

Bacterial Stalk Rot(*Dickeya zae*) of Maize: A New Critical Threat

Abstract: Bacterial stalk rot caused by *Dickeya zae* has emerged as significant threat to maize production globally. This pathogen previously known as *Erwinia chrysanthemi* pv *zae*, is very notorious for its ability to cause severe yield losses, sometimes upto 98.8% depending on the climatic conditions. This disease is particularly prevalent in tropical, subtropical and temperate regions, thriving in high temperature and moisture conditions. *Dickeys zae* has been characterized by morphological, molecular, physiological methods and virulent factor is found out to be pectolytic enzyme responsible for degradation of pectin present in cell wall component of plant. Bacterium survives in host debris and serves as primary source of inoculum. Symptoms can appear at any stages of crop growth from sowing to harvesting. Water soaking and rotting of basal stem mainly leaf sheath which emits foul smell. This pathogen have broad host range including sorghum, maize, rice, tomato, potato, brinjal, chilli and other grasses which makes its very difficult to manage this disease. It found to be severe in heavy rainfall area and stagnant water conditions by creating systemic disease infecting vascular tissues of plant. This pathogen can survive upto 3-6 months in soil having maize crop residues. Current control measures such as cultural, biological, physical, mechanical approaches have proven ineffective. No chemicals are found to be completely effective against this, although some are commercially available. This pathogen is soil borne and destructive in nature, complete resistance has not been identified. Therefore, identification and development of resistant cultivars are great strategy to combat bacterial stalk rot. Among all control measures, host plant resistance will be most economical and time consuming. Bacterial stalk rot is destructive pathogen causing great economic losses hence requires great attention to manage it effectively.

Keywords: Maize, Bacterial Stalk Rot, *Dickeya zae*, Host, Crop, Disease, Yield losses.

1. INTRODUCTION

Bacterial stalk rot (BSR) caused by *Erwinia chrysanthemi* pv. *Zae* is the most devastating disease of maize. This disease is widespread worldwide in Asia, Africa, North America, Central America, South America and Oceania. Three bacterial pathogens have been reported to cause stalk rot of maize namely, *E. dissolvens*, *E. chrysanthemi* pv. *zae* and *Pseudomonas syringae* pv. *lapse* (Sinha, 1966). Bacterial stalk rot disease was first reported by Prasad (1930) as *Erwinia dissolvens* whose symptoms resemble more closely with *E. chrysanthemi* pv. *zae*. *E. chrysanthemi* is causal agent of soft rot disease on wide range of plant species in tropical, subtropical and temperate region of the world. Waldee (1945) suggested a new

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genus *Pectobacterium* on the basis of the distinctive pectolytic activity of the *Erwinia*. In a comparative study of different strains of *Erwinia* species, Dickey (1979) on the basis of 12 physiological characters proposed a major taxonomic change that separated *E. chrysanthemi* into five species under the new genus *Dickeya* based on rRNA sequence. The pathogen has been re-classified as *Dickeya zea* (Samson *et al.*, 2005).

After Pseudomonadaceae, bacterial family Enterobacteriaceae comprises largest and diverse group of plant pathogens. It comprises of several genera such as *Brenneria*, *Dickeya*, *Enterobacter*, *Erwinia*, *Pantoea* and *Pectobacterium* (Samson *et al.*, 2005). *Dickeya* was previously known as *Erwinia chrysanthemi*, affecting a wide range of plant hosts throughout the world, particularly *Musa* spp., *Chrysanthemum* spp., *Dianthus* spp., maize, sorghum, potato, tomato, carrot, onion, pineapple, rice, hyacinth, and calla lily (Charkowski, 2018). In India, for the first time it was reported on sorghum crop in Pantnagar, Uttarakhand during 1987-88 and identified initially as *E. chrysanthemi* which was recently classified as *Dickeya dianthicola*. This group of pathogen is widely distributed all over the world in epidemic and sporadic manner. Several European countries, Indonesia, China, Netherlands, Belgium, Israel and France etc. have been affected by *Dickeya* resulting in yield loss of several crops. *Dickeya* affecting seed tubers, suckers, propagating materials of banana, potato and other vegetative crops.

Crop production is severely affected by various abiotic factors i.e. high and low temperature, water-logging, drought, salinity, acidity, nutritional imbalance etc. and biotic factors i.e. fungi, bacteria, virus, nematodes, mycoplasma, insects and rodents etc. (Gong *et al.*, 2014). Among them plant-pathogenic bacteria can reduce potential yield around the world (Singh *et al.*, 2017). Based on pathogen characteristics, symptoms, morphological, biochemical, molecular characteristics, viable management strategies need to be concerned in order to reduce the impact of disease on economic yield loss. Evaluation of the different inoculation methods to find out resistance genotype by germplasm screening is pre-requisite for the release of new resistant varieties of this disease. Efforts should be focused on maximizing yield to meet growing population need for food and fodder as researcher reported it as rapidly spreading disease from field to field through rain water. Bacterial stalk rot disease results in great yield losses during off season of maize crop. Maize hybrids with greater yield potential suffer more yield loss due to greater drain force of their ear head (Costa *et al.*, 2018).

