The 4th International Conference on Agriculture and Agro-Industry
ICAAI2022
Transforming Value Chains for Global Security
Mae Fah Luang University, Chiang Rai, Thailand
November 10 -11, 2022

Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

Original Research Article

Correlation between mycelium-inoculated detached leaf and field assessments of resistance to *Cercospora canescens* in mungbean

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ARTICLE INFO

Article history:
Received 11 November 2022
Received in revised form 18 July 2023
Accepted 31 July 2023

Keywords:
Cercospora leaf spot (CLS)
Detached leaf assay
Field screening
Inoculation
Mycelium
Vigna radiata

ABSTRACT

Cercospora leaf spot (CLS) caused by *Cercospora canescens* is a serious disease of mungbean (*Vigna radiata*) in Thailand. For the selection of resistant varieties in a breeding program, a rapid screening of numerous genotypes for CLS resistance is necessary. Most screening methods use spores as inoculum, but their productivity on culture media is low. The objective was to develop an efficient laboratory inoculation method using mycelium. We compared resistance/susceptibility levels of 19 mungbean genotypes from detached leaf inoculation using mycelium from the most virulent *C. canescens* isolates (SUT1, SUT4, PAK1, and PAK2) with field inoculations. The resistance levels of mungbean genotypes evaluated by the detached leaf inoculations were comparable to the field inoculations when comparing genotypes with a correlation coefficient of 0.822 ($p< 0.01$). In addition, the paired t-test revealed no statistically significant difference ($p> 0.05$) between the average disease severity scores obtained from the detached leaf assay and those from field evaluation. Both inoculation methods consistently identified mungbean genotypes V4718, V4785, V4758, and SUPER5 as resistant to CLS, which will be useful for future breeding programs. These results suggest that the mycelium-inoculated detached leaf assay was as effective as field inoculation for inducing disease symptoms and identifying resistant genotypes. Moreover, mycelium inoculum exhibited much simpler and faster to prepare in large quantities than spore inoculum, allowing effective and economical large-scale screening with multiple isolates to ensure reliability.

INTRODUCTION

Cercospora leaf spot (CLS) disease in mungbean (*Vigna radiata*) is caused by *Cercospora canescens*. This was first reported in 1960 by Munjai et al. from Delhi, India. It is a serious disease that can reduce yield by 50-80% depending on the stage of infection. It can incur up to 96% yield losses in uncontrolled conditions (Lal et al., 2001; Kaur, 2004; Chand et al., 2012). This disease is spread widely in Asia in such countries as India, Bangladesh, the Philippines, Thailand, and in all parts of the world (Pandey et al., 2009). Conidia infection can cause lesions on foliage with an average diameter of 3-15 mm. The symptoms are at first a brown spot which then turns to grey with a reddish-brown margin, with amphigenous fruiting becoming more abundant on the lower surface (Munjal et al., 1960). The disease mostly infects plants at 30-40 days old and causes premature defoliation, which affects the

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ISSN: 2408-1736

size of pods and grains (Grewal et al., 1980). Although chemical control is the most popular method to control CLS, the use of mungbean resistant varieties is an effective method which is the cheapest, with the most practical and effective features for controlling CLS.

The pathogen development for tolerance to resistant varieties is not acute because it takes a long time and may affect their important metabolic processes. However, to develop resistant varieties, a rapid, highly efficient, and reliable laboratory assay for screening a large number of mungbean genotypes for resistance to CLS is required. Most screening methods use conidia as an inoculum, but conidial production on culture media is typically low and may not be produced in some isolates. Moreover, preparing conidial inoculum is a time-consuming process, particularly when large amounts of inoculum must be prepared from numerous culture plates and sporulation capacity varies among fungal isolates. While mycelia grow more quickly and in larger quantities, they have been used as an inoculum in only a few studies (e.g., Singh et al., 2002; Chen & Wang, 2005). If mycelia are to be effective in causing symptoms, it would be much simpler to prepare inoculum for these screening assays. Furthermore, a detached leaf assay is an effective method for the rapid screening of large numbers of genotypes as well as for resistance assessment under highly controlled conditions. However, the detached leaf assay is only useful if it has a strong correlation with the results of a field evaluation. The objective of this study was to evaluate the effectiveness of the laboratory inoculation method compared with field evaluation by using mycelium of *C. canescens*.

