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Differences between the Bud End and Stem End of Potatoes in Dry Matter Content, Starch Granule Size, and Carbohydrate Metabolic Gene Expression at the Growing and Sprouting Stages

Bailin Liu,[†,‡,§] Guodong Zhang,[§] Agnes Murphy,[‡] David De Koeyer,[‡] Helen Tai,[‡] Benoit Bizimungu,[‡] Huaijun Si,[*,§] and Xiu-Qing Li[*,‡]

[†]State Key Laboratory of Crop Stress Biology for Arid Areas and College of Agronomy, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China

[‡]Potato Research Centre, Agriculture and Agri-Food Canada, 850 Lincoln Road, Post Office Box 20280, Fredericton, New Brunswick E3B 4Z7, Canada

[§]Gansu Provincial Key Laboratory of Arid Land Crop Science, Gansu Key Laboratory of Crop Genetic and Germplasm Enhancement, Gansu Agricultural University, Lanzhou, Gansu 730070, People's Republic of China

ABSTRACT: Potatoes usually have the tuber bud end dominance in growth during tuber bulking and in tuber sprouting, likely using carbohydrates from the tuber stem end. We hypothesized that the tuber bud end and tuber stem end coordination in carbohydrate metabolism gene expression is different between the bulking dominance and sprouting dominance of the tuber bud end. After comparing the growing tubers at harvest from a green vine and the stage that sprouts just started to emerge after storage of tubers at room temperature, we found the following: (1) Dry matter content was higher in the tuber stem end than the tuber bud end at both stages. (2) The starch granule size was larger in the tuber bud end than in the tuber stem end. (3) The tuber bud end had higher gene expression for starch synthesis but a lower gene expression of sucrose transporters than the tuber stem end during tuber growing. (4) The tuber stem end at the sprouting stage showed more active gene expression in both starch degradation and resynthesis, suggesting more active export of carbohydrates, than the tuber bud end. The results indicate that the starch accumulation mechanism in the tuber bud end was different between field growing and post-harvest sprouting tubers and that tubers already increased dry matter and average starch granule sizes in the tuber bud end prior to the rapid growth of sprouts.

KEYWORDS: *Solanum tuberosum L., tuber growth, potato sprouting, tuber ends, dry matter, starch granules, real-time quantitative polymerase chain reaction, starch metabolism, gene expression*

INTRODUCTION

In botany, a potato tuber is a modified, bulking shoot that converts and stores photosynthetic products mainly in the form of starch. The tuber bud end is the apical end of the shoot, and the tuber stem end (connected with the mother plant by a stolon) is the basal end of the shoot. Dry matter content, starch granule size, and starch metabolic gene expression of a tuber are likely most different between the bud end and the tuber stem end. Carbohydrate metabolism in tubers is expected to considerably affect the growth, yield, and sprouting of tubers, but its difference at the tuber bud end and tuber stem end coordination level is unknown between the two ends of tubers in growing tubers and sprouting tubers. Coordination among different parts of plant storage organs or propagules during germination or sprouting is relatively difficult to study in true seeds because of their small size (unless using *in situ* approaches). However, tuber and root crops (such as potato and sweet potato) provide ideal model materials for such an analysis on the difference and coordination between the apical end and the basal end during the propagule growth and sprouting.

Seed potatoes for sprout induction and table potatoes for daily use in the kitchen are usually stored at room temperatures until the early stage of sprouting, although cool storage is recommend for kitchen use to minimize sprouting. The tuber stem end is obviously essential during tuber growth because it is the connected part with the mother plant, but its role at the beginning of sprouting is unclear. Although the pattern of sprouting is under genetic control and is also influenced by the physiological age of the tuber, sprouts on stored potatoes usually occur with dominance at the bud end, also referred to as apical dominance.[1,2]

Knowledge of the endogenous biological change in potato tubers may assist sprout control during potato storage or for the early and healthy emergence of sprouts after planting seed potatoes. In one crop season countries, such as in North America, seed tubers are usually held at 4 °C for the whole winter until the planting time in May. Potatoes will gradually break their dormancy and start to sprout during this long period of storage. However, in regions with two potato crop seasons per year, such as in most regions of China and Korea,[3] seed potatoes often need to be stored at room temperature to accelerate sprouting, particularly for seed potatoes that are

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harvested in early July and to be planted in the middle of August. In these regions, potatoes are usually harvested directly from green vine plants (without a chemical top kill) to maximize the tuber growth before harvest. "Russet Burbank" and "Shepody" are two of the most important potato cultivars in both North America as well as China. The starch granule size and carbohydrate metabolism gene expression are rarely studied for sprouting tubers under room temperatures of these cultivars.

Various aspects of potato tuber endogenous changes during tuber growth, cold storage, and sprouting have been characterized, including, for example, changes in levels of growth regulators/hormones,[4−7] starch degradation enzymes,[8,9] and sugar and other carbohydrates.[10−13] Although starch degradation occurs during cold storage and sprouting,[8] it is unclear whether and how the starch granule size changes during storage and sprouting. Little information is available for the carbohydrate metabolic changes in tubers between the harvest (still growing and accumulating photosynthetic products) and sprouting (using only the carbohydrates within the tuber) for the tubers immediately entered the sprouting induction stage at room temperature after harvest. The extent of starch granule size differences between the tuber bud end and tuber stem end is unknown for any tubers, regardless of storage under cold or warm temperatures.

