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RESEARCH ARTICLE

Heterologous expression of two *Arabidopsis* starch dikinases in potato

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Starch phosphate esters influence physiochemical properties of starch granules that are essential both for starch metabolism and industrial use of starches. To modify properties of potato starch and understand the effect of starch phosphorylation on starch metabolism in storage starch, the starch dikinases from *Arabidopsis thaliana*, glucan water dikinase 2 and 3 (*AtGWD2* and *AtGWD3*), were heterologously expressed in potato tubers (*Solanum tuberosum*) from two genetic backgrounds: the amylose-containing clone Kardal and the amylose-free mutant *amf*. Modified starches showed altered granule morphology, but no significant changes in starch phosphate content were observed. Genes involved in starch metabolism did not show altered expression in the transgenic lines relatively to the control; however, sucrose synthase 4 (*SUSY4*) was upregulated in transgenic tubers with AtGWD2 expression. Taken together, these results demonstrated that expression of *AtGWD2* and *AtGWD3* are not suitable for engineering starch with a high phosphate content in potato tubers but can provide new insights in the process of starch phosphorylation in the amyloplast.

Keywords:
Glucan / Granule morphology / Starch metabolism / Storage starch / Transitory starch / Water dikinase

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1 Introduction

Starch is the primary energy reserve in higher plants and the most important resource for animal and human energy intake. Starch granules are mainly composed of amylose (~25%) and amylopectin (~75%) regardless of botanic origin and synthesized in plastids as two main types, transitory and storage starch. Transitory starch is produced in chloroplasts during daytime photosynthesis and remobilized into sugars for respiration and growth at night [1]. Storage starch is accumulated in amyloplasts of heterotrophic organs (*e.g.*, potato tubers, cereal seeds and cassava roots) and when required by the plant, hydrolyzed to support phases of growth [2].

In the past decades, substantial progress has been made to elucidate the metabolism of transitory starch by using the model plant *Arabidopsis thaliana*. A breakthrough came with the discovery of the essential role of starch phosphorylation in starch metabolism. It has been shown that phosphate groups are built into the starch granules, with variations in content within and among species, during starch

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Abbreviations: AGPase, ADP-glucose pyrophosphorylase; **AtGWD2,** glucan water dikinase 2 of *Arabidopsis thaliana*; **AtGWD3,** glucan water dikinase 3 *of Arabidopsis thaliana*; ***BAM,*** β-amylase; ***EF1α,*** elongation factor; **GBSSI,** granule-bound starch synthase I; **HPAEC-PAC,** high-performance anion-exchange chromatography with pulsed amperometric detection; **qRT-PCR,** quantitative RT-PCR; **SUSY4,** sucrose synthase 4; **StGWD1,** potato glucan water dikinase; **StGWD3,** potato phosphoglucan water dikinase; ***SEX4***, ***LSF1***, **and** ***LSF2***, starch phosphatases; **SSIII,** starch synthase III; ***SBEI*** **and** ***SBEII,*** starch branching genes; ***SP,*** starch phosphorylase; **UGPase,** UDP-glucose pyrophosphorylase

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biosynthesis for an efficient and accurate granule packing and physical amorphization prior to degradation [3]. The presence of phosphate groups leads to a local structural disturbance within the starch granule and by that facilitates the access of degrading enzymes and promote starch degradation. Phosphate groups are found attached to amylopectin chains at both C-6 (~60%) and C-3 (~30%) positions of the glucose residue [4, 5]. Two enzymes are responsible for this process, namely, glucan, water dikinase (GWD1) and phosphoglucan, water dikinase (GWD3).

GWD1 was shown to phosphorylate glucose residues exclusively at the C-6 position [6], while GWD3 phosphorylate glucose residues exclusively at the C-3 position with substrate specificity requirements for pre-phosphorylated starch [7, 8]. GWD3 was first discovered in *A. thaliana* and named AtGWD3. Very recently, GWD3 was also isolated and identified in potato (*Solanum tuberosum*), and named StGWD3, with 75.3% similarity to AtGWD3 (Supporting Information Fig. S1) [9]. Additionally, a third glucan, water dikinase termed AtGWD2 was also first discovered in *A. thaliana* and identified as an active cytosolic isoform of GWD1, as it lacks the transit peptide (Supporting Information Fig. S1) [10]. This gene is able to phosphorylate α-glucans in vitro and does not require pre-phosphorylation of substrate. Analysis of mutants suggested that AtGWD2 is not directly involved in transitory starch degradation. So far, only GWD1 has been extensively studied in various crops [*e.g.*, rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and potato], where much attention has been focused on the manipulation of gene expression or expression of orthologous gene to modify phosphate content in starch granules or investigate the effect of starch phosphorylation in plant primary metabolism [11–14].

