



Rapid screening technique for evaluation of maize genotypes against stalk rot complex caused by *Macrophomina phaseolina* and *Fusarium verticilloides*

GOPALA¹, ROBIN GOGOI², K S HOODA³, S N RAI⁴, A KUMAR⁵ and FIROZ HOSSAIN⁶

ICAR–Indian Agricultural Research Institute, New Delhi 110 012

Received: 28 May 2015; Accepted: 29 April 2016

ABSTRACT

In order to screen maize genotypes for resistance to post-flowering stalk rot (PFSR) complex caused by *Macrophomina phaseolina* (Tassi) Goid and *Fusarium verticilloides* (Sacc) Nirenberg under field conditions, toothpick method was used for creating artificial epiphytotics. In this study, 34 maize inbred lines were screened in field by toothpick method of inoculation. Of these accessions, representative 20 maize genotypes were screened by two new methods of inoculation of the cut stems under laboratory condition. Split stems were inoculated in the first method and un-split stems were inoculated by toothpick method in the second. The split method produced recordable data within 15 days of post-inoculation (DPI) where as the un-split stem inoculation method produced result at 20 DPI. Both the new techniques employed in the laboratory were faster in producing results as compared to the field screening of maize genotypes by the standard toothpick method which needs about 40 days for expression of PFSR symptoms. Split open method was better than the un-split method of cut stem inoculation *in vitro*. A new scale ranging from 1 to 16 cm was developed based on the existing 1-9 scale of PFSR for scoring disease severity *in vitro*.

Key words: Maize genotypes, New methods of inoculation, Rating scale, Screening, Stalk rot complex,

Maize (*Zea mays* L.) is one of the important cereal crop next to wheat and rice in the world. Among the cereal crops in India, maize with an annual production of around 21 million tonnes covering 8.5 million hectares ranks third in production with sixth rank in global maize production, contributing 2.4% of world production with almost 50% share in world harvested area. However, the country lags far behind in productivity (24.7 q/ha) against world average of 5.14 tonnes/ha (Anon. 2013).

Post-flowering stalk rot (PFSR) is one of the important disease among 61 diseases of maize occurring in India (Payak and Sharma 1981). PFSR is caused by a complex of pathogenic fungi, predominantly *Macrophomina phaseolina* and *Fusarium verticilloides*. Stalk rot is found to be prevalent in the plains only in the kharif crop when summer temperature becomes relatively high (30° to 35°C). The disease incidence, recorded in India time to time, ranged from 10 to 42% (Desai *et al.* 1991), 25 to 32% (Kumar *et al.* 1998) and 10.18 to 31.08% (Harlapur *et al.* 2002), 13.2 to 39.5% (Payak and Sharma 1985) that cause significant losses in yield.

¹Ph D Scholar (e mail: gplpatho33@gmail.com), ^{2,5}Principal Scientist (e mail: r.gogoi@rediffmail.com), ³Former Chief Technical Officer, Division of Plant Pathology. ⁴Principal Scientist (Plant Pathology), ICAR-Indian Institute of Maize Research, Pusa Campus, New Delhi 110 012, ⁶Senior Scientist, Division of Genetics.

Out of the various conventional and modern approaches, use of disease resistant varieties is the most cost-effective and safe, environment friendly means of disease management. To develop disease resistant varieties, screening of available genotypes against the pathogens was done under artificial epiphytotic condition and it yielded a set of stalk rot resistant germplasm in India (Shekhar *et al.* 2010, Hooda *et al.* 2012) and abroad (Clark and Foley 1985). In India, artificial epiphytotic condition for PFSR disease is created by inoculating the plants in the field just after flowering mainly by toothpick method of inoculation (Anon. 1983, 2012). But this method requires longer time for disease development and rotting symptoms in the inoculated stalks become prominent only at harvesting stage. Therefore a study was planned to develop a method for screening of the maize genotypes *in vitro* which is rapid and more efficient than the tooth pick method currently used in the field.

