Characterization of *Dickeya zeae* Causing Bacterial Stalk Rot of Maize and Advancing Efficient Inoculation Methods and Susceptible Check for Germplasm Screening

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Maize is a staple food in many regions and a vital agricultural commodity that supports diverse industries and providing essential carbohydrates, proteins, vitamins, and minerals, make it a critical component of food security worldwide. Bacterial stalk rot (BSR) caused by *Dickeya zeae* is considered the most destructive disease of maize widespread in tropical, subtropical and temperate region of the world. This bacterium has wide host range causing soft rot which make it difficult to manage the bacterium. Cultural and biological control approaches are ineffective, and no effective

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chemicals are available for the management of the disease. Therefore, identification and development of resistant cultivars could be an appropriate approach. For development of resistance in plant, identification of resistant source is pre-requisite. The present study was conducted for identification of susceptible genotypes and effective artificial inoculation methods to create a higher disease pressure under suitable environmental conditions which could be utilized for germplasm screening. Under glasshouse conditions, syringe inoculation was found to be the most appropriate method; therefore, it was used for screening of ten maize genotypes against BSR. Out of the 10 genotypes screened against BSR in the glasshouse, Chakrata Local was found to be the most susceptible and PSM 6 was most resistant. Under field conditions, seven inoculation methods were further evaluated for their efficacy in creating higher disease pressure in Chakrata Local and PSM 6. Syringe inoculation was found to be most consistent and effective method of inoculation under both glasshouse and field conditions followed by toothpick inoculation. The techniques identified in the present investigation are of considerable importance for development of artificial epiphytic conditions in the field for screening of appropriate resistant sources which could be used both in the farmers' field and breeding programs.

1. INTRODUCTION

Plant diseases pose a significant threat to global food security and the economy. Crops such as maize, wheat, rice, and soybeans are highly susceptible to a variety of pathogens, leading to adverse effects on crops, farmers, and the broader agricultural industry. Effective disease management hinges on the timely and accurate detection and diagnosis of plant diseases. This can prevent the spread of diseases, reduce crop losses, and minimize the reliance on harmful chemicals. Additionally, it enables farmers to adopt appropriate control measures, thereby enhancing production efficiency. Maize, in particular, is one of the world's most vital crops, cultivated in numerous countries and valued for its versatility. It serves multiple purposes, including as food, animal feed, bioethanol, and raw material for various industrial products. Given its importance, protecting maize from diseases is crucial for maintaining its productivity and supporting global agricultural sustainability. Bacterial stalk rot (*Dickeya zeae*) is the most devastating disease of maize, widespread in Asia, Africa, North America, Central America, South America, and Oceania. In India, losses vary from region to region. On basis of climatic condition, pathogen causes 21 to 98 per cent grain yield losses [1]. The disease was first reported by Prasad (1930) as *Erwinia dissolvens* whose symptoms resembled more closely with *E. chrysanthemi* pv. *zeae*, causal agent of soft rot on wide range of plant species. Dickey (1979) on basis of 12 physiological characters proposed a major taxonomic change that separated *E. chrysanthemi* into five species under new genus *Dickeya* based on rRNA sequence**.** The

pathogen has been re-classified as *Dickeya zeae* [2,3]. It is a gram-negative bacterium occurring singly or in pairs, motile, straight rod with rounded ends and non-spore forming. The size of bacterium varies from 0.8-3.2× 0.5-0.8 μm (average 1.8×0.6 μm) depending on growth conditions and carbon source of medium [4]. There are usually 8-11 peritrichous flagella [5]. *Dickeya* is plant pathogenic bacteria that are facultative anaerobes [6].

Major symptoms BSR of maize includes discoloration of leaf sheath which slowly spreads to the stalk and leaves. Appearance of water-soaked symptoms on stem and disintegration of pith tissue emitting foul smell. In severe condition, wilting of whole plant occurs. In *Kharif* season, crop has the most susceptible stage (tasseling or silking) coinciding with the annual monsoon rainfall aggravating disease development. A temperature of 35°C, 70% relative humidity and inoculum level of 2×10^8 cfu per ml are essential for disease development in 15 to 30 days old maize plants [7]. Presently, no effective chemicals are available for management of disease. However, it can be achieved by cultural practices and biological control, but these methods have not been found much effective [8]. Development of resistant cultivars is an appropriate approach for which identification of resistant sources is pre-requisite. For screening of germplasm, appropriate effective inoculation techniques are needed to produce higher disease pressure under field conditions which ensure maximum contact between the host and pathogen or delivery of pathogen inoculum at the targeted place to facilitate proper screening. Therefore, the

present investigation aimed to create artificial epiphytic condition through suitable inoculation technique for screening of maize germplasm.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Pathogenicity Test