The importance of starch phosphorylation is not limited to the relevance of starch metabolism in plant survival, but also due to its positive impact on the starch functionality. Unlike cereal starch, potato starch contains high phosphate content conferring a high swelling capacity to starch gels, which is desired in many industrial processes [15, 16]. In industry, starch is often chemically or physically modified to gain such properties; however, these modifications are expensive and frequently produce hazardous waste. Therefore, producing starches with enhanced properties *in planta* is needed to fully meet urgent demands for environmentally friendly starch production. To achieve this, a comprehensive understanding of starch phosphorylation is fundamental. To date, however, the role and molecular mechanism of starch phosphorylation are unclear in storage organs.

To explore the mechanism of starch phosphorylation in storage starch and produce starch with altered properties *in planta*, AtGWD2 and AtGWD3 were introduced into two potato genetic backgrounds, Kardal and *amf* mutants, respectively. The effects on starch phosphate content, granule morphology, properties, and starch metabolism pathway have been examined and are discussed.

2 Materials and methods

2.1 Plasmid construction

Two constructs, pBIN19/GWD2 and pBIN19/GWD3, were made for ectopic expression of AtGWD2 (GenBank: AAO42141) and AtGWD3 (GenBank: AY747068) in potato, respectively. All constructs were fully sequenced to verify their correctness. The construct pBIN19/SBD previous reported [17] was modified by adding an *Xba*I site to generate pBIN19/SBD-*Xba*I in order to clone each of the two GWD genes (details in Supporting Information Fig. S2).

For the assembly of the pBIN19/GWD2 construct, the AtGWD2 encoding fragment was generated by PCR amplification with the primers 5′-TGC**TCTAGA**GCA **G**AC TACAAAGACGATGACGATAAAACTAGTATGGCAACCT CTAAAT-3′ (FLAG-encoding sequence underlined) and 5′-GC**GTCGAC**GTTCAGTGGTGGTGGTGGTGGTGCTC GAGAACTTGGGGTCTAGCT-3′ (HIS-encoding sequence underlined), which contained *Xba*I and *Sal*I sites at 5′ ends, respectively (in bold). This amplified fragment, containing an N-terminal FLAG tag, AtGWD2 and a C-terminal HIS tag, was cloned into the same restriction sites of pBIN19/SBD-*Xba*I, generating the pBIN19/GWD2 plasmid. Construct pBIN19/GWD3 was obtained using the same procedure. A fragment containing FLAG tag, tGWD3, and a HIS tag was amplified by PCR amplification with the primers 5′-TGC**TCTAGA**GCA**G**ACTACAAAGACGATGAC GATAAAACTAGTATGGAGAGCATTGGCAGCCATTGT-3′ (FLAG-encoding sequence underlined) and 5′-GC**GTCGAC**GTT CAGTGGTGGTGGTGGTGGTGCAGAGGTTGTGGCCTTG ACTGAAC-3′ (HIS-encoding sequence underlined) (Fig. 1).

2.2 Transformation and regeneration

Constructs were introduced into both amylose-containing (Kardal) and amylose-free (*amf*) potato genetic backgrounds via *Agrobacterium*. Four transgenic series KDG2, KDG3, *amf*G2, and *amf*G3 were yielded in which G2, G3, KD, and *amf* represent AtGWD2, AtGWD3, Kardal, and *amf* background, respectively. Thirty independent transgenic lines were generated for each transgenic series. Each transgenic lines as well as control lines (untransformed plants and transformed plants with empty vector) were multiplied to five plants and grown in the greenhouse under standard conditions, 16 h light at 20°C and 8 h dark at 18°C. Mature tubers were harvested at the end of the life-cycle. No differences were detected between untransformed lines and transformed plantlets with empty vector, therefore, they were further referred to as control or UT.

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Figure 1. Schematic representation of the constructs pBIN19/GWD2 and pBIN19/GWD3. *AtGWD2* and *AtGWD3* were cloned in frame with GBSSI transit peptide to allow amyloplast targeting and were driven by GBSSI promoter for tuber expression. RB and LB stand for right and left borders, respectively. Kan and 3′NOS stand for kanamycin resistant gene and NOS terminator, respectively. The arrow represents the cleavage site of the transit peptide. FLAG and HIS are two tags for protein quantification and *Xba*I and *Sal*I are restriction enzymes.

