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Pathogenicity Test of *Sclerotium rolfsii*, a Causal Agent of Jerusalem Artichoke (*Helianthus tuberosus* L.) Stem Rot

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Abstract: Stem rot disease caused by *Sclerotium rolfsii* can cause severe yield losses of Jerusalem artichoke. Pathogenicity of *S. rolfsii* in Jerusalem artichoke has not been well-researched especially in the semi-arid tropics. The objective of this study was to test pathogenicity of different isolates of *S. rolfsii* in Jerusalem artichoke. Ten isolates of *S. rolfsii* and three varieties of Jerusalem artichoke (JA 89, HEL 65 and CN 52867) were assigned in factorial experimental design. Plants were inoculated by spreading 20 seeds (0.6 g) of sorghum-based inoculums around the stems at 6-8 leaf stage. A non inoculated treatment is also included as control. The interactions between isolate and variety were not significant for all characters. The isolates were significantly different for disease incidence, Area under Disease Progress Curve (AUDPC), days to permanent wilting, plant height and shoot dry weight. The reactions of most isolates were aggressive except for the isolates 2 and 7. HEL 65 exhibited low disease incidence and AUDPC.

Key words: Aggressive isolate, disease incidence, inoculum, stem rot, tuber crop

INTRODUCTION

Jerusalem artichoke is an under-utilized tuber crop, originating in North America (Cosgrove *et al*., 1991). Tubers of Jerusalem artichoke contain carbohydrate is beneficial to human health because it can prevent obesity, enhances immunity and reduces blood cholesterol and the risk of insulin-dependent diabetes mellitus (type 2) and heart disease (Orafti, 2005). Tubers of Jerusalem artichoke can be used as fresh or cooked vegetable for humans, ingredient substitute for antibiotic in animal feed and raw material for sugar, inulin and ethanol production (Denoroy, 1996).

Jerusalem artichoke production can be performed in a commercial scale. However, stem rot disease caused by *Sclerotium rolfsii* poses a treat to Jerusalem production. Substantial yield losses of Jerusalem artichoke caused by *Sclerotinia sclerotiorum* have been reported mostly in the temperate regions (Cosgrove *et al*., 1991; Cassells and Walsh, 1995). Yield losses caused by *S. rolfsii* have also been reported in the United States (Koike, 2004). The sclerotial disease caused by *S. rolfsii* primarily occur in warm climates, especially under a high temperature with humid condition. The pathogen of sclerotial disease causes damping-off of seedlings, stem canker, crown blight and rots on root, crown, bulb, tuber and fruit of various plant groups (Kwon *et al*., 2008). Sclerotial disease frequently affect a wide variety of plants, including most vegetables, flower, legumes, cereals, forage plants and weeds (Agrios, 2005).

Sclerotium rolfsii can result in yield of Jerusalem artichoke serious losses, especially when grown on land previously planted with Jerusalem artichoke or other hosts. It can cause a 60% plant loss in plots which had grown the two previous years (Mccarter and Kays, 1984). Initial symptoms of Jerusalem artichoke consisted of wilting of new shoots and leaves followed by browning and collapse of all foliage. Crown and lower stem tissues were colonized internally and externally by white, cottony mycelium. Tan, spherical sclerotia that measured approximately 1 mm in diameter formed on surfaces of the affected crowns and stems (Koike, 2004).

The susceptibility of potential host plants varied depending on the isolates of *S. rolfsii* (Farr *et al*., 1989). Variations in growth rate, number and days to first appearance of sclerotia by *S. rolfsii* isolates on Potato-Dextrose Agar (PDA), were observed among isolates collected from the tropical humid lowlands of Southeastern Nigeria (Okereke and Wokocha, 2007). There are several reports where various isolates of *S. rolfsii* have shown significant variations not only in their morphology but also in their pathological behavior (Sarama *et al*., 2002). However, pathogenicity of *S. rolfsii* in Jerusalem artichoke has not been well-researched

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especially in the semi-arid tropics. This information is very useful for an effective control of this pathogen. This work reports pathogenicity of different isolates of *S. rolfsii* in Jerusalem artichoke.

MATERIALS AND METHODS

Collection and isolation of *S. rolfsii*: The study was undertaken during March to April 2009. Ten isolates of *S. rolfsii* were collected from Jerusalem artichoke at different locations in Thailand. Isolate 1, 2 and 3 were collected from the Khon Kaen University farm (KKU farm) in different fields, Isolate 4, 5, 6 and 7 were collected from the Agricultural Exhibition Center of Khon Kaen University in different fields, Isolate 8 and 9 were collected from Petchaboon province in different fields and Isolate 10 were collected from Nakornratchasima province (Table 1). Plants showing typical symptoms of stem rot were collected. Single sclerotia were surface-sterilized using 70% alcohol for 1 min and rinsed with sterilized water for 1 min. The samples were then transferred to Potato Dextrose Agar (PDA) medium in Petri dishes and incubated at room temperature for 3 days. After incubation, the mycelium was purified by subculture. After purification *S. rolfsii* isolates were stored in test tube slants.

