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Influence of planting date and temperature on inulin content in Jerusalem artichoke (*Helianthus tuberosus* L.)

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Abstract

Lower temperatures during the dry season in tropical regions might affect inulin content and inulin yield of Jerusalem artichoke. The objective of this study was to determine the effect of planting dates during low temperature on inulin yield and content of Jerusalem artichoke. Two pot experiments were conducted during the dry seasons 2008/09 and 2009/10. Three genotypes were grown at seven planting dates. Planting Jerusalem artichoke during lower temperature periods (10-16 °C) reduced total dry weight and inulin content, whereas inulin content increased when planted during warmer periods (21-31°C). Jerusalem artichoke could be grown in all planting dates, but the most appropriate planting dates were in March. November to January should be avoided because the plants showed severe stunting with these planting dates. Moderate relationships between temperature sums and inulin content were observed in 2008/09 (r = 0.64; P < 0.01) and 2009/10 (r = 0.61; P < 0.01). The results revealed that temperature was important for producing high tuber yield having high inulin during the dry season in tropical regions.

Keywords: Genotype; fructan; temperature sums.
Abbreviations: D-planting dates; DF-degree of freedom; DMRT-Duncan's multiple range test; DP-degree of polymerization; G-genotypes; TDW- total dry weight; Y-year.

Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is native to North America (Cosgrove et al., 1991) and is an inulin producing crop. Inulin is a polydisperse fructan with a degree of polymerization (DP) of 2–60 or higher. Fructan is used mainly as dietary fibers in the food industry, and the fructose resulting from fructan hydrolysis is used as low calorie sweeteners (Denoroy, 1999). Recently, fructan also has other applications for functional food ingredients that are eligible for enhanced function claims and reduced risk of a colorectal cancer (D'Egidio et al., 1998; Frese, 1993; Roberdfroid, 2007a, b). Furthermore, dietary fructans cause an increase of the amine production in the intestine of animals preventing pasture-associated laminitis disease (Crawford, 2007; Meijer and Mathijssen, 1992; Muir et al., 2007; Valluru et al., 2008).
Jerusalem artichoke originated in temperate climates (Denoroy, 1996) and has been introduced to most parts of the world including semi-arid tropical regions such as Thailand (Pimsaen et al., 2010). A large amount of germplasm has been tested under tropical climates and some accessions are promising for commercial production in tropical regions (Jogloy et al., 2006; Pimsaen et al., 2010). In Southeast Asia, Jerusalem artichoke is grown in the rainy season and dry season. The dry season has lower temperature during October to February (11-16 °C), and this low temperature might affect inulin content and tuber yield of Jerusalem artichoke. Previous research found that temperature is an important factor affecting growth and yield of Jerusalem artichoke in temperate (Kocsis et al., 2007a, 2008) and tropic regions (Pimsaen et al., 2010). Growth and yield of Jerusalem artichoke grown in the tropics during low temperature period (Pimsaen et al., 2010) were much lower than those in the temperate regions (Kocsis et al., 2007b, 2008). In addition to growth, tuber yield and inulin are important agronomic traits of Jerusalem artichoke, and these characters are affected by photosynthesis (Schubert and Feuerle, 1997) and water stress (Conde et al., 1991; Monti et al., 2005; Valluru et al., 2008). Seasonal variation during tropical dry seasons might affect inulin content and inulin yield of Jerusalem artichoke. However, the effect of low temperature on inulin content and inulin yield at different planting times during the dry season has not been clearly investigated. A better understanding of the effect of planting date during low temperature for inulin yield and inulin content is important for suitable planting date management for both crop production and cultivar development. The objective of this study was to determine the effect of planting dates during low temperature on inulin yield and inulin content of Jerusalem artichoke.

Results

Temperature sums

The temperature sums (2288 °C to 4242 °C) in 2008/09 were higher than sums (2273 °C to 3699 °C) in 2009/10 (Table 2 and 3). Air temperatures in 2008/09 were also higher than those in 2009/10 (Fig. 1). Because of the differences in maturity, the highest temperature sums were observed in HEL 65 followed JA 89 and CN 52867.

