Lemoine et al., 2013). In addition to acting as osmolytes and signalling molecules to control the expression of several genes during abiotic stress, they provide the energy required for adaptation (for review see Rosa et al., 2009). In comparison to Razinieh, where sucrose is found in the leaves, we noticed that sucrose accumulates more persistently in the roots in Della **(Figure 3.4a)**. It is straightforward to assume that sucrose, the predominant transport form for carbohydrates, is efficiently translocated from the leaves to the roots in Della, while in Razinieh, this transport is more sluggish. Since roots rely on shoot-derived sucrose as an energy source, especially if challenged by abiotic stress (Li et al.,

2021; Milne et al., 2018), such sucrose accumulation in Della roots under salt stress is assumed as a sign of efficient adaptation. Such long-distance transport from leaves to roots as sink tissue requires loading and unloading the phloem involving SWEET and SUT proteins. As has been shown in rice under drought and salt stress, one would anticipate that transcripts of sucrose transporter genes would be elevated in response to salinity (Mathan et al., 2021). In contrast to Razinieh, Della exhibits a very rapid and noticeable buildup of fructose and glucose in the leaves, together with the rapid accumulation of sucrose in the root **(Figure 3.4a)**. In a study comparing perennial ryegrass with contrasting salt tolerance (Hu et al., 2013), the tolerant genotype accumulated sucrose in a manner similar to that seen in Della.

Sucrose transport to the root qualifies as an adaptive trait. This adaptive mechanism is only efficient when the photosynthesis process is protected even under salt stress. Closing the stomatal in response to the osmotic stress component limits CO_2 uptake, but oxygen from water splitting at photosystem II cannot be eliminated and accumulates, increasing from chances of binding RubisCO to oxygen, leading to photorespiration (Munns & Tester, 2008). Then in peroxisomes, Phosphoglycolate is converted to glyoxylate by the glycolate oxidase and reacts with glutamate into glycine and a-ketoglutarate (for review see Bauwe et al., 2010). The accumulation of photorespiratory intermediates such as glycine at the dispense of depleting glutamate is used as a metabolite marker of increased oxygenation of Rubisco.

From our metabolomic profile of amino acids (**Figure 3.4b**), we can assume that Della exploited an enhanced CO_2 recycling under salinity stress; meanwhile, Razinieh exhibited high rates of photorespiration as evidenced by the rapid and high glycine level in leaves. Increased glycine pool in C4 plants leaves such as *Amaranthus edulis* (Maroco et al., 2000) and maize under drought stress (Foyer et al., 1998) was interpreted as well as an increase in the rate of photorespiration.

It has been reported that salt tolerance in sorghum has been associated with maintaining high photosynthetic efficiency in sorghum as a C4 crop is linked with catalysing the decarboxylation of malic acid by NADP-Malate Dehydrogenase (NADP-ME) to liberate CO_2 for fixation via Rubisco (for review see Yang et al., 2020). The high expression of the gene encoding NADP-ME enzyme correlates with salinity tolerance (Sui et al., 2015), and overexpression of sorghum NADP-Malate Dehydrogenase can confer salinity tolerance to Arabidopsis as a host (Guo et al., 2018), assigning to this enzyme a key role for salinity tolerance.

The consequences of the high rate of photorespiration are reducing the transfer rate of sucrose from leaves to roots and will also result in the buildup of reactive oxygen species. Reduced NADPH will build up as a result of decreased carbon fixation, feeding back on electron transport, leading to a phenomenon known as hyperreduction, whereby the free electrons will bind to molecular oxygen (Allakhverdiev et al., 2000). ROS is produced when excess electrons are transferred to molecular oxygen O_2. Additionally, the peroxisome uses molecular oxygen as an acceptor during the conversion of glycolate to glyoxylate, resulting in hydrogen peroxide as an additional ROS (Bauwe et al., 2010).

To summarise, the slower rate of sodium ion transfer to the shoot in Della reduces the chances of photosynthetic apparatus impairment, which gain time to yield an elevated hexoses content in leaves, meanwhile transferring more sucrose to the roots. This condition suggests more sustained photosynthesis and better redox homeostasis in Della, which helps in tolerance to salinity.

4.1.3. Redox homeostasis helps in photosynthesis protection in Della against salt stress

Phenotypic traits of Della under salt stress indicate more vigorous redox homeostasis. For instance, chlorophyll content in Della is buffered better against salinity (**Figure 3.2c**), and, more strikingly, oxidative damage as reported by the level of MDA is strongly mitigated in Della **(Figure 3.3a)**. There is a particular enzyme signature correlating with this: First, the superoxide radicals produced at PSI are converted to hydrogen peroxide by a membrane-bound copper/zinc superoxide (Cu/ZnSOD), and then the resulting H_2O_2 is converted to water by a membrane-bound thylakoid ascorbate peroxidase (tAPX) in the so-called water-water cycle. In fact, we see a rapid increase in the activities of both enzymes in Della, but not in Razinieh (**Figure 3.3c**). At day 1, the significant SOD activity causes a temporary increase in peroxide steady-state levels, which is already was alleviated at day 3 (**Figure 3.3b**). Our results concur with previous studies, where targeting maize Cu/ZnSOD to chloroplasts of *Brassica campestris* suppressed leaf damage under salt stress conditions concomitant with a superior PSII activity (Tseng et al., 2007) and where overexpression of Arabidopsis *Apx1* gene rendered *Brassica juncea* more salt-tolerant (Saxena et al., 2020).

On the other hand, Razinieh also exhibits signatures indicating a more challenged redox homeostasis. CATs localised in peroxisomes are the principal antioxidative enzymes to scavenge

the H_2O_2 released during photorespiration (Apel & Hirt, 2004; Miller et al., 2010). This explains our findings with the high level of CAT activity **(Figure 3.3c)** in Razinieh leaves after 1 day from the stress. However, under prolonged salinity, CAT activity is collapsing, correlated with the marked increase of H_2O_2 levels after 6 days from the stress (**Figure 3.3b**). Thus, the rise in H_2O_2 levels in the two genotypes seems to originate from different functional contexts. In Della, H_2O_2 derives from the rapid activation of SOD and soon after is dissipated by POD. In Razinieh, hydrogen peroxide derives from the stronger photorespiration and needs to be dissipated by CAT. This can be sustained only for a certain time because at day 6, CAT levels drop, and hydrogen peroxide (as well as MDA as damage readout) surge unrestrained.

On the other hand, increasing GR and POD activities in Razinieh at later stage **(Figure 3.3c)** were considered stress markers. GR is a key enzyme in ascorbate glutathione cycle which catalyse the reduction of H_2O_2 via ascorbate oxidation. In addition, GR catalyses the rate-limiting step in this cycle by reducing glutathione disulphide (GSSG) to reduced glutathione (GSH) to regenerate ascorbate and balance cellular GSH/GSSG ratio (Ahmad et al., 2010b; Noctor et al., 2002). Consequently, GSH can scavenge ROS such as O_2^- and $\cdot OH$ to prevent accumulative oxidative damage. Concurrently, the ratio of $NADP^+$/NADPH increases, thereby ensuring the availability of $NADP^+$ to accept electrons from the over-reduced photosynthetic electron transport chain (Mushtaq et al., 2020). Thus, the high increase in GR in Razinieh leaves at later stage from the stress to our notion is an attempt to relieve oxidative stress by bringing down the electron flow to O_2.

Meanwhile, PODs scavenge H_2O_2 by catalysing the oxidation of phenolic substrates using H_2O_2 as an electron acceptor (Sakihama et al., 2002). In accordance with our findings with the elevated POD activity in Razinieh leaves after 6 days from the stress **(Figure 3.3c)** accompanied by high accumulation of total phenolic content (**Supplementary Figure S3.4a**). When stress causes increased H_2O_2 accumulation in the chloroplast, which exceeds the Asc–GSH cycle scavenging capability, H_2O_2 may escape to the vacuole, where it is scavenged by vacuolar PODs (Gupta et al., 2018). Similarly, the activity of leaf vacuolar PODs in *Catharanthus roseus* for scavenging H_2O_2 employing phenolic substrates in the vacuole (Ferreres et al., 2011). We propose that, in this way, POD are the important sink for the over-accumulated H_2O_2 in Razinieh leaves, utilising phenolics as a substrate under severe stress.

According to the results of the DPPH and ABTS free radical scavenging assays **(Supplementary Figure S3.5 a, b, Figure 3.3 f, g)**, it is indicated that a higher level of non-enzymatic antioxidants in Della could make the late activation of CAT, POD, and GR partially unnecessary. This is undoubtedly a more resource-efficient way than synthesising these enzymes.

Overall, the time gain from the slower transfer of sodium to the shoot allows Della to bolster its redox homeostasis more efficiently, such that photorespiration can be restrained, enabling a more efficient transfer of sucrose to the root, providing the resources for the sequestration of sodium in the vacuoles of the distant elongation zone (which in turn further dampens the transfer of sodium into the shoot).

4.1.4. Proline accumulation is essential for adaption to salt stress

Proline accumulation in cytosol is a crucial metabolic response under water-deprived conditions and high salinity for osmoregulation, cellular redox homeostasis (Haffani et al., 2014), and preventing protein turnover (He et al., 2017). Indeed, we had seen that proline was highly accumulating in leaves of both genotypes but more swiftly in Della in response to salt, and not so much in the roots **(Figure 3.4b)**. Under salt stress, proline synthesis was reported to take place mainly through consuming glutamate as a substrate and then utilising NADPH as a reducing agent in catalysed by 1-pyrroline-5-carboxylate synthetase (P5CS) enzyme, which increases $NADP^+$ availability to ease the over-reduction of PSI during the stress (Armengaud et al., 2004; Fichman et al., 2015; Hare & Cress, 1997). In concurrent with our findings, the transcripts for *SbP5Cs1* responsible for channelling glutamate to proline biosynthesis have increased (Figure 3.9 b) and with a significant reduction in glutamate under salt treatment **(Figure 3.4b)**. Interestingly, *SbP5Cs1* expression in Razinieh tended to accumulate higher at day 3, while in Della, the expression remained more or less stabilised between day 1 and 3 under the stress condition. Albeit, such high accumulation of transcripts did not result in a parallel increase in proline steady-state levels. Proline can scavenge ROS, giving rise to hydroxyproline (for review see Liang et al., 2013). Such continuous activity of a key limiting enzyme in proline synthesis is considered a marker for stress damage rather than stress adaptation. Unlike *SbP5Cs1* transcripts, the actual proline level is used as a marker for tolerance.

Similar to the rapid accumulation of proline in the leaves of wide rice cultivar *O. australiensis* that was correlated with the high tolerance to salt with maintaining relative water content and cell membranes integrity compared to the cultivated rice genotypes Pokkali and Nipponbare (Nguyen et al., 2021). The rapid accumulation of proline is also interpreted to play a role in adjusting osmolarity stabilising RWC in Della leaves (**Figure 3.2d**), because higher proline levels should lower the water potential in the stressed leaves (Sharma and Verslues, 2010), such that they remain turgescent. Since proline synthesis requires NADPH, it also helps to mitigate the overreduction of PSII under salinity, further reducing ROS production in photosynthetic electron transport (Hare and Cress, 1997).

We noted an interesting detail with respect to the temporal patterns of proline responses in the leaf in relation to the transfer of sodium ions from roots to shoots. The accumulation of proline is more pronounced and sustained in Della as compared to Razinieh, although the translocation of sodium ions to the shoot was more pronounced in Razinieh as compared to Della. If the expression of *SbP5Cs1* is responding to local sodium high level, the opposite finding would have been predicted.

4.1.5. Adaption or susceptibility: a condition of systemic signalling?

Our results show that salt adaption in Della leaves is swiftly initiated before sodium entry to the transpirational stream, which is a case that is more efficient in coping with salt stress. Then the challenged root initiates a rapid systemic signal. Although this signal is unknown, but it is interesting that after one hour of stress, the levels of jasmonic acid (JA) and its bioactive conjugate, JA-Ile, are already significantly enhanced in Della leaves. Meanwhile, JA responds promptly in the root but without a significant rise in JA-Ile **(Figure 3.10).** It is noteworthy that one of the modifications or conjugations of JA is the transformation into the volatile shape of methyl-jasmonate "MeJA". As a volatile form, MeJA could signal to the leaves when the roots are stressed to initiate different adaptive measures such as proline synthesis,.etc. As indicated in previous research on sorghum (Su et al., 2011), the sweet genotype "Rio" seedlings exhibited a strong induction in *SbP5CS1* expression in leaves after 2 h of 10 µm MeJA spray treatment in addition to identifying many MeJA-responsive motifs in *SbP5CS1* gene promoter sequence (Su et al., 2011). In a similar study as well, when the roots of rice and maize seedlings were stressed with salinity, a comparable systemic signal had also been hypothesised for G-protein-dependent

suppression of cell proliferation in the leaves (Urano et al., 2014). While the function of MeJA in salt tolerance has received extensive research (for review, see Delgado et al., 2021), its potential for systemic signalling appears to have received little attention.

Our study summarised a visualised working model (**Figure 4.1**) that supports the notion that a rapid systemic signal issued by Della roots might be the prime contributor to initiating the adaption to salt stress. Such systemic signal, which may include MeJA, enables anticipatory response to the impending ionic stress. This adaptation helps reduce photorespiration, which buffers a better photosynthesis rate during sodium toxicity, depending on the stimulation of proline synthesis and ROS-scavenging enzymes. Such a high rate of photosynthesis under salt stress is needed for allocating sucrose to the roots, which will consume the energy in vacuolar sodium sequestration in the distal elongation zone, which is crucial for hampering sodium transfer to the shoot, giving the shoot more time to initiate the adaptive response. It is unclear how much the activation of ABA signalling will ultimately stabilise this self-amplifying functional circuit. Despite that, the earlier upregulation in *SbNCED1* expression suggests a potential role of ABA, even if not within the studied time points. Our model suggests that the difference between the two genotypes resides in the temporal regulation of jasmonic acid methyltransferase. Overall, we believe that the difference between susceptibility and tolerance is a matter of timing, consistent with previous examples where salt stress in vitis has been studied regarding temporal dynamics and molecular genetics (for review see Ismail et al., 2014a). With this viewpoint, swift stress signalling becomes the focus of future research.

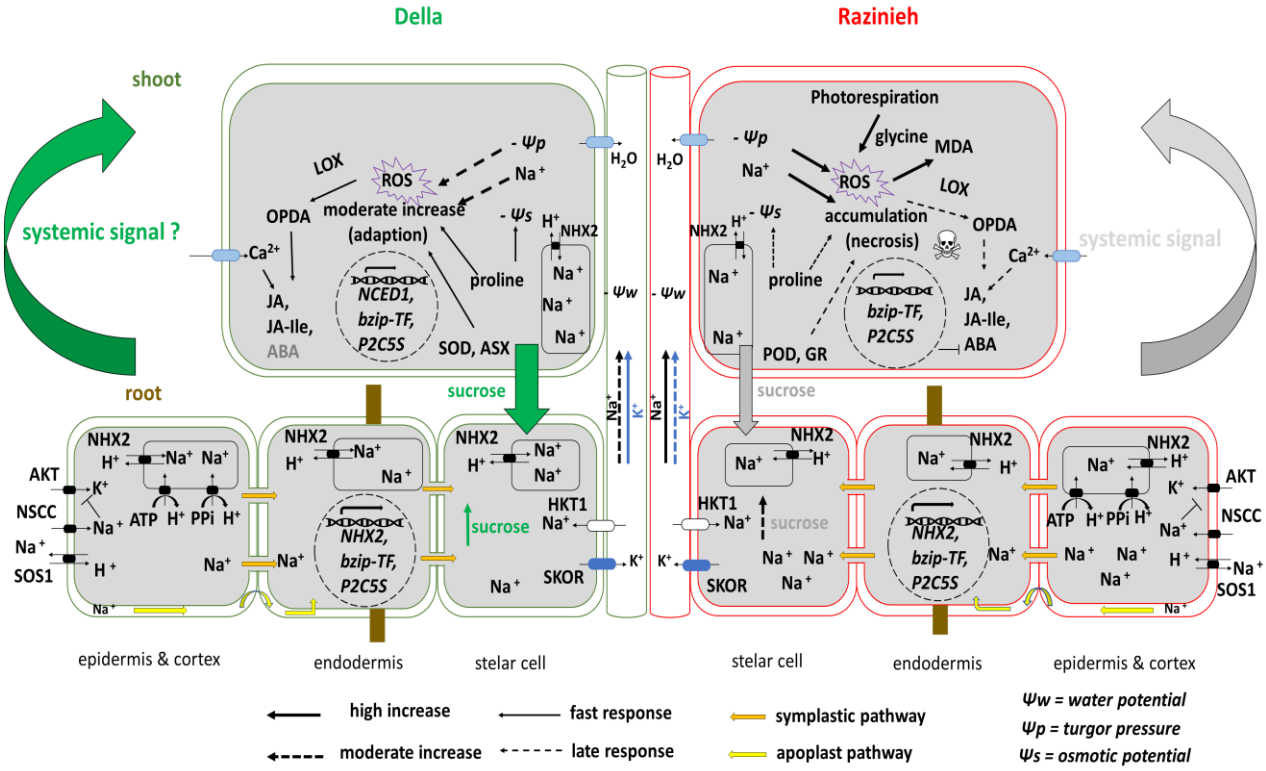

Figure 4.1. Visual model representing two contrasting sorghum genotypes to salt stress. The salt adaption in Della genotype is linked with with effecient sequestration of Na^+ in the vacuoles of root cortex cells and restricted translocation of Na^+ from root to shoot. In addition to rapid translocation of sucrose to the roots is coupled with efficient redox homeostasis and proline accumulation in leaves. Meanwhile, in Razinieh, glycine accumulation in leaves and H_2O_2 abundance were considered as markers of high photorespiration rate coupled with the observed leaf necrosis and MDA accumulation as a result of higher lipid peroxidation due to ROS spike. Published data in Abuslima et al., Frontiers in Plant Science (2022).

