South African Journal of Botany 115 (2018) 199–207

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Comparative study on carbon–nitrogen metabolism and endogenous hormone contents in normal and overgrown sweetpotato

W. Duan [a,b,1], Q. Wang [a,b], H. Zhang [a,b], B. Xie [a,b], A. Li [a,b], F. Hou [a,b], S. Dong [a,b], B. Wang [a,b], Z. Qin [a,b], L. Zhang [b,c,*]

[a] Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan 250100, China
[b] Scientific Observation and Experimental Station of Tuber and Root Crops in Huang-Huai-Hai Region, Ministry of Agriculture, Jinan 250100, China
[c] Shandong Academy of Agricultural Sciences, Jinan 250100, China

ARTICLE INFO

Article history:
Received 10 October 2017
Received in revised form 17 November 2017
Accepted 23 November 2017
Available online xxxx

Edited by J Van Staden

Keywords:
Overgrowth
C–N metabolism
Endogenous hormone
Sweetpotato

ABSTRACT

Sweetpotato is easily overgrown because of improved production conditions and soil fertility. In this study, normal and overgrown sweetpotato plants were obtained from the long vine cultivar J18 (J18) and the short vine cultivar X32 (X32) in a field experiment with different nitrogen (N) treatments (0, 120, and 240 kg N ha^{-1}). The carbon (C)–N metabolism and endogenous hormone contents were compared between normal and overgrown sweetpotato plants. Results revealed that the total C content and C/N ratios in leaves of the overgrown plants were significantly lower than those of the normal plants at 75 days after planting (DAP) and 105 DAP. Also, the sucrose phosphate synthase (SPS) activities significantly decreased whereas the nitrate reductase (NR) and glutamine synthetase (GS) activities significantly increased. In the overgrown plants, the sucrose content in leaves significantly decreased at 75 DAP and 105 DAP, whereas the free amino acid and soluble protein contents significantly increased at 50 DAP, 75 DAP and 105 DAP. The indole-3-acetic acid (IAA) and zeatin riboside (ZR) contents in leaves of the overgrown plants were significantly higher than those of the normal plants at 50 DAP, 75 DAP and 105 DAP. The changing of IAA and ZR contents in the stem tips of different growth types showed similar treads to those in leaves. The abscisic acid (ABA) content in the stem tips of the overgrown plants at 75 DAP and 105 DAP was significantly lower than that of the normal plants. The ZR and ABA contents in the storage roots of the overgrown plants at 50 DAP, 75 DAP and 105 DAP were significantly lower than those of the normal plants. Compared to the normal plants, the overgrown plants exhibited more vigorous N metabolism in leaves. Meanwhile, the levels of endogenous hormones that promote cell elongation and division in the leaves and stem tips increased whereas those restraining stem tip elongation and promoting storage root enlargement decreased, which could promote leaf hypertrophy and stem tip extension at above-ground parts and restrain storage root enlargement at under-ground parts. Thus, source–sink growth became incoordinate and sweetpotato underwent overgrowth.

1. Introduction

Sweetpotato is not only an important food crop but also a good feed crop and industrial raw material. This crop is largely produced in China, whose planting area and total production rank first worldwide (Zhang et al., 2009). As such, the continuous stability and improvement of sweetpotato production are essential for the sustainable development of agriculture and related industries in China (Abegunde et al., 2013). Soil N content increases as production conditions and soil fertility are enhanced. With large applications of N fertilizer, sweetpotato is easily overgrown even in moderately fertile soil. In overgrown plants, the growth of stems and leaves is not in coordination with the enlargement of root storage, and root yield is significantly decreased (Chen et al., 2012). C–N metabolism is a fundamental process in crops, and changes in their growth period directly affect various processes, such as photosynthetic product formation and transformation and protein synthesis (Schlüter et al., 2012; Young and Allen, 2013). C and N contents and C/N ratio can be used as indices for the intensity of C–N metabolism in crops (Zhu et al., 2009; Wang et al., 2010; Gan et al., 2011). The coordination of C–N metabolism in functional leaves is also the basis for the coordination of the source–sink relationship and is closely related to production levels (Coruzzi and Bush, 2001; Martin et al., 2002; Sun et al., 2013a, 2013b). However, differences in the C–N metabolism of normal and overgrown sweetpotato plants have been rarely investigated. Endogenous hormones as substances that transmit information regulate the growth and development of crops by promoting, restraining, and altering physiological processes (Bari and Jones,