The differences of carbohydrate metabolism gene expression between the tuber stem end and the tuber bud end in growing tubers are still unknown, although there are various studies of the dry matter contents in the two tuber ends in stored potatoes.[14,15] Therefore, the present study used first the tubers immediately after harvested from green vine plants. These harvesting stage tubes were still growing and actively metabolizing carbohydrates from the basal end to the apical end using the photosynthetic carbohydrates.

Because there is no longer any input of photosynthetic carbohydrates after the tubers were disconnected, the harvest stage tubers can also serve as the day zero of storage for the comparison to sprouting tubers. The residual carbohydrates from the green vine are expected to be consumed relatively quickly during the early stage of tuber storage after harvest. Therefore, the tuber must remobilize the carbohydrate resources within the tubers to support sprouting. Apical buds usually have sprouting dominance, which is known to be associated with a higher soluble sugar content in the apical bud region than in the lateral bud regions during sprouting of tubers from cold storage.[16] Sugar accumulation is also higher in the sprouting tubers than in tubers treated with a sprout inhibitor.[10] The dry matter content is different between the two ends in non-sprouted storage potatoes.[14,15] The carbohydrate metabolism is expected to be very active in both growing tubers (harvested from green plants) and sprouting initiation stage tubers. However, carbohydrate metabolism gene expressions can be very different because the carbohydrate sources of the two stages are different: the former uses carbohydrates translocated from the green vine, and the latter uses resources within the tubers. The gene expression differences between these two types of very active carbohydrate metabolisms (tuber growing and tuber sprouting) in the tuber ends are still unknown.

In the present study, we analyzed the dry matter content, starch granule size, and carbohydrate metabolism gene expression in comparison between the bud end and the stem end of tubers and between the harvest stage (developing tubers, day zero for sprout induction) and the sprout emergence stage (room temperature). Clear differences and coordination between the two regions were identified.

MATERIALS AND METHODS

Plant Material. Potato (*Solanum tuberosum* L.) tetraploid cultivars "Russet Burbank", "Shepody", "Yunshu107", and "Yunshu505" were used in this study. "Russet Burbank" and "Shepody" were long tuber cultivars and "Yunshu107" and "Yunshu505" are round tuber cultivars. "Russet Burbank" and "Shepody" were planted at the Potato Research Centre (recently changed the name to Fredericton Research and Development Centre) of Agriculture and Agri-Food Canada (AAFC, Fredericton, New Brunswick, Canada) in May 2013. Potato cultivars "Yunshu107" and "Yunshu505" were planted in the experimental farm of Gansu Agricultural University in May 2013 in the Gansu Province of China. The two Chinese cultivars were used for verifying whether other cultivars and plants in very different regions also have similar dry matter differences between the two ends as in Russet Burbank and Shepody. These two Chinese cultivars were not used in measuring starch granule size because the starch granule measurement system was not available in the Chinese laboratory. Plants were managed under standard agronomic practices without irrigation and were harvested in late September without top killing. Harvest was around 11 am to ensure that the plants had already conducted photosynthesis for several hours prior to the separation between the vine and the tubers. Approximately 30 tubers that were relatively uniform in size and shape were selected from six plants of each cultivar.

Tuber Storage, Sprouting, and Tuber Material Sampling. After harvest, three separate tubers were sampled. The sampling was repeated once with three additional tubers. In total, six to eight tubers per combination between cultivar and end were separately prepared immediately after harvest. Other tubers were stored at room temperature, and the two ends were sampled when the tubers were at the time of sprout emergence (2 mm). The tuber storage of "Shepody" and "Russet Burbank" was in the dark at room temperature (19 ± 1 °C, with a relative humidity of approximately 50%) for 70 days and were then stored under 24 ± 0.5 °C with an approximate 85% relative humidity in the dark for sprouting. The "Shepody" tubers were sprouted (2 mm apical buds) after 22 days in 24 °C, and "Russet Burbank" tubers were sprouted (2 mm apical buds) after 35 days in 24 °C with approximately 85% relative humidity. The "Yunshu107" and "Yunshu505" tubers were stored at room temperature (21 ± 2 °C) in the potato storage room at Gansu Agricultural University and showed signs of bud emergence approximately 1 month after storage. The minor difference (approximately 2 °C) in the potato storage temperature between AAFC and Gansu Agricultural University was because we tempted to follow the similar conditions used by commercial production in the regions and also because the research was conducted independently in the two laboratories. All tubers were washed, surface-sterilized with 70% ethanol and 1% sodium hypochlorite (v/v) Javel bleach solution, thoroughly washed with tap water, and then rinsed twice with distilled water prior to sampling from the tubers for dry matter content, starch granule size measurement, and RNA extraction.

The tuber stem end and bud end tissues were collected by taking the top 0.8−1 cm of the tissue off the bud end and 0.8−1 cm off the bottom of the tuber stem end. The stem end and bud end of each tuber was identified using a combination of markers that we made during harvest and the tuber eye directions. The major eyelid of a potato tuber eye is at the tuber bud end side. The sampling was repeated once with an additional three tubers. The samples of these six tubers were freeze-dried separately. The fresh weight of pieces of tuber bud end and tuber stem end were recorded after sampling. The samples were then dried at 60 °C (approximately 1 week) until the weight stabilized to allow for dry matter content determinations.

