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Research Article

A Tunisian wild grape leads to metabolic fingerprints of salt tolerance

Samia Daldoul [1,†] Mahmoud Gargouri [1,†] Christoph Weinert [2,†] Ali Jarrar [3] Björn Egert [2] Ahmed Mliki [1] and Peter Nick [3,*]

1 Laboratory of Plant Molecular Physiology, Center of Biotechnology of Borj-Cedria, Borj-Cedria PC5G+PV6, Tunisia
2 Institute for Safety and Quality in Fruits and Vegetables, Max-Rubner Institute for Nutrition, Karlsruhe 76131, Germany
3 Molecular Cell Biology, Joseph Gottlied Kölreuter Institute for Plant Sciences, Karlsruhe Institute of Technology, Karlsruhe 76131, Germany

*Author for correspondence: peter.nick@kit.edu
†These authors contributed equally.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/General-Instructions) is Ahmed Mliki.

Abstract

Soil salinity is progressively impacting agriculture, including viticulture. Identification of genetic factors rendering grapevine (*Vitis vinifera* L.) resilience that can be introgressed into commercial varieties is necessary for safeguarding viticulture against the consequences of global climate change. To gain insight into the physiological and metabolic responses enabling salt tolerance, we compared a salt-tolerant accession of *Vitis sylvestris* from Tunisia, "Tebaba", with "1103 Paulsen" rootstock widely used in the Mediterranean. Salt stress was slowly increased, simulating the situation of an irrigated vineyard. We determined that "Tebaba" does not sequester sodium in the root but can cope with salinity through robust redox homeostasis. This is linked with rechanneling of metabolic pathways toward antioxidants and compatible osmolytes, buffering photosynthesis, such that cell-wall breakdown can be avoided. We propose that salt tolerance of this wild grapevine cannot be attributed to a single genetic factor but emerges from favorable metabolic fluxes that are mutually supportive. We suggest that introgression of "Tebaba" into commercial varieties is preferred over the use of "Tebaba" as a rootstock for improving salt tolerance in grapevine.

Introduction

Climate change will increase the need for deficit irrigation, in most cases using water of low quality. This practice will accentuate the impact of salinity stress, since salt residues, left behind after evaporation of the water, accumulate in the soil. In fact, a systematic study, conducted on different crops, has demonstrated that the impact of salinity stress upon yield increases with the intensity of irrigation (Zörb et al. 2019). The yield losses depend on crop, soil type, and salinity of the irrigation water; but independently of these frame conditions, the problem is increasing, inevitably and progressively. Economic models estimate that alone the global losses by soil deterioration due to irrigation-dependent salinity range to some US$27,000 million per year (Qadir et al. 2014). Grapevine (*Vitis vinifera* L.), worldwide the fruit crop with the highest economic yield per area, is actually well adapted to drought, and therefore irrigation-dependent salinity should, at first sight, not be a major problem. However, the drier and hotter summers have fuelled the need for artificial irrigation, especially in semiarid regions. Prominent examples are the vineyards in the Mediterranean Basin (Costa et al. 2012), the Cuyo region in West Argentina (Guida-Johnson et al. 2017), or the Australian Murray–Darling Basin (Phogat et al. 2020). In California, deficit irrigation has already become so common that it has entered the standard training programs for farmers (Rieger 2021). Thus, salinity stress in viticulture must be considered as an emerging challenge.

Salinity stress can be subdivided into an osmotic and an ionic component (for a comprehensive review, see Munns and Tester 2008). The osmotic component can be understood in terms of biophysics. The drop of extracellular water potential

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causes water loss, and the responses resemble those to drought stress, such as stomatal closure, when roots are exposed to salt. The underlying systemic signals might be hydraulic as for drought stress (Christmann et al. 2007; for review, see Gorgues et al. 2022) or involve calcium waves (Choi et al. 2014). The ionic component of salt stress is caused by penetration of sodium through nonselective cation channels (NSCCs), perturbing functional surfaces, such as membranes and proteins. A particularly sensitive target is electron transport across the inner membranes of plastids and mitochondria because free electrons can be transferred to oxygen, leading to superoxide (reviewed in Foyer and Noctor 2005). Thus, salinity stress is accompanied by a severe loss of redox homeostasis, requiring the activation of enzymes able to detoxify, and secondary compounds able to scavenge, reactive oxygen species (ROS). To what extent ionic toxicity, especially that due to chloride anions, contributes to the cellular damage is not clear and has been questioned recently (Isayenkov and Maathuis 2019).

Life has come from the oceans. Thus, mechanisms to cope with and adapt to salinity, must have arisen early in evolution. In fact, many plants can tolerate salinity to variable degrees. Membrane transporters can constrain the influx of sodium ions, resecrete them, or sequester them into the vacuole (for reviews, see Schroeder et al. 2013; Shitan and Yazaki 2020). Export of sodium through the salt-overly sensitive (SOS) system (Shi et al. 2000; for review, see Ji et al. 2013) will reduce the intracellular concentration of noxious ions, but it will also go on cost of osmotic water loss. In contrast, the sequestering of sodium ions in the vacuole, driven by NHX sodium proton transporters in the tonoplast, allows to remove sodium from the cytoplasm while maintaining turgescence and, thus, the ability to grow. In fact, this approach has been successfully used to engineer salt tolerance in grapevine (Venier et al. 2018). A second mechanism to cope with salinity is to mitigate the cellular damage caused by ion toxicity. This group of responses is based not only on cellular adaptation including enzymatic and nonenzymatic antioxidants (for a classical review, see Apel and Hirt 2004; for a review focusing on salinity stress, see Gill and Tuteja 2015) but also on the formation of compatible solutes, such as glycine betaine or proline (reviewed in Chen and Murata 2002).

There is a third response type, though: salinity-induced programmed cell death (PCD) that can be observed in response to rapidly increasing salt stress (Andronis and Roubelakis-Angelakis 2010), which can be triggered in response to pertinently perturbed ROS homeostasis (for review, see Petrov et al. 2015). At first sight, it is hard to understand what the benefit of salinity-induced PCD might be because it will weaken rather than strengthen cellular survival. However, PCD must be understood in a holistic manner, on the level of the entire plant. Here, the controlled breakdown of individual organs and mobilization of their resources to other organs (for instance, the meristems) may help to compensate for the energy losses caused by stress adaptation (for review, see Baena-González 2010). Thus, PCD will support the survival of these recipient organs that, once the harsh stress episode has eased off, will regenerate the sacrificed part of the plant. Whether a plant cell that is challenged by salinity stress will respond by cellular adaptation or by cell death depends on the specific context of the stress condition, for instance, genotype or timing (for review, see Ismail et al. 2014).

With a damage threshold of ~2 to 4 $dS \cdot m^{-1}$, grapevine belongs to the moderately salt-sensitive crops (Zhang et al. 2002). If salinity crosses this threshold, there is an almost immediate drop of transpiration, followed by accumulation of sodium and chloride ions in the leaves and, from a few days after the onset of the stress period, leaf necrosis (Shani and Ben-Gal 2005). Since grapevines are predominantly grafted, selection of salt-tolerant rootstocks has been a central approach to render viticulture possible even under conditions of soil salinity (Popescu et al. 2015). Mechanisms of tolerance include ion sequestration in the root (Storey et al. 2003), or the accumulation of compatible solutes (Downton and Loveys 1981), contributing to a more resilient buffering of photosynthesis under salinity (Askri et al. 2012). These physiological phenomena are accompanied by massive changes of gene expression as revealed by transcriptomics. Salt-responsive transcripts are, for instance, involved in the metabolism of carbohydrates, lipids, and phenolic compounds, but also amino acids, or redox homeostasis (Guan et al. 2018; Das and Majumder 2019). Similar results were obtained with proteomic studies (Vincent et al. 2007).

While the quantity of data collected by such high-throughput approaches is impressive or even overwhelming, it remains often elusive, what the observed changes mean. These changes might be related to stress-related damage, but they might be, as well, manifestations of ensuing adaptation to salinity. To assign a functional role to a given entity usually requires a comparison, i.e. a contrasting pair. The comparison of suspension cell lines from the susceptible North American grapevine *Vitis riparia* and the tolerant *Vitis rupestris* (Ismail et al. 2012) revealed that the ability for cellular adaptation was accompanied by a swift but transient increase of JA-Ile, the bioactive form of jasmonic acid. This differential jasmonate accumulation was preceded by temporal differences in the uptake of sodium through the NCCS, oxidative burst, or the activation of transcripts involved in salt sequestration, jasmonate signaling, and metabolism of phenolic compounds, leading to the model of a temporal stress signature that decides whether the cell will adapt to salinity or enter PCD (Ismail et al. 2014).

Although a cellular system is convenient to dissect the temporal order of events and, thus, to infer causal relationships, it represents a strong reduction of the real-world situation in the vineyard. To mitigate this reductionism a bit, we ventured in the current study to transfer the strategy of a contrasting pair to the whole-plant level. As salt-tolerant genotype, we chose the wild accession "Tebaba" from Tunisia. This accession represents, so far, the Southernmost outpost known for the wild European grapevine, the

ancestral species of domesticated grapevine (Zoghlami et al. 2003), and is endowed with a considerable tolerance to salinity (Askri et al. 2012). The rootstock "1103 Paulsen" (1103P) is widely used all over the Mediterranean region and is the dominating rootstock in North Africa because it is fairly drought tolerant; however, it is more susceptible to salinity than "Tebaba."

To simulate a real-world situation, we administered salt stress in a form of a slowly increasing ramp mimicking the situation of an irrigated vineyard with continuously increasing soil salinity. We assessed several physiological parameters, the transfer of sodium and potassium into the aerial parts, the expression of phenylpropanoid synthesis genes and jasmonate signaling transcripts, and metabolic stress markers. We show that the tolerance of "Tebaba" is not caused by sequestration of sodium in the root system but by altered metabolism leading to a more robust homeostasis.

Results

Growth rate of "Tebaba" is more resilient to salt than in 1103P

To simulate the situation in an artificially irrigated vineyard, salt stress was administered in a gradient, where the concentration of NaCl was stepwise increased over 10 d from 0 to 150 mM and then kept constant over the subsequent 6 d (Fig. 1A). A mock control was subjected to the same procedure but omitting the salt in the medium (Fig. 1B). To monitor the physiological state of the treated plants, relative growth rates were followed over time (Fig. 2A). For "Tebaba," the growth rate in the mock control was constant but dropped progressively and steadily in response to salt stress. For 1103P (Fig. 2B), the growth rate was already lower in the mock control but, in addition, decreased even more substantially under salt stress. To compare the genotypes differing in their growth rates already in the controls (Fig. 2C, gray circles), we determined the time interval required to reach an inhibition of 70% of the growth rates in the mock controls (Supplemental Fig. S1). These I_{70} values were significantly different between the genotypes (Fig. 2C) with very low values for 1103P, while "Tebaba" could withstand salt stress for almost 10 d before reaching this I_{70}. The traditional Tunisian *vinifera* landraces "Houamdia" and "Razegui" were resilient as well, while the commercially important *vinifera* variety "Cabernet Sauvignon" ranged roughly in the middle between 1103P and "Tebaba." Based on these physiological parameters, we focused on the contrasting pair "Tebaba" and 1103P to understand the reasons behind this differential salt resilience.

The resilience of "Tebaba" is not caused by ion exclusion from the leaves

To protect photosynthesis against the impact of ionic stress, tolerant plants might exclude sodium from the transpiration stream. To test this possibility, we determined ion content in leaves of "Tebaba," "Houamdia," and 1103P in controls and salt-stressed plants, scoring at day 6 after reaching the plateau (Fig. 1). As to be expected, sodium content had increased over that seen in the control (Fig. 3A). However, this increase was more pronounced in "Tebaba" (by a factor of 2.4) as compared to 1103P (by a factor of 1.9) and "Houamdia" (by a factor of 1.25). Thus, the resilience was not linked to a reduced transfer of sodium into the leaves. While sodium stress often comes along with a decrease of potassium content, this was not observed in any of the 3 genotypes. Potassium content was even slightly increased, albeit not significantly, under sodium stress (Fig. 3B). The only difference between "Tebaba" and the other genotypes was noted with respect to bivalent cations. Both calcium (Fig. 3C) and magnesium (Fig. 3D) were increased in "Tebaba" under salt stress, while these ions either remained constant (in 1103P) or even decreased (in "Houamdia"). However, only the increase of calcium (by ∼22%) reached significance. In summary, the ion homeostasis in the leaf was mostly retained in all 3 genotypes and, thus, cannot qualify as cause for the observed difference in salt tolerance.

