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ORIGINAL PAPER

Development of screening techniques to evaluate response of jute (*Corchorus olitorius*) to *Macrophomina phaseolina*

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Abstract

Thirteen jute genotypes were evaluated for resistance against *Macrophomina phaseolina* (Mp) in the sick plot, and six of them were identified for stable differential disease reactions. We tested whether other screening methods could produce similar results in these host genotypes. An incubation temperature of 35 °C was optimum for Mp sclerotia germination (90.8%), and it was chosen for experiments involving sclerotia as inoculum. Infestation with 250 or more Mp sclerotia/g seed induced morbidity in ~ 40% seedlings in vitro. Inoculation at the root and leaf could not differentiate between susceptible (JRO 524) and resistant (JRO 204) cultivars. Infesting both growth media and seed with Mp sclerotia induced early (15 days post-inoculation, DPI) expression of disease symptoms. However, the host reactions were in great variation with those from the field screening. Mp inoculum consisting of growing mycelial tips was more aggressive producing 6.4 cm long stem lesion at 4 DPI as compared to 3.9 cm by 7-day-old inoculum. Inoculating at the middle of the main stem produced the largest lesion (5.7 cm long) compared to the basal (3.3 cm) and the tip (1.3 cm) inoculations 6 DPI. Plants at ≥ 70 days after sowing (DAS) produced larger stem lesions than younger plants. This technique grouped test genotypes similar to sick plot experiments, with minor variations. Stem inoculation method may be adopted for large-scale screening and also confirming the resistance reaction of jute towards Mp infection.

Keywords Soft rot · Charcoal rot · Artificial inoculation · Resistance screening

Introduction

Jute is a bast fibre crop commercially cultivated in the south-east Asian countries having tropical climate. Among the natural fibres, global production of jute is second to cotton. India and Bangladesh are major producers of jute, contributing more than 95% of the world's jute production. In India, cultivation is concentrated in the eastern part of the country in the states of West Bengal, Bihar, Odisha, Assam, and Uttar Pradesh. The majority of jute farmers belong to the small and marginal category with low landholdings. Hence, the cultivation is mostly traditional with little scope for mechanisation. Under such a situation loss due to diseases practically jeopardise the economic return from this crop.

Among several diseases, stem rot or, charcoal rot, is a major threat to jute cultivation. The average yield loss due to this disease is 10%. However, under severe epidemic conditions, the disease may cause 35–40% loss of fibre yield (Roy et al. 2008). It is caused by *Macrophomina phaseolina* (Mp), a plurivorous fungus. The disease appears in all jute growing areas in India and affects both the cultivated species of jute (*Corchorus capsularis* and *C. olitorius*). Jute plants are reported to be affected by this disease at all the growth stages—seedling to seed development (Roy et al. 2008). However, during our field visits, farmers informed us that the disease became severe after 60 days of plant age. The pathogen remains dormant in soil amidst crop debris. Disease incidence usually coincides with the onset of rain. Hence, it is difficult to be controlled with fungicide applications. Biological (Das et al. 2008; Shekhar et al. 2010; Srivastava et al. 2010) and cultural managements (Mawar and Lodha 2009) are tried against this pathogen. However, jute being cultivated during the summer season, long-duration soil solarisation is not practically feasible. And biological control is yet to become popular mainly due to the unavailability of an effective commercial formulation.

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Resistant cultivars are one of the most cost-effective and socially acceptable technologies for disease management (Coser et al. 2017). However, information on the resistance status of jute towards charcoal rot pathogen is scanty. Mandal et al. (2000) subjected several white jute (*C. capsularis*) accessions to field screening against the disease at a hot spot. However, the possibility of plant death due to other pests prevalent at the site (such as root-knot nematodes) cannot be eliminated. Also, uneven distribution of inoculum may lead to the selection of pseudo-resistant lines. Moreover, a high throughput screening methodology is desirable to accommodate a large number of accessions within a limited time frame. Hence, we compared several resistance screening methods to identify jute accessions with contrasting resistance reactions.

Materials and methods

Plant materials

Seeds of the different jute accessions were obtained from the gene bank of ICAR-Central Research Institute for Jute and Allied Fibres (ICAR-CRIJAF), Barrackpore, India. Seeds of the accession RS6 were donated by Dr. D. Sarkar, ICAR-CRIJAF. Unless mentioned otherwise, studies were conducted with the susceptible accessions JRC 412 and JRO 524. All the experiments were conducted at ICAR-CRIJAF, Barrackpore (22° 45′31″ N, 88° 25′ 42″E).

Pathogen

A virulent isolate of *M. phaseolina* (CJMR17) was used throughout the study. The active culture was maintained on potato dextrose agar (PDA; HiMedia Laboratories, India) by sub-culturing, with occasional passage through host tissues to avoid loss of virulence. For long-term preservation, it was grown on sterile wheat grains and stored at 4 °C. To isolate sclerotia, 7-day-old culture plates were flooded with sterile distilled water and the fungal growth was scrapped with a sterile scalpel. The resulting fungal suspension was sequentially passed through 149-µm and 50-µm sieves. Mycelial mass retained on the upper sieve was discarded, while sclerotia on the 50 µm sieve were collected by sterile water jet from a wash bottle applied on the reverse side of the sieve. Sclerotia concentration in this suspension was estimated using a haemocytometer.