MATERIALS AND METHODS

Infected stalks of maize plants bearing typical symptoms of charcoal rot and *Fusarium* stalk rot were collected from the maize field during *kharif* 2012. Small bits cut from the infected stalks were surface sterilized with 4% sodium hypochlorite for 1 min. A single bit was aseptically transferred to Petri plates containing sterilized PDA and incubated for 3-4 days at 27±2°C. The hyphal tips of fungal mycelia grown out from the infected bits were aseptically transferred to sterile PDA slants and incubated for about 10 days to get

the pure culture of the pathogen. The identity of *M. phaseolina* was confirmed by using compound microscope based on the characters like sooty black colour of the culture, numerous dark pin head size microsclerotia. Similarly, *F. verticilloides* was confirmed based on the features like pinkish white fungal colony and hyaline, curved near the tips, three to five septate. Micro conidia are abundant single celled and borne in chains.

Among various methods of field inoculation, the standard toothpick inoculation is commonly followed for these diseases under the coordinated programmes. Round bamboo toothpicks about 6.5 cm long were boiled three times (about 1 h each time) in tap water to remove gum and resin like toxic substances which might inhibit the growth of the test fungi. After each boiling these were thoroughly washed in fresh water and dried in the sun. After drying, by keeping the tapering end upward, toothpicks were loosely packed in bundles and put into screw capped glass jars/bottles. Prior to autoclaving, potato dextrose broth was added. The level of broth was adjusted to one-third length of the toothpicks and autoclaved. Subsequently, the sterilized toothpicks were seeded with fungus and incubated 28°C for one week. Abundant mycelial growth was spread on the toothpicks and inoculum became ready for use in about 10 days.

Seeds of 34 maize germplasm were collected from Winter Nursery Centre (WNC), Hyderabad and two maize inbred lines from Maize Genetic Lab, IARI (Table 1). The germplasm were raised in field during *khari*, 2013. Inoculation of the plants of 45-50 days old was done just after flowering by toothpick method (Anon. 1983 and 2012, Fig 1 A). Before inoculation, one jabber was made by driving/fixing a nail of toothpick size into a wooden handle. For inoculation of the plants, the lower internode (second or third) above soil level was selected. Then the pointed head of the nail was pushed carefully into the selected internode to make a hole of desired length (2cm). The round toothpick bearing inoculum was inserted into the hole (Fig 1) that effectively sealed the hole to prevent drying of the inoculum.

Typical symptoms like partial or whole plant drying appear in the inoculated plants about 20-25 days post-inoculation (DPI). The disease severity of PFSR was recorded at harvesting stage following 1-9 rating scale of Payak and Sharma (1983) with slight edition (Anonymous 2014) as depicted in Table 2. Per cent Disease Index (PDI) was calculated using the following formula of Mc kinney (1923),

$$\text{PDI (\%)} = \frac{\text{Sum of individual rating}}{\text{Total No. of leaves examined} \times \text{Maximum disease score/grade}} \times 100$$

On the basis of PDI, the inbred lines were classified as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S).

Out of the 36 PFSR resistant and susceptible genotypes raised for field inoculation, 20 inbred lines (Table 1) were selected based on the availability of sufficient plant population and better stature of the stem.

Table 1 List of maize inbred lines used for identification of post flowering stalk rot (PFSR) resistant and susceptible genotypes

Inbred lines	Pedigree
H-8	42048-2-21
H-62*	CML 44
H-61*	CML 384
BML-6*	BML 6
H-139*	LM 16
14933,-2*	T2STR1107
H 109*	HKI 191-1-2-5
H 10 *	951-7
P 503*	NC 392
DMSC-I	DMSC 1
H 75*	DMSC 36
P 320*	HKIPC-5-1
H 182	WIN POP 21
H 103	HKI 1040-5
P 408*	PFSR/51016-1
15026,	CM114
E684	CM 123
H 37	CM 144
P 364*	CM 500
H 100	HKI MBR-139-2
H 68	DMR QPM -03-104
14982,-5	DMR QPM-58-26
E 613	ITNA 004
P 373*	LM15
14902,-2	WIN SWEETCORN
H 186	WOSC
P 345	HKI C 322
E 618*	PFSR S3
18527*	CML 269
18758	DMSC 37-3
18768	MAS,MADU(SH2 SH2)
18833*	HKI PC 43
18855*	POP31DMR-88-3
18834*	HKI PC5
HM8 *	
PC4*	

*Genotypes used for *in vitro* inoculation study.

