

## GENE EXPRESSION PROFILING OF ADP-GLUCOSE PYROPHOSPHORYLASE (AGPase) IN SINK AND SOURCE ORGANS OF SOME CASSAVA VARIETIES WITH DIFFERENT STARCH CONTENTS IN VIETNAM

Nguyen Thi Minh Hong<sup>1,2</sup>, Le Thu Ngoc<sup>1</sup>, Nguyen Mau Hung<sup>1</sup>, Pham Bich Ngoc<sup>1,✉</sup>, Chu Hoang Ha<sup>1</sup>

<sup>1</sup>Institute of Biotechnology, Vietnam Academy of Science and Technology

<sup>2</sup>Hong Duc University

✉ To whom correspondence should be addressed. E-mail: pbngoc77@gmail.com

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### SUMMARY

Starch is the most widespread and abundant storage carbohydrate in plants. We depend upon starch for our nutrition, exploit its unique properties in industry, and use it as a feedstock for bio-ethanol production. Starch is stored in the form of osmotically inactive, water-insoluble granules in amyloplasts (storage starch) and chloroplasts (transitory starch). The biosynthesis of starch involves not only the production of the composite glucans but also their arrangement into an organized form within the starch granule. Understanding the specific functions played by individual isoforms of enzymes involved in starch biosynthesis pathways will provide important basis for regulation of starch production in plant. A transcript-level analysis of the genes which encode starch-synthesis enzymes is fundamental for assessment of enzyme function and the regulatory mechanism for starch biosynthesis in source and sink organs. In this work, the expression level of the genes encoding ADP-glucose pyrophosphorylase (AGPase) in two local varieties Do Dia Phuong (Do DF) and Trang Hoa Binh (Trang HB) as well as two imported varieties KM94 (Rayong1 X Rayong 90) and KM140 (KM98-1 x KM36) with different starch contents were evaluated by quantitative real-time PCR method. The result of transcript level analysis made the expression profiles of cassava AGPS and AGPL genes (encoding AGPase small and large subunits) during three development periods, 90, 180 and 270 DAP (day after planting). The transcriptional activities of these genes exhibited tissue-specific expression patterns. In particular, AGPS2 and AGPL1 transcripts were predominant in leaves, whereas expression of AGPS1, AGPL2, and AGPL3 appeared to be mostly confined to storage roots. Despite of having disparities between development stages, expression patterns of both AGPS2 and AGPL1 in leaves did not show significant differences amongst investigated cassava varieties. In contrast, transcriptional activities of AGPS1 and AGPL3 in tubers had patterns directly related to the starch contents of the cultivars. These results indicated that AGPS1 and AGPL3 genes likely play an important role in the starch biosynthesis pathway and have potential for regulation of starch production in cassava.

**Keywords:** *ADP-glucose pyrophosphorylase; cassava varieties; quantitative real-time PCR, starch biosynthesis, transcriptional level*

### INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a root crop belonging to section fruticosae of family Euphorbiaceae, Dicotyledonae. This crop is a high starch producer with levels between 73.7 and 84.9% of its total storage root dry weight. This attribute together with the unique properties of its starch creates demand for particular food and nonfood applications (Baguma, 2004). Starch is the end-product of photosynthesis in source tissues and is

stored as energy reserves in sink tissues. Starch granules are composed of two different glucosyl polymers called amylose and amylopectin. Amylose has an estimated molecular weight of  $10^5$ - $10^6$  daltons (Pezez, Bertoft, 2010) and consists of essentially linear  $\alpha$ -1,4 linked glucose chains with a low proportion of  $\alpha$ -1,6 linkages (branch points). Amylopectin has an estimated molecular weight of  $10^7$ - $10^9$  daltons and consists of shorter, linear  $\alpha$ -1,4 linked glucose chains with high degree of  $\alpha$ -1,6 branches (Yoo, Jane, 2002; Hostettler, 2014).

Transient starch and storage starch are two forms of starch available in plants. The chloroplasts in photosynthetic tissues such as leaves produce transient starch during photosynthesis and store it temporarily during the light period. Transient starch is converted into sucrose in the dark and which is translocated within the plant to supply the energy and carbon demand required for growth and development. Storage starch is a long-term carbon store in the plant which is synthesized in non-photosynthetic plastids called amyloplasts, found in tuberous tissues or seeds (Tetlow, 2006, 2011). Storage starch is extremely important to the plant metabolism of higher plants as a supplier of long-term energy requirement (Gerard *et al.*, 2001).

