

Identification of resistant sources of jute (*Corchorus olitorius* L.) against stem rot caused by *Macrophomina phaseolina* (Tassi) Goid

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Here a study was undertaken during 2006-08 to identify *C. olitorius* germplasm accessions tolerant to *M. phaseolina* at Ramie Research Station, Sorbhog, Assam known as 'the hot spot' for the disease. During first year, 293 germplasm accessions including short listed ones from earlier study and new collection were tested along with 2 check varieties viz. JRO 632 and JRO 524 and 19 were selected on the basis of per cent disease index (PDI). These 19 and 2 check varieties were further evaluated during 2007 and only 8 accessions were selected. These lines, namely, OIN 107, OIN 125, OIN 154, OIN 157, OIN 221, OIN 651, OIN 853 and OIJ 084 and two check varieties were grown for rigorous screening during 2008. The two check varieties showed higher PDI during three consecutive years. Only four accessions namely OIN 125, OIN 154, OIN 651 and OIN 853 showed moderately resistant reaction based on mean PDI 5.0 or less and were finally selected. The reaction of these 4 entries was also confirmed in pot culture tests.

Key words : *Macrophomina phaseolina*, stem rot, jute, *Corchorus olitorius*, resistance

INTRODUCTION

Stem rot of jute caused by *Macrophomina phaseolina* (Tassi) Goid. is economically the most important disease of jute affecting both yield and quality of fibre in both cultivated species, namely, *Corchorus olitorius* L. and *C. capsularis* L. Seed yield also declines both quantitatively and qualitatively due to this disease. Ten to fifteen per cent yield loss has been estimated in different jute growing regions of India. Stem rot is the common name but the pathogen attacks any part of the plant at any stage of growth right from germination to harvest producing symptoms, like, damping-off, seedling blight, leaf blight, stem rot, collar rot and root rot. The disease is prevalent in all the jute growing areas not only in India but also in all other countries where jute is grown.

The disease is seed, soil as well as air borne and continues to damage the crop starting from germination to crop maturity. Hence, management of the disease becomes tricky and involves manipulation

of soil condition, seed treatment and foliar application of fungicides or judicious combination of all. The chemical and other control measures do not always give the desired result due to some reasons or the other for which use of resistant/ tolerant variety appears to be the most important tool in present day disease management programme and is the most vital component of the integrated disease management strategy. But so far, no resistant/ tolerant variety is available in jute in either of the two species at least at the cultivators' level (Mandal *et al.* 2000; Mahapatra *et al.* 1994; Kar *et al.* 2009). All the jute and mesta germplasms available at CR1JAF were evaluated against the major diseases at Budbud and Barrackpore condition (Saha *et al.*, 1994; Mahapatara *et al.*, 1994; Mandal *et al.*, 2000). A large number of germplasm accessions of both *C. olitorius* and *C. capsularis*, on preliminary field evaluation, have been recorded free from *M. phaseolina* infection. The short listed *C. capsularis* lines are further tested under high inoculum pressure at the hot spot of Ramie Research Station (RRS). Sorbhog in Assam and 9 are finally identified as tolerant. But in *C. olitorius*, no further study has been done so far. A great degree

of morphological and pathological variability exists among the isolates of *M. phaseolina* from different jute growing regions of India, of which the isolate from Sorbhog was the most virulent one (Mandal *et al.*, 1998). Pathogenic variability of *M. phaseolina* has also been studied by various workers (Ahmed and Ahmed 2005; Ghosh and Sen 1973).

Tossa jute being most widely cultivated in all the jute growing areas, this study has been undertaken to identify source of resistance to *M. phaseolina* in *C. olitorius* accessions so that the resistant character, if any, may be utilized for further breeding purpose.

MATERIALS AND METHODS

The experiment was conducted in a sick plot for three years (2006-08) at Ramie Research Station, Sorbhog, Assam known as the 'hot spot' due to the high inoculum pressure, virulence of the pathogen present, conducive soil and favourable weather condition. The soil of the experimental plot was sandy loam and acidic in nature (pH 5.6). The inoculum density of *M. phaseolina* was 3×10^3 colony forming units at the surface up to 5 cm depth, gradually decreasing with the increase in depth. In the first year (2006), 293 germplasm accessions that include 164 earlier short listed from initial evaluation and the rest 129 new collections under National Agricultural Technology Projects were assessed in Incomplete Block Design for further short listing. The ruling varieties, viz., JRO 524 and JRO 632 were used as the standard check. Standard agronomic practices were followed except use of any plant protection chemical. Observation on disease incidence and severity was done following the disease scoring method described below.

