Identification and Validation of Resistance to Fusarium Wilt and Sterility Mosaic Disease in Pigeonpea

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Abstract

Fusarium wilt (FW) and Sterility mosaic disease (SMD) are the two important diseases of pigeonpea (Cajanus cajan) worldwide, and best managed through host plant resistance. The aim of the work was to identify and validate new sources to wilt and SMD in pigeonpea. Preliminary evaluation of 3000 germplasm and breeding lines was carried out at International Crop Research Institute for Semi Arid Tropics (ICRISAT) for FW and SMD resistance in sick plot during 2005/06 crop season to 2007/08. Sixty lines with < 10% FW and SMD incidence were selected from 3000 germplasm and breeding lines and these lines were evaluated repeatedly for three consecutive years during 2008/09, 2009/10 and 2010/11 crop seasons for their stability against both the diseases. Fifty-four lines were found resistant to FW and high level of resistance to SMD was found in all the 60 lines. Combined resistance to FW and SMD was found in 54 lines, of which, one line (ICPL 20108) was found asymptomatic. These resistant lines can be exploited for crossing with commercial cultivars to develop pigeonpea varieties with adequate levels of multiple resistances to enhance pigeonpea production in the Indian subcontinent.

Keywords: *Cajanus cajan*, Fusarium wilt, sterility mosaic disease, host plant resistance

Introduction

Pigeonpea [*Cajanas cajan* (L.) Millisp.] is an important food legume crop being cultivated in Indian subcontinents, eastern Africa and Central America. Diseases are the major constraints to the high yield potential of pigeonpea cultivars, among which Fusarium wilt and sterility mosaic diseases are the major constraints to pigeonpea production worldwide.

Fusarium wilt (FW), caused by *Fusarium udum* Butler, is a soil borne disease, and is reported from all the pigeonpea growing regions (Gwata *et al*., 2006). The disease symptoms usually appear when plants are at the pre- flowering and podding stage, but sometimes symptoms also appear in 1-2 month-old plants. In India alone, the annual yield loss due to this disease has been estimated at US $ 71 million (Kannaiyan *et al*., 1984; Reddy *et al*., 1993). However, increased incidence of the disease has been reported in recent years (personal observation).

Sterility mosaic disease (SMD) is caused by *Pigeonpea sterility mosaic virus* and transmitted by eriophyid mite, *Aceria cajani* (Jones *et al*., 2004). The disease is characterized by complete or partial cessation of flower production, mosaic symptoms on leaves, excessive vegetative growth, stunting and reduction in leaf size (Reddy *et al*., 1990). The SMD infection at an early stage (<45 day old plants) results in 95 to 100% loss in yield, while infection at late stages (>45 day old plants) causes 26 to 97% yield loss (Kannaiyan *et al*., 1984). Disease incidence is usually higher in perennial and ratooned crops. In India alone, losses due to SMD were estimated at 205,000 tons of grain valued at US$76 million annually (Kannaiyan *et al*., 1984), and in India and Nepal in 1993, losses were US$280 million (Reddy *et al*., 1993). Recent studies on the impact of FW and SMD are lacking but the diseases are endemic in the subcontinent and continue to be responsible for greater losses (Zote *et al*., 1991; Reddy *et al*., 1998).

Development and use of resistant cultivars is the only effective, economical and environmentally sound strategy for the management of these diseases. Several sources of resistance to FW and SMD have been identified. However, information on combined resistance to both FW and SMD is very limited.Therefore, the study was conducted to identify the combined resistance to FW and SMD in the pigeonpea germplasm and breeding lines that can be utilized in pigeonpea disease resistance breeding program.

Materials and methods

Plant material

More than 3000 accessions of pigeonpea germplasm and breeding lines were evaluated for FW and SMD resistance at ICRISAT, Patancheru from 2005/06 to 2007/08 crop seasons. These lines were evaluated in the respective sick plots for wilt and SMD. Based on the phenotypic data for resistance to FW and SMD, finally a set of 60 pigeonpea promising lines (<10% incidence) was selected. The seed of these 60 lines was multiplied in disease free fields and used for confirmation of resistance to both FW and SMD consecutively for three years in 2008/09 to 2010/11 crop season at ICRISAT.

