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The endogenous plant hormones and ratios regulate sugar and dry matter accumulation in Jerusalem artichoke in salt-soil

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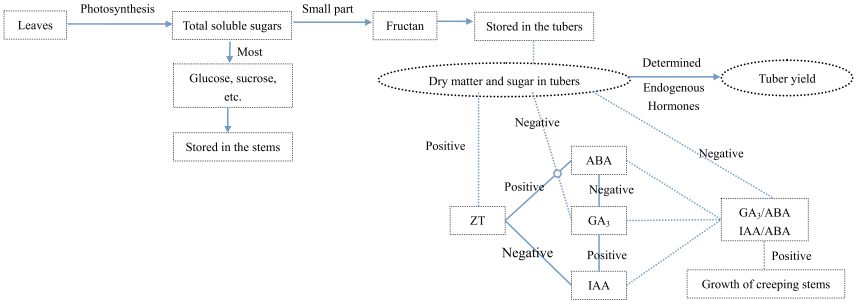
HIGHLIGHTS

- Jerusalem artichoke is tolerant to various environmental stresses.
- Jerusalem artichoke is an alternative as feedstock for biorefinery.
- The GA3/ABA and IAA/ABA were greater in NY-1 than QY-2 before tuber initiation.
- The GA3/ABA and IAA/ABA in QY-2 surpassed NY-1 during the tuber growth stage.
- A dynamic balance of endogenous hormones was important for tuber development.

GRAPHICAL ABSTRACT



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ABSTRACT

The changes in content of endogenous hormones in stolons and tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) regulate tuber growth, but the specific knowledge about the importance of balance among the endogenous hormones is lacking. Two varieties of Jerusalem artichoke (NY-1 and QY-2) were tested for the endogenous zeatin (ZT), auxins (IAA), gibberellins (GA_3) and abscisic acid (ABA) in regulating sugar and dry matter accumulation in tubers. The dry matter content and sugar accumulation in tubers were correlated positively with endogenous ZT and negatively with GA_3 content and GA_3/ABA and IAA/ABA content ratios. Throughout the tuber formation, ZT content was higher in NY-1 than QY-2 tubers, whereas ABA content was higher in QY-2 than NY-1 tubers. The content ratios GA_3/ABA and IAA/ABA were greater in NY-1 than QY-2 before tuber initiation, but QY-2 surpassed NY-1 during the tuber growth stage. The GA_3/ABA and IAA/ABA content ratios declined during tuber growth. The results suggested that a dynamic balance of endogenous hormones played an important role in tuber development.

1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is an agricultural and industrial crop with a great potential usage for food as well as manufacture of ethanol and other industrial products (Jin et al., 2013). Jerusalem

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Table 1
Salt-soil quality.

Salt content ($g \cdot kg^{-1}$)	pH	Soil texture	OC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	TK ($g \cdot kg^{-1}$)	AP ($mg \cdot kg^{-1}$)	AK ($mg \cdot kg^{-1}$)
2.5–3.2	7.69	Powder sand > 60%	33.42	4.15	2.72	26.63	17.48	124.35

artichoke is rich in carbohydrates, of which 70–90% is inulin. Inulin is suitable for diabetics and is used to replace sugar and flour for patients needing a special diet (Barta and Rosta, 1958). Jerusalem artichoke is hardy and has low production costs (Long et al., 2009), and could potentially be cultivated commercially in the relatively cold regions of the Nordic countries (Slimestad et al., 2010). It can yield >90 t ha^{-1} of fresh weight, which equates to 4 to 15 t ha^{-1} of carbohydrates (Swanton et al., 1992). Tubers can also be used as animal feed, for producing purified inulin and high fructose syrup, or for fermentation into bioethanol or other biochemicals by suitable microorganisms (Li et al., 2013; Long et al., 2016). However, Jerusalem artichoke can be considered a non-food crop due to poor digestibility of inulin and its low calorific value compared to other carbohydrate sources (Gunnarsson et al., 2014; Baldini et al., 2004); in contrast, it appears to be a potential feedstock candidate in view of large biomass production, particularly on poor soils and in extreme environments (Kays and Nottingham, 2007; Long et al., 2014).

A sufficiently high level of carbohydrate accumulation is essential for tuber formation. For example, potato tuberization is preceded by photosynthesis activation, assimilate accumulation in stalks, and intense transport to underground organs (Aksenova et al., 2012). It is well known that carbohydrate is stored in the form of inulin in Jerusalem artichoke tubers (Denoroy, 1996).

It is generally accepted that hormones (growth regulators) are the most important information system for transforming matter and energy into plant growth. Plant hormones play an important role in growth and organ formation. The possible roles of five categories of endogenous hormones have been extensively studied: auxins (IAA), zeatin (ZT), gibberellins (GA_3), abscisic acid (ABA) and ethylene (Abdala et al., 2002; Dermastia et al., 1996; Vreugdenhil and Dijk, 1989; Xu et al., 1998).

