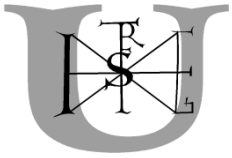

SZENT ISTVÁN UNIVERSITY

The effect of StubSNF1 protein kinase complex and the trehalose-6-phosphate synthase on potato yield and drought tolerance

Theses of PhD dissertation

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Introduction and Aims

The growth rates and final sizes of plant organs are determined by both genetic constraints and environmental factors. A number of growth-promoting and growth restricting factors have now been identified in plants, primarily in *Arabidopsis thaliana*. One factor that inhibits growth is trehalose (α,α-1,1-diglucose). In desiccation-tolerant plants, it accumulates under osmotic/dehydration stress and helps cells to survive by protecting membranes and proteins, but in other plants, trehalose is synthesised at an almost undetectable level. Although some data indicate that trehalose has a protective role during abiotic stresses, over-expression of enzymes involved in trehalose synthesis results in growth aberrations and development arrest in most of the species tested so far (FERNANDEZ et al., 2010).

It is generally accepted that sugars, such as sucrose, act as signalling molecules. However, there is only weak evidence for the direct role of trehalose as a signalling molecule. There is stronger evidence that signalling functions are carried out by trehalose-6-phosphate. Recent findings have shown that such properties could be related to an inhibiting effect of trehalose-6-phosphate on SnRK1 (sucrose non-fermenting-related kinases-1) (ZHANG et al., 2009; MARTINEZ-BARAJAS et al., 2011; DELATTE et al., 2011).

SnRK1, which takes its name from its fungal homologue SNF1 (sucrose nonfermenting-1), is a key transcriptional regulator that responds to carbon and energy supply. *SnRK1* genes have been cloned from many plant species, and it has been shown that the encoded proteins are very likely heterotrimeric enzymes similar to yeast SNF1, which consists of the serine/threonine kinase (SNF1), along with an activating subunit (SNF4) and one of the three cofactors (SIP1, SIP2, or GAL83) that is required for kinase function and confers substrate specificity (HEDBACKER and CARLSON, 2008). GAL83 orthologues interacting with the SNF1-related kinase subunit were identified in *Arabidopsis*, potato and tomato. SnRK1 is regulated transcriptionally and post-transcriptionally; it is activated by phosphorylation and inactivated by dephosphorylation, which is inhibited by low concentrations of AMP. Different types of proteins can interact with SnRK1s, leading to the emerging complexity of SnRK1 interactions (COELLO et al., 2011).

The potato genome possesses at least two *SnRK1* kinases, designated *PKIN1* and *StubSNF1* (MAN et al., 1997; LAKATOS and BÁNFALVI, 1997). The sequences of these proteins are 72% similar. Previously, we demonstrated that the StubSNF1 kinase, but not PKIN1, can interact with the potato orthologue of yeast GAL83, designated StubGAL83 (LAKATOS et al., 1999), and can complement the

Δsnf1 yeast mutation (LOVAS et al., 2003a). We also showed that antisense repression of StubGAL83 results in a delay in rooting and increases sensitivity to salt stress *in vitro*. Tuberisation of the antisense StubGAL83 lines was delayed and the size of the tubers was reduced, while the number of tubers per plant was increased (LOVAS et al., 2003b).

The aim of our recent work was dual: (1) to continue the functional analysis of the StubSNF1 complex by repressing the kinase subunit StubSNF1 and co-repressing it with StubGAL83; and (2) to test the influence of SnRK1 on TPS1 action by expressing the yeast *TPS1* gene in StubSNF1-repressed and StubGAL83-repressed backgrounds.

Materials and methods

Plant material, growth conditions and salt treatment

Transgenic derivatives of the *Solanum tuberosum* cv. Désirée with repressed StubGAL83 (aG1, aG5, aG6) and StubSNF1 (aS5, aS6) were generated previously from *Solanum tuberosum* cv. Désirée (LOVAS et al., 2003b; SÓS-HEGEDŰS et al., 2005). Plants were propagated *in vitro* from single-node stem segments on RM medium (MS medium without vitamins) containing 2% (w/v) sucrose and 50 mg l^{-1} kanamycin and 9 mg l^{-1} hygromycin when appropriate at 24°C under a 16 h photoperiod (ca. 5000 lux). Six-week old plants were transferred into pots and grown further under greenhouse conditions at 18-28°C. For salt sensitivity detection, apical stem segments derived from *in vitro* plants were transferred to RM medium supplemented with 50 mM NaCl in a 500-ml glass jar, and the appearance of roots was followed daily.