Resistance screening for fusarium wilt

Pigeonpea germplasm and breeding lines were evaluated in the wilt sick plot under artificial epiphytotic conditions at ICRISAT, Patancheru. A threshold level of the wilt pathogen (5×10^5 conidia m^{-2}) was maintained by incorporating chopped wilted pigeonpea plants in the sick plot every year (Nene *et al*., 1981). Each entry was planted in two rows of 4 m length with seed to seed spacing of 10 cm and row to row spacing of 75 cm. Cultivar ICP 2376 selected as a susceptible check, was planted after every 10 test rows to serve as an indicator/infector rows. The trial was conducted in randomized block design (RBD) with two replications.

Resistance screening for sterility mosaic disease

Resistance screening for SMD was done in the field under artificial epiphytotic condition at ICRISAT, Patancheru. Each entry was planted in two rows of 4 m length with seed to seed spacing of 10 cm and row to row spacing 75 cm. Each pigeonpea plant was inoculated at the two-leaf stage with viruliferous mites (*Aceria cajani*) by stapling SMD-affected pigeonpea leaves containing at least 5 live mites onto leaves of test plants (Nene and Reddy, 1976). An isolated pigeonpea SMD nursery was maintained on susceptible cultivar ICP 8863 at ICRISAT for the mass multiplication of SM inoculum for inoculation. At the time of inoculation, the SMD infected leaflet was collected and folded on the primary leaf in such a way that its lower surface comes in contact with a primary leaf of the test seedling and was then stapled with a small paper stapler for successful SMD infection. The test entries were evaluated in RCBD with two replications. The susceptible (ICP 8863) and resistant cultivar (ICP 2376) were planted after every 10 rows.

Data collection and statistical analysis

Data on FW and SMD infected plants were collected from each replication at seedling, flowering and pod formation stages. The disease incidence was calculated separately by using the following formula:

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

Based on the disease incidence, the test lines were grouped as resistant (0-10% incidence), moderately resistant (10-20% incidence), susceptible (20-40% incidence) and highly susceptible (>40% incidence). The arcsine transformation (Gomez and Gomez, 1984) was applied for per cent FW and SMD data. The arcsine transformed values were used for analysis of variance (ANOVA) using the GENSTAT statistical package (version 14.0 Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK). The ANOVAs were obtained in terms of block effects and the entry effects, considering replications as random and entry as fixed. For combining data across three years, Bartlett's test for homogeneity of error variance was done and found significant. Therefore, data of the three years were pooled and ANOVA was performed using a mixed model (considering the years as random and entries as fixed). The significance of main effects, year, entry and their interactions were tested against residual mean squares.

Results and discussion

Preliminary screening performed during 2005-2008 on more than 3000 pigeonpea germplasm and breeding lines at ICRISAT, Patancheru allowed the selection of 60 promising lines to be further evaluated during 2008-09, 2009-10 and 2010-11 for their consistent performance against both Fusarium wilt and sterility mosaic diseases.

Fusarium wilt resistance

The mean disease incidence for FW in the 60 germplasm and breeding lines varied between 0 - 77.5 %. 97.1% disease incidence in susceptible check ICP 2376 indicated the higher disease pressure in the wilt sick plot (Table 2). ANOVA indicated significant ($P< 0.001$) variation among the 60 germplasm and breeding lines for wilt resistance in all the three years as well as in the pooled data (Table 1). Pooled data for three years showed the non-significant interaction between year × lines for wilt. The mean square variance for line was very high, indicating that the differences in disease incidence were mainly contributed by the test lines. There was no significant effect of years on disease incidence, indicating the uniformity of *Fusarium udum* population in sick plot. Fifty eight lines were found resistant to wilt in which, seven (ICPB 2048, ICPLs 20108, 99013, 99014, 99016, 99090 and 99099) were asymptomatic (0%

Table 1. Analysis of variance for fusarium wilt and sterility mosaic disease incidence in the pigeonpea lines under field conditions

Source of variation	df	Mean squares: Fusarium wilt incidence	Mean squares: SMD incidence pooled
Year (Y)	2	101.6	129.5
Line (L)	61	1087.3*	699.1*
Y × L	122	40.9	34.1
Year 2008			
Replication	1	7.9	162.7
Line (L)	61	360.0*	229.7*
Year 2009			
Replication	1	11.9	1.9
Line (L)	61	461.6*	261.9*
Year 2010			
Replication	1	31.1	13.8
Line (L)	61	347.4*	275.7*

* Significant at P< 0.001

incidence), 47 resistant (<10% incidence) and 4 lines were found moderately resistant (10-20% incidence). Frequency distribution for FW incidence of 62 lines shows the consistency of resistant lines over the screened season during 2008/09 to 2010/11 (Figure 1).