2.3 Starch isolation and characterization

Tubers from five plants from the same clone were pooled together to minimize individual variation, and starch isolation was performed according to the procedure and analyzed as described in Ref. [18]. These included starch chain length distribution, particle size distribution, granule morphology, amylose content, phosphate content, and starch gelatinization properties. All analyses conducted for starch characteristics have been performed in duplicate unless indicated otherwise.

2.4 Quantitative RT-PCR (qRT-PCR) analysis

Total RNA was extracted from potato tuber samples according to Kuipers et al. (1994) and reverse transcribed using the iScript cDNA synthesis kit from BioRad. Transcription levels of all target genes were determined in triplicate using the comparative Ct method [19] and expressed relative to elongation factor *EF1α* [20]. The gene specific primers used in this study are listed in Supporting Information Table S1.

The expression level of *AtGWD2* and *AtGWD3* was determined for all transformants and control plants. The relative expression level of target genes was multiplied by a factor of 10^6 and then converted to log 10. The resulting value (v) was used to divide tranformants to different categories: undetectable (N, $v = 0$), low (L, $0 < v < 2$), medium (M, $2 \leq v < 3$), and high (H, $v \geq 3$) expressors.

Five or six high-expressors from each series and a few randomly picked control lines were further subjected to qRT-PCR for investigating the expression of key genes involved in starch metabolism. These genes were sucrose synthase (*SUSY4*), potato glucan water dikinase (*StGWD1*), potato phosphoglucan water dikinase (*StGWD3*), starch phosphatase (*SEX4*, *LSF1*, and *LSF2*), granule-bound starch synthase I (*GBSSI*), starch synthase III (*SSIII*), ADP-glucose pyrophosphorylase (*AGPase*), UDP-glucose pyrophosphorylase (*UGPase*), β-amylase (*BAM*), starch branching genes (*SBEI* and *SBEII*), and starch phosphorylase (*SP*).

2.5 Statistical analysis

Significant differences between modified starches and control samples in phosphate content, granule size, amylose content, and starch thermal properties were determined by using *t*-test. The least significant difference values were calculated at 1 or 5% probability.

3 Results

3.1 Transformants show different expression levels

Four transgenic series KDG2, KDG3, *amf*G2, and *amf*G3 were generated by introducing two constructs into potato genetic backgrounds, KD and *amf*. Plant architecture, tuber morphology, and yield of transgenic lines did not show consistent changes compared to that of control plants (data not shown).

The transformants of each transgenic series were divided into four classes based on the expression level of *AtGWD2*/*AtGWD3*: N, L, M and H represent undetectable (or none), low, medium, and high expressors, respectively (Fig. 2). Generally, more transformants were classified as the M- and H-expressors regardless of constructs and backgrounds. To illustrate, 54 and 21% of KDG2 transformants exhibited medium and high expression, respectively, while the same amount of *amf*G2 transformants (42%) were categorized as M- and H-expressors. The M- and H-expressors represented 30 and 48%, respectively, of the KDG3 transformants, whereas the corresponding figures were 42 and 26% for *amf*G3 series. No L-expressors were observed in KDG3 or *amf*G2 series.

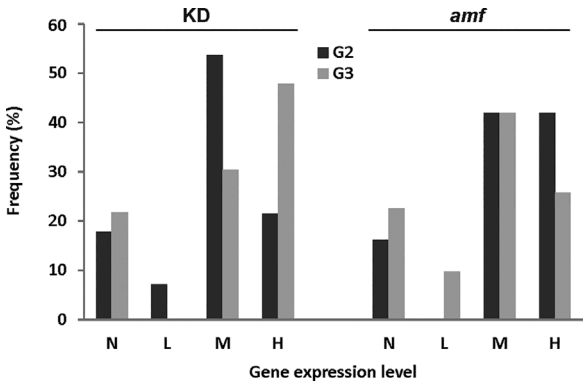

Figure 2. Distribution of the individual transformants over the classes of transgenes expression. N, L, M, and H stand for undetectable, low, medium, and high expressors.

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3.2 Phosphate content is unaffected in modified starches

Although small variations in phosphate content were observed for the different series of transformants, *t*-test analyses revealed no significant changes were found compared to the controls (Fig. 3).