Generation of double-transgenic lines

The *TPS1* gene of *Saccharomyces cerevisiae*, cloned in pBluescript II KS (STILLER et al., 2008), was re-cloned into the pBINAR-Hyg vector (for hygromycin resistance in plants) between the CaMV *35S* promoter and the *ocs* terminator. The 779-bp 3' fragment of *StubSNF1*, comprising the regulatory domain of the kinase, was cloned in antisense orientation in pBINAR-Hyg. Transgenic lines were obtained by leaf disc transformation, using *Agrobacterium tumefaciens* strain AGL0. Culture media contained 250 mg l^{-1} cefotaxime for the elimination of *A. tumefaciens* and 9 mg l^{-1} hygromycin for positive selection.

RNA gel-blot analysis

To screen regenerated plants for the expression of transgenes, total RNAs extracted from *in vitro* plants were separated on formaldehyde-agarose gels and blotted and hybridised exactly as described by LOVAS et al. (2003b). The full-length coding region of the *TPS1* cDNA was labelled by ^{32}P-dCTP with the random priming method, according to SAMBROOK et al. (1989). The *StubSNF1*-specific probe was obtained by PCR amplification of the fragment extending from bases 842-1196 of *StubSNF1* cDNA, a part of the regulatory domain, and labelled with ^{32}P-dCTP by the same procedure as the *TPS1* cDNA.

Enzyme activity assays

Partial purification and activity measurement of SnRK1s was performed exactly as described in detail by BECZNER et al. (2010) with the SAMS peptide (HMRSAMSGLHLVKRR) as a substrate. Protein concentrations of the extracts were determined by the BRADFORD (1976) method with BSA as a standard. Nitrate reductase (NR) activity in leaves was assayed, according to HAGEMAN and HUCKLESBY (1971) and HARRIS et al. (2000).

Results

Isolation and phenotypic characterisation of StubSNF1-repressed potato plants

StubSNF1-repressed (aS) transgenic *S. tuberosum* cv. Désirée lines were isolated earlier by expressing a part of the regulatory domain of *StubSNF1* cDNA in antisense orientation under the control of the constitutive CaMV *35S* promoter (SÓS-HEGEDŰS et al., 2005). The regulatory domain of *StubSNF1* has only 65% identity with the same domain of *PKIN1* at the nucleic acid level (LOVAS et al., 2003a). BECZNER et al. (2010) demonstrated that the repression in aS lines is specific to StubSNF1 and that the reduction of kinase activity in shoots of *in-vitro* plants is 30-45%.

In vitro-grown plants of several independent aS lines were transferred into pots and grown further under greenhouse conditions. The growth rate of the aS plants was lower and the final size of plants was smaller than that of wild-type (WT) plants. Tubers of aS plants harvested from pots were frequently distorted, elongated and prone to develop additional tubers. Interestingly, we observed that the colour of the tubers' skin was also changed (Fig. 1). The yield of aS lines was reduced by 20-30% compared to the WT control.

LOVAS et al. (2003b) showed that the repression of the StubGAL83 subunit of the StubSNF1 complex results in a delay in rooting and increases sensitivity to salt stress *in vitro*. We found the same alterations but in an even more pronounced form in aS lines. In rooting medium, the roots of aS lines were retarded in early development and characterised by stunted growth. In the presence of 50mM NaCl in rooting medium, appearance of roots was delayed or, in the majority of stem segments, even a complete prevention of root development has been occurred.

