Sub-project 3:

Sub-project 3: Basic and applied research supporting the varietal improvement of Jerusalem artichoke for drought tolerance, stem rot and leaf spot resistance, and plant growth promoting microorganism inoculants for enhancing the growth of Jerusalem artichoke

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The perennial sunflower *Helianthus tuberosus* L., known as Jerusalem artichoke or Sunchoke, was cultivated in Northeastern America before European contact. Its tubers were adopted as a source of food and forage when the species was transferred to the Old World in the early 1600s, and are still used today (Bock et al., 2014). Jerusalem artichoke is an inulin-containing crop and is a good source of dietary fiber that is useful for health (Slavin, 2013). Dietary fiber is an important component of human nutrition. It is the common name for all carbohydrate components occurring in foods that are non-digestible in the human small intestine. It is known that a deficiency of dietary fiber in food, provokes disturbance of the intestinal tract (Jaundzeikare and Beitane, 2014). As a carbohydrate-producing crop with soluble fiber property of inulin, Jerusalem artichoke can be used for the production of various value-added products such as high fructose syrup, bio-ethanol, medicine, and others.

Jerusalem artichoke was introduced into Thailand decades ago for research purposes. Khon Kaen University (KKU) introduced a large number of Jerusalem artichoke accessions for breeding under partial support from KKU and the Thailand Research Fund (TRF). As a result, Jerusalem artichoke varieties with improved yield and agronomic traits have been released and some promising breeding clones have been under evaluation. Other than the breeding of Jerusalem artichoke for better traits that are suitable for production and utilization as raw material for functional food products, the research has focused on good agronomic practices, germplasm evaluation, drought research, and disease resistance research. In this proposed phase, the advanced breeding clones will be evaluated in multi-location trials.

Although Jerusalem artichoke is a hardy plant that can be grown in a wide range of environments in both temperate and tropical regions, it is susceptible to drought (Schittenhelm, 1999). Yield losses have been estimated at 20% under mild stress (Conde et al., 1991; Losavio et al., 1997) and 90% under severe stress (Ruttanaprasert et al., 2016), and drought also affected inulin yield and inulin content of the crop (Aduldecha et al., 2016). The research on drought resistance in Jerusalem artichoke will be resumed in this proposed phase. In this phase, the research will focus on physiological traits related to drought tolerance, and responses of Jerusalem artichoke (*Helianthus tuberosus* L.*)* genotypes under terminal drought conditions.

Jerusalem artichoke production on a commercial scale also faces yield losses caused by diseases including stem rot and leaf spot. Therefore, the breeding of Jerusalem artichoke for disease resistance is the objective of Jerusalem artichoke breeding program at Khon Kaen University. In the earlier phases of the project, the research focused on stem rot caused by *Sclerotium rolfsii* (Junsopa et al., 2017; Junsopa et al., 2016). The research developed screening methods for the selection of resistant genotypes both in greenhouses and fields and verified the disease control methods using resistant varieties and biological control. Leaf spot also poses a threat to Jerusalem artichoke production in the tropics. In temperate regions, the disease is caused by bacteria (*Pseudomonas syringae* pv. tagetis) (Shane and Baumer, 1984). To the best of our knowledge, the causal pathogen of the disease has not been identified in the tropics. According to Shane and Baumer (1984), the disease reduced leaf area and plant stand and causes stunting of the plant. Yield reduction is caused by low plant stand and small plants. In this proposed phase, the research on stem rot is not continued and the research on the leaf spot of Jerusalem artichoke will be carried out. The research covers the detailed study of the causal pathogen, disease resistance screening and disease control.

Higher plants live in associations with micro-organisms, and these micro-organisms affect plants in different ways, such as commensalism, mutualism, amensalism and pathogenic consequences (Montesinos, 2003). Commensalism is a type of symbiotic relationship in which the micro-organisms do not influence host fitness. Mutualism means that both parties have benefited. Amensalism is referred to the benefit of only one party, and pathogenic means causing disease (Montesinos, 2003). The micro-organisms can live both intracellular and surface of plants.

Mycorrhiza is associated with the root zone of host plants and can increase the ability of water and nutrient uptakes and, thus, promotes the growth of host plants (Rosenblueth and Martinez-Romero, 2006). Growth-promoting bacteria are also associated with the root zone of host plants but the functions of these types of microorganisms are different. The bacteria are capable to solubilize nutrients that are available to plants and, thus, promote plant growth (Ruangsanka, 2014). The study on growth-promoting micro-organisms in Jerusalem artichoke was initiated in the earlier phase and the study will be continued in this proposed phase.

This sub-project consists of fourteen studies, and the details for each study are as follows.

3.1 Responses of total biomass, shoot dry weight, yield and yield components of Jerusalem artichoke (*Helianthus tuberosus* L.) varieties under different terminal drought duration

3.2 The variation of relative water content, SPAD chlorophyll meter reading, stomatal conductance, leaf area, and specific leaf area of Jerusalem artichoke genotypes under different durations of terminal drought in the tropical region
3.3 Photosynthetic and physiological responses to drought of Jerusalem artichoke genotypes differing in drought resistance
3.4 Net photosynthetic rate, transpiration rate and transpiration efficiency performances of Jerusalem artichoke (*Helianthus tuberosus* L.) genotypes under different durations of terminal drought
3.5 Inulin content and inulin yield of Jerusalem artichoke (*Helianthus tuberosus* L.) genotypes and relationship to net photosynthesis rate in different terminal drought duration
3.6 Identification of pathogenic fungal species causing leaf spot disease of Jerusalem Artichoke in Thailand
3.7 Variability of leaf spot resistance in Jerusalem artichoke (*Helianthus tuberosus* L.) accessions grown under the humid tropical region
3.8 Effective plant age for screening for field resistance to Alternaria leaf spot (caused by *Alternaria* spp.) under natural infection in Jerusalem artichoke (*Helianthus tuberosus* L.)
3.9 Biological control of Alternaria leaf spot caused by *Alternaria* spp. in Jerusalem Artichoke (*Helianthus tuberosus* L.) under two fertilization regimes
3.10 Bio-control of stem rot in Jerusalem artichoke (*Helianthus tuberosus* L.) in field conditions
3.11 Development of an oxalic acid assay to evaluate *Sclerotium rolfsii* resistance in Jerusalem artichoke
3.12 Determination of lethal dose and effect of gamma rays on growth and tuber yield of Jerusalem artichoke mutant
3.13 Genotypic variability of total phenolic compounds and antioxidant activity in Jerusalem artichoke (*Helianthus tuberosus* L.) germplasm
3.14 Efficiency of plant growth promoting microorganism inoculants for enhancing growth, production and tuber inulin accumulation of Jerusalem artichoke

References

Aduldecha, C., W. Kaewpradit, N. Vorasoot, D. Puangbut, S. Jogloy and A. Patanothai. 2016. Effects of water regimes on inulin content and inulin yield of Jerusalem artichoke genotypes with different levels of drought tolerance. Turkish Journal of Agriculture and Forestry 40: 335-343.

Bock, D. G., N. C. Kane, D. P. Ebert and L. H. Rieseberg. 2014. Genome skimming reveals the origin of the Jerusalem artichoke tuber crop species: neither from Jerusalem nor an artichoke. New Physiologist 201: 1021-1030.

Jaundzeikare, L., and I. Beitane. 2014. Comparison of Dietary Fibre Content in Different Fibre Sources. Available at: http://llufb.llu.lv/conference/foodbalt/2014/FoodBalt_Proceedings_2014-90-93.pdf. verified February 11, 2018.

Junsopa, C., S. Jogloy, W. Saksirirat, P. Songsri, T. Kesmala and B. B. Shew. 2017. Genotypic diversity of Jerusalem artichoke for resistance to stem rot caused by *Sclerotium rolfsii* under field conditions. Euphytica 213:164.

Junsopa, C., S. Jogloy, W. Saksirirat, P. Songsri, T. Kesmala, B. B. Shew and A. Patanothai. 2016. Inoculation with *Sclerotium rolfsii*, cause of stem rot in Jerusalem artichoke, under field conditions. European Journal of Plant Pathology 146: 47-58

Montesinos, E. 2003. Plant-associated microorganisms: a view from the scope of microbiology. International Microbiology 6(4): 221-223.

Rosenblueth, M., and E. Martínez-Romero. 2006. Bacterial endophytes and their interactions with hosts. Molecular Plant-Microbe Interactions 19: 827-837.

Ruttanaprasert, R., S. Jogloy, N. Vorasoot, T. Kesmala, R. S. Kanwar, C. C. Holbrook and A. Patanothai. 2016. Effects of water stress on total biomass, tuber yield and harvest index in Jerusalem artichoke. Agricultural Water Management 166: 130-138.

Ruangsanka, S. 2014. Identification of phosphate-solubilizing bacteria from the bamboo rhizosphere. Science Asia 40: 204-211.

Schittenhelm, S. 1999. Agronomic performance of root chicory, Jerusalem artichoke and sugarbeet in stress and non-stress environment. Crop Science 39: 1815-1823.

Shane, W. W. and J. S. Baumer. 1984. Apical chlorosis and leaf spot of Jerusalem artichoke incited by *Pseudomonas syringae* pv. tagetis. Plant Disease 68(3): 257-260.

Slavin, J. 2013. Fiber and prebiotics: Mechanisms and health benefits. Nutrients 5: 1417.

3.1 Responses of total biomass, shoot dry weight, yield and yield components of Jerusalem artichoke (*Helianthus tuberosus* L.) varieties under different terminal drought duration

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In most target areas of Jerusalem artichoke (JA) is produced under rainfed conditions in tropical areas of Thailand, the rainfall has a bimodal pattern with two peaks. Durations of terminal droughts largely depend on the end of the rainy season, mainly as a consequence of a decrease in regional precipitation. Therefore, crops grown in the late rainy season under natural environments are frequently subjected to prolonged and severe droughts in late growth stages, with water shortages as the limiting factor for crop growth and yield of JA. Many empirical studies of potted plants have reported that early droughts reduced yields by more than 86% under moderate drought (50% available water; AW) when compared with under well water (100% AW) (Ruttanaprasert et al., 2016).

Hence, the improvement of drought tolerance genotype is the appropriate method to alleviate the drought problem. Even though there have been numerous studies on drought tolerance for improved tuber yield in JA under field conditions, we are not aware of any studies conducted under terminal drought durations. Besides, the view of the breeder is considering the genotype with high yield potential under field capacity conditions and it is expected that this genotype can maintain a high yield under drought conditions or low yield reduction (Blum, 2005; Ruttanaprasert et al., 2014). This information should be useful for the selection of JA genotypes for the improvement of new varieties with high yield potential and low yield reduction in terminal drought-prone environments.

Objective

This study aims to estimate the effects of different durations of terminal drought on total biomass, tuber yield, harvest index (HI), and yield components, and to identify high-yield potential and low-yield reduction genotypes for the varietal improvement program.

Materials and Methods

A split-plot design with four replications was used for the experiment. Terminal droughts included three durations: no drought(SD0), drought from 60 days after transplanting (DAT) until harvest (SD1) and drought from 45 DAT until harvest (SD2), and were assigned in main plots. Subplots consisted of six JA genotypes (HEL256, JA37, HEL253, JA4, JA60, and JA125) that were selected based on different responses of biomass and tuber yield to long periods of drought from early growth stage until harvest (Ruttanaprasert et al., 2014).

Conventional tillage was performed to prepare the soil by a tractor. The plot size was 6 × 5 m with the plant spacing 50 x 30 cm. For each subplot, thirteen drip lines with emitter distances of 30 cm were installed at a depth of 10 cm below the soil surface between the plant rows. The seedlings had 1-2 pairs of leaves that were suitable for transplanting to the experimental field. The crop was managed properly for optimum growth including weed, insect, and disease control and fertilizer application. The amount of water for irrigation was calculated from the crop water requirement of JA plus the soil surface evaporation as described by Doorenbos and Pruitt (1992) and Singh and Russell (1981), respectively.

The data were recorded for soil physical and chemical properties, meteorological, soil moisture content, and relative water content (RWC). Plant data were recorded for total biomass, shoot dry weight, tuber yield, harvest index, and yield components.

Data for each year were analyzed according to a split-plot design. The combined analysis of variance over two years of data was done. The means were compared by the least significant difference (LSD) at the 0.05 probability level. A simple linear regression analysis was performed to estimate the reductions in all parameters (Ewing and Sandlan, 1995). All calculations were performed using MSTAT-C package (MSTAT-C Version 1.42.; East Lansing, Michigan: Michigan State University).

Results

There were no significant differences in soil water content at 45 DAT of both 30 and 60 cm for two years, according to the water was withheld beginning at 45 DAT until the harvest (data not shown). Significant differences among water treatments were observed at 60 DAT of both depths for two years. SD2 was significantly lower than SD0 and SD1 conditions. At 75 DAT, the soil moisture was significantly different in all water treatments. Similarly, RWC values were the same trend as those for soil water content, these results indicated that the plants were severely wilting plus lower soil moisture content under SD2 followed by SD1, and SD0, respectively.

Combined analysis of variance showed that overall interactions were significant for most traits, they were rather a small portion of the total variation to all characteristics when compared to the main effects (water treatment and genotype) (data not shown). Terminal drought conditions reduced total biomass, shoot dry weight, tuber dry weight, HI, tuber fresh weight, number of tubers per plant, and tuber size in both years (Tables 1 and 2). The results from this study indicated that HEL256, JA37, and JA4 had high potential for total biomass, tuber dry weight and tuber fresh weight in the year 2017/18, whereas in the year 2018/19, JA37 had the only one with high potential for total biomass, tuber dry weight and tuber fresh weight. The reductions in most parameters were more severe under SD2 than SD1 except for HI in 2017/18 and shoot dry weight in 2018/19. Moreover, genotypes were significantly different for the reductions (b-value) in most parameters in both years. For this investigation, genotypes could be classified into two groups; HEL256, JA37, and JA125 were classified into the group with high yield potential and high yield reduction, whereas JA60 and HEL253 were classified into the group with low yield potential and low yield reduction under no drought and terminal drought conditions, respectively (Figure 1).

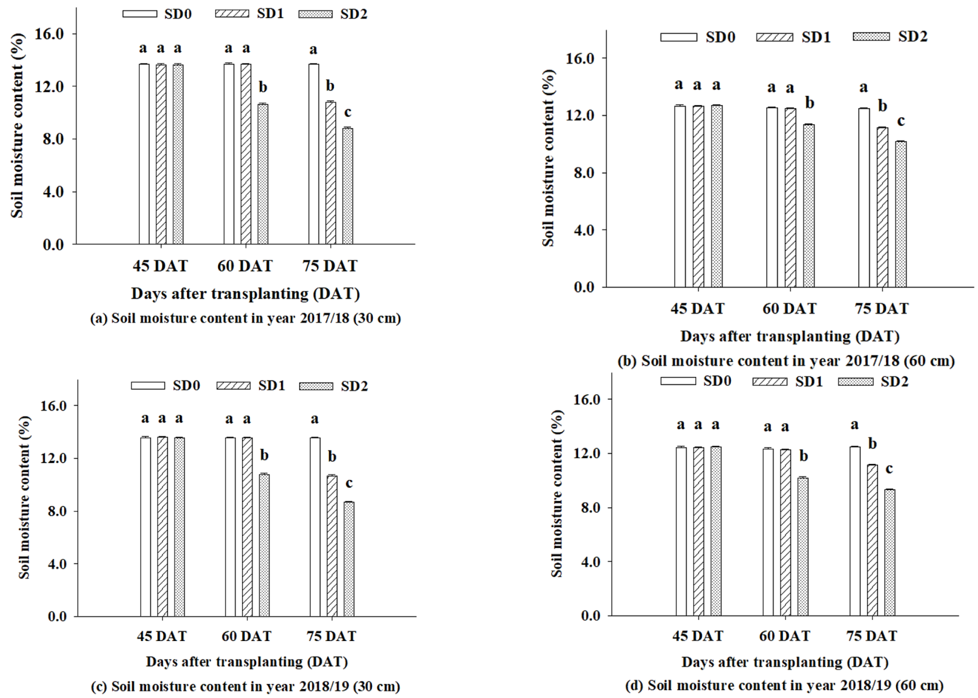

Figure 1. The soil moisture content at 45, 60, and 75 DAT of six JA genotypes grown under three water levels at the depths of 30 and 60 cm in years 2017/18 (a and b), and 2018/19 (c and d). The same letters indicated at each water level were not different for each date by the least significant difference at $p \leq 0.05$ probability level.

Table 1. Total biomass, shoot dry weight, tuber dry weight, regression coefficient (b-value) and coefficient of determinations (R^2) of six JA genotypes at harvest grown under three water levels in years 2017/18 and 2018/19.

Genotypes	Total Biomass (t ha^{-1})					Shoot Dry Weight (t ha^{-1})					Tuber Dry Weight (t ha^{-1})				
	SD0	**SD1**	**SD2**	**b-Value**	**R^2**	**SD0**	**SD1**	**SD2**	**b-Value**	**R^2**	**SD0**	**SD1**	**SD2**	**b-Value**	**R^2**
Year 2017/18															
HEL256	6.70^{a}	4.13^{a}	2.83^{a}	−1.94 **	0.88	2.02^{b}	1.76^{a}	1.16^{a}	−0.43 **	0.66	4.68^{ab}	2.36^{ab}	1.67^{ab}	−1.50 **	0.83
JA37	$5.76^{a,b}$	$3.64^{a,b}$	2.79^{a}	−1.49 **	0.87	1.16^{c}	1.03^{b}	$0.88^{b,c}$	−0.14 *	0.44	$4.61^{a,b}$	$2.61^{a,b}$	$1.90^{a,b}$	−1.35 **	0.88
HEL253	5.32^{b}	4.18^{a}	2.16^{b}	−1.58 **	0.87	2.28^{a}	1.88^{a}	1.16^{a}	−0.56 **	0.88	3.04^{d}	$2.30^{a,b}$	0.99^{c}	−1.02 **	0.84
JA4	6.52^{a}	$3.22^{b,c}$	2.94^{a}	−1.79 **	0.76	1.19^{c}	1.00^{b}	$0.94^{a,b}$	−0.12 *	0.35	5.33^{a}	$2.21^{a,b}$	1.99^{a}	−1.67 **	0.76
JA60	4.32^{c}	2.85^{c}	2.20^{b}	−1.06 **	0.63	0.82^{d}	0.84^{b}	0.66^{c}	-0.08^{ns}	0.16	$3.50^{c,d}$	2.02^{b}	1.54^{b}	−0.98 **	0.60
JA125	5.43^{b}	$3.62^{a,b}$	2.22^{b}	−1.60 **	0.94	1.11^{c}	0.97^{b}	0.66^{c}	−0.22 **	0.70	$4.32^{b,c}$	2.65^{a}	$1.56^{a,b}$	−1.38 **	0.92
Mean	5.68A	3.61B	2.52C	−1.58	0.83	1.43A	1.25A	0.91B	−0.26	0.53	4.25A	2.36B	1.61C	−1.32	0.81
F-test	**	**	*			**	**	**			**	*	**		
Year 2018/19															
HEL256	$6.47^{a,b}$	4.95^{a}	2.72^{a}	−1.88 **	0.90	2.42^{a}	1.68^{a}	$1.26^{a,b}$	−0.58 **	0.53	4.05^{b}	3.27^{a}	$1.46^{a,b}$	−1.29 **	0.81
JA37	7.44^{a}	3.83^{b}	2.65^{a}	−2.39 **	0.79	1.22^{b}	1.15^{b}	$1.04^{a,b,c}$	-0.09^{ns}	0.06	6.22^{a}	$2.68^{a,b}$	$1.61^{a,b}$	−2.31 **	0.79
HEL253	$5.14^{b,c}$	3.40^{b}	2.53^{a}	−1.30 **	0.86	2.03^{a}	1.51^{a}	1.35^{a}	−0.34 **	0.49	3.10^{b}	1.88^{c}	1.18^{b}	−0.96 **	0.76
JA4	$5.35^{b,c}$	3.60^{b}	2.73^{a}	−1.31 **	0.89	1.23^{b}	$1.00^{b,c}$	0.81^{c}	−0.21 *	0.35	3.11^{b}	2.60^{b}	1.93^{a}	−0.59 **	0.59
JA60	4.99^{c}	2.09^{c}	2.20^{a}	−1.39 **	0.69	0.97^{b}	0.81^{c}	0.82^{c}	-0.07^{ns}	0.13	4.03^{b}	1.27^{c}	$1.38^{a,b}$	−1.32 **	0.68
JA125	$5.51^{b,c}$	3.95^{b}	2.41^{a}	−1.55 **	0.77	1.36^{b}	$0.96^{b,c}$	$0.86^{b,c}$	−0.25 *	0.37	4.15^{b}	$2.99^{a,b}$	$1.55^{a,b}$	−1.30 **	0.79
Mean	5.82A	3.64B	2.54C	−1.64	0.82	1.54A	1.19B	1.02B	−0.26	0.32	4.11A	2.45B	1.52C	−1.30	0.74
F-test	**	**	ns			**	**	*			**	**	*		

Note**:** SD0 = no drought; SD1 = drought from 60 DAT; SD2 = drought from 45 DAT until harvest. ns, *, ** indicated non-significant, significant, and highly significant at $p \leq .05$ and $p \leq .01$ probability levels, respectively. Means followed by the same small letter in a column and means followed by a capital letter in each row were not significantly different by the least significant difference at $p < .05$ probability level.

Table 2. Harvest index, tuber fresh weight, number of tubers per plant, tuber size, regression coefficient (b-value) and coefficient of determinations (R^2) of six JA genotypes at harvest grown under three drought durations in years 2017/18 and 2018/19.

Genotypes	Harvest Index					Tuber Fresh Weight (t ha^{-1})					Number of Tubers (no. $Plant^{-1}$)						Tuber Size (g $Tuber^{-1}$ FW)			
	SD0	SD1	SD2	b-Value	R^2	SD0	SD1	SD2	b-Value	R^2	SD0	SD1	SD2	b-Value	R^2	SD0	SD1	SD2	b-Value	R^2
Year 2017/18																				
HEL256	0.70 ^{b}	0.57 ^{b}	0.59 ^{b}	−0.05 ^{ns}	0.31	18.63 ^{a}	8.40 ^{a,b}	5.05 ^{a,b}	−6.79 **	0.89	21.24 ^{b,c}	16.37 ^{b}	12.47 ^{b,c}	−4.38 **	0.62	13.46 ^{a}	7.76 ^{b}	6.20 ^{a,b}	−3.63 **	0.77
JA37	0.80 ^{a}	0.72 ^{a}	0.68 ^{a,b}	−0.06 **	0.86	18.19 ^{a}	9.48 ^{a}	5.75 ^{a,b}	−6.22 **	0.92	21.88 ^{b,c}	17.90 ^{b}	14.00 ^{b}	−3.94 **	0.83	12.46 ^{a}	8.03 ^{b}	6.13 ^{a,b}	−3.17 **	0.88
HEL253	0.57 ^{c}	0.55 ^{b}	0.45 ^{c}	−0.06 **	0.63	13.60 ^{b}	7.44 ^{b}	3.13 ^{c}	−5.23 **	0.90	15.22 ^{d}	12.16 ^{c}	7.93 ^{d}	−3.64 **	0.62	14.02 ^{a}	9.22 ^{b}	5.83 ^{a,b}	−4.09 **	0.73
JA4	0.82 ^{a}	0.68 ^{a}	0.68 ^{a,b}	−0.07 **	0.67	18.91 ^{a}	7.98 ^{a,b}	6.50 ^{a}	−6.21 **	0.81	30.44 ^{a}	25.23 ^{a}	21.03 ^{a}	−4.70 **	0.66	9.35 ^{b}	4.78 ^{c}	4.74 ^{b}	−2.30 **	0.66
JA60	0.80 ^{a}	0.71 ^{a}	0.69 ^{a}	−0.06 ^{ns}	0.29	11.83 ^{b}	7.06 ^{b}	4.74 ^{b}	−3.54**	0.86	23.75 ^{b}	18.45 ^{b}	12.44 ^{b,c}	−5.66 **	0.75	7.71 ^{b}	5.77 ^{c}	5.71 ^{a,b}	−1.00 ^{ns}	0.28
JA125	0.80 ^{a}	0.73 ^{a}	0.70 ^{a}	−0.05 **	0.46	17.07 ^{a}	9.31 ^{a}	4.46 ^{b,c}	−6.30 **	0.96	18.20 ^{c,d}	12.25 ^{c}	9.79 ^{c,d}	−4.21 **	0.83	14.06 ^{a}	11.47 ^{a}	6.91 ^{a}	−3.58 **	0.84
Mean	0.75A	0.66B	0.63B	−0.06	0.54	16.37A	8.28B	4.94C	−5.72	0.89	21.79A	17.06B	12.94C	−4.42	0.72	11.84A	7.84B	5.92C	−2.96	0.69
F-test	**	**	**			**	*	**			**	**	**			**	**	*		
Year 2018/19																				
HEL256	0.63 ^{c}	0.66 ^{b,c}	0.54 ^{a,b}	−0.05 ^{ns}	0.21	14.14 ^{b}	10.00 ^{a}	4.38 ^{a}	−4.88 **	0.93	24.73 ^{a}	17.60 ^{b}	9.58 ^{c}	−7.57**	0.90	8.70 ^{c}	8.58 ^{a,b}	6.90 ^{a}	−0.90 ^{ns}	0.26
JA37	0.83 ^{a}	0.70 ^{a,b}	0.62 ^{a}	−0.10 **	0.56	16.19 ^{a}	9.52 ^{a}	5.14 ^{a}	−5.52 **	0.86	16.96 ^{b}	16.98 ^{b}	14.24 ^{a,b}	−1.36 ^{ns}	0.22	14.61 ^{a}	8.45 ^{b}	5.46 ^{a}	−4.57**	0.75
HEL253	0.61 ^{c}	0.54 ^{d}	0.46 ^{b}	−0.07 ^{ns}	0.29	11.43 ^{c,d}	6.00 ^{b,c}	3.43 ^{a}	−4.00 **	0.92	14.95 ^{b}	11.23 ^{c}	11.69 ^{b,c}	−1.63 ^{ns}	0.17	11.61 ^{b,c}	8.59 ^{a,b}	4.77 ^{a}	−3.42**	0.70
JA4	0.72 ^{b}	0.54 ^{d}	0.47 ^{b}	−0.13 **	0.92	10.19 ^{d}	6.95 ^{b}	5.24 ^{a}	−2.48 **	0.81	27.71 ^{a}	25.26 ^{a}	15.41 ^{a}	−6.14 **	0.78	5.55 ^{d}	4.17 ^{c}	5.13 ^{a}	−0.21 ^{ns}	0.03
JA60	0.80 ^{a}	0.61 ^{c,d}	0.62 ^{a}	−0.09 *	0.43	13.24 ^{b,c}	5.04 ^{c}	5.05 ^{a}	−4.09**	0.69	16.44 ^{b}	8.80 ^{c}	10.43 ^{c}	−3.00 *	0.39	12.22 ^{a,b}	9.52 ^{a,b}	7.41 ^{a}	−2.40*	0.40
JA125	0.75 ^{a,b}	0.75 ^{a}	0.64 ^{a}	−0.06 ^{ns}	0.31	14.48 ^{ab}	8.86 ^{a}	4.95 ^{a}	−4.76 **	0.90	18.35 ^{b}	11.38 ^{c}	11.58 ^{b,c}	−3.38 *	0.44	12.34 ^{a,b}	11.83 ^{a}	6.43 ^{a}	−2.95 **	0.53
Mean	0.72A	0.63B	0.56C	−0.08	0.45	13.28A	7.73B	4.70C	−4.29	0.85	19.86A	15.21B	12.15C	−3.85	0.48	10.84A	8.52B	6.02C	−2.41	0.44
F-test	**	**	*			**	**	ns			**	**	**			**	**	ns		

Note: SD0 = no drought; SD1 = drought from 60 DAT; SD2 = drought from 45 DAT until harvest. ns, *, ** indicated non-significant, significant, and highly significant at $p \leq .05$ and $p \leq .01$ probability levels, respectively. Means followed by the same small letter in a column and means followed by a capital letter in each row were not significantly different by the least significant difference at $p < .05$ probability level.

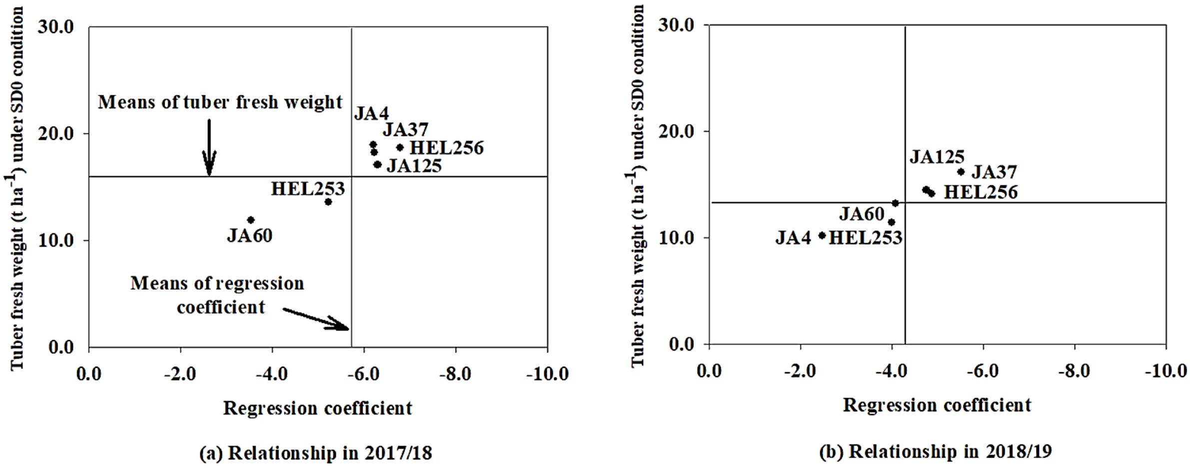

Figure 1. Regression coefficient (b-values) for tuber fresh weight of six JA genotypes under SD0 (no drought) in years 2017/18 (a) and 2018/19 (b).

Conclusions

Long-terminal drought durations decreased total biomass, tuber fresh weight, tuber dry weight, tuber number, and tuber size more than short-terminal drought duration in two years. The two groups of these genotypes (HEL256, JA37, and JA125 exhibited high yield potential and high yield reduction whereas JA60 and HEL253 exhibited low yield potential and low yield reduction under no drought and terminal drought conditions) should be used as parental genotypes to generate new varieties with high yield potential and low yield reduction for the terminal drought-prone environment.

References

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research 56: 1159-1168.

Doorenbos, J. and W.O. Pruitt. 1992. Calculation of Crop Water Requirement. In Crop Water Requirement. FAO of The United Nation, Rome, Italy, pp. 1-65.

Ewing, E. E., and K. P. Sandlan. 1995. Biomass partitioning in the potato: considerations for modelling. The Netherlands: Wageningen Pers.

Ruttanaprasert, R., P. Banterng, S. Jogloy, N. Vorasoot, T. Kesmala, R. S. Kanwar, C. C. Holbrook and A. Patanothai. 2014. Genotypic variability for tuber yield, biomass and drought tolerance in Jerusalem artichoke germplasm. Turkish Journal of Agriculture Forestry 38: 570-580.

Ruttanaprasert, R., S. Jogloy, N. Vorasoot, T. Kesmala, R. S. Kanwar, C. C. Holbrook and A. Patanothai. 2016. Effect of water stress on total biomass, tuber yield, harvest index and water use efficiency in Jerusalem artichoke. Agriculture Water Management 166: 130-138.

Singh, S., and M. B. Russell. 1981. Water use by maize/pigeon peas intercrop on a deep vertisol. In Proceedings of the International Workshop on Pigeonpeas, ICISAT Center Patancheru, Andhra Pradesh, India, 15-19 December 1981; Volume 1.