Preparation of plant material: Pathogenicity test was carried out under greenhouse conditions, using three Jerusalem artichoke varieties JA 89, HEL 65 and CN 52867 with 10 isolates of *S. rolfsii* collected from different agricultural and ecological areas. The experiment was set up in a 10×3 factorial in RCBD with four replications and non-inoculated controls of the three varieties were also included. There were 33 treatments totally. The fungal isolates were assigned as factor A and Jerusalem artichoke varieties factor B. Jerusalem artichoke varieties were incubated in burnt rice husk for germination for a week and then grown in plastic trays for a week until germination with two leaf stage. The plants were later transferred to pots with 3 inches in diameter containing sterilized sandy-loam and burnt rice husk (1:1). Plants were grown by individual (1 plant per pot) and there were five pots for each unit.

Table 1: Ten isolates was collected from various locations

Isolates	Location
1	KKU farm in different fields
2	
3	
4	A agricultural Exhibition Center of Khon Kaen University in different fields
5	
6	
7	
8	Petchaboon province in different fields
9	
10	Nakornratchasima province

Preparation of inoculum: Ten isolates of *S. rolfsii* from various locations that infect Jerusalem artichoke were maintained on Potato Dextrose Agar (PDA) medium in Petri dishes and stored in test tube slants. The isolates were later transferred to sorghum medium, the sorghum medium made by boiling sorghum grains for 15 min, separation cooked sorghum grains and the sorghum grains in bottle were autoclaved for 30 min, inoculated with mycelium of *S. rolfsii* and incubated at room temperature (30±4°C)". After two weeks of incubation, the inoculum was ready for use.

Pathogenicity test of *S. rolfsii* on different isolates: Plants were artificially inoculated by spreading 20 seeds (0.6 g) of sorghum-based inoculums around the stems at 6-8 leaf stage (modified from Block *et al*., 2007). A non inoculated treatment is also included as control. The inoculum is placed beneath the soil surface in close proximity of the stems of the plants. The water was supplied regularly to avoid stress.

Data collection: Data were recorded daily for number of infected plants and then converted to percent infected plants as (infected plants/total plants) ×100 at the end of study. The plants showing apparent wilting and rotting at the crowns were considered infected and the plants without these symptoms were considered healthy. Area under the Disease Progress Curve (AUDPC) was calculated from disease incidence based on the formula suggested by Davis *et al.* (1996) as follows:

$$AUDPC = \sum_{i=1}^{n}\left[(X_i + X_{i-1})/2\right](t_i - t_i - 1)$$

where, i is the number of days in which observations were made, n is the last days in which observations were made, x_i is disease incidence in days i and t_i is DAP on which disease incidence were measured in days I and days to permanent wilting for severity of each isolates.

Crop data were recorded daily until 23 days after inoculation for plant height (measured daily from inoculation) measured from stem to the last node of plant and shoot dry weight by oven-dried for 72 h at 80°C or until constant weight.

Statistical analysis: The data were subjected to analysis of variance according to a randomized complete block design for variety and isolate main effects and analysis of single degree of contrast was also carried out to test the difference between inoculated treatment and non-inoculated treatment. Least Significant Difference (LSD) was used to compare mean differences (Hoshmand, 2006) and other appropriate statistical procedures were employed.

Table 2: Effects of inoculated and un-inoculated treatment for disease incidence, area under disease progress curve (AUDPC), plant height and shoot dry weight

Treatments	Disease incidence (%)	AUDPC	Height (cm)	Shoot dry weight (g)
Inoculated	78.2a	1631.4a	11.1b	0.77b
Un-inoculated	0.0b	0.0b	14.9a	1.01a
LSD (0.05)	6.6	189.2	1.3	0.1

Means in the same column with the same letter are not significantly different by Least Significant Difference (LSD)

Table 3: Effects of various isolates of *S. rolfsii* on disease incidence, area under disease progress curve (AUDPC), days to permanent wilting, plant height and shoot dry weight

S. rolfsii *Isolate	Disease incidence (%)	AUDPC	Days to permanent wilting	Plant height (cm)	Shoot dry weight (g)
Isolate 1	88.3a	2033a	9.8c	9.8b	0.7c
Isolate 2	46.7b	757b	19.5a	13.3a	0.8b
Isolate 3	85.0a	1618a	13.3b	11.1b	0.7bc
Isolate 4	83.3a	1931a	10.6bc	10.0b	0.7bc
Isolate 5	90.0a	1911a	10.7bc	10.2b	0.7bc
Isolate 6	81.7a	1773a	11.5bc	10.1b	0.7bc
Isolate 7	41.7b	678b	19.5a	13.9a	1.0a
Isolate 8	91.7a	1948a	10.4bc	11.2b	0.8bc
Isolate 9	88.3a	1833a	11.2bc	11.1b	0.8bc
Isolate 10	85.0a	1833a	11.4bc	10.6b	0.7bc
LSD (0.05)	16.1	421.5	3.0	1.8	0.1