Sclerotia germination at different temperatures

A drop of sclerotium suspension in sterile distilled water was placed on a clean slide, and this was placed inside a Petri plate lined with moist filter paper. The whole unit was incubated at varying temperatures (20, 25, 30, 35, and 40 °C). The experiment was conducted following complete randomised design (CRD) with three replications and was repeated once. After 7 h of incubation, the slides were stained with lactophenol cotton blue (HiMedia Laboratories, India). The number of sclerotia germinated and the number of germ tubes per sclerotium were counted under a microscope (BX43, Olympus, Japan). Germ tube length was measured using a micrometre.

Determining aggressiveness of the pathogen

PDA plates were inoculated at the centre with an actively growing mycelial plug and incubated at 25 °C. Agar plugs containing the pathogen were removed from these plates at 1 cm away from the initial inoculation site at 1, 2, and 7 days after incubation. Jute plants at 60–70 DAS were used for inoculation. The stem-tape inoculation technique (Grezes-Besset et al. 1996) was followed with slight modifications. Bark epidermis at mid-length of the plant was wounded with a sterile scalpel. An agar block was placed on the inoculation site, mycelia facing the host, and it was wrapped with a strip of Parafilm® to avoid drying up and contamination. Control plants received sterile PDA plug in place of the pathogen. The experiment was laid in randomised complete block design (RCBD) with four replications and conducted twice. Lesion length was measured 2 and 4 days post inoculation (DPI).

Host inoculation with the pathogen

Seed inoculation

Pre-weighed jute seeds (cv. JRO 524) were surface sterilised by dipping in 0.1% $HgCl_2$ followed by several washings in sterile distilled water. Seeds were inoculated with Mp by adding sclerotium suspension and mixing well. To optimise pathogen concentration for producing disease symptoms, an experiment was conducted following CRD with three replications. Varied concentrations of sclerotia (0, 50, 100, 250, 500, 750, and 1000/g seed) were used. Infested seeds were placed on sterile 1% water agar plates. Plates were placed on laboratory benches receiving diffused sunlight. Each plate containing 10 seeds was considered as one replication. Development of disease symptoms and plant death were recorded regularly till 6 DAS.

Root inoculation

Surface sterilised seeds (JRO 204 and JRO 524) were germinated on germination paper inside a Petri plate and incubated on a rack receiving 16-h light at ambient temperature. Young seedlings (4 DAS) were placed directly on a PDA

plate containing 48-h growth of Mp in such a way that the roots remained in contact with the pathogen. To prevent desiccation of the seedlings, the lid was lined with a moist paper towel. After 2 h of incubation, the seedlings were transplanted into plastic pots containing sterile river sand. Each replication consisted of one pot containing 5 seedlings, and three replications were maintained. This experiment was conducted in CRD and was conducted twice. The disease symptom development was monitored regularly.

The root dip method of inoculation as proposed by Nene et al. (1981) was also tested with little modifications. Jute roots remain too fragile to handle during the early growth phase. Hence, plants were grown for 10 days (first true leaf emergence) and then the blotter paper method (Nene et al. 1981) was followed, arranged in CRD.

Stem inoculation

A field trial was conducted following RCBD to determine the susceptibility of the host at a particular age. Seeds were sown at 20-day intervals. The plants were inoculated at the middle of their lengths following the stem-tape inoculation method as described above, and the lesion lengths were measured at 2 and 4 DPI.

Jute plants of ≥ 70 DAS age were inoculated at three positions, viz., tip, middle, and base of the main stem under the field conditions. For the tip inoculation, the point just below an unopened whorl of leaves was selected. A point approximately at mid-length of a plant and a point 15–20 cm above the ground level were selected for the middle and base positions, respectively. The experiment was conducted during two consecutive years (2013 and 2014), every time with three replications. Each plant inoculated at a single position was considered as a replicate. Lesion lengths were recorded at 2, 4, and 6 DPI.

Selected jute genotypes, differing in their susceptibility towards stem rot disease under field evaluations, were also subjected to the stem inoculation method.

Leaf inoculation

To determine the susceptibility of different leaves, second to sixth fully opened leaves from the top (cv. JRO 524, 90–110 DAS) were selected. These were excised, put in tap water to prevent desiccation and brought to the laboratory. The leaves were wiped dry and placed on sterile 0.5% water agar plates. Leaves were pricked at the middle of the lamina with a sterile needle and inoculated by placing a pathogen mycelial plug. Sterile PDA plugs were placed on the control leaves. These were incubated inside an incubator at 25 °C. Lesion size was determined using ImageJ 1.51 (National Institutes of Health, USA) at 2 DPI. The experiment was conducted in CRD with three replications and repeated once more after a month.

Attached and excised leaves from cv. JRO 524 and JRO 204 were inoculated to determine varietal differences. Excised leaves were inoculated as above. However, under the field conditions, the inoculated leaves on plants were placed inside a transparent plastic bag to prevent washing out of agar block by rain. The experiment was conducted during two consecutive years (2014 and 2015) with four replications.