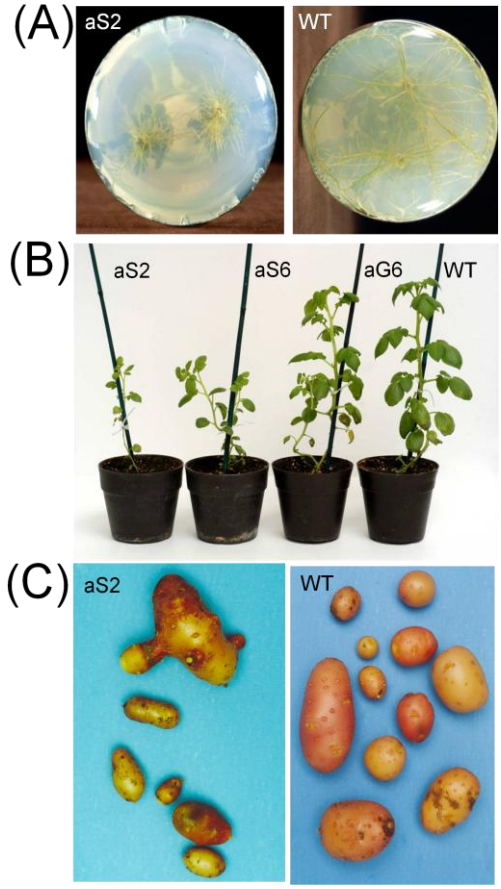

Figure 1. Phenotype of antisense StubSNF1 lines. (A) rooting in *in vitro* culture, (B) phenotype under greenhouse conditions, (C) tubers of greenhouse-grown plants (aS: antisense StubSNF1, aG: antisense StubGAL83, WT: wild type)

Isolation and characterisation of StubGAL83-StubSNF1 co-repressed potato plants

StubGAL83-StubSNF1 co-repressed aGaS lines were isolated by over-transformation of the antisense StubGAL83 line, aG6. This line was isolated previously, using the binary vector pCP60, which confers kanamycin resistance to the transgenic plants (LOVAS et al., 2003b). To obtain co-repressed lines, the same fragment of *StubSNF1* used to isolate the aS lines was cloned in antisense orientation into pBINAR-Hyg and transformed into aG6. Eighty-four hygromycin resistant lines were obtained. The phenotypes of the plants varied from dwarf to normal. Some lines developed very small, yellowish leaves *in vitro*. These lines retained their morphological characteristics when grown in pots. After four months

of growth in the greenhouse, tubers were harvested. Substantial variation in tuber yield was detected. Tuber yield of line aG6 was similar to that of the WT control. Twenty-two aGaS lines produced lower-, while 16 aGaS lines produced higher average tuber mass than WT. Two high tuber-producing lines, aGaS6 and aGaS7, were extensively propagated *in vitro*, and their yield was tested in three consecutive experiments. On average, aGaS6 had a 1.7-fold, while aGaS7 had a 1.2-fold increase in yield compared to WT (Fig. 2).

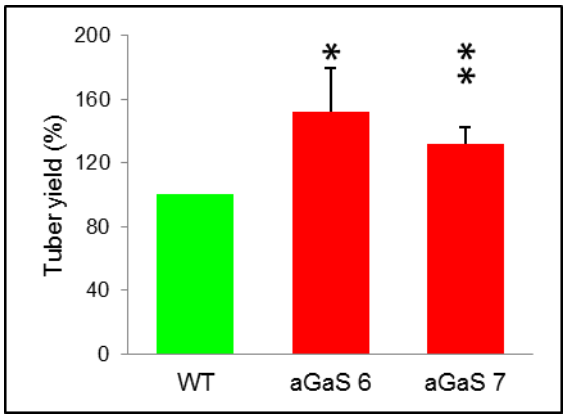

Figure 2. Tuber yield of StubGAL83-StubSNF1 double antisense (aGaS) potato lines (WT, wild type)

Salt sensitivity of the high-tuber-producer lines aGaS6 and aGaS7 was tested in the same way as in case of aS lines. Both aGaS lines were more sensitive to salt than WT or aG6 plants as indicated by the delayed appearance of roots on apical segments of *in-vitro* shoots in the presence of 50 mM NaCl in rooting medium. To test whether the increase in yield by StubSNF1 repression could be achieved only in aG6 or whether this is a more general phenomenon, two additional aG lines, aG1 and aG5, were over-transformed with the antisense *StubSNF1* construct. Sixty-three and 41 hygromycin resistant lines were isolated from the transformations of aG1 and aG5, respectively, of which 23 aG1 progenies and 10 aG5 progenies produced higher average tuber mass than the WT.

The level of repression of StubSNF1 expression at the mRNA level in aS lines was tested earlier by SÓS-HEGEDŰS et al. (2005). Although, *StubSNF1* is expressed in leaves at low levels in WT plants (LAKATOS et al., 1999) reductions in StubSNF1 mRNA levels could be detected in aS lines on RNA gel-blots. The same type of analysis was used to test the efficiency of StubSNF1 repression in aGaS lines. Four low tuber-producing lines and ten high tuber-producing lines derived from over-transformation of aG1, aG5 and aG6 were tested. Nevertheless,

compared to the level of expression in WT and aG lines, no visible differences in the amounts of StubSNF1 mRNA were detected in any of the aGaS lines tested, except for aGaS6 which was characterised by a slight reduction in StubSNF1 mRNA level.

