African Journal of Biotechnology Vol. 7 (11), pp. 1644-1649, 3 June 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.700
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Detection of latent infection by *Ralstonia solanacearum* in potato (*Solanum tuberosum*) using stems instead of tubers

Mwangi, J. K.[1], Nyende, A. B.[2], Demo, P.[3] and Matiru, V. N.[1]

[1]Jomo Kenyatta University of Agriculture and Technology Botany Department, P.O. Box 62000, Nairobi, Kenya.
[2]Jomo Kenyatta University of Agriculture and Technology Horticulture Department, P.O. Box 62000, Nairobi, Kenya.
[3]International Potato Center (CIP) P. O. Box 21571, Nairobi, Kenya.

Accepted 7 April, 2008

The potential of using stems for the detection of latent infection caused by *Ralstonia solanacearum (Rs)* was studied. Forty plants each were collected from four farms with bacterial wilt incidence below 4% in two growing seasons (season A and season B of 2005). The tubers of all the selected plants including 10 cm of the all lower stems were collected. Samples were taken to the laboratory for indexing against *R. solanacearu (Rs)* using ELISA techniques. The *Rs* status of each of the composite samples of all the tubers and of stems was determined and then correlation coefficients computed. There was a notable difference in the percentage number of samples per farm with particular categories of *R. solanacearum* status. When stems were compared to tubers for detection of *Rs*, an average r – value of 0.4 was obtained when r-values for the four different farms were averaged. The lowest r-value recorded was 0.2 while the highest was 0.5. When individual farms were considered it was only in one farm out of the four that r was not significant (p = 0.2). Overall the r-value was significant (p < 0.05). These results indicate that there is scope for adoption of stems as an alternative sample to tubers for indexing against *R. solanacearum* in potato tuber seed certification schemes more so in screening for presence of *R. solanacearum* in seed potato fields. However, although significant, the low r-value calls for more investigations to be done prior to final recommendation on use of stems from potato fields.

Key words: *Solanum tuberosum*, ELISA, *Ralstonia solanacearum*.

INTRODUCTION

Bacterial wilt (BW) is caused by *Ralstonia (Burkholderia, Pseudomonas) solanacearum* and is considered the second most damaging disease of potato worldwide. The disease is particularly damaging in humid climates and at low and medium elevations in tropical and subtropical regions (Hayward, 1991, 1996). In Kenya, the pathogen has been reported at both low and higher elevations (Nyangeri et al., 1984). Yield losses caused by bacterial wilt are estimated at 50 - 100 % in traditional potato production areas (Ajanga, 1993).

Bacterial wilt is, after late blight, the most important biotic constraint to potato production in Kenya (Barton et al., 1997). The disease was observed in Kenya in 1940 and has spread to altitudes as high as 3440 m (Bekele, 1996). Yield losses due to Bacterial wilt average about 50% with occasional losses of 75% on seed potato (Ajanga, 1993). It is therefore necessary that appropriate measures be taken without much delay. Bacterial wilt has remained a major constraint to potato production because most of the traditional potato fields are contaminated by *R. solanacearum* and good quality seed is unavailable to most farmers in Kenya (Michieka, 1993; Kinyae et al., 1994; Barton et al., 1997). When good quality seed is available, it is either too expensive or inaccessible to many potato growers (Kinyua et al., 2001). Most farmers recycle their own seed potato or purchase from nearby markets or neighbours (Barton et al., 1997). Such seed is of unknown quality with respect to tuber-borne diseases such as bacterial wilt and those

*Corresponding author. E-mail: jkmwangi2005@yahoo.com.

caused by viruses. Additionally, most potato growers hardly practice field sanitation or crop rotation procedures, perhaps due to limited land sizes and inadequate information to the farmers on the cause, spread, perpetuation and control options of bacterial wilt and other diseases (Barton et al., 1997). These factors have led to a general increase in incidence of various diseases and low potato yields (Nyangeri et al., 1984; Eden-Green, 1991; Ajanga, 1993). Since the pathogen is transmitted through tuber seed, the most effective means to control it is use of healthy planting materials (Adipala et al., 2001).

Bacterial wilt (BW) of potato is a devastating disease worldwide (Hayward, 1991). In cool conditions, such as those found in the tropics at altitudes above 2500 m, infected but symptom-less plant may harbour the bacterium and transmit it to progeny tubers as latent infection. This may lead to severe disease outbreaks when the tubers are grown at warmer sites (Ciampi et al., 1980; Hayward, 1991). Therefore, the use of healthy planting material is the most effective means to control the disease (Hayward, 1991). Original planting material used in formal or traditional multiplication schemes must therefore be tested for latent infection by *R. solanacearum*.