"Tebaba" is endowed with a more robust redox homeostasis

The sodium ions arriving in the leaves can enter the cells through nonspecific cation channels and permeate through the outer plastid membrane through porin homologs (Flügge 2000), such that they can interfere with electron transport leading to the formation of ROS (for review, see Mittler 2002). We monitored, therefore, malondialdehyde (MDA) as stable readout for lipid peroxidation, as well as hydrogen peroxide. While salinity induced MDA levels in all tested genotypes, they were more than doubled in 1103P compared to "Tebaba" and "Razegui" (Fig. 4A). Interestingly, the steady-state levels of hydrogen peroxide (Fig. 4B) did not follow this pattern. They increased 4-fold in "Tebaba." Instead, 1103P preserved the lower starting levels. The highest values were observed in "Razegui." To understand this, we measured the activities of superoxide dismutase (SOD), generating hydrogen peroxide (Fig. 4C), and those of catalase (CAT), dissipating hydrogen peroxide (Fig. 4D). SOD activity increased more strongly in 1103P (∼3.5-fold) over "Tebaba" (∼2-fold). The pattern for CAT activity was a mirror image—a steep increase in "Tebaba" by >15-fold of the mock control, a much milder induction in 1103P (∼4-fold of the control). "Razegui" resembled "Tebaba" with respect to the SOD response, but showed a sharp decline, when CAT activity was scored. In summary, oxidative damage as reported by MDA was substantially lower in "Tebaba" (and "Razegui") as compared to 1103P. A second salient feature was a high CAT activity in "Tebaba," while in 1103P, it was SOD that was strongly induced.

We used simple mathematical model (Fig. 5A) to estimate the steady-state levels of the short-lived superoxide (Fig. 5A), assuming a steady state for the concentration of hydrogen peroxide, such that its genesis (by SOD) and its dissipation

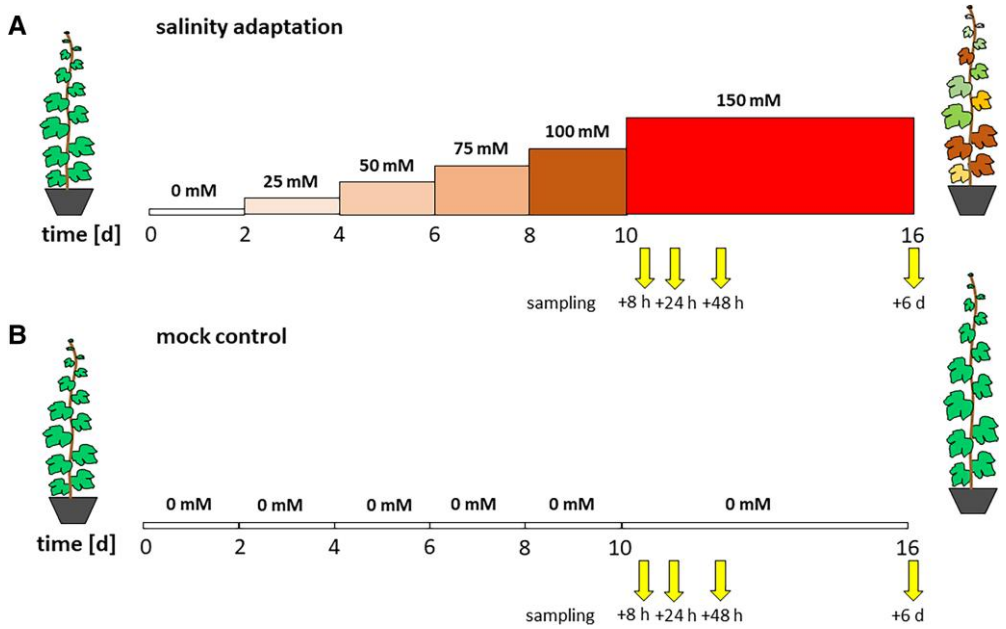

Figure 1. Experimental design for the study, where the stringency of salt stress was progressively increased **A)**, while in the mock control, plants were treated in the same way omitting the salt **B)**.

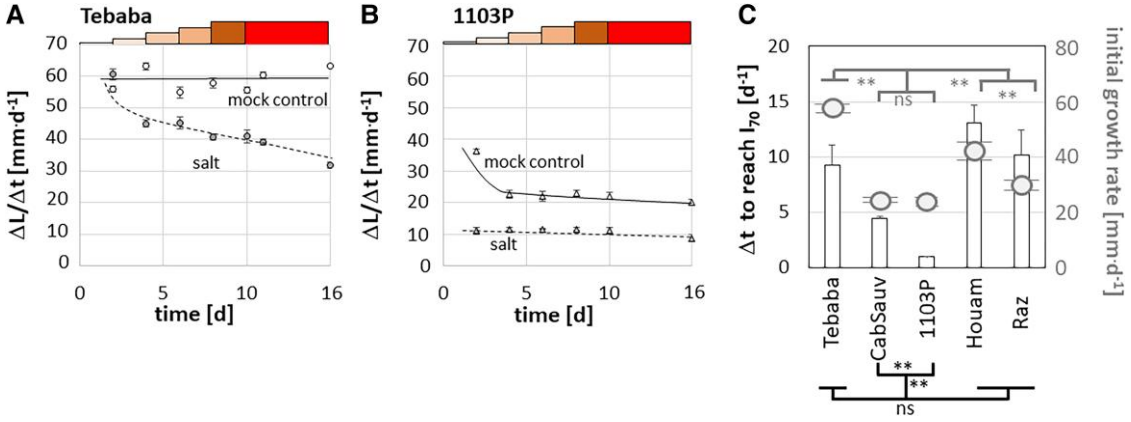

Figure 2. Time course of growth rate in Tebaba **A)** versus 1103P **B)**. The colour and height of the square code shows the progressive increase of NaCl. Data represent mean and SE from 2 experimental series with 3 individual plants per data point. **C)** Comparison of initial growth rates and salt susceptibility (represented by the time needed to inhibit growth to 70% of the initial level). In addition to Tebaba and 1103P, the commercial variety Cabernet Sauvignon (CabSauv), and the Tunisian landraces Houamdia (Houam) and Razegui (Raz) were measured. **, statistically different with $P < 0.01$; ns, not significantly different using a pairwise *t*-test.

(by CAT) could be equalized, and the ratio of superoxide over peroxide could be derived from the ratio of CAT over SOD activity. This estimation inferred a sharp (almost 20-fold) increase of superoxide under salinity for "Tebaba" but lower values in 1103P and "Razegui" (Fig. 5B). A survey for ROS and ROS-scavenging enzymes (Fig. 5C) highlights a steep increase of superoxide levels (inferred) in "Tebaba," linked with a strong increase of CAT activity (measured), while there is only a mild increase of peroxide levels (measured). In contrast, 1103P displays low superoxide levels, while SOD activity is elevated, and CAT activity is low. Also, in "Razegui," superoxide levels and CAT activities are low, but here, peroxide levels are elevated. It should be noticed that the activity of peroxidases has been ignored that would act to consume peroxide, such that the real levels of superoxide in "Tebaba" are supposed to be even higher. This contrasts with the only mild increase in MDA levels (Fig. 4A) in "Tebaba."

Stilbene synthase transcripts are strongly induced by salt stress in 1103P

The response of grapevine cell cultures to salinity involves the activation of jasmonate signaling leading to transcriptional activation of JASMONATE ZIM DOMAIN 1 (JAZ1) as well as an activation of genes involved in the phenylpropanoid

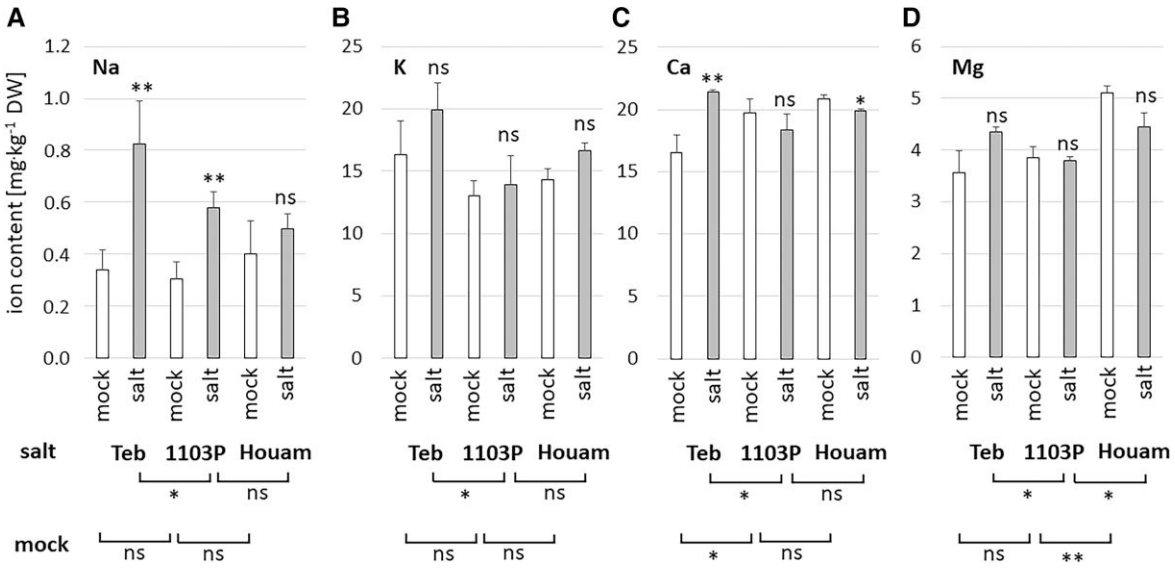

Figure 3. Ion content in leaves from Tebaba, 1103P, and Houamdia (Houam) determined by ICP-OES at the end of the experiment (day 6 after reaching the plateau). Contents of Na **A)**, K **B)**, Ca **C)**, and Mg **D)** are shown. Data represent mean and SE from 3 biological replicates per data point. **, statistically different with $P < 0.01$; *, statistically different with $P < 0.05$; ns, not significantly different using a pairwise *t*-test.

pathway (Ismail et al. 2012). Therefore, we measured steady-state transcript levels for *JAZ1*, for *phenyl ammonium lyase* (*PAL*) as first committed step of secondary metabolism, for *STILBENE SYNTHASE 27* (*STS*), the first committed step of the stilbenoid branch of phenylpropanoids, and for *CHALCONE SYNTHASE* (*CHS*), the first committed step of the concurrent flavonoid branch. For comparability, data were related to the mock control of "Tebaba" sampled at 8 h. We see a weaker and transient induction of *JAZ1* in "Tebaba" (Fig. 6A) but a stronger, earlier, and stable induction in 1103P (Fig. 6B). Transcripts for *PAL* were not modulated in "Tebaba" (Fig. 6C) but mildly and transiently induced in 1103P (Fig. 6D). In contrast, CHS transcripts showed a mild and transient induction in "Tebaba" (Fig. 6E) but remained noninduced in 1103P (Fig. 6F). The most salient difference, however, was seen for the STS transcripts. Here, there was a mild (~4-fold) and transient induction in "Tebaba" (Fig. 6G), while in 1103P, the induction rose to 45-fold but remained transient as well. Thus, the induction of STS by salinity is more pronounced in the susceptible genotype and, thus, qualifies as stress marker.

Phenylpropanoid channeling differs between 1103P and "Tebaba"

To get insight into metabolic differences linked with salt tolerance, we compared the genotypes using unbiased metabolome analysis by two dimensional Gas Chromatography coupled to Mass Spectrometry (GC × GC-MS) under control conditions or 24 h after reaching the terminal salt concentration. To detect genotypic differences that consisted a priori, we mapped the relative abundance of the respective metabolite compared to the control level in 1103P. To get insight into the responsiveness of a given metabolite, we scored, individually for each genotype, the relative change in abundance under salt stress compared to the level in the control. The phenylpropanoid pathway is strongly regulated in grapevine giving rise to important secondary compounds, some of which can act as ROS scavengers. Here, we detected several salient differences (Fig. 7).

Independently, salinity, quinic, and shikimic acids were elevated in "Tebaba" and channeled to the aromatic amino acids, phenylalanine, and tyrosine. In 1103P, these compounds were less abundant and shifted toward pyrogallol and gallic acid. A higher proportion of tyrosine was converted into tyramine, and tyrosol (probably as glucoside) in "Tebaba," again irrespective of salt stress. We did not detect cinnamomic acid, the desamination product of phenylalanine, but coumaric acid, the desamination product of tyrosine and its downstream products, caffeic acid and ferulic acid. For all 3 compounds, levels were higher in 1103P and increased further under salinity. In "Tebaba," ferulic acid was so scarce that it was not consistently detected. Coumaric acid was partially diverted to coutaric acid, which was more pronounced in 1103P, while "Tebaba" displayed less than half of the value. However, under salt stress, coutaric-acid levels in 1103P dropped to those seen in "Tebaba." Coumaric acid can fuel not only flavonoids but also epicatechine, which was more abundant in 1103P, but dropped drastically under salinity, while being mostly sustained in "Tebaba."

Overall, in "Tebaba," the shikimate pathway is more active and channeled toward aromatic amino acids, while the pyrogallol/gallic acid branch is suppressed. After desamination of the aromatic acids, "Tebaba" restrains the accumulation of coumaric and ferulic acids, while 1103P invests more into monolignols, indicative of lignification. Instead, "Tebaba" sustains epicatechine under salinity.