MATERIALS AND METHODS

Fungal isolation and growth

C. canescens isolation was performed by the following standard tissue isolation technique. The infected mungbean leaves were collected from mungbean fields in 5 locations, including Suranaree University of Technology Farm and Pak Thong Chai district in Nakhon Ratchasima province, as well as Nakhon Sawan, Petchabun, and Phichit provinces. Infected leaves were surface sterilized for 30 seconds in 0.5% (v/v) Clorox before being rinsed 3 times in sterile distilled water. Then, leaf pieces with a 0.5 to 1.0 cm width were cut from the infected area along with some healthy tissues. The leaf pieces were transferred onto potato dextrose agar (PDA) medium (Verma & Agnihotri, 1972) in sterilized petri dishes by an aseptic technique and were incubated at room temperature (27±2°C).

The 10 isolates collected in this study were obtained from SUT1 and SUT4 from Suranaree University of Technology Farm, PAK1 and PAK2 from Pak Thong Chai district, NW1 and NW2 from Nakhon Sawan, PB1 and PB2 from Petchabun, and PCH1 and PCH2 from Phichit provinces. The morphological characters of the fungus such as mycelia, conidiophores, conidia, and cercosporin production were studied under a microscope based on the characteristics of *C. canescens* according to Ellis & Martin (1882). The fungus was sub-cultured on PDA slants and incubated at 27±2°C for 14 days. Then, they were preserved in a refrigerator at 4°C and renewed once every month. The preserved culture was transferred to room temperature before use.

Inoculum preparation

To determine a suitable inoculum concentration for the detached leaf assay, the fungal culture plates were used to prepare mycelium inoculum by adapting the method of Guo et al. (2016). Agar disks were collected from the growing edges of isolate SUT1 at 14 days old by using a 5 mm cork borer. The different amounts of mycelium-containing agar disks (10, 30, 50, 80, and 100 pieces) were mixed with 5 mL of sterile distilled water in a sterile bottle using a shaker at 2,000 revolutions per minute (rpm) for 2 hours to homogenize the mycelia. The solution was filtered through 2 layers of muslin cloth. The prepared inoculum can be stored at room temperature for up to 5 days. These different concentrations of inoculum were tested in a resistant genotype (V4718) and a moderately resistant to susceptible genotype (CN72). Leaves of similar size (leaves from the second node counted from the top) of 21-day-old plants were collected and used. A completely randomized design (CRD) with 15 replications (a leaf piece/ replication) was used. The leaves were surface-disinfected with 1% (v/v) Clorox and rinsed with sterile distilled water, cut into 1 cm^2 pieces, and placed on water agar (WA; 1.5% (w/v) agar) with 25 mg/L streptomycin in a 90 mm diameter petri dish. One 5 µL droplet of mycelium inoculum of a fungal isolate was inoculated onto each leaf piece. Sterile distilled water without mycelia was used as a control. The inoculated leaves were incubated at 27°C for a 12-hour photoperiod (1,500 lumen/m^2) for 3 days to ensure successful infection. The visible lesions appeared 2 days after inoculation (DAI) but were evaluated after 3 days by rating the symptoms on a 1-5 scale according to Ngegba et al. (2017) (Table 1). Disease severity scores of various concentrations indicated that 80 mycelium discs/5 mL of DI water was suitable for evaluation in this assay and so was used for further experiment.

Table 1. Disease severity scores for Cercospora leaf spot of mungbean. Source: Ngegba et al. 2017 (modified).

