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ORIGINAL ARTICLE

Control of potato tuber dormancy and sprouting by expression of sense and antisense genes of pyrophosphatase in potato

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Abstract Inorganic pyrophosphate (PPi) is an enzyme involved in sugar metabolism in potato tubers. In our previous study, we isolated an inorganic pyrophosphatase (PPase) gene from potato and obtained the transgenic potato plants transformed with the sense and antisense *PPase* genes respectively. In the present experiment, the physiological indexes, tuber dormancy, and sprouting characteristics of the transgenic potatoes were analyzed and evaluated. The result showed that the PPase activity and the inorganic phosphate content of tubers were lower in the antisense transgenic plant lines but were higher in the sense transgenic plant lines, compared with wild-type tubers. Soluble sugars, such as glucose, fructose and sucrose increased in transgenic plants that had overexpression of the sense *PPase* gene, but decreased in the antisense transgenic plant lines, compared with wild-type tubers. Tuber sprouting time of the antisense transgenic plants were delayed for 2 and 3 weeks and reached the 100 % sprouting rate only after 14 and 16 weeks storage compared with the wild-type when tubers are stored under 25 and 4 °C, respectively. In contrast, tuber sprouting time of the sense transgenic plants was earlier by approximately 2 weeks than that of wild-type tubers under these storage temperatures.

Keywords Pyrophosphatase · Soluble sugars · Tuber · Dormancy · Sprouting · Potato

Abbreviations

Pi	Inorganic phosphate
PPase	Inorganic pyrophosphatase
PPi	Inorganic pyrophosphate

Introduction

Potato is an important crop in the world. Potato tubers, mainly the fresh tubers, can be used in many different ways (Sonnewald 2001). Potato tuber dormancy and sprouting is very important to potato cultivation, tuber production, and potato processing. Control of potato tuber sprouting is a major objective in potato breeding since sprouting can lead to major quality losses of stored tubers (Sonnewald and Sonnewald 2014). Potato tuber dormancy and sprouting are complex biological processes, which are influenced directly or indirectly by numerous environmental, physiological, and genetic factors during tuber production and storage. Until now, the main methods to inhibit potato tuber sprouting are using sprout inhibitors and/or storing tubers under low temperatures. These techniques are often problematic. Therefore, new strategies are needed. Engineering of responsible genes may regulate potato tuber sprouting.

Inorganic pyrophosphate (PPi) plays a vital role as a co-factor in sucrose metabolism and its fine regulation and cross talk between metabolic pathways (Sonnewald 2001).

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Sucrose metabolism requires PPi at two enzymatic steps. One is in the conversion of UDP-glucose to glucose-1-phosphate by the action of UDP-glucose pyrophosphorylase. Another is in the conversion of glucose-1-phosphate to fructose-6-phosphate (F6P) by the action of pyrophosphate-fructose 6-phosphate phosphotransferase (PFP). Sonnewald (2001) proposed that removing cytosolic PPi should inhibit sucrose breakdown and stimulate sucrose synthesis. Inorganic pyrophosphatase (PPase) is ubiquitous enzyme catalyzing the hydrolysis of PPi into two inorganic phosphates (Pi). PPi metabolism is one of key regulation sites in sucrose synthesis.

In order to elucidate the role of PPi in plant metabolism, Lahti (1988) isolated a *PPase* gene from *Escherichia coli*. Sonnewald (1992) transformed tobacco and potato with *PPase* gene from *E. coli* under the control of CaMV 35S promoter and targeted to the cytosol to decrease the PPi concentration. The result showed that sugar levels increased dramatically in source leaves of the transgenic tobacco and potato, which was speculated to be the result of stimulated sucrose biosynthesis in mesophyll cells and the reduced long distance transport of sucrose (Sonnewald 1992; Jelitto et al. 1992). Hajirezaei and Sonnewald (1999) obtained transgenic potato tubers that did not sprout even after a prolonged storage period of 2 years using expressing *E. coli PPase* gene under the control of the strong and constitutive chimeric ST-LS1/35S promoter, however, plant growth and tuber development of transgenic plants was impaired severely because of the unspecific removal of cytosolic pyrophosphate. The opposite results were found by the expression of *E. coli PPase* gene under the control of the tuber-specific patatin promoter B33 that showed accelerating the transgenic potato microtuber sprouting 6–7 weeks, and the early-sprouting phenotype is stable in generations (Farré et al. 2001). Therefore, the characteristics of tuber dormancy and sprouting could be regulated by controlling cytosolic PPi content, and manipulating cytosolic PPi content can represent a cost-effective and environmentally responsible strategy to control tuber sprouting (Sonnewald 2001).

