Macrothink Institute™

Journal of Agricultural Studies
ISSN 2166-0379
2017, Vol. 5, No. 1

Time Course of Starch Biosynthesis Enzymes Activity and Root Tuber Starch of Four Cassava Cultivars

Huifang Huang, Yanchun Luo, Qiang Huang, Yinong Tian, & Huimin Li

Guangxi Cassava Research Institute, Nanning, China

Guangxi Sub-tropic Crops Research Institute, Nanning, China

Xuewen Hou (Corresponding author)

Research Center of Plant Stress Biology, College of Life Sciences

South-China Agricultural University, Guangzhou , China

Tel: 86-20-8528-0194 E-mail: hxw1969@scau.edu.cn

Received: November 16, 2016 Accepted: February 12, 2017 Published: February 24, 2017

doi:10.5296/jas.v5i1.10810 URL: https://doi.org/10.5296/jas.v5i1.10810

Abstract

To understand the accumulation rule of cassava root tuber starch, the amylose, amylopectin and total starch content of fresh root tuber, the enzyme activities of sucrose synthase (SuS, EC 2.4.1.13) and sucrose phosphate synthase (SPS, EC 2.4.1.14) of leaves, the enzyme activities of ADP-glucose pyrophosphorylase (AGPase, EC 2.7.7.27), soluble starch synthase (SSS, EC 2.4.1.21) and starch branching enzyme (SBE, EC. 2.4.1.18) of tubers were assessed by using cultivars SC201, SC205, GR891 and GR911 leaves and root tubers during their growth period, respectively. The results as follows: the enzyme activity of leaf SPS and the synthesis direction of SuS showed the highest at August, 2010, however the enzyme activity of the decomposing direction of SuS formed parabolic curve; the enzyme activity of tuber AGPase showed an increased then decreased single peak curve, the enzyme activity of tuber SSS oscillating decreased, while the enzyme activity of SBE was relatively stable; the amylose, amylopectin and total starch contents of fresh cassava tuber were all gradually increased along with the growth period. This research would enrich our knowledge of the time course of amylose, amylopectin and total starch contents of fresh cassava tuber, and above related enzyme activities.

Keywords: ADP-glucose pyrophosphorylase, Cassava starch, Soluble starch synthase, Starch branching enzyme, Sucrose synthase, Sucrose phosphate synthase

1. Introduction

Cassava (*Manihot esculent* Crantz) was originally from Amazon tropical area, now is planted widely in more than 100 countries. Due to its large amount starch production, it is referred as granary of underground and king of starch (Balagopalan, 2002). The cassava starch has not only widely used as food (Cook and Cock, 1989), raw material for chemical industry (Pimentel et al., 2007), and also as the raw material of biomass energy (Osei et al., 2013). Therefor cassava tuber starch has attracted more and more attention. Systemic research on the accumulation rule of cassava root tuber starch, the activities of starch biosyntheses related enzymes of cassava, and the relationship between starch accumulation and related enzymes activities are necessitated. However, knowledge about this is inferior to other starch crops, such as rice, wheat, maize and potato (Wang and Wang, 2004; Tan et al., 2009). This knowledge will help to screen better cassava cultivars, and to regulate its starch accumulation and enzyme activity through suitable cultivation methods, and also to breed cultivars for higher starch production or starch composition optimized transgenic lines through regulating related enzyme activity by genetic modification (Carvalho et al., 2004; Ihemere et al., 2006). SC201, SC205, GR891 and GR911 are the four cassava cultivars widely planted in Guangxi nowadays. In this research, the root tuber starch content, the enzyme activities of leaf SuS and SPS, tuber AGPase, SSS and SBE were determined by using cultivars SC201, SC205, GR891 and GR911 root tubers as materials during their growth period.

2. Materials and methods

2.1 Materials and Management

Cassava cultivars SC201, SC205, GR891 and GR911 were cultivated in the experimental field (Red soil, moderate fertility) of Guangxi Sub-tropic Crops Research Institute, Nanning during March 9, 2010, row spacing 1×0.8 m, each block 7×11 plants, 3 trials randomized block design. Base manure: cassava starch residue 3 t/ha, cassava specific fertilizer 1250 kg/ha, topdressing KCl 450 kg/ha. Other field management was same as cassava field management (Pan et al, 2014).

2.2 The Collection of Experiment Materials

The cassava leaves and tuber materials were collected at July 9, August 10, September 8, October 9, November 8 and December 10 at one month interval, respectively. 1 plant was randomly picked from 1 block, 3 leaves and 3 representative root tubers were chosen, and surface cleaned. Samples were ground to powder with liquid nitrogen, and stored in -80℃ for enzyme activity determination.