The glasshouse experiment was conducted during December 2020 at GBPUA&T, Pantnagar, Uttarakhand, India. For isolation of suspected bacterium, leaf and stem tissues showing characteristic symptoms of the disease were collected from the field (Fig. 1). The diseased tissues were disinfected with 70 per cent alcohol [9]. Biochemical and physiological characterization was performed for further confirmation of the pathogen. The diseased stem tissue was cut into 2 halves with a sterilized razor blade, left for 30 minutes in sterile water for bacterial diffusion and 100 µl suspension was plated onto Petri plates containing Nutrient Agar (NA) medium. The pure culture of the bacteria was obtained by streaking a freshly grown single colony on a fresh nutrient agar plate and incubating at 28°C for two days. Pathogenicity test was performed by inoculating 21 days old susceptible maize variety (CM 600) by stem injection method, under glasshouse conditions. A diagonal hole, deep into pith was made with sterile pointed needle (2 mm diameter). One milliliter of bacterial suspension (1×10^8 cfu/ml) was injected into plant with hypodermic syringe. Suitable controls were maintained using distilled water. The pathogen was re-isolated from the inoculated plants showing typical symptoms like those observed in the field and compared with original cultures *in vitro*.

2.2 Morphological and Biochemical Test

Morphological test carried out using 100 µl of bacterial suspension streaked on three different selective medium such as Logan's medium, Nutrient glycerol manganese chloride medium (NGM), Crystal violet polypectate medium (CVP) and incubated at 27±1°C for 48 hours with three replications. In biochemical test, Potassium hydroxide test by mixing single colony of bacterium with 50 µl of 3% (w/v) KOH until even suspension obtained and checked for presence of string of slime. Catalase production test, bacterium covered with drops of hydrogen peroxide. Oxidase test for confirmation of cytochrome oxidase enzyme using oxidase filter paper disks impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-naphthol moistened with de-ionized water. A loopful of pure bacteria transferred aseptically on the disk and observed for colour change within 30 seconds. Gelatin liquification test, gelatin medium inoculated with bacterium for ability to produce an extracellular enzyme gelatinase and incubated at 27±1°C for 2 days. Agar surface flooded with 0.2 per cent mercuric chloride solution in 20% per cent.
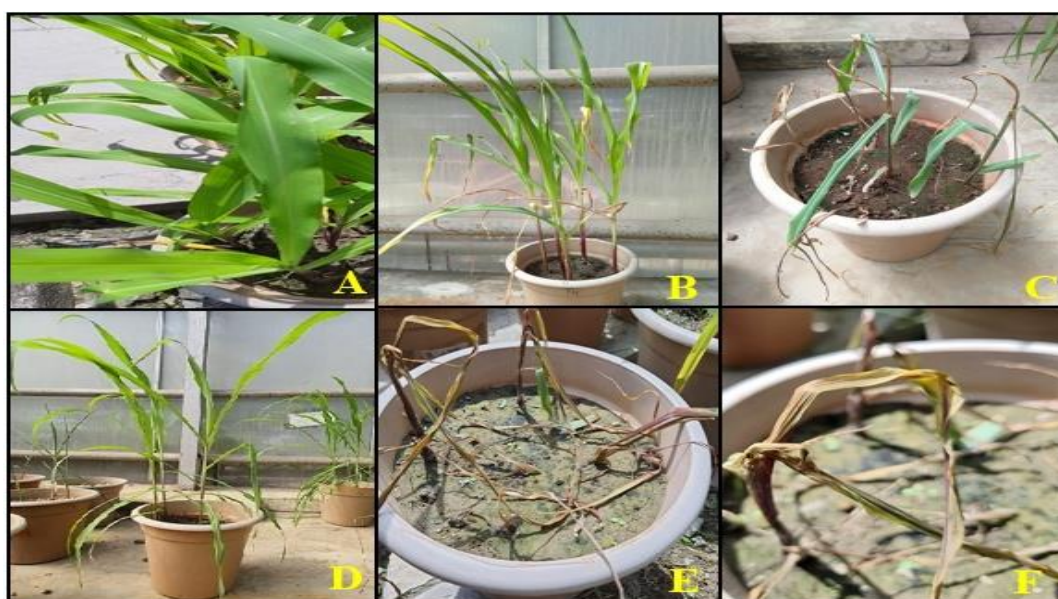

Fig. 1. Pathogenicity of stalk rot of maize under glasshouse condition
Before inoculation (A and D), After inoculation (B, C, E and F)

2.3 Molecular Characterization

Bacterial genomic DNA was isolated using CTAB method [10]. Amplification of genomic DNA was performed by polymerase chain reaction in Applied Biosystem Thermal Cycler. PCR was carried out with universal primer pair 27F and 1492R with total of 35 cycles [11]. PCR products were then sent for purification and sequencing by a commercial sequencing service provider Eurofins Genomics India Pvt. Ltd. The received sequences were blast using NCBI and phylogenetic analysis was done using MEGA 11 software.