Inoculating the plant growth media

Field soil and washed river sand, both at field capacity, were used as plant growth media for the pot experiments laid in CRD. These were sterilised by autoclaving at 121 °C for 1 h on two consecutive days. Plastic pots of 12-cm diameter were filled with ~ 400 ml (200/400 g dry soil/sand) of the medium. Soil/sand was inoculated with six concentrations of sclerotia (0, 100, 150, 200, 250, and 300/ml growth medium). Pots containing soil were fertilised with 50 ml of 1% urea, while the sand medium was irrigated with 1 × Hoagland solution and incubated at 35 °C for 3 days. Each pot was sown with six seeds and placed in a growth chamber programmed at 16/8-h light/darkness and 35 °C. Seed germination was counted 5 DAS. The final disease development was recorded at 40 DAS and expressed as per cent disease incidence (PDI). To determine infection success, roots were carefully removed from the pots, washed gently under tap water, and 2-cm length from collar region was excised. Infection by Mp was estimated through culturing these tissues on sterile PDA at 25 °C for 7 days. The number of positive samples was expressed in percentage.

Field screening

Thirteen selected *C. olitorius* accessions were screened under the sick plot conditions for three consecutive years (2016, 2017, and 2018) following RCBD. Test accessions were sown in 2-m-long rows, two rows per accessions, spaced at 40 cm. Three rows of the susceptible check JRC-412 were placed interspersed between the test lines to check uniformity of disease development. During sowing Mp inoculum (multiplied in autoclaved jute seeds) was added to the furrows. The experiment was regularly monitored to note disease symptom appearance, and thereafter, numbers of total and infected plants were counted at 10-day intervals till crop maturity. Percent disease incidence (PDI) was calculated based on this data. The area under disease progress curve (AUDPC) was calculated using the following formula (Vanderplank 1963):

$$AUDPC = \sum_{i=1}^{k} \left(x_i + x_{i-1}\right)$$

x_i = PDI at the end of *i*.
k = number of successive measurement.

Screening under control conditions

The optimum seed and soil inoculum levels (as determined from the above experiments) were combined for pot trials. Accordingly, seeds were infested with 500 sclerotia/g and sown in river sand containing 300 sclerotia/g. Each magenta box was sown with uniformly spaced 12 infested seeds. Other experimental conditions were similar to those mentioned earlier. Six *C. olitorius* accessions showing contrasting reactions towards Mp under the field screening were included in this experiment for confirmation. The experiment was conducted twice.

Statistical analysis

Experimental data were analysed using the online resource OPSTAT (http://14.139.232.166/opstat/). The PDI data were angular transformed before statistical analyses. Treatment means were compared based on least significant difference (LSD, $P = 0.05$).

Results

Role of temperature on sclerotium germination

Incubation temperature had significant influence on sclerotium germination (Table 1). Data from two repetitions followed similar trends. Pooled analysis of the data suggested that sclerotium germination was the highest at 35 °C, while incubation temperature 20 °C supported the minimum germination. The number of germtubes from one sclerotium varied between 9.7 and 13.1, while germtube lengths were 219–286 µm at incubation temperature 35 °C, indicating it as optimum conditions for early events of sclerotium germination.

Table 1 Effect of temperature on germination, germtube number, and germtube length of Mp sclerotia

Incubation temperature	Germination %[a,b]	Germtube/sclerotium[a]	Germtube length (µm)[a]
20 °C	0.4 (2.2d)	0.4e	2e
25 °C	73.0 (59.0c)	4.9d	104d
30 °C	82.2 (65.3b)	6.8c	147b
35 °C	90.8 (72.6a)	11.9a	260a
40 °C	87.0 (69.1b)	9.3b	129c

[a]Results from pooled analyses of two independent experiments are presented. Data points followed by the same letter do not differ significantly ($P = 0.05$). LSD values for germination%, germtube/sclerotium, and germtube length are 4.0, 1.0, and 15.0, respectively

[b]Figures in parentheses are angular transformed values of original data

Effect of inoculum's age on its aggressiveness

The pathogen produced smaller stem lesions as age of the inoculum increased from 1 to 7 days (Fig. 1; Supplementary Fig. 1). One-day-old inoculum consisted of young mycelia, mostly growing tips and the colony appeared white in colour. As the culture aged, 2- and 7-day-old inocula turned olive green and brown, respectively. Numerous sclerotia were present in the oldest inoculum. At 2 DPI stem lesion produced by the 1-day-old inoculum was the largest which gradually decreased with ageing of inoculum; however, lesion lengths produced by 2- and 7-day-old inocula were similar. At 4 DPI overall trend remained the same. Between the two consecutive observations, the lesion length due to the young mycelium increased 60%, while lesion size progressed 55–56% in case of other two inocula.

Seed inoculation

Artificially infested seeds recorded 83–97% germination within 2 days when placed on 1% agar. This was similar to those found in the untreated control (97%) indicating

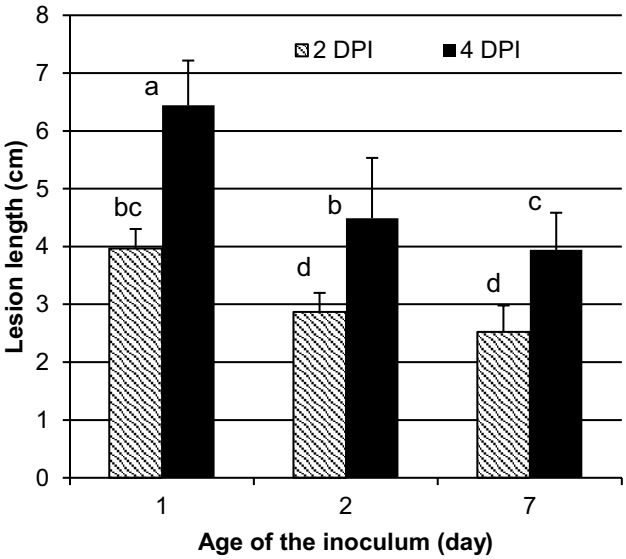

Fig. 1 Lesion length on stem produced by 1-, 2-, and 7-day-old colonies of Mp. Mean of two independent experiments are presented here. Bars topped by the same letter do not differ significantly (LSD = 0.5, $P = 0.05$). Error bars represent standard deviation of mean