The SAMS peptide (HMRSAMSGLHLVKRR) is a good substrate for plant SnRK1s and use of this peptide to measure SNF1 activity in different plant species, including potato, has become a widely accepted practice. Using the SAMS peptide as a substrate, BECZNER et al. (2010) detected 30-45% reduction in SNF1 kinase activity of aS lines. We used the same peptide to test the SNF1 kinase activities of aG and aGaS lines (Fig. 3). Compared to the SNF1 kinase activity of the WT leaves, a 42% reduction in activity was detected in aG6. Similar reductions in activity were found in other aG lines as well, indicating that StubGAL83 is a positive regulator of the StubSNF1 kinase complex. Compared to the SNF1 kinase activity detected in aG6, no significant change (*t*-test $P \leq 0.01$) in activity was found in any of the aGaS lines. The lowest activity was detected in the high tuber-producing line aGaS6. On an average, aGaS6 had only 36% of WT SNF1 kinase activity.

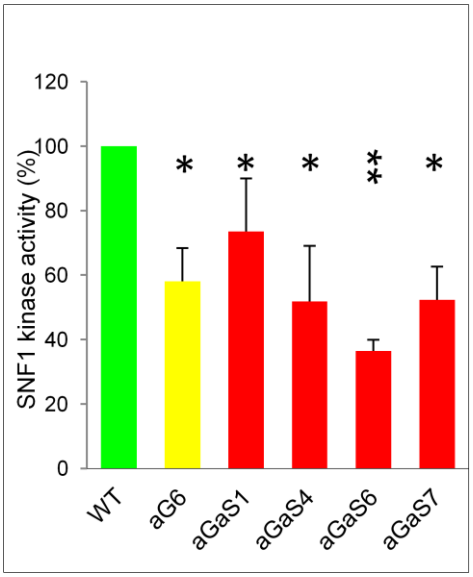

Figure 3. "SNF1"-activity of antisense lines. (aGaS1, aGaS4, double antisense lines with dwarf phenotype and low tuber yield; aGaS6, aGaS7: double antisense lines with high tuber yield; aG6, antisense StubGAL83 line; WT, wild type)

Effect of TPS1 expression in StubSNF1-, and StubGAL83-repressed potato plants

To obtain double-transgenic lines, the full-length *TPS1* gene was cloned into the binary vector pBINAR-Hyg behind the constitutive promoter CaMV *35S* and transformed into the wild-type potato cv. Désirée as a control (WT), as well as into its antisense StubSNF1 strain, *aS6*, and its antisense StubGAL83 strain, aG6. The putative transgenic plants were selected on regeneration and rooting medium containing hygromycin. In total, six WT-TPS1 (WTT), 25 aS6-TPS1 (aST) and 27 aG6-TPS1 (aGT) hygromycin-resistant lines were obtained. The WTT lines were propagated in the presence of hygromycin, while the double-transformed aST and aGT lines were propagated with both hygromycin and kanamycin in the medium.

Expression of *TPS1* in the hygromycin-resistant plants was tested by RNA gel-blot assay using the full-length *TPS1* cDNA as a probe. RNA was isolated from the shoots of all 58 *TPS1*-transformed lines, as well as from untransformed control plants grown *in vitro*. From this assay, 20 lines showing detectable hybridisation with *TPS1* cDNA were identified on RNA gel-blots. Three lines from each transformation, expressing the *TPS1* at different levels (Fig. 4), were selected for further experiments.

