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Stem Rot of Pearl Millet Prevalence, Symptomatology, Disease Cycle, Disease Rating Scale and Pathogen Characterization in Pearl Millet-Klebsiella Pathosystem

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The oldest and most extensively cultivated form of millet, known as pearl millet (*Pennisetum glaucum* (L.) R. Br. Syn. *Pennisetum americanum* (L.) Leeke), is raised over 312.00 lakh hectares in Asian and African countries. India is regarded as the significant hotspot for pearl millet diversity. In the Indian state of Haryana, where pearl millet is grown, a new and catastrophic bacterial disease known as stem rot of pearl millet spurred by the bacterium *Klebsiella aerogenes* (formerly *Enterobacter*) was first observed during fall 2018. The disease appears in form of small to long streaks on leaves, lesions on stem, and slimy rot appearance of stem. The associated bacterium showed close resemblance to *Klebsiella aerogenes* that was confirmed by a molecular evaluation based on 16S rDNA and gyrA gene nucleotide sequences. The isolates were also identified to be *Klebsiella aerogenes* based on biochemical assays, where Klebsiella isolates differed in D-trehalose and succinate alkalisation tests. During fall 2021-2023, the disease has spread all the pearl millet-growing districts of the state, extending up to 70% disease incidence in the affected fields. The disease is causing considering grain as well as fodder losses. The proposed scale, consisting of six levels (0-5), is developed where scores 0, 1, 2, 3, 4, and 5 have been categorized as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible disease reaction, respectively. The disease cycle, survival of pathogen, and possible losses have also been studied to understand other features of the disease.

Keywords **:** *Klebsiella*, pearl millet, stem rot

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For millions of impoverished individuals living in arid and semi-arid tropical countries, pearl millet [*Pennisetum glaucum* (L.) R. Br.], belonging to the family *Poaceae*, is one of the oldest staple millets. Pearl millet is the 5th most important cereal crop in the world after rice, wheat, maize and sorghum. According to the All India Coordinated Research Project on Pearl Millet (2022), it is grown on more than 30 million ha of land worldwide, with the majority of that land being in Africa (>18 million ha) and Asia (>10 million ha). It is vital for the food and energy security of rural populations, especially in rain-fed regions. Accord-

ing to Yadav et al. (2012), 90% of the world's pearl millet area is found in India and African countries. With a 130% rise in production in central and West Africa since 1980, it contributes 50% of the millet production globally (Chandra et al., 2017). India stands at first position for production, where Rajasthan is the country's top state. During 2021-2022, in India, it was grown across an area of 68.40 million hectares, with a productivity of 1,430 kg/ha and a production of 97.80 million tonnes. In Haryana, it was grown on 4.83 lakh hectare area with 11.19 lakh tonnes production and 2,318 kg/ha productivity (Ministry of Agriculture, Government of India, 2022). The primary pearl millet-growing districts in Haryana are Bhiwani, Mahendragarh, Rewari, Hisar, Charkhi Dadri, Jhajjar, Rohtak, and Palwal. The pearl millet in India is prone to different diseases such as, downy mildew or green ear disease by *Sclerospora graminicola*, rust by *Puccinia substriata* var. *indica*, smut by *Moesziomyces bullatus* and sugary disease or ergot of pearl millet by *Claviceps fusiformis*, pyricularia leaf spot or blast by *Magnaporthe grisea*, stem rot of pearl millet by *Klebsiella aerogenes*, bacterial leaf blight by *Pantoea stewartii* subspecies *indologenes*, and stunting, little leaf and phyllody disease of pearl millet by *Candidatus* Phytoplasma aurantifolia related strain 16SrII-D which concerns both the farmers and researchers. Out of these three diseases namely stem rot of pearl millet, bacterial leaf blight and stunting, little leaf and phyllody disease of pearl millet were recorded during 2021-2022 in India (Hemalatha et al., 2022; Malik et al., 2022; Mushineni et al., 2021). *Klebsiella* genus usually cause diseases in humans and animals, recently emerging as potent plant pathogen, has been found inciting different kind of diseases in crops *viz.* top rot in maize (Huang et al., 2016), stem rot of pearl millet (Malik et al., 2022) Klebsiella leaf streak in sorghum (Malik et al., 2023) and wilting in many plantations (Ajayasree and Borkar, 2018).

Our preliminary studies, based on the bacterial exudation from diseased plant tissues, colony morphology, gram staining, and microscopic studies, molecular, as well as physiological and biochemical traits of the isolates, strongly suggested that the causal agent of the stem rot should be *Klebsiella aerogenes* of *Enterobacteriaceae*. The *Enterobacteriaceae* family encompasses a large set of bacteria that have a number of properties and form large genera, united into a family, grouped by similar biochemical, morphological, and cultural traits. Biochemical activity and epitope changes characterize species and the intraspecific differences of enterobacteria. In 2017, *Enterobacter aerogenes* was awarded the binomial name *Klebsiella aerogenes* (Tindall et al., 2017). The binomial name was changed as a result of genome-based comparative bacterial phylogenetics (Wesevich et al., 2019).

