The influence of soybean new blade's sugar content variation to its apparent growth

Yiqiong Wu[1], and Yun Gong[2,*]

[1]School of Landscape Architecture, Zhejiang A&F University, Lin'an, China
[2]School of Engineering, Zhejiang A&F University, Lin'an, China

*Corresponding author e-mail: gongyun204@163.com

Abstract. To a certain extent, leaf sugar content shows the plant growth status. At the beginning of the rapid growth of the seedlings of soybean, internal physiological indexes, such as the correlation between sugar content and external apparent growth is not very clear. This experiment measured the soybean seedling stage in different gradient of the soluble sugar content and the correlation of the apparent growth indicators, including leaf weight, leaf width, leaf length, root length, number of radicle, etc. The correlation between soluble sugar content and the apparent growth studies to preliminary discussion on this issue is analyzed. The data shows that in the seedling stage, leaf soluble sugar content increase quickly, and the apparent growth has different correlation with indicators. The weight of leaf has a intimate relativity with soluble sugar content, but leaf width and length have weaker correlation with it. And the root length and number of young root show little correlation with soluble sugar content. Experiment results indicate that in soybean seedling stage, internal physiological indexes do have a specific relationship with external growth index in some degree.

1. Introduction

Glycine max (L.) Merr is generally called soybean, is an annual leguminous herb attributes to bean. What's more, it is one of the important food crops in China which has a five thousand years of cultivation history and it was called SHU in ancient times [1]. Soybean is one kind of comprehensive nutrient food, and compared with the same amount of pork, the protein content, and calcium content, iron content of soybean is 1 times, 33 times, 26 times more respectively, while the price is lower than pork. Soybean protein contains the all kinds of amino acids needed in the body, especially the essential amino acids, such as lysine, leucine, and threonine. Only the content of methionine is relatively few [2]. This is the opposite of general cereal. Therefore, soybean mixed with other grain to eat can complement each other and greatly improve the nutritional value of them. Soybean contains a lot of fat which are unsaturated fatty acids, especially the content of linolenic acid which plays a great role to prevent atherosclerosis is found most abundantly. Soybean also contains about 1.5% of phospholipids which are the basal components of cells and play an important role in maintaining the health of nerves, liver, bones and skin.

All aspects of agricultural production of soybean are paid great attention to because of high nutritional value of it. At present, the research of soybean at home and abroad mainly focuses on the

cultivation and transgenosis [2, 3]. There are some reports about the relationship between the internal physiological indexes and the external growth index. For instance, studies on the correlation between the sugar content of the beetroot and the growth amount of the plant have been carried out [4]. In addition, the changing trend of sugar contents during the growth of the soybean seedlings is preliminarily discussed [5]. These research results help us to understand the changes of some important nutrients during the growth of plants[6, 7]. However, these studies were mainly focused on a single index, and there was no report on the correlation between the apparent growth and the sugar content in the early stage of the development of soybean seedling leaves. The sugar content and growth of soybean leaves were measured at different time in this study, including leaf length, leaf weight, leaf width, plant height, and root length and so on. The main research methods of this thesis are documentation method and experimental method [8]. The discussion on the relationship between the sugar content and the apparent growth during the growth of soybean was based on a mathematical model which was established for the dynamic change of sugar content and its apparent growth in different time periods.

2. Expriment

2.1. Experiment Reagents and Instruments

723 Visible Spectrophotometers (Shanghai optical instrument factory), Electronic Analytical Balance (Beijing Jingke RitaTechnologies co., ltd), Thermostatic Water Bath (M344133, Beijing ZhongXi Yuanda Technology Co., Ltd.). Phenol Solution, Trichloroacetic acid (TCA), concentrated sulphuric acid, Hoagland nutrient solution, ether, acetone, anhydrous ethanol and other reagents are all analytical pure, purchased from Sinopharm Chemical Reagent Co., ltd. "Kenfeng series" soybean seeds purchased from Heilongjiang Province Academy of Agricultural Sciences and Crop Research Institute.

2.2. Experimental Procedure

2.2.1. Cultivation of soybean seedlings The cultivation methods of soybean can refer to the previous literature reports [9]. Buy "Kenfeng series" soybean seeds from seed company, disinfect with mercury solution (10 mL 0.1%) for 10 minutes, and then filter the mercury solution, wash soybean seeds with running water for half an hour, rinse with distilled water for three times. The seeds should be evenly placed in a Petri dish covered with two layers of wet filter paper, and then put into constant temperature incubator after covering. Accelerating germination for 48 h under the dark condition of 25 ℃, then select seeds of same germination to sow in plastic basin nylon net, use hoagland's nutrient solution to culture, the culture temperature is 25-28 ℃. Shine a light on them for 12 hours a day, transfer to the plastic basin (12 cm in diameter) when seedlings grow to the stage of seedling with one leaf. Single basin area (about 200 plants per pot). The culture solution should be changed every two days, then sample and measure each index after a week.

