Relationships between Leaf and Stem Soluble Sugar Content and Tuberous Root Starch Accumulation in Cassava

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Abstract

The purpose of this study was to examine the relationships between the soluble sugar content in the leaves and stems and the starch accumulation in the tuberous roots of four cassava cultivars: FuXuan01, GR891, SC124, and SC201. The results showed that the highest leaf soluble sugar content was in September, and the highest stem sugar content was in October, which played an important role in starch accumulation in the tuberous roots. The leaf and stem soluble sugar content and the tuberous root starch accumulation were positively correlated in the setting and growth stages but negatively correlated in the ripening stage. The soluble sugar content in the leaves went from high to low as the tuberous roots progressed from the setting stage to the ripening stage; starch accumulation in the root tubers followed the opposite pattern.

Keywords: Cassava, Leaves, Soluble sugar contents, Starch accumulation, Stems

1. Introduction

Cassava is a woody shrub that is extensively cultivated as an annual starch crop for its edible, tuberous root. One of the primary goals in cassava cultivation and breeding has been to obtain rich starch content in the root (Xing-lu 2001, Songhai 1994, Qiu-ying2000). Cassava has a long growth period, and cassava yielding and starch accumulation occur with the late stage of breeding. Studying the physiological mechanism of cassava starch accumulation in its late breeding stage and utilizing the most appropriate methods to increase starch accumulation is an effective way to obtain high starch content in the tapioca root. Shu-li *et al.* (2005) previously reported that in potato, rice, barley, wheat, corn, and sweet potato, starch accumulation relies largely on the synthesis, translocation and accumulation of the internal soluble sugar (Shu-li et al 2005). Further, it has been demonstrated that the soluble sugar in leaves also had an effect on the starch accumulation in the tuberous root (Xing-lu2006). Previous research has shown that the change of the soluble sugar content in wheat leaves and stems was correlated with the starch accumulation in the seeds and the trend was that increasing of soluble sugar content in leaves and stems in early stage and decreasing in late stage was good for starch accumulation in the seeds. (Jiang Dong2001, You-jun 2005, Jian-min2008, Bao-ren2007, Jin-zhi2008, Xiao-jie2008, Chun-yan2007, Hai-ying,2006, Xue-li2005, Chen-yang2005, WANG Jue2008, Wen-jing2007, Zhong-min2007, Xu-dong2003, etc). It has been reported that soluble sugar content in rice leaves is closely related to starch accumulation in the grain (ZHANG Jun2008, WANG Wei2006, Yan-fang2006), yet such relationships within the cassava plant have not been investigated, either in China or elsewhere. Therefore, the present study investigated the relationship between the change in soluble sugar in cassava leaves, stems and tuberous roots and the starch accumulation in tuberous roots under open field cultivation conditions, which provides a theoretical basis for physiological control in the breeding and the cultivation of high starch content and high yield cassava cultivars.

2. Materials and Methods

2.1 Trial cassava cultivars

The cassava cultivars for trial included two high-starch cassava cultivars (Fuxuan 01 and GR891) and two low-starch cultivars (SC124 and SC201).

2.2 Test design

The experiment was conducted in a mid-soil fertility sample plot at the teaching and scientific research base of the Agricultural College of Guangxi University. A randomized block design was utilized. Each block was repeated three times, and the area of each block was 42 m^2 with a planting specification of 80×80 cm. The

cultivars were planted in April 2007 and harvested in mid-January 2008, and the cultivation management was the same as that of normal open field cultivation.

2.3 Measured parameters and methods

2.3.1 Determining reducing sugar content

Reducing sugar content was measured using a method with 3,5-dinitrosalicylic acid (DNS) described in Analysis Techniques for Grain Quality of Cereals and Oils by Zhaofan (1985). Briefly, a 0.3-0.5 g dried sample (shattered, fineness: passing through 40-mesh) was weighed in a triangular flask. Twenty milliliters of distilled water was added, and the sample was heated in a 90-99℃ water bath for 20 min, then cooled down to room temperature. The extracted solution was transferred to a flask and mixed with distilled water to a total volume of 50 mL. The solution was filtered, and 1 mL of DNS reagent per 1 mL of the filtered solution was added. The mixture was heated in a boiling water bath for 5 min and then cooled down with tap water. Eight milliliters of distilled water was added, and the solution was mixed well. The absorbency at 520 nm wavelength was measured.

