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Sweet versus grain sorghum: Differential sugar transport and accumulation are linked with vascular bundle architecture

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ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench) is globally produced as a source of food, fiber, feed, and fuel. Sweet and grain sorghums differ in a number of important traits, including biomass production, stem sugar and juice accumulation. In this study, a sweet (KIT1) and a grain (Razinieh) genotype of sorghum were used to investigate major differences between sweet and grain sorghum in terms of stem-sugar accumulation. Differences in stem component traits such as internodes, stem anatomy, but also transcripts of key sucrose transporter genes and their response to salt stress were compared. While internodal traits were similar, differences on anatomical level were observed in internodes. Sugar accumulation was highest in the central internodes in both genotypes. However, phloem to xylem cross areas in internodes was correlated with the amount of sugar stored in stem. Sugar accumulation increased significantly under salinity in both genotypes. The expression of sugar-transporter genes *SbSUT1*, *SbSUT2*, and *SbSUT6* was higher in the leaves of KIT1 under normal conditions, but significantly increased in the stem of KIT1 under salinity stress. Nevertheless, transcriptional levels of *SbSUT* genes could not account for the big difference of sugar accumulation in stems between both genotypes. Thus, in addition to anatomical differences, additional (molecular) factors might regulate sugar accumulation in the stem.

1. Introduction

As the global population grows, the demand for food and fossil fuel will consequently increase, and therefore, the negative effect on climate changing will also continue to grow (Keairns et al., 2016). Two main strategies were proposed to confine the expected excess of carbon dioxide: the use of technological removal of carbon using carbon capture and storage devices or bio-engineered organisms, the second strategy aims to use the natural carbon sink i.e. C_4 plants, to absorb ambient carbon dioxide and transform it into biomass (Blätke and Bräutigam, 2019). In addition, a moderate shift from fossil energy to renewable plant biomass-derived energy could mitigate the effect of greenhouse gas emissions (Gielen et al., 2019). While the use of food crops for bio-energy is progressively seen critically, one approach to circumvent the "no food for fuel" dilemma is to use plants that are able to grow on marginal lands and, thus, do not compete with food crops.

Sorghum is a plant that has the capacity, by virtue of C_4 photosynthesis, to efficiently absorb carbon dioxide from the ambient atmosphere and transform it into multiple-use biomass, namely, food, fodder, fiber and bio-ethanol (Regassa and Wortmann, 2014). The use of sorghum for fuel has emerged because of several advantages, such as high-biomass yield, low input requirements, rich genomic resources, and good adaptation to the constraints typical for marginal land, such as water scarcity, salinity, and alkalinity (Regassa and Wortmann, 2014). Based on the major forms of use, sorghum can be divided into four major classes: sweet, grain, forage, and high biomass types (Shakoor et al., 2014; Murray et al., 2009). Grain sorghum is an important staple crop in Africa and China for its gluten-free grains and dry stem (Dicko et al., 2006; Felderhoff et al., 2012). Sweet sorghum, in addition to providing grain yield, accumulates large quantities of soluble sugars (mostly sucrose) in

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its stem, which represents a vegetative sink. Thus, sweet sorghum has the ability to produce high biomass and represents a promising source for bioenergy (Calviño and Messing, 2012; Mathur et al., 2017). The wide range of productive and morphological variations between and among grain and sweet sorghum gives the opportunity to tailor genotypes with specific qualities for production in a given environment (Naoura et al., 2019). The genetic architecture underlying the diversity of sorghum production, qualities and stress tolerance has been addressed by DNA molecular markers and transcriptome sequencing (Wang et al., 2009; Murray et al., 2009; Felderhoff et al., 2012; Bihmidine et al., 2015). The significant phenotypic and genotypic differences between sweet and grain sorghum enabled also strategies using sweet x grain sorghum mapping populations. This allowed to identify major QTLs associated with soluble solids, sugar yield, juice yield and other traits related to sugar accumulation in the stem (Murray et al., 2008a, b; Murray et al., 2009; Shiringani et al., 2010; Guan et al., 2011; Felderhoff et al., 2012; Disasa et al., 2018).

Interestingly, however, genetic analyses revealed striking genetic similarities between grain and sweet sorghum, where the genotypes of grain and sweet sorghum clustered together based on geography of origin, rather than by sugar-related differences (Ritter et al., 2007). This may be a result of the use of dominant markers based on complex traits i.e. whole-plant productivity, on rather than of phenotyping individual components of each trait, such as differences in internode growth. In addition, the use of different genotypes in different locations can increase the environmental variance which than overshadows genetic variance, which is further accentuated by the fact that inbred lines were rarely used (Tao et al., 1993; Ahnert et al., 1996). Furthermore, a comparison between the sweet sorghum line 'PR22′ and the grain sorghum genotype 'Rio' revealed a high level of sequence similarity between sweet and grain genotypes reflecting their historical relatedness, rather than their current phenotypic differences (Cooper et al., 2019).

Also at the molecular level, it is not trivial to discriminate the two types of sorghum. While grain and sweet sorghum would be expected to differ in terms of sugar transport, both seem to unload sucrose unloads apoplastically from phloem to parenchyma cells as inferred from stem anatomy (Bihmidine et al., 2015). Thus, sucrose transporters (*SUTs*) are required to import and/or export sucrose across cell membranes in stems in both types of sorghum. Interestingly, no expressional differences of *SbSUT* genes between grain and sweet sorghum were observed, even at early growth stage, or under stress conditions (Sui et al., 2015). Accumulation of stem sucrose was also correlated with cessation of leaf and stem growth at anthesis, decreased expression of genes involved in stem cell wall synthesis, and approximately 10-fold lower levels of *SbSUS4* transcripts. However, the expression of *SbSUS3* and *SbSUS4* genes was stable after anthesis (McKinley et al., 2016).

Even though sweet sorghum is highly genetically similar to grain sorghum at the structural level, key differences were found in regulatory genes as well as with respect to potential deletions and loss-of-function mutations in sugar metabolism genes that are likely to play important roles in stem sugar accumulation (Cooper et al., 2019). Thus, other factors are needed to explain the difference in stem storage capacity in sorghum genotypes. Recently, a combination of a genome wide association study, quantitative trait loci analysis for recombinant inbred lines, and genetic complementation revealed that the ability to store sugar in sweet sorghum is caused by a recessive loss-of-function in a NAC (synonymous with NAM, ATAF and CUC) transcription factor harboured by the locus Dry. The Dry locus is associated with collapse of secondary cell wall biosynthesis in parenchymatic cells leading to higher juice storage in sweet sorghum stems (Zhang et al., 2018).

Precise phenotyping coupled with the use of recombinant inbred lines or genome-wide association study were used to investigate the genetic architecture of sugar-related traits and genetic tradeoffs between grain and stem sugar (Murray et al., 2008a, b; Ritter et al., 2008; Shiringani et al., 2010; Felderhoff et al., 2012; Luo et al., 2020). Many QTL for structural and non-structural carbohydrate yields co-localised with loci for height and flowering time (Murray et al., 2008a, b). Lately, several studies have focused on individual differences in components of complex traits between grain and sweet sorghum (Sui et al., 2015; Bihmidine et al., 2015; Zhang et al., 2018). However, potential differences in the ratio of phloem to xylem cross ratio, and the effect of stress on sucrose transport in mature stem tissue were not addressed; furthermore, their relationship to whole plant production was not studied. This study aimed to investigate the differences between grain and sweet sorghum on different levels: whole-plant morphology, development of internodes as modular building blocks of the stem, stem vascular bundle structure, expression levels of sucrose transporters, and their response to salt stress. For this purpose, a comparative strategy was chosen, based on two contrasting genotypes, that had previously evaluated under conditions of temperate areas and were known to be different morphologically, genetically, and by geographical origin (Kanbar et al., 2019).

2. Materials and methods

2.1. Plant material

Seeds of the grain sorghum genotype 'Razinieh' and sweet sorghum genotype 'KIT1' were used in this study. The genotype Razinieh was a Syrian landrace and improved by the General Commission for Scientific Agricultural Research (GCSAR) in Syria using a bulk breeding strategy to enhance its biomass and grain productivities (Alhajturki et al., 2012), while KIT1 was developed by us using pedigree selection from the F_2 generation of the sweet ICSSH30 hybrid; the previous name for the genotype KIT1 was ICSSH30-11-ADP (Kanbar et al., 2019) (Fig. 1A).

2.2. Plant cultivation

Plants were grown at the Botanical Garden of Karlsruhe Institute of Technology (Karlsruhe, Germany) during the summers of 2017 and 2018. Seeds were planted in three randomised experimental blocks, with each block containing a plot for each sorghum genotype. Every plot was composed of six adjacent rows of five meters in length. The two outer rows served as buffers, while all measured parameters were taken from the plants in the two middle rows. Spacing between rows was 60 cm, between individuals within rows 25 cm. In total, 100 seeds were planted in each row. At the two-leaf stage, the seedlings were manually thinned to 20 individuals per row. The irrigation of plants was depending on rainfall throughout from the start of cultivation to harvesting except one time where the plants were irrigated after the rain stopped for more than 15 days. Temperature and rainfall were monitored for the duration of the experiment, and monthly average values are presented in Table S1. Based on soil analysis the recommended amounts of fertilizer were supplemented as 100 g / m^2 organic fertilizer (Hauert Hornoska® Special, Germany) and 90:60:40 kg/ha of NPK.

