Potato bacterial wilt management for quality seed potato production in Ethiopia

A training manual for agricultural extension experts, development agents, farmers' seed grower cooperatives and decentralized seed multipliers

DEC
2021

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POTATO BACTERIAL WILT MANAGEMENT FOR QUALITY SEED POTATO PRODUCTION IN ETHIOPIA

A TRAINING MANUAL FOR AGRICULTURAL EXTENSION EXPERTS, DEVELOPMENT AGENTS,
FARMERS' SEED GROWER COOPERATIVES AND DECENTRALIZED SEED MULTIPLIERS

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ISBN: 978-92-9060-629-1
DOI: 10.4160/9789290606291

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Citation:
Workayehu, M., Enyew, M., Abitew N. W. and Kakuhenzire, R. (2022). *Potato bacterial wilt management for quality seed potato production in Ethiopia: A training manual for agricultural extension experts, development agents, farmers' seed grower cooperatives and decentralized seed multipliers*. Lima, Peru: International Potato Center.

Design and Layout:
Communications Department

December 2021

CIP also thanks all donors and organizations that globally support its work through their contributions to the CGIAR Trust Fund: www.cgiar.org/funders

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ACRONYMS AND ABBREVIATIONS

BCA	Biological Control Agents
BW	Bacterial Wilt
CIP	International Potato Center
DLS	Diffused Light Store
EGS	Early Generation Seed
EPS	Extracellular polysaccharide
IPM	Integrated Pest Management
masl	Meter above sea level
NCM-ELISA	Nitro-Cellulose Membrane - Enzyme-Linked Immunosorbent Assay
NGO	Non-governmental organization
QTL	Quantitative trait loci
ToT	Training of Trainers
VBNC	Viable but nonculturable

FOREWORD

About 85% of Ethiopia's population residing in rural areas is engaged in agriculture as a major means of livelihood. However, the sector here is dominated by subsistence, smallholder farmers characterized by low productivity. Over the last decades, Ethiopia has been unable to produce adequate food for its rapidly growing population. In fact, natural calamities, food insecurity and famine seem to have become common phenomena and critical problems plaguing the country. Food insecurity is threatening to become chronic, and its underlying causes include, but are not limited to, a rapidly increasing population, low agricultural productivity, widespread environmental degradation, and recurrent droughts (International Potato Center, 2012). Thus, intensification of farming systems targeting the expansion of roots and tubers is imperative to forestall a human food insecurity catastrophe in the future.

Potato is a high-potential food security crop because it provides high yields of high-quality food per unit input with a short crop cycle (Teklemariam, 2014). Thus, it can play an important role in improving food security and cash incomes of smallholder farmers in Ethiopia. It is also a cheap source of quality food that can be supplied within a relatively short period compared to most locally adapted food crops.

Potato is the perfect food and one of the few that can sustain life on its own. It is a well-balanced major plant food with a good ratio of proteins and carbohydrates. It contains substantial amounts of vitamins, especially vitamin C, minerals, and trace elements. Moreover, it has the correct balance of protein calories and total calories. It is one of the cheapest sources of energy, with the highest production of protein per unit of land compared to major food crops such as rice, maize, and wheat.

Potato has long been regarded as a lowly, subsistence crop and is still an underexploited food resource in Ethiopia. Its huge potential to improve food security, income and human nutrition is increasingly being realized and explored by farmers, private investors, and policymakers. While the average national potato yield is far below attainable levels, ample opportunities exist to unleash its potential for increased productivity, food security and income generation (Teklemariam, 2014).

The Ethiopian government considers potato a strategic crop that can enhance food security and generate economic benefits for the country. As the population increases, enhanced potato productivity is likely to improve the livelihoods of smallholder potato farmers by meeting the growing food demand (Gildemacher et al*.,* 2009).

However, potato production faces many constraints including pests and diseases, especially potato bacterial wilt caused by *Ralstonia solanacearum*. Potato bacterial wilt continues to be an economically serious problem in field-grown potato in many tropical, sub-tropical and warmer areas of Ethiopia because it cannot be controlled by conventional agro-chemicals, and it persists in the soil for a long time. There are no registered chemicals in either Ethiopia or globally for the cost-effective management of this disease. Natural control through genetic resistance has not been successful, hence an integrated approach is recommended.

This manual for the integrated management of potato bacterial wilt was developed by reviewing training materials produced by different actors in the potato sub-sector and used as a tool in agricultural extension in the fight against the disease. It consolidates various aspects of potato production into a harmonized training material targeting agricultural extension experts, development agents and farmer seed group cooperative leaders.

This manual provides insights into the background of potato production in Ethiopia, pest and disease management, and economic and gender considerations in integrated management of potato bacterial wilt. It is expected to guide farmer trainers, entrepreneurs, and other stakeholders, including youth on how to grow a healthy potato crop with a business approach. The manual will serve as a reference for trainers in the industry and a guide in preparing training materials for farmers, training of trainers (ToTs) and youth.

Acknowledgement

The publication of this manual was funded by the German Federal Ministry for Economic Cooperation and Development (BMZ) commissioned by Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) through the Fund for International Agricultural Research (FIA) under the auspices of Seeds of Change Project (Grant number: 81235249).

1 INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops in Ethiopia with a high productivity per unit area and versatile uses. Potato in Ethiopia is grown thrice annually during the long rains (meher), short rains (belg) and as dry season irrigated crop. It is estimated that 970,000 tons are produced on about 70,362.22 hectares in the main season (CSA 2019/20). However, its estimated yield at 13.14 t/ha is low compared to its potential yield of 40 t/ha under recommended agronomic practices. The key causes of low yield are related to inadequate use of high-quality seed potato and quality inputs. Inadequate storage facilities on farm and at market centers have led to huge losses during potato marketing. Farmers do not store potatoes due to lack of technical knowledge, the need for immediate cash and poor quality of produce due to pests, diseases, or mechanical damage.

Potato is an important food security crop with great potential for poverty alleviation. It is one of the major root and tuber crops well adapted to the northern and central highlands of Ethiopia. The crop is a major source of food and cash for more than 1.07 million farming households (CSA 2019/20). However, its sustainable production is constrained by both abiotic and biotic stresses, among which late blight caused by *Phytophthora infestans* and bacterial wilt (BW) caused by *Ralstonia solanacearum* stand out (Bekele ,2017).

Bacterial wilt has spread to most potato producing countries globally. Its appearance in Australia and southeastern United States has led to extensive scientific research in these areas. In Latin America, the disease has been reported in all potato producing countries except Ecuador (Priou et al.,1999). The disease occurs throughout central and southern Africa and is a serious production constraint in Uganda, Ethiopia, Kenya, Madagascar, Rwanda, Burundi, Nigeria and Cameroon in potato, tomato and other important solanaceous crops (Priou et al.,1999). Though it is not a serious disease in Egypt, latent BW infection in ware potato tubers has resulted in a steep decline in exports to Europe (Seleim etal.,2014). In Southern Asia, BW occurs in the mid-hills of the Himalayas in India, Pakistan, Nepal, and Bhutan. Its occurrence in the mid-hills and plains of India have however been effectively controlled. In East and Southeast Asia, BW is important in Indonesia, the Philippines, southern Vietnam, Laos, Japan, and southern China (Priou et al.,1999).

In the early 1990s, BW became a serious threat to potato production in Europe mainly in Belgium, England, France, The Netherlands, Spain, Italy, and Portugal. It has also been reported in Russia. Earlier incidence in Sweden was followed by accelerated research and its eventual eradication. Given its wide geographic distribution, broad host range, rapid dispersal, and heavy economic losses it can cause, *R. solanacearum* has been classified as a quarantine pathogen in Europe and is listed as a “Bioterrorism Select Agent” in the United States.

Potato BW was first recorded in Ethiopia in 1956 and has since been a major production constraint and significant limitation to the production of clean potato planting material. The disease has progressed to disease-free areas, usually introduced through latently infected potato planting materials obtained from the informal seed system. Bacterial wilt has become a bigger threat than late blight to potato production in Ethiopia. It can decimate an entire crop and render the land unusable for most other solanaceous crops for many years. In warmer environments, the land can get endemically infected, making potato and tomato production impossible.

In Ethiopia, BW in potato is caused by *R. solanacearum* race 3 which is responsible for considerable losses in the potato and tomato industries (Yaynu, 1989). With growing popularity of potato cultivation in the country, BW has been rapidly spreading from highly infested regions to new, uninfected areas. This situation is exacerbated by the lack of a well-organized seed certification system, weak regulations governing seed distribution in the country and inadequate awareness about BW among seed potato producers and farmers.

The socio-economic importance of potato and the threat BW poses in Ethiopia requires an urgent policy to increase awareness about the disease, undertake research and implement practical steps to manage it. Bacterial wilt will continue to be an economically serious problem for field-grown potatoes in many agro-ecological zones in Ethiopia largely because of the lack of a well-defined mechanism to obtain clean seed, the absence of cost-effective chemical control measures and the persistence of the pathogen in the soil. Conventional BW management is limited due to the special biological adaptation features associated with *R. solanacearum* in potato and tomato cultivation.