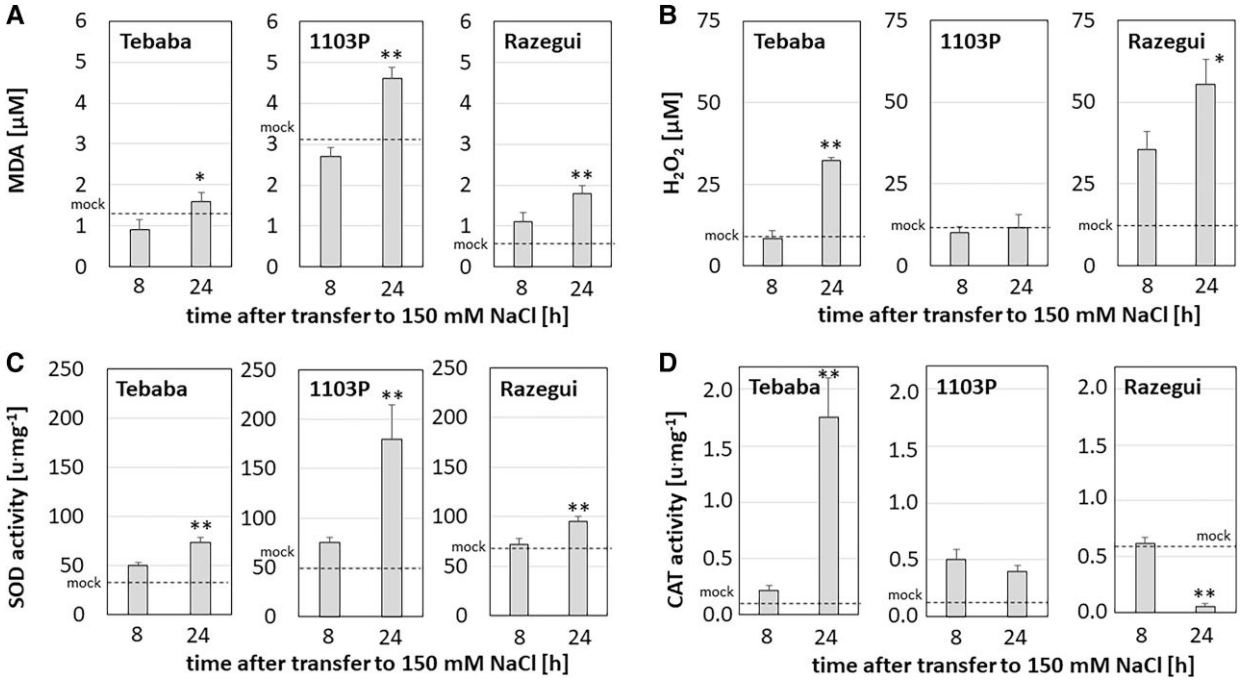

Figure 4. Markers for oxidative homeostasis in response to salinity stress in leaves of Tebaba, 1103P, and Razegui. **A)** MDA as marker for lipid peroxidation, **B)** steady-state levels of hydrogen peroxide, **C)** specific activity of SOD, and **D)** specific activity of CAT. Data represent mean and SE from 3 biological replicates per data point. **, statistically different with $P < 0.01$; *, statistically different with $P < 0.05$; ns, not significantly different using a pairwise *t*-test.

"Tebaba" displays elevated ground levels of proline

The amino-acid proline is a key factor for the adaptation to salt stress (for review, see Szabados and Savouré 2010). Indeed, differences in proline between the 2 genotypes were the most salient features among all these pathways (Fig. 8). Already under control conditions, proline was >3 times more abundant in "Tebaba" than that in 1103P. Salt stress induced proline ~3.5 times in both genotypes, which means that proline levels "Tebaba" under salinity were almost 1 order of magnitude higher than those in the 1103P control.

Interestingly, ground levels for the proline precursor glutamate were equal in both genotypes but were induced stronger in "Tebaba." Instead, glutamine levels were constant in both genotypes but substantially lower in "Tebaba." Likewise, γ-aminobutyric acid (GABA) was strongly reduced in "Tebaba" and did not increase under salinity, while in 1103P, there was a strong increase, contrasting with the pattern for glutamate. We asked ourselves, whether, under salinity, "Tebaba" recruits aspartate to fuel the increase in glutamate, but there was no corresponding increase of aspartate. Moreover, the conversion of aspartate into glutamate would require equimolar amounts of α-ketoglutarate. However, not only did this reaction partner show any strong decrease upon salt stress in "Tebaba," but it also even increased substantially. Also, for 1103P, a strong decrease of aspartate levels came with only a mild increase of glutamate.

A second fingerprint was the higher (more than twice) ground levels of glycine in "Tebaba." Glycine can be an output of peroxisomal detoxification of phosphoglycolate. This product of photorespiration is converted to serine and then, via glycerate, refuels the Calvin–Benson cycle. Alternatively, glycine can derive from serine formed as product of glycolysis. The fate of glycine and serine differs in the 2 genotypes. In "Tebaba," threonine accumulates strongly under salinity, much weaker in 1103P. Threonine accumulation feeds an increase in isoleucine, needed to convert jasmonic acid into the active hormone JA-Ile. Since "Tebaba" also sustains higher levels of α-linolenic acid, both precursors of JA-Ile are more readily available than those in 1103P. In contrast, 1103P induces serine much more strongly than "Tebaba." "Tebaba," in turn, shows elevated levels of glycerate, indicating that serine is rechanneled to the Calvin–Benson cycle, while much lower glycerate levels in 1103P indicate that serine is not converted efficiently under salinity. Thus, in "Tebaba," glycine is efficiently channeled back to carbon fixation via glycerate and also fuels the synthesis of isoleucine via threonine to provide a necessary precursor for the stress signal JA-Ile. Instead, 1103P fails to feed these 2 pathways that either contributes to an important adaptive signal (JA-Ile) or recycle the Calvin–Benson cycle (glycerate).

Central carbon metabolism and ascorbate cycle are more efficient in "Tebaba"

The metabolic analysis of sugars and related compounds yielded a high number of metabolites that allowed to highlight several differences between the 2 genotypes (Fig. 9). Some of these

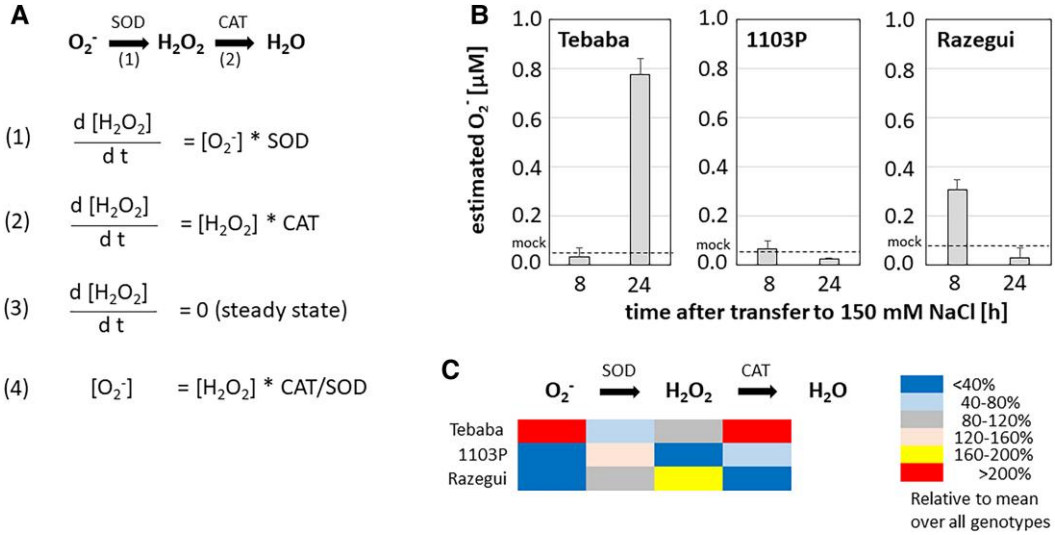

Figure 5. Estimation of steady-state levels for superoxide in response to salinity stress in leaves of Tebaba, 1103P, and Razegui. **A)** Model used for the estimation assuming steady state and the simplified reaction scheme shown at top. **B)** Steady-state levels of superoxide inferred using this model and the data shown in Fig. 4. Data represent mean and SE from 3 biological replicates per data point. **C)** Comparison of steady-state levels for superoxide and hydrogen peroxide and the activities of the antioxidant enzymes SOD and CAT relative to the mean value over the 3 genotypes to highlight differences between the genotypes.

differences were noted already prior to salt stress, others became manifest only in response to salinity. In the following, some of the salient phenomena will be described:

Ground levels for central metabolites of the Krebs cycle, such as succinate and fumarate, were elevated in "Tebaba." In contrast, GABA was almost twice as abundant in 1103P and exceeded under salinity those in "Tebaba" by >3-fold. A similar pattern, albeit at lower amplitude, was seen for citrate. Pyruvate levels were doubled in 1103P but dropped substantially under salinity. Overall, it seems that "Tebaba" runs the central carbon metabolism more actively than 1103P does and seems to cope with the elevated superoxide levels inevitably resulting from this more vigorous metabolism.

In this context, the Foyer–Halliwell–Asada pathway is relevant. Interestingly, we could not detect any ascorbate in none of the genotypes. Instead, dehydroascorbate, the product of hydrogen peroxide and ascorbate by the activity of ascorbate peroxidase was present, at elevated levels in "Tebaba," and only slightly decreasing under salinity. In "Tebaba," the downstream product threonate was increasing correspondingly, at the base of >2-fold increased ground levels. In grapevine, ascorbate can also be recruited to tartrate via gluconate (Cholet et al. 2016). Interestingly, ground levels for gluconate (which might also be glucuronate) are almost doubled in "Tebaba," and under salinity, gluconate levels in "Tebaba" rose further to reach 5 times more than those in 1103P. These higher gluconate levels do not translate into tartrate levels, though. Here, "Tebaba" has substantially lower levels. Interestingly, a considerable part of tartrate is converted to the volatile methylated tartrate in 1103P, while this compound is not consistently detectable.

We also searched for readouts for potential photorespiration and found, in 1103P, a strong increase of glycine and serine in response to salt (Fig. 8), while glycerate was dropping (Fig. 9), indicating that the Calvin–Benson cycle is in strong need for phosphoglycerate. In "Tebaba," glycine increased only mildly under salinity, and serine levels even dropped (Fig. 8). Likewise, resting levels for glycerate were 3 times higher and decreased only marginally under salinity. Thus, 1103P shows a clear signature for enhanced photorespiration, which is not seen in "Tebaba." The higher ground levels of glycine and serine in "Tebaba" are unlikely deriving from photorespiration but seem to arise from glycolysis (Kishor et al. 2020). This conclusion is also supported by the 2-fold increased ground levels of gluconate, glucose, and glucose-6-P for "Tebaba." Overall, "Tebaba" can command of higher carbohydrate resources and defend them more efficiently against salinity-induced photorespiration.

A mirror image was seen for the levels of xylitol (reporting pectin breakdown) and a different disaccharide, probably representing cellobiose (reporting cellulose breakdown), that were generally lower in "Tebaba" as compared to 1103P (Fig. 9). These differences are associated with a higher growth rate in "Tebaba," such that cell-wall polymers are built up rather than broken down (Fig. 2). Among the other metabolic differences, the higher ground levels of the inositols in "Tebaba," especially *myo*-inositol-phosphate was almost doubled over 1103P (Fig. 9). Salinity reduced *myo*-inositol-phosphate levels drastically, which was not observed in "Tebaba," such that this metabolite became <20% in 1103P as compared to "Tebaba." The higher ground levels of the inositols in "Tebaba" were accompanied by a substantially higher level of galactinol. These already high galactinol levels increased further under salinity, whereas they dropped in 1103P. Interestingly, in the absence of salt stress, the

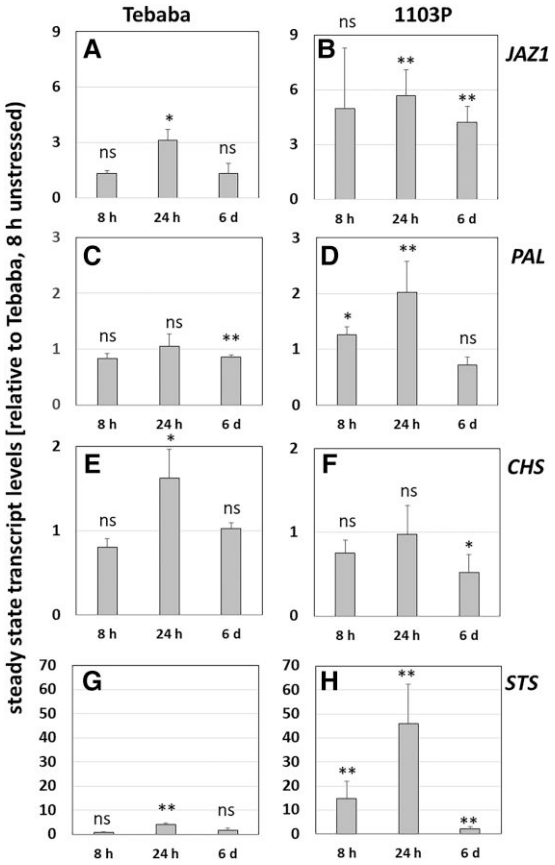

Figure 6. Expression of the jasmonate response factor *JAZ1* **A**, **B)**, and key genes of the phenylpropanoid pathway: **C**, **D)** PAL as first committed step of the pathway, **E**, **F)** CHS as entry point for flavonoids, and **G**, **H)** STS as entry point for stilbenes in leaves of Tebaba **A**, **C**, **E**, **G)** and 1103P **B**, **D**, **F**, **H)** at different time points after reaching the plateau. Data represent mean and SE from 3 biological replicates per data point, each in 3 technical triplicates and are given relative to the steady-state level of the respective transcript measured in the Tebaba mock control at 8 h. **, statistically different with $P < 0.01$; *, statistically different with $P < 0.05$; ns, not significantly different using a pairwise *t*-test against the Tebaba mock control.

galactinol derivative raffinose was lower in "Tebaba" but increased in response to salinity in both genotypes. A substantial reduction in the ground levels of allantoin indicates that "Tebaba" did not need to rely on this route of nitrogen mobilization.