2.2.2. Determination of total soluble sugar content and its standard curve soluble sugars mainly includes soluble monosaccharides and oligosaccharides which are soluble in water and ethanol. The sugar is dehydrated to produce furfural or hydroxymethyl furfural under the action of concentrated sulfuric acid, which can react with phenol to form an orange red compound. In the range of 10 to 100 mg, the color depth is directly proportional to the sugar content, and has a maximum absorption peak at the wavelength of 490 nm, so the total sugar content can be determined by colorimetry at the wavelength of 490 nm. Sulfuric acid - phenol method can be used for the determination of methylated sugar, pentose and polysaccharide, this method is simple and sensitive, and the color stability time is above 160 min. The measured data is absorbance, and then use the standard curve method, the sugar content can be obtained through the standard curve. Prepare a series of standard solutions of different concentrations, measure the absorbance of the standard solution respectively. Then, the standard curve

can be drawn, with the concentration as abscissa and the corresponding absorbance as ordinate. Measure the absorbance of the solution under exactly the same conditions, and obtain the solution concentration from the standard curve. Take six test tubes (20ml), numbered from 0-6, add solution and water according to table 1, then add phenol solution (1 mL 6％) to the test tube in sequence and shake well. Add 5 mL of concentrated sulfuric acid from test tube walls in 5-20 s time and shake well. The total volume of the color standard solution is 8 mL, place 30 min at room temperature, cooling and the color development reaction will happen. Then take the blank as the reference, colorimetric determination at 490 nm wavelength, take the sugar content as the abscissa, the light density as the ordinate, draws the standard curve, the standard line equation can be obtained.

Table 1 sugar content of glucose standard solution (ug)

tube number	1	2	3	4	5	6
glucose standard solu-tion (mL)	0.0	0.8	1.0	1.2	1.4	1.6
distilled water (mL)	2.0	1.2	1.0	0.8	0.6	0.4
sugar content (g)	0	40	50	60	70	80

2.2.3. Determination of apparent growth index The experiment will use vernier caliper to measure the leaf length, width and root length; and will use analytical balance to record the weight of young leaves and the number of radicles (Recording time from the stage of seedling with one leaf, take 5, 7, 9, 11, 13 and 15 days for the time gradient).

2.2.4. Determination of total soluble sugar content The method for determination of total soluble sugar is based on the experimental methods reported previously [13, 14], but in the specific operation process, we improved some experimental parameters, and achieved the desired results.

1) By taking new leaves of soybean, grinding, centrifugation, speed 3000 r/min, a total of three times. The first time was 15 minutes, take the supernate. The latter two was about 5 mins respectively, the supernatant was taken into the cone type colorimetric tube (25 mL). The final filtrate kept around 18 mL.

2) Constant volume sampling after 2 hours of water bath. After the bath, added 2 mL 6 mol/L sodium hydroxide and shaked well after cooling with running water. Constant-volumed with a 25 mL volumetric flask. Draw 0.2 mL of sample solution and add distilled water to make a total volume of two mL. Then add 1 mL 6% of phenol and 5 mL of concentrated sulfuric acid, shake well and cool to room temperature for 20 minutes. Use the spectrophotometer to measure the absorbance at 490 nm wavelength. Each measurement should take dual control, the content of polysaccharide was calculated by standard curve.

3. Results and discussion

3.1. Standard linear equation

Soluble sugar content X=(C×D)

X - total sugar content in each sample

C - the sugar content that shows on the standard curve (g)