One drawback within the potato seed production system in sub-Saharan Africa is lack of rigorous seed health testing and certification programme (Nortje, 1997; Kakurhenze et al., 2000). The classical detection method of tuber infection consists of incubating tubers for 3 to 4 weeks at 30°C and observing oozing from the eyes or stolon ends, or cutting the tubers to observe oozing from the vascular ring. However this method is time and space consuming and may not reveal low infection rates (Priou et al., 1999). Therefore a more economical and efficient detection technique for routine use in quarantine procedures and seed certification scheme is necessary. The aim of this study was to evaluate the potential use of stems in detection of latent infections due to *R. Solanacearum* for use in seed potato certification and quarantine procedures.

MATERIALS AND METHODS

Sample collections

Samples were collected from four different farms in Meru at two different seasons (short rain 2005 and long rain 2005/06 seasons).

Season one, sample one

Sampling was done in mid August, 2005 from a potato farm in Katheri location, Meru. The farm belonged to Margaret Meruh at GPS reading 2039 m N00002.226', E0370 34.530'. The crop was at three months age of variety Asante. Seed were bought from other farmers from the region. The potato crop was preceded by maize. Bacterial wilt incidence was below 1%. Plant population estimate was about 4870 plants.

Symptomatic plants

Five wilted plants were sampled. All the tubers from the wilted plants were dug up and put into khaki paper bags. The corresponding lower parts of the stems with the root system intact were also put into each bag and labelled accordingly. The upper parts of the stem were chopped off at about 10 cm from the soil surface.

Symptomless plants adjacent to symptomatic plants

Three adjacent symptomless plants for every wilted plant were sampled. All the tubers from each symptomless adjacent plant were placed into a khaki paper bag. The corresponding lower parts of the stem with root system intact chopped off at about 10 cm above the soil surface was also placed into the bag and the bag labelled accordingly.

Symptomless plants positioned away from symptomatic plants

Thirty plants per potato field were sampled. All tubers from plants with no symptoms of bacterial wilt and positioned such that no wilted plant was within a radius of two meters were dug up and put in well labelled khaki paper bags. The corresponding lower part of the stems with root system intact were chopped as above and put into the khaki paper bag.

Season one - sample two

sampling was done in a second farm belonging to Stephen Mworia at GPS reading 2051m, NOOo' 02.271', E0370 34.541. The crop was around 4 months old of the variety Asante. Bacterial wilt incidence was below 2%. Seeds had been bought from other farmers in the area. Maize preceded the sampled potato crop. Plant population was about 2540 plants. The same sampling procedures used in the first farm were applied.

All the samples were then transported to the KARI-NARL, Plant pathology section laboratory for ELISA analysis.

Season two - sample one and sample two

Sampling was done in mid January, 2006 from two potato farms at Katheri village Meru belonging to the same farmer at GPS reading 2046 m, NOOo' 02.186', E0370 34.461. Farm 1 had potato crop variety Asante intercropped with maize on approximately half an acre plot. Bacterial wilt incidence was below 3% in a plant population of about 4800 plants. Farm 2 had potato crop variety Komesha also intercropped with maize on approximately half an acre plot. Bacterial wilt incidence was below 4% in a plant population of around 5200 plants. Sampling was done in a similar manner to what was done in season one. All samples were taken to the laboratory for analysis.

Stems analysis

From each plant sample the main stems were separated from each other and any soil/dirt washed of thoroughly with tap water. Each of the main stems was then cut at the point of the uppermost roots and on the other end to get a 7 - 10 cm fragment. The segments were disinfected in 0.5% sodium hypochlorite for 3 min, then rinsed with sterile distilled water and allowed to dry on clean serviette tissue paper. Then 2 cm of the lower and upper portion of the fragment was chopped off and discarded. Four, 1 cm piece was cut from the remaining fragment and put in plastic crushing bags for

sample extraction. Three milliliters of sterile citrate buffer per gram of stem tissue were then added and the tissues crushed with a wooden roll. From each stem sub-sample, 0.5 millilitres of the aliquot was added to 0.5 milliliters of modified SMSA broth for enrichment. Then 0.2 millilitres of aliquots from each stem sub-sample per plant were mixed together in a plastic bag, making a composite sample from which aliquots were also placed into two SMSA broth tubes for enrichment. After enrichment, the sub-samples and composite samples were analyzed using DAS-ELISA techniques for the detection of *R. solanacearum* (Priou, 2004).