Overall, we see in "Tebaba" indicators for a more robust central carbon metabolism and ascorbate cycle. Instead, 1103P shows indications for a more pronounced catabolism, such as breakdown of cell-wall components or purines, and needs to invest more resources in detoxification of photorespiration products.

"Tebaba" can sustain a higher level of phytol

We were also able to monitor the response of 10 terpenoid metabolites (Fig. 10). Salient features were much lower levels of α-tocopherol and γ-tocopherol. Under salinity, the levels dropped in both genotypes to a similar extent. In contrast, the precursor phytol was >3 times elevated in "Tebaba" as compared to 1103P, and "Tebaba" was able to sustain phytol levels, while in 1103P, phytol dropped in response to salinity. The triterpenoid alcohols α- and β-amyrine and their precursor squalene were also much lower in "Tebaba." For the β-amyrine derivative erythrodiol and oleanic acid, the levels in "Tebaba" were so low that they could not be detected, contrasting with 1103P. Likewise, the sterol campesterol was so low in "Tebaba" that it could not be detected consistently. Interestingly, the squalene derivative neophytadiene showed the inversed pattern—here, the ground level was elevated in "Tebaba" and increased further under salinity, while it decreased in 1103P. Thus, phytol and neophytadiene were qualitatively different from the other measured terpenoids being elevated in "Tebaba," which corresponded to a better performance under salt stress.

Discussion

Grapevine as perennial and deep-rooting species is expected to cope more efficiently with the challenges of global warming compared to many annual crops. However, salinity is a pertinent and progressively accentuated challenge in many viticultural regions of the world. The motivation behind this study was to understand the pronounced salt resilience of the wild Tunisian grapevine genotype "Tebaba" to learn something about the underlying mechanisms. We combined physiological and metabolomic approaches in a comparative strategy to delineate salt-dependent stress responses from adaptive events. We observed that "Tebaba" sustained growth although sodium was readily translocated to the shoot. This was, not surprisingly, linked to a more robust redox homeostasis. The metabolomic analysis helped us to identify characteristic fingerprints that were linked with salt resilience. These included altered channeling of the phenylpropanoid pathway, a higher ground level of proline, a different metabolic fate of glycine, and a more robust central carbon metabolism, supported by a more efficient ascorbate cycle, such that "Tebaba" does not rely on catabolic breakdown of cell-wall material and, thus, can sustain a higher growth rate, even under salinity.

In the following, we will discuss to what extent the response of "Tebaba" reflects that what is known from other cases of salinity tolerance, what our comparative strategy tells us about metabolomic predictors for salinity damage versus adaptation, and where the ultimate drivers are to be sought. Last, but not least, we will discuss, how this knowledge can contribute to secure viticulture under salinity.

Salt exclusion versus salt adaptation—"Tebaba" is not an excluder

To prevent sodium ions to enter the root, or to sequester sodium in the root, such that it cannot reach the shoot and, thus, harm, photosynthesis, is a common strategy to cope with salinity (for a recent review, see Keisham et al. 2018). In fact, several transporter systems can contribute to reduce

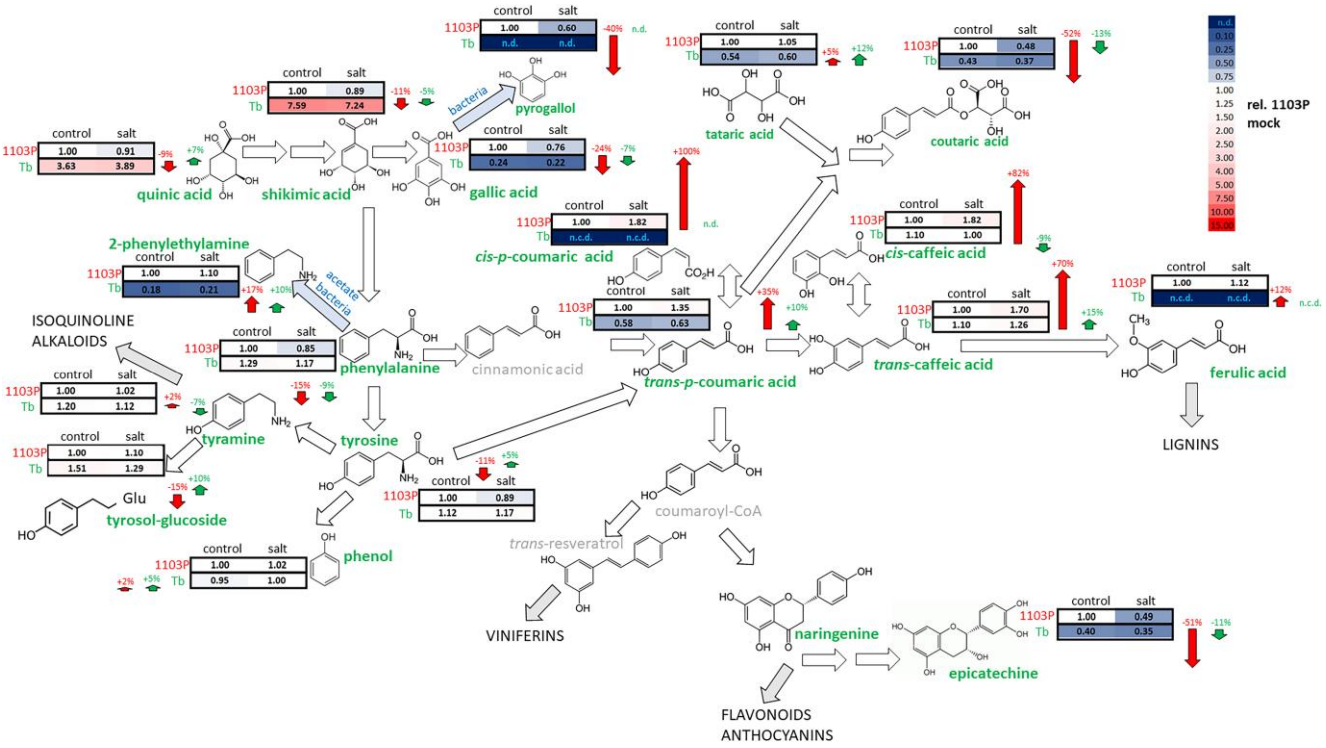

Figure 7. Response of the phenylpropanoid pathway to salt stress in leaves of 1103P and Tebaba (Tb) and scored at 24 h after reaching the plateau of 150 mM NaCl as measured by GC-GC × MS. Metabolite abundance is given relative to the 1103P mock control. The change induced by the salt treatment relative to the respective mock control is indicated by the arrow (red, 1103P; green, Tb). Data represent 3 biological replicates. n.d., not detected; n.c.d., not consistently detected.

sodium accumulation in the shoot, such as the SOS exporter system, the NHX1 transporters sequestering these ions in the vacuole (which will prevent damage to cytosolic targets but preserve turgescence), or HKT1 transporters retrieving sodium from the central cylinder. Sequestration of sodium can contribute to salt tolerance as shown by overexpressing a NHX1 transporter from *Arabidopsis* (*Arabidopsis thaliana*) in grapevine (Venier et al. 2018). Likewise, the comparison of 2 Sorghum (*Sorghum bicolor* L.) genotypes differing in salinity tolerance showed that tolerance was linked to efficient sequestration of sodium ions in vacuoles of the distal elongation zone as demonstrated by a fluorescent sodium fluorophore, CoroNa Green (Abuslima et al. 2022). However, our data (Fig. 3) do not support any role of sodium sequestration in the root or sodium retrieval from the xylem in salinity tolerance of "Tebaba." In fact, sodium transfer to the shoot is more pronounced in "Tebaba" as compared to 1103P. Actually, 1103P is known to be efficient in ion exclusion as compared to other rootstocks (Zhang et al. 2002), which is also consistent with our observation that sodium accumulation in 1103P is significantly lower than in "Tebaba" (Fig. 3A). Interestingly, potassium levels under salt stress are significantly higher in "Tebaba" over 1103P, which would predict that berry expansion during post-véraison can be buffered against salinity, because this process is strongly dependent on potassium influx into the vacuole (Hanana et al. 2007). Induction of the vacuolar sodium–proton antiporter *NHX1* by salt stress has been shown for both grapevine leaves (Saleh and Alshehada 2018) and grapevine cells (Ismail et al. 2012) and would provide a mechanism to explain our findings. However, this sequestration seems to take place in the leaves and not in the roots. While sodium sequestration in root vacuoles has been described to account for salt tolerance in barley (Wu et al. 2019), the tolerance of "Tebaba" is not based on avoiding sodium entry to the leaves. This conclusion is supported by a recent transcriptomics study on roots of the very same 2 genotypes, conducted under the very same conditions (Daldoul et al. 2022). Here, mainly genes involved in enzymatic and nonenzymatic control of ROS, cell-wall modeling, and sugar metabolism were found to be upregulated in "Tebaba" as compared to 1103P. Also, ion transporters involved in potassium/sodium homeostasis at the plasma membrane were found among the responsive genes. Missing on the list were the NHX genes, sodium–proton antiporters that can sequester sodium in the vacuole of the root cortex, thus mitigating ionic stress in the cytoplasm, while sustaining turgescence as prerequisite of growth (Ismail et al. 2012). Thus, unlike salt tolerance in Sorghum (Abuslima et al. 2022), sodium sequestration seems not to be a relevant factor for the salt tolerance of "Tebaba."

Metabolic signatures of salt tolerance?

Therefore, we searched for metabolic changes that allow "Tebaba" to sustain photosynthesis and, thus, growth, even

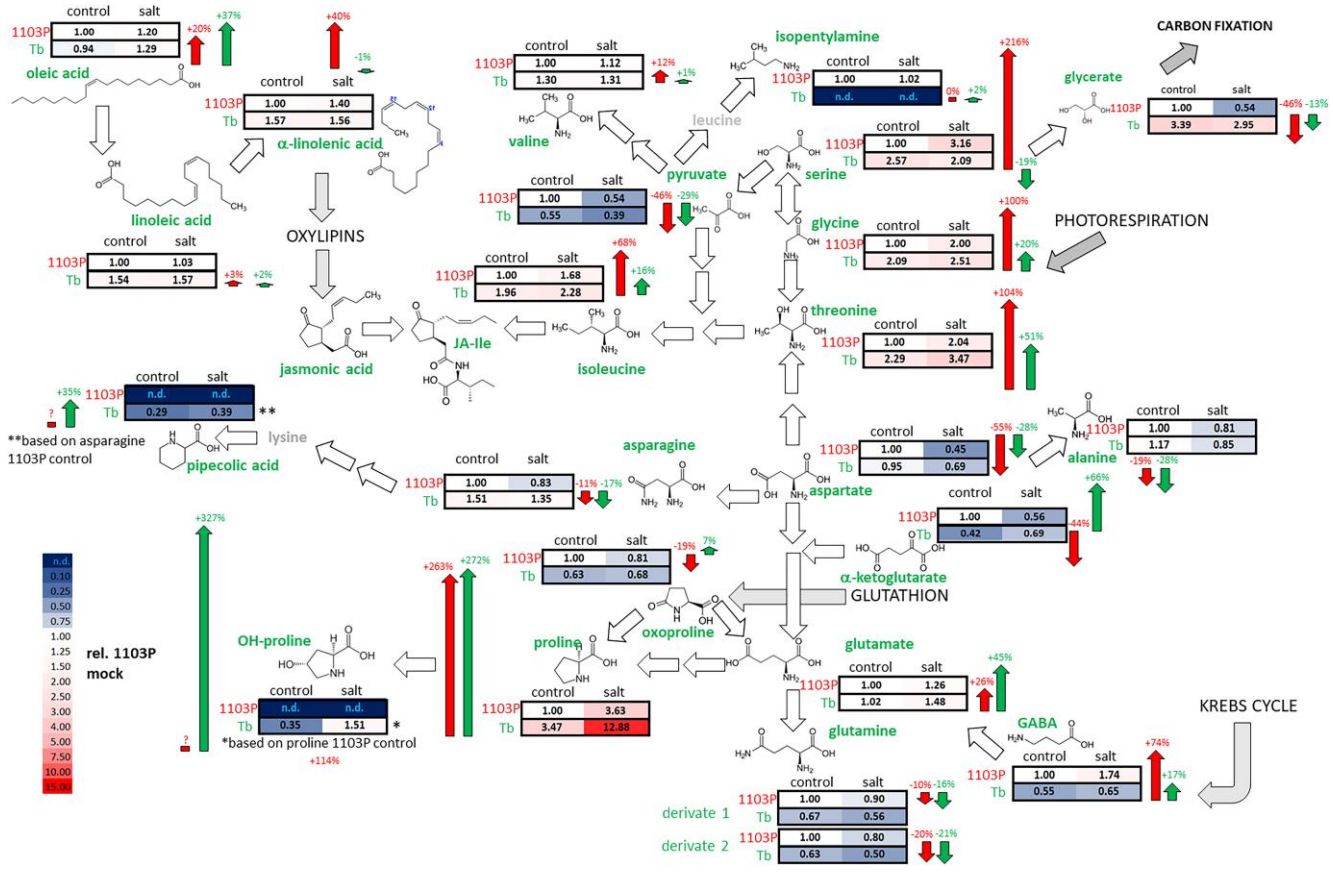

Figure 8. Response of amino-acid metabolism to salt stress in leaves of 1103P and Tebaba (Tb) and scored at 24 h after reaching the plateau of 150 mM NaCl as measured by GC-GC × MS. Metabolite abundance is given relative to the 1103P mock control. The change induced by the salt treatment relative to the respective mock control is indicated by the arrow (red, 1103P; green, Tb). Data represent 3 biological replicates. n.d., not detected; n.c.d., not consistently detected.