Sampling for starch assay: 1 plant was picked from 1 block, 3 representative root tubers were chosen, washed and surface dried. Sampled 100g from middle of the tuber, enzyme inactivated at 105 ℃ for 15 min, then 75 ℃ 24 h to constant weight. The ratio of dry weight to fresh weight marked as dry material content. Sample was powdered after weighting, and passed through 60 mesh sieves, fat extracted by Soxhlet's fat extraction apparatus 6h, then baked at 80 ℃ 2 h in baking oven, stored in desiccator for starch determination.

2.3 The Extraction of Cassava Leaves Crude Enzymes

About 1 g cassava leaf powder were ground with 3mL extraction buffer (pH 7.2 100 mmol/L Tris-HCl, 10 mmol/L $MgCl_2$, l mmol/L Na_2EDTA, 10 mmol/L β-mercaptoethanol, 2% ethylene glycol, l% PVP (Polyvinylpyrrolidone)) in mortar, then washed twice each with 1 mL extraction buffer, then collected into centrifuge tube, centrifuged 12000 rpm 15 min at 4 ℃, 2.5 mL supernatant was taken into dialysis tube, dialyzed in 200 mL dialysis buffer (20 mmol/L， pH 7.2 Tris-HCl, 2.5 mmol/L $MgCI_2$, l mmol/L Na_2EDTA, 5 mmol/L β-mercaptoethanol, 1% ethylene glycol) at 4 ℃ 12 h, new dialysis buffer changed at 6 h once. After dialysis, the volume of the enzyme extract was measured and used as crude enzyme for leaf SuS and SPS assay.

2.4 The Determination of Enzyme Activity of Cassava Leaves SuS Synthesis Direction

The method used here was modified from Yu (1999). Each assay using 4 tubes, 1 as control, other 3 are assay repeats, control added with 0.2 mL NaOH, assay tubes added with 0.2 mL above crude enzyme extract, then all 4 tubes were added with reaction solution (100 mmol/L pH 7.2 Tris-HCl, 10 mmol/L $MgCl_2$, 5 mmol/L UDPG (Uridine diphosphate glucose), 10 mmol/L D-fructose), incubating in 30 ℃ water bath to react upto 30 min, then the reaction was stopped at boiling water bath for 5 min, 0.2 mL 2 mol/L NaOH added and mixed, incubated in boiling water bath 10 min again, 1 mL of 0.1% m-dihydroxybenzene and 3.5 mL 30% HCl were added in after cooling down. Mixture was incubated in 80 ℃ water bath for 10 min, OD480 nm was measured after cooling down. Enzyme activity was calculated as μmol sucrose/gFW ħ.

2.5 The Determination of Enzyme Activity of Cassava Leaves SuS Decomposing Direction

The method used here was modified from Zhao (2003). Each assay using 4 tubes, 1 as control, other 3 are assay repeats, control added with 0.2 mL 2 mol/L NaOH, assay tubes added with 0.2 mL above crude enzyme extract, then all 4 tubes were added 0.4 mL reaction solution (100 mmol/L pH 7.2 Tris-HCl , 10 mmol/L $MgCl_2$, 5 mmol/L UDP (Uridine diphosphate), 50 mmol/L sucrose), incubated in water bath at 30 ℃ to reaction for 30 min. The reaction was stopped in boiling water bath for 5 min, then 0.2 mL 2 mol/L NaOH added and mixed, 0.5 mL dinitrosalicylic acid reagent was added, then incubating in boiling water bath for 5 min, 4 mL distilled water were added in after cooling down, colorimetric analysis at 540 nm. Enzyme activity was calculated as μmol glucose/gFW ħ.

2.6 The Determination of Enzyme Activity of Cassava Leaves SPS

The method used here was modified from Yu (1999). Each assay using 4 tubes, 1 as control, other 3 are assay repeat, control added with 0.2 mL 2 mol/L NaOH, assay tubes added with 0.2 mL above crude enzyme extract, then all 4 tubes were added with reaction solution(100 mmol/L pH 7.2 Tris-HCl, 10 mmol/L $MgCl_2$, 5 mmol/L UDPG, 10 mmol/L 6-P-fructose), incubate in 30 ℃ water bath for 30 min, then reaction was stopped in boiling water bath for 5 min, 0.2 mL 2 mol/L NaOH was added and mixed, treated in boiling water bath for 10 min again, 1 mL 0.1% m-dihydroxybenzene and 3.5 mL 30% HCl were added in after cooling down. Mixed and incubated in 80℃ water bath for 10 min, OD480 nm was measured after

cooling down. Enzyme activity was calculated as μmol sucrose/gFW ћ.