2.3. Phenotyping of plants and internodes

To determine the growth stage provides the optimum concentration of stored sugar in the stem sink tissues, sorghum plants were harvested at pre-flowering (flag leaf stage) and post-flowering stage or dough stage (when the seeds are still soft and immature, and embryogenesis was completed) to measure the sugar concentration based on degree Brix (Brix). Five random plants located in the centre of a plot were harvested from each replicate, recording days to flowering (day), leaf blade area (cm^2), plant height (cm), leaf number, biomass yield (t/ha), cane yield (g fresh weight / plant and t/ha), bagasse yield (t/ha), sugar concentration (Brix), juice yield (ml/plant and kl/ha), and the estimated sugar yield (g/plant and t/ha), over two consecutive years (2017 and 2018). All the recorded data are presenting the morpho-physiological parameters at post-flowering stage after confirming that sugar concentration was maximal at this stage in both genotypes.

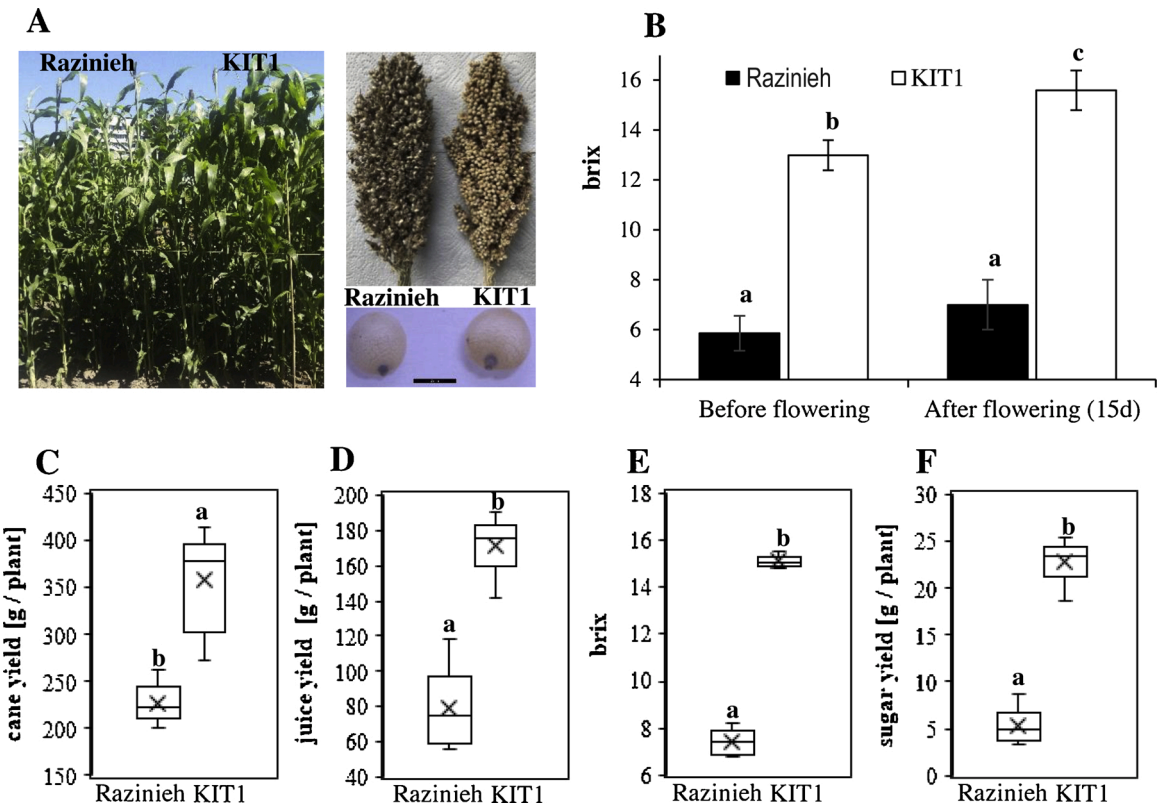

Fig. 1. Sugar-related traits of a sweet (KIT1) and a grain (Razinieh) sorghum genotype grown in two consecutive years (2017 and 2018) in South-West Germany. (A) plants, panicles and seeds of Razinieh and KIT1 at maturity, (B) Brix pre- and after flowering (at dough seed stage), (C) cane yield (g/plant), (D) juice yield (ml/plant), (E) Brix, and (F) sugar yield (g/plant). All the presented observations were taken at post flowering stage after determination that sugar concentration was maximal at this stage in both genotypes. Data indicate the mean of three or more biological replicates with error bars representing the standard error. ns: not significant. KIT1=white bars, and Razinieh = black bars. The black scale bar line in B is 2 mm.

In addition to entire plants, for each phytomer, the internode and the adjacent leaf blade were phenotyped in the same manner. In order 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 to crush the canes. Sugar concentration as Brix recorded with a manual refractometer (Model PAL, Atago Co. Ltd., Tokyo, Japan) for each individual cane. Sugar yield were estimated based on the formulas given in Alhajturki et al. (2012). The sugar juice was diluted, and sucrose, fructose, and glucose contents were measured by using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Separation was performed on a CarboPac PA-20 column (150 × 3 mm, Thermo Fisher Scientific, Waltham, USA). Water, 0.1 M aqueous NaOH, and 0.1 M aqueous NaOH + 0.2 M aqueous sodium acetate were used as eluents using a tertiary gradient program.

2.4. Vascular bundle architecture

To characterise vascular bundles of KIT1 and Razinieh, stem segments of 5 cm length were excised from the centre of the sixth internode using a sharp razor blade as used for making transverse sections. Stem sections were obtained from five randomly sampled plants for each replicate to reach a total of 15 plants for each genotype. Sections were fixed in 4 % (w/v) paraformaldehyde in 50 mM 1,4-piperazine diethane sulfonic acid (PIPES), pH 6.8 for 30 min, dehydrated in an increasing ethanolic series, embedded in paraffine through a xylol-paraffine series, sectioned by microtomy (thickness 25 µm), and stained by Fuchsine-Safranine-Astrablue. Details of the method are given in Supplementary data (Method S1). Sections were observed by brightfield microscopy (Axioscope, Carl Zeiss, Jena, Germany) and digital pictures recorded (AxioCam, Carl Zeiss, Jena, Germany). Five vascular bundles were selected randomly from the sections of each plant, making sure that their distance to the stem epidermis was comparable. Cross areas of lacuna/protoxylem cavity (A1), protoxylem (A2), the metaxylem (A3 and A4), and phloem (A5) areas (Fig. 3A) were marked by the freehand selection tool and measured using the area tool of ImageJ (https://imagej.nih.gov/ij/). In addition, the longest and shortest diameter of each area (Fig. 3A) were measured using the perimeter tool of ImageJ. The data were exported into EXCEL 2016. Additional five sweet sorghum genotypes were characterised for vascular bundle architecture to further test the relationships between vascular bundle areas (A1, A3, and A5) and sugar accumulation in the stem. The five sweet genotypes were two hybrids (ICSSH39 and ICSSH25) and three varieties (ICS22SS, NIJ2, ICSV25274). These five genotypes were grown and treated as described for KIT1 and Razinieh genotypes in the season 2018.

2.5. Sugar accumulation and ion compartmentalisation

Surface-sterilised seeds of both sorghum genotypes were sown into 7.5 l plastic pots filled with a mixture of a 1:1:1 peat moss:perlite:soil mixture and raised in the greenhouse at 25 ± 3℃ with a 12-h photoperiod. Each pot was considered as one experimental unit, and treatments were replicated three times (five plants per replicate) in a completely randomised design. Irrigation was performed to maintain 80 % of field capacity from cultivation day to flowering stage.

The mean relative humidity for crop growing periods was about 70 %, ranging from 65 to 75%. Light bulbs (400 W / 220 E40 55,000 lm) (SON-T AGRO, Philips) fixed at 3 m height were used in the greenhouse to maintain a lighting intensity of about 1000 µmol/m^2/s PAR at noon. A continuous flow of fresh air was maintained during the experimental

period to ensure that no CO_2 deficit developed. Salinity stress was imposed, when the head of each plant had fully emerged from the flag leaf, by adding daily to each pot 400 ml of a 100 mM NaCl solution over a period of 15 days. In parallel, a mock control was run, where the plants were treated in the same way by de-ionised water. Sugar concentration and juice volume were measured after the 15 days of salinity treatment by taking five individual cane samples from each genotype in each replicate. The mean of the five samples from each replicate was considered as an individual value for further analysis.

For measuring of ion content, leaves, stems and roots of control and treated plants were harvested after the 15 days of salinity treatment, then washed gently several times with de-ionised water, and subsequently incubated at 80℃ in a drying oven for three days. The dry tissues were homogenised using a mortar and pestle and collected in digestion tubes (Gerhardt, UK). The main elements Na^+, K^+, and Ca^{2+} were determined via ICP-OES (iCAP 7600, from Thermo Fisher Scientific). The samples (50 mg ± 0.01 mg) were dissolved in 9 ml nitric acid and 1 ml hydrofluoric acid at 250℃ for 12 h in a pressure digestion vessel DAB-2 (Berghof). After complexation with boric acid, the analysis of the elements was accomplished using four different calibration solutions and an internal standard (scandium Sc) (Bergfeldt et al., 2018).