With plants, the cloning of *PPase* gene cDNAs was reported various plants, including *Arabidopsis thaliana* (Kieber and Signer 1991), *Beta vulgaris* (Kim et al. 1994), tobacco (Lerchl et al. 1995), potato (du Jardin et al. 1995), rice (Sakakibara et al. 1996), and barley (Visser et al. 1998). We isolated a *PPase* gene cDNA (GenBank accession EF091820) from potato, which was 673 with 636 bp open reading frame (ORF) encoded a 211-amion acid polypeptide (Liu et al. 2008). The sense and antisense plant expression vectors were constructed by fusing the *PPase* gene with the constitutive expression promoter CaMV 35S, respectively, which were transferred to potato cultivar Gannongshu 2 through *Agrobacterium tumefaciens*-mediated transformation (Li et al. 2008). In the present paper, we reported the analysis results of physiological traits, dormancy, and sprouting of transgenic potato minitubers harvested from greenhouses.

Materials and methods

Plant materials

Experiments were carried out with the tetraploid cultivar Gannongshu 2 (G2) of potato (*Solanum tuberosum* L.). Two independent antisense transgenic plant lines, AS-G2-1-1 and AS-G2-1-2, and two independent sense transgenic plant lines, OE-G2-2-3 and OE-G2-2-4, were obtained by transformation of sense and antisense *PPase* gene under the control of the constitutive expression promoter CaMV 35S through *Agrobacterium tumefaciens*-mediated transformation (Liu et al. 2008; Li et al. 2008). These two groups of potato plants were propagated in vitro by subculture of single-nodal cuttings on MS medium supplemented with 3 % sucrose and 0.6 % agar. Plantlets were grown in 150 ml flasks under photoperiod of a 16 h white fluorescent light and 8 h dark cycle at 20 °C. The transgenic and control potato plants were transferred from flask to vermiculite in greenhouses under natural light at 25 °C and watered and fertilized weekly with a complete nutrient solution. Minitubers were harvested from 12 weeks-old plants and stored at room temperature (25 °C) and refrigerator (4 °C) under dark condition, respectively.

Assay of PPase activity and inorganic phosphate content

Minituber slices were prepared from intact minitubers and immediately frozen in liquid nitrogen and stored at −80 °C until use. PPase activity was assayed by measurement of inorganic phosphate release as described by Heinonen and Lahti (1981). Minituber slices were homogenized in liquid nitrogen and dissolved in 50 mM Tris–HCl (pH 8.0) and 3 mM $MgCl_2$. After centrifugation of 12,000 rpm for 10 min at 4 °C, 50 µl of the supernatant was assayed in 500 µl of 100 mM Tris–HCl (pH 8.0), 50 mM PPi and 3 mM $MgCl_2$ for 30 min at 30 °C. The reaction was stopped by the addition of 100 µl 1 M citrate. The release of inorganic phosphate was determined by adding AAM solution (1 v/v 10 mM ammonium molybdate, 1 v/v 5 mM H_2SO_4 and 2 v/v acetone), and the yellow color of solution was measured spectrophotometrically at 340 nm. Inorganic phosphate was assayed as described by Zhang and Tian (2007).

Determination of carbohydrates

Glucose, fructose, and sucrose were determined in the extracts of potato minitubers according to Plant Physiology Society of Shanghai (1985). Starch was measured as described by Zou (2000).

Evaluation of tuber dormancy and sprouting characteristics

Sprouting of minitubers stored at room temperature (25 °C) and refrigerator (4 °C) was monitored once a week. The length of main bud reached 0.5 cm which was defined as sprouting tubers. Each of the transgenic potato plant lines and the control line contained 30 minitubers and had three replicates.