There are four major groups of enzymes are involved in starch biosynthesis, ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (SBE) and starch debranching enzyme (DBE). These enzymes are found in several isoforms in many plants (Vrinten & Nakamura, 2000; Joen *et al.*, 2010). ADP-glucose has been identified as a precursor for starch biosynthesis, which is produced from glucose-1-phosphate (G-1-P) and adenosine triphosphate (ATP) by the catalytic activity of AGPase. Therefore, AGPase plays a critical role in the regulation of starch synthesis in plants because it not only catalyses the first dedicated step, but also is the rate-limiting step in starch biosynthesis pathway (Subasinghe, 2013). Antisense-mediated inhibition of AGPase expression has been shown to lead to a severe decrease in starch production in potato tubers (Muller-Rober *et al.*, 1992) as well as in cassava tuberous roots (Munyikwa *et al.*, 1998) and in *Vicia faba* seeds (Rolletschek *et al.*, 2002). In contrast, an increase in expression of AGPase due to introduction of a mutant bacterial *glgC* gene into potato resulted in 35% increase of starch in the tuber (Stark *et al.*, 1992).

The plant AGPase is a heterotetrameric enzyme composed of a pair of large subunits and a pair of small subunits, encoded by different genes (Salamone *et al.*, 2002). The large subunit is 54–60 kDa in size, and the small subunit is 51–54 kDa. Multiple isoforms of the subunits have been found in plants (Martin, Smith, 1995). Both subunits are required for maximal enzyme activity in plants (Frueauf *et al.*, 2001; Tiessen *et al.*, 2002). These requirements make the genetic manipulation of the plant AGPase more challenging, as it potentially requires modification of the expression or activity of

one or more AGPase genes in transgenic plants (Ihemere *et al.*, 2006).

In the present study, four cassava cultivars with various starch contents were used as plant materials to investigate differences in expression profiles of the genes encoding cassava AGPase in sink and source organs by quantitative real-time PCR. The purposes were to reveal the concrete function of these genes on starch biosynthesis, and the enzymatic mechanisms in gene levels responsible for the differences in starch synthesis of the four cassava varieties.

## MATERIALS AND METHODS

### Plant materials

Two local varieties as Do Dia Phuong (Do DF) and Trang Hoa Binh (Trang HB) and two imported varieties including KM94 (Rayong1 X Rayong 90) and KM140 (KM98-1 x KM36) were used. Cassava plants were grown in single rows along the furrows (25 m long, 1 m wide) with distances between the trees as 0.8 m and between rows as 1.2 m. Sample (including mature leaves and storage roots) collections were performed at 90, 180 and 270 days after planting (DAP).

### Starch content measurement

The starch content of cassava tubers grown was measured by collecting tubers just after planting 270 days. Starch concentrations were analyzed as described by Frederick & Leslie, 1949; McCready *et al.*, 1950.

### RNA isolation and cDNA preparation

Total RNA from cassava leaves and tubers were extracted using Plant RNA Reagent (Invitrogen) following manufacturer's instructions, and the extracts were then treated with RNase-free DnaseI (Thermo Scientific) to completely remove contaminating genomic DNA. Total 5 µg of leave or tuber total RNA were used for first-strand cDNA synthesis with the iScript cDNA Synthesis Kit (Bio-Rad) using oligo-dT13 primer.

### Identification of the the genes encoding cassava AGPase with DNA database search

To indentify the genes encoding AGPase involved in starch biosynthesis in cassava, in the first step, the databases of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>) were searched for

general entries of nucleotide sequences of target genes in plant. These sequences were then used as queries to search cassava genes in Phytozome v10.1 database (<http://www.phytozome.net/>). The Pfam tool was used to detect the conserved domains, while the exon/intron structure of each gene was collected from the databases of Phytozome and displayed with the help of Gene Structure Display Server (<http://gsds.cbi.pku.edu.cn/>).