Combined analysis of variance

Variations in total dry weight, inulin content and inulin yield were observed among planting dates (D) and Jerusalem artichoke genotypes (G) (Table 1). There were also variations in year (Y) for these traits. The interactions between year and planting date and between genotype and year were also significant for total dry weight, inulin content and inulin yield. The interactions between planting date and genotype (G × D) were not significant for total dry weight, inulin content and inulin yield while the second level interactions (Y × D × G) were significant for these traits.

Planting date effect on total dry weight, inulin content and inulin yield

In 2008/09, total dry weight was highest in Jerusalem artichoke planted in September, and lowest in Jerusalem artichoke planted in December for CN 52867 and HEL 65 genotypes, however, for JA 89 it was highest in March (Table 2). Inulin content was highest in Jerusalem artichoke planted in March, and lowest in December for all genotypes, whereas inulin yield was highest in Jerusalem artichoke planted in September, and lowest in December for most genotypes, except JA 89 which was highest in March. In 2009/10, total dry weight was highest in Jerusalem artichoke planted in September, and lowest in December, except for JA 89 which was highest in March (Table 3). Inulin content was highest in Jerusalem artichoke planted in March, and lowest in December for all genotypes, whereas inulin yield was highest in Jerusalem artichoke planted in September, and lowest in December, except JA 89 which was highest in March. The present study indicated that temperature affected total dry matter, inulin content and inulin yield. Weak and positive correlations were observed between temperature sums with total dry weight and inulin yield but moderately positive correlated with inulin content (r = 0.64**) in 2008/09 (Fig. 2A-C). However, the correlations were moderate and significant between temperature sums with total dry weight (r = 0.53*), inulin content (r = 0.61**) and inulin yield (r = 0.52*) in 2009/10 (Fig. 3A-C).

Discussion

The significant interactions for G × D and Y × D × G indicated that environmental effects play an important role in the variations for total dry weight, inulin content and inulin yield. These effects can confound the differences in genotypes. Some researchers reported that selection for superior genotypes based on yield is more difficult due to high interaction between genotype and environment. Therefore, the identification of superior genotypes would be difficult and extensive evaluation in multiple environments is necessary. Differences between years were observed for total dry weight, inulin content and inulin yield (Table 1). The difference was mainly due to higher temperature in the first year that caused higher inulin content and inulin yield. In multi-location trials, Pimsaen et al. (2010) found that the contribution of environmental effect was significant for tuber yield and shoot dry weight. In this study, genotype × year (G × Y) interaction was significant, but the interactions were much smaller than genotype × planting date interactions for all characters (Table 1). The results indicated that the effect of planting date was larger than year. Furthermore, the results indicated that planting dates with different temperature sums had large effects on total dry weight, inulin content and inulin yield. The reduction in total dry weight from November to February plantings seems to correspond to decrease in temperature sums from November to February plantings. Growing Jerusalem artichoke during periods with higher temperatures (21-31°C) could result in higher total dry weight, inulin content and inulin yield in all genotypes. Similarly, Kocsis et al. (2007a) and Kocsis et al. (2008) indicated that tuber yield and inulin yield increased with high temperature sums in temperate regions. Likewise, Kocsis et al. (2008) and Badea and Basu (2009) found that high temperatures increased sugar content. In addition, high temperature sums in some planting dates were generally associated with late maturity. Previous research revealed that late harvest can enhance inulin of chicory and Jerusalem artichoke (Baert, 1997; Saengthongpinit and Sajjaanantakul, 2005). Late harvest extends duration of inulin accumulation in Jerusalem artichoke tubers and results in high inulin. However, inulin content mainly depended on the cultivars (Baldini et al., 2004; Baert, 1997). Growing Jerusalem artichoke during lower temperature periods in December and January especially at early growth stages reduced total dry weight compared to growing Jerusalem artichoke during warmer periods (Table 2 and 3). The results support previous findings that shoot dry weight and tuber yield were reduced when Jerusalem artichoke was planted during low temperature in tropical regions (Jogloy et al., 2006). Likewise, Pimsaen et al. (2010) and Kocsis et al. (2007a, b) reported that lower temperature reduced plant growth and resulted in reduced total dry weight and tuber yield. In this study, low temperature during early growth stages of Jerusalem artichoke greatly reduced inulin content and inulin yield. Similarly, Kocsis et al. (2007a) and Kocsis et al. (2008) indicated that at temperate regions the temperature sums also affected inulin yield. Growing Jerusalem artichoke in the tropics during low temperature in November to January should be avoided in order to reduce yield loss cause by low temperature (always associated with short day length). Planting these genotypes of Jerusalem artichoke in March resulted in the highest inulin content and inulin yield. The information obtained in this study is useful for production planning of Jerusalem artichoke during the dry season in the tropics. The results indicated that high temperature promoted growth and accumulation of inulin in tubers whereas low temperature had detrimental effects on growth and inulin content. In a parallel study, Puangbut et al. (2011) also reported that warmer weather favored the accumulation of total soluble solid (brix value) in tubers. They also demonstrated that brix value was associated with inulin content across planting dates.