A plastic tray of size 40 cm length × 30 cm width was sterilized with 70% alcohol. Fresh saw dust was procured from the furniture shop and sterilized by autoclaving for three consecutive days. A layer (2 cm thickness) of sterilized saw dust was spread uniformly in the surface sterilized trays. Then a clean blotter paper was placed on the saw dust, sprinkled with sterilized water and covered with polythene sheet for maintenance of moisture. Freshly harvested maize stalks measured about 35 cm long with 3-4 internodes were collected and placed on the blotter paper after inoculation. Inoculation of the stalks was done by the following two methods.

Maize stalks collected from field were cleaned and split longitudinally into two equal halves (Fig 1 B). One

Table 2 Modified scale for scoring disease severity of PFSR in laboratory condition

Disease rating scale		Disease severity (%)	PDI*	Disease reaction
<i>In vitro</i> lesion length (cm)	<i>In vivo</i> (Payak and Sharma 1983)			
1	1.0	Healthy or trace/slight discolouration at the site of inoculation.	11.11	Resistant
2	2.0	Up to 50% of the inoculated internode is discoloured	22.22	(Score: ≤ 3.0) (PDI: ≤ 33.33)
3	3.0	51-75% of the inoculated internode is discoloured	33.33	
4				
5	4.0	76-100% of the inoculated internode is discoloured	44.44	Moderately resistant (Score: 3.1-5.0)
6				
7	5.0	Less than 50% discolouration of the adjacent internode	55.55	(PDI: 33.34–55.55)
8				
9	6.0	More than 50% discolouration of the adjacent internode	66.66	Moderately susceptible (Score: 5.1 – 7.0)
10				
11	7.0	Discolouration of three internodes	77.77	(PDI: 55.56 – 77.77)
12				
13	8.0	Discolouration of four internodes	88.88	Susceptible (Score: ≥ 7.0)
14				
15	9.0	Discolouration of five or more internodes and premature death of plant	99.99	(PDI: ≥ 77.77)
16				

small and shallow well of 5 × 5 mm size was made on the cut surface of the stem by scooping out of soft tissue/pith. A measured amount of inoculum was taken from 10 days old pathogen cultured in PDA and placed in the well. Both cut ends of the stem were covered with moist cotton swabs and placed on the blotter paper of saw dust laid tray. Then sprinkled with sterilized water and covered with a perforated polythene sheet for maintenance of humidity inside the tray so as to fasten the spread of the inoculum. Perforation of the polythene sheet was made before its use by pinching with a sharp pin in order to facilitate mild ventilation.

The lower most internode of the cut stem was inoculated by the method as described above in the field inoculation.

After inoculation, stems were placed in the tray and maintained as described in the split method.

The trays of above two methods were maintained in room temperature (32-35°C). Observations on disease severity were made at two days regular interval up to 15 days post inoculation (DPI) in case of split method and only once at 20 DPI in case of toothpick method by split opening of the stalk.

Scoring of disease reaction

For scoring of the disease severity of PFSR under lab condition, a new scale has been developed based on the 1-9 rating scale of Payak and Sharma (1983). The lesion length of the disease progress in the cut stems was measured with a scale in cm and scoring was done using the modified scale (Table 2).

Rhizopus oryzae Went & Primsen Geerligs, a non pathogenic fungus for maize crop was obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi. The fungus was inoculated by split method in order to know if any disease development occurs on the split maize stalks *in vitro*.