2.7 The Extraction of Cassava Tuber Crude Enzyme

About 1.0 g cassava tuber powder was added with 3 mL extraction buffer [100 mM HEPES (Hydroxyethyl piperazine ethanesulfonic acid, pH 7.5)，5 mM $MgC1_2$，5 mM DTT，2%(w/v) PVP], ground in pre-cooled mortar, extracted at 4 ℃ for 30 min, centrifuged at 4 ℃ l0000 g 15 min, the supernatant was used for enzyme activity assay.

2.8 The Determination of Enzyme Activity of Cassava Root Tuber AGPase

The method of OU-Lee and Setter (1985) was followed. 225 μL reaction buffer A [100 mM HEPES-NaOH (pH 7.4)，1.2 mM ADPG (Adenosine diphosphate glucose)，3 mM Na_2PPi，5 mM $MgC1_2$，4 mM DTT (Dithiothreitol)] and 100 μL above crude enzyme were mixed together, incubate in 30 ℃ water bath to react 20 min, the reaction was stopped at boiling water bath for 30 seconds, centrifuged 5 min at 10000 g, 250 μL supernatant was added into 750 μL reaction buffer B[100 mM HEPES(p H7.5)，6 mmol / L $NADP^+$, 1.5 IU/mL glucophosphomutase, 5 IU/mL glucose 6-phosphate dehydrogenase], mixed and incubated in 30 ℃ water bath to react 10 min, colorimetric analysis at 340 nm. The NADH content was calculated by the molar extinction coefficient (6220) of NADH at 340 nm.

2.9 The Determination of Enzyme Activity of Cassava Root Tuber SSS

The method used by Nakamura et al. (1989) was adopted. 180 μL reaction buffer A [50 mM HEPES-NaOH(pH 7.4)，1.6 mM ADPG，0.7 mg amylopectin，15 mM DTT] and 100 μL above crude enzyme were mixed together, incubate in 30 ℃ water bath to react 20 min, the reaction was stopped at boiling water bath for 5 min. After cooling down, 100 μL reaction buffer B [50 mM HEPES-NaOH (pH 7.5)， 4 mM PEP，200 mM KCl， 10 mM $MgCl_2$，and 1.2 U pyruvate kinase] was added in, incubate in 30 ℃ water bath to react 20 min, the reaction was stopped at boiling water bath for 5 min, centrifuged 5 min at 10000 g, 330 μL supernatant was added into 237 μL reaction buffer C[50 mmol/L HEPES-NaOH (pH 7.5)，10 mmol/L glucose，20 mmol/L $MgC1_2$，2 mmol/L NADP，1.4 unit hexokinase，0.35 unit glucose 6-phosphate dehydrogenase], mixed and incubated in 30 ℃ water bath to react 10 min, colorimetric analysis at 340 nm. The NADH content was calculated by the molar extinction coefficient (6220) of NADH at 340nm.

2.10 The Determination of Enzyme Activity of Cassava Root Tuber SBE

The method of Li et al. (1997) was followed. About 3 g cassava tuber powder was ground with 2 mL 0.05 mol/L citrate buffer (pH 7.0) in ice-cold mortar, the mortar was washed twice with 2 mL 0.05 mol/L citrate buffer, the buffer was collected together, centrifuged 20 min at 18000 rpm, the supernatant was used as crude enzyme. 1 mL above enzyme solution, 1 mL 0.2 mol/L citrate buffer (pH 7.0), 0.5 mL 0.l mol/L EDTA (with shaking, to inactivate α-amylase) and 0.1 mL 7.5 g/L soluble starch were mixed together, incubated in 37 ℃ water bath to react 40 min, the reaction was stopped by adding 4 mL 10% trichloroacetic acid, centrifuged 8 min at 3000 rpm, 0.3 mL iodine solution was added into the supernatant, the OD660nm was measured after 10 min. Use the 0 min as the control, enzyme activity is

represented by the decreased percentage of OD 660, so 1 U is OD 660 decrease 1% per min.

2.11 The Determination of Cassava Root Tuber Starch

The method of Jin et al. (2009) was followed. The amylose and amylopectin content of cassava root tuber were determined by dual-wavelength spectrophotometry, respectively. Then total starch content was calculated by adding amylose and amylopectin content together.

2.12 Data Processing

MS Excel was used to calculate the data for plotting; Software DPS 7.05 was used for statistical analysis and Duncan's new multiple range test was used for analysis. All data in the following figures are the mean of three replications. The small letters at the top of each column indicate the significant difference at $P<0.05$.