Materials and methods

Jerusalem artichoke genotypes and crop management

The experiments was conducted at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province, Thailand (latitude 16° 28´ N, longitude 102° 48´ E, 200 m above mean sea level). Three Jerusalem artichoke genotypes (CN 52867, JA 89 and HEL 65) representing three maturity classes (early, intermediate and late) were used in this study. Genotypes were planted in a pot experiment using seven planting dates of about 30-day intervals starting from September 20 in 2008 to March 20 in 2009. The experiments were repeated in 2009/10 and the planting dates were the same as those in 2008/09. The experiment was laid out in a completely randomized design (CRD) with four replications, and there were 4 pots per treatment and each pot was planted with a single plant. The pots were 28 cm high

Table 1. Mean square from the combine analysis of variance for total dry weight (TDW), inulin and inulin yield of three genotypes in two years at seven planting dates.

SOV	DF	TDW	Inulin content	Inulin yield
Year (Y)	1	9568.9**	42.0**	204.2**
Planting date (D)	6	27727.2*	214.9*	3276.4*
Y x D	6	6399.5**	50.2**	754.6**
Rep within Y and D	42	77.2	4.9	12.6
Genotype (G)	2	7991.3*	1223.5*	6541.4*
G x Y	2	413.3**	32.1**	342.9**
G x D	12	2064.1 ns	10.8 ns	346.1 ns
Y x D x G	12	1230.8**	24.8**	512.2**
Polled error	84	75.4	3.9	10.1

ns- not significant; * -Significant at $P < 0.05$ level; **- Significant at $P < 0.01$ level; DF-Degree of freedom; TDW-Total dry weight.

Table 2. Influence of planting dates and temperature sums on total dry weight (TDW), inulin content and inulin yield of three Jerusalem artichoke genotypes at seven planting dates during 2008/09.

