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Full Length Research Paper

Levels of *Sclerotium rolfsii* inoculum influence identification of resistant genotypes in Jerusalem artichoke

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Improvement of Jerusalem artichoke genotypes with resistance to stem rot caused by the soil borne fungus *Sclerotium rolfsii* is a sustainable means for controlling the disease. However, this crop is rather new to breeders. A consensus screening procedure for resistance to the disease is not yet available. The aim of this study was to determine the level of inoculum that provides the reliable and effective results for screening trials. In the experiment in Khon Kaen, Thailand, four levels of sorghum seed infested with *S. rolfsii* (1, 2, 3 or 4 seeds/plant) were tested with 10 Jerusalem artichoke genotypes. Plants inoculated with one sorghum seed had the lowest disease incidence, whereas plants inoculated with four sorghum seeds had the highest disease incidence. Most pairings of inoculum levels were statistically different for disease incidence except for two vs. three seeds. In addition, permanent wilting and area under disease progress curve occurred more rapidly with four seeds compared to the other inoculum levels. The highest variation among Jerusalem artichoke genotypes was observed in the plants inoculated with three sorghum seeds. Therefore, three sorghum seeds inoculum was suitable to identify Jerusalem artichoke genotypes resistant to *S. rolfsii*. Based on days to permanent wilting, resistant and susceptible genotypes were identified.

Key words: *Helianthus tuberosus* L., sunchoke, stem rot, disease incidence, genotypes, sorghum seed, area under disease progress curve.

INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is currently an important crop for production of healthy food (Danilčenko et al., 2008), as the crop produces substantial amounts of the carbohydrate inulin rather than starch in its tubers. Because inulin is absorbed by the human body at lower rates than starches it can prevent obesity, enhance immunity, and reduce blood cholesterol and the risk of insulin-dependent diabetes mellitus (type 2) and heart disease (Orafti, 2005). Jerusalem artichoke is also used to produce a variety of products such as animal feed (Zaky, 2009), and bio-ethanol (Yildiz et al., 2006).

Jerusalem artichoke originated in North America (Kays and Nottingham, 2008) but can also be grown commercially in the tropics (Pimsaen et al., 2010; Puangbut et al., 2011). In tropical regions, stem rot disease caused by *S. rolfsii* can be a severe disease of the crop (Sennoi et al., 2010). The first occurrence of *S. rolfsii* on Jerusalem artichoke was reported in the United States (Koike, 2004), but diseases incited by *S. rolfsii* are more prevalent in warm climates, especially under high temperature and high humidity (Kwon et al., 2008), and the pathogen infects a wide variety of host plants, including most vegetables, flowers, legumes, cereals, and forage plants as well as many weeds (Agrios, 2005).

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Selection and improvement of Jerusalem artichoke varieties with resistance to *S. rolfsii* may provide a sustainable means of disease management. Many attempts have been made to find resistant genotypes against *S. rolfsii* (Gorbet et al., 2004; Infantino et al., 2006; Akram et al., 2008). As Jerusalem artichoke is rather new to breeders and plant pathologists, an optimal screening procedure for resistance is not available. Previous work on disease resistance in Jerusalem artichoke studied only *Sclerotinia sclerotiorum* (Cassells and Walsh, 1995).

In a recent study at Khon Kaen University in Thailand as well as in earlier work on peanut, inoculum grown on agar and on sorghum seed was compared, and sorghum seed was shown to be more effective (Shokes et al., 1996). However, the question of how many *S. rolfsii*-infested seeds should be used per inoculated plant remains unresolved. The objective of this study was to determine levels of sorghum seed inoculum that provide the most reliable and effective results for evaluation *S. rolfsii* resistance of Jerusalem artichoke.