All data on the disease severity generated from the experiments conducted in field and laboratory were compared and efficiency of the new inoculation methods was assessed.

RESULTS AND DISCUSSION

Screening of maize genotypes in field : Out of the 34 maize genotypes screened against *M. phaseolina*, only four lines, viz. H37, E618, 18527 and 18758 were found resistant, 10 lines, viz. H 62, 14933, H 109, P 503, P 408, E 684, P 364, E 613, P 345, 18855, were moderately resistant, thirteen lines, viz. H 61, BML 6, H 10, P 320, H 182, 15026, H100, H 68, 14982, 18833, 18834, HM 8, PC 4 were moderately susceptible and only seven lines, viz. H 8, H 139, H 75, H103, P 373, H 186, 18768, were found susceptible. Whereas, against *F. verticilloides*, six lines, viz. H 109, P 503, 15026, H 37, P 345 and 18758 were found resistant, 13 lines, viz. H 62, BML 6, H 139, 14933, P 320, E 684, P 364, 14982, E 613, E 618, 18527, 18768 and 18855 were found moderately resistant, 11 lines, viz. H 61, H 10, H 75, H 103, P 408, H 100, H 68, P 373, 18833, 18834 and HM 8 were found moderately susceptible, four lines, viz. H 8, H 182, H 186 and PC 4 were found susceptible. Against complex of *M. phaseolina* and *F. verticilloides*, only five lines, viz. P 503, P 373, P 345, 18527 and 18758 were found resistant. 10 lines, viz. H 8, BML 6, H 139, 14933, P 320, E 684, P 364, 14982, E 613, E 618, 1885, and 18834 were moderately resistant, 11 lines, viz. H 62, H 61, H 139, H 109, H 10, H 182, H 103, P 408, H 37, HM 8 and PC 4 were moderately susceptible, seven lines, viz. H 75, 15026, H 100, H 68, H 186, 18768 and 18833 were susceptible. Symptom of PFSR was not observed in the lines HM 8 and PC 4 which were used as control plants (Table 3).

Screening of maize genotypes in laboratory: Twenty genotypes were screened *in vitro* against the pathogens

Table 3 Disease incidence of charcoal rot, fusarium stalk rot and complex recorded in field by toothpick method (at harvesting) using standard rating scale (score 1-9)

Inbred lines	Previous record	In field (Toothpick method)						Control (Sterilized toothpick)
		MP meanscore	Disease reaction	FV meanscore	Disease reaction	FV+MP meanscore	Disease reaction	
H 8	R	8	S	8	S	4	MR	
H 62	MR	4	MR	4	MR	6	MS	
H 61	R	6	MS	7	MS	6	MS	
BML 6	R	6	MS	4	MR	5	MR	
H-139	MR	9	S	4	MR	7	MS	
14933,-1	R	5	MR	5	MR	4	MR	
H 109	S	5	MR	3	R	7	MS	
H 10	MR	7	MS	6	MS	6	MS	
P 503	R	5	MR	2	R	2	R	
H 75	R	9	S	6	MS	9	S	
P 320	R	7	MS	5	MR	5	MR	
H 182	R	6	MS	9	S	6	MS	
H-103	R	8	S	7	MS	7	MS	
P 408	R	5	MR	7	MS	7	MS	
15026	R	8	MS	2	R	9	S	
E 684	R	5	MR	4	MR	4	MR	
H 37	MR	3	R	3	R	7	MS	
P 364	R	4	MR	5	MR	4	MR	
H 100	MR	7	MS	7	MS	8	S	
H 68	MR	7	MS	7	MS	8	S	
14982	R	6	MS	4	MR	4	MR	
E 613	S	5	MR	4	MR	5	MR	
P 373	S	9	S	6	MS	3	R	
H 186	S	9	S	9	S	9	S	
P 345	S	4	MR	1	R	2	R	
E 618	MR	3	R	5	MR	5	MR	
18527	R	3	R	4	MR	2	R	
18758	R	2	R	3	R	3	R	
18768	MR	9	S	4	MR	9	S	
18833	SMS	7	MS	7	MS	9	S	
18855	R	5	MR	4	MR	4	MR	
18834	R	6	MS	7	MS	6	MR	
HM-8	S	7	MS	7	MS	7	MS	
PC-4	S	7	MS	8	S	7	MS	

