Received: 15 November 2021 | Revised: 11 February 2022 | Accepted: 16 February 2022

DOI: 10.1111/ppl.13653

SPECIAL ISSUE ARTICLE

Physiologia Plantarum

Overexpression of the vacuolar sugar importer *Bv*TST1 from sugar beet in Camelina improves seed properties and leads to altered root characteristics

Gloria O. Okooboh[1] | Ilka Haferkamp[1] | Marzieh Valifard[1] | Benjamin Pommerrenig[1] | Amélie Kelly[2] | Ivo Feussner[2] | Horst Ekkehard Neuhaus[1]

[1]Plant Physiology, Faculty of Biology, University of Kaiserslautern, Kaiserslautern

[2]Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences and Göttingen Center for Molecular Biosciences (GZMB), University of Göttingen, Göttingen, Germany

Correspondence
Horst Ekkehard Neuhaus, Plant Physiology, Faculty of Biology, University of Kaiserslautern, Erwin-Schrödinger Straße, Kaiserslautern D-67653, Germany.
Email: neuhaus@rhrk.uni-kl.de

Funding information
Bundesministerium für Bildung und Forschung, Grant/Award Number: frame of the CAMPRO project (031B0343A) to IF; Deutsche Forschungsgemeinschaft, Grant/Award Number: Collaborative Research Center (Transregio-SFB) 171; Federal State of Rhineland Palatinate in the frame of the project BioComp

Edited by R. Le Hir

Abstract

Overexpression of the vacuolar sugar transporter *TST1* in Arabidopsis leads to higher seed lipid levels and higher total seed yield per plant. However, effects on fruit biomass have not been observed in crop plants like melon, strawberry, cotton, apple, or tomato with increased tonoplast sugar transporter (TST) activity. Thus, it was unclear whether overexpression of TST in selected crops might lead to increased fruit yield, as observed in Arabidopsis. Here, we report that constitutive overexpression of *TST1* from sugar beet in the important crop species *Camelina sativa* (false flax) resembles the seed characteristics observed for Arabidopsis upon increased TST activity. These effects go along with a stimulation of sugar export from source leaves and not only provoke optimised seed properties like higher lipid levels and increased overall seed yield per plant, but also modify the root architecture of *BvTST1* overexpressing Camelina lines. Such mutants grew longer primary roots and showed an increased number of lateral roots, especially when developed under conditions of limited water supply. These changes in root properties result in a stabilisation of total seed yield under drought conditions. In summary, we demonstrate that increased vacuolar TST activity may lead to optimised yield of an oil-seed crop species with high levels of healthy ω3 fatty acids in storage lipids. Moreover, since *BvTST1* overexpressing Camelina mutants, in addition, exhibit optimised yield under limited water availability, we might devise a strategy to create crops with improved tolerance against drought, representing one of the most challenging environmental cues today and in future.

1 | INTRODUCTION

Sugars and starch belong to the most prominent carbon storage products present in plants. As major carbohydrates, sugars represent molecules acting as prime energy reserves and that provide precursors for a wide range of primary and secondary metabolites synthesised, as well as for other storage products like cellulose, proteins or lipids (Ap Rees, 1988; Martin & Smith, 1995). Moreover, many studies reveal that sugar contents, composition, transport and compartmentation affect plant organ properties, storage product quality and impact

tolerance against biotic and abiotic stress stimuli (Conrath et al., 2003; Ho et al., 2019; Keller et al., 2021; Ko et al., 2021; Linke et al., 2002; Patzke et al., 2019; Pommerrenig et al., 2018, 2020). Because of this plethora of effects, it is not surprising that sugar levels are sensed in plants and corresponding signals are utilised to steer gene expression and development (Hanson & Smeekens, 2009; Tognetti et al., 2013).

In many plant cells, sugars accumulate to high levels in the large central vacuole occupying up to 85% of the cellular volume (Martinoia et al., 2007). We identified the carrier tonoplast sugar transporter (TST) as critical for vacuolar accumulation of glucose, fructose and sucrose in leaves as well as in sugar beet taproots (Jung et al., 2015; Wingenter et al., 2010; Wormit et al., 2006). In contrast to the Vacuolar Glucose Transporter1 (VGT1), which physiological function is mainly unresolved (Aluri & Büttner, 2007), the absence of TST in Arabidopsis leads to low monosaccharide levels in leaves (Wormit et al., 2006) and impaired accumulation of sugars in the cold (Klemens et al., 2014). Since these processes are a prerequisite to resist low environmental temperatures, disturbances of intracellular sugar homeostasis and compartmentation usually provoke impaired frost tolerance in plants (Alberdi & Corcuera, 1991; Hedrich et al., 2015; Patzke et al., 2019; Pommerrenig et al., 2018). Sugar transport across the vacuolar membrane, the tonoplast, is a dynamic process. Besides the VGT and TST type importers, the tonoplast contains several sugar exporters, e.g. the carriers Sugars Will Eventually be Transported 2, 16 and 17 (SWEETs) as well as the protein Early Response to Dehydration-Like 6 (ERDL) (Hedrich et al., 2015). While SWEET carriers act as facilitators, ERDL6 represents a proton-driven exporter (Chardon et al., 2013; Chen et al., 2015; Klemens et al., 2013, 2014; Poschet et al., 2011).

The notion that TST fulfils an important function for plant properties received further support by two independent observations. First, as revealed in Arabidopsis and cotton plants, TST activity is under the control of different types of protein kinases and corresponding phosphorylation of amino-acid residues, located in the large loop domain connecting transmembrane domain 6 and 7, stimulating vacuolar sugar import (Deng et al., 2020; Schulze et al., 2012; Wingenter et al., 2011). Second, a direct or indirect increase of TST activity in various mutants of different species leads to either higher total seed yield (Wingenter et al., 2010), or promotion of sugar levels in fruits and leaves (Cheng et al., 2017; Deng et al., 2020; Zhu et al., 2021).

It has been shown that sugar levels in fruits from various strawberry accessions correlate positively with TST expression in these tissues (Liu et al., 2020). In line with this, increased total sugar levels have been observed in fruits from apple, tomato, strawberry or melon mutants overexpressing *TST* genes (Deng et al., 2020; Zhu et al., 2021), while solely Arabidopsis mutants overexpressing *AtTST1* exhibited increased total fruit (seed) yield (Wingenter et al., 2010).

However, given that total crop harvest is one of the major goals of all breeding efforts (Wallace et al., 1993), it is extremely important to verify whether it is possible to increase total seed yield in crops by stimulation of TST activity. Such analyses gain further impact since intracellular sugar compartmentation affects stress tolerance and other basic properties of plant organs (Lastdrager et al., 2014; Pommerrenig et al., 2018; Thalmann & Santelia, 2017; Vu et al., 2020; Wang & Ruan, 2013). Accordingly, a detailed analysis of physiological consequences induced by overexpression of one of the main cellular sugar transporters, namely TST, is mandatory.

To this end, we aimed to overexpress the *TST1* gene from sugar beet (*Beta vulgaris*) in the oilseed crop species *Camelina sativa* (also named "false flax"). Camelina, like Arabidopsis is a member of the *Brassicaceae* family, has gained increasing interest as an alternative oil crop during the last decade (Faure & Tepfer, 2016; Vollmann & Eynck, 2015). This plant was used for plant oil production in Europe until the 1950ies, but was pushed aside by rapeseed (*Brassica napus*) from that time on (Zubr, 1997). Camelina-derived oil is used for industrial biofuel production, and especially in recent times, this crop regained attention since its storage oil is rich in healthy ω3-fatty acids (Feussner, 2015; Murphy, 2011). Both the high synteny of the hexaploid Camelina genome with the Arabidopsis genome (Kagale et al., 2014) and the possibility to create Camelina mutants via Agrobacterium or CRISPR-Cas9 (Lu & Kang, 2008; Morineau et al., 2017) further make this species a suitable candidate to check whether overexpression of *TST* might also stimulate total harvest in a crop species.

As an outcome of our efforts, we concluded that overexpression of *BvTST1* in Camelina not only led to increased lipid contents of single seeds and higher seed yield per plant but also stimulated shoot growth. Moreover, we observed that *BvTST1* overexpressing plants showed increased numbers of lateral roots (LR) and larger primary roots (PR), especially under drought conditions. Latter properties correlate with increased drought tolerance leading to a markedly higher seed yield when Camelina mutants grew under conditions of limited water availability.

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

Camelina sativa, var. Céline wild types and *BvTST1* overexpressing plants were cultivated on standard soil (ED-73, Patzer; https://www.einheitserde.de/) in a growth chamber under long-day conditions (16/8 h light/dark regime) at 22°C, 200 μmol quanta m^{-2} s^{-1} and 60% relative humidity. Generally, seeds were stratified in the dark at 4°C for 24 h prior to germination at 22°C. For cold experiments, plants were grown for 4 weeks under standard conditions thereafter transferred to cold temperature for 4 days, at 4°C. For drought stress, 40% field capacity was applied to soil grown plants as earlier described (Valifard et al., 2021).

For optical inspection of root architecture, ½ MS medium (pH 5.7, KOH, 0.5% sucrose) plates were used. To induce drought, ½ MS medium was supplemented with polyethylene glycol (PEG) corresponding to −0.7 or −1.2 MPa. Growth on agar plates was allowed for 6 days in total; 2 days under control conditions (no PEG) followed by transfer to new agar plates and four additional days of growth under either control- or drought stress conditions. To check

for re-initiation of LR growth under drought, plants were grown for 2 days under control conditions (no PEG), then transferred to new agar plates under either control- or drought stress conditions for 7 days. For growth experiments under sterile conditions, seeds were surface sterilised in 5% sodium hypochlorite before sowing.

2.2 | Generation of *BvTST1* overexpressing lines

The *pUBQ:BvTST1* construct was generated using the Gateway-specific destination vector pUB-Dest (Grefen et al., 2010). For this, the *BvTST1* coding sequence (inserted in pBSK) was amplified by PCR using *BvTST1*-specific primers harbouring *attB1* and *attB2* sites (Table S1), cloned via BP reaction into pDONR/ZEO (Invitrogen), and integrated by LR reaction into pUB-Dest. For the generation of Camelina lines overexpressing *BvTST1*, Agrobacterium-mediated transformation was used as described (Liu et al., 2012; Lu & Kang, 2008). Camelina transformation was carried out either by floral dip or vacuum infiltration method.

2.3 | Gene expression analysis via quantitative real-time PCR

RNA was extracted from 50 mg plant material – previously frozen in liquid nitrogen and finely ground – with the NucleoSpin® RNA Plant-Kit (Macherey-Nagel). For cDNA synthesis, RNA was reverse transcribed with the qScript cDNA Synthesis Kit (Quantabio). Primers used for gene expression analysis by qRT-PCR are listed in Table S1. *CsActin* and *CsTubulin* served as reference genes. Alternatively, the relative accumulation of mRNA was visualised by Northern blot analysis as previously described (Thulke & Conrath, 1998).

2.4 | Sugar quantification

For sugar extraction, 1 ml of 80% ethanol was added to 100 mg of frozen in liquid N_2 finely ground plant material, mixed and incubated for 30 min at 80°C in a thermomixer at 500 rpm. After centrifugation at 16,000 *g* (10 min at 4°C), the clear supernatant was used for sugar quantification using NADP-coupled enzymatic tests (Stitt et al., 1989).

2.5 | Amino acid quantification

For proline quantification, finely pulverised leaf samples were extracted with 80% ethanol as described above. For derivatisation, 20 µl of the supernatant was mixed with 60 µl borate buffer (0.2 M boric acid, pH 8.8) and 20 µl AQC (6-aminoquinolyl-*N*-hydroxysuccnimidyl carbamate. Watrex), prior to incubation at 55°C for 10 min. Quantification was done with HPLC, and chromatograms were analysed using the Chromeleon 6.7 software (Thermo-Fisher).