MATERIALS AND METHODS

Experimental setup

Five Jerusalem artichoke genotypes (HEL 278, HEL 246, HEL 280, JA 1 and HEL 65) that exhibited a high level of resistance to *S. rolfsii* in our previous work and five genotypes (CN 52867, HEL 62, JA 102, JA 37 and JA 122) that were most susceptible were used in this study. Four levels of inoculum density (1, 2, 3 or 4 *S. rolfsii*-infested sorghum seeds/plant) were tested with the 10 Jerusalem artichoke genotypes in a factorial design in randomized complete block (RCBD) with four replications. There were four plants in each treatment unit. The experiments were carried out in an open-sided greenhouse at Khon Kaen University (KKU) Agronomy Farm, Khon Kaen, Thailand. The first run of the experiment was carried out in September 2010 and the second run was done in October 2010. Temperatures in the first and the second runs ranged from 23 to 29°C and 21 to 31°C, respectively. Average of all day's relative humidity was 91% for the first experiment and 95% for the second experiment.

Preparation of plant materials

Tubers of Jerusalem artichoke were cut into small pieces with 2 to 3 active buds, and incubated for one week in charred rice husks to facilitate germination under open-side greenhouse. Water was regularly applied in order to avoid drying out of the medium used. The germinated tuber pieces were then transferred to plug trays for one week until each seedling had two leaves. They were later transferred to experimental pots (8 × 8 × 9 cm) containing steamed soil and charred rice husks (1:1). The soil used in the experiment belonged to the Roi-et series (Re; fine-loamy, mixed, subactive, isohyperthermic Aeric Kandiaquults).

Preparation of *S. rolfsii* inoculum and inoculation method

Isolate 1 of *S. rolfsii*, which was obtained from a KKU field in Khon Kaen, Thailand, and was very aggressive on Jerusalem artichoke as determined by previous screening assays (Sennoi et al., 2010), was used in the trials. The isolate was transferred to potato dextrose agar (PDA) medium in petri dishes and incubated at room temperature (25 ± 2°C) for 3 days. After incubation, mycelium plugs were transferred to steamed sorghum seeds and incubated at room temperature for 10 days; the inoculum was then ready to use. At the 6- to 8-leaf stage, plants were inoculated by placing infested sorghum seeds at the crowns of stems at determined levels (1, 2, 3 and 4 sorghum seeds/plant). Cotton wool was used to cover infested sorghum seeds in order to maintain moisture.

Data collection

Number of infected plants and lesion length (cm) were recorded every two days after inoculation. Days to permanent wilting was observed daily after inoculation. Number of symptomatic plants was later converted to disease incidence (% plants exhibiting symptoms). Area under disease progress curve (AUDPC) was calculated from disease incidence according to the formula suggested by Marcel et al. (2008).

Statistical analysis

Error variances between two experiments were tested for homogeneity. For analysis of variances, data with homogeneity of variance were pooled for the two experiments and assessed for factor main effects and interactions (Hoshmand, 2006). The interaction of genotype × level of inoculum was significant for all traits; therefore, each inoculum level was analyzed separately for each parameter. Least significant difference (LSD) was used to compare mean differences. All calculations were done using STATISTIX8 software program. Simple linear regression was used to determine the relationship between inoculum level and disease incidence, lesion length, days to permanent wilting and area under progress curve.

RESULTS

Base on high F-ratio values for genotypes and low coefficient of variation, data collected for disease incidence and lesion length were reported 3 and 5 days, respectively, after inoculation. Significant differences ($P<0.01$) between the two runs of the experiment were found for disease incidence, lesion length, days to permanent wilting and area under disease progress curve (AUDPC) (Table 1). The first experiment had higher disease incidence (88%) than the second experiment (67%) (data not shown). Genotypes were also significantly different ($P<0.01$) for disease incidence, lesion length, days to permanent wilting and AUDPC at all inoculum levels. Differences in the levels of inoculum were significant ($P<0.01$) for disease incidence, lesion length, days to permanent wilting and AUDPC. Genotype × level of inoculum interactions were also significant ($P<0.01$) for all four dependent variables.

