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Screening Tomato Line Resistance to Controlling *Sclerotium rolfsii* in Cambodia

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Abstract:- Tomatoes are now one of the most commercially important vegetables in Cambodia, where they are the fourth most important agriculture crop and the leading tomato growers. In Cambodia, the climate conditions are favorable for the disease due to high temperatures, humid conditions, and acidic soil, these factors can make the disease survive in the soil for several years in the absence of the plant host. Southern blight on tomato disease, caused by *Sclerotium rolfsii*, was studied in Cambodia in 2010 and demonstrated that southern stem rot caused the plant lesion on the stem near the soil line, resulting in wilting and the plant dying.

Fifty tomato lines, including two local check varieties, were evaluated for disease resistance, and data on growth and yield were measured and analyzed. Apart from this, two screenings had also been done under greenhouse conduction during the dry and wet seasons in order to find the resistant lines. First, the disease was collected, identified, and cultured in the laboratory, and then an amount of 1g of *Sclerotium rolfsii* was applied directly to the soil on the tomato plant at the age of 30 days. The disease score was evaluated on days 6, 12, and 18 after inoculation. Based on the result of southern blight screening, 4 lines showed a consistent result of being highly disease-resistant (AVTO 1314, AVTO 1715, AVTO 1716, and AVTO 1616). The interactions between the genotypes and growing conditions for all yield traits were significant.

Keywords:-*Sclerotium rolfsii, resistant variety,Fifty tomato lines and Cambodia variety.*

I. INTRODUCTION

Tomato (*Solanum lycopersicum*) is an edible fruit and vegetable that belongs to the Solanaceae family (Estan et al., 2005). Tomato are a popular crop in Cambodia, because they are easy to grow, have a short growing season, and have a high marketing demand. At the same time, growing tomato always faces many problems, like suffering from insects and a variety disease including viruses, bacteria, and fungi known as rotten root fungi that cause heavy damage on tomato (Kry et al., 2022). Many methods have been applied to control this pathogen, such as crop rotation, chemical, and biological. Ray G. et al. (2005) reported that disease levels have been reduced by the application of ammonium nitrate either before planting or as three side dressings at monthly intervals while the crop is growing.

Southern blight (*Sclerotium rolfsii*) is one of the major diseases of many different plant species, especially vegetable crops such as tomatoes, peppers, melon, and watermelon(Punja, 2005). It was known as southern stem rot because the plant lesion on the stem near the soil line develops quickly, resulting in wilting and making the plant die (Taylor and Rodriguez, 1999). In Cambodia, the climate conditions are favorable for the disease due to high temperatures, humid conditions, and acidic soil. These factors can make the disease survive in the soil for several years in the absence of the plant host (Edmund et al., 2003).

Even though this disease can be controlled by some chemical fungicides or cultural practices, most farmers in Cambodia did not take preventative measures before the disease occurred and were only willing to control it when it had already infected the field and caused economic losses. The best choice for farmers when it comes to disease is to use resistant varieties so that they can avoid the calamity of the disease outbreak, which leads to failure for their crop production, particularly during the wet season.

In addition, two screenings under greenhouse conduction during the dry and wet seasons had also been carried out in search of the resistant lines. At the age of 30 days, 1g of *Sclerotium rolfsii* was inoculated directly to the soil on the tomato plant after the disease had been first collected, identified, and grown in the lab. The disease score was assessed six, twelve, and eighteen days following the vaccination. Based on the results of the southern blight screening, four linesAVTO 1314, AVTO 1715, AVTO 1716, and AVTO 1616showed a consistent outcome of being extremely disease-resistant. For breeding and experimentation, the four resistant lines that outperform other lines the greatest will be chosen and treated as the male parental line.

Due to the limited availability of local tomato varieties in Cambodia and the fact that the majority of farmers use hybrid seed from various companies, as well as travel restrictions during the covid crisis, this research was conducted to screen the tomato line for controlling *Sclerotium rolfsii* using 1) germplasm of 50 lines. 2) Variety registration (1 variety) is necessary because the majority of lines are in the screening stages and breeding will take place the following year. As a result, additional activities and data are required to ensure that the variety is uniform, stable, and valuable for use (AVRDC, 2003). As a result, some study indicators should be taken into consideration that are based on the actual situation and specific need.

ISSN No:-2456-2165

II. METHODOLOGY

A. Site location and experimental condition

The experiment conducted at Kbal Koh research experimentation and Royal University of Agriculture in both laboratory and filed. In field, the tomato plants planted in pot under net house condition and the pathogen will be confirmed testing in the vitro.