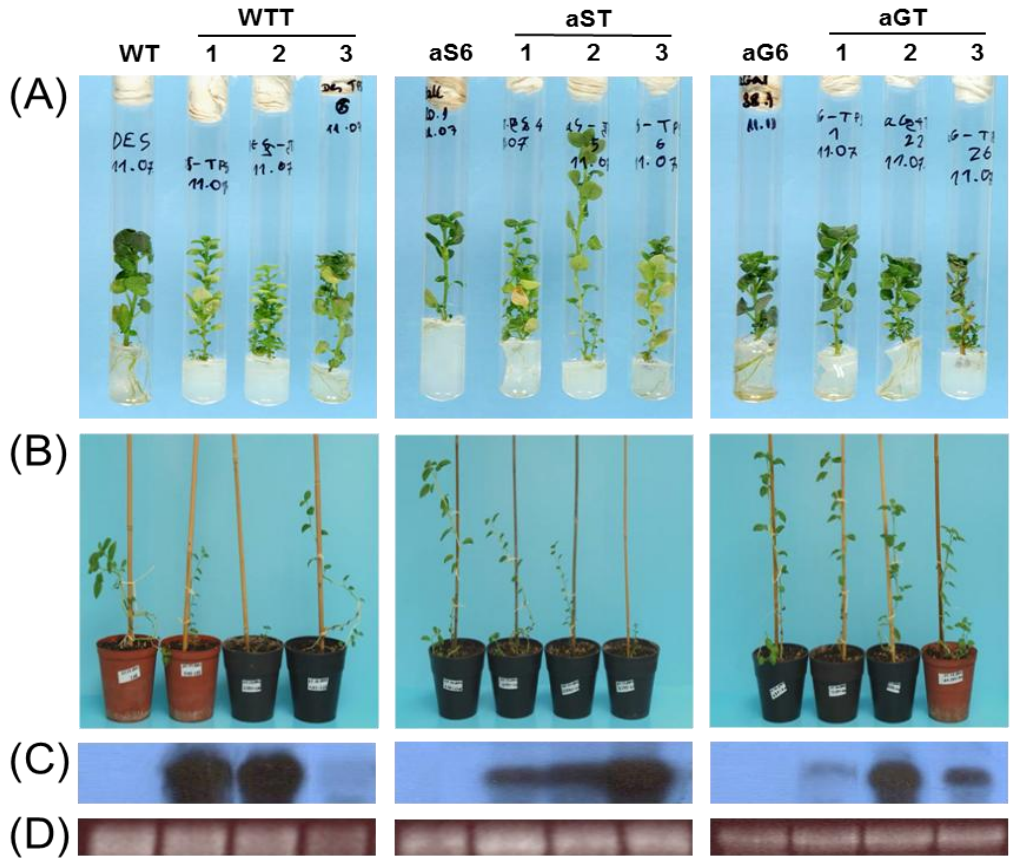

Figure 4. Phenotypes of *in vitro*-grown *TPS1*-expressing plants. (A) Plants grown in tubes, (B) Plants grown in greenhouse, (C) RNA gel-blot analysis using the full-length *TPS1* cDNA as the hybridisation probe, (D) Ethidium bromide-stained 25S rRNA bands shown as loading controls (WT, wild-type; WTT, *TPS1*-expressing derivatives of WT; aS6, antisense StubSNF1; aST, *TPS1*-expressing derivatives of aS6; aG6, antisense StubGAL83; aGT, *TPS1*-expressing derivatives of aG6)

The nine selected *TPS1*-expressing lines and their controls were propagated *in vitro*. It was found that even a low level of *TPS1* expression in WT and aS6 backgrounds resulted in serious morphological aberrations. The roots of the WTT and aST lines were retarded in development. The plants were bushy and showed early senescence. In contrast, two aGT lines, aGT1 and aGT2, did not show phenotypic changes compared to the aG6 control. The lower leaves of the line aGT3, however, started to turn yellow earlier than the lower leaves of aG6 did (Fig. 4). The same morphological differences were detected when the *in-vitro* plants were transferred to pots and grown further in the greenhouse. The WTT and aST plants grew very slowly and had no or only a few small tubers by the end of the vegetative period. The two aGT plants, which were similar to aG plants *in vitro*,

developed at a rate similar to aG plants *ex vitro*, and produced the same amounts of tubers as the aG6 plants in pots. The aGT3 line showed earlier senescence than the control.

Nitrate reductase activity of transgenic potato plants

It was previously demonstrated that GAL83 orthologues in plants interact with nitrate reductase (NR) and negatively regulate its activity (POLGE et al., 2008; LI et al., 2009). This result prompted us to investigate the NR activity of our transgenic plants. In the first set of experiments, NR activities of the WT, aG, aS and aGaS lines were determined from the source leaves of greenhouse-grown plants. Because it is well known that the activity of NR is up-regulated in light and down-regulated in darkness (STITT et al., 2002), samples were collected four hours after sunrise and four hours after sunset.