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

For gene expression studies, mature leaf and stem samples were collected from greenhouse-grown plants at 1 day, 10 days, and 15 days of salinity treatment, immediately frozen in liquid nitrogen, and stored at −80 °C until processed. Stem tissues were harvested from the middle part of the sixth internode, leaf tissues from the centre of the leaf blade adjacent to the sixth internode after omitting the mid-rib. Total RNA was isolated using the Spectrum™ Plant Total RNA Kit (Sigma, Germany) according to the instructions of the manufacturer from a small amount of tissue ground to a powder (Tissue Lyzer, Qiagen, Hilden, Germany). The extracted RNA was reversely transcribed into cDNA by *M-MuLV Reverse Transcriptase* (New England Biolabs, Frankfurt am Main) using 1 µg of total RNA as a template. Real-time (qPCR) was performed with the CFX96 Touch™ Real-Time PCR Detection System from Bio-Rad Laboratories GmbH (Munich) using a SYBR Green dye protocol according to Svyatyna et al. (2014). Transcript levels between the different samples were compared using the ΔCt method (Livak and Schmittgen, 2001). Three biological replicates were performed for each treatment. Three technical replicates were conducted from each biological replication. All *SbSUT* gene-specific primers were designed according to previously published by Bihmidine et al. (2015) (Table S2).

2.7. Statistical analyses

Statistical analyses were performed using the procedures PROC MEANS and PROC GLM implemented in the SAS v9.4 software (SAS Institute Inc., Cary, NC, USA). The mixed model analysis of variance (PROC MIXED) was used to test for significant differences between KIT1 and Razinieh, with the genotypes and years used as the fixed and random effects, respectively. Tukey's honest significance test was used to carry out post-hoc comparisons of differences among means, applying a significance threshold of $p < 0.05$. Pearson's correlation coefficient (PROC CORR) was used to reveal inter-trait correlation.

3. Results and discussion

3.1. More sugar in sweet sorghum in temperate climate, especially during grain filling

Using a contrasting pair of a sweet (KIT1) and a grain (Razinieh) sorghum genotype, we determined at which developmental stage sucrose accumulation in the stem is maximal (Fig. 1B). Our experiments showed that KIT1 exhibited a significantly higher Brix, especially during the early stage of grain filling (15 days after flowering). However, as compared with Razinieh, the Brix were higher in KIT1 in both, pre- and post-flowering, stages (Fig. 1B). This pattern would be expected for a sweet sorghum genotype and is in accordance with sweet sorghum literature, since stem tissues are thought to serve as a terminal sink tissue where sucrose accumulates during the post-flowering stage (Kumar et al., 2011; Oyier et al., 2017). In contrast, the grain sorghum Razinieh did not show a significant difference in Brix between pre- and post-flowering stages (Fig. 1B). Also this result is expected, since in grain sorghum the grain dominates as terminal sink, while there is only a low capacity of sucrose storage in the stem (Dicko et al., 2006; Ritter et al., 2007; Morey et al., 2018). Many sweet sorghum genotypes such as Della accumulate high levels of sucrose in stems at post-flowering stage, similar to sugarcane (Jackson et al., 1980; Murray et al., 2009; Wang et al., 2009)

Stem-sugar concentration may be quantitatively measured by high performance liquid chromatography (HPLC). Likewise, since the soluble sugars are predominantly sucrose, sugar content can be inferred refractometrically (Murray et al., 2009). The readout (degree Brix, which equals w/v % in case of sucrose) is widely accepted as a reflection of sucrose percentage in a solute, and the positive and significant correlation between Brix and sucrose level in sorghum syrup has been demonstrated across different genotypes of sweet sorghum (Morey et al., 2018).

Also in our case, the dominant form of sugar in stem juice of both genotypes is sucrose for both genotypes (Fig. 2). In fact, the sucrose content assessed by HPLC correlated tightly with the refractometric readout in degree Brix ($r = 0.89$, $P < 0.01$), while the correlation with measured glucose or fructose content, while being positive, but not significant ($r = 0.77$, $P < 0.06$). Based on these results, we used refractometry as reliable and convenient readout for sucrose content.

3.2. Morphological plasticity is more pronounced in Razinieh

To understand key morphological differences between grain and sweet sorghum, some morpho-physiological traits were recorded, during the dough stage, over two consecutive years (Fig. 1C-F and Table 1). The sweet sorghum genotype KIT1 out-performed the grain genotype Razinieh not just with respect to biomass yield, and bagasse yield, but also in cane yield, juice yield, sugar concentration as Brix, and total leaf area (Fig. 1C-G, Table 1). These results are generally in accordance with previous morphological evaluations of KIT1 and Razinieh (Alhajturki, 2012; Kanbar et al., 2019). Likewise, other comparative studies between grain and sweet sorghum genotypes reported the overall biomass superiority of sweet over grain sorghum (Ritter et al., 2007; Qazi et al., 2012; Bihmidine et al., 2015). Based on the observed phenotypes (plant height and sugar content as degree Brix) and known origins, sweet and grain sorghums remain distinct (Murray et al., 2009).

This superiority has been suggested to be directly linked to the larger leaf area and the longer persistence of the green state in sweet sorghum, and, hence higher capacity of biomass production (Qazi et al., 2012; Bihmidine et al., 2015). However, the relationship between source (leaf) and sink (stem or grain) in sorghum has remained to be addressed. Another essential point is that KIT1 always required a longer period of time to reach flowering compared with Razinieh (Table 1). Thus, KIT1 has a longer period of biomass production (Kanbar et al., 2019). However, compared with subtropical, semi-arid conditions, both genotypes required more time to reach flowering and, therefore, produced longer stems in temperate areas (Alhajturki et al., 2012). In spite of the phenotypic plasticity in both genotypes across different locations, KIT1 always maintained its superior performance not only just over Razinieh, but also other sorghum genotypes (Alhajturki et al., 2012; Kanbar et al., 2019).

The environmental variance across two years in one location showed that Razinieh recorded significant difference between the two consecutive years of evaluation for all sugar-related traits except for cane yield

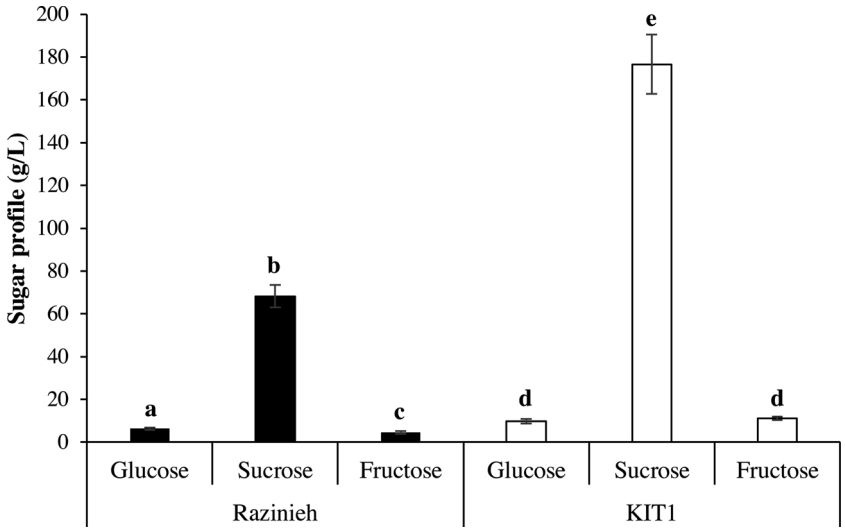

Fig. 2. Juice sugar composition profile of a sweet (KIT1) and a grain (Razinieh) sorghum genotype grown in 2018 season as analyzed using HPLC. Significant differences at 0.05 between the two genotypes are indicated with different letters (ANOVA, Tukey HSD test, $P \leq 0.05$). The measurement unit is g/L.

and number of leaves (Table 1), while for KIT1, the differences, with exception of cane yield, were not significant (Table 1). Cane yield depends on length and diameter of stem as well as the juice content in the stem. The higher variance suggests, therefore, that stem parameters show a higher plasticity in Razinieh as compared with KIT1. The genetic base of multicomponent traits and their plasticity is rather complex, nevertheless, it allows to infer how different trajectories of evolution affect the overall performance of plants (Turner et al., 2016). Hence, it was decided to investigate the morphological differences in stem components between KIT1 and Razinieh with closer scrutiny.

3.3. *Internode weight rather than length can be used as predictor for sugar yield*

Cane and juice yields in sorghum are mainly determined by those properties of the stem that concern its capacity to act as sink for soluble sugars. To further pinpoint this capacity, we measured and analyzed, for each internode individually (from base to top), sugar concentration, length, weight, juice content and area of the adjacent leaf were measured and analysed, for each internode individually (from base to top). For time limitations, this experiment could be conducted only in the second season (2018). In KIT1 sugar concentration, fresh weight of internodes, juice content per internode, and leaf area were significantly higher, at a relatively similar length of internodes (Fig. 3A-D, H). Thus, the superiority of KIT1 over Razinieh on the level of the entire stem is due to its heavier, more juicy, more sugars internodes and their greater diameter. This result is consistent with previous observations, where sweet sorghum was shown to produce more weight and to exhibit higher sugar concentration reflected as higher refraction (in degree Brix) compared with grain sorghum (Murray et al., 2008a, 2008b; Murray et al., 2009; Wang et al., 2009; Shiringani et al., 2010; Guan et al., 2011; Felderhoff et al., 2012; Disasa et al., 2018; Bihmidine et al., 2015; Luo et al., 2020).