Mp infestation did not influence seed germination through the manifestation of pre-emergence damping-off. This was true for seeds sown in the soil. In Petri plates, seedling mortality gradually increased as sclerotium load was enhanced (Fig. 2). However, it should be noted that variation within a treatment was high. A pathogen dose of 500 sclerotia/g caused the highest (53.3%) mortality 7 DPI, which was similar to those found with 250, 750, and 1000 sclerotia/g seed. In the pot experiments, seedling mortality varied between 0 and 38.9% at 20 DAS. The variation was high, and data (not shown) suggested no specific trend.

Root inoculation

Inoculated seedlings became morbid starting from 2 DPI. Symptoms were quick, soft rot of stem; unlike slow, dry rot observed under the field conditions. Progress of seedling mortality was almost similar in case of two cultivars used in the study (Table 2). Under the field conditions, JRO 524 shows higher susceptibility to stem rot disease compared to JRO 204. However, the root inoculation failed to differentiate between these two cultivars and at 7 DPI both recorded similar plant death.

The root dip method of inoculation resulted in similar root damage in both the cultivars (Supplementary Fig. 2). Roots turned brown, and lesion often extended on collar upward. Unlike the control seedlings, no new root formation or root growth could be observed in inoculated seedlings. Within 7 DPI, the seedlings started dying.

Table 2 Mortality of jute (cv. JRO 204 and JRO 524) seedlings due to root inoculation with Mp

Jute cultivars	Seedling mortality (%)[a]		
	2 DPI	4 DPI	7 DPI
JRO 204	15.0 (22.5d)	38.3 (38.2c)	63.3 (52.8a)
JRO 524	13.3 (21.1d)	53.3 (46.9b)	65.0 (53.8a)

[a]Figures in parentheses are angular transformed values of original data. Data points followed by the same letter do not differ significantly (LSD = 4.8, $P = 0.05$)

Stem inoculation

It was observed that susceptibility of the plants, as determined by lesion size, increased with age (Fig. 3). Lesion length measured at 4 DPI differentiated between the treatments more efficiently than data from 2 DPI. A plant at 70 DAS developed the largest stem lesion upon inoculation. At 4 DPI average lesion length on 30-day-old plants was 29.1% less compared to those of 70 DAS plants. With the passage of time, 20–30% of the youngest plants showed partial recovery of these lesions (data not shown). Callus growth was observed at the lesion margin, and the lesion size reduced gradually. However, such was not observed in the 70 DAS plants and many (~ 50%) broke at or dried above the lesion.

In another experiment, it was observed that lesion size was significantly influenced by their positions on the main stem (Fig. 4). During the period of observations, lesion lengths increased gradually. However, lesions at the tip

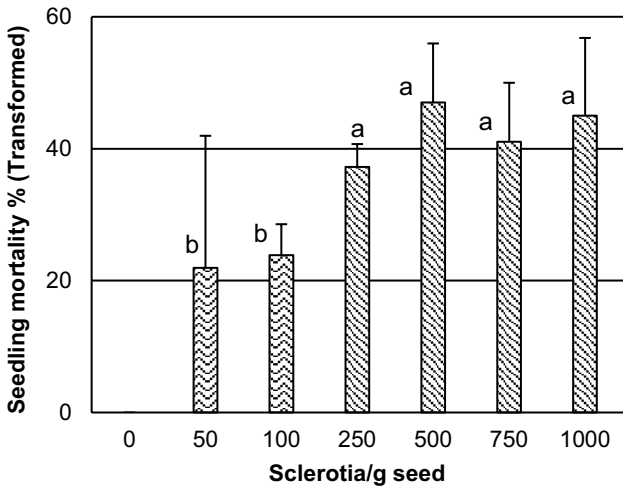

Fig. 2 Mortality of JRO 524 seedling due to seed infestation with variable sclerotia load. Bars topped by different letters are significantly different (LSD = 17.2, $P = 0.05$). Error bars indicate standard error of mean

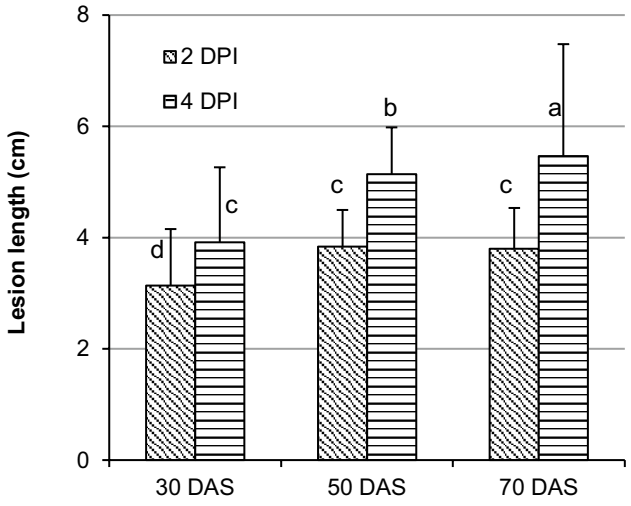

Fig. 3 Lesion lengths on jute stem (cv. JRO 524) of 30-, 50-, and 70 DAS age after inoculation with Mp. Lesions were measured at 2- and 4-DPI. Data points topped by the same letters are not significantly different (LSD = 0.4, $P = 0.05$). Error bars represent standard deviation of mean