#### Primer design

The primers for real-time PCR were designed

based on conserved regions of each gene using Primer3 software (<http://bioinfo.ut.ee/primer3-0.4.0/>) and were checked for their compliance to the recommendations of the ABI guidelines for Real-Time PCR Primers and amplicons (Real-Time PCR handbook, University of Illinois, Urbana-Champagne, IL, USA). Subsequently, the primers generated and the original Phytozome sequences were analyzed using BLAST program to verify the absence of homologies between the primers and other reported cassava sequences.

**Table 1.** Target genes for analysis of expression profile and primer sequences.

No	Primers	Primer nucleotide sequences	Amplified genes	Access Number
1	18S_F1	ATGATAACTCGACGGATCGC	18S rRNA (reference gene)	GenBank: AB233568.1
2	18S_R1	CTTGGATGTGGTAGCCGTTT		
3	AGPS1_F	GCCAAGGGTAGTGTCCAA	AGPS1	Phytozome: Manes.12G067900
4	AGPS1_R	GACATTATACACCAGGGCAT		
5	AGPS2_F	CCAAGATGGTTGAGGCTGAT	AGPS2	Phytozome: Manes.13G058900
6	AGPS2_R	ACTACCCTTTGCAGCCAGAA		
7	AGPL1_F	TCCAGACTTTTGCAGTGTGC	AGPL1	Phytozome: Manes.03G182100
8	AGPL1_R	ACTGTCCTTGGGTCCCTCTT		
9	AGPL2_F	TGAGAAACCCAAGGGAGATG	AGPL2	Phytozome: Manes.18G019600
10	AGPL2_R	CCAAAGTCATTGCATGATGG		
11	AGPL3_F	TCCGGAGATCATCTTTACCG	AGPL3	Phytozome: Manes.11G085500
12	AGPL3_R	CAATTCGTCCCCTGTTGTCT		

#### Quantitative real-time PCR

Aliquots of the first-strand cDNA mixtures corresponding to 20 ng of total RNA served as the templates for quantitative real-time RT-PCR analysis with SsoAdvanced SYBR Green Supermix (Bio-Rad). For each analysis, 20 µl of the reaction mixture was prepared, including 10 µl of SYBR Green Supermix (Bio-Rad), 0.5 µl of each gene-specific primer (250 nM final concentration), 8 µl of deionized water and 1 µl of cDNA. All reactions were conducted in a CFX96 real-time PCR thermocycler (Bio-rad) with PCR conditions as follows: 95°C for 5 minutes; then 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds and 72°C for 30 seconds; followed by a melt curve of 95°C for 15 seconds, 60°C for 30 seconds and 15 seconds at 95°C.

#### Data treatment and analysis

The quality of amplification from each real-time PCR reaction was assessed through the dissociation curves generated by the CFX Software (version 3.0) incorporated to the CFX96 real-time PCR thermocycler and the Ct data were collected. Transcription levels of the genes of interest were estimated by  $\Delta C_t$  relative quantification method using a reference gene (18S rRNA). Being a variation of the Livak method, this method uses the difference between reference and target Ct values for each sample:

$$\text{Ratio (target/reference)} = 2^{C_t(\text{reference}) - C_t(\text{target})} \text{ (Bio-Rad, 2006).}$$

RESULTS AND DISCUSSIONS

**Cassava collection and cultivation**

In our previous studies, based on the data collection involving in agro-biological indexes from Hung Loc Agriculture Research Center, we collected four varieties in which two local varieties Do DF, Trang HB and two productive varieties KM94, KM140 with different fresh root yield (ton/ha), dry matter content (%), starch content (%) for the starch related gene expression analysis.

The cassava variety KM94 is a hybrid between Rayong1 and Rayong90. It belongs to “bitter” cassava type and the lower part of the stem bends. KM94 has purple shoots, non-branching in delta regions but primary branching in mountainous areas. This variety has high root uniformity, white interior root flesh, fresh root yield of 28,1 ton/ha, starch content of 27,4- 29%