Conversely, similar patterns were observed between KIT1 and Razinieh. Both genotypes exhibited higher values for Brix in the central internodes, especially from the fourth to the eighth internode (Fig. 3A), while accumulating more weight in the basal internodes, from the second to the fourth or fifth internode (Fig. 3B). This heavier weight for basal internodes was correlated with a higher juice content (Fig. 3C). Thus, the middle internodes although storing less juice, nevertheless produced the highest sugar concentration (in terms of Brix). Our result is consistent with earlier observations which showed that it was the central internodes in sorghum stems that displayed the highest readout for Brix (Hoffmann-Thoma et al., 1996; Bihmidine et al., 2015; Shukla et al., 2017). A common feature of three sweet sorghum cultivars (Keller, NK 405 and Tracy) were the significantly lower sucrose contents (in degree Brix) in the upper- and lowermost three internodes at the time of anthesis (Hoffmann-Thoma et al., 1996). A lower sucrose concentration was observed also in the last top internodes and the peduncle of the landrace IS2848 (Gutjahr et al., 2013). The sweet sorghum cultivars Della and Rio showed similarities in internode sugar concentration dynamics, whereas total sugar concentrations were markedly increased in both genotypes after anthesis and the upper and lower internodes had lower sugar concentrations than the middle internodes in both genotypes (Li et al., 2019)

This can be attributed by the area of the respective adjacent leaves. Both genotypes exhibited similar patterns of leaf area distribution if followed from the basal internode to the panicle. The biggest leaf areas were observed between the third to the sixth internodes in both genotypes (Fig. 3H). This correlation between area of the adjacent leaf with it's the sucrose content of the internode is congruent with previous reports in sorghum (Rosenthal and Vanderlip, 2004) and maize (Debruin et al., 2013). A positive and highly significant correlation was observed between individual leaf area and sugar concentration in terms of Brix ($r = 0.85$, $p < 0.001$) in the corresponding internode (Fig. 3I). Using a recombinant inbred line population consisting of 176 $F_{4:5}$ lines was developed from a cross between 'BTx623' (a grain sorghum) and 'Rio' (a sweet sorghum), significant and positive correlations were found between Brix, cane yield and leaf yield (Murray et al., 2008b).

The situation was different for Razinieh, however. Here, internodes were longer, when followed from the second to the fifth, but this was not accompanied by higher juice yield per internode, contrasting with previous studies were a positive correlation between sugar concentration and stem length had been reported (Burks et al., 2015). While a positive, significant association was measured between Brix, internode weight ($r = 0.80$, $p < 0.0001$), and juice content ($r = 0.85$, $p < 0.0001$), no significant relation between sugar content (in degree Brix) and internode length (r=-1.17, $p < 0.5$) were observed (Fig. 3E-G). The diameter of the 6th internode was also, exemplarity, checked and found it to be greater in KIT1 as compared with Razinieh (Table 1). Thus, it could be concluded that the major difference between KIT1 and Razinieh may be explained by the diameter of internodes. The greater diameter of internodes may infer to bigger area of parenchyma storage cells in KIT1 and consequently different anatomical structures for xylem and phloem.

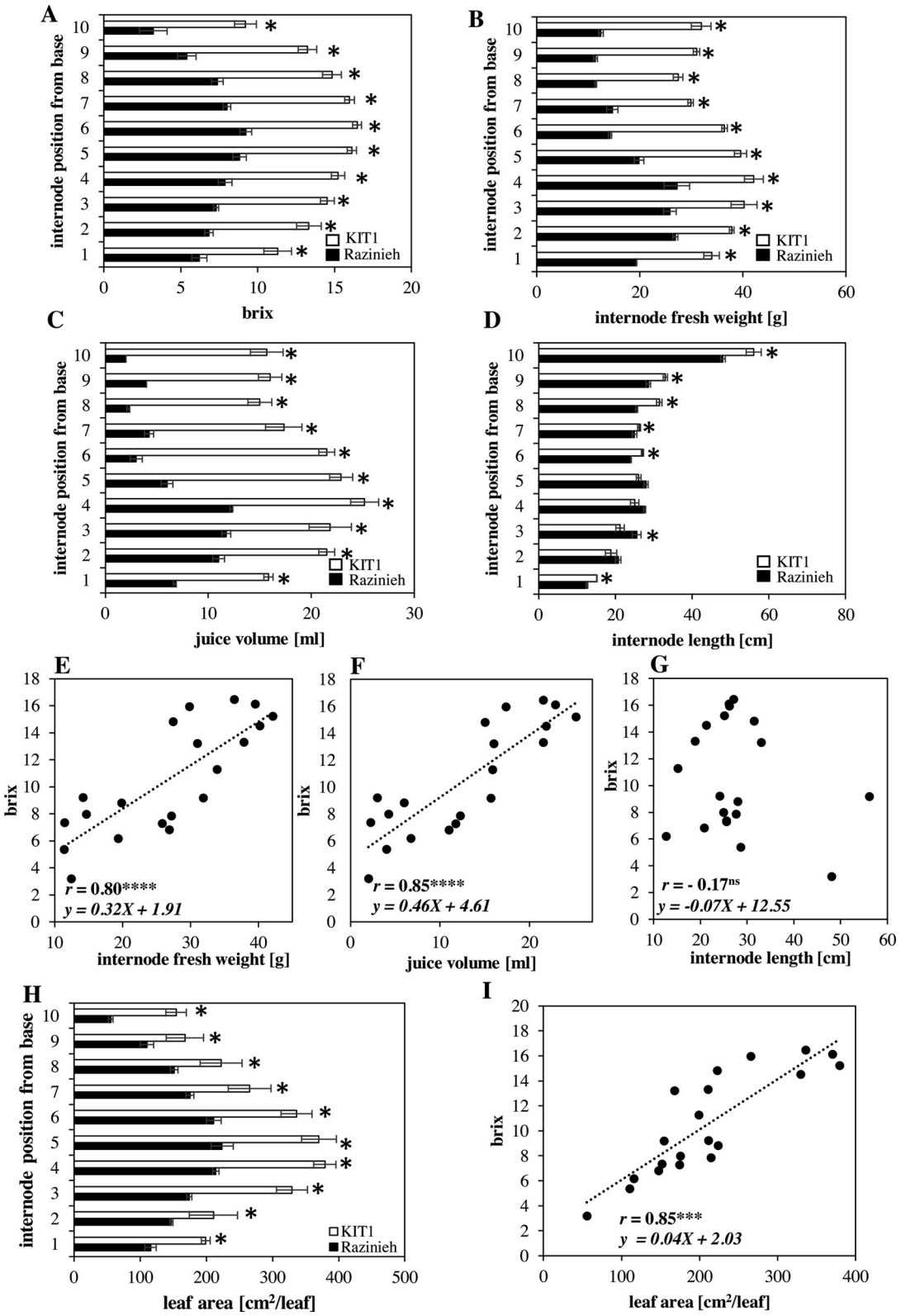

Fig. 3. Differences in stem component parameters and sugar accumulation between a sweet (KIT1) and a grain (Razinieh) sorghum genotype grown in 2018 season in South-West Germany. (A) Brix reading per internode. (B) fresh weight [g] per internode. (C) Juice volume [ml] per internode. (D) length [cm] of internodes. Relationship between Brix and (E) internode weight, (F) internode juice volume, and (G) internode length. (H) Differences in individual leaf area for each phytomer unit between KIT1 and Razinieh. (I) Relationship between individual internode Brix and individual leaf area. The two genotypes have the same number of internodes (10 internodes). Data indicate the mean of three or more biological replicates (five samples in each replicate) with error bars representing the standard error. ns: not significant. KIT1 = white bars, and Razinieh = black bars.

Table 1
Mean values of morpho-physiological traits of KIT1 and Razinieh genotypes at post-flowering growth stage (dough stage) in two consecutive years (2017 and 2018) in South-West Germany.

Year	Genotype	Days to flowering (day)	Plant height (cm)	Leaf number	Stem diameter (cm)	Total green leaf area (cm^2)	Biomass yield (t/ha)	Cane yield (t/ha)	Juice yield (Kl/ha)	Bagasse yield (t/ha)	Brix	Sugar yield[a] (t/ha)
2017	Razinieh	72.0a	271.0a	9.7a	2.3a	2291.0a	57.1a	46.8a	19.5a	22.9a	7.8a	1.4a
	KIT1	86.7b	297.3b	10.0a	3.6b	2563.3b	77.3b	64.4b	33.2c	23.1a	15.0b	4.4b
2018	Razinieh	75.0c	282.7c	10.0a	2.1c	1578.3c	49.6c	44.0a	12.0b	19.8b	7.0c	0.7c
	KIT1	84.7b	292.0b	9.3a	3.9d	2635.7b	82.3b	78.6c	35.4c	23.4a	15.2b	4.7b

[a] Sugar yield was estimated as descried in our previous works (Alhajturki et al., 2012; Kanbar et al., 2019).

3.4. The genotypic differences in sugar accumulation are tightly linked with differences in vascular bundle anatomy

Despite the importance of vascular bundle architecture in sorghum stem yield, little attention has been paid so far to anatomical differences in vascular bundle architecture in grain and sweet sorghum. The two genotypes were comparatively phenotyped on a quantitative level, five randomly picked vascular bundles, selected from the sections of the sixth internode of each plant (Fig. 4A), as it was found to contain the highest sugar concentration in terms of sugar content (measured as degree Brix), irrespective of the genotype (Fig. 3A).