3.3 Granule morphology and size are altered in modified starches

Light microscopy analysis showed that the shape of starch granules from both KDG2 and KDG3 tranformants was irregular (Fig. 4D and G) in contrast to the regular oval shape observed in the UT-KD (Fig. 4A). Further analyses with SEM revealed that KDG2 and KDG3 starch granules have bumps on the surface (Fig. 4E, F, H, and I), while the surface of starch granule from UT-KD was uniformly smooth (Fig. 4B and C). In the *amf* background, the control starch exhibited regular round-shaped granules (Fig. 4G–I). Starch granules from *amf*G2 transformants displayed helical shell structure occurring mostly with cracks and craters (Fig. 4J–L), whereas those from *amf*G3 transformants showed an elongated shape with craters on the surface of the granule (Fig. 4P–R).

Starch granules with altered morphology were observed for KD transformants at all levels of gene expression. About 70 and 47% of the starch granules showed altered morphology in H-expressors from KDG2 and KDG3, respectively, which was markedly higher than those observed in L- and M-expressors (Fig. 5A). In the *amf* background, altered granules from both *amf*G2 and *amf*G3 series were mainly found in the H-expressors at a frequency of 21 and 54%, respectively (Fig. 5A).

t-test analysis revealed that the median granule size of KDG2 and KDG3 starches was significantly increased compared to that of control starch ($p < 0.05$, Fig. 5B). In *amf*G2 and *amf*G3 no significant change in the granule size was observed relative to the control (Fig. 5B).

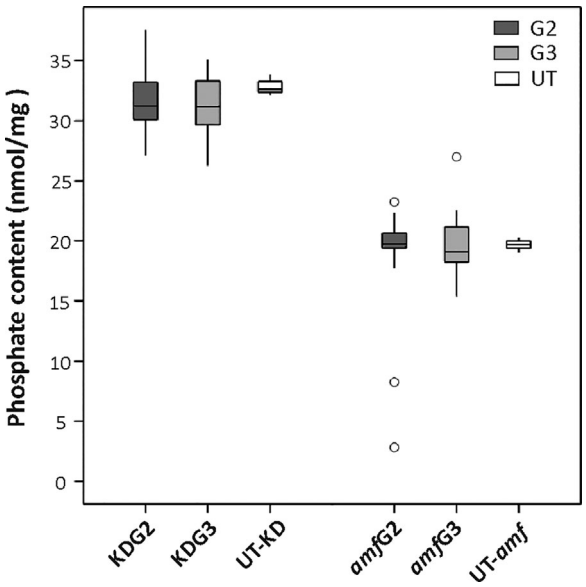

Figure 3. Box-plot representing the phosphate content of starches from all transformants in both genetic backgrounds and respective untransformed controls (UT-KD and UT-*amf*). The measurements were performed on starches of transformants except N-expressors (lines with undetectable expression of the transgene). Boxes in the plot include values in the 25–75% interval, internal lines represent the median, black circles indicate outliers, and bars represent extremes.

3.4 Neither starch fine structure nor properties were altered

No significant changes in chain length distribution were detected in modified starches compared with the control starches in both backgrounds (data not shown). Moreover, amylose content and gelatinization properties did not show significant differences between modified starches and control starches (Table 1).

3.5 *SUSY4* was up-regulated in transformants carrying the *AtGWD2* gene

Transcript quantification showed that the expression of *SUSY4* was substantially upregulated in AtGWD2 transgenic tubers in both backgrounds (Fig. 6A and B), whereas no significant differences were found in the expression level of the other starch enzymes *StGWD1*, *StGWD3*, *LSF1*, *LSF2*, *SEX4*, *GBSSI*, *SSIII*, *AGPase*, *UGPase*, *BAM*, *SBEI*, *SBEII*, and *SP* regardless of constructs and backgrounds (Fig. 6A–D).

4 Discussion

Unlike GWD1, GWD2 locates in companion cells of the phloem rather than chloroplast and it lacks the transit peptide. In our study, AtGWD2 was targeted to the amyloplast and one would expect it would have a similar activity as GWD1, as their sequences are 50% identical. However, no increase in phosphate content was observe in tuber starch granules. In fact, the function of AtGWD2 has not yet been elucidated in vivo. In *Arabidopsis*, AtGWD2 is thought to be active in phloem sieve elements' plastids. These sieve element starches have different structure from storage starches [21]. The environment in the plastids of *Arabidopsis* phloem sieve elements might be distinct from that in the potato amyloplast (*e.g.*, pH, salt concentrations, etc.) and this might explain the fact that starch was not further phosphorylated in GWD2 transgenic lines relatively to the control.