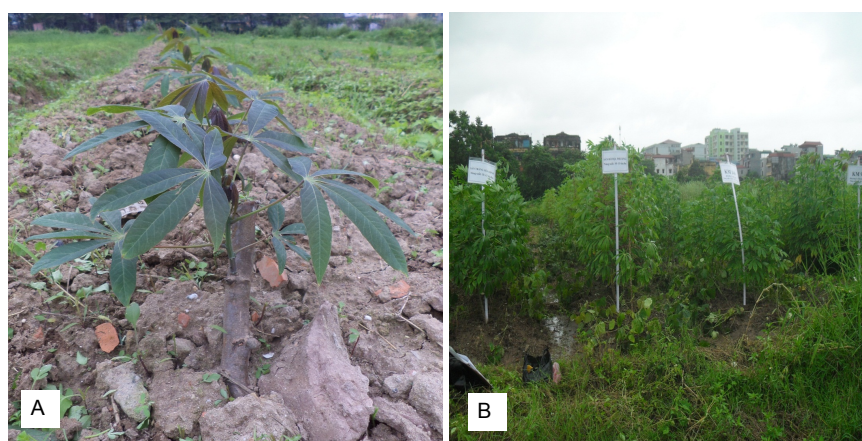
and harvest time of 10 – 12 months after planting.

The cassava variety KM140 is a hybrid of KM98-1 x KM36 cross. Suitable harvest time is about 7-10 months after planting. Average fresh root yield of 35,0 ton/ha (under intensive cultivation conditions in Dong Nai and Tay Ninh provinces, yield can reached 40-50 tons/ha). Cassava variety KM140 has been cultivated across the country with approximately 150000 hectares. For example, Tay Ninh, Dong Nai, Binh Thuan, Gia Lai and Binh Dinh provinces applied KM140 with dozens of hectares per family and got the yield of 30- 80 ton/ha.

Trang HB and Do DF are local varieties and grown popularly in the midlands, mountainous in duration 10-12 months with delicious tubers, suitable for eating or sliced dried or reserves, yielded 10-13 tons/ha and 10-15 tons/ha, respectively.

**Table 2.** Characteristics of planting cassava cultivars.

Cultivars	KM94	KM140	Do Dia Phuong	Trang Hoa Binh
Height (cm)	154.2	135.7	162.9	142.1
Characteristics	Bend, branching at the base, purple shoots	Straight, non-branching	Straight, non-branching, petioles	non-red Straight, non-branching at the base
Harvest duration (days)	340	340	340	340
Actual productivity (tons/ha)	28 – 30	33,4 – 35	10 – 13	10 – 15
Theoretical output (tons/ha)	26.5	30	16.66	18.3
Starch contents (mg/g) after 270 days	449.9	475.3	574.7	389.7



**Figure 1.** Cassava cultivation in Co Nhue experimental Centre: 3 weeks (A) and 9 months (B) after planting

In this study, two local varieties in Northern of Vietnam as Do DF and Trang HB and two varieties KM94 and KM140, the most planted cultivars in Southern of Vietnam were grown in Co Nhue Experimental Station, Hanoi from February 2013 to January 2014. In the growing conditions, there were differences between real productivity and theoretical output of two local cassava varieties (Do DF, Trang HB) and two high productivity varieties (KM94 and KM140). After 270 days of cultivation, the theoretical output of two local varieties was significantly increased as compared with the actual yields. Meanwhile, the contrary results were recorded at both KM94 and KM140 (table 2). Beside, Do DF cultivar showed the highest value at not only the average plant height but also the starch content, 162.9 cm and 547.7 mg/g, respectively. These consequents might be caused by the climate and environments. KM94 and KM140 are usually grown in the south area of Vietnam, where the temperate is stable and warm. Their original characteristics may hardly be adapted with the difficult condition of Vietnam' northern environment, which is tropical monsoon climate area with harsh winter period. On the other hand, two local varieties (especially Do DF) mainly distributed in the north area, are much more adaptable with climate in Co Nhue Experimental Station than KM94 and KM140.

#### **Expression patterns of the genes encoding cassava AGPase during development stages**

The growth and development of cassava can be divided into four periods: root generation and budding period; root system development; leaf development and stem elongation; and tuber development. Root generation and budding period usually lasts 3 weeks. During this period, in the suitable conditions of moisture and temperature, cassava cuttings germinate and develop into seedlings. The next two months are period of root system development not only in quantity but also in length, and rootlets are differentiated into the thick and thin roots. Thick roots are mostly roots at meristem zones which will easily differentiate into tubers in favorable weather and nutrition conditions. During the root development period, the growth rate of stems and leaves remains slow, and caulicles grow mainly based on reserve substances in the cuttings. From the fourth to the sixth month is the development period of stems and leaves. Stems rise up and thicken by wood formation. The total number as well as area