3. Results and Discussion

3.1 The Time Course of Cassava Leaf SuS Synthesis Direction and Decomposing Direction Activity

The carbohydrate synthesized from cassava leaf photosynthesis is the source of cassava tuber starch. Cassava leaf SuS plays an important role in the distribution of the photosynthesized carbohydrate (Zheng et al., 2011), thereby affects the cassava tuber starch accumulation. The cassava leaf SuS synthesis and decomposing direction activities were determined during the growth period (Figure 1 and 2). For the cassava leaf SuS synthesis direction activity, all the four cassava cultivars' enzyme activities were highest at August among the growth period determined, then decreased gradually during the succeeding months. For the cassava leaf SuS decomposing direction activity, it decreased initially then increased gradually to form a parabolic curve during the growth period. Taking together, the function of SuS is for the sucrose synthesis only in August, however for sucrose decomposition for other months. Poovaiah et al (2015) increased the biomass of switchgrass through overexpression of switchgrass sucrose synthase gene *PvSUS1*. This suggested that cassava starch might be increased through similar approach.

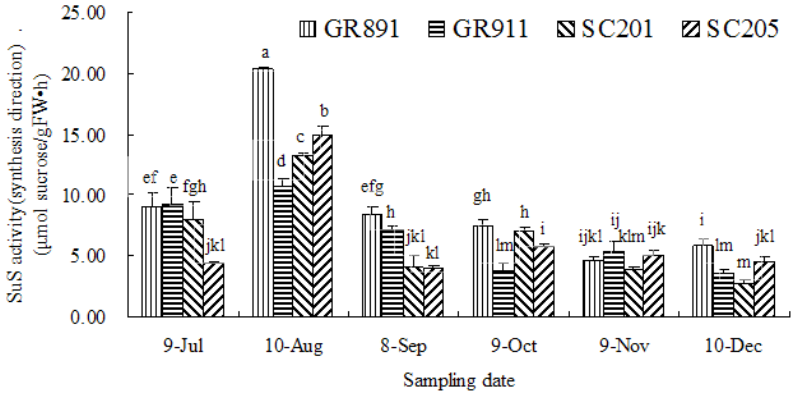

Figure 1. The time course of cassava leaf SuS activity of synthesis direction

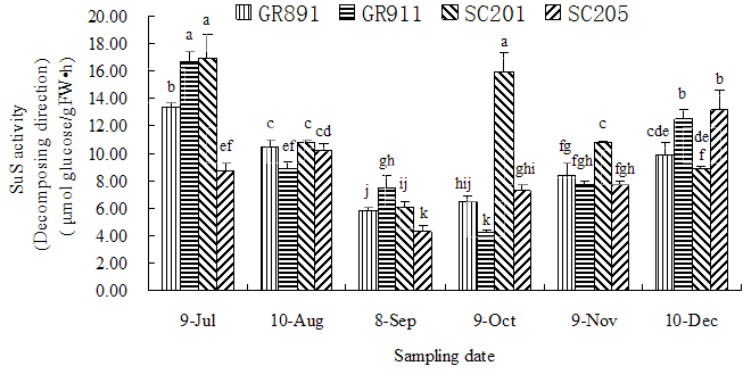

Figure 2. The time course of cassava leaf SuS activity of decomposing direction

3.2 The Time Course of Cassava Leaf SPS Enzyme Activity

SPS is another enzyme for the synthesis of sucrose. As known sucrose is the carbohydrate transported from source to sink. The higher SPS activity will favor the synthesis of sucrose, thus promote the carbohydrate to be transferred from source (cassava leaves) to sink (cassava tuber) for starch biosynthesis (Lutfiyya et al., 2007). All the four cassava cultivars' SPS activities were highest at August among the growth period; this showed that August is much more important than other months for tuber starch accumulation (Figure 3). For the high starch content cultivar GR891, the SPS activities were almost highest among the four cultivars during the growth period.