causing PFSR disease by split method and un-split method of inoculation. In case of split method, against *M. phaseolina* only one lines, viz. P364 was found resistant, seven lines, viz. H 61, BML 6, H 139, 14933, H 109, P 503 and 18855 were recorded as moderately resistant, eight lines, viz. H 62, H 10, H 75, P 320, P 408, 18833, 18834 and HM 8 were moderately susceptible, four lines, viz. P 373, E 618, 18527 and PC 4 were susceptible. Against *F. verticilloides* 14 lines, viz. H 62, H 61, BML 6, H-139, 14933, H 109, P 503, P 320, P 408, P 364, 9373, E 618, 18527 and 18834 were found resistant, H 75, 18855 and PC 4 were moderately resistant, H 10, 18833 and HM 8 were found moderately susceptible. Five lines, viz. 14933, H 109, P 364, E 618 and

18527 were found resistant to the complex of *M. phaseolina* and *F. verticilloides*, nine lines, viz. H 61, BML 6, P 503, H 75, P 408, P 373, 18855, 18834 and PC 4 were moderately resistant, six lines, viz. H 62, H 139, H 10, P 320, 18833 and HM 8 were moderately susceptible to this complex. None of the lines were susceptible to *F. verticilloides* as well as to the complex.

In un-split method, eight lines, viz. H 61, H 139 14933, H 10, P 503, H 75, P 364 and 18833 were found resistant and 10 lines, viz. H 62, BML 6, P 320, P 408, P 373, 18527, 18855, 18834, HM 8 and PC 4 were moderately resistant against *M. phaseolina*, Only one line each, viz. H 109 and E 618 was moderately susceptible and susceptible, respectively,

Table 4 Disease severity of charcoal rot, fusarium stalk rot and their complex recorded *in vitro* by split method (1-15 DPI) and un split method (at 20 DPI) using new rating scale (1-16 cm)

Inbredlines	AICRP record	Split method						Un-split method						
		MP Lesion length	Reaction	FV meanscore	Reaction	FV+MP meanscore	Disease reaction	N.P	MP meanscore	Disease reaction	FV meanscore	Disease reaction	FV+MP meanscore	Reaction
H-62	MR	11.75	MS	4.00	R	10.25	MS	0.66	9.00	MR	9.00	MR	9.00	MR
H-61	R	7.77	MR	3.11	R	5.60	MR	0.33	4.00	R	8.00	MR	4.00	R
BML 6	R	7.22	MR	2.90	R	4.60	MR		8.00	MR	6.00	MR	12.00	MS
H-139	MR	7.33	MR	4.00	R	9.00	MS		2.00	R	5.00	MR	4.00	R
14933	R	6.55	MR	1.50	R	3.50	R		3.00	R	4.00	R	6.00	MR
H 109	S	7.77	MR	3.00	R	4.10	R	0.70	10.00	MS	9.00	MR	5.00	MR
H 10	MR	11.25	MS	12.00	MS	11.00	MS		3.00	R	4.00	R	6.00	MR
P 503	R	8.33	MR	1.60	R	8.00	MR		3.00	R	3.00	R	3.00	R
H 75	R	11.55	MS	7.00	MR	8.50	MR		3.00	R	4.00	R	5.00	MR
P 320	R	12.00	MS	3.00	R	10.50	MS		8.00	MR	7.00	MR	6.00	MR
P 408	R	11.25	MS	1.00	R	9.37	MR		8.00	MR	9.00	MR	6.00	MR
P 364	R	3.00	R	1.75	R	1.00	R	0.55	2.00	R	1.00	R	1.00	R
P 373	S	12.75	S	2.00	R	5.60	MR		5.00	MR	4.00	R	6.00	MR
E 618	MR	15.87	S	1.75	R	2.50	R	0.70	21.00	S	5.00	MR	8.00	MR
18527	R	20.75	S	1.75	R	1.00	R		5.00	MR	6.00	MR	2.00	R
18833	MS	12.55	MS	8.25	MS	8.50	MS		3.00	R	5.00	MR	7.00	MR
18855	R	8.00	MR	7.00	MR	5.00	MR		5.00	MR	4.00	R	4.00	R
18834	R	12.00	MS	4.00	R	5.50	MR	0.55	9.00	MR	6.00	MR	8.00	MR
HM 8	S	10.66	MS	11.00	MS	10.30	MS		5.00	MR	7.00	MR	9.00	MR
PC 4	S	15.25	S	5.50	MR	4.75	MR	0.66	8.00	MR	4.00	R	4.00	R

