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Effects of water regimes on inulin content and inulin yield of Jerusalem artichoke genotypes with different levels of drought tolerance

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Abstract: Drought is a recurring problem of Jerusalem artichoke (*Helianthus tuberosus* L.) production under rainfed conditions. A better understanding of the responses of Jerusalem artichoke genotypes to drought can lead to the improvement of inulin content and inulin yield in Jerusalem artichoke and its production planning. The objective of this study was to compare the responses of five Jerusalem artichoke genotypes (JA60, JA125, JA5, JA89, and HEL65) to three water regimes (100% available water (AW; field capacity), 50% AW, and 25% AW) for inulin content and inulin yield. The experimental results showed that water regimes and Jerusalem artichoke genotypes were significantly different for inulin content and inulin yield. The interactions between Jerusalem artichoke genotypes and water regimes were also significant for inulin content and inulin yield. Under field capacity level, the results from the experimental years (2012 and 2013) showed that JA125, JA5, JA60, and HEL65 had the highest inulin content. Under 50% and 25% AW, JA5 and HEL65 had high inulin content, while JA5 had the highest inulin yield. The results indicated that if irrigation is available, JA5 should be planted because of its high inulin content under well-irrigated conditions. However, JA5, JA125, and JA60 are highly recommended under rainfed conditions because of their high inulin content under drought conditions. The results were limited to a pot experiment, and further field experiments are still required.

Key words: Water management, available water, tuber dry weight

1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuber crop native to North America, and it is a valuable source of inulin (Azis et al., 1999; Muir et al., 2007). Inulin-type fructans are a linear polydisperse carbohydrate material with a degree of polymerization of 2–60 or higher (Denoroy, 1996), consisting of β(2,6)-fructosyl-fructose units with one glucose unit at the reducing end (Frese, 1993; Muir et al., 2007). The fructose content in tubers ranges from 70% to 87% of all the reducing sugars of the tuber, depending on the cultivar characteristics and the time of harvest. The crop can be produced commercially in the tropics. Inulin is used for functional food products, animal feed, ecotourism, and raw material for high fructose syrup and ethanol (Denoroy, 1996). Jerusalem artichoke has been introduced to most parts of the world, including semiarid tropical regions such as Thailand (Pimsaen et al., 2010). A large number of accessions have been tested in tropical climates, and some accessions are promising for commercial production in tropical regions (Jogloy et al., 2006; Pimsaen et al., 2010; Ruttanaprasert et al., 2014).

Drought is a major problem for Jerusalem artichoke production in rainfed areas, as it reduces inulin accumulation in tubers (Conde et al., 1991; Schittenhelm, 1999; Monti et al., 2005). Under rainfed conditions, drought greatly reduced stalk dry weight, tuber dry weight, sugar yield, and inulin yield of Jerusalem artichoke, and differential responses of Jerusalem artichoke genotypes to drought for these characters were observed (Kocsis et al., 2007, 2008). A supply of 50% of the water requirement reduced tuber yield by 20% (Conde et al., 1991) and 22.8% (Losavio et al., 1997). Among inulin-containing and sugar-containing crops, Jerusalem artichoke is more susceptible to water stress than sugar beet or root chicory (Schittenhelm, 1999).

Water stress leads to increased fructan content in numerous plant species (Kocsis et al., 2008). Root chicory displayed resistance to water stress, but the resistance was

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obtained at the expense of growth, which in turn led to a significant decrease in inulin production (Vandoorne et al., 2012). Percent of total sugar increased up to 10% with irrigation establishment of 50% water requirement when compared to 100% water requirement (Kerepesi et al., 1998).

Most reports on the effects of drought on inulin content and inulin yield in Jerusalem artichoke were conducted in temperate regions with few varieties. Although a few studies from the tropics are available, the studies focused on tuber production and biomass rather than inulin content and inulin yield. Drought in the tropics is usually more severe than that in temperate regions, as it is associated with heat stress and a short photoperiod, which causes earlier maturity and smaller plants. The effect of drought on inulin content and inulin yield in tropical areas has not been well researched.

