BIOLOGICAL CONTROL OF BACTERIAL WILT OF POTATO IN KENYA AND PAKISTAN

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FINAL TECHNICAL REPORT

[DFID RNRKS CPP R6629]

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REPORTING STRUCTURE

The following DFID RNRKS CPP funded projects are reported

1996-99: Biological control of bacterial wilt disease in Kenya and Pakistan

Add-on: Socio economic study of potential for incorporation of BCA into IPM systems of smallhold farms in East Africa
Add-on: Establishment of on-farm production for home seed production
Add-on: Biological Control of bacterial wilt in Kenya: Preliminary efficacy trials in South Africa to circumvent legislative delays in Kenya

1999-00: Extension: Biological control of bacterial wilt disease in Kenya and Pakistan

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ABBREVIATIONS

ARC VOPI	Agricultural Research Council, Roodeplaat Vegetable and Ornamental Plant Institute
AHI	African Highland Initiative
BCA	Biological control agent
BCA Ω5.1	BCA Omega (Ω) 5.1 developed by CAB Bioscience for KARI
BCA ARC	*Pseudomonas resinovorans* BCA developed by ARC VOPI
CIP	International Potato Centre
DFID	Department for International Development
DFID RNRKS CPP	DFID, Renewable Natural Resources and Knowledge Systems, Crop Protection Programme
hrp	Hypersensitive response and pathogenicity
KARI	Kenya Agricultural Research Institute
KARI BC	KARI Biosafety Committee
KARI NARL	KARI National Agricultural Research Laboratories
KARI NPRC	KARI National Potato Research Centre
KIOF	Kenya Institute of Organic Farming
ICM	Integrated Crop Management
INR	Institute of Natural Resources
MALD&M	Ministry of Agriculture, Livestock, Development & Marketing
NPPS	National Plant Protection Service (RSA)
NCST	National Council of Science and Technology
NCST NBC	National Biosafety Council
PRAPACE	Regional Potato ans Sweet Potato Improvement Program in Eastern and Central Africa
PROINPA	Promocion e Investigacion de Productos Andinos
RSA	Republic of South Africa
SAGENE	South African Committee for Genetic Experimentation
SSPS	Small-scale (on-farm) Seed Production System
WT	Wild type (*R. solanacearum*)

1. EXECUTIVE SUMMARY

Bacterial wilt disease (*Ralstonia solanacearum*) imposes a severe constraint on yield and sustainability of potato production in Kenya and other sub-Sahara African countries. Availability of land and seed free of bacterial wilt and other pests are major constraints.

Previous research under DFID CPP R5310 developed a biological control agent (BCA) against bacterial wilt disease of potato. The BCA was a genetically modified non-pathogenic mutant of the wild type (WT) organism. This project aimed to further the outputs of R5310 by establishing the agricultural potential of the BCA in Kenya and to identifying a 'window' within farmer systems for its application. The applicability of these technologies to potato cropping in other sub-Sahara African countries, Republic of South Africa (RSA) and Pakistan were assessed.

BCA efficacy assessments were performed in the United Kingdom [CABI Bioscience] and the Republic of South Africa [ARC-VOPI] under contained-use conditions. These assessments substantiated efficacy data obtained under R5310 and strongly indicated that the BCA would be effective on diverse potato varieties and against *R. solanacearum* biovar 2a populations of other countries. Antibiotic marker-based methods for monitoring soil BCA and WT populations were developed to assess impact and biosafety of the BCA. These studies showed BCA populations to decline in soils independently of crop rotation imposed, and thus soil BCA populations are unlikely to persist in agricultural soils. No evidence of gene transfer between BCA and WT populations was observed. These data strongly supported the testing of the BCA in-country (Kenya) and accordingly counterparts at KARI received instruction in bacteriology and GMO working practices in preparation for an in-country testing phase.

