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A Review of Banana *Xanthomonas* Wilt (BXW) Current Control Measures and Genome Editing as a Potential Intervention

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Abstract: Bananas are important to millions of people around the world as a source of food and income. Many children especially from the African continent eat mashed bananas as their first solid food during weaning. Banana *Xanthomonas* wilt (BXW) is a huge threat to banana production and if nothing is done to control the disease, 50% of production could be lost. Different interventions towards the control of the wilt have been tried, including cultural, biological, chemical, and genetic modifications. However, measures are inadequate in their capacities. The cultural methods are hindered by inconsistencies by farmers while administering. Biological, chemical, and genetic modifications face the challenge of resistance that might arise due to pathogen evolution. In addition, genetic modification attracts non-acceptance due to the novel genes introduced into the crop and the misconceptions created by interested groups. There is, therefore, a need to embrace new technological advances like gene editing (GE) which is viewed as the future of creating resistance in crops against diseases because, unlike genetic modifications, the novel genes are removed through the cell's natural processes. GE technology utilizes clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) to target genes intending to create resistance by knocking the susceptible genes out or by activating the expression of the defense genes. This review gives a synopsis of BXW's current control measures and the potential that GE has to address the disease more adequately.

Keywords: Gene Editing, CRISPR-Cas9, Banana *Xanthomonas* Wilt

1. Introduction

Bananas are important to millions of resource-poor farmers in the tropics and the subtropics [1]. Bananas originally came from South East Asia but have their popularity and use have been growing steadily over the years [2]. Bananas are the fourth most important crop in the world trailing rice, wheat, and maize [3]. The cultivated bananas are from the two *Musa* groups of *Musa accuminata* and *Musa balbisiana* [4]. The global banana production is estimated at 130 million tons but the production is steadily increasing. India is leading the world in the production of bananas and is currently is at 11 million tons annually [5].

Banana production is however challenged by decreasing soil fertility, increased drought, reduced biodiversity of banana germplasm, and pests and diseases [6]. Banana *Xanthomonas* wilt (BXW) is one of the devastating diseases of bananas and it can claim up to 50% of the farmer's production. The disease originated in Ethiopia in a banana relative called *enset*. The disease has however spread to other places in the world [7]. There is therefore a need to protect banana production from the losses caused by the BXW disease. Any effort towards control of BXW disease will go a long way toward realizing food security among the many people around the globe who depends on the crop. The technology developed for the control of BXW must however be effective and readily embraced by the end consumers and the regulatory frameworks. This review is therefore a

synopsis of BXW disease, the approaches toward control, and the advancement of gene editing as the most appropriate control measure.

2. Pathogen Diagnostic Tools

The banana *Xanthomonas* pathogen comes from a group of bacteria that is only found in plants and plant materials [8]. In glucose-rich media or environments, the bacteria produce *xantha* gum which is an extracellular polysaccharide. *Xantha* affects the plant by causing significant blockage of the plant tissues leading to wilting [9]. In the comparison of the banana *Xanthomonas* wilt (BXW) with the other groups of *Xanthomonads*, BXW is much slower in growth than for example Ralstonia, Burkholderia, and Pseudomonas. The BXW bacterial is therefore not very well able to compete with other bacteria outside the host and its thought that the slow growth enables the bacteria to live longer if it is released by an infected plant into the soil. Studies to understand the survival of BXW bacteria has been set before. The initial studies showed that the bacteria can live in chopped plant debris for up to 6 months in soil [10]. The studies about the bacteria were important because once the pathogen's way of survival is understood, then a proper recommendation for disease management measures can be made for instance whether fallow or crop rotation. Recent studies on the semi-selective medium are contributing to a better understanding of the pathogen's survival and epidemiology and even the species of insects that are involved in the transmission of the disease [11]. As concerns the detection of the pathogen there has been recent progress in the development of surgical tests for the pathogen. Polyclonal and monoclonal antibodies have been developed successfully for the detection of banana *Xanthomonas* pathogen. [12]. Studies to develop PCR-based protocols have also been carried out and this has contributed to an in-depth understanding of the pathogen's population structure [13].