Significant differences were found in the cross areas of xylem and phloem between Razinieh and KIT1 (Fig. 4B-E), and between Razinieh and other five sweet sorghum genotypes (ICS22SS, NIJ2, ICSSH39, ICSV25274, and ICSSH25) (Fig. S2). While Razinieh showed significantly greater diameters and cross areas for xylem vessels, its phloem cross area was significantly smaller compared with KIT1 (Fig. 4A-E) and other five sweet sorghum genotypes (Fig. S2A,C–F). Namely, Razinieh had bigger lacunae (area A1 in Fig. 4A; Fig. S2C), protoxylem (area A2 in Fig. 4A; Fig. S2D), and metaxylem vessels (areas A3-4 in Fig. 4A; Fig. S2E). Since the cross areas of phloem and xylem depend on length and diameter of the internode, the ratio of phloem to xylem diameter was measured in the 6^{th} internodes. Here, KIT1 showed significantly higher ratio of phloem to xylem diameter compared with Razinieh (Fig. 4E). The same results were obtained when Razinieh was compared with the other five different sweet sorghum genotypes (Fig. S2G). Thus, KIT1 and other five sweet sorghum genotypes assign more vascular cross area in the stem towards the phloem, less to the xylem. To the best of our knowledge, this is the first study discussing the relationship between vascular bundle architecture and sugar accumulation in the stem. It has been proposed that xylem development is regulated by the water demand in the leaves, depending on their degree of photorespiration (Salih et al., 1999). This would imply that larger leaf areas should correspond to larger xylem areas. However, in our case, the opposite was observed (Fig. S1). Although KIT1 displayed a leaf area almost twice as the leaf area recorded by Razinieh, it did not exhibit a larger but a significantly smaller xylem cross area in its internode. Instead, the larger cross area of the phloem in KIT1 may refer to a higher capacity of sugar mobilisation from leaves to stem.

As to be expected from this hypothesis phloem cross area showed significant correlation with both sugar concentration ($r = 0.79$; $p < 0.0001$) and leaf area ($r = 0.95$; $p < 0.0001$) (Fig. 4F, G). Instead, xylem cross area was negatively correlated with sugar concentration (r=-0.95; $p < 0.0001$) and leaf area (r=-0.89; $p < 0.0001$) (Fig. 4H, I). To support the hypothesis, relationship between of sugar concentration with phloem and xylem cross areas were performed on the data of seven genotypes including Razinieh and KIT1. The results showed that sugar concentration had significant positive correlation with phloem cross area ($r = 0.68$; $p < 0.0001$) and significant negative correlation with xylem area A1 (r=- 51; $p < 0.001$) and xylem area A3 (r=- 88; $p < 0.0001$) (Fig. 2S, H–J). Hence, our data do not support the hypothesis of xylem diameter and leaf area proposed earlier (Salih et al., 1999). While, the number of grain and sweet sorghum genotypes involved in this study is less to generalize the relationship between vascular bundle structure and amount of sugar accumulation, further more research is needed with involving diverse and large number of sweet and grain sorghum genotypes.

3.5. Effect of salt stress on sucrose content

The next step was testing our hypothesis that differences in stem phloem to xylem area ratios not only correlate with sugar transport capacity, but also contribute to resilience against abiotic stress. Salinity stress, composed of osmotic and ionic stress was used, because it is of agronomic relevance, and because sugars as osmotically components might be part of the adaptive mechanism. Some C_4 grasses degrade, when they experience stress condition, sucrose to fructose to create a storage pool in their stem (Halford et al., 2011). Consequently, their sucrose storage is decreased. Also in sorghum some genotypes have been reported to exhibit decreased sucrose content (measured as degree Brix) in response to salinity stress, while others increased their sugar concentration (Vasilakoglou et al., 2011). Our results show clearly that the sugar content (in degree Brix) increased significantly from 15.6 to 17.5 ($P < 0.05$) in KIT1 and from 7.0 to 9.2 ($P < 0.05$) in Razinieh after 15 days of treatment in comparison to control (Fig. 5A). The higher values found in KIT1 indicated a superior capacity for sucrose mobilisation and storage in stem tissues, although this ability did increase under salinity in both genotypes. Increased sucrose mobilisation requires that, under stress conditions, photosynthesis in leaves, the activity of sucrose transport increase, while sucrose breakdown by invertase in stem tissues decreases. In fact, the activity of sucrose invertase in mature parenchyma storage cells of sorghum stem is suppressed (Tarpley and Vietor, 2007), such that sucrose degradation is minimal in mature tissues of the stem (Rossouw et al., 2010).

To understand if the soluble solids concentration increased due to a loss of water from the stem, juice volume was measured under both control and salinity treatments (Fig. 5B). The juice volume increased significantly in KIT1 from 127 ml per plant under control to 184 ml per plant under salinity stress. No significant change was observed for the juice volume in Razinieh (control = 27.5 ml/plant; salinity stress = 30.5 ml/plant). However, salinity stress reduced green leaf weight in both genotypes, but KIT1 had still higher green leaf weight (>1-fold) under salinity treatment for 15 days (Fig. 5C). The imposition of strong water or salt stresses in sorghum has been demonstrated to be accompanied to an increase in the sugar levels of the stem, which may help in osmoregulation under stress conditions (Gill et al., 2001). The total soluble sugar increased in sorghum sap with increasing salinity level at seedling stage (Ibrahim, 2004). Accumulation of sugars is also related to salinity-tolerant mechanisms in many plant species (Gupta and Huang, 2014). The increase in cellular osmolarity due to the accumulation of compatible solutes was accompanied by the influx of water into cells, thus providing the turgor required for cell expansion (Hussain et al., 2003). Studies have shown that when plants grow in salinity soil, the plant tissue sugar concentration increases (Munns, 1993). The sweet sorghum genotype KIT1 was also more efficient in maintaining Na^+/K^+ ratios under salt treatment (discussed in section 3.7).

In conjunction with the “stay-green” trait that prolongs the productive time of leaves and delays senescence, some sorghum genotypes have

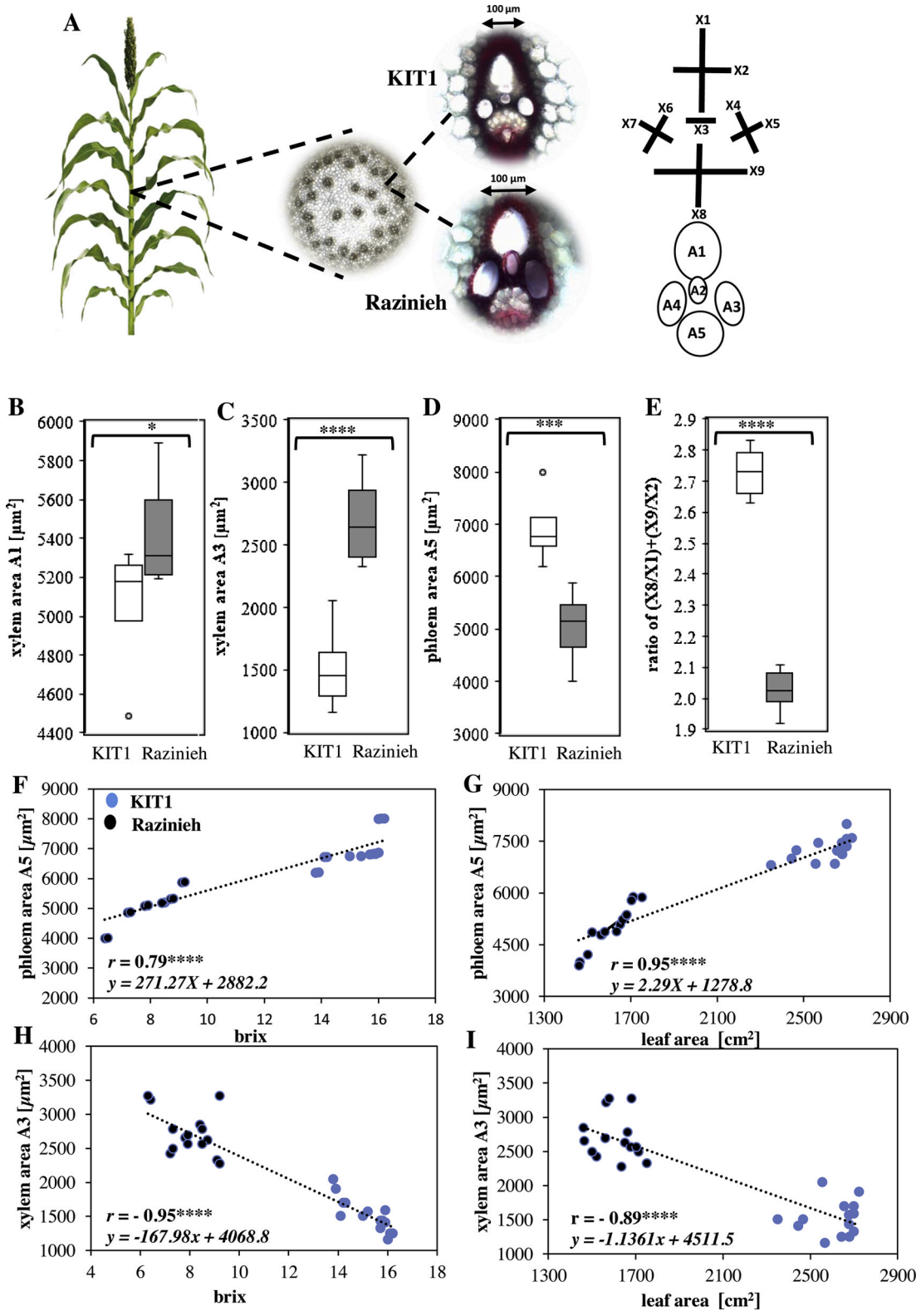

Fig. 4. Cross-sections of sixth internode and relationship between the circumferences and diameters of phloem and xylem of vascular bundles with Brix reading and total leaf areas of a sweet (KIT1) and a grain (Razinieh) sorghum genotype. (A) Fully transverse section from the middle of the sixth internode are shown, along with a vascular bundle architecture for each genotype A1, protoxylem vessel area; A2, cavity (protoxylem) area; A3 and A4, Metaxylem areas; A5, Phloem area. X1 and X2, two diameters of lacuna area; X3, diameter of protoxylem area; X4/ X5 and X6/X7, diameters of two similar metaxylem areas, respectively. X8 and X9, diameters of phloem area. Scale bars: 100 µm. (B) Xylem area A1. (C) Xylem area A3. (D) Phloem area A5. (E) Total ratios of (X8/X1)+(X9/X2). (F–G) Relationship of phloem area A5 with Brix and total leaf area, respectively. (H–I) Relationship of xylem area A3 with Brix and total leaf area, respectively. Data indicate the mean of five or more biological replicates (five vascular bundles were selected randomly from each plant samples in each replicate) with error bars representing the standard error. ns: not significant. KIT1 = white bars, and Razinieh = brown bars.