Genotypes/ Planting dates	Temperature sums (°C)	TDW (g plant^{-1})	Inulin content (%)	Inulin yield (g plant^{-1})
CN 52867				
20-Sep 08	2623	112.0 ± 0.7 a	68.7 ± 0.8 ab	69.7 ± 0.7 a
20-Oct 08	2409	58.9 ± 1.4 d	66.8 ± 1.0 abc	41.6 ± 0.8 c
20-Nov 08	2288	88.3 ± 1.1 b	66.5 ± 0.5 abc	50.4 ± 0.4 b
20-Dec 08	2759	41.6 ± 0.4 e	64.1 ± 0.4 bc	26.0 ± 0.5 e
20-Jan 09	3468	72.9 ± 1.2 c	62.9 ± 0.5 c	40.1 ± 0.7 c
20-Feb 09	3697	62.3 ± 0.5 cd	68.3 ± 0.3 ab	36.6 ± 0.9 cd
20-Mar 09	3511	60.1 ± 1.1 cd	71.4 ± 0.4 a	33.7 ± 0.5 d
Mean	2965	70.9	67.0	45.4
JA 89				
20-Sep 08	2786	133.5 ± 1.0 b	67.0 ± 0.9 b	64.6 ± 0.4 b
20-Oct 08	2506	62.5 ± 0.6 d	66.7 ± 0.7 bc	32.6 ± 1.0 de
20-Nov 08	2350	70.5 ± 0.7 cd	63.2 ± 0.8 cd	42.5 ± 1.2 c
20-Dec 08	2816	42.6 ± 1.0 e	69.5 ± 0.3 ab	23.8 ± 1.1 f
20-Jan 09	3385	67.8 ± 0.9 cd	54.7 ± 0.6 e	29.3 ± 1.3 ef
20-Feb 09	3783	79.4 ± 0.2 c	62.5 ± 0.6 d	35.4 ± 1.1 d
20-Mar 09	3821	149.2 ± 0.6 a	71.3 ± 0.4 a	82.0 ± 0.2 a
Mean	3064	86.5	65.0	45.7
HEL 65				
20-Sep 08	2786	128.5 ± 1.0 a	64.5 ± 0.6 ab	60.2 ± 1.2 a
20-Oct 08	2506	51.7 ± 0.9 c	62.0 ± 0.9 ab	23.2 ± 1.0 c
20-Nov 08	2673	67.0 ± 0.9 b	59.4 ± 0.7 b	37.1 ± 1.1 b
20-Dec 08	2903	50.1 ± 0.8 c	61.7 ± 0.1 b	22.4 ± 1.2 c
20-Jan 09	3385	63.3 ± 1.0 b	60.0 ± 0.4 b	22.9 ± 1.0 c
20-Feb 09	3872	38.5 ± 0.7 d	63.3 ± 0.6 ab	23.2 ± 1.0 c
20-Mar 09	4242	68.0 ± 0.5 b	67.1 ± 0.2 a	50.3 ± 0.9 ab
Mean	3195	66.7	61.3	37.0

Data are presented as mean ± Standard error; Means in the same column with the same letters are not significantly different (at $p < 0.05$) by DMRT; TDW-Total dry weight

and 31 cm in diameter and each pot was filled with 0.017 m^3 of soil consisting of burnt rice husk and soil at the ratio of 1:1 by volume. Pre-sprouted seed tubers were used as planting materials. To prepare the sprouted seed tubers, the tuber were cut into small pieces each of which had 2-3 buds/piece. The tuber pieces were incubated in plastic bags containing moistened coconut peat at the bottom and the top of the bags for 7 days. The plastic bags were kept opened for good aeration. The tuber pieces with active buds and roots were further transferred to plug plastic trays containing a mixture 1:1 soil: burnt rice husk medium for about 7 days for germination. The two leaf-sprouted seedlings were then suitable for uniform transplanting to the pots. One seedling was transplanted per pot. Fertilizer formula 15-15-15 of N-P_2O_5-K_2O was applied at 30 days after transplanting at a rate of 2 g per pot^{-1}. Terraclor (quintozene 24 % W/V EC) was applied monthly for 3 months after transplanting at the rate 25 mL/water 20 L for the control of stem rot (*Sclerotium rolfsii*).

Weather data

Weather data were obtained from the nearest meteorological station (Fig. 1). The seasonal means of minimum and maximum air temperatures were between 22 and 34 °C in the year 2008/09, and 22 and 31 °C in the year 2009/10. The results revealed that air temperature was slightly higher in 2008/09 than that in 2009/10.