2.4 Development of Growth Curve

Inoculation of 10 ml Nutrient broth medium with 100 µl of bacterial suspension having 0.7 to 0.9 OD value at 660 nm and incubated at 27±1°C, 150 rpm with three replications for each treatment [12]. Observations concerning colony-forming unit (cfu) @ 10^{-8} dilutions recorded at 0, 12, 24, 36, 42 and 48 hours of time interval by serial dilution method. The growth curve for *Dickeya zeae* developed at different time intervals, at 27±1°C on nutrient broth media. The initial phase or lag phase of bacterium showed less mean growth, log phase had exponential growth by utilizing all available nutrient sources in the medium, stationary phase showed constant growth followed by decline phase due to unavailability of the nutrients, also known as death phase.

2.5 Inoculum Preparation

For evaluation of different inoculation methods and germplasm screening bacterial cell suspension was prepared from 48 hours old culture. Inoculum was mass multiplied on nutrient broth (NB) media. Flasks containing 250 ml NB were inoculated with 1 ml of bacterial suspension and incubated in BOD incubator cum shaker for 36 hours at 27±1°C and 150 rpm. Inoculum used for inoculation was adjusted to 1 $\times 10^8$ cfu/ml by adding sterile distilled water.

2.6 Glasshouse Experiment

Surface-sterilized maize seeds were planted in pots containing 5 kg sterilized moist soil. After thinning, 5 plants per pots were maintained and 21 days old plants were used for the experiment. In glasshouse, day/night temperature 32/25°C, 12 hr photoperiod, and >90% RH were maintained.

In glasshouse conditions, efficacy of different inoculation methods i.e. syringe, tooth pick, cotton swab and whorl spray inoculation against four genotypes were tested (PSM 6, Rasi 4212, CM 600, and Chakrata Local). After inoculation, high humidity maintained for disease development and observed for symptoms. Number of plants showing symptoms out of total plant were observed by splitting stem into two halves by sharp knife. The trial was conducted in two factor completely randomized block design (CRD) with four replications.

For screening germplasm against susceptibility to BSR, 10 different genotypes of maize viz. ADV 7022, IIMR PBT POOL, RCRMH 41, CM 500, CM 600, PSM 6, Early Composite, Surya, Chakrata Local and Rasi 4212 inoculated with syringe inoculation to obtain most susceptible cultivar that can be used as susceptible check. Trial was conducted as completely randomized design (CRD) with four replications.

2.7 Field Experiment

The field experiment was conducted during *Kharif* and *Rabi* seasons of 2021–22 at two different plots of Norman Borlaug Crop Research Centre, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India with two different maize genotypes Chakrata Local and PSM 6 sown at spacing of 75 x 20 cm. Atrazine @1 kg/ha was applied before seedling emergence along with 1-2 inter culture operations for prevention of weed growth. Thinning was done after 25 days of sowing to maintain distance of 20 cm between plant to plant. At the pre-silking stage, 45 days old plants were artificially inoculated using seven different inoculation methods i.e. syringe, toothpick, stem cotton swab, node cotton swab, node sabudana, whorl sabudana and spray inoculation. The experiment was conducted in a factorial randomized block design (RBD) and replicated thrice.

2.8 Disease Assessment

Disease incidence and disease severity (PDI) was recorded near the dry silk stage of maize. Disease incidence was based on number of plants infected out of the total plants assessed. For disease severity randomly ten plants from each replication of a particular treatment were selected and rated for disease reaction on a 1-9 rating scale [13] (Table 4). The disease incidence and percent disease index were calculated using the following formulae:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plant}}{\text{Total number of plant}} \times 100$$

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Number of plants observed} \times \text{Maximum rating grade}} \times 100$$

2.9 Statistical Analysis

Data was analyzed statistically using Computer Programme SPSS and STPR using Completely randomized design (CRD) and Randomized block design (RBD). Data were subjected to angular transformation whenever required. The treatment was compared by means of critical differences (CD) at 5% level of significance.

3. RESULTS

3.1 Morphological and Biochemical Characterization

Bacterial identification was done by various physiological and biochemical properties on basis of Bergey's manual of determinative bacteriology (1994). Morphological study through binocular compound microscope performing gram staining showed pink to reddish colored, rod-shaped cells. On nutrient agar media, shiny colonies with round convex raised centre, entire or sculptured with irregular margin depending on the moisture content were observed. Logan's media show red to pink colored colonies with deep red centre, colourless border. Translucent, slightly convex colonies with regular edges producing blue pigment indigoidine on NGM media. Blue pigment (indigoidine) used as chemotaxonomic trait for rapid identification of *Dickeya zeae* [14]. Slightly rough, concave colonies with deep pit formation on CVP medium [15]. Deep, cup-shaped cavities and characteristic colony morphology are two identification marks of soft rot Dickeya (Fig. 2).