Tubers analysis

From each plant sample, tubers were thoroughly washed in tap water to remove soil/dirt. The tubers were then disinfected in 0.5% sodium hypochlorite for 3 min and then rinsed in sterile distilled water and allowed to dry on clean serviette tissue. From each plant sample, each tuber separately had five cross-sections cut and vascular rings removed from the stolon end. Five vascular rings from each tuber were put into a plastic bag. Each tuber constituted a sub-sample of the main plant sample. Three millilitres of sterile citrate buffer per gram of tuber tissue were added and the tissues crushed with a wooden roll. Half a millilitres of extract from each sub - sample was put into 0.5 millilitres of SMSA - broth tube for enrichment. For each lot of tubers from one plant, 0.2 millilitres aliquots from each sub sample were placed into a plastic bag and mixed to form a composite sample. 0.5 millilitres each for the composite samples were placed into two SMSA-broth tubes for enrichment. To ascertain the sensitivity of ELISA the enriched sub - samples from season one were indexed against *R. solanacerum* through NCM-ELISA technique (CIP 2001), while the enriched sub - samples from season two were indexed against *R. solanacerum* through DAS - ELISA technique (Priou, 2004). This was to ensure that two different sets of results - one based on visual judgement and the other based on ELISA reading were obtained for analysis. Analysis of samples of season one and two were done at different times.

Data analysis

Data on the *R. solanacearum* status of each sample was recorded. Data for 2005 samples were transformed by converting the values to base of the natural logarithm (e). Correlation coefficients of each set of data (2005 and 2006) were calculated and analyzed through Fisher Z - test.

RESULTS

Bacterial wilt status for the samples

There were significant differences observed in the per-centages for the bacterial wilt status of the samples in each category per farm. Farm - A of year 2005 had a significantly low number of samples positive for bacterial wilt in both stems and tubers at 10.0%, while farm - B of the same year had a significantly high number of samples in the same category at 58.0%. Significant differences were observed in the bacterial wilt positive samples in stems only category where farm - A of 2005 had the highest percent of 32.0%, while farm B of the same year had the lowest percent of 2.0%. There was a significant difference in the percentage number of samples positive in tubers only with farm - B of 2005 having a significantly high percentage of 36.0% and farm - A of the same year having a significantly low percent of 2.0%. There was also a significant difference within the farms for samples negative to bacterial wilt in both stems and tubers where farm B of 2005 had a significantly low percent of 4.0% while the other farms had above 50.0% with farm – A of 2006 having the highest percent of 57.5% (Table 1).

Correlation analysis results

There were significant differences in the r - values from the four farms with farm A of 2006 having significantly high r - value of 0.5 compared to farm B of 2005 ($r = 0.2$). Farm B of 2006 also had a significantly higher r - value of 0.5 compared to farm B of 2005 ($r = 0.2$). The r - value was not significant in farm B of 2005 ($p > 0.05$), while in the other three farms the r - values were significant ($p < 0.05$). The p- value was significantly high in farm B of 2005 ($p > 0.05$) (Table 2).

The scatter and regression graph for farm A 2005 indicates that positive correlation exist between stems and tubers in the detection of *R. solanacearum* (Figure 1).

The scatter and regression graph for farm B 2005 indicates that positive correlation also exists between stems and tubers in the detection of *R. solanacearum* (Figure 2).

The scatter and regression graph for farm A 2006 indicates that positive correlation exist between stems and tubers in the detection of *R. solanacearum* (Figure 3).

The scatter and regression graph for farm B 2006 also indicates that positive correlation exists between stems and tubers in the detection of *R. solanacearum* (Figure 4).

DISCUSSION

One of the main constraints to developing strategies to efficiently control bacterial wilt has been the absence of rapid and accurate methods to detect the pathogen in large numbers of plants on a routine basis (French et al., 1995).

The aim of the third experiment was to determine the suitability of using stems instead of tubers in testing for latent infection by *R. solanacearum.* When the samples from four farms were analyzed, four sets of samples based on status were identified. There were those positive in stems and tubers, those positive only in stems, those positive only in tubers, and those negative in both stems and tubers (Table 1). From three farms the percentage number positive in stems only was higher than those positive in tubers only. In one farm those positive in tubers only were significantly higher than those positive in stems only. The mean percentage for each

Table 1. Percentage number of samples for each *Ralstonia solanacearum* status for samples from four farms collected from Meru for bacterial wilt correlation analysis between tubers and stems.