of leaves increase rapidly. Photosynthesis becomes stronger and dry matters are accumulated. From the sixth month onwards, storage roots begin to thrive in parallel with a reduction in growth rate of stems, leaves and rootlets. The growth of tuber can be divided into 3 stages: tuber formation stage corresponding to 1-3 months after plating when the growth rate of tubers is slow, middle stage (4-7 months after planting) when tubers grow quickly, and late stage (8-10 months after planting) when tuber starch tubers accumulate starch but their growth rate is slow down (Hoang Kim *et al.*, 2008).

Based on the growth and development period of cassava as above, we chose 3 timelines 90 days, 180 days and 270 days after planting (DAP) for sample collecting to evaluate expression levels of the genes of interest by real-time PCR. In this study, cassava 18S rRNA (GenBank: AB233568.1) was used as reference gene to normalize the transcription levels of target genes. Two plantlets were chosen from each cultivar (plantlet number 3 and 5 presenting 2 biological replicates), and each real-time PCR reaction was replicated twice for technical error.

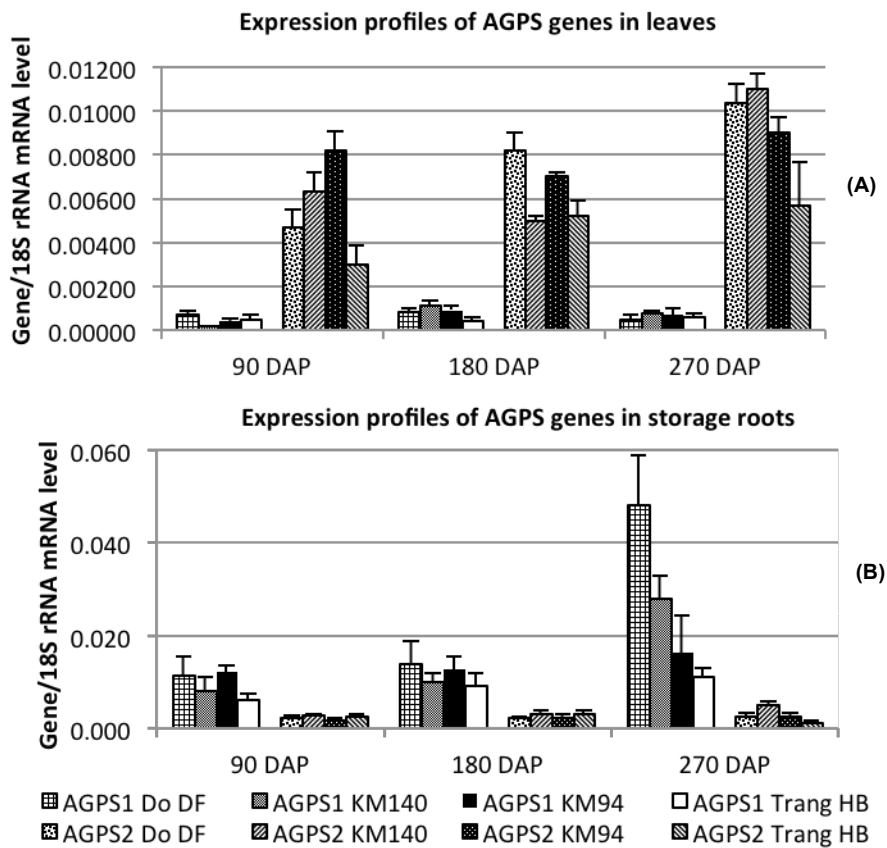
As mentioned above, plant AGPase is a heterotetrameric enzyme composed of two each of the larger regulatory subunits (AGPL) and the smaller catalytic subunits (AGPS). These two subunits are encoded by different genes. Our results of database research showed that cassava has a total of 5 genes encoding AGPase: two for AGPS (AGPS1 and AGPS2) and three for AGPL (AGPL1, AGPL2, and AGPL3) (Table 1).