in the presence of sodium. To do so under salinity will inevitably lead to a considerable oxidative burden, because sodium will lead to hyperreduction of electron transport over the thylakoid. The accumulating electrons will then end up on the molecular oxygen that has been generated by water splitting in photosystem II (Allakhverdiev et al. 2000). The 2 genotypes address this problem by different mechanisms: While 1103P is strongly inducing SOD activity (Fig. 4C), "Tebaba" seems to cope with a strong increase of steady-state superoxide levels (Fig. 5B), indicative of photosynthetic electron transport remaining uncompromised. Still, MDA levels in "Tebaba" remain buffered, reporting a lower level of lipid peroxidation as readout for salt-induced damage (Fig. 4A). If it is not a slowdown of electron transport nor the induction of SOD activity that renders "Tebaba" more tolerant, there must be metabolic changes that can compensate the oxidative challenge. In fact, our nontargeted metabolomics approach detected several differences that seem to be relevant in this context (Fig. 11).

Phenolic compounds

The more active shikimate pathway in "Tebaba" seems to be used to support higher ground levels of tyramine and especially its derivative tyrosol glucoside (Fig. 11). Tyramine is generated from tyrosine by a tyrosine decarboxylase (TyDc) and is often hydroxylated further to give rise to dopamine, a well-known mitigator of salt stress (Li et al. 2015). However, in our study, we did not detect dopamine but rather the concurrent tyrosine derivative tyrosol. Both compounds exert a strong antioxidant activity in vitro (Yen and Hsieh 1997), and tyrosol has also been implicated to be involved in stress resilience, during a metabolic study, where the stress metabolome of the cold tolerant Chinese species *Vitis amurensis* was compared to the more susceptible *V. vinifera* (Chai et al. 2019). The activation of the pathway depends on the key enzyme TyDc. Overexpression of this enzyme in apples (*Malus domestica* L.) was able to induce tolerance to alkalinity, an especially harsh variation of salinity (Liu et al. 2022). What compound was responsible in this context is not known, but overexpression of TyDc in potato (*Solanum tuberosum* L.) was reported to result in accumulation of tyrosol glucoside (Landtag et al. 2002). Thus, a straightforward working hypothesis would assume that activity or expression level of TyDc is constitutively elevated in "Tebaba," enabling a superior redox balance due to elevated levels of tyrosol glucoside. Instead, 1103P recruits the shikimate pathway for monolignols as evident from the higher levels of ferulic acid. This may be

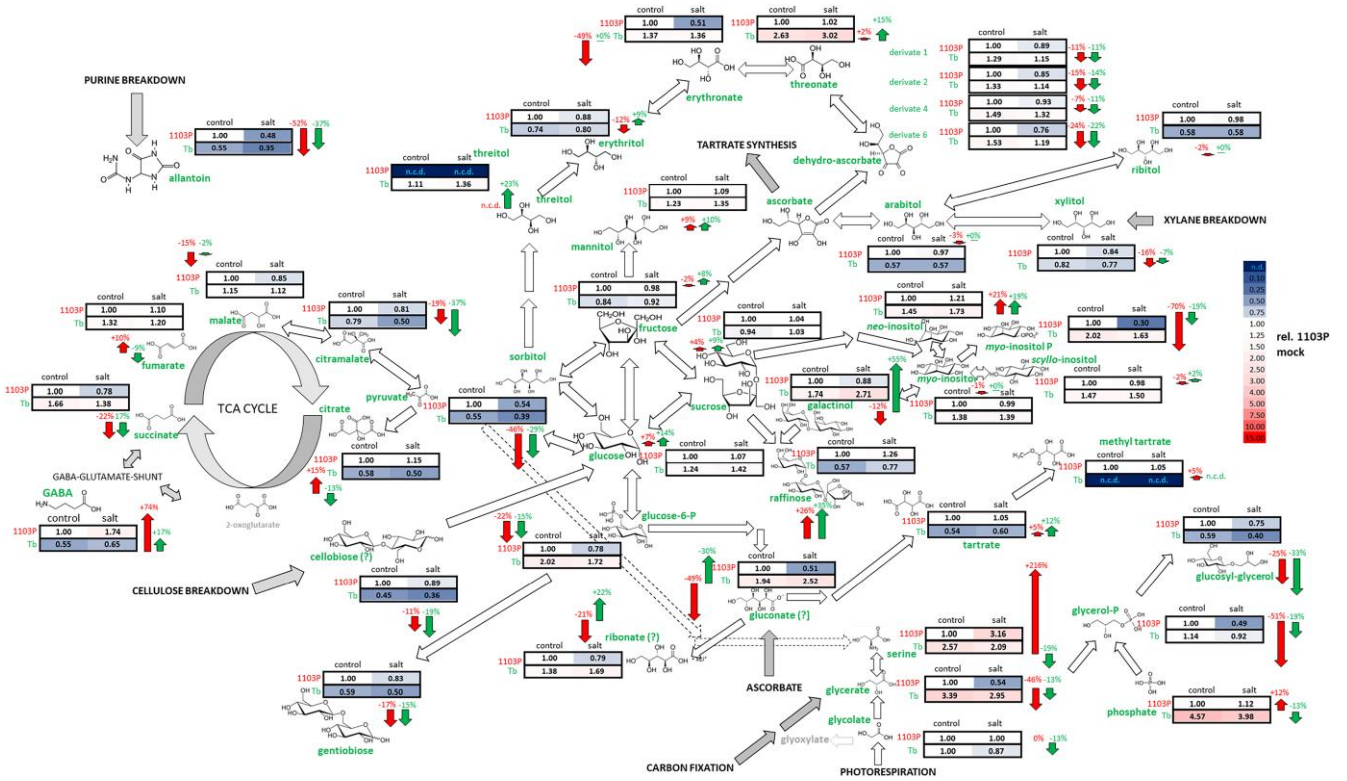

Figure 9. Response of sugar metabolism to salt stress in leaves of 1103P and Tebaba (Tb) and scored at 24 h after reaching the plateau of 150 mM NaCl as measured by GC-GC × MS. Metabolite abundance is given relative to the 1103P mock control. The change induced by the salt treatment relative to the respective mock control is indicated by the arrow (red, 1103P; green, Tb). Data represent 3 biological replicates. n.d., not detected; n.c.d., not consistently detected.

linked with an induction of *caffeoyl-O-methyl transferase* (COMT) by jasmonate signaling (Lee et al. 1997), which would explain, why higher *JAZ1* transcript levels correspond to higher ferulate levels in 1103P and why both parameters are lower in "Tebaba." This leads the question how the caffeic-acid precursor *p*-coumaric acid is utilized in "Tebaba." Partitioning toward coutaric acid, the adduct with tartaric acid is not the reason (Fig. 7). Thus, *p*-coumaric acid is recruited for a different pathway. The key factor might be CHS, whose transcripts are significantly induced in "Tebaba" but not in 1103P (Fig. 6, E and F). The resulting metabolites might be more efficiently detected by analytics alternative to 2D-GC several compounds of this pathway known to exert antioxidant activity and to support salt tolerance (rice [*Oryza sativa* L.], Chutipaijit et al. 2011; sugarcane [*Saccharum officinarum* L.], Wahid and Ghazanfar 2006; thale cress [*A. thaliana* L.], Lotkowska et al. 2015).

The glutamate–proline connection

"Tebaba" accumulates more proline (Fig. 11), already prior to stress (Fig. 8). Proline allows to lower the intracellular water potential, such that the cell remains turgescent, but in addition to this function as compatible osmolyte, it buffers the photosynthetic electron transport against ROS (Szabados and Savouré 2010). During comparative studies, higher accumulation of proline in the leaves is associated with higher salt tolerance on both, rice (Nguyen et al. 2021) and Sorghum (Abuslima et al. 2022) and, thus, qualifies as adaptive marker. For grapevine as well, proline content was increasing with the stringency of salt stress, and the amplitude of this increase corresponded to the resilience to stress during a comparative study on Iranian genotypes differing in their salt tolerance (Mohammadkhani et al. 2013). Since "Tebaba" is not an ion excluder, the readiness by which it accumulates proline might be a central factor in its salinity tolerance. A comparative transcriptomic strategy with a *sylvestris* genotype from Southern Spain (Carrasco et al. 2022) has uncovered osmotin as 1 of the upregulated factors, a protein reported to improve water use efficiency by causing an upregulation of proline (reviewed in Kumar et al. 2015). To what extent osmotin is responsible for the salinity tolerance of "Tebaba" remains to be elucidated. The induction of proline under salinity is linked to see a stronger increase of glutamate in "Tebaba," linked with lower levels of glutamine and of GABA reporting that the GABA shunt that is activated under salinity (reviewed in Che-Othman et al. 2017) does not play a role here, contrasting with the situation in the sensitive 1103P.

Sucrose fuels antioxidants safeguarding pectins

Mitochondria are central targets for salinity stress. Especially the succinate dehydrogenase complex II, generating

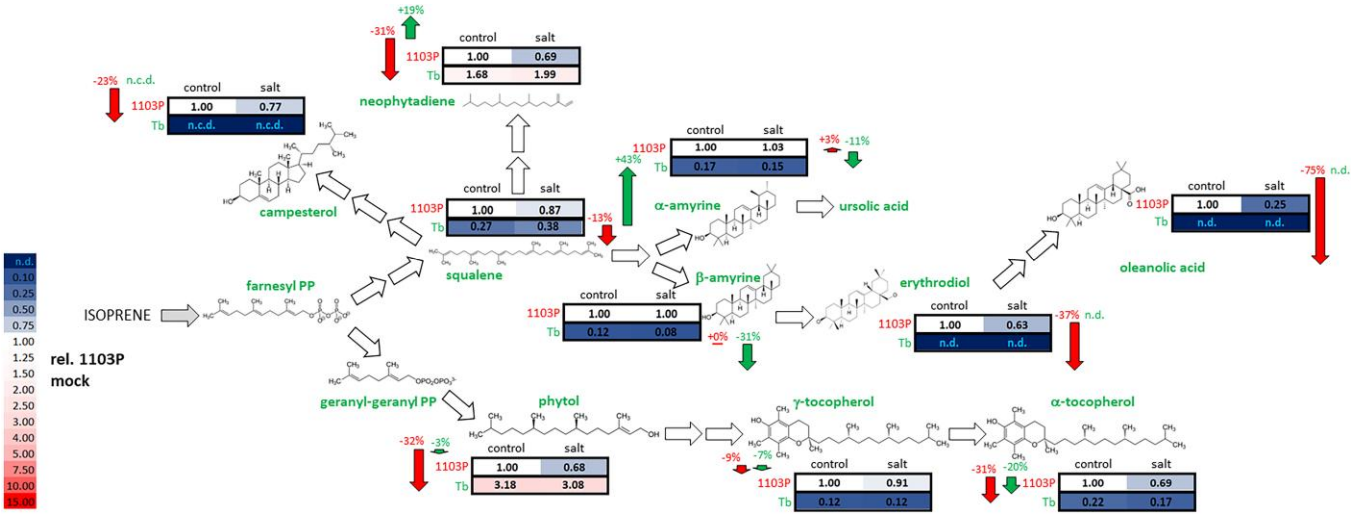

Figure 10. Response of terpenoid metabolism to salt stress in leaves of 1103P and Tebaba (Tb) and scored at 24 h after reaching the plateau of 150 mM NaCl as measured by GC-GC × MS. Metabolite abundance is given relative to the 1103P mock control. The change induced by the salt treatment relative to the respective mock control is indicated by the arrow (red, 1103P; green, Tb). Data represent 3 biological replicates. n.d., not detected; n.c.d., not consistently detected.

fumarate, represents a sensitive target, because it will lead to accumulation of superoxide that needs to be efficiently scavenged as to avoid cellular damage. In fact, higher superoxide steady-state levels had been inferred from modeling redox homeostasis (Fig. 5). If redox homeostasis is challenged by salt stress, accumulating superoxide can activate retrograde signaling, such that the alternative oxidase pathway needs to be activated, accompanied by a more active GABA shunt (for review, see Bandehagh and Taylor 2020). "Tebaba" also seems to command a more active ascorbate cycle, supporting detoxification of hydrogen peroxide (higher dehydroascorbate levels). This is accompanied by a reduced recruitment of ascorbate for tartrate synthesis but instead channeling into gluconate and ribonate. Rather than being converted to tartrate, gluconate can also be metabolized to ribonate. This seems to be the preferential route in "Tebaba." Higher levels of gluconate and ribonate have been identified as hallmark of tolerance in *Arabidopsis* (Hill et al. 2013). The more efficient, nonenzymatic scavenging of peroxidase in "Tebaba" is accompanied by a higher activity of CAT (Fig. 5C). Also, the higher ground levels of *myo*-inositol might be linked with a more robust redox homeostasis because this compound can generate, together with UDP glucose, galactinol, a scavenger for hydroxyl radicals (Nishizawa et al. 2008). This pathway, which is specific for plants, can also lead to raffinose, a route that is used less intensely in "Tebaba," which might be linked with the fact that galactinol as a more efficient compatible osmolyte (Sengupta et al. 2015).