As shown in Figure 5A, the activity of NR in the high-tuber-producing lines aGaS6 and aGaS7 was highest, about two to three times higher than that in the WT in light. No significant difference was found between NR activities of WT, aG6, aS5 and aS6. NR activity was lowest in the low-tuber-producing aGaS4 plants, in which it was below the detection limit in both light and darkness (Fig. 5A,B). Darkness significantly reduced the NR activity in all lines, however, with a less extent in aGaS6 and aGaS7 than in the other lines. In these two high tuber-producing lines the NR activity remained relatively high during the dark period and did not decrease below 0.12±0.03 in aGaS6 or below 0.04±0.003 μmol NO_2^- mg^{-1} protein h^{-1} in aGaS7 (Fig. 5B).

Antisense repression of StubGAL83 attenuated the morphological aberrations triggered by *TPS1* expression in the WT background (Fig. 4A,B). As such, we were also interested in the NR activities of WTT and aGT lines. Figure 5C shows a significant decrease in the NR activity in WTT1, WTT3 and aGT1 lines but not in WTT2, aGT2 or in aGT3 compared to WT in light. In darkness, no NR activity was detected in any of the WTT or in aGT3 plants, while the NR activity in aGT1 and aGT2 was similar to that in aG6. Based on the sum of day and night activities, aGT1 and aGT2, the two lines showing no morphological aberrations (Fig. 4C,D), were the most similar to the WT plants.

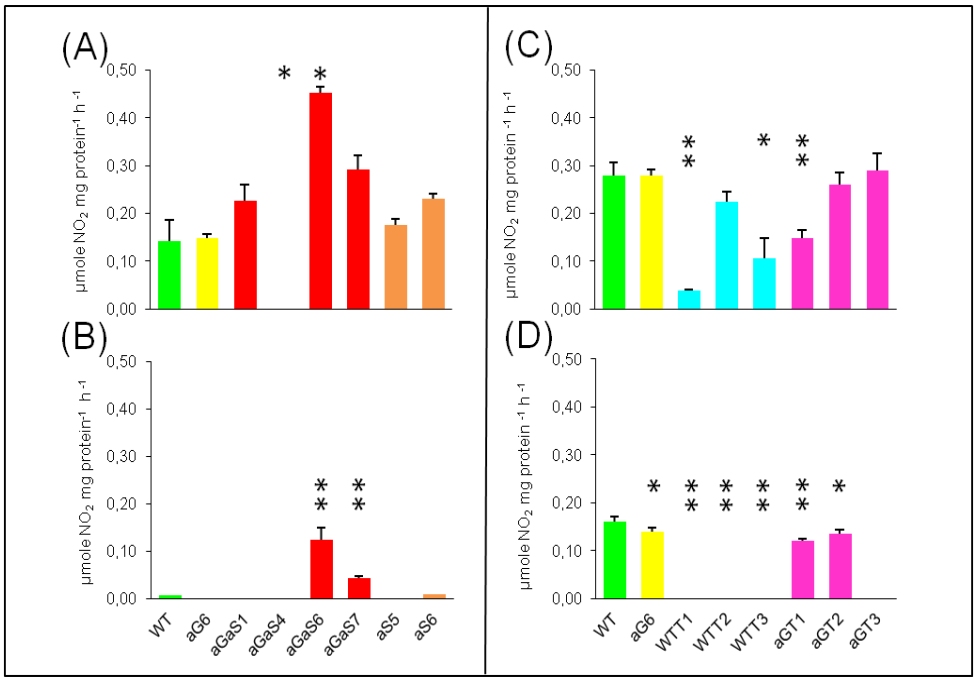

Figure 5. Nitrate reductase (NR) activities in leaves of potato plants grown in pots in the greenhouse. (A) NR activities of aS and aGaS lines compared to WT and aG6 in light, (B) NR activities of aS and aGaS lines compared to WT and aG6 in darkness, (C) NR activities of WTT and aGT lines compared to WT and aG6 in light, (D) NR activities of WTT and aGT lines compared to WT and aG6 in darkness. Bars and error bars are the mean ± SE of two or three independent measurements using 0.5 g leaf tissue derived from each of three plants for each measurement. Two asterisks depict differences significant at P=0.01, while one asterisk depicts differences significant at P=0.05 (t-test) compared to WT. Samples were collected four hours after sunrise and four hours after sunset. Experiments shown in A and B and those in C and D were carried out in different seasons.