4.2. Chapter 2: How sugar transport contributes to the response to salt stress

Sucrose transport and partitioning are controlled physiological processes in plants that can be modified during stressful environmental conditions (Lemoine et al., 2013; Xu et al., 2018). Maintaining sugar homeostasis across the tissues of crop plants is ideal for optimum plant performance; thus, much of research aims to understand better how abiotic stress affects sucrose transport, particularly in crop plants. For this context, we are interested in studying salt stress response mechanisms in *Sorghum bicolor* L as a sugar-producing crop. Grain and sweet sorghum types exhibit a wide variety of productive and morphological differences, providing the potential to select genotypes with particular traits for the desired products in a particular environment (Naoura et al., 2019).

Here, we quantified sucrose, glucose, and fructose in leaves, internodes, and root tissues of Della (sweet) and Razinieh (grain) sorghum genotypes under salt stress conditions. Then we quantified the expression of selected genes related to sucrose metabolism and transport at the flag leaf stage under control and salt treatment. We found that the differential expression of sucrose transporter genes was detected mostly in sink tissues such as flag internodes and roots of both genotypes. We showed differential activation of different transporters to different predicted signals. We found that *pSbSWEET13* promoter was activated under the effect of ABA, unlike *pSbSUT6* promoters was activated under the effect of MeJA. We hypothesised that salt stress-mediated increases in ABA and MeJA levels promoted induction of *SbSWEET13* and *SbSUT6* in a fine tunned manner to control sucrose partitioning from source to different sink tissues.

4.2.1. The two genotypes show variations in stem phenotypic traits and biomass partitioning

First, we determined different morpho-physiological parameters of Della and Razinieh at the flag leaf stage under salt stress. Our results showed that the superiority of Della over Razinieh at the level of the entire stem is due to its heavier and juicy internodes **(Supplementary Figure S3.8a-c).** In addition to the higher sugar content in stem internodes of Della compared to Razinieh as indicated by °Brix value in both control and salt-treated plants **(Figure 3.11b).**

This pattern is consistent with previous literature on sweet sorghum and would be expected when the stem tissues are believed to act as a terminal sink tissue for sugar accumulation (Kumar et al., 2011; Oyier et al., 2017). Similar to sugarcane, many sweet sorghum cultivars, including Della, accumulate significant quantities of sucrose in stems after the initiation of the flag leaf stage (Murray et al., 2009; Wang et al., 2009). The results shown in Razinieh are also expected and consistent with previous literature (Kanbar, Shakeri, et al., 2021) on the same genotype because, in grain sorghum, as previously described, the grains filling is the terminal sink and the stem only has a little capacity for sugar storage (Dicko et al., 2006; Morey et al., 2018; Ritter et al., 2007). Similar to other comparative research between grain and sweet sorghum genotypes revealed that sweet sorghum out-performed grain sorghum in terms of overall biomass (Bihmidine et al., 2015; Qazi et al., 2012; Ritter et al., 2007)**.**

A similar contrasting pattern was also observed between the two genotypes under salinity. Della exhibited higher values for °Brix in the central internodes, especially from the fifth to the ninth internode, especially under salinity treatment (°Brix ≈12.5); meanwhile, Razinieh displayed the highest readout for °Brix in the last top internodes from tenth to twelfth or thirteenth (°Brix ≈ 5.5) under salt treatment **(Figure 3.11b)**. The two genotypes showed more weight accumulating in the basal internodes, from the second to the sixth or seventh internode **(Supplementary Figure S3.8c)**, again with significant superiority to Della. The higher juice content in Della internodes was correlated with the heavier weight for base-middle internodes and the adjacent green leaf area **(Supplementary Figures S3.8c, S3.9b).** The biggest leaf areas were observed of those adjacent to the third to the ninth internodes in Della, but in Razinieh from the fifth to twelfth internodes (**Supplementary Figure S3.9b**).

Our findings are in accordance with previous studies that the highest readout for °Brix was seen at middle internodes (the centre of sorghum stems) (Bihmidine et al., 2015; Shukla et al., 2017)**.** The internode sugar concentration dynamics of the sweet sorghum cultivars Della and Rio are similar, as indicated in previous studies (Li et al., 2019). Whereas the total sugar concentrations significantly increased in both genotypes after anthesis, the upper and lower internodes had lower sugar concentrations than the middle internodes (Li et al., 2019). This can be explained by the area of the corresponding adjacent leaves, the persistence of the green state, and the higher ability for biomass production (Bihmidine et al., 2015; Qazi et al., 2012)**.** This relationship between sucrose

concentration per internode and the green area of the adjacent leaf is consistent with other studies on maize and other genotypes of sorghum (Debruin et al., 2013; Kanbar, Shakeri, et al., 2021).

4.2.2. Sucrose mobilisation to the roots is prioritized over middle internodes in Della under salinity

Salinity stress, composed of osmotic and ionic components, and sugars as an osmoprotectant might be part of the adaptive mechanism to prevent tissue damage under disturbed water potential (Cramer et al., 2007; Hummel et al., 2010).

Our results show that sugar content (°Brix) increased significantly from 15.6 to 17.5 ($P < 0.05$) in Della after 30 days and from 7.0 to 9.2 ($P < 0.05$) in Razinieh after 10 days of treatment in comparison to control **(Figure 3.11b)**. The higher values found in Della suggest a superior capacity for sucrose mobilisation and sugar storage in stem tissues, which increases more under salinity.

To understand the dynamics of sucrose mobilisation and to see whether the soluble solids concentration increased due to sucrose accumulation in the stem tissues or cleavage to increase the pool of sugars content, we measured sucrose, glucose, and fructose contents in leaves, internodes, and root tissues. Our results show a clear tendency for sucrose synthesis in leaves of Della under salt stress after 30 days of treatment ($p < 0.05$), which was at the expense of glucose and fructose significant decrease ($p < 0.05$). The most striking result was increasing sucrose content in the middle internode of both genotypes under salt treatment ($p < 0.05$) but superior to Razinieh **(Figure 3.13)**.

Increased sucrose mobilisation under stressful conditions necessitates increased leaf photosynthesis, increased sucrose transport activity, and decreased sucrose breakdown. In the stem, sucrose content can also be altered by varying rates of hydrolysis by sucrose synthases (SUS) and invertases (INV) and synthesis by sucrose phosphate synthase (SPS) (McKinley et al., 2016). In the presence of salt stress, the sugar level in the stem of sorghum increases, which may improve osmoregulation (Gill et al., 2001). However, some C4 grasses convert sucrose to fructose and glucose to form a storage pool in the stem (Halford et al., 2011), and then their capacity to store sucrose is consequently diminished (Kanbar, Shakeri, et al., 2021).

This is related to the high transcripts level of *SbSUS3*, and *SbSUS4* in the middle internodes of Della under salinity. According to (McKinley et al., 2016), sucrose synthase (SUS) hydrolyzes sucrose to produce UDP-glucose (and fructose) to be consumed as substrates for cell wall biosynthesis. Also consistent with previous studies, for example, the high expression of *SbSUS1* in the internodes of the sweet sorghum genotype "SIL-05" had two consequences (Verma et al.. 2011). The first is that *SbSUS1* supplies the development of internodes sink structure with the necessary resources (such cellulose), which increases the stem ability to store sucrose later. In sugarcane, *SUS1* gene is expressed most strongly in developing internodes and least strongly in mature internodes (Verma et al., 2011).

The second is that the hexose content of SIL-05 stem increases as a result of the high activity of SbSUS1. The stem of SIL-05 has a remarkable high hexose (glucose and fructose) content compared to other high-Brix sorghum plants (Kawahigashi et al., 2013; Makita et al., 2015). Therefore, the significantly high level of *SbSUS1* expression in the stem may be related to SIL-05 high hexose concentration. Interestingly in middle internodes, glucose and fructose contents are significantly higher ($p < 0.05$) in Della than in Razinieh in both control and salt treatment.

Simultaneously, *SbSPS1* transcripts were upregulated in the middle internodes of both genotypes but with superiority to Razinieh under salinity **(Figure 3.14)** in parallel with high accumulation of sucrose also in Razinieh; moreover, both *SbSPS1* and *SbSPS4* genes showed higher expression relative to *SbSUS3*, and *SbSUS4*, so the contribution of these genes to overall internodes sucrose accumulation is suggested but still needs futher research.

Under stress, plants need more water and mineral nutrients; as a result, root growth is prioritized over leaves and shoot growth (Durand et al., 2016) which demands a force of carbon partitioning from leaf to root tissue. In Della, sucrose was preferably more accumulated in the roots **(Figure 3.13)** in response to salinity ($p < 0.05$), which is related to the ability to withhold sodium in the roots **(Figure 3.12a)** compared to Razinieh. According to previous reports, excess carbon is often sent to sink tissues after consumption for leaf growth, respiration, and osmotic adjustment (Hummel et al., 2010). In salt-stressed indica rice types, an elevated sucrose partitioning to the root tissue was also noticed (Mathan et al., 2020). Nevertheless, it has been demonstrated that enhanced drought adaptation in wheat plants results from increased carbon mobilisation to roots

(Nicolas et al., 1985). Additionally, under the early stages of drought stress, prioritised carbon partitioning of assimilates to roots has also been clarified in bentgrasses (DaCosta & Huang, 2006).

4.2.3. Differential expression of sucrose transporter genes

To investigate whether the expression of sucrose transporter genes has a role in the differential sucrose partitioning between the two genotypes under salt treatment, the expression of predicted *SbSUTs* and *SbSWEET*s genes was monitored **(Figure 3.14)**.

Sucrose is the primary sugar produced after photosynthesis (Gnansounou et al., 2005; Kühn & Grof, 2010). Sucrose is either stored in vacuoles for short-term or exported through long-distance phloem transport. The produced sucrose in leaves (source tissues) will then be transferred to sink tissues through the phloem (McCormick et al., 2006; Yang et al., 2020). Sucrose takes an apoplastic route in sorghum to be transferred from the phloem to sink tissues (Qazi et al., 2012; Tarpley & Vietor, 2007), and sucrose transporters such as SbSWEETs and SbSUTs carry out the loading and unloading processes during the long-distance transfer of sucrose through the phloem **(Figure 4.2)**.

Since the expression of *SbSUT1*, *SbSUT2*, *SbSUT4*, *SbSUT5*, *SbSUT6*, *SbSWEET6*, and *SbSWEET13* genes showed quantifiable levels in grain and sweet sorghum tissues (Cooper et al., 2019; Milne et al., 2013), the expression of these genes was analysed. Furthermore, the gene encoding for the *SbbZIP-TF-TRAB1* was included in our analysis **(Figure 3.14)**.

Under control conditions, the expression levels of *SbSUT1* and *SbSUT4* genes revealed that these genes are more strongly expressed in leaves and internodes of both genotypes. In addition, under salt treatment, the expression of *SUT1* was significantly increased in the flag internode of both genotypes, although at both control and salt treatment, the expression level was higher in Razinieh.

Consistent with the previous report, SUT1 is one of these SUTs and is very specialised in sucrose transport in monocotyledonous plants (Reinders et al., 2006; Slewinski et al., 2009). In Sweet sorghum genotypes, internodes express less *SUT1* than grain genotypes, which could be due to the decrease in sucrose phloem retrieval and an increase in efflux into storage parenchymal cells (Qazi et al., 2012). In addition, SUT4 functions in the tonoplast membrane to release sucrose from mesophyll vacuoles to their cytoplasm, which increases sucrose availability for phloem loading

(Milne et al., 2013; Schneider et al., 2012; Schulz et al., 2011)**.** Slowed photoassimilate export in knockdown SUT4 rice mutant demonstrates the importance of this function for SUT4 (Eom et al., 2011).

On the other hand, we noticed a relatively higher expression level of *SbSUT2* at ground level in sink tissues such as flag internodes compared to the roots **(Figure 3.14)** and vice versa compared to *SbSUT6*, whereas the latter showed a high expression level under both control and salt treatments in the roots more than other studied tissues. This suggests differential regulation of these two genes.

In addition, the stabilised level of *SbSUT2* transcripts in flag internode tissues of Razinieh under salt stress **(Figure 3.14)** unlike Della (which shows downregulation) suggests that this gene might participate in the direct mobilisation of sucrose to support inflorescence development. Meanwhile, *SbSUT6* is suggested as the main player in sucrose mobilisation to the roots.

The same is valid for *SbSWEET13*, and *SbSWEET6*. The high upregulation of *SbSWEET13* in flag internode in Della under salt treatment suggests sucrose accumulation in phloem apoplast, meanwhile the high expression of *SbSWEET6* in roots suggests that this gene plays a role in sucrose importing in phloem apoplasm which consequently is taken up by SbSUT6 to enter the cytoplasm of root parenchyma cells **(Figure 4.2)**.

Interestingly the increase in the transcripts level of *SbbZIP-TF-TRAB1* in the middle and flag internode was found to correlate with the significant increase in *SbSWEET13* transcripts in the same tissues under salt treatment **(Figure 3.14)**. These results are consistent with the study of salt and drought stress on rice (Mathan et al., 2020). It has been found that an abscisic acid (ABA)-responsive transcription factor OsbZIP72 directly binds to the promoters of the sucrose transporter genes "*OsSWEET13* and *OsSWEET15*" and activates their expression, consequently modifying sucrose transport and distribution in rice under stressful conditions (Mathan et al., 2020).

In summary, more research is needed to clarify whether this variation in sucrose transport dynamics is post-transcriptional regulated by sucrose transporter function or subcellular localisation (Kanbar, Shakeri, et al., 2021). Differentiating between sorghum types at the molecular level is not simple. Although sugar transport in grain versus sweet sorghum genotypes should vary, however; stem anatomy suggests that both appear to unload sucrose apoplastically

from phloem to parenchyma cells (Bihmidine et al., 2015). Cooper et al. (2019) presented a new reference genome based on the archetypal sweet sorghum line "Rio" and compared it to the existing grain sorghum reference; he found that genomic similarity between sweet and grain sorghum depends on their historical relatedness more likely than their current phenotypic differences.

4.2.4. Isolation and analysis of the promoters *pSWEET13, pSUT2,* and *pSUT6* reveal motifs associated with abiotic stress signalling

To dissect signals that are activating sucrose transporters activity, we used the approach to overexpress promoters of three candidate genes from both genotypes: *p*SWEET13, *pSUT2*, and *pSUT6* under control of the CaMV-35S promoter in sorghum protoplasts suspension. We found that *pSWEET13* promoter of Della was significantly responsive to ABA treatment **(Figure 3.15a)**, and this responsiveness was high pronounced compared with the *pSWEET13* promoter of Razinieh.

It has been demonstrated that ABA increases the expression of a number of genes involved in sugar metabolism, signalling, and SUT transporters as well (Gong et al., 2015; Jia et al., 2015). As a result, ABA may serve as the connection between abiotic stress and sucrose transport. We hypothesised that ABA-responsive transcription factors mediated the induction of *SbSWEET13* under salinity stress. For example, The ABRE-binding bZIP transcription factors are one of the ABA responsive regulators that are essential for plants to adjust to abiotic stress (Choi et al., 2000). According to previous reports, the rice genes *OsbZIP72*, *OsbZIP46CA1*, *OsbZIP23*, *OsbZIP72*, and *OsbZIP16* were found to be correlated with tolerance to abiotic stresses (Lu et al., 2009; Mathan et al., 2020; Tang et al., 2012). Additionally, it has been demonstrated that ABF/AREB transcription factors participate in the accumulation of sugar (Ma et al., 2017).

Although the freqeuncy of bZIP binding sites with ACGTG is the same between both *SbSWEET13* alleles from Della and Razinieh, but Della shows higher expression levels of both *SbSWEET13 and SbbZIP-TF-TRAB1* in flag internode under salinity. These results also suggested that *SbbZIP-TF-TRAB1* mediated modulation of abiotic stress response probably involves sucrose transport and

dynamics across the tissues. However, the potential binding of *SbbZIP-TF-TRAB1* to *SbSWEET13* promoter still needs to be experimentally confirmed.