Data analysis

Data are analyzed using Microsoft Excel 2003. ANOVA and Duncan's multiple range test were using the statistical analysis system (SAS).

Results

PPase activity and inorganic phosphate content in the transgenic potato tubers

PPase activity and Pi content of growing tubers were higher than stored tubers at 4 and 25 °C. In the antisense transgenic plant lines AS-G2-1-1 and AS-G2-1-2, PPase activities and Pi contents in growing tubers, stored tubers at 4 °C, and stored tubers at 25 °C for 90 days were lower than those in wild-type tubers (Tables 1, 2). In the sense transgenic plant lines OE-G2-2-3 and OE-G2-2-4, PPase activities and Pi contents in growing tubers and stored tubers (at 4 and 25 °C) for 90 days were higher than wild-type tubers (Tables 1, 2).

Effects of transgenic PPase gene on carbohydrate contents in the transgenic potato tubers

Overexpression of *PPase* gene in the sense transgenic potato tubers led to an increase of soluble sugars, such as glucose, fructose and sucrose. Glucose content was 14.11–29.16 % higher in the transgenic lines than wild-type (G2) tubers. Fructose content was 78.22–107.29 % higher in tubers of the transgenic lines than in wild-type tubers. In the sense transgenic plant lines OE-G2-2-3 and OE-G2-2-4, sucrose content in growing tubers, tubers stored at 4 °C (90 days), and tubers stored at 25 °C (90 days) were 32.15 and 37.35, 31.96 and 32.81 %, and 28.65 and 26.61 % higher than wild-type tubers, respectively (Table 3). In contrast, glucose, fructose, and sucrose contents of tubers of the antisense transgenic plant lines was decreased compared with wild-type tubers, and the highest reduction was 33.74, 51.73, and 39.80 %, respectively for the three types of sugars (Table 3).

There were no remarkable differences in starch content of growing tubers between the sense transgenic plants and wild-type plants, while there was 15.27 and 15.99 % decreases of starch content of tubers in the antisense transgenic plant lines AS-G2-1-1 and AS-G2-1-2, respectively. Similar results were obtained in tubers that were stored for 90 days at 4 °C. While in tubers stored for 90 days at 25 °C, there was 17.06 and 16.56 % decreases of starch content of tubers in the sense transgenic plant lines OE-G2-2-2 and OE-G2-2-4, respectively; whereas no significant difference in the antisense transgenic plants was found compared with wild-type tubers (Table 3).

Characterestics of tuber dormancy and sprouting of the transgenic potatoes

Tubers harvested from wild type plants started to sprout after 7 weeks storage at 25 °C and reached the 100 % sprouting frequency (all tubers were spourted) after 12 weeks of storge, while tubers of the antisense transgenic

Table 1 PPase activity in the transgenic potato tubers (μmol/min/g FW)

Plant lines[a]	Growing tubers	Tubers stored at 4 °C	Sprouting tubers stored at 25 °C
G2 (CK)	2.26 ± 0.17 Bb[b]	2.08 ± 0.23 bB	2.03 ± 0.32 bB
AS-G2-1-1	1.84 ± 0.11 cC	1.74 ± 0.29 cC	1.43 ± 0.11 cC
AS-G2-1-2	1.80 ± 0.05 cC	1.77 ± 0.13 cC	1.47 ± 0.25 cC
OE-G2-2-3	3.02 ± 0.33 aA	2.82 ± 0.25 aA	2.60 ± 0.21 aA
OE-G2-2-4	2.99 ± 0.12 aA	2.86 ± 0.12 aA	2.73 ± 0.15 aA

[a] G2: the potato cultivar Gannongshu 2. AS-G2-1-1 and AS-G2-1-2: the two independent antisense transgenic plant lines. OE-G2-2-3 and OE-G2-2-4: the two independent sense transgenic plant lines

[b] Values represent the mean ± standard error (SE) from three replicates. Each replicate contained 30 tubers. Data followed different small and capital letters in the same column indicated a significant difference at $P < 0.05$ and $P < 0.01$, respectively, by Duncan's multiple range test

Table 2 Inorganic phosphate (Pi) content in the transgenic potato tubers (μmol/g FW)