From the regression analysis, the plants inoculated with one sorghum seed had the lowest disease incidence (62.5%), whereas the plants inoculated with four sorghum seeds had the highest disease incidence (92.5%) (Figure 1a). Most pairings of seed treatments were statistically different for disease incidence except for the plants

Table 1. Mean squares from combined ANOVA for disease incidence (at 3 days after inoculation), lesion length (at 5 days after inoculation), days to permanent wilting and area under disease progress curve (AUDPC).

SOV	df	Disease incidence	Lesion length	Days to permanent wilting	AUDPC
Experiment (E)	1	34031.3**	2.3**	37.8**	303195*
Rep/experiment	6	134.1	0.1	2.1	9945
Genotype (G)	9	4397.6**	4.2**	12.7**	79010**
G × E	9	5094.6**	0.8**	2.4**	18699**
Inoculum level (I)	3	12085.9**	2.0**	12.3**	129607**
I × E	3	2536.5**	0.1	0.4**	22617**
G × I	27	1366.9**	1.1**	1.2**	7810**
G × I × E	27	1979.5**	0.7**	0.9**	5841**
Pooled error	234	110.1	0.1	0.4	2135
C.V. (%)		13.5	22.8	20.3	9.9

** Significant at $P<0.01$.

Table 2. Mean squares from combined ANOVA for days to permanent wilting at different level of *Sclerotium rolfsii* inoculum in Jerusalem artichoke.

SOV	df	1 seed	2 seeds	3 seeds	4 seeds
Experiment (E)	1	16.2**	7.2*	9.1*	6.6**
Rep/experiment	6	0.9	0.7	0.8	0.4
Genotype (G)	9	4.5**	4.4**	4.5**	3.0**
G × E	9	2.0**	1.5**	1.4**	0.2
Pooled error	54	0.4	0.4	0.2	0.3
F-ratio for genotypes		11.6	12.3	21.4	11.1
C.V. (%)		19.7	21.1	17.7	23.2

*,** Significant at $P<0.05$ and $P<0.01$, respectively.

inoculated with two seeds (76.5%) vs. those inoculated with three seeds (79.1%). Lesion length ranged from 1.1 to 1.5 cm (Figure 1b). Plants inoculated with one sorghum seed had the shortest lesions (1.1 cm), but lesion length did not differ significantly from the plants inoculated with three sorghum seeds (1.2 cm) nor did two seeds differ significantly from four seeds (1.4 and 1.5 cm, respectively). Days to permanent wilting ranged from 2.2 to 3.2 (Figure 1c). Treatment with one sorghum seed required 3.2 days to permanent wilting. The other inoculum levels with two, three and four sorghum seeds required 2.8, 2.6 and 2.2 days to permanent wilting, respectively. One seed treatment gave the lowest AUDPC, whereas the highest AUDPC was observed in four seed treatment (Figure 1d). The correlation coefficient between seed treatment and AUDPC was positive and significant ($R^2 = 0.98$).

The highest variation among Jerusalem artichoke genotypes was observed for days to permanent wilting in the plants inoculated with three sorghum seeds as indicated by high F-ratio (21.4) (Table 2). Disease incidence and AUDPC in the plants inoculated with three sorghum seeds also had the highest variation (data not shown).

Inoculation with three sorghum seeds resulted in the greatest range of variation in disease response among Jerusalem artichoke genotypes. Using three sorghum seeds per plant, disease incidence ranged from 50 to 100% among genotypes (Figure 2a). Genotypes HEL 278, JA 1, HEL 65, CN 52867 and JA 37 showed the lower of disease incidence (50 to 68.8%), whereas genotypes HEL 246, HEL 280, HEL 62, JA 102 and JA 122 had the higher disease incidence (90.6 to 100%).

The genotypes that required the longer time (2.8 to 3.3 days) to reach permanent wilting were HEL 278, HEL 246, HEL 280, JA 1, HEL 65, CN 52867 and JA 37. In contrast, genotypes HEL 62, JA 102 and JA 122 required only 1.4 to 2.3 days to reach permanent wilting (Figure 2b). Higher AUDPC were observed for HEL 62, JA 102 and JA 122 (540.6 to 565.6), and lower AUDPC were observed for HEL 278, HEL 246, HEL 280, JA 1, HEL 65, CN 52867 and JA 37 (403.1 to 487.5) (Figure 2c).