Chipping. The potato chipping used the following procedure: longitudinal slices from the center of the tuber were taken with a rotary slicer set to give slices of 1.0 mm in thickness. Slices were placed in a colander, rinsed with cold water to remove excess starch, and then

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Table 1. Primers Used for qRT-PCR Analysis

gene	abbreviation	gene sequence ID in NCBI		sequence 5′−3′
ubiquitin/ribosomal fusion protein	Ubi3	L22576	F[a]	TCCGACACCATCGACAATGT
			R	CGACCATCCTCAAGCTGCTT
sucrose synthase 2	Susy	AY205084.1	F	GGGTTGCACTTGCTATTCGT
			R	GGTTGGTTTGGGAAATGATG
ADP-glucose pyrophosphorylase	AGPase	X55155.1	F	CCACTGCATTCGGTCTCATG
			R	CAATTGCTCTCCTTGCGGTT
isoamylase isoform 1	SDBE	AY132996.1	F	GCTGCTGTTGATAGTGGACG
			R	ACAGCGAAATTCACACCACC
sucrose transporter	ST	X69165	F	CGCCTGGTGTTAAAATCGGT
			R	CCATGGCCCTCCTACTAGTG
α-amylase	α-amy	GQ406048.1	F	CTCATTGACTGGATGCGGTG
			R	TGCAGTTGAAAGTTGTCCCG
β-amylase	β-amy	AF393847.1	F	CTGAAGGGGTGATGGTGGAT
			R	CCGGCCTGATCTATCTGTGT
acid invertase	AI	DQ478950.1	F	AAACTCCGCCTCCCATTACA
			R	GAGAAACACCTCTTGACGGC
starch phosphorylase	SP	X52385.1	F	AGGAACCAGATGCTGCTCTT
			R	GGACTGCCAATTTCAAGCCA
granule-bound starch synthase	GBSS	EU403426	F	TTGGCACACAGCTCTCATTC
			R	GGCCTTGGTAGGCAATGTTA

[a]F, forward primer; R, reverse primer.

placed on paper towels to remove excess water. Samples were deep-fried in vegetable oil (canola oil, Spectra Foods, Canada) at 177 °C (350 °F) until bubbling, removed, and drained.

Starch Granules. The starch granule size was measured by the previously described method.[17] Tuber end pieces were squeezed with a garlic press, and the resulting starch juice was collected in a 1.5 mL Eppendorf tube. After the juice was mixed well by tapping on the tube wall with a finger, 20 μL of juice was immediately transferred to 80 μL of water in a new microfuge tube. A 10 μL diluted starch juice sample was directly transferred to a microscope slide chamber (see the study by Li et al.[17] about how to make the well-like chamber using a punched tape) and covered with a coverslip. The microscopic observation (20× objective and 10× eyepiece lens) was conducted using a Carl Zesis light microscope equipped with a polarizer and AxioVisionRel 4.7 software. Three to six images were randomly taken. The length of starch granules were measured in micrometer using the image processing software AxioVisionRel 4.7. The data were exported using AxioVisionRel 4.7 software to a Microsoft Excel file. The starch granules that were equal to or larger than 8 μm were used to calculate the average length of starch granules, because the starch granule average length measure in this way was highly reproducible.[17] For "Russet Burbank", growing tubers and sprouted tubers of 5539 and 4452 starch granules in bud end and 8270 and 8601 in stem end were measured, respectively. For "Shepody", growing tubers and sprouted tubers of 7429 and 6620 starch granules in bud end and 6596 and 7428 in stem end were measured, respectively.

RNA Extraction. For growing tubers and sprouted tubers, the bud end and stem end of each variety were sliced from three tubers, mixed together, and then frozen by immersion in liquid nitrogen. RNA was isolated using RNeasy Plant Mini Kit (catalog number 74904, Qiagen) according to the directions of the manufacturer. RNA extraction was repeated once using three other tubers. Extracted RNA was stored at −80 °C until use.

Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) Analysis of Gene Expression. Real-time qRT-PCR was conducted to estimate the gene expression related to starch metabolism. The potato *ubi3* gene was used as the qPCR control.[18] The gene-specific primers were designed on the basis of the corresponding potato nucleotide sequences using Primer 3 (version 4.0.0) (Table 1). The first-strand cDNAs were synthesized from 500 ng of total RNA with the Quantitec Reverse Transcription Kit (catalog number 205311, Qiagen) in a 40 μL reaction volume, including gDNA Wipeout buffer supplied from the same kit. The real-time PCR was performed on a Sequence Detection System (ABI) in a 10 μL reaction volume with the QuantitecSYBR Green PCR Kit (catalog number 204054, Qiagen), using 1 μL of a 5-fold diluted cDNA solution described above as the template, 5 μL of 2 × SYBR Green master mix, and 1 μM of each primer. The thermal cycling conditions were 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. All of the reactions were performed in triplicates (three technical repeats). The relative expression levels for each gene were calculated using the $2^{-\Delta C_t}$ method in comparison to the control gene and log normalization.[19]

Statistical Analysis. The significance of the difference in dry matter between the two ends was determined by the two-way analysis of variance (ANOVA) (SAS 9.3). The significance of the starch granule size difference between the two ends was determined using the *t* test (two tails, unpaired) statistical package in Excel 2010 for each cultivar.

