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EVALUATION OF DIFFERENT DISEASE INOCULATION TECHNIQUES FOR BANDED LEAF AND SHEATH BLIGHT DISEASE OF MAIZE (*Zea mays* L.) IN SRI LANKA

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ABSTRACT

Banded leaf and sheath blight disease, caused by *Rhizoctonia solani*, is a serious disease in maize cultivation. Identification of disease resistant/tolerant germplasm/varieties is the primary and essential component of a successful breeding programme. In Sri Lanka, screening for this disease has been done by the detached leaf disk method. However, this is not practical as the leaf disks become yellow and dry before scoring. An experiment was conducted during *Maha* 2012/13, *Yala* 2013, *Maha* 2013/14, and *Maha* 2014/15 seasons at the Field Crops Research and Development Institute, Mahailluppallama, with the objective of identifying a nondestructive, accurate, and rapid screening technique for the disease using three maize varieties. Five inoculation methods, namely spraying mycelium suspension into soil, incorporation of sclerotia into soil, injecting mycelium suspension into sheath, inserting sclerotia into sheath, and the detached leaf disk method, were practiced in the first two seasons. Thereafter, four inoculation methods, namely inserting sclerotia into the sheath, inserting culture grown on paddy straw, inserting culture grown on sorghum grain, and the floating leaf disk method, were practiced in the next seasons. Number of days taken to appear symptoms, disease incidence, and disease severity index were recorded. According to the results, inserting sclerotia into the sheath showed rapid symptom development in all the varieties. However, the disease incidence and disease severity index of the inbred variety Ruwan were greater than other local and exotic hybrids used. Among the tested methods, inserting sclerotia into the sheath is significantly ($p<0.05$) superior to other methods tested and can be adopted as a rapid varietal screening technique for banded leaf and sheath blight disease in Sri Lanka.

Key words: Banded leaf and sheath blight (BLSB), *Rhizoctonia solani*, Sclerotia, Screening technique

INTRODUCTION

Maize (*Zea mays* L.) is ranked as the second most important cultivated cereal and the foremost feed grain in Sri Lanka (Karunarathna, 2006). The extent cultivated and annual production under maize in Sri Lanka were about 67,372 ha and 208,275 t in 2013 respectively (Agstat, 2014). The yield of maize cultivation is highly affected by biotic stresses like foliar diseases, stalk rots, ear rots, and viral diseases caused by different pathogens. Among the maize diseases, Banded Leaf and Sheath Blight (BLSB) is becoming a major disease of Maize cultivation in Sri Lanka. As reported by Sivakumar and Sharma (2007), Bertus (1972) the disease was first reported in Sri Lanka as Sclerotial disease. Presently, the disease is common in most maize growing countries. BLSB mainly occurs in tropical and sub-tropical regions, where high humidity, high temperature, long rainy and sunless weather provide favorable conditions for the disease (Sharma *et al*., 1993; Asano *et al*., 1987). Climatic conditions in Sri Lanka are highly favorable for its infection and spread. BLSB is caused by *Rhizoctonia solani* (*Thanatephorus cucumeris*), and the disease appears on leaves and sheaths as characteristic ashy grey concentric spots and spreads to cover larger areas of infected leaves and husks showing light brown, cottony mycelium with small, round, black sclerotia (The CIMMYT Maize Program, 2004). The disease causes direct losses resulting in premature death of the plant, stalk breakage, and ear rot, besides causing indirect losses by reducing the yield by 40-100% (Singh and Sharma, 1976).

Since the pathogen is soil-borne, drenching with fungicides is expensive and impractical, and breeding for host resistance is a key feature in disease management. Therefore, identification of disease resistant/tolerant germplasm is essential for the successful disease resistant breeding programme. Since natural infections vary from time to time due to many reasons, the use of artificial inoculation methods can provide more uniformity in the screening process, facilitating the elimination of highly susceptible genotypes from the breeding program (Shekhar and Kumar, 2012).