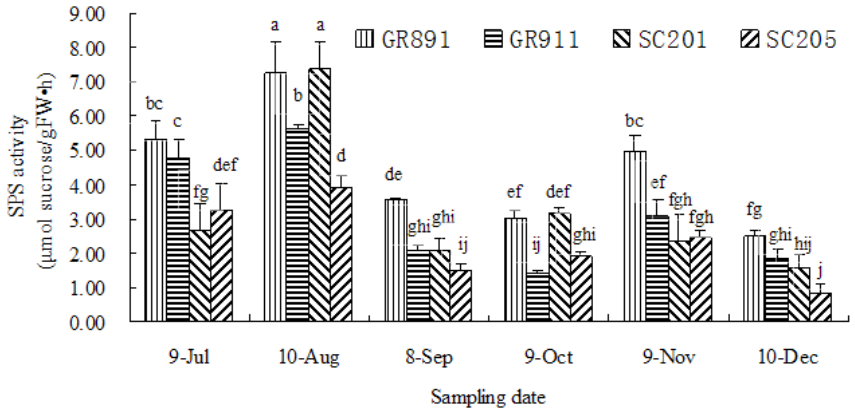

Figure 3. The time course of cassava leaf SPS activity

3.3 The Time Course of Cassava Root Tuber AGPase Enzyme Activity

As well known, the reaction catalyzed by AGPase is the key and rate-limiting step of starch biosynthesis (Huang et al., 2011). The rate of starch accumulation has direct relationship to its enzyme activity. The enzyme activity of AGPase in August, September and October were all higher than other months (Figure 4), it indicated that these three months played important role for the cassava root starch production. The AGPase activity of cultivar GR891 was the highest among the four cultivars determined. These results were consistent with that the starch content of GR891 was also the highest among the four cultivars.

Tan et al. (2009) studied the transcription and enzyme activity of AGPase during the formation of wheat seed by using fluorescent quantitative PCR and enzyme activity assay

method. It was found that they all formed a single peak curve. This result was consistent with the cassava root tuber AGPase activity determined in this study (Figure 4). This indicated that there was a starch formation peak during the accumulation of starch. Kang et al (2013) increased the starch content and grain weight of common wheat through overexpression of the cytosolic AGPase large subunit gene. This study hinted that AGPase is important in starch biosynthesis, and this approach could be utilized for increasing cassava starch accumulation through molecular breeding.

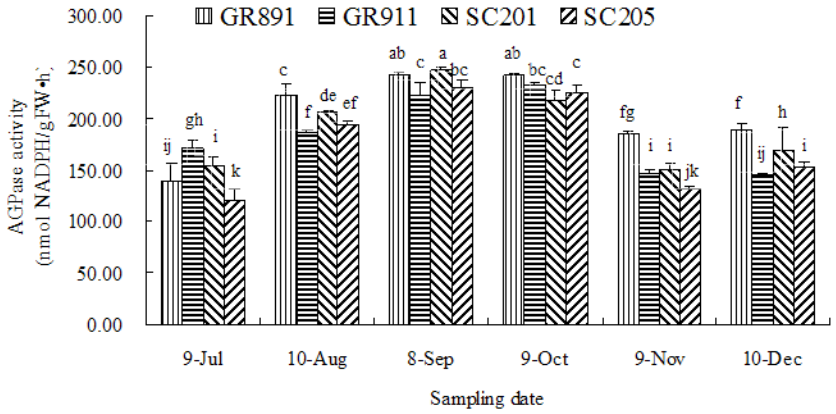

Figure 4. The time course of AGPase activity of cassava root tuber

3.4 The Time Course of Cassava Root Tuber SSS Enzyme Activity

It was known that cassava root tuber SSS mainly involved in the biosynthesis of amylopectin, so its activity determines the amylopectin content (Zhang et al., 2008). The SSS activities of four cultivars were all decreased gradually along the growth period (Figure 5). It is postulated from this result that the amylopectin biosynthesis rate is higher at early stage than late stage.

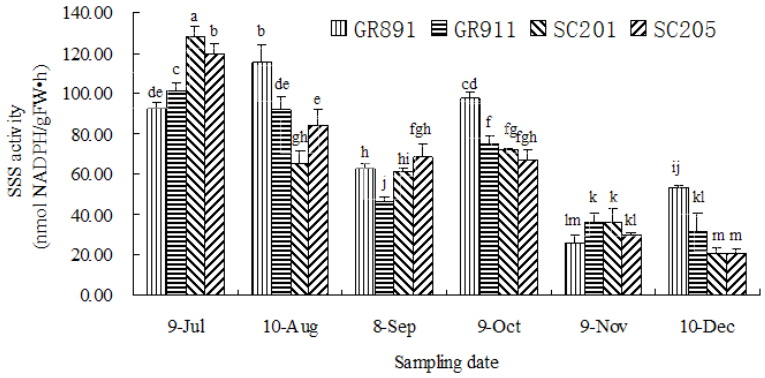

Figure 5. The time course of SSS activity of cassava root tuber

3.5 The Time Course of Cassava Root Tuber SBE Enzyme Activity

Cassava root tuber SBE is also involved in the biosynthesis of amylopectin, however it is mainly to modify the fine structure of amylopectin (Xia et al., 2011). Although the cassava root tuber SBE activity of four cultivars oscillated during the growth period, the range was not too much (Figure 6). Taking the amylopectin content of fresh weight continually increasing into consideration (Figure 8), it is postulated that SBE might not play an important role in the formation of amylopectin, but to its fine structure modification.

