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Running title: Improving potato yield by overexpression AtPAP2

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Title:

Heterologous expression of *AtPAP2* in transgenic potato influences carbon metabolism and tuber development

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Abstract

Sugar supply is important for the vegetative growth and reproductive development of plants. Changes in carbon flow and sink/source activities could affect floral, architectural, and reproductive traits of plants. In potato, overexpression (OE) of the purple acid phosphatase 2 of Arabidopsis (*AtPAP2*) resulted in earlier flowering, faster growth rate, increased tubers and tuber starch content, and higher photosynthesis rate. There was a significant change of sucrose in leaves, petioles and sink of the overexpressors, which were consistent with the upregulated expression of sucrose transporter 1 (StSUT1) in the OE lines. Low sucrose content in the source leaves could boost the photosynthesis rate by less sugar inhibition. Meanwhile, the expression levels and enzyme activity of sucrose-phosphate synthase (SPS) were also significantly up-regulated in the AtPAP2 OE lines, which could supply more sucrose for export and anabolism. Since co-immunoprecipitation assays did not detect direct interactions between AtPAP2 and SPS and StSUT1, the expressions of SPS and StSUT1 were upregulated in AtPAP2 overexpression lines through an indirect mechanism.

Keywords

Potato, *AtPAP2*, photosynthesis, tuber yield, sugar efflux

1. Introduction

Photosynthate supply and sink strength have been demonstrated experimentally to be the major determinants of crop yield [1]. Assimilated carbon from photosynthesis supplies both energy sources for metabolism and building blocks for complex carbohydrates. Photoassimilate is further partitioned within the mesophyll cells and transported, mainly in the form of sucrose, from source to sink tissues to support plant growth and development. The plant growth rate depends on the photosynthetic fixation capacity and on how efficiently the fixed carbon is utilized in biosynthetic processes which support growth. Accordingly, the distribution of carbon assimilates assists in balancing photosynthetic activity in the source leaves and photoassimilate utilization and storage in sinks. During this process SPS activity, which usually determines sucrose synthesis rates, and sucrose transporters (SUTs) required for sucrose phloem loading, have been demonstrated to be regulated by protein phosphorylation status [2].

Purple acid phosphatases (PAPs) are a family of acidic binuclear metalloenzymes which

hydrolyze phosphate esters and anhydrides under acidic conditions. Many plant PAPs were shown to be induced by Pi starvation and involved in phosphorus metabolism[3]. Our previous studies demonstrated that overexpression (OE) of AtPAP2, an Arabidopsis PAP with an additional C-terminal hydrophobic motif, located at the outer membrane of both chloroplasts and mitochondria [4], drastically enhanced the growth rate and seed yield of *Arabidopsis thaliana* and *Camelina sativa* [5, 6]. These results imply that AtPAP2 can potentially regulate plant carbon metabolism.

2. Materials and Methods

2.1 Plant materials and growth conditions

Potatoes (*Solanum tuberosum var. Bintje*) were provided by Prof. M. L. Chye of the University of Hong Kong and potatoes (*Solanum tuberosum 'Desirée'*) were from the Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm. Potato was maintained in tissue culture with 16-/8-h day/night cycles on Murashige and Skoog medium [7] in growth room, which contained 2% (w/v) sucrose. The top 5 internode of one-month-old WT potatoes were used for transformation. In addition, transgenic potato plants were first grown in a growth chamber (150 µmol/m2 s1 and 75% relative humidity (RH)) under 16 h light (22℃) / 8 h dark (18℃) light period for several weeks before they were transferred to greenhouse (Light intensity varied between a minimum of 90 and a maximum of 200 µmol/ m2 s1 PPFD and 75% RH) with the day/ night condition of Hong Kong and Germany and watered twice every week before tuber collection. Tubers of all plants were collected after growing in soil for about 4 months.

2.2 Extraction of total plant RNA and quantitative RT-PCR analysis

Total RNA was isolated from fresh leaves by using Trizol reagent (Invitrogen). To generate full-length cDNA for quantitative RT-PCR, reverse transcription was performed using the M-MLV reverse transcriptase (Promega, Hong Kong). Full length *AtPAP2* cDNA was amplified by Pfx DNA polymerase (Invitrogen) and subcloned into pBA002 by *Xho*I and *Sac*I (Table S1) for potato transformation.

Quantitative RT-PCR reaction was carried out in the presence of SYBR Green with HotGoldStar DNA polymerase (Eurogentec) in Rotor Gene 3000 cycler (LTF Labortechnik) using Rotor Gene software (version 4.6.94). An aliquot of 0.2 µL cDNA of the 10 µL RT reaction was used for each reaction. Relative quantification of transcript amounts was

calculated in relation to the respective ubiquitin transcript level and given as percentage of ubiquitin. Primers (Table S1) were designed according to published papers [8] and produce 50- to 150-bp amplicon using Primer5 software. Quantitative RT-PCR data were corrected by calculation of the PCR efficiency individually using the *LinReg* PCR software[9].