Publication

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3.2 The variation of relative water content, SPAD chlorophyll meter reading, stomatal conductance, leaf area, and specific leaf area of Jerusalem artichoke genotypes under different durations of terminal drought in the tropical region

Chaimala A., S. Jogloy, N. Vorasoot, C.C. Holbrook, C.K. Kvien and S. Laohasiriwong

In a typical northeast environment, Jerusalem artichoke (JA) is popularly grown under rain-fed conditions in the late-rainy season and drought duration mostly appears at the terminal growth stage (tuber bulking; T4). The severity of terminal drought durations is expected to vary from year to year depending on the end time for rainfall and distribution that occurred specifically in each region. Unsurprisingly, early drought is an abiotic stress factor affecting tuber yield, and quality and reflects altering physiological responses of JA genotypes (Puangbut et al., 2017). Thus, drought tolerance varieties are the ultimate objective of safeguarding crop gain under drought environments.

To date, the easy, rapid, and non-destructive measurable of physiological traits is the surrogate trait that might be related to drought tolerance, increasing the challenge to plant breeders to help speed up improvement (Boontang et al., 2010). According to the previous studies, drought tolerance traits were based on consideration of tuber yields and yield components resulting in the slow progress of genotype selection. In turn, physiological traits were addressed and there were lower interactions between genotype × environment (G × E) in comparison to yield relative traits (Nigam et al., 2005).

In research in root chicory, Vandoorne et al. (2012) reported that the physiological traits for LA, SLA, SC, and RWC were lower in stressed plants than in controlled plants. So, it leads to a hypothesis that a change in those physiological traits probably contributes to high yield potential under well water conditions and low yield reduction under terminal drought conditions. This hypothesis did not still find an answer in any research on JA.

Objective

This work assessed the responses of JA genotypes experienced to different durations of terminal drought for RWC, SCMR, SC, LA, and SLA, to investigate the significant contributor of physiological traits to yield and yield reduction and determined the genotypic diversity of physiological traits.

Materials and Methods

A field experiment was carried out at Khon Kaen University during the late-rainy season from October to January (2017/18 and 2018/19). A split-plot design with four replications was used. The main plots were three irrigation treatments, no-drought condition (maintain soil moisture content at field capacity level (FC)) (SD0), drought performed 60 days after transplanting (DAT) till harvest for a short-duration of terminal drought (SD1), and drought performed since 45 DAT till harvest times for long-duration of terminal drought (SD2). Six JA genotypes were arranged in subplots. They were selected based on the difference in yield potential (FC conditions) and yield reduction (under long-period drought conditions at the early growth stage from 15 DAT through harvest) in total biomass and tuber dry weight.

After soil preparation, a subsurface drip irrigation system was installed to supply water throughout the experiment. Water was sequentially supplied to reach FC level through 60 cm in-depth to provide uniform soil moisture favorable for the establishment of the crop after transplanting. The amount of water supply for every other day was calculated based on the crop water requirement (Doorenbos and Pruitt, 1992) and soil surface evaporation (Singh and Russell, 1981). The crop was managed properly for optimum growth including weed, fertilizer, insect, and disease.

The data were recorded for soil physical and chemical properties, meteorological, soil moisture content, and RWC. Physiological data were measured for SCMR, SC, and SLA. Yield data were collected for LA, tuber fresh weight, tuber dry weight, shoot dry weight, total biomass, and harvest index (HI).

The significance of differences was performed using a split-plot analysis of variance (ANOVA) (Gomez & Gomez, 1984). Simple linear regression was used to determine the reductions for all characters across three irrigation treatments. Multiple linear regression was used to determine the relative contributions of all physiological characteristics to yield and yield reduction in each irrigation treatment.

Results

Different terminal drought durations had a significantly reduced RWC, SC, LA, and SLA, whilst slightly increased SCMR higher under SD2 than SD1 (data not shown). The present study, it was also observed that there were differential responses of JA genotypes for physiological characters studied under three irrigation treatments and years.

Based on multiple linear regression analysis of 2 years summarized that two traits of SCMR and SC had the highest contribution to the reductions of HI and tuber fresh weight, whilst LA, SC, and SLA contributed the highest portion to the reduction of total biomass (Table 1).

Our finding pointed out that HEL256 and JA37 displayed high tuber fresh weight and SLA (Figure 1a, 1b) and there were exhibited high tuber fresh weight and medium RWC (Figure 1c, 1d) under SD1. Furthermore, JA4 and JA37 performed the greatest under SD2 with high tuber fresh weight and medium RWC (Figure 1e, 1f). In the case of HEL253 and JA60, it is identified as drought-tolerant genotypes because these maintain a low reduction in tuber fresh weight which displayed low SC (Figure 2a, 2b). This result showed that these selected parents could be applied for recombination breeding with parents with high-performing economic yield under no-drought and terminal drought for further development of drought-tolerant accessions.

Table 1. Contribution of all physiological traits to agronomic traits and agronomic trait reductions under three irrigation treatments of six Jerusalem artichoke genotypes at 75 days after transplanting (DAT) in 2017/18 and 2018/19.

Physiological Traits	Years	Explained by Regression[1] (%)											
		Total Biomass (t ha^{-1})				Shoot Dry Weight (t ha^{-1})				Tuber Dry Weight (t ha^{-1})			
		SD0	SD1	SD2	Reduction	SD0	SD1	SD2	Reduction	SD0	SD1	SD2	Reduction
Regression	2017/18	66.0*	97.9**	51.9^{ns}	92.2**	98.0**	100.0**	99.8**	81.4**	59.7^{ns}	88.9**	97.7**	48.8^{ns}
RWC		0.0^{ns}	0.0^{ns}	33.6^{ns}	0.0^{ns}	0.0**	0.0^{ns}	0.0^{ns}	0.0^{ns}	30.2^{ns}	44.0**	44.4**	17.3^{ns}
SCMR		0.0^{ns}	14.0**	11.0^{ns}	1.0^{ns}	2.3**	0.6**	89.0**	0.0^{ns}	3.6^{ns}	17.1*	0.0^{ns}	0.3^{ns}
SC		7.2*	83.3**	7.3^{ns}	23.9**	0.0^{ns}	0.1**	0.1**	0.0^{ns}	23.8^{ns}	15.4*	0.3^{ns}	15.8^{ns}
LA		2.2^{ns}	0.5*	0.0^{ns}	55.7**	95.5**	95.9**	9.7**	8.7*	0.0^{ns}	12.4*	24.8^{ns}	15.4^{ns}
SLA		56.6*	0.1*	0.0^{ns}	11.6*	0.2**	3.4**	1.0**	72.7**	2.1^{ns}	0.0^{ns}	28.2^{ns}	0.0^{ns}
Regression	2018/19	99.2**	63.6*	72.1*	97.2**	95.5**	99.5**	99.6**	83.0**	98.8**	46.8^{ns}	69.5*	93.3**
RWC		0.1^{ns}	0.0^{ns}	5.2^{ns}	0.0^{ns}	0.0^{ns}	0.00^{ns}	2.0**	0.0^{ns}	0.0^{ns}	15.1^{ns}	34.8*	0.7^{ns}
SCMR		0.0^{ns}	0.4^{ns}	60.0*	2.5*	3.2**	0.3**	85.3**	0.2^{ns}	37.1**	0.9^{ns}	0.0^{ns}	16.0*
SC		1.3^{ns}	53.9*	0.0^{ns}	36.0**	0.0**	0.0**	0.0^{ns}	4.9*	25.7**	0.0^{ns}	3.6^{ns}	71.1**
LA		44.6**	0.9^{ns}	6.8^{ns}	38.5**	91.8**	97.7**	2.1**	10.4*	24.2**	6.9^{ns}	18.7^{ns}	5.5*
SLA		53.2**	8.4^{ns}	0.1^{ns}	20.2*	0.5**	1.5**	10.2**	67.5**	11.8*	23.9^{ns}	12.4^{ns}	0.0^{ns}
		Harvest Index				Tuber Fresh Weight (t ha^{-1})							
Regression	2017/18	94.0**	98.3**	78.0*	83.9**	62.8*	94.3**	98.5**	98.6**				
RWC		7.3*	0.0^{ns}	0.0^{ns}	26.8*	21.6*	32.9**	42.6**	0.0^{ns}				
SCMR		79.1**	93.9**	7.3^{ns}	53.2**	0.0^{ns}	31.8**	0.0^{ns}	3.8^{ns}				
SC		0.0^{ns}	0.2**	3.2^{ns}	0.0^{ns}	4.1^{ns}	29.1**	1.9^{ns}	55.4**				
LA		7.5*	3.4**	7.9^{ns}	3.3*	4.1^{ns}	0.0^{ns}	20.5**	37.7**				
SLA		0.1^{ns}	0.8**	59.6*	0.6*	33.0*	0.5^{ns}	33.5**	1.7^{ns}				
Regression	2018/19	97.0**	68.8*	100.0**	90.4**	91.5**	73.8*	95.2**	92.3**				
RWC		1.6^{ns}	1.3^{ns}	0.0^{ns}	0.0^{ns}	0.0^{ns}	32.5*	85.5**	2.0*				
SCMR		81.5**	36.5*	13.5^{ns}	41.4**	36.7**	0.0^{ns}	0.0^{ns}	15.6*				
SC		0.0^{ns}	23.6*	2.2^{ns}	14.9^{ns}	43.4**	1.7^{ns}	1.7*	73.3**				
LA		12.1**	7.4^{ns}	39.5**	6.8^{ns}	0.1**	28.3*	3.0*	0.0^{ns}				
SLA		1.8^{ns}	0.0^{ns}	44.8**	27.3^{ns}	11.3**	11.3*	5.0*	1.4*				

Note*:* LA, leaf area; RWC, relative water content; SC, stomatal conductance; SCMR, SPAD chlorophyll meter reading; SD0, no-drought condition; SD1, short terminal drought; SD2, long terminal drought; SLA, specific leaf area. ns, *, ** indicated non-significant, significant, and highly significant at $p \leq .05$ and $p \leq .01$ probability levels, respectively.[1] Regression was computed by using multiple regression (n=6).

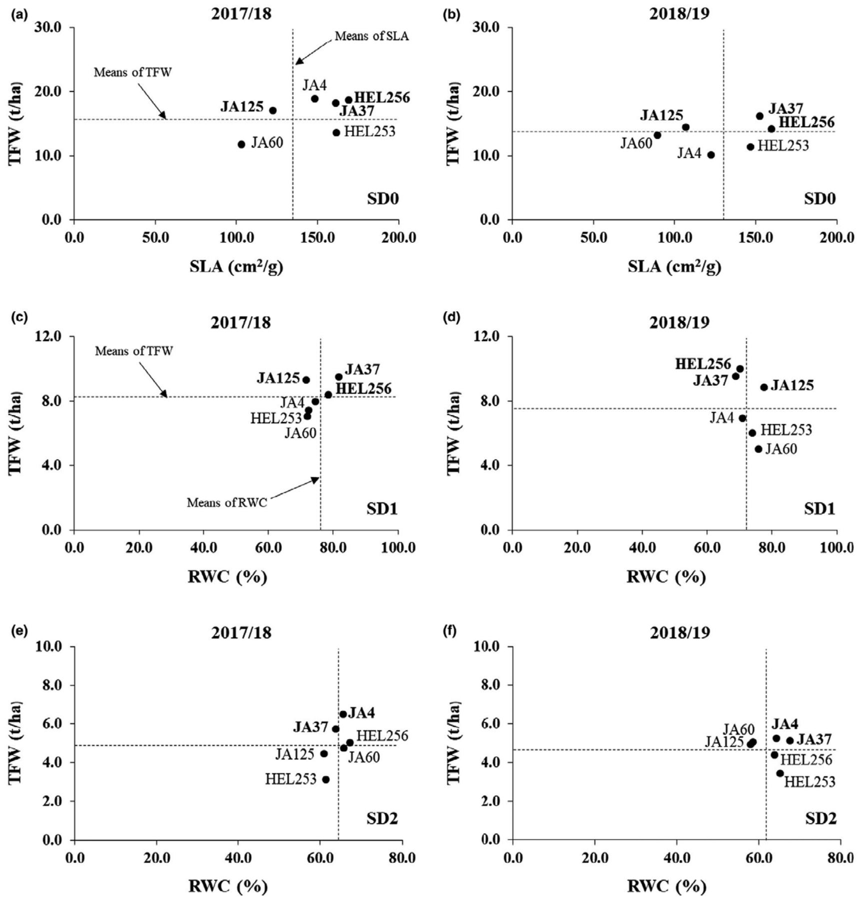

Figure 1. Means comparison among six Jerusalem artichoke genotypes between tuber fresh weight (TFW) and specific leaf area (SLA) (a and b) under SD0, and relative water content (RWC) (c–f) under SD1 and SD2 in 2 years. SD0 = no-drought; SD1 = short terminal drought; SD2 = long terminal drought.

Conclusions

Different terminal drought durations had a significantly reduced RWC, SC, LA, and SLA, whilst slightly increased SCMR higher under SD2 than SD1. The results showed that the best genotypes that performed high economic yield potential (SD0) were identified in JA125 with low SLA, HEL256, and JA37 with high SLA, and there was also a high economic yield with medium RWC under SD1. Genotypes that were superior under SD2 were JA4 and JA37, which best performed with high economic yield with medium RWC. Contrasting genotypes of JA60 and HEL253 were identified as drought tolerance due to exhibiting a low reduction in economic yield with medium SC under terminal drought. These promising genotypes could be selected to generate drought tolerance in terminal drought-prone areas.

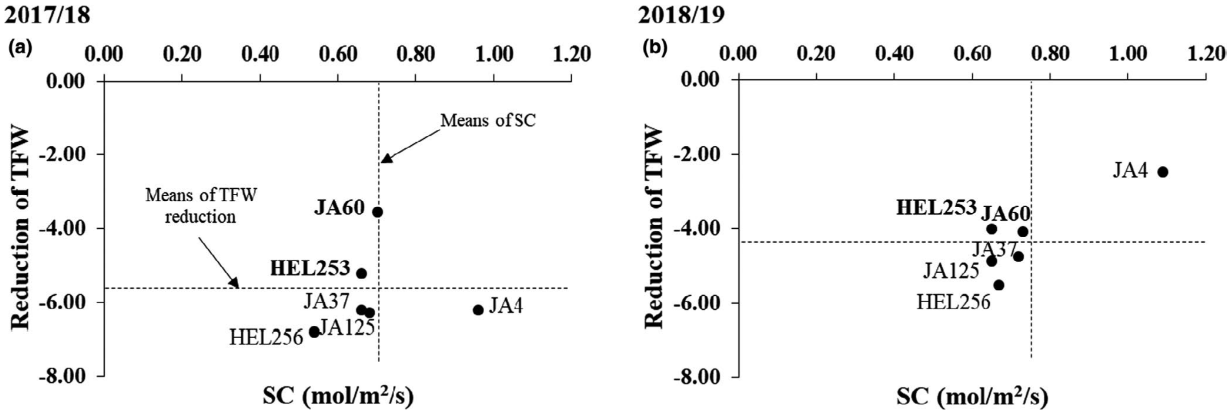

Figure 2. Means comparison among six Jerusalem artichoke genotypes between tuber fresh weight (TFW) reduction and stomatal conductance (SC) (a and b) under three irrigation treatments in 2 years.

References

Boontang, S., T. Girdthai, S. Jogloy, C. Akkasaeng, N. Vorasoot, A. Patanothai and N. Tantisuwichwong. 2010. Responses of released cultivars of peanut to terminal drought for traits related to drought tolerance. Asian Journal of Plant Science. 9: 423-431.

Doorenbos, J., and W.O. Pruitt. 1992. Calculation of Crop Water Requirement. In Crop Water Requirement. FAO of The United Nation, Rome, Italy, pp. 1-65.

Gomez, K. A. and A. A. Gomez. 1984. Statistical procedures for agricultural research, 2nd ed. New York: John Wiley and Sons.

Nigam, S.N., S. Chandra, K. R. Sridevi, M. Bhukta, A.G.S. Reddy, N. R. Rachaputi, G. C. Wright, P. V. Reddy, M. P. Deshmukh, R. K. Mathur, M. S. Basu, S. Vasundhara, P. V. Varman and A. K. Nagda. 2005. Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Annals of Applied Biology 146: 433-439.

Puangbut, D., S. Jogloy and N. Vorasoot. 2017. Association of photosynthetic traits with water use efficiency and SPAD chlorophyll meter reading of Jerusalem artichoke under drought conditions. Agricultural Water Management 188: 29-35.

Singh, S., and M. B. Russell. 1981. Water use by maize/pigeon peas intercrop on a deep vertisol. In Proceedings of the International Workshop on Pigeonpeas, ICISAT Center Patancheru, Andhra Pradesh, India, 15-19 December 1981; Volume 1.

Vandoorne, B., A. S. Mathieu, W. V. den Ende, R. Vergauwen, C. Périlleux, M. Javaux and S. Lutts. 2012. Water stress drastically reduces root growth and inulin yield in *Cichorium intybus* (var. sativum) independently of photosynthesis. Journal of Experimental Botany 63: 4359-4373.

Publication

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3.3 Photosynthetic and physiological responses to drought of Jerusalem artichoke genotypes differing in drought resistance

Puangbut, D., S. Jogloy, N. Vorasoot and P. Songsri

Drought stress adversely affects both morpho-physiology and photosynthesis of Jerusalem artichoke (Zhang et al., 2011). Plant physiological and photosynthetic responses to drought among Jerusalem artichoke genotypes possessing different levels of drought resistance are still lacking. Information on photosynthetic and physiological traits contributing to high tuber yield under water deficit might reveal the underlying plant mechanism to drought and could be adopted as selection criteria for breeders to increase the effectiveness of Jerusalem artichoke improvements for drought resistance.

Objective

The objective of this study was to investigate the physiological and photosynthetic responses to drought of six Jerusalem artichoke genotypes differing in drought-resistance levels and evaluated the contribution of physiological and photosynthetic traits to tuber yield under drought conditions.

Materials and Methods

The field experiment was conducted in the dry season (November to March) for two growing years (2015/2016 and 2016/2017) at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province, Thailand. The experiment was laid in a split plot in a randomized complete block design (RCBD) with four replications. Two irrigation levels: full irrigation with 100% evapotranspiration (100%ET) and deficit irrigation with 50% ET were assigned as the main plot whereas six Jerusalem artichoke genotypes: KT 1, KT 2, KT 50-4, JA 5, JA 60, JA 125 were assigned as sub-plot. Water was supplied uniformly at field capacity (FC) at 0-35 cm depth from 0 to 30 DAT for crop establishment. At 31 DAT, the water deficit treatment was imposed by withholding water at 50% of evapotranspiration (ET) until the harvest stage. The irrigated treatment was maintained at 100% ET throughout the crop growth cycle. The calculation of the amount of water applied was based on crop water requirement (Doorenbos and Pruitt, 1992) and soil surface evaporation (Singh and Russel, 1981).

Relative water content (RWC) was recorded at 30, 60 and 90 DAT. SPAD chlorophyll meter reading (SCMR), leaf area (LA) and leaf gas exchange measurements were recorded at 30 and 90 days after transplanting (DAT). Tuber yield and biomass were collected at 90 DAT. Analysis of variance in a split-plot design was performed for each character within each year. Homogeneity of variance was tested for all characters (Hoshmand, 2006), and a combined analysis of the variance of two-year data was then performed. Differences between means were tested by least significant difference (LSD). These analyses were performed with Statistix 10 software program.

Results

Relative water content (RWC) showed no significant difference between the two water regimes during the end of full irrigation in both years (Figure 1). RWC differed significantly between fully irrigated and drought treatments at 60 and 90 DAT. Water deficit reduced RWC to 15% and 20% of fully irrigated treatment at 60 and 90 DAT, respectively.

The interaction effects of genotype and year (G × Y) and G × Y × W were not significant for all traits, indicating that the performance of all tested genotypes on physiological and photosynthetic traits was relatively constant across years and water regimes (data not presented).

SPAD chlorophyll meter reading (SCMR) of each tested genotype under 50% ET was slightly higher than that under 100% ET at 90 DAT. Genotype JA 125 had the highest SCMR under both 100% ET (49.93) and 50% ET (53.17), whereas three genotypes KT 1, KT 2, and KT 50-4 had the lowest SCMR both 100% ET and 50% ET (Table 1). Water deficit reduced the leaf area with KT 1, KT 2, and KT 50-4 having the highest LA while JA 60 and JA 125 had the lowest LA at 90 DAT (Table 1). Drought significantly reduced LA of both resistant and susceptible genotypes. However, the resistant genotypes could retain high leaf area when they were subjected to water deficit at 50% ET.

The imposition of a water deficit at 50% ET reduced net photosynthetic rate (P_n) and Stomatal conductance (g_s). Drought-resistant genotypes (JA 60 and JA 125) showed fewer reductions in P_n and g_s (Table 2). The results indicated that the stomata of drought-resistant genotypes remained open under water-deficient conditions. In addition, the drought-resistant genotype showed a relatively high P_n and less reduction in transpiration rate under drought conditions. The present study suggests that irrigation at 50% ET may be sufficient for Jerusalem artichoke growth and promote high photosynthesis efficiency

Water deficit at 50%ET significantly decreased biomass production and tuber yield. KT 50-4 showed low reductions in biomass and tuber yield, while KT 1 revealed large reductions in biomass (49.57%) and tuber yield (Table 3). These results indicated that a drought-resistant genotype could retain biomass and tuber yield under water deficit conditions.

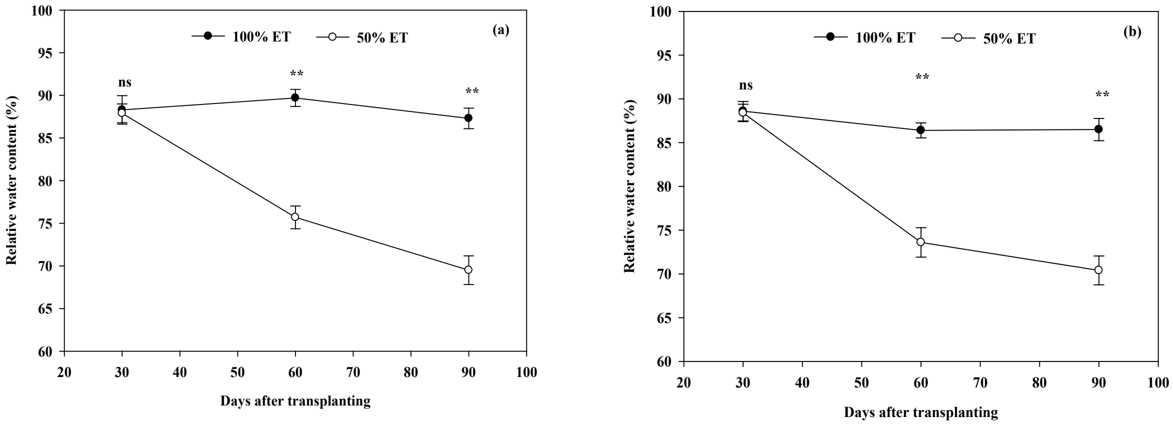

Figure 1. Relative water content of six Jerusalem artichoke genotypes under 100% evapotranspiration (100%ET) and deficit irrigation (50%ET) at 30, 60 and 90 days after transplanting in year 2015/16 (a) and 2016/17 (b). Means of four replications ± SD. ET – evapotranspiration.

Table 1. SPAD chlorophyll meter reading (SCMR) and leaf area of six Jerusalem artichoke
genotypes under 100% evapotranspiration (100%ET) and deficit irrigation (50%ET) conditions at 90 days after transplanting (DAT).

Genotypes	SCMR			Leaf area (cm^2 plant^{-1})		
	100% ET	50%ET	Increase (%)	100% ET	50%ET	Reduction (%)
KT 1	37.90 c	40.12 d	5.49	3,059 a	2,102 b	31.28
KT 2	38.78 c	42.01 d	7.62	3,441 a	2,697 a	21.62
KT 50-4	39.35 c	42.72 c	7.73	2,840 a	2,376 b	16.34
JA 5	40.83 b	43.72 c	6.64	1,972 b	1,433 c	27.33
JA 60	45.47 a	48.76 b	6.76	1,540 b	1,200 c	22.08
JA 125	49.93 a	53.17	6.20	1,584 b	1,250 c	21.09
Mean	42.04	45.08	6.74	24,06	1,843	23.29

Means in the same column with the same letters are not significantly different by LSD (p <0.05).

Table 2. Net photosynthetic rate (P_n) and stomatal conductance (g_s) of six Jerusalem artichoke genotypes under 100% evapotranspiration (100%ET) and deficit irrigation (50%ET) conditions at 90 days after transplanting (DAT).

Genotypes	P_n (μmol m^{-2} s^{-1})			Stomatal conductance [mol (H2O) m^{-2} s^{-1}]		
	100% ET	50%ET	Reduction (%)	100% ET	50%ET	Reduction (%)
KT 1	22.13	19.60 c	11.71	0.14 c	0.07 c	50.00
KT 2	22.30	19.81 c	11.21	0.14 c	0.07 c	50.00
KT 50-4	23.60	21.30 b	9.75	0.14 c	0.08 bc	42.86
JA 5	26.44	23.82 b	9.85	0.24 a	0.09 bc	62.50
JA 60	27.80	25.12 a	9.64	0.19 b	0.15 a	21.10
JA 125	29.30	27.23 a	7.06	0.13 c	0.11 b	15.40
Mean	25.26	22.18	9.87	0.16	0.10	40.31

Means in the same column with the same letters are not significantly different by LSD (p <0.05).

Table 3. Biomass and tuber yield of six Jerusalem artichoke genotypes under 100% evapotranspiration (100%ET) and deficit irrigation (50%ET) conditions at harvest stage.

Genotypes	Biomass (t ha^{-1})			Tuber yield (t ha^{-1})		
	100% ET	50%ET	Reduction (%)	100% ET	50%ET	Reduction (%)
KT 1	4.70 b	2.37 b	49.57	3.50 b	1.60 b	54.29
KT 2	4.71 b	2.80 a	40.55	3.80 a	2.00 b	47.37
KT 50-4	5.21 a	3.21 a	38.39	4.10 a	2.60 a	36.59
JA 5	3.60 c	2.11 c	41.39	3.20 b	1.81 b	43.75
JA 60	3.90 c	2.20 c	43.59	3.10 b	1.60 b	48.39
JA 125	4.00 c	2.31 b	42.25	2.80 c	1.50 b	46.43
Mean	4.35	2.50	42.62	3.42	1.85	46.13

Means in the same column with the same letters are not significantly different by LSD (p <0.05).

Conclusions

The present study revealed a significant response to water deficit for physiological and photosynthetic traits among Jerusalem artichoke genotypes differing in drought resistance levels. In general, drought-resistant genotypes could retain leaf area (LA), net photosynthesis rate (Pn), and stomatal conductance (gs) and increase the SCMR value.

Genotype KT 50-4 showed high LA and low gs, while genotypes JA 60 and JA 125 had high SCMR, Pn and gs. KT 50-4 showed high tuber yield potential, high LA, and low yield reduction under water deficit, being an ideal genotype of Jerusalem artichoke for drought-resistant breeding. These traits could be used as selection criteria for improving actual yield under drought conditions.

References

Doorenbos, J., and W. O. Pruitt. 1992. Calculation of Crop Water Requirement. In Crop Water Requirement. FAO of The United Nation, Rome, Italy, pp. 1-65.

Hoshmand, A. R., 2006. Design of Experiments for Agriculture and the Natural Sciences. Chapman & Hall, Florida

Singh, S., and M. B. Russell. 1981. Water use by maize/pigeonpeas intercrop on a deep vertisol. In: Proceedings of the International Workshop on Pigeonpeas, ICRISAT Center, pp.271–282.

Zhang, M., Q. Chen, S. Shen. 2011. Physiological responses of two Jerusalem artichoke cultivars to drought stress induced by polyethylene glycol. Acta Physiol. Plant. 33: 313–318.

Publication

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3.4 Net photosynthesis rate, transpiration rate, transpiration efficiency performance of Jerusalem artichoke (*Helianthus tuberosus* L.) genotypes under different durations of terminal drought

Chaimala A., S. Jogloy, N. Vorasoot, C.C. Holbrook and C.K. Kvien

The previous reports reveal that short photoperiod along with low temperatures in the late rainy season has led to the crop-enhancing tuber growth rate, inulin accumulation, and quick phenological developmental processes, in comparison to the early rainy season (Puangbut et al., 2015a; 2015b). Unfortunately, most target areas of JA are produced under rainfed areas in which terminal drought is recurrent and frequently occurs at the difference of terminal growth stage.

In water-limited control environments in the greenhouse conditions for JA, drought-resistant traits associated with P_n were attended to study under early drought conditions with prolonged drought periods (Puangbut et al., 2017). Furthermore, the studies of Puangbut et al. (2022) explored the contribution of physiological traits of

SCMR, stomatal conductance (g_s), and P_n to the tuber yield under mid-season drought in field conditions.

No studies have been toward to use of physiological characteristics for P_n, E, and TE that tend to contribute to higher yield potential under optimal conditions and lowest yield reduction under drought conditions in JA genotypes when encountering different terminal drought conditions. Hence, physiological characteristics should be used as surrogate traits for drought tolerance screening along with high yielding, which is required to speed up plant breeding improvement.

Objective

The main focus of this study was to determine the effect of P_n, E, TE, and to investigate the association between these traits with economic yield and HI under different durations of terminal drought in three different groups for drought tolerance of JA genotypes.

Materials and Methods

The experiment was undertaken under field conditions, located at Khon Kaen University during the late rainy season (ranges from October to January) in 2017/18 and 2018/19. The experimental treatments were set up as a split-plot design in four replications. The main plots consisted of three water levels, including optimal conditions (SD0), short duration of terminal drought (SD1), and long duration of terminal drought (SD2). Within each water level, six JA genotypes (HEL256, JA37, HEL253, JA4, JA60, and JA125) were planted as subplots.

Thirteen drip lines with an emitter distance of 30 cm were placed between the rows of the plants and embedded at 10 cm below the soil surface in each subplot. Within each subplot, a plant spacing of 50 x 30 cm between hills was established. Seedling preparations and crop managements were as described by Chaimala et al. (2020). Crop water requirements and soil surface evaporation were calculated to follow as described by Doorenbos and Pruitt (1992) and Singh and Russell (1981), respectively for supplying the water to individual sub-plots every couple of days.

The data were recorded for soil physical and chemical properties, meteorological, soil moisture content, and relative water content. The P_n, E, TE, and SLA were investigated for physiological characteristics. Data were recorded for tuber fresh yield. Analysis of variance for split plot design for all traits. The mean comparison was done by LSD at $P<0.05$ and < 0.01. To establish correlations between the overall parameters, simple linear correlations were established.

Results

The variety and water level had a significant effect on all studied traits (data not shown). The water level was a great source of the total variation for P_n (79.1%), E

(73.3%), TE (38.1%), dry weight of leaf (25.2%), and stem (11.6%). Also, variety contributed to a large portion of the total variation for P_n, E, TE, leaf, and stem dry weight by 12.3%, 13.9%, 42.2%, 48.9%, and 56.9%, respectively. Terms of the decreased values of P_n and E were shown more during encountered long duration.

than short durations of terminal drought. On the one hand, higher increases in TE were found under SD1 than SD2. Corresponding to values in an average of two years, terminal drought-stressed at SD1 reduced P_n by 34% and tended to be more reduced 51% under SD2 compared to SD0 in all JA genotypes (Table 1). Moreover, terminal drought-induced E values dropped from about 42% under SD1 to reached 55% under SD2 of all genotypes compared with SD0. Conversely, TE was induced to raise higher under SD1 than SD2 among six JA genotypes consistently for two years confirming by SD1 has stimulated an increase TE by 15% and whilst increase of 8% under SD2 conditions compared with SD0.

