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RELATIONSHIP BETWEEN DRY MATTER CONTENTS AND METABOLIC ENZYMES DURING POTATO TUBER ENLARGEMENT

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Abstract

To explore the relationship between the dry matter contents of potato tubers and metabolic enzymes, an experiment was designed. Ten individual indicators, including metabolic enzymes, could be reduced to 4 comprehensive indicators by using a principal component analysis. Using these 4 components as independent variables (X) and the dry matter content as the dependent variable (Y), the following linear regression equation was obtained: $Y = -51.802 - 1.022X_5 - 0.034X_6 + 0.872X_9 + 0.286X_{10}$ ($R^2 = 0.889$, $P = 0.012$). In a comparison between the dry matter contents (22.74, 16.58 and 20.72%) calculated according to the equation and the measured value (22.96, 17.09 and 19.75%), the absolute error was < 1% and the estimation accuracy was > 95%. These results indicated that the linear regression equation can be used to predict accurately and quickly the dry matter contents during the tuber enlargement stage.

Introduction

Dry matter is an important indicator of the nutritional content and processing quality of potatoes. Improving the dry matter contents of tubers is an important objective for breeder varietal exploration (Chen and Chen 2015). The synthesis of carbohydrates (dry matter, starch, sugar and protein) is closely related to metabolic enzyme activities in plant during its fruit and seed development (Farrar *et al.* 2000). Metabolic enzyme levels are determined by the photosynthetic capacity of plants and the fruit sink strength (Ho 1988). To determine sink strength, the metabolic enzymes (important indicators) are determined by the level of sucrose metabolism in sink cells and its storage capacity (Sun *et al.* 1989, Farrar 1993). Sucrose synthase and starch synthase are key enzymes in the synthesis of carbohydrates in crop grains. In the grain-filling stage, sucrose synthase and starch synthase promote the synthesis of starch and other carbohydrates in grains, increasing grain weight and crop yield (Ian and Johannes 1994, Dong *et al.* 2003, Gao *et al.* 2003, Xu *et al.* 2015). On the other hand, during dry matter accumulation in peanut leaves verily shoots to sucrose phosphate and soluble sugar syntheses in promoting the rapid accumulation of dry matter in growing pods (Cui *et al.* 2010).

The accumulation of potato dry matter begins in the tuber formation stage, with the most rapid accumulation taking place in the tuber enlargement stage (Chen and Chen 2015) as a direct activity of leaf metabolic enzyme. In this study, leaf metabolic enzymes and tuber dry matter contents during the potato tuber enlargement stage were evaluated. In particular, a principal component analysis (PCA) and multivariate linear regression were used to refine metabolic enzyme information and to establish a mathematical model for dry matter content. The model provides a convenient and rapid method for determining the intrinsic relationship between the dry matter contents and metabolic enzymes; it also lays a theoretical foundation for early generation selection and cultivation physiology in potato breeding.

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Materials and Methods

In April 2018, three test materials of potato (lines 15013 and 15122 as well as a processing variety named Atlantic) were planted at the experimental base of Jilin Agricultural University, China. A RCBD was used with 3 replicates and row spacing of 0.25 m × 0.8 m.

A chlorophyll content analyzer (SPAD-502, Zhejiang Top Yunnong Technology Co. Ltd., Hangzhou, China) was used to determine the SPAD value for the third functional leaf under the growing point. After obtaining values, the leaves were removed and stored in liquid nitrogen at –80°C for metabolic enzyme determination. Tubers of the same plant were obtained to measure the dry matter contents.

To determine metabolic enzyme activity, 0.5 g of leaves was ground in liquid nitrogen. Then, 3 ml of 5 mmol/l PBS (pH 7.4) was added for homogenization. The resulting mixture was centrifuged at 4°C for 20 min (2,000 - 3,000 r/min), and the supernatant was used to determine metabolic enzyme activity according to the measurement and calculation methods specified in the enzyme kit (R&D, Shanghai Enzyme linked Biotechnology Co., Ltd., Shanghai, China).

Tubers of the same plant were obtained to measure the dry matter contents. To determine the dry matter contents, each tuber was rinsed with water, dried, and sliced. Portions of the slices were weighed, dried in an oven at 105°C for 30 min, and further dried at 80°C until a constant weight was obtained. Dry matter (%) = dry weight/fresh weight × 100.

Data compilation and multivariate statistical analyses were conducted using Microsoft Excel 2013, DPS9.5 and SPSS 19.0.