2.3 Agrobacterium-mediated transformation and Southern Blotting analysis

The full-length coding region of the AtPAP2 cDNA (AT1G13900) was subcloned into the binary vector pBA002 downstream of the cauliflower mosaic virus (CaMV) 35S promoter (pBA002-CaMV35: AtPAP2). The vector was then introduced into *Agrobacterium tumefaciens* strain GV3101 and internodal explants from 4-week-old WT plantlets were used for transformation [10]. Southern blot analysis was carried out as described [11]. The probes were SB-PAP2-f and SB-PAP2-r (Table S1).

2.4 Western blotting analysis

Potato leaves were finely ground in a 1.5-ml Eppendorf tube containing 200 µl of ice-cooled extraction buffer (50 mM Tris-HCl, pH7.4 containing 150 mM NaCl, 1mM EDTA, 0.2 mM PMSF) and incubated on ice for 30 min with occasional mixing. The protein extract was separated by centrifugation at 10,000 x g for 30 min at 4℃. The supernatant was transferred to a new 1.5-ml Eppendorf tube and the protein concentration was determined by the Bradford assay method using the Bio-Rad Protein Assay Kit. Proteins (25 µg) were resolved by SDS-PAGE, transferred to Hybond-C nitrocellulose membranes, immunodetected and then the proteins were visualized by the Enhanced Chemiluminescence (ECL) method (Amersham Biosciences).

2.5 Measurement of leaf assimilation rate

The leaf assimilation rates of potato were measured using a portable photosynthesis system (LI-COR, LI-6400, Nebraska, USA) in the morning (8.30 to 12:30 AM) under a fixed blue-red light-emitting diode (LED) light source. Nine measurements were made for each three fully expanded intact leaves from the tip of 65 to 67-day-old potato and at least 3 plants of each line were used for measurement. Light curves were measured on 6 cm^2 leaf area using the instrument's auto program function. Measurements were taken in darkness, to determine leaf respiration, and at actinic light intensities of 0, 125,250, 500, 750 and 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 25℃ cuvette temperature and a CO_2 concentration of 400ppm. Relative humidity was set

to 75%.

2.6 HPLC analysis of sugar content

For measurement of sucrose, glucose and fructose of plant tissues, an aliquot of 0.1 g freeze-dried tissue powder was dissolved in 1 ml of 70% (v/v) ethanol, incubated at 70℃ for 90 min and centrifuged at 13,000 × g for 10 min. After passing through a 0.22 mm filter, A volume of 10 µl sample was injected into a CarboPac PA 1 column (4 x 250 mm) connected to a Dionex LC 20 Chromatography system and the sugar contents were analyzed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [12]. Standard curves were prepared by 0.0-0.1 mg/ml sucrose, fructose and glucose in 70% ethanol. The levels of starch in the tubers were determined as described previously [13].

2.7 SPS activity assay

SPS activity was assayed by the anthrone test [14]. Samples were incubated for 20 min at 25℃ in 50 µl pre-balanced buffer (50 mM HEPES-KOH pH 7.5, 20 mM KCl, and 4 mM $MgCl_2$) containing (a) V_{max} assay: 12 mM UDP-Glc and 10 mM Fru6P (in a 1:4 ratio with glucose-6-phosphate (Glc6P), (b) $V_{limiting}$ assay: 4 mM UDP-Glc and 2 mM Fru6P (in a 1:4 ratio with Glc6P) and 5 mM KH_2PO_4.

2.8 Pull-down assay

Plant materials were ground in liquid nitrogen and incubated in ice-cold buffer (50 mM HEPES-KOH pH 7.5, 150 mM NaCl, 50 mM NaF, 5 mM NaPPi, 1 mM dithiothreitol, 1 mM PMSF, 1 mM benzamidine) for 15 min and then they were centrifuged at 12, 000 x g for 20 min. One milligram extracted protein was added to 5 µg GST-14-3-3 [15] on 50µl GST beads with gentle agitation for 2 h at 4℃. The beads were then centrifuged at 2,000 x g for 2 min at 4℃ and washed five times with pull-down washing buffer (50 mM HEPES-KOH pH 7.5, 150 mM NaCl, 1 mM dithiothreitol). The washed beads were eluted (50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0, 150 mM NaCl) and the supernatant was subjected to SDS-PAGE for silver-staining and Western blotting.

2.9 Co-immunoprecipitation (Co-IP) assay

Twenty microliter of soluble proteins in inhibitor buffer (1x PBS, pH 7.4, 1 mM PMSF, 1 mM $NaVO_4$, 50 mM NaF, with COMPLETE™ Protease Inhibitor Cocktail (Roche)) was

incubated with 10 μg first antibody for 2 h at 4℃, and then mixed with 20 μl Protein-G Sepharose (10 ml of 50% slurry in 1 X PBS/20% ethanol (GenScript Co.) for 2 h . The pellets were washed three times in 1 ml inhibitor buffer and boiled in 20 μl SDS sample buffer [16] and used for SDS-PAGE and Western blotting.