In addition, all genotypes also had highly significant differences in P_n, E, and TE for the two years of three water levels (Table 1). HEL256 and HEL253 exhibited low E and high TE values, all these changes contributed to maintaining a low reduction in tuber fresh weight under SD2, regardless of lower P_n under SD1 and SD2.

The result clearly showed that a high positive correlation was found between P_n and tuber fresh weight (r = 0.76; $p \leq .05$ in 2017/18 and r = 0.85; $p \leq .05$ in 2018/19) at SD1 and the highest positive correlation (r = 0.93; $p \leq .01$ in 2017/18 and r = 0.94; $p \leq .01$ in 2018/19) at SD2, but not at SD0 (Figures 1a and 1b). The correlation coefficient between P_n and the reduction of tuber fresh weight at SD2 was highly significant (r = -0.76; $p \leq .05$ in 2017/18 and r = -0.86; $p \leq .05$ in 2018/19) and negative at SD1 in 2018/19 (Figure 1d) but not at SD1 in 2017/18 (Figure 1c). It was indicated that the selection of genotypes for high P_n might help to improve tuber fresh weight and retain low tuber fresh weight reduction under different drought durations at SD2.

Similarly, a high positive correlation was found between TE and tuber fresh weight at SD2 (r = 0.80; $p \leq .05$ in 2017/18 and r = 0.86; $p \leq .05$ in 2018/19) and between TE and reduction in tuber fresh weight (r = 0.88; $p \leq .05$ in 2017/18 and r = 0.79; $p \leq .05$ in 2018/19) (Figure 2). In this sense, the correlation between TE and tuber fresh weight was not significant at SD0 and SD1 in both years (Figures 2a and 2b). The correlation between TE and reduction of tuber fresh weight was also not significant at SD1 of both years (Figures 2c and 2d). It was pointed out that the selection of genotypes for medium TE might contribute to improving tuber fresh weight and maintaining a low reduction in tuber fresh weight at SD2.

In addition, the correlation between Tr and tuber fresh weight and between Tr and reduction of tuber fresh weight was not significant under three water levels of both years (data not showed).

The result demonstrated that P_n was positively associated with SCMR under different drought duration at SD1 (r = 0.81; $p \leq .05$ in 2017/18 and r = 0.79; $p \leq .05$ in 2018/19) and SD2 (r = 0.82; $p \leq .05$ in 2017/18 and r = 0.83; $p \leq .05$ in 2018/19) (Figure 3). In this case, the association between P_n and SCMR was not significant at SD0 in both years. The results are apparent that improvements in P_n could be accomplished by selecting high SCMR under different drought durations. Furthermore, the high negative association (r = -0.85; $p \leq .05$ in 2017/18 and r = -0.82; $p \leq .05$ in 2018/19) between P_n and SLA at SD1 in both years and not found at SD0 and SD2 of both years (Figure 4). This indicates that the selection of high P_n genotypes at SD1 could be achieved by screening for low SLA.

Table 1. Net photosynthetic rate, transpiration rate, and transpiration efficiency of six Jerusalem artichoke genotypes at 75 days after transplanting planted under three water levels in 2017/18 and 2018/19

Genotypes	**Net Photosynthetic Rate [µmol m^{-2} s^{-1}]**			**Transpiration Rate [mmol (H_2O) m^{-2} s^{-1}]**			**Transpiration Efficiency [mmol (CO_2) mol (H_2O)]**		
	SD0	**SD1**	**SD2**	**SD0**	**SD1**	**SD2**	**SD0**	**SD1**	**SD2**
				2017/18					
HEL256	27.4dA	16.8eB	11.9eC	6.9aA	2.2eB	2.1eB	4.0dC	7.6aA	5.7aB
JA37	30.6bA	19.8cB	7.1fC	6.6cA	4.7aB	1.6fC	4.6cA	4.2eC	4.4bB
HEL253	26.8eA	15.5fB	13.6dC	4.3fA	2.6dB	2.5dB	6.2aA	6.0bB	5.4aC
JA4	25.0fA	22.8aB	19.3aC	6.3eA	4.6bB	4.3aC	4.0dC	4.9dA	4.5bB
JA60	29.9cA	18.4dB	14.9cC	6.5dA	3.7cB	2.8cC	4.6cC	5.0dB	5.3aA
JA125	33.1aA	20.3bB	18.6bC	6.7bA	3.7cB	3.5bC	4.9bB	5.5cA	5.3aA
Mean	28.8	18.9	14.2	6.2	3.6	2.8	4.7	5.5	5.1
F-test	**	**	**	**	**	**	**	**	**
				2018/19					
HEL256	27.0dA	16.5eB	11.7eC	7.1aA	2.5eB	2.2eC	3.8eC	6.6aA	5.3aB
JA37	29.9bA	19.5cB	7.1fC	6.7cA	4.9aB	1.7fC	4.5cA	4.0eB	4.2eB
HEL253	26.6eA	15.4fB	13.3dC	4.5fA	2.9dB	2.6dC	5.9aA	5.3bB	5.1bC
JA4	24.5fA	22.6aB	19.1aC	6.5eA	4.8aB	4.3aC	3.8eC	4.7dA	4.4dB
JA60	29.0cA	18.3dB	14.7cC	6.6dA	3.8cB	2.9cC	4.4dB	4.8cA	5.1bA
JA125	32.9aA	20.3bB	17.1bC	6.9bA	3.8bB	3.6bC	4.8bB	5.3bA	4.7cB
Mean	28.3	18.8	13.8	6.4	3.8	2.9	4.5	5.1	4.8
F-test	**	**	**	**	**	**	**	**	**

** = highly significant at $p \leq 0.01$ by LSD testing the effect of water levels
Means followed by the same lowercase in the vertical and means followed by the same uppercase in the horizontal are no significant differences according to LSD ($p \leq 0.05$)
SD0: Optimal condition, SD1: Short-drought duration, SD2: Long-drought duration

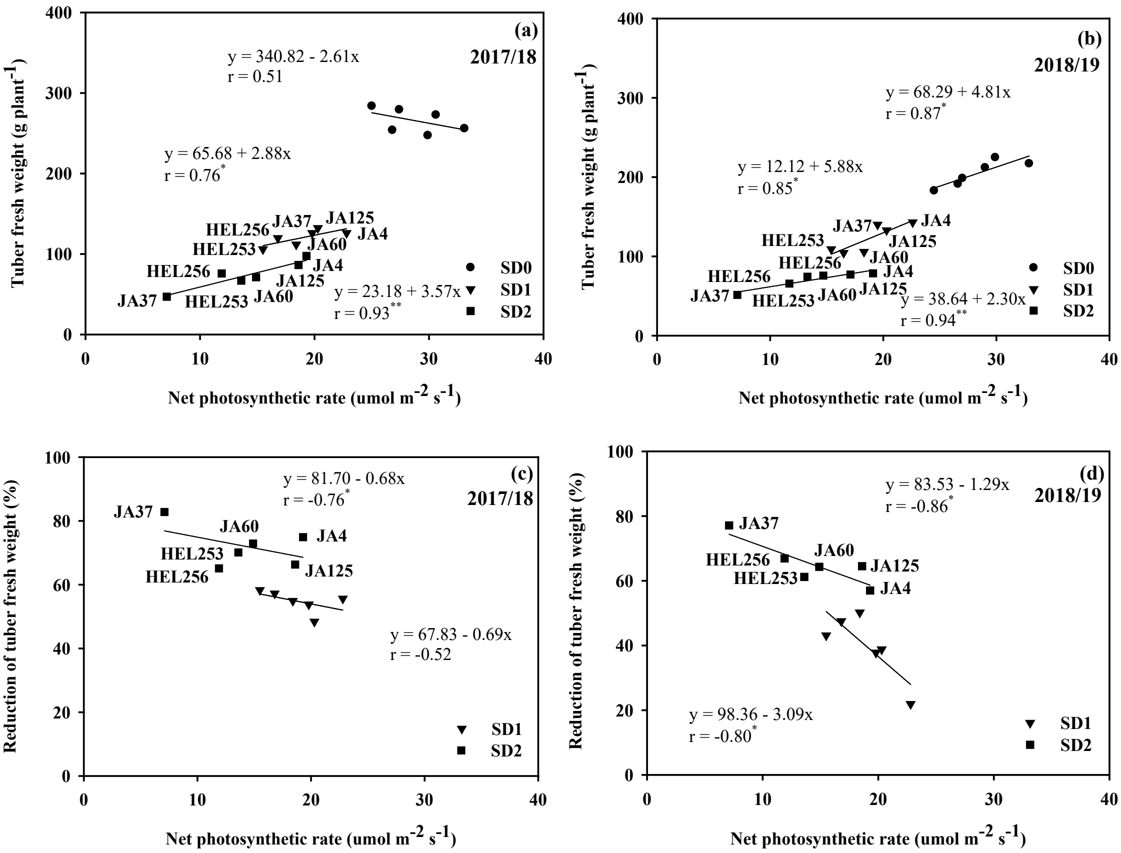

Figure 1. Relationship between net photosynthetic rate with tuber fresh weight (a,b) and reduction of tuber fresh weight (c,d) of six Jerusalem artichoke genotypes under optimal conditions (SD0), short-drought duration (SD1), and long-drought duration (SD2) in 2017/18 and 2018/19. *, ** = significant and highly significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Conclusions

Different drought durations at SD1 and SD2 significantly reduced P_n, Tr, and tuber fresh weight in all Jerusalem artichoke genotypes. All traits studied were more declined at SD2 compared with SD1. By contrast, the values of TE increased at SD1 and SD2. Significant differences in each Jerusalem artichoke genotype for all traits were obtained under both optimal conditions SD0 and different drought durations SD1 and SD2. In our study, JA125 and JA4 exhibited high P_n and tuber fresh weight under different drought durations at SD1 and SD2. High correlations between P_n, tuber fresh weight, and reduction of tuber fresh weight were obtained under different drought durations. The result concluded that drought-tolerant genotypes JA125 and JA4 could be promoted as a potential parental source for generating new progenies with high tuber yield productivity under drought stress. Importantly, SCMR and SLA could be used as physiological traits for indirect selection to improve P_n and drought tolerance in breeding programs for Jerusalem artichoke.

References

Chaimala, A., S. Jogloy, N. Vorasoot, B. Toomsan, N. Jongrungklang, T. Kesmala, C. C. Holbrook and C. K. Kvien. 2020. Responses of total biomass, shoot dry weight, yield and yield components of Jerusalem artichoke (*Helianthus tuberosus* L.) varieties under different terminal drought duration. Agriculture 10: 1-16.

Doorenbos, J. and W. O. Pruitt. 1992. Calculation of Crop Water Requirement. In Crop Water Requirement. FAO of The United Nation, Rome, Italy, pp. 1-65.

Puangbut, D., S. Jogloy, N. Vorasoot and A. Patanothai. 2015a. Growth and phenology of Jerusalem artichoke (*Helianthus tuberosus* L.). Pakistan Journal of Botany 47: 2207-2214.

Puangbut, D., S. Jogloy, N. Vorasoot and A. Patanothai. 2015b. Responses of growth, physiological traits and tuber yield in *Helianthus tuberosus* to seasonal variations under tropical area. Scientia Horticulturae 195: 108-115.

Puangbut, D., S. Jogloy and N. Vorasoot. 2017. Association of photosynthetic traits with water use efficiency and SPAD chlorophyll meter reading of Jerusalem artichoke under drought conditions. Agricultural Water Management 188: 29-35.

Puangbut, D., S. Jogloy, N. Vorasoot and P. Songsri. 2022. Photosynthetic and physiological responses to drought of Jerusalem artichoke genotypes differing in drought resistance. Agricultural Water Management 259: 107252.

Singh, S., and M.B. Russell. 1981. Water use by maize/pigeon peas intercrop on a deep vertisol. In Proceedings of the International Workshop on Pigeonpeas, ICISAT Center Patancheru, Andhra Pradesh, India, 15-19 December 1981; Volume 1.

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This manuscript was expected to publishing in Agronomy MDPI (Impact factor = 3.94: Q1).

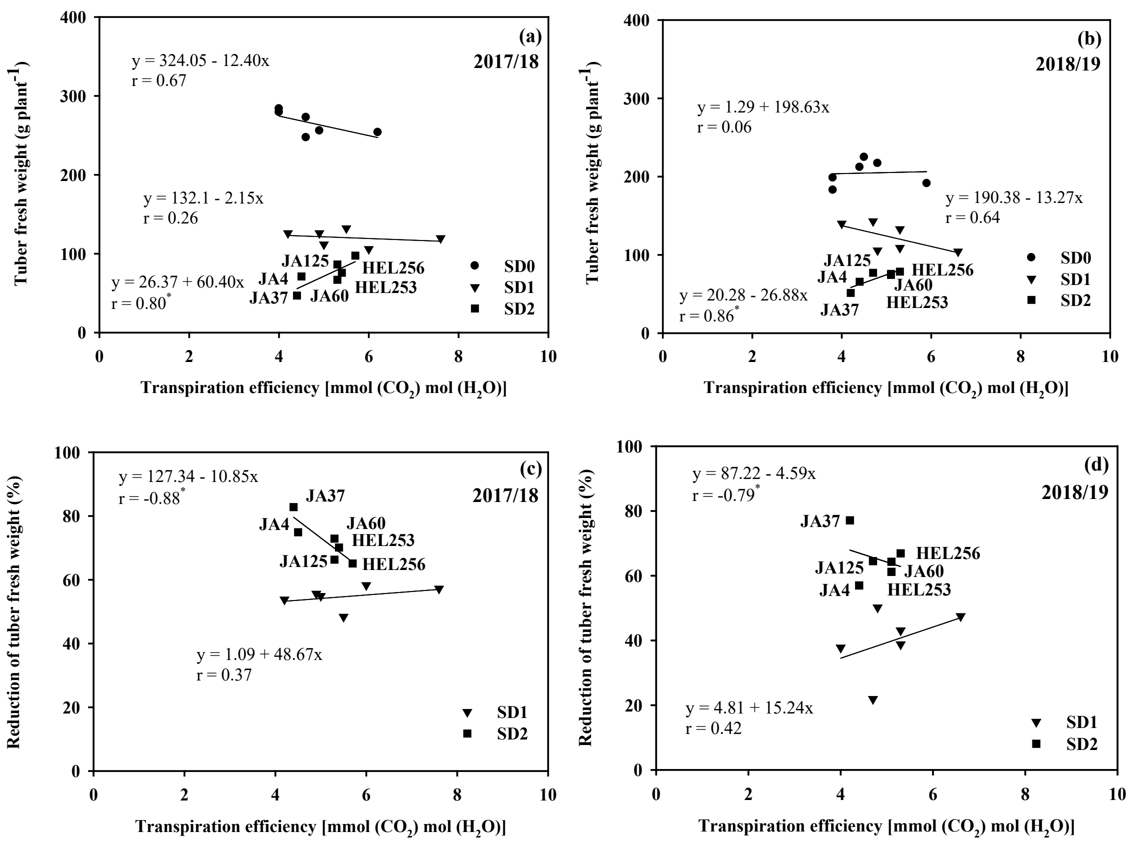

Figure 2. Relationship between transpiration efficiency with tuber fresh weight (a,b) and reduction of tuber fresh weight (c,d) of six Jerusalem artichoke genotypes under optimal conditions (SD0), short-drought duration (SD1), and long-drought duration (SD2) in 2017/18 and 2018/19. * = significant at $p \leq 0.05$

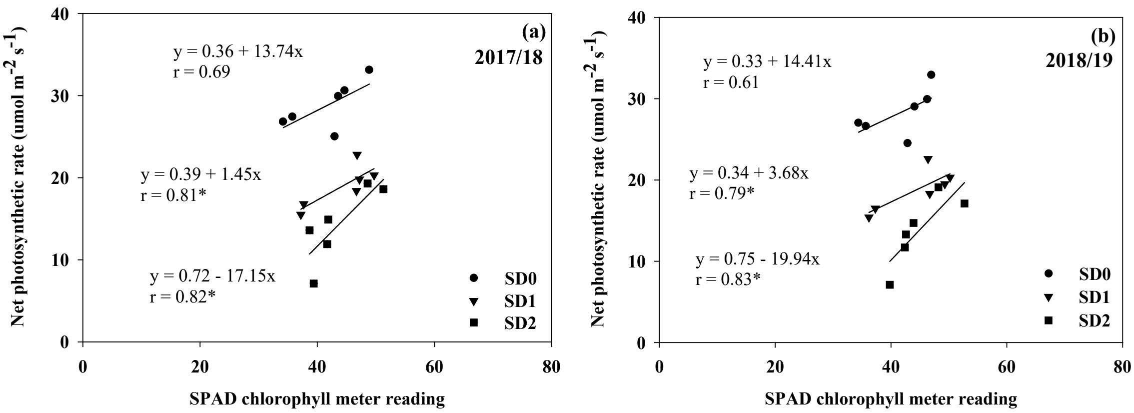

Figure 3. Relationship between net photosynthetic rate with SPAD chlorophyll meter reading of six Jerusalem artichoke genotypes under optimal conditions (SD0), short-drought duration (SD1), and long-drought duration (SD2) in 2017/18 (a) and 2018/19 (b). * = significant at $p \leq 0.05$

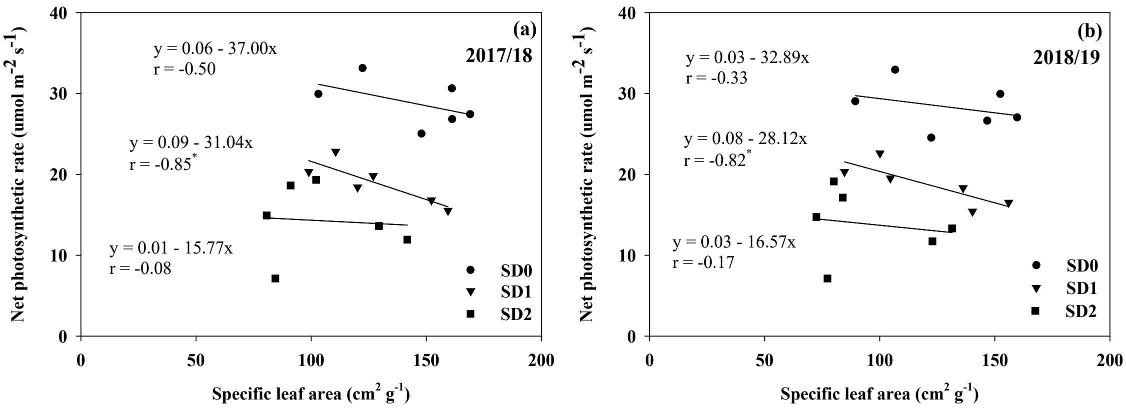

Figure 4. Relationship between net photosynthetic rate with specific leaf area of six Jerusalem artichoke genotypes under optimal conditions (SD0), short-drought duration (SD1), and long-drought duration (SD2) in 2017/18 (a) and 2018/19 (b). * = significant at $p \leq 0.05$

3.5 Inulin content and inulin yield of Jerusalem artichoke (*Helianthus tuberosus* L.) genotypes and relationship to net photosynthesis rate in different of terminal drought duration

Chaimala A., S. Jogloy, N. Vorasoot, C. C. Holbrook and C. K. Kvien

From the report of previous studies, the most suitable season for the high tuber yield and inulin production in Jerusalem artichoke (JA) cultivars is the late-rainy season with irrigation. Unfortunately, terminal drought can occur during the different terminal growth stages at tuber formation (T3) and tuber bulking (T4) of JA genotypes and affect inulin production, particularly in the late rainy season. Where irrigation is not available, drought-tolerant genotypes could help ameliorate drought problems as a sustainability method for enhancing inulin production.

The previous study suggested that drought-tolerant genotypes selected for water use efficiency of inulin yield could contribute to higher inulin yield under limited water conditions (Puangbut et al., 2015). The functional soluble sugars involving sucrose, fructose, and glucose may directly contribute to enhancing the inulin concentration in tolerant genotypes than in more sensitive ones (Valluru and den Ende, 2008). In *Cichorium intybus* (var. sativum), high-efficiency P_n in drought-tolerant plants caused an increase in the accumulation of soluble sugar concentrations and supported high inulin contents (Vandoorne et al., 2012).

Regardless, inulin content and inulin yield are dependent upon many factors such as temperature, genotype, drought duration, and P_n (Monti et al., 2005). Nevertheless, JA genotype responses on commercial inulin content and inulin yield under different durations of terminal drought have not been reported. Literature is also lacking on the association between inulin productions with P_n and SCMR.

Objective

This study aimed to investigate the effect of different durations of terminal drought on inulin content and inulin yield of JA genotypes. We also investigated the associations between inulin productions with P_n and SCMR.

Materials and Methods

Field experiments were performed at Khon Kaen University in the late-rainy season from October to January in 2017/18 and 2018/19. A split-plot design with four replications was used. Three water levels were laid out as main plots. SD0 was an optimal condition. SD1 was drought starting at 60 days after transplanting (DAT) (short duration of terminal drought), and SD2 was drought starting at 45 DAT until harvest (long duration of terminal drought). Six JA genotypes were arranged randomly as the subplots.

The healthy and uniform seedlings were then selected for transplanting to the field experiment. The soil in the experimental plot was prepared by conventional procedures. The plot size was 5 × 6 m and each subplot was comprised of twelve rows with a spacing of 0.5 x 0.3 m. Water was applied to each subplot based on crop water requirements (Doorenbos and Pruitt, 1992) and soil surface evaporation (Singh and Russell, 1981).

The data were recorded for soil physical and chemical properties, meteorological, soil moisture content, and relative water content. The P_n and SCMR were investigated for physiological characteristics as well as tuber fresh and dry weight, inulin content, and inulin yield was determined for plant data.

Results

Significant differences between years (Y), were observed for inulin content ($p < .01$), and inulin yield ($p < .05$) (data not shown). Water level (W) and genotype (G) were also significant ($p < .01$) for all parameters. The interactions between Y × W, Y × G, W × G and Y x W X G were also significant for all parameters. Additionally, the genotype had the greatest contribution (96.8%) to the total variation for inulin content, as well as the water level, had the largest contribution (58.0%), and the genotype had the second-largest contribution (17.5%) to the total variation for inulin yield.

In this study, there were four genotypes with high yield potential (JA125, JA37, JA60, and HEL256) that displayed high inulin content under three water levels for two years. In our study, JA125, JA37, and HEL256 exhibited high inulin yield in both well-irrigated conditions and terminal drought conditions for SD1 and SD2 (Table 1). Furthermore, JA4 and JA60 had high inulin yields under SD2. Even if inulin content was slightly increased either in SD1 or SD2, inulin yield was reduced because of a decrease in tuber dry weight under the terminal droughts.

Inulin content was increased by an average of 2.3% under SD1 and 3.3% under SD2 for years 2017/18, 2.3% under SD1, and 2.9% under SD2 for years 2018/19 when compared with SD0. Inulin yield was decreased under terminal drought-stressed in 2017/18, under SD1 and SD2 where it was reduced by an average of 42.8% and 60.5%, respectively. For 2018/19, inulin yield decreased an average of 39.7% and 62.7% under SD1 and SD2, respectively (Table 1).

Highly significant ($p < .01$) and positive correlations were found between P_n and inulin content only under SD0 (r = 0.92 in years 2017/18 and r = 0.88 in years 2018/19 (Fig. 1a and 1d). Positive relationships and significance ($p < .05$) were observed between SCMR and inulin content under SD1, r = 0.73 and r = 0.63 in the years 2017/18 and 2018/19, respectively (Fig. 2b and 2e). Similarly, a positive correlation between SCMR and inulin content was also observed under SD2, r = 0.72 ($p < .05$) in the years 2017/18, but not in the years 2018/19 (Fig. 2c and 2f).

Table 1. Inulin content and inulin yield of six Jerusalem artichoke genotypes at harvest under three water levels observed in the years 2017/18 and 2018/19.

Genotypes	Inulin content (%)			Inulin yield (t ha^{-1})		
	SD0	SD1	SD2	SD0	SD1	SD2
2017/18						
HEL256	70.9d	71.1d	72.2d	331.7a	168.2ab	120.8a
JA37	74.5b	76.0b	76.2b	343.1a	198.3a	145.1a
HEL253	59.7e	59.5f	59.9f	181.3c	136.6b	59.4b
JA4	57.9f	63.1e	63.6e	308.2ab	139.8b	126.7a
JA60	72.1c	73.9c	74.4c	252.5b	149.2b	115.1a
JA125	78.8a	80.1a	81.6a	340.1a	212.0a	127.3a
Mean	69.0C	70.6B	71.3A	292.8A	167.4B	115.7C
F-test	**	**	**	**	**	**
2018/19						
HEL256	71.9d	72.1d	73.2d	291.3b	235.6a	106.9ab
JA37	75.3b	77.0b	77.0b	468.8a	206.3ab	123.7a
HEL253	59.8e	60.2f	60.4f	185.6c	113.8c	71.3b
JA4	59.2e	63.3e	63.8e	184.3c	164.8b	122.9a
JA60	73.1c	74.4c	74.5c	294.5b	95.1c	102.6ab
JA125	79.0a	80.7a	81.6a	327.8b	241.4a	126.7a
Mean	69.7C	71.3B	71.7A	292.1A	176.2B	109.0C
F-test	**	**	**	**	**	*

Note: SD0 = no drought; SD1 = drought from 60 DAT; SD2 = drought from 45 DAT until harvest. *, ** indicated significant and highly significant at $p \leq .05$ and $p \leq .01$ probability levels, respectively. Means followed by the same small letter in a column and means followed by a capital letter in each row were not significantly different by the least significant difference at $p < .05$ probability level.

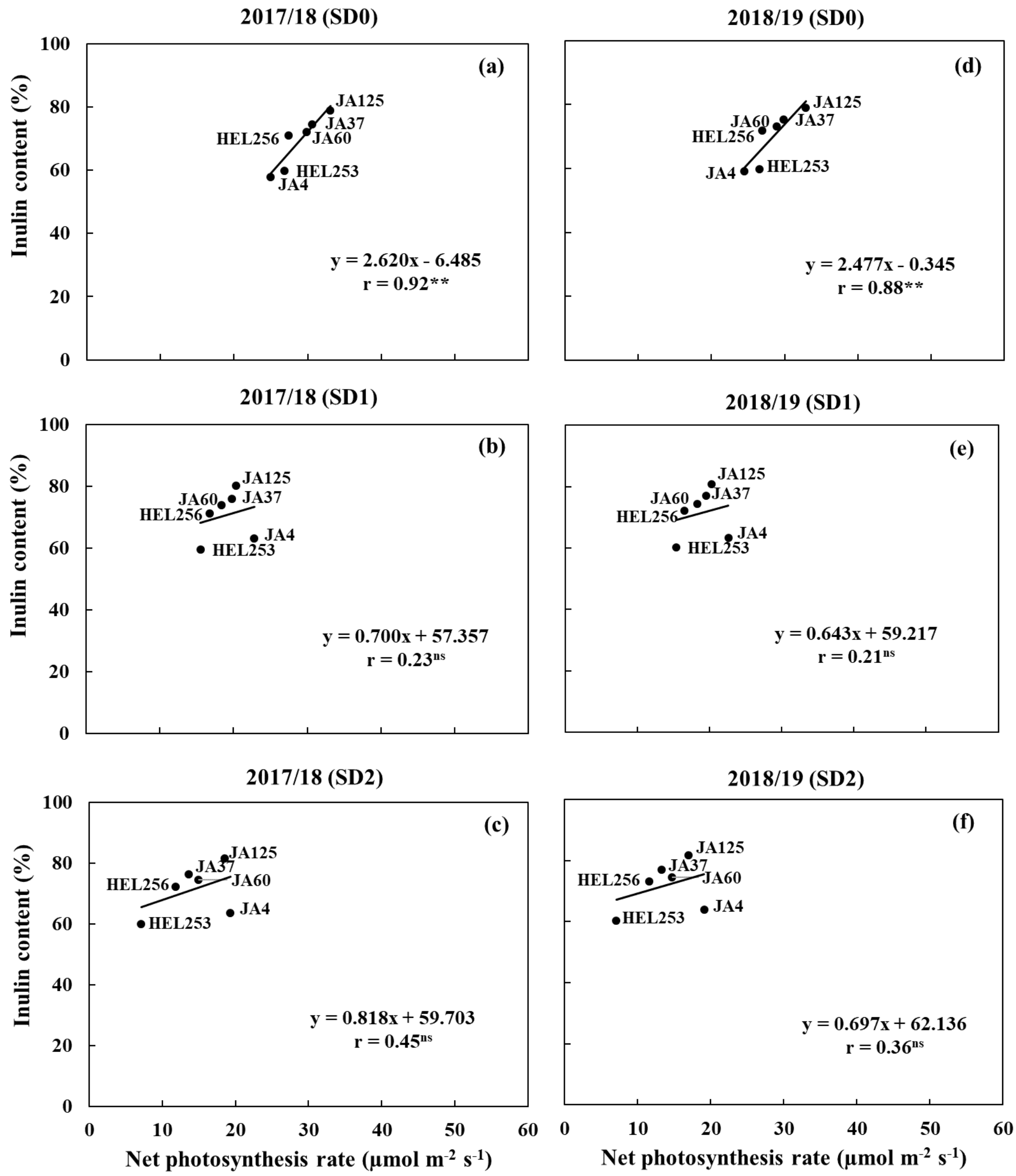

Figure 1. Relationship between net photosynthetic rate and inulin content of six Jerusalem artichoke genotypes under three irrigation treatments (SD0 = optimal conditions; SD1 = short duration of terminal drought; SD2 = long duration of terminal drought) in 2017/18 (a-c, respectively) and 2018/19 (d-f, respectively). r = correlation coefficients (n = 6). ns, ** = not significant and highly significant at $p < .01$, respectively.

Current results of the correlations implied that P_n has putatively contributed to increasing inulin content under optimal conditions. SCMR had a positive contribution to increased inulin concentration under SD1.

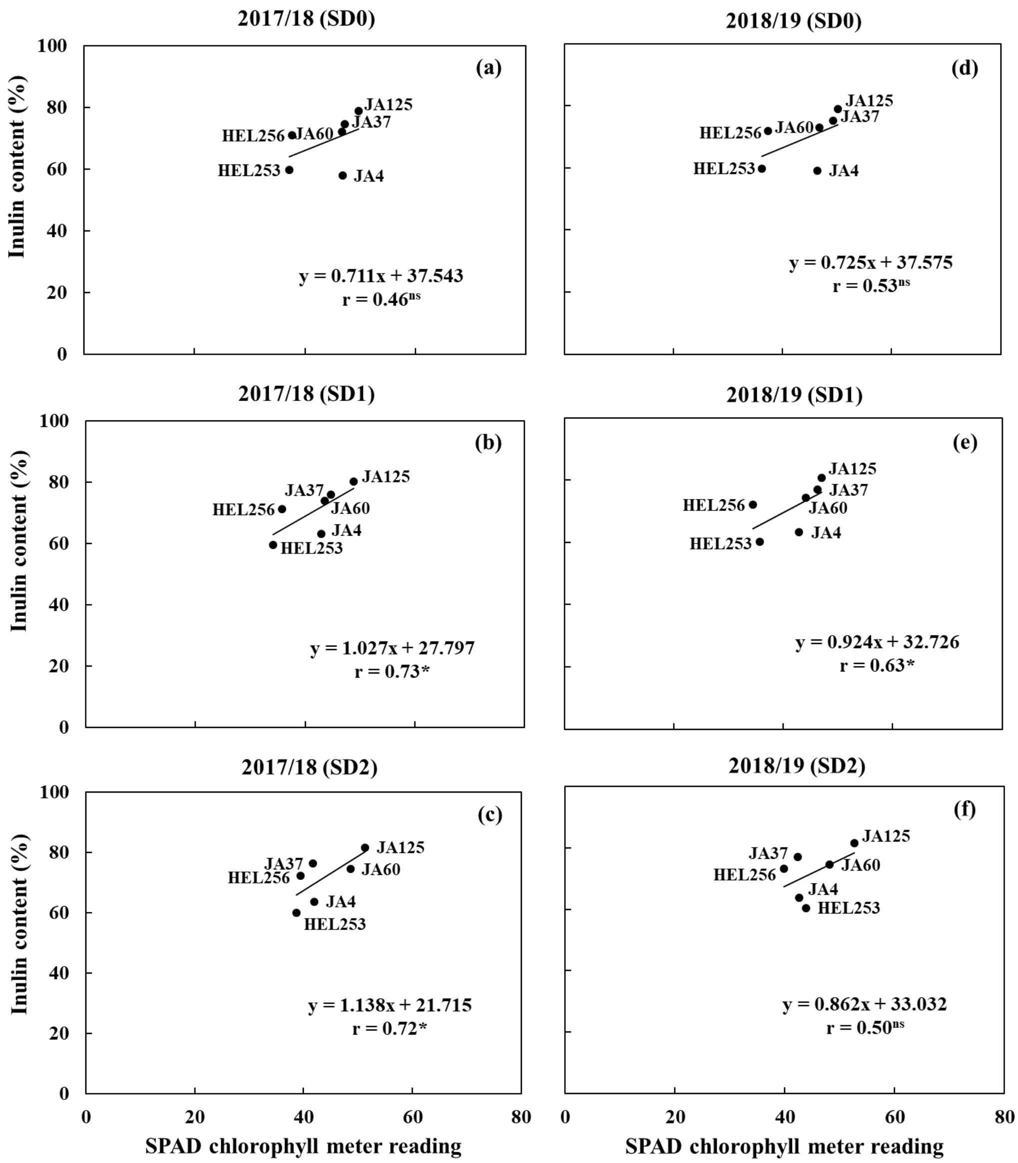

Figure 2. Relationship between SPAD chlorophyll meter reading and inulin content of six Jerusalem artichoke genotypes cultivated under three irrigation treatments (SD0 = optimal conditions; SD1 = short duration of terminal drought; SD2 = long duration of terminal drought), in 2017/18 (a-c, respectively) and 2018/19 (d-f, respectively). r = correlation coefficients (n = 6). ns, * = not significant and significant at $p < 0.05$, respectively.