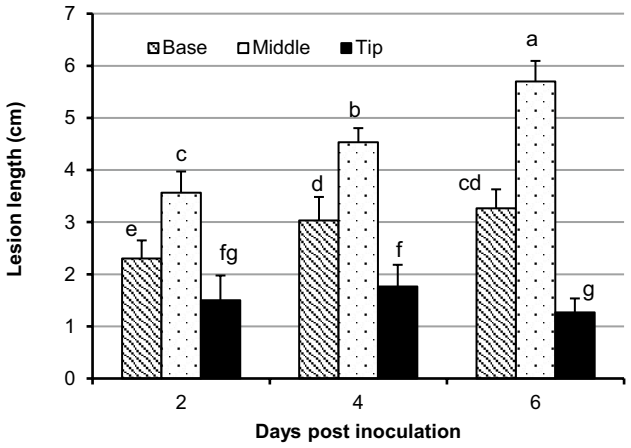

Fig. 4 Development of lesion on jute stem upon inoculation with Mp. Inoculations were made at the tip, middle, or base of a stem. Error bars represent standard deviation of mean. Bars topped with the same letter are not significantly different (LSD = 0.4, $P = 0.05$)

registered a reduction at 6 DPI after an initial increase at 4 DPI. Plants often developed callus tissues around lesions at the tip. This healing mechanism caused a reduction of lesion size at 6 DPI compared to 4 DPI. For inoculations at the base and the middle of a stem, the increase in lesion length was almost linear; slopes were variable though. The coefficient of linear function of lesion development at the middle was much steeper than the other one, the slope being more than double. Accordingly, lesions produced at the middle of a stem were the maximum at 6 DPI.

Screening of selected jute genotypes through stem inoculation resulted in variable lesion sizes. At 6 DPI significant differences in lesion lengths were observed (Table 3). On this basis, the genotypes could be placed into three categories. OIJ-272 and JRO 524 produced the largest lesions, while OIN-154-1, RS6, and OIN-456 formed the smallest lesions. The difference between the two extreme groups was ~ 34%. JRO 204 belonged to the intermediate group. The lesion size of JRO 204 was more close to the resistant group (difference 7.8%) than the susceptible ones (~ 23.4%).

Leaf inoculation

Inoculated leaves in the Petri plates developed lesions as the pathogen grew on it (Supplementary Fig. 3). The infected leaves became very soft and often it became very difficult to shift these from their place. Leaf lesions on older plants were smaller compared to those from 90 DAS plants. However, lesion size did not vary significantly between the leaves. Hence, all the leaves, second to sixth from the top, were equally susceptible to Mp.

Table 3 Screening of selected jute genotypes using three different methods

Host genotypes	Sick plot (PDI)[a]	Stem lesion (cm)[b]	Growth chamber (PDI)[c]
RS6	2.6 (9.0a)	5.9 a	15.3 (16.4a)
OIN-154–1	2.8 (9.5a)	5.8 a	32.0 (34.0b)
JRO 204	4.2 (11.8b)	6.4 b	20.3 (21.7a)
JRO 524	10.0 (18.3c)	7.5 c	37.6 (37.7b)
OIN-456	19.6 (26.2d)	5.9 a	32.0 (33.3b)
OIJ-272	19.5 (26.2d)	8.3 c	47.2 (45.3c)

[a]Figures in parentheses are angular transformed values of percentage data. Each data represent mean of three independent experimentations. Data points followed by the same letter do not differ significantly (LSD = 1.6, $P = 0.05$)

[b]Lesion lengths followed by the same letter do not differ significantly (LSD = 1.1, $P = 0.05$)

[c]Data represent mean of two independent experiments. Mp inoculum was added on seed and in growth medium (sand). Figures in parentheses are angular transformed values of percentage data. Data points followed by the same letter do not differ significantly (LSD = 10.6, $P = 0.05$)

Leaf lesion could not decipher varietal differences between JRO 204 and JRO 524 (Fig. 5). However, the size of the leaf lesions varied depending on the conditions of the experiments, i.e., field and laboratory. The attached leaves (field) developed 12–23% larger lesions compared to excised ones (laboratory). Variations were also observed between the repetitions of experiments (data not shown).