Dickeya zea is having wide habitats because of its great adaptability in different environments; for example, from plants to soil, from plant to plant, from host plant to non-host plant, from surface or irrigation water to plants, from water to soil, and vice versa (Charkowski, 2018). It is found that this pathogen can even initiate disease from lower inoculum potential, fast spreading rate through the plant's vascular tissue, have more aggressiveness and requires very high optimum temperatures for disease

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development leading to greater damage in area of high temperature. In recent past maize is also become susceptible host for this pathogen worldwide besides including 16 dicotyledonous families of plants in 11 orders and 10 monocotyledonous families in five orders (Samson *et al.*, 2005). Other plant hosts especially ornamentals are likely also to play a great role. It causes hypersensitive response in tobacco plant producing catalase and lecithinase but oxidase negative (Prokic *et al.*, 2020). Due to wide host range of *Dickeya* spp., there is lot more chances that *Dickeya* pathogens is transferred between different plant species (including potato, other crops and wild species), a problem that may intensify with the introduction and spread of new plant species and increased use of irrigation water (that may harbour such pathogens), both as a consequence of climate change. Close consideration thus needs to be given to potential infection pathways, as well as the adaptability of these pathogens to other plant hosts, environments and climatic conditions.

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This study will be provide detailed insights on pathogen taxonomy and biology, symptoms development, dissemination, host range and distribution, ecology and survival, diagnosis and disease control strategies. As if now, no effective biological, chemical management strategies are available with controlling loss due to disease. Resistance breeding and some advanced technique such as bacteriophage typing methods are in experiment for getting wider command on minimizing infectivity rate. Phage particles are abundant in nature which integrates with bacterial genome in case of temperate phage while it destroys bacterium in lytic phage; still very less is known about its importance in controlling this bacterial disease. Studies were undertaken with a view to identify and explore about prophage present in bacterial genome. In total 37 intact prophage and 48 putative prophage were found using RAST but till date it has not been used in *Dickeya* (Toth *et al.*, 2011). This pathogen does not causes severe outbreak but considerable yield loss has been found in susceptible cultivars (Ivanovic *et al.*, 2022).

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2. TAXONOMY AND BIOLOGY

Domain	:	<i>Bacteria</i>
Phylum	:	<i>Proteobacteria</i>
Class	:	<i>Gammaproteobacteria</i>
Order	:	<i>Enterobacteriales</i>
Family	:	<i>Pectobacteriaceae</i>
Genus	:	<i>Dickeya</i>
Species	:	<i>zeae</i>

The genus *Erwinia* was named after the founder and father of phytobacteriology, Erwin Frank Smith, and was recognized by Winslow *et al.* (1917) to comprise the plant pathogenic enterobacteria. In 1917, the genus *Erwinia* was recognized to enclose the entire members of the plant pathogenic

“Enterobacteriaceae” including both *Erwinia* have strong pectolytic activity, causing soft rots in plants (the “carotovora” group e.g. *Erwinia chrysanthemi* and *E. carotovora*) and non-pectolytic, causing wilt or necrotic diseases (the “amylovora” group e.g. *E. amylovora*). In India, initially three bacterial pathogens have been reported to cause stalk rot of maize (*Zea mays* L.) namely, *E. dissolvens* (syn. *Pseudomonas dissolvens* Rosen), *E. chrysanthemi* pv. *zeae* (syn. *E. carotovora* var. *zeae* Sabet) and *Pseudomonas syringae* pv. *lapsa* (syn. *P. lapsa* Starr and Burkholder) (Sinha, 1975). The bacterium was first named as *Erwinia carotovora* f. sp. *zeae* by Sabet (1954) on the basis of its similarity to *E. carotovora* (Jones) in cultural, morphological and biochemical characteristics and its ability to attack maize and other graminaceous host. Thind (1970) reported that the bacterium is closer to *E. chrysanthemi* Burkholder, McFadden and Dimock than to *E. carotovora*. In the 8th Edition of *Bergey's Manual* (Buchanan and Gibbons, 1974) it was classified as *E. chrysanthemi* corn pathotype. Dye *et al.* (1980) have listed it as *E. chrysanthemi* pv. *zeae* (Sabet). A major taxonomic change separated *E. Chrysanthemi* into five species under the new genus *Dickeya* based on rDNA sequence (Zhang *et al.* 2014). The bacterium now reclassified as *Dickeya zeae* under pectolytic *Erwinia* spp.