Year-farm	Farm no.	Positive in tubers and stems	Positive in stems only	Positive in tubers only	Negative in both stems and tubers
2005-A	1	10.0	32.0	2.0	56.0
2005-B	2	58.0	2.0	36.0	4.0
2006-A	3	12.5	22.5	7.5	57.5
2006-B	4	17.5	25.0	2.5	55.0
Mean		24.5	20.4	12.0	54.5
$L.S.D._{0.05}$		35.9	20.5	25.8	46.5

Table 2. Correlation coefficient factors between stems and tubers for samples from four farms collected from Meru for bacterial wilt detection correlation analysis between tubers and stems.

Year-farm	Farm no.	Calculated r- value	P- value
2005-A	1	0.4	0.01
2005-B	2	0.2	0.17
2006-A	3	0.5	0.00
2006-B	4	0.5	0.00
Mean		0.4	0.04

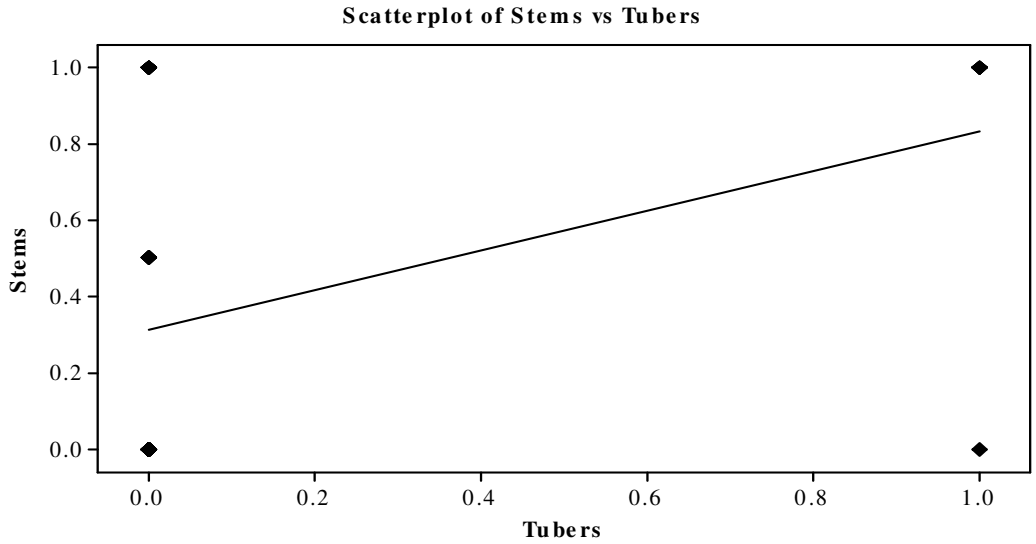

Figure 1. Scatter and regression graph for stems vs. tubers in detection of *Ralstonia solanacearum* for farm A in 2005.

particular category were however not significantly different (Table 1). These observations point to the fact that an infected plant can have both healthy tubers and diseased stems or diseased tubers and healthy stems. In relation to this, further research should be done to determine the relationship between inoculums (infection) source and the detection of *R. solanacearum* in either stems or tubers.

When r – values were considered, there was none indicating perfect positive correlation for the four farms which had a range of 0.2 – 0.5 (Table 2). On average, the correlation coefficient indicated some positive correlation, which is an indication that there is scope for adoption of stems as a sample instead of tubers for testing of latent infection due to *R. solanacearum* in seed certification schemes. This would be through developing a protocol for screening seed potato fields for presence of latent infection due to *R. solanacearum.* If achieved, this would save the loss incurred when classical method is used, as the fate of the crop will be determined before harvesting.