Scales	Rating	Description
1	No infection	No visible symptoms
2	Low infection	1-25% of total leaf area is covered by lesions
3	Moderate infection	26-50% of the total leaf area is covered by lesions
4	Severe infection	51-75% of the total leaf area is covered by lesions
5	Very severe infection	76-100% of the total leaf area is covered by lesions

Fungal pathogenicity analysis

The 10 isolates from 5 locations were evaluated for pathogenicity to select the most virulent isolates. Two, which were moderately resistant to susceptible mungbean genotypes (CN72 and CN84-1), and 3 resistant mungbean lines (V4718, V4758, and V4785) were grown in pots under greenhouse conditions. Leaves from the second node (counting from the top) of 21-day-old plants were collected and used for the detached leaf assay. The leaf pieces were placed on water agar (WA; 1.5% (w/v) agar) with 25 mg/L streptomycin in a 90 mm diameter petri dish and inoculated with one droplet of 5 µL of *C. canescens* inoculum (80 mycelium disks/ 5 mL), while the control leaves were inoculated with DI water. A CRD with 9 replications (a leaf piece/replication) was used.

The inoculated leaves were kept for 3 days at 27°C in a 12-hour photoperiod (1,500 lumen/m^2). Disease severity was rated at 3 DAI as previously described. The most virulent four isolates of *C. canescens* (SUT1, SUT4, PAK1, and PAK2) were selected for further use in the experiment.

To compare the effects of inoculating detached leaves at different plant developmental stages, leaves of 21-day-old plants (vegetative stage) and 45-day-old plants (reproductive stage) of 5 mungbean genotypes (CN72, CN84-1, V4718, V4785, and V4758) grown under greenhouse condition were inoculated with mycelium inoculum. Inoculum from each of four *C. canescens* isolates (SUT1, SUT4, PAK1, and PAK2) was prepared separately at 80 mycelium discs/5 mL of DI water according to the methods previously described. Equal amounts of solution from each of the four isolates were mixed together well before being used as an inoculum. The leaf pieces were inoculated with the inoculum as previously described. A CRD with 9 replications (a leaf piece/ replication) was used. Disease severity was assessed 3 DAI. The results demonstrated that the resistance status within the genotype was mainly identical at both developmental stages and the 45-day-old plants (reproductive stage) were therefore used for the next experiment.

Comparison of detached leaf and field assessments

The 19 mungbean genotypes with varying resistance/ susceptibility levels to *C. canescens* (four resistant genotypes; V4718, V4758, V4785, and SUPER5, one susceptible genotype; EGMD-6D, three moderately resistant genotypes; CN72, CN84-1, and KING, and eleven breeding lines; O2-31, O2-37, O2-39, P01, P05, H3, H4, B1, B2, D5, and G1) and four virulent isolates of *C. canescens* (SUT1, SUT4, PAK1, and PAK2) were used in this study. All plants were grown in an experimental field in Pak Thong Chai district, Nakhon Ratchasima province. There were 3 rows per genotype, 50 cm apart, with 20 cm between plants. Each row consisted of 10 plants. The genotype CN72 was planted as a guard row around the field.

The 45-day-old plants were used for both detached leaf and field assessments. For both detached leaf and field assessments, mixed inoculum from four isolates with the optimal mycelium concentration (80 mycelium discs/5 mL of DI water) was used. One day prior to inoculation, we watered the plants to increase the moisture content of the experimental field. A CRD with 9 replications (a plant/replication) was used for both detached leaf and field assessments. Nine plants per genotype were randomly selected (3 plants from each row) and tagged for use in both detached leaf and field assessments. One leaf per plant of the selected plants (9 plants/genotype) were collected and kept for detached leaf assessment. The inoculation method for the detached leaf assay was carried out according to the method described previously. Disease severity was assessed at 3 DAI by rating on the same scale as in Table 1.

For the field assessment, mycelium inoculum (same preparation as in the previous detached leaf assay) was used. The guard row was inoculated by spraying the mycelium inoculum on the leaf surface with a mist sprayer until it drained to ensure that there was a uniform infection spread to the genotypes being tested. Disease severity was rated from the sampled plants at 15 DAI.

Statistical methods

All experiments were conducted in a CRD. The mungbean genotypes were classified as resistant (scores 1.0-2.4), moderately resistant (scores 2.5-3.4), and susceptible (scores 3.5-5.0). The disease severity scores from all experiments were analyzed by using analysis of variance (ANOVA) by SPSS software (version 16; SPSS Inc.; Chicago, IL, USA). The Pearson's correlation coefficient was used to analyze the relationships between the detached leaf assay and field evaluations by SPSS software. SPSS was also used to perform paired t-test analysis in order to compare the average disease severity scores between the detached leaf assay and field evaluation.