3. Results and Discussion

Overexpression of AtPAP2 in Arabidopsis and Camelina has been shown to improve plant growth and seed yield [5, 6]. Potatoes feature a very large sink organ (tuber) for carbon and utilize a well-defined apoplastic phloem loading mechanism with sucrose transporters, and are highly suitable for further investigations of the biological functions of AtPAP2. We generated four transgenic lines in potatoes with high and mild expressions of AtPAP2 proteins (Fig. S1 and S2). All three high AtPAP2 expressing potato lines (OE1, OE4 and OE7) grew faster and produced more lateral branches than the WT and the mild AtPAP2 overexpression line (OE10; Fig. 1, Fig. S3). The first harvest plants were grown in greenhouse from April 2010 to August 2010 under LD conditions. The second and the third harvest plants were grown in greenhouses in Hong Kong (Fig. S4) and in Golm (Table 2, Fig. S5), respectively. All three high expression OE lines displayed earlier flowering than the WT (Table 1). Floral and tuberization transitions in potatoes are controlled by two different FT-like paralogues that respond to environmental cues [17]. As OE lines exhibited earlier flowering (Table 1) and produced more tubers (Table 2), it would be interesting to examine if FT expression is driven by high sugar supply from source tissues.

Genetic manipulation of sucrose transporter (SoSUT1) in potato has been documented to result in a shift in carbon partitioning in both leaves and tubers and improved assimilation rates [18]. Our OE lines generally exhibited higher photosynthetic rates (10%-20%) than the WT (Fig. 2) and higher StSUT1 expression level (Fig. 4). After 4 months of growth in the greenhouse, tubers were collected when the plants were totally senescent. In both harvests (Fig. S4 and S5), all three high AtPAP2 expression lines (OE1, 4, and 7) produced more tubers than the WT and the biomass of aboveground organs was also higher in the OE lines (Table 2). In a separate experiment using a different potato cultivar (*Solanum. tuberosum cv. Desirée*), highly similar results were obtained, suggesting that the observation was independent of the cultivar. Compared with the WT, overexpression of AtPAP2 increased the tuber yield per plant ~2 to 3-fold in *Solanum tuberosum var. Bintje* and 2-fold in *Solanum*

tuberosum 'Desirée', respectively. The increase in tuber yield was due to an increase in dry weight and starch content (Table 2). In total, the five greenhouse trials all produced highly similar results.

Increased sink demand (via systemic signals) and decreased photoassimilate levels in source leaves (via an alleviated feedback repression of photosynthesis by sugar sensing) can both enhance photosynthetic activity [19]. Therefore, a high performance liquid chromatography method was next used to evaluate sugar contents of leaves immediately following the measurement of the rate of photosynthesis. The sucrose contents of all OE lines were decreased by ~60%, with glucose and fructose also being decreased (Fig. 3). These results suggested an inverse relationship between AtPAP2 expression and the contents of sucrose, glucose and fructose in potato leaves. For comparison we analyzed the sugar content of the petioles (phloem) of the top 2 to 3 fully-expanded leaves of 68-day-old potatoes. In contrast to the leaves, the sucrose levels of the petioles were increased by 1.5 to 2 fold in the petiole, while glucose and fructose contents increased moderately (Fig. 3). The higher concentration of sucrose in the petioles is consistent with its higher sucrose transport activity. Overexpression of SoSUT1 in potato resulted in a shift in carbon partitioning in both leaves and tubers and improved photosynthesis rate [18]. Improved rates of sugar efflux via the leaf petioles would stimulate petioles loading and lower mesophyll carbohydrate levels and thus relieve inhibition of photosynthetic activity. Consistent with this hypothesis the sugar levels in tubers of the three higher *AtPAP2* overexpression lines tubers were greatly increased (Table 2).

The rate of sucrose synthesis controlled by SPS was shown to correlate with the rate of photosynthesis and with the rate of export from leaves [20]. The activity of the SPS is inhibited by binding with the 14-3-3 protein which is regulated by the SnRK1 protein kinase. In previous studies transgenic Camelina and Arabidopsis overexpressing *AtPAP2* exhibited higher SPS activity in the leaves [5]. Moreover, transgenic tomato, potato, Arabidopsis and tobacco expressing various *SPS* genes were documented to exhibit increased biomass and photosynthesis rate. As shown in Table 3, SPS activity was enhanced in the leaves of AtPAP2 OE plants in both optimal V_{max} and limiting V_{limit} capacities. Thus, both a higher photosynthetic rate (Fig. 2) and an increased SPS activity in OE lines can provide more sucrose for growth. Western blotting analysis using an anti-SPS antibody (Agrisera, Sweden) indicated that SPS accumulation was remarkably enhanced in the OE lines. However, the protein expression levels of nitrate reductase (NR) and fructose bisphosphatase (FBPase)