Different techniques have been developed for the artificial inoculation of *Rhizoctonia solani* to maize plants in the world (Madhavi *et al.*, 2011, Shekhar and Kumar, 2012; Chen *et al*., 2013). Floating leaf disk method had been practiced to screen maize varieties against BLSB disease in Sri Lanka (Anonymous, 1994). However, this method

is associated with many unfriendly features, like the difficulty of detaching plant leaves from the same growth stage withering and drying off leaf disks before taking disease counts after inoculating the leaf disks. Hence, this experiment was carried out with the objective of identifying an effective, non-destructive and rapid screening technique for maize varieties against BLSB disease.

MATERIALS AND METHODS

Pot experiments were conducted using sterilized soil during the *Maha* 2012/13, *Yala* 2013, *Maha* 2013/14, and *Maha* 2014/15 seasons at the Field Crops Research and Development Institute, Mahailluppallama, using three varieties namely Ruwan (local inbred), Sampath (local hybrid) and Pacific 984 (commonly growing exotic hybrid). Fungicides were not applied during the experimental period, and insecticides were applied to control the stem borer. The experiment was conducted adopting two factor (varieties and inoculation techniques) factorial randomized completely block design with three replicates. Other agronomic practices were done as per the recommendations of the Department of Agriculture, Sri Lanka.

Isolation of the pathogen

BLSB disease affected maize sheaths were collected, and pathogens were isolated from lesions of disease affected maize sheaths on Potato Dextrose Agar (PDA). *Rhizoctonia solani* does not produce spores but produces sclerotia, which are hard, compact masses of fungal hypae (Singh, 2008), and the pathogen was identified based on the morphology of mycelium and sclerotia on culture medium as described by Barnett (1962). Isolates of the pathogen were subcultured and stored in the PDA for later use.

Inoculation of the pathogen

Five inoculation methodsnamely, spraying mycelium suspension into soil (T_1), incorporation of sclerotia into soil (T_2), injecting mycelium suspension into sheath (T_3), inserting sclerotia into sheath (T_4), and floating leaf disk method (T_5), were practiced during *Maha* 2012/13 and *Yala* 2013 to introduce *Rhizoctonia solani* to the plants one month after planting.

In T_1 treatment, *Rhizoctonia solani* mycelium culture broth (1cm diameter, 5 mycelial disks per 10 mL of water) was prepared using culture grown on PDA and sprayed

into pot soil very closer to the plant. Five sclerotia grown on PDA were incorporated into the soil very closer to the plant in treatment T_2. In T_3 treatment, mycelium culture solution was injected into the third internode of the sheath of every plant using a syringer. Five to six sclerotia were inserted into the third internode of the sheath as the T_4 treatment, and in the T_5 treatment, similar growth stages of fully opened leaves were collected from each plot, cut into 5 cm wide pieces, and floated in petri-plates containing distilled water. Then, around 0.5 cm diameter *Rhizoctonia solani* mycelium broth droplets were placed on the centre of each leaf disk (Anonymous, 1994).

The inoculated plants and leaf disks were observed daily on the development of sheath blight symptoms. Water was sprayed twice a day for potted plants after introducing the pathogen to enhance disease development. Days for symptoms to appear were taken, and Disease Incidence (DI) was calculated.

$$\text{DI \%} = \frac{\text{No. of infected plants per treatment}}{\text{Total number of plants per treatment}} \times 100$$

Inserting sclerotia into the sheath technique showed the best results among five techniques during *Maha* 2012/13 and *Yala* 2013. Trial was repeated with the existing floating leaf disk method, inserting sclerotia into sheath technique, and two new methods that other countries are using. Hence, four screening techniques, inserting sclerotia into sheath (T_1), inserting culture grown on paddy straw (T_2), inserting culture grown on sorghum grain (T_3), floating leaf disk method (T_4), and control (T_5) were practiced during *Maha* 2013/14 and *Maha* 2014/15 one month after planting.

As described in Madhavi *et al*. (2011), T_2 treatment was practiced. Dried paddy straw was chopped into 5 cm pieces and soaked in water overnight. After draining the water, the straw was sterilized for 30 minutes at 16 lb pressure. *R. solani* was inoculated on sterilized paddy straw and incubated for 15 days. Then, culture grown on paddy straw bit was inserted at the 3rd internode of the maize plants using a forcep.