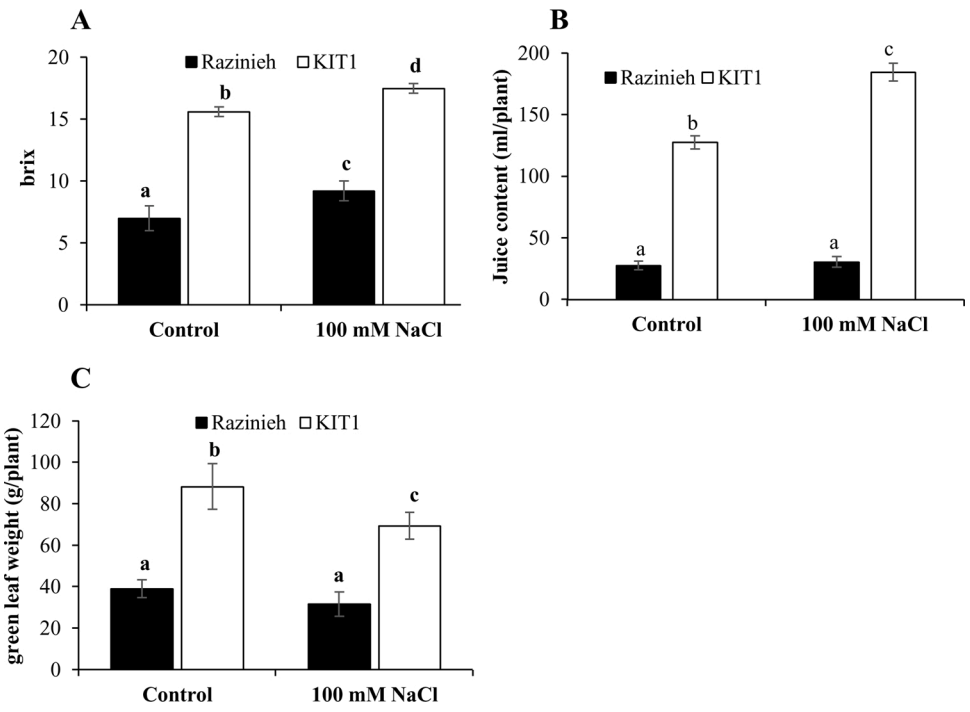

Fig. 5. Effects of salinity stress on Brix, and juice content in the stem and the green leaf weight of a sweet (KIT1) and a grain (Razinieh) sorghum genotype after 15 days of NaCl treatment (100 mM NaCl) at post-flowering stage. Values are means ± SE from three independent biological replicates (number of samples from each replicate is five plants). Significant differences at 0.05 between the two genotypes are indicated with different letters (ANOVA, Tukey HSD test, $P \leq 0.05$). KIT1 = white bars, and Razinieh = black bars.

therefore the ability to produce and conduct sucrose under stress condition (Ghate et al., 2017). This is consistent with a study conducted by Sui et al. (2015), high Brix sorghum genotypes that were salt tolerant, also showed significant up-regulation of genes involved in photosynthesis and sucrose synthase, while genes encoding sucrose invertase were downregulated. However, in the same study, *SbSUTs* transcripts were not modulated in response to salinity, independent of genotype.

3.6. Differential expression of SbSUTs and SbNHX1

In order to investigate whether a differential expression of *SbSUT* genes can help to obtain a deeper insight into the reason why KIT1 has higher Brix than Razinieh, the expression of six predicted *SbSUT* genes was monitored (Braun and Slewinski, 2009). Since only *SbSUT1*, *SbSUT2* and *SbSUT6* genes showed quantifiable expressional levels in grain and sweet sorghum tissues (Bihmidine et al., 2015; Cooper et al., 2019), the expression of these three genes were checked. Furthermore, the gene encoding for the sodium-proton antiporter *SbNHX1* was included into our analysis. Cooper et al. (2019) presented a new reference genome based on an archetypal sweet sorghum line 'Rio' and compared it with the current grain sorghum reference, revealing a high level of genomic similarity between sweet and grain sorghum reflects their historical relatedness, rather than their current phenotypic differences. They also found that 276 genes that appear to have been deleted in sweet genotype Rio. The most interesting putative deletions observed in Rio were three known sucrose transporter genes: *SbSUT4*, *SbSWEET3–3*, and *SbSWEET8–2*.

Under normal conditions, analysis of expression levels of *SbSUT1* and *SbSUT2* genes revealed that these genes are more strongly expressed in leaves of KIT1. However, the expression of *SbSUT1* gene was higher in stems of Razinieh, while, *SbSUT2* expression was comparable in stem tissue for both genotypes (Fig. 6A-B). The expression of *SbSUT6* gene in leaves was also found upregulated developmentally in KIT1. However, the expression of this gene was very low in stem tissues, for both genotypes (Fig. 6C). As a result, the expression levels of these genes could not account for the significant difference in sugar storage in stems of KIT1 and Razinieh. Our result is consistent with what was previously observed by Bihmidine et al. (2015). Nevertheless, these genes may have a significant role in sucrose mobilisation in leaves. The expression of *SbSUT1*, *SbSUT2* and *SbSUT6* genes were higher in PR22 (grain genotype) internodes, and higher in Rio (sweet genotype) leaves at soft dough stage under normal condition (Cooper et al., 2019).

Consequently, the higher expression of *SbSUT1* and *SbSUT2* genes in leaves tissues of KIT1 may be linked with its bigger leaf area compared with grain sorghum (Milne et al., 2013). In addition, the higher level of *SbSUT1* transcripts in stem tissues of Razinieh during the first ten days indicates that this gene might be involved in direct mobilisation of sucrose to grains. However, a higher expression of the *SbSUT1* gene is not necessarily correlating with higher sugar concentrations as genotype dependent differences have been found (Qazi et al., 2012; Milne et al., 2013; Bihmidine et al., 2015). In addition, parallel symplastic pathways are unlikely to contribute to sucrose storage, because phloem tissues in mature internodes are symplastically isolated and the sucrose unloads apoplastically into storage cells (Bihmidine et al., 2015). These results rather suggest another independent pathway responsible for sucrose accumulation in stem storage cells. The sweet sorghum genotype KIT1 requires a significantly longer period of time to reach flowering and senescence (late-flowering) compared with Razinieh (early-flowering). This means that KIT1 can build up storage capacity in parenchyma cells over a longer period as compared with Razinieh. Although expression of *SbSUT* genes did not show major differences during the first 15 days after flowering. Further experiments should clarify, whether this difference in carbon transport dynamics might depend on post-transcriptional regulation of sucrose transporter, such as their activity or subcellular localisation. The expression level of *SbNHX1* was relatively low and comparable in both genotypes and organs under control conditions (Fig. 6D). This result is totally expected, up-regulation of *SbNHX1* is a specific hallmark for the salinity stress in sorghum (Kumari et al., 2018).

The salinity responses of the *SbSUT* transcripts were complex and not only dependent on the respective member of the *SbSUT* family, but also on the organ (leaf versus stem), and the genotype (Fig. 6). Therefore, the pattern will be described separately for the genotypes. For KIT1, *SbSUT1* transcripts in the stem increased moderately and slowly, by a factor of 2, reached from day 10 of the salt treatment (Fig. 6A). In the leaf, there was a sharp drop by a factor of 8 during the first day, followed by full