The objective of this study was to compare the responses of Jerusalem artichoke genotypes with different drought resistance levels to limited soil moisture for inulin content and inulin yield. This information is useful for crop production in drought prone areas.

2. Materials and methods

2.1. Plant material and experimental design

Five Jerusalem artichoke varieties (JA60, JA125, JA5, JA89, and HEL65) were used in the experiment. These varieties had different drought resistance levels based on tuber yield under drought stress in previous evaluations (Ruttanaprasert et al., 2014). The varieties also differ in their maturity, tuber yield, and agronomic traits.

The field experiment was conducted at the field crop research station of Khon Kaen University, Khon Kaen, Thailand (16°28′N, 102°48′E; 200 m above sea level). A 3 × 5 factorial experiment was set up in a rainout shelter, and the treatments were arranged in a randomized complete block design with four replications over 2 years (May to September 2012 and May to September 2013). Factor A consisted of three water regimes including field capacity (FC), 50% available water (AW), and 25% AW; factor B included five Jerusalem artichoke genotypes with different drought tolerance levels.

2.2. Pots and plant preparation

Plastic containers with a diameter of 35 cm and height of 25 cm were loaded with dry soil (20 kg). The soil was divided into two equal layers for better control of bulk density (1.61 g cm^{-2}). Plastic tubes were installed in a low soil layer to supply water to the containers, and half of the water was also used to irrigate the soil surface of the containers.

Seed tubers were used as planting material. The seed tubers were cut into small pieces with 2 or 3 buds and soaked in a solution of carboxamide (10 g in 20 L of water) for 40 min**.** The tuber pieces were presprouted in charred rice husk mixed with *Trichoderma* (3:1) under ambient conditions for 4 to 7 days. These sprouted tubers were transferred to germinating plug trays with a mixed medium containing charred rice husk, *Trichoderma*, and soil (3:2:2) for 7 days for complete sprouting. *Trichoderma* was applied to each hill base. The healthy and uniform seedlings with 3 or 4 leaves were then ready for transplanting. The carboxamide and *Trichoderma* were used to control the stem rot diseases caused by *Sclerotium rolfsii*.

Weeds were controlled manually after transplanting to keep weeds at a minimum. The fertilizer formula 15-15-15 of $N\text{-}P_2O_5\text{-}K_2O$ at the rate of 2 g pot^{-1} (156.25 kg ha^{-1}) was applied at 15 days after transplanting (DAT). Pests and diseases were controlled by application of wood vinegar two times a week (5 mL in 1 L of water) until harvest.

2.3. Water management

The water supplied to the pots was divided into two fractions. The first fraction was given to the soil surface and the second fraction was given through the tubes installed 10 cm below the soil surface. Prior to planting, water was supplied to all the pots at FC level (20.5%), and the soil moisture was maintained at FC level until 10 DAT for uniform plant establishment. The irrigation treatments were initiated after 14 DAT. Soil water levels were maintained at 20.5% for FC, 13.9% for 50% AW, and 10.6% for 25% AW from 14 DAT until harvest. The difference from the predetermined levels was not lower or higher than 1%.

Water irrigation was added to the containers based on the crop water requirement for maintaining specified soil moisture levels. The water supplied to individual containers was equal to the sum of water used by the crop and soil surface evaporation. Irrigation water was calculated from crop water requirements (Doorenbos and Pruitt, 1992) and surface evaporation (Singh and Russell, 1981), respectively. Crop water requirement was calculated daily using the methods described by Doorenbos and Pruitt (1992):

$$ET\ crop = kc \times ETo\ ,$$

where is the crop water requirement (mm day^{-1}), is evapotranspiration of the reference crop, and *kc* is the coefficient of the crop at different growth stages. The crop coefficient (*kc*) of the Jerusalem artichoke was not found in the literature, and the *kc* of sunflower was used (Monti et al., 2005; Ruttanaprasert et al., 2014).