SSS is an important enzyme for starch biosynthesis, especially for amylopectin. SBE can further modify the structure of amylopectin. The enzyme activity change style of cassava root SSS (Figure 5) and SBE (Figure 6) were not conformed well to those of maize (Zuo et al., 2011) and wheat (Wang and Wang, 2004). The reason of this inconsistence is not known so far and worth to study further.

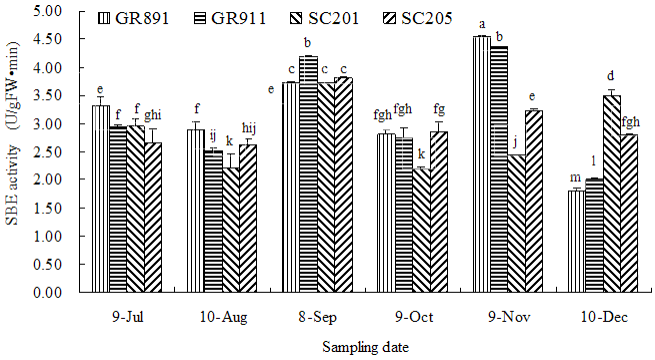

Figure 6. The time course of SBE activity of cassava root tuber

3.6 The Time Course of Cassava Root Tuber Starch

The amylose and amylopectin content of cassava root tuber were determined by dual wavelength spectrophotometry, respectively (Figure 7 and 8), and the total starch content of fresh weight was calculated (Figure 9). The amylose, amylopectin and total starch content of fresh cassava root tuber of four cultivars were continually increased along with the growth period (Figure 7, 8, 9). GR891 and SC205 were two cultivars which were known as high-starch content and planted widely in Guangxi. From our results, the amylose, amylopectin and total starch content of GR891 and SC205 were actually higher than those of GR911 and SC201, and GR891 was the highest among the four cultivars determined.

The amylose, amylopectin and total starch content of fresh cassava root tuber were all increased gradually along with the growth period (Figure 7, 8, 9). The correlation between the starch accumulation and the determined enzyme activities is not very well. As we know, the starch biosynthesis is very complex process, and it is influenced by many biofactors (Martin and Smith, 1995). Although, SuS, SPS, AGPase, SSS and SBE are important enzymes for starch biosynthesis, they are only part of the whole starch biosynthesis net.