D -dilution ratio

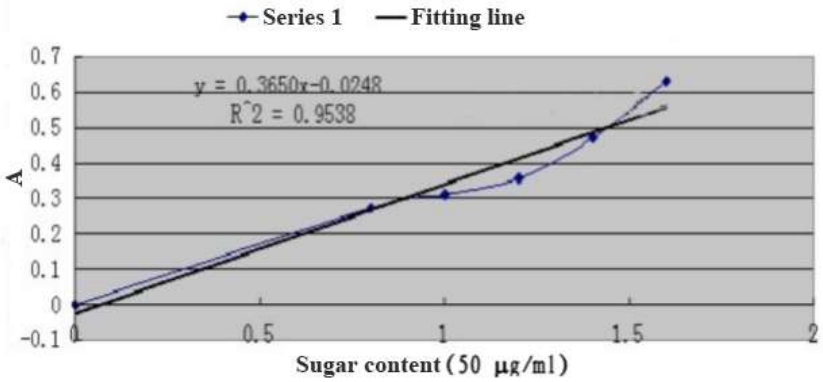

Figure 1 standard linear equation

3.2. The changing characteristics of the growth amount of the newly-growing soybean leaves

Table 2 Data summary table of growth amount

Days (d)	5	7	9	11	13	15
Leaf length (CM)	0.99	1.46	2.31	3.01	3.48	3.50
Leaf width (CM)	0.62	0.92	1.79	2.34	2.86	3.52
Leaf weight (g)	0.0183	0.0425	0.074	0.121	0.173	0.21
Root length (CM)	4.84	5.49	5.51	10.10	7.12	8.20
The number of radicles (root)	15	16	29	45	34	36

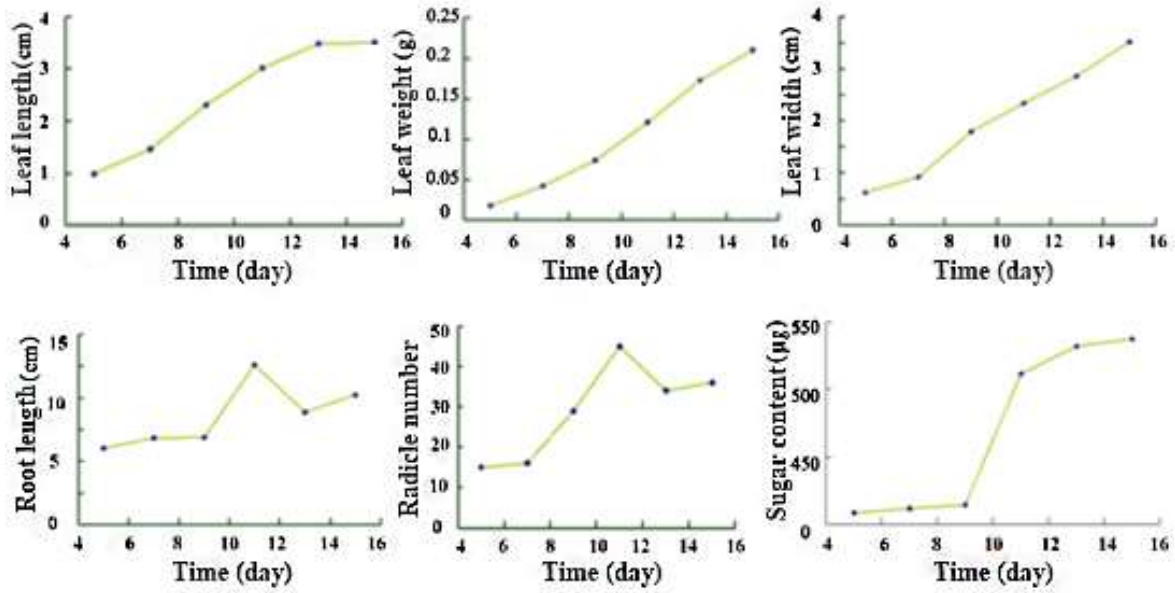

Figure 2 The dynamic changes of leaf width, leaf weight, leaf length, root length, the number of radicles and sugar content of leaves over time