were indistinguishable from the WT (Fig. 4). Moreover, the levels of 14-3-3 protein and the amount of phosphorylated SPS that was capable of binding 14-3-3 were unaltered in the OE lines. These results indicated that only unphosphorylated SPS was greatly enhanced in the OE lines, which would be anticipated to result in a far greater *in vivo* SPS enzyme activity. To determine if there was direct interaction between AtPAP2 and SPS, the crude protein extracts of potato leaves were immuno-precipitated by the anti-SPS antibody. The bound proteins were then detected by an anti-AtPAP2 antibody via Western blotting. No direct interaction between AtPAP2 and SPS was observed (Fig. S6). Besides, the expression level of SnRK1 was not significantly changed (Fig. 4), and no protein interaction between SnRK1 and AtPAP2 could be detected by the yeast two-hybrid assay (data not shown). Hence, the higher SPS activity in the OE lines was attributed to a higher expression level of SPS protein, rather than through activation by post-translational modification. Therefore, overexpression of AtPAP2 appears to indirectly regulate the expression and enzyme activity of SPS, thus affecting sucrose synthesis, flower time and tuberization.

To examine how AtPAP2 overexpression might affect sugar partitioning in leaves and petioles, the expression levels of sugar transporters in leaves were examined. Although the expression level of *StSUT1* transcript did not change significantly, its protein level was significantly elevated in the OE lines. StSUT1 is essential for long-distance transport of sucrose and plays a role in phloem loading in mature leaves [21]. Its higher expression in the leaf of OE lines may lead to a higher sucrose level in petioles and tubers but a lower leaf sucrose content (Fig. 3). In contrast, the transcription levels of *StSUT2* and *StSUT4* were greatly decreased (Table 4). Sucrose transporters are known to be regulated by phosphorylation [22], however, co-immunoprecipitation assays did not reveal any direct interaction between AtPAP2 and StSUT1 (Fig. S6).

Many plant PAPs mediate phosphorus acquisition and redistribution based on their ability to hydrolyze phosphorus compounds [3]. AtPAP2 is a phosphatase anchored on the outer membrane of chloroplasts and mitochondria [4]. Theoretically, overexpression of a phosphatase in cytosol may supply additional phosphate as a counter-exchange substrate for the triose phosphate/phosphate translocator (TPT) on chloroplasts to facilitate higher export of triose phosphates to cytosol for sucrose synthesis. This, however, is unlikely the reason for higher tuber yield in the AtPAP2 OE lines. First, the Pi content was not significant changed in our OE lines (Table. S2). Second, overexpression of a soluble *E. coli* pyrophosphatase,

(PPase) which could generate more phosphates by hydrolyzing pyrophosphates, in the cytosol of leaf cells did not lead to increase in tuber yield [23].

Changes in carbon flow and sink/source activities could affect floral, architectural, and reproductive traits of plants. In potato, the tuber yield could be improved by simultaneously genetically modifying source and sink strengths by sucrose transporter [18] . The potato sink strength is defined as the ability to attract photoassimilates, and the sink strength of growing potato tubers was assumed to be limited by metabolism and/or starch synthesis [24]. Sink strength had been concluded to be a more important factor than source strength on tuber yield [23]. In that study, the tuber yield was enhanced in the PGN and AGN lines. Sugar contents in phloem were higher but the leaf starch was lower in the transgenic lines, reflecting an increase in sink strength in tuber could redistribute the carbohydrates from source (leaf starch) to tuber starch. The authors also produced transgenic lines with enhanced source strength by overexpressing *E. coli* PPase in potato leaf [23]. While some transgenic lines exhibited higher PPase activities, higher sugars and lower starch in leaves, the tuber yield did not increase. The author therefore concluded that tuber yield is sink-limited, and that an additional enhancement of source capacity could further increase yield [23]. However, it should be noted that the photosynthesis rates of the AGN lines were not enhanced, and the sugar contents in the petioles and tubers of the PPase overexpression lines were not measured. Thus the potential of increasing tuber yield by enhancing photosynthesis rate in source leaf and/or sugar export from source leaf cannot be ruled out.

In this study, overexpression of AtPAP2 could improve source capacity by improved photosynthesis rate, elevated SPS activity, and sugar efflux rate in the leaves. Increased photosynthesis in the source tissues could also potentially improve the adenylate pools in the potato leaves [25]. Increased adenylate pools have previously been demonstrated to increase the potato tuber yield in experiments in which the activity of the plastidial adenylate kinase was altered [26]. ATP and adentylate pools were shown to be significantly increased in the rosette leaves of AtPAP2 OE Arabidopsis lines [9]. Meanwhile, the expression levels and enzyme activity of SPS were also significantly elevated in the AtPAP2 OE lines, which could be expected to supply more sucrose for export and anabolism. Similar to the situation observed following up-regulated expression of the sucrose transporter [18], overexpression of AtPAP2 resulted in an increased sugar efflux, improved photosynthesis and faster plant growth rate. In addition, coupled to the accumulation of soluble sugars in the tuber, the total

tuber yield and tuber starch content were improved in our OE lines. These data indicate an enhanced sink strength. We believe both (limited by sink and promoted by source) are valid as nature in generally does not follow all-or-none principle. We deduced that overexpression of AtPAP2 improves the source capacity and sink strength of potatoes by indirectly regulating the expression of SPS and sucrose transporters.