New scientific achievements

1. We demonstrated that the antisense inhibition of StubSNF1 in potato results in developmental aberrations, decreased salt stress tolerance and decreased tuber yield, which is often accompanied by abnormal tuber shape and colour.

2. We designed and characterized StubGAL83-StubSNF1 double antisense (aGaS) potato plants by over-transformation of the line aG6 with an antisense StubSNF1 construct. We showed that in certain lines, this type of genetic modification increases the size and tuber yield of the plants under optimal greenhouse conditions. The aGaS potato plants, however, display increased sensitivity to salt stress.

3. We generated yeast *TPS1*-expressing potato plants in wild-type, StubSNF1-, and StubGAL83-repressed background. We found that repression of StubGAL83, but not repression of StubSNF1 attenuated growth aberrations caused by *TPS1* expression.

4. We provided evidence that the increased plant size and yield in StubGAL83-StubSNF1 co-repressed plants as well as the attenuation of aberrations caused by *TPS1* expression are related to increased nitrate reductase activity.

Conclusions and suggestions

SnRK1s are central regulators of metabolism and development in plants (COELLO et al., 2011). Changes in starch and soluble sugar contents were observed in the model plant *Arabidopsis* over-expressing AKIN10, the catalytic subunit of SnRK1. Modifications of the activities of essential enzymes such as NR or ADP-glucose pyrophosphorylase (AGPase) and of the expressions of several sugar-regulated genes were also observed (JOSSIER et al., 2009). In addition, it was found that over-expression of the catalytic subunit of AKIN10 in *Arabidopsis* antagonises the embryonic-to-vegetative and vegetative-to-reproductive phase transitions and alters cotyledon, silique and floral organ development (TSAI and GAZZARRINI, 2012). In potato, tuber-specific antisense repression of the SNF1 kinase activity resulted in decreased expression of sucrose synthase and modulated the post-translational redox activation of AGPase in tubers (PURCELL et al., 1998; TIESSEN et al., 2003). In this study, we demonstrated that constitutive repression of the catalytic subunit of the StubSNF1 complex resulted in stunted growth and reduced tuber yield. Our findings therefore further support the essential role of SnRK1s in plant development.

Two isoforms of *SnRK1, PKIN1* and *StubSNF1*, were previously detected in potato (MAN et al., 1997; LAKATOS and BÁNFALVI 1997). BECZNER et al. (2010) demonstrated specific repression of the corresponding kinase in antisense PKIN1 (aP) and antisense StubSNF1 (aS) lines generated by constitutive expression of the non-conserved regulatory domains in antisense orientation. Using the SAMS peptide, a widely accepted substrate for measuring SNF1 activity in different plant species, BECZNER et al. (2010) detected a 25-50% reduction in kinase activity in aP and aS lines compared to the WT control. We have measured the SAMS peptide phosphorylation activity of the antisense StubGAL83 line aG6 and found 42% reduction in kinase activity compared to WT. This indicates that StubGAL83 is a positive regulator of the kinase. The same conclusion can be drawn from the results of the salt sensitivity test, which showed enhanced salt sensitivity of both the aG and aS lines. The inhibitory effect of salt on rooting, however, was stronger in aS than in aG lines. Furthermore, both the phenotypic aberrations caused by StubGAL83-, and StubSNF1 repression affected tuber development. Nevertheless, while repression of StubGAL83 only delayed tuberisation, but did not result in yield reduction or phenotypic alterations of tubers, repression of StubSNF1 significantly reduced the growth rate of plants and led to decreased yield, deformed shape and abnormal color of tubers (LOVAS et al., 2003b; Fig. 1).

Three aG lines, aG1, aG5 and aG6, were over-transformed with the *StubSNF1*-specific antisense construct. Three categories of double-transgenic lines were obtained from each transformation: (1) slow-growing lines with altered leaf morphology, producing only a few small tubers or no tubers at all; (2) plants similar to the controls; and (3) plants growing faster and larger and producing more and larger tubers than the control. Amounts of StubSNF1 mRNA were tested in 14 lines with contrasting phenotypes, but no alterations in mRNA levels were detected except in the high-tuber-producing line aGaS6, in which a slight reduction in StubSNF1 mRNA level was observed. Contrary, reduction of StubSNF1 mRNA level was detectable earlier in aS lines (SÓS-HEGEDŰS et al., 2005) in which the same region of *StubSNF1* was expressed in antisense orientation as in aGaS lines. Considering the essential role of SnRK1s in development and the reduced SNF1 kinase activity of aG plants this finding indicates that further significant reduction of StubSNF1 may be lethal. Nevertheless, small differences in StubSNF1 mRNA levels may exist between the individual aGaS lines. Resolution of the RNA gel-blot analysis that we used, however, may not be high enough for detection of small differences in gene expression. In addition, it is also possible, that in our aGaS lines there is a translational inhibition instead of transcriptional (BRODERSEN et al., 2008). The different mode of gene silencing in the different aGaS lines may be an alternative or additional explanation for the different phenotype of aGaS lines.