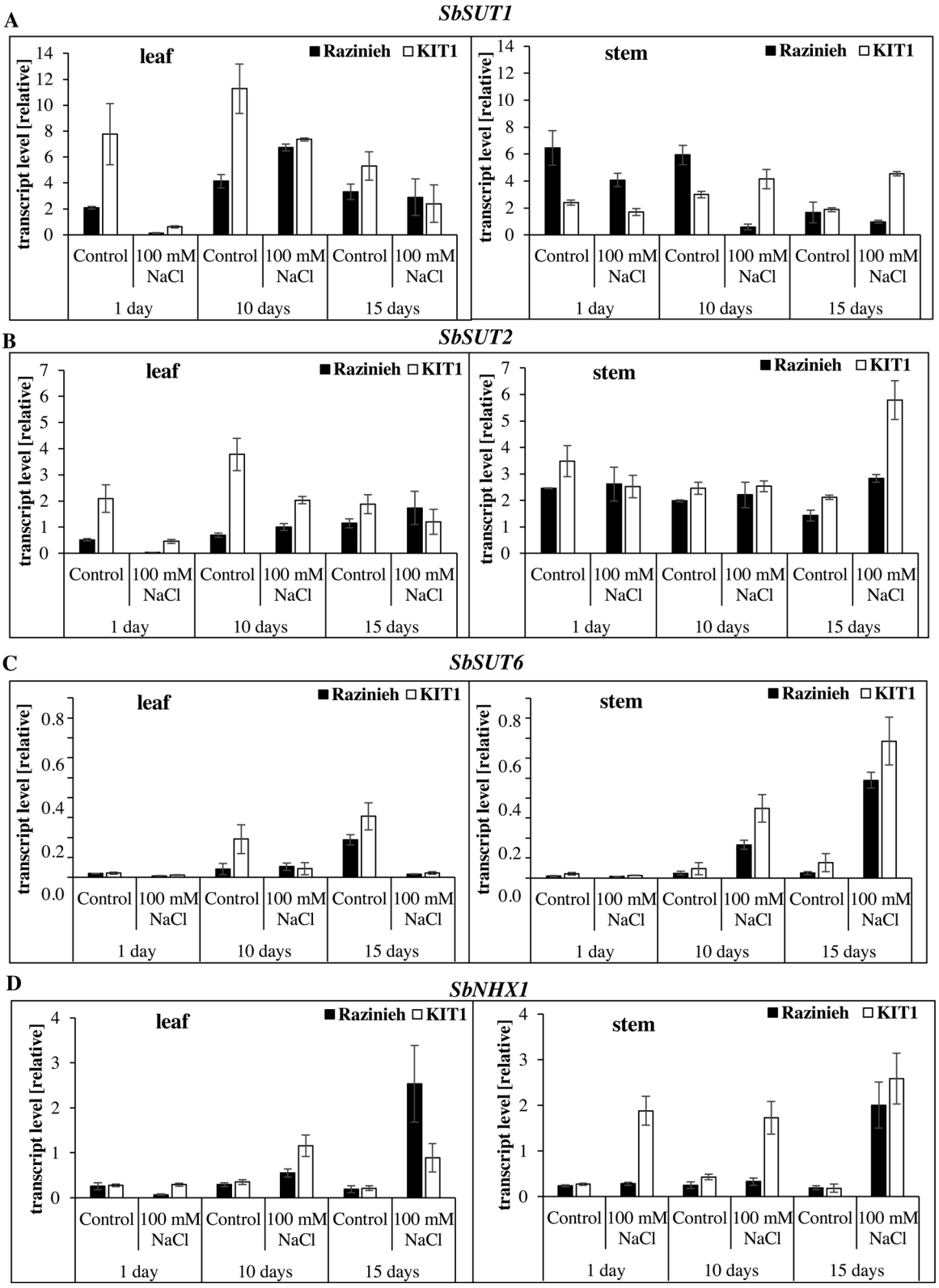

Fig. 6. Relative gene expression analysis of *SbSUT1*, *SbSUT2*, *SbSUT6*, and *SbNHX1* in a sweet (KIT1) and a grain (Razinieh) mature leaves and stems at 1, 10, and 15 days of control and salinity treatment (100 mM NaCl) at post-flowering stage. (A–D) The gene expression of *SbSUT1*, *SbSUT2*, *SbSUT6*, and *SbNHX1*, respectively. Three biological replicates were performed for each treatment. Three technical replicates were conducted from each biological replicate. Values are means ± SE from three independent biological. KIT1 = white bars, and Razinieh = black bars.

recovery till day 10 and a slow and moderate dissipation after day 10. In contrast, *SbSUT2* (Fig. 6B) in the stem, did not change during the first ten days of treatment with 100 mM of NaCl, but roughly doubled, if scored at day 15 of the treatment. In the leaves, its expression pattern was similar to the *SbSUT1* gene, with an intermediate downregulation, a recovery at day 10 and a subsequent mild decline, however, these changes occurred at a much lower amplitude as compared with teh *SbSUT1* gene. The most drastic regulation was seen for *SbSUT6* transcripts (Fig. 6C), although the overall expression of this gene was much lower than for *SbSUT1* and *SbSUT2*. Here, the levels in the stem rose by a factor of almost 10-fold over those seen in the control, while in the leaves, *SbSUT6* transcripts were reduced by at least 5-fold below those seen in the control. *SbNHX1* was strongly upregulated in the stem by a factor ~5 already from day 1 and stayed at this level throughout the subsequent period (Fig. 6D). In the leaves, a somewhat smaller increase was seen, but later (only from day 10 after the onset of salt stress).

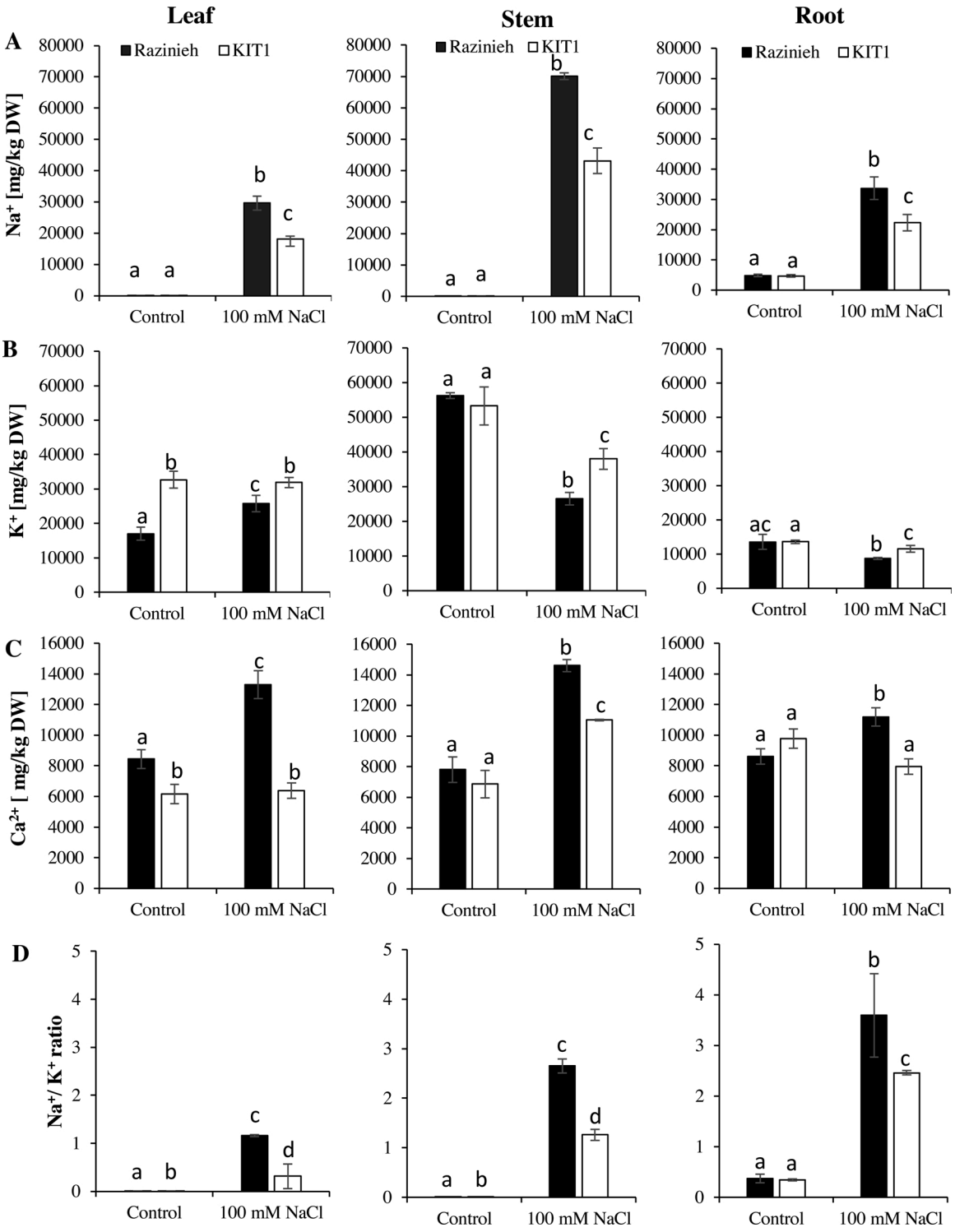

Fig. 7. Ion concentrations of leaves, roots, and stems of a sweet (KIT1) and a grain (Razinieh) sorghum genotype at post-flowering stage under control and salinity stress (100 mM NaCl) treatments for 15 days. (A–C) Na^+, K^+, and Ca^{2+} concentrations in the leaves, roots, and stems. Values are means ± SD of three replicates. Bars with different letters are significantly different (ANOVA, Tukey HSD test, $P \leq 0.05$). KIT1 = white bars, and Razinieh = black bars.

The patterns observed for Razinieh were similar overall, but were less persistent (stem, *SbSUT1*), lower in amplitude (stem, *SbSUT2*, *SbSUT6*), or delayed (all tested *SbSUTs* in the leaves), if compared with KIT1. There was one remarkable difference with respect to *SbNHX1*. Here, the accumulation in the leaves was significantly more pronounced in leaves of Razinieh as compared with leaves from KIT1 (Fig. 6D), while accumulation in the stem, although reaching similar levels as KIT1, was delayed and seen only at day 15 after the onset of salt stress. In summary, the induction of *SbSUT* genes in the stem of both sorghum genotypes under salinity stress conditions cannot account for the observed differences of sucrose accumulation that are recorded by the values for Brix, neither with respect to time nor with respect to amplitude. Thus, it is not transcriptional control of SUT transporters that matters, but rather a regulatory mechanism acting downstream of transcription. A straightforward hypothesis would be that the differences in vascular architecture allow to integrate sucrose transporters more efficiently in KIT1 as compared with Razinieh.