Content of reducing sugar (%) = $[(A \times 50)/(1 \times W \times 10^3)] \times 100 = 5A/W$

Where: A— reducing sugar content in the sample solution to be measured obtained by referring to the standard curve (mg)

W—weight of the sample (g)

50—total volume of the sample solution (mL)

1—volume of the sample solution to be measured (mL)

10^3—milligram converted from gram

100—converted percentage composition

2.3.2 Determining sucrose content

To measure sucrose content, the anthrone colorimetric method described in the Experimental Handbook of Plant Physiology by Shanghai Society for Plant Physiology (1985) was applied. Briefly, 0.1 g of dried sample (shattered, fineness: passing through 100-mesh) was weighed in a 10 mL centrifuge tube, and 6-7 mL of 80% ethanol was added. The sample was heated in an 80℃ water bath for 30 min and centrifuged (3000r/min) for 5 min. The supernatant was collected and the extraction was repeated twice (3000r/min for 10 min each). Supernatant was collected into a flask and 80% ethanol was added to a final volume of 50 mL. Then 1 mL of the extracted solution was added to a test tube and heated in a boiling water bath until it condensed to 0.05-0.1 mL (not greater than 0.1 mL to avoid longer color developing time). Next, 0.1 mL of 30% KOH was added and the solution was incubated in boiling water for 10 min. After the solution cooled down to room temperature, 3 mL of anthrone reagent was added, and the solution was incubated at 40℃ for 10-15 min. The absorbency at 620 nm wavelength was measured.

Sucrose content (%) = $\{[C \times (V/a)]/(W \times 10^6)\} \times 100$

where: C—glucose content obtained by referring to the standard curve (μg)

V—total volume of the extracted solution (mL)

a—volume of sample solution for color developing (mL)

W—weight of sample (g)

2.3.3 Total soluble sugar content measurement

To measure total soluble sugar content, the anthrone colorimetric method described in Research Method in Crop Physiology by Xianzheng (1992) was applied. One-tenth of a gram dried sample (shattered, fineness: passing through 100-mesh) was weighed in a 10 mL centrifuge tube, to which 6-7 mL of 80% ethanol was added. The sample was heated in an 80℃ water bath for 30 min, then centrifuged (3000r/min) for 5 min. The supernatant was collected, and the extraction was repeated twice (3000r/min for 10mins each). The supernatant was collected into a flask, and 80% ethanol was added to total volume of 50 mL. Then, 1 mL of solution was taken, and 1.5 mL of water was added, followed by 6.5 mL of anthrone reagent. The sample was mixed and incubated at room temperature (18-30℃) for 15 min to allow color developing. The absorbency at 620 nm wavelength was measured after the sample was cooled down.

Content of total soluble sugar (%) = $\{[C \times (V/a)]/(W \times 10^6)\} \times 100$

Where: C—glucose content obtained by referring to the standard curve (μg)

V—total volume of the extracted solution (mL)

a—volume of sample solution for color developing (mL)

W—weight of sample (g)

2.3.4 Determining starch content in tuberous roots

The residual sugar content measurement was rinsed with 20－30 mL distilled water in a 50 mL flask. The solution was gelatinized in boiling water for 15 min and cooled down. Two milliliters of cold 9.2mol/L perchloric acid was added, and the mixture was gelatinized again in boiling water for 15 min. Distilled water was added to a final volume of 50 mL, and the solution was mixed. Five mL of anthrone reagent was added to 2 mL of the supernatant. The solution was boiled for 10 min. The absorbency at 620 nm wavelength was measured after cooling.