The procedure and formulation of an application to allow contained-use and field trail testing of the BCA in Kenya and the subsequent review of that application has provided a test case for the emerging national biosafety policy of Kenya. Commendable resolve by all concerned has resulted in this application reaching the final stages of the review process by the National Biosafety Committee. Approval for testing the BCA in RSA under contained conditions was gained by the ARC-VOPI demonstrating wider acceptance of the BCA strategy. In consideration of these experiences, development of an analogous BCA strategy against bacterial wilt on potato in Pakistan was considered premature as the national GMO legislation in Pakistan was still under development.

An socio-economic review of potato production in Kenya concluded certified seed was insufficient to meet national demand and farmer selection of seed was not optimal for yield. An on-farm small-scale seed production system (SSPS) for seed production was proposed that countered these constraints and that also afforded a window for the application of the BCA (and other control strategies) on-farm. On-farm trials over 6 growing seasons have shown the SSPS to be an effective system for seed production in terms of land inputs, but at a premium of increased seed inputs, and for maintaining or increasing ware yields. Optimisation assessments on the spacing of the SSPS seed cultivation indicate further substantial improvements on land/seed input parameters can be achieved presenting a win-win scenario for on-farm seed production.

The implementation of BCA and SSPS technologies will help maintain on-farm potato seed health and increase the 'flush-out' rate of degenerate on-farm seed. These factors will improve ware yields where soil fertility and water availability are not yield-limiting factors.

2. BACKGROUND

The potato is one of the major food and cash crops in sub-Sahara Africa and the Republic of South Africa (RSA). Kenya and Uganda for example have a total area of 98,000 and 60,000 ha under potato per year with a per capita consumption of 23 and 19 kg per year. In these countries the potato producing regions are characteristically the populous regions of the highlands, dominated by smallholder farms that operate intensive, low input agricultural practices. Emerging local markets for chips and crisps are making potato an increasingly attractive crop for smallholders, although home consumption remains important for securing local food security. By contrast potato production in the RSA, which is the largest potato producing country in Africa, is characterised by large-scale commercial practices that are highly mechanised. However, the dominance of these large-scale enterprises detracts from a significant number of smallholder practices that operate in a similar way to that described as typical to Kenya and Uganda. Here again potato is a valued crop for both cash and home consumption, and the stimulation of smallholder enterprises is a priority of the government of the RSA.

On-farm yields achieved by smallholders have been shown to be very variable and much lower than achieved under optimal conditions. An indication of the level of under-achievement can be gained by comparison of the current average on-farm potato yield in Kenya of less than 10 t/ha to research station yield figures of over 40 t/ha (Lung'aho et al., 1997). Such low yields have been attributed to near-continuous potato cultivation that leads to increased incidence of diseases and pests, a shortage of disease-free seed and a decline in soil fertility. Diseases such as potato blight (*Phytophthora infestans*), bacterial wilt (*Ralstonia solanacearum*) and viruses are recognised as primary constraints to production (Lemaga et al., 1997; Barton et al., 1997).

Bacterial wilt and virus are recognised as being primarily seed-borne, and hence the production of disease-free seed is of paramount importance. Despite this, certified seed production in Kenya and Uganda is vastly insufficient to meet national demand. By contrast in the RSA, production of certified seed is not a constraint, however, marketing is orientated to large-scale practices that exclude smallhold enterprises. These constraints severely limit the availability of certified seed of old and new varieties to smallholders and force the smallhold farmer to select seed from their previous harvest, local market or 'neighbour' that are of unknown health status (Barton et al., 1997). This is compounded by the common practice of commercial middlemen to select only the marketable product from a harvest, leaving mainly the diseased and undersized (<25mm) tubers from which to select seed. Whereas visibly diseased tubers can be selected against, latent infections go unseen and undersized tubers have been shown to give reduced yields due to poor tolerance to drought and/or frost (Steyn, 1997). It has also been suggested that undersized tubers have a higher risk of latent disease, such as bacterial wilt, since the small size is likely to reflect the unhealthy status of the parent plant.

