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Seed Tuber Cycle and Latent Infection for the Spread of Potato Bacterial Wilt *Ralstonia solanacearum* (Smith) a Threat for Seed Production in Ethiopia

Bekele Kassa and Abebe Chindi

Ethiopian Institute of Agricultural Research, Holetta Agricultural Research Center, P.O. Box 2003, Addis Ababa, Ethiopia

Corresponding Author: Abebe Chindi, Ethiopian Institute of Agricultural Research, Holetta Agricultural Research Center, P.O. Box 2003, Addis Ababa, Ethiopia Tel: +251-11-2370295

ABSTRACT

The study was conducted to estimate yield and quality losses attributed to potato seed tuber cycle and latent infection at Shashemene Ethiopia. Four consecutive seasons (2009B to 2011A) at five 'kebeles' using three improved potato varieties of Jalene, Gudenie and Awash and two local varieties, 'Nech abeba' and 'Agazer' was done using previous harvest seed for the following season. The results indicated that seed tuber cycle significantly ($p < 0.05$) affected the progress of the disease on the local varieties as compared to improved varieties. The incidence of the disease in the field and the incidence of latent infection in tubers was highly significant ($p < 0.001$) between varieties and among progeny tubers produced in different seasons. In the inception of the trial year, in the field, in the first cycle, the local variety had significantly ($p < 0.05$) higher (20.93%) BW incidence than the improved varieties (3.98%), whereas in the 4th cycle the disease incidence increased to 26 and 53%, respectively. In the 4th cycle of progeny tubers, the incidence increased by 75 and 50% from the 1st cycle, respectively. Under ware potato production, in the first cycle, the yield loss was 4 and 7% in improved and local varieties whereas, in the 4th cycle, it increased to 21 and 32%, respectively. When potato was produced for seed, in the first cycle, the loss was 5.34 and 20.78% on improved and local varieties but in the 4th cycle it increased to 28.6 and 67.34%, respectively. Therefore, the study revealed that source of seed and year after year seed revolving were found to be the major factor and the main path for the dissemination of BW disease.

Key words: Potato seed cycle, tuber yield loss, seed health, *Ralstonia solanacearum*, dissemination

INTRODUCTION

Ethiopia is one of the major potato producing countries in Africa as 70% of its arable lands in the highlands are suitable for potato production (FAOSTAT, 2008). As put by Gildemacher *et al.* (2009a) the Northwestern, Central and Eastern highlands of the country are under potato production. The same authors pointed out virus diseases and potato Bacterial Wilt (BW) as the most important seed borne potato diseases in Eastern Africa including Ethiopia. The use of low seed potato quality is another factor contributing to reduction in potato yield in potato producing countries of Sub-Saharan Africa (Fuglie, 2007). Due to these and other biotic and abiotic factors, average potato yield potential (8 t ha^{-1}) of sub Saharan countries is very low as compared with the

word average of 16 t ha⁻¹ (Gildemacher *et al.*, 2009b). Bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi *et al.*, 1995) is one of the most important diseases that limit potato production worldwide in general, and in Ethiopia in particular. The disease also constitutes a serious damage to the cultivation of many other solanaceous crops (potato, tobacco, pepper and eggplant) in tropical, sub-tropical and temperate regions (Hayward, 1991). Specifically, Bekele (1996) reported that BW, caused by *Ralstonia solanacearum*, is the second most important yield reducing biotic factor in Ethiopia after late blight. The findings of Hayward (1991) showed the severity of BW in reducing average potato yield in the tropical and subtropical regions worldwide. As (Kassa and Bekele, 2008) found out BW is the most widely spread disease in potato producing areas of the country. The case is getting series as the emergence of highland strains makes the disease to be more economically important and affects the expansion of the potato industry.