There are no commercially registered chemicals in Ethiopia and globally that can be used to control BW in potato. Though breeding for *R. solanacearum* resistance has not been successful, some potato cultivars are less susceptible than others and can give appreciable yields in the presence of the disease. This manual strives to enable users to understand basic BW integrated management options for seed potato producers to eradicate or prevent the spread of this serious disease if this agro-enterprise is to be sustainable. Both growers and extension agents must be aware of the risks associated with BW and take precautions to prevent its introduction and spread.

2 NATURE OF BACTERIAL WILT (*RALSTONIA SOLANACEARUM*)

2.1 Potato BW and the causative agent

Potato BW is caused by a soil-borne bacterium, *R. solanacearum* (formerly known as *Pseudomonas solanacearum*). It is a serious disease of potato and other solanaceous crops in many developing countries in temperate, sub-tropical, and tropical zones of the world. An aerobic, non-spore-forming, rod-shaped, gram-negative bacteria, *R. solanacearum* is usually an immobile soil-borne plant pathogen but may ordinarily live as a saprophyte in the soil (Fig. 1E) in the absence of a suitable host. It mainly infects potato or any host system through the roots by penetrating microscopic wounds caused by the emergence of lateral roots or mechanical root injury. The common causes of root wounds are transplanting, nematodes and injury by insect or cultivation equipment that allow the bacteria living in the root zone in free water or root exudates (Fig. 1A) to enter the plant. Once the bacteria enter the host plant, infection occurs immediately. The bacterium colonizes the cortex and makes its way towards the xylem vessel, moving passively in the plant sap, from where it rapidly spreads to the entire plant (Fig. 1A&B). The bacterium finally colonizes the xylem vessels (Fig. 1C), multiplies and forms bacterial masses that prevent free water flow from the roots to the leaves, resulting in characteristic plant wilting that starts from a single leaflet to the leaf, branch, whole stem and eventually to the entire plant (Fig. 1D).

***R. solanacearum* infection in plant hosts**

Figure 1 (A to E) illustrates the process of *R. solanacearum* infection in plant hosts.

A) Bacteria are attracted to the host root exudates, attach themselves to the root surface and penetrate roots via natural openings, cracks and wounds.

B) Bacteria then make their way through the cortex (the tissue beneath the skin) to the xylem vessels (the conductive tissue through which the sap moves from the ground to aerial plant parts).

C) Bacteria pass through the pits in the xylem tissue walls and colonize vessel lumen. (Inset: Inside view of a xylem vessel with bacteria infiltrating through the pit to the vessel lumen.)

D) Bacteria enter the vessels, multiply and fill the lumen with cells and mucilage (inset: polysaccharides). This impairs free water flow leading to wilting.

E) Due to excessive wilting, the host dies. With time, the plant parts wither and the bacteria are released into the soil until another suitable host becomes available.

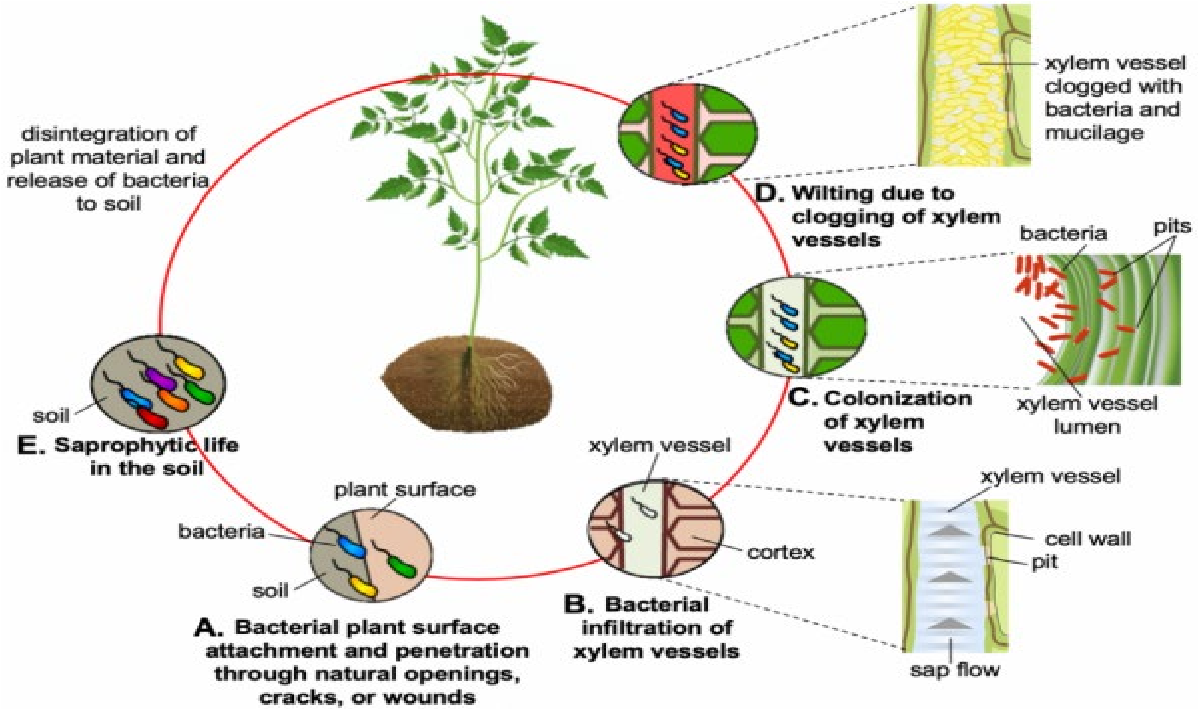

Fig. 1. Different phases of *Ralstonia solanacearum* life cycle: pathogenic (B-D) and saprophytic (E) (Alam and Rustgi, 2020).

Disease epidemiology

Ralstonia solanacearum is often described as a soil-borne pathogen, though its survival as a free-living organism in bare soil is usually short-lived at low temperatures. However, it can live significantly longer in alternative and wild host plants, or on root exudates as a saprophyte on non-host plants species without causing any disease. *Ralstonia solanacearum* has been known to survive in a viable but nonculturable (VBNC) forms under stress conditions in soil and water (Kong et al., 2014). However, the epidemiological relevance of this pathogen behavior is unclear although it is speculated to be a transient state between stressful and conducive conditions that allows it to perpetuate itself.

Bacterial wilt under adequate moisture supply is most severe at temperatures ranging between 24°C and 35°C, although the PIIB1 strain is colder tolerant than other strains (Stevenson, 2001). High soil moisture or periods of wet weather or rainy conditions are associated with high disease incidence and severity. The entry into host plants is usually through root injury, from where the bacteria move in the water stream to colonize the xylem. Once in the xylem, the bacteria adhere by polar attraction to the vessel walls or invade the lumen. The blocking of xylem vessels by bacterial cells and their extracellular polysaccharide (EPS) metabolites is the major cause of wilting observed in infected susceptible plant hosts.

Ralstonia solanacearum can also be transmitted mechanically during pruning operations or when cuttings are taken for propagation. Long distance transportation of vegetative propagating material (e.g., seed potato, rhizomes of ginger and turmeric and banana suckers) can carry latent infections which spread the disease to new areas. While natural infection of true seed is rare, it has been reported in groundnut in Indonesia and China. Although contaminated seed have been reported in other hosts (including tomato, capsicum, eggplant and soyabean), seed infection and transmission have not been adequately substantiated.

Currently, R. *solanacearum* transmission in potato is mainly through water run-off, irrigation, soil adhering to contaminated farm tools. However, movement of latently infected vegetative plant parts pose the greatest risk in most host plants than disease transmission via true seed. In contrast, some strains of *R. solanacearum* and *R. syzygii* that cause Moko and blood disease in banana and Sumatra disease in clove are transmitted by insects, including pollinating flies, bees, wasps, thrips, and xylem-feeding spittlebugs of *Hindola* spp. in cloves. These transmission routes of bacterial pathogens have the potential to spread the disease over long distances in a short time.

The potato BW pathogen may alternatively survive in leftover plant debris such as stems and cut tubers, which can serve as a source of inoculum for the appropriate host. Race 3 of *R. solanacearum* biovar 2, the cause of BW in potato and tomato, can survive for up to two years in soil under fallow conditions (Kakuhenzire et al., 2013). Graham et al. (1979) reported the survival of *R. solanacearum* race 3 in plant debris for almost three years after the harvest of an infected potato crop. It can also survive for up to three years in bare fallow soils, infested soil, or water, forming a reservoir of inoculum for the pathogen, and longer in soils cropped with non-solanaceous and non-host crops among cereals and legumes.

Race 3 of *R. solanacearum* is adapted to low temperatures and occurs predominantly in temperate, sub-tropical and cool tropical highlands. It can survive during the winter in temperate regions in semi-aquatic weeds or in plant debris or as saprophytes in the rhizosphere of non-host plants. These act as reservoirs for the pathogen which is released when conditions become favorable for it to resume its pathogenic cycle. In potato farming systems, it is difficult to eradicate volunteer crops; hence land infected with BW is likely to remain so if volunteer potato is present after the initial outbreak. Therefore, eliminating all volunteer potatoes in such a field is key to reducing soil-borne *R. solanacearum* infestation.