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Figure Legends

Fig 1. Growth phenotypes of 40-day-old plants. All three high AtPAP2 OE lines produced more branches than the WT.

Fig 2. Assimilation rates of potato plants. Assimilation rates (μmol CO_2 m^{-2} s^{-1}) were measured on leaves 3-4 from the top of 65- to 68-d-oldplants. Assimilation rates at different actinic light intensities (0-1000 μmol photons m^{-2} s^{-1}) were normalized to leaf area.

Fig 3. Sugar contents in leaves, petioles and tubers of second generation plants. The leaves of OE lines exhibited low sucrose, glucose and fructose contents. However, the petioles of OE lines contained higher sucrose, glucose and fructose content and the tuber OE lines shows higher sucorse, glucose and fructose contents. Within each column, the values are the fold change of OE lines compare with wild type. DW- dry weight, FW- fresh weight.

Fig. 4. Western blotting analysis of proteins involved in the sucrose synthesis. Total soluble protein extracts were from 70-day-old second generation plants in the middle of day.

Vector is the transgenic potato lines by empty vector pBA002; SPS, sucrose phosphate synthase; NR, nitrate reductase; StSUT1, *Solanum tuberosum* sucrose transporter 1; cFBPase, cytosolic fructose-1,6-bisphosphatase; Anti-cFBPase, anti-NR and anti-SPS antibodies were obtained from Agrisera; Anti-StSUT1 antibody was provided by Dr. Christina Kuhn (Humboldt University Berlin, Germany); AtPAP2, anti-AtPAP2 specific antibody; Anti-14-3-3 antibody was from Prof. Carol MacKintosh. Excess 14-3-3 recombinant protein was loaded on the GST beads and mixed with 0.5 mg total plant protein extracts, the washed and bound proteins were eluted for Western blotting analysis using the anti-SPS antibody.

Table 1. Flowering time of the transgenic potatoes

Solanum tuberosum var. Bintje						
	WT	Vector	OE1	OE4	OE7	OE10
Flowering time (day)	81 ± 0.7^{a}	89 ± 0.5^{b}	43 ± 0.8^{d}	44 ± 0.9^{d}	45 ± 1.3^{d}	73 ± 0.8^{c}
S.tuberosum cv. Desirée						
	WT	OE1	OE2	OE3		
Flowering time (day)	54.5 ± 1.6^{a}	38 ± 1.6^{b}	37.7 ± 1.4^{b}	38 ± 1.2^{b}		

Second generation potatoes (*Solanum tuberosum var. Bintje*) were grown from September 2010 to December 2010 in greenhouse (SD, Hong Kong). Potatoes (*S.tuberosum cv. Desirée)* were grown under LD conditions [16 h light period (100-200 µE $m^{-2}s^{-1}$), 25℃ and 8 h darkness, 20℃, greenhouse, Potsdam-Golm, Max Planck Institute for Molecular Plant Physiology]. Throughout the light-dark cycle relative humidity was kept at 50 %. The values marked by different letters (a, b, c) are significantly different ($p < 0.05$), n = 4~6.

Table 2. Tuber yield of the transgenic potatoes

Second generation (*S. tuberosum var. Bintje*, SD)						
	WT	Vector	OE1	OE4	OE7	OE10
Tuber number/plant	2^{a}	2^{a}	3 ± 0.8^{b}	3^{b}	2.8 ± 0.5^{ab}	2.0 ± 0.8^{ab}
Average weight/tuber, FW	7.4 ± 1^{a}	12.5 ± 1.3^{ab}	18.6 ± 2.9^{cd}	15.3 ± 3.0^{bc}	21.6 ± 2.8^{d}	11.4 ± 5.2^{ab}
Tuber weight/plant, FW	14.8 ± 2^{a}	25.1 ± 2.6^{ab}	55.9 ± 16.4^{c}	45.9 ± 9.1^{bc}	60.2 ± 16.7^{c}	20.7 ± 8.7^{a}
Root Biomass/plant, DW	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	1.4 ± 0.4^{b}	0.5 ± 0.2^{a}	1.1 ± 0.3^{b}	0.4 ± 0.3^{a}
Aboveground Biomass /plant, DW	2.9 ± 0.3^{a}	2.9 ± 0.7^{a}	5.1 ± 0.6^{c}	3.7 ± 0.7^{b}	4.6 ± 0.6^{bc}	2.9 ± 0.5^{a}
Leaf Biomass/Plant, DW	1.8 ± 0.3^{a}	1.8 ± 0.6^{a}	2.7 ± 0.2^{ab}	2.3 ± 0.7^{ab}	2.7 ± 0.7^{ab}	1.9 ± 0.3^{a}
Petioles Biomass/plant, DW	1.1 ± 0.2^{a}	1.1 ± 0.2^{a}	2.4 ± 0.5^{c}	1.6 ± 0.4^{ab}	2.0 ± 0.2^{bc}	1.1 ± 0.3^{a}
Third generation (*S. tuberosum var. Bintje*, LD)						
	WT	OE1	OE3	OE4	OE7	OE10
Tuber number/plant	3	15	15	22	18	20
Tuber weight (g)/plant, FW	107.5	258.5	270.2	300.9	275.9	241.3
Tuber yield and starch content of a different cultivar (*S.tuberosum cv. Desirée*, LD)						
	WT	OE1	OE2	OE3		
Tuber number/plant	6.3 ± 1.4^{a}	11.5 ± 1.8^{b}	9.8 ± 0.8^{b}	10.3 ± 1.4^{b}		
Tuber weight (g/plant), FW	176.0 ± 20.7^{a}	281.4 ± 17.8^{b}	267.8 ± 14.5^{b}	252.5 ± 17^{b}		
Tuber weight (g/plant), DW	25.0 ± 2.9^{a}	53.9 ± 3.4^{b}	52.7 ± 2.9^{b}	48.0 ± 3.2^{b}		
Tuber Water content (mg/g), FW	857.8 ± 8.2^{a}	806.6 ± 4.1^{b}	803.0 ± 13.2^{b}	811.1 ± 15.2^{b}		
Starch content (µmol Glucose /g), FW	351.7 ± 7.7^{a}	416.8 ± 9.9^{b}	429.9 ± 8.9^{b}	419.13 ± 5.3^{b}		