2.6 | Phloem exudates analysis

To quantify carbon export from source leaves, sugar levels were determined in leaf exudates as described earlier (Wingenter et al., 2010). Essentially, leaves from 4- to 5-week-old plants were cut under water, 4 h after onset of light. Three leaves from each plant were transferred into an Eppendorf reaction vessel filled with 400 µl of 20 mM EDTA solution (pH 7.5). Export was allowed for 4 to 6 h in a water-saturated atmosphere in the dark. Subsequently, leaves were removed, weighed and the solution transferred to the vacufuge concentrator to evaporate the water. Fully dried sediments were resuspended in 200 µl dd H_2O thereafter, sugar contents were determined using NADP-coupled enzymatic tests (Stitt et al., 1989).

2.7 | Seed analysis

For determination of the entire plant seed weight, Camelina plants were individually cultivated under standard growth conditions. Seeds were harvested at maturity and the biomass was subsequently weighed.

2.8 | Seed lipid quantification and fatty acid analysis

Fatty acid analysis was performed as described earlier (Marmon et al., 2017) with the following modifications: Crushing the seeds before derivatisation was omitted (Ma et al., 2020), and for each plant, 3× 10–15 seeds were dried overnight at 105°C and incubated together with 150–250 µg of tripentadecanoin standard in 2 ml of a methanol/toluene (2:1, v/v) solution containing 2.5% (v/v) H_2SO_4 (95%–97%) and 2% (v/v) dimethoxypropane at 80°C for 3 h. Given values are the trimmed means of individual plants after averaging the technical replicates for each plant and removing the highest and the lowest values per genotype.

3 | RESULTS

3.1 | Camelina gains increased TST activity by ectopic expression of the *TST1* gene from sugar beet (*Beta vulgaris*)

To create *Camelina sativa* mutants with increased activity of the vacuolar TST protein, we stably integrated the *BvTST1* gene from sugar beet (*Beta vulgaris*) via Agrobacterium-mediated gene transfer into the wild type genome. To this end, we chose the sugar beet homologue *BvTST1* (Jung et al., 2015) to prevent the possibility of the co-suppression phenomenon, which seems likely to occur when using instead the structurally related homologous Arabidopsis gene *AtTST1*.

The transformation efficiency of Camelina is substantially lower than those known for Arabidopsis (Liu et al., 2012). We screened

12 independent transformants for the presence of the recombinant *BvTST1* sequence using RT-qPCR. The three overexpressing lines, *BvTST1#1*, *#2* and *#12*, exhibited the highest relative *BvTST1* transcript levels and were chosen for further analysis (Figure S1A; please note: all transgenic plants analysed below were homozygous for the transgene as confirmed by BASTA resistance). The presence of recombinant *BvTST1* mRNA in the transgenic Camelina lines has been confirmed by Northern-blot analyses using a radioactive probe specific for *BvTST1* mRNA, which does not react with mRNA from wild type plants (Figure S1B).

To reveal whether the expression of the *BvTST1* gene in Camelina leads to increased TST activity, we quantified levels of the major vacuolar located sugars glucose, fructose and sucrose in leaves from 4-week-old plants. When grown at 21°C, wild types contained 6.5 μmol glucose, 0.3 μmol fructose and 7.1 μmol sucrose per g fresh weight (FW) (Figure 1A). All three *BvTST1* overexpressing Camelina lines contained similar levels of glucose and fructose, while lines *BvTST1 #2* and *#12* exhibited increased sucrose levels compared to wild types, reaching 9.5 and 10.8 μmol g^{-1} FW, respectively (Figure 1A). Starch levels in all three transgenic Camelina lines were similar to those of wild types and ranged between 64.8 to 74.4 μmol C6/g FW (Figure 1B).

It is known that cold stress conditions induce increased sugar levels in plants and that Arabidopsis *tst1-2* double knock-out plants lack the ability to accumulate sugars in vacuoles upon cold treatment (Klemens et al., 2014). Thus, checking for altered sugar homeostasis in *BvTST1* overexpressing Camelina plants grown at low temperatures was tempting.

Similar to other species, the exposure of Camelina to low temperature leads to a marked increase of glucose, fructose and sucrose in leaves. After 4 days at 4°C, glucose accumulated about eight-fold when compared to the level at 21°C, fructose reached about 18 μmol g^{-1} FW and sucrose levels rose to 30 μmol g^{-1} FW (Figure 1C). Interestingly, while *BvTST1* lines *#1*, *#2* and *#12* showed only moderately altered sugar levels at 21°C (Figure 1A), all three overexpressing lines exhibited significantly higher glucose and sucrose levels in the cold when compared to corresponding contents in wild types. Glucose levels in cold treated *BvTST1* overexpressing plants ranged between 61.2 to 65.9 μmol g^{-1} FW, and sucrose ranged between 39.9 to 46.4 μmol g^{-1} FW. *BvTST1 #12* exhibited slightly higher fructose levels when compared to wild types (23.4 μmol g^{-1} FW instead of 18.7 μmol g^{-1} FW in WT), while the concentration of the latter type of sugar appeared only slightly higher in the other two *BvTST1* overexpressing lines when compared to cold treated wild types (Figure 1C).

3.2 | *BvTST1* overexpressors show increased biomass accumulation and early flowering

It has been shown that overexpression of *AtTST1* in Arabidopsis promoted germination and subsequent development of young seedlings (Wingenter et al., 2010). To check for similar effects in

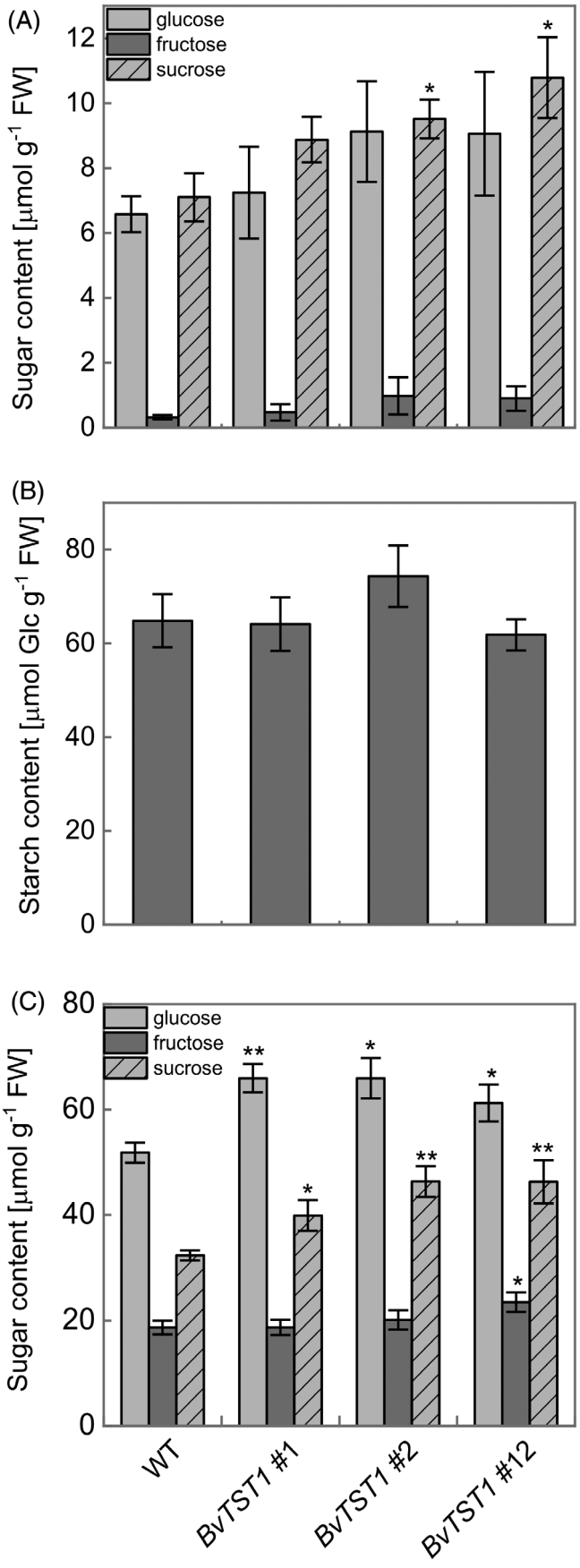

FIGURE 1 Legend on next page.

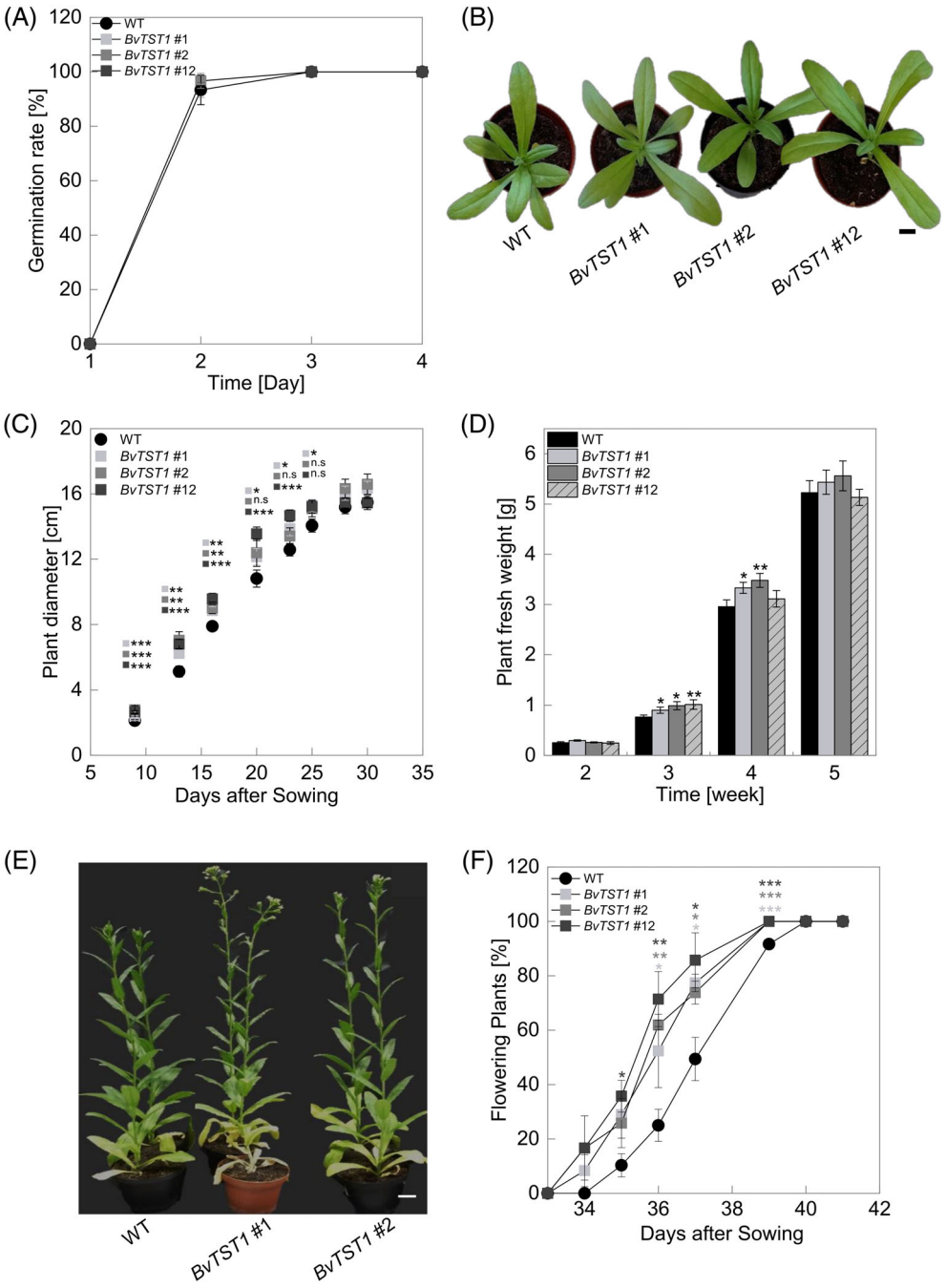

FIGURE 2 Germination, growth and development analysis of wild types and *BvTST1* overexpressing plants. (A) Germination rates. (B) Representative image of 3-week-old wild types and three independent *BvTST1* overexpressing lines, bar = 2 cm. (C) Plant diameters. (D) Fresh weights. (E) Representative image of 5-week-old plants of wild types and *BvTST1 #1* and *#2*, bar = 2 cm. (F) Representative phenotypes of flowering plants. Plants were cultivated on soil under standard growth conditions. Data represent means ± SE of $n = 6$ individual pots with 20 seeds each for (A), and $n = 26$ individual plants for (C), (D) and (F). Statistical analysis of differences between wild types and overexpressing plants were calculated using the Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

FIGURE 1 Carbohydrate levels in leaves of Camelina wild types and *BvTST1* overexpressing plants. Four-week-old plants, grown on soil under standard conditions (16 h light/ 8 h dark) were used for analysis. For cold experiments, plants were moved to 4°C for 4 days. All samples analysed were harvested at the end of light phase. (A) Soluble sugar contents. (B) Starch contents. (C) Soluble sugar contents from cold treated plants. Data represent mean values of $n = 4$ to six plants ± SE. Statistical analysis of differences between wild types and overexpressing plants were calculated using Student's *t*-test (*$P < 0.05$, **$P < 0.01$)

BvTST1 over-expressing Camelina plants, we compared their growth pattern with that of wild type plants.