RESULTS

Dry Matter Content. The shapes of the analyzed tubers of four cultivars, including two long tube cultivars (Russet Burbank and Shepody) and two round tuber cultivars (Yunshu107 and Yunshu505) are shown in Figure 1. The tubers were apparently healthy without obvious diseases or

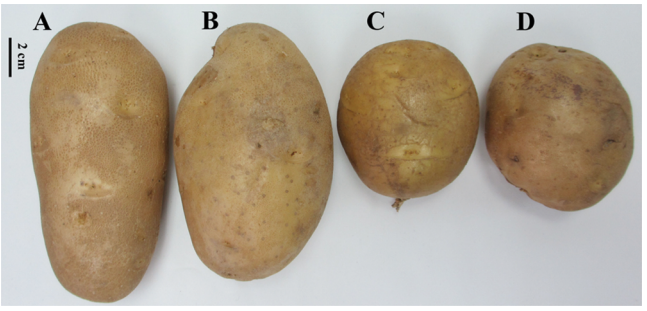

Figure 1. Shape, size, and general appearance of potato tubers of the four cultivars: (A) "Russet Burbank" (RB), (B) "Shepody" (Sh), (C) "Yunshu505", and (D) "Yunshu107".

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secondary growth regions (Figure 1). We did some chipping of the three tubers per cultivar at each stage. The purposes of chipping were to show the bud lengths, bud distribution, and existence or absence of dark-color ends in these tubers that we stored at room temperatures. The sprout size was approximately 2 mm in length, as shown clearly on the chips (Figure 2). Although the chip color is not a focus of this study, we do

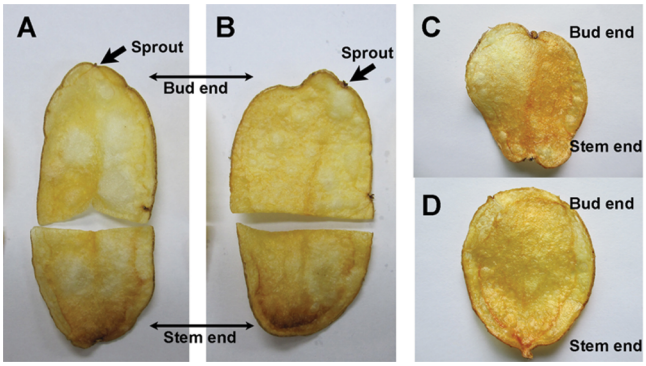

Figure 2. Potato chips, showing the size and position of the sprouts and the color distribution: (A) "Russet Burbank", (B) "Shepody", (C) "Yunshu107", and (D) "Yunshu505". The sprouting stage studied was at the emergence of sprouts, approximately 2 mm in length in "Russet Burbank" and "Shepody" and approximately 1−1.5 mm in "Yunshu107" and "Yunshu505". Note that the tiny sprouts had unlikely consumed a significant amount of carbohydrates.

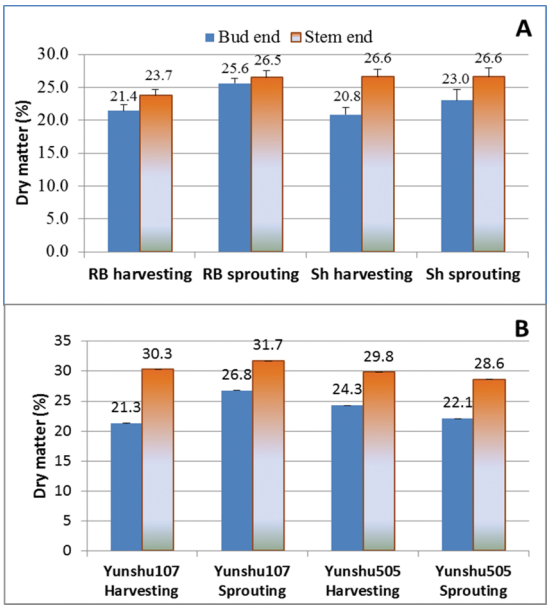

Figure 3. Dry matter contents in the bud end and stem end of tubers from cultivars "Russet Burbank" (RB), "Shepody" (Sh), "Yunshu107", and "Yunshu505". RB and Sh had long tubers, and "Yunshu107" and "Yunshu505" had round tubers. Each dry matter value is the mean from 3 to 6 tubers. Bar = standard error of biological repeats (tubers). The dry matter contents were different ($p < 0.001$) between the tuber stem end and tuber bud end according to two-way ANOVA (cultivar and ends, by the statistical software SAS 9.3).

observed a relatively darker color at the tuber stem end than in the tuber bud end in all four cultivars, with a clear dark stem end in the chips from the "Russet Burbank" and "Shepody" (Figure 2). The issue of dark stem end in chips of "Yunshu107" and "Yunshu505" was not as obvious as in Shepody (Figure 2); however, because the storage conditions were different in the two laboratories, the colors were not directly comparable between the chips of "Yunshu107" and "Yunshu505" and the chips of "Russet Burbank" and "Shepody".

Dry matter content was higher at the tuber stem end than at the bud end in all four cultivars (Russet Burbank, Shepody, Yunshu107, and Yunshu505) in both harvest stage tubers and room-temperature-stored tubers (panels A and B of Figure 3). The difference in dry matter between the tuber stem end and tuber bud end was smaller in "Russet Burbank" than in "Shepody" (Figure 3A). In comparison to the harvest stage, the sprouting stage dry matter content in the bud end significantly increased in three cultivars, except the cultivar "Yunshu505" (Figure 3). The dry matter content at the stem end was higher than that of the tuber bud end in all four cultivars and at both the harvest stage and the sprouting stage (Figure 3).