Escalante and Langille (1998) found that GA_3 increased stolon length, but reduced tuber number, diameter and biomass. Xu et al. (1998) assumed that the endogenous GA_1 content was high during stolon elongation and decreased when stolons started to swell under tuber-inducing conditions, whereas it remained high under non-inducing conditions. Carrera et al. (2000) elevated the GA_3 oxidase overexpression led to a longer duration of short-day photoperiods to form tubers by transgenic potato plants, whereas antisense inhibition of this enzyme resulted in tuberization happening earlier than in control plants.

ABA may be related closely to tuber formation, with a late increase in ABA causing tuber expansion (Meng et al., 1997), but there are reports suggesting ABA is not an important factor in tuber formation (Claver, 1970). Application of exogenous ZT was also reported to increase speed of tuber induction either in *in vitro* system or by direct application to stolons of developing potato (Malkawi et al., 2007).

The aims of this work were to characterize accumulation and transportation of dry biomass and sugars in whole plants, monitor changes in endogenous IAA, GA_3, ZT and ABA contents, and evaluate the relationships among endogenous hormones, sugar and dry matter accumulation in tubers of two Jerusalem artichoke varieties, NY-1 and QY-2.

Table 2
Accumulation and distribution of dry matter in NY-1 and QY-2 varieties of Jerusalem artichoke.

Variety	Organ		July 30th	August 14th	September 02th	September 17th	October 08th	October 28th	November 15th	November 28th
NY-1	Leaves	Dry matter ($g \cdot plant^{-1}$)	139 ± 9.91 ab	168 ± 14.57 a	172 ± 11.06 a	125 ± 14.68 b	63 ± 6.35 c	23 ± 4.63 d	–	–
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	1.93	0.21	−2.22	−4.16	−1.98	–	–
		Distribution (%)	35.1	36.8	35.9	25.6	13.1	5.2	–	–
	Stalks	Dry matter ($g \cdot plant^{-1}$)	214 ± 16.38 cd	242 ± 6.49 bc	255 ± 13.58 b	314 ± 11.15 a	337 ± 9.35 a	251 ± 13.42 b	182 ± 7.94 c	75 ± 4.36 d
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	1.87	0.68	2.81	1.53	−4.30	−3.83	−8.23
		Distribution (%)	54.0	53.0	53.2	64.0	70.2	55.7	38.2	16.8
	Roots	Dry matter ($g \cdot plant^{-1}$)	43 ± 4.37 a	47 ± 4.10 a	46 ± 6.06 a	43 ± 3.21 a	46 ± 7.21 a	41 ± 3.53 a	43 ± 4.10 a	37 ± 3.06 a
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	0.27	−0.05	−0.14	0.20	−0.25	0.11	−0.46
		Distribution (%)	10.9	10.3	9.6	8.8	9.6	9.1	9.0	8.3
	Tubers	Dry matter ($g \cdot plant^{-1}$)	–	*	6 ± 0.33 d**	8 ± 0.88 d**	34 ± 4.41 d	135 ± 16.09 c	251 ± 25.31 b	335 ± 10.84 a
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	–	–	0.10	1.73	5.05	6.44	6.46
		Distribution (%)	–	–	1.3	1.6	7.1	30.0	52.7	74.9
QY-2	Leaves	Dry matter ($g \cdot plant^{-1}$)	98 ± 8.33 b	155 ± 10.41 a	79 ± 6.39 b	24 ± 3.76 c	–	–	–	–
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	3.80	−4.00	−2.62	–	–	–	–
		Distribution (%)	37.8	41.8	22.6	7.7	–	–	–	–
	Stalks	Dry matter ($g \cdot plant^{-1}$)	128 ± 15.01 f	182 ± 10.97 cd	232 ± 7.84 ab	250 ± 4.26 a	211 ± 11.70 bc	166 ± 9.61 de	141 ± 11.89 ef	77 ± 9.21 f
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	3.60	2.63	0.86	−2.60	−2.25	−1.39	−4.92
		Distribution (%)	49.4	49.1	66.3	79.9	83.7	79.4	71.2	59.7
	Roots	Dry matter ($g \cdot plant^{-1}$)	33 ± 2.89 ab	34 ± 4.33 ab	39 ± 4.33 a	38 ± 4.91 ab	37 ± 9.24 ab	29 ± 1.20 ab	30 ± 4.93 ab	21 ± 4.91 b
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	0.07	0.26	−0.05	−0.07	−0.40	0.06	−0.69
		Distribution (%)	12.7	9.2	11.1	12.1	14.7	13.9	15.2	16.3
	Tubers	Dry matter ($g \cdot plant^{-1}$)	–	–	*	1 ± 0.44 c**	4 ± 0.58 c	14 ± 3.18 b	27 ± 2.03 a	31 ± 2.60 a
		Daily increment ($g \cdot plant^{-1} \cdot d^{-1}$)	–	–	–	–	0.20	0.50	0.72	0.31
		Distribution (%)	–	–	–	0.3	1.6	6.7	13.6	24.0

Note: Different letters indicated significant differences at 5% level according to Duncan's multiple range test among different sampling dates for a particular variety. *Rare stolon; **stolon + tuber — the leaves have dropped or the tubers have not formed yet. Dry matter data are the means ± SE (n = 3).