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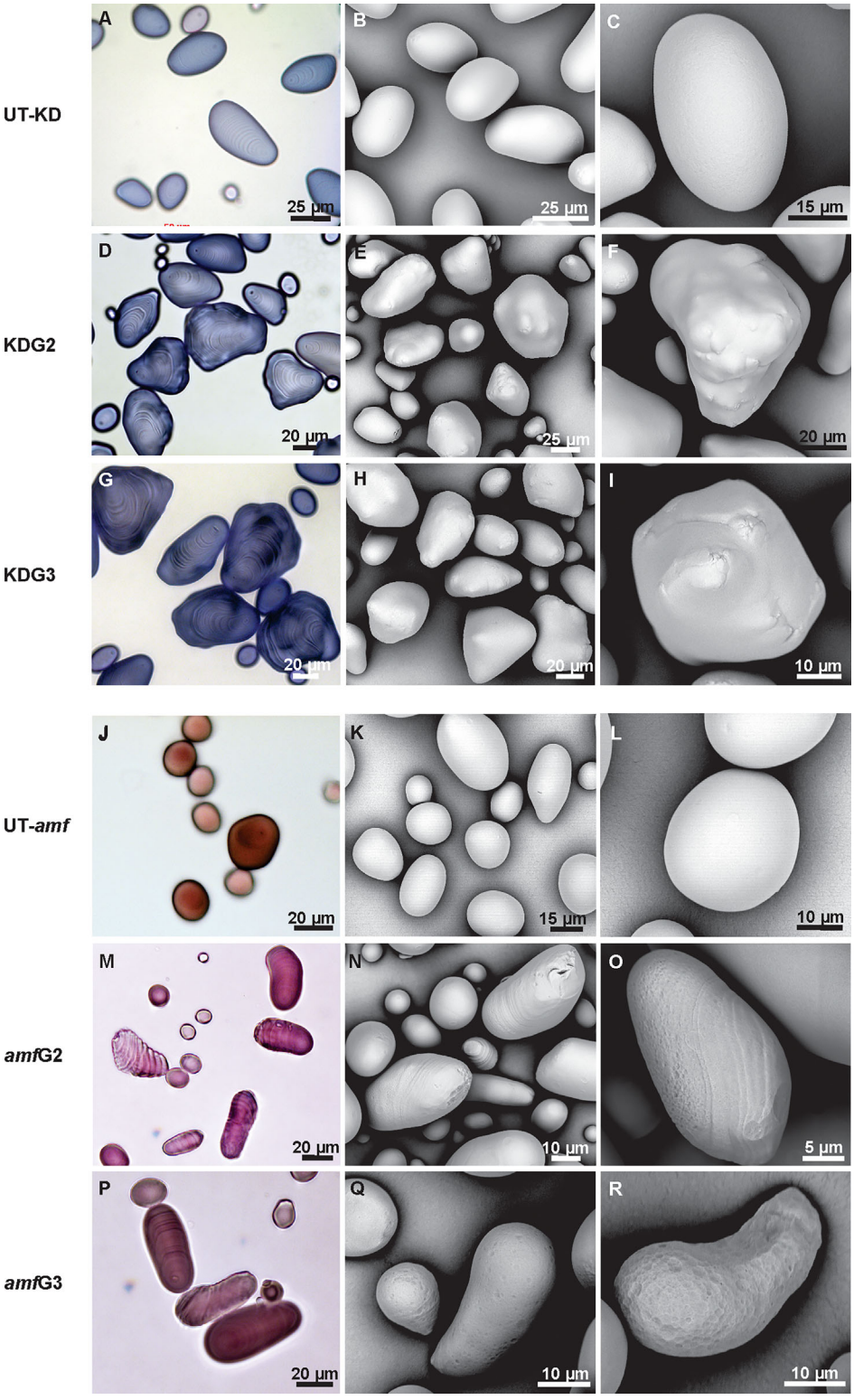

Figure 4. Granule morphology of starches from transformants and controls. Light microscopy (left column of the panel) and scanning electron microscopy (middle and right columns of the panel) were used to visualize starch granule morphology from UT-KD (A–C), KDG2 (D–F), KDG3 (G–I), UT-*amf* (J–L), *amf*G2 (M–O), and *amf*G3 (P–R). Starch granules were stained with a 20× diluted Lugol solution for light microscopy.