Tubers > 1g was measured. Samples were collected from 4 - 6 independent plants after the decay of the shoots following a 4-month growth period. The second generation potato (*S. tuberosum var. Bintje*) was grown in short day condition in Hong Kong and the third generation was grown in long day condition in University of Potsdam, Golm. Potatoes (*S.tuberosum cv. Desirée)* were grown under controlled conditions from November 2013 to February. 2014 [16 h

light period (100-200 µE $m^{-2}s^{-1}$), 25℃ and 8 h darkness, 20℃, greenhouse, Potsdam-Golm, Max Planck Institute for Molecular Plant Physiology]. Throughout the light-dark cycle relative humidity was kept at 50 %. 100mg fresh sample of developing tuber was lyophilized before measurement of dry weight and water content. Compared with that of the wild type, tubers of OE lines showed decreased water content but improved tuber weight and starch content. Starch content was measured by 10 mg fresh potato tubes. Within each column, the values marked by different letters (a, b, c) are significantly different ($p < 0.05$), n = 4~6. DW, dry weight (g); FW, fresh weight (g).

Table 3. SPS Enzyme activity analysis

µM sucrose/µg protein/hour	WT	Vector	OE 1	OE 4	OE 7	OE 10
V_{max}	139 ± 4^{a}	147 ± 4^{ab}	168 ± 6^{c}	163 ± 13^{c}	162 ± 9^{c}	155 ± 6^{c}
V_{limit}	73 ± 3^{a}	83 ± 5^{b}	97 ± 2^{c}	93 ± 7^{c}	93 ± 6^{bc}	90 ± 5^{bc}

The third and the fourth fully mature leaves were used for SPS enzyme activity analysis after the photosynthesis measurement. Within each column, the values marked by different letters (a, b) are significantly different ($p < 0.05$), n = 5.

Table 4. Relative transcriptional levels of sucrose transporters

	WT	Vector	OE1	OE4	OE7	OE10
StSUT1	1.00 ± 0.01^{a}	1.31 ± 0.01^{b}	1.53 ± 0.01^{b}	1.07 ± 0.02^{a}	1.21 ± 0.01^{ab}	1.03 ± 0.01^{a}
StSUT2	1.00 ± 0.01^{a}	1.02 ± 0.01^{a}	0.36 ± 0.01^{b}	0.25 ± 0.01^{b}	0.23 ± 0.01^{b}	0.07 ± 0.01^{c}
StSUT4	1.00 ± 0.02^{a}	0.74 ± 0.01^{ab}	0.57 ± 0.02^{b}	0.58 ± 0.01^{b}	0.45 ± 0.02^{b}	0.08 ± 0.01^{c}

The third and the fourth 70-days-old mature leaves were used for real-time PCR analysis. RQ, the gene relative expression to WT was calculated using the equation: $2^{-(\Delta\Delta CT)}$. Within each column, the values marked by different letters (a, b, c) are significantly different ($p < 0.05$), n = 3.