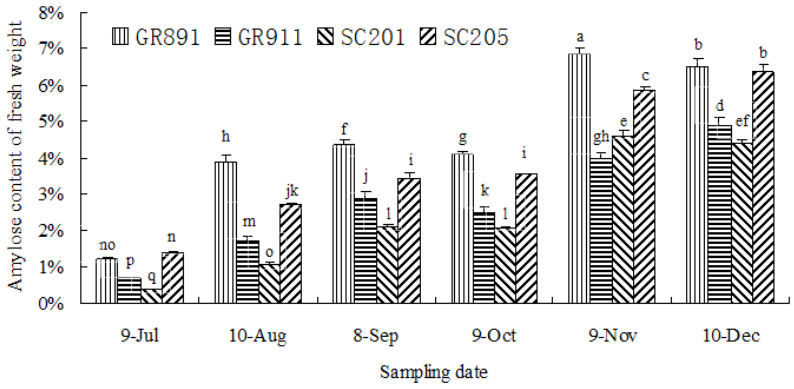

Figure 7. The time course of amylose content of fresh cassava root tuber

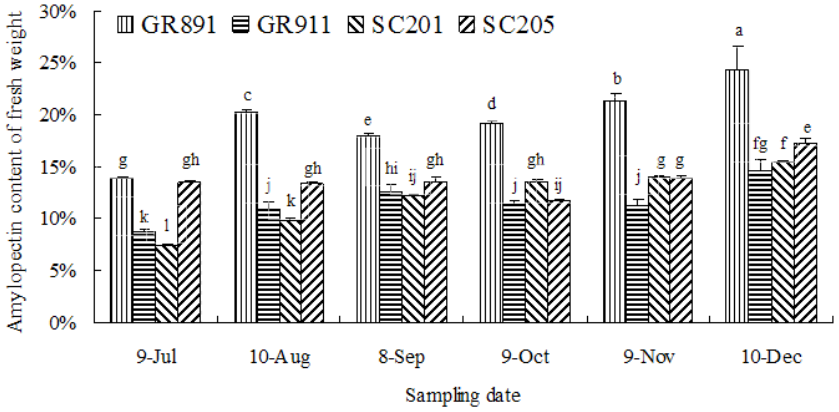

Figure 8. The time course of amylopectin content of fresh cassava root tuber

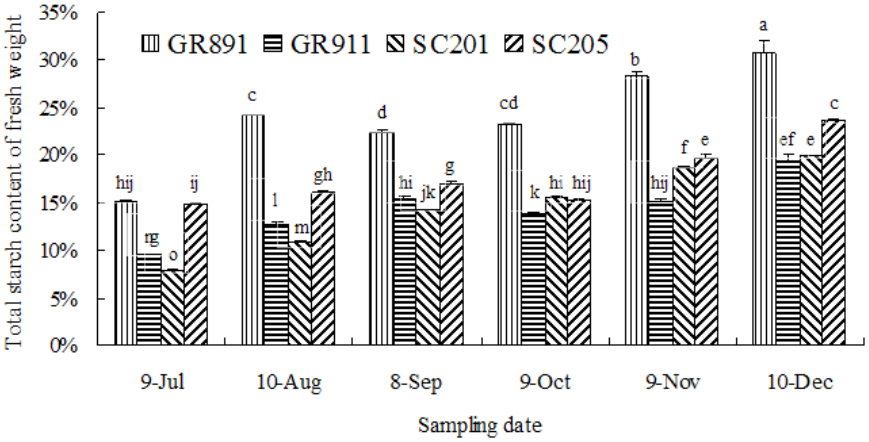

Figure 9. The time course of total starch content of fresh cassava root tuber

4. Conclusion

The time course of four cassava cultivars' root tuber starch content, and starch biosynthesis related enzymes of cassava leaves and root tuber were determined during their growth period. The trends of SuS decomposing direction, SuS synthesis direction, SPS, AGPase, SSS, SBE activities were different from each other; however the amylose, amylopectin and total starch content of cassava root tuber gradually increased along with the growth period. And the amylose, amylopectin and total starch content of GR891 and SC205, especially GR891 was higher than others. This result is consistent with that GR891 and SC205 are well-known high starch content cultivars. The changing trends of cassava root tuber starch accumulation and its related biosynthesis enzymes were becoming clear through this experiment.

Acknowledgments

This work was supported by Guangxi scientific research and technological development foundation (09203-03).

References

Balagopalan, C. (2002). *Cassava utilization in food, feed and industry*. In Cassava Biology, Production and Utilization. Oxford, New York: CABI Publishing, pp: 301–318. https://doi.org/10.1079/9780851995243.0301

Carvalho, L.J.C.B., de Souza, C.R.B., de Mattos Cascardo, J.C., Bloch, C., & Campos, L.

(2004). Identification and characterization of a novel cassava (*Manihot esculenta* Crantz) clone with high free sugar content and novel starch. *Plant Molecular Biology*, *56*, 643–659. https://doi.org/10.1007/s11103-004-4873-9

Cook, R., & Cock, J. (1989). Cassava crops up again. *New Scientist*, *122*, 63–68.

Huang, B.Q., Chen, J., Zhang, J.J., Liu, H.M., Tian, M.L., Gu, Y., Hu, Y.F., Li, Y.P., Liu, Y.H., & Huang, Y.B. (2011). Characterization of ADP-glucose pyrophosphorylase encoding genes in source and sink organs of maize. *Plant Molecular Biology Reporter*, *29*, 563–572. https://doi.org/10.1007/s11105-010-0262-5

Ihemere, U., Arias-Garzon, D., Lawrence, S., & Sayre, R. (2006). Genetic modification of cassava for enhanced starch production. *Plant Biotechnology Journal*, *4*, 453–465. https://doi.org/10.1111/j.1467-7652.2006.00195.x

Jin, Y. H., Zhang, K. L., Zhang, X. C., & Du, J. H. (2009). Determination of amylose and amylopectin in wheat and wheat malt by dual-wavelength spectrophotometry. *Journal of the Chinese Cereals and Oils Association*, *24*, 137-140.

Kang, G. Z., Liu, G. Q., Peng, X. Q., Wei, L. T., Wang, C. Y., Zhu, Y. J., Ma, Y., Jiang, Y. M., & Guo, T. C. (2013). Increasing the starch content and grain weight of common wheat by overexpression of the cytosolic AGPase large subunit gene. *Plant Physiology and Biochemistry*, *73*, 93-98. https://doi.org/10.1016/j.plaphy.2013.09.003

Li, T. G., Shen, B., Cheng, N., & Luo, Y.K. (1997). Effect of Q-enzyme on the chalkiness formation of rice grain. *Acta Agronomica Sinica*, *23*, 338–344.