Unlike late blight, BW cannot be controlled by using chemicals as there are no chemicals developed so far for the purpose (Martin and French, 1985). In order to minimize the damage caused by BW, a number of integrated approaches have been recommended and used. One of the approaches recommended and being used is crop rotation agronomic practice. It has been more effective in controlling race 3 than race 1 (International Potato Center, 1996) may be due to the survival period in soil or plant debris of 1-3 years. Race 1 can survive longer than this period and thus, Lemay *et al.* (2003) recommended crop rotation of 5-7 years. Another integrated approach recommended by Messiha (2006) was the use of farm yard manure (except compost) and inorganic fertilizer (NPK). This has reduced the survival period of the *Ralstonia* for 50% in Egyptian and Dutch soils. The author justified that K and Ca rich soils enable plants to develop resistance to BW and consequently resulted in less incidences. Apart from cultivars bred for resistance, some varieties that were not bred for resistance behave as resistant and or/tolerant in specific situations. French (1994) and Vander Zaag (1986) found out Achat variety as highly resistant in Brazil, in Mexico, whereas Molinera variety, which was bred for resistance, and Lopez variety, which was not, behaved as equally resistant. According to the same source, in Rwanda, several varieties behaved as resistant or tolerant. Hence, host resistance has proven to be useful to control the potato strain, and is potentially the most effective way to control the disease especially as component in integrated disease management (Bekele and Lemaga, 2001). The destructiveness of this pathogen and its exceptional ability to survive in soil (Hayward, 1991) plant debris and hair roots of plant hosts (Graham *et al.*, 1979; Granada and Sequeira, 1983) as well as its means of dissemination contribute to massive crop loss (Kelman, 1998). Tusiime *et al.* (1996) described that the presence of a variety of weed species that are symptom less carriers of the pathogen where (Kelman, 1998) stated that infested seed tubers and extensive soil infestation are the main contributing factors for higher tuber yield losses.

Moslem Khani *et al.* (2005) revealed that BW is mainly both seed-borne and soil-borne disease as the dominant way of spread is through the use of infected tubers as planting materials which are hard to recognize at the latent infection, or planting on an infected soil. According to Lemay *et al.* (2003) the pathogen can move from plant to plant in the soil, hence not localized. Other than infected seed and soil the pathogen can be spread through contaminated water and other hosts (weeds) in the Solanaceae family. Tubers harvested from polluted soils are potentially infected and convey the pathogen latently and hence tubers with latent infection are taking the largest share in dissemination of the disease as well as for huge tuber yield losses. In addition, Hayward (1991) reported that the pathogen is mainly transmitted through tuber seed. Whereas, Ciampi *et al.* (1980) pointed out that cool condition favor spread of the disease on plant without

exhibiting visible symptoms. This results in latent infection in vascular tissues of progeny tubers and it is the silent carrying of the disease by potato tubers. Hence, the use of healthy planting material is the most effective means to control the disease. Therefore this paper looks into the recycled seed effect on total production and disease incidence and quantifies the losses attributed to latently infected potato tuber as seeds.

MATERIALS AND METHODS

The study was conducted in Shashemene District, East Arsi, on farmer's field to investigate the tuber yield and quality losses of potato due to BW for four successive seasons during (2009B, 2010A, 2010B and 2011A). This district was selected in consultation with respective agricultural offices as they are potential areas for potato production and also source of BW disease for the country. The study was executed using a participatory research approach on farmer's field. Shashemene is located at 7°12' North latitude and 38°36' East longitude and having an elevation of 1700-2600 meters above sea level (masl) with an annual rain fall of 1200 mm. It has an annual minimum and maximum temperature in the range of 12-27°C, respectively. The site is suitable for potato production, and BW pressure is generally high due to successive potato production year after year using same farm and previously harvested tubers year after year. The selection of participating farmers and communities was based on the presence of BW in the field plots to be used and perform recommended cultural practices for potato production. An improved potato varieties, Jalene (CIP-384321.19), Gudenie (CIP-386423.13) and Awash (CIP-378501.3) which are less susceptible to BW and local varieties (Nech abeba and Agazer) relatively susceptible to the disease were used. A spacing of 75 cm between rows and 30 cm between plants were used; hilling at planting; rouging of volunteer potatoes; and post emergence cultivation were exercised by farmers. The study was laid out in a randomized complete block design where each farmer's field (farm) was considered as a replication within a season; the number of fields per season was five but, one of the experimental field was not handled properly by the group hence it was excluded.

