

Original Research Article

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Studies on Screening of BC₃F₁ Population Against Sorghum Down Mildew in Maize (*Peronosclerospora sorghi*)

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ABSTRACT

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An experiment was carried out during *Rabi*, 2013 at Eastern Block of the Central Farm Unit, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India to identify resistant progenies in BC₃F₁ population against sorghum downy mildew (SDM) incited by *peronosclerospora sorghi*. Sorghum downy mildew is one of the most serious diseases in maize producing areas throughout the world. *P. sorghi* (SDM) is a factor that limits maize production in several countries of Asia (Rifin 1983). Therefore, there is a need to develop the new maize cultivars with resistance to SDM in order to enhance the yield. In this present study, experiments were undertaken under vigorous artificial infection conditions in spreader row technique during *Rabi*, 2013 for characterization of responses of 22 back cross progenies to the SDM; in which 16 progenies were confirmed as phenotypically resistant to sorghum downy mildew viz., UMI 79/936-C1-3-2, UMI 79/936-C1-3-4, UMI 79/936-C1-7-2, UMI 79/936-C1-29-8, UMI 79/936-C1-29-9, UMI 79/936-C1-29-13, UMI 79/936-C1-29-23, UMI 79/936-C1-29-35, UMI 79/936-C1-29-36, UMI 79/936-C1-67-3, UMI 79/936-C1-67-12, UMI 79/936-C1-67-25, UMI 79/936-C1-101-12, UMI 79/936-C1-101-13 and UMI 79/936-C1-101-14. Resistant lines will be serve as basis material for developing single cross and double cross hybrids for resistance against sorghum downy mildew in maize.

Introduction

Maize (*Zea mays* L.) plays a unique role in world agriculture as a food, feed and industrial crop. It is the world's third most important crop after rice and wheat (Hoisington and Melchinger, 2005). All parts of the crop can be used as food and non-food products. It is utilized as food for human consumption, as feed for livestock and as a raw material for

industry (FAO, 1992). The demand for maize is increasing as it is becoming more favoured as a major food and feed source due to its higher productivity, lower labour demands, easy processing, ease of digestibility and cheaper cost than other cereals. By 2025, maize will be the crop of greatest production globally and by 2050 the demand for maize in developing world will double (Rosegrant *et al.*, 2008).

The major maize production constraints include both abiotic and biotic factors such as drought, weeds, pests and diseases. Biotic stresses are one of the most limiting factors for stable crop production worldwide. Maize is susceptible to many biotic components, including viruses, fungi, and bacteria. Downy mildews are important maize diseases in many tropical regions of the world. Krishnappa *et al.*, (1995) in Karnataka conducted a survey revealed that the incidence of the disease ranged from 10 to 90% and the yield losses are as heavy as 30 – 40%.

The disease is known by two names, downy mildew and crazy top based on two types of symptoms in maize that develop as a result of systemic infection. Symptoms of downy mildew on maize caused by various pathogen species can vary depending on plant age, prevailing climatic conditions, and host germplasm. Infection of maize plants at the seedlings stage (less than 4 weeks old) results in stunted and chlorotic plants and premature plant death. Leaves on older plants display characteristic symptoms of downy mildews which include mottling, chlorotic streaking and lesions, and white stripe leaves that eventually shred. Downy growth is often observed on both leaf surfaces, but is more common on the lower leaf surfaces. Infected plants have leaves that narrower and more erect compared to healthy leaves. Infected plants are often stunted, fillers excessively and have malformed reproductive organs (tassel and ears). Infected plants may not seed, while tassels may busy growth.

Therefore, there is a need to develop the new maize cultivars with resistance to SDM in order to enhance the yield. Though the disease can be controlled by cultural practices such as the eradication of infected plants, deep ploughing, adjusting the time of planting and use of systemic fungicides like metalaxyl for seed treatment (Odovody and Frederiksen,

1984a) or foliar application (Odovody and Frederiksen, 1984b), their effectiveness on disease incidence is variable in most cases. The major concerns are cost and the buildup of chemical resistance in the pathogen. Considering all, the use of resistant varieties is a more cost-effective and an environmentally safe alternative for managing the problem of SDM in maize (Rathore and Jain, 2000).

Materials and Methods

Maintenance and screening of Maize genotypes

The experiments were conducted in Eastern Block of the Central Farm Unit, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during *Rabi* 2013. BC₃F₁ population was used in the present study. It is derived from crossing the inbred UMI 79 which is susceptible for sorghum downy mildew and UMI 936(w) which has resistance for sorghum downy mildew and backcrossing progenies with UMI79. Twenty two BC₃F₁ progenies were used for screening under sick plot conditions by spreader row technique. The disease assessment was done at 30 days after plant emergence.