Starch Granules. Images of starch granules are shown in Figure 4. The granule shape was similar in "Russet Burbank" and "Shepody". The starch granule images have the characteristic maltese cross pattern because the images were taken using crossed polarizers. The maltese cross pattern is also very similar, except that the average length of granules was greater in "Russet Burbank" than in "Shepody" (Figure 5). In both "Russet Burbank", the average length of starch granules was consistently greater in the tuber bud end than in the tuber stem end before and after sprouting (Figure 5). Starch granule size increased in the tuber bud end at the sprouting stage in "Russet Burbank" but remained similar in "Shepody" (Figure 5).

Gene Expression Profiling of Developing Tuber Ends. The developing tubers at the harvest stage were clearly characterized by stronger gene expression of starch synthesis genes in the tuber bud end than in the tuber stem end in both "Russet Burbank" and "Shepody". These genes included ADP-glucose pyrophosphorylase (AGPase) and granule-bound starch synthase (GBSS) (Figure 6). Genes involved in both starch synthesis and degradation, such as starch phosphorylase (SP), starch-debranching enzyme (SDBE), and sucrose synthase (Susy), also showed similar or higher activities in the tuber bud end than the tuber stem end, whereas sucrose transporter (ST) showed higher activity in the tuber stem end (Figure 6). Genes, including acid invertase (AI), α-amylase (α-amy), and β-amylase (β-amy), that were known to be mainly responsible for sucrose cleavage (AI) and starch degradation (α-amy and β-amy) were much more active in the tuber stem end than in the tuber bud end. The tuber stem ends of "Russet Burbank" and "Shepody" were found to have higher expression for sucrose transport and starch degradation genes (acid invertase and amylases) but lower expression for starch synthesis genes (e.g., ADP-glucose pyrophorephorylase and granule-bound starch synthase) than in those genes found in the tuber bud ends. The net activity in gene expression suggests that the tuber bud end was a strong sink for starch accumulation in the growing tubers at harvest.

Gene Expression in the Ends of Tubers Starting Sprouting. At the sprouting stage, the tuber stem end had strong gene expression for both starch synthesis (AGPase and GBSS) and starch degradation (AI, α-amy, and β-amy) (Figure 7). Because no new carbohydrate supply from the outside photosynthesis was available post-harvest to the tuber, this high activity of starch metabolism gene expression in the tuber stem end likely resulted in net degradation of starch, whereas the

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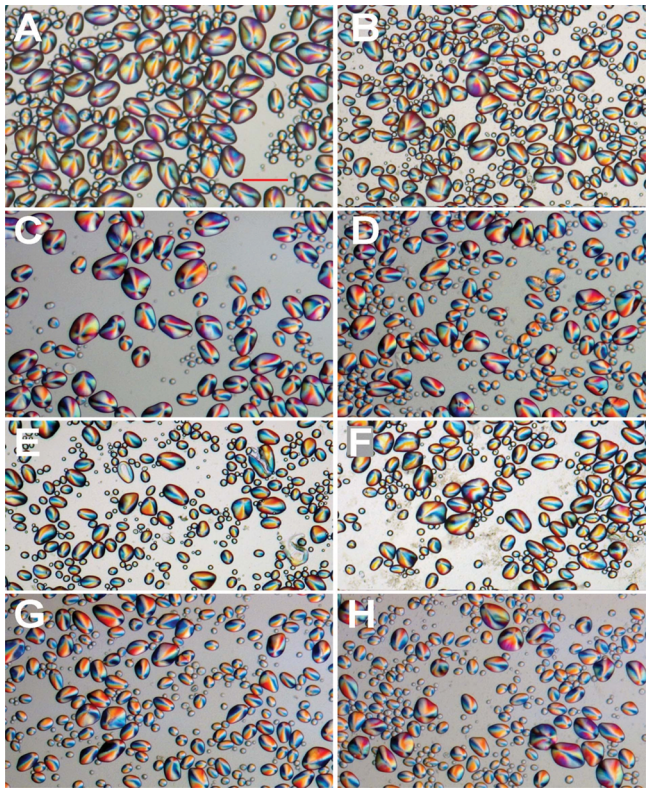

Figure 4. Starch granules image under a light microscope: (A) "Russet Burbank" tuber bud end in growing tubers at harvest, (B) "Russet Burbank" stem end in growing tubers at harvest, (C) "Russet Burbank" tuber bud end at the sprouting stage, (D) "Russet Burbank" tuber stem end at the sprouting stage, (E) "Shepody" tuber bud end in growing tubers at harvest, (F) "Shepody" tuber stem end in growing tubers at harvest, (G) "Shepody" tuber bud end at the sprouting stage, and (H) "Shepody" tuber stem end at the sprouting stage. All images are in the same magnification, and the size bar (shown in panel A) represents the length of 100 μm.