Dickeya zeae is a gram-negative bacterium which occurs singly or in pairs, motile, straight rod with rounded ends and non-spore forming bacteria. The size of this bacterium varies from 0.8-3.2×0.5-0.8 µm (average 1.8×0.6 µm) depending on the growth conditions and carbon source present in the medium (Kharayat and Singh, 2013). There are usually 8-11, peritrichous flagella (Dickey, 1981). *Dickeya* is merely plant pathogenic bacteria that are facultative anaerobes (Agrios, 2005). It produces young colonies as convex, smooth, circular and entire, or sculptured with irregular margins, depending on the moisture present in the nutrient agar medium. After 4-5 days colonies develop into the raised centre, lobed periphery and round which later becomes feathery or almost coralloid (EPPO, 1992). The non-fluorescent strain causing hypersensitive response in tobacco plants producing enzyme lecithinase and catalase but did not produce arginine dehydrolase and oxidase. All strains reduced nitrate showing variable growth was observed in medium containing 5% NaCl. ERIC-PCR analysis shows presence of more genetic diversity of this pathogen. Furthermore, phylogenetic analysis based on recA gene sequence analysis proved pathogen as *Dickeya zeae*. Molecular identification is more reliable method for pathogen identification.

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3. SYMPTOMATOLOGY AND DIAGNOSIS

Bacterial stalk rot causes both local and systemic symptoms, ranging from tissue maceration to wilts and blights. It appears as a tan to dark brown, water soaked, soft or slimy disintegration of pith tissues at a single internode. Premature withering and drying-up of the tips of the primary leaves, soon trailed by the lower nodes of the plants. Infection generally leads to maceration and rotting of

parenchymatous tissue of the affected plant parts but wilting and leaf chlorosis are common early symptoms in vegetatively grown crops due to systemic infection of the young plant from inoculum in infected seed material (Pérombelon and Kelman, 1980). The external symptom includes maceration of the stalk and basal internodes, resulting in the softening and later on turn into a dry mass of shredded and disjoined fibers. Initially it starts with discoloration of leaf sheath which spread towards stalk, stem and plants topples down with foul smell. This pathogen possess macerating enzyme i.e. pectinase, cellulase isozymes, protease isozymes, xylanases and phospholipases which helps in stem tissue maceration as a result plant cell wall depolymerizes followed by necrosis of whole plant (Robert-Baudouy *et al.* 2000).

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Research studies shows that systemic infection of *Dickeya zeae* is mainly based on their two abilities such as iron acquisition and production of blue pigment, indigoidine (Reverchon *et al.*, 2002). In soil and environment due to scarcity of iron as an essential element, microorganisms possess ability to sequester iron molecule by low molecular weight and high affinity iron-chelating agents called siderophores which captures Fe³⁺ ions. In a plant -bacteria interaction, the successful competition for iron between the two organisms could determine the outcome of an invasion (Enard *et al.*, 1988). The bacterium enters the host through natural opening, abrasions or wounds created by specific feeding insects. The infected stem pith disintegrates and show slimy soft-rot symptoms with foul-smell and eventually the whole plant wilts (Hseu *et al.* 2008). Due to severe infection cobs filling are not perfect and plants were killed before physiological maturity. Infection generally exaggerated with high temperature and humidity. A frequent rain produces more prevalent symptoms (Fig. 1).

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Fig. 1: Symptomatology of the bacterial stalk rots of maize caused by *D. zeae*

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4. ECOLOGY AND SURVIVAL

The soft rot pathogen (*Dickeya zeae*) survive on dead plant organs such as tubers, roots, thick leaves and stem cuttings but period of the survival varies from different environmental conditions. The period of survival was positively correlated with an increase in moisture or 90% moisture (Kumar *et al.*, 2017b). Cloudy weather with high humidity and free water favour the spread and penetration of the bacteria as well as promote disease development or disease epidemics (Saxena *et al.*, 1991). 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. Nutrient rich soil and the application of the high nitrogen fertilizer favor disease development (Saxena and Lal, 1981). Studies suggested that maximum infection of stalk rot was observed, having high disease incidence with the use of sewage or run off water generally used for irrigation purpose in India. It is found severe in warmer and high humidity condition, which prevails in most maize-growing areas 3-4 weeks after sowing.

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5. HOST RANGE

Dickeya zeae have a wide host range of plant species in the tropical, subtropical and temperate regions of the world (Bradbury, 1986). It attacks tubers of potato and sweet potato, onion bulbs, bean pods, roots of carrot, turnip, radish and sugar beet, fruits of tomato, brinjal, chillies and papaya and plants of pearl millet, sorghum, brinjal, potato, tomato, tobacco and cabbage (Thind, 1970; Prasad and Sinha, 1977). The primary hosts are: *Zea mays* (corn), *Sorghum bicolor* (sorghum), *Sorghum sudanensis* (Sudan grass). The secondary hosts include: *Ananas comosus* (pineapple), *Brachiaria mutica* (tall panicum), *Chrysanthemum* spp. (*Chrysanthemum*), *Dahlia* spp. (dahlia), *Daucus carota* (carrot), *Dianthus caryophyllus* (carnation), *Ipomoea batatas* (sweet potato), *Musa* spp. (plantain), *Oryza sativa* (rice), *Saccharum officinarum* (sugarcane), *Saintpaulia ionantha* (African violet), *Solanum tuberosum* (potato), *Nicotiana tabacum* (Tobacco).