Jute stem rot scoring methodology

A rating scale was developed for precise evaluation of germplasm lines for resistance to stem rot considering all the types of symptoms produced in jute. The stem rot disease was scored observing size of lesion (1 - 4), position of lesion on the stem (1-4) and lesion type (1 - 8) with maximum value of 16 (= 4+4+8) and minimum of 3 (= 1 + 1 + 1), number of stem rot infected plants (Number x score value) and dead plants by root rot infection (Num-

ber x 16).

(a) Lesion size, namely, minor dots or lesion size of less than 0.5 cm was scored with 1.0, lesion size of 0.6 - 1.0 cm" was rated as 2.0, likewise, lesion size of 1.1 - 2.0 cm with 3.0 and lesion size of more than 2.0 cm² with 4.0.

(b) Position of lesion on the first quarter of the stem from the top of the plant carried 1.0 point, similarly, on the second quarter it was 2.0, on the third quarter it was 3.0 and on the last quarter it was 4.0.

(c) Lesion type covering less than 10 % of the stem diameter was given 1.0 point, in the same way, lesion covering 10.1 - 25.0 % of the stem diameter was 2.0, 25.1 - 40.0 % was 4.0 and more than 40.0 % carried 8.0 points.

(d) Then per cent disease index (PDI) was calculated by sum of all numerical ratings multiplied by 100 and dividing it with number of plants observed multiplied by highest value.

(e) Accessions showing less disease on the basis of PDI were selected and further tested in with three replications.

Selected accessions were further evaluated under pot culture condition using three different times of inoculation with *M. phaseolina*, i.e., (i) soil inoculation at 7 days before sowing, (ii) simultaneous soil inoculation and sowing and (iii) soil inoculation at 7 days after sowing seeds in three replications with JRO 524 and JRO 8432 as susceptible checks.

RESULTS AND DISCUSSION

During 2006, the PDI in the first observation ranged from 0- 49.6 and that of second observation was from 4.1 to 86.0. Out of 293 germplasm lines tested, based on PDI (average of the first and second observations), which ranged from 7.6 to 67.8, six entries namely OIN 1152, OIN 1153, OIN 1154, OIN 1160, OIN 1164 and OIN 1165 showed PDI above 50. Only 19 accessions (Table 1) showing mean PDI less than 24 were selected for further evaluation during second year. Nineteen accessions, namely, OIN 070, OIN 084, OIN 107, OIN 125, OIN 146, OIN 154, OIN 157, OIN 176, OIN 221, OIN 651, OIN 853, OIJ 064, OIJ 084, OIJ 087, OIJ 153, OIJ 163, OIJ 181, OIJ 232 and OMU 014 showed least

stem rot disease, i.e., mean PDI of below 24, and seeds of these accessions were collected for further evaluation during the second year. The germplasm lines showing PDI more than 24 were rejected.

Among the selected 8 accessions, highest PDI of 23.54 was observed in OIN 221 and it was preceded by OIN 107 and OIN 154 with PDI of 20.6 and 20.5, respectively (Table 2). Of the eight accessions selected, lower disease in the two acces-

Table 1 : Percentages of root rot, stem rot and mean per cent disease index (PDI) of 19 (+2 checks) selected jute germplasm lines evaluated at hot spot, Ramie Research Station, Sorbhog, Assam during 2006

Accession number	Root Rot(%)	Stem rot (%)	Mean PDI*	Accession number	Root Rot(%)	Stem rot (%)	Mean PDI*
OIN 070	2.67	3.57	13.50	OIN 221	0	10.97	15.66
OIN 125	2.29	11.49	17.38	OIN 853	5.88	45.88	21.25
OIN 107	0	7.14	14.88	OMU 014	0	22.09	7.66
OIN 157	1.28	7.69	12.50	OIJ 232	3.79	30.37	20.09
OIN 154	0	19.04	12.20	OIJ 181	8.43	24.09	20.93
OIN 146	1.20	13.25	17.28	OIJ 087	5.40	14.86	12.83
OIN 176	3.94	9.21	14.67	OIJ 084	0	11.90	10.41
OIJ 064	12.04	31.32	23.71	OIN 084	1.14	3.44	17.99
OIJ 153	0	13.75	13.24	JRO 632	2.27	59.09	35.16
OIJ 163	2.63	10.52	16.61	JRO 524	6.43	89.19	46.81
OIN 651	6.81	11.36	12.14				

*PDI based on mean of first and second PDI values.