On the other hand, we found that MeJA was able to activate *pSbSUT6* promoter in this system **(Figure 3.15c)**, (despite that both alleles showed the same level of responsiveness). This finding is consistent with the observation that both alleles have the same number of MeJA responsive elements. In addition, this places SbSUT6 in the context of functioning in root tissues because, according to (Yang et al., 2017), jasmonates were found to regulate growth responses in sorghum roots, unlike ABA, which plays a main role in the leaves.

Interestingly, we found that *pSbSUT2* promoter activities were diminished under ABA and PEG treatments **(Figure 15b)** in accordance with the significant decrease in *pSbSUT2* expression in Della under salt treatment. In summary, the differential responsiveness of *pSbSUT2*, *pSbSUT6*, and *pSbSWEET13* promoters indicates a possible fine-tuning of *SbSUTs* and *SbSWEETs* expression under abiotic stress conditions.

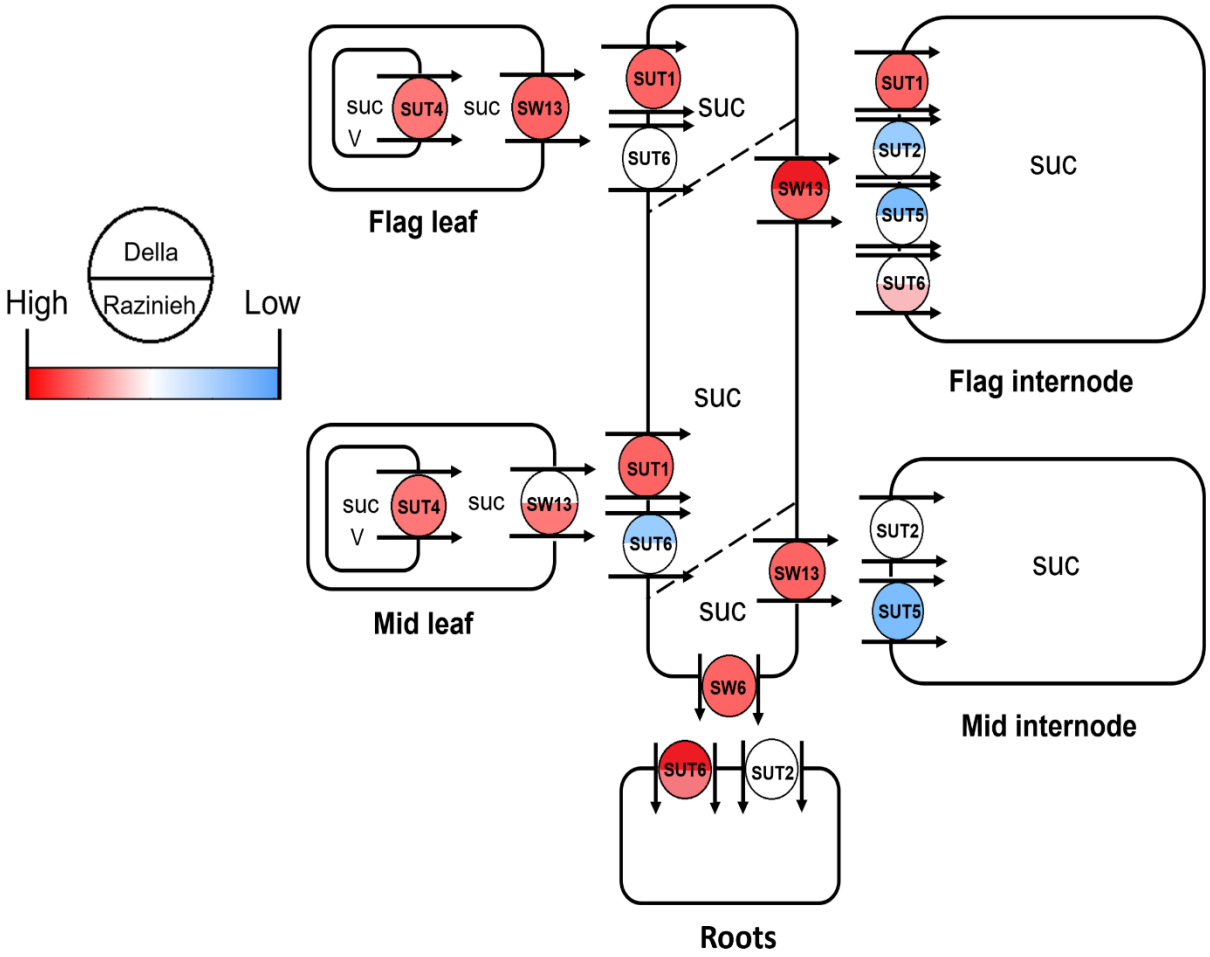

Figure 4.2. Predicted source–transport–sink pathway in two sorghum genotypes under salt stress**.** Sucrose (suc) is exported from source leaves vacuoles (V) by SbSUT4. SWEETs (SW13) efflux sucrose to the apoplasm in source leaves. SbSUT1 loads the phloem. SWEETs (SW13, SW6) unload sucrose to the apoplasm in sink tissues (stems, roots). SbSUT1,SbSUT2, SbSUT5, and SbSUT6 load sucrose into stem sinks. SbSUT6 load sucrose into root sinks. The relative expression of each corresponding gene is compared between the two genotypes in colour codes.

5. Concluding remarks and outlook

The dissertation presented several main points that extended our understanding of the adaptive responses in cereals such as sorghum to salt stress and provided a model to study how the alteration of sugar profile can help to cope with the stress in a sugar-producing crop. In the following, we are summarizing the central findings.

I) In the first part of the thesis, we sought to investigate the adaptive mechanisms of salt stress in sorghum, and the following headings represent the mechanisms deployed by the salt-tolerant sorghum genotype "Della":

1. Restrained translocation of sodium from root to shoot

   We found that the salt-tolerant genotype "Della" behaved similarly to the salt-susceptible "Razinieh" in the level of sodium accumulation in the roots. However, Della showed a preferential less accumulation of Na^+ in the shoot (by ≈7-folds less compared to Razinieh), giving time to the leaves to cope with the salt stress, while Razinieh leaves exhibited necrosis because of the increased accumulation of Na^+ in the shoot. This difference is correlated with superior sequestration of Na^+ in the vacuoles of the elongation root zone in Della rather than Razinieh.

2. Rapid and transient activity of enzymatic antioxidants

   We found a conceptual result that the same general players (such as enzymatic antioxidants activity) communicate various responses depending on the temporal signature and the context. For instance, both genotypes showed a significant increase in the activity of enzymatic antioxidants under salt stress, but there were specific differences in the timing and the amplitude of the response. It seems that salt stress tolerance is correlated with quick and transient activation of SOD, ASX, and POD, While increased but delayed activation of GR and POD under stress serve as indications for stress damage.

3. Swifter accumulation of sucrose and proline

   The pronounced redox homeostasis in the shape of sucrose and proline accumulation under salinity was more evident and swifter in "Della" the salt-tolerant genotype than in "Razinieh"

the salt-susceptible genotype. Although the accumulation of proline in leaves was visible in both genotypes, it happened earlier and to a higher amplitude in Della. In addition, we noticed that sucrose translocation to the roots was more persistent in Della but seemed sluggish in Razinieh. This is consistent with the notion that under abiotic stress, roots rely on shoot-derived sucrose as an energy source; hence, such sucrose accumulation in roots of the salt-tolerant genotype is assumed as a sign of efficient adaptation to salt stress. Furthermore, when comparing the temporal patterns of proline accumulation in the leaves to the movement of sodium ions from roots to shoots, we noted that the translocation of sodium ions to the shoot was more pronounced in Razinieh, while in Della, the buildup of proline is more swiftly pronounced as an anticipative adaption.

4. Reduced photorespiration rate as monitored by high sugars content and lower glycine levels in the leaves

   The most sharp and visible increase was the rapid and robust buildup of glucose and fructose in Della leaves, which was much slower and less noticeable in Razinieh leaves. In addition, from the metabolomic profile of amino acids, we can assume that Della utilized more efficient CO_2 recycling under salinity stress; in contrast, Razinieh exhibited high rates of photorespiration as indicated by the rapid and high glycine level in leaves. This adaptive mechanism is only effective when the photosynthesis process is protected even under salt stress.

5. Rapid systemic signal originating in the roots is proposed to travel faster than sodium to the leaves

   We conclude from our results that salt adaption in Della leaves swiftly initiated before sodium entry into the transpirational stream, which is more efficient in coping with salt stress. We were able to summarize our findings in a visual working model consistent with the idea that a swift systemic signal sent by Della roots might be the main factor in starting the adaptive response to salt stress. Such a systemic signal might include MeJA, which enables anticipatory response to the approaching ionic stress. This anticipative adaption includes activating proline synthesis and ROS-scavenging enzymes and reducing photorespiration, which buffers a greater photosynthetic rate during sodium toxicity. Under salt stress, a high rate of photosynthesis is

required to allocate sucrose to the roots, which will then use that energy to sequester sodium in vacuoles in the distal elongation zone. This process is essential for preventing sodium transfer to the shoot, which gives the shoot more time to start the adaptive response.

II) In the second part of the thesis, we investigated sugar partitioning in the two sorghum genotypes at the flag leaf stage under salt stress, and the following are the main findings:

1. Total soluble sugars build up in stem internodes, accompanied by sucrose partitioning to the roots under salt stress

   Measuring total soluble sugars content in terms of (^{O}Brix; total dissolved sugars in a solution) in sorghum internodes indicates a substantial accumulation of total soluble sugars in Della under salt stress rather than Razinieh. This is consistent with the notion that different forms of soluble sugars are needed as building materials for structural sugars, such as cellulose and hemicellulose, which will provide the stem internodes with biomass and structural integrity. At the same time, the non-structural sugars (soluble sugars) can control different metabolic processes. They act as signalling molecules and osmoprotectants under stressful conditions and participate in the antioxidative system.

   In addition, measuring sucrose content in different plant parts suggests a strong influence of salt stress on sucrose mobilisation, particularly to Della root tissues, indicating substantial parted metabolic shifts to root tissues in salt-tolerant genotype even under different life stages.

2. Sucrose transporters (SUTs) play a prominent role in sucrose unloading in sink organs

   Apoplastic phloem loading and unloading in sorghum are mediated by sucrose transporters, including SUT proteins. Consistent with our findings regarding the high expression of *SbSUT4* in source leaf tissues, which may contribute to phloem loading. In contrast, *SbSUT6* was expressed most strongly in sink tissues suggesting a possible role in facilitating sucrose import into sink storage pools, particularly in the root tissues. The activation of *pSbSUT6* promoter by MeJA suggests that sugars unloading to sink tissues is controlled by endogenous jasmonates content.

These outputs pave the way to perceive the complexity of the several levels of signalling and adaption to salt stress; however, different approaches can be followed in the future:

I) To dissect upstream signalling, we can investigate the response of the same contrasting pair of sorghum genotypes to osmotic stress with respect to jasmonate and Abscisic acid signalling.

II) To achieve sustainability by deploying marker-assisted breeding, making use of mechanisms of salt tolerance and the efficient sucrose mobilisation from the sweet sorghum and salt-tolerant genotype “Della”.

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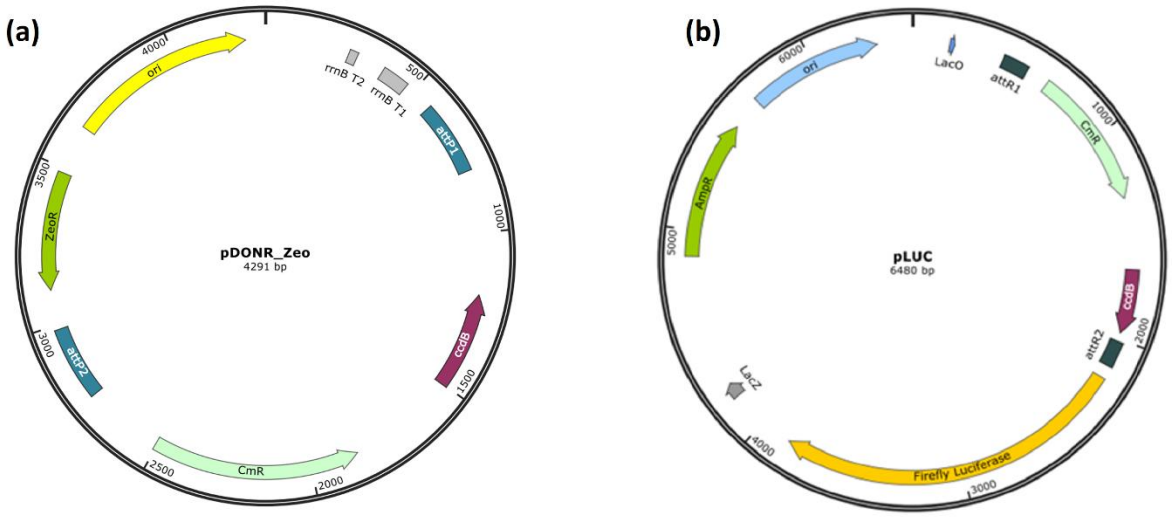
7. Supplementary

Supplementary Table S2.1. List of primers used for qPCR

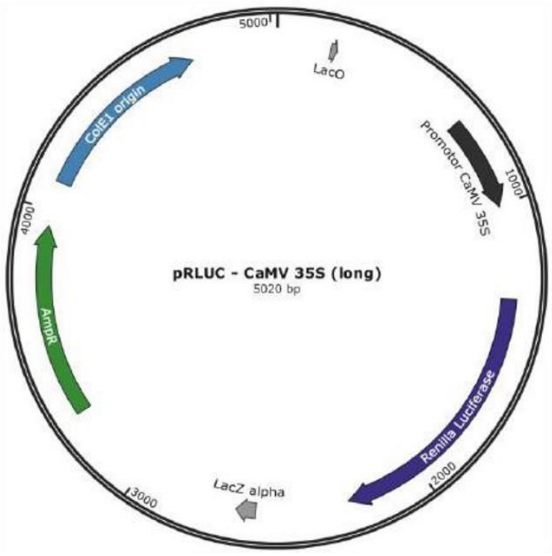
Gene name	Accession No.	Forward(5'-3' prime)	Reverse(5'-3' prime)
SbUbiquitin	XM_002439293.2	CAAGGAGTGCCCCAACAC	TGGTAGGCGGGTAAAGCAAA
SbNHX2	XM_002461123.2	TTGGCACCAAGAGACGAACA	CGCCATTGCTAACGCATCAT
SbHKT1	XM_002457691.2	GCAATGTGGGATTCAGCACC	CTTGAGCCTGCCGTAGAACA
SbSOS1	XM_002443629.2	AGTGGCAGCTCTACCTCTCA	TCGTCGCTTGAGTTGTCCTC
SbNCED1	XM_002466708.2	GTTGCTGGCTTGGCTCCTAT	ATCGGGACAGAAACCGTCAC
SbSAPK1	XM_002465139.2	GGAAAGGTTGCTGATGTTTGG	CGCCGACAAGCATCACATAT
SbbZIP-TF-TRAB-LIKE 1	XM_021465104.1	CTCCACGACCTTCTCCACAC	CTCCACGACCTTCTCCACAC
SbP5CS1	XM_021455806.1	CGAATGGCTGAACTCCCCTT	GGCACCGCAGAGAGAGTATC
SbGAPDH	XM_021449348.1	AGGGTATCATGGGCTACGTG	AGTTGTCGTTCAGGGCAATC
SbSUT1	KP685701.1	GTGCTCCTGTAATCTTTGTGTCC	ACTATACTGCACATTGATTGATCG
SbSUT2	KY287230.1	GCACATGCATTGAATGAACC	TTCGCATTTGGAAATTCCTC
SbSUT4	KY287232.1	ATGCAATGGCTGCTAGTCGT	CACCGCCGAACATTTGATCC
SbSUT5	XM_002454013.2	CCCGTAGTGTTGCGGAGTC	CCAATGGATCGGAAAATAAAG
SbSUT6	KY287234.1	CTCCTCTTCTGCTCCGTCGC	GAACACCATGAGGTTGCTGA
SbSWEET13	XM_002442074.2	CTCTCCATCGCAAGCAAGCA	GCTAAGGGTTGGATAAACGGG
SWEET6	XM_002455874.2	CGCTCATCCGCTTCGACC	GGGCAGCTCCACGTTCTT
SbbZIP-TF-TRAB1	XM_021454381.1	CAGTGGTGCTTGGGATCTGT	ACTACATAAGCGGCGGAAGG
SbSPS1	XM_002458946.2	ACGCGACAAGACTTCAGGTT	ATCGGTATCGCCATGTTCCC
SbSPS4	XM_002441477.2	TCCTGTTTTGGCTTCTCGCT	TTTGTGCACGCCTCCAAGTA
SbSUS3	XM_002465258.2	AAGCTTGAGAGGCGGGAGAC	TGAGCAAGCTACGAGCACCA
SbSUS4	XM_021449494.1	TGCTGCCCCCTCTATTTATTGG	GAGGAGAAGGTGGCACCAAG
SbCINV1	XM_002453920.2	AGGCGATGTTGAAGCCTGTT	CCCGGTTTGGAAGTCAAGGA
SbCINV2	XM_002452587.2	TGCTTTCCGAGTTCCTACTGG	TCCTTATTTCCCCGACCAAACT

