Original Research Article

Characterization of Dickeyazeae causing bacterial stalk rot of maize and advancing efficient inoculation methods and susceptible check for germplasm screening

ABSTRACT

Bacterial stalk rot(BSR) caused by *Dickeyazeae* is consideredthe most destructive disease of maize. Cultural and biological control approaches are ineffective, and no effective chemicals are available for the management of the disease.Therefore, identification and development of resistant cultivars could be an appropriate approach. 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 and PSM 6 were found to be the most susceptible. 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. Thetechniques 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.

Keywords**:** Bacterial Stalk Rot (BSR)**,***Dickeyazeae*,

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1. Introduction

Bacterial stalk rot (*Dickeyazeae*)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, pathogencauses 21 to 98 per cent grain yield losses (Thind and Payak 1978). The disease wasfirst 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 *Dickeyazeae*(Samson et al. 2005; Prokićet al. 2020). 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 (Kharayat and Singh 2013). There are usually 8-11 peritrichous flagella (Martinez et al. 2014). *Dickeya* is plant pathogenic bacteria that are facultative anaerobes (Agrios 2005).

Major symptoms BSR of maize includes discoloration of leaf sheath which slowly spreads to the stalk and leaves.Appearance ofwater-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 x 10^8 cfu per ml are essential for disease development in 15 to 30 days old maize plants(Kumar et al. 2017). 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 (Aysan et al. 2003). Development of resistant cultivars is an appropriate approach for whichidentification of resistant sources is pre-requisite. For screening of germplasm, appropriate effective inoculation techniquesare 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 ofmaize germplasm.

2. Materialsand 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 (Janse 2005).Biochemical and physiological characterizationwas performed for further confirmation of the pathogen.The diseased stem tissue was cut into 2 halves with asterilized razor blade, left for 30 minutes in sterile water for bacterial diffusion and 100 µl suspensionwas plated onto Petri plates containing Nutrient Agar (NA) medium. The pure culture of the bacteria was obtained by streaking a freshly grown single colonyon 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 likethose observed in the field and compared with original cultures*in vitro.*

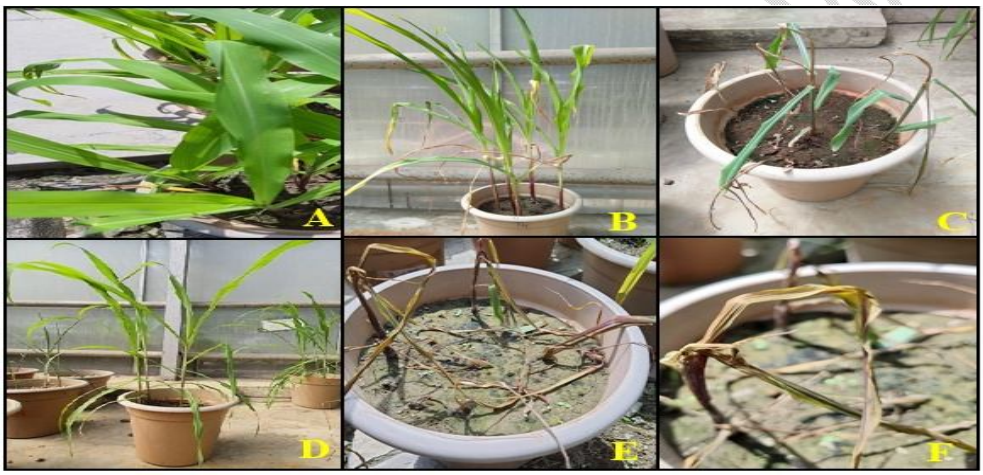

Fig 1: Pathogenicity of stalk rot of maize under glasshouse condition.
Before inoculation (A and D), After inoculation (B, C, E and F)

2.2 Morphological and biochemical test

Morphological test carried out using 100 µlof 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 ℃ 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.