The distribution of *R. solanacearum* race 3 in temperate areas is due to its close association with potato and its ability to survive under cold temperatures where potato is commonly cultivated. Its ability to survive in sheltered sites, infected debris, and pockets of infestation in deep soil layers may serve as additional sites for the long-term survival of the bacterium (Graham and Lloyd, 1979). High soil moisture and low temperatures appear to favor the long-term survival of *R. solanacearum* in vegetation-free soil.

2.2 Geographical distribution

The *R. solanacearum* species complex is widely designated as a quarantine organism in many countries to prevent its movement across borders. Phylotype PIIB1 strain has spread from its origin to many areas worldwide, presumably in seed potato trade (Elphinstone, 2005). However, this strain has never been reported in potato in the USA, despite it having been introduced from infected geraniums (Williamson et al., 2002) and Phylotype II strains found on potato and other hosts in the southern states.

Phylotype, I strain of *R. solanacearum* are regarded to be of Asian origin, and predominantly belong to race 1 or biovar 1, 3, 4 and 5. Phylotype II strains are thought to be of South American origin and mainly belong to races 2 and 3 or biovar 1, 2 and 2T. Phylotype III appears to have evolved in Africa and Phylotype IV in Indonesia.

Phylotype PIIB1 strain has been designated as a select agent in the USA because of its perceived potential as a severe threat to agriculture. Moko disease-causing Phylotype II strains mainly occur in South and Central America and Caribbean countries. They also appear to have spread to the Philippines, where the same strain has been found on cooking banana (ABB and BBB genotypes), causing bugtok or banana BV disease.

2.3 Environmental conditions conducive to BW disease development

Bacterial wilt disease development in potato is most favoured between 25°C and 37°C favor (Stevenson, 2001). The optimum temperature for race 3 is 27°C, which makes it less dangerous in temperate and highland tropical regions. It is usually not harmful in areas where mean soil temperature is below 15°C or in cool highland areas. When the temperature is low, infection may remain latent until conditions are more favorable. This makes it difficult to visibly recognize infected fields or planting material in those fields unless it is diagnosed with sensitive tools. In optimum temperature environments, high soil moisture, slightly acidic soils, and poor and low fertility soils favor infection. Disease incidence and severity are highest when temperature and soil moisture are high, such as during rainy periods or high irrigation. Once infection has occurred, symptoms will often be more severe under hot and dry conditions, with intermittent moisture supply hastening wilting in infected plants.

2.4 Host range

The host range of *R. solanacearum* has been constantly expanding due to rudimentary and exhaustive agricultural practices at the global level together with climate change associated global warming. Its economically important host range includes banana and plantain, cucurbits, eggplant, eucalyptus, ginger, groundnut, mulberry, tobacco, tomato, and many ornamental plants. Ralstonia *solanacearum, R. pseudo solanacearum* and *R. syzygii* each comprise strains that were originally designated as race 1 and occur in tropical areas all over the world, attacking more than 250 hosts in 54 plant families. A few ornamentals and some common solanaceous weed species commonly found in most Ethiopian agro-ecologies that are infected by *R. solanacearum* include bittersweet (*Celastrus orbiculatus*), black nightshade (*Solanum nigrum*), thorn apple or jimson weed (*Datura stramonium*) and stinging nettle (*Urticadioica*).

Race I infect a wide range of plant species including potato, tomato, eggplant, tobacco, chili, peanut, and several weeds in warm areas and at low elevations in the tropics. Race 3 mainly affects potato at high elevations. Some *R. solanacearum* genotypes within Phylotype II (sequevars IIA-6, IIA-24, IIA-41, IIA-53, IIB-3, IIB-4, and IIB-25), originally described as race 2, cause Moko or banana BW disease of Musa spp. (banana and plantain) and Heliconia (Priou et al.,1999). *Ralstonia syzygii* comprises three subspecies: subsp. *syzygii* infecting cloves, subsp. *celebesensis* that causes banana blood disease and subsp. *Indonesian* found on solanaceous crops (potato, tomato, and chili pepper) as well as clove in Indonesia.

Strains within each of the four phylotypes of *R. solanacearum* can cause BW or brown rot of potato. However, sequevar 1 within Phylotype IIB (PIIB1), formerly known as race 3/biovar 2, is most associated with potato. This genotype has a lower optimum plant temperature (27°C) than most other genotypes (35°C). It often manifests as latent (symptomless) infections at high altitudes in the tropics, sub-tropics and temperate potato growing areas. This strain can also cause BW of tomato and can survive perennially in black nightshade which acts as a secondary host. For example, strain PIIB1 survives absence of suitable hosts in underground stolon's of *Solanum dulcamara* (woody nightshade) that grows along some European rivers and water systems, spreading the disease to potato crops when the contaminated river or surface water is used for irrigation (Janse et al., 1998). The same strain has also spread internationally on geranium cuttings produced in Africa and Central America (Williamson et al., 2002).

2.5 Signs and symptoms of BW infection

The potato BW disease pathogen can remain latent without showing any symptoms in the field on both host and non-host crops and weeds, impacting fresh tuber yields in future potato crops. Under cool conditions, infected potato plants may harbor the bacterium without exhibiting symptoms but retain the pathogen in progeny tubers, resulting in severe disease outbreaks if the resulting seed is grown in warmer and moist conditions. Once the attack happens under optimum conditions, *R. solanacearum* infection symptoms occur in both above and below ground parts of the potato plant.

2.5.1 Symptoms above the ground

Symptoms of *R. solanacearum* infection above the ground manifest as wilting, stunting, and yellowing of foliage. However, under optimum conditions the disease develops rapidly, and the entire plant can wilt quickly without yellowing. Plant wilting resembles symptoms of lack of water among all *R. solanacearum* phylotypes in most infected hosts. The youngest leaves usually wilt first, occurring at the warmest time of the day. Wilting may be consistently visible in only one stem or on one side of the infected plant or even a part of a leaf, depending on side of the plant where vascular infections are restricted. Leaves may become bronzed or chlorotic and epinasty (facing downwards) may occur. Wilting may be observed on 1-2 leaves in young plants during the heat of the day, but sometimes such plants appear to recover at night, but the infection remains. In severe infection, the plant will not recover though the leaves may remain green.

The whole plant may wilt rapidly if environmental conditions are favorable for both plant and pathogen growth. As the disease develops, a brown discoloration of the xylem vessels in the stem transverse section may be observed. In severe infections, a creamy, slimy mass of bacterial exudate may be observed from vascular bundles when a succulent stem of an infected plant is cut transversely. When such cut ends are held vertically for a few minutes, thin threads of ooze will flow from the cut ends as they slowly separate. If such a cut stem is vertically suspended in a glass of clear water, a white and milky stream of bacterial cells and slime will flow from the vascular system into the water in 5-15 minutes. This cannot be demonstrated by other wilt-causing pathogens in potato.

2.5.2 Symptoms below the ground

External symptoms may not always be visible on aerial plant parts or infected tubers. However, in severe infections, bacterial ooze collects on tuber eyes or stolon end, causing soil to adhere to those areas when it dries. Symptoms in infected tubers may or may not be visible depending on the state of development of the tuber or disease in relation to the prevailing temperature and soil moisture. However, infected symptomatic tubers cut in cross-section show brownish discoloration of the vascular ring and neighboring tissues. In extreme infections, the tubers will completely rot. Infected but non-rotting tubers may produce slimy, sticky, pale yellow ooze from the eyes and heel end rendering them unusable. A creamy exudate usually appears spontaneously from the vascular ring at the cut surface. When a cut stem or tuber vascular tissue is placed in water, threads of bacterial ooze exude.

2.6 Vascular flow test to detect BW infection

- Make a 4–6-cm-long incision at the center of the stem from the base of a suspected infected plant.
- Tie the piece with a string or attach it to a paper clip.
- Suspend the piece of stem vertically in a glass full of clean, clear water, as illustrated in Figure 2, with the stem base facing the bottom of the glass.
- Observe the base of the cut stem for 10-15 minutes. If the plant is infected with BW, white smoky threads will flow from the stem base to the glass bottom.

Fig. 2. Pictorial presentation of steps in *Ralstonia solanacearum* infection vascular flow test.

The vascular flow test may not reveal the presence of *R. solanacearum* in very early stages of BW when infection occurs late in the crop growth cycle and when the infected plant is water deficient. The test may also not be adequate to detect *R. solanacearum* when the potato plant does not show wilt symptoms; moreover, other pathogenic infections besides *R. solanacearum* can also cause plant wilting. Vascular flow may also not be suitable amenable to mass testing of samples. Therefore, more sensitive and throughput techniques such as serological such as NCM-ELISA or molecular based methods are more desirable.