Supplementary Table S2.2. List of primers used for promoters amplifying, sequencing, and GATEWAYcloning

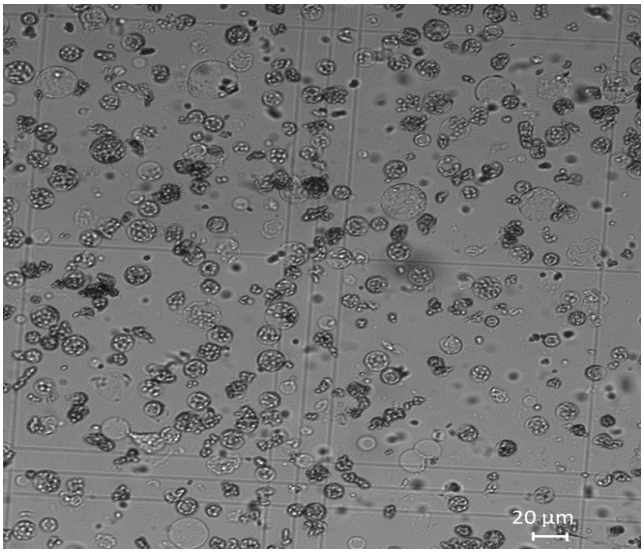
Gene name	Accession No.	Forward(5'-3' prime)	Reverse(5'-3' prime)
pSbSWEET13	XM_002442074.2	CGGTGAGGAGTCAAACACAA	AGGGGTGCTGCAGAGATAGG
pSbSUT2	KY287230.1	CTCTCTGCCACGGTGAAGTC	GAGGCTGACGAGCTCCATCT
pSbSUT6	KY287234.1	GTCAGTTCTAGCTCCACTGTCA	GAGACCATGCAGGCCAAGAA
attB-pSbSWEET13	XM_002442074.2	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGGGGTGCTGCAGAGATAGG	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGGGGTGCTGCAGAGATAGG
attB-pSbSUT2	KY287230.1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTCTCTGCCACGGTGAAGTC	GGGGACCACTTTGTACAAGAAAGCTGGGTCGAGGCTGACGAGCTCCATCT
attB-pSbSUT6	KY287234.1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGTCAGTTCTAGCTCCACTGTCA	GGGGACCACTTTGTACAAGAAAGCTGGGTCGAGACCATGCAGGCCAAGAA



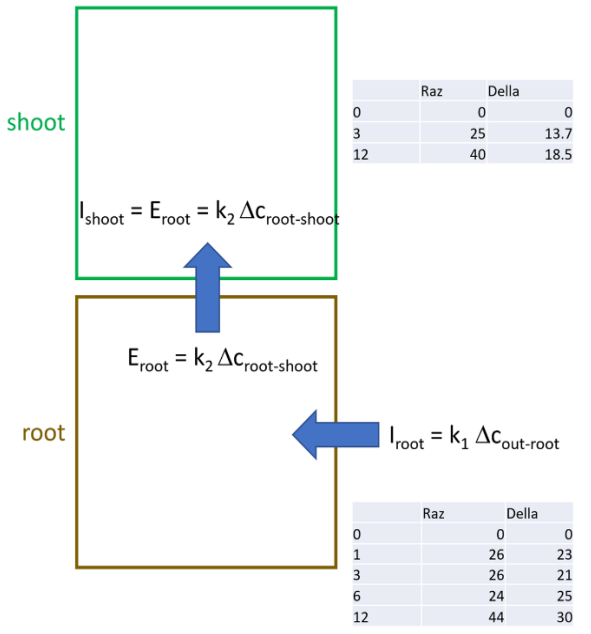
Supplementary Figure S2.1. Maps showing the elements of plasmids used in the Gateway cloning system (BP and LR recombination reactions; a) map of the donor vector pDONR™/Zeo- reference: Thermo Fisher Scientific Inc., Waltham, MA, USA, b) map of pLUC: destination vector for luciferase assay (Horstmann et al., 2004).



Supplementary Figure S2.2. pRLUC – CaMV 35S (long): control plasmid for standardisation in dual luciferase assay; constitutively p35S promoter coupled to Renilla luciferase gene - (Horstmann et al., 2004).



Supplementary Figure S2.3. The image of sorghum protoplasts as obtained by (AxioImager Z.1.), scale bar = 20 µm.



	Raz	Della
0	0	0
3	25	13.7
12	40	18.5

	Raz	Della
0	0	0
1	26	23
3	26	21
6	24	25
12	44	30

Steady state in the root between day 1 and day 6:
Here: $E_{root} = I_{root}$ hence

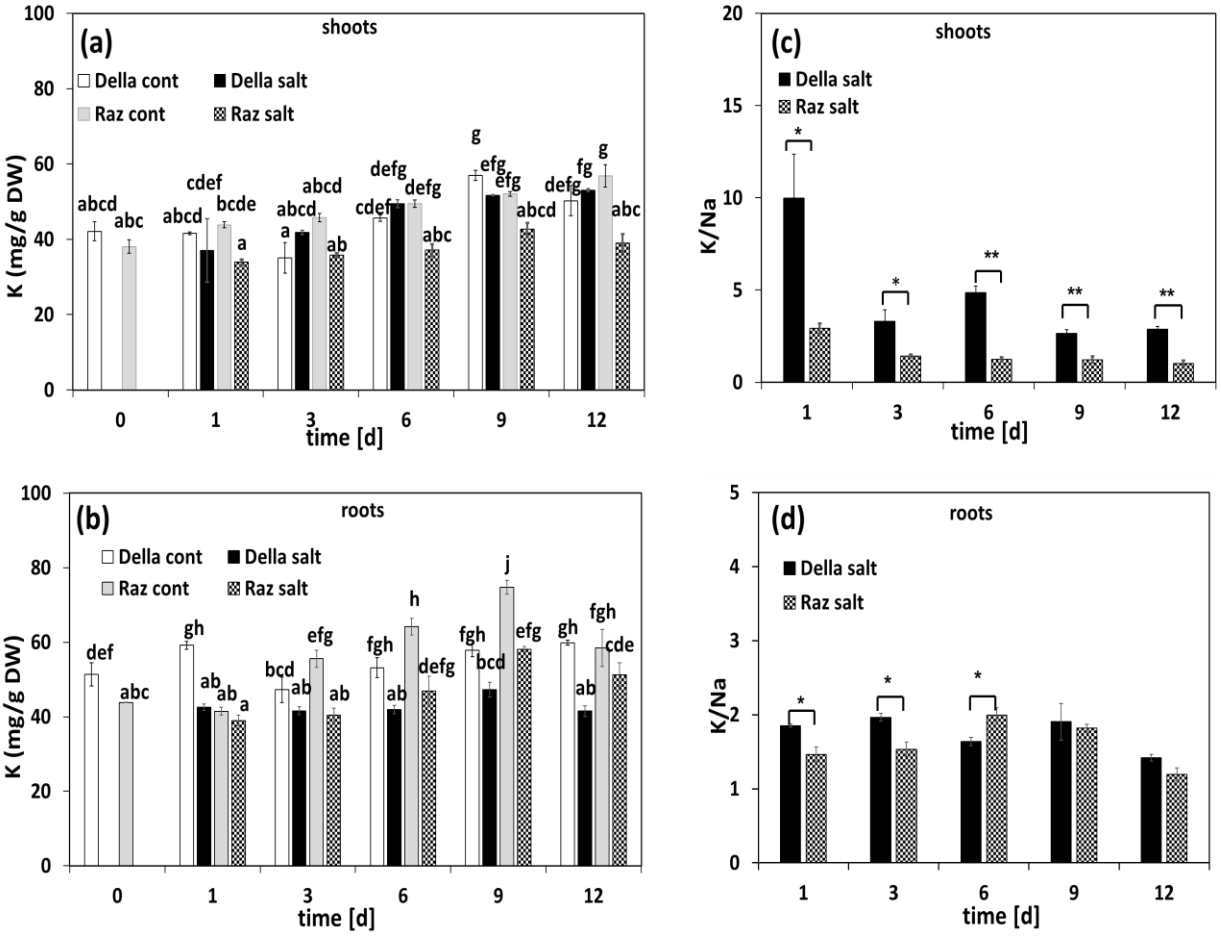
$k_2 Dc_{root\text{-}shoot} = k_1 Dc_{out\text{-}root}$

or $k_2 = k_1 Dc_{out\text{-}root} / Dc_{root\text{-}shoot}$

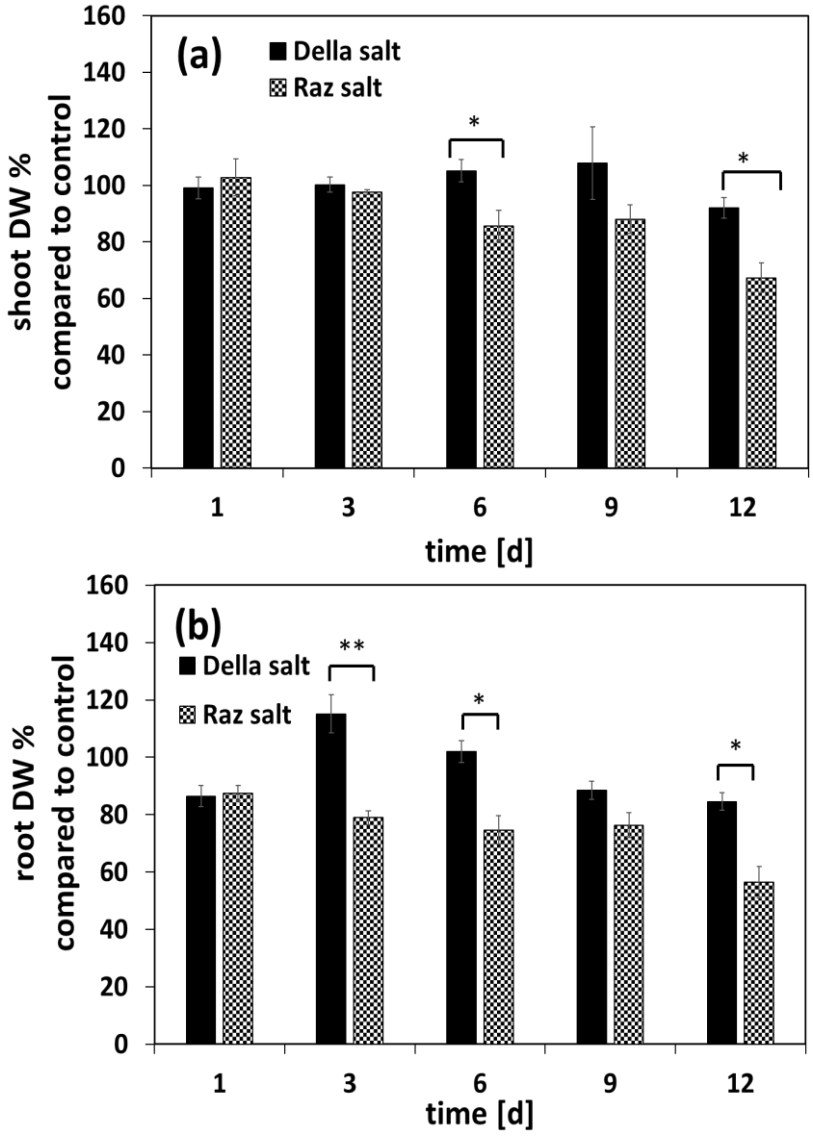
C_{out} is sodium in the bath (100 mM), c_{root} is the mM sodium concentration at d3, c_{shoot} is the mM sodium concentration at d3

To determine k_1, you can use the slope in the root between day 0 to day 1, assuming that c_{root} is ~0

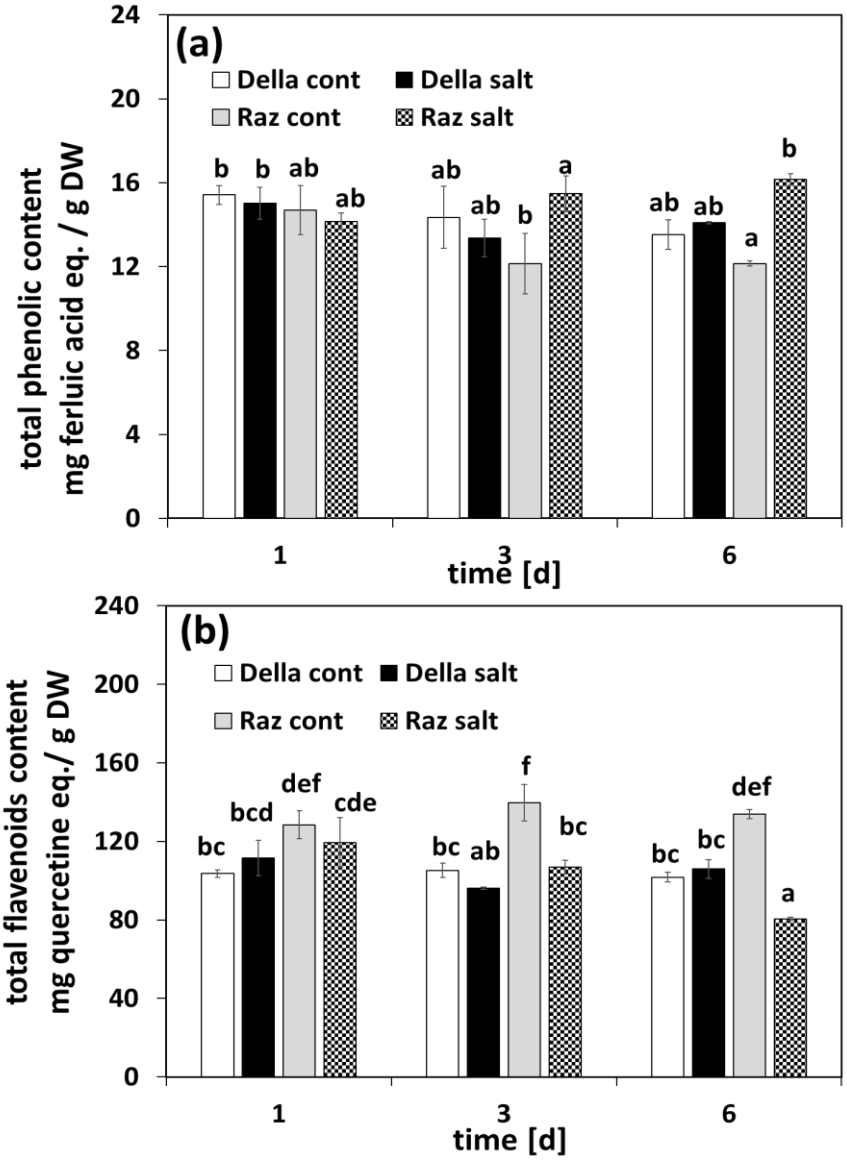
Supplementary figure S3.1. Mathematical model representing the coefficient for sodium transfer from root to shoot.



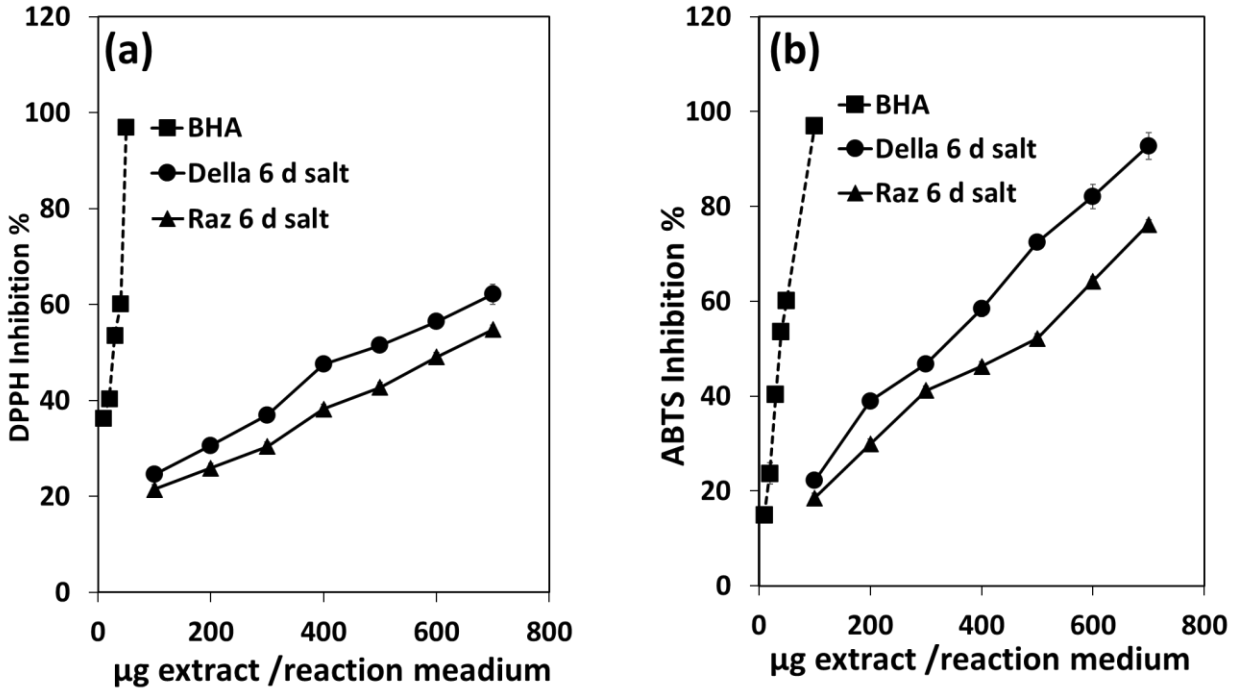
Supplementary Figure S3.2. Content of potassium ions in (a) shoots and (b) roots of sorghum genotypes Della and Razinieh (c) K/Na ratio in the shoots and (d) K/Na ratio in the roots. Thirteen days old Della (white bars), and Razinieh (grey bars) seedlings were stressed in aqueous NaCl (100 mM) solution for 1, 3, 6, 9, and12 days. Values represent the mean of at least three independent experiments ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05). Asterisks indicate a statistically significant difference between genotypes, as determined by Student's *t*-test (*P< 0.05, and **p<0.01).