Spreader row technique in the sick plot for screening BC₃F₁ maize genotypes against SDM

Screening against SDM was carried out during December to January by taking advantage of monsoon season, which is conducive for pathogen development. Artificial epiphytotic conditions were created by planting spreader rows of a susceptible maize genotype, CM 500 (Shetty and Ahmad, 1980, Krishnappa *et al.*, 1995, Setty *et al.*, 2001, George *et al.*, 2003, Nair *et al.*, 2004, Yen *et al.*, 2001 and Nair *et al.*, 2005) 30 days prior to sowing of test entries. Spreader row technique adopted by

Craig *et al.*, (1977) was followed for screening the maize genotypes against SDM in the field.

Planting of CM 500 seeds as spreader row in the sick plot

Sick plot maintained in Department of Millets, Centre for Plant Breeding and Genetics, at Tamil Nadu Agricultural University, Coimbatore was used for screening the BC₂F₁, BC₃ F₁ and BC₃F₂ progenies. Mono-cropping of downy mildew susceptible entries has been followed and at the end of growing season infected leaf debris containing oospores of *P. sorghi* have been incorporated in the soil by ploughing mainly to increase oospore content of the soil.

In sick plot, ridges were formed in 3m length with 60 cm ridges. The seeds of SDM susceptible inbred CM500 were sown in sick plot in every 11th row leaving 10 rows in between to accommodate test entries 30 days later and also on all four sides of sick plot. This time gap (30 days) between sowing of spreader row and test entries allowed disease development in spreader rows.

Conidial inoculums preparation and spraying on spreader row entries

Being obligate parasite, conidia of *P. sorghi* were harvested from fresh, infected plants for inoculations. The method of conidial inoculums preparation used in this study was adopted from Cardwell *et al.*, 1994 and by utilizing the natural spore producing cycle of the fungus, which involved spray operation in the middle of the night (Siradhana *et al.*, 1975 and Renfro *et al.*, 1979).

Conidia were obtained from three week old systematically infected maize plants. Maize leaves infected with *P. sorghi* showing visible conidial growth were collected from the infected field in the early evening. Infected

leaves were wiped with wet absorbent cotton to remove old 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 abaxial leaf surface faced upwards. Another tray lined with moist blotting paper was used to close the tray containing infected leaf materials. 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 muslin cloth to remove conidiophores and other leaf particles. The concentration was adjusted to 6×10^5 per ml using a hemocytometer.

The resulting spore suspension was placed into backpack sprayers and taken to the field. The spraying was taken from 3.30 to 4.30 AM on ten days old spreader row (CM 500) plants. This method utilizes the natural spore producing cycle of pathogen. The test entries were planted after ensuring hundred per cent disease establishment in the spreader rows. In that way test entries were exposed to infection by both oospores from the soil and conidia from spreader rows.

Disease assessment in maize genotypes against SDM

The disease reaction was assessed at thirty days after plant emergence of test entries in spreader row technique (under sick plot). The number of infected plants and total number of plants in each row were recorded. The percentage of incidence of downy mildew was calculated as per standard procedure (Lal and Singh, 1984).

The rating scale was followed as below:

Percentage Downy Mildew incidence (per cent)	
Reaction	
0-10	Resistance(R)
>10-30	Moderately Resistance(MR)
>30-50	Moderately Susceptible(MS)
>50	Susceptible(S)

Results and Discussion

The present investigation was carried out to select the back cross progenies resistant against sorghum downy mildew through field screening under sick pot. Phenotypic screening was done to confirm the introgression of SDM locus in recombinant lines. (Resistant -0-10%, moderately resistant - >10-30%, moderately susceptible >30-50%, Susceptible - >50%) (Yen *et al.*, 2001 and Nair *et al.*, 2004 and 2005). Twenty two BC₃F₁ backcross progenies evaluated under sick plot conditions by spreader row technique (Table.1).