Table 2 : Percentages of root rot, stem rot and mean disease index (PDI) of 8 (+2 checks)* selected jute germplasm lines evaluated at hot spot, Ramie Research Station, Sorbhog, Assam during 2007

Accession number	Root rot (%)	Stem rot (%)	Mean PDI
OIN 125	10.46	8.13	12.71
OIN 157	6.30	11.61	15.90
OIN 107	14.04	15.49	20.64
OIN 853	7.70	14.71	11.82
OIN 154	13.05	11.42	20.59
OIN 221	18.89	13.68	23.54
OIN 651	6.31	16.48	12.04
OIJ 084	12.88	8.297	15.47
JRO 632	9.53	27.96	26.96
JRO 524	12.03	31.10	33.68

During second year, the mean PDI ranged from 11.8 to 33.6. All the short listed accessions had lower PDI than the check varieties. The highest PDI (33.6) was recorded in the ruling variety JRO 524 followed by JRO 632 (26.9). Based on their PDI value, eight accessions, viz., OIN 107, OIN 125, OIN 154, OIN 157, OIN 221, OIN 651, OIN 853 and OIJ 084 had been selected for final evaluation. These accessions showed PDI less than 24 and considered tolerant to stem rot. Lowest PDI was shown in case of OIN 853 (11.8) and it was followed by OIN 651 and OIN 125 with respective PDI of 12.0 and 12.7.

sions (OIN 125 and OIN 853) compared to that of first year might be due to disease escape for some reason or other. Rest eleven accessions showing higher disease in terms of PDI were discarded.

In the final evaluation, of the eight short listed accessions and two check varieties, it was observed (Table 3) that none of them were resistant, i.e., PDI equal to or less than 1.0. Four accessions, viz., OIN 125, OIN 154, OIN 651, and OIN 853 had PDI lower than 5.0 and these were graded as moderately resistant. Amongst the rest, OIJ 084 and OIN 107 were moderately susceptible (PDI = 7.9 and 8.9, respectively) and OIN 157 and OIN 221 were susceptible (PDI 10.6 and 10.0, respectively).

The two check varieties, viz., JRO 524 and JRO 632 are the ruling variety all over jute growing areas of India. Both of them showed higher PDI during three consecutive years of testing. JRO 632 showed the PDI from 35.1 in 2006, 26.9 in 2007 to 21.6 in 2008 while JRO 524 was more susceptible to this isolate of *M. phaseolina* causing stem rot with PDI ranging from 46.8 in first year, 33.6 in second year to 30.9 in final year (Tables 1, 2 and 3). Year wise progress in selection of *C. olitorius* germplasm tolerant to *M. phaseolina* was also shown in Table 4.

In pot culture experiment, four entries, namely, OIN 125, OIN 154, OIN 651 and OIN 853 showed resistant reaction under all three different inoculated conditions compared to two susceptible checks. No stem rot was observed in these four entries when

Among the three different times of inoculation with *M. phaseolina*, soil inoculation at 7 days before sowing was most effective in causing infection of stem rot in the susceptible checks as the added inoculum got extra time to establish and multiply in

Table 3 : Final evaluation of the eight selected *C. olitorius* accessions against *M. phaseolina*

Accessions	Stem Rot	Root Rot	Total	PDI*	**Disease Reaction Grade	Remarks
OIJ 084	235.2	80	315.2	7.91	MS	Not selected
OIN 107	252.0	64	316.0	8.92	MS	Not selected
OIN 125	92.1	48	140.1	4.29	MR	Selected
OIN 154	70.8	48	118.8	4.72	MR	Selected
OIN 157	194.4	80	274.4	10.65	S	Not selected
OIN 221	228.0	80	308.0	10.09	S	Not selected
OIN 651	66.0	48	114.0	4.74	MR	Selected
OIN 853	68.5	32	100.5	3.73	MR	Selected
JRO 524 (Navin)	551	128	679	30.97	HS	Used as check
JRO 632	361	144	505	21.67	HS	Used as check

(Baisakhi Tossa)

$$*PDI = \frac{\text{Sum total of numerical ratings}}{\text{Number of plants observed} \times \text{Highest value}} \times 100$$

**Disease Reaction Grade : PDI :- 0-1= Resistant (R), 1.1-5.0 5.1-10.0= Moderately susceptible (MS), 10.1-20.0=Susceptible susceptible (HS) Moderately Resistant (MR), (S) and Above 20.0=Highly susceptible (HS).