The analysis of relative AGPS expression in tubers and leaves through development stages (Fig. 2) showed that: AGPS2 transcripts were predominant in leaves, whereas expression of AGPS1 appeared to be mostly confined to storage roots. The expression of AGPS2 in leaves of KM140 and KM94 shared the same pattern: it was vigorously expressed at the early stage (90 DAP), slightly declined at 180 DAP and increased at 270 DAP. On the contrary, in Do DF and Trang HB, AGPS2 expression levels increased gradually through development stages. Compared to 90 DAP, the transcription levels of AGPS2 in leaves of Do DF, KM140 and KM94 at 270 DAP were about 2.2, 1.75 and 1.9-fold higher, respectively. However, there was small disparities detected in AGPS2 expression in Trang HB leaves between these stages. The highest AGPS2 transcript was recorded in KM140 leaves at 270 DAP, but it was not

significantly different from those of the other cultivars (Fig. 2A).

In addition, real-time PCR results showed the different AGPS1 expression levels in tuber amongst investigated cassava varieties between development stages. Despite of low AGPS1 transcripts from 90 until 180 DAP, they increased dramatically at 270 DAP (Fig. 2B). Do DF and KM140, the two high starch content types, showed significant increase in AGPS1 expression levels at 270 DAP, which were approximately 4.2 and 3.5- fold higher, respectively, as compared with those at 90 as well as 180 DAP. In

lower starch content varieties, KM94 and Trang HB, these ratios were calculated about 1.3 and 1.8, respectively. Especially, in tubers, the average transcriptional activity of AGPS1 had a pattern directly related to the starch contents reported for each cultivar. The maximum expression level of AGPS1 in tuber was found in Do DF which is the most starchy type. The results revealed that it was approximately 1.7, 2.9 and 4.3 times higher in comparison with those of KM140, KM94 and Trang HB, respectively.



**Figure 2.** Relative AGPS expression in leaves (A) and tubers (B) of cassava varieties at 90, 180 and 270 DAP.

The analysis results of AGPL expression (Fig. 3A) showed that: AGPL2 transcripts were not detected, and AGPL3 was not significantly active in leaves of all cassava cultivars. In contrast, AGPL1 transcripts on average were expressed over 11-38 times more than AGPL3 during development stages, which indicated that AGPL1 plays main role in the

synthesis of transitory starch in cassava leaves. However, no significant difference in AGPL3 expression between the four varieties was recorded.

In tubers, AGPL2 and AGPL3 transcripts were predominant, while those of AGPL1 were scarce. AGPL2 was expressed in early stage (90 DAP), but

its transcripts sharply declined, average 1.8 and 3.7 times at 180 and 270 DAP, respectively. Furthermore, AGPL2 expression pattern did not exhibit significant difference between cultivars. Conversely, in tuber development and starch accumulation stages (180 and 270 DAP), AGPL3 transcripts had a dramatic increase, about 2.3 and 3 times higher, respectively, as compared with those

in early stage. Moreover, transcriptional activity of AGPL3 in cassava tubers showed a pattern based on the starch levels of the cultivars. The maximum expression level of AGPL3 was found in Do DF variety with highest starch content at 270 DAP. It was approximately 1.2, 1.4 and 1.9 times higher than those of KM140, KM94 and Trang HB, respectively (Fig. 3B).