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Fig. 1

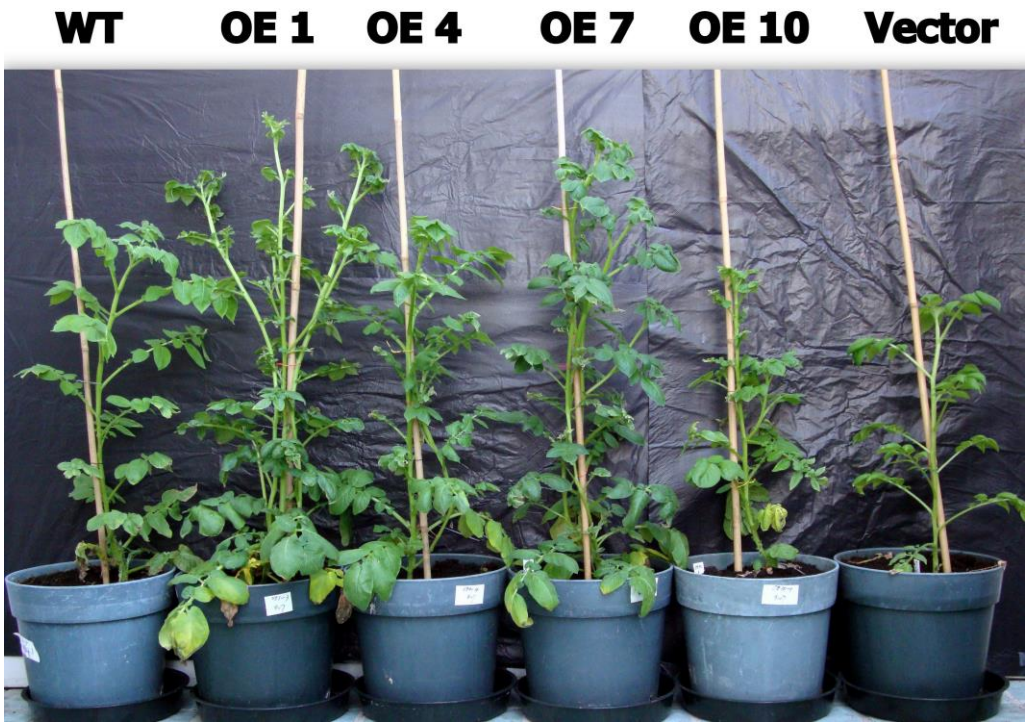

WT
OE 1
OE 4
OE 7
OE 10
Vector

Fig. 2

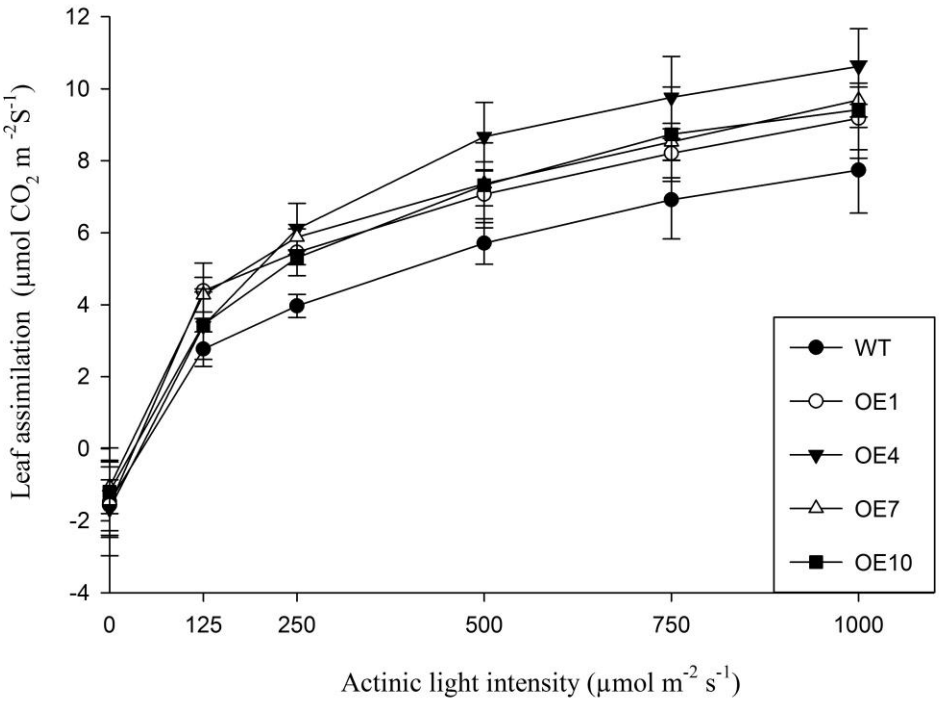

Leaf assimilation (µmol CO2 m-2S-1)
12
10
8
6
4
2
0
-2
-4
0
125
250
500
750
1000
Actinic light intensity (µmol m-2 s-1)
WT
OE1
OE4
OE7
OE10