These factors highlight the inherent problems of ware production, and emphasise the need to improve seed-tuber management. Curiously, seed production practised in Africa shows few differences to ware production, both operating at a planting density of 44,500 tubers/ha. This contrasts to seed production practised in Europe where planting densities of over 60,000 tubers/ha are recommended. The objective of the higher planting density is to maximise seed production (25-55 mm sized tubers) per unit area of land through suppressing the production of oversized tubers (>55 mm). This disparity in farming practice may reflect a reticence by farmers to commit solely to seed production as the farmer then lacks the flexibility to take advantage of differences in market prices between ware and seed-tubers (N. Mienie pers comm.). This is an important consideration in extending seed production systems to the regional farmers as the approach developed has to be accepted by farmers and seen as beneficial to them individually.

Improved seed management in isolation is unlikely to break the bacterial wilt disease cycle as *R. solanacearum* is also known to survive as a soil saprophyte. This is particularly true for countries such as Kenya and Uganda where effective rotation of crops is not common and soil populations can be maintained at high levels. In addition to clean seed production, control practices for bacterial wilt have centred on plant breeding, field sanitation, crop rotation and use of bacteriocides, and are generally implemented using a ICM approach. Unfortunately, these approaches have either proved ineffective or impractical to implement, and bacterial wilt remains a significant constraint. Accordingly, alternative methods for control involving appropriate technology at a low cost to the farmer are urgently needed. Various recent studies have indicated that biological control of bacterial plant diseases can be achieved using antagonistic or competitive bacteria such as non-pathogenic strains of the pathogen or other bacterial species. The former approach was piloted by INRA on *R. solanacearum* race 1 of tomato using defined *hrp* mutants of the wild type bacterium characterised by retaining a fluidal morphology on TZC medium (Frey et al., 1994). The mutating element was an Omega cassette that shared homology to the *hrp*O gene and was specifically designed to create a stable mutation through possession of transcription and translation terminator sequences that prevent subsequent genomic transfer events (Fellay et al 1987; Frey et al 1994). The non-pathogenic omega mutants were therefore appropriate for environmental release consistent with the use of a BCA. This technology therefore showed significant promise and by virtue of the conserved nature of the *hrp* region the Omega cassette was able to transform other races of *R. solanacearum.* Accordingly, under DFID CPP R5310 a BCA strategy to control race 3 in Kenya was initiated using the Omega cassette technology. This research was conducted by CABI Bioscience in collaboration with KARI and INRA. Preliminary BCA efficacy trials undertaken in the UK under contained-use conditions recorded levels of protection against bacterial wilt in the order of 30% (R5310 Final Technical Report). The mechanism by which control occurs remained to be fully elucidated, however, induction of host resistance or competitive exclusion of the pathogen has been suggested. Subsequent work is now needed to validate these findings under field conditions and, of equal importance, to identify a niche within farmer systems where the BCA can be cost effectively applied.

3. PROJECT PURPOSE

Bacterial wilt disease (*Ralstonia solanacearum*) imposes a severe constraint on yield, quality and sustainability of potato production in Kenya and other sub-Sahara African countries. Previous research under DFID CPP R5310 developed a BCA against bacterial wilt disease. This project aimed to further the outputs of R5310 by establishing the agricultural potential of the BCA in Kenya and to identify a window for its application in smallholder farmer systems. Implementation of the technology is centred about an ICM strategy. It is ultimately the objective to increase the yield, quality and sustainability of potato production by smallhold farmers in peri-urban regions of Kenya through the reduction of major potato disease and pest constraints.

The applicability of the ICM strategy to potato cropping in other sub-Sahara African countries, Republic of South Africa and Pakistan was also assessed.