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6. IMPACT AND DISTRIBUTION

Europe has infestation of *Dickeya* spp as emerging problem in potato crops with the proposed name '*D. solani*', which spreads across European countries via trade in seed tubers and is causing increasing economic losses (Toth *et al.*, 2011). Some weeds including *Solanum dulcamra* (bittersweet nightshade) growing nearby agriculture field also support soft rot bacterium growth. Interaction of *Dickeya solani* is also found to have interaction with this weed plant and *Solanum tuberosum* hostplant (Fikowicz and Czajkowski, 2018). These soft rot pathogens degrade succulent fleshy plant organs including roots, tubers, stem cuttings, and leaves including potato. Members of the *Dickeya* genus, generally feed on the tissues of living plants, are able to survive long times only in association with plant material, and multiply only in association with a suitable host plant. An outbreak of bacterial soft rot has occurred in the

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northeastern region of the United States. Multiple species of *Pectobacterium* and *Dickeya* are causal agents, resulting in losses to commercial and seed potato production in the Northeastern and North Central United States (Curland *et al.*, 2021).

This disease has been reported to attack corn crops in Asia and Europe such as India, Korea, Thailand, Philippines, Nepal, Mexico, Serbia, and China. In Indonesia, this disease was first reported to attack corn in the West Sulawesi region by the Mamuju Class II Quarantine Station. *Dickeya zeae* were also found to be associated with pineapple soft rot in East Lampung, Indonesia (Patandjengi *et al.*, 2021). Pineapple infected with bacterial strain BRIP64263 were isolated from infected tissue and found to be as Gram negative soft-rotting bacterium capable of growth at 41 °C, and based on its culture properties it was identified as *Dickeya*. This strain was compared with other putative *Dickeya* strains affecting banana (BRIP64262) and potato (BRIP29490) and after that the pineapple strain was placed in phylotype I of *D. zeae*, whereas the banana strain was placed in phylotype II (Young *et al.*, 2022). The results of molecular identification indicated that this disease is previously known as *Erwinia chrysanthemi* pv. *zeae* that previously reported attacked pineapple and aloe vera in Indonesia.

7. PATHOGEN CHARACTERIZATION

There are different selective medium such as Logan's medium, Nutrient glycerol manganese chloride medium (NGM), Crystal violet polypectate medium (CVP) producing characteristics growth pattern. Several biochemical tests such as Potassium hydroxide, 3% (w/v) KOH can be checked for absence or presence of string of slime. Catalase production with few drops of hydrogen peroxide for production of gas bubbles which indicates a reaction due to the evolution of O₂ gas (Reiner, 2010). Oxidase test for confirming the presence of cytochrome oxidase enzyme using oxidase filter paper disks of 10 mm diameter impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and α-naphthol (York *et al.*, 2004). A loopful of pure bacteria transferred aseptically on the disk need to be observed for colour change within 30 seconds. Gelatin liquification test observation for production of an extracellular enzyme gelatinase (Leboffe and Pierce, 2010).

Biochemical tests reveal positive results for nitrate reduction, H₂S production, citrate utilization, indole, Voges Proskauer's, and malonate utilization. Carbon utilization tests show that the isolates utilize various carbon sources, including arabinose, xylose, rhamnose, cellobiose, melibiose, sacharose, and raffinose. A pair of *fliC* gene-based primers, specific to *Dickeya zeae*, was designed to identify the pathogen. These isolates were successfully amplified at 230 base pairs, confirming their association with maize stalks rot. Antibiotics, fungicides, and bacterial antagonists were screened against virulent *D. zeae* isolates under in vitro conditions. Streptomycin inhibited *D. zeae* growth most effectively. Among bio-control agents, *Bacillus amyloliquefaciens* DSBA-11 displayed the maximum area of inhibition zone.

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8. HOST-PATHOGEN INTERACTION

IHF (Integration Host Factor), a nucleoid-associated protein, plays a crucial role in regulating gene expression. IHF subunits (IHF α and IHF β) influence nucleoid structure and DNA bending. IHF binding sites were predicted throughout the genome, affecting the expression of various genes. Virulence factors are controlled and regulated by Integration host factor such as VFM (Virulence Factor-Modulating) quorum-sensing signal. Putrescine quorum-sensing signal is also influenced by IHF. Phytotoxin and Indigoidine production are also controlled by IHF. Transcriptional Regulators such as Fis, SlyA, and FlhD interacts with IHF. Secretion systems from type I to type VI are affected by IHF. Understanding IHF's role in *D. zeae* pathogenesis provides insights for preventing and controlling plant soft rot disease.

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9. MANAGEMENT STRATEGIES

Enhancing plant resistance against *Dickeya zeae*, the bacterium causing bacterial stalk rot in maize, is crucial for sustainable crop management.