Biochemical test *viz.* KOH confirmatory test, string of slime lifted with sterile loop, due to liberation of cellular DNA leading to viscous slime formation, confirming gram negative nature [16]. Catalase production test, gas bubbles releases due to evolution of O_2 gas from hydrogen peroxide similar to findings of Reiner [17]. Catalase enzyme helps protecting bacteria by breaking H_2O_2 into H_2O and O_2 and neutralizes its bactericidal effects. In oxidase test, no colour changes produced within 30 seconds of bacterium transferred aseptically on disk. Due to absence of cytochrome oxidase enzyme, *Dickeya zeae* failed to oxidizes the test reagent [18]. In gelatin liquification, bacterium liquefied gelatin and growth surrounded by a clear zone when agar surface flooded with 0.2% mercuric chloride solution in 20% HCl. Leboffe and Pierce [19] obtained similar growth pattern of bacteria mentioned presence of gelatinase enzyme (Fig. 3). This enzyme usually acts on surface of medium, necessary to pour medium uniformly in petriplates otherwise it leads to false negative interpretation (Table 5).

3.2 Molecular Identification of the Isolates

Test bacterium was subjected to 16s ribosomal RNA (rDNA) genomic sequencing by the Sanger-Nicolson method, using universal primer 27f and 1492r. The sequencing results were obtained and multiple alignment was done. Sequencing and BLAST analysis of the resulting PCR products showed that the isolate has closest belonging to *Dickeya zeae* (GenBank Accession No. OR398800). The phylogenetic analysis with MEGA software was done forming clade with *D. zeae* strains (Fig. 4).

Fig. 2. Growth of *Dickeya zeae* on different media (A) Nutrient agar, (B) Logan's medium, (C) NGM media and (D) CPV medium
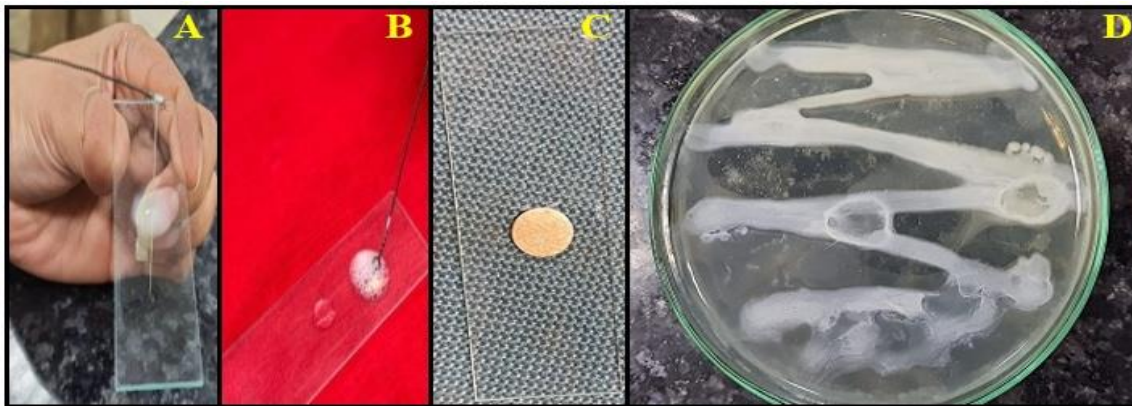
**Fig. 3. Biochemical activities of *Dickeya zeae* on (A) 3% KOH, (B) Catalase,
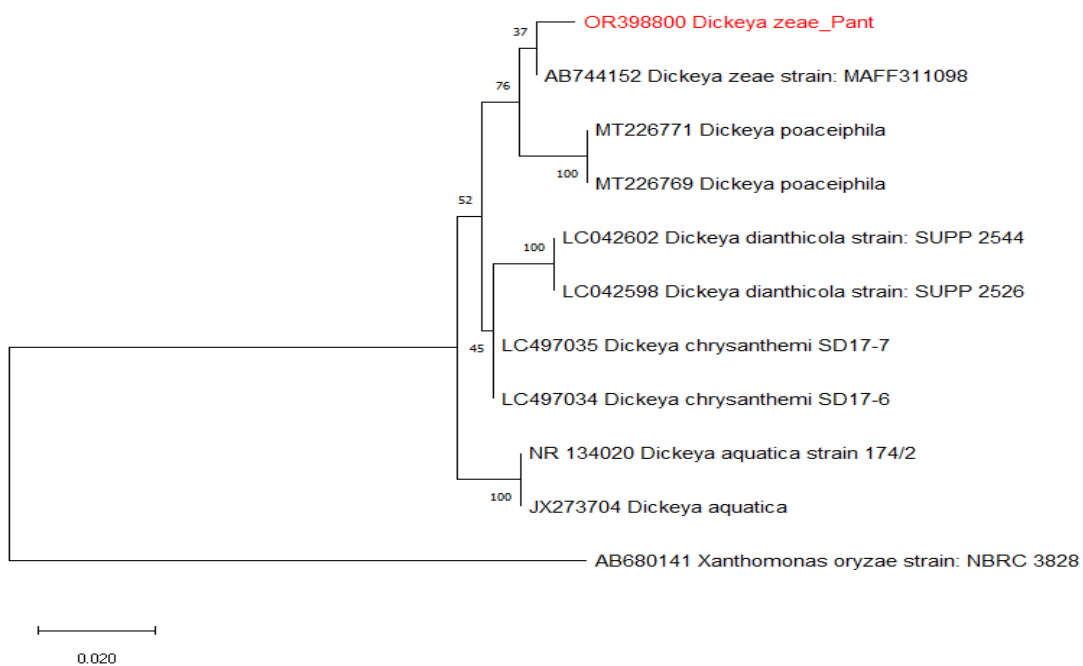(C) Oxidase and (D) Gelatin liquification**