Conclusion

The results showed that SD1 and SD2 conditions significantly enhanced the inulin content of all genotypes while inulin yield was diminished for two years. The physiological traits for P_n and SCMR were correlated with inulin content and inulin yield and could be used as surrogate traits to select drought-tolerant genotypes. Four genotypes of HEL256, JA37, JA60, and JA125 showed high P_n and SCMR with a significant positive correlation with inulin content under SD0 and SD1, respectively. Among these genotypes, JA37, JA4, JA60, and JA125 exhibited high P_n resulting in high inulin yield under SD2. Among these genotypes, JA37, JA4, JA60, and JA125 exhibited high P_n resulting in high inulin yield under SD2. The current study indicated that SCMR could be beneficially applied as a surrogate trait to identify drought-tolerant genotypes with high inulin accumulation under SD1.

References

Doorenbos, J., and W. O. Pruitt. 1992. Calculation of Crop Water Requirement. In Crop Water Requirement. FAO of The United Nation, Rome, Italy, pp. 1-65.

Monti, A., M. T. Amaducci, G. Pritoni and G. Venturi. 2005. Growth, fructan yield, and quality of chicory (*Cichorium intybus* L.) as related to photosynthetic capacity, harvest time, and water regime. Journal of Experimental Botany 56: 1389-1395.

Puangbut, D., S. Jogloy, N. Vorasoot, S. Srijaranai, C. C. Holbrook and A. Patanothai. 2015. Variation of inulin content, inulin yield and water use efficiency for inulin yield in Jerusalem artichoke genotypes under different water regimes. Agricultural Water Management 152: 142-150.

Singh, S., and M. B. Russell. 1981. Water use by maize/pigeon peas intercrop on a deep vertisol. In Proceedings of the International Workshop on Pigeonpeas, ICISAT Center Patancheru, Andhra Pradesh, India, 15-19 December 1981; Volume 1.

Valluru, R., and W. V. den Ende. 2008. Plant fructans in stress environments: emerging concepts and future prospects. Journal of Experimental Botany 59: 2905-2916.

Vandoorne, B., A. S. Mathieu, W. V. den Ende, R. Vergauwen, C. Périlleux, M. Javaux and S. Lutts. 2012. Water stress drastically reduces root growth and inulin yield in *Cichorium intybus* (var. sativum) independently of photosynthesis. Journal of Experimental Botany 63: 4359-4373.

Publication

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3.6 Identification of pathogenic fungal species causing leaf spot disease of Jerusalem Artichoke in Thailand

Viriyasuthee W., S. Saepaisan, W. Saksirirat, M. L. Gleason, R. S. Chen and S. Jogloy

Jerusalem artichoke (*Helianthus tuberosus* L.) currently becomes an important crop for the production of healthy food as it contains inulin in its tubers which is regarded as a dietary fiber (Kays and Nottingham, 2008). Jerusalem artichoke is now distributed to all parts of the world including in the tropics such as in Thailand. However, the production of Jerusalem artichoke in the tropics faces severe yield loss caused mainly by diseases (Sennoi et al., 2010). Under growing conditions in Thailand, leaf spot is a newly emerging disease of Jerusalem artichoke in the tropical region (Viriyasuthee et al., 2019). The disease is new to Jerusalem artichoke growers, phytopathologists, and agronomists and the authors did not find a report on the disease in the open literature.

Objective

The objective of this study is to examine the relationship between disease resistance parameters and plant ages in Jerusalem artichoke.

Materials and Methods

Collection and Isolation

Leaves of Jerusalem artichoke that showed symptoms of leaf spot were collected from four locations in Thailand including Field Crop Research Station, Khon Kaen University, Khon Kaen during the rainy season 2014, Nong Ruea, Khon Kaen (Northeast of Thailand), Phatthana Nikhom, Lopburi, Mung, Samutsakhon (Central Plain of Thailand) during rainy season 2017. Isolation of fungal pathogens was conducted mostly using the tissue transplanting method.

Pathogenicity test

Two methods for pathogenicity tests including inoculation with mycelium and inoculation with conidial suspensions were used in this study. Nine isolates from Jerusalem artichoke were used for mycelium inoculation. Healthy leaves were detached from Khon Kaen University research farm. Five-day-old mycelial plugs colonized (5 mm diameter) were placed on a single detached leave and a total of five plugs were used for each isolate. Non-colonized PDA plugs were also used as controls. The symptomatic leaves were re-isolated as previously described to confirm Koch's postulates.

Three isolates including KK1, KK2 and KK3 were used for inoculation of Jerusalem artichoke using conidial suspensions. Healthy leaves were detached from the field, and they were inoculated with conidial suspensions at a concentration of 1×10^5

conidial/ml. The suspension was sprayed on the leaves. Sterilized distilled water was also used as control. The symptomatic leaves were re-isolated as previously described to confirm Koch's postulates.

Morphological characterization

The colonies from single spores were used for morphological characterization of the pathogen. Purified fungal colonies from each isolate were cut by cork borer and transferred onto plastic petri dishes containing half PDA. The sample was incubated at 22 °C under cool white fluorescent light with 8/16 h periods of light/dark for 7 days and sizes of the colonies were then examined. The colonies apparatus were examined including the diameter of cultures.

Molecular analysis

DNA extraction

Each isolate of the fungus that infected Jerusalem artichoke was cultured on PDA and incubated at 25°C for 5 days. After incubation, the active mycelia were used for DNA preparation using Prepman Ultra reagent (Applied Biosystem) following the manufacturer's recommendations for fungal DNA isolation.

Polymerase chain reaction conditions and Sequence

The polymerase chain reaction (PCR) was carried out to amplify four loci. The ITS region was amplified with the primers ITS1 and ITS4. The RPB2 region was amplified with RPB2-5F2 and fRPB2-7cR. and the TEF1 gene was amplified with the primers EF1-728F and EF1-986R.

The PCR reaction for each primer pair was performed in Thermal cycler with a total volume of 25 µL. PCR mixtures for ITS, TEF and RBP2 loci consisted of 2 µL genomic DNA, 1X GoTaq® Flexi buffer (Promega Corporation, USA), 2 mM $MgCl_2$, 20 µM of each dNTP, 0.1 µM of each primer and 0.5 Unit GoTaq® DNA Polymerase (Promega Corporation, USA). Conditions for PCR amplification consisted of an initial denaturation step of 2 min at 95 °C followed by 30 cycles of 1 min at 94°C, 1 min at 58°C, 1 min 72°C and 5 min at 72°C for ITS. For RBP2 and TEF PCR amplification started at the step of 5 min at 95°C followed by 34 cycles of 1 min at 95 °C, 2 min at 60°C, 1.5 min 72°C and 10 min at 72°C.

PCR products were verified by electrophoresis in 1% agarose gels in 1.0 TBE buffer stained with ethidium bromide. The gels were visualized under UV light using a UV transilluminator. The PCR products were purified using a purified kit (GFX PCR DNA and Gel Band) and sequenced by DNA facility at Iowa State University. DNA sequencing was run by Applied Biosystems 3730xl DNA Analyzer. The DNA sequences were compared with DNA sequences in a database of GenBank using the nucleotide Blast program and the alignment was refined using the Bioedit program.

Results

Symptoms of leaf spot and Isolation of Alternaria sp.

The disease caused severe damage to leaves. For Khon Kaen University, Khon Kaen during the rainy season of 2014, The disease symptom first developed a small yellow spot and spread on leave all plants. Later that spot developed to be a small brown spot and a yellow halo appeared around the spot (Figure 1. A-B). The spots expanded and fused. The lower leaves were defoliated but still stick on the plant. The symptoms started from the lower leaves and spread to the top leaves. The fungal agents were collected in 4 isolations include with KK1, KK2, KK3 and KK5. For Nong Ruea, Khon Kaen during the rainy season of 2017, The lesions showed a small brown spot and yellow halo some of the wounds fused (Figure 1. C-D). The fungal agents were collected in 2 isolations include with KK6 and KK7. For Phatthana Nikhom, Lopburi during the rainy season of 2017, the symptom developed from the margin of the leaf and brown spot. The fungal agents were collected in 2 isolations include with LBR1 and LBR2. For Mung, Samutsakhon during the rainy season of 2017, the symptom showed pretty similar with Khon Kaen University, Khon Kaen area but lower leaves weren't defoliated (Figure 1. E). The fungal agent was collected 1 isolation that was BK2. Leaf damage by the disease reduced photosynthetic area and ultimately reduced yield.

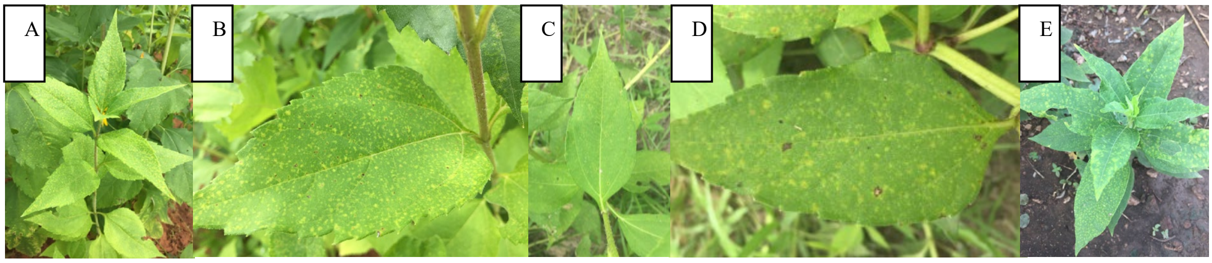

Figure 1. The symptom of leaf spot disease in Jerusalem artichoke. A-B Khon Kaen University, Khon Kaen, C-D Nong Ruea, Khon Kaen and E Mung, Samutsakhon

Pathogenicity test

Inoculation with mycelium caused the disease symptoms on nine isolates of Jerusalem artichoke. For 4 isolations that were collected during the rainy season of 2014, the Pathogenicity test did in the same year. At early as three days after inoculation, the leaves inoculated with mycelium showed disease symptoms, whereas the leaves inoculated with blank media control did (Figure 2. A). Re-isolation of the same pathogen yielded similar results. For 5 isolations that were collected during the rainy season of 2017, the Pathogenicity test did in the same year. The results from the pathogenicity test showed disease symptoms and re-isolation got the same pathogen when inoculated. Inoculation with conidial suspension that in 2014 developed the symptoms on the detached leaves five days after inoculation and on the intact plants seven days after inoculation. The symptoms with dark brown spots and yellow halo observed on the detached leaves were similar to those observed in the fields, indicating the putative pathogen (Figure 2. B). The same fungus used to inoculate was re-isolated from the newly formed leaf spots confirming Koch's postulates.

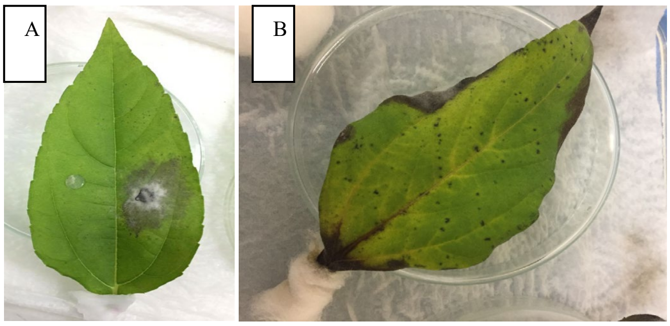

Figure 2. Pathogenicity test of leaf spot disease in Jerusalem artichoke. A inoculation with mycelium, B inoculation with conidial suspension

Morphological and culture

The resulting isolates were cultured on half potato dextrose agar medium for 7 days at 22 °C under cool white fluorescent light with 8/16 h periods of light/dark. The sizes of the culture varied from 6.8-1.5 cm (diameter) (Figure 3).

Sequence analysis

The resulting sequences from ITS, RPB2 and TEF were divided into three groups. The first group included KK1, KK3, KK5, KK7 and BK2 which were closed with *A. alternata*. The second group included KK6 and KK2 This group was clustered with *A. tenuissima*. The last group consisted of LBR1 and LBR2 which were grouped with *A. helianthi*. (Table 1).

Conclusion

To our knowledge, this is the first report of *A. alternate*, *A. tenuissima* and *A. helianthi* causing a leaf spot disease in Jerusalem artichoke. *Alternaria* sp. causing leaf spot has diversity in Thailand.

References

Kays, S.J. and Nottingham, S.F. Biology and Chemistry of Jerusalem Artichoke Helianthus tuberosus L.; Taylor & Francis: Boca Raton, FL, USA, 2008.

Sennoi, R., S. Jogloy, W. Saksisirat and A. Patanothi. 2010. Pathogenicity test of *Sclerotium rolfsii*: A causal agent of Jerusalem artichoke (*Helianthus tuberosus* L.) stem rot. Asian Journal of Plant Sciences, 9(5): 281–284.

Viriyasuthee W., Saksirirat W., Saepaisan S., Gleason M.L., and Jogloy S. 2019. Variability of Alternaria leaf spot resistance in Jerusalem artichoke (*Helianthus tuberosus* L.) accessions grown in a humid tropical region. Agronomy 9, 286.

Manuscript

Viriyasuthee W., S. Saepaisan, W. Saksirirat, M.L. Gleason, R.S. Chen and S. Jogloy. Identification of pathogenic fungal species causing leaf spot disease of Jerusalem Artichoke in Thailand. Agronomy (Impact factor 2.259 Q1)

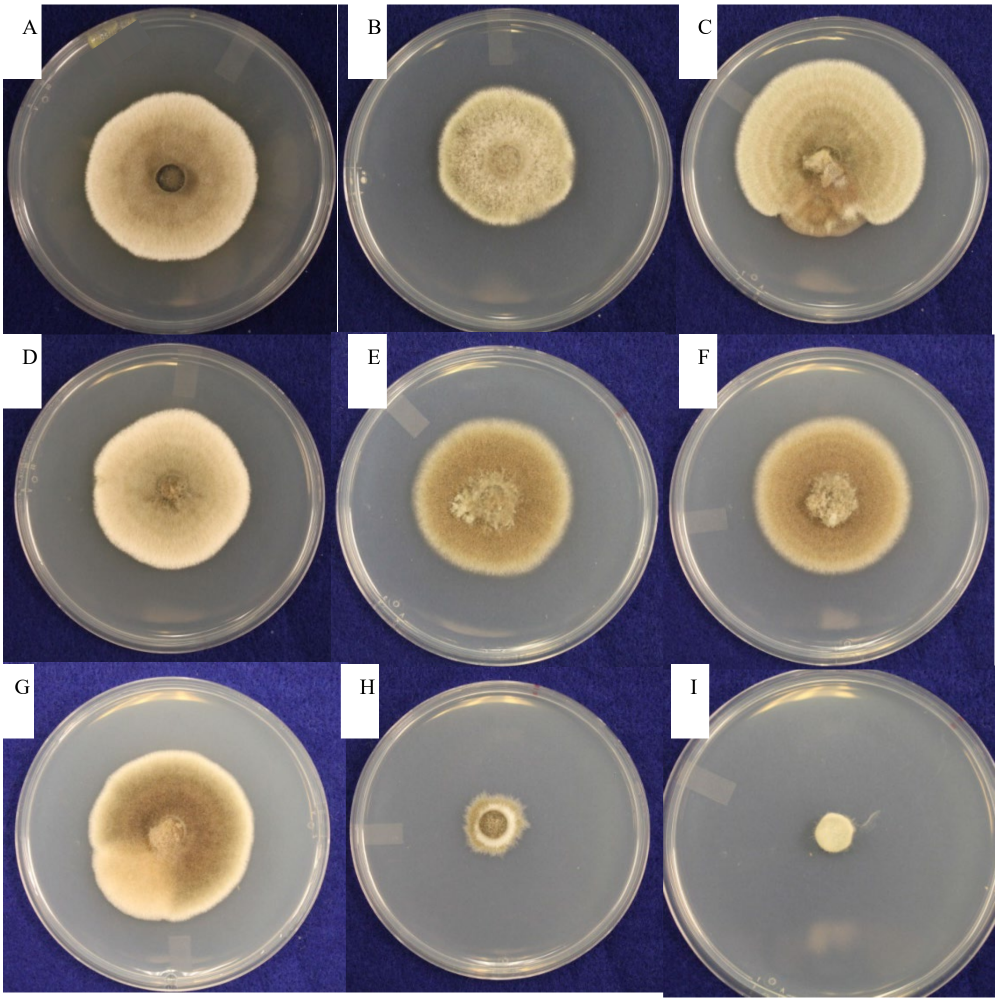

Figure 3. Cultures of *Alternaria* spp. A KK1 (Ø 5.9cm), B KK2 (Ø 4.5cm), C KK3 (Ø 6.8cm), D KK5 (Ø 5.5cm), E KK6 (Ø 5.6cm), F KK7 (Ø 5.3cm), G BK2 (Ø 5.7cm), H LBR1 (Ø 2.3cm), I LBR2 (Ø 1.5cm).

Table 1. Identification of fungal isolation between morphological characteristic and molecular techniques.

Isolations	Identity			
	Morphological characteristic	Molecular techniques(accession number from GenBank)		
		ITS	RBP2	TEF
KK1	*A. alternata*	*Alternaria* sp. (KR094462)	*A. tenuissima* (LC134327)	*A. alternata* (MF741188)
KK2	*A. tenuissima*	*Alternaria* sp. (HQ630970)	*A. tenuissima* (LT707523)	*A. tenuissima* (LC136865)
KK3	*A. alternata*	*Alternaria* sp. (AY154699)	*A. tenuissima* (KY230164)	*A. alternata* (MF741188)
KK5	*A. alternata*	*Alternaria* sp. (HQ630970)	*A. tenuissima* (LT707523)	*A. alternata* (MF741188)
KK6	*A. tenuissima*	*Alternaria* sp. (AY154699)	*A. tenuissima* (KY230164)	*A. tenuissima* (LT707524)
KK7	*A. alternata*	*Alternaria* sp. (AY154699)	*A. tenuissima* (KY230164)	*A. alternata* (LC132709)
BK2	*A. alternata*	*Alternaria* sp. (KR094462)	*A. tenuissima* (LT707523)	*A. alternata* (MF741188)
LBR1	*A. helianthi*	*A. helianthi* (AY154713)	*A. helianthi* (KC609349)	-
LBR2	*A. helianthi*	*A. helianthi* (AY154713)	*A. helianthi* (KC609349)	-

3.7 Variability of Alternaria leaf spot resistance in Jerusalem artichoke (*Helianthus tuberosus* L.) accessions grown in a humid tropical region

Viriyasuthee W., W. Saksirirat, S. Saepaisan, M. L. Gleason and S. Jogloy

Alternaria leaf spot is an emerging disease of Jerusalem artichoke (*Helianthus tuberosus* L.) in tropical regions. Methods for control of leaf spots incited by Alternaria species have been investigated in sunflowers and many other crops. The disease can be controlled by several methods such as the use of resistant varieties, chemical control by fungicide applications (Mesta et al., 2011), and biological control (Liu et al., 1995). However, the lack of known resistant germplasm sources is an important constraint to the development of Jerusalem artichoke varieties with resistance to Alternaria leaf spots.

Objectives

The objectives of this study were to identify genotype variability of Jerusalem artichoke genotypes for resistance to Alternaria leaf spot under field conditions and to investigate the relationships among resistance characters, yield, and yield components for the selection of resistant varieties.

Materials and Methods

Ninety-six accessions of Jerusalem artichoke were received from the North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, USA, the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Stadt, Seeland, Germany, the Plant Gene Resources of Canada (PGRC) Agriculture and Agri-Food Canada, Saskatoon, Sasketchewan, Canada, and a commercial variety from Khon Kaen University, Khon Kaen, Thailand. These accessions were evaluated in a randomized complete block design (RCBD) with three replications in the early rainy season from March to June 2014 and the late rainy season from September to December 2014 at the experimental farm, Faculty of Agriculture, Khon Kaen University.

Disease score was assessed using the method described by Mayee and Datar, (1986)

for Alternaria leaf blight, where 0 = leaves free from infection, 1 = small irregular spots covering the leaves, 3 = small irregular brown spots with concentric rings covering 1%–10% leaf area, 5 = lesions enlarging, irregular brown with concentric rings covering 11%–25% leaf area, 7 = lesions coalesce to form typical blight symptoms covering 26%–50% leaf area, and 9 = lesions coalesce to typical blight symptoms covering >51% leaf area.

Disease incidence (DI) was calculated as follows Anfok, (2000)

$$DI\ (\%) = (\text{number of infected plants} / \text{total number of plants}) \times 100$$

The disease severity index (DSI) was calculated as follows Anfok, (2000)

$$\text{DSI (\%)} = \Sigma\,[(\text{rating score} \times \text{number of plants in rating}) / (\text{total number of sampled plants} \times \text{highest rating})] \times 100$$

The area under the disease progress curve (AUDPC) was calculated for disease incidence (AUDPC-DI) and disease severity index (AUDPC-DSI) over time from 31 to 82 days after transplanting using the formulae as follows Ojiambo et al. (1998)

$$\text{AUDPC} = \Sigma\,[(X_i + X_{i+3}) / 2] \times (t_{i+3} - t_i)$$

where X_i is disease incidence or disease severity on a day i, X_{i+3} is disease incidence or disease severity on day i+3, t_i is disease incidence or disease severity assessment on day i, and t_{i+3} is disease incidence or disease severity assessment on day i and i+3.

The plants in each plot, without border row plants, were harvested at maturity.

Three plants in each plot were sampled randomly from harvested plants and used for the determination of yield components (number of tubers/ plant and tuber size).

Results

Data from three disease resistance groups were selected for presentation (Tables 1 and 2). Disease incidence of testing entries in the early rainy season ranged from 0 to 100% with an average of 88.2% (Table 1) and ranged from 0 to 100% with an average of 59.7% in the late rainy season (Table 2). The disease severity index in the early rainy season ranged from 0 to 77.8% with an average of 38.7% ranging from 0 to 100% with an average of 11.1% in the late rainy season. Disease scores in the early rainy season ranged from 0 to 7 with an average of 3.5 and ranged from 0 to 9 with an average of 1.0 in the late rainy season. AUDPC-DI in the early rainy season ranged from 350 to 4571 with an average of 1679 and ranged from 0 to 3250 with an average of 1344 in the late rainy season. AUDPC-DSI in the early rainy season ranged from 150 to 2655 with an average of 580 and ranged from 0 to 3072 with an average of 274 in the late rainy season.

In the early rainy season, Jerusalem artichoke accessions could be classified into distinct groups based on reaction to the disease. The selected resistant group included HEL335, JA86, HEL256, HEL317, JA20, and HEL308 and the susceptible group consisted of JA132, JA19, HEL288, JA95, HEL293, JA2, and HEL246 (Table 1). In the late rainy season, the selected resistant group comprised JA86, HEL256, HEL335, HEL308, JA15, and HEL317, and the susceptible group included JA5, JA117, JA95, JA93, JA109, HEL293, and HEL246 (Table 2).

Five Jerusalem artichoke genotypes showed low disease parameters for both seasons HEL335, HEL256, HEL317, HEL308, and JA86 (Tables 1 and 2).

For yield and yield components, the number of tubers/plants in the early rainy season ranged from 6 to 89 with an average of 29 (Table 3) and the number of tubers in the late rainy season ranged from 14 to 78 with an average of 34 (Table 4). Tuber size in the early rainy season ranged from 1.5 to 15.6 g/tuber with an average of 5.7 g/tuber and ranged from 3.6 to 44.1 g/tuber with an average of 17.5 g/tuber in the late rainy season. Tuber yield in the early rainy season ranged from 28.1 to 365.2 g/plant with an average of 149.3 g/plant, and tuber yields in the late rainy season ranged from 123.6 to 913.6 g/plant with an average of 493.9 g/plant.

In the early rainy season, JA9, JA8, JA18, JA116, JA46, JA27, JA58, JA49, JA59, and JA71 formed a group with low yield and yield components, whereas HEL243, JA134, JA15, JA6, HEL280, HEL257, JA123, JA122, HEL278, and JA95 formed a group with high yield and yield components (Table 3). In the late rainy season, JA21, JA76, JA27, JA35, JA22, JA6, JA9, JA49, JA59, and JA117 were classified as the group with low yield and yield components, whereas JA129, JA60, JA111, JA58, HEL278, JA102, JA120, HEL65, HEL280, and JA37 were classified as the group with high yield and yield components (Table 4).

Conclusions

Variation of Jerusalem artichoke genotypes for Alternaria leaf spot was grouped into three groups including resistant, moderately resistant, and susceptible. HEL335, HEL256, HEL317, HEL308, and JA86 were resistant genotypes and HEL 293 and HEL 246 were classified as susceptible genotypes. These groups can be used as sources of resistance and susceptible check, respectively, for the breeding of leaf spot disease resistance. HEL278 and HEL280 had the highest yield and yield components in both seasons. These genotypes can be used as sources for breeding programs to improve yield and yield components in Jerusalem artichoke. Selection of Jerusalem artichoke for high yield and desirable yield components with Alternaria resistance is possible because of no correlation between agronomic traits with leaf spot disease resistance.

References

Anfok, G. H., 2000. Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum*. Mill cv. Vollendung) to cucumber mosaic virus. Crop Protection 19: 401-405.

Liu, D., N. A. Anderson, and L. L. Kinkel. 1995. Biological control of potato scab in the field with antagonistic *Streptomyces scabies*. Phytopathology 85: 827-831.

Mayee, C. D., and V. V. Datar. 1986. Sunflower diseases. Parabhan, India: Marathwad Agricultural University.

Mesta, R. K., V. I. Benagi, S. Kulkarni and M. P. Basavarajappa. 2011. Management of Alternaria blight of sunflower through fungicides. Karnataka Journal of Agricultural Science 24: 149-152.

Ojiambo, P. S., R.D Narla, P.O. Ayiecho, and J. O. Nyabundi. 1998. Effect of infection level of sesame (*Sesamum indicum* L.) seed by *Alternaria sesame* on severity of Alternaria leaf spot. Tare 1: 125-130.

Table 1. Selected varieties of resistant, moderately resistant and susceptible Jerusalem artichoke evaluated in early rainy seasons.

Groups	No	Varieties	DI (%) [a,b]	DSI (%) [a,b]	Score [a,b]	AUDPC-DI [b]	AUDPC-DSI [b]
Resistant	1	HEL335	0.0 C	0.0 H	0.0 E	600 SU	167 Q-S
	2	JA86	33.3 BC	18.5 E-H	1.7 DE	800 Q-U	322 L-S
	3	HEL256	33.3 BC	18.5 E-H	1.7 DE	650 R-U	194 Q-S
	4	HEL317	33.3 BC	3.7 GH	0.3 E	1183 L-U	331 L-S
	5	JA20	33.3 BC	11.1 F-H	1.0 DE	889 O-U	248 P-S
	6	HEL308	33.3 BC	18.5 E-H	1.7 DE	350 U	150 S
	1	JA12	66.7 AB	37.0 B-G	3.3 C-E	1133 L-U	448 G-S
Moderately resistant	2	[JA102× JA89]-8	66.7 AB	22.2 C-H	2.0 C-E	2528 D-K	599 D-P
	3	HEL280	66.7 AB	29.6 B-G	2.7 C-E	1081 M-U	346 L-S
	4	JA134	66.7 AB	29.6 B-G	2.7 C-E	1200 K-U	433 H-J
	5	JA98	66.7 AB	29.6 B-G	2.7 C-E	1617 H-U	613 D-P
	6	JA102	66.7 AB	37.0 B-G	3.3 C-E	567 TU	256 N-S
	7	HEL66	66.7 AB	37.0 B-G	3.3 C-E	567 TU	256 N-S
	1	JA132	100.0 A	63.0 A-C	5.7 A-C	2433 D-L	949 C-H
Susceptible	2	JA19	100.0 A	48.1 A-E	4.3 B-D	2271 E-N	968 C-H
	3	HEL288	100.0 A	40.7 A-F	3.7 B-D	3540 A-D	1119 B-F
	4	JA95	100.0 A	55.6 A-D	5.0 B-D	3286 B-F	1159 B-E
	5	HEL293	100.0 A	48.1 A-E	4.3 B-D	3467 A-E	1889 B
	6	JA2	100.0 A	55.6 A-D	5.0 B-D	3164 B-G	1196 B-D
	7	HEL246	100.0 A	77.8 A	7.0 A	4154 AB	2655 A
		Min	0.0	0.0	0.0	350	150
		Max	100.0	77.8	7.0	4571	2655
		Mean	88.2	38.7	3.5	1679	580
		CV (%)	32.5	30.8	23.0	38.2	23.8
		F test	1.6**	2.3**	2.4**	5.1**	4.4**

** Significant at $P < 0.01$. Data are presented as minimum, maximum and mean values that were calculated from 96 varieties in the early rainy season, values with different letters within the same column are significantly different at $P < 0.05$ by DMRT.

[a] Disease incidence (DI), disease severity index (DSI) and disease scores at 76 days after transplanting in the early rainy and late rainy season.

[b] Disease incidence, disease severity index, disease scores, areas under disease progress curve of disease incidence (AUDPC-DI) and areas under disease progress curve of disease severity index (AUDPC-DSI) were transformed by square root.