3.7. Differential compartmentalization of Na^+, K^+, and Ca^{2+}

The sodium-proton antiporter *NHX* is central for the sequestration of sodium in the vacuole (Falhof et al., 2016). The differential expression of *SbNHX1* in leaves of Razinieh and stem of KIT1 indicates differences in Na^+ sequestration or transport in these genotypes. Thus, the next step was to look at ion accumulation in leaves, stem and roots in both genotypes after 15 days of NaCl treatment. As to be expected from a salt treatment, all tissues had accumulated significant amounts of Na^+ after 15 days of 100 mM NaCl. However, the two genotypes differed significantly: Razinieh accumulated significantly higher concentrations of Na^+ in leaves, stem and roots compared with KIT1 (Fig. 7A). This was linked with a concomitant decrease of K^+, especially in leaves and stem under stress treatment compared with KIT1 (Fig. 7B). Thus, KIT1 is endowed with a higher capacity to maintain Na^+/K^+ homeostasis (Fig. 7D). Since the influx of Na^+ into plant cells causes, through plasma membrane depolarisation, an efflux of potassium, which plays a major role in cell turgidity especially in roots and leaves (Demidchik et al., 2014), the loss of K^+ efflux under salinity stress leads to a retardation of plant growth, a lower production, and can be in extreme cases even lethal. Thus, a robust buffering of Na^+/K^+ ratio is a hallmark for salt tolerance (Assaha et al., 2017).

The loss of ionic balance in Razinieh was also accompanied by significantly higher concentrations of Ca^{2+} in leaves compared with KIT1, both, under stress and in control conditions (Fig. 7C). Also in stem and roots of Razinieh, significantly higher concentrations of Ca^{2+} compared with KIT1 were observed, but here only under stress treatment (Fig. 7C). This might be linked with activation of voltage-dependent calcium-influx channels in consequence of the membrane depolarisation caused by Na^+ influx (Demidchik et al., 2001).

In summary, KIT1 has a higher capacity to cope with salt stress compared with Razinieh. While the transcript patterns of sugar transporters were not able to account for the observed differences in sugar concentration, there is a good congruence between the expression pattern of *SbNHX1* in leaves of Razinieh (Fig. 6D) and the higher Na^+ and Ca^{2+} accumulation in leaves of this genotype (Fig. 7A). More experiments should be done to further understand whether ion compartmentalisation in sorghum feeds back on the activity of sugar transporters and, hence, sugar accumulation patterns.

3.8. Model for stem sugar accumulation, individual leaf areas, and role of SbSUTs, and SbNHX1 genes in phloem loading and unloading of sucrose

The following theoretical model was proposed to explain the pattern of sugar accumulation, distribution of the leaf areas across the stem, cross areas for phloem and xylem areas, and the expression of *SbSUT*

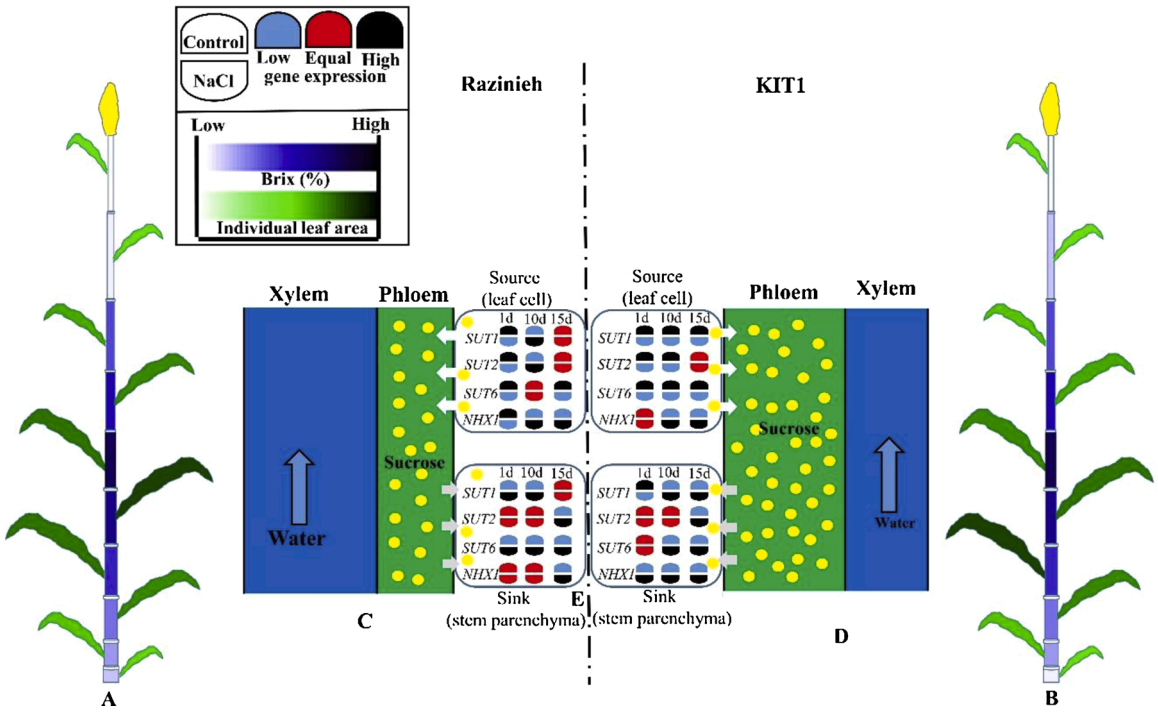

Fig. 8. Schematic models to explain the patern of sugar accumulation in the stem, the distrbution of the individual leaf area in each phytomer unit, and the role of *SbSUT*, and *SbNHX1* genes in phloem loading and unloading of sucrose. (A–B) The model links the higher sugar accumulation in the central internodes and their gradual decrease towards the top and bottom of the stem in both genotypes with the distribution of individual leaf areas along the phytomers. (D-C) The relation between the amplitude of sugar flow, and vascular architecture. Here, the larger phloem cross area in the stem, linked with a greater stem diameter, is causing higher sucrose accumulation in the stem, and is correlated with a smaller xylem area as compared with Razinieh. (E) Relative gene expression analysis of *SbSUT1*, *SbSUT2*, *SbSUT6*, and *SbNHX1* in a sweet (KIT1) and a grain (Razinieh) mature leaves and stems at 1, 10, and 15 days of control and salinity treatment (100 mM NaCl) at post-flowering stage.

genes in the source (leaves) and the storage tissues (stem) of a grain and a sweet sorghum genotypes (Fig. 8). The model links the higher sugar accumulation in the central internodes and their gradual decrease towards the top and bottom of the stem in both genotypes (Fig. 8A-B) with the distribution of individual leaf areas along the phytomers, where the leaves located in the centre of the stem show the highest area in both genotypes. This link is reflected by a highly significant and positive correlation between individual leaf area and sugar concentration measurable as degree Brix.

The second explanatory factor of the model is the relation between the amplitude of sugar flow, and vascular architecture. Here, the larger phloem cross area in the stem (Fig. 8D), linked with a greater stem diameter, is causing higher sucrose accumulation in the stem, and is correlated with a smaller xylem area as compared with Razinieh, which consequently has a smaller phloem cross area in the stem, a thinner stem diameter, and, hence, a lower sugar accumulation in the stem (Fig. 8C). An implication of this model would be a highly positive and significant relation of phloem cross area with total leaf area, and with sugar concentration manifest as Brix, while xylem cross area should be related inversely. Both implications of the model are strongly confirmed by the experimental data. The high expression of the *SbSUT1* gene in the source tissue (leaves), especially in KIT1, and the lower expression in sink tissues under control conditions is matching with the spatial patterns of sugar accumulation and with the differences between the genotypes consistent with a role of *SbSUT1* of sugar loading into the leaf phloem (Fig. 8E). Thus, the pattern of the *SbSUT* genes generally cannot account for the observed patterns of sugar accumulation observed under salt stress, indicating that, here, post-transcriptional mechanisms (such as integration of the transporters into the membrane, or the partitioning of phloem versus xylem differentiation) are more important. In contrast to the sugar pattern under salt stress, the partitioning of NaCl is well predicted by the transcript patterns for the transporter *SbNHX1*, for instance, with respect to the early induction in KIT1 stems or the delayed expression in Razinieh stem and leaf.

4. Conclusion

The sweet sorghum genotype KIT1 accumulated more sucrose in stem tissues in both, pre- and post-flowering, stages under normal and salinity stress conditions. The genotype KIT1 showed significantly heavier, sugars and juicy internodes, bigger phloem area and smaller xylem area compared with Razinieh. Phloem to xylem cross areas in internodes was correlated with the amount of sugar stored in stem. Razinieh was less efficient in maintaining Na^+/K^+ ratios under salt treatment. Transcriptional regulation of sucrose transporter genes could not give insight into the mechanism of differential sugar distribution in the two genotypes.

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CRediT authorship contribution statement

Adnan Kanbar: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources (seeds), Data curation, Writing - original draft. **Ehsan Shakeri:** Experimental work in the 2018 season. **Dema Alhajturki:** Formal analysis, Writing - review & editing. **Michael Riemann:** Designing the primers, Writing - review & editing. **Marco Tomasi Morgano** and **Dieter Stapf:** Ion analysis. **Peter Nick:** Supervision, Funding acquisition, Resources, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2021.113550.

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