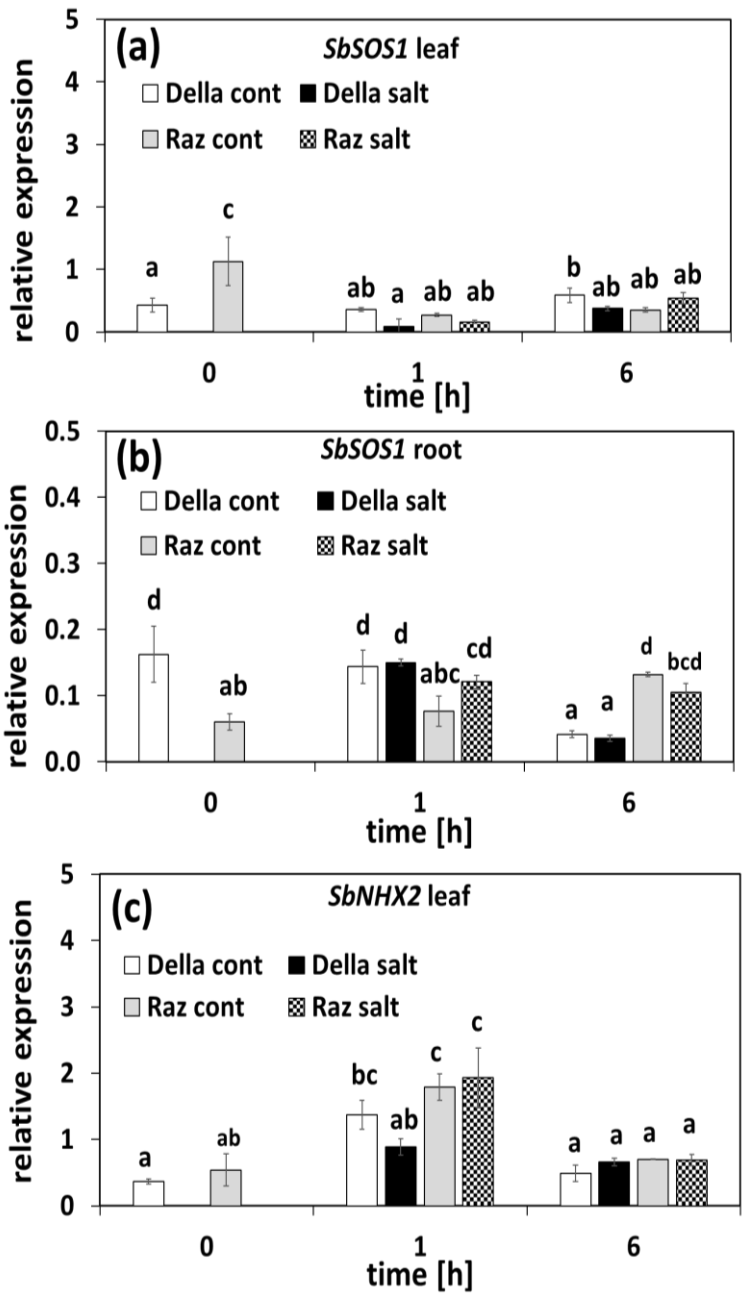
Supplementary Figure S3.3. The dry weight (%) of shoot (a) and root (b) under stress compared to each corresponding control of Della and Razinieh thirteen-days-old seedlings subjected to 100 mM NaCl solution for 1, 3, 6, 9, and 12 days. Values represent the mean of at least five independent experiments ±SE. Asterisks indicate a statistically significant difference between genotypes, as determined by Student's *t*-test (**P*< 0.05, and ***p*<0.01).



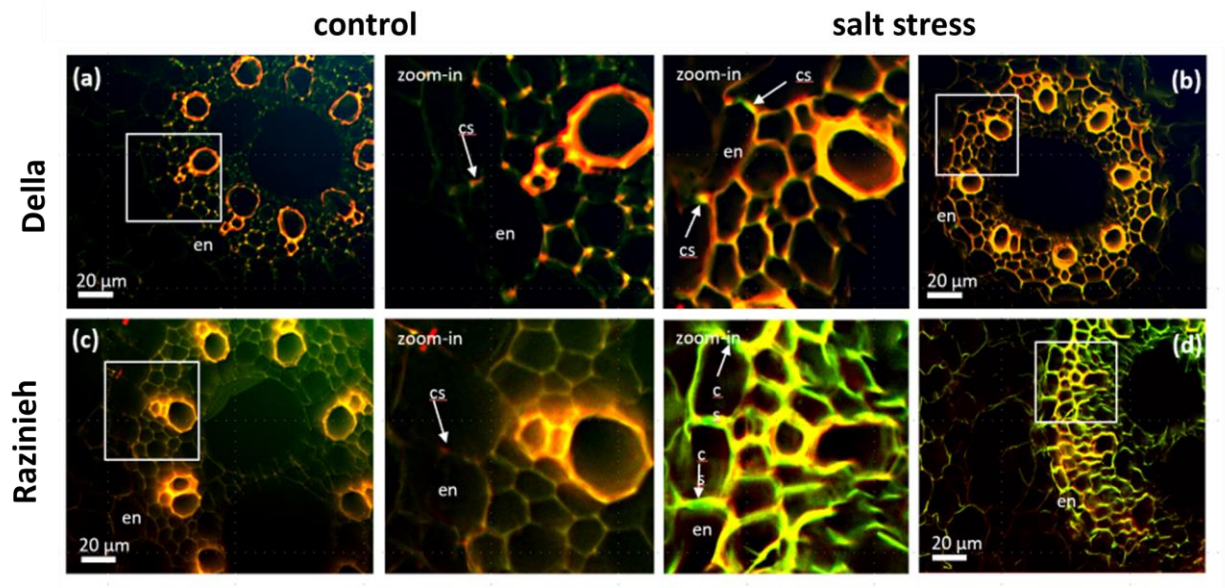
Supplementary Figure S3.4. (a) The level of total polyphenolics contents, and (b) the level of total flavonoids contents in thirteen-days-old seedlings shoots treated with 100 mM NaCl solution for 1, 3, and 6 days. Values represent the mean of at least three independent experiments ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05).



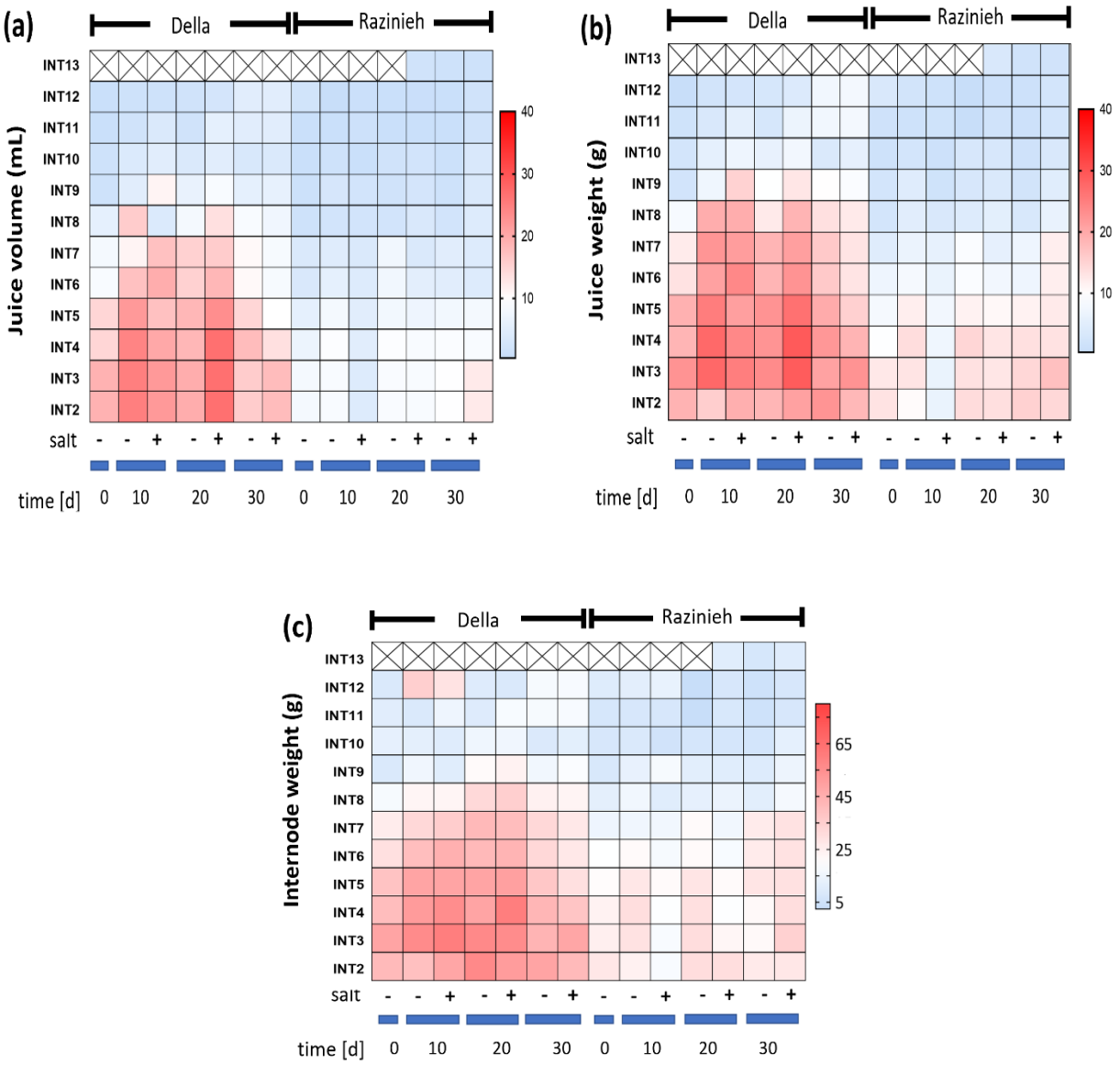
Supplementary Figure S3.5. The scavenging activity of methanolic extract of sorghum seedlings shoots treated with 100 mM NaCl for 6 days against (a) the synthetic free radicals 2, 2-diphenyl-1 - picrylhydrazyl (DPPH) and (b) 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) using Butylated hydroxyanisole (BHA) as internal standard. Values represent the mean of at least three independent biological replicates ±SE.



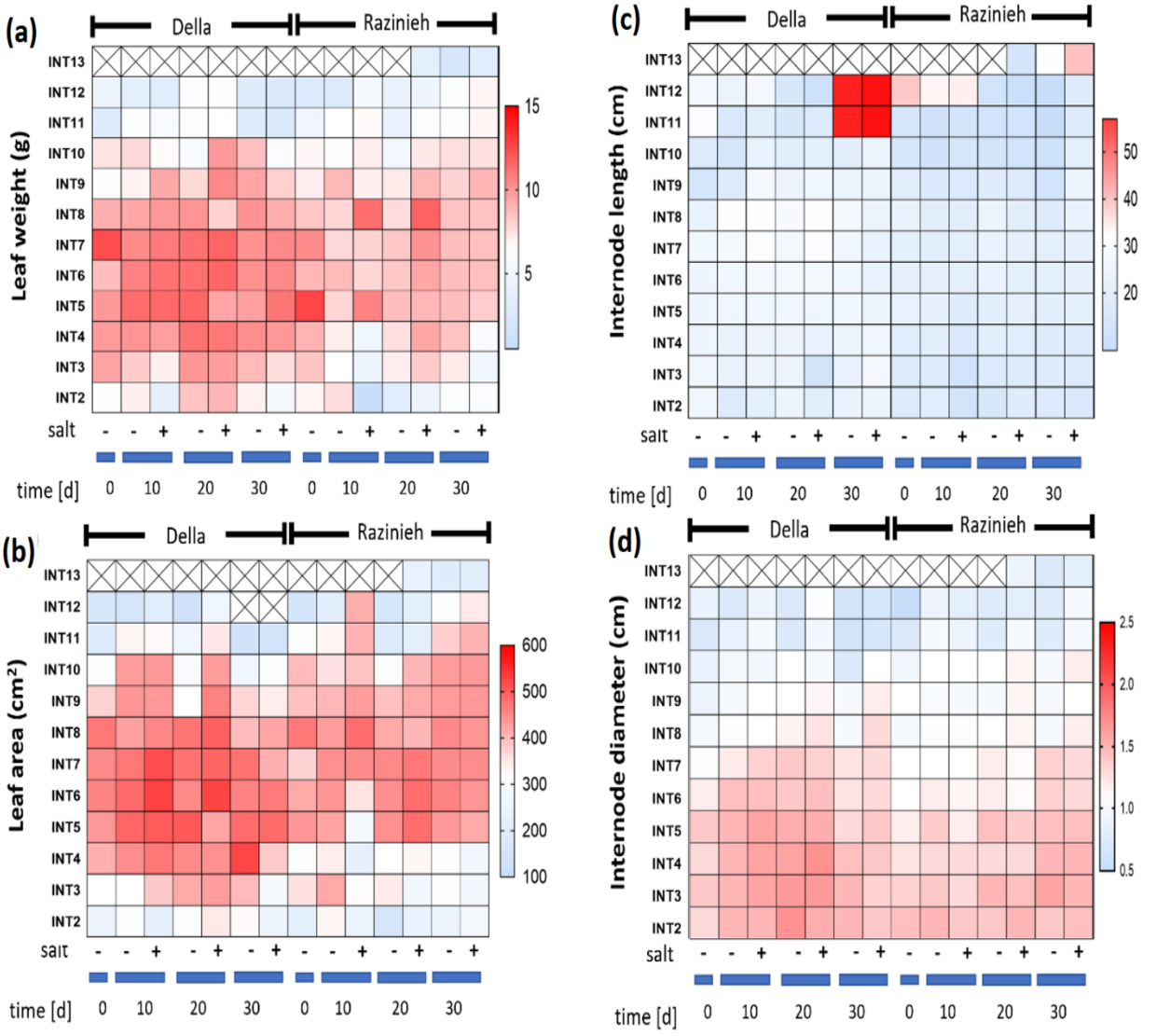
Supplementary Figure S3.6. The steady-state transcripts level of salt stress-related genes (a) *SbSOS1* in leaf, (b) *SbSOS1* in roots, and (c) *SbNHX2* in 2 leaf. Thirteen-days-old seedlings were treated with 100 mM NaCl for 1 and 6 h. Values represent the mean of at least three independent biological samples ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05).