Soil data and temperature sums

The soil were collected from field and mixed thoroughly to assure uniformity. Soil was mixed with burnt rice husk. The mixed soil data analysis revealed a pH of 6.7, an organic matter of 1.16, and total nitrogen content of 0.04. Available P

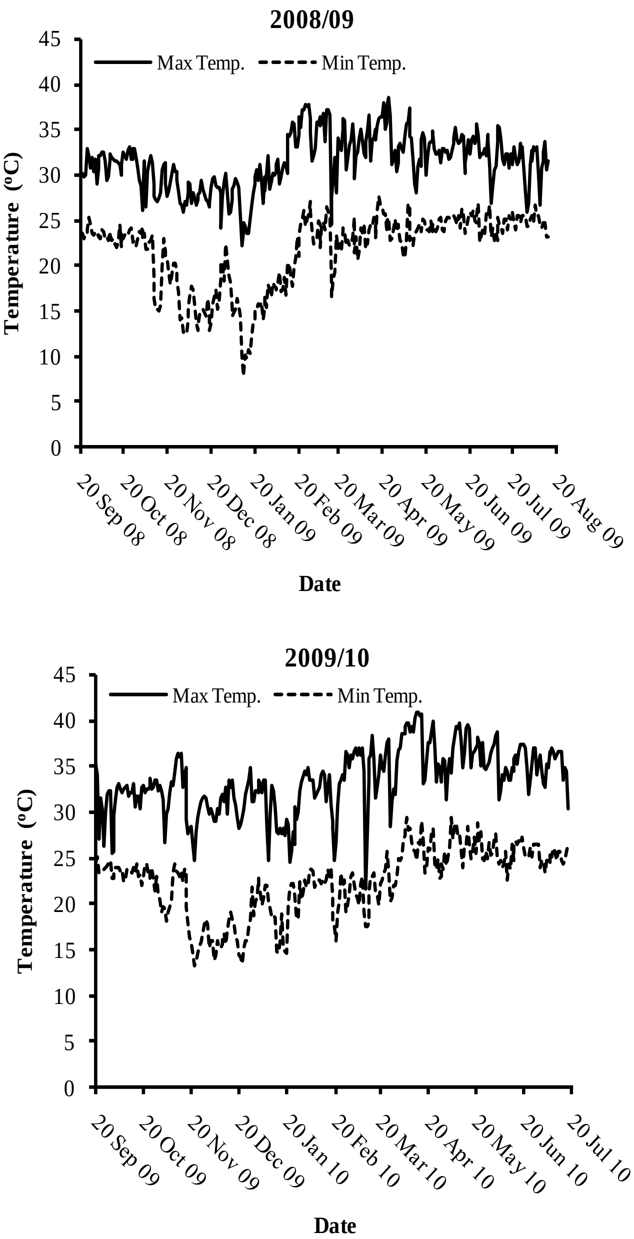
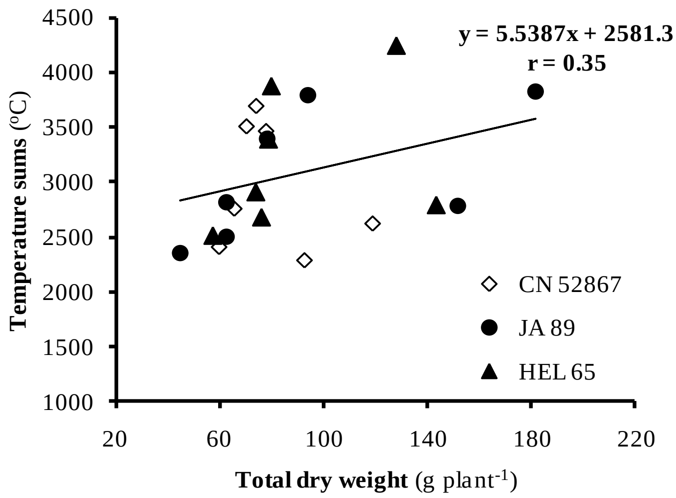


Fig 1. Maximum Temperature and Minimum Temperature in 2008/09 and 2009/10.