3. Transmission of the Pathogen

Effective infection of the host plant by a bacterium requires a series of events which includes the movement of the bacterium towards the plant, establishment of contact with the host, penetration of the bacterium to the host, and finally proliferation of the bacterium once inside the host [14]. Observations that have been made on infection pattern from infected fields shows infection of the lower plant parts (mats, roots, and cut petioles) indicating that the infection is soil-borne [15]. The other disease pattern is an infection of the inflorescence possibly by inoculum that has been dispersed by insects and maybe aerosols [16]. The transmissions from plant to plant is suspected to be executed by insects or mechanically through the use of infected farm implement. It has also been claimed that moles and rats can transmit the disease as they dig tunnels from one plant to another [17]. The claim that large animals like cattle and goats once they move through infected fields can aid in the dispersal of the disease has not been well established. Other organisms that cannot be considered transmitters of the disease are nectar-collecting bats and birds.

The entrance of the disease into the plant is thought to result from the mechanical injuries caused to plants by nematodes and insects. During the removal of excess infected suckers, injuries are created, and then there is continued oozing of the bacteria. The continued accumulation of the bacteria oozes is enough pool of bacteria that is picked by the insects and other vectors. The injured and diseased plants once they fall off are a source of inoculum in soil that is spread through soil water [18].

Other banana bacterial diseases like *E. amylovora* and soft rot *Erwinias* have been reported to get disseminated through rainfall splashes, there is no evidence that banana *Xanthomonas* wilt is disseminated through aerosols [19]. Although BXW has not been reported to be disseminated through rainfall splashes, it is highly likely that when there are heavy winds during rainfall, the bacterial oozes on a plant can be transmitted to open injuries of a healthy plant. Water droplets in cyclonic conditions have been noted to carry and disperse bacteria cells over a long distance [20]. On the contrary, it has been noted that bacterial inoculum transfers under stormy conditions are not able to cause disease over a long distance [21]. There is however need to carry out studies to be able to ascertain the role played by aerosols in the spreading of bacterial diseases, especially in farms where plants are heavily dense.

Bananas with persistent bracts are not infected by BXW; for instance, those bananas that are grown in Ethiopia. However, it has been reported that bananas with and without persistent bracts have been infected in the Eastern Democratic Republic of Congo (DRC) [22]. This, therefore, suggests that the differences in infection for those bananas with or without bracts could be a result of other factors like altitude and insect vectors. The cultivars that seem to resist the disease are not resistant but rather escape the disease through their floral morphology.

4. Cultural Methods and Their Limitations

In the management of bacterial diseases, early detection and destruction of the infected materials remain an effective step in controlling the disease spread [23]. This, therefore, calls for methods that reduce the inoculum for the disease and disease management measures that reduce the spread of the pathogen to new plant hosts. The banana wilt spread is through the banana buds, it is therefore important that farmers conduct timely removal of the male buds so that they can interrupt the transmission cycle of the disease. Timely removal of the buds has to be carried out when the last hand of the bunch is formed. This will not only prevent the flowers from being infected by the wilt but will also encourage the formation of a bigger more filled fruit [24]. In addition, early removal of the pseudostem that is infected prevents the

spreading of the disease to the suckers. It is therefore important that the fields where the bananas are grown are kept clean through sensitization of the farmers. The other important aspect is to control the movement of the banana materials as this is one of the ways that the bacterial disease moves into new places [25]. One of the recommendations for control of the disease in a banana is that farmers need to plant disease-free planting materials, clean farm equipment that has been in contact with diseased plant materials, and practice rotation of crops. Some farmers also leave their fields fallow for 6 months to avoid re-infection with soil-borne pathogens. Phytosanitary measures have a high potential for controlling the disease however, they are labor-intensive and so many small-scale farmers are not able to adopt them consistently [26].

The major challenge in the control of BXW is that bananas are widely grown by resource-poor farmers. The farmers are therefore not able to afford cleaning planting materials developed through micro-propagation from various laboratories. The farmers, therefore, borrow suckers from neighbors even in areas where the disease exists. This significantly leads to the increased spreading of the disease. The farmers too have the habit of leaving the roughed-up diseased material to rot on the farm. The diseased materials even as they rot are a source of inoculum for more infections [27]. Control measures such as de-budding and destruction of infected pseudostems face the challenges of a lack of well-structured and sufficient eradication programs in developing countries. This is especially so due to the poor funding of such programs. Approaches like de-budding are labor-intensive and the majority of the farmers are not able to follow it through due to advanced age or infirmity.