Lutfiyya, L. L., Xu, N. F., D'Ordine, R. L., Morrell, J. A., Miller, P. W., & Duff, S. M. G. (2007). Phylogenetic and expression analysis of sucrose phosphate synthase isozymes in plants. *Journal of Plant Physiology*, *164*, 923-933. https://doi.org/10.1016/j.jplph.2006.04.014

Martin, C., & Smith, A. M. (1995). Starch Biosynthesis. *The Plant Cell*, *7*, 971–985. https://doi.org/10.1105/tpc.7.7.971

Nakamura, Y., Yuki, K., Park, S. Y., & Ohya, T. (l989). Carbohydrate metabolism in the developing endosperm of rice grains. *Plant Cell Physiology*, *30*, 833–839.

Osei, G., Arthur, R., Afrane, G., & Agyemang, E. O. (2013). Potential feedstocks for bioethanol production as a substitute for gasoline in Ghana. *Renewable Energy*, *55*, 12–17. https://doi.org/10.1016/j.renene.2012.12.012

OU-Lee, T. M., & Setter, T. L. (1985). Enzyme activities of starch and sucrose pathways and growth of apical and basal maize kernels. *Plant Physiology*, *79*, 848–851. https://doi.org/10.1104/pp.79.3.848

Pan, H., Luo, Y. C., Zheng, H., Yu, B. H., Lu, S. Q., & Li, J. (2014). Effects of different compound fertilizers on qualitycharacteristics and yield of cassava. *Journal of Southern Agriculture*, *44*, 2023-2026.

Pimentel, T. A. P. F., Durães, J. A., Drummond, A. L., Schlemmer, D., Falcão, R., & Sales,

M. J. A. (2007). Preparation and characterization of blends of recycled polystyrene with cassava starch. *Journal of Materials Science*, *42*, 7530–7536. https://doi.org/10.1007/s10853-007-1622-x

Poovaiah, C. R., Mazarei, M., Decker, S. R., Turner, G. B., Sykes, R. W., Davis, M. F., & Stewart, C. J. (2015). Transgenic switchgrass (*Panicum virgatum* L.) biomass is increased by overexpression of switchgrass sucrose synthase (*PvSUS1*). *Biotechnology Journal*, *10*, 552–563. https://doi.org/10.1002/biot.201400499

Tan, C. X., Guo, J., Chen, J., Feng, C. N., Guo, W. S., Zhu, X. K., Li, C. Y., & Peng, Y. X. (2009). Study on gene expression involved in starch synthesis and activities of enzymes in wheat grain. *Jounal of Triticeae Crops*, *29*, 24–30.

Wang, F., & Wang, X. Z. (2004). Study on the dynamic changes of starch synthesis and their related enzymes in wheat. *Journal of Triticeae Crops*, *24*, 57–60.

Xia, H., Yandeau-Nelson, M., Thompson, D. B., & Guiltinan, M. J. (2011). Deficiency of maize starch-branching enzyme I results in altered starch fine structure, decreased digestibility and reduced coleoptile growth during germination. *BMC Plant Biology*, *11*, 95–108. https://doi.org/10.1186/1471-2229-11-95

Yu, J. X. (1999). The determination of sucrose synthase and sucrose phosphate synthase activities. In The experimental guide of modern plant physiology, edited by Fan SQ and Liang SW, published by Chinese scientific publication corporation, pp126.

Zhang, X. L., Szydlowski, N., Delvallé, D., D'Hulst, C., James, M. G., & Myers, A. M. (2008). Overlapping functions of the starch synthases SSII and SSIII in amylopectin biosynthesis in *Arabidopsis*. *BMC Plant Biology*, *8*, 96–114. https://doi.org/10.1186/1471-2229-8-96

Zhao, Y., Wei, Z. M., & Ma, F. M. (2003). Influence of ammoniacal nitrogen on sucrose synthase and sucrose phosphate synthase in sugar beet. *Sugar Crops of China*, 1–5.

Zheng, Y., Anderson, S., Zhang, Y. F., & Garavito, R. M. (2011). The structure of sucrose synthase-1 from *Arabidopsis thaliana* and its functional implications. *Journal of Biological Chemistry*, *86*, 36108–36118. https://doi.org/10.1074/jbc.M111.275974

Zuo, Z. P., Tian, F. L., Jiang, P., Wang, J., Ma, D. C., Ma, Q., Li, D., Sun, Q. Q., & Dong, S. T. (2011). Comparison of kernel starch accumulation and related enzyme activities among six maize cultivars of different yield types. *Acta Agronomica Sinica*, *37*, 529–536. https://doi.org/10.3724/SP.J.1006.2011.00529

Copyright Disclaimer