Sterility mosaic disease resistance

Based on the mean SMD incidence of three years (2007/08 to 2010/11)high level of resistance (<10 % incidence) was found in 60 lines.SMD incidence varied from 0.0 to 4.5 %.Ten lines (ICPLs 20108, 20111, 20112, 90011, 99088, 99089,99094, 99100, 99101 and 99102) were found asymptomatic (0% incidence) and 50 lines resistant (<10% incidence) to SMD. The Year × Line interaction was found to be non-significant in the pooled data for all years; hence the data were analyzed separately for each year. Since the effect of the year was not significant, it appears that difference in the disease reaction was due to the genotypic effect in the germplasm and breeding lines.

Combined wilt and sterility mosaic disease resistance

Combined resistance to FW and SMD found in 58 lines, one line (ICPL 20108) was found asymptomatic (0%

Table 2. Fusarium wilt and sterility mosaic disease reaction of pigeonpea breeding lines under field conditions at ICRISAT, India

Genotype	Wilt incidence (%) 2008	2009	2010	Pooled	[a]SMD incidence (%) 2008	2009	2010	Pooled
1	2	3	4	5	6	7	8	9
ICP 7201	3.2	7.2	6.8	5.7	1.5	1.9	0.0	1.1
ICP 7977	7.1	2.1	2.5	3.9	2.1	0.0	0.0	0.7
ICP 12012	7.2	6.5	5.9	6.5	3.8	0.0	0.0	1.3
ICP 12320	13.4	14.5	15.0	14.3	0.0	3.4	0.0	1.1
ICP 13092	3.0	1.0	4.6	2.9	0.7	0.0	0.0	0.2
ICP 14282	4.7	5.6	3.1	4.5	2.5	0.0	0.0	0.8
ICPB 2043	5.1	6.6	4.5	5.4	1.3	3.2	0.0	1.5
ICPB 2048	0.0	0.0	0.0	0.0	3.5	0.0	0.0	1.2
ICPB 2078	11.9	21.9	14.1	15.9	1.6	1.7	0.0	1.1
ICPB 2092	5.4	1.7	0.0	2.4	3.1	0.0	0.0	1.0
ICPB 2162	77.0	92.2	63.3	77.5	4.9	4.1	0.0	3.0
ICPL 20095	4.2	6.9	4.1	5.1	2.5	0.0	0.0	0.8
ICPL 20104	6.1	1.9	4.6	4.2	0.0	1.9	2.9	1.6
ICPL 20108	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ICPL 20111	5.5	0.0	3.4	3.0	0.0	0.0	0.0	0.0
ICPL 20112	3.1	0.0	2.5	1.9	0.0	0.0	0.0	0.0