4. RESEARCH ACTIVITIES

4.1. BIOLOGICAL CONTROL AGENT (BCA) EFFICACY AND FORMULATION

4.1.1. EFFICACY OF BCA

This research aimed to demonstrate the efficacy of the BCA against bacterial wilt disease (*Ralstonia solanacearum* race 3 biovar 2a) of potato in Kenya.

BCA efficacy assessment undertaken at CABI Bioscience UK Centre [Egham]

Experimental outline:

Potato variety	–	Desiree mini-tubers (supplied by Scottish Agricultural Service Agency)
BCA	–	Omega 5.1
WTs	–	K3, K9, K41 [Biovar 2a; Kenya]
Pot size and soil	–	15 cm diam. pots (3 litres volume) with John Innes No1 compost
Treatments	–	Treatment 1: No treatment Treatment 2: BCA only Treatment 3: WT only Treatment 4: BCA + WT
Inoculation of tuber	–	BCA applied 7 days prior to planting as water soak (approx. 10^9 cfu ml^{-1}; 30mins) followed by standard chitting
Inoculation of soil	–	Inoculum of WT and BCA grown on CPG plates (48 hr growth at 30ºC); equal volumes of 10^7 and 10^9cfu ml^{-1} inoculum of WT (equal ratio of isolates) and BCA, respectively, absorbed in vermiculite (1 volume of inoculum to 9 volumes of vermiculite) and brought to saturation with H_2O, prior to dispersal in soil according to treatments; vermiculite to soil ratio adjusted to 1:10 in all treatments; tubers planted on day of soil inoculation
Growth conditions	–	14 hr day 10 hr night cycle; min/max temp 20-25ºC
Experimental design	–	Randomised complete block design; 8 replicates; 3 pots per replicate, planted to 3 tubers per pot
Disease scoring	–	Disease was recorded as either present or absent, 1 or 0, respectively, for each plant; each pot therefore had a potential maximum score of 3
Statistical analysis	–	Hypothesis testing on the effect of BCA on disease incidence was undertaken at onset, exponential and plateaux phases of the disease progress curve by generalised regression analysis modelling binomial proportions e.g. by logits (Genstat).

BCA efficacy assessment undertaken at ARC VOPI

Experimental outline:

Potato variety	–	BP-1 mini-tubers
BCA	–	Omega 5.1 [BCA Ω5.1]; *Pseudomonas resinovorans* [BCA ARC]
WTs	–	111, 47, 49 [Biovar 2a; RSA]
Pot size and soil	–	15 cm diam. pots (3 litre volume) with local compost

Treatments	–	Treatment 1: No treatment Treatment 2: BCA Ω5.1 only Treatment 3: BCA ARC only Treatment 4: WT only Treatment 5: BCA Ω5.1 + WT Treatment 6: BCA ARC + WT
Inoculation of tuber	–	BCAs applied 7 days prior to planting as perlite based formulation (approx. 10^9 cfu ml^{-1}) followed by standard chitting (see formulation section)
Inoculation of soil	–	Inoculum of WT and BCA grown on CPG plates (48 hr growth at 30ºC); equal volumes of 10^7 and 10^9cfu ml^{-1} inoculum of WT (equal ratio of isolates) and BCA, respectively, absorbed in vermiculite (1 volume of inoculum to 9 volumes of vermiculite) and brought to saturation with H_2O, prior to dispersal in soil according to treatments; vermiculite to soil ratio adjusted to 1:10 in all treatments; tubers planted on day of soil inoculation
Growth conditions	–	12 hr day 12 hr night cycle; min/max temp 20-25ºC
Experimental design	–	Randomised complete block design; 2 replicates; 25 pots per replicate, planted to 1 tuber per pot
Disease scoring	–	Disease was recorded as either present or absent, 1 or 0, respectively, for each plant
Statistical analysis	–	Hypothesis testing on the effect of BCA on disease incidence was undertaken at onset, exponential and plateaux phases of the disease progress curve by generalised regression analysis modelling binomial proportions e.g. by logits (Genstat).