Plant lines[a]	Growing tubers	Tubers stored at 4 °C	Sprouting tubers stored at 25 °C
G2 (CK)	17.09 ± 1.58 bB[b]	15.75 ± 1.16 bB	13.70 ± 0.83 bB
AS-G2-1-1	15.11 ± 1.09 cC	12.75 ± 1.24 cC	10.47 ± 1.26 cC
AS-G2-1-2	14.96 ± 2.17 cC	12.58 ± 2.24 cC	10.45 ± 0.67 cC
OE-G2-2-3	19.60 ± 2.09 aA	17.62 ± 1.07 aA	16.26 ± 0.85 aA
OE-G2-2-4	19.29 ± 2.16 aA	17.60 ± 0.92 aA	16.55 ± 0.95 aA

[a] G2: the potato cultivar Gannongshu 2. AS-G2-1-1 and AS-G2-1-2: the two independent antisense transgenic plant lines. OE-G2-2-3 and OE-G2-2-4: the two independent sense transgenic plant lines

[b] Values represent the mean ± standard error (SE) from three replicates. Each replicate contained 30 tubers. Data followed different small and capital letters in the same column indicated a significant difference at $P < 0.05$ and $P < 0.01$, respectively, by Duncan's multiple range test

Table 3 Carbohydrates contents in the transgenic potato tubers

Plant lines	Parameters	Growing tubers	Tubers stored at 4 °C	Sprouting tubers stored at 25 °C
G2 (CK)	Glucose (mg/g FW)	8.54 ± 0.21 bB[a]	13.46 ± 1.42 bB	14.67 ± 1.32 bB
	Fructose (mg/g FW)	8.68 ± 0.34 bB	9.43 ± 0.72 bB	10.02 ± 1.20 bB
	Sucrose (mg/g FW)	20.00 ± 1.51 bB	25.72 ± 1.63 bB	26.91 ± 2.46 bB
	Starch (% FW)	23.26 ± 1.83 aA	19.79 ± 0.65 aA	17.94 ± 0.68 bA
AS-G2-1-1	Glucose (mg/g FW)	5.87 ± 0.96 cC	9.59 ± 0.61 cC	9.72 ± 1.08 cC
	Fructose (mg/g FW)	4.19 ± 0.44 cC	6.72 ± 0.52 cC	7.50 ± 0.26 cC
	Sucrose (mg/g FW)	12.50 ± 1.12 cC	19.56 ± 1.25 cC	20.92 ± 2.46 cC
	Starch (% FW)	19.71 ± 1.12 bB	17.82 ± 1.25 bA	16.41 ± 0.61 aA
AS-G2-1-2	Glucose (mg/g FW)	6.01 ± 0.45 cC	9.87 ± 0.12 cC	9.99 ± 1.21 cC
	Fructose (mg/g FW)	4.89 ± 0.66 cC	6.92 ± 0.22 cC	7.49 ± 0.25 cC
	Sucrose (mg/g FW)	12.04 ± 1.12 cC	19.06 ± 1.92 cC	20.64 ± 1.86 cC
	Starch (% FW)	19.54 ± 1.62 bB	17.57 ± 1.12 bA	16.76 ± 1.42 aA
OE-G2-2-3	Glucose (mg/g FW)	10.22 ± 0.34 aA	16.55 ± 0.82 aA	16.74 ± 1.02 aA
	Fructose (mg/g FW)	15.71 ± 0.51 aA	18.49 ± 1.31 aA	19.61 ± 0.83 aA
	Sucrose (mg/g FW)	26.43 ± 2.16 aA	33.94 ± 1.82 aA	34.62 ± 1.26 aA
	Starch (% FW)	24.83 ± 1.03 aA	20.06 ± 0.86 aA	14.88 ± 0.95 cB
OE-G2-2-4	Glucose (mg/g FW)	11.03 ± 0.76 aA	15.98 ± 0.86 aA	17.03 ± 1.02 aA
	Fructose (mg/g FW)	15.47 ± 0.62 aA	18.44 ± 0.65 aA	20.77 ± 1.33 aA
	Sucrose (mg/g FW)	27.47 ± 1.06 aA	34.16 ± 1.92 aA	34.07 ± 2.65 aA
	Starch (% FW)	24.78 ± 2.32 aA	20.87 ± 225 aA	14.97 ± 0.63 cB