RESULTS AND DISCUSSION

In the laboratory, a detached leaf evaluation was conducted using mycelium, a suitable concentration of which evaluated by comparing various concentrations (10, 30, 50, 80, and 100 mycelium discs/5 mL of DI water) in the resistant genotype (V4718) and the moderately resistant to susceptible genotype (CN72). It was found that 80 mycelium discs/5 mL of DI water was the most suitable for the evaluation because this concentration can clearly identify the different levels of resistance between the two mungbean genotypes. At the lower and higher concentrations, the disease severity scores were not well differentiated between two genotypes (Table 2). Therefore, the 80 mycelium discs/5 mL of DI water were used for a pathogenicity analysis as well as detached leaf and field assessments of the 19 mungbean genotypes.

Table 2. Differential responses of mungbean genotypes on detached leaf assay against *Cercospora canescens* isolate SUT1 at different concentrations of mycelium inoculum.

Inoculum conc. (pieces/ 5mL)	Genotypes			
	CN72		V4718	
	Scores	RI	Scores	RI
10	1.00 ± 0.00	R*	1.00 ± 0.00	R
30	2.00 ± 0.00	R	1.36 ± 0.11	R
50	2.40 ± 0.05	R	1.53 ± 0.13	R
80	3.23 ± 0.07	MR	2.00 ± 0.07	R
100	3.73 ± 0.07	S	3.23 ± 0.07	MR

Cercospora leaf spot resistance levels based on disease severity scores as follows: 1.0-2.4 = resistant (R), 2.5-3.4 = moderately resistant (MR), and 3.5-5.0 = susceptible (S). Conc. = concentrations. RI = resistance levels.

In this study, 10 isolates of *C. canescens* belonging to different locations (isolates SUT1 and SUT4 from Suranaree University of Technology Farm, PAK1 and PAK2 from Pak Thong Chai district, NW1 and NW2 from Nakhon Sawan, PB1 and PB2 from Petchabun, and PCH1 and PCH2 from Phichit provinces) were evaluated for pathogenicity to select the most virulent isolates for further use in the experiment. When inoculated on five mungbean genotypes varying in resistance levels (CN72, CN84-1, V4718, V4758, and V4785), isolates SUT1, SUT4, PAK1, and PAK2 were found to be highly virulent, while the remaining isolates were moderately virulent (Table 3). These results suggest that there are variations in the pathogenicity of *C. canescens*. Similarly, Iqbal & Mukhtar (2014) reported that *Macrophomina phaseolina*, a fungus causing charcoal rot in mungbean, has pathogenic variation and a broad host range, which enable it to survive better.

To evaluate the effects of the plant developmental stages on the detached leaf assay, five mungbean genotypes (CN72, CN84-1, V4718, V4785, and V4758) were inoculated with mycelium inoculum at 2 different stages (21 days after sowing (DAS) (vegetative stage) and 45 DAS (reproductive stage)). It was demonstrated that resistance within the genotype was mainly

identical at both developmental stages (Table 4). These results indicate that relative disease resistance is consistent with genotypes regardless of the stages of plant development. Guo et al. (2016) also found that the resistance of boxwood plants inoculated with *Calonectria pseudonaviculata* mycelium at various developmental stages remained stable over time. Therefore, mycelium screening assays can be performed in the laboratory at any stage of plant development with accurate results. The early laboratory screening assay using the leaves at the vegetative stage will be helpful to reduce the time required for a resistance evaluation in the mungbean breeding programs.

When the disease severity of 19 mungbean genotypes was evaluated at 45 DAS using mycelium inoculum detached leaf assay with four virulent *C. canescens* isolates, the average disease severity scores of the mungbean genotypes was significantly different (p< 0.01), varying from 2.34 (resistant) to 4.36 (susceptible) (Table 5). The susceptible genotype (EGMD-6D) was found to have the highest disease severity score (4.36). While all resistant genotypes (V4718, V4785, V4758, and SUPER5) possessed low disease severity scores (2.34, 2.35, 2.48, and 2.48, respecttively), all the moderately resistant genotypes (CN72, CN84-1, and KING) and most of the breeding lines were moderately resistant to CLS (Table 5). Nevertheless, four breeding lines (H3, H4, D5, and G1) were found to be susceptible. These findings suggest that *C. canescens* mycelium can be used as an inoculum to determine mungbean resistance to CLS. Our results are similar to those reported by Guo et al. (2016), which suggested that mycelium inoculum can determine the resistance of boxwood plants to blight (*C. pseudonaviculata*) under laboratory conditions, with a correlation coefficient of 0.91 (p< 0.01) between the detached leaf and whole plant assays.