Table 2. Selected varieties of resistant, moderately resistant and susceptible Jerusalem artichoke evaluated in late rainy seasons

Groups	No	Varieties	DSI (%) [a,b]	DSI (%) [a,b]	Score [a,b]	AUDPC-DI [b]	AUDPC-DSI [b]
Resistant	1	JA86	0.0 B	0.0 G	0.0 G	0 Z	0 h
	2	HEL256	0.0 B	0.0 G	0.0 G	100YZ	11gh
	3	HEL335	0.0 B	0.0 G	0.0 G	400 U-Z	44 Z-h
	4	HEL308	33.3 AB	3.7 FG	0.3 FG	450 T-Z	50 V-h
	5	JA15	33.3 AB	3.7 FG	0.3 FG	650 P-Z	74 U-h
	6	HEL317	33.3 AB	3.7 FG	0.3 FG	1050 L-W	117 P-g
Moderately resistant	1	HEL243	66.7 AB	7.4 E-G	0.7 E-G	600 Q-Z	67 S-h
	2	HEL316	66.7 AB	7.4 E-F	0.7 E-G	700 O-Z	78 G-h
	3	HEL61	66.7 AB	7.4 E-G	0.7 E-G	1100 L-W	144 O-f
	4	JA20	66.7 AB	7.4 E-G	0.7 E-G	1500 G-O	167 G-a
	5	JA134	66.7 AB	7.4 E-G	0.7 E-G	1600 F-N	200 G-W
	6	HEL65	66.7 AB	7.4 E-G	0.7 E-G	1700 F-N	211 F-V
	7	JA113	66.7 AB	7.4 E-G	0.7 E-G	1700 F-N	278 E-P
	1	JA5	100.0 A	33.3 BC	3.0 BC	2150 C-J	717 CD
Susceptible	2	JA117	100.0 A	48.1 B	4.3 B	2550 A-E	983 BC
	3	JA95	100.0 A	33.3 BC	3.0 BC	2550 A-E	1094 B
	4	JA93	100.0 A	55.6 B	5.0 B	2450 B-F	1117 B
	5	JA109	100.0 A	55.6 B	5.0 B	3100 AB	1300 B
	6	HEL293	100.0 A	100.0 A	9.0 A	3100 AB	2889 A
	7	HEL246	100.0 A	100.0 A	9.0 A	3250 A	3072 A
		Min	0.0	0.0	0.0	0.0	0
		Max	100.0	100.0	9.0	3250	3072
		Mean	59.7	11.1	1.0	1344	274
		CV (%)	57.3	40.5	20.5	30.9	27.7
		F test	4.2**	8.5**	11.2**	12.2**	19.4**

** Significant at $P < 0.01$. Data are presented as minimum, maximum and mean values that were calculated from 96 varieties in the early rainy season, values with different letters within the same column are significantly different at $P < 0.05$ by DMRT.
a Disease incidence (DI), disease severity index (DSI) and disease scores at 76 days after transplanting in the early rainy and late rainy seasons.
b Disease incidence, disease severity index, disease scores, areas under the disease progress curve of disease incidence (AUDPC-DI) and areas under the disease progress curve of disease severity index (AUDPC-DSI) were transformed by square root.

Table 3. Selected varieties of yield and yield component Jerusalem artichoke evaluated in early rainy season

Groups	Entry	Varieties	Number of Tubers/Plant	Tuber Size (g/tuber)	Tuber Yield (g/plant)
Low tuber yield	1	JA9	14 g-l	2.0 X-a	28.1 l
	2	JA8	12 i-l	2.3 W-a	28.4 l
	3	JA18	14 g-l	2.5 V-a	33.7 kl
	4	JA116	21 X-h	1.7 Za	35.0 j-l
	5	JA46	13 h-l	2.9 U-a	35.8 l-l
	6	JA27	20 X-h	1.8 Y-a	37.5 h-l
	7	JA58	12 j-l	3.7 Q-a	38.1 h-l
	8	JA49	26 P-b	1.5 a	39.6 h-l
	9	JA59	32 J-T	2.2 W-a	48.2 g-l
	10	JA71	18 a-j	2.9 U-a	53.3 f-l
High tuber yield	1	HEL243	25 R-d	10.3 B-E	252.7 B-G
	2	JA134	29 M-Z	9.0 C-H	257.1 B-F
	3	JA15	37 G-N	6.9 E-S	257.7 B-F
	4	JA6	44 D-H	5.9 G-W	257.9 B-F
	5	HEL280	51 C-D	5.2 J-a	263.0 B-E
	6	HEL257	39 E-L	7.2 E-Q	280.0 B-D
	7	JA123	36 H-P	7.8 D-N	282.4 B-D
	8	JA122	22 V-h	13.4 AB	289.7 BC
	9	HEL278	36 H-Q	8.5 C-K	300.3 B
	10	JA95	45 D-G	8.0 D-L	365.2 A
		Min	6	1.5	28.1
		Max	89	15.6	365.2
		Mean	29	5.7	149.3
		CV (%)	17.1	31.6	23.4
		F test	22.8**	7.6**	13.7**

** Significant at P < 0.01. Data were presented minimum, maximum and mean values were calculated from 96 varieties in early rainy season, values with different letters within the same column are significantly different at P < 0.05 by DMRT.

Table 4. Selected varieties of yield and yield component Jerusalem artichoke evaluated in late rainy season

Groups	Entry	Varieties	Number of tubers/plant	Tuber size (g/tuber)	Tuber yield (g/plant)
	1	JA21	18 a-h	10.0 U-d	123.6 c
	2	JA76	43 E-R	3.6 d	151.5 bc
Low tuber yield	3	JA27	47 C-L	3.9 d	165.9 a-c
	4	JA35	16 d-h	15.5 I-d	248.1 Z-c
	5	JA22	15 e-h	17.5 G-d	256.0 Y-c
	6	JA6	60 B-D	4.7 cd	266.7 X-c
	7	JA9	22 W-h	12.2 N-d	268.7 X-c
	8	JA49	30 M-h	13.9 K-d	278.3 W-c
	9	JA59	28 Q-h	11.2 Q-d	292.1 V-c
	10	JA117	52 C-H	5.7 b-d	298.2 V-c
	1	JA129	52 C-G	13.2 L-d	684.0 A-I
	2	JA60	34 K-a	27.2 B-N	701.9 A-H
High tuber yield	3	JA111	26 S-h	27.8 B-L	712.7 A-G
	4	JA58	29 O-h	25.4 C-S	733.0 A-F
	5	HEL278	23 W-h	32.5 A-F	745.8 A-E
	6	JA102	34 J-a	24.6 D-V	816.9 A-D
	7	JA120	22 W-h	40.3 AB	833.7 A-C
	8	HEL65	42 E-T	28.4 B-K	836.1 A-C
	9	HEL280	36 H-Y	24.7 D-U	861.0 AB
	10	JA37	34 J-a	26.4 B-P	913.6 A
		Min	14	3.6	123.6
		Max	78	44.1	913.6
		Mean	34	17.5	493.9
		CV (%)	23.2	41.3	26.6
		F test	7.4**	4.8**	4.6**

** Significant at P < 0.01. Data were presented minimum, maximum and mean values were calculated from 96 varieties in early rainy season, values with different letters within the same column are significantly different at P < 0.05 by DMRT.

Publication

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3.8 Effective plant age for screening for field resistance to Alternaria leaf spot (caused by *Alternaria* spp.) under natural infection in Jerusalem artichoke (*Helianthus tuberosus* L.)

Viriyasuthee W., S. Saepaisan, W. Saksirirat, M. L. Gleason, R. S. Chen and S. Jogloy

Jerusalem artichoke is a tuber crop in the genus Helianthus that originated in temperate regions of North America (Cosgrove et al., 1991). However, the production of Jerusalem artichoke in the tropics can be subject to severe yield loss because of drought (Ruttanaprasert et al., 2014), stem rot (Sennoi et al., 2013) and Alternaria leaf spot (Viriyasuthee et al., 2019). Alternaria leaf spot is an emerging disease of Jerusalem artichoke in these regions. The disease causes severe leaf damage, lowers photosynthesis and can reduce yield by up to 80% (Dudhe and Bharsale, 2005). Improvement of Jerusalem artichoke resistance to Alternaria leaf spot requires effective screening techniques. The age of the plant has a major influence on disease resistance. For Jerusalem artichoke, correlations between seedling and adult plant resistance were found for resistance to stem rot caused by *Sclerotium rolfsii* (Sennoi et al., 2013; Junsopa et al. 2018).

Objective

The objective of this study is to examine the correlation between disease resistance parameters in seedlings and adult plants in Jerusalem artichoke.

Materials and Methods

The experiment was conducted at the Khon Kaen University Agronomy Farm, Khon Kaen, Thailand, from March to June 2017. The first trial was conducted 15 days earlier than the repeat trial. The experiment was set up using a 6×3 factorial in an RCBD with four replications. Six Jerusalem artichoke varieties, including a resistant group (JA15, JA86 and JA116) and a susceptible group (HEL246, HEL293, and JA109) (Viriyasuthee et al., 2019) were evaluated at three different plant ages (20, 40, and 60 DAT). Thirty cm-diameter, 25 cm-tall pots were used with one plant per pot. The experimental unit consisted of 3 pots.

Disease assessment was done at 2-day intervals from the date of transferring potted plants until 20 days later. The disease score was assessed according to a qualitative rating scale modified by Mayee and Datar (1986) for Alternaria leaf spot as described by Viriyasuthee et al. (2019).

Results

Analysis of variance for individual experiments for DI, DS, AUDPC-DI, and AUDPC-DS showed a significant interaction of variety × plant age, so data were presented in two-way tables of varieties and plant ages.

Regarding experiment 1, the 60-DAT plants had the highest DI, DS, AUDPC-DI, and AUDPC-DS across the six varieties, followed by 40 and 20 DAT, respectively (Table 1). JA15 had the lowest DI for plant ages 20 and 40 DAT. JA15, JA86, and JA116 had the lowest DS and AUDPC-DS for all plant ages. HEL246 had the highest DS and AUDPC-DS for all three plant ages, whereas JA109, JA86, JA116, and JA15 showed lower DS and AUDPC-DS than HEL246. HEL246, HEL293, JA109 had the highest AUDPC-DI whereas JA86, JA116, and JA15 showed low AUDPC-DI.

Concerning experiment 2, the results were similar. Plants at 60 DAT had the highest DI, DS, AUDPC-DI, and AUDPC-DS across all six varieties, followed by 40 and 20 DAT, respectively (Table 2). JA15 had low DI for all plant ages whereas the other varieties had a high DI (up to 100%). JA15, JA116, JA109, and JA86 showed low DS and AUDPC-DS at all plant ages except JA109 for AUDPC-DS, whereas HEL246 and HEL293 had the highest DS and AUDPC-DS for all plant ages. JA 15 had the lowest AUDPC-DI. The other varieties showed high AUDPC-DI for all plant ages, except for JA116 which had a low value at 20 DAT.

DI data from the two experiments revealed that correlations between plant ages were moderately positive and significant; 0.62, between DI20 and DI40, 0.47 between DI20 and DI60 and 0.62 between DI40 and DI60 (Table 3). The correlation between three plant ages for DS was highly positive and significant; 0.92 between DS20 and DS40, 0.88 between DS20 and DS60, and 0.97 between DS40 and DS60. The correlations between AUDPC-DI at 20 with 40 DAT (r=0.60) and 20 DAT with 60 DAT (r=0.50) were moderately positive and significant whereas the correlations between AUDPC-DI at 40 DAT with 60 DAT were significant (r=0.80). The correlation between the three plant ages For AUDPC-DS was significant (r=0.88-0.98).

Conclusions

In conclusion, the correlations of DS, AUDPC-DI, and AUDPC-DS between 40 and 60 DAT were highly positive and significant. Therefore, screening for resistance to Alternaria leaf spot of Jerusalem artichoke at 40 and 60 DAT should yield similar findings. In application, for an accelerated breeding program, screening 40-day-old plants would be recommended for breeders intending to select for resistance to this important fungal disease, since they can select resistant plants and generate crosses during a single cropping year.

References

Cosgrove, D. R., E.A Oelke, J. D. Doll, D. W. Davis, D. J. Undersander, and E.S. Oplinger. 2018. Jerusalem artichoke Alternative Field Crops Manual. Available online: http://www.hort.purdue.edu/newcrop/afcm/ jerusart.html (accessed on 2 April 2018).

Dudhe, M.Y., and S. S. Bharsakle. 2005. Screening of germplasm line for resistance to Alterneria blight and necrosis disease in sunflower. Crop Protection Products 1: 84-87.

Junsopa, C., S. Jogloy, W. Saksirirat, P. Songsri T. Kesmala, and B. B. Shew.2018. Association of seedling and adult plant resistance to *Sclerotium rolfsii* in Jerusalem artichoke (*Helianthus tuberosus* L.) under field conditions. European Journal of Plant Pathology151: 251-255.

Mayee, C.D., and V.V. Datar. 1986. Sunflower diseases. Parabhan, Indi Marathwad Agricultural University

Ruttanaprasert, R., P. Banterng, S. Jogloy, N. Vorasoot, T. Kesmala, R. S. Kanwar. 2014. Genotypic variability for tuber yield, biomass and drought tolerance in Jerusalem artichoke germplasm. Turkish Journal of Agriculture 38: 570-580.

Sennoi, R., S. Jogloy, W. Saksirirat, P. Banterng, T. Kesmala, and A. Patanothai. 2013. Evaluation of seedling and adult plant stages resistance *Sclerotium rolfsii* Sacc. in Jerusalem artichoke (*Helianthus tuberosus* L.) SABRAO Journal of Breeding Genetics 45: 324-331.

Viriyasuthee, W., W. Saksirirat, S. Saepaisan, M. L. Gleason, and S. Jogloy.2019. Variability of Alternaria leaf spot resistance in Jerusalem artichoke (*Helianthus tuberosus* L.) accessions grown in a humid tropical region. Agronomy 9: 268.

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Table 1. Means of disease parameters of six Jerusalem artichoke varieties at three plant ages in experiment 1

Varieties	Disease incidence[a]			Disease severity[a]			Area under disease progress curve for disease incidence			Area under disease progress curve for disease severity		
	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT
Resistant varieties												
JA15	25 b	0 b	100 a	3 d	0 e	11 e	325 b	33 c	1267 b	36 d	4 e	141 e
JA86	100 a	100 a	100 a	11 c	13 d	33 d	734 b	1184 b	1800 a	81 d	152 d	444 c
JA116	100 a	100 a	100 a	11 c	11 d	33 d	717 b	1233 b	1300 b	80 d	137 d	330 d
Susceptible varieties												
HEL246	100 a	100 a	100 a	78 a	96 a	100 a	1800 a	1800 a	1800 a	1289 a	1437 a	1511 a
HEL293	100 a	100 a	100 a	78 a	78 b	96 b	1633 a	1800 a	1800 a	1048 b	1119 b	1486 a
JA109	100 a	100 a	100 a	35 b	35 c	50 c	1800 a	1800 a	1800 a	487 c	517 c	683 b
Mean	88 B	83 B	100 A	36 C	39 B	54 A	1168 C	1308 B	1628 A	504 C	561 B	766 A

Means followed by the same capital letter in the same row or small letter in the same column do not differ significantly according to least significant difference (LSD) at $P < 0.05$.

[a] Data of disease incidence and disease severity at 18 days after inoculation in experiment 1.

Table 2. Means of disease parameters of six Jerusalem artichoke varieties at three plant ages in experiment 2

Varieties	Disease incidence[a]			Disease severity[a]			Areas under disease progress curve of disease incidence			Areas under disease progress curve of disease severity		
	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT	Plant age 20 DAT	Plant age 40 DAT	Plant age 60 DAT
Resistant varieties												
JA15	0 b	25 b	25 b	0 c	3 d	3 d	0 c	450 b	450 b	0 e	50 e	50 d
JA86	100 a	100 a	100 a	11 b	24 c	33 c	467 b	1467 a	1800 a	52 d	250 d	483 c
JA116	0 b	100 a	100 a	0 c	33 b	56 b	0 c	1800 a	1800 a	0 e	554 c	728 b
Susceptible varieties												
HEL246	100 a	100 a	100 a	56 a	78 a	100 a	1800 a	1800 a	1800 a	737 a	1309 b	1700 a
HEL293	100 a	100 a	100 a	54 a	80 a	100 a	1800 a	1800 a	1800 a	696 b	1433 a	1726 a
JA109	100 a	100 a	100 a	11 b	33 b	56 b	1800 a	1800 a	1800 a	98 c	495 c	743 b
Mean	67 B	88 A	88 A	22 C	42 B	58 A	978 B	1520 A	1575 A	264 C	682 B	905 A

Means followed by the same capital letter in the same row or small letter in the same column do not differ significantly according to least significant difference (LSD) at $P < 0.05$.

[a] Data of disease incidence and disease severity at 18 days after inoculation in experiment 2.

Table 3. Correlation coefficient (r) of disease parameters of six Jerusalem artichoke varieties and three plant ages

	DI20	DI40	DS20	DS40	AUDPC-DI20	AUDPC-DI40	AUDPC-DS20	AUDPC-DS40
DI40	0.62**							
DI60	0.47**	0.62**						
DS40			0.92**					
DS60			0.88**	0.97**				
AUDPC-DI40					0.60**			
AUDPC-DI60					0.50**	0.80**		
AUDPC-DS40							0.88**	
AUDPC-DS60							0.85**	0.98**

** Significant at P < 0.01 probability level.
DI20, DI40 and DI60 = disease incidence at 20, 40 and 60 DAT, respectively.
DS20, DS40 and DS60 = disease severity at 20, 40 and 60 DAT, respectively.
AUDPC-DI20, AUDPC-DI40 and AUDPC-DI60 = areas under the disease progress curve of disease incidence at 20, 40 and 60 DAT, respectively.
AUDPC-DS20, AUDPC-DS40 and AUDPC-DS60 = areas under the disease progress curve of disease severity at 20, 40 and 60 DAT, respectively.

3.9 Biological control of alternaria leaf spot caused by *Alternaria* spp. in Jerusalem artichoke (*Helianthus tuberosus* L.) under two fertilization regimes

Viriyasuthee W., S. Saepaisan, W. Saksirirat, M. L. Gleason, R. S. Chen and S. Jogloy

Jerusalem artichoke (*Helianthus tuberosus* L.), also known as sunchoke, a native of North America, can provide multiple benefits as a functional food and source of bioethanol and animal feed (Puttha et al., 2012). A fungal leaf spot disease incited by an *Alternaria* spp. has emerged as a threat to Jerusalem artichoke production in Thailand (Viriyasuthee et al., 2019). The conventional approach to the control of Alternaria leaf spot is spray applications of synthetic chemical fungicides. In addition, biocontrol methods against Alternaria leaf spot can provide alternatives to synthetic chemical fungicides that are much less damaging to people and the environment. To our knowledge, alternatives to chemical fungicides for control of Alternaria leaf spot have not been investigated in Jerusalem artichoke, but are worth exploring to mitigate environmental damage and reduce the risk of development of fungicide resistance.

Objective

The objectives of this study were to evaluate the efficacy of integrating resistant genotypes of Jerusalem artichoke with *T. harzianum* to control Alternaria leaf spot caused by *Alternaria* spp. under two fertilization regimes and to determine whether *T. harzianum* induces the activity of chitinase and β-1,3-glucanase activity in Jerusalem artichoke leaves.

Materials and Methods

A field trial at the experimental farm (Khon Kaen University, Thailand) was arranged as a 6×3 factorial in a randomized complete block design with four replications. Six Jerusalem artichoke varieties included three resistant against Alternaria leaf spot (JA15, JA86 and JA116) and three susceptible to the disease (HEL246, HEL293 and JA109) (Viriyasuthee et al., 2019). The three disease control methods included applications of *T. harzianum* (isolate T9), sprays of propiconazole fungicide at a rate of 30 ml/20L of water (375 ppm), and a control that received neither T9 nor propiconazole.

T. harzianum populations in soil and colonization of roots and leaves were assessed in plots of JA15, JA116, HEL246 and JA109, both for the non-inoculated control and *T. harzianum* application treatments.

Leaf samples of Jerusalem artichoke were collected from JA15, JA116, HEL246 and JA109 in non-inoculated control and *T. harzianum* inoculated treatments at 7 DATS, 15 and 30 DAT. Different plants in each subplot were sampled on each sampling date so that no plant was sampled on more than one date. Leaves were extracted for crude protein and analyzed for the activity of chitinase and β-1,3-glucanase.

Disease assessment was performed at 3-day intervals from 20 to 81 DAT. Twelve plants per subplot, except border row plants, plants for enzyme activity analysis and plants for monitoring of *T. harzianum* colonization of roots and leaves, were assessed individually for the number of symptomatic plants and disease score (an index of disease severity). The disease score was determined by the qualitative rating scale developed by Mayee and Datar (1986).

Five plants in each subplot, excluding border row plants, were sampled at maturity and used for the determination of yield components, number of tubers per plant and tuber size.

Results

The *Trichoderma* and fungicide treatments showed a low reduction in disease severity (0-9% and 0-10% for *Trichoderma* application and fungicide sprays, respectively), an area under the disease progress curve for disease incidence (AUDPC-DI), and an area under the disease progress curve for disease severity (AUDPC-DS) across all genotypes (Table 1). In the low-fertilization trial, HEL293 had the highest disease severity, AUDPC-DI, and AUDPC-DS under all three disease control methods, whereas JA86, JA116, and JA15 showed relatively low disease severity, AUDPC-DI, and AUDPC-DS.

Under the high-fertilization regime, the *Trichoderma* treatment, JA86 had the lowest disease incidence among the genotypes (Table 2). For disease control methods, the *Trichoderma* and fungicide treatments showed a low reduction in disease severity (0-10% and 0-5% for *Trichoderma* application and fungicide sprays, respectively), AUDPC-DI, and AUDPC-DS across all genotypes. HEL246 and HEL293 had the highest disease severity, AUDPC-DI, and AUDPC-DS, whereas JA86, JA116, and JA15 showed the lowest disease severity, AUDPC-DI, and AUDPC-DS under all disease control methods.

Under both fertilization regimes across genotypes, *Trichoderma* inoculation resulted in a higher number of *Trichoderma* propagules than non-inoculated control (data not shown). Among genotypes-plot, soil adjacent to HEL246 had the highest number of *Trichoderma* propagules. For root and leaf colonization, the *Trichoderma* treatment resulted in 99-100% incidence of colonization, whereas the non-inoculated control had no colonization (data not shown).

The *Trichoderma* treatment had higher chitinase activity than the non-inoculated control treatment only at 7 days after transferring the seedling (DATS) under high fertilization regime (data not shown), but lower at 15 days after transplanting (DAT) under a low fertilization regime. No significant difference was observed at 7 DATS and 30 DAT under a low fertilization regime and 15 and 30 DAT under a high fertilization regime (data not shown). For genotypes, HEL246 had the highest chitinase activity at 7 DATS and higher than non-inoculated control under both fertilization regimes (Figure 1 a, b).

The *Trichoderma* treatment had higher β-1,3-glucanase activity than the non-inoculated control at 7 DATS under both fertilization regimes (data not shown), but lower at 30 DAT under the high fertilization regime. No significant difference was observed at 15 and 30 DAT under the low fertilization regime and 15 DAT under the high fertilization regime across genotypes. At 7 DATS, JA15 and HEL246 and JA109 had the highest β-1,3-glucanase activity, with the *Trichoderma* application under two fertilization regimes (Figure 1c, d). However, only HEL246 had higher β-1,3-glucanase activity than the non-inoculated control under both fertilization regimes.

No significant differences among disease control methods were found for tuber yield (Table 3), or yield components (number of tubers per plant and tuber size) across fertilization regimes (Table 4) For combined analysis of variance, significant differences were found for the interaction of genotypes × fertilization regime in tuber yield, therefore the results were presented in a two-way table.

Among genotypes, JA109 had the highest number of tubers per plant, whereas JA86 had the lowest number of tubers per plant (Table 4). JA86 had the largest tuber size, whereas JA116 and JA109 had the smallest tuber size. Under the low-fertilization regime, JA86 and HEL293 had the highest tuber yield, whereas JA116 and JA109 had the lowest tuber yield under both fertilization regimes (Table 3).

Conclusions

In conclusion, resistant genotypes of Jerusalem artichoke alone showed low disease severity, AUDPC-DI and AUDPC-DS and similar efficacy for suppression of Alternaria leaf spot as integrated control of resistant varieties and *Trichoderma* application or propiconazole sprays. Application of *T. harzianum* T9 or propiconazole sprays showed a low reduction of disease severity (10% or less) and AUDPC-DS of Alternaria leaf spot in susceptible genotypes, especially HEL246 and HEL293 under two fertilization regimes. The application of *T. harzianum* induced an increase of chitinase and β-1,3-glucanase only at the seedling stage in HEL246. Neither host resistance nor the biocontrol or fungicide strategies impacted yield or yield components of Jerusalem artichoke.

References

Mayee, C.D., and V. V. Datar. 1986. Sunflower diseases. Parabhan, Indi Marathwad Agricultural University

Puttha, R., S. Jogloy, P. P. Wangsomnuk, S. Srijaranai, T. Kesmala, and A. Patanothai. 2012. Genotypic variability and genotype by environment interactions for inulin content of Jerusalem artichoke germplasm. Euphytica 183: 119-131.

Viriyasuthee, W., W. Saksirirat, S. Saepaisan, M. L. Gleason, and S. Jogloy.2019. Variability of Alternaria leaf spot resistance in Jerusalem artichoke (Helianthus tuberosus L.) accessions grown in a humid tropical region. Agronomy 9: 268

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Table 1. Means of disease parameters under low- fertilization regime of six Jerusalem artichoke genotypes and three control methods

Genotypes	Disease incidence (%) [a]				Disease severity (%) [a]			
	Non-inoculated control	*Trichoderma*	Fungicide	Mean	Non-inoculated control	*Trichoderma*	Fungicide	Mean
JA15	100 a	100 a	100 a	100 A	39 d	33 e	33 e	35 D
JA86	67 b	0 c	50 b	39 B	7 fg	0 h	6 g	4 F
JA116	100 a	100 a	100 a	100 A	11 f	11 f	11 f	11 E
HEL246	100 a	100 a	100 a	100 A	78 a	69 b	68 b	72 B
HEL293	100 a	100 a	100 a	100 A	78 a	75 a	75 a	76 A
JA109	100 a	100 a	100 a	100 A	56 c	56 c	56 c	56 C
Mean	94 A	83 B	92 A		45 A	41 B	42 B	

Genotypes	Areas under disease progress curve for disease incidence				Areas under disease progress curve for disease severity			
	Non-inoculated control	*Trichoderma*	Fungicide	Mean	Non-inoculated control	*Trichoderma*	Fungicide	Mean
JA15	2606 c	2575 c	2550 c	2577 C	485 e	453 e	448 e	462 C
JA86	413 ef	0 g	230 fg	214 E	46 f	0 f	26 f	24 D
JA116	700 d	525 de	579 de	601 D	78 f	58 f	64 f	67 D
HEL246	5506 a	5528 a	5416 a	5483 A	1840 a	1534 bc	1501 c	1625 A
HEL293	5581 a	5559 a	5341 a	5494 A	1811 a	1523 c	1609 b	1648 A
JA109	3950 b	3944 b	3769 b	3888 B	1189 d	1160 d	1138 d	1163 B
Mean	3126 A	3022 B	2981 B		908 A	788 B	798 B	

Means followed by the same small letters were not significantly different treatment in combination according to least significant difference (LSD) at $P < 0.05$.

Means followed by the same capital letters were not significantly different for each of main effects, according to least significant difference (LSD) at $P < 0.05$.

[a] Disease incidence and disease severity at 77 days after transplanting

Table 2. Means of disease parameters under high- fertilization regime of six Jerusalem artichoke genotypes and three control methods

Genotypes	Disease incidence (%) [a]								Disease severity (%) [a]							
	Non-inoculated control		*Trichoderma*		Fungicide		Mean		Non-inoculated control		*Trichoderma*		Fungicide		Mean	
JA15	100	a	100	a	100	a	100	A	33	e	33	e	33	e	33	C
JA86	100	a	63	b	100	a	88	B	15	f	7	h	11	g	11	E
JA116	100	a	100	a	100	a	100	A	16	f	11	g	11	g	13	D
HEL246	100	a	100	a	100	a	100	A	70	a	64	bc	66	b	67	A
HEL293	100	a	100	a	100	a	100	A	71	a	61	c	66	b	66	A
JA109	100	a	100	a	100	a	100	A	56	d	56	d	56	d	56	B
Mean	100	A	94	B	100	A			44	A	39	C	40	B		
Genotypes	**Areas under disease progress curve for disease incidence**								**Areas under disease progress curve for disease severity**							
	Non-inoculated control		***Trichoderma***		**Fungicide**		**Mean**		**Non-inoculated control**		***Trichoderma***		**Fungicide**		**Mean**	
JA15	2150	f	2006	f	2150	f	2102	D	402	f	374	f	397	f	391	D
JA86	820	g	225	i	641	h	562	E	97	g	25	g	71	g	64	E
JA116	627	h	519	h	532	h	559	E	77	g	58	g	59	g	65	E
HEL246	5469	a	5444	ab	5281	b	5398	A	1864	a	1453	bc	1576	b	1631	A
HEL293	5453	ab	5459	a	4958	c	5290	B	1801	a	1383	c	1410	c	1532	B
JA109	4213	d	3956	e	3981	e	4050	C	1208	d	1067	e	1050	e	1108	C
Mean	3122	A	2935	B	2924	B			908	A	727	B	761	B		

Means followed by the same small letters were not significantly different treatment in combination according to least significant difference (LSD) at P <0.05.

Means followed by the same capital letters were not significantly different for each of main effects, according to least significant difference (LSD) at P < 0.05.

[a] Disease incidence and disease severity at 77 days after transplanting.

Table 3. Means of tuber yield (g/ plant) under two fertilization regimes of six Jerusalem artichoke genotypes and three control methods

Factors	Low-fertilization regime		High-fertilization regime	
Genotypes				
JA15	182	bc	273	b
JA86	243	a	278	ab
JA116	107	d	150	c
HEL246	189	bc	330	a
HEL293	222	ab	321	ab
JA109	150	cd	171	c
Treatments	**Across fertilization regimes**			
Control (non-inoculated)	213	a		
Trichoderma	219	a		
Fungicide	221	a		

Means followed by the same small letters in the same column were not significantly different according to least significant difference (LSD) at P < 0.05.

Table 4. Means of the number of tubers/ plant and tuber size (g/ tuber) averaged over two fertilization regimes of six Jerusalem artichoke genotypes and three control methods

Factors	Number of tubers/ plant		Tuber size (g/ tuber)	
Genotypes				
JA15	15.7	b	14.4	b
JA86	8.9	c	30.2	a
JA116	16.5	b	7.7	c
HEL246	16.3	b	15.8	b
HEL293	17.6	b	15.4	b
JA109	20.7	a	7.8	c
Treatments				
Control (non-inoculated)	16.3	a	14.5	a
Trichoderma	15.0	a	15.9	a
Fungicide	16.5	a	15.3	a

Means followed by the same small letters in the same column were not significantly different according to least significant difference (LSD) at P < 0.05.