On the other hand, assuming that AtGWD2 is capable of phosphorylating potato starch during starch synthesis, the question remains as to whether it is possible to increase the phosphate content of potato starch without changes in starch molecular structure. It has been shown that potato tuber starches are already highly phosphorylated (up to 33 nmol/mg) compared with transitory and cereal endosperm starches (less than 1 nmol/mg) [3, 7]. To

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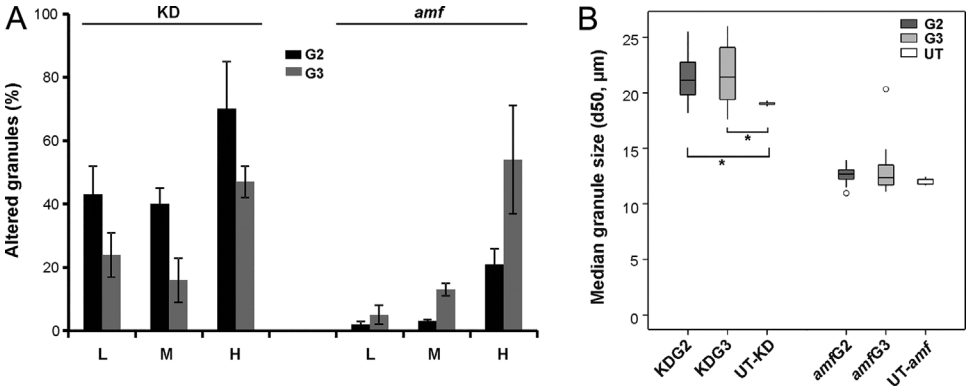

Figure 5. (A) Percentage of granules with altered morphology in transformants with different gene expression level: low (L), medium (M), and high (H). A population of 100 starch granules was counted in triplicate. The values are expressed as the mean ± SD. (B) Boxplot presenting median granule size (d50) of modified starches and controls. Analyses were performed on all starches except N-expressors (lines with undetectable expression of the transgene), which are 25, 29, 22, and 21 lines for KDG2, KDG3, *amf*G2, and *amf*G3 series, respectively. Boxes in the plot include values in the 25–75% interval. Internal lines, unfilled circles, and bars represent the median, outliers, and extremes, respectively. Statistical significance was analyzed using *t*-test ($^{*}p < 0.05$).

date, potato starches with increased phosphate content have been attained as a side effect due to altered amylopectin structure or decreased amylose content. It is likely that there are limiting factors for the incorporation of phosphate groups in starch rather than the phosphorylating enzyme itself. Therefore, the measured phosphate content might be the maximum amount achievable in potato starch, which could possibly explain why transformants with *AtGWD2* expression do not lead to increase in starch phosphate content. We suggest that a further increase in phosphate content is only possible when more phosphorylation sites are available and these result from changes in starch molecular structure. A similar phenomenon has been observed in earlier studies, where additional copies of the granule-bound starch synthase (*GBSS*) gene introduced into potato plants did not increase amylose content above the wild-type level [22, 23]. The authors reasoned that the restricted amount of amylose content was due to the limited space existed to deposit amylose molecules in starch granules.

Table 1. Overview of characteristics determined for the representative starches in both backgrounds

Clone	Class	*P* (nmol/mg)	AM (%)	d50 (µm)	T_o (°C)	T_c (°C)	ΔH (J/g)
UT-KD	–	32.8 ± 0.8	19.0 ± 0.6	19.1 ± 0.2	66.5 ± 0.3	79.9 ± 0.6	19.6 ± 1.3
KDG2-24	N	32.8 ± 0.2	18.8 ± 1.0	21.0 ± 0.3**	66.7 ± 0.2	80.1 ± 0.5	18.8 ± 1.2
KDG2-25	L	33.7 ± 0.1	18.6 ± 1.1	24.4 ± 0.3**	66.8 ± 0.5	79.1 ± 0.7	18.3 ± 0.3
KDG2-15	M	29.4 ± 0.4**	18.1 ± 0.9	21.4 ± 0.2**	65.8 ± 0.2**	79.9 ± 0.2	20.0 ± 0.1
KDG2-07	H	33.5 ± 0.5	18.8 ± 0.2	20.4 ± 0.3**	66.8 ± 0.1	80.6 ± 0.3	19.5 ± 0.3
KDG3-28	N	33.8 ± 0.4	18.7 ± 0.3	22.0 ± 0.2**	66.5 ± 0.1	79.6 ± 0.2	17.7 ± 0.0
KDG3-25	M	32.3 ± 0.6	18.6 ± 0.3	24.2 ± 0.6**	67.7 ± 0.2**	81.6 ± 0.7**	19.2 ± 0.7
KDG3-06	H	31.0 ± 0.2*	18.5 ± 0.1	21.0 ± 0.7**	67.0 ± 0.2*	80.0 ± 0.5	18.4 ± 1.1
UT-*amf*	–	19.7 ± 0.4	3.2 ± 0.4	12.0 ± 0.3	73.1 ± 0.1	85.7 ± 0.1	16.0 ± 0.2
*amf*G2-02	N	19.2 ± 0.1	3.5 ± 0.2	13.1 ± 0.2**	72.7 ± 0.3*	84.8 ± 0.2**	15.8 ± 0.4
*amf*G2-05	M	19.6 ± 0.2	4.2 ± 0.4	12.8 ± 0.7*	73.2 ± 0.2	86.1 ± 0.1**	16.4 ± 0.5
*amf*G2-23	H	22.4 ± 0.2**	3.7 ± 0.0	12.2 ± 0.5	72.9 ± 0.1	86.4 ± 0.3**	16.2 ± 0.4
*amf*G3-08	N	20.5 ± 0.1*	3.5 ± 0.2	11.1 ± 0.6*	73.7 ± 0.5*	86.1 ± 0.2*	16.2 ± 0.1
*amf*G3-25	L	18.6 ± 0.0**	3.2 ± 0.5	14.9 ± 0.8**	72.2 ± 0.3**	85.4 ± 0.1**	16.4 ± 0.0
*amf*G3-10	M	19.0 ± 0.3*	4.1 ± 0.4	11.5 ± 0.3	72.9 ± 0.0	85.9 ± 0.5	15.7 ± 0.4
*amf*G3-24	H	21.0 ± 0.0**	3.1 ± 0.0	11.7 ± 0.2	73.8 ± 0.1**	86.2 ± 0.3*	16.4 ± 0.2