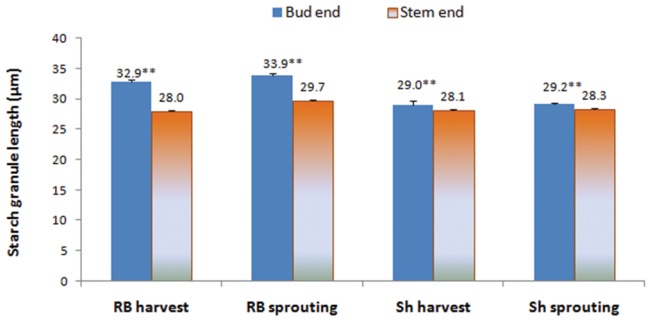

Figure 5. Starch granule average length. The values represent the means from three tubers. RB, "Russet Burbank"; Sh, "Shepdoy". Number of measured starch granules at the bud end: RB harvest, 5539; RB sprouting, 4452; Sh harvest, 7429; and Sh sprouting, 6620. Number of measured starch granules at the stem end: RB harvest, 8270; RB sprouting, 8601; Sh harvest, 6596; and Sh sprouting, 7428. Bar = standard error of starch granule lengths using all of the granules represented by the column. The standard error bars are small because of the large number of starch granules. The difference between the two ends within each cultivar−stage combination was highly significant (**, $p < 0.01$) according to Student's t test.

tuber bud end had a similar gene expression in starch synthesis as the tuber stem end but a much lower starch degradation gene expression than the tuber stem end. Thereby, the tuber bud end should have more accumulation than degradation of starch (Figure 7), likely using the sucrose transported from the tuber stem end. Sucrose transporter gene expression was similar in the two ends of "Russet Burbank" but was higher in the tuber stem end than in the tuber bud end in "Shepody", also suggesting an export of sucrose generated by starch degradation from the tuber stem end (ST in Figure 7). In "Russet Burbank", the net degradation of starch was obvious in the tuber stem end, whereas in "Shepody", the net mobilization of dry matter from the tuber stem end to the tuber bud end appeared to be slower than in "Russet Burbank", according to the gene expression pattern (Figure 7).

DISCUSSION

In the present study, we found the following about dry matter: (i) The dry matter content was higher in the tuber stem end, regardless of potato field (in China or Canada), tuber shape (long or round), and stage (harvest or sprout). (ii) The dry matter content in the tuber bud end clearly increased during storage in three of the four cultivars and made the difference between the two ends smaller at the sprouting stage than at the harvest stage (Figure 3). The dry matter content contributes to the quality of both table potatoes and processing potatoes and provides energy reserves for sprout growth. The dry matter data of late developing (harvest stage) potatoes of "Russet Burbank" and "Shepody" were higher at the tuber stem end than at the tuber bud end (Figure 3). This provides indirect support to the previous study of cold-stored potatoes of the same cultivars.[15] The high dry matter at the tuber stem end is likely because the photosynthetic product arrived at the tuber stem end first during field growth of the plants. The difference in dry matter between the two ends became smaller at the sprout emergence stage than the harvest stage (Figure 3), which is likely because some starch was metabolized and remobilized to other parts of the tubers, including the tuber bud end. Dry matter was usually found to be higher at the tuber stem end than the tuber bud end during cold storage[20−22] or higher than the middle sections of tubers[15] or the core tissue.[14] The present study, by presenting data for each cultivar, added the finding that round tubers also had higher dry matter in the tuber stem end and that dry matter tended to accumulate to the tuber bud end during storage prior to sprouting (Figure 3).

The present study also found that starch granules increased their average size at the tuber bud end during storage prior to sprouting compared to the developing tubes at harvest in "Russet Burbank" (Figure 5). We used the average length of starch granules of three tubers for each end at each stage and did not use the same tubers for both harvest and sprouting stages because each tuber could only be used once and was no longer suitable for storage after being cut during measurement of starch granules. This average length approach is known to be highly reproducible with very high correlation ($r = 0.975$; $p < 0.0001$) between tubers harvested in different years in a study of 14 potato cultivars.[24] It is known that nano structures of starch granules are quite stable, even after somatic hybrids after protoplast fusion between cultivated and wild potatoes;[23] however, the starch granule average length varies among cultivars in tuber and root crops, such as potato[17,24,25] and sweet potato.[26] The average length of starch granules was found to be slightly larger in "Russet Burbank" than in "Shepody" (Figure 5), which is in agreement with previous whole tuber studies that the average size of "Russet Burbank" starch granules[24] was larger than that of "Shepody".[27] "Russet