Surface evaporation was calculated as described by Singh and Russell (1981):

$$S.E. = \beta\left(\frac{Eo}{t}\right),$$

where *S.E.* is the soil evaporation (mm), *b* is the light transmission coefficient measured depending on crop cover, is the evaporation from a class A pan (mm day^{-1}), and t is the days from the last irrigation.

During the imposition of the irrigation treatments, soil moisture content was monitored by gravimetric method at 7-day intervals. Additional water was also supplied to the treatments weekly for correction of soil moisture if soil moisture levels were lower than the predetermined treatments.

2.4. Data collection

The weather data including humidity, evaporation, and maximum and minimum temperatures were recorded daily from transplanting until harvest by a weather station located at 100 m from the experimental field. Soil moisture content was recorded by gravimetric method at 30, 60, and 90 DAT. The soil for both years was loamy sand, and soil chemical and physical properties were also analyzed.

In each experimental unit, relative water content (RWC) was measured at 30, 60, and 90 DAT to estimate plant water status. RWC was measured following Kramer (1980) using the second leaf from the top of the main stem and five plants from each experimental unit. The leaf was bored by a disk borer (1 cm^2) in the leaf area. Leaf fresh weight was measured immediately in the laboratory. Saturated weight was determined by putting the leaf sample in water for 8 h, blot-drying the outer surface, and then measuring leaf saturated weight. The leaf samples were then oven-dried at 80 °C for at least 72 h or until the leaf weights were constant, and leaf dry weight was recorded. RWC was calculated as:

$$RWC = \frac{Fresh\ weight\text{–}Dry\ weight}{Saturated\ Dry\ weight} \times 100\ .$$

Plants in two containers from each experimental unit were harvested at maturity. The mature plants, as determined by defoliation and stem browning of 50%, were cut at the soil surface and separated into leaves, stems, tubers, and roots. The tubers and roots were washed in tap water to remove the soil medium. The samples were oven-dried at 80 °C for at least 72 h or until the weights were constant.

Inulin content was analyzed using methods described previously (Saengkanuk et al., 2011). The tubers were longitudinally sliced into thin pieces at the middle of the tubers. Fifty grams of sliced tuber was 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 h. To extract inulin, 2 g of dried sample was mixed with distilled water at 80 °C for 20 min. The solution was cooled to room temperature and filtered through a 0.45-μm membrane filter. The extracts (500 μL) were pipetted into a 25-mL volumetric flask containing 3% hydrochloric acid and diluted to 25 mL with water. The mixtures were then heated at 80 °C in a water bath for 45 min. After cooling, the solutions were kept in plastic bottles until analysis by spectrophotometer. Fructose was determined by spectrophotometer using periodate reaction (Saengkanuk et al., 2011). Inulin content was based on fructose measurement, ignoring trace amounts of glucose and reducing free fructose, glucose, and sucrose. Inulin analysis was shown as percentage of inulin content on a dry weight basis, and inulin yield was computed by the following formula (Puangbut et al., 2011):

$$Inulin\ yield = inulin\ content$$
$$(\%) \times tuber\ dry\ weight\ \left(\frac{g}{plant}\right).$$

2.5. Statistical analysis

Analysis of variance was performed for each character in each water year. Error variances for the two years were tested for homogeneity by Bartlett's test (Hoshmand, 2006). The data for each parameter with homogeneous variance were combined, and least significant difference ($P \leq 0.05$) was used to compare means. All calculations were performed using Statistix 8 (http://statistix.software.informer.com/8.0/). Simple correlations were computed to determine the relationship between tuber dry weight and inulin content and inulin yield of Jerusalem artichoke genotypes with differential drought tolerance in each year. The graphs and tables were designed with Microsoft Excel.

3. Results

3.1. Meteorological conditions

The data for meteorological conditions were recorded daily in 2012 and 2013 for rainfall, maximum temperature (T-max), minimum temperature (T-min), relative humidity, and evaporation. T-max values in 2012 ranged between 26.5 and 36.5 °C, and T-min ranged between 21.3 and 26.5 °C (Figure 1).