DISCUSSION

In our trials, higher levels of inoculum resulted in severer disease. In other investigations, chickpea seedling

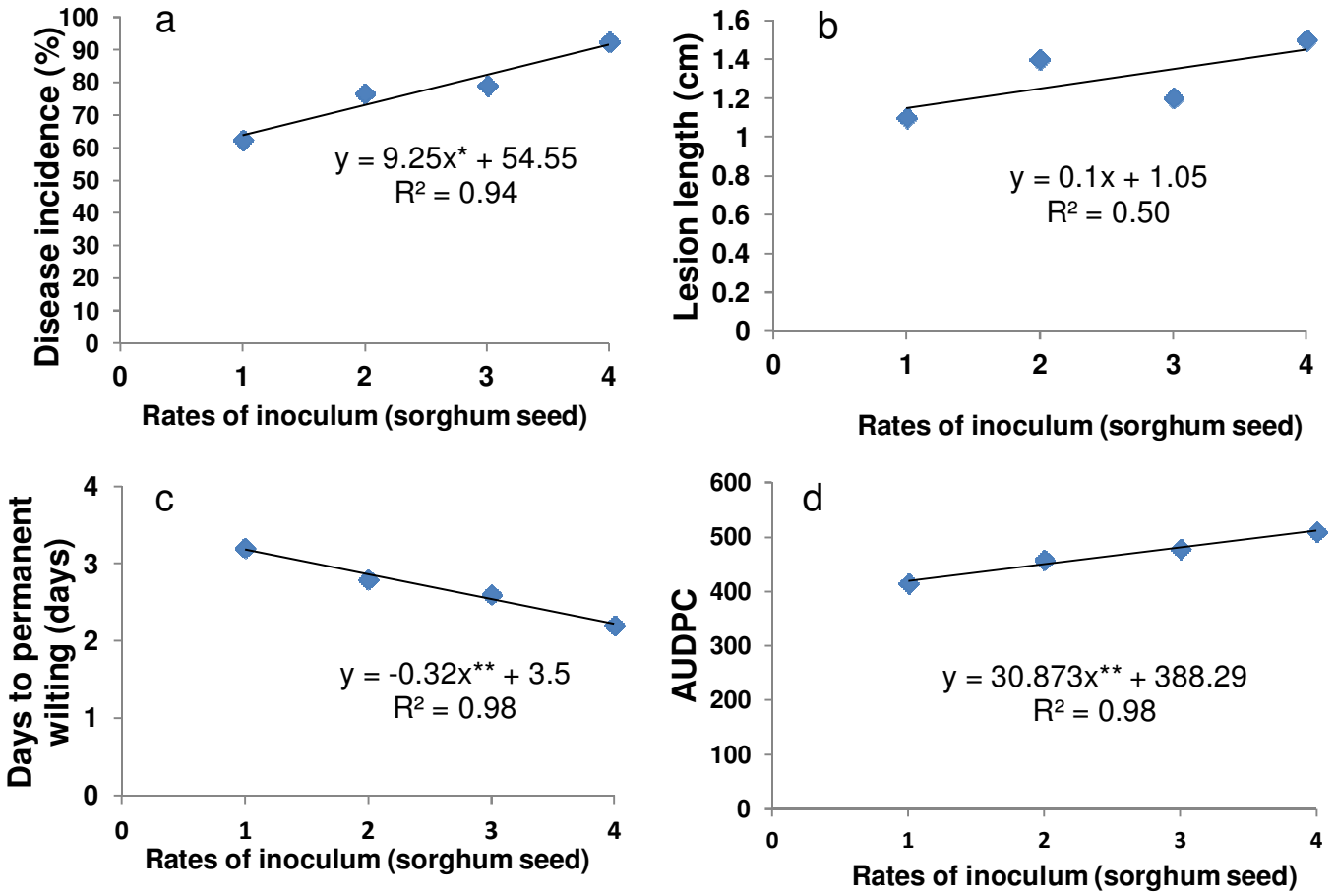

Figure 1. Disease incidence (**a**), lesion length (**b**), days to permanent wilting (**c**) and area under disease progress curve (AUDPC) (d) of Jerusalem artichoke at four different levels of *S. rolfsii*-infested seeds per plant; *, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