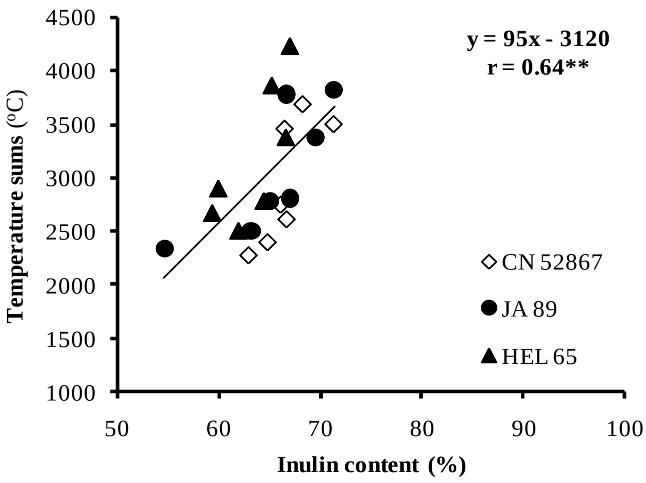
and extractable K were 176 and 96 ppm, respectively. The proportions of sand, silt, and clay in the soil were 75, 18, and 7%, respectively. Temperature sums for each genotype in each planting date were calculated from day after transplanting to harvest (Table 2 and 3). Temperature sums were calculated using the method described by Kocsis et al. (2007a).

Total dry weight

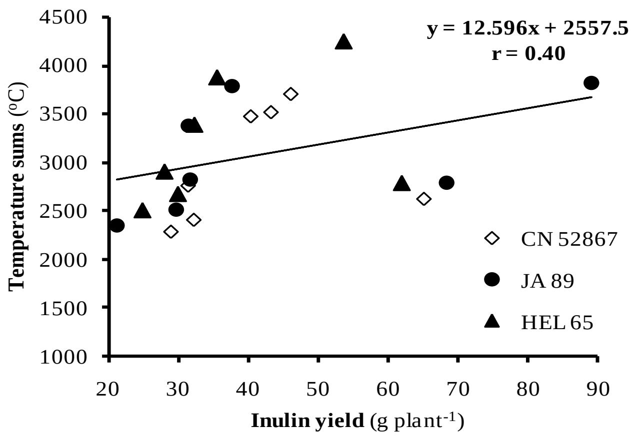
The plants were harvested at maturity depending on genotype. Shoot were separated into stalks and leaves and underground parts were separated into roots and tubers. All plant parts were oven-dried at 80 °C for at least 72 hours or until the weight was constant, and tuber dry weight and shoot dry



(A)



(B)



(C)

Fig 2. Relationship between temperature sums and total dry weight (A), inulin content (B) and inulin yield (C) of three Jerusalem artichoke genotypes at seven planting dates during 2008/09.

Table 3. Influence of planting dates and temperature sums on total dry weight (TDW), inulin content and inulin yield of three Jerusalem artichoke genotypes at seven planting dates during 2009/10.