9.1 Cultural practices:

Crop rotation of maize with non-host crop such as legumes and rice will help to break the continuity of disease cycle by reducing the survival of pathogen in field. Complete destruction of infected plant debris such as maize straw, leaves etc. to reduce pathogen inoculum which prevent the pathogen from overwintering. Pathogen free planting material and avoiding the use of contaminated tools and equipment is necessary to prevent spread of bacterium. Avoid excess irrigation as stagnant water leads t perpetuation of pathogen. Excess moisture and humidity creating congenial condition hence proper drainage facility is required. Vermicomposting, composting and organic matter will improve the soil health supporting beneficial organisms that can suppress the pathogen. Careful monitoring of the temperature and humidity is necessary because high relative humidity favors *D. zeae* growth and careful management of irrigation is also necessary. Incorporation of organic matter into the soil will improve its health. Compost or well-rotted manure will also enhance microbial diversity and beneficial soil microorganisms having antagonistic potential.

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9.2 Biological control: Antagonistic bacteria such as *Pseudomonas fluorescens* have shown effectiveness against *Dickeya zea*. These beneficial bacteria can inhibit the growth of the pathogen through the production of antibiotics and competition for nutrients. Bacteriophages are viruses that specifically infect and kill bacteria. Research is ongoing to identify bacteriophages that can target *Dickeya zea* without harming beneficial bacteria. Plant Growth-Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and promote growth while also providing protection against pathogens. They can induce systemic resistance in plants, making them more resilient to infections. Incorporating organic matter and compost that contain beneficial microorganisms can enhance soil health and suppress *Dickeya zea*. This approach helps create a more balanced microbial community that can outcompete with the pathogen.

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9.3 Resistant breeding: Since there is no effective chemical for pathogen management, development of resistance cultivars could be an appropriate approach. For development of the resistance in the plant, identification of resistant source is pre-requisite. For screening germplasm an appropriate inoculation technique is required, which possess an effective artificial inoculation method to produce higher disease under field conditions. An effective inoculation method should ensure the maximum contact between host and pathogen or delivery of the pathogen inoculum at particular targeted place viz. pith tissue of stem, internal spaces of leaf, sheath region of plant, internodal region and root tissue etc. For the germplasm screening against various pathogens there are certain inoculation method viz. stem injection, tooth-pick and leaf whorl spray inoculation, can be used. But the efficacy of these methods varies in terms of order in which the pathogen effectively reached to the target sites as well as to produce maximum disease, so this become a major limitation for which different inoculation methods are preferred by various workers.

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Development of an effective inoculation technique for the disease assessment is one of the important parts of the germplasm screening. Suitable inoculation method should be ensured for the purpose of introduction and contact between host and pathogen as well as to produce maximum disease under field condition. For disease development and assessment, a variety of inoculation methods such as stem injection, injection infiltration, tooth- pick, whorl inoculation, foliar spray and grain inoculation can be used. However, the efficacy of various methods for disease development varies. Concentration of the inoculum is most important factors for the successful development of the disease in the host. Infection of maize plant with *Erwinia chrysanthemi* (5×10^7 /ml) using whorl inoculation method resulted in consistent rotting and collapsing of 3 week old corn plants (Hartman and Kelman, 1972). In leaf whorl inoculation technique care should be taken not to disturb the plant after inoculation so that maximum amount of bacterial suspension can be retained on whorl of leaves and spraying without causing any injury with help of hand atomizer.

Hossain and Logan, (1983) proved the pathogenicity of black leg of potato caused by *Erwinia carotovora* f.sp. *antroseptica* using two methods for inoculating potato tubers, one by dipping them in an aqueous suspension of bacteria and the other by inserting the end of a tooth pick charged with undiluted bacteria and found that pathogen inoculation with tooth prick method gives better results compared to dipping in suspension. Tooth pick method is time taking and care should be taken not to insert tooth pick too deeply in order to avoid any splitting of stalk. Drought condition should not be prevailed during toothpick insertion. Artificial inoculation by stem pricking and leaf pricking methods of inoculation with *Erwinia chrysanthemi* have been found effective in symptom development of the bacterial stalk rot of sorghum (Hseu *et al.* 2008). Care should be taken while inserting hypodermic needle as vascular tissue will be damaged resulting in plant injury. The toothpick inoculation technique has been used to screen germplasm against sorghum and maize pathogens (Bramel-Cox and Claflin, 1989, Clements *et al.* 2003).

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During Kharif 2012 and 2013 disease survey were carried out in different maize growing areas of Punjab which revealed that maximum disease incidence (70%) and severity (20%) was recorded on Dekalb Double hybrid during Kharif 2012, while maximum disease incidence (25%) and severity (5%) was recorded on DKC 9106 hybrid of maize in 2013 (Kumar *et al.* 2015a). Sinha and Prasad (1975) found partial resistance in CM 600, CM 104 and CM 105 and their crosses. Saxena and Lal (1980, 1981a) established a correlation between the time of infection, disease incidence and yield loss and reported that plants infected before flowering showed complete yield loss whereas plants showing wilting within 15 days after flowering exhibited 85 per cent yield loss.