The germination efficiency of seeds from *BvTST1* overexpressing plants was identical to that of wild types (Figure 2A). Two days after the transfer of cold-stratified seeds into warm conditions (21°C), about 96% of all seeds showed a radicle and after 3 days, all seeds were germinated. Since the same germination stadium was reached after 3 days at 21°C, we conclude that the germination efficiency of Camelina seeds under the chosen conditions is generally close to 100%.

Optical inspection showed that 3 weeks post-germination *BvTST1 #1*, *#2* and *#12* mutant plants exhibited a larger diameter when compared to corresponding wild types (Figure 2B). To analyse a putative dynamic of this phenomenon, we quantified the average plant diameter during the entire growth period. Seven days after germination (DAG), the plant diameter of wild types and the three *BvTST1* overexpressing lines were similar and reached about 2.5 cm (Figure 2C). However, at 13-DAG, the three overexpressing lines exhibited a larger plant

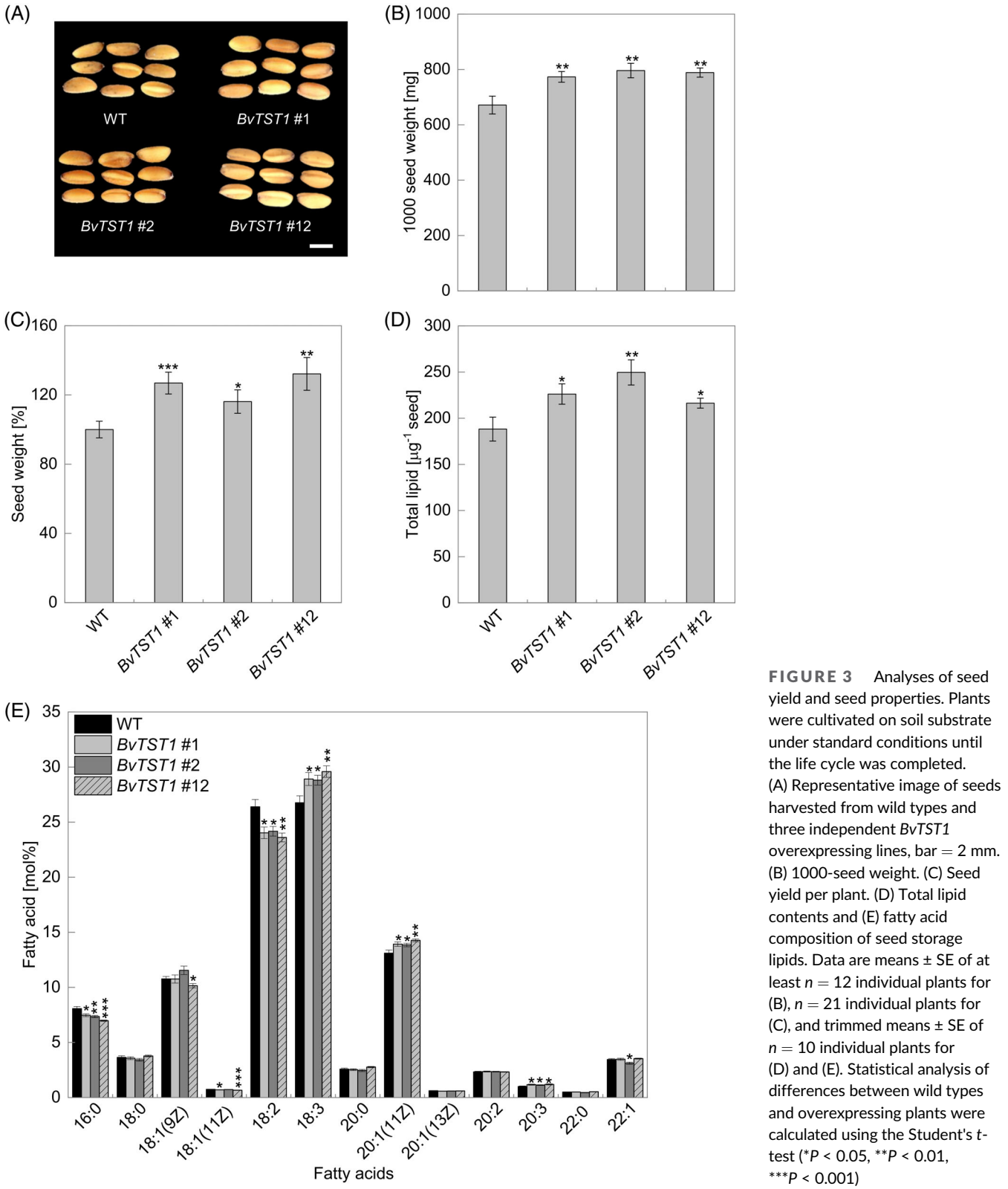

FIGURE 3 Analyses of seed yield and seed properties. Plants were cultivated on soil substrate under standard conditions until the life cycle was completed. (A) Representative image of seeds harvested from wild types and three independent *BvTST1* overexpressing lines, bar = 2 mm. (B) 1000-seed weight. (C) Seed yield per plant. (D) Total lipid contents and (E) fatty acid composition of seed storage lipids. Data are means ± SE of at least $n = 12$ individual plants for (B), $n = 21$ individual plants for (C), and trimmed means ± SE of $n = 10$ individual plants for (D) and (E). Statistical analysis of differences between wild types and overexpressing plants were calculated using the Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

diameter when compared to wild types (Figure 2C). This tendency was observed until 25-DAG (Figure 2C), while from 30-DAG on all plants analysed exhibited a diameter of about 15.5 to 16.6 cm (Figure 2C). This increased plant diameter between 13- and 25-DAG (Figure 2C) was also present when the plant biomass was quantified (Figure 2D). Two weeks post-germination, all four plant lines exhibited a similar FW of about 0.26 to 0.30 g, while 3 weeks after germination, the three *BvTST1* lines showed about 17.7 to 32.3% higher FW when compared to wild types (Figure 2D). After 4 weeks, *BvTST1 #1* and *#2* plants still exhibited higher FWs than present in wild types, and after 5 weeks, all four plant lines exhibited nearly identical FWs ranging between 5.0 to 5.6 g per plant (Figure 2D).

Interestingly, we observed that *BvTST1* lines *#1*, *#2* and *#12* exhibited earlier flowering when compared to corresponding wild types (Figure 2E). Until 33-DAG, none of the plant lines showed emerging inflorescences (Figure 2F). However, between 34 and 39-DAG, all three *BvTST1* overexpressing lines exhibited, on average, a higher number of flowering individuals when compared to wild types (Figure 2F). At 40-DAG, all plant lines had reached the flowering status (Figure 2F).

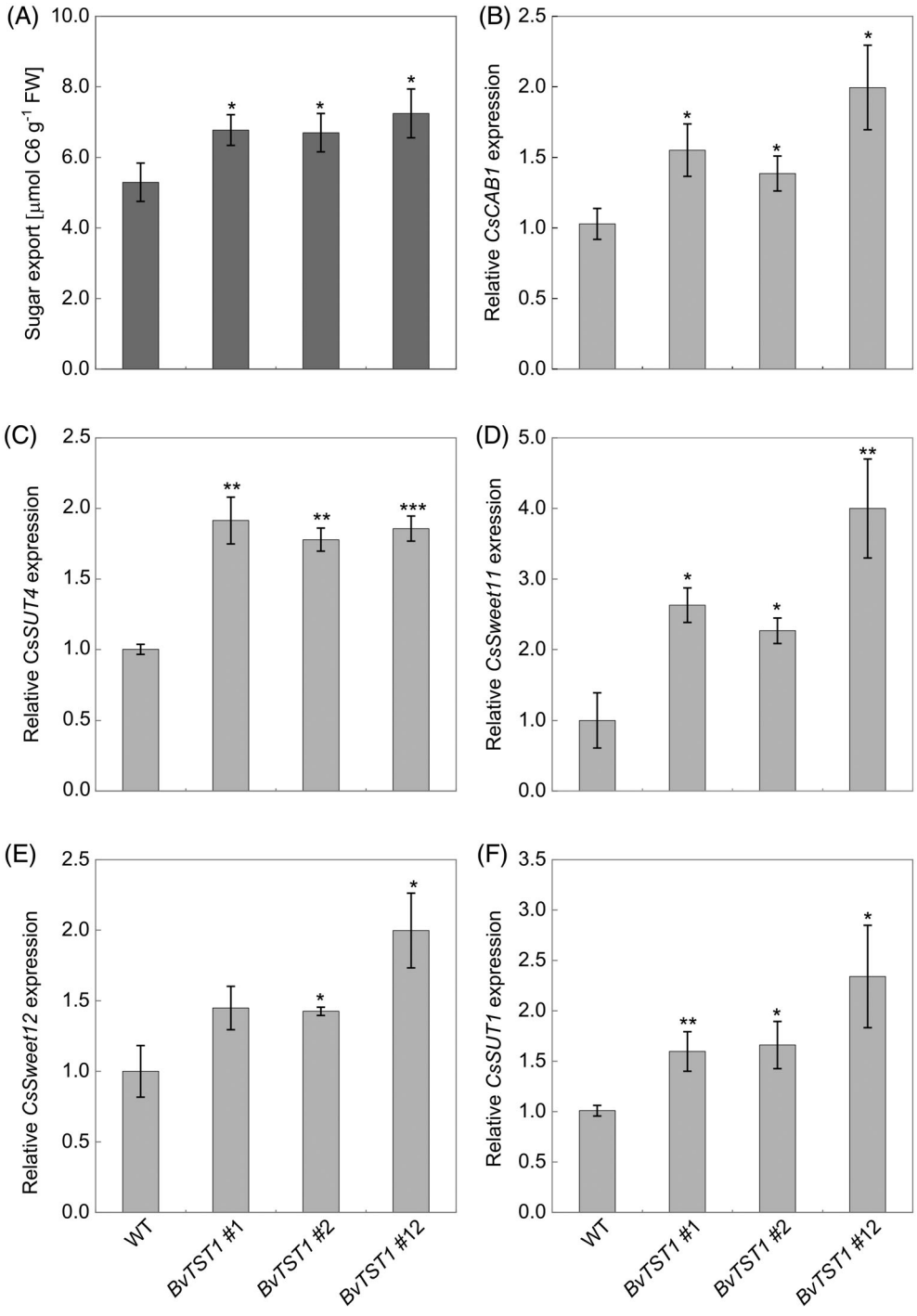

FIGURE 4 Phloem exudate quantification, expression analysis of photosynthesis and sugar transport related genes from leaves of wild types and three independent *Bv*TST1 overexpressing lines. Plants were grown for 5 weeks on standard conditions before samples were harvested. (A) Sugar contents of phloem exudates from detached, fully expanded Camelina leaves. Leaves were detached 4 h after onset of light and exudation was continued for 4–6 h in the dark. (B) Expression of *CsCAB1* in leaves. (C) Expression of *CsSUT4* in leaves. (D) Expression of *CsSWEET11* in leaves. (E) Expression of *CsSWEET12* in leaves. (F) Expression of *CsSUT1* in leaves. Data represent means ± SE of $n = 3$ independent experiments for (A), $n = 3$ or 4 individual plants for (B), to (E), and $n = 7$ individual plants for (F). Statistical analysis of differences between wild types and overexpressing lines were calculated using the Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

3.3 | *BvTST1* overexpressing lines exhibit increased seed yield and higher storage lipid levels

Overexpression of *AtTST1* in Arabidopsis leads to increased seed yield (Wingenter et al., 2010). However, so far, it is unclear whether a similar effect can also be observed on an oil-producing crop species exhibiting increased TST activity. To this end, we harvested seeds from fully dried inflorescences and analysed the respective total seed biomass from individual plants of the four plant lines.