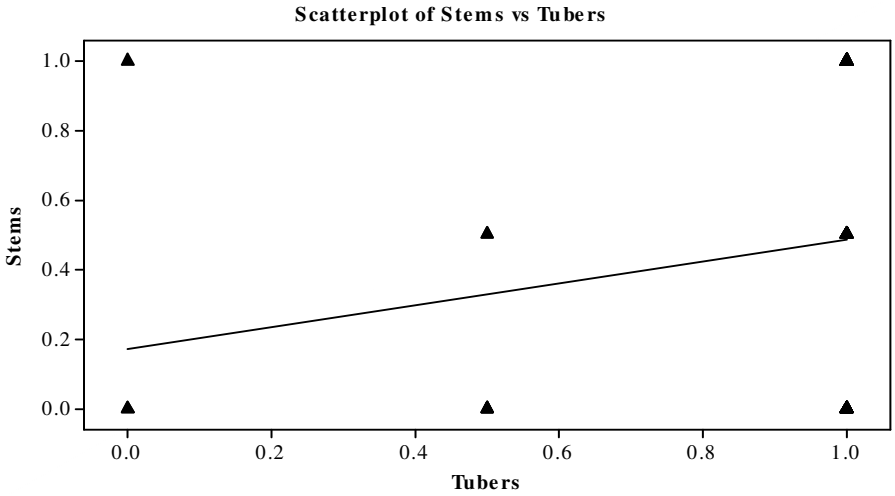

Figure 2. Scatter and regression graph for stems vs. tubers in detection of *Ralstonia solanacearum* for farm B in 2005.

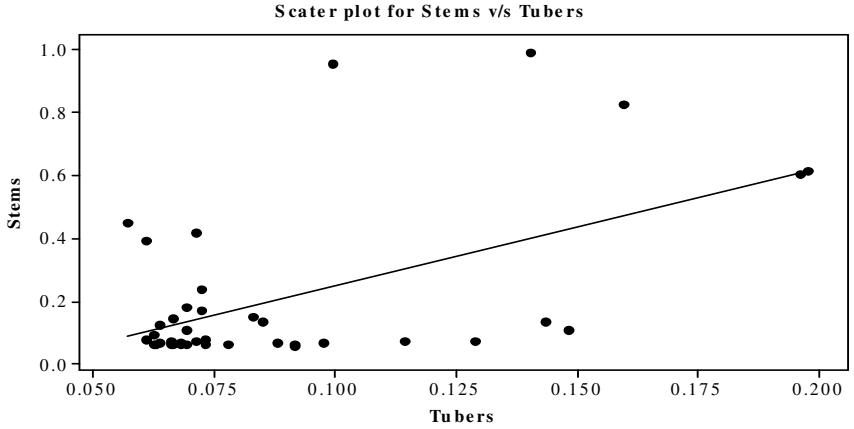

Figure 3. Scatter and regression graph for stems vs. tubers in detection of *Ralstonia solanacearum* for farm A in 2006.

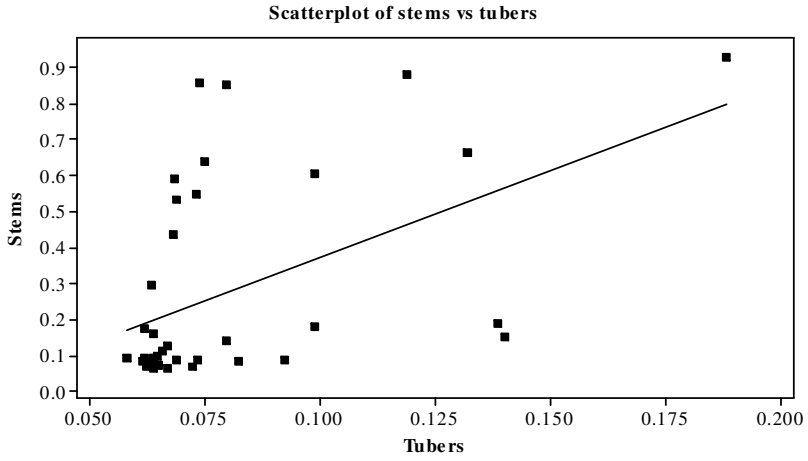

Figure 4. Scatter and regression graph for stems vs. tubers in detection of *Ralstonia solanacearum* for farm B in 2006.

Conclusions and recommendations

From the study on suitability of using stems in detection of *R. solanacearum*, it can be concluded that there was a positive correlation between tuber and stem testing. However, the correlation coefficient factors were between 0.2 – 0.5, which is low considering that bacterial wilt is a quarantine disease. This may be as a result of the fact that an infected plant can contain both healthy and clean tubers, as well as both healthy and clean stems. The outcome of the study therefore indicates that care should be taken in the analysis of results from both stem and tuber testing. There is, however, scope for the adoption of stem testing to develop a protocol for screening of seed potato fields for bacterial wilt infections. This would be useful in seed certification schemes and would save valuable seed potato and allow for the results to be available faster.