to the charcoal rot causing fungus (*M. phaseolina*). Against *F. verticilloides*, eight lines, viz. 14933, H 10, P 503, H 75, P 364, P 373 and 18855 were resistant, twelve lines, viz. H 62, H 61, BML 6, H 139, H 109, P 320, P 408 E 618, 18527, 18833, 18834 and HM 8 were found moderately resistant, but there was no any moderately susceptible and susceptible lines. To the PFSR complex, H 61, H 139, P 503, P 364, 18527, 18855 and PC 4 were found resistant, twelve lines, viz. H 62, 14933, H 109, H 10, H 75, P 320, P 408, P 373, E 618, 18833, 18834 and HM 8 were moderately resistant and only one line BML 6 was moderately susceptible. No susceptible line was recorded against *M. phaseolina* and *F. verticilloides* complex (Table 4).

Infectivity of non pathogen: Out of 7 entries inoculated with the non pathogenic fungus *Rhizopus oryzae*, the observed lesion length was varying from 0.33 to 0.77 cm. The size of these lesions is very less in comparison to the rotting lesions developed by the pathogenic fungi *M. phaseolina* and *F. verticilloides* which varied from 7 to 15.5 cm.

Performance of new screening technique: Table 5 shows comparison of the three inoculation methods. *In vitro* comparison of split method (SM) and un-split method (USM) revealed that out of 20 entries 10 showed 50% complete matching, 8 entries showed 40% marginal matching and 2 entries showed 10% non matching. In case of the comparison of *in vitro* split method with *in vivo* toothpick method (TPM), 50% complete matching was found in 10 entries, 45% marginal matching in 9 entries and 5% non matching in only one entry. *In vitro* un-split method when compared with *in vivo* toothpick method, it showed 35% complete matching in 7 entries, 40% marginal matching in 8 entries and 25% non matching in 5 entries. Comparison of *in vitro* split method and toothpick method (AICRP) revealed 6 entries with 30% complete matching, 12 entries having 60% marginal matching and 2 entries showing 10% non matching. Eight entries showed 40% complete matching, another 8 entries had 40% marginal matching and 4 entries exhibited 20% non matching while *in vitro* un-split method was compared with toothpick method (AICRP). The toothpick method of *in vivo* and AICRP was also compared and found 25% complete matching in 5 entries, 45% marginal

matching in 9 entries and 30% non matching in 6 entries.

In the present study, 34 inbred maize lines were selected based on All India Coordinated Research Programme on Maize (AICRP) and grouped as the disease resistant and susceptible lines. All these maize inbred lines were screened in field by toothpick method of inoculation. Under laboratory condition, reaction to the PFSR disease of 20 entries was reconfirmed by two new methods of inoculation of the cut stems. Split stems were inoculated in the first method and un-split stems were inoculated by toothpick method in the second. The disease severity was recorded in the laboratory by using a new scale (1-16 cm) developed based on the 1-9 scale of Payak and Sharma (1983). As a result, most of the lines exhibited disease reaction varying from resistant (score 2) to moderately resistant (score 5) against *M. phaseolina*, *F. verticilloides* and their complex. Earlier comprehensive study was made to screen out maize genotypes for resistance sources to charcoal rot disease caused by *M. phaseolina* by following toothpick method of inoculation. Adopting the same technique, Shekhar *et al.* (2010) reported three resistant lines namely PFSR-13-5, JCY2-2-4-1-1-1-1 and JCY3-7-1-2-1-b-1 against post flowering stalk rot. In the succeeding period, Hooda *et al.* (2012) also identified 121 lines possessing resistance against PFSR as well as multiple diseases.