According to Dickinson and Mocquot (2008), *Klebsiella aerogenes* is a rod-shaped, gram-negative, catalase-positive, citrate-positive, indole-negative, facultative anaerobic organism that forms a creamish white colony on nutrient agar media. There are *Klebsiella* organisms in soil, water, and on plants, and some strains are thought to be a natural component of the human gastrointestinal tract's flora. In addition to being known to incite various types of rots in various plants, such as top rot in maize, *Klebsiella* has also been identified to cause diseases in humans and animals (Huang et al., 2016). Establishing relationship between the amount of disease present and its effects on crop yield and quality is one of the most challenging aspects of disease assessment. The impact of disease on crop yield depends upon number of factors like inoculum source, disease incidence, and overall disease severity. Pathological experiments on pearl millet were carried out to assess the pathogen's presence, prevalence, pathogenicity, identification, characterization, disease cycle, and yield loss relationship based disease rating scale.

Materials and Methods

The infected plant parts collected from Hisar, Bhiwani, and Rewari districts were used for different assays. The three isolates (VMKA101 (Hisar), VMKA102 (Bhiwani), and VMKA103 (Rewari)) were obtained by streaking method (Janse, 2005) and incubated on slant containing NA media at 27 ± 2°C in BOD. Pathogenicity tests were performed with all three isolates on 15-day-old seedlings of pearl millet genotype 7042S. For pathogenicity, leaf whorl inoculation (10 ml suspension/whorl) was done by injuring plant parts and soaking them in a bacterial suspension (1×10^7 colony forming unit/ml). Inoculated plants were grown at 35 ± 2°C temperature and more than 80% relative humidity. After proven of pathogenicity detailed work was carried out on different aspects.

Survey for stem rot of pearl millet. The intensive survey was carried out during fall 2019-2022 in major pearl millet-growing districts of Haryana viz. Hisar, Bhiwani, Mahendragarh, Rewari, Jhajjar, and Rohtak to record the incidence of stem rot disease on pearl millet. The survey was conducted in respective fields by randomly selecting trials from each district. In the selected fields, 20 plants were randomly assessed for disease appearance.

Symptoms of stem rot of pearl millet. Accurate evaluation of symptoms is key in new manifestations that are being evaluated. In case of plant diseases, it often requires observations, descriptions, interpretations, and verifications through pathogenicity. The diseased plants were observed to understand the pathological processes and pertinence of abnormal findings under natural and artificial conditions, and then described on visual basis. All the collected information was interpreted after substantiation through laboratory analysis of different plant parts exhibiting presence of disease-causative *Klebsiella aerogenes*.

Thereafter, it was reviewed on different pearl millet genotypes and subjective symptoms were summarized.

Disease cycle of stem rot of pearl millet. The comprehensive knowledge of disease cycle is crucial for developing different parameters and disease management strategies. It is also very helpful in handling the different aspects of host and pathogen. In order to develop disease cycle, pathogen was monitored for presence, survival, and development among different plant parts, soil, debris, and water. For the purpose 1 g/ml of plant tissues/soil/water was examined for presence on nutrient agar medium under *in vitro* conditions.

Disease rating scale. In any pathosystem rating disease severity is critical for accurate and reliable assessments. Indeed, instruction on use of the rating scale is critical too, as error may result from misuse, as has been noted (Bock et al., 2013; Forbes and Korva, 1994). It is important to demonstrate repeated basic procedures as of other pathosystems to confirm outcomes. For assessment of grain as well as dry fodder losses occurred by newly reported stem rot of pearl millet, field experiments were conducted during *Kharif* 2019 to 2022 at the research farm, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. The pearl millet germplasm 7042S was grown in six plots (8 rows of 5 m length) under randomized block design with four replicates following suitable agronomic practices to assess the grain and dry fodder losses. For inoculation, strain VMKA 101 was used following leaf whorl inoculation method. In each plot, 20 plants showing identical symptoms were observed for varied symptoms to form the disease rating scale. At the time of harvesting, the grain and dry fodder yield of these 20 plants were also recorded separately and compared with the grain and dry fodder yield of protected healthy 20 plants per plot. Different kind of symptoms observed were interpreted in relation to losses and different levels of disease were graded in six (0-5) different categories to develop the disease rating scale. Validation was performed by four seasons information.

Biochemical characters of *K. aerogenes*. Biochemical tests viz. indole production, methyl red, Voges Proskauer's test, citrate utilization, arabinose, mannitol, rhamnose, sucrose, glucose, adonitol, lactose, and sorbitol tests of all the three isolates were performed by following standard methods (Holt et al., 1994). Microbial identification and antimicrobial susceptibility test results for *K. aerogenes* were imported from the VITEK 2 system. Variables evaluated included biochemical and antimicrobial susceptibility test results. In order to analyze the individual biochemical results, this compaction process was reversed in Microsoft Excel to extract the bionumber into the 48 individual component results. Statistical analyses were performed using WHONET 5.6, SaTScan 9.0, SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), and SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). A total of 47 tests were done for the identification and biochemical characteristics with the help of VITEK 2 system (Biomerieux, Durham, NC, USA).