Phytol is sustained safeguarding chlorophyll

"Tebaba" can rely on a much higher pool of phytol for chlorophyll synthesis, while 1103P needs to convert phytol to generate the antioxidant tocopherols. A higher steady-state level of phytol had been reported earlier as feature of salt tolerance in wild over domesticated soybean (Zhang et al. 2016). A second important redistribution of metabolism was manifest by a much lower level of α- and β-amyrine and their precursor squalene in "Tebaba" and a corresponding increase of the concurrent pathway toward the antioxidant neophytadiene (Bhardwaj et al. 2020). Triterpenoids, such as the amyrines, have been reported to increase in response to salt stress, for instance in mangroves and are thought to sustain membrane fluidity under salinity. In fact, among the tested triterpenoids, β-amyrine was the most effective in rescuing salt sensitive of the sterol biosynthesis yeast mutant *GIL77* (Inafuku et al. 2018). It seems that 1103P is relying on this last resort to rescue survival under salinity, while "Tebaba" instead can invest the squalene to form neophytadiene which helps to sustain redox balance. Overall, the 2 genotypes channel terpenoids differently (Fig. 11); "Tebaba" seems to safeguard phytol for chlorophyll synthesis and uses neophytadiene for redox balance. Instead, 1103P needs to invest squalene to produce triterpenoids as to rescue membrane fluidity, such that it forms less neophytadiene and, as a consequence, needs to convert a part of phytol into the antioxidant tocopherols. In summary, 1103P needs to pay a high price in terms of lower phytol levels to secure membrane integrity and redox balance, while "Tebaba" can spare squalene for neophytadiene synthesis, such that it does not rely on tocopherols for redox balance.

Why we need a comparative approach to make sense of metabolic differences?

To cope with salinity stress, plants have evolved several mechanisms that are often acting in concert. Despite numerous efforts to pinpoint "the gene of salt tolerance," such a gene

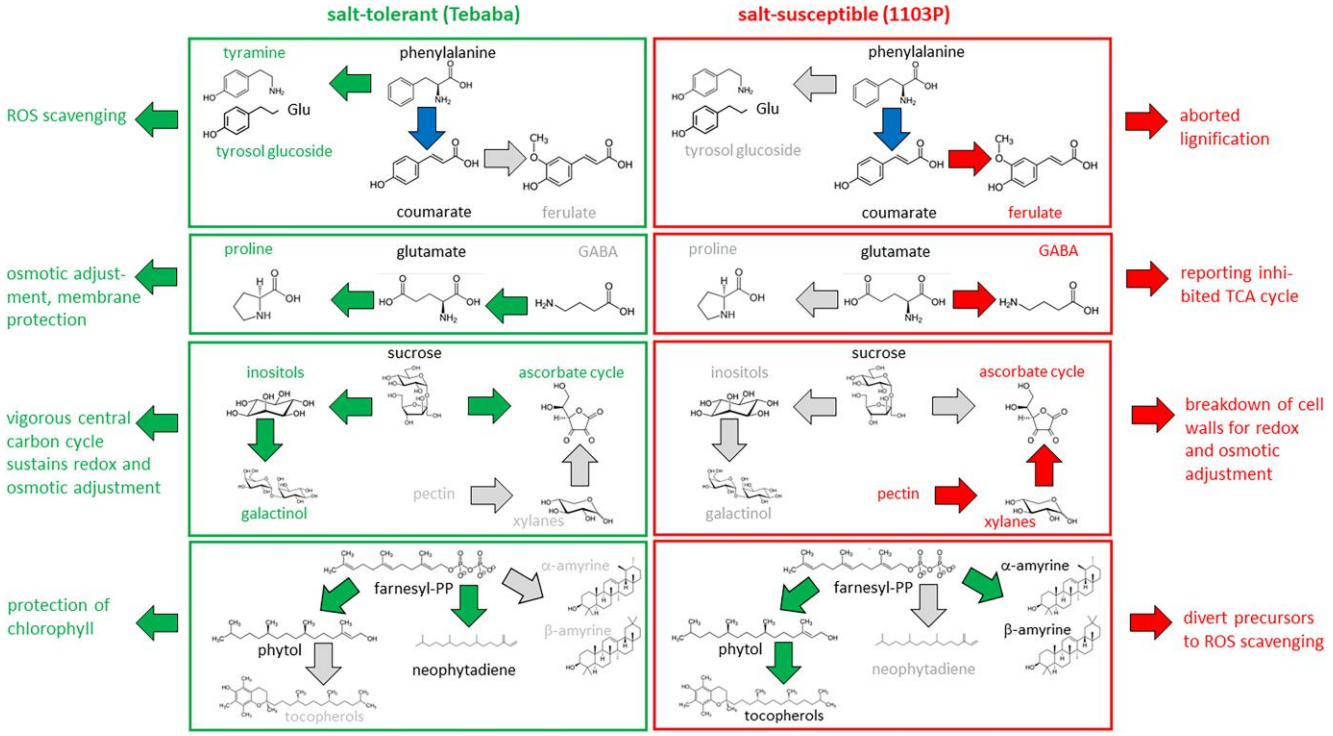

Figure 11. Model for the central metabolic signatures for salt tolerance versus salt susceptibility in grapevine emerging from the comparison of metabolic responses in Tebaba versus 1103P.

has not been found. It is progressively clear that salt tolerance emerges from the activity of numerous factors that need to be orchestrated in time and space. When the molecular response to salinity is addressed by transcriptomics and metabolomics, the number of significant changes ranges usually in the hundreds, if not in the thousands. Whether these changes are manifestation of the cellular damage imposed by salinity or whether they are involved in the adaptation to this challenge is not at all obvious and usually hard to deduce based on an omic snapshot alone, as detailed it may be. One needs information originating from realms beyond this snapshot to arrive at conclusions. For instance, the temporal sequence of events can help to infer the causal chain—cellular responses that occur early after the onset of the stress episode are often reflecting signaling events or immediate perturbations of metabolic homeostasis, while later events are more likely to be linked with adaptation, or, in case of susceptibility, with irreversible damage. To order the cellular responses in time, a design, where salinity is increased in a sudden step to get a clear point zero, would be the most straightforward option. However, in the current experimental setup, we tried to mimic the situation in the vineyard, where soil salinity increases over time. While being closer to the real-world situation, this design is not suited to infer temporal patterns but rather reflects steady-state situations. However, we used a design, where susceptible (1103P) and resistant ("Tebaba") genotypes were compared side by side. The use of such a contrasting pair allows to sort the observed events with respect to salt damage and salt adaptation. Those events that are more pronounced in "Tebaba" reflect adaptive processes; those events that are more pronounced in 1103P must be consequences of a more pronounced susceptibility. In fact, this approach allowed us to define several metabolic signatures of salt tolerance leading to the question, whether they are established independently or whether there is a master player for salt tolerance.

Redox homeostasis—the ultimate driver of salt resilience in grapevine

Salinity-induced damage and adaptation are multifaceted phenomena, such that it is far from trivial to infer what is cause and what is effect. Unlike the situation in Sorghum, where sodium sequestration into the vacuole of the root elongation zone seems to be a major factor for salt tolerance (Abuslima et al. 2022), neither the current study nor the data from the root transcriptome (Daldoul et al. 2022) provide any evidence that sodium exclusion from the shoot might be a major factor for the tolerance seen in "Tebaba." There might not be something like a single ultimate driver for salt tolerance but rather patterns of metabolic activity that are more efficient in buffering physiological homeostasis. Nevertheless, it can be useful to define criteria for such an ultimate driver: (i) It should be either early or upstream. (ii) It should be more active in the tolerant over the susceptible genotype. (iii) It should address metabolic decisions that are self-amplifying, such that even a minor initial difference will lead to a robust improvement of output.

Using these criteria, 4 metabolic decisions can be discerned that qualify as ultimate drivers (Fig. 11).

Phenylalanine channeling toward tyrosol as powerful antioxidant (Yen and Hsieh 1997) contributes to the robust redox balance in "Tebaba," while 1103P partitions phenylalanine toward ferulic acid, a typical hallmark for cell-wall modeling under salinity (Oliveira et al. 2020).

Glutamate channeling toward proline helps not only buffering osmotic potential but also protecting the conformation of challenged membranes and proteins in "Tebaba" (Szabados and Savouré 2010). In contrast, 1103P needs the glutamate to feed the GABA shunt to compensate perturbations of the challenged Krebs cycle (Che-Othman et al. 2017).

Buffered photosynthesis helps to fuel the ascorbate cycle and to sustain galactinol as effective antioxidants in "Tebaba" (Nishizawa et al. 2008), while 1103P needs to rely on pectin breakdown to forage ascorbate. Pectin breakdown has been identified as crucial promoter for salt susceptibility in rice (Liu et al. 2014), and, thus, the high xylene levels in 1103P must be seen as indicator for salt damage.

Recruiting the terpenoid toward phytol helps "Tebaba" replacing damaged chlorophyll and, thus, sustaining photosynthesis under salinity, while 1103P needs the farnesyl PP to generate β-amyrine to protect its membranes (Bhardwaj et al. 2020).

Of course, these drivers are interwoven by self-amplifying feedback loops. The higher accumulation of tyrosol will buffer ROS stabilizing membranes, such that terpenoids can be used for phytol synthesis, which will buffer photosynthesis, such that sucrose can fuel the ascorbate cycle, which in turn will improve redox homeostasis even further. In contrast, channeling of the phenylpropanoids toward lignin will come with a weaker redox balance, such that the central carbon cycle needs to activate the GABA shunt, such that less proline can be formed, which puts membranes under strain, requiring farnesyl PP to be used for amyrine synthesis, rather than to complement phytol, leading to lower photosynthetic activity, such that the ascorbate cycle cannot be fed by sucrose, but needs to rely on pectin breakdown.

In other words, which of these drivers is more crucial than others is hard to tell, because by their mutual interactions, even small initial improvements in 1 of these drivers will lead to more robust outputs of the other drivers that will return as further support for the "initial" driver. Salt tolerance would then emerge as a physiological process that is sustaining itself by mutual support, while bad performance of 1 or several of these drivers deploys a vicious circle. Tolerance of damage emerges as a kind of physiological phase transition, which cannot be attributed to a single factor, but needs a synchronized interplay of different activities. To get insight into this synchronized interplay and the underlying feedback regulation, the metabolite pattern should be accompanied by transcriptomic studies to identify adaptive and damage markers. In this context, it is interesting that transcript for the STS (VIT_16s0100g00990), which was strongly elevated in 1103P under salinity, is generally induced during leaf senescence and berry withering (Grape eFP Browser, https://bar.utoronto.ca/efp_grape/cgi-bin/efpWeb.cgi) indicating that this gene is activated under conditions of impaired homeostasis and, thus, can be used as a stress marker.

What can we do with this knowledge?

The current study has defined some of the mechanisms responsible for the salt tolerance in "Tebaba." On this base, 2 possible strategies can be envisaged. "Tebaba" might be either used as rootstock or as genetic resource for introgression into commercial varieties. For different reasons, the rootstock strategy does not seem to be promising. First, as European species, "Tebaba" is most likely lacking the resistance to *Phylloxera*, which is the main reason, why grapevine is grafted. This might be circumvented by introgression of "Tebaba" into commercial rootstock varieties. However, since the salt tolerance of "Tebaba" is not based on sequestration of sodium in the root, it is doubtful that it would lead to salt tolerance of the scion. Our data show that sodium is readily transferred into the shoot, which would also be the case for a scion grafted on a "Tebaba" rootstock. To make use of salt tolerance resulting from more robust redox homeostasis, the scion itself needs to be changed. Such introgression strategies have been successful for resistance against downy and powdery mildew due to resistance factors from North American and Siberian wild grapevine species. This introgression can be supported and accelerated by marker-assisted breeding (for review, see Eibach et al. 2007). By several rounds of backcrossing, it is, thus, possible to retain the oenological characteristics of a variety, while improving its resilience. In case of "Tebaba" that belongs to the same species as the commercial varieties, this should be much easier, because it does not show the foxiness characteristic of North American wild grapevines. Since the pressure from salinity will rise rapidly during the next decades, this will also contribute to consumer acceptance of novel flavors.