Representative starches with different expression level in each series and respective control are presented.
Data (mean ± S.D.) are the average of two or three independent measurements. *P*, total phosphate content; AM, apparent amylose content; d50, median granule size; T_o and T_c, starch gelatinization temperature; ΔH, gelatinization enthalpy. Statistical analysis of significant differences between each starch sample and the control was calculated using *t*-test ($^{*}p < 0.05$; $^{**}p < 0.01$).

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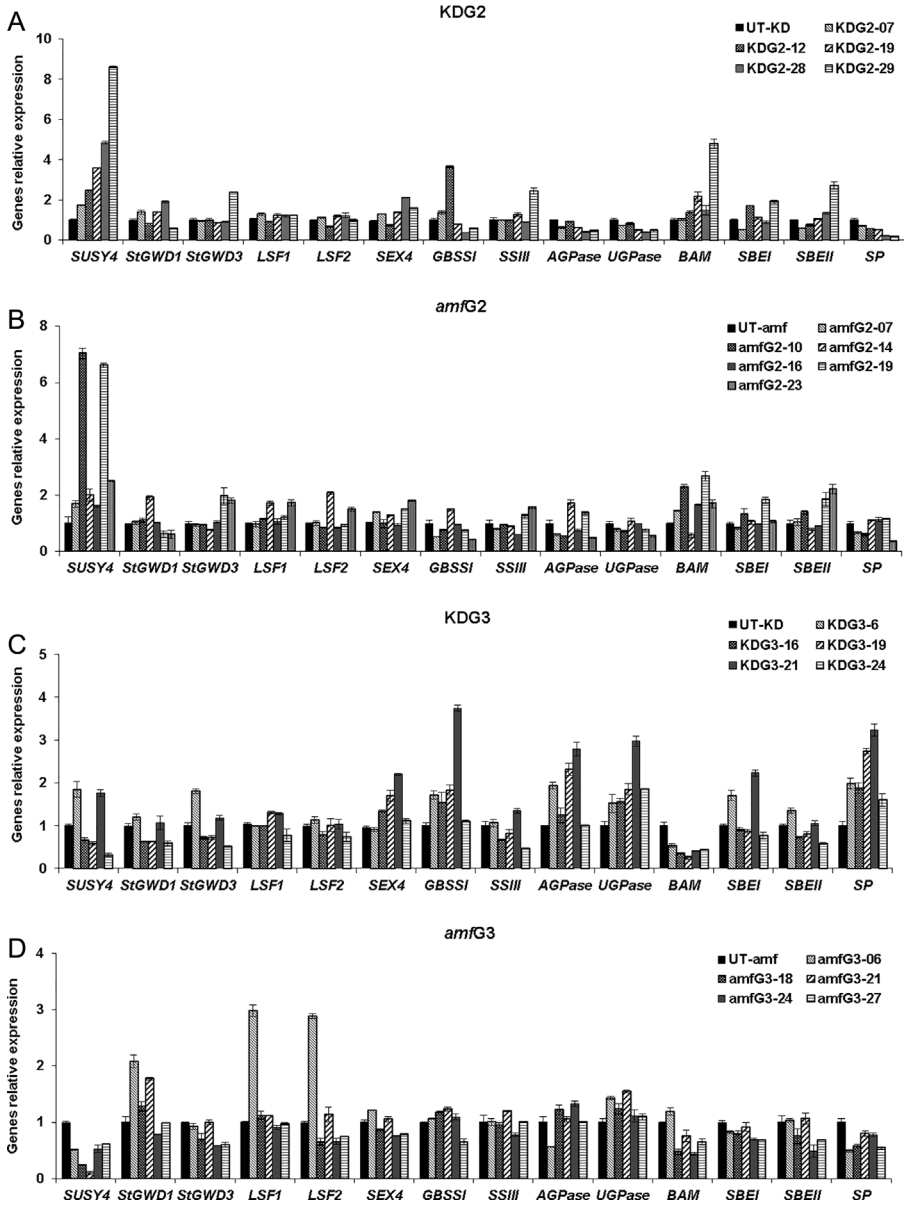