mortality caused by *S. rolfsii* increased with an increase in inoculum load (Hussain et al., 2006), but differences in the concentrations of sclerotia by weight between 0.1 and 0.5 g, by number of infested wheat between 1 grain and 5 grains and mycelium by weight between 3 and 20 g were not statistically significant. Similarly, increase in disease incidence of a lettuce crop was proportional to density of *Sclerotinia sclerotiorum* sclerotia used as inoculum (Chitrampalam et al., 2010). High inoculum concentration led to increased disease severity (Sugha et al., 1991).

Three main effects including environment, genotype and inoculation level were important for the variations in disease incidence, lesion length, permanent wilting point and AUDPC. Environment was more important for disease incidence, permanent wilting and AUDPC. Genotype was more important for lesion length, whereas inoculation level also contributed to high variations for disease incidence, days to permanent wilting and AUDPC although the contributions were somewhat lower than environment for disease incidence, days to permanent wilting and AUDPC and lower than genotype for lesion length and days to permanent wilting.

In this study, the effect of inoculation level is the main focus followed by genotypic differences. The questions underlying the experiment are that what inoculation level is more suitable for screening of Jerusalem artichoke genotypes and what traits are more appropriate for identification of resistant genotypes. Higher inoculation level gave higher disease incidence, lesion length and AUDPC but it gave lower days to permanent wilting.

However, more suitable inoculation level was observed for three sorghum seeds because this seed rate had high variations among Jerusalem artichoke genotypes for disease incidence, days to permanent wilting and AUDPC as indicated by high F-ratios.

Sorghum seed inoculum is efficient for mass production of inoculum and can be used for screening a large number of plants. *S. rolfsii*-infested cereal grains were generally used in greenhouse (Pande et al., 1994) and field screening in peanut (Gorbet et al., 2004). Infested millet and oat inoculum were also used in screening of *S. sclerotiorum* resistance in sunflower (Gulya, 2004).

In our previous study, Jerusalem artichoke genotypes showed higher variation in days to permanent wilting than