Disease severity data obtained from the field and laboratory experiments were summarized based on their average disease reaction presented in the (Table 6). Efficiency of the new inoculation methods was determined by comparing the disease reaction of the three inoculation methods namely split and un-split method adopted *in vitro* and toothpick method of the field. As a result, three matching groups namely complete-, marginal- and non-matching were drawn out from the results of different combinations of the inoculation methods. Of these, split method of inoculation was found best for *in vitro* screening of maize genotypes for PFSR disease. Since, this method is able to show highest per cent (50%) of complete matching and only 5% non-matching. In addition this method can provide the results within 15 days. It was followed by the un-split method by which germplasm screening can be completed by 20 days *in vitro*. In contrast, the currently followed inoculation

Table 5 Comparison of the inoculation methods based on the PFSR disease incidence in maize inbreds *in vitro* and *in vivo* recorded by new rating scale (1-16 cm) and standard scale (1-9 score)

Matching combinations of inoculation methods	Matching group					
	Complete matching		Marginal matching		Non matching	
	Total entries (20)	% Matching	Total entries (20)	% Matching	Total entries (20)	% Matching
SM (<i>in vitro</i>) × USM (<i>in vitro</i>)	10	50	8	40	2	10
SM (<i>in vitro</i>) × TPM (<i>in vivo</i>)	10	50	9	45	1	5
USM (<i>in vitro</i>) × TPM (<i>in vivo</i>)	7	35	8	40	5	25
SM (<i>in vitro</i>) × TPM (AICRP)	6	30	12	60	2	10
USM (<i>in vitro</i>) × TPM (AICRP)	8	40	8	40	4	20
TPM (<i>in vivo</i>) × TPM (AICRP)	5	25	9	45	6	30

SM: Split method, TPM: Toothpick method, USM: Un-split method, AICRP: All India Coordinated Research Project on Maize

Table 6 Summary of post-flowering stalk rot (PFSR) disease reaction observed in different inoculation techniques under laboratory and field condition

Inbreds lines	Lab method		Field method	Disease reaction as per AICRP report
	Split	Un-split	Toothpick	
H-62	MR	MR	MR	MR
H-61	MR	R	MS	R
BML-6	MR	MR	MR	R
H-139	MR	R	MS	MR
14933,-1	R	R	MR	R
H 109	R	MR	MR	S
H 10	MS	R	MS	MR
P 503	MR	R	R	R
H 75	MR	R	S	R
P 320	MR	MR	MR	R
P 408	MR	MR	MS	R
P 364	R	R	MR	R
P 373	MR	MR	MR	S
E 618	MR	MR	MR	MR
18527	MR	MR	R	R
18833	MS	MR	MS	MS
18855	MR	R	MR	R
18834	MR	MR	MS	R
HM-8	MS	MR	MS	S
PC-4	MS	R	MS	S

R: Resistant, MS: Moderately susceptible, MR: Moderately resistant, S: Susceptible, AICRP: All India Coordinated Research Project on Maize.

procedure developed by Payak and Sharma (1983) requires a longer time of about 40 days for expression of plant drying symptoms due to PFSR and data are possible to record only at the time of crop harvesting.

In case of some entries like H 109 and PC 4, the disease reaction data obtained from newly developed *in vitro* inoculation methods did not match with the disease reaction reported in the AICRP on maize. However, field data of present study recorded in those lines were in perfect match either with the disease reaction of split method or with the un-split method. The AICRP reports were mainly considered for sorting out of a set of PFSR resistant and susceptible inbred lines, but not for any comparison with the disease reactions resulted in both *in vitro* and *in vivo*.