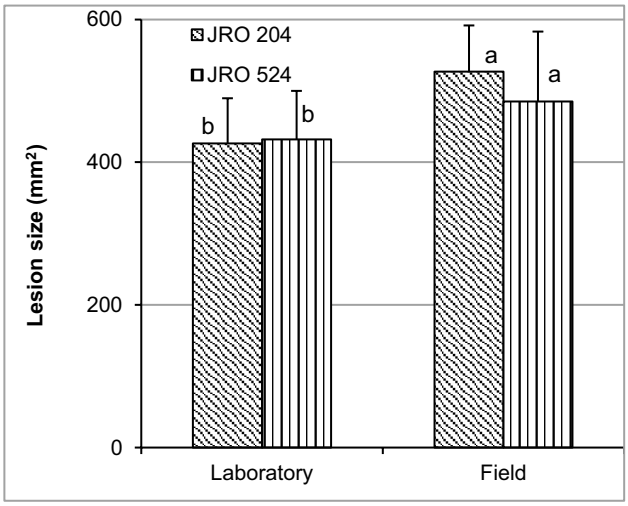

Fig. 5 Area of leaf lesion caused due to inoculation with Mp on two cultivars (JRO 204 and JRO 524) under laboratory and field conditions. Bars topped with the same letter are not significantly different at $P = 0.05$. LSD value for two experimental conditions is 30.9. Error bars represent standard deviation of mean

Soil inoculation

Pooled data from four experiments indicated disease incidence was similar in both the growth media (Table 4). Seed germination varied between 88.9 and 100% and was not significantly different among the treatments. Typical dry rot symptoms started appearing from 10 DAS. Affected plants developed brown streak on the stem at the collar region which later turned brown lesion, stem shrank, and ultimately it died. Disease incidence gradually increased with the rise of inoculum load. At 40 DAS, the highest disease incidence was observed when 1.2×10^5 sclerotia were used in a pot (at 300 sclerotia/mL growth media) and this was followed by the next lower pathogen load (1.0×10^5 scelrotia/pot). However, host infection by Mp, as indicated by pathogen isolation, was similar at both these treatments (Table 4). Lower pathogen concentration further reduced host infection rate.

Screening under the sick plot conditions

Stem rot symptoms started appearing from ~ 50 DAS under the field conditions. Brown lesions appeared at the collar or any other position on the stem. However, seldom any lesion was noticed towards the stem tip. Affected plants dried up and later often broke down at the lesion. The thirteen accessions differed in their disease reactions (Fig. 6). During the initial phase of the disease development (56–87 DAS), variations between the replications were high leading to an unacceptable coefficient of variations. All the accessions did not follow similar trends during three consecutive years. Nevertheless, the perusal of data suggests that RS6 and OIN- 154-1 were always less affected, while OIJ-272 and OIN-456 were consistent with high PDI and AUDPC values. Two released cultivars JRO 524 and JRO 204 also had contrasting reactions (final AUDPC: 767, 839, 690 and 437, 447, 361, respectively, during 2016, 2017 and 2018). It was also noted that symptom appearance was early (56–58 DAS) in the susceptible accessions compared to the resistant ones. Over the years, RS6 and OIN 154-1 remained disease-free till 67–87 DAS. However, once disease appeared on a particular accession, no difference could be observed between the susceptible and resistant groups in terms of the slope of the disease progress curve.

Table 4 Percent disease incidence (PDI) due to inoculation with Mp sclerotia in the growth media and isolation success of the pathogen from experimental plants

Sclerotia/mL growth medium	PDI[a]	Isolation success (%)[b]
0	0.0 (0.0e)	0.0 (0.0c)
100	18.1 (24.0d)	58.3 (50.1b)
150	28.9 (32.1c)	52.8 (46.6b)
200	34.0 (35.3b,c)	69.7 (57.1b)
250	42.9 (40.9b)	88.9 (76.1a)
300	56.7 (49.1a)	88.9 (76.1a)

[a]Mean data from four independent experiments (two each using soil and sand as growth media) are presented. Figures in parentheses are angular transformed values of original PDI. Values followed by the same letter do not differ significantly ($P = 0.05$, LSD = 6.5)

[b]Means from two independent experiment conducted using sand as growth medium are presented. Figures in parentheses are angular transformed values of original data. Values followed by the same letter are statistically similar ($P = 0.05$, LSD = 13.0)

Disease development with seed and growth medium infestation

Application of inoculum through seed infestation and in the plant growth media resulted in early disease development. Disease symptoms started appearing during 15 DAS. Initial symptoms were necrotic brown streak at the collar region. The final disease assessment was made between 21 and 25 DAS (Table 3). Beyond this period, most of the plants developed disease symptoms or died, thereby masking the genotype effects. Disease incidence varied between the experimental repetitions; however, overall trends remained similar. Pooled data analysis of two experiments indicated that the test accessions could be grouped into three broad categories. OIJ-272 was susceptible, while RS6 and JRO 204 were resistant to Mp. OIN-456, OIN-154-1, and JRO 524 were intermediate types in terms of disease incidence.

Discussion

The present paper compares different inoculation techniques with Mp to screen selected jute genotypes to identify a robust method producing results akin to actual field conditions. Disease development is a complex process that depends on proper epiphytotic conditions apart from a susceptible host and virulent pathogen (Billones-Baaijens et al. 2013). Under optimum conditions, the disease reactions become the sole function of the genetic makeup of host and pathogen. In this endeavour, we first determined the effects of external conditions such as temperature on pathogen's growth.

Diseases incited due to Mp are prevalent in the warm tropical climate; incidences in the cooler temperate regions are not uncommon though (Zveibil et al. 2012). Warm temperature (35 °C) increases survival of the primary inoculum (sclerotium) in soil and contributes to disease development (Zveibil et al. 2012). Our data suggest that an incubation temperature of 35 °C induced the highest sclerotia germination (Table 1). This is concurred by similar observations of Viana and de Souza (2002).