B. Sample collection

From a field of diseased tomatoes in the Kien Svay area of the province of Kandal, about 10 samples of contaminated tomato plants were taken. The following water-soaked lesion on the crown and lower stem tissue was the sample selected for isolation. Yellowing and wilting of the leaves served as a telltale sign of the disease. Following the plant's total collapse, individuals died. Fan-shaped white mycelium growth on the bottom stem, leaf litter, and soil.

C. Land preparation

53 lines of tomato were chosen to test in the experiment and sown in a plastic pot. The seeds of tomatoes were raised in sterile soil. At the age of 28 days, the tomato seedlings were transferred into the plot, which is 20cm deep and 27 diameters in size and contains 10kg of sterile soil and 100g of compost.

D. Characteristic evaluation of the lines under filed condition

All 53 tomato varieties and lines were sown on December 15, 2020. It was designed without replication and contained 53 plots; each plot resembled an individual line consisting of 8 plants. The row and plant spacing was 60cm by 50 cm, with 50cm between beds. All lines were evaluated in order to study their morphological characteristics, environmental adaptability, and pest resistance. It was cultivated in an open field at the Kbal Koh Vegetable Research Station. Data collection includes sowing date, transplanting date, 100% flowering date, plant height, number of fruits per plant, weight of fruits per plant, fruit length, fruit width, and number of locules. All the characteristics of each line will be considered when selecting parental lines for breeding purposes (results in Tables 3 and 4).

Table 1: List of varieties/line for characterization

No	WorldVeg No	International code/source	No	WorldVeg No	International code/Source
1	AVTO1288	CLN3552B	28	KK5	Kbal koh
2	AVTO1314	CLN3212C	29	KK6	Kbal koh
3	AVTO1315	CLN3241Q	30	SKK1	Kbal koh
4	AVTO1464	FMTT1733E	31	SKK2	Kbal Koh
5	AVTO1702	CLN3853C	32	SKK3	Kbal Koh
6	AVTO1705	CLN3902C	33	SKK4	Kbal Koh
7	AVTO1706	CLN3961D	34	Tbk 1	Kompong cham
8	AVTO1409	CLN3641A	35	Tbk 2	Kompong cham
9	AVTO1306	CLN3451D	36	KTh	Kompong thom
10	AVTO1711	CLN3938A	37	AVTO1003	CLN3125L
11	AVTO1712	CLN3938B	38	AVTO1008	CLN3078C
12	AVTO1715	CLN3938E	39	AVTO1010	CLN3070J
13	AVTO1716	CLN4018A	40	AVTO1903	CLN4066G
14	AVTO1717	CLN4018B	41	AVTO1914	CLN4079L
15	AVTO1718	CLN4018C	42	AVTO1907	CLN4066E
16	AVTO1719	CLN4018D	43	AVTO1910	CLN4079J
17	AVTO0301	CLN2498D	44	AVTO1915	CLN4079M
18	AVTO1616	CLN3900B	45	AVTO1911	CLN4079K
19	AVTO1219	CLN3241H-27	46	AVTO1912	CLN4079E
20	AVTO1429	FMTT1733D	47	AVTO1913	CLN4079D
21	AVTO1707	CLN3961C	48	AVTO1909	CLN4078A
22	AVTO1424	CLN3682C	49	AVTO1921	CLN4032C-8
23	AVTO1713	CLN3938C	50	AVTO1919	CLN3938K-8
24	KK1	Kbal Koh	51	AVTO1954	CLN3940C
25	KK2	Kbal Koh	52	AVTO1828	CLN4123A
26	KK3	Kbal Koh	53	AVTO1829	CLN4123B
27	KK4	Kbal Koh			

E. Experimental Design

The experiment was conducted at Kbal Koh vegetable research station. This experiment was designed in Completely Randomized Design (CRD) with 5 replications and 53 treatments in the plot under greenhouse condition. One treatment contained 9 plots and one plant per plot. The row spacing is 0.5 meter.

F. Growing and Maintenance of Healthy Tomato Seedlings

All 53 lines of seeds were sown on a damp paper towel for a night with low temperatures. The seeds were placed on moist soil by filling the tray within 12 inches, then 1-2 seeds

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were put in each hole and covered with soil gently. The heat mat was covered on the plastic tray, and the plastic bags were wrapped to speed the incubation. After 4 days, uncover the tray and water it twice a day.