2.7 NCM-ELISA test

Nitro-cellulose membrane - enzyme-linked immunosorbent assay (NCM-ELISA) has been found to be a more sensitive, specific, cheap and a mass testing technique that overcomes the challenges of classical diagnostic techniques. However, certain steps must be taken before the diagnosis is conducted.

2.7.1 Sample collection

A composite sample of tubers is collected in the field or seed potato store. The number of tubers collected per field or store will depend on the field size or tonnage of stored seed, respectively. Seed tuber samples may be collected from potato fields just before dehaulming or during as randomly selected from heaps or from stores. A seed lot is defined as comprising tubers of one variety harvested from the same field and originating from the same seed source. In a vegetative crop or before harvesting, one tuber is collected from each sampled plant. The sample plants in the field are selected in a zigzag or other random fashion. For one hectare of basic seed, 250 plants are sampled or 250 tubers for every 25 tons of stored seed tubers. Each composite sub-sample of no more than 25 tubers is collected in a net bag, carefully labeled, and stored at room temperature before it is processed for BW testing. After sample collection in each field or store, the tools and equipment used are immediately disinfected (Fig. 3) before collecting the next sample, even if they are from the same field or store.

Fig. 3. Disinfecting a hoe before collecting a sample (left) and sample collection (right).

2.7.2 Sample preparation

Each composite tuber sample is thoroughly washed in tap water to remove soil or dirt. It is then disinfected in 1% household bleach for five minutes and rinsed in sterile distilled or boiled water and allowed to dry on clean bags or soft tissue paper (Fig. 4).

Each tuber is then cut in cross-section about a third of the distance from the heel end to expose the vascular ring. Fragments of the vascular ring are extracted using a cuticle remover and put in a plastic bag labeled with the number corresponding to that on the unprocessed sample prepared for *R. solanacearum* extraction and culturing. A step-by-step procedure for *R. solanacearum* extraction, culturing and final detection using NCM-ELISA is elaborated in Priou (2001).

2.7.3 Incubation, enrichment and dot blotting

The NCM-ELISA process is primarily used to identify *R. solanacearum* or detect latent infections in asymptomatic hosts (Fig. 5). However, in latent infections, a low bacterial infection load may not be detected in a sample without first culturing and enrichment. Fragments from the extracted vascular bundle from tuber samples are crushed in an extraction medium. An aliquot of a supernatant is incubated in a semi-selective medium to culture and multiply the few *R. solanacearum* cells in asymptomatic samples before conducting ELISA to increase the sensitivity of the test and detect latently infected samples.

Fig. 4. Sample preparation to extract *R. solanacearum* from potato tubers prior to pathogen detection using NCM-ELISA.

The enrichment procedure increases bacterial cells and reduces the population of saprophytes that would compete with *R. solanacearum* without the enrichment step. During enrichment, the tuber sample extracts are incubated in semi-selective medium, South Africa (SMSA) agar for at least 48 h at 37°C with regular shaking. After the enrichment step, each sample extract is dot-blotted on the NCM membrane as double spots (Fig. 5).

The *R. solanacearum* antigens are detected using *R. solanacearum*-specific antibodies in ELISA (Priou, 2001). A positive reaction revealed by a purple coloration indicates the presence of *R. solanacearum* in the sample, assuming there has been no contamination or cross reactions. Results of the test samples are compared with positive and negative control NCM strips that are included in the kit as reference on the level of infection. A higher intensity of purple coloration of the reaction reveals a higher concentration of bacterial cells and therefore high infection severity. The results are accordingly tabulated qualitatively as positive or negative depending on the purpose of the test.

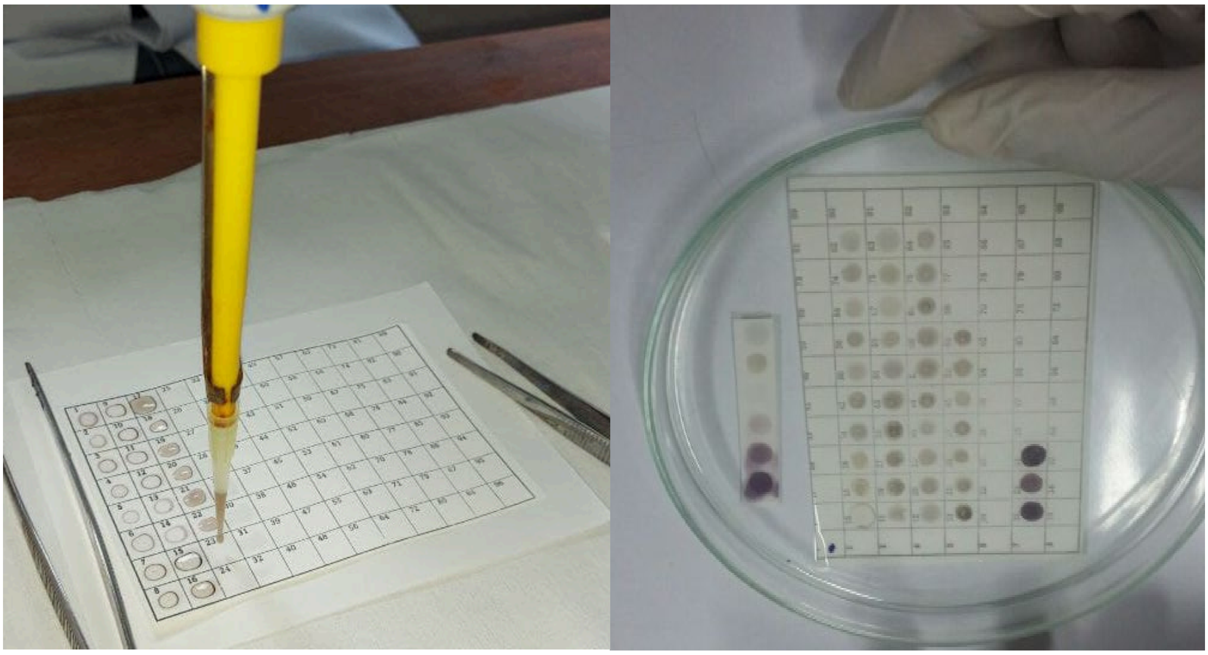

Fig. 5. Loading samples (dot blotting) on the NCM membrane (left) and results of the ELISA test (right).

3 INOCULUM SOURCE, TRANSMISSION AND SPREAD OF BACTERIAL WILT DISEASE

Potato BW due to *R. solanacearum* can spread in fields through various channels, but mainly via latently infected tubers, contaminated soil, irrigation and flood water, contaminated farm tools and equipment, infected plant parts, plant debris, weeds, animals, and other farm activities (Stevenson, 2001). Latently infected tubers and irrigation water are the major sources of inoculum. Infected planting material is the main mode of the bacteria's spread to locations and during seasons, particularly in vegetatively propagated crops. Seed potato produced in cool climates such as tropical highlands may not show any symptoms, but when planted in warmer locations, severe BW may develop. Hence the transportation of infected seed from distant regions in Ethiopia to new, disease-free areas of the country by traders, private actors, and non-governmental organizations (NGOs) is a probable cause of the recent widespread occurrence of BW.

The spread of *R. solanacearum* within a potato crop occurs because of the pathogen's passive movement from plant to plant along plant roots and water streams. Further spread happens during cultivation, weeding and hilling up. In general, the principal inoculum sources of BW infection are infected planting materials and infested soil.

3.1 Infected seed tubers

Infected seed tubers are the most common source of *R. solanacearum* inoculum, especially as latent infection. Seed tubers produced in cool climates such as tropical highlands at altitudes exceeding 2500 meter above sea level (m.a.s.l) may not show infection symptoms. When planted at lower and warmer elevations, disease development may be rapid and severe. Infected seed is the principal vehicle of transmission locally and internationally.

Heavily infected tubers usually rot away and contaminate the land in which they were produced than apparently healthy seed tubers in latent form that are moved together with the pathogen to new areas (Shamsuddin et al., 1989). This manner of *R. solanacearum* spreading has been known for many years, however, its importance in containing *R. solanacearum* spread has probably been not fully appreciated especially in smallholder potato growing communities. The few well-documented cases of long-range *R. solanacearum* dispersal will increase awareness of the potential of its dissemination in latently infected seed.

3.2 Contaminated or infested fields

Planting clean seed potato tubers in infected fields will result in BW disease expression, but only later in the cropping season and under favorable conditions. Locally, the disease can spread among plants via soil adhering to shoes, farm tools and water movement in the soil. Cultural practices such as planting on ridges reduces interplant BW spread because it promotes better soil water drainage and limits root damage during weeding and hilling up.

3.3 Irrigation and flood water

Using *R. solanacearum*-infected water for irrigation in disease-free farms will spread BW inoculum. Flooding through rainwater from high slopes with infected farms to lower non-infected fields will spread BW to new areas or disease-free potato fields. Bacterial wilt can also spread by irrigation water from ponds and surface streams.

It is therefore important to protect irrigation dams and channels from run-off and surface or flood water that may carry *R. solanacearum* inoculum from infected fields or other water sources.