Sclerotia produced by Mp mainly aid this pathogen's survival. This thick-walled structure acts as a resting propagule

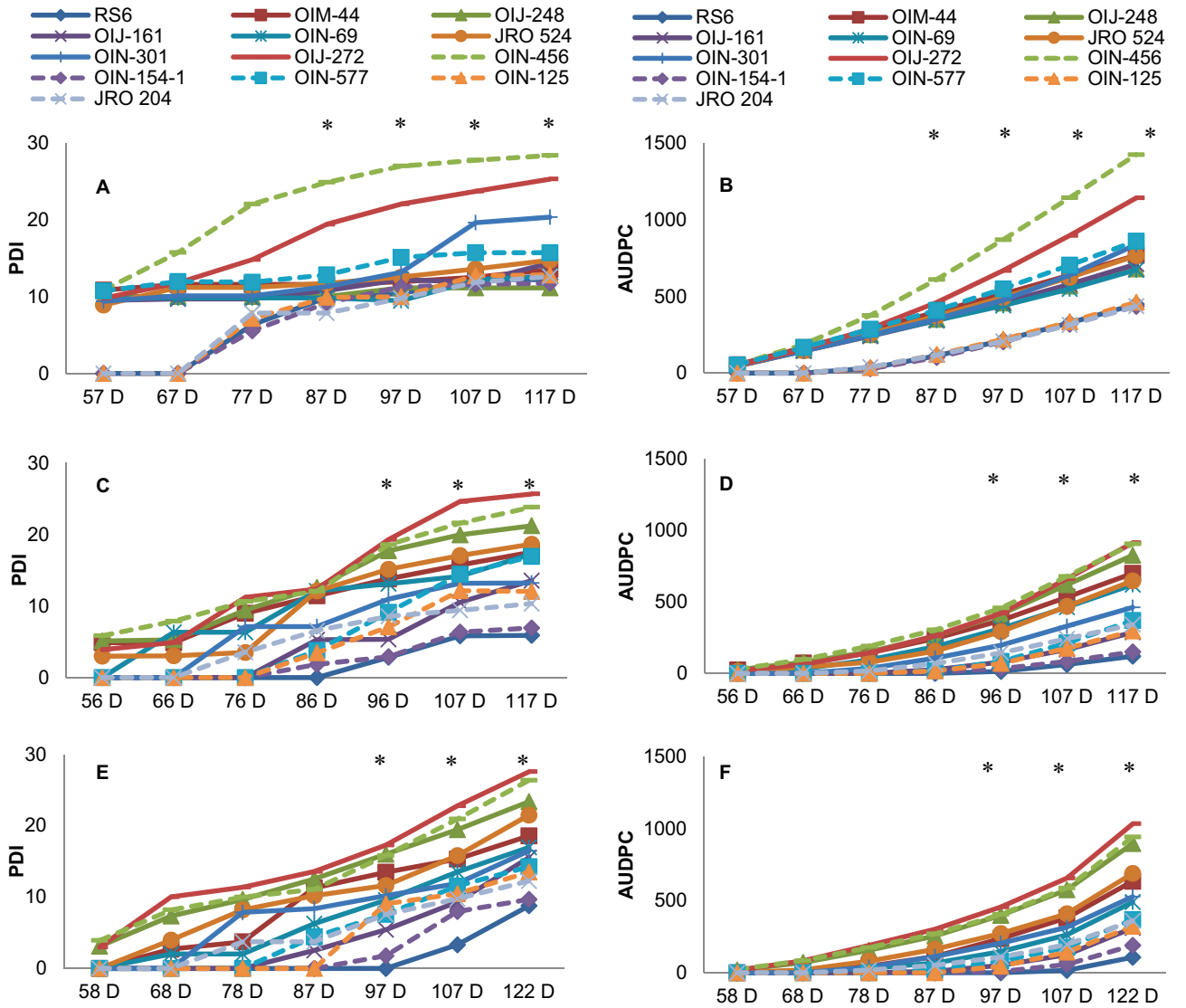

Fig. 6 Depiction of disease development through PDI (**A**, **C**, **E**) and AUDPC (**B**, **D**, **F**) on different jute genotypes under the sick plot conditions during 2016 (**A**, **B**), 2017 (**C**, **D**), and 2018 (**F**, **F**). *Significant differences between the treatment means ($P = 0.05$) LSD values: for PDI, 2016 are 3.3, 3.3, 3.7, and 3.1 at 87, 97, 107, and 117 DAS, respectively; for PDI, 2017 are 5.2, 2.5, and 3.7 at 96, 107, and 117 DAS, respectively; for PDI, 2018 are 3.3, 5.3, and 3.3 at 97, 107, and 122 DAS, respectively; for AUDPC, 2016 are 66.4, 80.7, 96.2, and 102.3 at 87, 97, 107, and 117 DAS, respectively; for AUDPC, 2017 are 137, 144, and 148 at 96, 107, and 117 DAS, respectively; and for AUDPC, 2018 are 126, 140, and 164 at 97, 107, and 122 DAS, respectively

and helps perennation under unfavourable conditions. Nutrient stored in sclerotia provides the pathogen an edge over other competing soil inhabitants. As a result, the pathogen can survive as long as 15 years in an infested soil (Baird et al. 2003). However, new infection is initiated by actively growing freshly produced germtubes, often from few cells on the surface of a sclerotium. The role of sclerotia in disease development is thus limited as a source of such infection hyphae (Gupta et al. 2012). Our observations also suggest that inoculum containing sclerotia were less effective in initiating stem lesions compared to the actively growing hyphal tips.