* Corresponding author at: Shandong Academy of Agricultural Sciences, No.202 Gongyebei Road, Jinan 250100, Shandong, China.
E-mail addresses: duanwenxue2010@163.com (W. Duan), zhanglm2016saas@163.com (L. Zhang).
[1] First author: Crop Research Institute, Shandong Academy of Agricultural Sciences, No. 202 Gongyebei Road, Jinan 250100, Shandong, China.

https://doi.org/10.1016/j.sajb.2017.11.016
0254-6299/

2009; Peleg and Blumwald, 2011; Zhang et al., 2016). For instance, endogenous hormone contents in leggy seedlings significantly differ from those in normal types. In leggy seedlings, increasing endogenous hormone in indole-3-acetic acid (IAA), gibberellic acid-3 (GA_3) and zeatin riboside (ZR) contents possibly promote cell elongation and division (Zhou, 2010). In sweetpotato, endogenous hormone in IAA, ZR, abscisic acid (ABA) and gibberellic acid (GA) contents are closely related to the formation and development of the source–sink organ and the regulation of its production (Liu et al., 2013; Wang et al., 2014; Hou et al., 2015). Nevertheless, the variation of endogenous hormone contents in different organs of normal and overgrown sweetpotato plants has been rarely described. In this study, normal and overgrown sweetpotato plants were cultivated through a field experiment by applying different levels of N fertilizer. The C and N contents and the C/N ratio in leaves, key products and enzymatic activities associated with C–N metabolism in leaves, and endogenous hormones in different organs were analyzed and compared between the normal and overgrown sweetpotato plants. Our study could help elucidate the underlying overgrowth mechanism and provided a basis for the development of techniques to control sweetpotato overgrowth and promote its high-yield cultivation.

2. Materials and methods

2.1. Experimental design

Field experiments were conducted at the experimental station (36°7′ N, 118°2′ E) of Shandong Academy of Agricultural Sciences, China, in 2014. The soil type was classified as light loam. The average annual temperature and rainfall in the study area are 13.0 °C and 746.8 mm, respectively, with 60%–70% of rainfall occurring in the summer from June to August. The top 20 cm soil layer in the experimental field before sowing contained 12.1 g kg^{-1} of organic matter, 1.0 g kg^{-1} of total N, 73.5 mg kg^{-1} of alkali-hydrolyzable N, 27.0 mg kg^{-1} of available phosphate, and 115.8 mg kg^{-1} of available potassium.

The long vine cultivar J18 and the short vine cultivar X32 were chosen for the experiment. Three N rates were applied: 0 (N0), 120 (N1), and 240 kg N ha^{-1} (N2). The types of the fertilizers included urea (contained 46.4% N), calcium triple superphosphate (contained 46% P_2O_5), and potassium sulfate (contained 50% K_2O). Fixed amounts of P_2O_5 (75 kg ha^{-1}) and K_2O (150 kg ha^{-1}) were added with varying amounts of N fertilizer as the basal fertilizer. All plants were planted on June 17, 2014 and were harvested on October 20, 2014. Each treatment was replicated thrice in plots measuring 27 m^2 in a completely randomized block design. Each plot was 6 m long, and the sweetpotato seedlings were spaced 75 cm apart within the rows. Thus, each plot consisted of 6 adjacent rows of 144 plants. The other cultivation managements were the same as those in a normal field.

2.2. Sampling methods

Six plants were randomly uprooted to collect storage roots and above-ground organs at 50, 75, and 105 days after planting. The main stems were determined, and the fourth and fifth functional leaves of the main stem were taken for dried and fresh samples. The dry samples were oven-dried at 75 °C to a constant weight and then preserved in a desiccator to determine the sucrose and free amino acid contents. A part of the fresh sample was placed in an ice box to examine nitrate reductase (NR) activity. The remaining parts of the samples were immediately frozen in liquid N and then stored at −80 °C until used for the evaluation of sucrose phosphate synthase (SPS) activity, glutamine synthetase (GS) activity, soluble protein content, and endogenous hormone content. The stem tip samples were collected from the top to the base of the first leaf petiole in the main stem. Storage roots were uprooted, and the central parts of the representative storage roots were chosen and chopped. All of the stem tips and storage roots were frozen in liquid N and then stored in a refrigerator at −80 °C to determine their endogenous hormone content.