2. Materials and methods

2.1. Samples and chemicals

We selected two varieties of Jerusalem artichoke [identified as *Helianthus tuberosus* L. cv. Nanyu No. 1 (NY-1) and cv. Qinyu No. 2 (QY-2) in Jinhai farm of Dafeng City (Jiangsu Province, China, 32°59′N, 120°49′E). The plot size was 5 m in length and 4 m in width with three replicates. The uniformly Jerusalem artichoke tubers was planted in March 18th. Plant row spacing was inter-row (60 cm) and intra-row distance between plants (40 cm). The soil properties showed in Table 1 (Edward et al., 2015). Samples were collected at eight stages (July 30th, August 14th, September 2nd, September 17th, October 08th, October 28th, November 15th, and November 28th) from the field experimental base. In our study we divided the tuber formation process into three stages according to Aksenova et al. (2012) with some modifications. 1. Stolon formation and growth (NY-1: Sep. 02–Sep. 17; QY-2: Sep. 17–Oct. 08); 2. Induction of tuberization and tuber initiation (NY-1: Sep. 17–Oct. 08; QY-2: Oct. 08–Oct. 28) and 3. Tuber growth (NY-1: Oct. 08–Nov. 28; QY-2: Oct. 28–Nov. 28). We delimited the tuber growth periods by the state of >70% of adventitious roots because of the poor synchronization of Jerusalem artichoke tuber development. Samples of stolons and tubers of three plants in each plot were separated and rinsed with clean water. The latest six expanded leaves of three plants in each plot were also selected as the samples and clipped. Each sample was packed in individual sterile kraft bags (Chenery et al., 2012). Then some of the samples of stolons, tubers and leaves immediately frozen in liquid nitrogen, and were then stored at −70 °C. The other samples were dried to constant weight at 80 °C after enzyme deactivation at 105 °C for 15 min, milled into powder and stored in dry environment for further analyses.

The hormone standard samples were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and stored at −20 °C in the dark. The fructose, glucose, sucrose, 1F-fructofuranosylnystose, nystose and 1-kestose standard samples were obtained from Wako Pure Chemical Industries, Ltd. (Japan), and were stored at 4 °C. All other analytical-grade chemicals and chromatographic chemicals were obtained from Shoude Experimental Equipment Co., Ltd. (Nanjing, China).

2.2. Analytical methods

Samples of all plant parts were dried to a constant weight at 80 °C before weighing. Daily increment and distribution were calculated as follows:

$$\text{Daily increment}\left(\text{g}\cdot\ \text{plant}^{-1}\cdot\text{d}^{-1}\right)=\frac{w'-w_o}{D},$$

$$\text{Distribution}\ \%=\frac{w'}{w}\cdot\%$$

w': average dry weight of the organ at time t_2. w_0: average dry weight of the organ at time t_1. w: average total plant dry weight. D: number of days between the samplings ($t_2 - t_1$).

Total soluble sugars were quantified using colorimetric method based on phenol-sulfuric acid (Dubois et al., 1956). The dried and milled samples (0.50 g) were extracted with 10 mL of water at 90 °C three