T_3 treatment was practiced, referring to Shekhar and Kumar (2012). Sorghum grains were water soaked for 24 hours, dispensed 40 g in 250 mL conical flask after removing excess water, and autoclaved at a pressure of 15 lb for 30 minutes. Suspension of *R. solani* was inoculated on sorghum grains and incubated at 27 ºC for 10 days. The

impregnated grains were inserted at the 3rd internode of the maize plants using a forcep. T_1 and T_4 treatments were practiced as in previous seasons.

The inoculated plants and leaf disks were observed daily for the development of sheath blight symptoms. Water was sprayed twice per day on the potted plants after introducing the pathogen to enhance disease development. Disease development was measured using the following scale.

0 - No disease symptoms
1 - Lower 1/8 stalk portion affected
3 - Lower 1/4 stalk portion affected
5 - Lower 1/2 stalk portion affected
7 - Lower 3/4 stalk portion affected
9 - Whole stalk affected

The disease severity index (DSI) was calculated using the formula given below.

$$DSI = \frac{\text{Total sum of numerical ratings}}{\text{No. of observation x maximum disease rating scale}} \times 100$$

DI was taken in the first two seasons to identify the technique, and DSI was calculated in the next season to analyze the technique and confirm it using the severity of the infection. Data were analyzed using SAS Statistical Analysis software.

RESULTS AND DISCUSSION

Normality test was run for the DI and DSI calculated using disease counts of the trial and all the data sets were not followed the normal distribution (Shapiro-Wilk test statistics $p< 0.05$). Therefore, non-parametric approach was practiced in the SAS analysis using the CATMOD procedure.

Leaf disks discolored and withered without giving clear symptoms in T_5 treatment in both seasons. According to Table 1, T_4 treatment showed better results and showed significant differences ($p < 0.05$) among the other techniques. It showed disease symptoms within 4 days and 100 % DI within 11 days in all the varieties. Other techniques took 11 days to show sheath blight disease incidences and 19 days after inoculation to show 100 % DI. Except few BLSB lesions in T_4 treatment, others did not show symptoms due to

dry weather in *Yala* 2013. There were significant differences ($p < 0.05$) in varieties at the initial counts, and high DI was observed in the inbred variety Ruwan. Disease incidences of BLSB in T_4 treatment among varieties during *Maha* 2012/13 are shown in Figure 1. This explains that the inbred variety used is more susceptible to the sheath blight disease than the hybrid varieties used.

Table 1. DI of maize BLSB after inoculation during *Maha* 2012/13

Treatment	DI %				
	4 DAI	8 DAI	11 DAI	14 DAI	19 DAI
Technique					
Spraying mycelium suspension into soil (T_1)	0.0 ^{b}	0.0 ^{b}	76.1 ^{b}	91.9 ^{ab}	100.0 ^{a}
Incorporation of sclerotia into soil (T_2)	0.0 ^{b}	0.0 ^{b}	73.7 ^{b}	86.4 ^{b}	100.0 ^{a}
Injecting mycelium suspension to sheath (T_3)	0.0 ^{b}	0.0 ^{b}	74.8 ^{b}	96.0 ^{a}	100.0 ^{a}
Inserting sclerotia to sheath (T_4)	28.2 ^{a}	48.9 ^{a}	100.0 ^{a}	100.0 ^{a}	100.0 ^{a}
Floating leaf disk method (T_5)	No clear symptoms				
P (Variety)	0.001	0.319	0.023	0.069	
P (Technique)	0.001	0.0001	0.006	0.012	
P (Variety x Technique)	0.001	0.341	0.695	0.325	

DAI- Days after inoculation

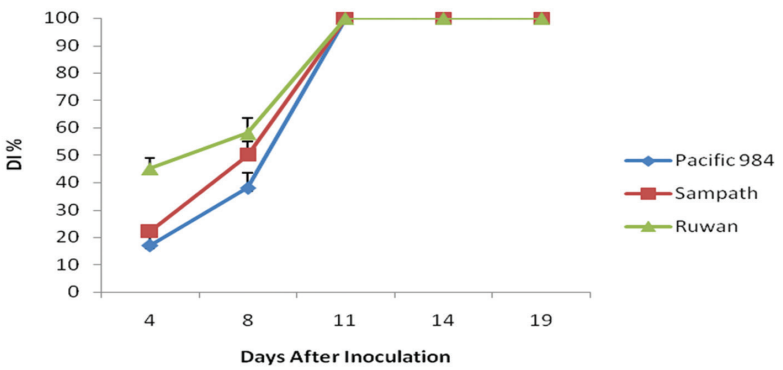

Figure 1. DI of maize BLSB in inserting sclerotia to sheath technique among varieties during *Maha* 2012/13