During study, two lines, viz. HM8 and PC4 were maintained as control where symptoms of PFSR were not observed, since only the sterilized toothpicks devoid of any inoculum were used for stalk inoculation. This has confirmed that the insertion of plain toothpick is not enough to create the damage (rot) in the maize stalks, but the use of inoculum of the pathogenic fungi is indispensable.

In conclusion, the disease severity of PFSR is recorded in the field by observing the disease symptoms on the whole/individual plant. Hence, for recording data in field, Payak and Sharma (1983) prescribed 1-9 scale. But this scale is not applicable in the *in vitro* experiments, since cut

stems of maize plants bearing three internodes were inoculated for disease development. Therefore a separate scale has been developed based on the existing scale of Payak and Sharma to measure the lesion length of PFSR *in vitro*. The new scale having 16 keys from 1 to 16 cm has been equally distributed into four categories of disease severity, viz., resistant, moderately resistant, moderately susceptible and susceptible reaction. This scale could be efficiently used for recording severity of PFSR *in vitro* within 20 days following split and un-split method of inoculations.

ACKNOWLEDGEMENT

Authors are thankful to the Winter Nursery Centre (Indian Institute of Maize Research), Rajendranagar, Hyderabad for providing maize inbred lines.

REFERENCES

- Anonymous. 1983. The techniques of scoring for resistance to diseases of maize in India. All India Co-ordinated Maize Improvement Project, IARI, New Delhi, p133.
- Anonymous. 2012. *Inoculation Methods and Disease Rating Scales for Maize Diseases*. Shekharm and Kumar Sangit (Eds). Directorate of Maize Research, ICAR, New Delhi.
- Anonymous. 2013. *Agricultural Statistics at a Glance*. Directorate of Economics and Statistics. Department of Agriculture and Cooperation, Ministry of Agriculture, GOI. www.agricoop.com (visited on April 31, 2013).
- Anonymous. 2014. Guidelines for infection methods. *Proceedings of 57th Annual Maize Workshop*, All India Coordinated Maize Improvement Project, Udaipur, p 87, 59-60.
- Clark R L and Foley D C. 1985. Stalk rot resistance and strength of maize stalk from the plant introduction collection. *Plant Disease* 69: 419-22.
- Desai S, Hegde R K and Desai S. 1991. A preliminary survey of incidence of stalk rot complex of maize in two districts of Karnataka. *Indian Phytopathology* 43: 575-6.
- Harlapur S I, Wali M C, Prashan M and Shakuntala N M. 2002. Assessment of yield losses in maize due to charcoal rot in Ghataprabha Left Bank Canal (GLBC) command area of Karnataka. *Karnataka Journal of Agricultural Science* 15: 590-1.
- Hooda K S. 2012. Identifying sources of multiple disease resistance in maize. *Maize Journal* 1: 82-4.
- Kumar M, Lal H C and Jha M. 1998. Assessment of yield loss due to post flowering stalk rots in maize. *Journal of Applied Biology* 8: 90-2.
- Payak M M and Sharma R C. 1981. Influence of some environmental and host factors on *Pythium* stalk rot of maize. *Indian Phytopathology* 33: 10-5.
- Payak M M and Sharma R C. 1983. Disease rating scales in maize in India. (*In*) *Techniques of Scoring for Resistance to Diseases of Maize in India*. All India Co-ordinated Maize Improvement Project, IARI, New Delhi, pp 1-4.
- Payak M M and Sharma R C. 1985. Maize diseases and approaches to their management in India. *Tropical Pest Management* 31: 302-10.
- Shekhar M, Kumar S, Sharma R C and Singh R. 2010. Sources of resistance against post-flowering stalk rot of maize. *Archives of Phytopathology and Plant Protection* 43: 259-63.