In the field experiment, disease symptoms first appeared at 10 DAI on the susceptible genotype (EGMD-6D). At 15 DAI, average disease severity scores varied significantly (p< 0.01) among nineteen mungbean genotypes. The severity of the disease ranged from 1.00 (resistant) to 3.67 (susceptible) (Table 5). It was found that the field results were consistent with the laboratory results from the detached leaf evaluation using mycelium. The severity scores of the disease were mostly higher on detached leaves, possibly due to higher disease pressure on detached leaves than on field plants, but both methods reliably identified the level of disease of most mungbean genotypes, especially those that were resistant or susceptible. Moreover, the disease symptoms from Table 6 confirms that both methods show consistent disease lesions, which can be visually observed and easily rated to identify the severity of the symptoms. These results indicate that the mycelium inoculation enabled us to evaluate the CLS resistance of plants under field or laboratory conditions. Four resistant genotypes (V4718, V4785, V4758, and SUPER5) were classified as resistant, while EGMD-6D and two breeding lines (H4 and D5) were classified as susceptible using both laboratory and field disease assessments. Two moderately resistant genotypes (CN84-1 and KING) and six breeding lines(O2-31, O2-37, O2-39, P01, B1, and B2) were classified as moderately resistant by both methods. However, one moderately resistant genotype (CN72) and three breeding lines (P05, H3, and G1) were found to have minimal variation between both methods. Different rates of disease development may have been observed in different genotypes due to the genetic background of mungbean genotypes (Chueakhunthod et al., 2020). Iqbal & Mukhtar (2014) also reported that there was pathogenic variability among *M. phaseolina* isolates associated with mungbean genotypes. The existence of genetic diversity in mungbean and pathogenic variability of pathogens suggests that resistant varieties should be developed by pyramiding several resistance genes.

Table 3. Differential responses of mungbean genotypes on detached leaf assay against 10 isolates of *Cercospora canescens*.

Geno-types	Isolates																			
	SUT1		SUT4		PAK1		PAK2		NW1		NW2		PB1		PB2		PCH1		PCH2	
	Score	RI	Score	RI	Score	RI	Score	RI	Score	RI	Score	RI	Score	RI	Score	RI	Score	RI	Score	RI
V4718	1.76 a	R	2.00 a	R	1.81 a	R	1.24 a	R	1.17 a	R	1.28 a	R	1.56 a	R	1.17 a	R	1.28 a	R	1.44 a	R
V4758	2.22 b	R	2.36 b	R	2.26 b	R	2.07 b	R	1.95 b	R	2.00 b	R	2.05 b	R	1.90 b	R	1.83 b	R	1.35 a	R
V4785	2.28 b	R	2.37 b	R	2.22 b	R	2.09 b	R	1.97 b	R	2.13 bc	R	1.58 a	R	1.65 b	R	1.76 b	R	1.81 b	R
CN72	3.26 c	MR	3.19 c	MR	3.33 c	MR	3.31 c	MR	2.24 bc	R	2.31 c	R	2.33 b	R	2.28 c	R	2.34 c	R	2.40 c	R
CN84-1	3.37 c	MR	3.32 c	MR	3.35 c	MR	3.28 c	MR	2.42 c	R	2.33 c	R	2.38 b	R	2.35 c	R	2.23 c	R	2.24 c	R

Isolates of *Cercospora canescens* were obtained from infected mungbean leaves in the fields at Suranaree University of Technology Farm (SUT1 and SUT4), Pak Thong Chai district (PAK1 and PAK2), Nakhon Sawan (NW1 and NW2), Petchabun (PB1 and PB2), and Phichit (PCH1 and PCH2) provinces.