Less BLSB symptoms were observed in the control treatment during *Maha* 2013/14 even though the plants were not inoculated (Table 2). This may be due to splashing off the mycelium portions while watering or physical spreading while the experiment works and data collection. Hence, safe handling of research works had been practiced

during *Maha* 2014/15. T_1 treatment showed characteristic and uniform symptoms in most of the plants, while the other techniques did not show uniform and rapid symptoms. This can be due to failures in the development and spreading of mycelium. Large seed size of the sorghum grain caused problems while inserting them into the sheath. Result shows that the DSI% of varieties in different seasons varied. Plant house conditions and the microenvironment surrounding the plants were favorable for disease development in *Maha* 2014/15. Hence, proper environment management is important for rapid disease development.

Table 2. DSI of maize BLSB after inoculation during *Maha* 2013/14 and 2014/15

Treatment	*Maha* 2013/14	*Maha* 2014/15
Technique		
Inserting sclerotia into sheath (T_1)	25.35 [a]	55.7 [a]
Inserting culture grown on paddy straw (T_2)	10.80 [b]	18.4 [b]
Inserting culture grown on sorghum grain (T_3)	11.42 [b]	15.1 [b]
Floating leaf disk method (T_4)	No symptoms	
Control (T_5)	1.85 [c]	0.0 [c]
P (Variety)	0.093	0.594
P (Technique)	0.001	0.0001
P (Variety x Technique)	0.355	0.449

According to Table 3, all the varieties show the same response for the inserting sclerotia to the sheath technique, giving higher DSI values. When selecting a technique for varietal screening, it should perform well in all the varieties among the techniques tested. Results revealed that the technique of inserting sclerotia into the sheath shows disease severity prominently without depending on the varieties. Considering the three varieties used, the inbred variety Ruwan showed slightly higher DSI value than other local and exotic hybrid varieties. Similar results were observed in *Maha* 2012/13 saying the susceptibility of Ruwan variety for the BLSB was higher than that of Sampath and Pacific 984. But all the tested varieties were susceptible for the BLSB. Results of the other studies also stated that highly resistant germplasm were extremely poor (Yang *et al.*, 2003 and Zhao *et al*., 2006) to the BLSB disease of Maize. Since the BLSB is becoming a problem in maize cultivation, hybrid breeding programmes should prefer to use the moderately susceptible/resistant lines as parents, and the identified technique is giving

more disease severities in a favorable environment and accurate germplasm screening.

Table 3. DSI variation of maize BLSB in different varieties during *Maha* 2013/14 and 2014/15 based on inoculation technique

Technique	DSI %					
	***Maha* 2013/ 14**			***Maha* 2014/ 15**		
	Sampath	**Ruwan**	**Pacific 984**	**Sampath**	**Ruwan**	**Pacific 984**
T_1	25.1 [a]	30.6 [a]	20.4 [a]	50.5 [a]	62.0 [a]	54.6 [a]
T_2	11.6 [b]	9.3 [b]	11.6 [a]	17.6 [b]	17.6 [b]	19.9 [b]
T_3	10.6 [b]	14.4 [b]	9.3 [a]	16.2 [b]	16.7 [b]	12.5 [b]
T_4	No symptoms in floating leaf disk method					
T_5 (Control)	1.8 [c]	2.8 [c]	0.9 [b]	No res.	No res.	No res.
P	0.001	0.001	0.001	0.001	0.001	0.001

No res. – No response

CONCLUSIONS

Among the inoculation techniques used, inserting sclerotia into the maize sheath was significantly superior to other techniques tested. Therefore, the above technique can be adopted as a rapid screening technique for evaluating maize germplasm/varieties against banded leaf and sheath blight disease in Sri Lanka.

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