5. Biological Control and Limitations

There is currently no biological control for *Xanthomonas vasicola PV. musacearum.* However, some of the biological controls like *P. putida*, *P. fluorescens*, and *P. syringae* upon use showed a significant reduction in the bacterial spot of tomato (*Xanthomonas campestris PV. vesicatoria*) applied under green-house conditions [28]. Consistently foliar spraying of *Pseudomonas syringae* was also found to significantly suppress bacterial spot of tomato that is caused by *Xanthomonas campestris pv. vesicatoria* [29]. Application of *Pseudomonas fluorescens* and *Bacillus pumilus* to seed and root was also found to significantly suppress bacteria spots in field trials conducted in Alabama [29]. Although significant suppression of the bacteria disease has been reported in biological control, inconsistencies in performance between experimental conditions and the field have been reported [30]. The inconsistencies have been caused by the varied biotic and abiotic factors in the field. In addition, the survival of the efficacy in biocontrol is affected by varying environmental factors like agricultural practices, plant genotype, and resistance to the pathogen [31]. Off-target effects can occur if the biocontrol can affect important microorganisms which might lead to extinction, therefore, affecting a component of the ecosystem. Some fungi used as biocontrol could affect a wide range of hosts including mammals. Application of bio-controls should therefore be carried out with the evaluation of potential virulence to non-target organisms [32].

6. Chemical Control of BXW and Their Limitations

Although there are currently no chemicals available for BXW control, chemicals have been used for drenching soil or killing infected banana stems [29]. For many years the control of bacteria diseases using chemicals was digging out the infected matt and applying methyl bromide [33]. However, the effect of methyl bromide on the Ozone layer made many countries ban its use. An alternative to methyl bromide called Dazomet (Basomid® granular 97%) has been used to sterilize soil to control bacterial diseases like Moko/Bugtok diseases [34]. Formalin too has been reported to be used in drenching soils around Ralstonia-infected Cavendish bananas resulting to lower bacteria counts [35]. Herbicides too can effectively be used to destroy the infected banana plants. This is carried out by injecting an infected mature banana plant with 1.2 ml of 2, 4-Dichlorophenoxyacetic acid (2, 4-D). The working of 2,4-D is that two weeks after it has been applied to a plant, the pseudostem of the plant falls off at the base. At the time of falling the chemical has reached the base of the pseudostem and it has started rotting. This method of using 2,4-D is easier than physically burying the infected plants [36].

Although chemicals have been used to some extent in controlling the spread of BXW and other bacterial diseases, chemicals are not well perceived by the consumers and other food outlet chains [37]. One great disadvantage of chemical use is that they contaminate groundwater, affect animal health, enters into the food chain, and are harmful to the person spraying the pesticides. Some countries in the Europe Union (EU) like Sweden, Netherlands, and Denmark in the mid-1980s decided to decrease the use of agrochemicals to 50% [38]. The use of agrochemicals provides suppression of the pathogen in some crops, but they must be sprayed around 8-10 times for the suppression to be sustained. This kind of management is very expensive to the farmers and in some instances, the chemicals are resisted by the pathogens.

7. Genetic Modification for Resistance to BXW

Genetic modification is one of the promising technology that has been applied in the control of BXW. The technique allows breeders to work with their genes of choice rapidly to produce new varieties [39]. The technique combines both traditional breeding and transgenic perspective to develop new varieties that have multiple resistance to pathogens. Several approaches to genetic engineering exist today which include particle bombardment [40, 41] and agrobacterium

transfer of genes using embryonic cell suspensions [42, 43]. Genetic engineering using cell suspensions is a lengthy process and expensive. Gene transfer has been achieved using the shoot tips of plants. The process applies to many types of cultivars as it does not involve disorganization of the cell cultures but rather uses micro-propagation. The technique produces several homogenous populations within a short period and therefore offers an alternative to embryonic cell suspensions. In Uganda, bananas have been transformed for resistance against BXW [44, 45]. The transgenes encode for plant ferredoxin-like protein (*Pflp*) and hypersensitive reactive assisting protein (*Hrap*). The transgenes work from two approaches i.e iron depletion which is an antibiotic perspective and eliciting hypersensitive reaction brought by triggering the Harpin, therefore, blocking the pathogen from advancing. The confined field trials conducted to evaluate the transgenic bananas in Uganda showed the bananas were resistant to BXW [44].

