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# Comparing the Efficiency of Field and Glass House Screening Techniques for Promotion of Sorghum Downy Mildew Infection in Maize

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**ABSTRACT: Sorghum Downy Mildew (SDM) is one of the most devastating diseases affecting maize which causes yield losses even up to 100 per cent under favourable disease epiphytotic conditions. A sound screening method is essential to identify the resistant sources which constitutes the first step in any plant breeding programme. In the present investigation, field and glass house screening techniques were compared for promotion of SDM infection in maize. Level of infection recorded in glass house trial was slightly higher than recorded in sick plot trials. Hence, screening of maize genotypes under glass house against SDM infection was found to be the most efficient technique in inducing severe SDM infection in maize. The highly susceptible sorghum genotype, DMS 652 showed resistance in response to SDM both in sick plot and glass house trials which indicated the prevalence of maize race in maize sick plot which is one of the races of *P. sorghi.* Of nine maize inbred lines, UMI 936(w) was highly resistant to infection by SDM pathogen followed by UMI 102 and UMI 285. These inbreds can be used in future for crop improvement programme to evolve a SDM resistant composites and hybrids.**

**Keywords:** Maize, Sorghum Downy Mildew, Screening methods, resistance.

## INTRODUCTION

Maize is one of the four prime crops of the world. It is mainly utilized as food in the developing countries and as livestock feed in the developed countries. Because of its' utilization for variety of value-added products maize becomes an important crop in recent years. However, increasing incidence of pest and diseases is one of the main factors limiting productivity in maize. Of these, Sorghum Downy Mildew (SDM) caused by *Peronosclerospora sorghi* is one of the most devastating diseases and has the ability for epiphytotics on susceptible genotypes under favorable environmental conditions. Although *P. sorghi* usually infects both maize and sorghum (named as the sorghum strain), there are some strains that infect only maize (named as the maize strain) (Bock *et al*., 2000). The effective and cheapest method of controlling SDM disease is the development of resistant varieties / hybrids in maize. In any breeding programme for disease resistance, the initial step is to screen all the available germplasm against disease pathogen. Anahosur and Hegde (1979) compared the five different techniques for screening sorghum genotypes against SDM in the field and revealed that 'Infector row' planting was the most reliable technique for assured screening. Schmitt and Freytag (1974) also reported that conidial spray inoculation at seedling stage was most efficient in inducing severe downy mildew infection in corn and sorghum. Narayana and his coworkers (1995) compared six inoculation techniques for artificial promotion of SDM in green house for screening sorghum genotypes. Among the six inoculation method evaluated in the green house they obtained maximum downy mildew incidence of 100 percent when seedlings at the first leaf stage were spray-inoculated. Cardwell *et al*. (1997 & 2006) have developed 'Direct seed inoculation' method for screening maize genotypes against SDM and they identified direct inoculation of pre-germinated seeds of spreader rows as a consistent method in promoting SDM infection in susceptible maize lines. A sound screening technique forms the platform for identification of resistant sources for disease resistance breeding programme. Therefore, an attempt was made to compare field and glass house screening techniques for promotion of sorghum downy mildew infection in maize and also to identify the strain of *P. sorghi* prevailing in sickplot of Department of Millets, TNAU, Coimbatore based on pathogenicity to sorghum and maize.

## MATERIALS AND METHODS

The maize genotypes selected for the present research programme consisted of nine maize inbred lines maintained by sib mating at Maize unit, Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. SDM disease susceptible checks *viz*, CM 500 (maize genotype) and DMS 652 (sorghum genotype) are also included for pathogenicity test. The source and details of the maize inbred lines are given in Table 1.