**Fig. 4. Phylogenetic tree derived from 16s rRNA of *Dickeya zeae* by using neighbour joining
model in MEGA (version 11), based on partial sequence of dnax gene**

3.3 Development of Growth Curve

In present study, the bell-shaped growth pattern observed with three distinct phases i.e. lag, log and decline phase were evident. The duration of lag phase observed between 0-24 hours (0-42 cfu/ml), log phase between 24-36 hours (42-179 cfu/ml) and decline phase after 36 hours as cfu/ml declined from 179 to 35.5 cfu/ml after 42 hours of inoculation, further reduced to 19 cfu/ml after 48 hours (Fig. 5). Standard curve studies revealed that the pathogen had log phase at 27hrs under controlled favorable conditions (Hooda et al. 2018). We have also recorded the

maximum number of bacterial cell (179 cfu/ml) at 36 hours after incubation.

3.4 Glasshouse Experiment

3.4.1 Pathogenicity test

Collected diseased samples were confirmed as bacteria by ooze test visible inside microscope. Pathogenicity test performed for proving Koch postulates and plants were observed with rapid soft rot of parenchymatous tissues, water-soaked lesions, changes in leaf morphology, chlorosis and curling of leaf from margin (Fig. 6).

Symptoms resembled exactly similar like noticed under field conditions.

3.4.2 Evaluation of different inoculation method

Different maize genotypes were evaluated through different inoculation methods to obtain susceptible genotype and best inoculation method (Fig. 7). The recorded observation based on per cent disease incidence and disease severity which varied with different inoculation methods and genotypes. Experiment results pertaining to different inoculation methods, evaluation against four genotypes presented in Table 1. Maximum mean incidence (45.98%) recorded in syringe inoculation followed by tooth pick method (36.53%) at par with stem cotton swab method (29.24%). No disease incidence in spray inoculation method. Among genotypes, maximum disease incidence observed in Chakrata local (40.26%) followed by CM 600

(31.28%). Minimum incidence observed in PSM 6 (13.27%). Maximum severity (50.56%) recorded in syringe inoculation followed by tooth pick method (45.89%) at par with stem cotton swab method (43.25%). Among genotypes, maximum severity observed in Chakrata local (45.12%) followed by CM 600 (34.40%).

3.4.3 Evaluation of different genotypes

Experiment results pertaining to evaluation of different genotypes against incidence and severity of BSR presented in Table 2. Maximum mean disease incidence (58.05%) recorded in Chakrata local followed by CM 600 (51.03%) found to be at par with Surya (48.14%). Minimum incidence was observed in PSM 6 (16.55%) at par with Rasi 4212 (19.72%). Maximum mean severity found in Chakrata local (72.79%) at par with CM 600 (71.47%) while Minimum PDI observed in PSM 6 (39.17%) (Fig. 8).
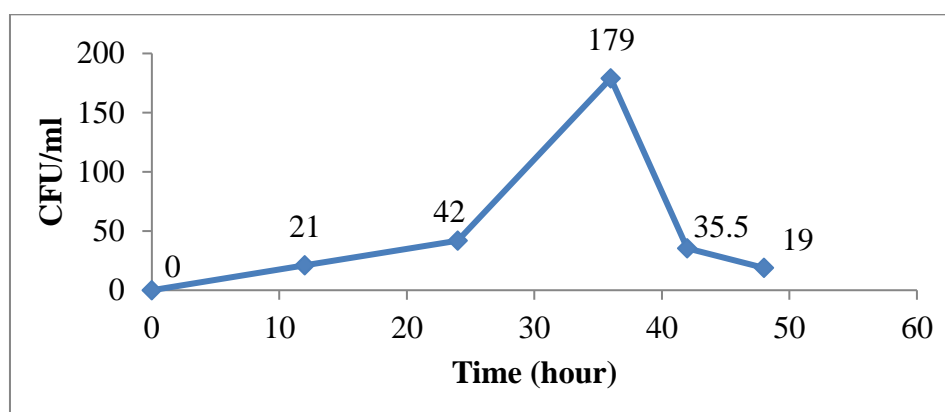