While the current study was focusing on the vegetative phase, the question has to be posed, to what extent the results can be transferred to berry development. The literature report does not lead to a straightforward answer, since both negative (for instance, Walker et al. 2008; Stevens et al. 2011) and positive (for instance, Mosse et al. 2013; Liu et al. 2019) effects of salinity have been described. However, the real situation seems less discrepant as it appears at first sight, since comparative studies show that the reported positive effects, such as increased sweetness of berries, accumulation of aromatic compounds, or enhanced coloration through anthocyanins, were observed only for moderate salinity (20 to 60 mM NaCl), while for stringent salt stress (100 mM NaCl and above), the negative impacts, such as berry shriveling, or sugar reduction prevailed (Li et al. 2013). In the long run, even mild salinity will accumulate over time, which means that even the potential improvements of berry size and quality reported for moderate salinity will not be sustainable but soon overrun by the harsh deterioration observed for stringent salinity (see, for instance, Suarez et al. 2019).

Since Tebaba is not a salt excluder, such that introgression of salinity resilience into rootstock varieties does not seem to be a feasible strategy, future studies need to address the metabolic response in berries under mild but prolonged salinity. In this context, the channeling of phenylpropanoid metabolism toward tyrosol and the channeling of glutamate toward proline are of particular interest.

Materials and methods

Plant material and stress application

The study was focused on a comparison between the salt-tolerant *Vitis sylvestris* genotype "Tebaba" originating from the North of Tunisia and the commercial rootstock "1103P," along with the commercially relevant variety *V. vinifera* cv. 'Cabernet Sauvignon', and 2 traditional *vinifera* landraces from Tunisia, "Houamdia" and "Razegui." Details on the background of these genotypes are given in Supplemental Method S1. Wood cuttings were rooted for 2 mo and then transferred into 10-L pots of sand in a greenhouse and used for the salt-stress experiment 3 mo later. To mimic the situation in the vineyard, where soil salinity increases continuously progressively during a stress episode, we used a ramp, where salinity was increased in steps to reach a final concentration of 150 mM, which was then maintained, sampling fully expanded leaves in a standardized manner (Fig. 1A) and compared by a mock control (Fig. 1B). The physiological state of the plants was monitored by daily measuring shoot length. Details of cultivation and treatment are given in Supplemental Method S1.

Determination of ion content

Samples were dried for 2 d in a drying oven at 48 °C. Aliquots of 50-mg dried leaf material was homogenized to a powder (TissueLyser, Qiagen) and digested in concentrated HNO_3 (2 ml) supplemented by 0.5 ml of 30% *v*/*v* hydrogen peroxide in 50-ml digestion tubes (Gerhardt, UK) in a heating block (DigiPrep jr, S-prep) for 2 h at 110 °C. After cooling, the walls of the digestion tube were rinsed twice with 0.5 ml of hydrogen peroxide. The final volume of each sample was adjusted to 20 ml with 1% *v*/*v* HNO_3. The contents of sodium and potassium were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES, 715ES, Varian, radial mode). Data represent means and standard errors from 3 biological replicates. To calibrate the quantification, blanks were generated by mock digestion of the solvents in the same way but omitting the plant sample.

Monitoring redox homeostasis

Lipid peroxidation as a readout for oxidative damage was determined by measuring the reaction product MDA according to the standard protocols by Heath and Packer (1968) and Hodgson and Raison (1991). Activities of CAT, SOD, and the steady-state levels of hydrogen peroxide were measured according to Beauchamp and Fridovich (1971) and Ruch et al. (1989). Minor modifications to the published methodology to fresh grapevine leaves as material are described in Supplemental Method S2.

Quantifying stress-related transcripts

Steady-state transcript levels for salt-stress markers (Ismail et al. 2012) were measured by reverse-transcription quantitative PCR (qPCR) in leaf material shock frozen in liquid nitrogen and transferred on dry ice from the lab of the Tunisian partner, where the stress experiment had been conducted, to the lab of the German partner. Steady-state transcript levels were measured for *PHENYLALANINE AMMONIA LYASE* (*PAL*), as first committed step of phenylpropanoid synthesis; *STS*, as major member of the stilbene synthase family responsive to salinity stress; *CHS* as first committed step of flavonoid synthesis, and the jasmonate signaling gene *JAZ1*, as salt responsive indicator for jasmonate signaling using the primers listed in Supplemental Table S1 as described by Khattab et al. (2021) using *elongation factor 1α* and *actin* as internal standards (Reid et al. 2006). Data were compared to the first sampling point (8 h) of the mock treatment for "Tebaba" (8 h) and represent 3 biological replicates, each in technical triplicate.

Metabolite analysis

Metabolites were extracted from leaf material that had harvested into liquid nitrogen and kept at −80 °C till transport from Tunisia to Germany on dry ice. They were analyzed by GC × GC-qMS using a common nonpolar × medium-polar column setup. Details of sample preparation, quality control, data analysis, and visualization are given in Supplemental Method S3. The components of the GC × GC-MS system are given in Supplemental Table S2, the details of the GC × GC method along with the consumables used for analysis in Supplemental Table S3, and the settings for MS analysis in Supplemental Table S4.

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers: XM_002284888, (VvEF1-α), XM_002268220 (VvPAL), X76892 (VvSTS27), AB066274 (VvCHS1), and JF900329 (VvJAZ1).

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Author contributions

S.D., C.W., and A.J. have conducted the experiments, C.W. and M.G. have performed the metabolic analysis, A.M. has initiated and conceived the study, A.M., M.G., and P.N. have

organized funding for this study, and P.N. has generated the figures and written the paper.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Growth rates of grapevine accessions.

Supplemental Method S1. Details of plant material and stress application.

Supplemental Method S2. Methodological details of monitoring redox homeostasis.

Supplemental Method S3. Methodological details of metabolite analysis.

Supplemental Table S1. Oligonucleotide primers used for qPCR.

Supplemental Table S2. Components of the GC × GC-MS system.

Supplemental Table S3. GC × GC method and consumables.

Supplemental Table S4. MS settings.

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Conflict of interest statement. None declared.

Data availability

Data are stored on the server of the Steinbuch Centre for Computing of the Karlsruhe Institute of Technology and are made available upon reasonable request.

References

Abuslima ER, Kanbar A, Raorane ML, Eiche E, Hause B, Riemann M, Nick P. Gain time to adapt—mechanisms of salt adaptation in Sorghum. Front Plant Sci. 2022:**13**:1008172. https://doi.org/10.3389/fpls.2022.1008172

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* Sp. Plant Physiol. 2000:**123**(3):1047–1056. https://doi.org/10.1104/pp.123.3.1047

Andronis EA, Roubelakis-Angelakis KA. Short-term salinity stress in tobacco plants leads to the onset of animal-like PCD hallmarks in planta in contrast to long-term stress. Planta 2010:**231**(2):437–448. https://doi.org/10.1007/s00425-009-1060-x

Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. 2004:**55**(1):373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Askri H, Daldoul S, Ammar AB, Rejeb S, Jardak R, Rejeb MN, Mliki A, Ghorbel A. Short-term response of wild grapevines (*Vitis vinifera* L. ssp. *sylvestris*) to NaCl salinity exposure: changes of some physiological and molecular characteristics. Acta Physiol Plant. 2012:**34**(3):957–968. https://doi.org/10.1007/s11738-011-0892-8

Baena-González E. Energy signaling in the regulation of gene expression during stress. Mol Plant. 2010:**3**(2):300–313. https://doi.org/10.1093/mp/ssp113

Bandehagh A, Taylor NL. Can alternative metabolic pathways and shunts overcome salinity induced inhibition of central carbon metabolism in crops?. Front Plant Sci. 2020:**11**:452. http://dx.doi.org/10.3389/fpls.2020.01072

Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971:**44**(1):276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Bhardwaj M, Sali VK, Sugumar M, Vasanthi HR. Neophytadiene from *Turbinaria ornata* suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and Sprague–Dawley rats. Inflammation 2020:**43**(3):937–950. https://doi.org/10.1007/s10753-020-01179-z

Carrasco D, Zhou-Tsang A, Rodriguez-Izquierdo A, Ocete R, Revilla MA, Arroyo-García R. Coastal wild grapevine accession (*Vitis vinifera* L. ssp. *sylvestris*) shows distinct late and early transcriptome changes under salt stress in comparison to commercial rootstock Richter 110. Plants 2022:**11**(20):2688. https://doi.org/10.3390/plants11202688

Chai F, Liu W, Xiang Y, Meng X, Sun X, Cheng C, Liu G, Duan L, Xin H, Li S. Comparative metabolic profiling of *Vitis amurensis* and *Vitis vinifera* during cold acclimation. Hortic Res. 2019:**6**(1):8. https://doi.org/10.1038/s41438-018-0083-5

Che-Othman MH, Millar AH, Taylor NL. Connecting salt stress signalling pathways with salinity-induced changes in mitochondrial metabolic processes in C3 plants. Plant Cell Environ. 2017:**40**(12):2875–2905. https://doi.org/10.1111/pce.13034

Chen THH, Murata N. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr Opin Plant Biol. 2002:**5**(3):250–257. https://doi.org/10.1016/S1369-5266(02)00255-8

Choi W-G, Toyota M, Kim S-H, Hilleary R, Gilroy S. Salt stress-induced Ca^{2+} waves are associated with rapid, long-distance root-to-shoot signaling in plants. Proc Natl Acad Sci U S A. 2014:**111**-(17):6497–6502. https://doi.org/10.1073/pnas.1319955111

Cholet C, Claverol S, Claisse O, Rabot A, Osowsky A, Dumot V, Ferrari G, Gény L. Tartaric acid pathways in *Vitis vinifera L.* (cv. Ugni blanc): a comparative study of two vintages with contrasted climatic conditions. BMC Plant Biol. 2016:**16**(1):144. https://doi.org/10.1186/s12870-016-0833-1

Christmann A, Weiler EW, Steudle E, Grill E. A hydraulic signal in root-to-shoot signalling of water shortage. Plant J. 2007:**52**(1):167–174. https://doi.org/10.1111/j.1365-313X.2007.03234.x

Chutipaijit S, Cha-um S, Sompornpailin K. High contents of proline and anthocyanin increase protective response to salinity in *Oryza sativa* L. *spp. indica*. Aust J Crop Sci. 2011:**5**:1191–1198.

Costa JM, Lopes CM, Rodrigues ML, Santos TP, Francisco R, Zarrouk O, Regalado A, Chaves MM. Deficit irrigation in Mediterranean vineyards. Acta Horticult. 2012:**931**(931):159–170. https://doi.org/10.17660/ActaHortic.2012.931.17

Daldoul S, Hanzouli F, Hamdi Z, Chenenaoui S, Wetzel T, Nick P, Mliki A, Gargouri M. The root transcriptome dynamics reveals new valuable insights in the salt-resilience mechanism of Mediterranean wild grapevine (*Vitis vinifera* subsp. *sylvestris*). Front Plant Sci. 2022:**13**:1077710. doi 10.3389/fpls.2022.1077710

Das P, Majumder AL. Transcriptome analysis of grapevine under salinity and identification of key genes responsible for salt tolerance. Funct Integr Genomics. 2019:**19**(1):61–73. https://doi.org/10.1007/s10142-018-0628-6

Downton WJS, Loveys BR. Abscisic acid content and osmotic relations of salt-stressed grape vine leaves. Aust J Pysiol. 1981:**8**:443–452. https://doi.org/10.1071/PP9810443

Eibach R, Zyprian E, Welter L, Töpfer R. The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis 2007:**46**:120–124. 10.17660/ActaHortic.2009.827.96

Flügge UI. Transport in and out of plastids: does the outer envelope membrane control the flow? Trends Plant Sci. 2000:**5**(4):135–137. https://doi.org/10.1016/S1360-1385(00)01578-8

Foyer HC, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 2005:**17**(7):1866–1875. https://doi.org/10.1105/tpc.105.033589

Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2015:**48**(12):909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gorgues L, Li X, Maurel C, Martinière A, Nacry P. Root osmotic sensing from local perception to systemic responses. Stress Biol. 2022:**2**(1):36. https://doi.org/10.1007/s44154-022-00054-1

Guan L, Haider MS, Khan N, Maazullah N, Jiu ST, Fiaz M, Zhu XD, Zhang KK, Fang JG. Transcriptome sequence analysis elaborates a complex defensive mechanism of grapevine (*Vitis vinifera* L.) in response to salt stress. Int J Mol Sci. 2018:**19**(12):4019. https://doi.org/10.3390/ijms19124019

Guida-Johnson B, Abraham EM, Cony M. Soil salinization in irrigated drylands: prospects for restoration in Cuyo, Argentina. Rev Fac Cienc Agrar. 2017:**49**:205–215.