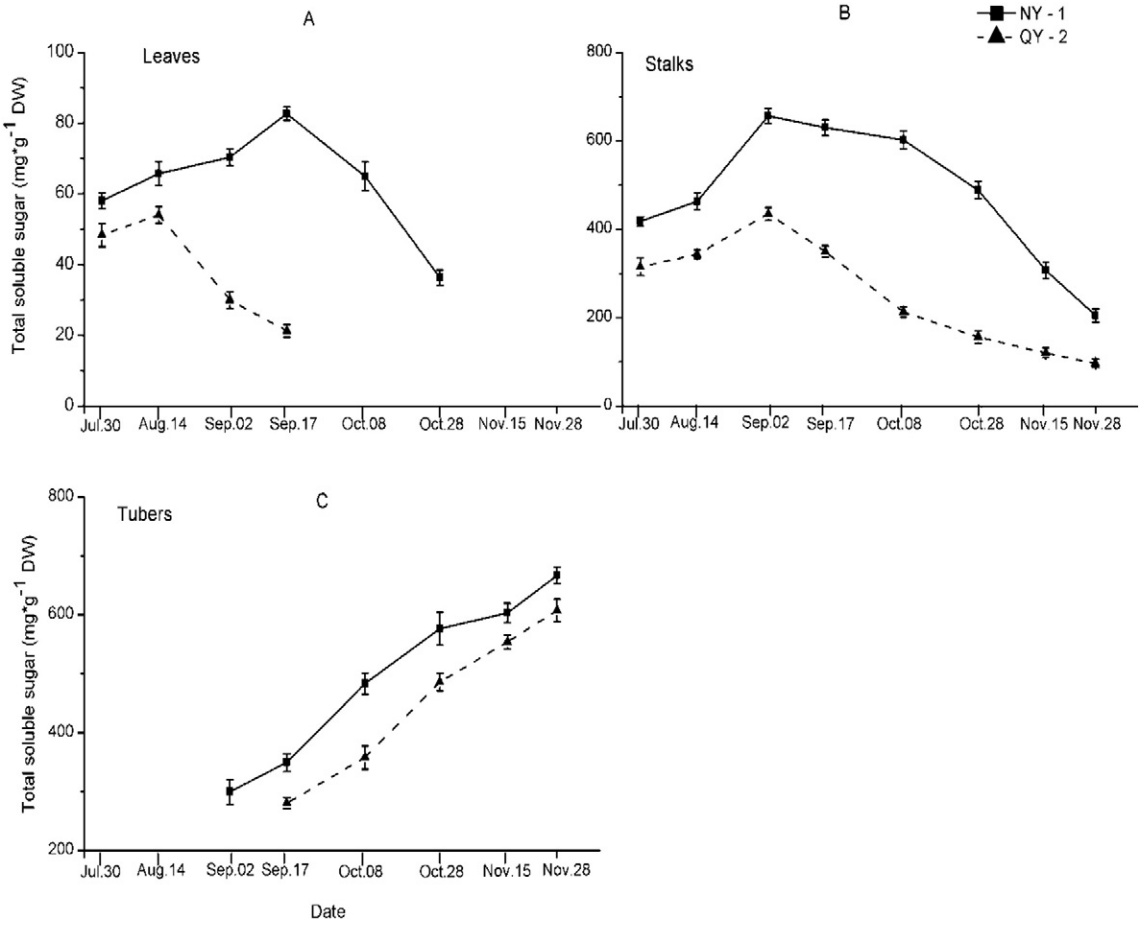

Fig. 1. Changes in total soluble sugar content in various organs of two varieties of Jerusalem artichoke: leaves (A), stalks (B) and tubers (C). Data are means ± SE (n = 3). The Y-axis has a different scale in (A) than the other two graphs.