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9.5 Chemical management: On bacterial stalk rot of sorghum researcher found that zones of inhibition formed when bacterium placed upon found on antibiotic impregnated discs, seeded on agar medium, showed highest sensitivity to senoclin, chloramphenicol, kanamycin, gentamicin, carbenicillin, cefotaximine, tetracycline, amikacin, and tobramycin. Penicillin family members observed to be ineffective for controlling this bacterium. Copper-based fungicides or bactericides can act as preventive measures. Presently, no effective chemicals are available for the management of the disease. Bacterial stalk rot is found to be highly sensitive to chlorine (Thind and Payak, 1972). Efficient control of the disease can be done by using bleaching powder ($\text{CaOCl}_2 \cdot \text{H}_2\text{O}$ containing 33% chlorine), is achieved by drenching the basal stalk region when the plants are knee high length. However, management of bacterial stalk rot of maize can be achieved by other approaches including cultural practices and biological control but these methods are not found to be very effective.

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10. FUTURE PERSPECTIVES

Dickeya zeae is not only a problem for maize but also causes soft rot in many plant species, including essential crops such as potato, rice, and banana. The global impact of the pathogen necessitates international

collaboration in research and management efforts. Understanding the epidemiology of *Dickeya zae* is crucial for managing the disease. Climate change is expected to influence the distribution and severity of the pathogen, with studies predicting an increase in disease spread in certain regions due to changes in environmental variables like temperature and precipitation. Effective management strategies are needed to control outbreaks. This includes developing resistant maize varieties, improving cultural practices, and using biological control agents. Research is ongoing to identify the modes of transmission and measures to control the disease. Comprehensive molecular analyses of *Dickeya zae* isolates provide valuable insights for future crop improvement strategies. Such studies can help in understanding the pathogen's virulence factors and resistance mechanisms, which are essential for developing targeted control measures. The challenges lie in the implementation of these strategies, especially in developing countries where resources may be limited. Continuous monitoring, research, and adaptation of management practices will be essential to mitigate the impact of *Dickeya zae* on maize and other crops.

11. CONCLUSION

The stalk rot of maize responsible for severe destruction and yield loss of corn plant caused by bacterium *Dickeya zae* is matter of concern due to huge economic loss. This pathogen is reported from the entire world including those found in the corn field of Pantnagar in Uttarakhand. This bacterium is gram negative in nature and its wide host range is major constrain in its management. It spread from field to field through rainwater and its run off. Unavailability of effective chemical, biological, cultural control measures is threat for its management therefore resistant host finding through germplasm screening would be best alternative to bacterial stalk rot management. Identification of resistant source is pre requisite for development of resistant cultivar which could be an appropriate approach for its management. Based on these facts, ecological studies about this disease, its distribution pattern, symptoms, characterization of pathogen and its destructive impact need to be concern wisely. Pathological behavior should be noticed to check virulence of bacterial isolate found from maize field causing stalk rot disease. Molecular studies should be focused for accurate knowledge of bacterium species found.

Comment [d44]: Both conclusion and future perspectives can be merged into a single section

References

1. Agrios, G.N., Plant Pathology. 5th edition. Academic Press. San Diego, California, USA. 2005, 922.
2. Allen, D.J., Hebane, C.L.N. and Raji, J.A., Screening for resistance to bacterial blight of cowpea. *Tropical Pest Management*, 1981, 27, 218-224.