G. Potato Dextrose Agar Preparation

In the experiment, PDA was used to culture *Sclerotium rolfsii* sporulation. The PDA was prepared which consisted of 200g of potato, 20g of dextrose, 16g of selective agar and 1L of distilled water and PDA was sterile in autoclave for 30 minutes in 121oC. After Sterile, PDA was transferred to Petri-dish.

H. Isolation the Pathogen

After selecting the wilting plant root, part of the plant root that showed the symptom was cut into 5-mm sections. The specimen of roots that had diseased portions was cut to sterility for 1 minute in 0.1 percent chloride solution, followed by 1 minute in alcohol and 1 minute in distilled water. Then transferred to culture on potato dextrose agar media. After 14 days of inoculation, the spores were isolated under the microscope to assure the presence of *S. rolfsii.* The mycelium of *Sclerotium rolfsii* was verified according to morphology and reproductive structure.

I. Transferred Pathogen stock with Sorghum

Sorghum grains (500g) were proliferated with the Sclerotium rolfsii pathogen and then soaked overnight in water for the pot experiment. A 500 ml Erlenmeyer flask with a capacity for 100g of soaked sorghum grains was filled to the brim with non-absorbent cotton. After that, the flasks were autoclaved for 20 minutes at 121°C and 15 Psi to sterilize them. After being sterilized, the sorghum seeds were placed in flasks, which were then incubated for 15 days at 27°C 2°C to stimulate proper mycelial growth. Each flask included a 5 mm mycelial disc from a pure culture of *Sclerotium rolfsii* that was 7 days old.

J. Inoculation of Sclerotium rolfsii

Tomato seedling reaches the age of 30days old. First, the sclerotium rolfsii was multiplied on sorghum grains and then we applied 1g of sclerotium rolfsii directly in the soil per pot and spray the water to the plant to make it more moisture. Then the water regularly applied twice per day to maintain soil moisture. The data collection start at 6 days, 12 days and 18 days after disease inoculation. the disease score was evaluated by scoring the plant wilting, yellowing or death, mycelia or sclerototia production on the soil surface or on the stem, stem affected area and lesion length by rating from 1 to 9 scale.

K. Disease incidence

Table 2: The scoring of wilting leaf infected by the *Sclerotium rolfsii* (Nene and Thapliyal1982)

No	Disease Rating	Description
0	0	No infection
1	1	1-20% wilted foliage
2	2-3	21-40% wilted foliage
3	4-5	41-60% wilted foliage
4	6-7	61-80% wilted foliage
5	8-9	81-100% wilted foliage

Disease incidence **(%).** The disease incidence was determined by using the following formula.

$$\text{Disease incidence } (\%) = \frac{No.of.infected\ plant}{No.of\ plants\ examined} \times 100$$

n= Number of infected leaves

r1 _ r3 = Disease severity category

N= Total number of examined leaves

L. Data collection

The day of flower 50% and 100%, plant height, fruit weight, percentage of survival plant, disease severity, disease incidence, marketable yield and loss yield collected.

ISSN No:-2456-2165

III. RESULT AND DISCUSSION

A. Top eleven tomato lines

Table 3: Top 11 tomato lines base on yield performance

N0	Variety /Line	Fruit length (cm)	Fruit width (cm)	Number of locules	Fruit thickness (mm)	Weight per fruit (g)	Number of Fruit/Plant	Yield (t/ha)
30	SKK1	3.4 ± 0.3	2.0 ± 0.2	2	2.9 ± 0.6	9.2 ± 2.3	173 ± 7	63.34
32	SKK3	4.5 ± 1.1	2.7 ± 0.2	3	3.8 ± 0.8	17.3 ± 3.2	78 ± 8	54.12
10	AVTO1711	5.7 ± 0.6	7.0 ± 1.0	6	4.7 ± 1.2	170.6 ± 56	8 ± 4	52.08
12	AVTO1715	6.8 ± 0.9	5.9 ± 1.7	6	5.0 ± 1.3	133.8 ± 42.4	8 ± 2	44.68
16	AVTO1719	4.8 ± 0.8	5.3 ± 1.2	5	4.1 ± 0.9	52.3 ± 21.5	21 ± 4	43.18
9	AVTO1306	6.0 ± 0.8	5.8 ± 0.6	3	5.7 ± 1.3	99.3 ± 24.5	10 ± 3	40.83
31	SKK2	4.1 ± 0.3	2.5 ± 0.1	2	3.8 ± 0.5	16.5 ± 5.2	62 ± 6	40.73
14	AVTO1717	5.2 ± 0.6	5.5 ± 1.5	5	5.0 ± 1.0	103.9 ± 15.8	9 ± 3	36.43
15	AVTO1718	4.4 ± 0.6	4.5 ± 1.2	5	3.4 ± 1.1	49.3 ± 34.4	16± 5	31.63
2	AVTO1314	5.1 ± 1.1	5.3 ± 1.0	5	4.0 ± 1.5	75.2 ± 33.2	10 ± 6	29.29
8	AVTO1409	5.0 ± 0.3	5.3 ± 0.9	5	4.4 ± 1.2	97.6 ± 37.3	7 ± 4	29.21