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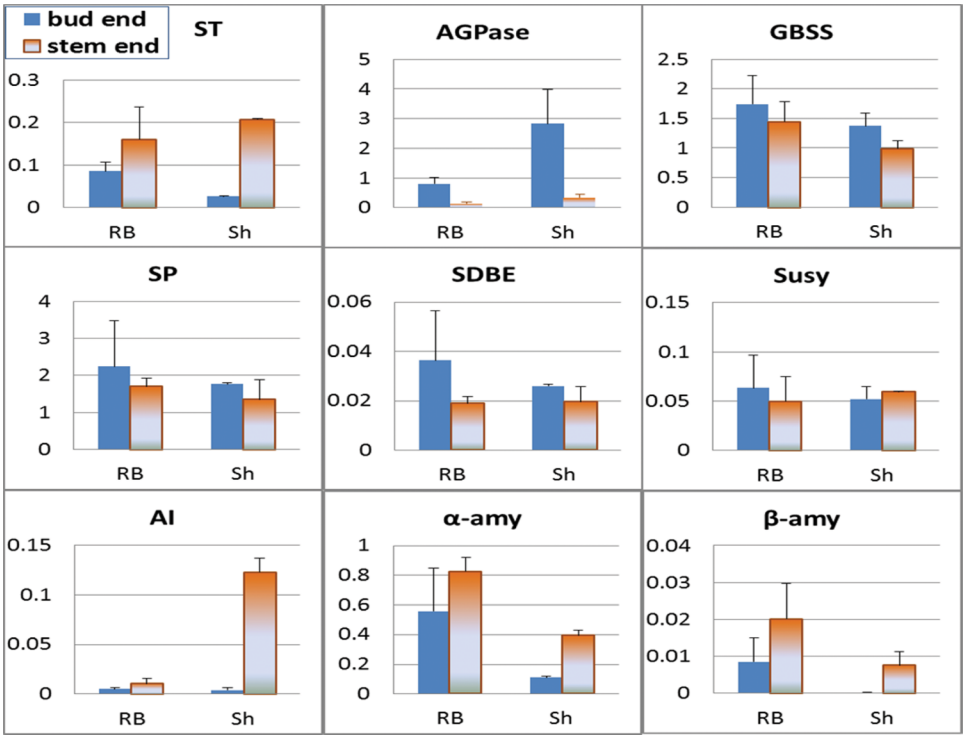

Figure 6. Real-time qRT-PCR analysis of the bud end and stem end of growing tubers from a green vine at harvest in "Russet Burbank" (RB) and "Shepody" (Sh). See Table 1 for the names of the enzymes. The *y* axis is in $2^{-\Delta C_t}$. Gene expression values in the panels are means from two PCR experiments that each used RNA from three tubers (six tubers total per cultivar). Each PCR experiment had three technical repeats. Note that the gene expression of enzymes responsible for starch synthesis (AGPase, GBSS, SP, and SDBE) was stronger in the tuber bud end than in the tuber stem end.

Burbank" is a later maturing cultivar than "Shepody". It was expected that the size change of starch granules was not exactly the same at harvest for "Russet Burbank" and "Shepody" tubers because of their differences in maturation. It is likely that some photosynthetic sugars in "Russet Burbank" were used by the growth of the starch granules after tubers were harvested, which may partly explain why the average starch granule size increased after storage (Figure 5).

Among potato cultivars, the average starch granule size and the specific gravity (a different way to describe dry matter) of whole tubers were positively correlated.[24,25] The present study was not about the whole tubers but about the tuber end regions. Even though further verification is required, the increase of both dry matter and starch granule size at the tuber bud end during storage in "Russet Burbank" and "Shepody" in the present study is in agreement with the previous finding of a potential positive correlation between the two traits.

We studied the gene expression activity of various enzymes (Figures 6 and 7) because these enzymes are known to be involved in starch synthesis and metabolism. Photosynthetic sucrose was translocated by a sucrose transporter (ST), and the ST gene expression was found to be very active in sink tuber phloem and influences tuber development.[28] During tuber development, the photosynthetic sucrose entered the cytosol of tuber parenchyma cells and was converted by sucrose synthetase to fructose and UDP-glucose.[29] Among the two sucrose cleavage enzymes (invertase and sucrose synthase), sucrose synthase is responsible for the direction to starch accumulation.[30,31] Sucrose converted to glucose 1-phosphate (Glc1-P) after subsequent phosphorylation and interconversion. Glc1-P serves as the substrate in the amyloplast for the first committed step in starch synthesis, catalyzed by AGPase.[32,33] The activities of enzymes of AGPase increased during tuber development. Inhibition of AGPase in potato resulted in the abolition of starch formation in tubers, proving that AGPase has a unique role in starch biosynthesis in plants.[34] GBSS is largely responsible for the synthesis of the amylose component of starch. Potato tubers with GBSS activity inhibited by the expression of antisense RNA reduced amounts of amylose.[35] Debranching enzyme (isoamylase) functions during starch synthesis to suppress the initiation of glucan molecules in the plastid stroma.[36] Starch may be degraded either hydrolytically or phosphorolytically, and degradation of starch probably requires the concerted action of several enzymes.[37] A range of starch-degrading enzyme activities has been reported in potato tubers, including cold-induced activity of α-amylase and β-amylase[38] and starch phosphorylase.[9] Amylase likely plays an important role in potato starch degradation during cold storage and sprouting.[8] In potato tubers, the total acid invertase activity generally reflected sugar changes.[39] The glucose content was positively correlated with the total activity of acid invertase among potato cultivars[40] and was confirmed using the transgenic approach.[41,42] Cycles of storage starch cleavage−resynthesis can occur in tubers with successive starch relocalization to the sites of further active consumption during bud sprouting.[9] In the present study, at the harvest stage, the gene expression of the sucrose transporters was more active in the cells of the stem end but the gene expression of starch synthesis enzymes, including AGPase and GBSS, were more active in the cells of the tuber bud end than in the stem end (Figure 6). The starch degradation−resynthesis activities at the gene expression level