Table 4 : Year wise progress in selection of *C. olitorius* germplasm tolerant to *M. phaseolina*

Accessions		Tested in 1 st year (2006)	Advanced for 2 nd year (2007)	Tested in the final year(2008)	Selected tolerant
Short listed accessions	OEX*	6	0	0	0
	OIJ*	37	7	1	0
	OIM*	5	0	0	0
	OIN*	192	11	7	4 (OIN Nos. 125, 154,651 and 853)
	OMU*	4	1	0	0
New Collection		49	0	0	0
Check varieties		2	2	2	0
Total		293+2 = 295	19+2 = 21	8+2 = 10	4

* OEX= *Olitorius* Exotic, OIJ- *Olitorius* 1JO collections, OIM=*Olitorius* Improved, OIN-*Olitorius* Indigenus, OMU= *Olitorius* Mutant

they were inoculated 7 days before sowing, whereas, two checks showed 23.3 % stem rot. Simultaneous sowing and inoculation of *M. phaseolina* resulted in 15 % stem rot in two check varieties but no symptoms of stem rot in these four entries. But when *M. phaseolina* was inoculated 7 days after sowing of jute seeds, similarly, these four entries were not infected with stem rot but two checks exhibited 10 % stem rot infected plant. This result confirmed the resistance of these four entries against *M. phaseolina* as same was also observed at hot spot location of Sorbhog (Assam).

the soil. In JRO 524 and JRO 8432, 23.3 % stem rot was observed when soil inoculation was made 7 days before sowing. But, only 15 % plant of both the varieties were infected with *M. phaseolina* when they were inoculated during sowing time. Inoculation done 7 days after sowing was the least effective method of inoculation of this fungus as it caused only 10 % stem rot infection in both JRO 524 and JRO 8432 (Table 5).

In case of jute, no true resistant variety is available till date in either of the species *C. olitorius* or *C.*

Table 5 : Reactions of 4+2 entries to stem rot caused by *Macrophomina phaseolina* in pots culture under three different inoculated conditions

Name of the entry	Soil inoculation 7 days before sowing*		Soil inoculation During sowing*		Soil inoculation 7 days after sowing*		Remarks
	Number of plants inoculated	% of infected plants	Number of plants inoculated	% of infected plants	Number of plants inoculated	% of infected plants	
OIN 125	40	0	40	0	40	0	Resistant
OIN 154	40	0	40	0	40	0	Resistant
OIN 651	40	0	40	0	40	0	Resistant
OIN 853	40	0	40	0	40	0	Resistant
JRO 524	40	23.33	40	15	40	10	Susceptible
JRO 8432	40	23.33	40	15	40	10	Susceptible

* Replicated three times.

capsularis though the degree of resistance/susceptibility has been observed to vary from variety to variety and also within the variety from place to place, which might be due to the pathotypes present in a particular area or the soil and climatic condition of the place. The most ruling varieties JRO 524 and JRO 632 of *C. olitorius* and JRC 212 and JRC 321 of *C. capsularis* showed differential reaction at different places and also at the same place with pathogen isolates obtained from different places (Mandal *et al*, 1998). When the whole set of *C. capsularis* germplasm available at CRIJAF were assessed against the pathogen quite a good number were totally free from the disease under field condition at Barrackpore as well as at Budbud in West Bengal (Saha *et al*, 1994; Mahapatra *et al*, 1994). But when those short listed *C. capsularis* accessions were further exposed to more vulnerable situation under high inoculum pressure at Sorbhog in Assam, most of them were severely attacked by the disease. Only nine entries, out of 196, confirmed their resistant reaction. Those entries were CIM 036, CIM 064, CIN 109, CIN 358, C1N 360, CIN 362, CIN 371, C1N 386 and CIN 439 (Mandal *et al*, 2000).

Some accessions of wild species of *Corchorus*, like *C. aestuans*, *C. fascicularis*, *C. pseudo-olitorius*, *C. tridens* and *C. trilocularis* showed very high degree of tolerance against the disease under Barrackpore condition (Pulve *et al*, 2003 and 2004; Sinha *et al*, 2003). Out of 14 *C. capsularis* and 27 *C. olitorius* lines, JRO 514 and IR 1 were highly resistant to *M. phaseolina* (Thakur Ji, 1973). De and Mandal (2008) developed a simple inocula-

tion technique of *M. phaseolina* by leaf tip cut and wet cotton swab on resistant CIM 036 and susceptible JRC 412 and the method may be very conveniently used for screening of large number of germplasm against *M. phaseolina* under artificial / Laboratory condition.

The identified disease tolerant lines will form base material for utilization for further breeding for disease resistance as resistant donors by repeated back crossing as recurrent parents. The DNA of these tolerant lines may also be studied for their phylogenetic relationship with other accessions through Unweighted Pair Group Method with Arithmetic Mean analysis (UPGMA) or by appropriate method. The DNA sequence responsible for stem rot tolerance may be identified, studied and amplified with suitable probe and analyzed. New transgenic jute may also be developed for *M. phaseolina* resistance using sequence responsible for that. Mechanism of resistance to *M. phaseolina* may also be studied at the biochemical and molecular level.

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