Starch content (%) = $\{[C\times(V/a) \times 0.9]/ (W\times 106)\} \times 100$ where: 0.9-the starch coefficient from glucose conversion;

C- glucose value (μg) obtained by referring to the standard curve;

V—total volume of the extracted solution (mL)

a—volume of sample solution for color developing (mL)

W—weight of sample (g)

3. Results and Analysis

3.1 Changes in total soluble sugar content in different parts of various cassava cultivars

Soluble sugars, including monosaccharides and oligosaccharides, are the main photosynthetic product and the main substrate for metabolism (Oscar G.,2010). Carbohydrate content accounts for 90-95% of the dry matter in a plant. Soluble sugar is the major form of carbohydrate that can be reciprocally transformed and reused by plants. The high content of soluble sugar in cassava leaves facilitates the translocation of photosynthetic products from the stem to the tuberous root as well as the nutrient accumulation in the root. This is one of the reasons for the decreased content of total soluble sugar in the reproductive growth period of cassava.

There were no major differences in the development trend of soluble sugar in the leaves of different cultivars (Fig.1). The soluble sugar content in cassava leaves increased rapidly from August to September, then dropped. The content of soluble sugar after October was much lower in Fuxuan 01 than in other cultivars.

The change in soluble sugar content in cassava stems developed in a single-peak curve (Fig. 2). The soluble sugar content of Fuxuan 01, GR891 and SC124 reached their peaks in October while that of SC201 was highest in November and then dropped afterwards. The soluble sugar content of the high-starch cultivar GR891 was much lower than that of other cultivars.

Additionally, the change in the content of soluble sugar of tuberous roots had two wave peaks during the measuring period, one of which occurred in September and the other in November (Fig.3). The soluble starch content in high-starch cultivars was significantly lower than those of the low-starch cultivars, indicating that the high-starch cultivars had better photosynthesis ability than the low-starch cultivars and ensuring the forming of starch in the tuberous roots.

3.2 Changes in sucrose content in different parts of different cassava cultivars

Sucrose is the major form of saccharide for long distance transportation in the sieve tubes of higher plants. By comparing the soluble sugar and sucrose contents in the cassava leaves and stems, it is apparent that the changes of sucrose contents and soluble sugar contents in the leaves and stems of different cassava cultivars maintained similar trend lines (Fig. 4 and Fig. 5). Specifically, the sucrose contents in the tuberous roots of various cassava cultivars was decreased in August and September and rapidly bounced back until reaching the peak in October-November followed by a quick drop（Fig. 6). During the recovery, the sucrose content in low-starch cultivars was significantly higher than that of the high-starch cultivars, indicating a quicker starch accumulation rate in the latter. This resulted in a continuous transformation of reducing sugar from sucrose.

3.3 Changes in reducing sugar contents in different parts of different cassava cultivars

All monosaccharides and most disaccharides and oligosaccharides have reducing properties and can become reducing sugars. Reducing sugar is the major soluble sugar in leaves, accounting for over 74% of the total sugar. Cassava leaves contained the richest reducing sugar content followed by the stems and the tuberous roots, and the reducing sugar content in the tuberous roots of high-starch cultivars was lower than those of the low-starch

cultivars. The contents of the reducing sugar in the stems and tuberous roots of different cultivars were decreased during the measured period (Fig. 7-9). This may be due to the enhanced ability to utilize and transform the reducing sugar, along with the increased starch content in the tuberous roots throughout the growth period.

3.4 Changes in starch contents and accumulation rates in tuberous roots of different cassava cultivars

The root is the main harvest product of cassava, and its essential component is starch. The starch content of different cassava cultivars varies depending on growth periods. This is an indicator of the differences in their individual photosynthetic rates, as well as the ability of carrying, transporting and unloading starch in the tissue pools. Further, it also indicates that there are differences in the ability to transform sugar into starch in the tuberous roots. The starch content of different cassava cultivars increased gradually, among which GR891 contained the richest starch (Table 1). Analysis results showed that the differences in starch contents in tuberous roots between high-starch and low-starch cassava cultivars during this measured period reached significant levels.