Molecular characters of *K. aerogenes*. DNA was extracted from three isolates using a genomic DNA kit according to the manufacturer's instructions, and the quality as well as quantity of the DNA samples were checked by measuring their A260/A280 ratios using a NanoDrop-2000 (Thermo Fisher Scientific, Waltham, MA, USA) and The DNA samples were stored at –20C until use. Molecular analysis of three isolates was performed using two sets of primers (universal 16S rRNA gene and genus-specific gyrA gene). The gyrA fragment (F: 5′-CGCGTACTATACGCCATGAACGTA-3′; R: 5′-ACCGTTGATCACTTCGGTCAGG-3′) has been adopted as *Klebsiella* genus-specific gene (Brisse and Verhoef, 2001). The quality and quantity of the isolated genomic DNA were analyzed using NanoDrop and resolved in 1% (w/v) agarose gel. Thereafter, visualized in gel documentation to confirm a single band of high-molecular-weight DNA. The fragment 16S rDNA was amplified using 27F and 1492R primers (polymerase chain reaction [PCR] amplification was carried out with conditions of predenaturation at 96°C for 4 min, followed by 30 cycles of 30 s at 94°C, 30 s at 57°C and 1 min at 72°C and a final extension step at 72°C for 10 min). Similarly, the gyrA gene was amplified using 09510F and 09510R primers (PCR conditions were predenaturation at 95°C for 3 min, 35 cycles of 30 s at 94°C, 30 s at 50.95°C and 1 min at 72°C and a final

extension step at 72°C for 5 min). The forward and reverse DNA sequencing reaction of purified PCR amplicons (16S rDNA and gyrA) was carried out using BDT v3.1 cycle sequencing kit on a genetic analyzer to generate gene sequences. The obtained sequences of both genes were compared with the available nucleotide sequences in the NCBI using the blast 2.2.9 system (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

Results

The newly reported stem rot diseases in pearl millet in Haryana, India is caused by *Klebsiella aerogenes*. In this study, a total of three bacterial isolates were obtained from different locations in Haryana, India, during cultivation year 2018-2019. All these isolates were pathogenic in pearl millet after artificial inoculation in the controlled conditions, and therefore strain VMKA 101 was used for different aspects of disease.

Survey for stem rot of pearl millet. An extensive disease survey for stem rot of pearl millet was carried out to know the disease incidence and severity in pearl millet-growing regions of Haryana. Maximum prevalence of the disease was noticed in Hisar, Bhiwani, Rewari, and Mahendragarh locations having an altitude of 215, 225, 245, and 262 m, respectively. The field surveys carried out in rainy seasons during 2019-2022 revealed the prevalence of typical stem rot disease in farmers' fields, representing up to 70% disease incidence in the infected fields. The disease was noticed as presence of few to numerous leaf streaks on leaves, brown to black water-soaked lesions, and slimy rot at stem. Maximum prevalence was observed in the Mahendragarh district of Haryana in all the surveyed years. The disease severity was recorded by using proposed 0-5 scale which was maximum (23.80%) in Mahendragarh, followed by Rewari (20.80%), Bhiwani (20.20%), and Hisar (14.20%), whereas it was minimum (6.40%) in Jhajjar district.

Symptoms of stem rot of pearl millet. The bacterium is systemic inside plant and induces symptoms on different parts of plant. Small to long streaks on leaves are the first symptom of the disease. Sooner, there is spike in number of these leaf streaks. More streaks lead to withering of leaves. The stem then developed water-soaked lesions, which later became brown to black. Severely diseased plants are dead, exhibiting hollowing of the stem and drying of leaves. The infected stem pith become disintegrated and shows slimy rot symptoms. In severely affected fields, pearl millet clumps topple down. The symptoms may vary on different genotypes. In some cases, there may be direct rotting of stem without showing any streak on leaves.