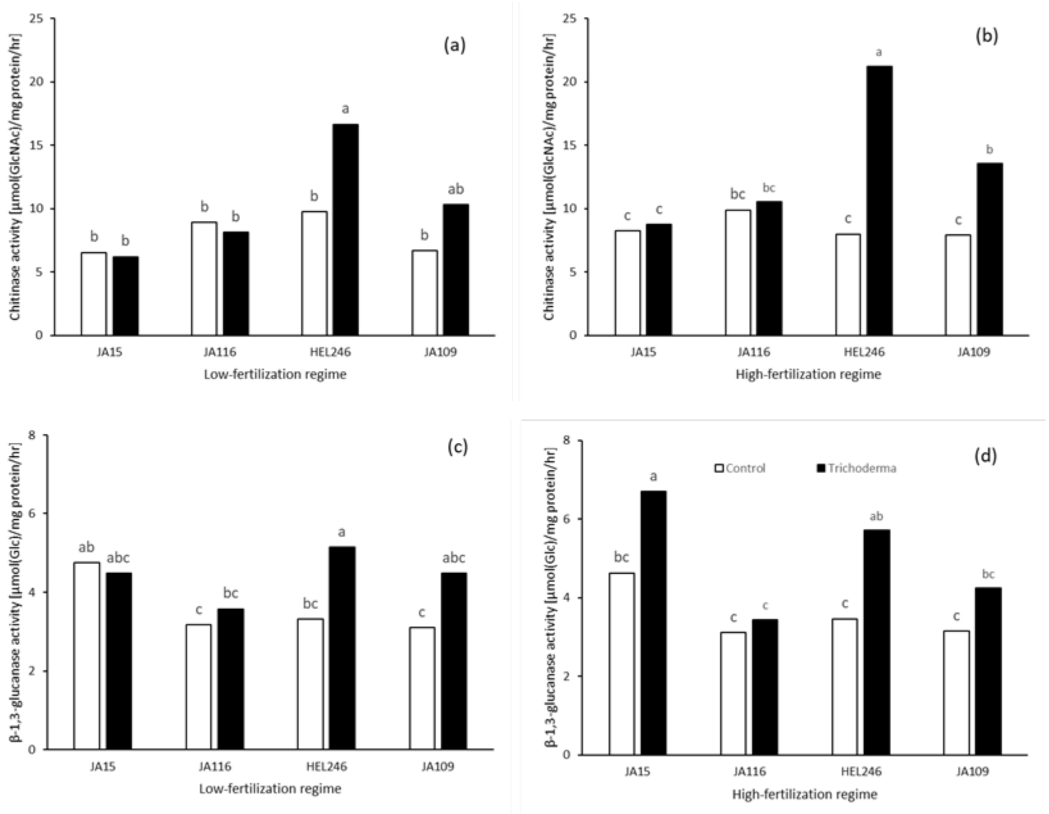


Figure 1. Enzyme activity of *Trichoderma* application and non-*Trichoderma* application for four Jerusalem artichoke genotypes at seven days after transferring seedling (DATS) under two fertilization regimes: (a) Chitinase activity at low fertilization regime; (b) Chitinase activity at high fertilization regime; (c) β-1,3-glucanase activity at low fertilization regime; (d) β-1,3-glucanase activity at high fertilization regime. Different letters indicate significant differences at $P < 0.05$ among treatments.

3.10 Bio-control of stem rot in Jerusalem artichoke (*Helianthus tuberosus* L.) in field conditions

Junsopa, C., W. Saksirirat, S. Saepaisan, P. Songsri, T. Kesmala, B. B. Shew and S. Jogloy

Jerusalem artichoke (*Helianthus tuberosus* L.) produces edible tubers that contain inulin. The levels of inulin in tubers range from 7 to 30% of fresh weight and about 50% of dry weight (Kays and Nottingham, 2008). Jerusalem artichoke (JA) is used as a functional food, feed additive, or as bioethanol.

Stem rot caused by *S. rolfsii* Sacc. [syn. Althelia rolfsii (Curzi) C.C. Tu & Kimbr.] is a major disease problem for JA production in the tropics (Sennoi et al., 2010). The pathogen can infect both tubers and stems of JA, causing 60% of yield loss in temperate regions (McCarter and Kays, 1984). In the tropical area of Thailand, disease incidences of up to 32% have been reported (Junsopa et al., 2016). It is important to control the disease by methods that reduce potential pesticide residues. Antagonistic microorganisms such as *Trichoderma* spp., arbuscular mycorrhizae, *Bacillus subtilis*, *Streptomyces* spp., and *Pseudomonas* spp. are widely applied for biocontrol of various plant diseases. The modes of action of the biocontrol agents include competition for nutrients, parasitism, production of antibiotic and volatile metabolites, and inducement resistance.

Trichoderma spp. has been used to control wilt, stalk, and tuber rots caused by *Sclerotinia sclerotiorum* and *Rhizoctonia solani* in JA. They have also reduced the percentage of tuber rot caused by *S. rolfsii* and increased the survival of JA. Other bio-control organisms that have reduced the incidence of stem rot in JA include *Saccharomyces cerevisiae*, *Trichoderma viride*, *B. subtilis*, and *Pseudomonas fluorescens*. Under greenhouse conditions in Thailand, *T. harzianum* T9 and *Glomus clarum* KKURA0305 controlled *S. rolfsii* in JA; similar results were reported in a subsequent study with *T. harzianum* T9, *B. firmus* BSR032, and *G. clarum*.

Although these studies indicate that biological control of stem rot is possible, antagonistic organisms are often specific to the target pathogens in each environment. Therefore, indigenous antagonistic organisms may be more effective for the control of the target disease than non-native antagonists.

Objective

The objective of this study was to evaluate the efficacy of selected indigenous strains of *T. harzianum* T9 and *B. firmus* BSR032 for control of *S. rolfsii* under field conditions.

Materials and Methods

A factorial experiment was conducted in July–October 2015 at Khon Kaen University, Thailand. Factors A included four JA genotypes with 2 resistant genotypes (HEL246, HEL65) and 2 susceptible genotypes (JA12 and JA47). Factor B was *T. harzianum* T9 and an uninoculated control, and factor C was *B. firmus* BSR032 and an uninoculated control. The 16 treatment combinations were arranged in a randomized complete block design with four replications. This field test was planted in two environments (unfertilized field and fertilized field) on the same research station.

The research areas were prepared using conventional tillage. Small plots (2 × 5 m) were then created. Uniform seedlings with 4 to 6 leaves were transplanted on the beds of a four-row plot (space 50 x 50 cm). Plots were hand-weeded and applied fertilizer to the fertilized field 15 days after transplanting (DAT) and 25 DAT with 15-15-15 fertilizer (156.125 kg ha-1). A mini sprinkler was set up and used for irrigation 2 times a week.

Isolates of *T. harzianum* T9 and *B. firmus* BSR032 kindly provided by Department of Entomology and Plant Pathology, Khon Kaen University were used in this experiment. *T. harzianum* T9 was cultured on potato dextrose agar (PDA) and incubated at 25 ± 2ºC for 3 days. Active mycelia were cut into small pieces using a cork borer with the diameter of 0.5 cm. Four mycelial plugs were put in sterilized 400g sorghum grains in a polypropylene bags and incubated at room temperature (30±2ºC) for 5 days (Sennoi et al., 2013a; Charirak et al., 2016). The spore suspensions were prepared from sorghum grains inoculum and then were counted with a hemacytometer and adjusted to a concentration of 1×10^9 spores ml^{-1}.

B. firmus BSR032 was cultured on nutrient agar (NA) and incubated at 25±2ºC for 48 hours. Colonies were transferred to nutrient broth (NB) and incubated for 24 hours. After incubation, bacterial suspension was determined using a spectrophotometer at 600 nm and adjusted to 0.1 OD to obtain the concentration of 1.62×10^9 cfu ml^{-1} (Maneesuwan and Sirithorn, 2013).

The stem rot pathogen, *S. rolfsii* was cultured on PDA and incubated at 25±2ºC for 3 days. Agar plugs were cut with 0.5 cm diameter cork borer and four plugs were added to cooled autoclaved bags of sorghum grains prepared as described above. Inoculum was incubated at room temperature (30±2ºC) for 7 days (Junsopa et al., 2016).

At 38 DAT, 16 plants in two middle rows of each plot were inoculated with the designated bio-control treatment. Before inoculation both of antagonistic and pathogen at 15:00, mini-sprinkler irrigation was applied for 30 minutes to increase humidity. Spore suspension of *T. harzianum* T9 was applied at the crown of each plant at approximately 10 ml per plant. Inoculation of *B. firmus* BSR032 at the rate of 10 ml per plant was carried out at the same time and with the same method. Seven days after inoculation (or 45 DAT) of biocontrol agents, plants were inoculated with *S. rolfsii*. Three sorghum grains of inoculum were buried in the soil around the crown of the plant, at 1 cm below the soil surface.

Soil samples (30 cm depth) were collected twice, before planting and at 20 days after fertilizer application. The samples were analyzed for pH, cation exchange capacity (CEC), electrical conductivity (EC), organic matter (OM), total nitrogen, available phosphorus, exchangeable potassium, exchangeable calcium, and soil texture. Meteorological data were recorded for air relative humidity, temperature, and rainfall throughout the experimental period.

The assessment of disease incidence was done at 3-day intervals (1-46 days after inoculation, DAI). Disease severity was evaluated using a disease score of 0–5 (0 = healthy plant, 1 = lesion without wilting, 2 = 1–2 leaves wilting, 3 = more than 2 leaves wilting, 4 = damped off and 5 = plant dead) was used (Sennoi et al., 2013b). Disease scores were converted to a disease severity index (SI) as follows (Anfok, 2000);

SI = [Σ (rating scores × number of plants receiving each score) × 100%]/ (number of plants rated × highest rating among all plants).

The data on disease incidence and severity index were analyzed by Statistix8 software (Statistix8, 2003). An analysis of variance and combined analysis for the two environments, unfertilized and fertilized fields, were used to determine the significance of the main effects and interactions. The difference in treatment means was calculated using the Least Significant Difference (LSD) test.

Results

Total rainfall was 447 and 434 mm for environment 1(fertilized field) and environment 2 (unfertilized field). Temperatures and relative humidity in the two environments were similar. The minimum temperatures ranged from 20.5 to 26.0 ºC, maximum temperatures ranged from 25.0 to 39.5 ºC, and relative humidity ranged between 76 to 98%, in both environments. Soil properties in both environments were similar. After fertilizer was added, most soil nutrients were slightly higher in the fertilized field than in the unfertilized field (data not shown).

Environments, Jerusalem artichoke genotypes, and the biological control organisms significantly affected disease incidence. The environments differed in disease incidence, with higher levels of disease incidence (33.7% vs. 27.3%) and severity (28.3% vs. 23.5%) observed in environment 1 compared to environment 2 (data not shown).

T. harzianum T9 reduced disease incidence in two environments, but disease severity was not reduced in environment 2 (Figure. 1A, B). *B. firmus* BSR032 reduced disease incidence in environment 1 and severity index in environment 2 (Figure. 1C, D). The combination of *T. harzianum* T9 and *B. firmus* BSR032 was less effective than either alone (Table 1).

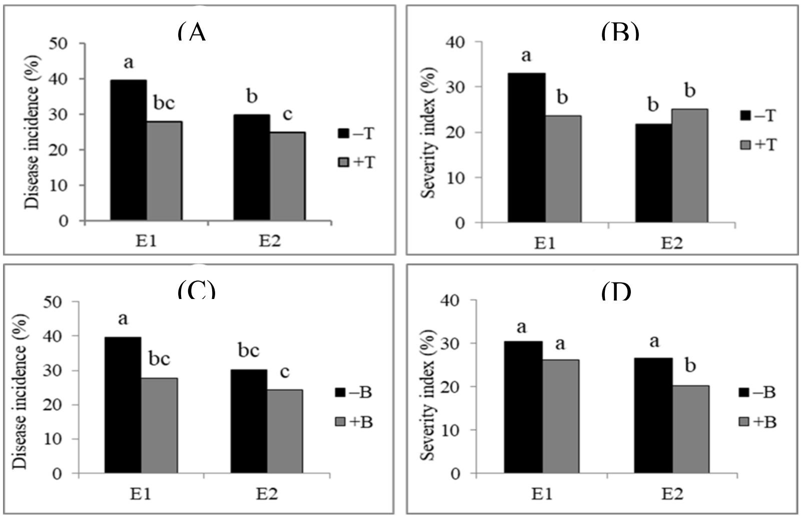


Figure 1. Incidence (A, C) and severity index (B, D) of stem rot caused by *S. rolfsii* in the presence (+T, +B) and absence (–T, –B) of *T. harzianum* T9 and *B. firmus* BSR032, respectively under two environments with fertilized field (E1) or unfertilized field(E2), means (from n=32 observations) with the same letter(s) are not significantly different.

Table 1. Effect of *T. harzianum* T9 and *B. firmus* BSR032 on incidence and severity index of stem rot in Jerusalem artichoke caused by *S. rolfsii*

Treatments	Disease incidence (%)	Severity index (%)	% reduction	
			Disease incidence	Severity index
Control	45.9 a	34.0 a	0	0
T. harzianum T9	24.0 c	23.1bc	47.6	32.0
B. firmus BSR032	23.4 c	20.8 c	48.9	39.0
T. harzianum T9 + *B. firmus* BSR032	28.7 b	25.6 b	37.4	24.7

Means from four varieties and two environments (unfertilized and fertilized fields), means with the same letter(s) in the same column are not significantly different at 5% level by LSD.

Among the JA genotypes, HEL246 had less disease incidence than the other three genotypes (Table 2), whereas HEL246 and JA47 had less disease severity index than the rest of the genotypes. Disease incidence of without the application of *T. harzianum* T9, HEL246 had less disease incidence than the other genotypes whereas with *T. harzianum* T9 added, HEL246 and JA47 had less disease incidence than the other genotypes (Figure 2A). For the disease severity index, HEL246 and JA47 had less disease severity index than other genotypes with or without *T. harzianum* T9 added (Figure 2B). HEL246 had less disease incidence than the other genotypes with and without *B. firmus* BSR032 (Figure 2C), likewise HEL246 and JA47 had less disease severity index than the other genotypes with and without *B. firmus* BSR032 added (Figure 2D).

Disease incidence and disease severity index in environment 1 were higher than in environment 2 (Figure 3). HEL246 and JA47 had less disease incidence than HEL65 and JA12 under environment 1, whereas HEL246 and HEL65 had less disease incidence than JA47 and JA12 under environment 2 (Figure 3A). HEL246 and JA47 had less disease severity index than HEL65 and JA12 under environment 1 and environment 2(Figure 3B).

Table 2. Incidence and severity index of stem rot caused by *S. rolfsii* on four varieties in Jerusalem artichoke

Varieties	Disease incidence (%)	Severity index (%)
HEL246	22.0 c	19.9 b
HEL65	32.6 b	34.3 a
JA47	30.0 b	16.9 b
JA12	37.4 a	32.3 a

Means from four bio-control treatments and two environments(unfertilized and fertilized fields), means with the same letter(s) in the same column are not significantly different at 5% level by LSD.

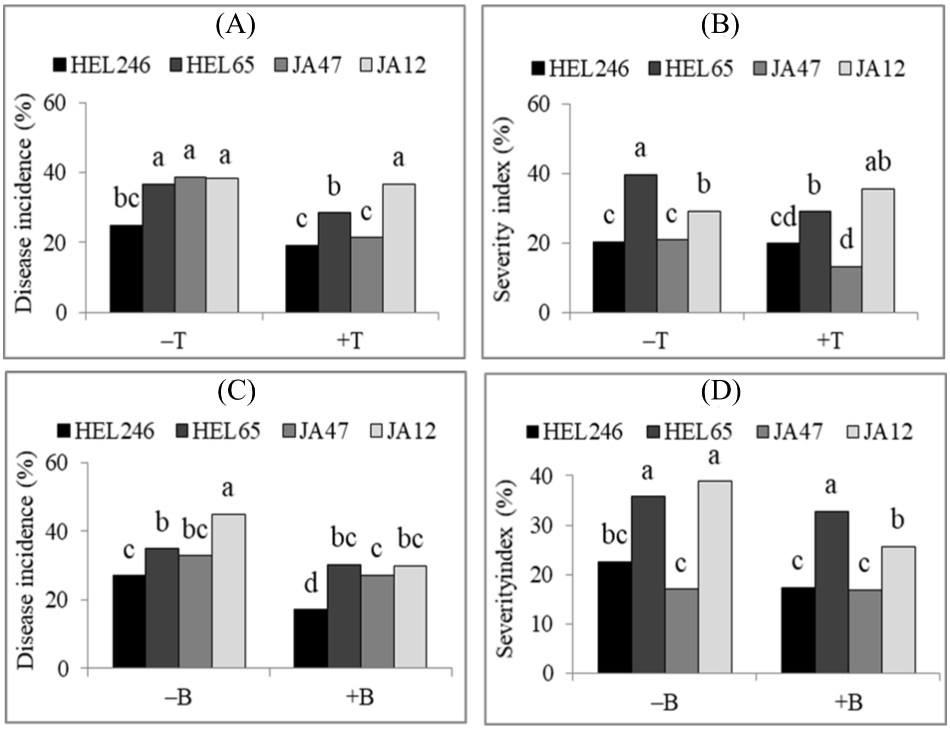


Figure 2. Incidence (A, C) and severity index (B, D) of stem rot caused by *S. rolfsii* on four cultivars in Jerusalem artichoke in the presence (+T, +B) and absence (–T, – B) of T. harzianum T9 and B. firmus BSR032, respectively. Means (from n=16 observations) with the same letter(s) are not significantly different at 5% level by LSD.

Conclusions

T. harzianum T9 or *B. firmus* BSR032 or host plant resistance could be a good choice for biological control of stem rot caused by *S. rolfsii* in JA. They reduced the incidence and severity index of disease in JA. Consideration of plant genotype, environment, and combination of the biocontrol agents is necessary because *T. harzianum* T9 and *B. firmus* BSR032 are specific with plant genotypes and environments. Further enhancement of control may be possible with continued efforts to select improved resistant genotypes, and biocontrol strains, and find good compatible combination strains of *Trichoderma harzianum* and *Bacillus firmus*.

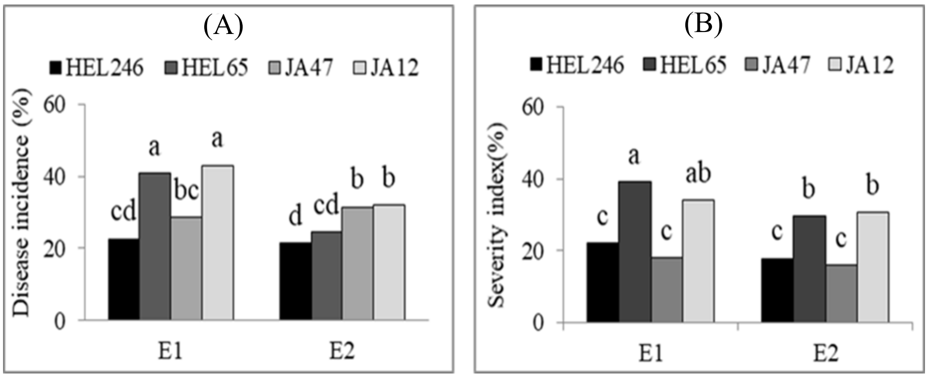


Figure 3. Incidence (A) and severity index (B) of stem rot caused by *S. rolfsii* on four varieties of Jerusalem artichoke under fertilized field (E1) with the application of fertilizer formula 15-15-15 at 156 kg ha^{-1} and unfertilized field (E2). Means (from n=16 observations) with the same letter(s) are not significantly different at 5% level by LSD.

References

Anfok, G. H. 2000. Benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum*. Mill cv.Vollendung) to cucumber mosaic virus. Crop Protection 19(6): 401-405.

Junsopa, C., S. Jogloy, W. Saksirirat, P. Songsri, T. Kesmala, B. B. Shew, and A. Patanothai, A. 2016. Inoculation with *Sclerotium rolfsii* cause of stem rot in Jerusalem artichoke under field conditions. European Journal of Plant Pathology146: 47–58.

Kays, S. J. and S. F. Nottingham. 2008. Biology and chemistry of Jerusalem artichoke *Helianthus tuberosus* L. Taylor & Francis, Florida, FL, USA.

McCarter, S. M., and S. J. Kays. 1984. Disease limiting production of Jerusalem artichokes in Georgia. Plant Disease. 68: 299–302.

Punja, Z. K., J. D. Carter, G. M. Campbell, and E. L. Rossell. 1986. Effects of calcium and nitrogen fertilizers, fungicides, and tillage practices on incidence of *Sclerotium rolfsii* on processing carrots. Plant Disease. 70: 819–824.

Sennoi, R., S. Jogloy, W. Suksisirat, and A. Patanothai. 2010. Pathogenicity test of *Sclerotium rolfsii*, a causal agent of Jerusalem artichoke (*Helianthus tuberosus* L.) stem rot. Asian Journal of Plant Science. 9(5): 281–284.

Publication

Chutsuda, J., W. Saksirirat, S. Saepaisan, P. Songsri, T. Kesmala, B. B. Shew, and S. Jogloy. 2021. Bio-control of stem rot in Jerusalem artichoke (*Helianthus tuberosus* L.) in field conditions. The Plant Pathology Journal 37 (5): 428-436. (Impact factor = 2.31: Q2)

3.11 Development of an oxalic acid assay to evaluate *Sclerotium rolfsii* resistance in Jerusalem artichoke

Sennoi, R., S. Jogloy and M. L. Gleason

Jerusalem artichoke has become a new crop with high potential for inulin production in Thailand (Puttha et al., 2012) and as a biomass crop in China (Yang et al., 2015). Stem rot caused by the soil-borne fungus *Sclerotium rolfsii* is a significant problem for Jerusalem artichoke production worldwide and yield losses up to 60% have been reported (McCarter and Kays, 1984). The use of resistant cultivars is potentially a sustainable way to control the disease. Available methods for screening accessions for resistance to *S. rolfsii* are impractical because they are labor-intensive, time-consuming, and difficult to conduct reliably. The development of simpler and more effective methods would provide an advantage for breeders in developing commercially acceptable cultivars with high levels of resistance.

Objective

The objective of this study was to assess the feasibility of developing an oxalic acid method for screening Jerusalem artichoke for resistance to *S. rolfsii*.

Materials and Methods

Plant materials were prepared from seeds of Jerusalem artichoke as described above. Stems of genotypes PI 650103, PI 547238, PI 547230, PI 650095 and PI 650091 were cut transversely at the soil line using a surface-sterilized scalpel. Mature leaves and petioles were removed from the stem except for two apical, fully-developed leaves. Each plant was placed immediately in a test tube containing oxalic acid solution (5 ml of 20, 30, or 40 mM oxalic acid). The amount and concentrations of OA were applied based on the protocol of Wegulo et al. (1998). As a control, an excised stem of each genotype was placed in a test tube containing sterile distilled water (5 ml) for each replication. Test tubes containing Jerusalem artichoke stems were arranged in test tube racks in a randomized complete block design with five replications. Blocking was done due to the slight difference in the light of each shelf in the dew chamber. For each genotype, there were two test tubes per concentration for each replication. After treatment, the test tubes were placed in a dew chamber at 100% relative humidity and 27 °C (Xu et al., 2009). The dew chamber was set up with 14h light and 10h dark (Wegulo et al., 1998). The experiment was conducted twice. Lesion length was measured daily 1 to 7 days after treatment (DAT) (Wegulo et al., 1998). Error variances between the two trials of the excised-stem assay were tested for homogeneity; data sets passing the homogeneity of variance criterion were subjected to a combined analysis of variance for the two trials. The least significant difference (LSD) was used to compare mean differences. All calculations were done using STATISTIX 8 software program (Analytical Software, Tallahassee, Florida, USA).

Results

In the replicated trials in which excised stems were immersed in oxalic acid solutions, discoloration and softening at the bases of the stems were noted on all accessions at all evaluation dates and concentrations in both runs of the experiment. The two runs of the experiment did not differ significantly for lesion length among accessions at 1, 3, 4, 5, 6, or 7 DAT (Table 1). Therefore, the data were pooled for the two experiments. Jerusalem artichoke accessions were significantly different ($P < 0.01$) for lesion length at 3 to 7 DAT. Significant differences ($P < 0.01$) among concentrations of oxalic acid were observed for lesion length at 1 to 7 DAT, and higher concentrations of oxalic acid and longer duration of evaluation time resulted in increased lesion length. Lesion lengths ranging from 4.4 to 15.5 cm were observed across oxalic acid concentrations and evaluation dates. The longest lesions were observed for an oxalic acid concentration of 40 mM at 7 DAT, and the shortest lesions were found for an oxalic acid concentration of 20 mM at 1 DAT.

Table 1. Mean squares for overall ANOVA of stem lesion length in Jerusalem artichoke (*Helianthus tuberosus*) evaluated at 1, 3, 5 and 7 days after treatment (DAT). Excised stems of Jerusalem artichoke were immersed in a range of concentrations of oxalic acid, and length of stem discoloration from the excised end was measured. Means shown represent two runs of the experiment.

Source of Variation	d.f.	Lesion length						
		1 DAT	2 DAT	3 DAT	4DAT	5 DAT	6DAT	7 DAT
Experiment (E)	1	1.94ns	4.16*	1.06ns	0.47ns	0.10ns	0.17ns	0.02ns
Rep within experiment	4	0.61ns	0.74ns	1.04ns	0.28ns	0.33ns	0.89ns	0.64ns
Accessions (A)	4	1.82**	1.77**	19.95**	108.68**	177.94**	199.39**	139.21**
Concentrations (C)	2	10.58**	22.65**	19.72**	89.66**	12.25**	7.83**	13.63**
A × C	8	0.34ns	0.26ns	3.23**	9.45**	7.48**	18.82**	15.36**
Pooled error	126	0.27	0.31	0.38	0.54	0.51	0.46	0.47
CV (%)		10.92	10.1	7.86	6.76	5.16	4.84	4.59

ns, *, **Non-significant, significant at 0.05 and 0.01 probability level

Jerusalem artichoke accessions responded differently to oxalic acid treatment in this experiment. Accessions PI 650091 and PI 547238 had shorter stem lesions than accessions PI 547230, PI 650095 and PI 650103 for most concentrations and observation dates. Lesion length in all Jerusalem artichoke accessions increased most rapidly between 2 and 5 DAT (Figure 1).

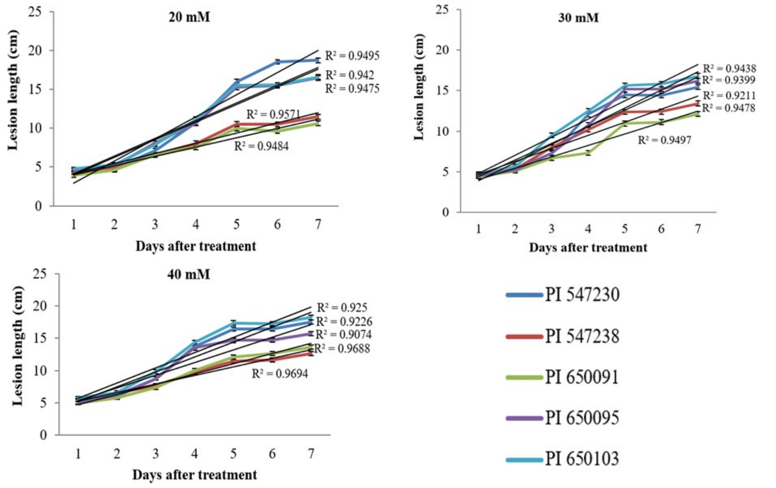


Figure 1. Effect of oxalic acid on lesion length of excised stems of five accessions of Jerusalem artichoke evaluated at 1 to 7 days after treatment in different concentrations of oxalic acid**.** Means shown represent two separate runs of the experiment**.**

Responses of Jerusalem artichoke accessions to oxalic acid concentrations at 6 DAT are shown in Figure 2. Higher concentrations of oxalic acid tended to result in longer lesion lengths for accessions PI 650103 and PI 650091. Conversely, PI 650095 had a shorter lesion length when oxalic acid concentration was higher. Variation in lesion length among accessions was determined by F-test value and coefficient of variation (CV) (Table 2). Treatment with 20 mM oxalic acid evaluated at 6 DAT showed the highest variation for lesion length in five Jerusalem artichoke accessions. Lesion lengths ranging from 9.6 to 18.5 cm were observed in Jerusalem artichoke accessions immersed in 20 mM oxalic acid and evaluated at 6 DAT. PI 650091 had the shortest lesions, whereas PI 547230 had the longest lesions (Figure 3).

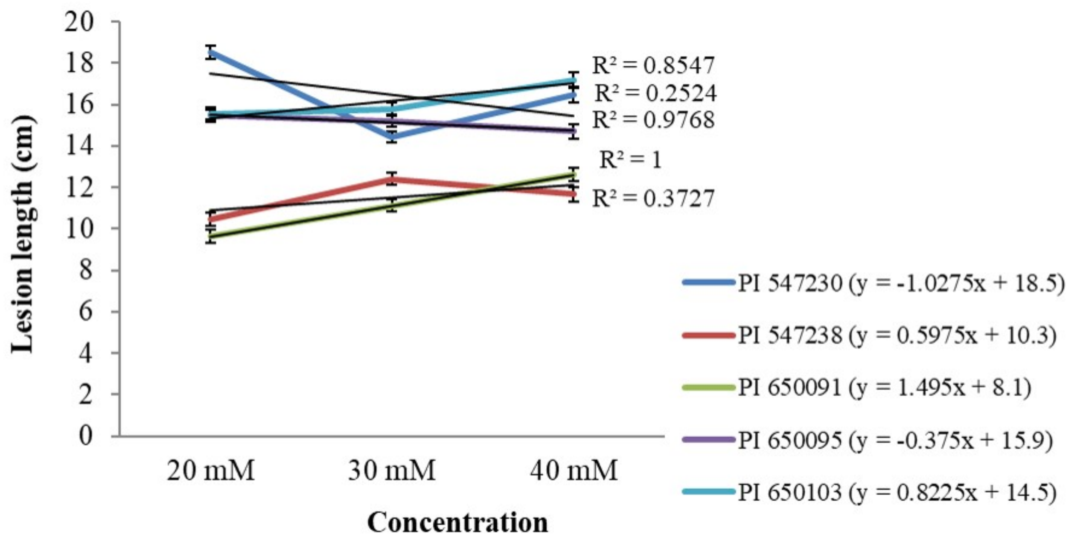


Figure 2. Lesion length of stem rot in five accessions of Jerusalem artichoke at 6days after treatment with different concentrations of oxalic acid.

Table 2.F-ratios for lesion length in Jerusalem artichoke accessions treated with three concentrations of oxalic acid evaluated at 1, 2, 3, 4, 5, 6 and 7 days after treatment (DAT). Excised stems were immersed in a range of concentrations of oxalic acid, and length of stem discoloration from the excised end was measured. Values shown represent two runs of the experiment.

Oxalic acid concentrations	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
20 mM	3.19*	1.95ns	11.62**	47.35**	137.29**	290.24**	267.21**
CV (%)	12.82	11.96	9.05	8.05	5.88	5.01	4.62
30 mM	0.92ns	1.64ns	30.82**	90.09**	93.05**	99.94**	89.88**
CV (%)	10.16	10.22	7.89	6.44	4.73	4.51	4.50
40 mM	4.07**	3.30*	24.94**	78.54**	148.32**	97.44**	96.19**
CV (%)	10.69	9.31	7.41	6.41	4.70	5.26	4.97

ns, *, **Non-significant, significant at 0.05 and 0.01 probability level

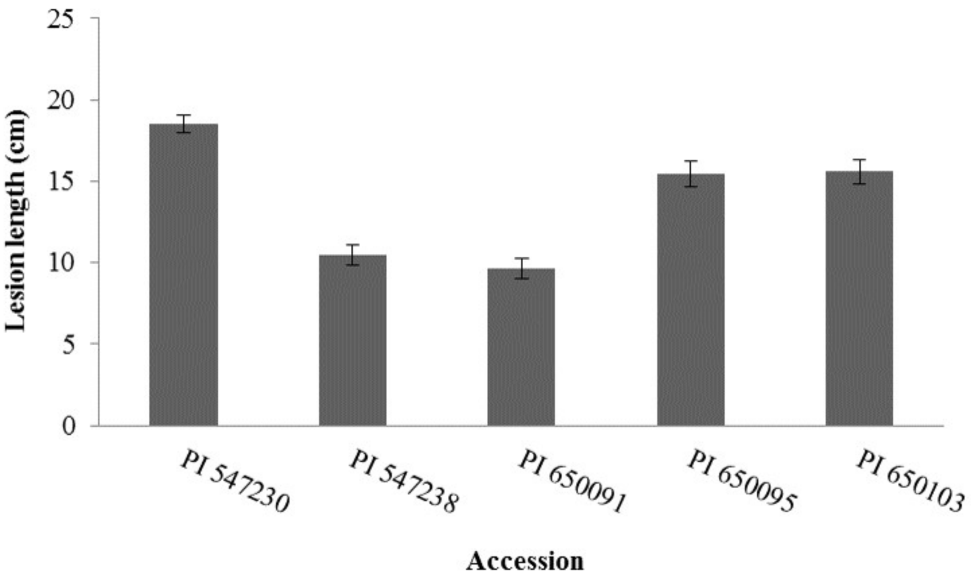


Figure 3. Lesion length of excised stems of five accessions of Jerusalem artichoke at 6 days after treatment with 20 mM oxalic acid. Means shown represent two runs of the experiment.