Continued

Table 2 *continued*

1	2	3	4	5	6	7	8	9
ICPL 20117	4.4	1.7	5.3	3.8	0.0	0.0	1.9	0.6
ICPL 20118	2.0	0.0	3.0	1.7	0.0	1.6	0.9	0.8
ICPL 20121	1.9	2.8	2.2	2.3	0.0	1.5	0.5	0.7
ICPL 20133	8.6	3.7	5.6	5.9	5.0	5.7	2.9	4.5
ICPL 20139	3.3	0.0	2.3	1.9	0.0	0.0	3.6	1.2
ICPL 20181	2.5	0.0	5.5	2.7	2.7	4.7	4.4	3.9
ICPL 90011	15.0	16.8	11.6	14.4	0.0	0.0	0.0	0.0
ICPL 94062	1.2	2.0	0.0	1.1	3.3	0.0	0.0	1.1
ICPL 96053	1.8	0.0	1.3	1.0	0.0	0.0	2.2	0.7
ICPL 96058	3.7	5.8	7.3	5.6	0.0	3.3	0.7	1.3
ICPL 96061	5.7	3.8	0.0	3.2	2.8	2.0	0.0	1.6
ICPL 99004	3.3	4.2	2.8	3.4	0.0	2.1	5.6	2.5
ICPL 99008	16.4	17.3	17.7	17.1	0.0	5.6	0.0	1.9
ICPL 99009	3.2	1.8	1.3	2.1	0.0	3.1	0.0	1.0
ICPL 99010	3.2	0.0	4.9	2.7	3.2	2.0	2.5	2.6
ICPL 99011	1.7	3.6	5.4	3.5	0.0	4.2	3.5	2.6
ICPL 99013	0.0	0.0	0.0	0.0	0.0	4.7	0.0	1.6
ICPL 99014	0.0	0.0	0.0	0.0	0.0	2.7	2.4	1.7
ICPL 99015	7.3	7.2	2.6	5.7	3.0	0.0	0.0	1.0
ICPL 99016	0.0	0.0	0.0	0.0	0.0	5.4	2.7	2.7
ICPL 99046	2.9	2.0	2.6	2.5	0.0	2.0	0.0	0.7
ICPL 99048	1.9	6.5	0.0	2.8	1.7	0.0	0.0	0.6
ICPL 99050	2.9	1.7	5.7	3.4	0.0	0.0	1.7	0.6
ICPL 99054	2.9	5.8	5.4	4.7	0.0	0.0	1.0	0.3
ICPL 99055	2.6	5.9	4.2	4.3	2.6	0.0	0.0	0.9
ICPL 99087	7.5	7.7	5.9	7.0	0.0	2.0	1.5	1.2
ICPL 99088	5.7	4.2	6.2	5.4	0.0	0.0	0.0	0.0
ICPL 99089	9.2	6.0	0.0	5.1	0.0	0.0	0.0	0.0
ICPL 99090	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.5
ICPL 99091	4.0	3.4	0.0	2.5	0.0	3.3	0.0	1.1
ICPL 99092	5.2	6.7	2.3	4.7	4.7	3.8	0.0	2.8
ICPL 99094	3.6	1.7	0.0	1.8	0.0	0.0	0.0	0.0
ICPL 99095	9.6	5.6	2.7	5.9	1.9	0.0	0.0	0.6
ICPL 99096	6.4	4.9	4.3	5.2	4.6	0.0	0.0	1.5
ICPL 99099	0.0	0.0	0.0	0.0	3.9	6.7	0.0	3.6
ICPL 99100	6.0	2.5	4.0	4.2	0.0	0.0	0.0	0.0
ICPL 99101	6.0	3.4	2.4	3.9	0.0	0.0	0.0	0.0
ICPL 99102	2.3	1.7	0.0	1.3	0.0	0.0	0.0	0.0
ICPR 2671	3.0	1.5	8.3	4.3	1.9	5.0	0.0	2.3
BGR 3	3.4	8.8	3.7	5.3	0.0	3.8	0.0	1.3
MAL 17	1.0	0.0	4.3	1.7	2.5	0.0	0.0	0.8
MAL 19	6.3	0.0	0.0	2.1	1.3	0.0	0.0	0.4
PH 860	2.9	3.0	0.0	2.0	0.7	0.0	0.0	0.2
SIPS 1	54.7	57.0	63.4	58.3	3.9	5.6	0.0	3.2
Controls								
ICP 2376	98.8	97.8	94.6	97.1	0.0	0.0	0.0	0.0
ICP 8863	2.0	3.9	5.8	3.9	95.9	98.8	100.0	98.2
LSD ($P = 0.05$)[b]	7.51	5.29	5.79	3.55	11.01	10.12	6.24	2.3