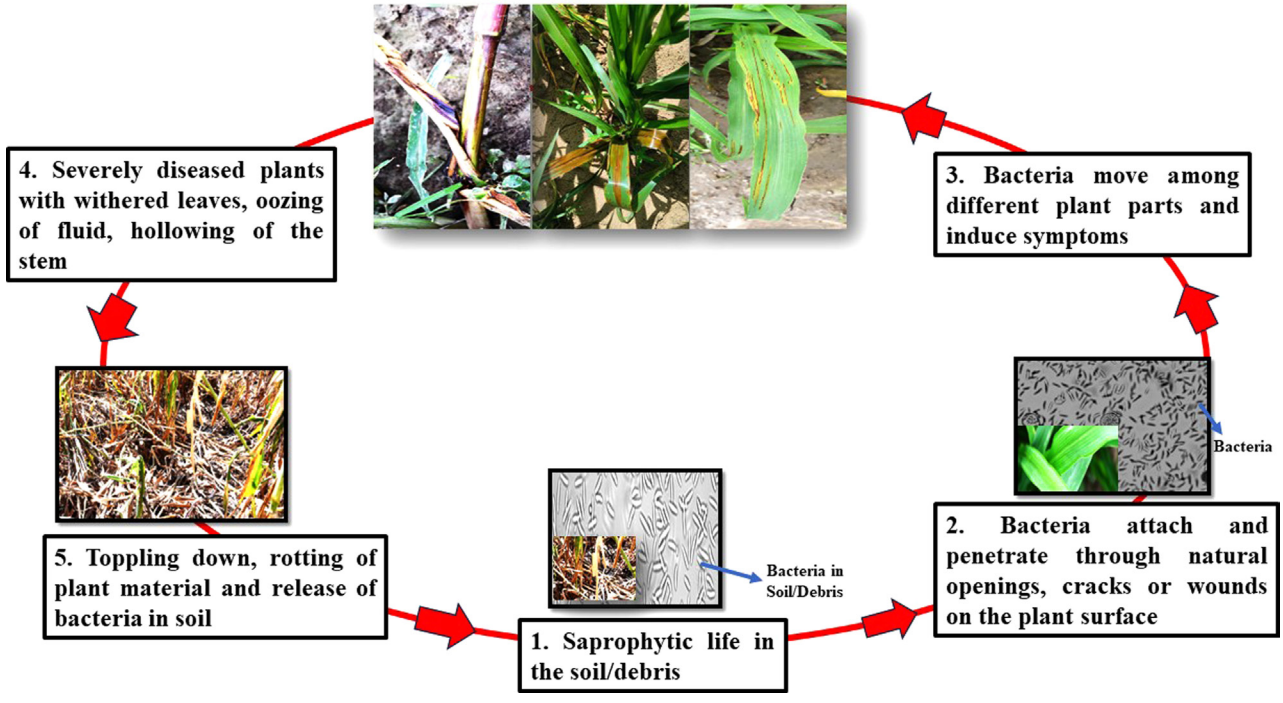

Fig. 1. Different stages in the disease cycle of stem rot of pearl millet.

Disease cycle of stem rot of pearl millet. The disease cycle is an important aspect to define chain of events involved in disease development. The disease cycle of stem rot of pearl millet is presented in Fig. 1. *Klebsiella* organism occurs in soil, water , and also on plants, and some strains are considered a part of the normal flora of the human gastrointestinal tract. Stem rot is caused by *Klebsiella aerogenes,* a soilborne bacteria and an economically significant disease of pearl millet. The plant invasion of this soilborne pathogen is favored by high temperatures and high soil moisture content. The bacterium enters the germinating seedling or young plants through natural openings, mechanical wounds, cracks, root tips and then invades the leaf or stem and prefers proliferation that results in different symptoms over different plant parts. The bacterium could be isolated from leaf, midrib, lower, and upper stem portions. After the plant dies, the pathogen remains in the plant debris and survives with plant matter. This can survive for a period of more than three years. The bacterium, also survives in the soil in the absence of a host. Long-term survival takes place only in association with plant tissues. This soil and infected debris serve as a primary source of new infections. If the soil is near or in a watercourse, it can also serve as a medium of pathogen dissemination.

Disease rating scale. The grain and dry fodder losses were assessed for four consecutive years, and have been presented in Tables 1 and 2. The grain loss percentage varied from 0 to 62.13% as per the disease advancements, whereas stover yield losses were up to 62.78%. Yield losses and symptoms were parted into six different categories and streamlined accordingly with one another. The plants characterized visually under disease rating scale '0' had the maximum grain and dry fodder yield. In disease rating scale '1', the reduction in grain yield varies from 2.20% to 4.12% and the disease severity varies between 1% and 20%. The plants which were characterized under disease rating scale '5' which is maximum grade, the grain yield reduction was also maximum (55.67% to 62.13%). The disease severity for the disease rating scale was between 81% and 100%. In case of dry fodder yield, the maximum average reduction in yield was in plants which were characterized under disease rating scale '5'. Similarly, in other disease rating scales, the reduction in dry fodder yield varied (Tables 1 and 2). In both the yield parameters (grain as well as dry fodder yield), the maximum yield reduction was in plants which are under disease rating scale '5', and minimum was in healthy plants under disease rating scale '0'. This category was characterized as highly resistant.