RI = resistance levels.

Data followed by the same letter in each column do not differ significantly (p> 0.05) according to Duncan's multiple range test.

Cercospora leaf spot resistance levels based on disease severity scores as follows: 1.0-2.4 = resistant (R), 2.5-3.4 = moderately resistant (MR), and 3.5-5.0 = susceptible (S).

Table 4. Disease severity of *Cercospora canescens* on detached leaf assay in the laboratory with isolates SUT1, SUT4, PAK1, and PAK2 inoculation of 5 mungbean genotypes on leaves of 21- and 45-day-old plants.

Genotypes	Detached leaf assay using 21-day-old plants		Detached leaf assay using 45- day-old plants	
	Scores	Resistance levels	Scores	Resistance levels
V4718	1.70 ± 0.16 a	R	2.34 ± 0.06 a	R
V4758	2.23 ± 0.06 b	R	2.48 ± 0.07 a	R
V4785	2.24 ± 0.06 b	R	2.35 ± 0.05 a	R
CN72	3.27 ± 0.03 c	MR	3.16 ± 0.09 b	MR
CN84-1	3.33 ± 0.02 c	MR	3.09 ± 0.05 b	MR
CV (%)	28.02		15.17	

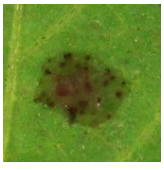
Data followed by the same letter in each column do not differ significantly (p> 0.05) according to Duncan's multiple range test. Cercospora leaf spot resistance levels based on disease severity scores are as follows: 1.0-2.4 = resistant (R), 2.5-3.4 = moderately resistant (MR), and 3.5-5.0 = susceptible (S). CV = Coefficient of variation

Table 5. Disease severity of *Cercospora canescens* on detached leaf assay in the laboratory and field inoculations of 19 mungbean genotypes.

Genotypes	Detached leaf assay		Field	
	Scores	Resistance levels	Scores	Resistance levels
V4718	2.34 ± 0.06 a	R	1.00 ± 0.00 a	R
V4758	2.48 ± 0.07 a	R	2.33 ± 0.08 d	R
V4785	2.35 ± 0.05 a	R	1.83 ± 0.22 c	R
SUPER5	2.48 ± 0.06 a	R	1.33 ± 0.08 b	R
EGMD-6D	4.36 ± 0.06 h	S	3.50 ± 0.14 gh	S
CN72	3.16 ± 0.09 de	MR	2.33 ± 0.17 d	R
CN84-1	3.09 ± 0.05 cde	MR	2.67 ± 0.08 de	MR
KING	3.28 ± 0.03 e	MR	3.33 ± 0.08 gh	MR
O2-31	2.94 ± 0.01 bc	MR	2.67 ± 0.08 de	MR
O2-37	2.80 ± 0.04 b	MR	2.67 ± 0.08 de	MR
O2-39	2.97 ± 0.03 bcd	MR	3.17 ± 0.08 fg	MR
P01	2.87 ± 0.02 b	MR	2.83 ± 0.08 ef	MR
P05	2.82 ± 0.01 b	MR	2.33 ± 0.08 d	R
H3	3.68 ± 0.03 fg	S	3.17 ± 0.08 fg	MR
H4	3.57 ± 0.05 f	S	3.67 ± 0.08 h	S
B1	3.29 ± 0.03 e	MR	3.33 ± 0.08 gh	MR
B2	2.86 ± 0.05 b	MR	2.83 ± 0.22 ef	MR
D5	3.81 ± 0.06 g	S	3.67 ± 0.08 h	S
G1	3.82 ± 0.04 g	S	3.33 ± 0.08 gh	MR
CV (%)	9.16		16.38	

Data followed by the same letter in each column do not differ significantly (p> 0.05) according to Duncan's multiple range test. Cercospora leaf spot resistance levels based on disease severity scores are as follows: 1.0-2.4 = resistant (R), 2.5-3.4 = moderately resistant (MR), and 3.5-5.0 = susceptible (S). CV = Coefficient of variation.

Table 6. Disease severity of *Cercospora canescens* in detached leaf assay and field conditions.