Results and Discussion

During potato growth, the application of calcium (Benhong *et al.* 2003) and phosphorus (Zhang and Tian 2007) were reported to increase the activity of sucrose synthase and sucrose phosphate synthase in leaves, which promotes the synthesis of soluble sugar and sucrose and increase the accumulation of starch and dry matter in tubers. The present results indicated that there are correlations between tubers contents and metabolic enzymes. For example, soluble sugar was significantly correlated with sucrose synthase and nitrate reductase ($p < 0.05$) and with glutamine synthetase ($p < 0.01$). Soluble sugar is a substrate for dry matter accumulation and its quantity is closely related to the dry matter content. During potato growth and development, SS, SPS, glutamine synthetase, and nitrate reductase catalyze the synthesis of soluble sugar (Tom *et al.* 1990, Xin 2008, Fleisher *et al.* 2013), promoting the rapid accumulation of dry matter.

PCA reduces the complexity of large amounts of data by a dimensionality reduction method while retaining most of the information (Miodrag 2011). A large number of indicators are converted into a smaller number of comprehensive evaluation indicators, thereby reducing the correlation between the original indicators and improving the reliability of evaluations (Zhou *et al.* 2003, Wang *et al.* 2011). The number of principal components (PCs) is determined according to Eigen values and the cumulative contribution of the extracted PCs; it is usually accepted that PCA Eigen vectors with Eigen values greater than 1 and a cumulative contribution of above 80-85% can be included as PCs (Schippers 1976). To ensure the accuracy of experimental data interpretation, in this study, PCs were included when Eigen values exceeded 0.5. Using this threshold, four PCs corresponding to four metabolic enzymes (X_5, X_6, X_9 and X_{10}) were retrieved, and these accounted for 51.51, 22.51, 11.67 and 7.13% of the total variance, respectively amounting to a cumulative contribution of 92.82% (Table 2). Based on an Eigen value curve analysis (Fig. 1), the four metabolic enzymes qualified as PCs in accordance with the inclusion criteria. Using the four metabolic enzymes as independent variables (X_5, X_6, X_9, and X_{10}) and the

Table 1. Correlation between potato dry matter content and metabolic enzymes.

Index	X	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
X	1	0.61	0.72*	0.47	0.48	0.82**	0.79*	0.41	0.60	0.74*	0.77*
X_1		1	0.57	0.52	0.44	0.11	0.68*	0.62*	0.72*	0.24	0.64
X_2			1	0.38	0.02	0.70*	0.84**	0.28	0.51	0.69*	0.47
X_3				1	0.30	–0.04	0.69*	0.37	0.32	0.10	0.61
X_4					1	0.08	0.38	0.59	0.42	0.31	0.32
X_5						1	0.52	0.10	0.40	0.95**	–0.24
X_6							1	0.50	0.60	0.69*	0.60
X_7								1	0.81**	0.29	0.72*
X_8									1	0.50	0.56
X_9										1	–0.03
X_{10}											1

X: Dry matter; X_1: Chlorophyll; X_2: Sucrose synthase; X_3: Sucrose phosphate synthase; X_4: Glutamate synthetase; X_5: Starch synthase; X_6: Nitrate reductase; X_7: Glutamine synthetase; X_8: Free amino acid; X_9: Soluble sugar; X_{10}: Soluble protein. $^{*}p < 0.05$; $^{**}p < 0.01$.

dry matter content of tubers as a dependent variable (*Y*), the regression equation $Y = –51.802 – 1.022X_5 – 0.034X_6 + 0.872X_9 + 0.286X_{10}$ was obtained, with $R^2 = 0.889$ and $P = 0.012$, indicating a satisfactory goodness of fit.

The dry matter content calculated using the regression equation was compared to the measured value. The absolute errors in the prediction were all less than 1% and in the range of 0.22 to 0.97% for different potato genotypes, with an estimation accuracy above 95% (Table 3). Moreover, the difference was significant (p = 0.00), as verified by a t test (Table 4), proving that

Table 2. PCA results for potato dry matter content and metabolic enzymes.