Figure 6. The expression of genes encoding key enzymes involved in starch metabolism in the high-expressors of (A) KDG2, (B) *amf*G2, (C) KDG3, and (D) *amf*G3 transgenic series. Following genes were investigated: sucrose synthase 4 (*SUSY4*), glucan, water-dikinase 1 and 3 (*StGWD1* and *StGWD3*), starch phosphatase (*LSF1*, *LSF2*, and *SEX4*), granule-bound starch synthase I (*GBSSI*), starch synthase (*SSIII*), ADP-glucose pyrophosphorylase (*AGPase*), UDP-glucose pyrophosphorylase (*UGPase*), β-amylase (*BAM*), starch branching genes (*SBEI* and *SBEII*), starch phosphorylase (*SP*). The expression of target genes was expressed relative to the expression of elongation factor *EF1α*. Data (means ± SD of three technical replicates) are expressed as the mean fold change relative to the control.

Similar to the results observed in the AtGWD2 transformants, the AtGWD3 transformants did not show significant change in the phosphate content of tuber starches. The explanations provided above may also apply in this case. It is worth noting that higher starch phosphate content has been obtained in *Arabidopsis* by overexpression AtGWD3 [24], however, transitory starches, the substrate for AtGWD3, are considerably different from storage starches, such as amylose/amylopectin ratio, size, and chemical structure of both these components.

The granule morphology was altered in modified starches compared to the control (Fig. 4). This is likely due to the presence of the newly introduced proteins in granules during starch synthesis, interfering with lateral interactions of amylopectin side chains and thereby interrupting the optimal crystalline packing of starch granules. This phenomenon has been observed in previous studies by introducing granule-bound proteins into amyloplasts [25–27]. The larger granules observed in KD modified starches may be partly attributed to the irregular geometrical shape of the granules.

The expression of key genes involved in the starch metabolism pathway generally did not show substantial alterations in any of the transgenic series, except for *SUSY4*. *SUSY4* expression increased substantially in AtGWD2 transformants compared to that of controls regardless of the genetic background (Fig. 6A and B). An early study has shown that SUSY4 is a major determinant of tuber sink strength and the suppression of this gene leads to an inhibition of starch accumulation in potato tubers [28]. In previous research, the inhibition of GWD1 expression led to

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the downregulation of a series of key genes including *SUSY4*, thus we hypothesized that starch phosphorylation affects starch metabolism partly by regulating the flux into the amyloplast [18]. Further experiments are needed to examine if the expression of AtGWD2 directly or indirectly triggers such regulation in potato tuber. In addition, KDG3 transformants showed a consistent increase in the expression of *GBSSI*, *AGPase*, *UGPase*, and *SP* compared to the control. It has been reported that the expression level of *GWD1* in potato tubers affects that of key genes involved in the starch metabolic pathway [18]. It is, therefore, likely that AtGWD3 may play a similar role in the regulation of starch metabolism and this hypothesis remains to be elucidated.

5 Conclusions

In our study, modified starches showed severe changes in granule morphology, but no significant changes in starch phosphate content. These results indicate that *AtGWD2* and *AtGWD3* are not suitable for engineering starch with a high phosphate content in potato tubers. Further analysis of properties of these enzymes and comparison with other effective glucan water dikinases will help us understand starch phosphorylation in storage starch and how to develop enzymes that efficiently modify starch phosphate content.

The authors have declared no conflict of interest.

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