Five more plants were randomly collected to determine the fresh weights of above-ground and storage root at 15 day intervals after 45 days of planting. T/R was calculated on the basis of the ratio of the fresh weight of above-ground parts to the fresh weight of storage roots. The number of storage roots per plant, the weight of storage roots per plant, and the fresh yield of storage roots were determined in the harvest period.

2.3. Variable measurements

The total C content in functional leaves was determined in accordance with methods previously described by Gan et al. (2011) and Sun et al. (2013a, 2013b). The total N content in functional leaves was identified by using a Kjeldahl apparatus. C/N was calculated on the basis of the ratio of the total C content to the total N content (Cornelissen et al., 2003; Sun et al., 2013a, 2013b).

SPS activity was determined in accordance with the methods described by Tsai et al. (1985) and Douglas et al. (1988). Extracts were prepared from 0.5 g of leaves ground in liquid N_2 in a chilled mortar by using 7 mL of Hepes–NaOH extraction buffer (pH 7.5). The crude extracts were transferred to a 10 mL centrifuge tube and centrifuged at 10,000 ×*g* and 4 °C for 10 min. The liquid supernatant was used as the enzyme solution. The SPS activity was further examined in accordance with the methods described by Wardlaw and Willenbrink (1994) with slight modifications. In brief, the reaction solution containing 50 μL of enzyme solution, 50 μL of buffer, 40 μL of 50 mmol L^{-1} Uridine 5′-diphosphoglucose disodium salt (UDPG), and 20 μL of 100 mmol L^{-1} 6-phosphate fructose was heated in a water bath at 30 °C for 30 min and then heated in a boiling bath for 1 min. The reaction solution was added to 100 μL of 2 mol L^{-1} sodium hydroxide (NaOH), mixed evenly, and heated in a boiling bath for 10 min. After 2.0 mL of 30% hydrochloric acid (HCl) and 1 mL of 1% resorcinol were added, the reaction solution was heated in a water bath at 80 °C. Absorbance was obtained at 480 nm for sucrose phosphate production after the reaction solution was cooled. Enzyme activity was expressed as sucrose phosphate production.

NR activity was determined in accordance with the method described by Luo et al. (2006). For enzyme extraction, the samples were ground in liquid N and 5 mL of an extraction buffer was added to 1 g of fresh tissue. For the NR assay, the extraction buffer contained 25 mmol L^{-1} potassium phosphate buffer (pH 8.8), 10 mmol L^{-1} cysteine, and 1 mmol L^{-1} ethylene diamine tetraacetic acid (EDTA). After continuous grinding until it thawed, the supernatant was centrifuged at 20,000 ×*g* for 20 min at 4 °C. The reaction mixture contained 0.4 mL of the extracted aliquots, 1.2 mL of a 0.1 mmol L^{-1} potassium phosphate buffer (pH 7.5), 0.1 mmol L^{-1} potassium nitrate (KNO_3), and 0.4 mL of 0.25 mmol L^{-1} nicotinamide adenine dinucleotide (NADH). Controls were made by adding 0.1 mmol L^{-1} potassium phosphate buffer (pH 7.5) instead of NADH. After 30 min the NO^-_2 produced was colorimetrically measured at 540 nm after addition of 10 g kg^{-1} sulphanilamide in 2 mol L^{-1} HCl and 0.2 g kg^{-1} N-(1-naphthyl)-ethylene-diammonium dichloride (NED). NR activities are expressed as μg NO^-_2 h^{-1} g^{-1} FW.

GS activity was examined in accordance with the methods described by Zhang et al. (1997) and Xu and Zhou (2006). Enzymes were extracted with 100 mM (pH 7.6) Tris–HCl buffer. The reaction solution for enzyme activity evaluation contained 0.6 mL of imidazole-HCl buffer extract, 0.4 mL of sodium glutamate solution, 0.4 mL of 30 mmol L^{-1} adenosine triphosphate disodium (ATPNa) solution (pH 7.0), and 0.5 mol L^{-1} magnesium sulfate ($MgSO_4$) solution. The enzyme extracts in 1.2 and 0.2 mL of reaction solutions were heated in a water bath at 25 °C for 5 min, and the reaction was started after 0.2 mL of hydroxylamine reagent was added to a water bath at 25 °C for 15 min. Afterward,