Conclusions

The OA stem immersion assay protocol may require further optimization. Suitable concentration and time for evaluation are important for the development of a reliable screening assay. In this study, the optimal concentration was 20 mM, and the optimal evaluation time was 6 DAT

References

McCarter, S.M., and S.J. Kays. 1984. Disease limiting production of Jerusalem artichoke in Georgia. Plant Disease 68: 299–302.

Puttha, R., S. Jogloy, P. P. Wangsomnuk, S. Srijaranai, T. Kesmala and A. Patanothai. 2012. Genotypic variability and genotype by environment interactions for inulin content of Jerusalem artichoke germplasm. Euphytica 183: 119 –131.

Wegulo, S. N., X. B. Yang and C. A. Martinson. 1998. Soybean cultivar responses to *Sclerotinia sclerotiorum* in field and control environment studies. Plant Disease 82: 1264–1270.

Xu, Z., M. L. Gleason and D. S. Mueller. 2009. Development of rapid method using oxalic acid to assess resistance among hosta cultivars to petiole rot caused by *Sclerotium rolfsii* var. *delphinii*. Online. Plant Health Program.

Yang, L., Q. S. Hea, K. Corscaddena and C. C. Udenigweb. 2015. The prospects of Jerusalem artichoke in functional food ingredients and bio-energy production. Biotechnology Reports 5:77-88.

Publication

Sennoi, R., S. Jogloy and M.L. Gleason. 2021. Development of an oxalic acid assay to evaluate *Sclerotium rolfsii* resistance in Jerusalem artichoke. SABRAO Journal of Breeding and Genetics. 53 (1): 70-78. (SNIP =0.30: Q3)

3.12 Determination of lethal dose and effect of gamma rays on growth and tuber yield of Jerusalem artichoke mutant

Songsri, P., S. Jogloy, C. C. Holbrook and D. Puangbut

Jerusalem artichoke is obligated out crosser and produces seeds only when cross-pollinated. Seed production varies with clones (Konvalinková, 2003) and usually wild clones produce more seed per flower than cultivated clones. Self-incompatibility has been demonstrated to reduce population size and leads to a reduction of genetic diversity and may increase the expression of inbreeding depression (Busch and Schoen, 2008; Silva et al., 2016). Induced mutations by gamma-ray irradiation can increase the genetic variation of economically important traits in germplasm. Gamma radiation is a tool that can be used to increase genetic variation for different plant characteristics such as plant height, flower shape and flower color in horticulture plants (Aslam et al., 2016). Determination of LD50 is very important for mutation breeding. Limited information is available on the determination of lethal dose and effects of gamma-ray irradiation on the growth and yield of Jerusalem artichoke.

Objective

The objectives of this study were to determine the LD50 of Jerusalem artichoke and measure the effects of gamma rays on growth and tuber yield.

Materials and Methods

A pot experiment was carried out under a rainout shelter in the post-rainy season from September to December 2016 at the Field Crop Research Station of Khon Kaen University. The experimental design was a 2×7 factorial in a completely randomized design (CRD) with four replications with two Jerusalem artichoke genotypes as factor A and seven gamma radiation doses including control (0 Gy) as factor B. Two commercial genotypes of Jerusalem artichoke grown in Thailand were used in this study. CN 52867 was donated by the Plant Gene Resource of Canada (PGRC) and HEL 65 was donated by the Leibniz Institute of Plant Genetics and Crop Plant Research of Germany (IPK). Tubers with axillary buds for the two genotypes were treated with gamma radiation at the doses of 0, 5, 10, 15, 20, 25 and 30 Grays (Gy) at the department of Applied Radiation and Isotop, Kasetsart University in Bangkok. The next day irradiated tubers were cut into small pieces each of which had one bud. The tuber pieces were incubated in coconut peat media under ambient conditions for 7 days or until a sprout developed from the bud on the tuber (VE stage). Sprouted seed tubers of two Jerusalem artichoke were grown in plastic pots 32 cm in diameter and 28 cm in height. The healthy seedlings were selected and transplanted into pots as one seedling per pot. Fertilizer of formula 15-15-15 was applied 30 days after transplanting (DAT) at a rate of 156 kg per ha^{-1} (2 g per pot)

The LD50 was determined using Microsoft Excel 2010 based on the number of survival plants at the different doses of gamma radiation. A regression equation was used to determine the LD50. Data were recorded for a percentage of germination at 10 days after incubation (DAI). Germination was confirmed when a sprout developed from a bud on a tuber and the percentage of emergence was computed. After that, they were transferred to plug plastic trays. After 7 days of growth in plug plastic trays, sprouted seed tubers which had two leaf-sprouted seedlings (V2) were transplanted to pots. The number of surviving plants was recorded at 7, 14, and 21 days after transplanting and then the percentage of survival plants was calculated.

The number of branches was recorded at 8 weeks after radiation. Tubers were harvested at maturity depending on genotypes. Tubers were washed in tap water to remove the soil and then tuber fresh weight was determined.

Individual analysis of variance was performed for each character following a factorial in CRD. The Least Significant Difference (LSD) test was used to compare means using a STATISTIX8 software program.

Results

The results indicated that plantlet survival decreased with increasing doses of gamma radiation (Figure 1). The LD_{50} of Jerusalem artichoke genotypes was 22 and 27 Gy for HEL 65 and CN 52867, respectively. There were no deaths in the control treatment (0 Gy), while treatments with irradiation levels higher than the LD_{50} value caused a high number of plantlet deaths. An irradiation dose above 30 Gy caused complete death for both genotypes.

Plant mutants from the 20 and 25 Gy treatments had a higher stem branching than the other gamma-ray doses (Table 1 and Figure 2) and usually plant mutants produce more branches (6) than normal plants (1 or 2).

Plant height differed significantly among gamma irradiation doses at 4, 6 and 8 weeks after irradiation (Table 2). The results indicated irradiation at 25 Gy resulted in reduced plant height compared to lower gamma irradiation doses in both HEL 65 and CN 52867 genotypes.

The difference in gamma ray doses had a significant effect on fresh tuber yield in both HEL 65 and CN 52867 genotypes (Figure 3). The results revealed that irradiation at 5 Gy promoted fresh tuber yield compared to the control and other gamma irradiation doses in both HEL 65 and CN 52867 genotypes. Irradiation at 5 Gy increased fresh tuber yield an average of 10% over the control.

With an increase in the radiation dose at 10, 15, 20 and 25 Gy, the fresh tuber yield decreased an average of 13%, 17%, 11% and 15% over the control. The results indicated that 15 and 10 Gy of gamma dose had higher reduced tuber yield for CN 52867 and HEL 65, respectively. The results demonstrated that a high dose of radiation decreased the tuber yield of Jerusalem artichoke.

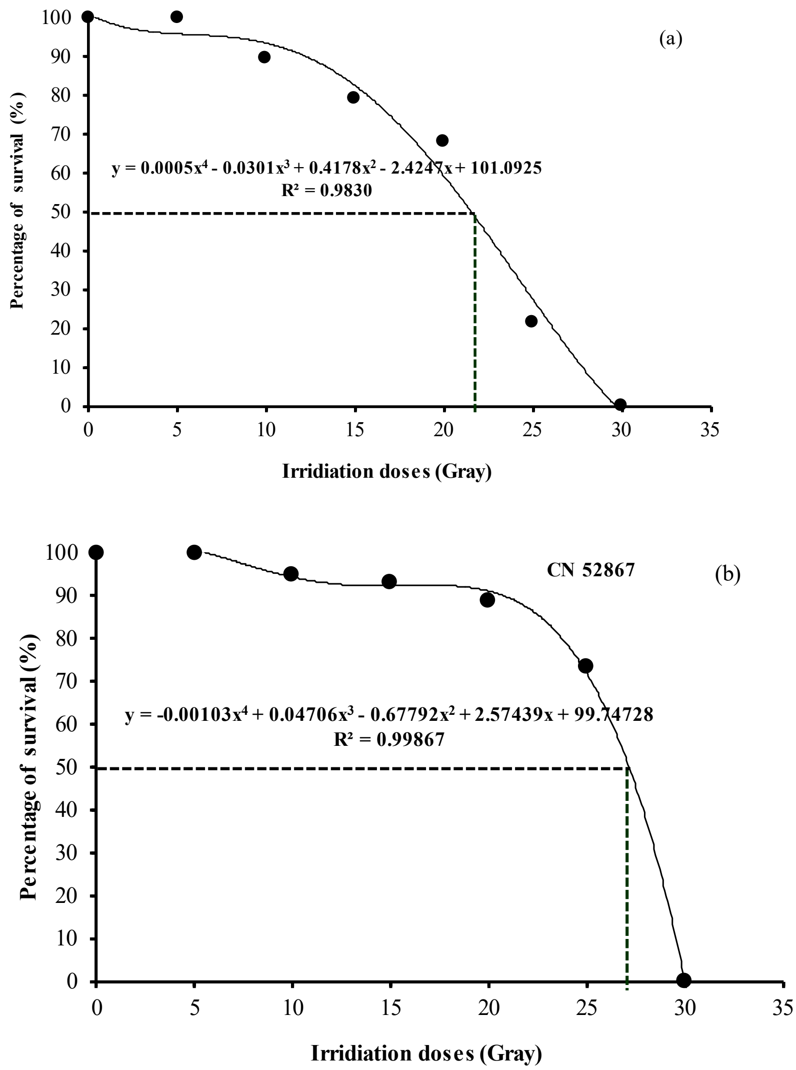
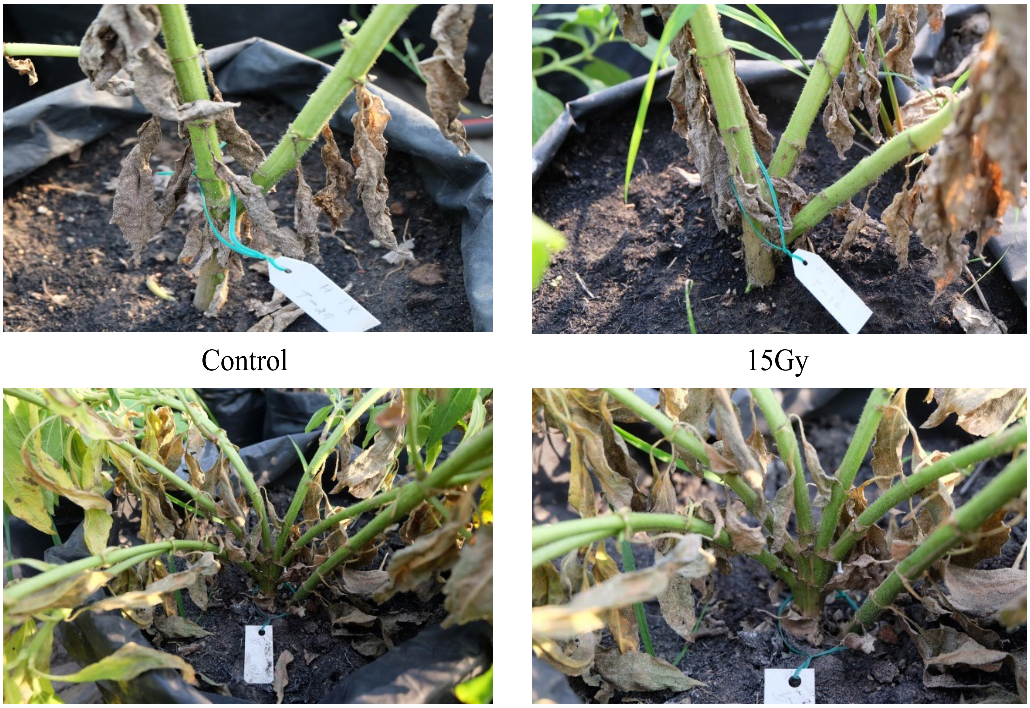


Figure 1. Lethal dose 50 (LD_{50}) of Jerusalem artichoke genotype HEL 65 (a) and CN 52867 (b)

Table 1. Number of stem branching of HEL 65 and CN 52867 genotypes at 4 weeks after irradiation at different irradiation doses.

Irradiation doses	number of stem branching	
	HEL 65	CN 52867
0 Gy	2.0 b	1.0 c
10 Gy	2.2 b	1.8 b
15 Gy	3.0 b	2.0 b
20 Gy	6.0 a	3.8 a
25 Gy	6.0 a	4.0 a
Mean	3.8	2.6

Means in the same column with the same letters are not significantly different by LSD (P≤ 0.05)



Control 15Gy

20 Gy 25 Gy

Figure 2. Effect of gamma irradiation on branching on the main stem of Jerusalem artichoke genotypes

Table 2. Effect of gamma irradiation on plant height of Jerusalem artichoke genotypes at 2, 4, 6 and 8 weeks after irradiation

	2 week		4 week		6 week		8 week	
Irradiation doses	HEL 65	CN 52867	HEL 65	CN 52867	HEL 65	CN 52867	HEL 65	CN 52867
0 Gy	8.1 a	9.9 a	43.0 a	63.0 a	90.8 a	90.4 a	96.7 a	92.8 a
5 Gy	8.3 a	9.0 a	36.4 a	49.8 b	95.6 a	76.8 d	96.0 a	79.6 c
10 Gy	7.4 b	7.3 b	30.4 b	35.4 c	88.1 ab	76.4 d	94.7 a	77.6 c
15 Gy	7.1 b	6.1 c	24.6 c	32.7 c	84.0 b	73.2 d	95.7 a	74.0 c
20 Gy	6.7 b	7.4 b	20.9 c	41.0 bc	78.3 b	79.5 b	90.2 b	81.4 b
25 Gy	5.4 c	5.9 c	15.4 d	18.1 d	58.6 c	65.7 d	73.5 c	70.7 d
Mean	7.2	7.6	28.5	40.0	82.6	77.0	91.1	79.4

Means in the same column with the same letters are not significantly different by LSD (P ≤ 0.05

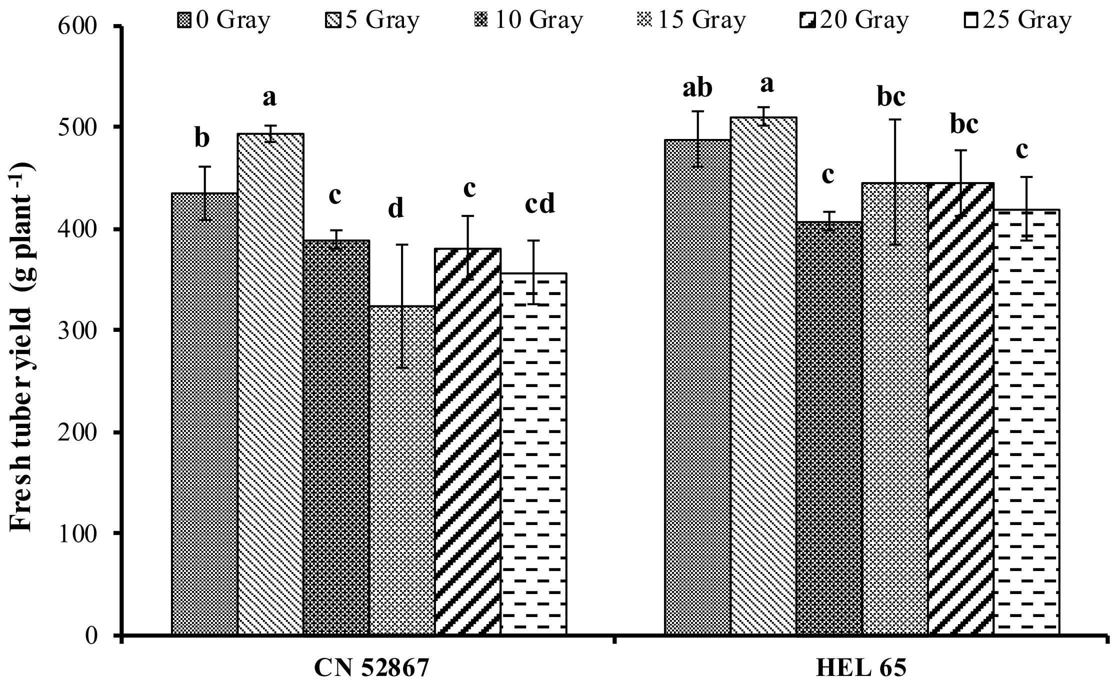


Figure 3. Effect of gramma irradiation on fresh tuber yield of Jerusalem artichoke genotypes at harvest. Means of three replicate ± SD

Conclusions

The LD50 of Jerusalem artichoke was determined based on the percentage of surviving plants. The LD50 of HEL 65 and CN 52867 genotypes were 22 and 27 Gy, indicating that Jerusalem artichoke had low radio sensitivity to gamma rays. Gamma irradiation at 20 and 25 Gy increased the number of shoots compared to the control treatment, which decreased plant height. The present study revealed that tuber yield increased with an irradiation dose of 5 Gy for both genotypes. A low dose can cause changes in morphological characteristics and increased tuber yield of Jerusalem artichoke.

References

Aslam, F., S. Naz and S. Javed. 2016. Effect of radiation on morphological characters of different cultivars of lilium and genetic analysis of mutants through molecular markers. Journal of Animal and Plant Sciences 26(6):1819-1827.

Busch, J. W., and D. J. Schoen. 2008. The evolution of self-incompatibility when mates are limiting. Trends in Plant Science13:128-135.

Konvalinková, P. 2003. Generative and vegetative reproduction of *Helianthus tuberosus*, an invasive plant in Central Europe. In: L. E. Child, J. H. Brock, G. Brundu, K. Prach, K. Pysěk, P.M. Wade and M. Williamson, eds., Plant invasions: Ecological Threats and Management Solutions. Backhuys, Leiden, pp. 289-298.

Silva, J. L., A. C. Brennan, J. A. Mejías. 2016. Population genetics of self-incompatibility in a clade of relict cliff-dwelling plant species. AoBPLANTS 8: doi.org/10.1093/aobpla/plw029

Publication

Songsri, P., S. Jogloy, C. C. Holbrook and D. Puangbut. 2019. Determination of lethal dose and effect of gamma rays on growth and tuber yield of Jerusalem artichoke mutant. SABRAO Journal of Breeding and Genetics. 51 (1): 1-11. (SNIP =0.30: Q3)

3.13 Genotypic variability of total phenolic compounds and antioxidant activity in Jerusalem artichoke (*Helianthus tuberosus* L.) germplasm

Ruttanaprasert R., R. Sennoi, R. Puttha, S. Jogloy and T. Senawong*

Jerusalem artichoke (*Helianthus tuberosus* L.) originated in North America and is an underutilized crop for human health. The tuber of Jerusalem artichoke comprises approximately 80% water, 15% carbohydrate, and 1 to 2% protein. The tuber of Jerusalem artichoke is being the most important source of inulin. Moreover, it contains many effective compounds including coumarins, unsaturated fatty acids, polyacetylene derivatives, sesquiterpenes and phenolic compounds. Phenolic compounds are secondary plant metabolites that have several benefits for human health (Pan et al., 2009) and biological control in the plant (Dykes and Rooney, 2007). Peels of Jerusalem artichoke tubers were found to contain a relatively high level of total phenolics (39-129 gallic acid equivalent (GAE) /100 g fresh weight) (Slimestad et al., 2010). Kapusta et al. (2013) found that among the caffeoylquinic acids, the 3-o-caffeoylquinic acid showed the highest concentration in the Jerusalem artichoke tuber. Furthermore, Tchone et al. (2006) also found that 100 g tuber had a high concentration of some phenolic compounds such as chlorogenic acid (20-5,000 mg), gentistic acid (30-3,000 mg) and salicylic acid (30-7,000 mg). Twenty-two phenolic compounds are found in the tuber of Jerusalem artichoke such as gallic acid (1 - 140 mg/100 g dry weight), chlorogenic acid (20 – 5,000 mg/100 g dry weight) and p-coumaric acid (1 - 40 mg/100 g dry weight) (Tchone et al., 2006). Those compounds have protection roles against the development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Pandey and Rizvi, 2009). Furthermore, the Jerusalem artichoke has great potential in terms of antioxidant capacity. DPPH radical scavenging activity of Jerusalem artichoke tuber ranged from 7.14 to 11.79% (Kim et al., 2010).

Nowadays, Jerusalem artichoke is received more attention from agronomists and plant breeders because a whole plant is an important source of several value-added products for human consumption as a pharmaceutical. In the past, a few studies were conducted to demonstrate the genetic diversity of phenolic compounds and antioxidant activity in Jerusalem artichoke germplasm. Therefore, this present work was carried out to determine the variability of phenolic compounds in tubers of twenty-five Jerusalem artichoke genotypes. The information obtained in this work may help breeders to select Jerusalem artichoke genotypes with high phenolic compounds and antioxidants. The Jerusalem artichoke genotypes with high phenolic compounds and antioxidants in tubers could be applied for producing pharmaceutical products in the future.

Objective

The objective of this study was to determine the variability of phenolic compounds in tubers of twenty-five Jerusalem artichoke genotypes.

Materials and Methods

Twenty-five genotypes of Jerusalem artichoke were evaluated for phenolic compounds. The field experiment was set up in a randomized complete block design with three replications from March to June 2018 at the Agronomy Field, Faculty of Agriculture, Khon Kaen University, Thailand. The crop was planted in 2-row plots with 4 m in length and spacing 50 × 20 cm.

The crop was harvested at the maturity stage and the data were recorded for tuber dry weight, individual tuber dry weight, and the number of tubers per plant. The tubers were analyzed for total phenolic contents. Total phenolic content was determined by the

Folin–Ciocalteu's reaction method (Chen et al., 2014) and phenolic content was calculated as gallic acid equivalent (mg GAE/10 g of dry weight samples) (Khaopha et al., 2012).

Antioxidant activity was determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging activity assay. The method was described by measuring the capacity of bleaching a black-colored methanol solution of DPPH radicals and ferric reducing antioxidant power (FRAP) method as reported by Benzie and Strain (1996). The 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging capacity was measured according to the method of Re et al. (1999) with slight modification.

Individual analysis of variance was conducted for each parameter according to randomized complete block design (RCBD). Duncan's multiple range test (DMRT) was used to compare means when the differences among genotypes are significant ($p \leq 0.05$). Simple correlations were used to determine the relationships between tuber dry weight, individual of tuber dry weight, number of tubers per plant, total phenolic content and antioxidant activity by DPPH FRAP and ABTS methods. All calculations were done using MSTAT-C package (Bricker, 1989). A data matrix of the 25 genotypes of Jerusalem artichoke was made using the means of yield, phenolic content and antioxidant activity. The cluster analysis based on Ward's method and squared Euclidian distance was done and the dendrogram was constructed.

Results

Analysis of variance showed highly significant differences between Jerusalem artichoke genotypes for tuber dry weight, individual tuber dry weight, number of tubers per plant, total phenolic content, and antioxidant activity determined by FRAP and ABTS method except for antioxidant activity determined by DPPH method (Table 1.). Significant differences among Jerusalem artichoke varieties were observed for tuber dry weight, individual tuber dry weight, total phenolic content, and antioxidant activity determined by FRAP and ABTS method ($P \leq 0.01$) (Table 2). Tuber dry weights ranged from 30.48 to 186.69 g/plant. Mean for tuber dry weight was 86.01 g/plant. JA 89, CN52867, KT504, HEL 53 and JA 6 had high tuber dry weights. Mean for individual tuber dry weight was 4.23 g/tuber. HEL 53, HEL 65, JA 76, CN52867 and HEL231 had high individual tuber dry weights. The number of tubers per plant ranged from 8.00 to 35.33 tubers. Mean for the number of tubers per plant was 22.55 tubers. JA 109, JA 5, JA 6, KT 504 and JA 89 had a high number of tubers per plant. Total phenolic contents ranged from 4.53 to 51.97 g/plant. The mean for phenolic content was 86.01 g/plant. KT504, JA 3, JA 76, HEL 65, and JA 89 had high phenolic content. Mean for antioxidant activity determined by FRAP method was 28.78 µmol Fe^{+2}/100 g. KT504, JA67, HEL 257, JA 89 and JA 6 had high antioxidant activity determined by FRAP method. Antioxidant activities determined by ABTS method ranged from 54.65 to 67.06%. Mean of antioxidant activity determined by ABTS method was 59.04%. CN52867, HEL 62, HEL 65, JA 67, JA 76, HEL 53 and JA 6 had high ABTS antioxidant activities.

Positive and highly significant correlations were found between tuber dry weight and total phenolic content ($r=0.34$, $P \leq 0.01$), between tuber dry weight and individual tuber dry weight ($r=0.54$, $P \leq 0.01$), between tuber dry weight and number of tubers per plant ($r=0.40$, $P \leq 0.01$) and between tuber dry weight and FRAP antioxidant activity ($r=0.33$, $P \leq 0.01$). In addition, positive and significant correlations were found between total phenolic content and the number of tubers per plant ($r=0.23$, $P \leq 0.01$). There was a highly significant linear correlation between total phenolic content and FRAP antioxidant activity ($r= 0.58$, $P \leq 0.01$). Positive significant correlations were found between individual tuber dry weight and ABTS antioxidant activity ($r=0.28$, $P \leq 0.05$). Conversely, negative and significant correlations were found between individual tuber dry weight and the number of tubers per plant ($r= -0.47$, $P \leq 0.01$) and between the number of tubers per plant and ABTS antioxidant activity ($r= -0.26$, $P \leq 0.05$).

A dendrogram based on tuber dry weight, phenolic content, DPPH and FRAP could divide 25 Jerusalem artichoke genotypes into six clusters (R-square = 0.80-0.92) (Figure 1). Group A consisted of 3 genotypes (CN52867, HEL 53 and JA 89). All genotypes had high tuber dry weight, medium values of phenolic content, DPPH and FRAP. Group B consisted of 6 genotypes (HEL 231, JA 109, HEL 256, HEL 65, HEL 257, HEL 67). Most of them had medium values of tuber dry weight, the medium value of phenolic content and high value of DPPH and FRAP. Group C consisted of 5 genotypes (HEL 253, JA 122, JA 1, JA 125 and JA 6). Most of them had medium values of tuber dry weight, low values of phenolic content, high values of DPPH and low values of FRAP. Group D consisted of 5 genotypes (HEL 62, JA 4, JA 37, JA 60 and JA 36). Most of them had low values of tuber dry weight, medium values of phenolic content, medium values of DPPH and medium values of FRAP. Group E consisted of 5 genotypes (JA 15, JA 3, JA 76, JA 21 and JA 5). Most of them had medium values of tuber dry weight, medium values of phenolic content, low values of DPPH and low values of FRAP. Group F had one genotype, KT504, which had high of tuber dry weight, high phenolic content, and high antioxidant activity determined by DPPH and FRAP.

Table 1. Mean squares for tuber dry weight, individual tuber dry weight, number of tubers per plant, total phenolic content, and antioxidant activity determined by DPPH, FRAP and ABTS methods of 25 Jerusalem artichoke genotypes grow under field conditions from March to June 2018.

Source of variation	DF	Tuber dry weight (g/plant)	Individual tuber dry weight (g/tuber)	Number of tubers per plant (tuber/ plant)	Total phenolic content (mg GAE/ g of dry weight)	Antioxidant activity		
						DPPH (%)	FRAP (µmol Fe^{+2}/ 100 g)	ABTS (%)
Replication	2	184.26 (0.33)	1.772 (1.05)	9.613 (0.33)	1.44 (0.05)	154.66 (10.73)	24.56 (0.29)	5.72 (1.16)
Genotypes	24	4208.52 (89.39)**	11.6325 (82.78)**	180.08 (74.84)**	236.08 (92.69)**	34.83 (29.00)^{ns}	579.00 (81.05)**	21.70 (52.89)**
Error	46	241.96 (10.28)	1.136 (16.17)	29.863 (24.82)	9.25 (7.27)	36.20 (60.27)	66.67 (18.67)	9.43 (45.94)
Total	72							
CV (%)		18.09	25.22	24.24	26.53	8.94	27.37	5.20

ns,** = non significant and significant at P ≤ 0.01 level, respectively.

Numbers within the parentheses are percentages of the sum of squares to the total sum of squares.

Table 2. Means for tuber dry weight (g/plant), Individual tuber dry weight (g/tuber), Number of tubers per plant (tuber/plant), phenolic content (mg GAE/ g of dry

weight) and antioxidant activity determined by DPPH method and FRAP method of 25 Jerusalem artichoke genotypes grow under field condition during March to June 2018.

Variety	Tuber dry weight (g/plant)		Individual tuber dry weight (g/tuber)		Number of tubers per plant (tuber/ plant)		Phenolic content (mg GAE/g of dry weight)		Antioxidant activity				
									DPPH (%)	FRAP (µmol Fe^{+2}/100 g)		ABTS (%)	
KT50-4	147.97	bc	4.94	c-f	32.67	a-c	51.97	a	69.87	71.63	a	58.47	c-g
JA89	186.69	a	6.18	b-e	30.33	a-d	13.29	b-d	69.20	39.20	b-d	58.08	c-g
JA76	87.12	e	6.79	b	14.00	g-j	13.61	bc	64.73	17.03	f-h	61.07	b-e
JA67	81.24	e-g	3.57	f-h	23.33	d-f	8.67	c-h	74.77	49.20	b	62.13	a-d
JA60	47.55	h-j	2.59	h-j	19.00	f-i	8.16	e-h	65.67	20.20	e-h	58.49	c-g
JA6	121.97	d	3.60	f-h	34.33	ab	10.59	c-g	70.30	39.00	b-d	60.27	b-f
JA5	81.84	ef	2.33	h-j	35.33	a	4.53	h	64.07	16.30	f-h	57.87	c-g
JA4	64.79	e-h	2.46	h-j	26.33	b-f	6.74	f-h	63.60	25.60	d-h	58.79	b-g
JA37	58.65	f-i	3.03	g-j	19.67	f-i	12.19	b-e	65.77	26.20	d-h	58.81	b-g
JA36	30.48	j	1.62	j	18.67	f-i	9.02	c-h	67.17	25.20	d-h	58.22	c-g
JA3	89.42	e	3.99	f-h	25.33	c-f	15.83	b	62.40	16.73	f-h	56.31	e-g
JA21	56.01	g-j	2.59	h-j	21.67	d-h	5.77	gh	62.10	10.93	h	54.65	g
JA15	78.92	e-g	2.65	h-j	29.33	a-e	11.22	b-f	60.60	11.77	gh	59.17	b-g
JA125	73.62	e-g	3.62	f-h	20.33	f-i	6.07	gh	68.10	17.53	f-h	56.13	e-g
JA122	80.66	e-g	3.95	f-h	20.67	e-i	7.19	f-h	69.43	29.80	c-g	59.08	b-g
JA109	64.26	e-h	1.80	ij	35.33	a	11.10	b-f	70.77	38.43	b-e	57.35	d-g
JA1	87.98	e	3.56	f-h	24.67	c-f	7.97	e-h	70.73	23.97	d-h	55.97	fg
HEL65	59.86	f-i	7.34	b	8.00	j	13.39	b-d	68.57	33.40	b-f	62.54	a-c
HEL62	37.55	ij	4.54	d-g	8.00	j	8.53	d-h	63.37	20.30	e-h	63.54	ab
HEL53	131.11	cd	9.92	a	13.67	h-j	9.32	c-h	66.33	21.37	d-h	60.47	b-f
HEL257	86.54	e	3.95	f-h	22.67	d-g	10.21	c-g	70.33	48.10	bc	58.11	c-g
HEL256	66.35	e-h	3.54	f-i	22.00	d-h	12.79	b-e	69.43	37.03	b-e	56.57	e-g
HEL253	87.19	e	4.43	e-g	19.67	f-i	9.62	c-g	68.53	26.80	d-h	57.88	c-g
HEL231	82.19	ef	6.41	bc	12.67	ij	10.03	c-g	70.30	33.50	b-f	58.95	b-g
CN52867	160.21	b	6.25	b-d	26.00	b-f	8.82	c-h	66.47	20.23	e-h	67.06	a
Means	**86.01**		**4.23**		**22.55**		**11.47**		**67.30**	**28.78**		**59.04**	

Means in the same column followed by the same letter(s) are not significantly different at P≤0.01 probability level by Duncan's multiple range test (DMRT).