Optical inspection of representative seeds from all plant lines provided the impression that seeds from each *BvTST1* overexpressing mutant were slightly larger when compared to seeds from wild types (Figure 3A). Quantification of the 1000-seed weight confirmed that seeds from all three *BvTST1* overexpressing Camelina lines exhibited increased biomasses. 1000 seeds from *BvTST1* #1 exhibited a weight of 773 mg, representing an increase of 15% when compared to wild types, 1000 seeds from *BvTST1* #2 exhibited a weight of 796 mg, representing an increase of 18% when compared to wild types and seeds from *BvTST1* #12 reached a weight of 789 mg, representing an increase of about 17.4% when compared to wild types (Figure 3B). Moreover, it turned out that all three *BvTST1* overexpressing lines exhibited between 16% (*BvTST* #2) to 32% (*BvTST* #12) total seed yield per plant when compared to corresponding wild types (Figure 3C).

Similar to the increased seed biomass per plant of *BvTST1* lines (Figure 3C), the total lipid levels in mutant seeds increased compared to wild types. The total lipid levels/seed rose similarly to the 1000 seed weight increased in *BvTST1* overexpressor lines. Seeds from wild types contained about 188 μg lipid/seed, while seeds from *BvTST1* #1 contained 226 μg lipid/seed (+20%), followed by seeds from *BvTST1* #2 with 250 μg lipid/seed (+33%) and seeds from *BvTST1* #12, which approached 216 μg lipid/seed. The latter value corresponds to an increase of +15% compared to wild type seeds (188 μg lipid/seed) (Figure 3D).

A detailed analysis of the individual fatty-acid (FA) composition in triacyl-glycerols (TAG, storage lipids) revealed that the relative contents of 16:0, 18:2, 18:3, 20:1 and 20:3 in seeds from *BvTST1* overexpressing lines differed from corresponding wild types, while the relative contribution of the other FAs to total TAGs appeared to be similar in all plant lines (Figure 3E). Most marked changes by overexpression of *BvTST1* have been observed in respect to the levels of 18:2 and 18:3 FAs, since all three *BvTST1* overexpressors showed about 8 to 10.6% less 18:2 FAs, while the 18:3 FA level appeared to be increased in these mutant lines by about 7.6% to 10.6%, when compared to corresponding wild type values (Figure 3E).

3.4 | *BvtTST1* overexpressing lines exhibit increased capacity for long-distance sugar transport

Increased shoot growth and higher seed yield (Figures 2 and 3) point to the possibility that leaves from *BvTST1* overexpressing lines exhibit increased source to sink transport of sugars. To monitor for such a phenomenon, we first quantified sugar transport in detached Camelina source leaves. To get more information on the molecular

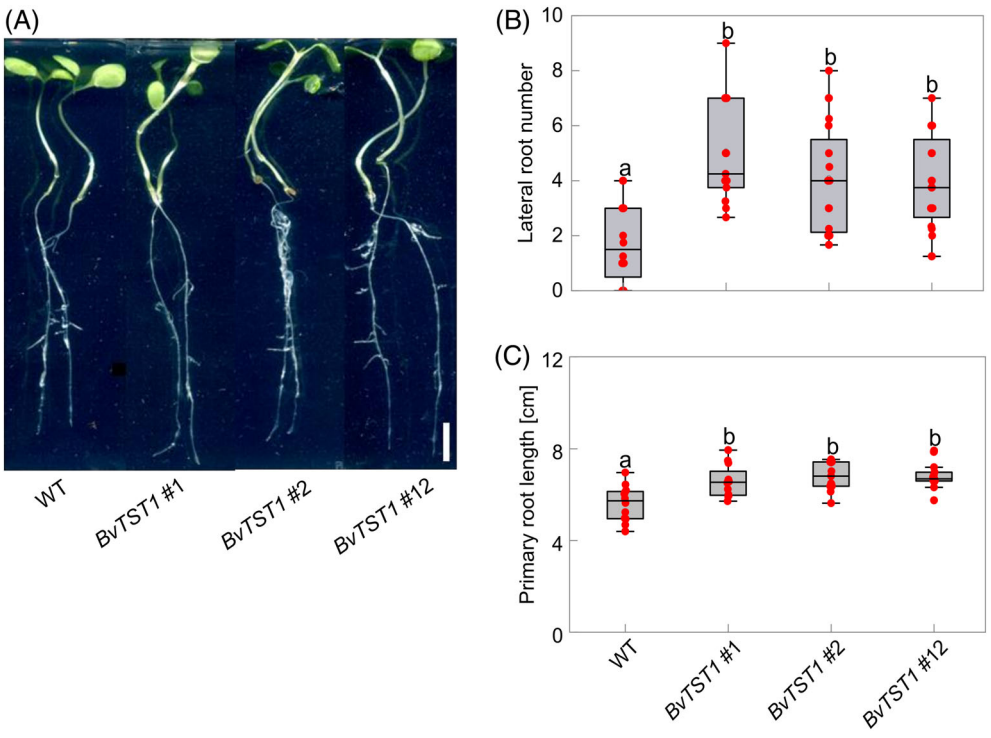

FIGURE 5 Root development analysis of wild types and *Bv*TST1 overexpressing seedlings grown on ½ MS medium. (A) Images of Camelina wild types and *BvTST1* lines grown on agar plates, bar = 2 cm. (B) Lateral root number. (C) Primary root length. For (B) and (C), data represent the means ± SE of four pools each consisting of four seedlings for all lines analysed. Different letters above boxes denote significant differences according to *one-way* ANOVA with *post-hoc* Tukey HSD test ($P < 0.05$)

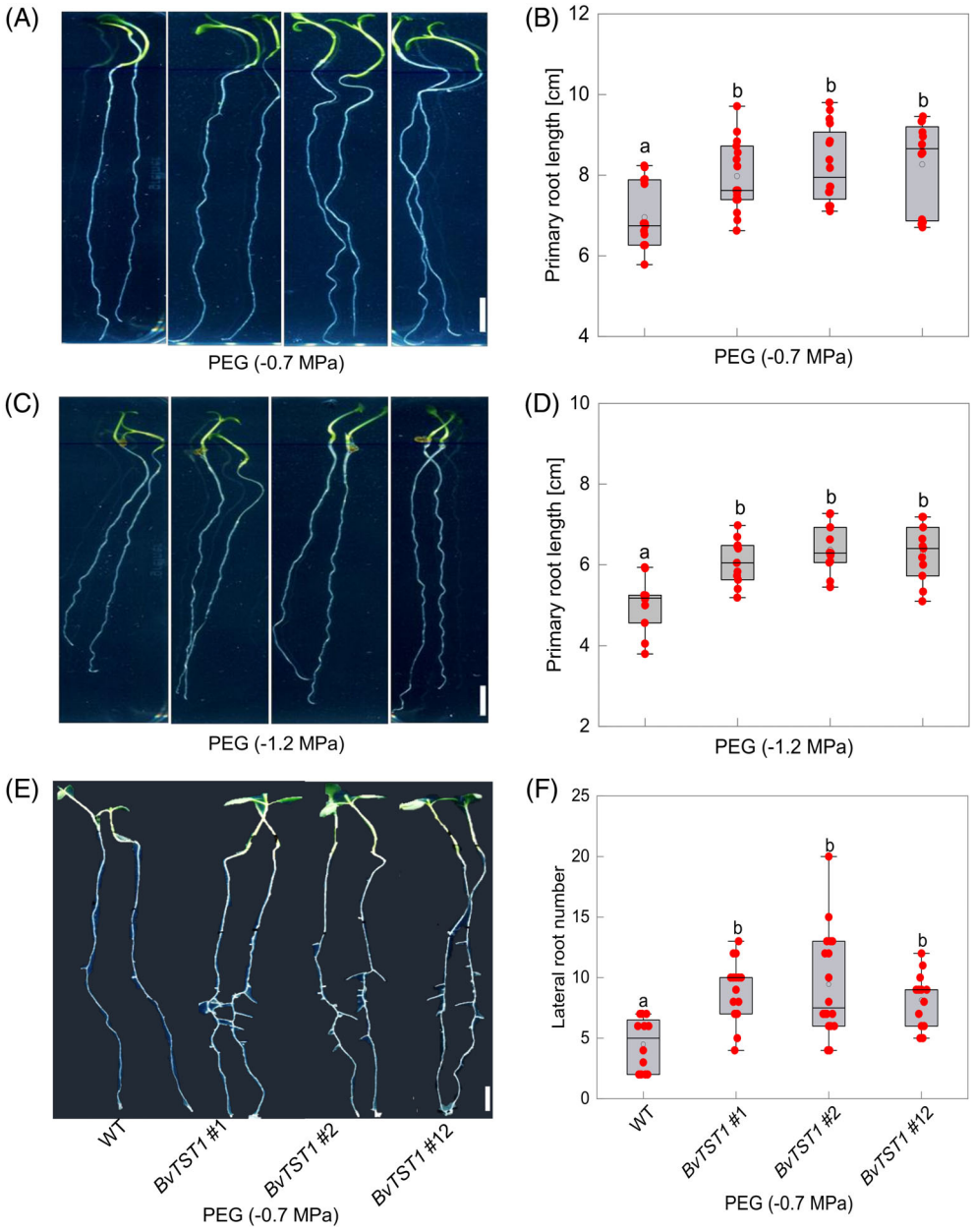

FIGURE 6 Effect of drought stress induced by polyethylene glycol (PEG) treatment on root development of wild types and overexpressing plants grown on agar plates. Plants were treated with PEG 2 d after sowing. (A) Images of 4-day PEG treated seedlings grown at −0.7 MPa. (B) Quantification of PR length of PEG treated seedlings grown at −0.7 MPa. (C) Images of 4-day PEG treated seedlings grown at −1.2 MPa. (D) Quantification of PR length of PEG treated seedlings grown at −1.2 MPa. (E) Re-initiation of lateral root development of seedlings grown for 7 days at −0.7 MPa. (F) Quantification of lateral roots on seedlings grown for 7 days at −0.7 MPa. Bar = 2 cm. For (B), (D) and (F), data represent the means ± SE of four pools each consisting of four seedlings for all lines analysed. Different letters above boxes denote significant differences according to *one-way* ANOVA with *post-hoc* Tukey HSD test ($P < 0.05$)

mechanisms connected to an increased sugar export capacity of *BvTST1* overexpressing leaves, we quantified mRNAs coding for various proteins involved in either, photosynthesis or sugar transport.