3. Anderson, R.C. and Gardner, D.E., An evaluation of the wilt-causing bacterium *Ralstonia solanacearum* as a potential biological control agent for the alien Kahili ginger (*Hedychiumgardnerianum*) in Hawaiian forests. *Biol. Control*, 1999, 15(2), 89-96.
4. Bachanan, R. E., and N. E. Gibbons., "Bergeys manual of determinative bacteriology Eighth edition." Williams and Wilkins Co., 1974, 471-477.
5. Balram, C., Sharma, A., Sivathasan, C., and Lee, E. J., Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic–genotypic correlates. *Br. J. Clin. Pharmacol.*, 2003, 56(1), 78-83.
6. Bradbury, J.F., Guide to plant pathogenic bacteria. CAB International Mycological Institute. Surrey, England. 1986, 332.
7. Bramel-Cox, P.J. and Claflin, L.E., Selection for resistance to *Macrophomina phaseolina* and *Fusarium moniliforme* in sorghum. *Crop Sci.* 1989, 29, 1468-1472.
8. Charkowski, A.O., The Changing Face of Bacterial Soft-Rot Diseases. *Annu RevPhytopathol.* 2018, 56, 269-288.
9. Clements, M.J., Klein Schmidt, C.E., Maragos, C.M., Pataky, J.K. and White, D.G., Evaluation of inoculation techniques for Fusarium ear rot and fumonisin contamination of corn. *Plant Dis.*, 2003, 87, 147-153.
10. Costa, R.V., Simon, J., Cota, L.V., Silva, D.D., Almeida, R.E., Lanza, F.E., Lago, B.C., Pereira, A.A., Campos, L.J., & Figueiredo, J.E., Yield losses in off-season corn crop due to stalk rot disease. *PesquisaAgropecuáriaBrasileira.* 2019, 54, 157-161.
11. Curland, R.D., Mainello, A., Perry, K.L., Hao, J., Charkowski, A.O., Bull, C.T., McNally, R.R., Johnson, S.B., Rosenzweig, N., Secor, G.A., Larkin, R.P., Gugino, B.K., Ishimaru, C.A, Species of *Dickeya* and *Pectobacterium* Isolated during an Outbreak of Blackleg and Soft Rot of Potato in Northeastern and North Central United States. *Microorganisms*, 2021, 9(8), 1733.
12. Dickey, R. S., *Erwinia chrysanthemi*: reaction of eight plant species to strains from several hosts and to strains of other *Erwinia* species. *Phytopathol.*, 1981, 71, 23-29.
13. Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A. and Schroth, M. N., International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Plant Pathol.*, 1980,59, 153-168.
14. Enard, C., Franza, T., Neema, C., Gill, P. R., Persmark, M., Neilands, J. B., and Expert, D., The requirement of chrysobactin dependent iron transport for virulence incited by *Erwinia chrysanthemi* on *Saintpaulia ionantha*. *Plant and Soil*, 1991, 130(1/2), 263-271.

15. Fikowicz-Krosko, J., and Czajkowski, R., Systemic colonization and expression of disease symptoms on bitter-sweet nightshade (*Solanum dulcamara*) infected with a GFP-tagged *Dickeya solani* IPO2222 (IPO2254). *Plant dis.*, 2018, 102(3), 619-627.
16. Gong, F. P., Yang, L., Tai, F., Hu, X. and Wang, W., "Omics" of Maize Stress Response for Sustainable Food Production: Opportunities and Challenges. *J. Integr. Biol.*, 2014, 18(12), 714-732.
17. Goszczynska, T., Botha, W.J., Venter, S.N. and Coutinho, T.A., Isolation and identification of the causal agent of brown stalk rot, a new disease of maize in South Africa. *Plant Dis.*, 2007, 91, 711-718.
18. Hartman, J. R. and Kelman, A., An improved method for inoculation of corn with *Erwinia* spp. *Phytopathol.*, 1972, 63, 658-663.
19. Hooda, K. S., Bagaria, P. K., Khokhar, M., Kaur, H. and Rakshit, S., Mass Screening Techniques for Resistance to Maize Diseases. ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana, 2018, 60-61.
20. Hossain, M. and Logan, C. A., Comparison of inoculation methods for determining potato cultivar reaction to black leg. *Ann. App. Biol.*, 1983, 103, 63-70.
21. Hseu, S. H., Kuo K. C., Lin, H. F. and Lin, C. Y., Bacterial stalk rot of sorghum occurred in Kimmen area caused by *Erwinia chrysanthemi*. *Plant Pathol. Bulletin*, 2008, 17, 257-262.
22. Ivanović, D., Bakteriozeka kukuruza. In R. Almaši, F. Bača, A. Bošnjaković, D. Čamprag, G. Drinić, D. Ivanović, et al. (Eds.), Bolesti, štetočine i korov kukuruza i njihovog uzrobovanje. Beograd-Zemun: Institut za kukuruz Zemun Polje. 2002, 156-161.
23. Kharayat, B. S. and Singh, Y., Characterization of *Erwinia chrysanthemi* isolates inciting stalk rot disease of sorghum. *Afr. J. Agric. Res.*, 2015, 10(22), 2309-2314.
24. Klement, Z., Rapid detection of the pathogenicity of phytopathogenic Pseudomonads. *Nature*. 1968, 199, 299-300.
25. Kumar, A., Hunjan, M. S., Kaur, H., Rawal, R., Kumar, A. and Singh, P.P., A review on bacterial stalk rot disease of maize caused by *Dickeya zea*. *J. App. Nat. Sci.*, 2017a, 9(2), 1214-1225.
26. Kumar, A., Hunjan, M. S., Singh, P. P. and Kaur, H., Statuses of bacterial stalk rot of maize in Punjab. *Plant Dis. Res.*, 2015b, 30, 97-99.
27. Kumar, A., Hunjan, M.S., Kaur, H. Rawal R. and Singh, P.P., Studies on survival of *Dickeya zea* causing agent of bacterial stalk rot disease of maize. *Int. J. Agri. Sci.*, 2017b, 9 (8), 3913-16.
28. Leboffe MJ, Pierce BE., Microbiology laboratory theory and application, 3rd ed. Morton Publishing Company, Englewood, CO., 2010.
29. Loyer, M.W. and Hamilton, M.A., Interval estimation of the density of organisms using a serial-dilution experiment. *Biometrics*, 1984, 40, 907-916.