[a] Values represent the mean ± standard error (SE) from three replicates. Each replicate contained 30 tubers. Data followed different small and capital letters in the same parameter of the transgenic potato plant lines and the control in the same column indicated a significant difference at $P < 0.05$ and $P < 0.01$, respectively, by Duncan's multiple range test

plants remained unsrpouted for two more weeks compared with wild-type tubers and reached 100 % sprouting frequency only after 14 weeks of storage. Tubers harvested from the sense transgenic plants started to sprout after 5 weeks storage at 25 °C and reached the 100 % sprouting frequency after 10 weeks of storage, which was 2 weeks earlier than the wild-type tubers (Figs. 1, 2). As for tubers stored at 4 °C, the sprouting time was delayed for three weeks, compared with tubers stored at 25 °C, and reached the 100 % sprouting frequency after 15, 16, and 12 weeks of storage for the wild-type tubers, the antisense transgenic tubers, and the sense transgenic plant tubers, respectively (Fig. 3).

Discussion

Potato tuber dormancy is defined as the physiological state of tubers in which autonomous sprout growth will not occur, even when tubers are placed under ideal conditions for

Fig. 1 Comparison of tuber sprouting between the transgenic potato tubers and the wild-type potato tubers. Tubers of the sense transgenic plant line OE-G2-2-3 and the antisense transgenic plant line AS-G2-1-1 and minitubers of the control G2 were stored at 25 °C for 45 days

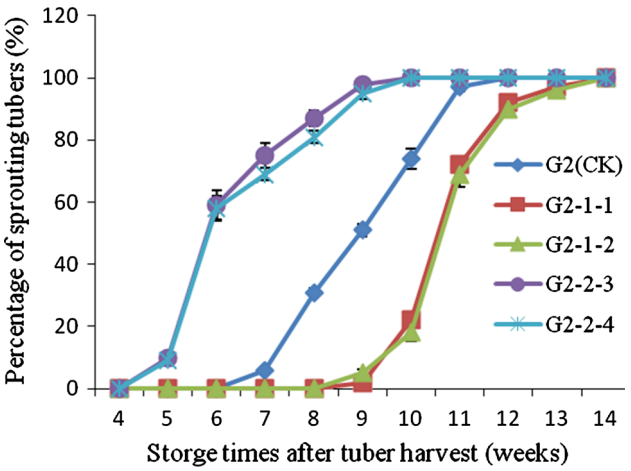

Fig. 2 Sprouting times of tubers from the transgenic lines and the control line at 25 °C. G2: potato cultivar Gannongshu 2. AS-G2-1-1 and AS-G2-1-2: the two independent antisense transgenic plant lines. OE-G2-2-3 and OE-G2-2-4: the two independent sense transgenic plant lines. Values represent the mean ± standard error (SE) from three replicates. Each replicate contained 30 tubers

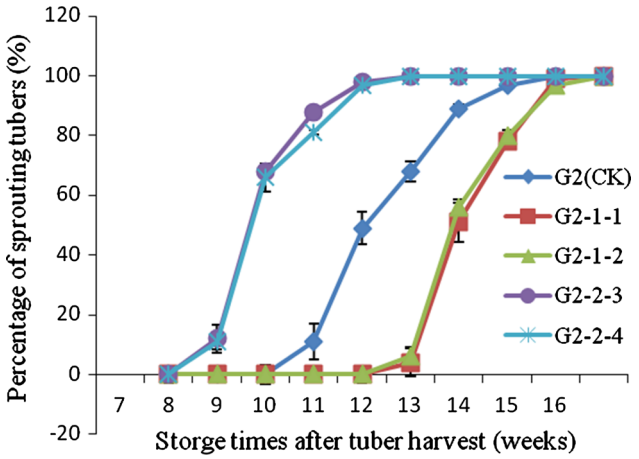

Fig. 3 Sprouting times of transgenic potatoes and the control at 4 °C. G2: the potato cultivar Gannongshu 2. AS-G2-1-1 and AS-G2-1-2: the two independent antisense transgenic plant lines. OE-G2-2-3 and OE-G2-2-4: the two independent sense transgenic plant lines. Values represent the mean ± standard error (SE) from three replicates. Each replicate contained 30 tubers

sprouting (Lang et al. 1987). The duration of tuber dormancy period depends on potato genotypes and the environmental conditions of tuber growing and storage (Sonnewald 2001).