4. Discussion

Previous findings showed that the starch accumulation in the tuberous roots had a positive correlation with the soluble sugar content in cassava leaves and a negative correlation with the reducing sugar content in leaves as well as the sucrose contents in leaves (LUO Xing-lu, 2002). It has also been shown that high-yield crops required not only strong carbon assimilation in leaves but also effective photosynthetic product transportation and distribution from leaves to the pool end. Cassava is a $C_3 - C_4$ intermediate plant with very high photosynthetic efficiency(EL-Sharkawy et al,1990). Photosynthesis produces sucrose in the leaves. The soluble sugar content in the leaves of both high and low starch cassava cultivars is the highest in the whole plant, indicating that the source leaves are of very strong capacity for timely transport of nutrients, which facilitates the transport of sugar to the tuberous root pools so as to provide sufficient substrate supply for starch synthesis.

Jiang Dong et al. (2001) and Youjun et al. (2005) reported studies on the changes in soluble sugar contents in wheat canes, Yet no studies on the relationship between the change in soluble sugar in cassava cane and starch accumulation in the tuberous root have been reported. The present study was conducted to investigate the soluble sugar content in the stems of different cassava cultivars. Results showed the contents of soluble sugar in stems of different cassava cultivars presented single-peak curves characterized by a rising trend in the early stage. The peaks of soluble sugar contents of the sampled Fuxuan 01, SC124 and SC121occured in September while that of the high-starch GR891 cultivar occurred in October, which was the lowest content among all cultivars. This indicates that there is a difference in the soluble sugar contents in different tissues and cassava cultivars.

Soluble sugar is the substrate for starch synthesis and there is a close relationship between the soluble sugar content and starch synthesis in plants. Previous studies have demonstrated this phenomenon. It has long been maintained that there was a relationship between starch synthesis and soluble sugar content in plants, however, such a relationship is complicated and not well understood SHEN Songhai et al,1994; Binbin Lu et al,2005). Results hereby show that, in general, the high-starch cassava cultivars Fuxuan 01 and GR891 contained lower soluble sugar in tuberous roots than that in the low-starch cassava cultivars SC124 and SC201, indicating that the former cultivars had a better pool-source relationship,. Therefore, the high-starch cultivars had more starch transformation from soluble sugar, which leads to lower content of soluble sugar in the tuberous roots.

Reducing sugar and sucrose are the two main soluble sugars in cassava, of which the former is the immediate product of photosynthesis as well as the prerequisite substance for sucrose synthesis, while and the latter is the major form of organics in internal transport. During the first stage of the later growth period (October 19 - November 19), as the cassava cultivars continued to grow, the sucrose content in leaves increased while the reducing sugar content decreased; they both dropped in the stems. During the second stage (November 19 - December 19), the sucrose content in the leaves dropped while the reducing sugar content relatively increased; the sucrose content in stems decreased while the reducing sugar content increased except for that of the high-starch cultivar Fuxuan 01. This pattern suggests that leaves transform reducing sugar into sucrose rapidly during the first stage and transport it to the stems and roots for starch synthesis, leading to the decrease of sucrose in leaves during the second stage. Due to weak starch synthesis ability in the tuberous roots, the sucrose to be transported to the roots also decreased during the second stage (harvest). As a result, the rate of transformation from reducing sugar into sucrose in the leaves decreased, which explains the relative increase reducing sugar in leaves during the later stage. Therefore, maintaining a continual sucrose supply in the tuberous roots is a prerequisite for high starch accumulation in the roots. In conclusion, enhancing the photosynthetic ability in leaves, as well as the distribution and storage ability of photosynthesis products in the nutritive organ may be a most effective way to increase starch yield in the roots of cassava.