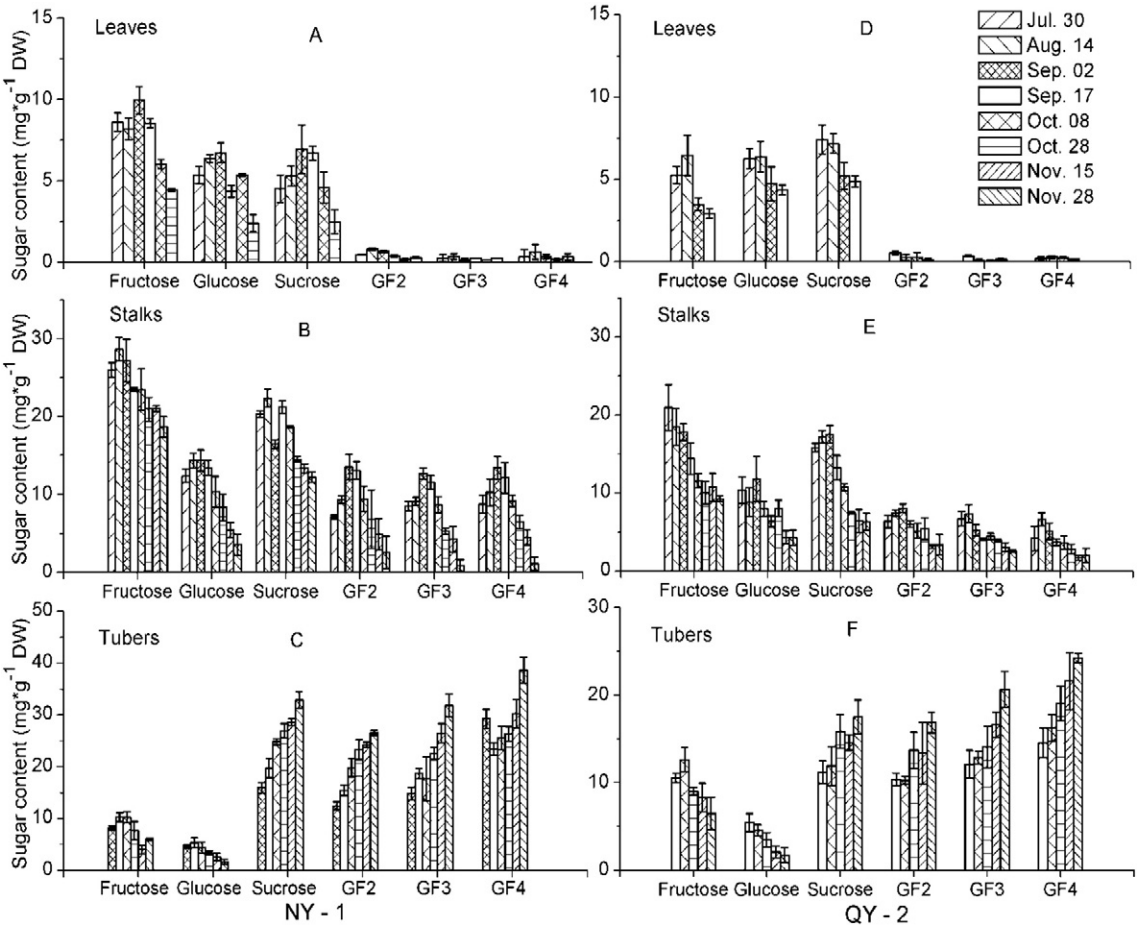

Fig. 2. Changes in fructose, glucose, sucrose, 1-kestose (GF2), nystose (GF3) and 1F-fructofuranosylnystose (GF4) content in various organs of two Jerusalem artichoke varieties (A to C = NY-1 and D to F = QY-2). Data are means ± SE (n = 3). The Y-axis scale differs for different organs.

times, filtered through a four-layer gauze and made up with water to a specified content. *A*1.0-mL aliquot of this crude extract was mixed with 1.0 mL of 5% *v*/v aqueous phenol solution and 5.0 mL of concentrated sulfuric acid at room temperature, and measured at 490 nm by a UV spectrophotometer (UV-755B, Shanghai Jingke, China) with glucose solution as standard.

For analysis of individual sugars (fructose, glucose, sucrose, 1-kestose, nystose and 1F-fructofuranosylnystose), 1.0 mL of crude extract was filtered through 0.45-μm microfiber filter and measured by high performance liquid chromatography equipped with evaporative light scattering detection (Agilent 1200, USA Alltech ELSD 3300, USA) (Li et al., 2014). The separation was carried out on a Prevail™ Carbohydrate ES Column (W250 × 46 mm, i.d. 5 μm) with gradient elution using acetonitrile: water (0–15 min: 75:25 v/v, 15–30 min: 65:35, 30–40 min: 50:50, 40–42 min: 50:50, 42–55 min: 75:25). Drift tube temperature of an ELSD system was set to 82 °C, and nitrogen flow rate was 2.0 L min^{-1} (Li et al., 2014).

Identification and quantification of endogenous hormones (IAA, GA_3, and ABA) was done according to Hou et al. (2008) and ZT was done according to Ge et al. (2004) and Muller and Hilgenberg (1986) with some modifications. A 1.00 g sample was ground to fine powder in the presence of liquid nitrogen, then extracted at 4 °C for 12 h with 20 mL of 80% (v/v) methanol solution and sealed using a preservative film. The extract was centrifuged at 5438*g* for 15 min at 4 °C, and the residue was re-extracted twice in the same way. The methanol solution extracts were combined and evaporated into the aqueous phase by a Pressure Blowing Concentrator. The aqueous residue was adjusted to 20 mL with water before passing through a preconditioned C18 SPE column (Sep-Pak waters USA; 500 mg, 6 mL). Both cartridges were washed with 1 mL of 20% v/v methanol containing 0.1% v/v formic acid, and the retained phytohormones were eluted with 1 mL of 80% v/v methanol (Hou et al., 2008). Prior to high performance liquid chromatography analysis, this reconstituted eluate was filtered using a 0.45-μm microfiber filter (Ge et al., 2004). The real samples were injected (injection volume: 10 μL) into a reverse phase column (Zorbax Eclipse XDB-C18 4.6 * 250 mm and i.d. 5 μm; Agilent, USA). The analysis was carried out by isocratic elution of solvent consisting of 0.6% v/v methanol and acetic acid (45:55 v/v) and was measured at 254 nm. A column thermostat was set at 30 °C, and the flow rate was 1.0 L min^{-1} throughout the separation.

2.3. Statistical analysis

Microsoft Excel and SPSS (15.0 for Windows, USA) were used for one-way analysis of variance (ANOVA) and Duncan's test of means to determine (i) significant differences ($p \le 0.05$) between different sampling dates, (ii) independent-samples *t*-test for the varieties effect, and (iii) correlations. Graphs were drawn using Origin 8.0.

3. Results

In the four consecutive months from the end of July to the end of November, the aerial dry matter accumulation in the two varieties of Jerusalem artichoke reached the maximum values during the induction of tuberization and the tuber initiation stage. The change in root dry matter was not significant. During the tuber growth stage, the dry matter

distribution into tubers increased (particularly in NY-1 that reached 75% distribution ratio). In contrast, dry matter distribution in QY-2 tubers was low (24%) with stalk receiving 60%. The whole-plant dry matter accumulation was significantly higher in NY-1 than QY-2 (NY-1 tuber dry matter yield was 335 $g \cdot plant^{-1}$, which was about an order of magnitude higher than QY-2) (Table 2).