2.3 Molecular characterization

Bacterial genomic DNA was isolated using CTAB method (Williamet al. 2012). 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 1492Rwith total of 35 cycles(Frank et al. 2008). 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

Inoculationof10 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(Kumar et al. 2016). 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 *Dickeyazeae*developed at different time intervals, at 27±1ºC 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 $x10^8$ cfu/ml by adding sterile distilled water.

2.6 Glasshouseexperiment

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 methodsi.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 intwo factor completely randomized block design 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 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 6sown at spacing of 75 x 20 cm.Atrazine @1kg/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 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(Hooda et al. 2018)(Supplementary Table 1). The disease incidence and percent disease index were calculated using the following formulae:

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

Total number of plant

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

3. RESULTS

3.1 Morphological and biochemical characterization

Bacterialidentification was done by various physiological and biochemical properties on basis ofBergey's manual of determinative bacteriology (1994). Morphologicalstudy 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 *Dickeyazeae*(Olabiyi 2010). Slightly rough, concave colonies with deep pit formation on CVP medium (Helias et al. 2011). Deep, cup-shaped cavities and characteristic colony morphology are two identification marks of soft rot Dickeya (Fig. 2).

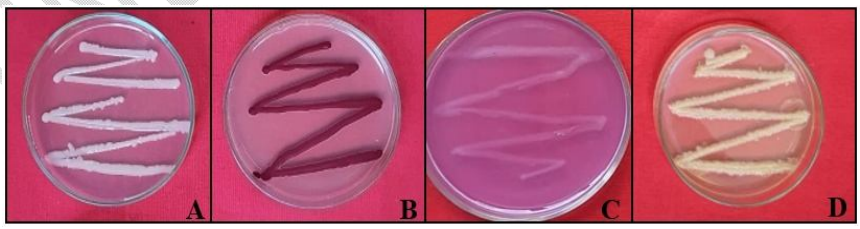

Fig. 2: Growth of *Dickeyazeae*on different media (A) Nutrient agar, (B) Logan's medium, (C) NGM media and (D) CPV medium

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 (Priyanka2023). Catalase production test,gas bubbles releases due to evolution of O□ gas from hydrogen peroxide similar to findings of Reiner (2010). 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,*Dickeyazeae* failed to oxidizes the test reagent (York et al. 2004). 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 (2010) 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 (Supplementary Table 2).

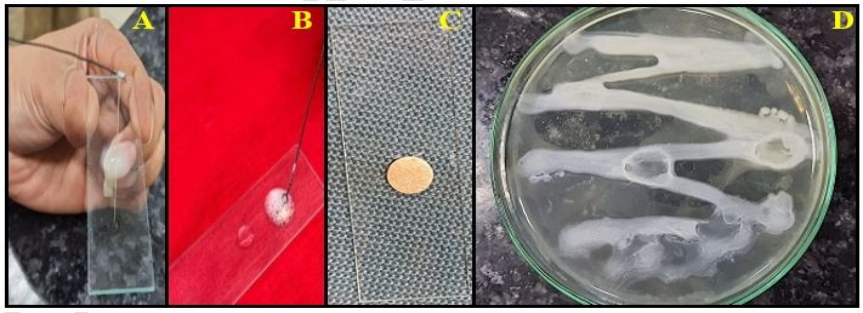

Fig. 3: Biochemical activities of *Dickeyazeae*on (A) 3% KOH, (B) Catalase, (C) Oxidase and (D) Gelatin liquification

3.2 Molecular identification of the isolates

Test bacterium was subjected to 16s ribosomal RNA (rDNA) genomic sequencing by theSanger-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 isolatehas closest belonging to *Dickeyazeae*(GenBank Accession No. OR398800). The phylogenetic analysis with MEGA software was done forming clade with *D. zeae* strains (Fig. 4).