From the correlation results, it was recommended that further research be done to establish the relationship between inoculums' source and detection of *R. solanacearum* in stems and tubers before adopting the use of stems as alternative samples to tubers for indexing against *R. solanacearum* for latent infections in potato seed certification programs. However, there is a strong indication that a protocol for screening seed potato fields using stems can positively be developed.

ACKNOWLEDGEMENTS

Our gratitude goes to the International Potato Centre (CIP) who funded this research project. JKM is also indebted to Dr. Z. M. Kinyua, Miriam Otipa, Joseph Kinoti, and the entire technical staff of KARI-NARL-Plant Pathology Section for the technical support and advice offered during my laboratory work at their station and through whose efforts, I was able to successfully carry out the field sampling and laboratory experiments.

REFERENCES

Adipala E, Tusiime G, Patel BK, Berga L, Olanya M (2001). Bacterial wilt management: A dilemma for Sub-Saharan Africa. Afr. Crop Sci. Proc. 5: 433-437.

Ajanga S (1993). Status of bacterial wilt of potato in Kenya. In: Hartman GL, Hayward AC (eds): Bacterial Wilt. ACIAR Proceedings 45: 338 - 340.Canberra, Australia.

Barton D, Smith JJ, Kinyua ZM (1997). Socio-economic inputs to biological control of bacterial wilt disease of potato in Kenya. ODA RNRRS Crop Protection Project ZA0085, United Kingdom, p. 23.

Bekele K (1996). Incidence and distribution of major potato diseases in 1993 and 1994 off-season in central Ethiopia. Abstract. The 4th annual conference of the crop protection society of Ethiopia. 23-24 May 1996. Addis Ababa, Ethiopia, p. 15.

Ciampi L, Sequeira L, French ER (1980). Latent infection of potato tubers by *Pseudomonas solanacearum*. Am. Potato J. 57: 377-386

CIP (2001). CIP-NCM-ELISA kit for the detection of *Ralstonia solanacearum* in potato. Instruction Manual.

Eden-Green SJ (1991). Final Report of a study tour on bacterial wilt (*Pseudomonas solanacearum*) and other bacterial diseases of tropical crops. NRI Project No. X0095. NRI, Chatham, Kent, UK.

French ER, Gutara L, Aley P, Ephistone J (1995). Culture media for Pseudomonas *Solanacearum*. Ann. Rev. Phytophathol. 29: 65-87.

Hayward AC (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Ann. Rev. Phytopathol. 29: 65-87.

Hayward AC (1996). Bacterial wilt caused by *Pseudomonas solanacearum* in Asia and Australia: An overview. In: Proceedings of an International Workshop on Bacterial Wilt Diseases in Asia and South Pacific. G. J. Persley, Ed. ACIAR Proc. 13: 15-24.

Kakuhenze R, Hakiza JJ, Berga L, Tusiime G, Alacho F (2000). Past present and future strategies for integrated control of bacterial wilt in Uganda. Afr. Potato Assoc. Conf. Proc. 5: 353-359.

Kinyae PM, Lung'aho C, Kanguha E, Njenga DN (1994). The status of seed potato in Meru, Nyambene, Nyandarua and Laikipia Districts. Second KARI/CIP Technical Workshop on collaborative research, Nairobi. January 1994.

Kinyua ZM, Smith JJ, Lung'aho C, Olanya M, Priou S (2001). On-Farm Successes and Challenges of Producing Bacterial Wilt-Free Tubers in Seed Plots in Kenya. Afr. Crop Sci. J. 9(1): 279-285.

Michieka AO (1993). Screening CIP potato germplasm for resistance to *Pseudomonas solanacearum* E.F. Smith. Proceedings of the workshop on Bacterial wilt of potato caused by *Pseudomonas solanacearum*. Bujumbura, Burundi. Feb 22-26, pp. 27-31.

Nortje PF (1997). Seed potato production in South Africa. An overview. African Potato Assoc. Conf. Proc. 4: 271-273.

Nyangeri JB, Gathuru EM, Mukunya DM (1984). Effect of latent infection on the spread of bacterial wilt of potatoes in Kenya. Trop. Pest Manage. 30: 163-165.

Priou S, Guttara L, Fernadez H, Aley P (1999). Sensitive detection of *Ralstonia solanacearum* in latently infected tubers and soil by post-enrichment ELISA.CIP programmer 1997-1998. International Potato Center, Lima, Peru, pp. 111-121.

Priou S (2004). DAS-ELISA kit for the detection of *Ralstonia solanacearum* in stems of potato. Instruction for use. International Potato Center. Lima Peru.