**Table 1: Details of Maize inbred lines used in this study.**

| Sr. No. | Genotypes | Particulars | Grain color |
|---|---|---|---|
| | | **Maize genotypes** | |
| 1. | UMI 79 | Selection from Pioneer 102 | Orange |
| 2. | UMI 176 | Selection from V46 | Yellow |
| 3. | UMI 432 | Derivative of UMI 25 x UMI 103 | Yellow |
| 4. | UMI 467 | Selection from K1 | Yellow |
| 5. | UMI 13 | Selection from CM 111 | Yellow |
| 6. | UMI 57 | Selection from DMR pool – Taiwan-3 | Yellow |
| 7. | UMI 102 | Selection from EH 431873 | Yellow |
| 8. | UMI 936 (w) | Selection from DMR pool – Taiwan 524 | White |
| 9. | UMI 285 | Selection from Suwan 1 – Indonesia composite | Yellow |
| | | **Checks** | |
| 1 | CM 500 | Maize genotype highly susceptible to SDM | Yellow |
| 2 | DMS 652 | Sorghum genotype highly susceptible to SDM | Yellow |

**Screening of maize genotypes against SDM.** Nine maize inbred lines were screened against SDM infection during September, 2005 and September, 2006 in the sick plot by spreader row technique and also in the glass house by seedling spray inoculation technique during February, 2007. The procedures adopted for the above two screening methods are as follows.

**Spreader row technique followed in the sick plot for screening maize genotypes against SDM.** Disease screening against SDM was carried out during September to November, 2005 and 2006 by taking advantage of monsoon season, which was conducive for pathogen development. Artificial epiphytotic conditions were created by planting spreader rows of a susceptible maize genotype, CM500 (Shetty and Ahmand, 1980; Krishnappa *et al*., 1995; Setty *et al*., 2001; George *et al*., 2003; Nair *et al*., 2004; Yen *et al*., 2004; Nair *et al*., 2005; Nagabhushan *et al*., 2014) 30 days prior to sowing of test entries. Spreader row technique (Craig *et al*., 1977) was followed for screening the maize inbred lines against SDM in the field.

Sick plot has been maintained in New Area, Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Every year mono-cropping of downy mildew susceptible maize entries would be followed in the sick plot and at the end of growing season, infected leaf debris containing oospores of *P. sorghi* would be incorporated in the soil by ploughing. This regular operation would increase the oospore content of the soil. In sick plot, ridges were formed in 3m length with 60cm space between ridges. The seeds of highly susceptible maize inbred line (CM500) were sown in sick plot in every 6$^{th}$ row leaving 5 rows in between in order to accommodate test entries 30 days later and also on all the four sides of sick plot in order to increase the disease inoculam. This time gap (thirty days) between sowing of spreader row entry (CM500) and test entries allowed disease development in spreader row entry and hence sufficient disease inoculum will be available for test entries for effective screening.

Since *P. sorghi* is an obligate parasite, conidia of *P. sorghi* were harvested from fresh and infected plants for inoculations. The method of conidial inoculum preparation used in the present study was followed from Cardwell *et al*., (1994) and by utilizing the natural spore producing cycle of the fungus, which involved conidial spraying operation in the middle of the night (Siradhana *et al*., 1976; Renfro *et al*. (1979)). Conidia were collected from three week old systemically infected maize leaves. Maize leaves infected with *P. sorghi* showing white visible conidial growth were obtained from the infected field in the previous day evening. The abaxial surface of infected leaves were wiped with wet absorbent cotton to remove aged and matured downy mildew conidia produced previously and they were wiped again using tissue paper to remove moisture from the leaf surface. These SDM infected leaves were spread in a single layer over a tray lined with moist blotting paper in such a way that lower leaf surface faced upwards. Another tray lined with moist blotting paper was used to close the tray containing infected leaf materials in order to enhance relative humidity. These trays were incubated at 20°C in the dark for six to seven hours for sporulation, until 3.00 AM. At this time, conidia were harvested by washing the sporulated leaves in chilled distilled water (5°C) using a camel hairbrush. The conidial suspension was filtered through a double layered of muslin cloth to remove conidiophores and other leaf particles. The

conidial concentration was adjusted to $6x10^5$ per ml using a hemacytometer. The resulting spore suspension was placed into backpack sprayers and taken to the field. The spraying of conidial suspension was taken in the field from 3.30 to 4.30 AM onto ten days old spreader row (CM500) plants. This screening method utilizes the natural spore producing cycle of disease causing pathogen.