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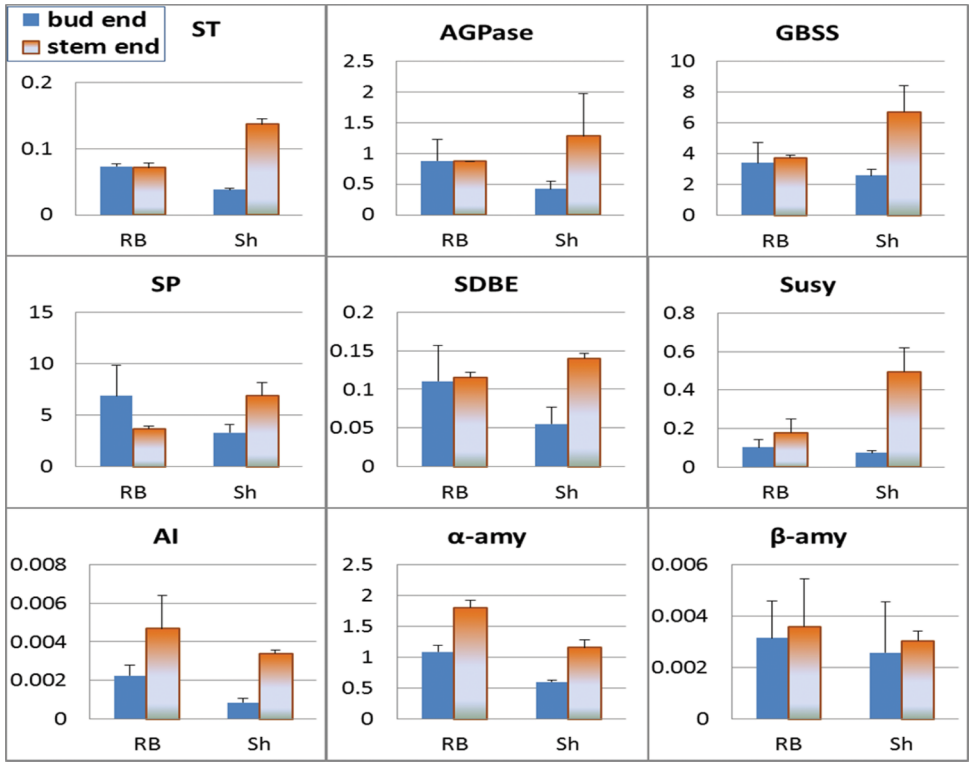

Figure 7. Real-time qRT-PCR analysis of the bud end and stem end of tubers at the sprouting time in "Russet Burbank" (RB) and "Shepody" (Sh). See Table 1 for the names of the enzymes. The *y* axis is in $2^{-\Delta C_t}$. Gene expression values in the panels are means from two PCR experiments that each used RNA from three tubers (six tubers total per cultivar). Each PCR experiment had three technical repeats. Note that gene expression was featured by active degradation (AI, α-amy, and β-amy) and resynthesis (AGPase, GBSS, SP, and SDBE) in the stem end, while the tuber bud end had less activity in starch degradation, suggesting a net export of carbohydrates from the tuber stem end.

were more active at the tuber stem end than at the tuber bud end at the sprout emergence stage in potato tubers (Figure 7).

The expression patterns of these starch synthesis−metabolism enzyme genes showed coordination in the present study. Acid invertase activity was very active in the tuber stem end (Figures 6 and 7), and this is in agreement with previous studies that the dark color on the tuber stem end of chips in potatoes from plants under stress was associated with increased tuber stem end acid invertase activity.[43,44] The present study also found that "Russet Burbank" and "Shepody", two of the most important cultivars in potato production, also have tuber stem end fry darkening issues (as shown in the chips in Figure 2) in potatoes stored at room temperature. Starch degradation enzymes were more active in the tuber stem end than in the tuber bud end, and sucrose transporter gene and Susy were more active at the time of harvest and less active during storage (Figures 6 and 7). At the sprouting stage after approximately 3 months of storage in the present study, the photosynthetic sucrose was expected to be far from depleted. The relatively higher gene expression activity in the tuber stem end at the sprouting stage for both starch degradation and resynthesis without external supplies of carbohydrates (Figure 7) suggests a possible net loss of carbohydrates in the tuber stem end. The starch resynthesis in the tuber stem end during storage was likely stimulated by the availability of substrate generated during starch degradation. This gene expression pattern fits well with the dry matter content increase at the tuber bud end during storage (Figure 3).

In this study, we found the following: (1) The bud end of developing tubers had strong starch synthesis gene expression, which likely attracted photosynthetic sucrose. (2) Gene expression in sprouting tubers suggested a net export of carbohydrates from the tuber stem end to the tuber bud end. (3) The potato tuber stem end had higher dry matter content and larger starch granules than the tuber bud end in growing tubers at harvest from a green vine. (4) The potato bud end already increased dry matter content and the average starch granule size prior to the rapid growth of sprouts. These changes suggest that the gene expression for carbohydrate metabolisms was very different between the field growing stage and the sprout growing stage of tubers, despite starch accumulation at the tuber bud end at both stages. The present study also found the readiness and already started remobilization of resources from the tuber stem end to the tuber bud end to support sprout growth at the sprout emergence stage. This knowledge may help develop strategies for improving tuber yield in the field, controlling sprout growth of stored potatoes, monitoring starch changes for starch-processing potatoes, and reducing tuber stem end darkening in frying of kitchen potatoes.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: hjsi@gsau.edu.cn.

*E-mail: xiu-qing.li@agr.gc.ca and/or xiu-qing.li@canada.ca.

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