Detached leaves from wild types exported within 5 h of the experiment about 5.3 μmol C6/g FW, leaves from all three *BvTST1* overexpressing lines showed stimulated sugar export. Leaves from *BvTST1 #1* exported sugars equivalent to 6.8 μmol C6/g FW, and leaves from *BvTST1 #2* and *#12* exported sugars equivalent to 6.7 and 7.3 μmol C6/g FW, respectively (Figure 4A).

The expression of *CsCAB1*, coding for the chlorophyll$_{A/B}$ binding protein1, was slightly increased in leaves of *BvTST1* overexpressors, reaching about a two-fold increase in *BvTST1 #1* when compared to the corresponding mRNA in wild types (Figure 4B). Similar to this, the expression levels of the vacuolar sugar exporter *CsSUT4* and the plasma membrane located sucrose transporters *CsSWEET11* and *12* were higher in *BvTST1* overexpressing leaves when compared to wild types (Figure 4C–E). In Arabidopsis, both SWEET transporters (SWEET 11 and 12) are involved in sucrose transport into the phloem and in the vasculature (Chen et al., 2012; Le Hir et al., 2015). In Arabidopsis and other vascular plants, the apoplasmic loading of sucrose into the phloem sieve element/companion cell (SE/CC) complex is mediated by SUC2/SUT1 type carriers (Gould et al., 2012; Srivastava et al., 2008; Truernit & Sauer, 1995). Interestingly, similar to the expression of the other transporter genes monitored (Figure 4B–E) *CsSUT1* expression in leaves from *BvTST1* overexpressing lines was increased when compared to corresponding values in wild types and ranged from 1.6-fold in *BvTST1 #1* to 2.3-fold in *BvTST1 #12* (Figure 4F).

3.5 | Overexpression of *BvTST1* leads to altered morphology of Camelina roots

The composition and levels of sugars affect plant organ development, partly due to the close interaction of the cellular carbohydrate status with hormone homeostasis and regulation of gene expression (Kiba et al., 2019; Rellán-Álvarez et al., 2016; Valifard et al., 2021; Zürcher et al., 2016). However, so far, it has not been analysed whether altered TST1 activity affects root properties. Thus, to check for an effect of *BvTST1* overexpression on Camelina root properties, we grew wild types and the three mutant lines under either control or drought stress conditions on vertical ½ MS-agar plates and analysed root characteristics via optical inspection.

It turned out that *BvTST1* overexpressing lines had an increased number of LR when compared to corresponding wild types (Figure 5A,B). Latter plants exhibited on average only about 2.0 LR per PR, while *BvTST1* overexpressing lines exhibited on average between 3.9 to 5.0 LR per PR (Figure 5B). After 6 days of growth, the average PR length of the *BvTST1* lines was slightly larger than PR length on wild types (Figure 5A). Under latter conditions, PRs from *BvTST1* plants reached 119% of the PR length exhibited by wild types (Figure 5A,C).

To check whether drought stress promotes specific alterations of the root architecture, we repeated this experiment in the presence of polyethylene glycol (PEG), mimicking drought corresponding to an osmotic potential of either −0.7 or −1.2 MPa (Michel, 1983) (for details see also Valifard et al., 2021). As given above, after 6 days of growth under control conditions (no PEG), wild type PRs exhibited an average length of 5.6 cm, while *BvTST1* overexpressing plants showed a slightly larger average PR length between 6.5 to 6.8 cm (Figure 5A,C).

Under drought, corresponding to −0.7 MPa, the relative PR length differences wild types and *BvTST1* overexpressing lines appeared similar to control conditions, as PRs from mutants exhibited an increased PR length of about +19% (Figure 6A,B). However, under stronger drought conditions of 1.2 MPa, the relative differences of PR lengths between these plant lines became larger as mutant PR reached e.g. up to 6.4 cm (*BvTST1 #2*), representing a 28% greater length when compared to the average wild type PRs (Figure 6C,D).

Above, we demonstrated that *BvTST1* overexpressors grown under control conditions exhibited a higher number of LRs than present in wild types (Figure 5A,B). Interestingly, in contrast to growth under control conditions (Figure 5A,B), Camelina does not show any LR after 4 days of growth under drought stress (Figure 6A,C). However, after three additional days under drought, Camelina is able to

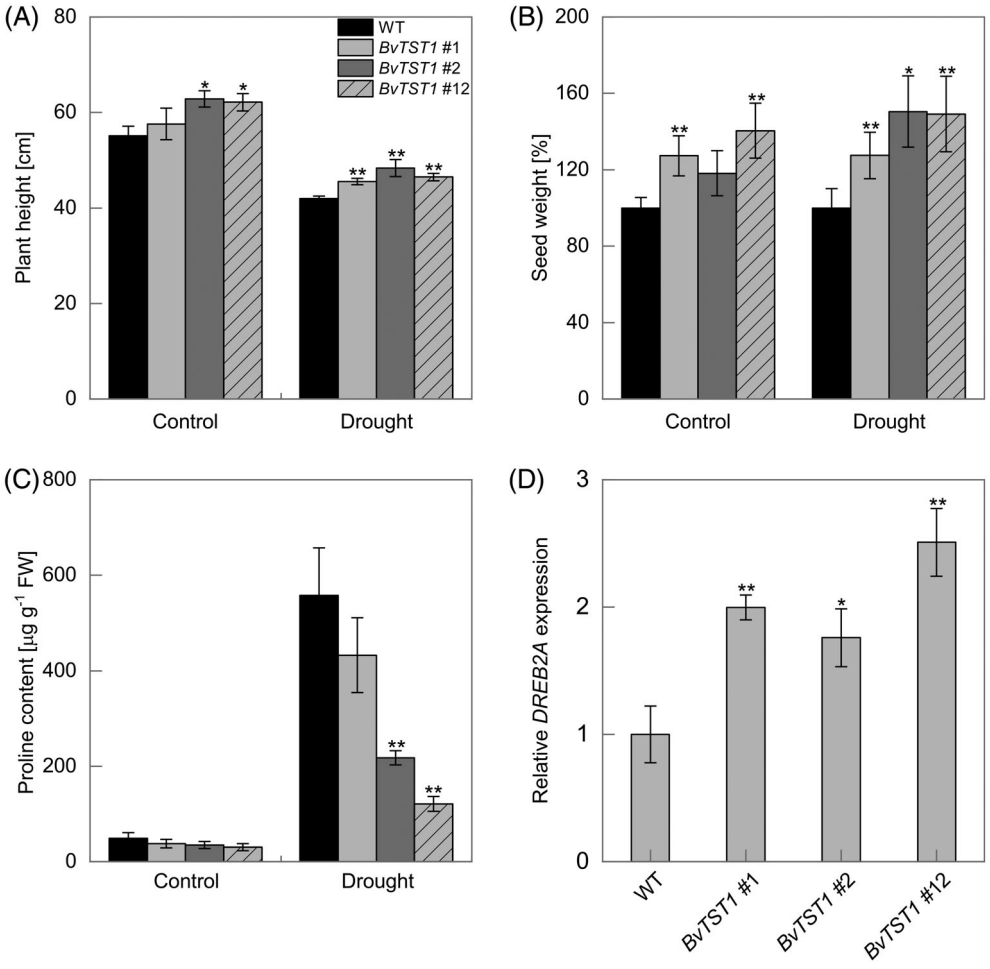

FIGURE 7 Effect of drought stress on development, seed yield, proline levels and expression of the drought related gene *DREB2A*. (A) Plant height of 6-week-old wild types and *Bv*TST1 overexpressing lines. (B) Total seed yield per plant. (C) Leaf proline contents of wild types and *Bv*TST1 overexpressing lines under control or drought stress conditions. Drought stress was applied for 10 days. (D) Expression of the gene coding for a drought related transcription factor, *DREB2A* in leaves of 10 days drought stressed plants. Data represent means ± SE of $n = 4$ individual plants for (A), at least 15 to 20 individual plants for (B), $n = 6$ individual plants for (C), and $n = 4$ individual plants for (D). Statistical analysis of differences between wild types and *Bv*TST1 overexpressing lines was calculated using the Student's *t*-test (*$P < 0.05$, **$P < 0.01$)

reinitiate LR growth leading on average to about 4.5, remarkable short LRs (Figure 6E,F). Interestingly, under those conditions all three *BvTST1* overexpressor Camelina lines showed an increased ability to reinitiate LR formation when compared to wild types, leading in average to 7.8 to 9.5 LR per mutant plant, and all LR on mutants appeared markedly longer than LR on correspondingly grown wild types (Figure 6E,F).

3.6 | Expression of the *BvTST1* gene leads to improved drought tolerance of Camelina

To determine whether altered root architecture leads to modified drought stress tolerance, we grew all plant lines on soil at either well-watered conditions (control) or under drought conditions (Figure 7). We already demonstrated above that the average plant height of wild types was slightly less than that of *BvTST1* overexpressors (Figure 2E). After 6 weeks of growth under well-watered control conditions, wild types reached an average height of about 55 cm, while *BvTST1 #2* and *#12* mutants reached, on average, 61 and 60 cm, respectively (Figure 7A). Under drought conditions, wild type plant height was decreased to 41.9 cm, and the average plant height of all three *Bv*TST1 overexpressors was slightly, but statistically significant, larger when compared to corresponding wild types (Figure 7A).

As given before, *Bv*TST1 overexpressor plants grown under control conditions showed increased seed biomass of about 12% to 32% compared to seed biomass harvested from wild types (Figure 3A). Interestingly, the relative differences between seed biomasses harvested from wild types and *BvTST1* overexpressor plants were even larger when grown under drought stress conditions. All three *BvTST1* overexpressing lines showed significantly increased seed biomasses, ranging between 42.6% in the case of *Bv*TST1 #1 to 49% in the case of plants from *Bv*TST1 #2 and #12, when compared to seed yield of drought-stressed wild types (Figure 7B).

Proline levels can be taken as a molecular readout for drought stress (Liang et al., 2013). Interestingly, the increased seed yield of *Bv*TST1 overexpressor lines correlates with lower leaf proline levels when compared to wild types. Under control conditions, all plant lines contained similar levels of proline in leaves, ranging between 30.6 to 49 $\mu g\ g^{-1}$ Fw (Figure 7C). In contrast, under drought conditions, when proline in wild type leaves accumulates to about 558 $\mu g\ g^{-1}$ Fw (Figure 7C), the corresponding levels in *Bv*TST1 overexpressors appeared to be lower. *Bv*TST1 overexpressor line #1 exhibited slightly but statistically not significantly lower proline levels compared to wild types. However, *Bv*TST1 overexpressing lines #2 and #12 showed markedly lower proline levels when compared to wild types, namely only 218 and 120 $\mu g\ g^{-1}$ FW, respectively (Figure 7C).

In general, altered expression of transcription factors might contribute to the tolerance properties (Singh & Laxmi, 2015). Among these, the transcription factor DREB2A is known to be involved in the induction of drought tolerance in Arabidopsis (Sakuma et al., 2006). Compared to wild types, the levels of mRNA coding for the DREB2A protein in *Bv*TST1 overexpressing lines were significantly higher and reached about 2 to 2.5-fold of the *DREB2A* mRNA levels observed in drought-stressed wild types (Figure 7D).

4 | DISCUSSION

Sugar levels and cellular sugar compartmentation is critical for plant yield and important plant properties (Pommerrenig et al., 2018; Ruan, 2012, 2014). In most types of vascular plant cells, the vacuole represents the largest organelle (Martinoia et al., 2007) and is the main cellular side for storage of a wide range of solutes, including several types of sugars (Martinoia et al., 2012). Accordingly, vacuolar import and export of sugars represent a dynamic process and several sugar carriers, mediating transport of glucose, fructose and sucrose, have been demonstrated to reside in the vacuolar membrane, the tonoplast (Hedrich et al., 2015).