The challenges faced by genetic engineering are the regulatory and acceptance hurdles especially in developing countries [46]. The potential consumers of the technology have been fed with misleading information, especially from the media. The misconception facing genetic modification is the fact that the novel genes used for transformation are argued to remain in the final food product and anti-GMO technology insists that the genes might have negative repercussions on the consumer. There is, therefore, a need to come up with a technology that erases the novel genes to improve the acceptance of the commodities by consumers.

8. Status of Genetic Editing

Recently, advances in gene editing (GE) technology have raised hope in the search for improved bananas. GE can be applied in banana breeding programs as it makes efficient and precise changes in the banana genome to develop resistance to diseases [47]. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) is one of the methods that has gained acceptance as a genome-editing tool because it has high precision in alteration of plant genome and multiplexing [48]. The technique is acceptable mainly because it involves editing the existing genome to mimic the natural cell processes without adding any foreign genes. The application of the technique has been reported in many plants and organisms [49]. Although the earliest application of the technology was in seed crops, it has also been recently applied in vegetative-produced crops such as cassava, potatoes, and bananas [50]. The first report of gene editing was conducted in the banana variety 'Rasthali' where a single gRNA was used to target *the phytoene desaturase* (*PDS*) gene and 59% of mutation frequency was achieved [51]. The second advancement was multiplexing of gRNA and targeting *PDS*, the study achieved 100% mutation frequency [52]. The next study targeted to disrupt the integrated endogenous of the banana *streak virus* [53].

9. Advances in Genome Editing for Control of BXW

Recently it has been demonstrated that the genes identified in the BXW-resistant *Musa balbisiana* can be utilized to develop a banana that is resistant to BXW by using CRISPR-Cas9 [54]. It has also been demonstrated that knocking out the banana orthologue of the downy mildew resistance 6 *(MusaDMR6)* enhances resistance to BXW [54]. The *downy mildew* resistance *6 (DMR6)* is identified as a susceptibility gene that encodes 2-oxoglutarate Fe(II)-dependent oxygenase (2OGO). The gene is up-regulated when there is pathogen infection. The genes *DMR6* together with its paralog *DMR6*-Like Oxygenase1 (DLO1) work by suppressing plant immunity and are co-expressed during pathogen infection [55] Phylogenetic analysis of the 2OGO gene family identified seven *AtMR6* orthologues in *Musa balbisiana* and *Musa acumina*ta that belonged to the same clade Q9FLV0.1. Upon further analysis of one of the *AtDMR6* as a putative candidate for enhancing resistance in bananas. The candidate gene showed up to 100% resistance towards BXW in the greenhouse evaluations [54]. The *dmr6* mutants might provide a broad-spectrum resistance to varied bacterial infections. The *dmr6* broad spectrum of resistance together with developing CRISPR/Cas9 therefore could be a strategy for developing resistance by targeting *MusaDMR6* and causing mutations as a way of controlling BXW. This strategy of developing resistance provides a more appreciative solution than genetic modification as there are no novel genes inserted into the banana genome.

10. Conclusion

The current methods for controlling BXW face a myriad of challenges including inconsistencies and labor intensity. The notable challenge especially for cultural, biological, and chemical controls is that the farmers are poor in resources and therefore are not able to consistently adhere to control measures prescribed by research. Genome editing comes across as the most promising as it does not add any new genes to the plant. This therefore might make the regulators consider lessening the regulatory requirement. The technology might also be appealing to consumers as they might embrace the product more readily than genetically modified foods. Investment in GE in the control of BXW in the future is, therefore, a worthwhile endeavor.

Conflict of Interest

The authors declare no conflict of interest.

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