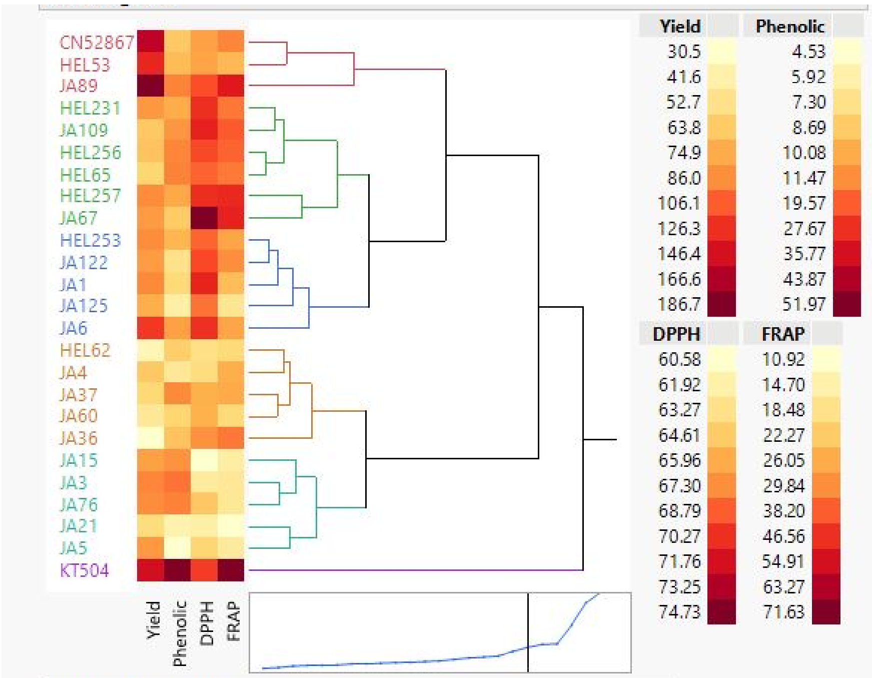


Figure 1. Dendrogram of genetic relationships among 25 Jerusalem artichoke genotypes grow under field condition during March to June 2018. Two ways Ward's cluster analysis based on tuber dry weight (g/plant), phenolic content (mg GAE/g of dry weight) and antioxidant activity determined by DPPH method and FRAP.

Conclusion

The results showed that there were significant genetic variations in tuber dry weight, phenolic content, and antioxidant activity determined by FRAP and ABTS methods in this set of Jerusalem artichoke accessions. The relationships of these parameters with tuber dry weight, including phenolic content, and antioxidant activity determined by FRAP method could serve as indexes to directly select Jerusalem artichoke genotypes with high phenolic compound and antioxidant activity. Jerusalem artichoke genotypes were classified into six groups based on tuber dry weight, phenolic content, and antioxidant activity determined by DPPH method and FRAP method. KT504 was identified as the accession with high values of tuber dry weight, phenolic content and antioxidant activity determined by DPPH and FRAP methods, and this genotype might be used as a material source for the pharmaceutical industry.

References

Bricker, A. A., 1989. MSTAT-C user's guide. Michigan State University, East Lansing.

Chen, F., X. Long, Z. Liu, H. Shao, L. Liu, F. Chen, X. Long, Z. Liu, H. Shao and L. Liu. 2014. Analysis of phenolic acids of Jerusalem artichoke (*Helianthus Tuberosus* L.) responding to salt-stress by liquid chromatography/tandem mass spectrometry. The Scientific World Journal 2514: 568043 doi: 10.1155/2014/568043

Dykes, L., and L. W. Rooney. 2007. Phenolic compounds in cereal grains and their health benefits. Cereal Food World 52: 105-111.

Kapusta, I., E. S. Krok, D. B. Jamro, T. Cebulak, J. Kaszuba, and R. T. Salach. 2013. Identification and quantification of phenolic compounds from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Journal of Food, Agriculture and Environment, 11(3 and 4), 601-606.

Khaopha, S., T. Senawong, S. Jogloy, and A. Patanothai. 2012. Coparison of total phenolic content and composition of individual phenolic acids in testae and testa-removed kernels of 15 Valencia-type peanut (*Arachis hypogaea*) genotypes. African Journal of Biotechnology, 11(92), 15923-15930.

Kim, D., Fan, J. P., Chung, H. C. and Han, G. D. 2010. Changes in extractability and antioxidant activity of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers by various high hydrostatic pressure treatments. Food Science and Biotechnology, 19(5), 1365-1371.

Pandey, K.B. and S.I. Rizvi. 2009. Plant polyphenols as dietary antioxidants in humanhealth and disease. Oxidative Medicine and Cellular Longevity 2:270–278.

Pan, L., Sinden, M. R., Kennedy, A. H., Chai, H., Watson, L. E., Graham, T. L. and Kinghorn, A.D. 2009. Bioactive constituents of *Helianthus tuberosus* (Jerusalem artichoke). Phytochemistry Letters, 2(1), 15–18.

Slimestad, R., Seljasen R., Meijer, K. and Skar. S. L. 2010. Norwegian-grown Jerusalem artichoke (*Helianthus tuberosus* L.): Morphology and content of sugars and fructo-oligosaccharides in stems and tubers. *Journal of the Science of Food and Agriculture, 90*, 956–964.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-evan, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), 1231-1237.

Tchone, M., G. Barwald, G. Annemuller, and L. G. Fleischer. 2006. Separation and identification of phenolic contents in Jerusalem artichoke (*Helianthus tuberosus* L.). Sciences des Aliments 26(5): 394–408.

Benzie, F. and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry. 239(1): 70-76.

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3.14 Efficiency of plant growth promoting microorganism inoculants for enhancing growth, production and tuber inulin accumulation of Jerusalem artichoke

S. Boonlue, N. Riddech and W. Mongkolthanaruk

Plant growth-promoting microorganisms (PGPM play effective roles as symbionts- receiving food from plants for colonization in the rhizosphere or inside plants and promoting plant growth by solubilization of nutrients, producing phytohormones and inducing plant immune response. The major group of PGPM is rhizobacteria; recently, endophytic bacteria and mycorrhiza are reported as PGPM. Rhizobacteria are living bacteria in legume-rhizobia symbiosis; while endophytic bacteria are plant growth-promoting bacteria that live inside plant hosts. They possess the abilities of phosphate and potassium solubilization, nitrogen fixation, induction of plant tolerance and disease defenses. In a mycorrhizal association, plants are promoted into growth by an increase of plant nutrient uptake, particularly phosphorus as well as nitrogen, potassium and micronutrient. In addition, Arbuscular mycorrhizal fungi (AMF) have been shown to offer other benefits to host plants such as enhanced tolerance or resistance to soil pathogens and improved plant resistance to non-biotic stress including drought and metal in the polluted soil. Because of this wide range of benefits to the host, PGPMs, particularly co-cultures are attractive to an organic farm as a biofertilizer for the promotion of growth in many crop plants.

In the previous study, *Pantoea ananatis* 4.14, an endophytic bacteria isolated from leaves of Jerusalem artichoke, exhibited high phosphate and potassium solubilization, ACC deaminase production and nitrogen fixation, but it did not have antifungal activity against *Sclerotium rolfsii*. This strain could promote the growth of Jerusalem artichoke in HEL65 variety by increasing in height, weight and chlorophyll. In contrast, this strain did not promote the tubers significantly (Khamwan et al., 2018). A rhizobacterium, named *Pseudomonas* sp. C2-114, was isolated from the rhizosphere soil of growing Jerusalem artichoke. This strain had the properties of nitrogen fixation, phosphate and potassium solubilization, IAA and siderophore production, and antagonistic activity against *Sclerotium rolfsii* (Sritongon et al., 2017). In addition to AMF, the dual culture between *Glomus* sp.1 KKU-Wh and *Klebsiella variicola* UDJA102x89-9 (phosphate solubilizing bacteria; PSB) had a significant effect on plants growth parameters including leaves area, height, fresh weight of leaves, shoot and root, and dry weight of leaves and shoot higher than those from uninoculated control. Moreover, the number of stolon and fresh weight of tuber, and tuber inulin were significantly higher than those from an uninoculated plant (Nacoon et al., 2020).

Thus, *Pantoea ananatis* 4.14, *Pseudomonas* sp. C2-114, *Klebsiella variicola* UDJA102x89-9 and *Glomus* sp. 1 KKU-Wh are candidates of PGPM for investigation of the effect of co-inoculation on Jerusalem artichoke growth. The goal of this study was to produce the bio-fertilizer by using the combinations of plant growth promotion microorganisms helping together to induce the growth of Jerusalem artichoke in the optimum conditions. The potential strains, *Glomus* sp. 1 KKU-Wh (AMF), *Pseudomonas* sp. C2-114 (rhizobacteria) and *Pantoea ananatis* 4.14 (endophytic bacterium), were used as single inoculums and co-inoculums for cultivating Jerusalem artichoke in the field. The output of this study may lead to an understanding of relationships between strains and the ability to apply effective inoculums in field conditions.

Objective

The objective of this study were to (1) produce a bio-fertilizer of plant growth-promoting bacteria by immobilizing microbial cells on agricultural waste, (2) test the chemical, physical and microbiological properties of bio-fertilizer. This study was to find out the new formula of biofertilizer that was able to promote the growth of Jerusalem artichoke

Materials and Methods

Bio-fertilizer formula producing

The bio-fertilizer was produced by using a mixture of three species of plant growth-promoting microorganisms which can promote the growth of Jerusalem artichoke including Rhizobacteria (*Pseudomonas* sp. C2-114), Endophytic bacteria (*Pantoea ananatis* 4.14) and phosphate solubilizing bacteria (*Klebsiella variicola* UDJA102x89-9). The combination of microbial cells was immobilized on the agricultural waste as the carrier which consisted of soil and burnt rice husk (ratio 9:1 w/w). In the first experiment, the production of a new formula for bio-fertilizer was performed. The formula of microbial fertilizer was done by varying the different compositions and modified as shown in Tables 1, 2, 3, respectively. The pellet-formed bio-fertilizer was set it up by using the machine at the Agricultural Engineering Center, Faculty of Engineering, Khon Kaen University.

Determination of the solubilization property of bio-fertilizer

One g of bio-fertilizer sample was transferred into Erlenmeyer flask. Sterilized distilled water (DW) was added to the sample and mixed well by stirrer at 200 rpm to dissolve bio-fertilizer pellets until the fertilizer was completely solubilized. The solubility time of each bio-fertilizer formula was recorded.

Total microorganisms counting by spread plate technique

Five grams of sample was added into a plastic bag and then blended at 260 rpm for 120 sec by a stomacher (Seward, UK). The samples were diluted in 10-fold serial dilution with distilled water and then were plated on NA agar using the spread plate technique. The plates were incubated at 30 ^{o}C for 24 hours. The colony was counted in the unit of CFU/mL. The percentage of survival was calculated by the number of log CFU/mL multiplied by 100 and then divided by the number of log CFU/mL at the initial time.

Determination of pH and electrical conductivity (EC)

Five grams of sample were transferred into a plastic bag and then were blended at 260 rpm for 120 sec by stomacher (Seward, UK). The samples were left to be static; the pH and EC were measured by pH meter and EC meter, respectively.

Determination of bacteria releasing from the pellet

Ten grams of bio-fertilizer were added into a flask and then mixed with 5 mL of sterilized distilled water. After that, the samples were kept at room temperature and were taken in 0, 2, 4, 6, 8, 10, 12 and 14 days to determine the number of bacteria released in the bio-fertilizer solution. The samples were diluted in 10-fold serial dilution with distilled water and then spread on NA agar using the spread plate technique. The plates were incubated at 30 ^{o}C for 24 hours. The bacterial colony was counted in CFU/mL.

Results

Bio-fertilizer formula producing

In the first formula of bio-fertilizer production (Testing No.1), the composition was as shown in Table 1, the result found that the bio-fertilizer was not set in the form of a pellet well, it was sticking together due to the excess of a percentage of water content. Therefore, this formula was modified for the second bio-fertilizer production (Testing No.2) as shown in Table 2. The result showed that the forming of a pellet of bio-fertilizer was easier to set it up by hand, and the composition of the bio-fertilizer was well coagulation. In addition, to allow for better formability of bio-fertilizer granules, it was developed by adding a high-viscosity dextrose component and this dextrose was added as a carbon source for microorganisms growth, this formula is shown in Table 3 (Testing No.3). After the forming of pellet by hand, the bio-fertilizer granules were dried at room temperature and the water solubility activity was determined. The result showed that formula No. 8 has the fastest water solubility, followed by formula No. 2 and formula No. 3, respectively (data was shown in Table 4). From the water solubility test, bio-fertilizer formula No. 2 was developed as a bio-fertilizer granule this formula was less used tapioca starch as the one of components, but it was still good at showing the water solubility. This could reduce the cost of biofertilizer production.

Table 1. Component of bio-fertilizer formulas (Testing No. 1).

No.	Components	Formula (g/mL)				Total weight (g)
		1	2	3	4	
1	Soil/Burt rice husk (g)	67	66	64	62	64.75
2	2 % PEG powder (mL)	10	10	10	10	10
3	Cassava Starch (g)	1	2	4	6	3.25
4	Inoculum (mL)	10	10	10	10	10
5	Water (mL)	10	10	10	10	10
	Total	98	98	98	98	

Table 2. Component of bio-fertilizer formulas (Testing No. 2)

No.	Components	Formula (g/mL)				Total weight (g)
		1	2	3	4	
1	Soil/Burt rice husk (g)	78	75	73	70	296
2	2% PEG powder (mL)	10	10	10	10	40
3	Cassava Starch (g)	2	5	7	10	24
4	Inoculum (mL)	10	10	10	10	40
	Total	100	100	100	100	400

Table 3. Component of bio-fertilizer formulas (Testing No. 3).

No.	Components	Formula (g/mL)								Final (%)	Total weight (g)
		1	2	3	4	5	6	7	8		
1	Soil/Burt rice husk (g)	78	75	73	70	78	75	73	70	70-78	592
2	20 % of PEG powder (mL)	10	10	10	10	10	10	10	10	2	80
3	Cassava Starch (g)	2	5	7	10	2	5	7	10	2-10	48
4	Inoculum (mL) (dextrose 1%)	10	10	10	10	0	0	0	0	0.1	40
5	Inoculum (mL) (dextrose 10%)	0	0	0	0	10	10	10	10	1.0	40
	Total	100	100	100	100	100	100	100	100		800

The solubilization activity of bio-fertilizer

The biofertilizer formula No. 2 (Soil/Burnt rice husk: 75 g, 20 % PEG powder: 10 mL, Cassava Starch: 5 g, Inoculum microorganisms in dextrose 1%: 10 mL) was tested. The result found that the biofertilizer formula No.2 was not set it up in the form of a pellet due to it containing excessive moisture. Therefore, soil and burnt rice husk (ratio 1:1 v/v) was added more into formula No.2 to reduce soil moisture and repeated the biofertilizer production (shown in Figure 1 P1). The result shows that the biofertilizer was shown in short pellets form. Therefore, to make the pellet in a stable form, the production was repeated second and third times in the machine. (Figures 1P2 and 1P3). But during that, during the production process, the temperature of the biofertilizer process increased, which might affect the survival of microorganisms. Therefore, triple time of the pellet forming process was chosen in our study to maintain the microbial content in the pellet. After the pelleting step, the biofertilizer was dried at room temperature as shown in Figure 2.

Determination of bacterial number by total plate count

The microbial inoculums of PGPBs consisted of 3 types of bacteria which were *Klebsiella variicola* UDJA102x89-9 (PSB), *Pseudomonas* sp. C2-114 (rhizobacterium) and *Pantoea ananatis* 4.14 (endophytic bacterium). The initial amount of the mixture of bacteria after processing the pelleted formation was 2.72×10^{9} CFU/mL. The number of PGPBs decreased approximately to 10% after 15 days in all samples (Figure 3). The P2 and P3 showed continuously reduced in the cell number; while the P1

Table 4. Water solubilization property of each biofertilizer formula.

Formula No.	Water solubilization time (second)
1	23.51±1.79 b
2	16.99±2.29 c
3	17.44±1.71 c
4	23.97±1.32 b
5	32.79±1.66 a
6	32.73±1.53 a
7	17.37±1.25 c
8	5.88±0.90 d

Different letters in the column present significant differences at $p<0.05$ according to the least significant difference test.

Figure 1. Pelleted bio-fertilizer formed by a fertilizer pellet machine at the Faculty of Engineering with different times of the pelleted process. P1 = the first time of pellet production, P2 = the second time of pellet production, P3 = the third time of pellet production.

Figure 2. The character of the pelleted of bio-fertilizer after it was dried at room temperature**.** P1 = the first time of pellet production, P2 = the second time of pellet production, P3 = the third time of pellet production.

exhibited the increase in the cell number at 60 days after that it began to reduce. However, the number of bacterial cells remained at more than 65% at 120 days of all samples. Colonies observed on the agar plates showed mainly 3 bacterial strains throughout the experiment (Figure 4). The results indicated that the pellets of bio-fertilizer in this form did not show any effect on bacterial cell survival. The prototype of the biofertilizer of PGPBs is exhibited in Figure 5.

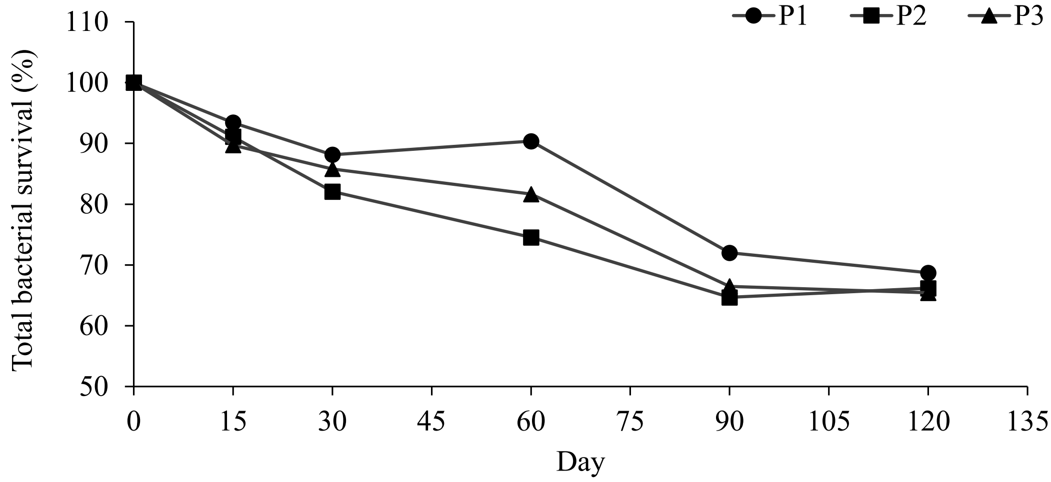
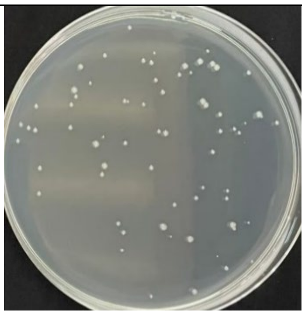
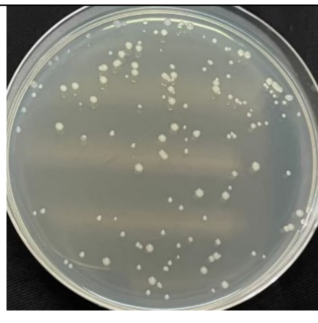


Figure 3. Percentage of bacterial survival in different processes of pellet bio-fertilizer. The samples were stored at room temperature for 120 days. P1 = the first time of pellet production, P2 = the second time of pellet production, P3 = the third time of pellet production.

Day	**P1**	**P2**	**P3**
0			
15			
30			

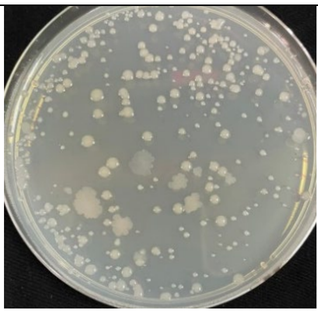
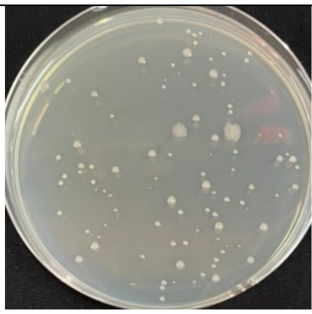
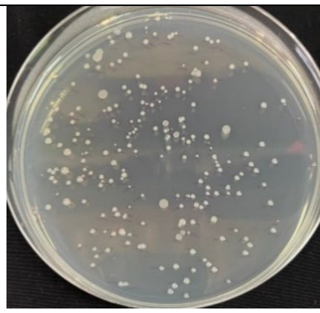
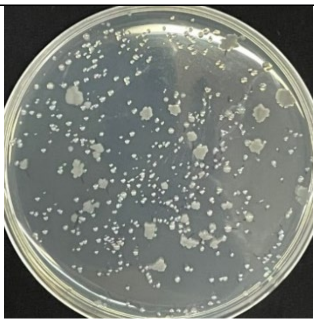
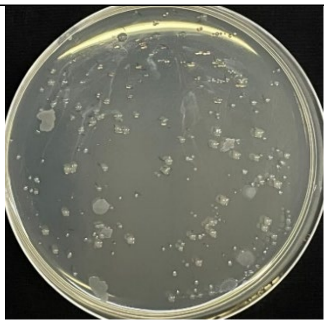
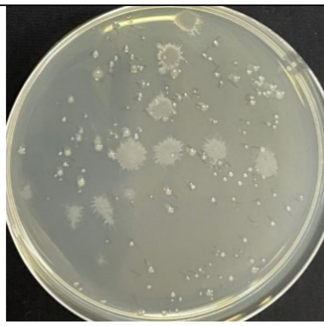
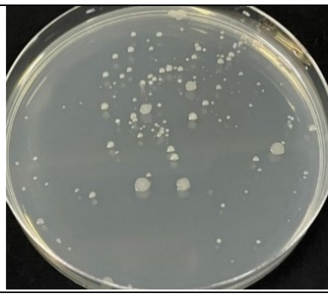
60			
90			
120			

Figure 4. Colony of bacteria on NA plate from different types of pellet biofertilizer at 0, 15, 30, 60, 90 and 120 days after production. P1 = the first time of pellet production, P2 = the second time of pellet production, P3 = the third time of pellet production.

P1 **P2** **P3**

Figure 5. Pelleted bio-fertilizers with different times of pelleted process as called P1 = the first time of pellet production, P2 = the second time of pellet production, P3 = the third time of pellet production.

Determination of pH and electrical conductivity (EC)

The bio-fertilizers of mixed PGPBs measured changes in pH values during 4 months of shelf life at room temperature. The initial pH of all samples was presented the weakly acid, consequently, the pH was a little raised and then dropped. The average pH of all samples was neutral pH (Table 5).

Table 5. Chemical properties of the bio-fertilizer values during 4 months at room temperature.

Days	**pH value**			**EC (μS/cm)**		
	P1	**P2**	**P3**	**P1**	**P2**	**P3**
0	6.79	6.47	6.17	198.8	201.0	198.5
15	6.90	7.05	7.41	359.0	349.0	192.1
30	7.76	7.49	7.25	347.0	344.0	355.0
60	7.54	7.45	7.77	367.0	362.0	378.0
90	7.04	6.90	6.84	360.0	355.0	357.0
120	6.91	6.90	6.93	341.0	339.0	345.0

P1, P2 and P3 mean the PGPBs with carriers were mixed and pressed one, two and three times respectively by a machine.

Bacteria releasing determination

The pellets of PGPBs obtained 3 types of bacteria, incubated in distilled water at room temperature for 0, 2, 4, 6, 6, 10,12 and 14 days after the pellet forming processing. The result showed that the initial amounts of the mixture of bacteria were released into supernatant at log 6.21, 6.74 and 6.75 CFU/mL of P1, P2 and P3, respectively. The number of the PGPBs released was approximate to log 9 CFU/mL after 2 days in all samples until 6 days. The number of bacteria releasing in all samples (P1, P2 and P3) and was showed a continuous reduction in the cell number from 8 until 14 days (Figure 6). This result indicated that PGPBs could release from the biofertilizer pellets throughout the incubation period, which showed that these bio-fertilizer pellets are suitable to use as a bio-fertilizer and may have a good function on plant growth promotion.

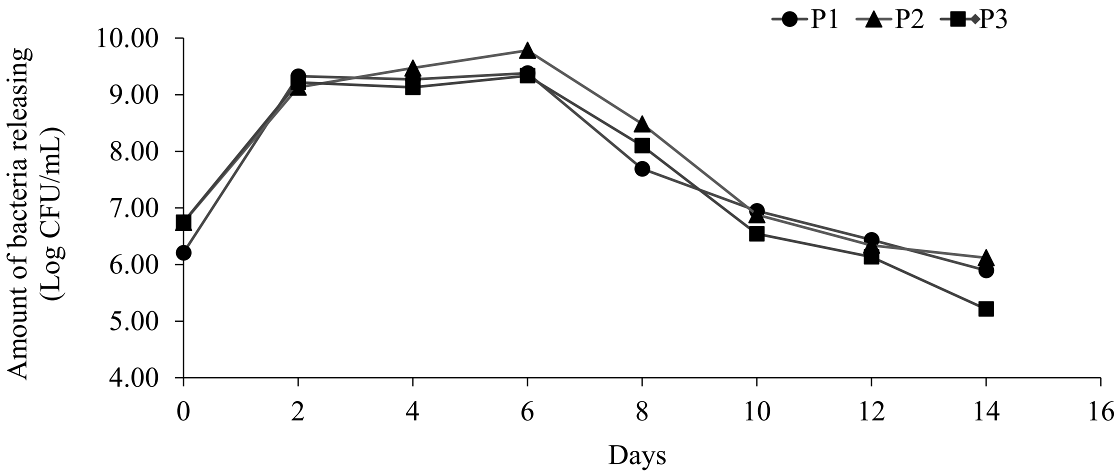


Figure 6. The amount of bacteria released from pelleted bio-fertilizers at different times of the pelleted process is called P1 = the first time of pellet production, P2 = the second time of pellet production, P3 = the third time of pellet production. The samples were stored at room temperature for 14 days.

Conclusion

The bio-fertilizer was produced in the form of pellet obtaining of *Klebsiella variicola* UDJA102x89-9 (PSB), *Pseudomonas* sp. C2-114 (rhizobacterium) and *Pantoea ananatis* 4.14 (endophytic bacterium). The biofertilizer formula No.2 consisted of the components; Soil/Burnt rice husk: 75 g, 20% of PEG powder: 10 ml, Cassava Starch: 5 g, Inoculum microorganisms in dextrose 1%: 10 ml and added more with soil and burnt rice husk (ratio 1:1 v/v) until the total weight of 100 g. This formula of bio-fertilizer was selected to form pellets in the fertilizer-forming production process. During 120 days incubation period, all 3 pelleted biofertilizer formulas (P1, P2 and P3) showed the potential the maintaining of PGPBs' survival, the average pH of all samples was neutral pH. In addition, bacteria could release well in all pellets formed.

Reference

Khamwan, S., Boonlue, S., riddech, N., Jogloy, S., & Mongkolthanaruk, W. (2018). Characterization of endophytic bacteria and their response to plant growth promotion in Helianthus tuberosus L. *Biocatalysis and Agricultural Biotechnology*, *13*(December 2017), 153–159. https://doi.org/10.1016/j.bcab.2017.12.007

Nacoon, S., Jogloy, S., Riddech, N., Mongkolthanaruk, W., Kuyper, T. W., & Boonlue, S. (2020). Interaction between Phosphate Solubilizing Bacteria and Arbuscular Mycorrhizal Fungi on Growth Promotion and Tuber Inulin Content of Helianthus tuberosus L. *Scientific Reports*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-61846-x

Nacoon, S., Seemakram, W., Ekprasert, J., Mongkolthanaruk, W., & Riddech, N. (2022).

Promoting growth and production of sunchoke (*Helianthus tuberosus*) by co-inoculation with phosphate solubilizing bacteria and arbuscular mycorrhizal fungi under drought, (October). https://doi.org/10.3389/fpls.2022.1022319

Sritongon, K., Mongkolthanaruk, W., Boonlue, S., Jogloy, S., Puangbut, D., & Riddech, N. (2017). Rhizobacterial candidates isolated from Jerusalem artichoke (Helianthus tuberosus L.) rhizosphere for host plant growth promotion. *Chiang Mai Journal of Science*, *44*(1), 83–93.

Publications

Suebrasri, T., S. Harada, S. Jogloy, J. Ekprasert, and S. Boonlue. 2020. Auxin-producing fungal endophytes promote growth of sunchoke. Rhizosphere 16: 100271. (Impact factor=3.49: Q1)

Suebrasri, T., A. Somteds, H. Harada, S. Kanokmedhakul, S. Jogloy, J. Ekprasert, S. Lumyong, and S. Boonlue. (2020). Novel endophytic fungi with fungicidal metabolites suppress sclerotium disease. Rhizosphere 16: 100250. (Impact factor=3.49: Q1)

Riddech, N., S. Jogloy, W. Mongkolthanarak, S. Boonlue, N. Duangdech, and T. Phibunwatthanawong. 2021. Effects of plant growth promoting bacteria on the growth and yield of Jerusalem Artichoke (*Helianthus tuberosus*) under water limitation condition. Chiang Mai Journal of Science 48(2): 354-372. (Impact factor= 0.523: Q4)

Khaekhum, S., J. Ekprasert, T. Suebrasri, W. Mongkolthanaruk, N. Riddech, S. Jogloy, and S. Boonlue. 2021. The first member of *Exserohilum rostratum* beneficial for promoting growth and yield of sunchoke (*Helianthus tuberosus* L.). Rhizosphere 19: 100379. (Impact factor=3.49: Q1)

Nacoon, S., S. Jogloy, N. Riddech, W. Mongkolthanaruk, J. Ekprasert, J. Cooper, and S. Boonlue. 2021. Combination of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth and production of *Helianthus tuberosus* under field condition. Scientific Reports 11: 6501. (Impact factor 4.996: Q1)

Khaekhum, S., J. Ekprasert, T. Suebrasri, W. Seemakram, W. Mongkolthanaruk, N. Riddech, S. Jogloy, and S. Boonlue. 2021. Co-Inoculation of an endophytic and arbuscular mycorrhizal fungus improve growth and yield of *Helianthus tuberosus* L. under field conditions. Journal of Fungi 7(11): 976. (Impact score=5.724: Q1)

Nacoon, S., W. Seemakram, J. Ekprasert, S. Jogloy, T.W. Kuyper, W. Mongkolthanaruk, N. Riddech, T. Somdee, and S, Boonlue. 2022. Promoting growth and production of sunchoke (*Helianthus tuberosus*) by co-inoculation with phosphate solubilizing bacteria and arbuscular mycorrhizal fungi under drought. Frontiers in Plant Science .13: 10.3389/fpls.2022.1022319. (Impact factor 6.627: Tier 1)

Namwongsa, J., Jogloy, S., Vorasoot, N., Boonlue, S., Riddech, N., Mongkolthanaruk, W. (2019). Endophytic bacteria improve root traits, biomass and yield of *Helianthus tuberosus* L. under normal and deficit water conditions. J. Microbiol. Biotechnol. 29(11): 1777–1789. (Impact factor=1.99: Q2)