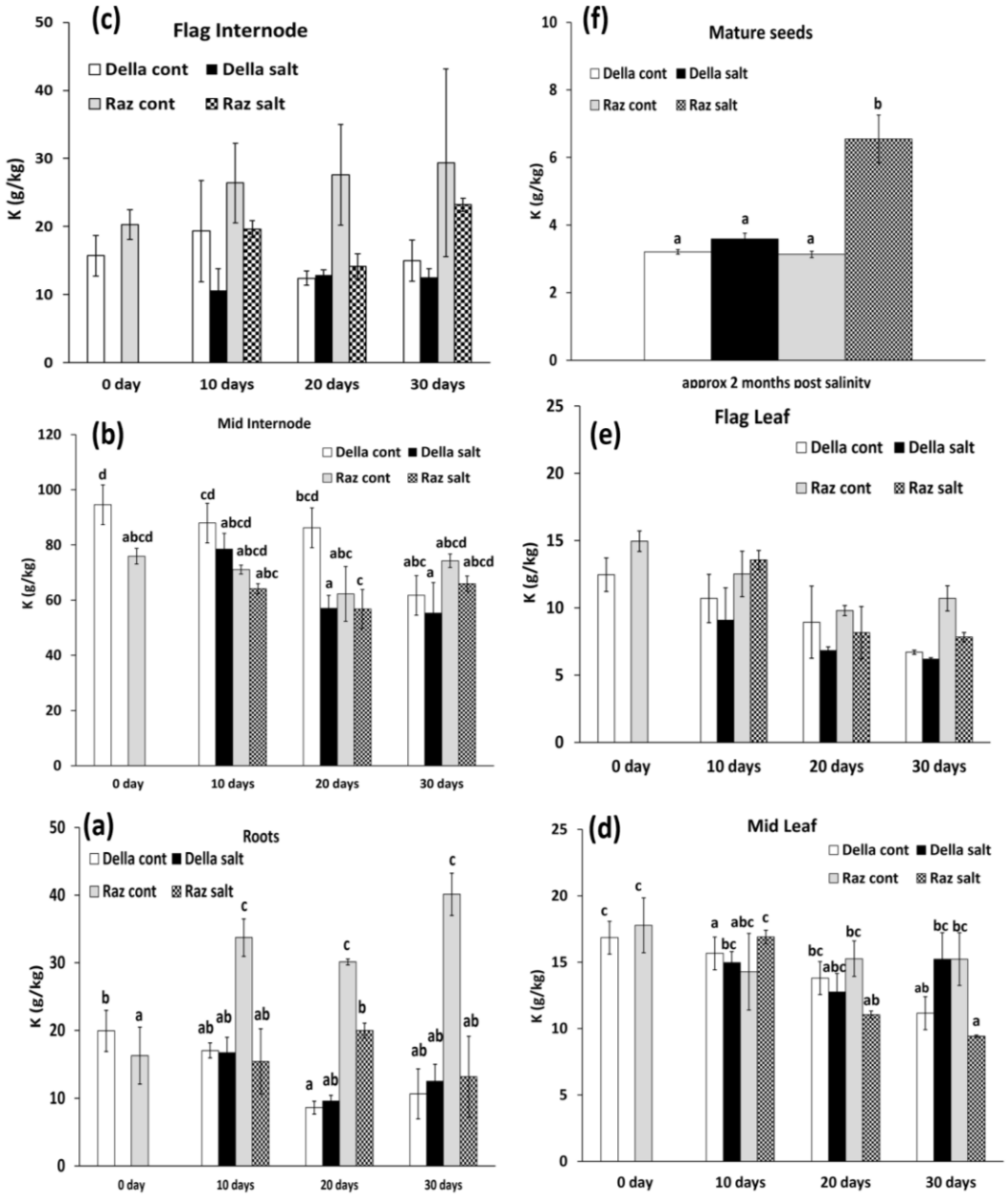
Supplementary Figure S3.7. Visualisation of Casparian strips development under salt stress. 15 µm thick cross sections were cut by a microtome from paraffin-fixed adventitious roots of thirteen-days-old seedlings subjected to a 100 mM NaCl solution for 6 days and the sections were stained with berberine aniline blue and viewed using blue light. Arrows show Casparian strips in the endodermis (en). (a) control Della, (b) stressed Della, (c) control Razinieh, and (d) stressed Razinieh.



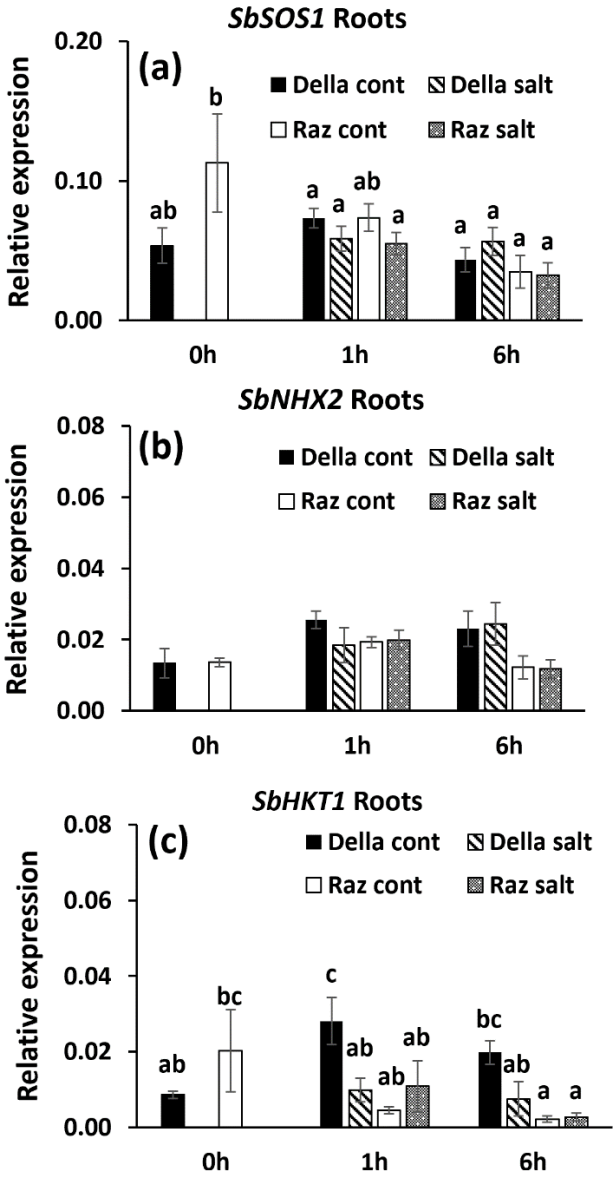
Supplementary Figure S3.8. Heatmaps representing the differences in sugar-related parameters and stem biomass in each internode Della and Razinieh sorghum genotypes grown in 2019 season in South-West Germany: (**a**) Internode Juice volume (mL), (**b**) Internode juice weight, (**c**) internode weight. Values are means of three replicates. Boxes with x indicate the absence of the internode.



Supplementary Figure S3.9. Heatmaps representing the differences in leaves and internodes phenotypic parameters of Della and Razinieh sorghum genotypes grown in 2019 season in South-West Germany: **(a)** Leaf weight (g), **(b)** Leaf area (cm^2), **(c)** internode length (cm), and **(d)** Internode diameter (cm). Values are means of three replicates. Boxes with x indicate the absence of the internode.



Supplementary Figure S3.10. Potassium ion concentration in roots, internodes, leaves and grains of a sweet (Della) and a grain (Razinieh) sorghum genotypes at flag leaf stage under control and salt treatment (100mM NaCl) after 0, 10, 20, and 30 days of treatment. Values represent the mean of at least three independent biological replicates ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05).



Supplementary Figure S3.11. The steady-state transcripts level of salt stress-related genes (a) *SbSOS1*, (b) *SbNHX2*, and (c) *SbHKT1* in the roots of a sweet (Della) and a grain (Razinieh) sorghum genotypes at flag leaf stage under control and salt treatment (100mM NaCl) after 0, 1, and 6 h treatment. Values represent the mean of at least three independent biological samples ±SE. Different letters show significant differences between different genotypes and treatments according to Duncan's test (P<0.05).

Supplementary Figure S3.12. The alignment and *cis*-elements for SWEET13, SUT2, and SUT6 promoters of Della and Razinieh sorghum genotypes, compared to the reference genome BTX623.

SWEET13 promoter

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Della       CGGTGAGGAGTCAAACACAAAGTTTTATAATTGCTCAACATATGCCGTTTTATTTGAGAA    60
Razinieh    gtgcaggcagtcaaaCACAAAGTTTTATAATTGCTCAACATATG--------------CC    46
BTX623      CGGTGAGGAGTCAAACACAAAGTTTTATAATTGCTCAACATATGCCGTTTTATTTGAGAA    60
              *   * **********************************

                                          Dehydration responsive element(DRE)
Della       AAGTGTGGTGTTCTGATGCTGACCAGTGGCACACAGCCACAAGATTAAAAACAGACCGAC    120
Razinieh    GTTTGtGgTGTTCTGATGCTGACCAGTGGCACaCAgcCACAAGATTAAAAACAGACCGAC    106
BTX623      AAGTGTGGTGTTCTGATGCTGACCAGTGGCACACAGCCACAAGATTAAAAACAGACCGAC    120
              ************************************************************

Della       AGATTCAAGACAATATATCATTAAATCAGTTCGTAGATTAGATCGTCGCCTATGTTCATA    180
Razinieh    AGATTCAAGACAATATATCATTAAATCAGTTCGTAGATTAGATCGTCGCCTATGTTCATA    166
BTX623      AGATTCAAGACAATATATCATTAAATCAGTTCGTAGATTAGATCGTCGCCTATGTTCATA    180
            ************************************************************

Della       GGAGAAAATATTCTTGGCCACCCGTGCCAACTGTTTGGAGACCACCAAAACCAAGTCTTG    240
Razinieh    GGAGAAAATATTCTTGGCCACCCGTGCCAACTGTTTGGAGACCACCAAAACCAAGTCTTG    226
BTX623      GGAGAAAATATTCTTGGCCACCCGTGCCAACTGTTTGGAGACCACCAAAACCAAGTCTTG    240
            ************************************************************

Della       AGAAACCACCTTATATAGAATAGCACAAGTACATAGTGAATGGATAGCTAGATGCGCGTA    300
Razinieh    AGAAACCACCTTATATAGAATAGCACAAGTACATAGTGAATGGATAGCTAGATGCGCGTA    286
BTX623      AGAAACCACCTTATATAGAATAGCACAAGTACATAGTGAATGGATAGCTAGATGCGCGTA    300
            ************************************************************

Della       GCAAGTCTTGTGCAATCAGGTGTCTAATTGTCTTAAGCCACACAATTTACTAACTTGCCT    360
Razinieh    GCAAGTCTTGTGcaATCAGGTGTCTAATTGTCTTAAGCCACACAATTTACTAACTTGCCT    346
BTX623      GCAAGTCTTGTGCAATCAGGTGTCTAATTGTCTTAAGCCACACAATTTACTAACTTGCCT    360
            ************************************************************
```

```
Della          GATGTGCTTGGCAAGATTATCCTTGGCCAATACAACTCCTCTTTGTAAGTACCATAAAAA    420
Razinieh       GATGTGCTTGGCAAGATTATCCTTGGCCAATACAACTCCTCTTTGTAAGTACCATAAAAA    406
BTX623         GATGTGCTTGGCAAGATTATCCTTGGCCAATACAACTCCTCTTTGTAAGTACCATAAAAA    420
               ************************************************************

Della          GATTTTGATTTTTTGATGGGGCCTTCAGTTTCCATAAACTTTTGTTGAAATTGGTATCGA    480
Razinieh       GATTTTGATTTTTTGATGGGGCCTTCAGTTTCCATAAACTTTTGTTGAAATTGGTATCGA    466
BTX623         GATTTTGATTTTTTGATGGGGCCTTCAGTTTCCATAAACTTTTGTTGAAATTGGTATCGA    480
               ************************************************************

Della          TTAACATCACTGAGAATTTTCCATTTGGGTGTAAATTCCAATGGAAACATGTAGGTCCTG    540
Razinieh       TTAACATCACTGAGAATTTTCCATTTGGGTGTAAATTCCAATGGAAACATGTAGGTCCTG    526
BTX623         TTAACATCACTGAGAATTTTCCATTTGGGTGTAAATTCCAATGGAAACATGTAGGTCCTG    540
               ************************************************************

Della          TGACAATTGTATCTATAACTCTGATAAAGCAAAACCCCACTAGCTATTTCTCTTGACATT    600
Razinieh       TGACAATCGTATCTATAACTCTGATAAAGCAAAACCCCACTAGCTATTTCTCTTGACATT    586
BTX623         TGACAATTGTATCTATAACTCTGATAAAGCAAAACCCCACTAGCTATTTCTCTTGACATT    600
               ******* ****************************************************

Della          TGAGGCGTCCACCTCAGCAGTCCATTATGATTTGCCACTATCAATTTCTCTTGACATGCA    660
Razinieh       TGAGGCGTCCACCTCAGCACTCCATTATGATTTGCCACTATCAATTTCTCTTGACATGCA    646
BTX623         TGAGGCGTCCACCTCAGCAGTCCATTATGATTTGCCACTATCAATTTCTCTTGACATGCA    660
               ****************** ****************************************

Della          CCTCAGTAGCCCACTATCATTTGTAATTAAAGTTCACTAGCAC-AAGAATTATGATATCC    719
Razinieh       CCTCAGCAGCCCACTATCATTTGTAATTAAAGTTCACTAGCACCAAGAATTAtgATATCC    706
BTX623         CCTCAGTAGCCCACTATCATTTGTAATTAAAGTTCACTAGCAC-AAGAATTATGATATCC    719
               ****** ********************************** ***************

               MeJA-responsive element
Della          ACGTCAGCCAAATATATTATCCACAATGGATACAATCTTTAAGATTATACTTTACACCCG    779
Razinieh       ACATCAGCCAAATATATTATCCACTATGGATACAATCTTTAAGATTATACTTTACACCCG    766
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BTX623          ACGTCAGCCAAATATATTATCCACAATGGATACAATCTTTAAGATTATACTTTACACCCG     779
                ** ******************** ***********************************

Della           CGGCAATGTGTGTGGGGAATCCTTCTAGTATTAGTAATGCGTGGTAAATGGTTGTTCCAA     839
Razinieh        CGGCAATGTGTGTGGGGAATCCTTCTAGTATTAGTAATGCGTGGTAAATGGTTGTTCCAA     826
BTX623          CGGCAATGTGTGTGGGGAATCCTTCTAGTATTAGTAATGCGTGGTAAATGGTTGTTCCAA     839
                ************************************************************

Della           GCTACTTAGGATTTGTATAATATGCTTATTGTCCTCCCAAAACCTCAATTGCATACTGTT     899
Razinieh        GCTACTTAGGATTTGTATAATATGCTTATTGTCCTCCCAAAACCTCAATTGCATACTGTT     886
BTX623          GCTACTTAGGATTTGTATAATATGCTTATTGTCCTCCCAAAACCTCAATTGCATACTGTT     899
                ************************************************************

Della           ATAAAGACTTGGATACTGTTCTTACAAAGTTGTGTTTCGAAGCCGTTTGTCCTTCTAGAA     959
Razinieh        ATAAAGACTTGGATACTGTTCTTACAAAGTTGTGTTTCGAAGCCGTTTGTCCTTCTAGAA     946
BTX623          ATAAAGACTTGGATACTGTTCTTACAAAGTTGTGTTTCGAAGCCGTTTGTCCTTCTAGAA     959
                ************************************************************

Della           TCTCAATTGCCAAATCATAAATAAATTATTTTATTGTTAGGTTACAAGATATAACTTGGT     1019
Razinieh        TCTCAATTGCCAAATCATAAATAAATTATTTTATTGTTAGGTTACAAGATATAACTTGGT     1006
BTX623          TCTCAATTGCCAAATCATAAATAAATTATTTTATTGTTAGGTTACAAGATATAACTTGGT     1019
                ************************************************************

Della           ACTAAAATCACAAATTGCCAATGGAATGAAGTAAATGTGTCTTTGGAGTCGAAAGTTGTG     1079
Razinieh        ACTAAAATCACAAATTGCCAATGGAATGAAGTAAATGTGTCTTTGGAGTCGAAAGTTGTG     1066
BTX623          ACTAAAATCACAAATTGCCAATGGAATGAAGTAAATGTGTCTTTGGAGTCGAAAGTTGTG     1079
                ************************************************************

Della           AAGATCAGTTTTGCTGTTGTGTGGCGGCTAGCTCCTACTCGATCTTAATTAGTTAATGAT     1139
Razinieh        AAGATCAGTTTTGCTGTTGTGTGGCGGCTAGCTCCTACTCGATCTTAATTAGTTAATGAT     1126
BTX623          AAGATCAGTTTTGCTGTTGTGTGGCGGCTAGCTCCTACTCGATCTTAATTAGTTAATGAT     1139
                ************************************************************
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Della          GTATGCGAATGAAGCAGGTTGGAGAGTTCCACGGGCAGTGGATAACAATTCGGAGTGTAC    1199
Razinieh       GTATGCGAATGAAGCAGGTTGGAGAGTTCCACGGGCAGTGGATAACAATTCGGAGTGTAC    1186
BTX623         GTATGCGAATGAAGCAGGTTGGAGAGTTCCACGGGCAGTGGATAACAATTCGGAGTGTAC    1199
               ************************************************************

Della          TACCCCAGCACTACTAGCCCATATATTGTGCTCATAAAGCTTGCACAAAGCTAACTCTTT    1259
Razinieh       TACCCCAGCACTACTAGCCCATATATTGTGCTCATAAAGCTTGCACAAAGCTAACTCTTT    1246
BTX623         TACCCCAGCACTACTAGCCCATATATTGTGCTCATAAAGCTTGCACAAAGCTAACTCTTT    1259
               ************************************************************