Localised inoculation with Mp at different positions of stem and variable plant age presented interesting results. Lesion size was the maximum when inoculation was done at the mid–length of a stem. An actively growing jute plant exhibits strong apical dominance. Most of the commercial cultivars of jute grow faster than other field crops. For example, jute cultivar JRO 524 attains a height of 192 cm at 70 DAS, effectively registering a growth rate of ~ 3 cm/day (Palit et al. 2004). Sustaining such apical growth rate is

achieved under the influence of plant hormone auxin, apart from lower sugar availability to the lateral buds (Kebrom 2017). Auxin synthesis occurs at the shoot and root apices which later got transported to distant parts (Adamowski and Friml 2015). This maintains a gradient of auxin within plant tissues, higher in the growing fronts and gradually lower in portions away from tips. Mah et al. (2012) observed that several genes involved in auxin synthesis, transport, and signalling were differentially modulated in model plant *Medicago truncatula* due to Mp infection. They also established that exogenous auxin application was effective in induction of defence response against Mp. Chowdhury et al. (2014) also reported from an in vitro study that actively growing sesame root tips were free from Mp infection. This was universal for both susceptible and resistant genotypes. Apart from auxin, the interplay of ethylene, jasmonic acid, and salicylic acid also determine the pathogenicity of Mp in crop plants (Schroeder et al. 2019). Arabidopsis mutant in terms of ethylene, jasmonic acid, and salicylic acid signalling and biosynthesis exhibited enhanced susceptibility to Mp. Cell wall strengthening enzymes like peroxidase (POX) and phenylalanine ammonia lyase (PAL) also determine the pathogenicity against Mp (Khan et al. 2018). Meshram et al. (2013) observed that the basal portion of the jute stem exhibited higher PAL activity compared to the middle part. This is commensurate with our observations of larger lesion development at the middle portion of jute stem due to Mp inoculation. These pieces of evidence suggest differential lesion development upon Mp inoculation in jute stem was due to complex interplay of plant hormones, plant developmental stage variation especially lignin, and other cell wall deposition. Nevertheless, our study has standardised that inoculation at the middle of stem length of a mature ($\geq$ 70 DAS) jute plant for better phenotypic expression. Inoculation at the stem base as proposed by Grezes–Besset et al. (1996) or at the stem apex (Twizeyimana et al. 2012) produced smaller lesions in our experimental conditions. Larger lesions provide higher resolution and better differentiation between the treatments. This is evident from the two stem inoculation experiments to compare plant ages and inoculation sites. Hence, screening of selected lines through stem lesion development was done by inoculating at the middle of the stem and measuring lesion length at 6 DPI.

Screening of host genotypes under sick plot conditions often resembles the reactions obtained under commercial fields. However, it demands resources in higher degrees. The usefulness and acceptance of alternative screening methods hence depend on the ease of the technique and more importantly, its ability to distinguish between the host genotypes in accordance with their field reactions. Infecting jute leaves or roots failed to differentiate between the host genotypes having contrasting susceptibility; hence, not useful. Among the different techniques evaluated in the present study, stem tape inoculation and a combination of soil–seed infestation methods could effectively differentiate among the host genotypes. Field screening (Fig. 6) under the sick plot conditions for three consecutive years and the stem inoculation method (Table 3) grouped RS6 and OIN-154-1 as resistant. However, OIN-456 (susceptible as per field screening) also found a place here. Repetitions of the experiment did not change the outcome. On the other hand, JRO 524 and OIJ-272 represented the susceptible group. Intermediate JRO 204 was rightly grouped close to the resistant types (Table 3). Soil and seed infestation method inside a growth chamber placed RS6 and OIJ-272 to two extremes (Table 3). However, reactions of other genotypes were not akin to those under the field conditions. Higher pathogen load under the growth chamber conditions compared to those found in the field conditions (Mihali and Alcorn 1982; Pickel et al. 2020) may be a plausible reason for such deviation. A whole crop season (90 days) greenhouse screening of sunflower against Mp (Siddique et al. 2021) used three times lower sclerotia load. But, further reduction in inoculum load produced inconsistent results in our hands, forbidding the use of lower doses. Calibration of inoculum hence is an important step suggested by Pickel et al. (2020) for such methods. However, even with the optimised concentration of Mp sclerotia, all differences between results obtained under the sick plot and the growth chamber conditions could not be obliterated. In the commercial field, stem rot in jute becomes prevalent after 60 DAS; disease reaction is hence expressed in mature plants. In contrast, necrotic streak or morbidity was recorded till 26 DAS under the growth chamber conditions, effectively determining seedling resistance of the test genotypes. Resistance against Mp is governed by a complex molecular mechanism, still not fully understood. Hence, often different sets of molecular markers are identified depending on screening environments (Coser et al. 2017). Mihaljcenic (1978) tested four screening techniques in sunflower under field and greenhouse conditions. However, none of these produced satisfactory results. Among the several methods tested by us in the present study, many either failed to distinguish between contrasting genotypes or, results were different than those found under the sick plot conditions. Nevertheless, the outcome from stem inoculation of adult plants mostly matched field screening reactions. Moreover, the method is easy, robust, and can be performed under both field and greenhouse conditions to screen a large population. To the best of our knowledge, this is the first comparison of different screening methods to determine the resistance status of jute against Mp. We are presently applying these findings to establish the genetic basis of resistance against stem rot disease in a segregating population of jute.

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