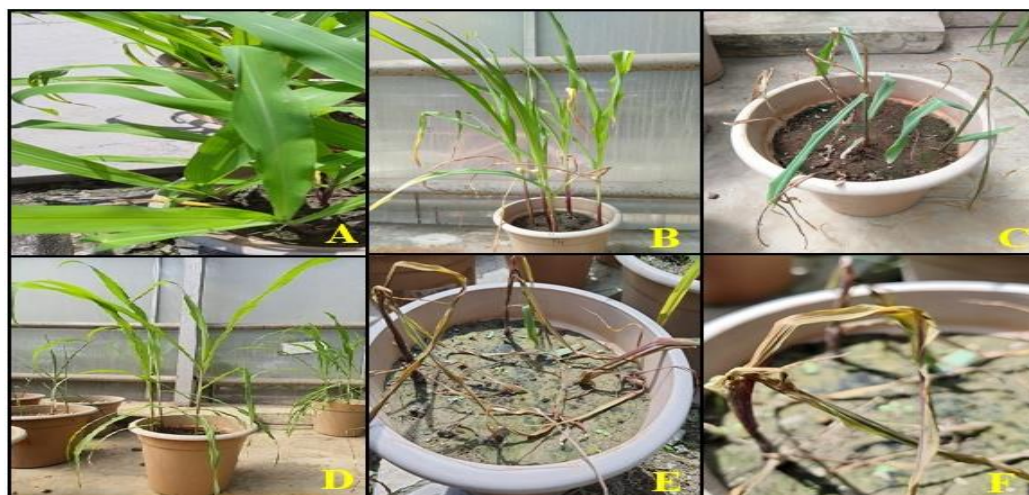**Fig. 5. Growth curve of *Dickeya zeae* on Nutrient broth medium at 10^{-8} dilution**

**Fig. 6 Pathogenicity of stalk rot of maize under glasshouse condition
Before inoculation (A and D), After inoculation (B, C, E and F)**

Table 1. Incidence and severity of maize stalk rot caused by *Dickeya zeae* in different genotypes by different inoculation methods under glass house conditions

Inoculation method (B) Germplasm (A)	Incidence of maize stalk rot (%)					Severity of maize stalk rot (Percent disease index)				
	CM 600	Chakrata local	PSM 6	Rasi 4212	Mean (B)	CM 600	Chakrata local	PSM 6	Rasi 4212	Mean (B)
Syringe	63.67 (59.18) *	73.33 (64.96)	20.00 (18.55)	40.00 (39.21)	45.98	69.37 (54.00)*	81.48 (68.49)	28.89 (24.45)	48.89 (44.30)	50.56
Tooth pick	52.33 (49.90)	66.67 (59.18)	13.33 (12.70)	20.00 (18.55)	36.53	61.44 (52.41)	74.81 (61.11)	25.19 (21.10)	41.48 (40.05)	45.89
Stem cotton swab	46.67 (41.06)	60.00 (53.12)	6.67 (5.85)	20.00 (18.55)	29.24	59.74 (51.19)	68.89 (59.11)	22.96 (20.61)	38.52 (36.34)	43.25
Spray inoculation	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00
Mean (A)	31.28	40.26	13.27	21.92	-	34.40	45.12	22.79	30.67	-
CD @ 5%										
Method of inoculation (A)	8.44					3.33				
Genotype (B)	3.42					1.33				
(A×B)	11.8					4.66				
SE(m)										
Method of inoculation (A)	3.57					1.42				
Genotype (B)	1.57					0.19				
(A×B)	5.13					1.61				
CV %	9.55					8.02				

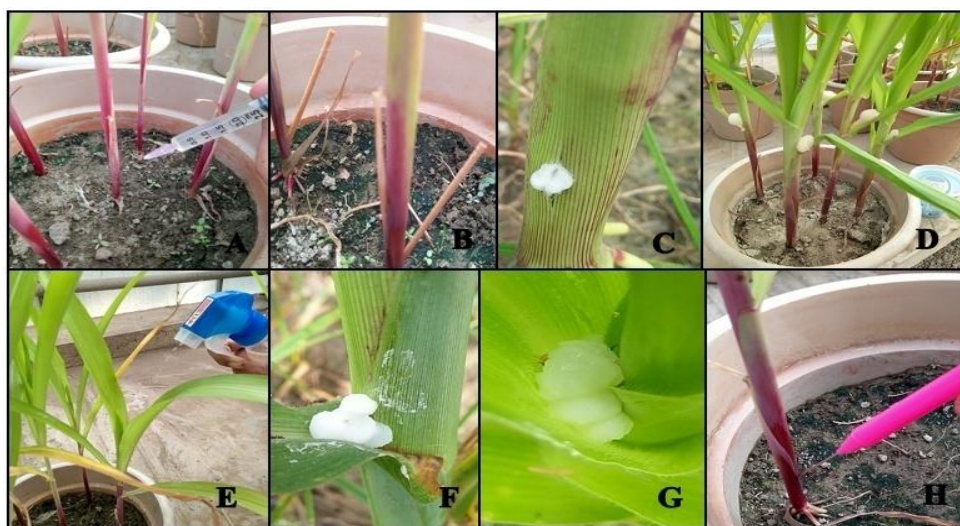*Figures in the parenthesis are angular transformed values*