*Isolates 1, 2 and 3 were collected from Khon Kaen University agronomy farm (KKU farm) in different fields. Isolates 4, 5, 6 and 7 were collected from the Agricultural Exhibition Center of Khon Kaen University in different fields. Isolates 8 and 9 were collected from Petchaboon Province in different fields. Isolate 10 was collected from Nakornratchasima Province. Means in the same column with the same letters are not significantly different by Least Significant Difference (LSD)

RESULTS

Analysis of variance indicated that the interactions between isolate and variety were not significant for disease incidence, Area under Disease Progress Curve (AUDPC), days to permanent wilting, plant height and shoot dry weight (data not reported). The results indicated that the varieties responded consistently across isolates for these characters. Inoculated plants had disease incidence and AUDPC higher than did un-inoculated plants, but they were lower for plant height and shoot dry weight (Table 2). The disease incidence and AUDPC were 78.2% and 1631.4 in inoculated plants, respectively, whereas infected plant was not found in un-inoculated plants.

The isolates were significantly different for disease incidence (percent infected plants), AUDPC, days to permanent wilting, plant height and shoot dry weight (Table 3). Disease incidences ranging from 41.7 to 91.7 % and AUDPC ranging from 678 to 2033 were observed among the fungal isolates. Days to permanent wilting were also found varying between 9.7 to 19.4 days after inoculation. The reactions of most isolates were aggressive except for the isolates 2 and 7. These isolates had the lowest disease incidence and AUDPC and they also took more days to reach permanent wilting. The plants inoculated with these isolates had taller plant height and heavier shoot dry weight than did the plants inoculated with other isolated. The aggressive isolates were rather similar in disease reactions in terms of disease incidence, AUDPC and days to permanent wilting.

Table 4: Effects of *Sclerotium rolfsii* isolates on disease incidence, area under disease progress curve (AUDPC), days to permanent wilting, plant height and shoot dry weight of three Jerusalem artichoke varieties

Variety	Disease incidence (%)	AUDPC	Days to permanent wilting	Plant height (cm)	Shoot dry weight (g)
JA 89	82.5a	1640b	12.6b	12.7a	0.9a
HEL 65	65.5b	1316c	14.9a	8.6b	0.7b
CN 52867	86.5a	1938a	10.9c	12.0a	0.8b
LSD (0.05)	8.8	230.9	1.6	1.0	0.1

Means in the same column with the same letter are not significantly different by Least Significant Difference (LSD)

Significant differences among varieties were recorded for disease incidence, AUDPC and days to permanent wilting (Table 4). HEL 65 exhibited better resistance to *Sclerotium rolfsii* because it had lower disease incidence and lower AUDPC and it took more days to reach permanent wilting. Significant differences among varieties were also observed for plant height and shoot dry weight. These characters did not show any relationship with disease reactions.

DISCUSSION

The disease reactions of isolates were consistent across Jerusalem artichoke varieties for disease incidence and Area under Disease Progress Curve (AUDPC). The results clearly indicated that the isolates giving high disease incidence were readily identifies. However, inoculation was necessary for sufficient disease pressure for the accurate evaluation as inoculated plants had significantly higher disease incidence than did un-inoculated plants. The disease incidences ranging of 41.7 to 91.7% were rater suitable for evaluation.

Eight out of ten isolates showed aggressive reactions for disease incidence and AUDPC and the differences in disease reactions also resulted in differences in days to permanent wilting, plant height and shoot dry weight. Two isolates showed significantly lower disease reactions. These isolates were collected in different fields from KKU farm and the Agricultural Exhibition Center of Khon Kaen University, respectively. This is an indicative of the difference in pathogenicity among isolates from the different areas. The isolates 1, 2 and 3 collected from KKU farm but at different fields showed different pathogenicity patterns. However, the isolates 4, 5, 6 and 7 collected at different locations also showed differences in disease reactions. Similar results were also reported in onion. The isolate of *S. rolfsii* from Guanajuato was regarded

more virulent than the isolate from Morelos (Flores-Moctezuma *et al*., 2006). The susceptibility of host plants varied depending on the isolates of *Sclerotium rolfsii* (Farr *et al*., 1989). The isolates from various hosts and geographical areas, even within the same area, showed variation in growth rate, numbers and size of sclerotia and mycelial compatibility (Punja and Grogan, 1983).

Jerusalem artichoke varieties also showed significant differences in disease reactions. HEL 65 exhibited lower disease incidence and AUDPC than did JA 89 and CN 52867, indicating more resistance to the disease and taking more time to reach permanent wilting. It may be used as resistance check for evaluate resistance difference among Jerusalem artichoke varieties. However, these result need to confirm because the variety of Jerusalem artichoke are not sufficient variation.

The disease characters were well associated with plant growth characters. Thus, the disease characters are more suitable than plant growth characters in discriminating the differences among isolates because they are simple and easy to evaluate. The aggressive isolates can be used in screening Jerusalem artichoke germplasm for *S. rolfsii* resistance.

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