According to Table 3, the top eleven tomato lines based on yield performance revealed significant differences between each line. The SKK 1 line demonstrated the highest with 63.34 t/ha, followed by SKK3, AVTO1711, AVTO1715, AVTO1719, AVTO1306, SKK2, AVTO1717, AVTO1718, AVTO1314, and AVTO1409 lines with 54.12 t/ha, 52.88 t/ha, 44.68 t/ha, 43.18 t/ha, 40.83 t/ha, 40.73 t/ha, 36.43 t/ha, 31.63 t/ha, 29.29 t/ha, and 29.21 t/ha, respectively. For the number of fruits per plant, there was also a significant difference among all tomato lines. The highest number of fruits per plant was found in the SKK1 line with 173 ± 7 followed by SSK3, SKK2, AVTO1719, AVTO1718, AVTO1314, AVTO1306, AVTO1717, AVTO1711, AVTO1715, and AVTO1409 with 78 ± 8, 62 ± 6, 21 ± 4, 16 ± 5, 10 ± 3, 9 ± 3, 8 ± 4, 8 ± 2 and 7 ± 4, respectively. On the other hand, there was a highly significant difference ($P \leq 0.01$) in the total yield.

Based on the result, the performance of the top eleven tomato line use of disease-resistant cultivars or varieties is always a highly favored approach of disease management or control, according to the results and the performance of the top eleven tomato lines. Unfortunately, there aren't many common host plant species of *S. rolfsii* that have high-level cultivars or variants resistant to this fungus. However, this field of study is still being explored. Hosta, peanut, and cowpea cultivar research were recently conducted. In 2003 greenhouse research, the susceptibility of 18 Hosta cultivars to *S. rolfsii* infection and the development of disease was assessed. The severity of the symptoms varied, but no reports of total resistance to *S. rolfsii* were made. There are variations in disease susceptibility between some cultivars, according to recent inoculation research using peanuts and cowpeas. Additionally, early findings from a recent lab experiment using transgenic carrots suggested a decreased susceptibility to *S. rolfsii*.When conditions are favorable for the pathogen and inoculum levels are high, management of Southern blight is challenging. The most effective strategy of control is to prevent the disease by choosing fields that are free of S. rolfsii. A two-year or longer crop rotation to a non-host crop, such as corn or small grains, will aid in preventing the build-up of inoculum and disease issues. In addition, two screenings were conducted in a greenhouse during the dry and wet seasons to identify the resistant lines. Before applying 1g of sclerotium rolfsii directly to the soil on the tomato plant at the age of 30 days old, the illness was first collected, identified, and cultured in the lab. The disease score was assessed six, twelve, and eighteen days following the inoculation. Based on the results of the southern blight screening, four lines (AVTO 1314, AVTO 1715, AVTO 1716, and AVTO 1616) consistently shown a high level of disease resistance. These four resistant lines, which outperform other lines in terms of resistance, will be chosen as the main parental line for breeding and experimentation.

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B. Disease score

Table 4: Result of disease score of (Southern blight) for 3 times periods under greenhouse condition