[a]Mean of two replications; [b]Trial least significant difference

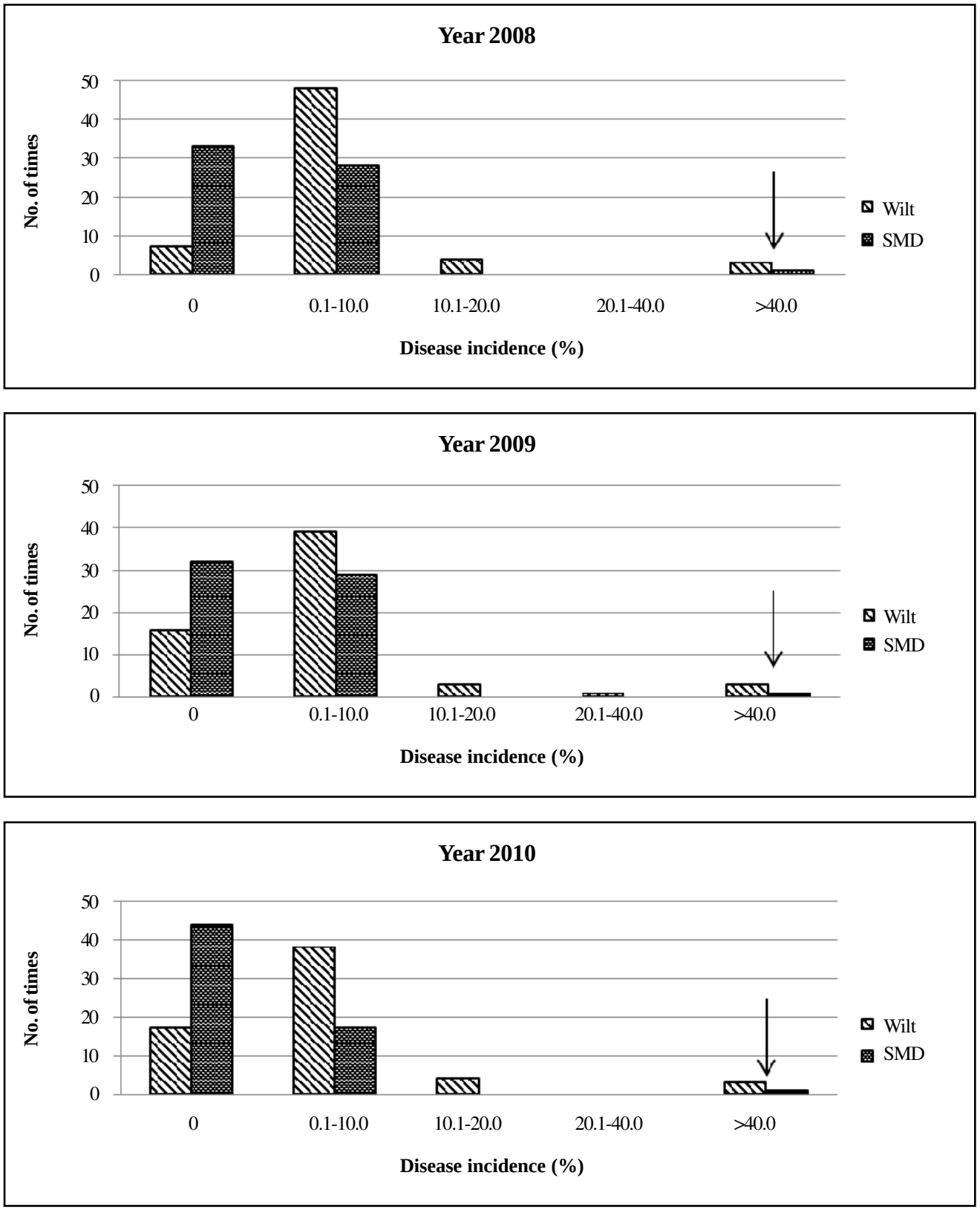

Figure 1. Frequency of distribution for wilt and sterility mosaic disease incidence of 62 pigeonpea lines evaluated in the field of ICRISAT during season 2008 to 2010

incidence), 53 were resistant and 4 were moderately resistant to both FW and SMD. Performance of all the lines including resistant and susceptible check lines for both the diseases in different years was consistent as shown in Table 2.

In this study, 60 lines that have been identified as resistant to wilt and SMD, were subsequently evaluated for three years in the sick plot at ICRISAT. However, most of the pigeonpea cultivars grown were the selection from landraces with a narrow genetic base (Singh *et al*., 1990). As a result the search continues for the sources of high level of resistance for these diseases. Considerable effort has been made by ICRISAT in developing wilt and SMD resistant pigeonpeas, adapted to cultivation in the Asia and Africa (Nene and Sheila, 1990; Zote*et al*., 1995; Reddy *et al*., 1998; Rangaswamy *et al*.,2005; Gwata *et al*., 2006; Sharma and Pande, 2011; Sharma *et al*., 2012). Resistant accessions identified in this study can be exploited for cultivation in wilt and SMD endemic areas and also in resistance breeding programme for their broad-spectrum resistance at different locations to test the genotype × environment interaction.

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