4.1.2. PATHOGENICITY OF *R. SOLANACEARUM* BIOVAR 2A ISOLATES REPRESENTATIVE OF PERU, COLOMBIA AND OTHER COUNTRIES ON DIVERSE POTATO VARIETIES, AND IMPACT ON BCA EFFICACY

This research aimed to evaluate 1) pathogenicity of *R. solanacearum* biovar 2a isolates representative of world-wide geographic regions against *S. tuberosum* and *S. andigena* varieties and 2) the efficacy of the BCA on these host/pathogen combinations. The assessments were undertaken at CABI Bioscience UK Centre [Egham].

Pathogenicity assessment on *R. solanacearum* biovar 2a isolates representative of Peru, Colombia and other countries on *S. tuberosum* and *S. andigena* varieties

Experimental outline:

Plates 1a & b present an analysis of genomic fingerprint data of *R. solanaearum* biovar 2a isolates of South America. The rep-PCR data defines 3 broad groupings that correspond to isolates that predominate from Peru, Colombia and other South American countries. *R. solanacearum* biovar 2a isolates outside of South America corresponded with isolates typical of South American countries other than Colombia and Peru [data not shown[1]].

[1] This research was an output of DFID CPP R5310 and presented at the 2nd International Bacterial Wilt Symposium in Guadeloupe 22-27 June 1997.

Based on these data WT isolates representative of the 3 groupings were selected and assessed for pathogenicity against varieties of *S. tuberosum* and *S. andigena*[2]. The primary objective was to investigate host/isolate interactions.

Experimental outline:

Potato variety	–	*S. tuberosum* var 800224 and 800226; *S. andigena* var Waych'a and Imilla Negra; plants raised by tissue culture
WTs	–	Bol 1 [Biovar 2a; Bolivia]; K54 [Biovar 2a; Kenya]; R303 [Biovar2a; Colombia]; CIP161 [Biovar 2a; Peru]; CIP202 [Biovar 2a; Peru]
Pot size and soil	–	10 cm diam. pots (0.5 litre volume) with John Innes No1 compost
Treatments	–	Treatment 1: No treatment [Var 1] Treatment 2: WT only [Var 1, isolate 1] Combinations repeated for all variety/isolate combinations
Inoculation	–	Potato varieties grown for 4 weeks prior to inoculation; Inoculum grown on CPG plates (48 hr growth at 30ºC); 20ml 10^7 cfu ml^{-1} drench applied to each pot
Growth conditions	–	14 hr day 10 hr night cycle; min/max temp 20-25ºC
Experimental design	–	Randomised complete block design; 4 replicates; 5 pots per replicate, planted to 1 plant per pot
Disease scoring	–	Disease was recorded as either present or absent, 1 or 0, respectively, for each plant
Statistical analysis	–	Hypothesis testing on host/strain interactions was undertaken at the exponential and plateaux phase of the disease progress curve by generalised regression analysis modelling binomial proportions e.g. by logits (Genstat).

BCA efficacy assessment against *R. solanacearum* biovar 2a isolates representative of Peru and other countries (except Colombia)

Constraints of contained-use growthroom space and difficulty of tissue culturing *S. andigena* varieties prevented a full assessment on host/isolate combinations and efficacy of BCA.

Experimental outline:

Potato variety	–	*S. tuberosum* var 800224 and 800226; plants raised by tissue culture
BCA	–	Omega 5.1
WTs	–	K54 [Biovar 2a; Kenya]; CIP161 [Biovar 2a; Peru]
Pot size and soil	–	10 cm diam. pots (0.5 litre volume) with John Innes No1 compost
Treatments	–	Treatment 1: No treatment Treatment 2: WT [K54] Treatment 3: BCA + WT Combinations repeated for CIP161; no BCA only treatment

[2] *Solanum andigena* vars were donated by PROINPA for research purposes only (pers comm. E. Fernadez-Northcote)