3.4 Nematodes

Nematodes are potato pests. By themselves nematodes do not contribute to the spread of *R. solanacearum* but facilitate infection by puncturing plant roots to provide entry routes for the pathogen. This synergistic interaction between the root-knot nematode (Meloidogyne spp.) and *R. solanacearum* on a variety of hosts is widely known to enhance attacks by other pathogens. Potato root infection by nematodes is expressed as root-galling index and is often positively correlated with BW incidence as a percentage of wilting potato plants. This is interpreted as the effect of increased wounding of the root system, providing points of entry for the pathogen. Nematodes may also modify plant root tissues that become more suitable for bacterial colonization. Considering the global importance of potato BW and its occurrence with root-knot nematodes, potato breeding programs should consider developing varieties with dual resistance to both organisms (Jatala et al., 1987).

3.5 Infected tools and farm machinery

Using contaminated farm tools and equipment or infested soil adhering to tools, boots and draught animal hooves that have not been washed and disinfected, facilitates the spread of *R. solanacearum* in potato production (planting, plowing, earthing up, digging, harvesting, grading, transporting, etc.).

3.6 Farm workers and animals

People entering potato production farms with contaminated or infested soil adhering to boots, shoes, feet or hooves of farm animals can spread *R. solanacearum* to clean areas. The movement of rodents, porcupines and cows feeding on foliage or roots can also contaminate healthy potato plants.

3.7 Dust particles in the wind

Wind can spread *R. solanacearum* inoculum in dust particles from an infested potato field to the nearest disease-free farms, surface water, irrigation ponds or even farm equipment.

3.8 Determinants of pathogenicity and resistance of R. solanacearum

Factors determining pathogenicity and virulence in the bacteria have been recently reviewed (Meng, 2013). Available field resistance to the *R. solanacearum* species complex is limited and tends to be unstable under different environmental conditions and/or strain variability. Traditional breeding has not yielded resistant cultivars yet because of the difficulty in transferring multiple unknown genes from wild sources with polygenic resistance into cultivars, without also transferring undesirable linked traits. Furthermore, high-level resistance approaching full immunity to host colonization as well as to disease development is needed to avoid the risk of pathogens spreading in symptomless latent forms.

Most studies on the genetic basis of resistance to BW have been conducted in model plants such as *Arabidopsis thaliana* and *Medicago truncatula* (Huet, 2014). Quantitative trait loci (QTL) have been identified that include R genes that encode proteins that recognize bacterial effector a virulence (AVR) protein, triggering resistance to the bacterium.

The transfer of selected R genes from *A. thaliana* into tomato conferred immunity to the *R. solanacearum* strain with the corresponding AVR gene (Narusaka et al*.,* 2013). Both broad-spectrum and strain-specific QTLs have been identified in tomato (Wang et al., 2013), tobacco (Qian et al., 2013) and eggplant (Salgon et al., 2017). The discovery of a possible resistance or virulence (R/Avr) gene for gene resistance mechanisms is particularly interesting since known bacterial effectors can be used to screen for homologous resistance genes in related crops, including potato.

4 MANAGEMENT OF POTATO BACTERIAL WILT

Bacterial wilt disease limits the production of potato, especially seed potato, worldwide. It has been recently detected in temperate regions and tropical zones at altitudes exceeding 3000 masl, threatening seed production areas previously assumed to be 'safe' from the disease. In the absence of a developed formal seed potato production and certification scheme and quarantine measures in Ethiopia, BW has spread to many potato producing areas previously free of the disease. It is important to recognize that there are no appropriate, practical, and cost-effective chemical control measures against potato BW. Though no single control method has been completely effective against the disease, some level of control has been possible through a combination of phytosanitary and cultural practices, chemical and biological control, and host resistance in some areas. Policymakers need to ensure that the quality of early generation seed (EGS) used to produce certified, disease-free seed potato is safeguarded by appropriate tests that can detect early *R. solanacearuzm* infection. Therefore, WB in seed potato can be managed by following integrated cultural approaches, rigorous testing (internal) and seed certification.

4.1 Preventing BW infection under indoor conditions

Early generation seed potato can be produced in indoor conditions such as screenhouses and greenhouses which are usually covered by insect-proof nets or/and solar energy-resistant plastic sheets. While screenhouses may allow rainwater in, greenhouses do not and must be artificially watered all the time. At Adet Agricultural Research Center, 11 green/screenhouses are currently being used to produce clean and disease-free breeders' seed, and three more seed tuber generations to distribute to farmer cooperatives to increase potato production and productivity. Similarly, other research centers in Ethiopia in agro-ecologies where potato is important have indoor seed potato production facilities to grow breeder seed and EGS. The following measures should be in place to produce disease-free tubers under indoor conditions:

- Sterilize the solid culture medium (soil/sand/manure/compost) including forest soil or any medium that must be used as growing substrate. Soil solarization using transparent plastic and mulches for 60 days prior to planting can reduce or eliminate BW inoculum. However, the solid plant growth media for indoor seed production should preferably be steam sterilized. In aeroponic and hydroponic systems, the water may be cheaply sterilized using ultraviolet light before it is used in feed to the plants.
- Use disease-free propagation materials (plantlets, seed, cuttings and minitubers) in pots. All nuclear planting material must be tested for *R. solanacearum* infection.
- Disinfect containers, pots and tools in the greenhouse before and after use to prevent the possible spread of *R. solanacearum* and other diseases among screenhouses.
- Fence areas reserved for screenhouses to prevent the free movement of people and animals that might enter the area with contaminating *R. solanacearum* inoculum.
- Disinfect all footwear and hand tools with chemicals or wear cleaned footwear before entering the screenhouse compound.
- Disinfect cutting knives and tools during the preparation of cuttings. Avoid the sharing of equipment and tools between screenhouses.
- Use only disinfected water in hydroponic and aeroponic greenhouses where recirculating liquid media is used to prevent the multiplication and spread of *R. solanacearum* and other plant pathogens. In any

case, clean water must be used for irrigation purposes in any kind of indoor seed potato production facility.

- Workers must wash their hands and feet before entering the screenhouse or at the start of a day's work.
- Display biosecurity signs on gates and fences of the greenhouse area to warn visitors to the facility. Visitors must seek the permission of the department of potato research or farm manager before visiting the production screenhouses.
- Inspect potato plants in screenhouses regularly and remove or pathologically test those that may appear unhealthy.
- Destroy the entire crop even if a single plant in a screenhouse is found with BW especially if the plants are not grown in individual pots. Place the unit under quarantine for one year under strict observation.
- Train screenhouse workers in indoor crop production hygiene practices. They should know the trouble spots, what to look for and how to report unusual pests and diseases.

4.2 BW management in open fields

Potato BW in Ethiopia is largely caused by race 3 biovar 2 of *R. solanacearum*. This race has a narrow host range and can be successfully controlled by stringently applied integrated pest management (IPM) strategies. However, potato BW is difficult to control for the following reasons:

- There is no cost-effective chemical control. Once infections set in there may not be is no treatment to salvage any plant in a healthy state.
- Its causative pathogen has many alternative hosts beyond crops in the Solanaceae family.
- The BW pathogen is soil-borne; thus, it can survive freely in soils in the absence of hosts.
- The BW pathogen has many biological variations, each behaving differently under different environmental conditions, increasing its adaptation and survival.
- Latently infected vegetative propagating materials carry *R. solanacearum;* hence the pathogen is easily transferred from one cropping season or location to another.

Nevertheless, the following control measures that can help minimize the spread of the disease.

4.2.1 Eliminate sources of infection

The pathogen may survive in vegetative propagules (potato tubers, rooted stem, and apical cuttings), infected plant parts, alternate crop hosts (e.g., tobacco, eggplant, and pepper) and weed hosts (e.g., nightshade, stinging nettle), soil, irrigation water, contaminated farm tools and equipment. Known alternate plant hosts for *R. solanacearum* should be avoided in crop rotation strategies or destroyed. Farm, tools, and equipment should be sanitized before use as a routine practice.

4.2.2 Crop rotation

Once a seed potato production site has been selected, it is important to draw a crop rotation and fallowing plan for the whole farm (Shamsuddin et al., 1978). Crop rotation is important in potato production because it helps halt the build-up of pests and diseases associated with potato and other related crops. Adopting a suitable rotation plan reduces the spread of potato pests and diseases between cropping seasons. It also ensures a more balanced use of plant nutrients in the soil.

Farmers should ensure that they grow potato only in virgin or previously fallowed land or on land where potato and its related crops have not been grown for two or more cropping seasons. Land where volunteer plants from solanaceous crops are persistently present should be avoided since they usually act as reservoirs or bridging hosts for most potato pests and diseases.

Designing a potato crop rotation program

Step 1: Inquire about the farm and market/use of alternative or rotation crops

- Seek information on the history of the farm. What was grown there before?
- What pests and diseases have been observed on the farm in the past?
- Observe the topography and drainage of the farm (sloping or flat).
- Observe the soil types (sandy, black cotton, high in clay or loam soil).
- Seek information on climatic conditions such as rainfall, temperature, wind, etc.
- Determine the household food requirements or what other crops are in demand in the local market.