Progenies free from disease were selected as resistant progeny. Moderately resistant, moderately susceptible and susceptible progenies were excluded from breeding operation. Out of twenty two progenies sixteen progenies were confirmed as phenotypically resistant to sorghum downy

mildew viz., UMI 79/936-C1-3-2, UMI 79/936-C1-3-4, UMI 79/936-C1-7-2, UMI 79/936-C1-29-8, UMI 79/936-C1-29-9, UMI 79/936-C1-29-13, UMI 79/936-C1-29-23, UMI 79/936-C1-29-35, UMI 79/936-C1-29-36, UMI 79/936-C1-67-3, UMI 79/936-C1-67-12, UMI 79/936-C1-67-25, UMI 79/936-C1-101-12, UMI 79/936-C1-101-13 and UMI 79/936-C1-101-14 have been confirmed as phenotypically resistant to sorghum downy mildew. The disease pressure developed was found to be high as the susceptible check CM 500 showed 100% infection. Similarly CM500 showed 94% and 100% disease infection in kharif 2008 and 2009 respectively during work of Kashmiri (2010).

Similar research was carried out by yen *et al* 2001 on differential expression on differential responses of the same inbred lines to specific on mildew pathogens in different countries. A collaborative study recently undertaken under the AMBIONET (Asian Maize Biotechnology Network) program clearly demonstrated the utility of specific maize inbred lines, namely Nei 9008 (developed in Thailand) and P345 C3S3B-46-1-1-1-1-2-B (developed by CIMMT –ARMP) in term of resistance to *P.Sorghum* in Mandya (India), *P.heteropogoni* in Uaipur (India), *P.zea* in Thailand, *P.Mais* in Indonesia and *P.Philipinensis* at Phiippines (yen *et al* 2001).

Plate.1 Resistant progenies of BC₃F₁ Population against Sorghum Downy Mildew in Maize



Table.1 Phenotyping of BC₃F₁ progenies against Sorghum Downy Mildew

S. no	Progeny no	Percentage of Disease Incidence	Phenotype (Disease Score)
1	UMI 79/936-C1-3-2	0	R
2	UMI 79/936-C1-3-4	0	R
3	UMI 79/936-C1-7-2	0	R
4	UMI 79/936-C1-7-3	50.00	S
5	UMI 79/936-C1-7-5	36.11	MS
6	UMI 79/936-C1-7-7	0.00	R
7	UMI 79/936-C1-7-8	45.16	MS
8	UMI 79/936-C1-29-4	41.67	MS
9	UMI 79/936-C1-29-8	0	R
10	UMI 79/936-C1-29-9	0	R
11	UMI 79/936-C1-29-13	0	R
12	UMI 79/936-C1-29-15	61.90	S
13	UMI 79/936-C1-29-23	0	R
14	UMI 79/936-C1-29-35	0	R
15	UMI 79/936-C1-29-36	0	R
16	UMI 79/936-C1-67-3	0	R
17	UMI 79/936-C1-67-12	0	R
18	UMI 79/936-C1-67-15	41.67	MS
19	UMI 79/936-C1-67-25	0	R
20	UMI 79/936-C1-101-12	0	R
21	UMI 79/936-C1-101-13	0	R
22	UMI 79/936-C1-101-14	0	R

Fig.1 Electron microscopic observation of mycelium and sporangia of *Perenoscleropora sorghi* from BC₃F₁ generation



A. Branched sporangiophore

B. Sporangia

C. 100 X Magnification

Leaves infected by SDM were collected from the susceptible progenies. The spores attached on the surface of the collected leaves were removed using ethanol to ensure the new spore formation. Then, the leaves were cut in to square shapes and the leaf squares were transferred onto moist cotton kept on a slide. The slide was incubated overnight in a dark and cool place to allow the formation of new spores. The following day morning, the white mycelium with spores found grown over the leaf, were gently removed from the leaf using the cellophane tape and cellophane tape was pasted on a new slide. Then, the slide was observed under the electron microscope to confirm

whether the pathogen is *Peronoscleropora sorghi*.

To conclude, sixteen progenies viz., UMI 79/936-C1-3-2, UMI 79/936-C1-3-4, UMI 79/936-C1-7-2, UMI 79/936-C1-29-8, UMI 79/936-C1-29-9, UMI 79/936-C1-29-13, UMI 79/936-C1-29-23, UMI 79/936-C1-29-35, UMI 79/936-C1-29-36, UMI 79/936-C1-67-3, UMI 79/936-C1-67-12, UMI 79/936-C1-67-25, UMI 79/936-C1-101-12, UMI 79/936-C1-101-13 and UMI 79/936-C1-101-14 have been confirmed as resistant to sorghum downy mildew, these progenies can be backcrossed with recurrent parent UMI 79 to recover the recurrent parent background genome along with resistance. These progenies may be served as the basis materials to develop the Near Isogenic lines (NILs) resistant to sorghum downy mildew which could further be used for developing sorghum downy mildew resistant single cross maize hybrids

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