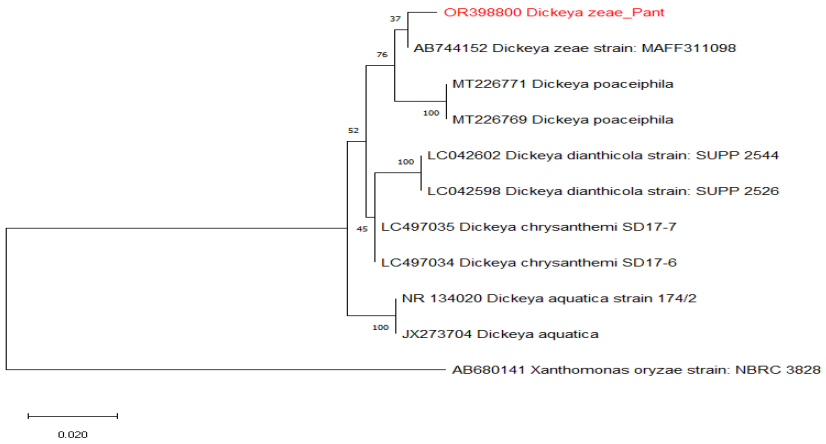

Fig. 4: Phylogenetic tree derived from 16s rRNA of *Dickeyazeae* by using neighbour joining model in MEGA (version 11), based on partial sequence of dnax gene.

3.4 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.

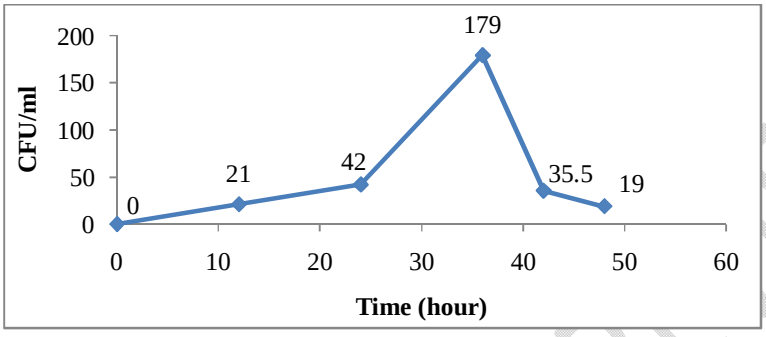

Fig. 5: Growth curve of *Dickeyazeae* on Nutrient broth medium at 10^{-8} dilution

3.5 Glasshouse experiment

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 withrapid 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.

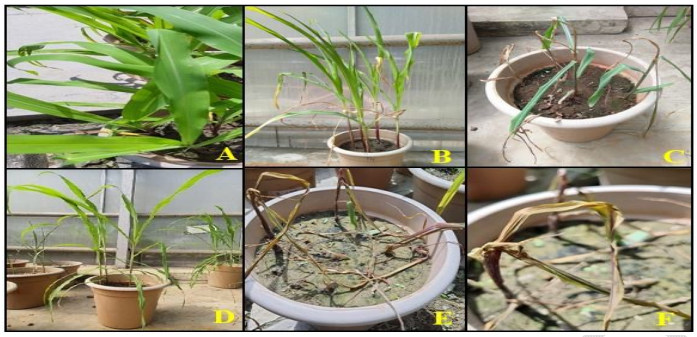

Fig 6: Pathogenicity of stalk rot of maize under glasshouse condition.
Before inoculation (A and D), After inoculation (B, C, E and F)

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%).

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

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 8: Screening of the maize germplasm against bacterial stalk rot of maize caused by *Dickeyazeae*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

3.6 Field experiment

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.

4. Discussion

Bacterial stalk rot disease is severe disease all over the world. In India, distinctsymptoms 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 andmainly 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 (Kumar et al. 2017).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 (Hseu et al. 2008).

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 (Sobowale 2011; Singh P and Singh Y 2019). 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 (Kutama et al. 2011; Sobowale 2011). 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 (Ahamad et al. 2015). Thind and Payak (1985) 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 genotypeand 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 *Dickeyazeae*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.

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Table 1: Incidence and severity of maize stalk rot caused by *Dickeyazeae* 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

Table 2: Screening of maize germplasm against *Dickeyazeae* 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

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

Genotype (B) / Method of inoculation (A)	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
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