  This is important in determining the rotation crops and farm profits.
- What is the rooting depth of various crops to be used in the rotation program?
- What are the feeding habits of the rotational crop (heavy feeders, light feeders, moderate feeders and givers)?
- What plant residues result from the harvest of the various crops?
- Is there a need to diversify enterprises for risk management and better farm income?

Step 2: Designing a crop rotation plan

After collecting basic information about the farm, design a crop rotation plan with the participation of the farmer. Seek the assistance/advice of an agricultural extension officer to obtain local expertise in the area. Developing the plan involves dividing the farm into at least four blocks and allocating a different crop per cropping season to each. The crops are rotated on the plots making sure a crop from the same family is not planted repeatedly on the same plot in successive seasons or years. A five-season rotation plan for four plots is suitable for small farms. A rotation block with natural fallow where possible will be beneficial. An example of a crop rotation plan without fallowing is presented in Table 1.

Table 1. Example of a crop rotation plan for a typical smallholder farm in Ethiopia to manage potato bacterial wilt (season refers to a full cropping cycle)

Cropping season				
First	Second	Third	Fourth	Fifth
Potato	Teff	Faba bean	Niger seed	Potato
Potato	Maize	Field pea	Lentil	Potato
Potato	Wheat	Chickpea	Cabbage	Potato

Source: Farmers field school (FFS) training manual by Adet Agricultural Research Center, Amhara.

Rotate crops with pasture, cereal and non-solanaceous crops for at least three years. One season's rotation of potato with beans, cabbage and carrot has been shown to significantly reduce the incidence of BW by 22.1, 15.4 and 27.3%, respectively compared to a monocrop potato which had 45.2% incidence (Lemaga et al., 2001). Several studies demonstrated that crop rotation helps to significantly reduce BW (Lemaga, 2001; Lemaga et al., 2001; Lemaga et al. 2005) but did not eliminate it and similarly was long term natural fallows (Kakuhenzire et al., 2013). However, crop rotation and improved fallows involving plants such as faba bean, vetch, lupins and *Clotolaria* sp. that produce bactericidal and bacteriostatic metabolites have the potential to eliminate soil inoculum of *R. solanacearum* and prevent infection when healthy seed is used (Kakuhenzire et al., 2013). These crops or plant species additionally improve soil fertility through nitrogen fixation and or herbage production (Kakuhenzire *et al*., 2013).

4.2.3 Use quality or disease-free seed

Obtain seed potato only from reputable sources, and preferably certified seed. Plant seed should be free from seed-borne diseases. Quality seed potato can be obtained from cooperatives that have been approved by seed inspection and quarantine services. Do not use seed potato from the local market whose source cannot be traced. To ensure freedom from latent infection, seed tubers should originate from areas where the disease has not been known to occur or the probability of its occurrence is highly unlikely.

4.2.4 Seed treatment with hot air/water

The potato BW pathogen can safely survive in potato seed tubers. However, infected but scarce and valuable seed tubers could be disinfected with heat treatment. Small quantities of valuable infected tubers can be exposed to hot air at 44ºC (112ºF) with 75% relative humidity for 30 minutes (Tsang et al., 1998). Producers can apply heat treatment to seed tubers in a seed preparation facility using a hot air blower and a humidifier. However, seed treated in this manner should be screened for freedom from infection or other means should be used to recover healthy samples of the infected seed.

4.2.5 Plant new and clean seed potato healthy fields

Bacterial wilt can be a seed, soil, or a water-borne disease. It can remain in latent and infective- free soil for up to two years after crop harvest and in water for up to four years (Álvarez et al., 2008; Hong et al., 2008) in the absence of a host. Therefore, while selecting a site to plant new and quality assured seed, one should:

- Choose a field and neighboring area with no history of BW disease.
- Select land that has a gentle gradient with good drainage.
- Avoid areas that are free of crossflows of water from other possibly infected fields.
- Avoid routinely damp and poorly drained soils or areas with excessive moisture retention, such as swamp land.

Certified or quality seed potato should be planted in healthy fields without a history of quarantine pests such as *R. solanacearum.* Planting quality (disease free) seed in infested or contaminated soil will lead to infecting and wasting of new seed. Disease-free seed and non-infected fields are prerequisites to prevent infection and spread of BW.

4.2.6 Host resistance

While host resistance is the cheapest and environmentally safe way of managing most pests and diseases, the approach is not so easy to achieve and comes with trade-offs against other preferred traits. *Solanum phureja* has been used in potato breeding as the major source of resistance to BW but has not been found suitable for all environments (Schmiediche, 1986).

The combining ability of parents is an apparent feature of resistance to BW in progeny. Widening the genetic base for both resistance and adaptation to the environment, particularly heat tolerance, is very important in breeding for resistance to BW in potato. Genetic engineering techniques are being used to introduce lysozyme, cecropins and other potent antibacterial proteins derived from insects into potato as a way of augmenting resistance to *R. solanacearum* and other bacterial diseases.

Resistance is the most practical pest and disease control component for most smallholder farmers. However, an acceptable level of resistance will depend on the use of the potato produced. When the produce is for consumption, a certain percentage of infection may be acceptable. However, any infection of *R. solanacearum* in seed potato production is not tolerated since a few infected seed tubers are enough to set off a massive disease cycle in a short time over a wide area.

4.2.7 Intercropping

Intercropping has been used in some developing countries to reduce pathogen population in the soil and root to-root pest transmission (Fig. 6). Growing potato with beans can lower the incidence of BW than intercropping with maize because beans have a dense root system and grow quickly (Autrique and Potts, 1987). Maize on the contrary grows slowly and has a more dispersed root system despite a large canopy. In some developing countries where the use of clean seed and long crop rotations are not practical solutions to the problem of *R. solanacearum* biovar 2 (race 3) in potato, intercropping has been used to reduce soil populations of the pathogen and root-to-root transmission (Ciampi-Panno et al., 1989).

Fig. 6. A potato-maize-faba bean intercrop in a smallholder potato field in Ethiopia.

It is important to recognize that while intercropping may reduce the incidence of BW infection, it may not prevent latent infection; this could compromise quality seed potato production if used as a main tool in BW management in both seed and ware potato production (Kakuhenzire et al., 2013).

4.2.8 Control volunteer plants

Removing/uprooting self-sown potato which grows after crop harvest can reduce *R. solanacearum* inoculum in the soil as it serves as a reservoir for the bacterium. It is therefore advisable that before fallowing a piece of land as a tool for BW management, all ground keepers and volunteer potato or other *R. solanacearum* host plants must be eliminated for crop rotation and fallowing to be effective.

4.2.9 Crop management practices

Avoid injury to potato plant roots and stolons during cultivation, earthing up and weeding because bacteria can easily enter the plant body through wounds. To avoid root injury during weeding and earthing up, potato should be planted on ridges and cultivation in a growing crop should be minimized. Removing weeds like nightshade and thorn apple grown along drain channels and in paddocks after growing potato on a piece of land reduces the reservoir of *R. solanacearum* inoculum in the infected field.

4.2.10 Nematode control

Nematodes reside in the soil and infect multiple plant species, although there are host-pest preferences. There are nematodes commonly associated with potato. In their feeding process, nematodes open routes for bacteria to enter the plant roots through injuries. Nematodes therefore must be controlled to reduce their interaction with *R. solanacearum* (Priou et al., 1999). Major nematode control methods include soil fumigation, rotation with cereals, application of high quantities of organic amendments which are free of *R. solanacearum* and planting nematode-resistant varieties.

4.2.11 Avoid deep plowing

Most organisms/pathogens survive harsh conditions in deep and cool soil layers if they can get maintenance levels of oxygen. Graham and Lloyd (1979) found that race 3 of *R. solanacearum* survived better (82 days) in deeper soil layers (55–65 cm) than in shallow soil (10–15 cm) (10 days). Thus, disease incidence was reduced under minimum tillage during the growing season. In shallow soils however, frequent tilling between growing seasons may reduce *R. solanacearum* inoculum.

4.2.12 Soil solarization

Solarization of the soil before planting with transparent plastic mulch for 60 days can reduce BW incidence in previously infested fields (Vinh et al. 2005). Soil solarization used in combination with biological control agents (BCAs) such as *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp. (growth-promoting bacteria) has been shown to efficiently reduce tomato wilt incidence (Anith et al., 2000; Kumar and Sood, 2001; Chen et al., 2013; Yuliar et al., 2015; Marian et al., 2019). However, the cost and practicability of this method may reduce its use among most smallholder farmers in developing countries, including Ethiopia.

4.2.13 Organic matter amendment

Adding organic amendments to soil has a direct impact on plant health and crop productivity (Lemaga et al., 2001). Organic amendments are advantageous because they improve the physical, chemical, and biological properties of soil, with positive impacts on plant growth. The degradation of organic matter in soil can also directly affect the viability and survival of soil-borne pathogen by restricting available nutrients, reducing metabolic oxygen, and releasing natural chemical substances (metabolites) which may have growth inhibiting or biocidal properties (Messiha et al., 2007; Kakuhenzire et al., 2013). However, if organic matter is to be used in potato or tomato cultivation for soil fertility improvement, it is critical to ensure that:

- Diseased plants and tubers are not included among waste meant for composting.
- Diseased potato plants are harvested only for domestic use and not for seed.
- Farmyard manure and compost are fully decomposed to avoid spreading the disease.