Some members of the large SUGAR WILL EVENTUALLY BE TRANSPORTED (SWEET) and EARLY RESPONSE TO DEHYDRATION6-LIKE (ERDL) protein families are involved in vacuolar sugar export (Chardon et al., 2013; Chen et al., 2015; Guo et al., 2014; Klemens et al., 2014; Poschet et al., 2011). However, according to the current data so far available, only VGT1 (VACUOLAR GLUCOSE TRANSPORTER1) (Aluri & Büttner, 2007) and TST (TONOPLAST SUGAR TRANSPORTER)-type proteins (Cho et al., 2010; Jung et al., 2015; Wormit et al., 2006) function as efficient importers. This is because the latter transporters exploit the existing pH gradient across the tonoplast to accumulate sugars in the organelle lumen against a concentration gradient (Aluri & Büttner, 2007; Schulz et al., 2011; Wingenter et al., 2011).

The physiological function of VGT1 is still enigmatic (Aluri & Büttner, 2007), *inter alia* since VGT1 loss-of-function mutants hardly show any peculiarity when compared to wild types, and because the isoform VGT3, now named pSuT, resides in the chloroplast (Aluri & Büttner, 2007; Patzke et al., 2019). In contrast to this both, down- or upregulation of TST activity in various species provoke marked biochemical and physiological responses. E.g., *TST* loss-of-function Arabidopsis lines show impaired sugar accumulation in cold conditions (Klemens et al., 2014; Wormit et al., 2006), while increased TST activity in melon, tomato and apple plants leads to higher cellular sugar levels (Cheng et al., 2017; Zhu et al., 2021).

Remarkably, increased TST activity in the seed oil-storing model plant Arabidopsis induces a larger size of individual seeds, higher seed lipid levels and an increased overall seed yield per plant (Wingenter et al., 2010). However, increased biomasses of fruits have not been observed in all other species analysed so far, namely melon, cotton, apple and tomato mutants exhibiting increased TST activities (Cheng et al., 2017; Deng et al., 2020; Zhu et al., 2021). These observations in various species questions whether stimulation of TST activity in crops might lead to an increased harvest of plant storage organs. Moreover, since sugar levels and especially intracellular sugar compartmentation not only affect fruit properties (Lastdrager et al., 2014; Wang & Ruan, 2013), but also architecture and properties of roots (Takahashi et al., 2003; Valifard et al., 2021) we studied here in addition to seed

characteristics also specific properties of roots from Camelina mutants overexpressing the vacuolar sugar importer *BvTST1*.

Overexpression of *BvTST1* in Camelina leads to larger seeds, increased storage lipids in mature seeds and higher seed yield per plant (Figure 3A–D). These characteristics fully concur with corresponding observations on *AtTST1* overexpressing Arabidopsis mutants (Wingenter et al., 2010). However, increased biomass and seed yield of *BvTST1* overexpressing Camelina does not resemble data raised on fruits from melon, strawberry and tomato mutants with increased TST activities (Cheng et al., 2017, Zhu et al., 2021). All *TST* overexpressing mutants of the latter species exhibit higher fruit sugar levels, while increased fruit size or higher fruit yield per plant have not been observed. The molecular reason for this discrepancy between various plant species overexpressing *TST* is unclear. However, especially the chosen promotors seem not to be the reason for such discrepancy. This is, because similar to *BvTST1* overexpression in Camelina, which is driven by the ubiquitin promoter (see Materials and Methods), overexpression of the heterologous melon *TST1* homologue in strawberry also occurred under control of a constitutive and strong promotor, namely the Cauliflower 35S promotor (Cheng et al., 2017). However, strawberry fruits accumulate sugars at high levels, while both Arabidopsis and Camelina seeds represent fruits with low sugars but high lipid and protein contents.

In contrast to TST mutants from strawberry and melon, increased TST activity in tomato plants is the indirect consequence of a stimulated vacuolar glucose export induced by overexpression of the apple (*Malus domestica*) protein ERDL6 (Zhu et al., 2021). *Md*ERDL6 is the closest homologue to the Arabidopsis vacuolar glucose exporter *At*ERDL6 (Zhu et al., 2021), and both proteins function as proton/glucose symporters exporting glucose into the cytosol (Poschet et al., 2011; Zhu et al., 2021). Thus, increased TST activity in tomato fruits is a consequence of altered sugar signalling caused by glucose accumulation in the cytosol (Zhu et al., 2021), lifting the degree of metabolic complexity even higher than already present in TST overexpressing Arabidopsis, Camelina or melon plants ((Figure S1) and (Cheng et al., 2017; Wingenter et al., 2010)).

Similar to the situation in melon, strawberry, apple and tomato with increased TST activity the overexpression of the TST1 activating CBL interacting Protein Kinase6 (CIPK6) from cotton (*Gossypium hirsutum*) in either cotton or Arabidopsis leads to higher leaf sugar levels without an effect on fruit yield (Deng et al., 2020). In the case of *BvTST1* overexpressing Camelina, we observed increased sugar levels in leaves solely under conditions of cold temperature but not under conditions of ambient temperature (Figure 1A,C), which resembles closely the observations made on *AtTST1* overexpressing Arabidopsis plants (Wormit et al., 2006). It is well known that TST proteins can become activated by phosphorylation, either via CIPK6 (Deng et al., 2020) or via the protein kinase VIK1, belonging to the large mitogen-activated protein triple kinase family (Wingenter et al., 2010). Since phosphorylation of TST is leading to increased transport activity in reconstituted and in *in vivo* systems (Deng et al., 2020; Schulze et al., 2012; Wingenter et al., 2011), we speculate that this type of modification of TST proteins is able to stimulate transporter activity stronger, when compared to the sole overexpression of the endogenous or the recombinant gene.

Higher sugar levels in leaves of cold treated *BvTST1* overexpressing Camelina mutants (Figure 1C) is a clear indication that the additional presence of *Bv*TST1 protein leads to higher pumping of sugars into the vacuole. However, we assume that even under ambient temperature, where *BvTST1* overexpressing Camelina plants show similar total sugars levels as present in wild types (Figure 1A), the sugar compartmentation in the mesophyll cell is altered, as seen in Arabidopsis overexpressing *AtTST1* (Wingenter et al., 2010). The latter conclusion is due to the observation that *CAB1* mRNA accumulation in leaves from *BvTST1* overexpressing Camelina plants is increased when compared to wild types (Figure 4B). Since *CAB1* mRNA accumulation is strongly inhibited during the accumulation of glucose in the cytosol (Koch, 1996) we assume that mesophyll cells of *BvTST1* lines exhibit decreased glucose concentrations in the cytosol and higher glucose levels in the vacuole when compared to wild types. Since a similar process is also present in Arabidopsis mutants overexpressing the endogenous *TST1* gene (Wingenter et al., 2010) we propose that both, *AtTST1* and *BvTST1* exhibit, when overexpressed *in planta*, highly similar biochemical properties, in particular the ability to import glucose. In fact, when heterologously expressed in Xenopus oocytes both carriers are able to transport glucose in counter exchange with H^+ (Jung et al., 2015; Schulz et al., 2011), which explains that increased TST activity causes a cytosolic decrease of glucose.

Another remarkable characteristic of *BvTST1* overexpressing Camelina mutants is their early flowering phenotype (Figure 2E,F). This property is likely not due to an acceleration of early plant development since seed germination of mutant and wild type plants is nearly identical (Figure 2A). Instead, this phenomenon speaks for an increased sugar export capacity of source leaves into sink structures, like e.g. the flower meristem. This assumption is likely since (1) it is known that increasing sucrose levels in inflorescence meristem cells induce flower induction (Wahl et al., 2013) and (2) by the demonstrated increased export of sugars from source leaves from *BvTST1* overexpressing plants (Figure 4A). The latter observation is fully in line with increased levels of *SUT1* mRNA in leaves of *BvTST1*-lines (Figure 4C), coding for the main phloem sucrose loader (Gould et al., 2012; Truernit & Sauer, 1995) and, moreover, resembles similar observations made on *AtTST1* overexpressing Arabidopsis mutants (Wingenter et al., 2010). We also observed, that *BvTST1* overexpressing Camelina plants exhibited higher expression of the *SWEET11* and *SWEET12* genes (Figure 4D,E). This finding provides further explanation why these mutants exhibit increased sugar export capacity from source leaves (Figure 4A), since both facilitators mediate sucrose export from parenchyma cells into the SE/CC complex (Chen et al., 2012) which is a prerequisite for subsequent proton coupled import into the phloem via SUC2/SUT1 (Sauer & Stolz, 1994).

Similar to the effect of TST1 overexpression in Arabidopsis, the overexpression of *BvTST1* in Camelina leads to higher seed lipid levels, as well as to an increased 1000 seed weight and increased overall seed harvest per plant (Figure 3A–D). It appears interesting that not

only the total lipid level per seed is increased, but also the levels of several fatty acids, especially 18:2 and 18:3 in storage lipids of *BvTST1* overexpressing lines, differs in comparison to wild type seeds (Figure 3E). Without detailed analyses of metabolic fluxes and enzyme properties, it is impossible to find an explanation for this phenomenon. However, we have to keep in mind that the synthesis and composition of storage lipids and their corresponding FAs in Camelina, as in other species, depends upon the concerted action of many different enzymes, like isoforms of acyltransferases or fatty-acid desaturase isoforms (Lee et al., 2021; Marmon et al., 2017). Thus, given that increased TST1 activity leads to higher concentrations of fatty-acid precursors in developing seeds, we assume that the individual catalytic response of each enzyme depends upon its individual substrate affinity and its specific activity in respective cells. In this context, it is worth mentioning that a similar observation has been made on another plant storage product, namely starch. Although the sole metabolic precursor for starch synthesis is the nucleotide sugar ADP-glucose, altered levels of this metabolite not only correlate directly with starch levels in storage organs but explicitly change the corresponding amylose to amylopectin ratio (Geigenberger et al., 2001; Lloyd et al., 1999; Tjaden et al., 1998). The latter effect is also due to different $K_{M(ADPGlc)}$ values of the soluble and granula-bound starch synthases involved in starch synthesis (Seung, 2020).

That *BvTST1* overexpressing Camelina mutants exhibit increased capacity to provide sugars to sink tissues is further indicated by analysing the speed of the plant development and biomass accumulation after seed germination (Figure 2B–D), and by comparing the development of the root architecture of wild types and mutants (Figure 5). Latter plants, when grown without drought stress, exhibit an increased number of LRs and slightly larger PRs when compared to wild types (Figure 5A–G). It is known that increased sugar levels in roots generally stimulate LR formation (Takahashi et al., 2003). Therefore, the observation that *BvTST1* Camelina plants exhibit a markedly higher number of LRs when compared to wild types (Figure 5A,B) further supports the conclusion that the stimulation of long-distance photosynthate transport in mutants (Figure 4A) is the reason for improved sink tissue development.

Generally, drought stress impairs LR development and promotes PR growth (Deak & Malamy, 2005; Xiong et al., 2006). This effect also presents when Camelina roots develop under drought stress conditions (Figure 6A,C) and represents a conserved morphogenetic reaction of vascular plants to reach deeper soil horizons still containing residual water (Koevoets et al., 2016). Interestingly, under drought conditions, the relative difference of PR length between *BvTST1* overexpressing plants and wild types is higher under conditions of optimal water supply (Figures 5C and 6B,D).

To interpret this relative difference of the root architecture, we have to keep in mind that drought-stressed plants activate the sugar transfer from source leaves to roots, which selectively promotes PR growth (Durand et al., 2016). In that context, it seems worth mentioning that drought stress induces the expression of the *SWEET11* gene. The corresponding transport activity, which is connected to phloem loading, turned out to be critical for photosynthate transfer into roots and maintains an appropriate root growth (Durand et al., 2016). Moreover, in Arabidopsis, both SWEET11 and 12 have been shown to be involved in xylem development (Le Hir et al., 2015), representing the long-distance transport system for water. Thus, our observation that all *BvTST1* overexpressing lines exhibit higher expression of the homologous genes *CsSWEET11* and *CsSWEET12* (Figure 4D,E) when compared to wild types, is fully in line with the proposed important function of SWEET11 activity for drought tolerance and water supply (Durand et al., 2016; Le Hir et al., 2015). Interestingly, the increased export of sucrose from source leaves (Figure 4A) seems to be further promoted by stimulation of *CsSUT4* expression in *BvTST1* overexpressing mutants (Figure 4C), known to be an efficient vacuolar sugar unloader (Schneider et al., 2012).