Della          ATGCAACGACCATATTATCATCTTTATTAGTATATGTCAGCTTATAAAAATACAATTATT    1319
Razinieh       ATGCAACGACCATATTATCATCTTTATTAGTATATGTCAGCTTATAAAAATACAATTATT    1306
BTX623         ATGCAACGACCATATTATCATCTTTATTAGTATATGTCAGCTTATAAAAATACAATTATT    1319
               ************************************************************

Della          CATCATGTCCAGAAATTGTCAGTGGAAAGTATCTTTGAATTATATTCCATACTTAATTGT    1379
Razinieh       CATCATGTCCAGAAATTGTCAGTGGAAAGTATCTTTGAATTATATTCCATACTTAATTGT    1366
BTX623         CATCATGTCCAGAAATTGTCAGTGGAAAGTATCTTTGAATTATATTCCATACTTAATTGT    1379
               ************************************************************

                                                ABRE, ABA-responsive element
Della          TTGGGGTTAGATGTGAGATTGGGGAGGCTATAGTGCACGCTGCAGACGTGTACACAATGT    1439
Razinieh       TTGGGGTTAGATGTGAGATTGGGGAGGCTATAGTGCAGGCTGCAGACGTGTACACAATGT    1426
BTX623         TTGGGGTTAGATGTGAGATTGGGGAGGCTATAGTGCACGCTGCAGACGTGTACACAATGT    1439
               ********************************** **********************

Della          CACAACCCACATGTATACCAAGGCATGCGTGCCACCTCCTATATAAAGCCCCCAACAGCC    1499
BTX623         CACAACCCACATGTATACCAAGGCATGCGTGCCACCTCCTATATAAAGCCCCCAACAGCC    1499
Razinieh       CACAACCCACATGTATACCAAGGCATGCGTGCCACCTCCTATATAAAGCCCCCAACAGCC    1486
               ************************************************************

Della          AGCGTATCATTGCCAGAGTTTCTGACAACAACTCAGCTGAGAACTCCTTGCAGAGCTCTT    1559
Razinieh       AGCGTATCATTGCCAGAGTTTCTGACAACAACTCagCTGAGAACTCCTTGCAGAGCTCTT    1546
BTX623         AGCGTATCATTGCCAGAGTTTCTGACAACAACTCAGCTGAGAACTCCTTGCAGAGCTCTT    1559
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Della           CGATCTTGCCCATAGCACCTGCAACTGTTAGTTCAGTTGTGTCGCAATGGCAGGCCTATC     1619
Razinieh        CGATCTTGCCCATAGCACCTGCAACTgttAGTTCAGTTGTGTCGCAaTGGCAGGCCTATC     1606
BTX623          CGATCTTGCCCATAGCACCTGCAACTGTTAGTTCAGTTGTGTCGCAATGGCAGGCCTATC     1619
                ************************************************************

Della           TCTGC 1624
Razinieh        TCTGC 1611
BTX623          TCTGC 1624
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SUT2 promoter

MeJA-responsive element

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Della         CTCcCTGCCACggTGAAGTCTTCTTGACGCAGGCAGCTAGaACCCCTAGCCAGCGCGGCG    60
Razinieh      CTCtCTGcCACGGtGAAGTCTTCTTGACGCAGGCAGCTAGAACCCCTAGCCAGCGCGGCG    60
BTx623        CTCTCTGCCACGGTGAAGTCTTCTTGACGCAGGCAGCTGGAACCCCTAGCCAGCGCGGCG    60
              *** ******************************** ********************

Della         CTTGTCGGCGGCTTTGCCCCTCTGTGGCACTTGATCATCTTTGgtTATCCCCATCATCTT    120
Razinieh      CTTGTCGGCGGCTTTGCCCCTCTGTGGCACTTGATCATCTTTGGTTATCACCATCATCTT    120
BTx623        CTTGTCGGCGGCTTTGCCCCTCTGTGGCACTTGATCATCTTTGGTTATCACCATCATCTT    120
              ********************************************** **********

Della         CTTTTGCTCtATCACTTGGTATGTACCAACCTCATCTACACTTAGCATGAAGGTTAGTAC    180
Razinieh      CTTTTGCTCTATCACTTGGTATGTACCAACCTCATCTACACTTAGCATGAAGGTTAGTAC    180
BTx623        CTTTTGCTCTATCACTTGGTATGTACCAACCTCATCTACACTTAGCATGAAGGTTAGTAC    180
              ************************************************************

Della         TTAGGTTTCAtCAATTATCCAAAACCaTACTAGAACTTTCAATCTCATCCTTTTTGGTAA    240
Razinieh      TTAGGTTTCATCAATTATCCAAAACCATACTAGAACTTTCAATCTCATCCTTTTTGGTAA    240
BTx623        TTAGTTTTCATCAATTATCCAAAACCATACTAGAACTTTCAATCTCATCCTTTTTGGTAA    240
              **** ********************************************************

Della         TTGATGACAACCCATTTACAAAGAGTTTGAACAAAATTTTCTTGGATTTCATGTGTGCTT    300
Razinieh      TTGATGACAACCCATTTACAAAGAGTTTGAACAAAATTTTCTTGGATTTCATGTGTGCTT    300
BTx623        TTGATGACAACCCATTTACAAAGAGTTTGAACAAAATTTTCTTGGATTTCATGTGTGCTT    300
              ************************************************************

Della         GCCCAAATATTTTACCATGTGTAAAGGTTATGGACAAGTTCCATGAACTTAAATTGGTAG    360
Razinieh      GCCCAAATATTTTACCATGTGTAAAGGTTATGGACAAGTTCCATGAACTTAAATTGGTAG    360
BTx623        CCCCAAATATTTTACCATGTGTAAAGGTTATGGACAAGTTCCATGAACTTAAATTGGTAG    360
              ************************************************************

Della         CAATTACTCCTCCTACATATGTGCTAAGAGTTTAGATTTGAAAGCCTACACATATGTTTG    420
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Razinieh      CAATTACTCCTCCTACATATGTGCTAAGAGTTTAGATTTGAAAGCCTACACATATGTTTG    420
BTx623        CAATTACTCCTCCTACATATGTGCTAAGAGTTTAGATTTGAAAGCCTACACATATGTTTG    420
              ************************************************************

Della         AATATGAAATATAGGAGTCAATTTCTATCAAATAATGCTAAGGTGTAAAAAATAGACCTT    480
Razinieh      AATATGAAATATAGGAGTCAATTTCTATCAAATAATGCTAAGGTGTAAAAAATAGACCTT    480
BTx623        AATATGAAATATAGGAGTCAATTTCTATCAAATAATGCTAAGGTGTAAAAAATAGACCTT    480
              ************************************************************
     ABRE, ABA-responsive element
Della         TGAAACGTGACACCAATACCTTGGTATGTTTTTTTCTCTCATAGTAAATCAACATAAACA    540
Razinieh      TGAAACGTGACACCAATACCTTGGTATGTTTTTTTCTCTCATAGTAAATCAACATAAACA    540
BTx623        TGAAACGTGACACCAATACCTTGGTATGTTTTTTTCTCTCATAGTAAATCAACATAAACA    540
              ************************************************************
                                ABRE, ABA-responsive element
Della         TCACCACAAGCTAAAATTCAGCGAAAGGAACGTGGATGTGCTGGAACTCAGCTGCAGGTG    600
Razinieh      TCACCACAAGCTAAAATTCAGCGAAAGGAACGTGGATGTGCTGGAACTCAGCTGCAGGTG    600
BTx623        TCACCACAAGCTAAAATTCAGCGAAAGGAACGTGGATGTGCTGGAACTCAGCTGCAGGTG    600
              ************************************************************

Della         CTGCGCCTGCGCTGATCTTTTCCTTATTGATGCTCTGCAgAGGGCATAGCTGGCGCTAAT    660
Razinieh      CTGCGCCTGCGCTGATCTTTTCCTTATTGATGCTCTGCAGAGGGCATAGCTGGCGCTAAT    660
BTx623        CTGCGCCTGCGCTGATCTTTTCCTTATTGATGCTCTGCAGAGGGCATAGCTGGCGCTAAT    660
              ************************************************************

Della         CTTTGTGGACGAGGGGATCGCTTTTGCAGTTGGGTTGGGCTTTGTTTAGATCCAAAAACT    720
Razinieh      CTTTGTGGACGAGGGGATCGCTTTTGCAGTTGGGTTGGGCTTTGTTTAGATCCAAAAACT    720
BTx623        CTTTGTGGACGAGGGGATCGCTTTTGCAGTTGGGTTGGGCTTTGTTTAGATCCAAAAACT    720
              ************************************************************

Della         TTTTGGATTTTGATACTGTAGCACTTTCGTTTTTATTTGACAAAGCAACTAGACTTAAAA    780
Razinieh      TTTTAGATTTTGATACTGTAGCACTTTCGTTTTTATTTGACAAAGCAACTAGACTTAAAA    780
BTx623        TTTTGGATTTCGATACTGTAGCACTTTCGTTTTTATTTGACAAAGCAACTAGACTTAAAA    780
              **** ***** **********************************************
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Della         GATTCGTCTCGTGATTTACAAGTAAACTGTGCAATTAGTTATCTTTTTTTTATCTATATT     840
Razinieh      GATTCGTCTCGTGATTTACAAGTAAACTGTGCAATTAGTTATCTTTTTTTTATCTATATT     840
BTx623        GATTCGTCTCGTGATTTATAAGTAAACTGTGCAATTAGTTATCTTT-TTTTATCTATATT     839
              ****************** ************************** *************

Della         TAATGTTCCATGCATGTGCCGCAAGATTTGATGTGATGGGAAATCTTGTAAAGTTTTGGG     900
Razinieh      TAATGTTCCATGCATGTGCCGCAAGATTTGATGTGATGGGAAATCTTGTAAAGTTTTGGG     900
BTx623        TAATGTTCCATGCATGTGCCGCAAGATTTGATGTGATGGGAAATCTTGTAAAGTTTTGGG     899
              ************************************************************

Della         TTTTTGGGTGTATGTAAACAAAGCCTTAGGGTCGGTAGCAAGTAAAATGAACTGACCGTG     960
Razinieh      TTTTTGGGTGTATGTAAACAAAGCCTTAGGGTCGGTAGCAAGTAAAATGAACTGACCGTG     960
BTx623        TTTTTGGGTGTATGTAAACAAAGCCTTAGGGTCGGTAGCAATTAAAATGAACTGACCGTG     959
              ************************************** ******************

Della         GAGCGGAACTCGAGAACTTGGCTGATTGGTAGTGCTAGTGCAAAGCTACCTACTACTGCG     1020
Razinieh      GAGCGGAACTCGAGAACTTGGCTGATTGGTAGTGCTAGTGCAAAGCTACCTACTACTGCG     1020
BTx623        GAGCCGAACTCGAGAACTTGGCTGATTGGTAGTGCTAGTGCAAAGCTACCTACTACTGCG     1019
              **** ********************************************************

Della         CACGTAAAAGGTTGCACTTGAGTCGTGAATTGCAGACAGCACAATGTGAATGAACATGCA     1080
Razinieh      CACGTAAAAGGTTGCACTTGAGTCGTGAATTGCAGACAGCACAATGTGAATGAACATGCA     1080
BTx623        CACGTAAAAGGTTGCACTTGAGTCGTGAATTGCAGACAGCACAATGTGAATGAACATGCA     1079
              ************************************************************

Della         ACAACAATAATAAGCAGCTGTAGTTGTGTGTCATGTACCCAAAGATAGGTACGGAGTAGT     1140
Razinieh      ACAACAATAATAAGCAGCTGTAGTTGTGTGTCATGTACCCAAAGATAGGTACGGAGTAGT     1140
BTx623        ACAACAATAATAAGCAGCTGTAGTTGTGTGTCATGTACCCAAAGATAGGTACGGAGTAGT     1139
              ************************************************************
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                            Dehydration responsive element (DRE)
Della          TTTATTCCTAGTCCCATGACACTGACCGACAGAAGCTCTCACCAAACAAACAATGGGAGT     1200
Razinieh       TTTATTCCTAGTCCCATGACACTGACCGACAGAAGCTCTCACCAAACAAACAATGGGAGT     1200
BTx623         TTTATTCCTAGTCCCATGACACTGACCGACAGAAGCTCTCACCAAACAAACAATGGGAGT     1199
               ************************************************************

Della          CAGCTGTCTGAGTTTCTTTGGTTGTGTGTAATCCATCAGTGATGATGACTCCGGgAGCGC     1260
Razinieh       CAGCTGTCTGAGTTTCTTTGGTTGTGTGTAATCCATCAGTGATGATGACTCCGGGAGCGC     1260
BTx623         CAGCTGTCTGAGTTTCTTTGGTTGTGTGTAATCCATCCGTGATGATGACTCCGGGAGCGC     1259
               ************************************* **********************

Della          caATCGCGCACCACAACGCACTTCACATCGGAGGAACTGGAAAaCTGGGTTGGGTCGAGT     1320
Razinieh       CAATCGCGCACCACAACGCACTTCACATCGGAGGAACTGGAAAACTGGGTTGGGTCGAGT     1320
BTx623         CAATCGCGCACCACAACGCCCTTCACATCGGAGGAACTGGAAAACTGGGTTGGGTCGAGT     1319
               ******************* ****************************************

Della          CGCGAATGGCAGTGCGCCAGgCAATGGGAGTCAACAACAGGACGCACACGCACGGAGTGA     1380
Razinieh       CGCGAATGGCAGTGCGCCAGGCAATGGGAGTCAACAACAGGACGCACACGCACGGAatGa     1380
BTx623         CGCAAATGGCAGTGCGCCAGGCAATGGGAGTCAACAACAGGACGCACACGCACGGAGTGA     1379
               *** **************************************************** ***

Della          caGgCGTCGGCGCGAGGCTGCTGCTGGaaTGCTGGCtGgcGCATCACGCGCAATTTCCCT     1440
Razinieh       CAGGCGTCGGCGCGAGGCTGCTGCTGGAGTGCTGGCTGGCGCATCACGCGCAATTTCCCT     1440
BTx623         CAGGCGTCGGCGCGAGGCTGCTGCTGGAGTGCTGGCTGGCGCATCACGCGCAATTTCCCT     1439
               **************************** *******************************

Della          CCCATTtCATTTTTCTACTCCTCCCTCCCCTCCCCTcccctttCctTccCttcAcaggC     1500
Razinieh       CCCATTTCATTTTTCTACTCCTCCCTCCCC-----TCCCCTTTCCTTcCCCTTCACCGGC     1495
BTx623         CCCATTTCATTTTTCTACTCCTCCCTCCCCTCCCCTCCCCTTTCCTTCCCCTTCACCGGC     1499
               ******************************     ********************* ***

Della          AggCacGCagGGCAcGGCAcGgCacggCAGGcTTcTctcCctGATctgccctgccgagcC     1560
Razinieh       AGGCACGCACGGCACGGCACGGCACGGCAGGCTTCTCTCCCTGATCTGCCCTGCCCTGCC     1555
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BTx623          AGGCACGCACGGCACGGCACGGCACGGCAGGCTTCTCTCCCTGATCTGCCCTGCCCTGCC    1559
                ********* ****************************************  ***

                                          Dehydration responsive element (DRE)
Della           ctgcctGGAGTGGAGTCTCgCtcTcctggtAcTcCAgcccgcCcTGCCCTcaGAcccGA    1620
Razinieh        CTGCCTGGAGTGGAGTCTCGCTCTCCTCGTACTCCAGCCCGCCCTGCCCTCCGACCCCGA    1615
BTx623          CTGCCTGGAGTGGAGTCTCGCTCTCCTCGTACTCCAGCCCGCCCTGCCCTCCGACCCCGA    1619
                ************************** ********************** ********

                Dehydration responsive element (DRE)
Della           ccGACCACCGCTGCCGCAGCGACACcTACACGCCCGCCCGCcGcGgcTGAGCCTCAAC-c    1679
Razinieh        CCGACCACCGCTGCCGCAGCGACACcTACACGCCCGCCcGCCGCGGCTGAGCCTCAACcg    1675
BTx623          CCGACCACCGCTGCCGCAGCGACACCTACACGCCCGCCCGCCGCGGCTGAGCCTCAAC-C    1678
                ********************************************************


Della           cCAGATCtCAcGCCGCGGCCACCAGATatgcGgcgcgcccgCCAtGGACGCcGGCaCCGG    1739
Razinieh        cCAGATCTCACGCCGCGGCCACCAGATCTGCGGCGCGCCCGCCATGGACGCCGGCACCGG    1735
BTx623          CCAGATCTCACGCCGCGGCCACCAGATCTGCGGCGCGCCCGCCATGGACGCCGGCACCGG    1738
                ************************** ********************************