30. Patandjengi, B., Junaid, M., and Muis, A., The presence of bacterial stalk rot disease on corn in Indonesia: A review. In *IOP Conference Series: Earth and Environmental Science*, 2021, 911(1), 012058.
31. Pérombelon, M. C. M. and Kelman, A., Ecology of the soft-rot *Erwinias*. *Ann. Rev. Phytopathol.* 1980, 12, 361-387.
32. Prasad, M. and Sinha, S. K., Survival and retention of infectivity of bacterial stalk rot pathogen of maize and its perpetuation on varied cropping pattern. *Plant Soil*, 1977, 47, 245-248.
33. Prokić & N. Zlatković & N. Kuzmanović & M. Ivanović & K. Gašić & Ž. Pavlović & A. Obradović., Identification and characterization of *Dickeya zeae* strains associated with maize stalk soft-rot in northern Serbia. *Eur J Plant Pathol* A., 2020, 90 (3), 236-247.
34. Rangarajan, M. and Chakravarti, B.P., Bacterial stalk rot of maize in Rajasthan. Effect on seed germination and varietal susceptibility. *Indian Phytopathol.* 1970a, 23, 470-477.
35. Reiner, K., Catalase test protocol. *American Society for Microbiology*. 2010, 11, 1-6.
36. Reverchon, S., Rouanet, C., Expert, D. and Nasser, W., Characterization of indigoidine biosynthetic genes in *Erwinia chrysanthemi* and role of this blue pigment in pathogenicity. *J. bacterial*, 2002, 184(3), 654-665.
37. Robert-Baudouy, J., Nasser, W., Condemine, G., Reverchon, S., Shevchik, V., and Hugouvieux-Cotte-Pattat, N., Pectic enzymes of *Erwinia chrysanthemi*: regulation and role in pathogenesis. *Plant Microbe Interactions*, 2000, 221-268.
38. Samson, R., Legendre, J. B., Christen, R., Fischer, M., Achouak, W. and Gardan, L., Transfer of *Pectobacterium chrysanthemi* (Burkholder et al 1953) Brenner et al 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *Int. J. Syst. Evol. Microbiol.* 2005, 55, 1415-1427.
39. Saxena, S. C. and Lal, S., Assessment of yield losses due to bacterial stalk rot of maize. *Indian J. Mycol. Plant Pathol.* 1980, 12(3), 308-309.
40. Singh, N., Sharma, P. and Kamboj, M. C., Maize Scenario in Haryana: A Brief Review. *Int. J. Pure App. Biosci.* 2017, 5(6), 1616-1623.
41. Sinha, S. K. and Prasad, M., Varietal screenings of maize germplasm against stalk rot pathogen *Erwinia carotovora* f.sp. *zeae*. *Labdev J. Sci. Technol.*, 1975, 13, 128-133.
42. Srivastava, K. K. and Prasad, M., Studies on *in vitro* and *in vivo* activities of cellulase in maize-*Erwinia carotovora* pv. *chrysanthemi* host-pathogen system. In: Third International Symposium on Plant Pathology, *Indian Phytopathological Society*, New Delhi, December 14-18, 1981, 192.

43. Thind, B. S. and Payak, M. M., A review of bacterial stalk rot of maize in India. *Int. J. Pest Manag.*, 1985, 31(4), 311-316.
44. Thind, B. S. and Payak, M. M., Antibiotics and bleaching powder in the control of bacterial stalk rot of maize. *Hindustan Antibiotics Bulletin*, 1970, 15, 9-13.
45. Thind, B. S., Investigations on bacterial stalk rot of maize(*Erwinia carotovora var. zea Sabet*). Thesis, Doctor of Philosophy, Indian Agricultural Research Institute, New Delhi, India. 1970, 113.
46. Toth, I., van der Wolf, J., Saddler, G., LOjkowska, E., Helias, V., Pirhonen, M., Tsrer, L., and Elphinstone, J., *Dickeya* species: an emerging problem for potato production in Europe. *Plant Pathol.*, 2011, 60 (3), 385-399.
47. York, M. K., M. M. Taylor, J. Hardy, and M. Henry., Biochemical tests for the identification of aerobic bacteria, p. 3.17.39.1. In H. D. Isenberg (ed.), *Clinical microbiology procedures handbook*, 2nd ed. ASM Press, Washington, DC, 2004.
48. Young, A.J., Pathania, N., Manners, A., Heart rot of Australian pineapples caused by *Dickeya zea*. *Aus. Plant Pathol.*, 2022,51, 525–533.
49. Young, H., The toothpick method of inoculating corn for ear and stalk rot. *Phytopathol.*, 1943, 33, 16.
50. Zhang, J., Shen, H., Pu, X., Lin, B. and Hu, J., Identification of *Dickeya zea* as a causal agent of bacterial soft rot in banana in China. *Plant Dis.*, 2014, 98(4), 436-442.