Table 1. Relationship in grain yield reduction (%) and stem rot of pearl millet severity scale

Disease rating scale	Disease severity	2019		2020		2021		2022		Pooled mean	
		Grain yield (g/20 plants)	Reduction in grain yield (%)	Grain yield (g/20 plants)	Reduction in grain yield (%)	Grain yield (g/20 plants)	Reduction in grain yield (%)	Grain yield (g/20 plants)	Reduction in grain yield (%)	Grain yield (g/20 plants)	Reduction in grain yield (%)
0	0.0	399.03	0.00	383.77	0.00	392.67	0.00	383.13	0.00	389.65	0.00
1	1-20	383.77	3.81	371.43	3.22	384.07	2.20	367.23	4.12	376.63	3.33
2	21-40	369.33	7.43	348.33	9.21	364.23	7.24	353.43	7.73	358.84	7.90
3	41-60	340.33	14.78	296.90	19.20	342.10	12.81	336.83	15.63	329.04	15.58
4	61-80	268.60	32.50	210.20	45.03	285.40	27.24	239.97	37.34	251.04	35.54
5	81-100	176.23	55.67	159.27	58.51	148.10	62.13	152.50	60.00	159.03	59.07
CD (P = 0.05)		37.54	8.24	25.55	5.77	16.74	3.56	30.88	6.63	20.83	3.24
SE		11.76	2.58	8.00	1.81	5.25	1.12	9.67	2.08	6.53	1.46

CD, critical difference; SE, standard error.

Table 2. Relationship in stover yield reduction (%) and stem rot of pearl millet severity scale

Disease rating scale	Disease severity	2019		2020		2021		2022		Pooled mean	
		Dry fodder yield (kg/20 plants)	Reduction dry fodder yield (%)	Dry fodder yield (kg/20 plants)	Reduction dry fodder yield (%)	Dry fodder yield (kg/20 plants)	Reduction in dry fodder yield (%)	Dry fodder yield (kg/20 plants)	Reduction in dry fodder yield (%)	Dry fodder yield (kg/20 plants)	Reduction in dry fodder yield (%)
0	-Nil-	3.19	0.00	2.75	0.00	2.85	0.00	2.80	0.00	2.91	0.00
1	1-20	3.04	4.65	2.63	4.33	2.76	3.01	2.71	3.30	2.80	3.79
2	21-40	2.90	8.63	2.50	9.14	2.65	7.08	2.63	6.17	2.68	7.75
3	41-60	2.65	16.87	2.22	19.20	2.50	12.12	2.43	13.25	2.47	15.29
4	61-80	2.11	33.74	1.48	45.73	1.92	32.55	1.74	37.82	1.86	36.03
5	81-100	1.19	62.78	1.09	60.10	1.19	58.29	1.13	59.84	1.15	60.25
CD (= 0.05)		0.21	3.46	0.18	5.67	0.26	9.86	0.14	5.55	0.07	2.20
SE		0.06	1.72	0.05	1.77	0.08	3.08	0.04	1.74	0.02	0.69

CD, critical difference; SE, standard error.

The plants showing loss percentage of 0.0, 0-5, 6-10, 11-20, 21-50, and >50% have been categorized as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible disease reaction, respectively.

Through the observed yield loss data recorded for four consecutive years, we can conclude that the plants which have no streak on leaves and no lesion on stem are healthy and maximum yield was recorded, while in the plants which have 1-2 leaf streaks per leaf, 0-5% losses were incurred. In plants which have withering of leaves, and where water-soaked lesions appear on the stem, high percent losses in the yield were observed. The maximum or more than 50% losses in the yield were observed in the plants which have hollowing of the stem; drying of leaves and toppled down of plant/clump. Fig. 2 represents the disease rating scale for stem rot of pearl millet.

The disease rating scale (0-5) for stem rot of pearl millet severity is given (Table 3). The correlation matrix of reduction in grain and stover yield were found positively highly significantly correlated with disease severity i.e., reduction in grain yield (0.945**), stover yield reduction (0.940**) during the experiment. The multiple regression analysis of reduction in grain yield and stover yield reduction as the dependent variable with disease severity was analyzed through MS Excel. The linear regression analysis showed that simulations accounted for 89.31% of this variation (r^2), with a slope of 0.63 and an intercept of –7.01% in case of grain yield reduction while in case of stover yield reduction the simulations accounted for 88.29% of this variation (r^2), with a slope of 0.65 and an intercept of –7.24%.

Biochemical characters of *K. aerogenes*. All three isolates were positive for indole production, methyl red, Voges Proskauer's test, citrate utilization, arabinose, mannitol, rhamnose, and sucrose, whereas negative for glucose, adonitol, lactose, and sorbitol tests. The initial *K. aerogenes* isolates were used to calculate the frequency of positive results for the 47 tests (Table 4). While the majority of the tests (32 out of 47) had a percentage of positive results above 95% or below 5% (and thus valuable for species identification), some were highly variable, such as TyrA (50% positive) and SUCT (25% positive). All three isolates were similar in 45 biochemical responses. Hisar isolate differed in D-trehalose test giving negative reaction as compared to the rest. Only Rewari isolate was positive to succinate alkalisation test.