The temperatures in 2013 were slightly higher than those in 2012, ranging between 25.7 and 40.4 °C for T-max and between 22.3 and 27.9 °C for T-min. Daily pan evaporations ranged from 0.3 to 7.6 mm in 2012 and 0.5 to 8.8 mm in 2013. The relative humidity values were 60% to 92.0% and 58% to 95% in 2012 and 2013, respectively.

3.2. Soil type, soil moisture content, and plant water status

The soil was loamy sand in 2012 and 2013. Sand particles were 79.93% to 81.00%, silt particles ranged from 15% to 18%, and clay particles ranged between 2.07% and 4.00% (Table 1). Soil pH values were 6.4 in 2012 and 6.1 in 2013. Electrical conductivity (EC) was 0.02 dS m^{-1} in 2012 and 2013. Cation exchange capacity (CEC) values were 17.84 cmol kg^{-1} in 2012 and 7.76 cmol kg^{-1} in 2013. Soil organic

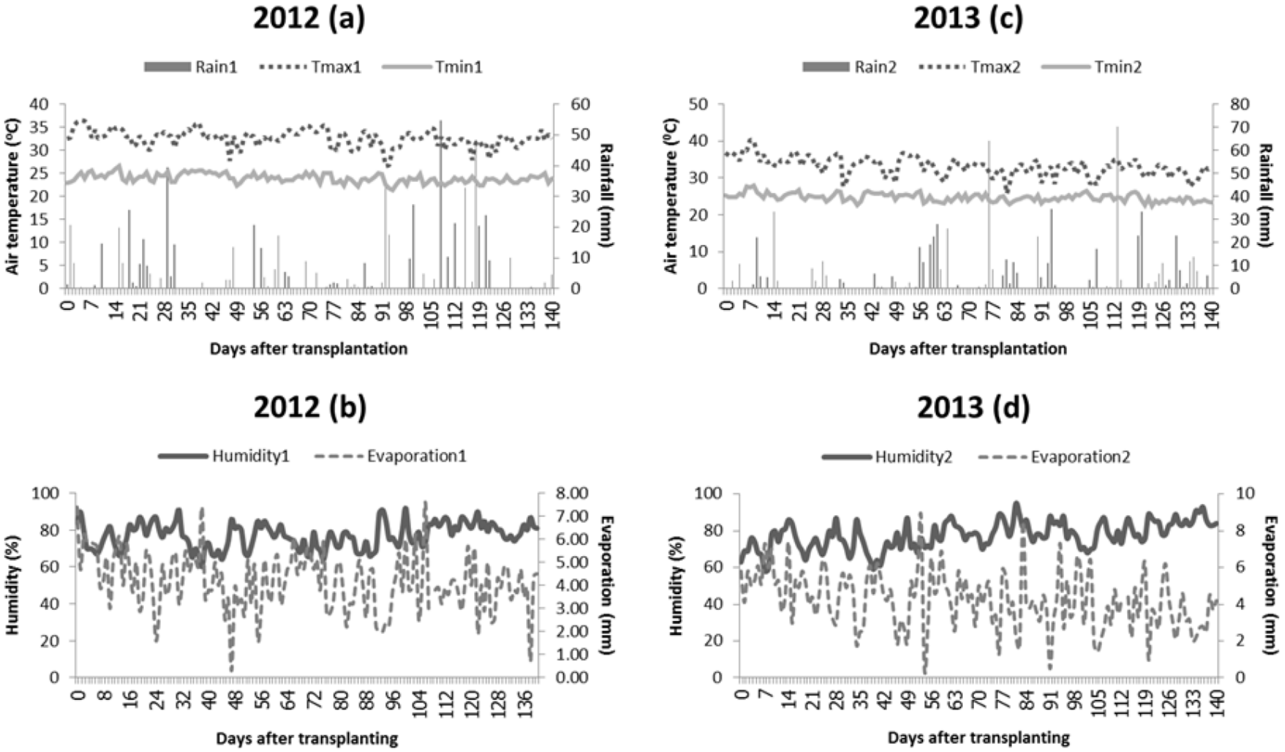

Figure 1. Rainfall (mm), maximum temperatures (T-max), minimum air temperatures (T-min) (°C), humidity (%), and evaporation (mm); 2012: (a), (b) and 2013: (c), (d).