Resistance levels	Geno-types	Methods	
		Detached leaf assay	Field
Resistant	V4718		
	SUPER5		
Moderately resistant	CN84-1		
	KING		
Susceptible	EGMD-6D		
	D5		

Pearson's correlation was used to assess the relationship between laboratory and field evaluations in all mungbean genotypes. The correlation between laboratory and field results was highly significant ($p< 0.01$), with a Pearson's correlation coefficient of 0.822 (Fig. 1). Furthermore, the paired t-test showed that average disease severity scores of detached leaf assay were not significantly different from those of field evaluation ($p> 0.05$). These results revealed that resistance to *C. canescens* in the laboratory was comparable to that observed in the field. In addition, the laboratory evaluation was sufficient for determining resistant and susceptible responses. The coefficients of variation (CV) in the field test were higher (16.38%) than in the laboratory evaluation (9.16%). This indicates that the laboratory screening assay was more precise in determining disease responses. Similar to the study of Poolsawat et al. (2012), higher CV (%) was observed in the field test compared to the laboratory evaluation of resistance to *Sphaceloma ampelinum* causing anthracnose in grapevine.

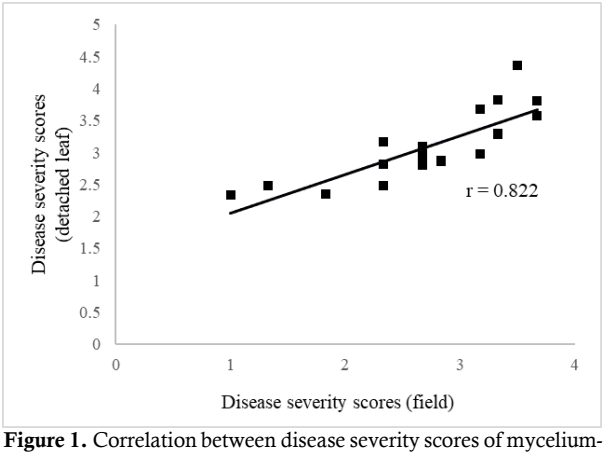


Figure 1. Correlation between disease severity scores of mycelium-inoculated detached leaf and field assessments of mungbean genotypes

To compare the costs associated with the mycelium-inoculated and conidial-inoculated detached leaf assays, the expenses for culture media, labor, and consumables were calculated. Evaluating the disease severity scores of 100 mungbean genotypes using the mycelium-inoculated detached leaf assay required an approximate expenditure of 15 U.S. dollars. Conversely, the conidial-inoculated assay incurred expenses amounting to approximately 23 U.S. dollars, as a greater number of culture plates were necessary to obtain an adequate quantity of conidial inoculum, suggesting that the mycelium-inoculated assay was more cost-effective. Moreover, the reduction of overall expenses associated with the mycelium-inoculated detached leaf assay can be accomplished through optimizing mycelium production to achieve maximum yields while minimizing resource requirements. In this regard, a study conducted by Jaichopsanthia et al. (unpublished data) demonstrated that mungbean leaf agar and lettuce leaf agar were the most effective culture media for promoting colony diameter in *C. canescens*, thereby supporting a higher yield of mycelium. Therefore, it is crucial to conduct further research to gain valuable insights and develop innovative approaches that make the assay more cost-effective and accessible for breeding programs.

CONCLUSION

In this study, the laboratory method of the detached leaf assay illustrated simplicity, easy preparation, cost effectiveness, and less time-consuming (< a week), allowing an efficient screening assay for CLS resistance in mungbean with a large number of genotypes. Thus, the laboratory method provided great screening assay performance in numerous mungbean genenotypes. These results suggest that mycelium can be used as an inoculum in laboratory and field disease assessments. The laboratory method of the detached leaf assay is effective and reliable for the screening of CLS resistance in mungbean varieties/lines and their breeding lines, which will be a useful method for developing resistant mungbean varieties in breeding programs.

ACKNOWLEDGEMENT

This work was supported by the Agricultural Research Development Agency (Public Organization) and Suranaree University of Technology, Thailand. The authors also express their sincere appreciation to Peter C. Bint for editing the manuscript.

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