Data collections and analysis: At harvest, data were recorded on yield, including total and marketable tuber yields, as well as rotten tubers due to BW. Farmers participated in collecting disease data and in harvesting, followed by discussions to evaluate the treatments. The relationship of mean tuber yield over disease incidence in the field was used to estimate tuber losses attributed by the disease to ware potato production scenario. In seed potato production scenario, the losses were determined based on the relationship of mean tuber yield over mean latent infection of the improved and the local varieties independently. Percent latent infection was calculated based on 20 randomly sampled plant which are looking healthy tubers from each treatment and sampled tubers were diagnosed for the presence of latent infection in the laboratory after the tubers were incubated at $2 \pm 32^{\circ}\text{C}$ that help to promote symptoms in the latently infected tubers (Rueda, 1990). Data on BW incidence were subjected to square root transformation before analysis using SPSS software.

Disease assessment: Each plot was assessed at weekly intervals to determine days to onset of first wilt symptoms. Subsequent counts of wilted plants were made at two weeks intervals from the onset of the disease. At each assessment, all plants that showed either complete or partial wilting were considered wilted. These were staked to avoid double counting in subsequent assessments and also to avoid the possibility of missing those that died completely during the growth period. The counts of symptomatic plants were expressed as a percent of the total number of plants that emerged. Late blight was controlled with one spray of Ridomil MZ 63.5% Wettable Powder fungicide for the

improved varieties and local varieties however, received additional two sprays of fungicide Dithane M-45 starting from the first visible symptoms since the local varieties are relatively very susceptible to late blight diseases.

RESULTS AND DISCUSSION

The progress of the disease was significantly ($p < 0.05$) increased as seed cycle repeated. In all cycles mean incidence of the disease was significantly higher in both local and improved varieties. In line with this study, Barton *et al.* (1997) stated the pathogen of BW is transmitted through seed tubers, recycle use of seed resulted in high disease incidence and yield losses. However, (Fig. 1a, b) depicts that the incidence was being higher by 10% on the local varieties as compared to the improved varieties. In both type of varieties, significantly higher mean disease incidence was recorded in the fourth tuber seed cycle as compared to the third and the second cycle. In the first cycle mean BW incidence was 9 and 13% and increased to 22 and 30% and 31 and 43% in the second cycle and third cycle of the seed on improved and local varieties, respectively. In the last season of the study the incidence reached to 41 and 52%.

Table 1 indicated that in the first season, the improved varieties gave the highest mean tuber yield compared to the mean yield of the local varieties. Use of tuber seeds from previous harvest for subsequent planting reflected in drastic declination of marketable tuber yield in the following

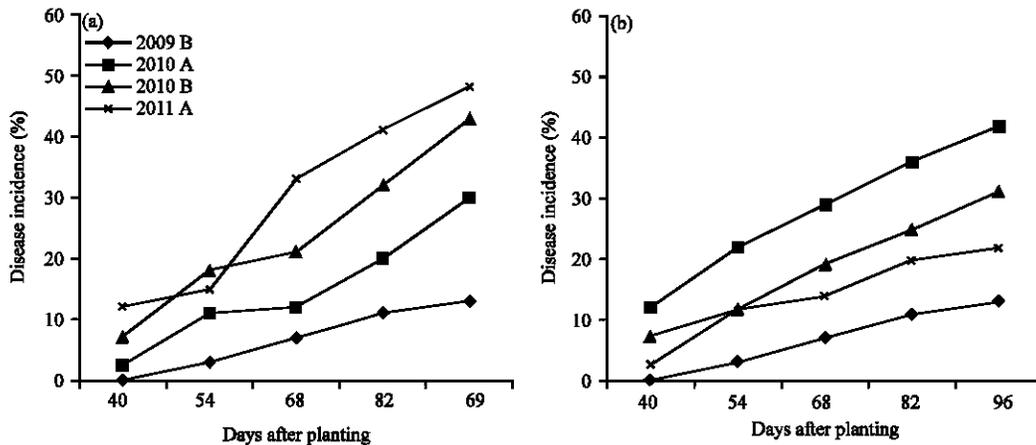


Fig. 1(a-b): Disease progress with time on (a) local and (b) improved varieties from 2009B to 2011A growing seasons

Table 1: Mean marketable tuber yield of three improved varieties and two local varieties in 2009B to 2011A, in the vicinity of Shashemene (yield data pooled from four farms)

Variety						
Yield t ha ⁻¹						
Season	Improved	Yield reduction (%)	Local	Yield reduction (%)	Mean	Yield reduction (%)
2009 B	39.1	-	31.2	-	35.15	-
2010 A	35.3	10.2	27.0	12.0	31.20	11.2
2010 B	31.6	19.2	22.4	29.1	27.0	23.2
2011 A	24.0	38.4	17.3	45.1	20.6	41.4
Mean	32.5		24.5			
CV (%)			13.5			

harvest. Mean marketable tuber yield reduced by 17.1, 28.6 and 40% on the improved varieties in the 2nd, 3rd and 4th seed cycle, while the corresponding percentage figure of mean yield on the local varieties were 12, 29 and 45. However, over all mean yield have been reduced by 20, 37 and 49, respectively.