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Table 1. The starch accumulating in the tuberous roots of different cassava cultivars (%)

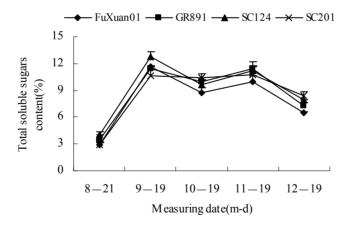| Cultivar	June19th	July19th	Aug. 19th	Setp. 19th	Oct. 19 th	Harvest period
FX01	12. 9	14. 5	15. 8	20. 8	27. 9	31. 9
GR891	13. 5	14. 9	16. 5	21. 3	28. 4	33. 8
SC124	12. 6	13. 9	15. 4	18. 4	23. 9	26. 2
SC201	12. 3	13. 2	15. 1	18. 3	23. 2	25. 8

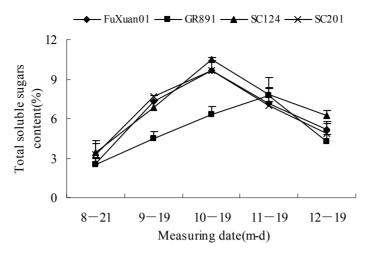Figure 1. The changes of soluble sugar in the leaves of different cassava cultivars

Figure 2. The change of soluble sugar contents in cassava stems of different cassava cultivars
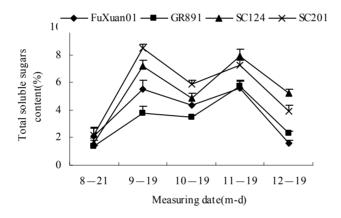

Figure 3. The change of the content of soluble sugar of tuberous roots of different cassava cultivars
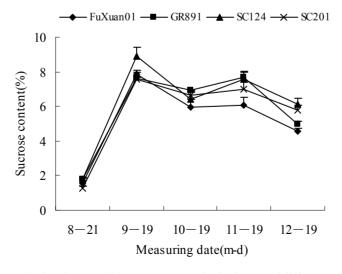

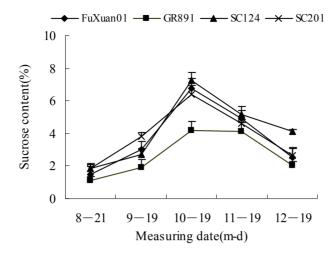Figure 4. The changes of sucrose contents in the leaves of different cassava cultivars

Figure 5. The changes of sucrose contents in the stems of different cassava cultivars
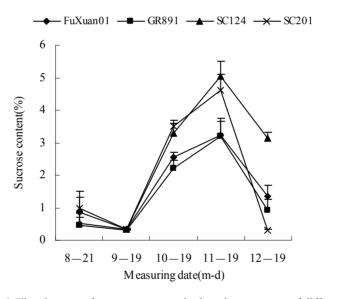

Figure 6. The changes of sucrose contents in the tuberous roots of different cassava cultivars
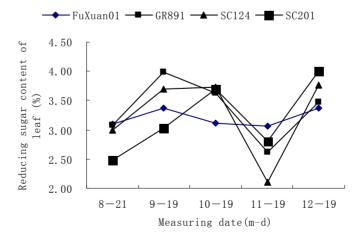

Figure 7. The changes of reducing sugar content in the leaves of different cassava cultivars
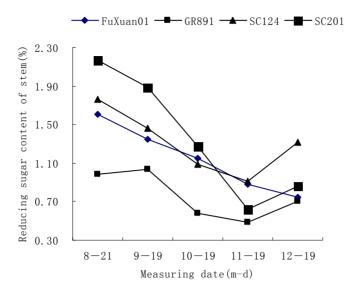
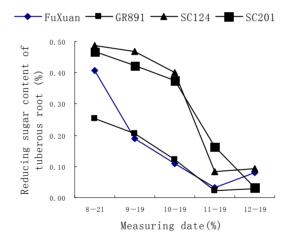Figure 8. The changes of reducing sugar content in the stems of different cassava cultivars

Figure 9. The changes of reducing sugar content in the tuberous roots of different cassava cultivars