Items		CI_1	CI_2	CI_3	CI_4
Eigen value		5.15	2.25	1.17	0.71
Contribution %		51.51	22.51	11.67	7.13
Cumulative contribution %		51.51	74.02	85.69	92.82
Eigen vector	X_1	0.82	–0.29	–0.04	–0.17
	X_2	0.76	0.38	–0.44	–0.16
	X_3	0.63	–0.38	–0.41	0.44
	X_4	0.52	–0.22	0.63	0.48
	X_5	0.45	0.87	0.09	0.02
	X_6	0.92	0.16	–0.26	0.20
	X_7	0.76	–0.33	0.42	–0.20
	X_8	0.83	–0.02	0.29	–0.36
	X_9	0.63	0.75	0.14	0.12
	X_{10}	0.73	–0.61	–0.22	–0.12

X: Tuber; X_1: Chlorophyll; X_2: Sucrose synthase; X_3: Sucrose phosphate synthase; X_4: Glutamate synthetase; X_5: Starch synthase; X_6: Nitrate reductase; X_7: Glutamine synthetase; X_8: Free amino acid; X_9: Soluble sugar; X_{10}: Soluble protein. $^{*}p < 0.05$; $^{**}p < 0.01$.

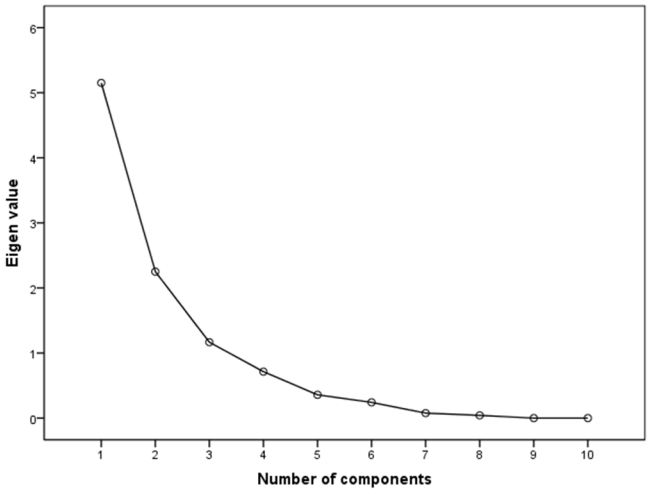

Fig. 1. A principle component analysis (PCA) of the refined metabolic enzymes.

the prediction results from the regression equation were reliable. This model-based method was similar to that developed by Dominique *et al.* (1987) for accurately predicting dry matter content in alfalfa. The reduction of the bias caused by the correlation between multiple indicators improves the accuracy (Miodrag 2011).

Table 3. Comparison of the potato dry matter content determined by the regression equation and the direct measurement.

Varieties	X_5	X_6	X_9	X_{10}	Calculated	Measured	Absolute error (%)	Estimation accuracy (%)
15013	11.56	127.06	50.32	164.39	22.74	22.96	0.22	99.04
15122	6.83	107.33	38.84	159.61	16.58	17.09	0.51	96.96
Atlantic	6.37	116.33	40.81	162.35	20.72	19.75	0.97	95.32

Table 4. Evaluation of the indirect method for the determination of the dry matter content.

Items	Mean	Standard deviation	Standard error	Confidence interval		t value	df	p
				Lower limit	Upper limit			
Absolute error								
Estimation accuracy	–96.55	1.94	0.65	–98.04	–95.05	–149.36	8	0.00

In a multivariate statistical analysis of leaf metabolic enzymes, starch synthase, nitrate reductase, soluble sugar, and soluble protein were included as independent variables and the dry matter content of tubers was used as the dependent variable to obtain the following regression equation: $Y = -51.802 - 1.022X_5 - 0.034X_6 + 0.872X_9 + 0.286X_{10}$ ($R^2 = 0.889$, $P = 0.012$). For three different potato genotypes, a standard error of <1% in the calculated dry matter content (22.74, 16.58 and 20.72%) relative to the measured dry matter content (22.96, 17.09 and 19.75%) was obtained, with an estimation accuracy above 95%. These relations indicated that the equation can be used for the accurate and rapid prediction of the dry matter content of potato, thereby providing a theoretical basis for potato breeding and cultivation.

Gao *et al.* 2003 and Dong *et al.* 2003 stated that dry matter of wheat was significantly correlated with its sucrose synthase, soluble sugar, soluble protein, and nitrate reductase ($p < 0.05$) and with starch synthase ($p < 0.01$). During the grain-filling stage, SS, ADPGPPase, SSS amylase and GBSS promoted starch accumulation and increased the dry matter content, which promoted amylose accumulation, further affecting wheat quality.

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