Table 1. Soil texture and chemical properties for pot experiment in 2012 and 2013.

Soil texture	2012	2013
Sand	80%	81%
Silt	18%	15%
Clay	2%	4%
Texture class	Loamy sand	Loamy sand
Soil chemical properties		
pH (1:2.5 H_2O)	6.4	6.1
EC (dS m^{-1})	0.02	0.02
CEC (cmol kg^{-1})	17.84	7.76
OM (mg g^{-1})	5.9	6.4
Total N (mg kg^{-1})	0.3	0.2
Available P (mg kg^{-1})	11.2	15.1
Exchangeable K (mg kg^{-1})	69	70
Exchangeable Ca (mg kg^{-1})	1005	823

matter (OM) ranged from 5.9 to 6.4 mg g^{-1}, and soil total nitrogen content ranged from 0.2 to 0.3 mg g^{-1}, whereas available phosphorus (P) values were between 11.21 and 15.14 mg kg^{-1}, exchangeable potassium (K) values were between 68.70 and 70.17 mg kg^{-1}, and exchangeable calcium (Ca) values were 1005 mg kg^{-1} in 2012 and 823 mg kg^{-1} in 2013.

Water regimes were also significantly different ($P \leq 0.01$) for RWC at 30, 60, and 90 DAT in 2012 and 2013 (Figure 2). The differences among water regimes for RWC were rather narrow compared to soil water content, ranging between 88.5% and 91.7% under well-watered conditions, 81.1% and 87.7% under mild water stress, and 70.3% and 84.8% under severe water stress in 2012. The

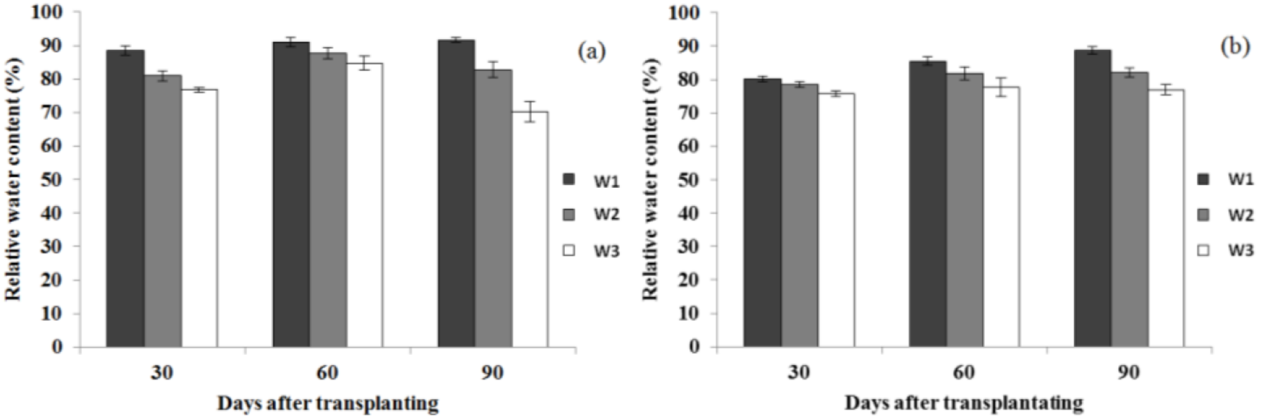

Figure 2. Leaf relative water content of 5 Jerusalem artichoke genotypes grown under 3 water regimes at 30, 60, and 90 days after transplanting in 2012 (a) and 2013 (b) (W1 = 100% AW (FC), W2 = 50% AW, W3 = 25% AW).

results were also similar in 2013, and RWC values ranged between 80.2% and 88.7% under well-watered conditions, 78.5% and 82.1% under mild water stress, and 75.8% and 77.6% under severe water stress.