3.3. Correlation between growth amount and sugar content

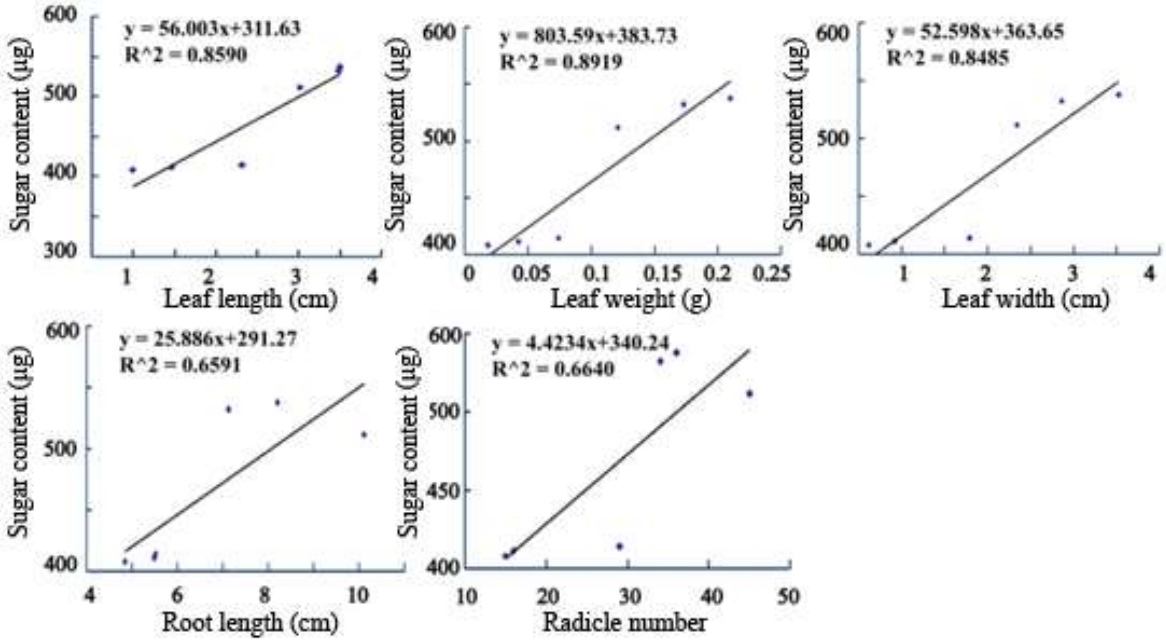

Figure 3 Correlation between the sugar content of the newly-growing soybean leaves and its leaf length, leaf weight, leaf width, root length and the number of radicles

3.4. Standard curve of total soluble sugar

According to the preparation scheme in the table 1, the standard curve (Figure 1) can be drawn by measuring the absorbance value at the wavelength of 490 nm, taking the sugar content as the abscissa and the absorbance as ordinate, and the equation is y = 0.3650x-0.0248. The standard equation provides an important basis for the determination of sugar content in different organs of soybean leaves.

3.5. Change rules of the growth amount of new soybean leaves

"Kenfeng series" of soybean seed began to grow to the phase of seedling with one leaf after five days, then we measured the soybean seedling leaf length, leaf weight, leaf width, root length, the number of radicles and the sugar content of leaves from the fifth day. It can be seen from Figure 2 that the leaf length, leaf weight, leaf width were steadily increasing in the 5-15 days, only the increment speed of leaf length noticeably slowed down, basically reached the platform, grow to 3.5 cm in 15 days. The leaf weight and leaf width had been going up all the time, which increased from 0.62 cm to 3.52 cm, and increased from 0.0183 g to 0.21 g respectively. The root length growed rapidly in 9-11 days, reached 10.10 cm, the number of radicles increased rapidly from the seventh day, and had been at a high level of quantity. The total sugar content did not change much from 5-9 days, however, the sugar content increased sharply from 9-11 days, and then flatten, the total sugar content increased from 408.3 g to 537.6 g within 15 days.

3.6. Correlation between growth amount and sugar content

We can see from Figure 3: The content of soluble sugar in the leaves of soybean seedlings increased within 15 days under normal conditions, increased from 408.3 g to 537.6 g while leaf length increased from cm to 3.5 cm, the leaf weight increased from 0.0183 g to 0.21 g, leaf width increased from 0.62 cm to 3.52 cm. The linear equation of soluble sugar content and leaf length, leaf width and leaf weight were obtained by linear fitting according to the experimental results: y=56.003x+331.61, R^2=0.8590; y=803.59x+383.73, R^2=0.8918; y=52.598x+363.65, R^2=0.8485. Analysed the equations and the

growth trend of total sugar and leaf length, leaf weight , leaf width can obtain: during the period of seedling, when the leaf length, leaf weight and leaf width increased, the sugar content in the leaves also increased over time, showed a positive correlation, and a strong correlation. During the 15 day test, the root length increased from 4.84 cm to 8.2 cm, the number of radicles also increased from 15 to 36. The linear equation of soluble sugar content and root length or the number of radicles can be obtained by linear fitting according to the experimental data: $y=25.886x+291.27$, $R^2=0.6591$; $y=4.4234x+340.27$, $R^2=0.6640$. Although with the increase of soluble sugar content,the root length and the number of radicles increased too, but it can be seen from the equation that the correlation between the sugar content and the root length or the number of radicles is very week.

4. Conclusion

The changes of apparent growth and total sugar content of soybean seedlings were measured in the early development process (5-15 days), and the relationship between the different apparent growth indexes and the total sugar content were linearly fitted. The results showed that with the increase of the leaf length, leaf weight and leaf weight, the sugar content in leaves also increased over time, and there is a linear relationship because of the large R square value. And for the root length or the number of radicles, the results of linear fitting with the total sugar content show that the R square value is small, illustrated that the root length and the number of radicles can account for the small change of total sugar content. Further experiments are needed, and other factors should be taken into account in future.

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