The results obtained from this study indicated that the fair uniformity of the pathogen distribution in the field, as it is believed to be the hotspot for the disease (Bekele, 1996) which inabels to investigate the role of varietal differences in response to the disease to the high land race 3 biovar 2-A strain of bacterial wilt. As to the previous reports (Rueda, 1990; French, 1994) the two groups of potato varieties showed their difference in reaction to the disease, which also reflected in latent infection in the progeny tubers and total and marketable tuber yield. In line with this study, Bekele *et al.* (2011) found that latent infection by BW was one way for disease dissemination, suggesting the need to consider strict quarantine measure and restrict the free movement of seed tubers. Moreover, Begum *et al.*(2012) found that due to soil borne nature of the pathogen, the disease out breaks occurs on the same areas even when susceptible varieties were re-grown after gap of 2-3 years of crop rotation with non-host crops. Soil temperature and soil type have been reported to play a role in the survival of *R. solanacearum* in the soil habitat (Van Elsas *et al.*, 2000). Significantly ($p < 0.05$) higher mean yield differences was recorded between the improved and local variety (Table 1). The improved and local varieties gave a mean tuber yield of 33 and 24 t ha⁻¹, respectively. Even though, there was significant ($p < 0.05$) differences in the yield of progeny tubers among varieties, tuber yield in both category (improved and local) of varieties decreased as the progeny tubers was used as a seed for the subsequent seasons. However, the tuber yield of local varieties were more affected compared to the improved varieties by the tuber seed cycle.

Table 1 indicated that percent latent infection in progeny tubers of local varieties was also significantly ($p < 0.05$) higher as compared to improved varieties. In the highlands of Ethiopia, crop senescence is not the criteria for harvest. Rather the field serve as a store for their produce and harvesting could often extended from June to December. During the last harvest, farmers collect tubers remained in the field and used as a tuber seed for following planting. In agreement with this study, Begum *et al.* (2012) indicated that, cropping sequence further inferred that BW is prevalent during high temperature and humidity within the months of June-July when most of solanaceous crops are near to harvesting stage. The largest proportion of tubers in the harvest are small sized with inferior quality and are sources of disease causing pathogens for the coming season (Tarekegne and Kassa, 1997). But farmers don't realize that, progeny of such tubers are low in productivity due to the accumulation of pathogens in the tubers. As a result of this type of seed system, the potato tuber yield per unit area declined year after year due to degeneration. In the same manner farmers in the mid altitude are often keep tubers from their harvest for the next planting in different ways, in some area they store in peats and some spread in flour in their living house and some acquire from the market. In all cases, farmers do cycle their tubers from season to season without any inspection for the health of the seed. Results of this study in which the tubers are cycled for four seasons showed that, the yield significantly reduced as the progeny tuber cycle repeated. In the first progeny the marketable mean yield reduced by 10 and 12% on improved and local variety, respectively. Whereas in the 2nd and 3rd cycle percent reduction increased to 19 and 38% in improved variety, crrospondingly 23 and 41% on the local variety. Mean tuber yield of the two groups of varieties reduced by 11, 23 and 41% in the 1st, 2nd and 3rd seed cycle, respectively. The relationship between mean tuber yield over incidence of bacterial wilt in the four season seed cycle (2009B to 2011A) had negative and strong relationship $y = -0.3826x + 35.609$ ($R^2 = 0.9923$) and