Tuber dormancy release and sprouting is associated with structural and metabolic changes as well as with major changes in gene expression pattern. Sucrose is one of substances regulating potato tuber dormancy and sprouting. PPi plays a important role as a co-factor in sucrose metabolism (Sonnewald 2001). PPi is produced by a wide range of biosynthetic reactions and is usually present in high amounts in the cytosol. The theoretical energy release by the cleavage of PPi was estimated to be about one-half of that of ATP hydrolysis (Weiner et al. 1987). PPase is a ubiquitous enzyme catalyzing the hydrolysis of PPi into Pi.

In order to elucidate the role of PPi and PPase in potato tuber dormancy and sprouting, we isolated a *PPase* cDNA (GenBank accession No. EF091820) from potato (Liu et al. 2008) and obtained the transgenic potato plants transformed with the sense and antisense *PPase* genes, respectively (Li et al. 2008). PPase activities and Pi contents of tubers were increased or decreased in the sense and antisense transgenic tubers, regardless of whether the storage was at 4 or 25 °C for 90 days (Tables 1, 2). Glucose, fructose and sucrose contents were also changes (Table 3); for which the changes in the PPase-overexpression tubers were similar with the results in the transgenic potato transformed with *PPase* gene from *E. coli* (Sonnewald 1992; Jelitto et al. 1992). The results from Hajirezaei and Sonnewald (1999) provided direct evidence that cytosolic pyrophosphate is essential for potato tuber sprouting. They obtained transgenic potato plants with a prolonged quiescence (likely dormant) period of tubers, and no sprouting even after a prolonged storage period of 2 years. They thought that reduced sucrose loading into the phloem and utilization in the developing sprout was the cause of inhibited sprout growth (Hajirezaei and Sonnewald 1999). There were several studies (Hajirezaei et al. 2003; Viola et al. 2007) suggested that sucrose availability was crucial for induction of sprout growth. In the late stages of tuber sprouting, induction of starch mobilization in parenchyma cells was found to be correlated with an accumulation of soluble sugars (Viola et al. 2007). In the present study, the starch content went down in the antisense transgenic plant lines in growing tubers and tubers stored at 4 °C,

while increased in the sense transgenic plant lines. In sprouting tubers stored at 25 °C, the starch contents went down in both sense and antisense transgenic plant lines, although sucrose content was decreased in the antisense and increased in the transgenic plant sense lines (Table 3). PPase activity was suppressed in the antisense transgenic plant lines AS-G2-1-1 and AS-G2-1-2 (Table 1), which resulted in enhancing sucrose breakdown and inhibiting sucrose synthesis (Table 3) and further inhibiting tuber sprouting (Figs. 2, 3). In contrast, PPase activity was enhanced in the sense transgenic plant lines OE-G2-2-3 and OE-G2-2-4 (Table 1), which resulted in inhibiting sucrose breakdown (Table 3) and further accelerated tuber sprouting (Figs. 2, 3).

In this research, the sense and antisense transgenic potato plants with both prolonged and shortened periods of tuber dormancy were obtained by genetic engineering of PPase gene. The transgenic potato plants will be used to further evaluate agronomic characteristics and application in potato breeding.

Conclusions

PPi plays a vital role as a co-factor in sucrose metabolism. PPase is a ubiquitous enzyme catalyzing the hydrolysis of PPi into Pi. Carbohydrate metabolisms of glucose, fructose, and sucrose were changed by genetic engineering of the *PPase* gene, further resulted in potato tuber dormancy period prolonged or shortened when tubers stored at 4 and 25 °C.

Author contribution statement Conceived and designed the experiments: Huaijun Si and Di Wang. Performed the experiments: Chongfen Zhang, Yikai Wen and Ning Zhang. Data analysis: Chongfen Zhang and Ning Zhang. Wrote the manuscript: Huaijun Si.

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