The stabilisation of seed production in *BvTST1* lines under drought (Figure 7B) might not only be due to a promotion of PR growth under such stress stimulus (Figure 6A–D). This increased stress resistance might also be due to the increased number of LRs in *BvTST1* plants as these plants reinitiate LR formation – especially under intense drought stress – more efficiently than wild types (Figure 6E,F). Although the inhibition of LR formation is a typical response of vascular plants under limited water supply (Deak & Malamy, 2005; Koevoets et al., 2016; Xiong et al., 2006), the loss of too many LRs also provokes impaired drought resistance (Yu et al., 2008). In addition, we showed recently that Arabidopsis mutants with decreased LR length under drought exhibited impaired tolerance towards limited water supply (Valifard et al., 2021). In other words, molecular mechanisms stabilising a certain size and number of LRs obviously contribute to increased drought tolerance, since those plants not only reach deeper soil horizons by increased PR length (Figure 6B), but might also exploit remaining water reservoirs in higher soil horizons (Figure 6E,F).

BvTST1 overexpressing Camelina plants experience less drought stress when compared to corresponding wild types as indicated by a slightly higher size (Figure 7A) and – most important – by a higher seed biomass under such challenging conditions (Figure 7B). Thus, it seems feasible that the larger PRs and more LRs of mutants (Figure 6B,F) allow these plants to exploit the soil more efficiently for remaining water. In other words, the reason why *BvTST1* overexpressing plants exhibit relatively higher seed biomasses than correspondingly stressed wild types is that they experience less drought stress. This assumption seems in particular justified since these Camelina mutants contain lower proline levels in leaves under drought (Figure 7C) and it is well known that proline accumulation can be taken as a general signal for stress in vascular plants (Liang et al., 2013). However, we cannot exclude that other, so far, unresolved molecular factors also contribute to the increased drought tolerance of *BvTST1* overexpressing Camelina mutants. E.g., the latter mutants show, for so far unknown reasons, higher expression of the *DREB2A* gene (Figure 7D). DREB2A is a transcription factor crucially involved in drought response (Nakashima et al., 2009) and mutants with higher mRNA coding for the DREB2A protein show increased drought tolerance (Sakuma et al., 2006).

5 | CONCLUSIONS

Overall, we conclude that overexpression of *BvTST1* in *Camelina sativa* improves plant properties regarding plant growth, seed size, seed quality and seed harvest and drought tolerance. Thus, stimulation of TST activity, which is possible by different approaches like direct overexpression of a corresponding gene (Figure S1) or post-translational modification of existing TST proteins (Deng et al., 2020) might avenue a strategy to improve the harvest of crop plants under conditions of low water availability. Thus, it might be worth checking the traits typical for *Bv*TST1 overexpressing *Camelina sativa* lines now under field conditions and, in case of confirmation, it might also be worth introducing such genetic construct into other crops, e.g. rapeseed.

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft (DFG) in the frame of the Collaborative Research Center (Transregio-SFB) 171 (*The Green Hub*, Project Z1) and the Federal State of Rhineland Palatinate in the frame of the project *BioComp* to EN and the Federal Ministry of Education and Research (BMBF) in frame of the *CAMPRO* project (031B0343A) to IF.

Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Gloria O. Okooboh conducted most experiments, Ilka Haferkamp supported cloning, Marzieh Valifard supported drought experiments, Benjamin Pommerrenig supported data interpretation, Amélie Kelly conducted lipid- and fatty acid analysis, Ivo Feussner supported data interpretation. Horst Ekkehard Neuhaus conducted data interpretation, wrote the manuscript and led the project.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as all new created data are already contained within this article.

ORCID

Gloria O. Okooboh https://orcid.org/0000-0002-3611-8401
Ilka Haferkamp https://orcid.org/0000-0002-7432-3190
Marzieh Valifard https://orcid.org/0000-0002-4851-8656
Benjamin Pommerrenig https://orcid.org/0000-0002-7522-7942
Amélie Kelly https://orcid.org/0000-0003-2142-9327
Ivo Feussner https://orcid.org/0000-0002-9888-7003
Horst Ekkehard Neuhaus https://orcid.org/0000-0002-5443-7123

REFERENCES

Alberdi, M. & Corcuera, L.J. (1991) Cold-acclimation in plants. *Phytochemistry*, 30, 3177–3184.

Aluri, S. & Büttner, M. (2007) Identification and functional expression of the *Arabidopsis thaliana* vacuolar glucose transporter1 and its role in seed germination and flowering. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 2537–2542.

Ap Rees, T. (1988) Hexose phosphate metabolism by nonphotosynthetic tissues of higher plants. In: Preiss, J. (Ed.) *The Biochemistry of Plants*, Vol. 14, 1988. New York: Academic Press, pp. 1–33.

Chardon, F., Bedu, M., Calenge, F., Klemens, P.A., Spinner, L., Clement, G. et al. (2013) Leaf fructose content is controlled by the vacuolar transporter SWEET17 in Arabidopsis. *Current Biology*, 23, 697–702.

Chen, H.Y., Huh, J.H., Yu, Y.C., Ho, L.H., Chen, L.Q., Tholl, D. et al. (2015) The Arabidopsis vacuolar sugar transporter SWEET2 limits carbon sequestration from roots and restricts Pythium infection. *The Plant Journal*, 83, 1046–1058.

Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R. et al. (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science*, 335, 207–211.

Cheng, J., Wen, S., Xiao, S., Lu, B., Ma, M. & Bie, Z. (2017) Overexpression of the tonoplast sugar transporter *Cm*TST2 in melon fruit increases sugar accumulation. *Journal of Experimental Botany*, 69, 511–523.

Cho, J.I., Burla, B., Lee, D.W., Ryoo, N., Hong, S.K., Kim, H.B. et al. (2010) Expression analysis and functional characterization of the monosaccharide transporters, OsTMTs, involving vacuolar sugar transport in rice (*Oryza sativa*). *New Phytology*, 186, 657–668.

Conrath, U., Linke, C., Jeblick, W., Geigenberger, P., Quick, W.P. & Neuhaus, H.E. (2003) Enhanced resistance to *Phythophthora infestans* and *Alternaria solani* in leaves and tubers, respectively, of potato plants with decreased activity of the plastidic ATP/ADP transporter. *Planta*, 19, 75–83.

Deak, K.I. & Malamy, J. (2005) Osmotic regulation of root system architecture. *The Plant Journal*, 43, 17–28.

Deng, J., Yang, X., Sun, W., Miao, Y., He, L. & Zhang, X. (2020) The calcium sensor CBL2 and its interacting kinase CIPK6 are involved in plant sugar homeostasis via interacting with tonoplast sugar transporter TST2. *Plant Physiology*, 183, 236–249.

Durand, M., Porcheron, B., Hennion, N., Maurousset, L., Lemoine, R. & Pourtau, N. (2016) Water deficit enhances C export to the roots in *Arabidopsis thaliana* plants with contribution of sucrose transporters in both shoot and roots. *Plant Physiology*, 170, 1460–1479.

Faure, J.-D. & Tepfer, M. (2016) Camelina, a Swiss knife for plant lipid biotechnology. *OCL*, 23, D503.

Feussner, I. (2015) Camelina—a promissing oilseed crop to contribute to the growing demand for vegetable oils. *European Journal of Lipid Science and Technology*, 117, 271–273.

Geigenberger, P., Stamme, C., Tjaden, J., Schulze, A., Quick, W.P., Betsche, T. et al. (2001) Tuber physiology and properties of starch from tubers of transgenic potato plants with altered plastidic adenylate transporter activity. *Plant Physiology*, 125, 1667–1678.

Gould, N., Thorpe, M.R., Pritchard, J., Christeller, J.T., Williams, L.E., Roeb, G. et al. (2012) *At*SUC2 has a role for sucrose retrieval along the phloem pathway: Evidence from carbon-11 tracer studies. *Plant Science*, 188, 97–101.

Grefen, C., Donald, N., Hashimoto, K., Kudla, J., Schumacher, K. & Blatt, M. R. (2010) A ubiquitin-10 promoter-based vector set for fluorescent protein tagging facilitates temporal stability and native protein distribution in transient and stable expression studies. *The Plant Journal*, 64, 355–365.

Guo, W.J., Nagy, R., Chen, H.Y., Pfrunder, S., Yu, Y.C., Santelia, D. et al. (2014) SWEET17, a facilitative transporter, mediates fructose transport across the tonoplast of Arabidopsis roots and leaves. *Plant Physiology*, 164, 777–789.

Hanson, J. & Smeekens, S. (2009) Sugar perception and signaling--an update. *Current Opinion in Plant Biology*, 12, 562–567.

Hedrich, R., Sauer, N. & Neuhaus, H.E. (2015) Sugar transport across the plant vacuolar membrane: nature and regulation of carrier proteins. *Current Opinion in Plant Biology*, 25, 63–70.

Ho, L.-H., Klemens, P.A.W., Neuhaus, H.E., Ko, H.-Y., Hsieh, S.-Y. & Guo, W.-J. (2019) *Sl*SWEET1a is involved in glucose import to young leaves in tomato plants. *Journal of Experimental Botany*, 70, 3241–3254.

Jung, B., Ludewig, F., Schulz, A., Meißner, G., Wöstefeld, N., Flügge, U.I. et al. (2015) Identification of the transporter responsible for sucrose accumulation in sugar beet taproots. *Nature Plants*, 1, 14001.

Kagale, S., Koh, C., Nixon, J., Bollina, V., Clarke, W., Tuteja, R. et al. (2014) The emerging biofuel crop *Camelina sativa* retains a highly undifferentiated hexaploid genome structure. *Nature Communications*, 5, 3706.

Keller, I., Rodrigues, C.M., Neuhaus, H.E. & Pommerrenig, B. (2021) Improved resource allocation and stabilization of yield under abiotic stress. *Journal of Plant Physiology*, 257, 153336.

Kiba, T., Takebayashi, Y., Kojima, M. & Sakakibara, H. (2019) Sugar-induced de novo cytokinin biosynthesis contributes to Arabidopsis growth under elevated CO_2. *Scientific Reports*, 9, 7765.

Klemens, P.A., Patzke, K., Deitmer, J., Spinner, L., Le, H.R., Bellini, C. et al. (2013) Overexpression of the vacuolar sugar carrier AtSWEET16 modifies germination, growth and stress tolerance in Arabidopsis. *Plant Physiology*, 163, 1338–1352.

Klemens, P.A., Patzke, K., Trentmann, O., Poschet, G., Büttner, M., Schulz, A. et al. (2014) Overexpression of a proton-coupled vacuolar glucose exporter impairs freezing tolerance and seed germination. *New Phytologist*, 202, 188–197.

Ko, H.-Y., Ho, L.-H., Neuhaus, H.E. & Guo, W.-J. (2021) Transporter *Sl*SWEET15 unloads sucrose from phloem and seed coat for fruit and seed development in tomato. *Plant Physiology*, 187, 2230–2245. https://doi.org/10.1093/plphys/kiab290

Koch, K.E. (1996) Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 47, 509–540.

Koevoets, I.T., Venema, J.H., Elzenga, J.T.M. & Testerink, C. (2016) Roots withstanding their environment: exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science*, 7, 1335.

Lastdrager, J., Hanson, J. & Smeekens, S. (2014) Sugar signals and the control of plant growth and development. *Journal of Experimental Botany*, 65, 799–807.

Le Hir, R., Spinner, L., Klemens, P.A.W., Chakraborti, D., de Marco, F., Vilaine, F. et al. (2015) Disruption of the sugar transporters *At*SWEET11 and *At*SWEET12 affects vascular development and freezing tolerance in Arabidopsis. *Molecular Plant*, 8, 1687–1690.