Genotypes/ Planting dates	Temperature sums (°C)	TDW (g plant^{-1})	Inulin content (%)	Inulin yield (g plant^{-1})
CN 52867				
20-Sep 09	2891	59.6 ± 0.9 e	75.5 ± 0.5 a	41.5 ± 0.3 c
20-Oct 09	2273	72.2 ± 0.8 d	66.3 ± 0.7 cd	41.8 ± 0.8 c
20-Nov 09	2660	59.1 ± 0.8 e	67.5 ± 0.4 bc	33.1 ± 0.4 d
20-Dec 09	2918	87.2 ± 0.9 c	63.5 ± 0.5 e	49.3 ± 0.8 b
20-Jan 10	3037	78.1 ± 1.0 cd	68.6 ± 0.7 b	42.9 ± 0.9 c
20-Feb 10	3127	130.8 ± 1.0 b	64.4 ± 0.8 de	38.9 ± 0.8 c
20-Mar 10	3370	156.5 ± 1.2 a	76.7 ± 0.4 a	61.2 ± 0.4 a
Mean	2897	91.9	68.9	44.1
JA 89				
20-Sep 09	3144	123.5 ± 0.9 b	67.7 ± 0.6 a	57.3 ± 0.1 a
20-Oct 09	2295	50.3 ± 0.4 d	64.8 ± 0.3 b	31.3 ± 0.3 e
20-Nov 09	2632	63.5 ± 0.2 d	64.5 ± 0.6 b	26.5 ± 0.5 f
20-Dec 09	3435	118.3 ± 0.8 b	60.0 ± 0.7 c	42.6 ± 0.6 c
20-Jan 10	3171	85.9 ± 0.8 c	64.8 ± 0.5 b	35.4 ± 0.8 d
20-Feb 10	3462	87.1 ± 0.9 c	59.9 ± 0.9 c	29.0 ± 0.6 e
20-Mar 10	3244	181.5 ± 0.8 a	68.4 ± 0.4 a	48.4 ± 0.7 c
Mean	3055	101.4	64.2	38.6
HEL 65				
20-Sep 09	3097	68.6 ± 1.1 b	59.6 ± 0.5 b	28.7 ± 0.4 b
20-Oct 09	2295	64.8 ± 1.0 bc	59.3 ± 0.6 b	30.3 ± 0.8 b
20-Nov 09	2660	52.8 ± 0.8 c	59.7 ± 0.6 b	22.4 ± 0.4 d
20-Dec 09	3463	68.4 ± 1.1 b	53.9 ± 0.5 c	26.8 ± 0.7 c
20-Jan 10	3330	65.1 ± 1.1 b	52.8 ± 0.6 c	22.6 ± 0.9 d
20-Feb 10	3621	74.5 ± 1.1 b	54.8 ± 0.7 c	22.3 ± 0.6 d
20-Mar 10	3699	130.1 ± 1.0 a	66.8 ± 0.3 a	35.9 ± 0.5 a
Mean	3167	74.9	58.1	27.0

Data are presented as mean ± Standard error; Means in the same column with the same letters are not significantly different (at $p < 0.05$) by DMRT; TDW-Total dry weight.