The total soluble sugar accumulation and the content of individual sugars were similar in QY-2 and NY-1 at each growth stages (Figs. 1, 2). Total soluble sugars accumulated in leaves and stalks before tuber initiation, and then declined gradually during the tuber growth stage. In tubers, the total soluble sugar content kept increasing throughout the growth and was higher than in stalks at the later stage of plant growth (Fig. 1). At the similar dry weight of tissue samples, total soluble sugar content in leaves, stalks and tubers was higher in NY-1 than QY-2.

Using HPLC-ELSD, we quantified six important components of total soluble sugars: fructose, glucose, sucrose, 1-kestose (GF2), nystose (GF3) and 1F-fructofuranosylnystose (GF4). Jerusalem artichoke leaves and stalks contained mainly fructose, glucose and sucrose. There was almost no GF2, GF3 and GF4 in leaves and relatively little existed in stalks, whereas tubers were rich in these compounds (Fig. 2). Fructose content was significantly higher in stalks than leaves, and was higher in NY-1 than QY-2. Both fructose and glucose decreased over time. Sucrose, GF2, GF3 and GF4 were present at higher contents in tubers than stalks, and these contents increased in tubers during the tuber growth stage.

The dynamics of changes in endogenous amounts of 4 hormones in stolons or tubers was similar during the whole tuber formation period in two varieties of Jerusalem artichoke (Fig. 3). GA_3 and IAA contents exhibited single peaks in stolons before tuber initiation, these peaks being higher in NY-1 than QY-2. Afterwards, a greater decline in GA_3 content occurred in NY-1 than QY-2 tubers, the latter ending up with higher GA_3 content than the former. IAA content in NY-1 tubers was always higher than that in QY-2 tubers. ABA content increased during tuber growth and was lower in NY-1 than QY-2 tubers at the last harvest. The ZT content was higher in NY-1 than QY-2 tubers.

ABA was significantly and positively correlated with ZT, but significantly and negatively with GA_3 in tubers of each Jerusalem artichoke variety (Table 3). In NY-1 tubers, IAA also showed a significant positive correlation with GA_3 and a significant negative correlation with ZT.

The GA_3/ABA content ratio declined throughout the tuber formation, whereas IAA/ABA content ratio slightly increased in the stolon-growth stage and then decreased afterwards (Table 3). The ZT/ABA content ratio was consistently lower than other ratios, and there was no obvious trend during tuber development. All the endogenous hormone content ratios were higher in NY-1 than QY-2 tubers, except for GA_3/ABA ratio at the tuber growth stage (Table 4).

In our studies, dry matter accumulation in Jerusalem artichoke tubers was related to carbohydrate content and both reached maximum at the end of tuber growth. However, there were significant differences of dry matter, total soluble sugars, sucrose, GF2, GF3 and GF4 in tubers between the two varieties (Table 5).

4. Discussion

In the present study above-ground biomass reached maximum in October for variety NY-1 and in September for QY-2. Similarly to NY-1, previous studies also observed the highest yield of above-ground biomass in October, followed by significant decreases afterwards (Gunnarsson et al., 2014). Indeed, there was a relationship between a decrease in above-ground biomass and an increase in tuber biomass in Jerusalem artichoke (Gunnarsson et al., 2014; Slimestad et al., 2010). In the present study, stolon formation occurred later in QY-2 than NY-1. Moreover, NY-1 tuber daily growth rate was initially slow and then picked up, whereas that of QY-2 remained relatively slow throughout (below 1 $g \cdot plant^{-1} \cdot d^{-1}$) (Table 2).