The nine maize inbred lines and DMS 652 were planted after confirming hundred per cent disease establishment in the spreader rows in three replications. Hence, the test entries were exposed to infection by both oospores from the soil and conidia from spreader rows.

**Seedling spray inoculation technique in the glass house for screening the maize genotypes against SDM.** Jones (1970), Schmitt and Freytag (1974) and Craig (1976) developed conidial spray inoculation of seedlings to evaluate responses of maize genotypes to SDM in the glass house. Procedure adopted for seedling spray inoculation method is as follows.

Transparent, UV (Ultra-violet) stabilized Silpaulin plastic sheets were used to cover in side the glass house in order to allow sun light to pass and also to maintain relative humidity created inside the glass house mainly to provide favorable environmental conditions (warm and humid) for downy mildew pathogen development. Screening of maize genotypes against SDM disease was carried out in the glass house available at Department of Cotton, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during February, 2007. Seeds were sown in trays containing soil from sick plot and allowed to germinate. At the plumule emergence stage when the first leaf was in the whorl (6 days after sowing) test entries were sprayed with conidial suspension adjusted to approximately $6 \times 10^5$ per ml of water and applied at a rate equivalent to approximately 1ml per plant using atomizer. In this similar way, conidial suspension spray was given for three consecutive days. Conidial suspension of SDM pathogen was prepared daily as described by Cardwell *et al*. (1994).

**Disease assessment in maize genotypes against SDM.** The downy mildew disease reaction was scored at 30 days after plant emergence of test entries in spreader row technique (under sick plot) and 21 days after seedling emergence of test entries in seedling spray inoculation technique (under glass house). The number of systemically infected plants and total number of plants in each test row were recorded. Per cent downy mildew incidence was calculated as per standard procedure (Lal and Singh, 1984) both in field and glass house trials. Per cent downy mildew incidence =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

The rating scale was followed as below.

| Per cent downy mildew incidence (%) | Reaction |
|---|---|
| 0 – 10 | Resistance (R) |
| >10 – 30 | Moderately resistance (MR) |
| >30 – 50 | Moderately susceptible (MS) |
| >50 | Susceptible (S) |

## RESULTS AND DISCUSSION

Sorghum downy mildew infection caused by *P. sorghi* is one of the significant devastating diseases of maize. Of various mean of disease management, host plant resistance is the most practical, eco-friendly and efficient method. For the development of host plant resistance in any crop, screening of available germplasm forms the platform for identification of resistance sources. Therefore, in the present study, the field (Sick plot method) and glass house (Seedling spray inoculation method) screening methods were compared to identify the reliable method in inducing SDM incidence in maize.

The susceptible check, CM 500 showed 100 per cent incidence (Table 2) during September, 2006. Among the test entries, sorghum downy mildew incidence ranged from zero (UMI 936(w)) to 88.13 per cent (UMI 79) over two years under sick plot conditions. On the basis of results obtained in two consecutive *rabi* seasons under sick plot method, the maximum downy mildew incidence was recorded by UMI 79 (88.13 per cent) which followed by UMI 432 (84.53 per cent) and UMI 467 (49.73 per cent). UMI 936(w) showed strong immune (0 per cent) response in both seasons under sick plot condition. The genotypes UMI 936(w) (0 per cent), UMI 102 (6.16 per cent) and UMI 285 (9.87 per cent) were resistant to infection by SDM pathogen, whereas, UMI 13 (12.99 per cent), UMI 176 (22.93 per cent) and UMI 57 (18.80 per cent) showed moderate resistant reaction to SDM pathogen in both seasons. Percentage downy mildew incidence under glass house ranged from 2.62 per cent (UMI 936(w)) to 100 per cent (UMI 79).

The positive correlation (r = 0.99) between the sorghum downy mildew infection in glass house and field screening methods indicated that the reactions of seedlings to sorghum downy mildew were similar under both conditions. This indicated the reliability of glass house trial for screening the maize genotypes against sorghum downy mildew. More over Seedling spray inoculation of nine maize inbred lines under glass house screening method resulted in increased level of infection (Table 2), whereas the same genotypes under field screening using the infector row method had shown slightly lower level of infection.