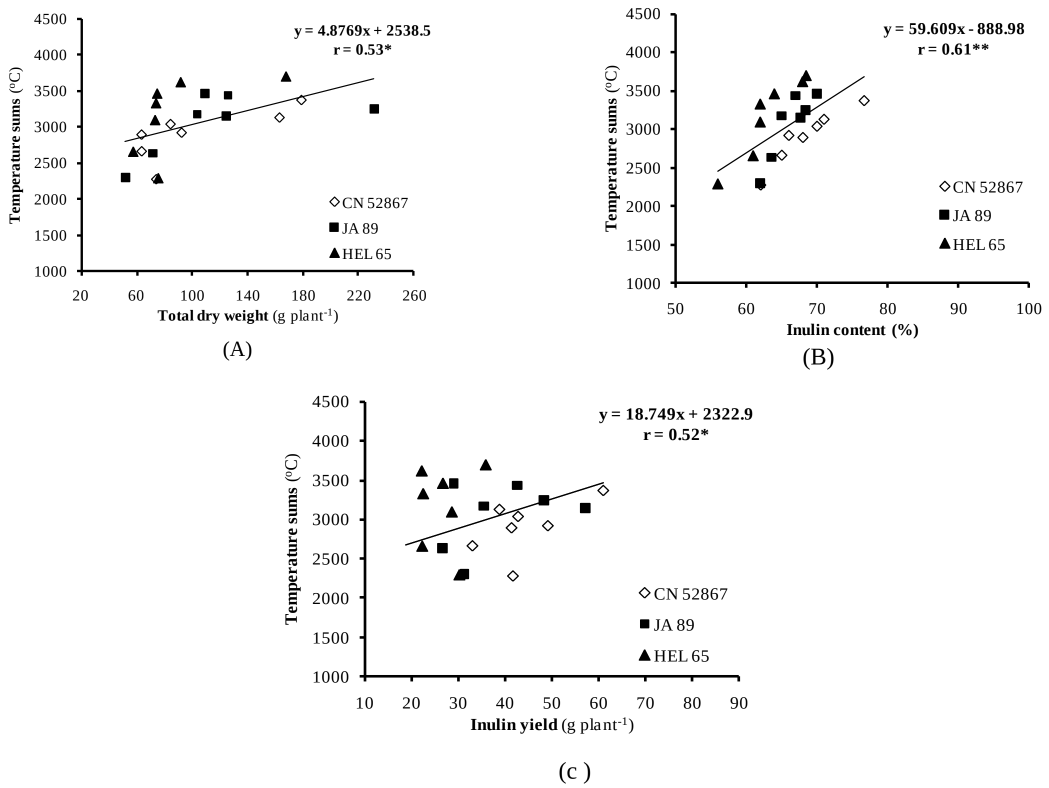


Fig 3. Relationship between temperature sums and total dry weight (**A**), inulin content (**B**) and inulin yield (**C**) of three Jerusalem artichoke genotypes at seven planting dates during 2009/10

weight were recorded. Total dry weight was computed by the following formula:
Total dry weight = shoot dry weight + tuber dry weight.

Sample preparation and inulin determination

Inulin content was analyzed using the methods described by Saengkanuk et al. (2011). The tubers were longitudinally sliced into thin pieces at the middle part of the tubers. Fifty grams of sliced tuber were soaked in absolute ethanol at 4 °C for 24 h and the samples were stored at -20 °C until analyzed. The samples were oven dried at 60 °C for 10 hours. To extract inulin, 2 g of dried sample was mixed with distilled water at 80 °C for 20 minutes. The solution was cooled to room temperature and filtered through a 0.45 µm membrane filter. The extracts (500 µl) were pipette into 25 ml volumetric flask containing 3% HCl and diluted to 25 ml with water. The mixtures were then heated at 80 °C in a water-bath for 45 minutes. After cooling, the solutions were kept in plastic bottles before being analyzed by spectrophotometer. Fructose was determined by spectrophotometer using priodate reaction (Saengkanuk et al., 2011). Inulin content was based on fructose measurement, ignoring trace amount of glucose and reducing free fructose, glucose and sucrose. Inulin analysis was shown as a percentage of inulin content on a dry weight basis and inulin yield was computed by the following formula (Puangbut et al., 2011):
Inulin yield = inulin content (%) × tuber dry weight (g plant^{-1}).

Statistical analysis

Individual analysis of variance was performed for each character in each planting date. Error variances for the two years were tested for homogeneity by Bartlett’s test (Hoshmand, 2006). Data were analyzed using STATISTIX 8 computer software with a model including year as random effects and planting date and genotype as fixed effects and for the combined analyses of variance were done for those characters whose error variances for the two years were homogeneous. Duncan’s multiple range test (DMRT) was used to compare means. Simple correlations were computed to determine the relationship between temperature sums and total dry weight, inulin content and inulin yield with differential planting dates in each year.

Conclusion

Differential responses of Jerusalem artichoke varieties to planting dates for total dry weight, inulin content and inulin yield also indicated the importance of selection for specific adaptation of Jerusalem artichoke varieties for the most suitable growing seasons which differ in temperature sums. Planting dates with warmer weather are advantageous as Jerusalem artichoke varieties grow better under these conditions and accumulate more inulin in tubers. Thus planting Jerusalem artichoke in the cooler months in tropic regions should be avoided. The obtained information on total dry weight and inulin content of Jerusalem artichoke dependent planting dates and temperature sums allow a definite choice of planting date for appropriate processing of tubers as source of inulin for application as prebiotic and functional food.

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