Della           GGGCGGcGGgcCAACGgCCATCCGCgtcccctAccacCACctccgagAcGGagaGaaGgA    1799
Razinieh        GGGCGGCGGgCCAACGGCCATCCGCGTGCcCTACCGCCACCTcCGCGACGCcGAGATGGA    1795
BTx623          GGGCGGCGGGCCAACGGCCATCCGCGTGCCCTACCGCCACCTCCGCGACGCCGAGATGGA    1798
                ************************** ******* ********* ****  **** ***

        MeJA-responsive element
Della           ggtcgTcAgCctC      1812
Razinieh        GctcGTCAGCcTC      1808
BTx623          GCTCGTCAGCCTC      1811
                * ***********
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SUT6 promoter

```
Della          GTCcCTTccAGCTCCACTgTCACTGCTGGAGCCAAATCCAACTTGATGTAAGTGATGAGT      60
Razinieh       GTcagTTCTAGCTCCACTGTCaCTGCTGGAGCCAAATCCAACTTGATGTAAGTGATGAGT      60
BTx623         GTCAGTTCTAGCTCCACTGTCACTGCTGGAGCCAAATCCAACTTGATGTAAGTGATGAGT      60
               ***  *** **************************************************

Della          GATCTGTGATAGTGATGATATGGATTTATACttGgTACGCCCACACTCATTTTCTAACAA      120
Razinieh       GATCTGTGATAGTGATGATATGGATTTATACTTGG-ACGCCCACACTCATTTTCTAACAA      119
BTx623         GATCTGTGATAGTGATGATATGGATTTATACTTGG-ACGCCCACACTCATTTTCTAACAA      119
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Della          GGCGTCTAGGAGAGGATAGGTTAGGGTGATTACAGGTCCAAGGTCCCATAAAAGGTGTTA      180
Razinieh       GGCGTCTAGGAGAGGATAGGTTAGGGTGATTACAGGTCCAAGGTCCCATAAAAGGTGTTA      179
BTx623         GGCGTCTAGGAGAGGATAGGTTAGGGTGATTACAGGTCCAAGGTCCCATAAAAGGTGTTA      179
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Della          GTAAAAAAAGTAGCAACATGTCTTGATGATTTagAGGTCTTGTCGATGTGATATTGAGGT      240
Razinieh       GtAAAaAAAGTAGCAACATGTCTTGATGATTTAGAGGTCTTGTCGATGTGATATTGAGGT      239
BTx623         GTAAAAAAAGTAGCAACATGTCTTGATGATTTAGAGGTCTTGTCGATGTGATATTGAGGT      239
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Della          GAGATTAGATCCCCGAATAGTAAGGGtTTGATGGCCTAACACTTCAAGATAGGTATTGCC      300
Razinieh       GAGATTAGATCCCCGAATAGTAAGGGTTTGATGGCCTAACACTTCAAGATAGGTATTGCC      299
BTx623         GAGATTAGATCCCCGAATAGTAAGGGTTTGATGGCCTAACACTTCAAGATAGGTATTGCC      299
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Della          CATAGTGTATCCATGTTCCACACAAGTTGTAGAATCACATCTTTTCGAAGCaaAACATAT      360
Razinieh       CATAGTGTATCCATGTTCCACACAAGTTGTAGAATCACATCTTTTCGAAGCAAAACATAT      359
BTx623         CATAGTGTATCCATGTTCCACACAAGTTGTAGAATCACATCTTTTCGAAGCAAAACATAT      359
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Della          TTAAAATAGGGAGTAGTTTGAATAAATTGGATATTTCgCTGAATTAtTAtcaAAAGAAAA      420
Razinieh       TTAAAATAGGGAGTAGTTTGAATAAACTGGATATTTCGCTGAATTATTATGAAAAGAAAA      419
BTx623         TTAAAATAGGGAGTAGTTTGAATAAATTGGATATTTCGCTGAATTATTATGAAAAGAAAA      419
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Della         GGACATTGCACTTgGAATCCTGGCCCTTCTTCAAAATtgAaatcggAaaCTtgAAgATTC      480
Razinieh      GGACATTGCACTTGGAATCCTGGCCCTTCTTCAAAATTGACGCAGGAAAGCTTGAAGATT      479
BTx623        GGACATTGCACTTGGAATCCTGGCCCTTCTTCAAAATTGACGCAGGAAAGCTTGAAGATT      479
              ************************************    *****  *  *   *

Della         GCGACGTGCGAGGCTTGCaTgTagcTTTTCTTCTTaaTGAAatACACATGaaGTCcGTtA      540
Razinieh      CGGACGTGCGAGGCTTGCAGA---GTTTTCTTCTTAATGAAATACACATAAAGTCGTGTT      536
BTx623        CGGACGTGCGAGGCTTGCAGAGTT---TTCTTCTTAATGAAATACACATGAAGTCG--TG      534
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Della         tgGGAGAAAAAAAatCGAAtATGCTCCAAGCGAGGAGGATCTCTGGAGATATGGGGGCTG      600
Razinieh      ATAGAAAAAAAAAATCAAATATGCTCCAAGCGAGGAGGATCTCTAGAGATATGGGGGCTG      596
BTx623        TTATAGAAAAAAAATCGAATATGCTCCAAGCGAGGAGGATCTCTGGAGATATGGGGGCTG      594
                  * ********** *************************** ***************

Della         GCAGGAGGAGGTAGAGATCCGGCAAGCGGGCAGTGTGGAGCTCCTGCGCCGTGGACTTGC      660
Razinieh      GCAGGAGGAGGTAGAGATCCGGCAAGCGGGCAGCGTGGAGCTCTTGCGCTGTGGACTTGC      656
BTx623        GCAGGAGGAGGTAGAGATCCGGCAAGCGGGCAGTGTGGAGCTCCTGCGCCGTGGACTTGC      654
              ******************************** ********* ***** **********

Della         CCGCCGCCAGTGGATCAAGGCTATAGGGGAGGAGGCGGCCGAGCCAAGGGAGGTGTTGTG      720
Razinieh      CCGCCGCCAGTGGATCAAGGCTATAAGGGAGGAGGCGGCCGAGCCAAGGAAGGTGTTGTG      716
BTx623        CCGCCGCCAGTGGATCAAGGCTATAGGGGAGGAGGCGGCCGAGCCAAGGGAGGTGTTGTG      714
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Della         GAGGGAGGCAGCaaTAGGGATGGTGCGGCACCGCATAGGAGGGAGAAGGGGCTGATCGAG      780
Razinieh      GAGGGAGGCAGCGATAGGGATGGTGCGACACCACATAGGAGGGAGAAGGGGCTGATCGAG      776
BTx623        GAGGGAGGCAGCAATAGGGATGGTGCGGCACCGCATAGGAGGGAGAAGGGGCTGATCGAG      774
              ************ ************** **** ****************************

Della         TGCATGGGAGGGGCAAGCAGGGAACACGACTCTGCGTTTTCCAAATCGCGCT-GAaCTCC      839
Razinieh      TGCATGGGAGGGGCAAGCAGGGAACACGACTCTGCGTTTTCCAAATCGCAGAGCAACTCC      836
BTx623        TGCATGGGAGGGGCAAGCAGGGAACACGACTCTGCGTTTTCCAAATCGCGCT-GAACTCC      833
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Della          CATTTCGTGCACCGTTGCGTTTTCCAACGCCAACGCATGTTCGTGTTCACCCCACCGTCG      899
Razinieh       CATTTCGGGCACCGTTGCGTTTTCCAACGCCAACGCATGTTCGTGTTCACCCCACCGTCG      896
BTx623         CATTTCGTGCACCGTTGCGTTTTCCAACGCCAACGCATGTTCGTGTTCACCCCACCGTCG      893
               ******* **************************************************

Della          CGACCCGTTCAAAGGCGGAAACAAACATACATGCCCTGCCAGTCTCAATGGAGTTTCATG      959
Razinieh       CGACCCGTTCAAAGGCGGAAACAAACATACATGCCCTGCCAGTCTCAATGGAGTTTCATG      956
BTx623         CGACCCGTTCAAAGGCGGAAACAAACATACATGCCCTGCCAGTCTCAATGGAGTTTCATG      953
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Della          AAAGTTTCATGCACATTAAATATGCTGATGTGGCGCTGTAGTAATGAAGAGAGAGATGAT      1019
Razinieh       AGAGTTTCATACACATTAAATATGCTGATGTGGCGCTGTAGTAATGAAGAGAGAGATGAT      1016
BTx623         AAAGTTTCATGCACATTAAATATGCTGATGTGGCGCTGTAGTAATGAAGAGAGAGATGAT      1013
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Della          AAGAGTTTCATGAAAGTAGAGAGAGTTTCATCCGCATAAAACTTCAATGCAATGTTTATA      1079
Razinieh       AATAGTTTCATGGGAGTAGAGAGAGTTTCATCCGCATAAAACTTCAATGCAACGTTTATA      1076
BTx623         AAGAGTTTCATGAAAGTAGAGAGAGTTTCATCCGCATAAAACTTCAATGCAATGTTTATA      1073
               ** *********  ************************************ *******

Della          AAATATGGATGTGTTGAAAACTGGGTCACGAAACTTTCATTGAAAATGATCTTAGTTTAT      1139
Razinieh       AAATATAGATGTGTTGAAAACTGGGTCATGAAACTTCCATTGAAAATGATCTTAGTTTAT      1136
BTx623         AAATATGGATGTGTTGAAAACTGGGTCACGAAACTTTCATTGAAAATGATCTTAGTTTAT      1133
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Della          GTGAGTTTCACTTCCACCACACAATATAGAGAGGGAAAACATGTGACAGCTGCGCTGTGA      1199
Razinieh       GTGAGTTTCACTTCCACCACACAATATAGAGAGAGGAAACATGTGACAGCTGCGCTGTGA      1196
BTx623         GTGAGTTTCACTTCCACCACACAATATAGAGAGGGAAAACATGTGACAGCTGCGCTGTGA      1193
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         MeJA-responsive element (DRE)
Della          TGCGCGTCACTTTTGCTTCTGTTATCATTCTCACCCGGTTGCTGCGGCATGCGGATAAGC      1259
Razinieh       TGCGCGTCACTTTTGCTTCTGTTCTCATTCTCACCCGGTTGCTGCGGCATGCGGATAAGC      1256
BTx623         TGCGCGTCACTTTTGCTTCTGTTATCATTCTCACCCGGTTGCTGCGGCATGCGGATAAGC      1253
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Della         TAGCGCTGCGGAGAAATCTAGCTGCATGCCCATTATCAAATCAAATTGAAGTTTCCTTGT      1319
Razinieh      TAGCGCTGCGGAGAAATCTAGCTGCATGCCCATTATCAAATCAAATTGAAGTTTCCTTGT      1316
BTx623        TAGCGCTGCGGAGAAATCTAGCTGCATGCCCATTATCAAATCAAATTGAAGTTTCCTTGT      1313
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Della         CCATGGCTGTTGCACTATATATTATGTTGTTTGTAACGAGGCTCAGCGAAACGACGACTA      1379
Razinieh      CCATGGCTGTTGCACTAT--ATTATGTTGTTTGTAACGAGGCTCAGCGAAACGACGACAA      1374
BTx623        CCATGGCTGTTGCACTATATATTATGTTGTTTGTAACGAGGCTCAGCGAAACGACGACAA      1373
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Della         GAGAGAACAACCCAACAGCTAGGCTGTGTCAAATAGGTCAACAGACCATCCCCACTGAGC      1439
Razinieh      GAGAGAACAACCCAACAGCTAGGTTGTGTCAAATAGGTCAACAGACCATCCCCACTGAGC      1434
BTx623        GAGAGAACAACCCAACAGCTAGGCTGTGTCAAATAGGTCAACAGACCATCCCCACTGAGC      1433
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Della         ACCACATACACTCTCCCTCTAAACTCTGATCAGAAGCGCCAATAATAATGCAAGGTCTCC      1499
Razinieh      ACCACATACACTTTCCCTCTAAACTCTGATCAGAAGCGCCAATAATAATGCAAGGTCTCC      1494
BTx623        ACCACATACACTCTCCCTCTAAACTCTGATCAGAAGCGCCAATAATAATGCAAGGTCTCC      1493
              ************ ********************************************

Della         ATCGAAATCATTGTTCAGAATCTGTTTTGTTAATTCCTTTCCTTGAGGACCATCCAAGCA      1559
Razinieh      ATCGAAATCATTGTTCAgAaTCTATTTGTTAAT-TCCTTTCCTTGAGGACCATCCAAGCA      1553
BTx623        ATCGAAATCATTGTTCAGAATCTGTTTTGTTAATTCCTTTCCTTGAGGACCATCCAAGCA      1553
              ********************** ***  * *  *************************

Della         AACA------------------------------------------------------      1563
Razinieh      AACAATACATATATTGGAGGCGGTTTGTTAATTCCTTTCCTTGAGGACCATCCAAGCAAA      1613
BTx623        AACA------------------------------------------------------      1557
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Della         ATACATATATATTGGAGGCGGCTGGCTAATTCTTTTCTGTTCCTTGCTCGATCTTTGGAA      1623
Razinieh      CAATACATATATTGGAGGCGGCTGGCTAATTCTTTTCTGTTCCTTGCTCGATCTTTGGAA      1673
BTx623        ATACATATATATTGGAGGCGGCTGGCTAATTCTTTTCTGTTCCTTGCTCGATCTTTGGAA      1617
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MeJA-responsive element

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Della         CTATGCTGGAAATTAAGCTGGCTACGCCGTCACGCTTCATTCGTCGTTCCCGCGCTCGGC      1683
Razinieh      CTATGCTGGAAATTAAGCTAGCTACGCCGTCACGCTTCATTCGTCGTTCCCGCGCTCGGC      1733
BTx623        CTATGCTGGAAATTAAGCTGGCTACGCCGTCACGCTTCATTCGTCGTTCCCGCGCTCGGC      1677
              ****************** ***************************************

Della         GCTCGTCGCTTTCCAACCCCATCCTTCCCGTCCGTGACCGTGATTATTTGCCGTGTGCTT      1743
Razinieh      GCTCGTCGCTTTCCAACCCCATCCTTCCCGTCCGTGACCGTGATTATTTGCCGTGTGCTT      1793
BTx623        GCTCGTCGCTTTCCAACCCCATCCTTCCCGTCCGTGACCGTGATTATTTGCCGTGTGCTT      1737
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Della         GTTTCGTTCGTTCCTGCTCCTCTCACTCCTCGCACCTTGCGCCGCCGCAGCCCGCAGCAG      1803
Razinieh      GTTTCGTTCGTTCCTGCTCCTCTCACTCCTCGCACCTTGCGCCGCCGCAGCCCGCAGCAG      1853
BTx623        GTTTCGTTCGTTCCTGCTCCTCTCACTCCTCGCACCTTGCGCCGCCGCAGCCCGCAGCAG      1797
              ************************************************************

                    MeJA-responsive element
Della         CCATGGACGACGGTGACGTCGGCGAGGAGGACGCCAACaAGCAGCGCCTCGAGCGGGCCA      1863
Razinieh      CCATGGACGACGGTGACGTCGGCGAGGAGGACGCCAACAAGCAGCGCCTCGAGCGGGCCA      1913
BTx623        CCATGGACGACGGTGACGTCGGCGAGGAGGACGCCAACAAGCAGCGCCTCGAGCGGGCCA      1857
              ************************************************************

Della         CCATGAACCTGGAGCGCGGCGTCGTCGCCGGCGAGAAGGgcGACGGCAGTGGCGGGAATG      1923
Razinieh      CCATGAACCTGGAGCGCGGCGTCGTCGCCGGCGAGAAGGGCGACGGCAGTGGCGGGAATG      1973
BTx623        CCATGAACCTGGAGCGCGGCGTCGTCGCCGGCGAGAAGGGCGACGGCAGTGGCGGGAATG      1917
              ************************************************************

Della         CGAGCCGGAAgCCGCCGATAGGCATCgTCCGGCTCTTCTTgGCCTGCATGGTCtg    1978
Razinieh      CGAGCCGGAAGCCGCCGATAGGCATCGTCCGGCTCTTCTTGGCCTGCATGGTCTC    2028
BTx623        CGAGCCGGAAGCCGCCGATAGGCATCGTCCGGCTCTTCTTGGCCTGCATGGTCTC    1972
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List of Publications:

Abuslima, E., Kanbar, A., Raorane, M., L., Eiche, E., Junker, B., H., Hause, B., Riemann, M., and Nick, P. (2022). Gain time to adapt: How sorghum acquires tolerance to salinity. *Front. Plant Sci.* 1–17. http://doi: 10.3389/fpls.2022.1008172

Kanbar, A., Mirzai, M., **Abuslima, E**., Flubacher, N., Eghbalian, R., Garbev, K., Bergfeldt, B., Ullrich, A., Leibold, H., Eiche, E., Müller, M., Mokry, M., Stapf, D., Nick, P. (2021). Starve to Sustain—An Ancient Syrian Landrace of Sorghum as Tool for Phosphorous Bio-Economy?. *Int. J.Mol. Sci. 22*, 9312. https://doi.org/10.3390/ijms22179312