Lee, K.-R., Jeon, I., Yu, H., Kimg, S.-G., Kim, H.-S., Ahn, S.-J. et al. (2021) Increasing monounsaturated fatty acid contents in hexaploid *Camelina sativa* seed oil by *FAD2* gene knockout using CRISPR-Cas9. *Frontiers in Plant Science*, 12, 1279.

Liang, X., Zhang, L., Natarajan, S.K. & Becker, D.F. (2013) Proline mechanisms of stress survival. *Antioxidants & Redox Signaling*, 19, 998–1011.

Linke, C., Conrath, U., Jeblick, W., Betsche, T., Mahn, A., Düring, K. et al. (2002) Inhibition of the plastidic ATP/ADP-transporter protein primes potato tubers for augmented elicitation of defense responses and enhances their resistance against *Erwinia carotovora*. *Plant Physiology*, 129, 1607–1615.

Liu, H.-T., Ji, Y., Liu, Y., Tian, S.-H., Gao, Q.-H., Zou, X.-H. et al. (2020) The sugar transporter system of strawberry: genome-wide identification and expression correlation with fruit soluble sugar-related traits in a *Fragaria* × *ananassa* germplasm collection. *Horticulture Research*, 7, 132.

Liu, X., Brost, J., Hutcheon, C., Guilfoil, R., Wilson, A.K., Leung, S. et al. (2012) Transformation of the oilseed crop *Camelina sativa* by Agrobacterium-mediated floral dip and simple large-scale screening of transformants. *In Vitro Cellular & Developmental Biology - Plant*, 48, 462–468.

Lloyd, J.R., Springer, F., Buléon, A., Müller-Röber, B., Willmitzer, L. & Kossmann, J. (1999) The influence of alterations in ADP-glucose pyrophosphorylase activities on starch structure and composition in potato tubers. *Planta*, 209, 230–238.

Lu, C. & Kang, J. (2008) Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by Agrobacterium-mediated transformation. *Plant Cell Repots*, 27, 273–278.

Ma, S., Du, C., Ohlrogge, J. & Zhang, M. (2020) Accelerating gene function discovery by rapid phenotyping of fatty acid composition and oil content of single transgenic T1 Arabidopsis and Camelina seeds. *Plant Direct*, 4, e00253.

Marmon, S., Sturtevant, D., Herrfurth, C., Chapman, K., Stymne, S. & Feussner, I. (2017) Two acyltransferases contribute differently to linolenic acid levels in seed oil. *Plant Physiology*, 173, 2081–2095.

Martin, C. & Smith, A.M. (1995) Starch biosynthesis. *Plant Cell*, 7, 971–985.

Martinoia, E., Maeshima, M. & Neuhaus, H.E. (2007) Vacuolar transporters and their essential role in plant metabolism. *Journal of Experimental Botany*, 58, 83–102.

Martinoia, E., Meyer, S., De Angeli, A. & Nagy, R. (2012) Vacuolar transporters in their physiological context. *Annual Review of Plant Biology*, 63, 163–213.

Michel, B.E. (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology*, 72, 66–70.

Morineau, C., Bellec, Y., Tellier, F., Gissot, L., Kelemen, Z., Nogué, F. et al. (2017) Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid *Camelina sativa*. *Plant Biotechnology Journal*, 15, 729–739.

Murphy, E.J. (2011) Versatile Camelina: The future of bioful and much more. *Inform*, 22, 604–608.

Nakashima, K., Ito, Y. & Yamaguchi-Shinozaki, K. (2009) Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiology*, 149, 88–95.

Patzke, K., Prananingrum, P., Klemens, P.A.W., Trentmann, O., Martins Rodrigues, C., Keller, I. et al. (2019) The plastidic sugar transporter pSuT influences flowering and affects cold responses. *Plant Physiology*, 179, 569–587.

Pommerrenig, B., Ludewig, F., Cvetkovic, J., Trentmann, O., Klemens, P.A.W. & Neuhaus, H.E. (2018) In concert: orchestrated changes in carbohydrate homeostasis are critical for plant abiotic stress tolerance. *Plant and Cell Physiology*, 59, 1290–1299.

Pommerrenig, B., Müdsam, C., Kischka, D. & Neuhaus, H.E. (2020) Treat and trick: common regulation and manipulation of sugar transporters during sink establishment by the plant and the pathogen. *Journal of Experimental Botany*, 71, 3930–3940.

Poschet, G., Hannich, B., Raab, S., Jungkunz, I., Klemens, P.A., Krüger, S. et al. (2011) A novel Arabidopsis vacuolar glucose exporter is involved in cellular sugar homeostasis and affects the composition of seed storage compounds. *Plant Physiology*, 157, 1664–1676.

Rellán-Álvarez, R., Lobet, G. & Dinneny, J.R. (2016) Environmental control of root system biology. *Annual Review of Plant Biology*, 67, 619–642.

Ruan, Y.L. (2012) Signaling role of sucrose metabolism in development. *Molecular Plant*, 5, 763–765.

Ruan, Y.L. (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. *Annual Review of Plant Biology*, 65, 33–67.

Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K. et al. (2006) Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell*, 18, 1292–1309.

Sauer, N. & Stolz, J. (1994) SUC1 and SUC2: two sucrose transporters from *Arabidopsis thaliana*; expression and characterization in baker's yeast and identification of the histidine-tagged protein. *The Plant Journal*, 6, 67–77.

Schneider, S., Hulpke, S., Schulz, A., Yaron, I., Holl, J., Imlau, A. et al. (2012) Vacuoles release sucrose via tonoplast-localised SUC4-type transporters. *Plant Biology*, 14, 325–336.

Schulz, A., Beyhl, D., Marten, I., Wormit, A., Neuhaus, E., Poschet, G. et al. (2011) Proton-driven sucrose symport and antiport are provided by the vacuolar transporters SUC4 and TMT1/2. *The Plant Journal*, 68, 129–136.

Schulze, W.X., Schneider, T., Starck, S., Martinoia, E. & Trentmann, O. (2012) Cold acclimation induces changes in Arabidopsis tonoplast protein abundance and activity and alters phosphorylation of tonoplast monosaccharide transporters. *The Plant Journal*, 69, 529–541.

Seung, D. (2020) Amylose in starch: towards an understanding of biosynthesis, structure and function. *New Phytologist*, 228, 1490–1504.

Singh, D. & Laxmi, A. (2015) Transcriptional regulation of drought response: a tortuous network of transcriptional factors. *Frontiers in Plant Science*, 6, 895–895.

Srivastava, A.C., Ganesan, S., Ismail, I.O. & Ayre, B.G. (2008) Functional characterization of the Arabidopsis *At*SUC2 Sucrose/H^+ symporter by tissue-specific complementation reveals an essential role in phloem loading but not in long-distance transport. *Plant Physiologst*, 148, 200–211.

Stitt, M., Lilley, R.M.C., Gerhardt, R. & Heldt, H. (1989) Determination of metabolite levels in specific cells and subcellular compartments of plant leaves. *Methods in Enzymology*, 174, 518–552.

Takahashi, F., Sato-Nara, K., Kobayashi, K., Suzuki, M. & Suzuki, H. (2003) Sugar-induced adventitious roots in Arabidopsis seedlings. *Journal of Plant Research*, 116, 83–91.

Thalmann, M. & Santelia, D. (2017) Starch as a determinant of plant fitness under abiotic stress. *New Phytologist*, 214, 943–951.

Thulke, O.U. & Conrath, U. (1998) Salicylic acid has a dual role in the activation of defense related genes in parsley. *The Plant Journal*, 14, 35–42.

Tjaden, J., Möhlmann, T., Kampfenkel, K., Henrichs, G. & Neuhaus, H.E. (1998) Altered plastidic ATP/ADP-transporter activity influences potato (*Solanum tuberosum*) tuber morphology, yield and composition of tuber starch. *The Plant Journal*, 16, 531–540.

Tognetti, J.A., Pontis, H.G. & Martínez-Noël, G.M. (2013) Sucrose signaling in plants: a world yet to be explored. *Plant Signaling & Behavior*, 8, e23316.

Truernit, E. & Sauer, N. (1995) The promoter of the *Arabidopsis thaliana* SUC2 sucrose-H^+ symporter gene directs expression of beta-glucuronidase to the phloem: evidence for phloem loading and unloading by SUC2. *Planta*, 196, 564–570.

Valifard, M., Le Hir, R., Müller, J., Scheuring, D., Neuhaus, H.E. & Pommerrenig, B. (2021) The vacuolar fructose transporter SWEET17 is critical for Arabidopsis root development and drought tolerance. *Plant Physiology*, 187, 2716–2730. https://doi.org/10.1093/plphys/kiab436

Vollmann, J. & Eynck, C. (2015) Camelina as a sustainable oilseed crop: Contributions of plant breeding and genetic engineering. *Biotechnology Journal*, 10, 525–535.

Vu, D.P., Martins Rodrigues, C., Jung, B., Meissner, G., Klemens, P.A.W., Holtgräwe, D. et al. (2020) Vacuolar sucrose homeostasis is critical for plant development, seed properties, and night-time survival in Arabidopsis. *Journal of Experimental Botany*, 71, 4930–4943.

Wahl, V., Ponnu, J., Schlereth, A., Arrivault, S., Langenecker, T., Franke, A. et al. (2013) Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana*. *Science*, 339, 704–707.

Wallace, D.H., Baudoin, J.P., Beaver, J., Coyne, D.P., Halseth, D.E., Masaya, P.N. et al. (1993) Improving efficiency of breeding for higher crop yield. *Theoretical and Applied Genetics*, 86, 27–40.

Wang, L. & Ruan, Y.-L. (2013) Regulation of cell division and expansion by sugar and auxin signaling. *Frontiers in Plant Science*, 4, 163–163.

Wingenter, K., Schulz, A., Wormit, A., Wic, S., Trentmann, O., Hörmiller, I.I. et al. (2010) Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signalling and seed yield in Arabidopsis. *Plant Physiology*, 154, 665–677.

Wingenter, K., Trentmann, O., Winschuh, I., Hörmiller, I.I., Heyer, A.G., Reinders, J. et al. (2011) A member of the mitogen-activated protein 3-kinase family is involved in the regulation of plant vacuolar glucose uptake. *The Plant Journal*, 68, 890–900.

Wormit, A., Trentmann, O., Feifer, I., Lohr, C., Tjaden, J., Meyer, S. et al. (2006) Molecular identification and physiological characterization of a novel monosaccharide transporter from Arabidopsis involved in vacuolar sugar transport. *Plant Cell*, 18, 3476–3490.

Xiong, L., Wang, R.-G., Mao, G. & Koczan, J.M. (2006) Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiology*, 142, 1065–1074.

Yu, H., Chen, X., Hong, Y.Y., Wang, Y., Xu, P., Ke, S.D. et al. (2008) Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. *Plant Cell*, 20, 1134–1151.

Zhu, L., Li, B., Wu, L., Li, H., Wang, Z., Wei, X. et al. (2021) *Md*ERDL6-mediated glucose efflux to the cytosol promotes sugar accumulation in the vacuole through up-regulating TSTs in apple and tomato. *Proceedings of the National Academy of Sciences of the United States of America*, 118, e2022788118.

Zubr, J. (1997) Oil-seed crop: *Camelina sativa*. *Industrial Crops and Products*, 6, 113–119.

Zürcher, E., Liu, J., di Donato, M., Geisler, M. & Müller, B. (2016) Plant development regulated by cytokinin sinks. *Science*, 353, 1027–1030.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Okooboh, G.O., Haferkamp, I., Valifard, M., Pommerrenig, B., Kelly, A., Feussner, I. et al. (2022) Overexpression of the vacuolar sugar importer *Bv*TST1 from sugar beet in Camelina improves seed properties and leads to altered root characteristics. *Physiologia Plantarum*, 174(2), e13653. Available from: https://doi.org/10.1111/ppl.13653