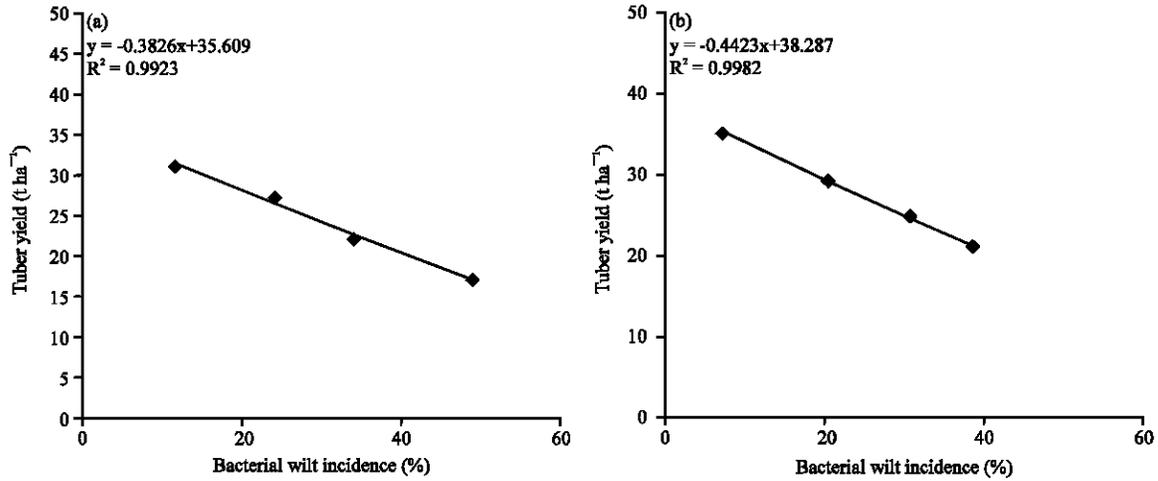


Fig. 2(a-b): Relationship between potato mean tuber yields over incidence of bacterial wilt in four cycles of progeny seed tubers (2009B to 2011A) of four locations using (a) local and (b) improved varieties at Shashemene

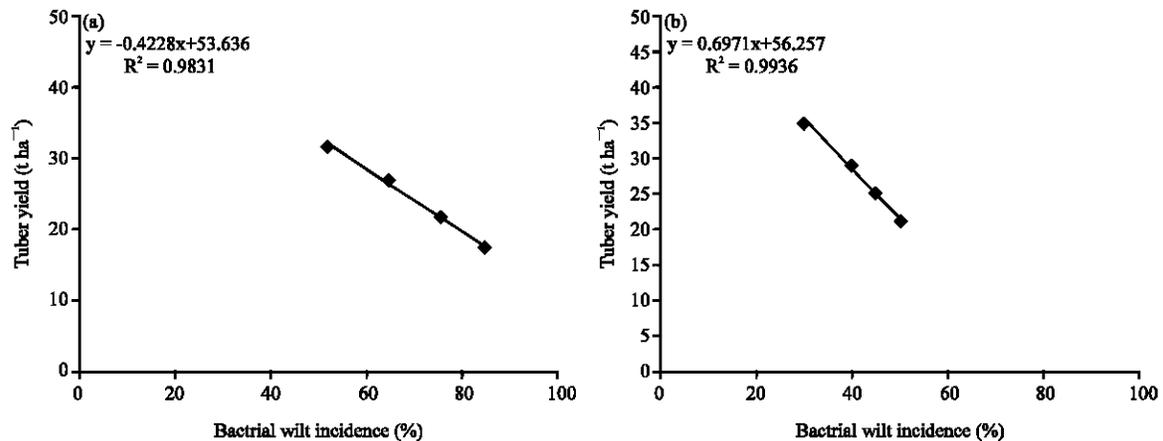


Fig. 3(a-b): Relationship between potato mean tuber yields over percent latent infection of bacterial wilt in four cycles of progeny seed tubers (2009A to 2011A) of four locations using (a) local and (b) improved varieties at Shashemene

$y = -0.4423x + 38.287$ ($R^2 = 0.9982$) over local and improved varieties (Fig. 2, 3). The mean yield loss was in the range of 7 to 32% on the local varieties and 4 to 21 % on improved varieties under the scenario of ware potato production. Tuber yield loss of local variety In the first progeny was estimated to 7 and increased by 50% loss in each cycle. The same trend of loss increment was also recorded in an improved variety.