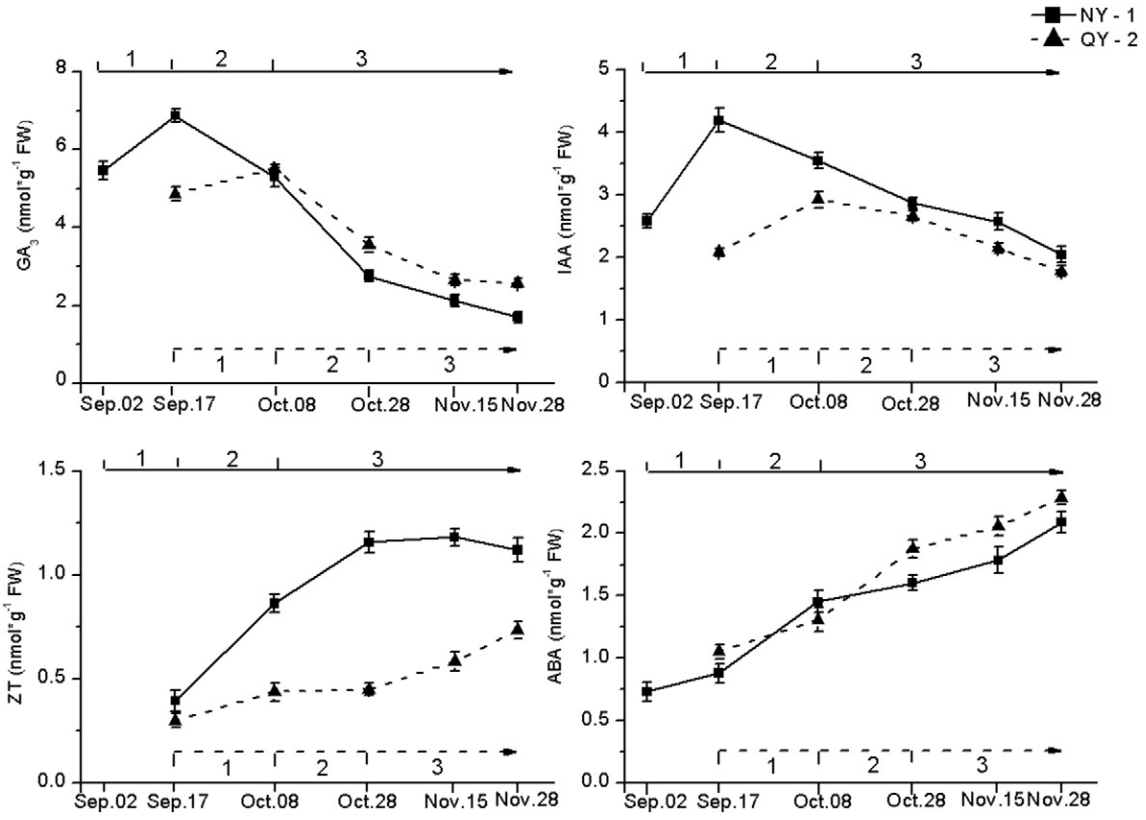

Fig. 3. Changes in content of endogenous hormones in Jerusalem artichoke stolons and tubers. In our study, numbers along the horizontal arrow (bottom of graph) indicate tuber formation stages: 1. stolon formation and growth; 2. induction of tuberization and tuber initiation; 3. tuber growth.

Table 3
The coefficient of correlation among endogenous hormone contents in NY-1 and QY-2 tubers.

NY-1 tubers				QY-2 tubers			
	IAA	GA_3	ZT		IAA	GA_3	ZT
GA_3	0.82*			GA_3	0.64		
ZT	−0.90*	−0.94*		ZT	−0.49	−0.80	
ABA	−0.57	−0.89*	0.90*	ABA	−0.42	−0.93*	0.92*

Note: * significant at 5% level, ** significant at 1% level.

We found a high proportion (and increasing continually before tuber initiation) of total dry matter in stalks. Tuberization initiated a change, with a shift in assimilate allocation from stalks to tubers (Slimestad et al., 2010). Interestingly, QY-2 was different to NY-1; at harvest, the dry matter in QY-2 was still mainly stored in stalks, and the tuber yield was relatively low (Table 2). Hence, NY-1 is considered a desirable variety with high above-ground biomass early and high tuber yield at harvest.

Leaf-generated photosynthates are transported to the underground parts in the form of sucrose, and are then transformed into highly-polymerized fructosans in tubers (Denoroy, 1996). Our results revealed that total soluble sugars generated in Jerusalem artichoke leaves as monosaccharides and disaccharides were mainly stored in stalks, with little polysaccharide formation before tuber initiation, followed by transport to tubers and conversion to fructosans (Figs. 2, 3). In our studies, a decrease in glucose and free fructose in tubers indicated formation of fructosans, with low glucose content indicative of tuber maturity (Fig. 3). Therefore, tubers are the main organ of fructan (particularly inulin) synthesis (Schubert and Feuerle, 1997). Furthermore, NY-1 showed greater total soluble sugar yield in above-ground biomass and fructosan yield in tubers compared with QY-2.

Several authors have reported the prominent role of endogenous hormones in tuber formation in potato; accordingly, we analyzed endogenous GA_3, ABA, ZT and IAA in the period from the stolon growth stage and tuber initiation to the tuber growth stage in Jerusalem artichoke. In the present study, GA_3 content increased during the stolon formation and growth stage; Abdala et al. (2002) also found high content of GA_3 during stolon growth. Fujino et al. (1995) reported that GA caused the microtubules and microfilaments oriented in a way that supported longitudinal cell extension and stolon elongation. On the other hand, the inhibitory effect of GA has been demonstrated in early studies. Xu et al. (1998) observed that with a decrease in the GA content, cell division in the subapical region of developing stolons was longitudinal, leading to cessation of stolon elongation. Hence, GA is considered an inhibitor of tuberization (Macháčková et al., 1998). In agreement, our results showed declining GA_3 content during the induction of tuberization and tuber initiation stage. During the tuber growth stage, the content of GA_3 in tubers kept on declining and then remained at a low level (Fig. 3).