**Fig. 7. Different methods of artificial inoculation under glasshouse conditions
A. Syringe, B. Tooth-pick, C. Stem cotton swab, D. Node cotton swab, E. Spray, F. Node
sabudana, G. Whorl sabudana and H. Stem pricking**

**Fig. 8. Screening of the maize germplasm against bacterial stalk rot of maize caused by
Dickeya zeae against syringe inoculation method under glasshouse condition, (A) ADV 7022,
(B) IIMR PBT POOL, (C) Surya, (D) CM 500, (D) Early composite, (E) RCRMH 41, (F) CM 600, (F)
Chakrata local, (F) PSM 6 and (F) Rasi 4212**

3.5 Field Experiment

3.5.1 Evaluation of inoculation methods against BSR

The different inoculation methods under field conditions were examined on maize plants to find out most appropriate artificial inoculation method for disease development (Table 3). Maximum incidence found in syringe inoculation (51.01%) followed by tooth pick (39.41%) while stem cotton swab (27.49%) and node cotton swab (20.97%) found to be at par. In syringe and tooth pick inoculation significantly higher disease incidence recorded in Chakrata Local (31.17%) compared to PSM6. Severity of maize stalk rot revealed maximum disease index (58.23) in syringe inoculation method followed by tooth pick (46.98) and stem cotton swab (37.81). Chakrata Local showed significantly higher percent disease index (38.85) as compared to PSM6 (29.20). However, inoculation methods with low severity (below 20), no significant differences observed among these two genotypes.

Table 2. Screening of maize germplasm against *Dickeya zeae* by syringe inoculation method under glass house conditions

Germplasm	Incidence of maize stalk rot (%)	Severity of maize stalk rot (Percent disease index)
ADV 7022	45(41.81)*	73.33(65.41)
IIMR PBT POOL	45(41.81)	71.85(65.03)
Surya	55(48.14)	57.04(53.38)
CM 500	35(34.7)	65.93(58.16)
Early composite	45(41.81)	75.56(69.75)
RCRMH 41	30(29.88)	64.44(58.01)
CM 600	60(51.03)	82.22(71.47)
Chakrata local	65(58.05)	86.67(72.79)
PSM 6	20(16.55)	40.00(39.17)
Rasi 4212	25(19.72)	60.00(55.47)
CD @ 5%	13.19	17.22
SE(m)	4.89	6.133
CV%	15.41	13.69

Figures in the parenthesis are angular transformed values

Table 3. Effect of different inoculation methods on incidence and severity of stalk rot of maize caused by *Dickeya zeae* under field conditions

Genotype (B)	Incidence of maize stalk rot (%)			Severity of maize stalk rot (Percent disease index)		
	Chakrata Local	PSM 6	Mean B	Chakrata Local	PSM 6	Mean B
Method of inoculation (A)						
Syringe inoculation	100.00(58.98) *	94.15(42.78)	51.01	78.75	64.28	58.23
Tooth pick	73.21(50.62)	63.28(39.01)	39.41	69.81	59.26	46.98
Stem cotton swab	52.78(28.78)	58.33(31.13)	27.49	51.23	46.91	37.81
Node cotton swab	24.09(21.45)	26.79(23.38)	20.97	48.77	47.53	33.58
Node sabudana	12.70(10.6)	5.56(3.73)	9.83	16.05	16.05	15.98
Whorl sabudana	4.55(3.01)	2.94(1.04)	2.37	11.11	12.35	10.73
Spray inoculation	8.33(7.70)	5.56(3.73)	5.59	11.73	13.58	12.67
Mean A	31.17	20.91	-	38.85	29.20	-
CD @ 5%						
Method of inoculation (A)	7.12			5.09		
Genotype (B)	2.95			2.11		
(A×B)	10.07			7.21		
SE(m)						
Method of inoculation (A)	2.30			1.65		
Genotype (B)	1.23			0.88		
(A×B)	3.53			2.53		
CV%	12.42			10.87		

Figures in the parenthesis are angular transformed values

Table 4. Disease rating scale for BSR of maize (Hooda *et al.*, 2018)

Rating scale	Degree of infection (per cent sensitivity)	PDI	Disease reaction
1	The infection is limited to a very small spot in the pith at the site of inoculation (<10%).	<11.11	Resistant(R)
2	Disease infection spreads in one-fourth of the length of the inoculated internodes in the pith and cortical tissue Rind not infected (10.1-20%).	22.22	(Score: < 3.0) (Severity: < 30%)
3	Disease infection spreads in half of the length of the inoculated internodes in the pith and cortical tissue. Rind not infected (20.1-30%).	33.33	(PDI: < 33.33)
4	Disease infection spreads in three fourth of the length of the inoculated internodes in the pith and cortical tissue (30.1-40%).	44.44	Moderately resistant (MR) (Score:3.1-5.0)
5	Disease infection covers entire length of the 55.55 inoculated internodes but does not cross the nodal plates. The rind is green and the symptoms are not visible externally, but the plant shows signs of wilting (40.1-50%).	55.55	(Severity: 30.1-50.0%) (PDI:33.34-55.55)
6	The nodal plates are crossed and the increasing 66.66 infection also covers the adjacent internode of the inoculated plants (50.1-60%).	66.66	Moderately susceptible (MS) (Score: 5.1-7.0) (Severity: 50.1-70.0%)
7	The disease spreads in two internodes. The pith and 77.77 cortical tissues are degenerated. The rind of the inoculated internodes is affected and the plant wilts (60.1-70%).	77.77	(PDI-55.56-77.77)
8	The disease spreads in three internodes. The pith and cortical tissues are degenerated. The rind of the inoculated internodes is affected and the plant wilts. Ear length and size is considerably reduced as compared to healthy plants (70.1-80%).	88.88	Susceptible (S) (Score: >7.0) (Severity: >70%)
9	The disease spreads in more than three internodes. The pith, cortical tissues and vascular bundles rot and disorganised. Rind discoloured, plants wilt and may topple down (>80%).	99.99	(PDI: >77.77)