Soil moisture contents for FC, 50% AW, and 25% AW at 30, 60, and 90 DAT in 2012 and 2013 are presented in Figure 3. The differences among water regimes were significant ($P \leq 0.01$) at all evaluation times in 2012 and 2013. Soil moisture contents at FC were highest, ranging from 19.5% to 21.1%, followed by soil moisture contents at 50% AW (12.3%–14.6%) and soil moisture at 25% AW (9.6%–10.8%), respectively.

3.3. Variations in inulin content and inulin yield and correlation between traits

Years were not significantly different for inulin content and inulin yield (Table 2). Water regimes were also not significantly different for inulin content, but they were significantly different ($P \leq 0.01$) for inulin yield. Genotypes were significantly different ($P \leq 0.01$) for inulin yield and inulin content. The interactions between water regime and year were not significant for inulin content and inulin yield. However, the interactions between water regime and genotype, year and genotype, and year, water regime, and genotype were significant ($P \leq 0.01$).

As the interactions between genotype and year were significant ($P \leq 0.01$) for inulin content and inulin yield, the data of the two years were reported separately (Table 3). The differences among water regimes were small, although they were significant, in 2012 and 2013. Although differences among genotypes were significant (Tables 2 and 3), the genotypes with consistently high inulin content across year and water regime could not be clearly identified due to the significant interactions between water regime and genotype and between year and genotype.

Large and significant ($P \leq 0.01$) differences among water regimes were observed for inulin yield in all Jerusalem artichoke genotypes in 2012 and 2013 (Table 3). Low soil moisture content caused a large reduction in inulin yield, and the reduction was more pronounced under severe drought stress.

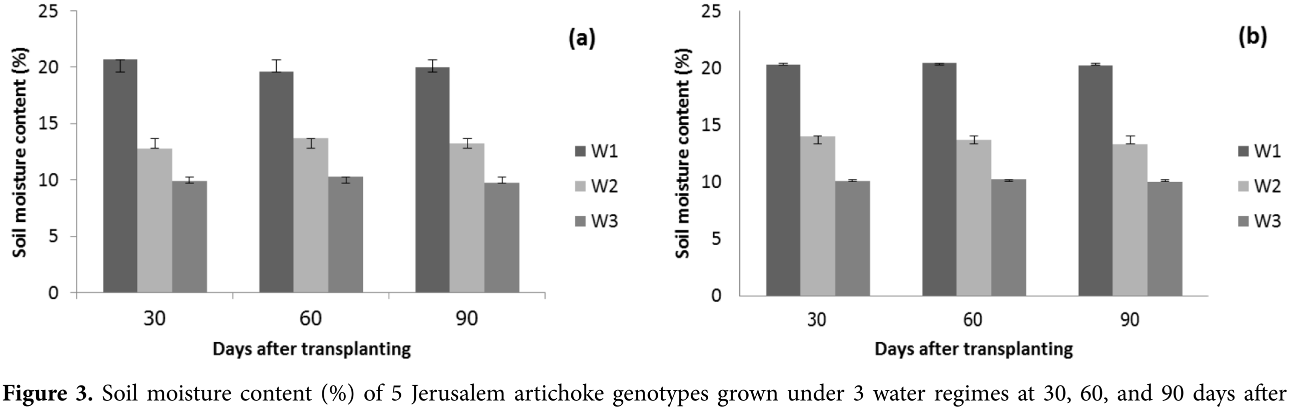

Figure 3. Soil moisture content (%) of 5 Jerusalem artichoke genotypes grown under 3 water regimes at 30, 60, and 90 days after transplanting in 2012 (a) and 2013 (b) (W1 = 100% AW (FC), W2 = 50% AW, W3 = 25% AW).

Table 2. Mean squares from the combined analysis of variance for inulin content and inulin yield of 5 Jerusalem artichoke genotypes grown under 3 water regimes (W1, W2, and W3) in 2012 and 2013.