A continuous slight increase in endogenous ABA content was consistent with the results on potato tubers whereby ABA inhibited stolon growth and promoted tuber initiation (Xu et al., 1998). In addition, our study showed that GA_3 and ABA contents were higher in QY-2 than NY-1 during the tuber growth stage, which suggested that low ABA as well as GA_3 content might be good for tuber growth. Furthermore, there was a significant negative correlation between GA_3 and ABA, and the cessation of stolon growth, tuber initiation and tuber growth occurred along with the content ratio GA_3/ABA dropping. Before tuber initiation, the GA_3/ABA content ratio was greater in NY-1 than QY-2; afterwards in the tuber growth stage, QY-2 overtook NY-1 (Tables 3, 4). Hence, there was an antagonistic effect between GA_3 and ABA, with high GA_3/ABA content ratio beneficial to stolon growth and the low ratio supporting tuber growth. In another study, a negative effect of ABA on the GA-mediated inhibition of tuberization was also reported (Modler et al., 1993).

ZT content increased during tuber development, with higher content in NY-1 than QY-2 tubers. ZT were not found in the early stolon formation stage; indeed, the role of ZT in stolon formation and growth is rarely reported (Fig. 3). Ewing (1987) reported that ZT stimulated tuber formation; similarly, we also observed slight accumulation of ZT during the tuber initiation and early growth stage. Thereafter, the content of ZT decreased in NY-1 but not QY-2 tubers. Hence, it seemed that ZT were not a tuberization regulator but rather a promoter of tuber initiation and early tuber growth. In addition, there was a significant positive correlation between ZT and ABA and the content ratio ZT/ABA almost was barely changed during the tuber formation.

Although IAA has been reported to be directly involved in the control of plant growth and morphogenesis, the role of IAA in tuber growth is still unclear. In our study, we observed that IAA content was positively correlated with GA_3 in tubers, with GA_3 content being relatively higher than that of IAA because GA_3 mediated stolon elongation and relatively low IAA maintained stolon apical dominance (Roumeliotis et al., 2012) (Fig. 3). With time, GA_3 content decreased slightly faster than that of IAA, and the changes in content ratio IAA/ABA during tuber growth were similar to GA_3/ABA. It appeared that IAA exhibited rather negative effects on tuber growth (similarly to GA_3) (Table 3). However, IAA content was higher in NY-1 than QY-2 tubers, and a higher content ratio IAA/ABA in NY-1 than QY-2 was associated with strong tuber development in the former (Fig. 3 and Table 4). According to Gukasyan et al. (2005), higher starch content and larger size of starch grains resulted in bigger potato tubers with IAA addition to medium. We would hypothesize that IAA favored tuber growth by promoting inulin accumulation in Jerusalem artichoke tubers.

5. Conclusions

It is suggested that tuber yield depends on sugar accumulation in tubers, with the endogenous hormones playing an important role in the

Table 4
The ratios of endogenous hormones in tubers of NY-1 and QY-2.

NY-1 tubers				QY-2 tubers		
	IAA/ABA	GA_3/ABA	ZT/ABA	IAA/ABA	GA_3/ABA	ZT/ABA
Sep. 02	3.6 ± 0.23 b	7.5 ± 0.48 a				
Sep. 17	4.9 ± 0.64 a	7.9 ± 0.61 a	0.5 ± 0.09 b	2.0 ± 0.17 a	4.7 ± 0.43 a	0.3 ± 0.05 a
Oct. 08	2.5 ± 0.08 c	3.7 ± 0.24 b	0.6 ± 0.06 ab	2.3 ± 0.41 a	3.3 ± 0.40 a	0.3 ± 0.01 a
Oct. 28	1.8 ± 0.07 cd	1.7 ± 0.04 c	0.7 ± 0.06 a	1.4 ± 0.06 b	1.9 ± 0.15 b	0.2 ± 0.02 a
Nov. 15	1.4 ± 0.01 d	1.2 ± 0.11 c	0.7 ± 0.06 a	1.0 ± 0.10 bc	1.3 ± 0.03 b	0.3 ± 0.03 a
Nov. 28	1.0 ± 0.10 d	0.8 ± 0.04 c	0.5 ± 0.02 b	0.8 ± 0.03 c	1.1 ± 0.07 b	0.3 ± 0.01 a

Note: Different letters indicated significant differences at 5% level according to Duncan's multiple range test among different sampling dates for a particular variety. Data are the means ± SE (n = 3).

Table 5
The significant differences in dry mass and sugar content between NY-1 and QY-2 tubers at harvest (28 November).

Dry matter	TSS	Fructose	Glucose	Sucrose	GF2	GF3	GF4
**	*	_	_	**	**	**	**

Note: * significant difference at 5% level and ** significant difference at 1% level according to independent-samples *t*-test. TSS: total soluble sugars; GF2: 1F-fructofuranosylnystose; GF3: nystose; GF4: 1-kestose.

process. The high content of endogenous ZT favored sugar accumulation in tubers, and the high GA_3/ABA and IAA/ABA content ratios were beneficial to stolon formation and growth, decreasing during tuber formation and sugar accumulation. This study confirmed that Jerusalem artichoke variety NY-1 has a good stalk yield, high sugar content in stalk, develops tubers early and has high tuber yield. It is suggested that application of plant growth regulators should be tested in practice to increase tuber yield.

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