Fig. 3

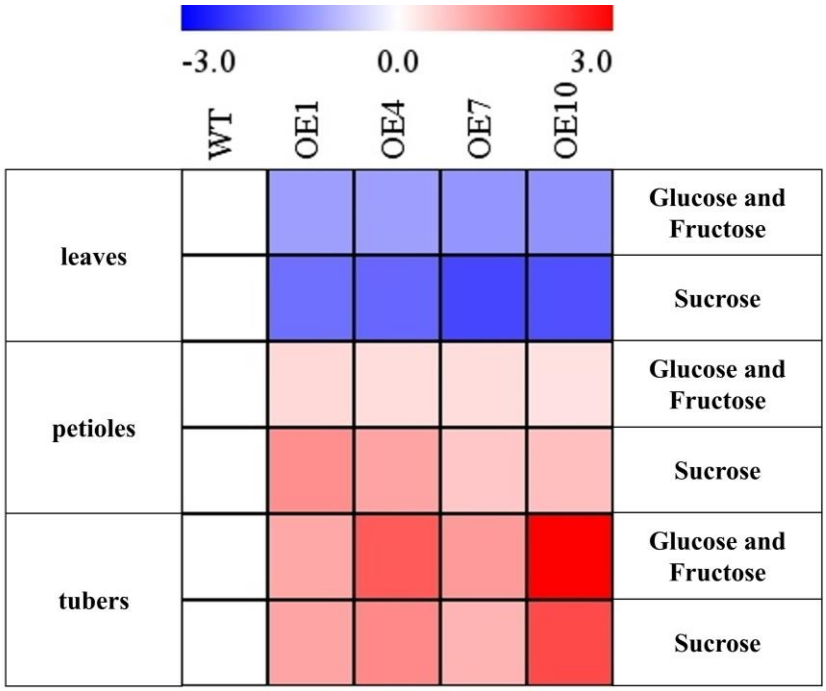
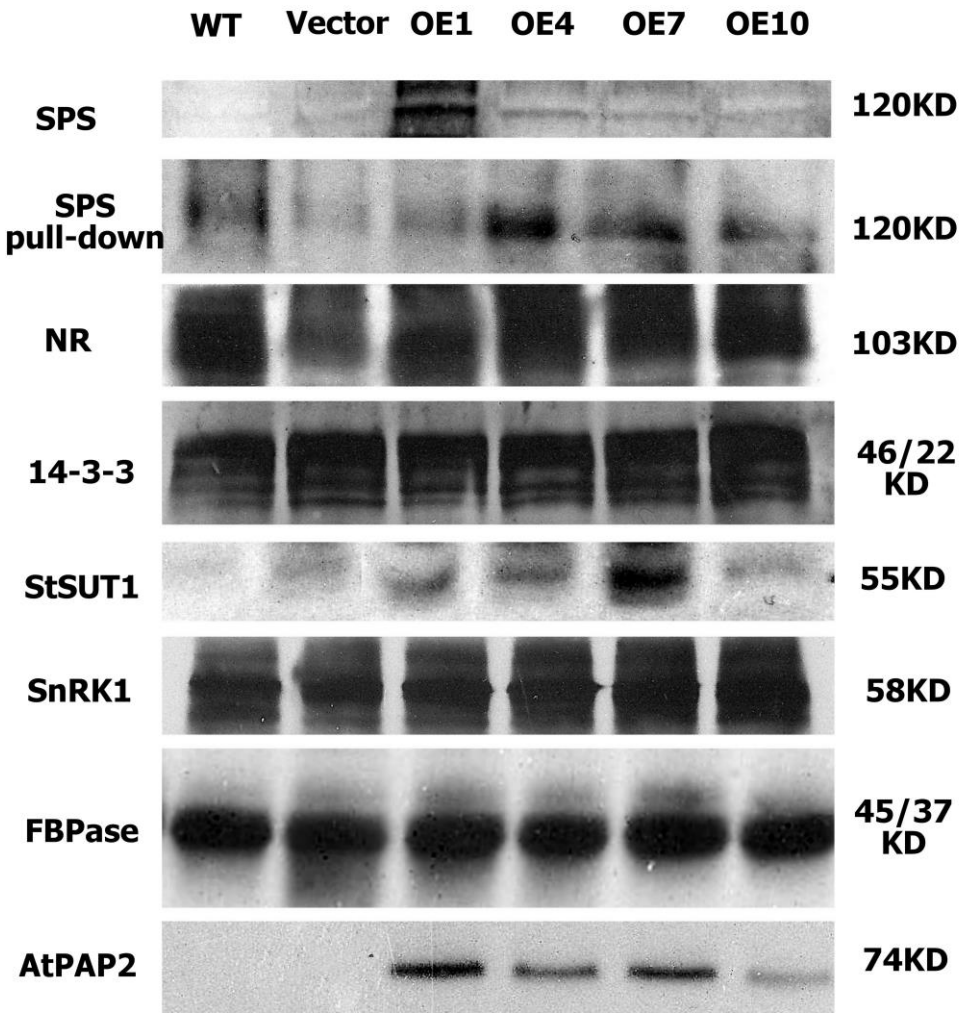

-3.0
0.0
3.0
WT
OE1
OE4
OE7
OE10
leaves
Glucose and Fructose
Sucrose
petioles
Glucose and Fructose
Sucrose
tubers
Glucose and Fructose
Sucrose

Fig.4


WT
Vector
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OE4
OE7
OE10
SPS
120KD
SPS pull-down
120KD
NR
103KD
14-3-3
46/22 KD
StSUT1
55KD
SnRK1
58KD
FBPase
45/37 KD
AtPAP2
74KD