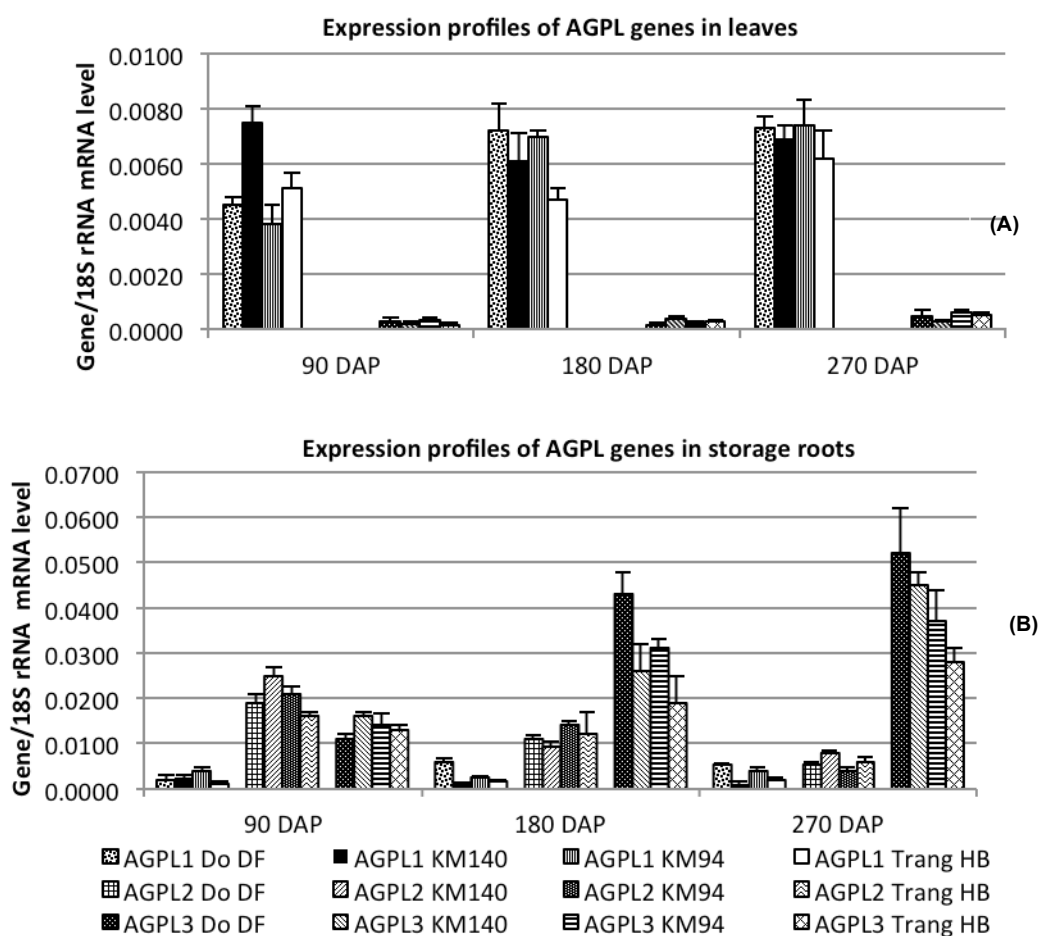


Figure 3. Relative AGPL expression in leaves (A) and tubers (B) of cassava varieties at 90, 180 and 270 DAP.

These results showed the variation of AGPase expression levels amongst cassava varieties as well as between different development stages. In either high or low-starch -content types, when cassava tubers were under starch accumulation period (270 DAP), transcriptional activity of the genes encoding AGPase were enhanced in both sink and source organs. In addition, the results presented here

indicated that AGPS1 combines with AGPL3 to form AGPase which is markedly expressed to play an important role in starch accumulation via ADP-glucose synthesis in cassava tubers.

In previous studies, Ohdan *et al.*, (2005) investigated the expression of the genes involved in starch synthesis in rice. These observations revealed that the pattern of gene expression is gene specific,

depending on each gene of each class during endosperm development, and that the genes which are highly expressed in the endosperm are quite different from genes that are markedly expressed in the leaf. There are intricate functional interactions between components of the starch biosynthetic, and the starch-biosynthesizing system in higher plants demands the concerted expression of many genes at any particular developmental stage of a particular tissue (Tetlow *et al.*, 2004; Ohdan *et al.*, 2005; Hennen-Bierwagen *et al.*, 2008). Since it is difficult to measure separately the protein level or activity of each enzyme in the enzyme complex, transcriptome analysis would be a more convenient tool to reveal the regulatory mechanism for starch synthesis that determines when and where multiple genes are co-expressed and what proteins may interact.

## CONCLUSION

The present results provide concrete evidence that the expressions of cassava AGPS and AGPL genes (encoding AGPase small and large subunits) involved in starch metabolism are highly coordinated temporally and spatially in both source and sink tissues of cassava and keep distinct expression patterns. In particular, AGPS2 and AGPL1 transcripts were predominant in leaves, whereas expression of AGPS1, AGPL2 and AGPL3 appeared to be mostly confined to storage roots. Transcriptional activity of these genes were markedly enhanced in both sink and source organs in the starch accumulation stage. Among these, transcriptional activities of AGPS1 and AGPL3 in tubers had patterns directly related to the starch contents of the cultivars, indicating their important role in the starch biosynthesis pathway and potential for regulation of starch production in cassava.