In line with the lack of significant alteration in gene expression no significant change in SNF1 kinase activity was detected in the double-transgenic aGaS lines compared to their single-transgenic ancestor, aG6. Nevertheless, aGaS6, the only aGaS line with detectable reduction in StubSNF1 mRNA level, possessed the lowest SNF1 kinase activity, which was 36% of the WT activity. The SAMS peptide used to measure the kinase activity is a substrate not only of StubSNF1 but also of PKIN1 (BECZNER et al., 2010). The residual activity measured in aGaS lines may have arisen, at least in part, from PKIN1. StubSNF1 and PKIN1, however, may have different functions; for example, StubSNF1 is able to complement the *Δsnf1* mutation of yeast while PKIN1, which cannot interact with StubGAL83, is unable to do so (LOVAS et al., 2003a). Therefore, PKIN1 cannot substitute for StubSNF1 in every respect. Since in different aGaS lines the level of StubSNF1 repression may be different, the contribution of PKIN1 and StubSNF1 to the measured SAMS peptide-kinase activity may also be different. Differences in PKIN1-StubSNF1 ratio may also contribute to the different phenotype of the plants.

The most intriguing question, however, is that of how the growth aberrations caused by the expression of TPS1 could be attenuated in certain aGT

lines and the tuber yield increased in some aGaS lines. It is known that the *Arabidopsis* orthologues of GAL83 can interact with NR and negatively regulate its activity (POLGE et al., 2008; LI et al., 2009). It was previously shown that the catalytic subunit of the complex, SNF1, can phosphorylate and inactivate NR (SUGDEN et al., 1999). Expression of StubGAL83 is activated in darkness (LOVAS et al., 2003b), while the expression of StubSNF1 is not induced in the dark (LOVAS A. unpublished result). We therefore suppose that SNF1 kinase activity is low during the day and high during the night in WT plants. The NR activity has the opposite diurnal cycle, being high during the day and low during the night in WT (Fig. 5). Antisense repression of StubGAL83 and StubSNF1 in aG and aS lines altered the proportion of the subunits, which could result in an even stronger inhibition of NR in transgenic than in WT plants during the night. We assume that the inhibition of StubSNF1 was strongest in the lines with relatively high NR activity during the night. Due to their high NR activities, these plants grow faster and produce higher average tuber mass. SnRK1s can also phosphorylate and inactivate sucrose phosphate synthase (SUGDEN et al., 1999) and regulate AGPase (TIESSEN et al., 2003). We demonstrated previously that the intensity and the light/dark periodicity of pyruvate kinase activity are altered in leaves of aS lines (BECZNER et al., 2010). Changes in carbohydrate metabolism may also contribute to the altered phenotype of the aGaS lines.

The sum of day and night NR activities of the morphologically altered WTT plants was reduced, while that of the morphologically normal aGT lines was similar to the controls. Night reduction of NR activity in WTT plants was especially conspicuous. We therefore concluded that the expression of *TPS1* fortified the negative effect of the StubSNF1 complex on NR activity. Reduction of StubSNF1 activity by antisense repression of StubGAL83 attenuated the fortification effect of TPS1 and resulted in plants with NR activities and phenotypes similar to those of WT plants.

TPS1 transgenic plants expressing the transgene, even at very low levels, in the WT background show increased drought tolerance, but grow more slowly and have lower CO_2 fixation rates than WT plants (STILLER et al., 2008). We isolated two aGT lines expressing *TPS1* at low (aGT1) and high (aGT2) levels that grew at similar rates and had the same phenotype as WT. Drought tolerance of the two aGT lines was tested in detached leaf assays, but no difference in the speed of water loss was observed. It therefore seems likely that the TPS1-triggered morphological changes are indispensable for increased drought tolerance in potato.

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