Hanana M, Cagnac O, Yamaguchi T, Hamdi S, Ghorbel A, Blumwald E. A grape berry (*Vitis vinifera* L.) cation/proton antiporter is associated with berry ripening. Plant Cell Physiol. 2007:**48**(6):804–811. https://doi.org/10.1093/pcp/pcm048

Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968:**125**(1):189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hill CB, Jha D, Bacic A, Tester M, Roessner U. Characterization of ion contents and metabolic responses to salt stress of different *Arabidopsis* AtHKT1; 1 genotypes and their parental strains. Mol Plant. 2013:**6**(2):350–368. https://doi.org/10.1093/mp/sss125

Hodgson RAJ, Raison JK. Lipid peroxidation and superoxide dismutase activity in relation to photoinhibition induced by chilling in moderate light. Planta 1991:**185**(2):215–219. https://doi.org/10.1007/BF00194063

Inafuku M, Basyuni M, Oku H. Triterpenoid modulates the salt tolerance of lanosterol synthase deficient *Saccharomyces cerevisiae*, GIL77. Saudi J Biol Sci. 2018:**25**(1):1–9. https://doi.org/10.1016/j.sjbs.2016.10.009

Isayenkov SV, Maathuis FJM. Plant salinity stress: many unanswered questions remain. Front Plant Sci. 2019:**10**:80. https://doi.org/10.3389/fpls.2019.00080

Ismail A, Riemann M, Nick P. The jasmonate pathway mediates salt tolerance in grapevines. J Exp Bot. 2012:**63**(5):2127–2139. https://doi.org/10.1093/jxb/err426

Ismail A, Takeda S, Nick P. Life and death under salt stress: same players, different timing? J Exp Bot. 2014:**65**(12):2963–2979. https://doi.org/10.1093/jxb/eru159

Ji H, Pardo JM, Batelli G, Van Oosten MJ, Bressan RA, Li X. The salt overly sensitive (SOS) pathway: established and emerging roles. Mol Plant. 2013:**6**(2):275–286. https://doi.org/10.1093/mp/sst017

Keisham M, Mukherjee S, Bhatla SC. Mechanisms of sodium transport in plants—progresses and challenges. Int J Mol Sci. 2018:**19**(3):647. https://doi.org/10.3390/ijms19030647

Khattab IM, Sahi VP, Baltenweck R, Grondard AM, Hugueney P, Bieler E, Dürrenberger M, Riemann M, Nick P. Ancestral chemotypes of cultivated grapevine with resistance to Botryosphaeriaceae related dieback allocate metabolism towards bioactive stilbenes. New Phytol. 2021:**229**(2):1133–1146. https://doi.org/10.1111/nph.16919

Kishor PBK, Suravajhala R, Rajasheker G, Marka N, Shridhar KK, Dhulala D, Scinthia KP, Divya K, Doma M, Edupuganti S, et al. Lysine, lysine-rich, serine, and serine-rich proteins: link between metabolism, development, and abiotic stress tolerance and the role of ncRNAs in their regulation. Front Plant Sci. 2020:**11**:546213. https://doi.org/10.3389/fpls.2020.546213

Kumar AS, Kumari HP, Kumar SG, Mohanalatha C, Kishor KPB. Osmotin: a plant sentinel and a possible agonist of mammalian adiponectin. Front Plant Sci. 2015:**6**:163. https://doi.org/10.3389/fpls.2015.00163

Landtag J, Baumert A, Degenkolb T, Schmidt J, Wray V, Scheel D, Strack D, Rosahl S. Accumulation of tyrosol glucoside in transgenic potato plants expressing a parsley tyrosine decarboxylase. Phytochemistry 2002:**60**(7):683–689. https://doi.org/10.1016/S0031-9422(02)00161-9

Lee JE, Vogt T, Hause B, Löbler M. Methyl jasmonate induces an O-methyltransferase in barley. Plant Cell Physiol. 1997:**38**(7):851–862. https://doi.org/10.1093/oxfordjournals.pcp.a029244

Li XL, ChR W, Li XY, Yao YX, Hao YJ. Modifications of Kyoho grape berry quality under long-term NaCl treatment. Food Chem. 2013:**139**(1–4):931–937. https://doi.org/10.1016/j.foodchem.2013.02.038

Li C, Sun XK, Chang C, Jia DF, ZhW W, Li CY, Ma FW. Dopamine alleviates salt-induced stress in *Malus hupehensis*. Physiol Plantarum. 2015:**153**(4):584–602. https://doi.org/10.1111/ppl.12264

Liu C, Huang LP, Liu ML, Hao SQ, Zhai H, Shao XJ, Du YP. Effects of seawater irrigation on fruit quality of grapevine, soil properties and microbial diversity. Scient Horticult. 2019:**253**:80–86. https://doi.org/10.1016/j.scienta.2019.04.022

Liu X, Jin Y, Tan K, Zheng J, Gao T, Zhang Z, Zhao Y, Ma F, Li C. MdTyDc overexpression improves alkalinity tolerance in *Malus domestica*. Front Plant Sci. 2022:**12**:625890. https://doi.org/10.3389/fpls.2021.625890

Liu H, Ma Y, Chen N, Guo S, Liu H, Guo X, Chong K, Xu Y. Overexpression of stress-inducible OsBURP16, the β subunit of polygalacturonase 1, decreases pectin content and cell adhesion and increases abiotic stress sensitivity in rice. Plant Cell Environ. 2014:**37**(5):1144–1158. https://doi.org/10.1111/pce.12223

Lotkowska ME, Tohge T, Fernie AR, Xue GP, Balazadeh S, Mueller-Roeber B. The *Arabidopsis* transcription factor MYB112 promotes anthocyanin formation during salinity and under high light stress. Plant Physiol. 2015:**169**(3):1862–1880. https://doi.org/10.1104/pp.15.00605

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

Mohammadkhani N, Heidari R, Abbaspour N, Rahmani F. Comparative study of salinity effects on ionic balance and compatible solutes in nine Iranian table grape (*Vitis vinifera* L.) genotypes. J Int Sci Vigne Vin. 2013:**47**:99–114. 10.20870/oeno-one.2013.47.2.1543

Mosse KP, Lee J, Leachman BT, Parikh SJ, Cavagnaro TR, Patti AF, Steenwerth KL. Irrigation of an established vineyard with winery cleaning agent solution (simulated winery wastewater): vine growth, berry quality, and soil chemistry. Agric Water Manag. 2013:**123**:93–102. https://doi.org/10.1016/j.agwat.2013.02.008

Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008:**59**(1):651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nguyen HTT, Bhowmik S, Long H, Cheng Y, Mundree S, Hoang L. Rapid accumulation of proline enhances salinity tolerance in Australian wild rice *Oryza australiensis* domin. Plants. 2021:**10**(10):2044. https://doi.org/10.3390/plants10102044

Nishizawa A, Yabuta Y, Shigeoka S. Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. Plant Physiol. 2008:**147**(3):1251–1263. https://doi.org/10.1104/pp.108.122465

Oliveira DM, Mota TR, Salatta FV, Sinzker RC, Končitíková R, Kopečný D, Simister R, Silva M, Goeminne G, Morreel K, et al. Cell wall remodeling under salt stress: insights into changes in polysaccharides, feruloylation, lignification, and phenolic metabolism in maize. Plant Cell Environ. 2020:**43**(9):2172–2191. https://doi.org/10.1111/pce.13805

Petrov V, Hille J, Mueller-Roeber B, Gechev TS. ROS-mediated abiotic stress-induced programmed cell death in plants. Front Plant Sci. 2015:**6**:69. https://doi.org/10.3389/fpls.2015.00069

Phogat V, Cox JW, Šimůnek J, Hayman P. Modeling water and salinity risks to viticulture under prolonged sustained deficit and saline water irrigation. J Water Clim Change. 2020:**11**(3):901–915. https://doi.org/10.2166/wcc.2018.186

Popescu CF, Bejan C, Dumitrica RN, Dejeu LC, Nedelea G. Rootstocks and wild grapevines responses to salinity. Vitis 2015:**54**:197–201 https://doi.org/10.5073/vitis.2015.54.special-issue.197-201 .

Qadir M, Quillerou E, Nangia V, Murtaza G, Singh M, Thomas RJ, Drechsel P, Noble AD. Economics of salt-induced land degradation and restoration. Nat Resour Forum. 2014:**38**(4):282–295. https://doi.org/10.1111/1477-8947.12054

Reid KE, Olsson N, Schlosser J, Peng F, Lund ST. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biol. 2006:**6**(1):27. https://doi.org/10.1186/1471-2229-6-27

Rieger T. California vineyards prepare for 2021 drought conditions. 2021; [accessed 2021 Aug 05]. www.winebusiness.com/news/?go=getArticle&dataId=243206

Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989:**10**(6):1003–1008. https://doi.org/10.1093/carcin/10.6.1003

Saleh B, Alshehada E. Gene expression profiling in Halwani and Baladi grapevine (*Vitis vinifera* L. cultivars under saline conditions. J Plant Biochem Physiol. 2018:**6**(03):3. https://doi.org/10.4172/2329-9029.1000222

Schroeder JI, Delhaize E, Frommer WB, Guerinot ML, Harrison MJ, Herrera-Estrella L, Horie T, Kochian LV, Munns R, Nishizawa NK, et al. Using membrane transporters to improve crops for sustainable food production. Nature 2013:**497**(7447):60–66. https://doi.org/10.1038/nature11909

Sengupta S, Mukherjee S, Basak P, Majumder AL. Significance of galactinol and raffinose family oligosaccharide synthesis in plants. Front Plant Sci. 2015:**6**:656. https://doi.org/10.3389/fpls.2015.00656

Shani U, Ben-Gal A. Long-term response of grapevines to salinity: osmotic effects and ion toxicity. Am J Enol Viticult. 2005:**56**(2):148–154. https://doi.org/10.5344/ajev.2005.56.2.148

Shi H, Ishitani M, Kim C, Zhu JK. The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. Proc Natl Acad Sci U S A. 2000:**97**(12):6896–6901. https://doi.org/10.1073/pnas.120170197

Shitan N, Yazaki K. Dynamism of vacuoles toward survival strategy in plants. Biochim Biophys Acta Biomembr. 2020:**1862**(12):183127. https://doi.org/10.1016/j.bbamem.2019.183127

Stevens RM, Harvey G, Partington DL. Irrigation of grapevines with saline water at different growth stages: effects on leaf, wood and juice composition. Aus J Grape Wine Res. 2011:**17**(2):239–248. https://doi.org/10.1111/j.1755-0238.2011.00145.x

Storey R, Schachtman DP, Thomas MR. Root structure and cellular chloride, sodium and potassium distribution in salinized grapevines. Plant Cell Environ. 2003:**26**(6):789–800. https://doi.org/10.1046/j.1365-3040.2003.01005.x

Suarez DL, Celis N, Anderson RG, Sandhu D. Grape rootstock response to salinity, water and combined salinity and water stresses. Agronomy. 2019:**9**(6):321. https://doi.org/10.3390/agronomy9060321

Szabados L, Savouré A. Proline: a multifunctional amino acid. Trends Plant Sci. 2010:**15**(2):89–97. https://doi.org/10.1016/j.tplants.2009.11.009

Venier M, Agüero CB, Bermejillo A, Filippini MF, Hanana M, Walker MA, Blumwald E, Dandekar AM. Analysis of salinity tolerance of *Vitis vinifera* 'Thompson Seedless' transformed with *AtNHX1*. Vitis 2018:**57**:143–150 https://doi.org/10.5073/vitis.2018.57.143-150

Vincent D, Ergül A, Bohlman MC, Tattersall EA, Tillett RL, Wheatley MD, Woolsey R, Quilici DR, Joets J, Schlauch K, et al. Proteomic analysis reveals differences between *Vitis vinifera* L. cv. Chardonnay and cv. "Cabernet Sauvignon" and their responses to water deficit and salinity. J Exp Bot. 2007:**58**(7):1873–1892. https://doi.org/10.1093/jxb/erm012

Wahid A, Ghazanfar A. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J Plant Physiol. 2006:**163**(7):723–730. https://doi.org/10.1016/j.jplph.2005.07.007

Walker RB, Read PE, Blackmore D. Rootstock and salinity effects on rates of berry maturation, ion accumulation and colour development in Shiraz grapes. Austr J Grape Wine Res. 2008:**6**(3):227–239. https://doi.org/10.1111/j.1755-0238.2000.tb00183.x

Wu HH, Shabala L, Zhou MX, Su NN, Wu Q, Ul-Haq T, Zhu J, Mancuso S, Azzarello E, Shabala S. Root vacuolar Na^+ sequestration but not exclusion from uptake correlates with barley salt tolerance. Plant J. 2019:**100**(1):55–67. https://doi.org/10.1111/tpj.14424

Yen GC, Hsieh CL. Antioxidant effects of dopamine and related compounds. Biosci Biotechnol Biochem. 1997:**61**(10):1646–1649. https://doi.org/10.1271/bbb.61.1646

Zhang XK, Walker RR, Stevens RM, Prior LD. Yield-salinity relationships of different grapevine (*Vitis vinifera* L.) scion-rootstock combinations. Aust J Grape Wine Res. 2002:**8**(3):150–156. https://doi.org/10.1111/j.1755-0238.2002.tb00250.x

Zhang J, Yang D, Li M, Shi L. Metabolic profiles reveal changes in wild and cultivated soybean seedling leaves under salt stress. PLoS One 2016:**11**(7):e0159622. https://doi.org/10.1371/journal.pone.0159622

Zoghlami N, Mliki A, Ghorbel A. Occurrence and discrimination of spontaneous grapes native to Tunisia by RAPD markers. Acta Hortic. 2003:**603-**(603):157–166. https://doi.org/10.17660/ActaHortic.2003.603.20

Zörb C, Geilfus CM, Dietz KJ. Salinity and crop yield. Plant Biol. 2019:**21**(S1):31–38. https://doi.org/10.1111/plb.12884