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## NGHIÊN CỨU SỰ BIỂU HIỆN CỦA NHÓM GEN MÃ HÓA ADP-GLUCOSE PYROPHOSPHORYLASE (AGPase) Ở CÁC CƠ QUAN QUANG HỢP VÀ DỰ TRỮ CỦA MỘT SỐ GIỐNG SẮN CÓ HÀM LƯỢNG TINH BỘT KHÁC NHAU Ở VIỆT NAM

Nguyễn Thị Minh Hồng<sup>1,2</sup>, Lê Thu Ngọc<sup>1</sup>, Nguyễn Mậu Hưng<sup>1</sup>, Phạm Bích Ngọc<sup>1</sup>, Chu Hoàng Hà<sup>1</sup>

<sup>1</sup>Viện Công nghệ sinh học, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

<sup>2</sup>Trường Đại học Hồng Đức

### TÓM TẮT

Tinh bột là loại carbohydrate dự trữ phổ biến nhất và phong phú nhất trong thực vật. Chúng ta phụ thuộc vào tinh bột để cung cấp dinh dưỡng cho cơ thể, khai thác đặc tính độc đáo của tinh bột trong công nghiệp và sử dụng tinh bột như là một nguyên liệu cho sản xuất ethanol sinh học. Tinh bột được lưu trữ dưới dạng hạt không tan, không hoạt tính ở amyloplasts (tinh bột dự trữ) và chloroplast (tinh bột tạm thời). Quá trình sinh tổng hợp tinh bột không chỉ liên quan đến việc sản xuất các thành phần glucan mà còn liên quan đến sự sắp xếp chúng vào một dạng có tổ chức trong hạt tinh bột. Hiểu được các chức năng cụ thể của các đồng vị riêng biệt của enzyme tham gia vào quá trình tổng hợp tinh bột sẽ cung cấp cơ sở quan trọng cho việc điều chỉnh sản xuất tinh bột trong thực vật. Việc phân tích sự biểu hiện ở cấp độ phiên mã của nhóm gen mã hóa các enzyme tổng hợp tinh bột là bước căn bản giúp đánh giá chức năng của mỗi enzyme và cơ chế điều tiết quá trình sinh tổng hợp tinh bột diễn ra tại các cơ quan quang hợp cũng như dự trữ. Trong nghiên cứu này, mức độ biểu hiện của các gen mã hóa ADP-glucose pyrophosphorylase (AGPase) giữa hai giống sắn địa phương là Đỏ Địa Phương (Đỏ DF) và Trắng Hòa Bình (Trang HB) cùng hai giống nhập nội là KM94 (Rayong1 X Rayong 90) và KM140 (KM98-1 x KM36) có hàm lượng tinh bột khác nhau được đánh giá bằng phương pháp real-time PCR định lượng. Kết quả của phép phân tích này đã cung cấp dữ liệu biểu hiện của các gen AGPS và GPL ở sắn

(mã hóa cho tiểu đơn vị nhỏ và lớn của AGPase) qua 3 giai đoạn phát triển là 90, 180 và 270 ngày sau khi trồng. Hoạt động phiên mã của các gen này thể hiện tính đặc hiệu mô rõ nét. Cụ thể là, sự phiên mã của các gen AGPS2 và AGL1 diễn ra chủ yếu ở lá, trong khi đó gen AGPS1 và AGL3 phần lớn biểu hiện ở rễ dự trữ. Mặc dù có sự chênh lệch giữa các giai đoạn phát triển, mô hình biểu hiện của cả hai gen AGPS2 và AGL1 trong lá không cho thấy sự khác biệt đáng kể giữa bốn giống sản nghiên cứu. Ngược lại, hoạt động phiên mã của gen AGPS1 cũng như AGL3 trong củ thể hiện mối tương quan tuyến tính với hàm lượng tinh bột của các giống sản. Những kết quả này cho thấy 2 gen AGPS1 và AGL3 rất có thể đóng vai trò quan trọng trong con đường sinh tổng hợp tinh bột và có tiềm năng ứng dụng trong việc cải thiện hàm lượng tinh bột ở sản.

**Từ khóa:** *ADP-glucose pyrophosphorylase, mức độ phiên mã, real-time PCR định lượng, sản, sinh tổng hợp tinh bột*