This is mainly because the production of disease inoculum is often determined by temperature and humidity, which may not always be favorable for sporulation and infection, leading to disease escape under field condition. Hence, screening under glass house against sorghum downy mildew was the most efficient method in inducing severe sorghum downy mildew infection in maize. This was in accordance with the findings of Craig (1976 and 1980); Schmitt *et al*. (1979); Narayana *et al*. (1995).

A sorghum genotype namely DMS 652 reported to be highly susceptible to sorghum downy mildew by many workers (Anahosur and Hegde 1979; Siradhana *et al*., 1980; Narayana *et al*., 1995) showed 0 per cent infection (resistance response) to SDM under both sick plot and glass house conditions. From these observations, it can be concluded that the race prevalent in maize sick plot was maize race, which is one of the races of *P. sorghi* which affects only maize but sorghum genotype. This was well supported by Kothari *et al*. (1980) from their observation that nowhere the disease has been recorded on any of the sorghum genotypes grown near maize fields having the sorghum downy mildew disease and they indicated the predominance of maize race which is one of the races of *P. sorghi* attacking maize only. Variability existing for pathogenicity in *P. sorghi* enhanced the potential for damage from sorghum downy mildew disease. So it is necessary to diversify the resistant sources against sorghum downy mildew in order to reduce the vulnerability of maize genotypes.

The screening carried out both under sick plot over seasons and glass house showed that out of nine maize inbreds, UMI 936(w) was highly resistant to infection by pathogen followed by UMI 102 and UMI 285. Maize inbred lines, UMI 79, UMI 432 and UMI 467 were highly susceptible to infection by SDM pathogen while UMI 176, UMI 13 and UMI 57 showed moderate reaction to the disease. Even though the maize inbred line, UMI 936 (w) recorded highly resistant reaction to SDM, it could not be directly used in the development of resistant maize hybrids as it segregates for seed colour in the $F_1$ harvested seeds. However, SDM disease resistant gene present in UMI 936(w) can be transferred to any yellow seeded maize genotype (recipient & recurrent parent) through repeated backcross breeding programme. These findings were supported by Hooda *et al*. (2012).

**Table 2: Downy mildew incidence recorded for nine maize genotypes both under sick plot and glass house screening techniques.**

| Sr. No. | Entries | Per cent downy mildew incidence (%) recorded over three replications | | | | | Reaction to downy mildew |
|---|---|---|---|---|---|---|---|
| | | Sick plot | | | Glass house (February, 2007) | Mean | |
| | | September, 2005 | September, 2006 | Mean | | | |
| | **Test Entries** | | | | | | |
| 1 | UMI 79 | 81.66 | 94.59 | 88.13 | 100.00 | 96.04 | S |
| 2 | UMI 176 | 15.86 | 30.00 | 22.93 | 27.23 | 25.11 | MR |
| 3 | UMI 432 | 83.05 | 86.00 | 84.53 | 88.02 | 88.85 | S |
| 4 | UMI 467 | 57.15 | 42.31 | 49.73 | 69.54 | 62.46 | S |
| 5 | UMI 13 | 15.97 | 10.00 | 12.99 | 25.55 | 20.81 | MR |
| 6 | UMI 57 | 19.23 | 18.37 | 18.80 | 27.46 | 24.75 | MR |
| 7 | UMI 102 | 2.56 | 9.76 | 6.16 | 7.77 | 7.46 | R |
| 8 | UMI 936(w) | 0.00 | 0.00 | 0.00 | 2.62 | 1.31 | R |
| 9 | UMI 285 | 3.73 | 16.00 | 9.87 | 10.04 | 9.98 | R |
| | **Checks** | | | | | | |
| 1 | CM 500 (Maize) | 92.00 | 100.00 | 96.00 | 100.00 | 98.67 | S |
| 2 | DMS 652 (Sorghum) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | R |
| Coefficient of correlation between sorghum downy mildew infection percentages in sick plot over seasons and the same recorded under glass house trial (r) is 0.99 | | | | | | | |

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