Table 5. Biochemical characteristics of test bacterium *Dickeya zeae* isolated from maize

Sl. No	Tests	Reaction	Response
1	Gram Reaction	Red bacterial colony	-
2	KOH Confirmatory Test	String of slime	+
3	Oxidase test	No colour change	-
4	Catalase test	Gas bubbles	+
5	Gelatin liquefaction	Clear zone formation	+
6	CPV medium	Deep pit formation	+
7	NGM medium	Blue pigment production	+
8	Logan's medium	Colony with red centre	+

4. DISCUSSION

Bacterial stalk rot disease is severe disease all over the world. In India, distinct symptoms were noticed in different places, with varying severity and occurrence. BSR is important concern in maize growing region in Tarai region of Uttarakhand, India. BSR is predominant in inflicting financial losses to the farmers. In order to effectively manage the disease and address the threat caused by the disease in Uttarakhand, India, an integrated approach utilizing disease resistant cultivar through germplasm screening must be devised. BSR emerged as an important disease in Pantnagar and the Himalayan foothills of Uttarakhand, India. The disease is characterized by wilting of apical leaves from margin and mainly affected stem showing water-soaked lesions that later turned reddish brown in color. Stem pith disintegrate and slimy soft-rot tissues were observed which eventually leads to wilting of entire plant. Losses are due to premature death of plant and harvest losses associated with stalk breakage. Lodged plant emit foul odour. The decay spreads rapidly downward and eventually there is complete rotting. Similar symptoms have also been observed from other locations [7]. The incidence and PDI show the status of the disease. The overall incidence and PDI of BSR were recorded higher in case of stem injection method of inoculation which has been also found effective in symptom development of bacterial stalk rot of sorghum [20].

Among inoculation methods, maximum severity of BSR was observed by syringe inoculation method and least severity in whorl sabudana and spray inoculation. Results of artificial inoculation with syringe and tooth pick inoculation gives more successful infection compared to other methods for screening germplasm against BSR [21,22]. Syringe inoculation as most promising method followed by tooth pick because in both the methods bacterial inoculum load is directly introduced into the close proximity of stem pith tissue. Investigation also confirmed in sorghum plants, stem injection by syringe inoculation method obtained maximum disease incidence and severity against test pathogen [23,21]. Leaf spray inoculation found ineffective in development of bacterial stalk rot due to fluctuation in climatic variations particularly the humidity prevailing during the period of inoculation. Researchers can use these artificial inoculation methods for screening different varieties bearing important traits of character in terms of economic importance. A suitable artificial inoculation method ensures successful infection of host which helps finding resistant and susceptible genotype against particular disease as an important approach for a breeding program. Resistant hosts used in economic production, susceptible genotype used as susceptible check during screening against a particular disease will enhance the understanding.

In India, resistance in some inbred lines, single crosses and hybrids has been identified through artificial inoculations. Among these, partial resistance reported in CM 600, CM 104 and CM 105 maize lines in the field against bacterial stalk rot [24]. Thind and Payak [25] also found CM 600 to be moderately resistant while in above experiment CM 600 found as susceptible genotype since cultivation for years causes some degree of susceptibility in this genotype and also the climate change may also influence the things in due course of time. Further seed production of CM 600 is being difficult for breeders hence finding alternative susceptible genotype is necessity for obtaining good source of resistance for developing high yielding stalk rot tolerant maize varieties. The present study also includes investigation of presence of saprophytic bacteria *Klebsiella* along with *Dickeya zeae* on maize. Hence, adoption of appropriate inoculation method in germplasm screening will ensure successful infection of pathogen, finding of susceptible host which can be helpful for breeders, pathologists and progressive researchers in the crop improvement programme.

5. CONCLUSION

Syringe inoculation method was found to be the best for artificially creating bacterial stalk rot disease of maize under glass house as well as field conditions. Chakrata local identified as the most susceptible genotype followed by CM 600 in greenhouse and field condition which can be used as susceptible check in germplasm screening against BSR of maize.

DISCLAIMER (ARTIFICIAL TELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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