The relationship of mean seed tuber over percent latent infection in tuber is presented in Fig. 2a and b. The relationship of mean tuber yield of varieties over latent infection demonstrated strong and significant ($P < 0.001$) relationship, $y = -0.4228x + 53.636$ ($R^2 = 0.9831$) and $y = -0.6971x + 56.257$ ($R^2 = 0.9936$) with local and improved varieties, respectively. Mean tuber

yield loss, on the local varieties, in the 1st seed cycle was 20.4% where as in the 4th cycle it increased to 67.34%. The same increment trend was also observed on the improved variety and the tuber loss of 5.34% was recorded in the 1st cycle where as in the 4th cycle it was as high as 5 fold from the 1st cycle.

According to the research findings of Hayward (1991) seed-borne wilt or latent infection has long been recognized as the principal method of dissemination of the potato strain, race 3, biovar 2-A. The movement of tuber seed from infected fields at worm location like Shashemene to cooler areas has apparently been reported to have high incidence of the disease in healthy appearing fields.

Such infected seed has often resulted in serious outbreaks of the disease appearing fields of north western and central Ethiopian highlands (Bekele, 1996; Kassa and Bekele, 2008). This study revealed that use of previous harvest as a seed for subsequent potato planting contributed to a serious tuber yield loss in terms of seed health and over all potato production as well as help for the dissemination of the pathogen. The results of this experiment agreed with the findings of French, 1994, in which latent infection in progeny tubers are the main vehicle for the spread of the disease. Hence, seed tubers should be checked for their health from the source so that latent infection could be monitored. That would result in good wilt control, higher yields, and reduced wilt spread.

The use of subsequent harvest as seed from season to season had negative and significant relationship between disease incidence, latent infection and tuber yield. The tuber loss increased as progeny cycle increased. Use of previous harvest as a seed for wear potato production attributed to a mean tuber yield loss of 21 and 32% in improved and local variety, respectively at the 4th cycle. Whereas, tuber yield loss in seed potato production scenario at the 4th seed cycle reached to 67.3 and 28.6% in local and improved varieties, respectively. These study results directly indicate how seed could be degenerate through time and attribute to huge tuber yield loss. Generally, the study strongly suggested that, farmers in low and mid altitude areas and having similar agro-ecologies with Shashemene should be used potato seeds produced in the high elevation potato fields where the weather condition is not conducive for the development of the pathogen so that the dissemination of the pathogen could be reduced and at the same time can minimize the yield loss what they are exercising. The study result of this work call for an immediate need to develop and materialize seed conspiracy at national and regional level. No single control measure is effective to prevent losses caused by the disease (Lemessa and Zeller, 2007; French, 1997). Barton *et al.* (1997) reported that most farmers recycle their own seed potato or purchase from nearby markets or neighbors. Hence, yield losses due to BW average about 50% with occasional losses of 75% on seed potato whereas yield losses caused by bacterial wilt are estimated at 50-100% in traditional potato production areas (Ajanga, 1993). Moreover, the pathogen is transmitted through tuber seed, the most effective means to control it is use of healthy planting materials (Adipala *et al.*, 2001).

CONCLUSION AND RECOMMENDATION

Bacterial wilt has no effective means of control because crop protection chemicals are ineffective and expensive and biological control agents are ineffective. In addition, phytosanitary methods such as quarantine are either expensive or difficult to apply and cultural methods such as crop rotations are largely impractical because the farms are too small to allow effective rotation, the pathogen has a wide host range and it persists for long time in the soil. The bacterial wilt is a great

threat to potato production in Ethiopia and farmers even reported to have lost their entire crop due to bacterial wilt. It becomes completely devastating the crop when the attack is at an early stage of crop development. Since, the pathogen spreads at a very fast speed in Ethiopia at present, enough attentions should be given in research and development strategies of potato producers. Especially quarantine regulations at regional level must, therefore, be enforced to prevent the spread of the disease into new disease-free regions of the country. Therefore, bacterial wilt was aggravated by recycling use of potato seed and farmers lack awareness on the use and advantage of healthy and quality seed, aggressive intervention is necessary to increase the awareness of farmers on seed quality maintenance in their farms. Moreover, field inspection and proper sanitary measures should be considered as training and advisory topics for farmers. The use of previous harvest as a seed for subsequent production contributed to a serious tuber yield loss in terms of seed health and over all ware potato production as well as help for the dissemination of the pathogen. Hence, seed tubers should be checked for their health from the source so that latent infection could be monitored. That would result in good wilt control, higher yields and reduced wilt spread. Generally seed conspiracy should be developed and materialized not only in the specific region but although throughout the country to control incidence of BW.

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