No	Line designation	Day 6th	Day 12th	Day 18th
1	AVTO1288	0.89	3.89	9.00
2	AVTO1314	0.00	0.00	0.00
3	AVTO1315	4.33	6.00	9.00
4	AVTO1464	0.00	0.89	2.00
5	AVTO1702	0.00	0.67	1.00
6	AVTO1705	1.33	2.67	5.00
7	AVTO1706	0.00	0.67	7.00
8	AVTO1409	0.00	1.44	9.00
9	AVTO1306	0.00	0.67	0.44
10	AVTO1711	0.00	1.00	1.00
11	AVTO1712	0.33	2.00	3.00
12	AVTO1715	0.00	0.00	0.00
13	AVTO1716	0.00	0.00	0.00
14	AVTO1717	1.11	2.89	5.00
15	AVTO1718	0.00	2.56	3.00
16	AVTO1719	0.00	0.78	1.78
17	AVTO0301	0.00	1.11	3.33
18	AVTO1616	0.00	0.22	1.00
19	AVTO1219	0.00	0.00	1.00
20	AVTO1429	0.44	2.00	3.00
21	AVTO1707	2.44	5.33	9.00
22	AVTO1424	2.78	6.00	9.00
23	AVTO1713	3.44	5.33	8.56
24	KK1	2.11	4.67	9.00
26	KK3	2.22	6.00	9.00
27	KK4	3.33	6.00	9.00
28	KK5	5.00	6.00	9.00
29	KK6	4.44	5.33	9.00
30	SKK1	1.33	2.00	9.00
31	SKK2	4.33	5.11	9.00
32	SKK3	2.22	6.44	9.00
33	SKK4	2.67	6.89	9.00
34	Tbk1	3.22	6.56	9.00
35	Tbk 2	2.44	5.11	9.00
36	KTh	2.89	3.89	9.00
37	AVTO1003	2.78	6.22	9.00
38	AVTO1008	3.89	9.00	9.00
39	AVTO1010	2.78	7.00	9.00
40	AVTO1903	3.78	8.67	9.00
41	AVTO1914	3.89	7.00	9.00
42	AVTO1907	3.33	6.00	9.00
43	AVTO1910	2.78	7.00	9.00
44	AVTO1915	2.00	9.00	9.00
45	AVTO1911	0.00	7.56	9.00
46	AVTO1912	0.00	6.00	9.00
47	AVTO1913	0.00	2.00	9.00
48	AVTO1909	1.44	2.56	9.00
49	AVTO1921	0.56	4.67	9.00
50	AVTO1919	0.00	3.22	9.00
51	AVTO1954	0.56	4.00	9.00
52	AVTO1828	0.00	1.33	9.00
53	AVTO1829	0.33	6.33	9.00
Mean		1.56 ± 0.56 **	3.99 ± 0.89**	6.77 ± 0.67**
Min		0.00	0.00	0.00
Max		5.00	9.00	9.00

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The mean and standard error with ** Significant at $P<0.01$, * Significant at $P<0.05$, ns Not significant at $P<0.05$

There were significant differences in the three time periods after disease inoculation. On day 6 after disease inoculation, the plant started to develop symptoms with a disease score ranging from 0 to 5 (Table 3). The overall mean disease score was 1.56. About 19 lines, all of them from AVRDC, did not show any symptoms. On day 12, the disease started to develop more symptoms ranging from 0 to 9, and some plants with a 9 score had already died at this stage. The overall mean was 3.99. Meanwhile, there were four lines that showed no symptoms (2. AVTO1314, 12. AVTO1715, 13. AVTO1716, and 19. AVTO1219). On the 18th, most plants died, the overall mean disease score was 6.77, and only three genotypes showed superior tolerance to southern blight with no sign of disease symptoms (2. AVTO1314, 12. AVTO1715, and 13. AVTO1716). With the exception of three genotypes that demonstrated superior tolerance to southern blight and showed no symptoms of the disease (2. AVTO1314, 12. AVTO1715, and 13. AVTO1716, which was moderately resistant with a mean disease score of 6.77, the lowest disease incidence was found in tomato 19 lines with 100% at day 18 after inoculation, most plants died, and the overall mean of disease score was 6.77. According to Alexandrov (2005), who reported that extremely low or high temperature causes a noticeable breakdown in the level of plant resistance to pathogens, environmental conditions like the high during the day temperature observed in the screen house may have been to blame for the breakdown of resistance of the various tomato varieties. In the study described, healthy tomato plants were experimentally inoculated with spore suspensions and sclerotia containing different fungal pathogens, which resulted in variable degrees of disease symptoms like leaf yellowing, leaf necrosis, crown wilt, stem rot, develop inhibition, and whole plant wilt. The ability of fungal infections to cause the occurrence of numerous disease symptom complexes in experimentally inoculated tomatoes was also noted in a study of a similar nature by Bem (2009). The most common occurrences of whole plant wilt, floral inhibition, leaf necrosis, and stem rot were seen in tomato plants that had been inoculated with *Sclerotium rolfsii*. Similar to this, Kwon and Park (2002) reported significant stem rot, crown rot, and whole plant blight in tomato plants infected with Fusarium rolfsii in Korea. Several studies (Bateman and Beer, 1965; Bateman, 1972; Hodgkinson, 1977; Franceschi and Horner, 1980; Punja et al., 1985) have attributed the remarkable success of S. rolfsii in the production of plant disease symptom complexes, to its ability to produce large quantities of oxalic acid and polygalacturonases which act in concert to achieve maximum toxicity and tissue damage, leading to the eventual death of infected plants.

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