4.2.14 Plant residue

Previous studies have reported the suppression of BW by plant residues derived from species such as chilies, clove (*Szygyuma romaticum*), eucalyptus (*Eucalyptus globules*), cole (*Brassica* spp.), etc. The possible mechanisms involved are mainly considered to be antimicrobial activities, followed by the indirect suppression of the pathogen through improved physical, chemical and biological soil properties.

4.2.15 Control infected sources of irrigation water, flooding and run-off

Do not allow contaminated irrigation water to run freely over or below the soil surface or return to the dam or stream from which it was pumped. Infected irrigation water will contaminate disease-free potato farms or soil with *R. solanacearum*. If furrow irrigation is used, water should be from a healthy source to minimize spreading the disease. Flooding and run-off should be controlled as they are likely to spread or promote the spread of BW to new farms.

4.2.16 Scouting for infected plants

Inspecting or regularly visiting a potato field to look for wilt symptoms throughout the growing stages of the crop is one way of managing disease spread in low intensity infections. In seed potato crops, the detection of infected plants should be reported to the quarantine or agricultural experts in a timely manner. Since there is no remedy once a potato plant or tuber has been infected with BW, accurate and early disease diagnosis are crucial for an effective management program. It is also important to recognize the difference between potato BW caused by *R. solanacearum* infection and wilting caused by *Verticillium, Fusarium* sp., water deficiency or mechanical root damage during tillage or by rodents. Wilting due to *R. solanacearum* exhibits the following typical signs:

- Fungi-induced potato plant wilting proceeds slowly in the host and produces more uniform symptoms through the plant than when infection is due to *R. solanacearum*.
- In BW, symptoms appear from the top of the plant and proceed downwards, whereas in *Fusarium* and *Verticillium* wilts, symptoms begin from the bottom leaves of the plant and progress upwards.

The biology of the causal organism and the host-pathogen interactions explain these visible differences. For example, the impairment of water transport to aerial plant parts in BW infections is due to the clogging of the conductive plant tissue with rapid *R. solanacearum* proliferation and mucilage production leading to water-flow blockage and plant wilting. In fungal-induced wilt, mycotoxins are the primary cause of initial wilting. When plant roots are physically damaged, for example by rodents, a physical examination by slightly pulling the plant as if uprooting it, will reveal physical or rodent damage.

4.2.17 Farm biosecurity signs

Install biosecurity signs on farm gates and fences to manage visitors coming to the fields on the farm and enforce all other quarantine measures among farm workers to prevent BW introduction and spread. Restrict entry to seed potato fields. Authorized visitors should be guided through the fields to mitigate unintentional introduction of *R. solanacearum* or any other seed potato pests and diseases.

4.2.18 Rogueing or removing diseased plants

Removing and destroying diseased potato plants with tubers and neighboring apparently healthy hills may reduce the spread of BW within potato fields, especially if the infection is isolated or sparse. However, a farmer should not remove or uproot visibly infected potato plants intended for seed production until a seed inspector or quarantine officer has visited the field to inspect and certify the crop's suitability for seed. A seed potato crop should show a form in which it was planted with as much uniformity in plant spacing and distribution as possible. Gaps in a seed potato crop renders it suspect to poor health. However, if BW infection is widespread, the farmer can directly downgrade the seed potato crop without seeking a third-party opinion.

Infected plants should be safely removed and destroyed. Rogued plants should be carried in a sack and disposed in a spot where potato is not likely to be planted or which is not a regular path for the movement of people or animals. It is advisable to add ash or lime at the spot where the sick plants were removed. This may reduce disease transmission to neighboring plants besides restricting the multiplication *R. solanacearum*.

4.2.19 Avoid sharing equipment

Do not use the same farm tools (plows, hoes, etc.) across potato fields. This is likely to transmit BW from infected fields to clean ones. If at all the tools must be shared, disinfect them before they are used among farmers. Simple farm tools such as hoes, knives, pangas, and ox-plows can be disinfected by flaming in ordinary fire (Fig. 7). Large equipment such as tractors should be cleaned with chemical disinfectants such as bleach.

4.2.20 Machinery, bags and other equipment

Hired machinery and equipment used on one farm should remain there until the operation is completed. The machine should be thoroughly cleaned and disinfected before it can be used on another farm (Fig. 7). Secondhand bags or half-ton bins to hold potatoes can be reused, but must be first washed, disinfected, and dried. Alternatively, bags could be discarded. Seed potato should never be packed in used bags even if they are cleaned and disinfected.

Fig. 7. Disinfecting local farm tools with fire during a farmer training in Ethiopia.

4.2.21 Clothing, footwear and boots

People working in EGS paddocks should wear clean clothing and boots when entering the work area. Footwear and boots should be cleaned with a suitable disinfectant. Visitors, contractors, and irregular workers should wear overalls, footwear and boots when entering seed potato production blocks.

4.2.22 Avoiding solanaceous crops in rotation

Avoid planting solanaceous crops such as potato, tomato, eggplant, pepper, and tobacco in field blocks with a history of BW infestation. They will maintain *R. solanacearum* inoculum for as long as possible until potato is reintroduced. In an infected potato crop, uproot all the infested potato plants with the roots and surrounding soil, and carry the infected material in a sealed plastic bag to a safe dumping site (Fig. 8). The surviving crop can be used for ware or table potato. Waste potato, crop residues and debris from such a field should not be disposed of in apparently clean blocks or fields. All diseased tubers should be safely destroyed, for example by burning or burying deep underground, and such produce should never be reserved as seed. Such a block should be put on the hold to grow seed until it is proven that the threat has been overcome.

Fig. 8. Removal and safe disposal of bacterial wilt-infected potato plants from the field.

4.2.23 Awareness creation

Potato farmers, particularly seed producers, should be trained on the risks from BW and improved potato production practices that reduce or prevent the spread of the disease. Farmers should be made aware of the importance and benefits of using clean seed and how to produce and secure it healthy. They should be trained in safe production practices such as crop sanitation and cultivation practices, removal of potato haulms, weeding, rouging volunteers, removing wilted potato plants, decontaminating farm tools, maintaining the safety of irrigation or flood water, disposal of infected tubers, etc. (Fig. 9). They should also be taught all the methods and techniques that prevent the introduction and establishment of *R. solanacearum* in clean fields. Farmers and extension agents should develop and follow strategies for preventing, containing, and managing BW in potato farming systems.

Fig. 9. Farmer field school participants learning how to identify bacterial wilt symptoms in potato fields and infected tubers.

4.2.24 Soil amendment

Soil amendments include fertility enhancement resources of both organic, inorganic, or mineral origins. Most mineral soil amendments are industrially made while organic ones are generated from vegetable matter. Adding calcium containing amendments like CaO, $CaCO_3$, manure and wood ash reduces infection and the impact of BW by inhibiting pathogen survival through changes in pH and nitrite accumulation in the soil (Dhital et al., 1997). It is known that *R. solanacearum* multiplication in the soil is suppressed if pH is maintained above 6.2. Organic fertilizer and biochar amendments are promising alternatives that suppress BW by increasing soil pH, electrical conductivity, organic carbon, and nitrogen availability and improving microbial activities that compete with the BW pathogen for space and nutrients.

4.2.25 Biological control

Potato BW can also be managed through BCA, such as antagonistic rhizobacteria (Ciampi-Panno et al*.,* 1989) and avirulent mutants of *R. solanacearum* (Trigalet and Trigalet-Demery, 1990). The mechanism of BW control with BCA may be due to induced plant resistance or active colonization of the rhizosphere with antagonistic soil bacteria and the production of bacteriocin or bacteriophage strains of *R. solanacearum* (Chen & Echandi, 1984). The impact of BCA may also be a result of protection by competitive exclusion (McLaughlin and Sequeira, 1988). However, there are indications that protection dependent on root surface colonization holds less promise than one based on the use of avirulent mutants of *R. solanacearum*.

Some *R. solanacearum* mutants are effective in preventing subsequent colonization by wild-type strains, but the degree of protection depends on maintaining an equal ratio between virulent to avirulent cells in the inoculum or less than 0.1 and the timing of the inoculation (Trigalet & Trigalet-Demery, 1990). It should be stressed that none of these approaches to biological control has reached a level of commercial application and they require more research.

4.2.26 Plant quarantine

Quarantine measures currently applied include a five-year ban on growing potato in fields identified with BW infection. In vegetatively propagated crops, the planting material must be indexed against *R. solanacearum* infection. Similarly, the movement of planting material in areas suspected to be infested with BW should be strictly restricted. Once the disease is discovered in an area, the acquisition of seed potato from such areas there should be stopped.

4.2.27 Storage

Bacterial wilt symptoms can appear in potatoes during storage. To prevent this, store healthy potatoes at low temperatures (less than 50 ºF) and scout them for the appearance of wilt symptoms (Alam and Rutsgi, 2020). Collect and eliminate infected potato tubers.