Molecular characters of *K. aerogenes*. The DNA of all

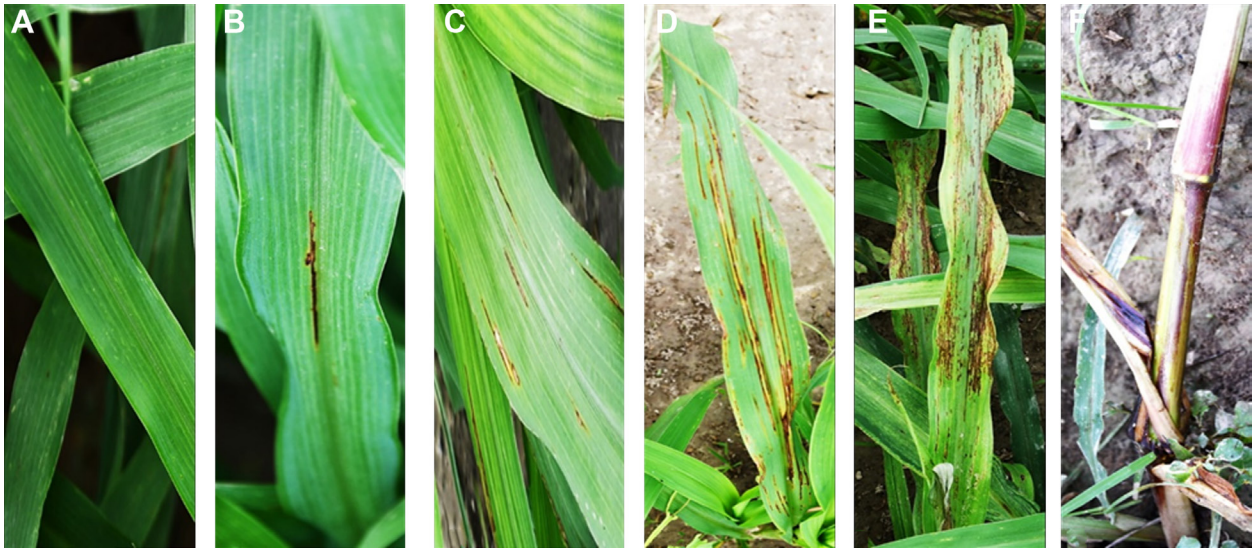

Fig. 2. Stem rot of pearl millet severity scale (0-5 scale). (A) No streak on leaves; no lesion on stem. (B) 1-2 leaf streaks per leaf. (C) 3-5 leaf streaks per leaf. (D) >5 leaf streaks per leaf but no withering. (E) >5 leaf streaks per leaf with withering of leaves. (F) Water-soaked lesions appear on the stem, hollowing of the stem and toppled down of plant/clump.

Table 3. The disease rating scale (0-5) for assessment of stem rot of pearl millet incited by *Klebsiella aerogenes*

Disease rating scale	Symptoms description	Disease reaction
0	No streak on leaves; no lesion on stem	Highly resistant
1	1-2 Streaks per leaf	Resistant
2	3-5 Streaks per leaf	Moderately resistant
3	>5 Streaks per leaf but no withering of leaves	Moderately susceptible
4	>5 Leaf streaks per leaf with withered leaves	Susceptible
5	Water-soaked lesions on the stem; rotting and hollowing of the stem and toppling down of plant/clump	Highly susceptible

three isolates gave a single band with a high molecular weight in each case i.e., 16S rRNA and gyrA gene. The fragment 16S rDNA was amplified using 27F and 1492R primers, where a single discrete PCR amplicon of 1,500 bp was observed in 1% (w/v) agarose gel. Similarly, the gyrA gene was amplified using 09510F and 09510R primers that conferred a single discrete band of 400 bp. The forward and reverse DNA sequencing reaction of purified PCR amplicons (16S rDNA and gyrA) was carried and generated gene sequences were used to form consensus sequences using aligner software. The obtained sequences of both genes exhibited higher homology to available *K. aerogenes* nucleotide sequences in NCBI. The 16S rDNA nucleotide sequences of isolates VMKA101, VMKA 102, and VMKA103 are annotated at NCBI as MZ_433194.1, MZ_540414.1, and MZ_801770.1, respectively. The fragment gyrA is deposited underneath Gene Bank accession nos. LR_ 607333.1; CP_035466.1 and CP_049600.1.