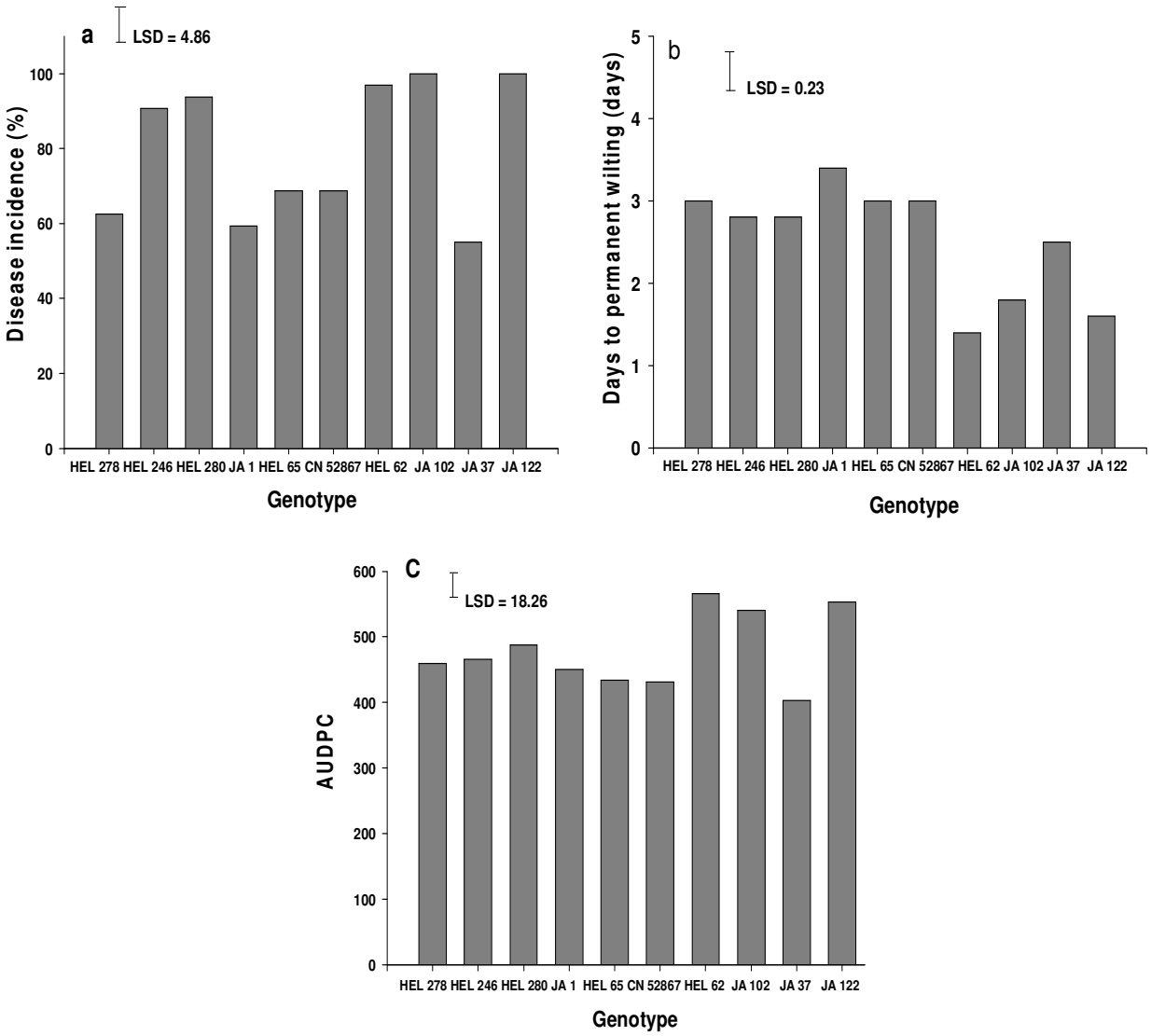

Figure 2. Disease incidence (**a**), days to permanent wilting (**b**) and area under disease progress curve (c) of Jerusalem artichoke genotypes after inoculation with three *Sclerotium rolfsii*-infested seeds per plant.

other traits (Sennoi et al., 2010). Therefore, this trait was used to identify resistance and susceptible genotypes. For the present study, the resistance ratings of genotypes were similar to those of the previous study based on days to permanent wilting except for CN 52867 and JA 37, showing good repeatability of the time to permanent wilting method, although, the inoculation methods of studies were different. A single *S. rolfsii*-infested sorghum seed was placed contacting a wound at the base of the stem. In contrast, the present study did not wound the stems. Stem wounding may destroy some structural defenses. For example, a thick and/or tough cuticle can increase resistance to a pathogen that enters the plant by direct penetration. The difference in methods of inoculation might be the main cause of the difference of the results between two studies. Since wounding does not normally accompany infection of Jerusalem artichoke in the field, inoculation without wounding should more accurately represent genotype resistance levels.

Higher disease incidence during the first experiment may have been due to the fact that temperature at the time of inoculation was 30.2°C compared to 34.5°C for the second experiment. Punja (1985) reported that the optimum temperature for *S. rolfsii* mycelium growth ranged from 27 to 30°C. In another trial, *S. rolfsii* mycelium did not grow at temperatures < 15°C or > 40°C and the optimum was 30°C (Kwon and Park, 2002).

Inoculation with three sorghum seeds obtained the highest variations in Jerusalem artichoke genotypes and provided replicable results for most genotypes. This method will be further used to evaluate Jerusalem artichoke genotypes for resistance to stem rot disease

caused by *S. rolfsii*.

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