Source of variance	df	Inulin content (%)		Inulin yield (g plant^{-1})	
Year	1	1.499	ns	1.18	ns
Rep within year	6	57.093		2.45	
Water	2	7.658	ns	4459.87	**
Year × water	2	0.395	ns	1.33	ns
Variety	4	568.879	**	254.52	**
Year × variety	4	218.180	**	17.14	**
Water × variety	8	72.688	**	82.77	**
Year × water × variety	8	138.251	**	10.45	**
Error (a)	84	19.575		1.87	
Total	119				
CV (%)		10.63		15.32	
CV (%) (a)		6.22		13.38	

ns, ** = nonsignificant and significant at P ≤ 0.001 probability levels, respectively.
W1 = 100% AW (FC), W2 = 50% AW, W3 = 25% AW.

Table 3. Effects of water regime on inulin content and inulin yield of 5 Jerusalem artichoke genotypes at harvest. W1 = 100% AW (FC), W2 = 50% AW, W3 = 25% AW.

Genotypes	Inulin content (%)						Inulin yield (g plant^{-1})					
	W1		W2		W3		W1		W2		W3	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
JA60	79b	64c	68b	66bc	76a	67b	21c	17c	8.7ab	9.1ab	1.2b	1.1b
JA125	85a	72b	72b	75a	69a	74a	25b	20b	10.3a	8.0bc	1.1b	1.1b
JA5	66c	82a	71b	76a	73a	76a	30a	35a	10.6a	11.0a	2.5a	2.4a
JA89	60d	60c	61c	63c	68a	65b	16d	16c	2.7c	2.3d	0.3c	0.4c
HEL65	66c	76b	81a	72ab	73a	74a	17d	19bc	7.8b	6.6c	0.9bc	1.2b
F-test	**		**		*		**		**		**	
C.V. (%)	10.45		8.68		7.10		12.60		19.92		34.87	

* and ** = significant at P ≤ 0.05 and P ≤ 0.001 probability levels, respectively. Values with different letters are significantly different.

JA5 had the highest inulin yield in 2012 and 2013 under well-watered conditions. JA60 and JA125 also had high inulin yield in 2012 and 2013, whereas JA89 and HE65 had high inulin yield under well-watered conditions in 2013 only. Under mild drought stress, JA5, JA125, and JA60 had consistently high inulin yield across years. Under severe drought, inulin yields of Jerusalem artichoke genotypes were very low, and the differences among genotypes for inulin yield were very small.

The correlation coefficients between inulin yield and tuber yield (Ruttanaprasert et al., 2016) were positive and significant for all water regimes in 2012 and 2013,