4.2.28 Plant spacing

Increasing row spacing can reduce bacterial wilt incidence in soil with low pathogen populations (Alam and Rutsgi, 2020). The recommended plant spacing to reduce wilt incidence and spread is between 1.5 and 2.5 feet.

4.2.29 Integrated control

An integrated approach to BW control has been advocated, particularly for potato, by combining resistance with proper agronomic and cultural practices. In Nagasaki, Japan, integrated control of BW in severely infested potato fields was achieved by combining soil fumigation with chloropicrin, the use of a relatively resistant cultivar and late planting to avoid high temperatures in the planting season (Katayam and Kumura, 1987). Therefore, agricultural extension agents and farmers need to ascertain the BW control strategies that best suit a situation and apply them to mitigate the impact of the disease (Table 2). The possible mix of BW disease control measures with their rankings to control *R. solanacearum* race 1 or race 3 are presented in Table 2 (Priou et al., 1999). The higher the rating for a factor, the greater the impact it has on reducing the negative effects of BW infection. While integrating various factors, achieving a total of at least 14 may be adequate to control BW or eliminate the pathogen.

Table 2. Factors to be considered in developing a strategy for the integrated management of potato bacterial wilt disease.

Factors to consider in a control strategy	Race 3	Race 1
Healthy seed	7	7
R. solanacearum-free soil	7	7
Rotation with non-host crops	5	3
Variety resistance or tolerance	4	2
Rogueing wilted potato plants	4	3
Rogueing volunteer plants	4	2
Minimal post-emergence cultivation	4	4
Remove potato haulms and harvested tuber leftovers	3	3
Nematode control	3	3
Bacterial wilt suppressive soils	2	2
Weed control	2	2
Control of flood water and run-off	2	1
Decontamination of tools and farm equipment	2	1
Field or soil solarization in vegetation-free fields	1	3
Flooding of paddy rice	1	3

4.3 Prevention of R. solanacearum infection in diffused light store (DLS)

Managing or controlling BW in a potato store helps maintain the quality of seed potato and reduces contamination and spread of infection from one farm to other areas. To control the spread of BW in DLS, the following precautions must be adhered to:

- Wash and disinfect the DLS floor and shelf with disinfectants.
- Store only disease-free seed potato in the DLS.
- Do not enter the potato store directly from the field with dirty or contaminated footwear.
- Wash hands with soap water on every visit to the store.
- Wear gloves to remove any infected or rotting tuber and possibly submit samples for *R. solanacearum* assessment or safely destroy the suspected infected tubers.
- Control potato tuber moth and other insects as they can accidently transfer the disease from infected to healthy tubers.
- Undertake post-harvest testing of seed potato tubers to reduce the spread of latent infections.
- Do not keep ware and seed potato in the same store as both differ in health standards and require different storage conditions.
- Install biosecurity signs at the entrance of DLS to manage visitors.

4.4 Gender considerations in managing and containing potato BW

In any farming system, different genders undertake various roles and responsibilities. Men, women, youth, children, elderly, people with disabilities, people with different religious beliefs, etc., in a potato farming community may routinely undertake specific roles, responsibilities, hobbies, etc. These differences in roles may impact a potato enterprise or pest and disease management systems. This underlines the need to examine specific routines, activities and actions that may influence the spread and distribution of BW. How each gender involved in the activities is likely to spread or prevent the spread of BW or any other potato disease needs to be examined. For example, while a man (husband) in farm family may consider volunteer potato or weedy vegetables as a threat to future crop production because they act as reservoirs of pests and diseases, a woman (wife) may cherish them as a source of food or fresh leafy vegetables.

In potato farming systems and value chain, attempts should be made to examine gender groups that are involved in seed and ware potato trade and distribution. These roles and responsibilities should be mapped to gender categories and how they may impact potato disease management. However, there are tasks and activities in potato farming that are not necessarily assigned to any specific gender but could still be instrumental in spreading BW disease. Below are different scenarios that may adversely spread the disease unintentionally and could be mapped by gender.

- A herdsperson grazing livestock around a potato waste dump and the animals feeding and trampling on potato waste that might be infected with *R. solanacearum* may carry them on their hooves or even in the gut to virgin areas.
- Pigs may be fed fresh but *R. solanacearum*-infected potato tubers from disease-infected fields.
- Children playing near a potato waste dump with *R. solanacearum*-infected potato tubers are likely to carry the infection on their feet or disperse whole tubers as they play.

- A woman disposing potato peels on a waste dump or open fields may be unaware that some of the tubers were latently infected with *R. solanacearum*.
- A male farm worker may take inadequately decomposed compost containing potato waste that might be infected with *R. solanacearum*.
- Young men and women carrying freshly harvested potato from a remote field to the roadside may drop some of the tubers which may be latently infected along the way.
- A potato trader who buys and sorts/grades ware potato may carelessly dispose waste tubers that might be infected with *R. solanacearum*.
- A local hotel or restaurant owner may pour wastewater from potato washings into surface flow that might eventually find its way into irrigation systems.
- A trader may buy ware potato that might be infected with *R. solanacearum* but may instead keep and distribute them as planting materials.
- Workers preparing a meal of potato in a field may use ware potato that might be latently carrying *R. solanacearum.*

Successful containment of potato BW will have to factor all these and more scenarios and possible precautions to prevent disease transmission. Therefore, during BW disease training and sensitization, all stakeholders and genders must be included. The large gains obtained because of dealing with big farmers can be lost due to actions of underrated or ignored actors. All the different actors in the potato value chain also need to be considered.

4.5 Critical interventions to prevent and eradicate potato BW

- Mobilize institutional support to control BW and prevent its spread in seed potato, preferably through seed certification and quarantine procedures.
- Promote cooperation between viable commercial seed and ware potato growers and other value chain actors to combat the spread of potato BW.
- Encourage and support the use of standard serological and molecular methods to ensure that latent BW infections in soil and seed tubers is detected and affected seed is not marketed.
- Train personnel in the seed potato sub-sector to adopt certification practices, adopt smart ICT tools and help farmer communities adopt innovative crop management technologies that would prevent the spread of BW.
- Not all seed producing cooperatives and individuals are equally knowledgeable about potato BW, its effects and management techniques. A well-organized training and experience sharing platform needs to be designed and used to mobilize potato farmers to act on preventing the spread of BW disease.
- Raise awareness among all stakeholders on potato farm hygiene practices through different information and communication approaches such as:
    - Radio and other multimedia information sharing systems,
    - Disseminate messages about BW disease management to potato producers and other stakeholders during farmer field days or farm visit days and,

- Prepare and distribute books, manuals and posters on potato BW disease to potato producers and other agricultural extension workers.

- Build strong and organized teams at all levels from federal government officials, quarantine officers, research institution, zonal and district agricultural extension, higher educational institutions, nongovernmental organizations, key stakeholders, and seed producing cooperatives as well as individual potato farmers to act on potato BW control.
- Strengthen BW disease management and legislation of seed certification and plant quarantine systems as a short-term plan. However, a long-term strategy should aim at developing wilt-resistant potato varieties and technologies to reclaim infested fields.
- Provide quick preliminary information to seed producers, policymakers, and other state bodies to mitigate BW disease by commissioning more studies and developing appropriate disease management approaches.
- Delay or curb BW outbreaks and reduce yield losses by changing agricultural practices, developing chemical and biocontrol measures, and breeding for resistant cultivars.
- Consider the roles and responsibilities of different actors in potato farming systems who may contribute to reducing the spread of potato BW and address the relevant genders to contribute to the mitigation of *R. solanacearum* spread.

5 CONCLUSION AND PROSPECTS

The relatively slow progress in understanding and controlling potato BW is partly a reflection of its geographical distribution mainly in the tropics and sub-tropics in developing countries, compared to Europe and North America. This goes to show the inadequate research efforts that have been directed at the disease in the developed world. While research on late blight and potato viruses in Europe and North America have attracted considerable research interest and investment because of their historical impact, potato BW caused by *R. solanacearum* has drawn less interest because it is not a key production constraint in those regions.

Greater investment in potato research for development focusing on potato BW management, broadening the genetic base of potentially BW-resistant germplasm, and improving the mechanisms for its exchange are essential. So are improving and standardizing BW disease resistance screening techniques and developing and adopting better methods of pathogen detection and differentiation. A greater understanding of *R. solanacearum* ecological relationship, soil survival, weed hosts and cropping systems interactions is equally important.

If global warming predictions due to the greenhouse effect are to come true, potato BW distribution may expand to higher latitudes and altitudes with dire consequences for international potato value chains. The rate of its spread will depend on the magnitude and rate of temperature increase. Climate change and variability will lead to changes in regions to which major crops and pasture plants are currently adapted, affecting the pest and disease systems, underlining the urgency for greater and more focused research efforts to control potato BW. An integrated scientific, social, economic, and demographic approach to research and development to contain potato BW at the community, zonal, regional, national and international levels is therefore paramount.

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