BLAST results of 16S RNA PCR amplicons showed up to 100% similarity to *Klebsiella aerogenes* (accession nos. NR102493.2, MT373521.1, MF682950.1, MF462979.1, MZ433194.1, etc.). High nucleotide homology to *K. aerogenes* gyrA gene sequences (accession nos. LR607333.1, CP035466.1, CP049600.1, etc.) was also detected. Molecular phylogenetic analysis of the 16s RNA sequences of isolated strains was carried out with *K. aerogenes*, *K. pneumoniae*, and *K. variicola* strains. A maximum likelihood phylogenetic analysis (Fig. 3) was conducted using the Tamura-Nei model and MEGA11 (Tamura et al., 2021) software. The tree with the highest log likelihood (–4,346.17) was selected. The final dataset contained 1,717 positions and 14 nucleotide sequences. The 16S RNA-based phylogenetic tree also revealed that the isolated strains are closely related to *Klebsiella aerogenes*. The evo-

Table 4. Frequency of positive test results for different isolates of *Klebsiella aerogenes* considering the 47 biochemical tests in the VITEK 2 ID-GNB Panel

Sr. no.	Biochemical details	Biochemical code	Hisar isolate	Bhiwani isolate	Rewari isolate
1	Ala-Phe-Pro-Arylamidase	APPA	–	–	–
2	H_2S production	H2S	–	–	–
3	Beta-Glucosidase	BGLU	+	+	+
4	L-Proline arylamidase	ProA	–	–	–
5	Saccharose/sucrose	SAC	+	+	+
6	L-Lactate alkalinisation	ILATk	+	+	+
7	Glyicine arylamidase	GlyA	+	+	+
8	2,4-Diamino-6,7-diisopropylpteridine	O129R	+	+	+
9	Adonitol	ADO	+	+	+
10	Beta-N-Acetyl-galactosaminidase	BNAG	+	+	+
11	D-Maltose	dMAL	+	+	+
12	Lipase	LIP	–	–	–
13	D-Tagatose	dTAG	–	–	–
14	Alpha-glucosidase	AGLU	–	–	–
15	Ornithine decarboxylase	ODC	+	+	+
16	Glu-Gly-Arg-Arylamidase	GGAA	–	–	–
17	L-Pyrrolydonyl-Arylamidase	PyrA	+	+	+
18	Glutamyl arylamidase pNa	AGLTp	–	–	–
19	D-Manitol	dMAN	+	+	+
20	Palatinose	PLE	+	+	+
21	D-Trehalose	dTRE	–	+	+
22	Succinate alkalisation	SUCT	–	–	+
23	Lysine decarboxylase	LDC	+	+	+
24	L-Malate assimilation	IMLTa	–	–	–
25	L-Arabitol	IARL	–	–	–
26	D-Glucose	dGLU	+	+	+
27	D-Mannose	dMNE	+	+	+
28	Tyrosine arylamidase	TyrA	+	+	+
29	Citrate-sodium	CIT	+	+	+
30	Beta-N-Acetyl-galactosaminidase	NAGA	–	–	–
31	L-Histidine assimilation	IHISa	–	–	–
32	ELLMAN	ELLM	–	–	–
33	D-Cellobiose	dCEL	+	+	+
34	Gamma-Glutamyl-tranferase	GGT	+	+	+
35	Beta-Xylosidase	BXYL	+	+	+
36	Urease	URE	–	–	–
37	Malonate	MNT	+	+	+
38	Alpha-Galactosidase	AGAL	+	+	+
39	Coumarate	CMT	–	–	–
40	L-Lactate assimilation	ILATa	–	–	–
41	Beta-Galactosidase	BGAL	+	+	+

(Continued)

Table 4. Continued

Sr. no.	Biochemical details	Biochemical code	Hisar isolate	Bhiwani isolate	Rewari isolate
42	Fermentation/glucose	OFF	+	+	+
43	Beta-Alanine arylamidase pNa	BAIap	–	–	–
44	D-Sorbitol	dSOR	+	+	+
45	5-Keto-D-gluconate	5KG	–	–	–
46	Phosphatase	PHOS	+	+	+
47	Beta-Glucuronidase	BGUR	–	–	–