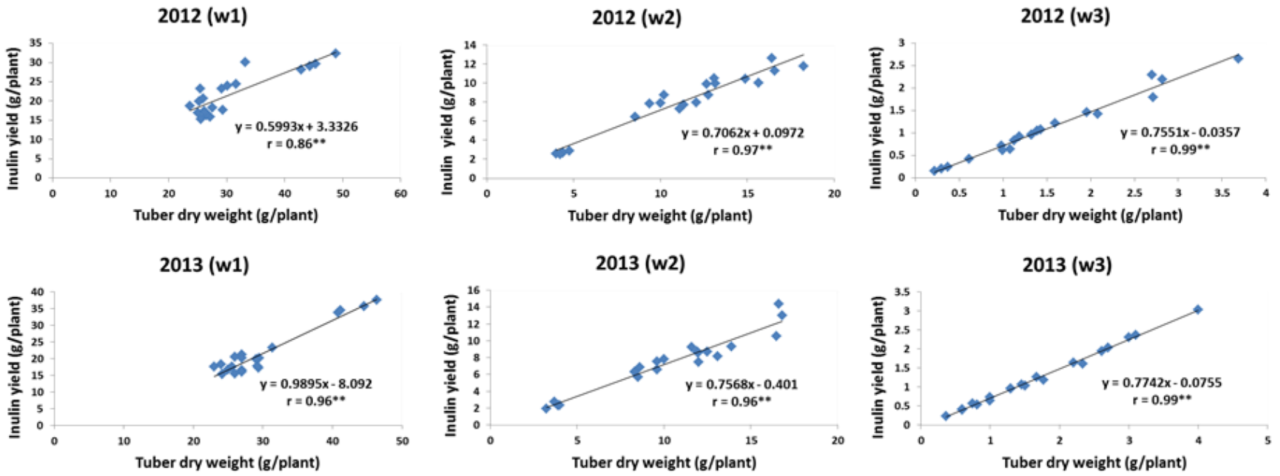

Figure 4. Relationship between tuber dry weight and inulin yield of 5 Jerusalem artichoke genotypes at harvest grown under 3 water regimes (W1 = 100% AW (FC), W2 = 50% AW, W3 = 25% AW) in 2012 and 2013. r = correlation coefficients (n = 20), ** = significant at P ≤ 0.01, respectively.

with r values ranging from 0.86 to 0.99 (Figure 4). The relationships between these traits were strongest for severe drought stress (W3), with r values of 0.99 in 2012 and 0.99 in 2013.

4. Discussion

Inulin content can be expressed based on dry weight and fresh weight. In a previous investigation, inulin content of Jerusalem artichoke tubers ranged from 8.16% to 13.46% of fresh weight (Brkljača et al., 2014) and 60.0% to 76.7% of dry weight (Puangbut et al., 2012). In this study, the range of inulin content was 60.0%–85.5% (Table 3), and the results were higher than the range in the previous study. Improvement of inulin content may be possible through breeding.

In this study, the differences among water regimes for inulin content were not significant. The results indicated that water regime had a small effect on inulin content. However, water regimes had a large effect on inulin yield.

In studies from temperate regions, reductions in tuber yield of 20% (Conde et al., 1991) and 22.8% (Losavio et al., 1997) were recorded when 50% of the water requirement was supplied to the crop (mild drought). As inulin yield was closely related to tuber yield (2012: W1; r = 0.86, W2; r = 0.97, and W3; r = 0.99; 2013: W1; r = 0.96, W2; r = 0.95, and W3; r = 0.99; Figure 4), reductions in inulin yield are expected to be similar to those in tuber yield. In temperate regions, limited irrigation seemed to be better than full irrigation in terms of water use efficiency. In tropical regions, however, drought is more severe than in the temperate regions as the crop also encounters heat and a short photoperiod (Ruttanaprasert et al., 2014). A more severe soil moisture deficit greatly reduced tuber yield in the tropics (Liua et al., 2012).

Although Jerusalem artichoke is known as a hardy plant, it is not drought-tolerant (Pimsaen et al., 2010). Severe drought can greatly reduce the yield of Jerusalem artichoke. However, Jerusalem artichoke genotypes can tolerate drought to some extent, and this tolerance could be due to its high yield potential under well-watered conditions, low reduction in yield under drought, or both high yield potential and low reduction (Ruttanaprasert et al., 2014). In this study, JA5, JA60, and JA125 are recommended for well-watered conditions, and these genotypes also had high yield under mild drought stress (Table 3). It should be noted that the results were based on a pot experiment, and further field experiments are required.

As for the correlations between tuber yield and inulin yield, there were positive and significant correlations for all water regimes, and the correlations under severe drought were stronger than under well-watered and mild drought conditions. The results indicated that inulin yield was dependent on tuber yield, especially under severe drought stress. Inulin content did not contribute significantly to inulin yield under drought stress, as drought showed very a small effect on inulin content, but it had very strong effect on inulin yield. Inulin yield was reduced greatly under drought, especially severe drought.

In conclusion, five Jerusalem artichoke genotypes with different levels of drought resistance based on tuber yield and biomass production were compared for their responses to different water regimes for inulin content and inulin yield. Water regime had a small effect on inulin content, but it had a large effect on inulin yield due largely to the great reduction in tuber yield. Differential responses among Jerusalem artichoke genotypes were observed for inulin yield, and the genotypes with high yield potential under well-watered conditions also had high inulin yield under drought, especially under mild drought conditions. Under severe drought conditions, reduction in inulin yield was high, and differences among Jerusalem artichoke genotypes were low. The genotypes with high yield under well-watered and drought conditions were identified.

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