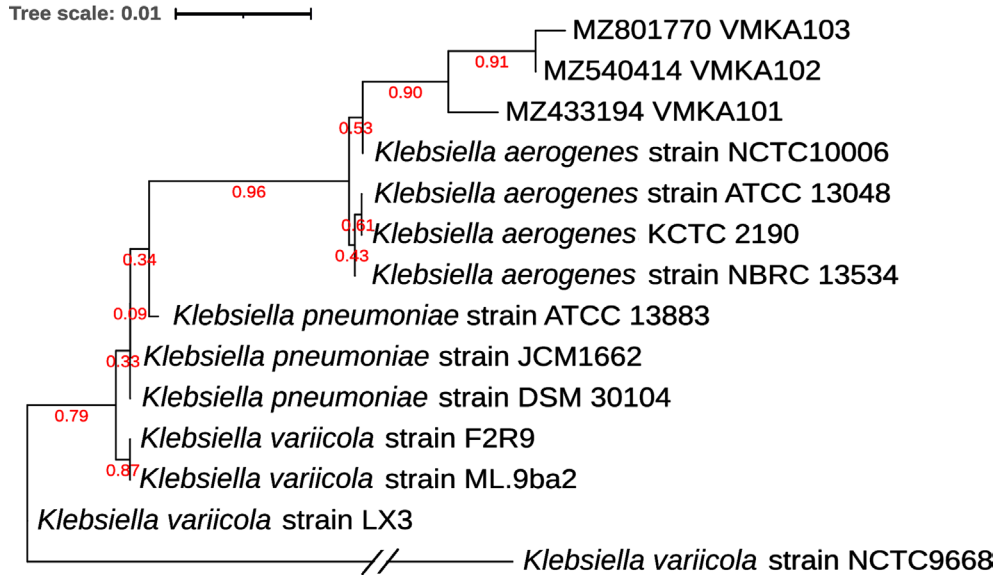

Fig. 3. Phylogenetic tree based on 16S rRNA genes from VMKA101, VMKA102, VMKA103 and close relatives.

lutionary history of 14 nucleotide sequences was inferred using the maximum likelihood method and Tamura-Nei model. The consensus tree obtained after 1,000 bootstrap replicates to represent the history. Branches with less than 50% replicate support collapsed. Bootstrap frequencies of replicates in which taxa clustered together in the tree are shown in red. Initial trees were generated using Neighbor-Join and BioNJ algorithms to a pairwise-distance matrix and the topology was selected with the best log-likelihood value. The final dataset contained 1717 positions and was analyzed in MEGA11 (Multiple sequence alignment and MEGA11 session file for these calculations are available on GitHub: https://github.com/navjeet0211/VMKA101).

Discussion

Millets are a group of cereal food grain crops adapted to cultivation over a range of tropical and subtropical climates, and can be grown with very low inputs. These millets are nutritionally rich and complete their life cycle within 4 months. In Indian conditions, pearl millet occupies the highest area among millet crops. Only fungal pathogen like *Sclerospora*, *Claviceps*, *Moesziomyces*, *Magnaporthe*, and *Puccinia* have been invading the crop for longer periods. Recently, two bacterial and one phytoplasma have been reported to cause new diseases, where one, stem rot of pearl millet by *Klebsiella aerogenes* (Malik et al., 2022) has emerged as potent threat to pearl millet cultivation in Haryana. The putative pathogen was confirmed as *Klebsiella aerogenes* by morphological, biochemical, and molecular tests. The genus *Klebsiella* has been found inciting different kind of diseases in different plantations (Ajayasree and Borkar, 2018; Huang et al., 2016; Malik et al., 2023). The bacterium survives saprophytically in the absence of host in soil or plant debris and becomes pathogenic on avail of congenial conditions, primarily attacking leaves and stem. Most of the plant pathogenic bacteria are known for long-term survival in soil (Buddenhagen, 1965; Crosse, 1971).

The diseased plants with water-soaked lesions and slimy stalk rot symptoms are more serious and resulted in up to 62.13% grain and 62.78% stover yield losses, respectively. *Klebsiella* is known to cause diseases in humans, animals, and plants, strong vigil is required on its multiplication on the plant biomass. It must be checked by consuming genetic resistance. Study of disease cycle apprehended longer survival of bacterium with plant tissues. Based on symptoms and yield loss relationship the 0-5 scale is developed where entries with rating score 0 (disease severity 0%), rating score 1 (disease severity 1-20%), rating score 2 (disease severity 21-40%), rating score 3 (disease severity 41-60%), rating score 4 (disease severity 61-80%), and rating score 5 (disease severity 81-100%), are categorized as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible, respectively.

All isolates are non-motile and urease-negative, similar found by Collins et al. (2004). Similarly, Ørskov (1995), observed that H_2S production is not produced but D-glucose, D-manitol, ssaccharose/sucrose are produced by *K. aerogenes*. Similar observation was found during biochemical analysis through VITEK 2.0. Oxidation of glucose to gluconate (mediated by glucose dehydrogenase) in the absence of added pyrroloquinoline quinone is a unique property of *K. pneumoniae* which is also shared by *K. mobilis* (*E. aerogenes*) (Bouvet et al., 1989). Biochemical profiles can distinguish among isolates of many other sequence types (Gibreel et al., 2012), but in present study, all three morphotypes exhibited mostly similar results, expect two tests. Hisar and Rewari isolates showed varied responses in relation to D-trehalose and succinate alkalisation tests, respectively. 16S rDNA-based nucleotide sequence deposited in NCBI GenBank (accession no. MZ433194.1) conferred its nearness to *Klebsiella aerogenes* (Hormaeche and Edwards, 1960) (Tindall et al., 2017). Molecular analysis and phylogeny of all three isolates from three different places conferred higher homology and genetic relatedness to *K. aerogenes*. Understanding of prevalence, survival, symptomatology, disease cycle, different characters of the pathogen, and assessments of yield losses with respect to disease rating scale will be useful in accessing stem rot of pearl millet and different factors touching the disease. For the further research on development of stem rot disease management strategies, research on biology, epidemiology and variability, etc. our findings are quite useful.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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