Inoculation	–	Potato varieties grown for 4 weeks prior to inoculation; Inoculum of WT (equal ratio of strains) and BCA grown on CPG plates (48 hr growth at 30ºC); BCA applied as 20ml 10^9 cfu ml^{-1} drench to each pot 7 days prior to inoculation with 20ml 10^7 cfu ml^{-1} of WT inoculum
Growth conditions	–	14 hr day 10 hr night cycle; min/max temp 20-25ºC
Experimental design	–	Randomised complete block design; 4 replicates; 10 pots per replicate, planted to 1 plant per pot
Disease scoring	–	Disease was recorded as either present or absent, 1 or 0, respectively for each plant
Statistical analysis	–	Hypothesis testing on the efficacy of the BCA was undertaken at exponential and plateaux phases of the disease progress curve by generalised regression analysis modelling binomial proportions e.g. by logits (Genstat).

4.1.3. CARRIER MATRIX FORMULATION OF BCA

This research aimed to identify a suitable matrix carrier for the BCA. The assessments were undertaken at ARC VOPI. Two aspects were researched:

Half-life of BCA in various matrix carriers

This assessment compared a perlite-based commercial product developed for rhizobial inoculums against other known inoculum carrier substrates.

Experimental outline:

BCA	–	Omega 5.1
Matrix carriers	–	Sterile perlite-based matrix, vermiculite, alginate beads, peat
Inoculation	–	BCA inoculum grown in broth and applied to matrixes; volume applied was dependent on matrix
Storage conditions	–	Matrixes were incubated for 1 week at 27ºC and thereafter stored without light at room temperature (20 – 25ºC)
Experimental design	–	No information was presented
Data recorded	–	BCA cfu cm^{-3} of matrix were determined at t = 0, 1, 2 and 5 weeks post inoculation by serial plating on SMSA
Statistical analysis	–	Non undertaken (raw data not presented)

Half-life of BCA on potato surface

This assessment looked at application methods for the BCA to tuber surfaces and survival during chitting

Experimental outline:

Potato variety	–	BP-1
Matrix carrier	–	Sterile perlite powder, perlite slurry, water
Inoculation	–	BCA inoculum grown in broth and applied to matrixes
Treatment	–	Tubers were dipped in inoculum preparations and stored under diffuse light at 20 – 25ºC (chitting) for 2 weeks
Experimental design	–	Randomised complete block design with 6 replicates

Data recorded	–	BCA cfu tuber^{-1} were determined at t = 0, 3, 5, 8 and 13 days post inoculation by serial plating on SMSA
Statistical analysis	–	Non undertaken (raw data not presented)

4.2. EPIDEMIOLOGY OF BCA AND *R. SOLANACEARUM* WT POPULATIONS IN SOIL, AND INTERACTION WITH POTATO AND ROTATION CROPS

Survival of BCA and *R. solanacearum* WT populations in soil, and interaction with potato

This research aimed to establish the persistence of the BCA in soil, the interaction with potato in comparison to the WT, and the potential for genetic exchange of the kanamycin marked omega cassette to WT *R. solanacearum* isolates. The assessment was undertaken at CABI Bioscience UK Centre [Egham].

Experimental outline:

Soil microcosms	–	15 diam. x 30 ht. cm free-draining plastic tubes; access porthole 10 cm from surface to allow soil sampling; Egham sandy loam soil (see Plate 2a)
BCA	–	Omega 5.1
WTs	–	K3, K9, K 41 [spontaneous rifampacin mutants; Biovar 2a; Kenya]
Potato variety	–	Desiree mini-tubers
Treatments	–	Treatment 1: Soil only Treatment 2: BCA only Treatment 3: WT only Treatment 4: BCA + WT Inoculation combinations planted with or without potato (5 mini-tubers per pot)
Inoculation of soil	–	Inoculum of WT and BCA grown on CPG plates (48 hr growth at 30ºC); equal volumes of 10^9 and 10^9cfu ml^{-1} inoculum of WT (equal ratio of isolates) and BCA, respectively, absorbed in vermiculite (1 volume of inoculum to 10 volumes of vermiculite) and brought to saturation with H_2O, prior to dispersal in soil according to treatments; vermiculite to soil ratio adjusted to 1:10 in all treatments; tubers planted on day of soil inoculation
Growth conditions	–	14 hr day 10 hr night cycle; min/max temp 20-25ºC
Sampling	–	3 soil sub-samples (1g) were taken from each treatment combination via the porthole and pooled (= approx 1 cm^3) prior to dispersal in a total volume of 10ml H_2O and serial dilution; cfu cm^{-3} were determined by enumeration on SMSA supplemented with kanamycin, rifamapacin or kanamycin and rifampacin (Plate 2b); samples taken at t = 0, 7, 14, 21, 33, 56, 77 and 112 days after planting
Experimental design	–	Randomised complete block design; 3 replicates
Data recorded	–	BCA and WT cfu g^{-1} soil
Statistical analysis	–	Hypothesis testing to determine effect of potato on BCA and WT populations, and interaction between BCA and WT populations was undertaken at t = 0, mid- and end-season growth stages by two-way ANOVAR in randomised blocks (Genstat)

Interaction of BCA and *R. solanacearum* WT with rotation crops

This research aimed to establish the interaction of the BCA and WT *R. solanacearum* with rotation crops associated with potato. There were 2 objective 1) to evaluate the persistence of BCA in soils under rotation crops of potato and 2) to evaluate the capacity of rotation crop of potato to support *R. solanacearum* in soil. The assessment was undertaken at CABI Bioscience UK Centre [Egham].

Experimental outline:

Soil microcosms	–	15 cm diam. pots; Sandy loam soil [Egham]
BCA	–	Omega 5.1
WTs	–	K3, K9, K 41 [spontaneous rifampacin mutants; Biovar 2a; Kenya]
Treatments	–	Soil was inoculated with equal populations of BCA and WT *R. solanacearum* and planted to maize, pea, potato and tomato (5 plants per pot); a no-plant control acted as a fallow treatment
Inoculation of soil	–	Inoculum of WT and BCA grown on CPG plates (48 hr growth at 30ºC); equal volumes of 10^9 and 10^9cfu ml^{-1} inoculum of WT (equal ratio of isolates) and BCA, respectively, absorbed in vermiculite (1 volume of inoculum to 9 volumes of vermiculite) and brought to saturation with H_2O, prior to dispersal in soil; vermiculite to soil ratio adjusted to 1:10 in all treatments; seeds/tubers planted on day of soil inoculation
Growth conditions	–	14 hr day 10 hr night cycle; min/max temp 20-25ºC
Sampling	–	At each sampling, 1 treatment combination (pot) was assessed; rhizosphere soil and root (5 plants) were weighed independently; soil fractions were serially diluted and plated onto SMSA supplemented with kanamycin or rifamapacin; samples taken at t = 3, 5 and 9 weeks growth
Experimental design	–	Randomised complete block design; 1 replicate planted to 5 plants; 3 sampling dates
Data recorded	–	BCA and WT cfu g^{-1} rhizosphere soil or cfu g^{-1} root
Statistical analysis	–	The absence of true replicates prevented a full statistical analysis of these data.

4.3. ASSESSMENT OF UPTAKE NEEDS FOR BCA IN KENYA, REPUBLIC OF SOUTH AFRICA (RSA) AND PAKISTAN

4.3.1. SOCIO ECONOMIC REVIEW ON THE NEED AND IMPLEMENTATION OF THE BCA

Objectives:

The specific objectives of this study were to:

* assess the constraints to the uptake of BCAs into integrated pest management (IPM) systems for smallholder potato production in Kenya;
* assemble production data for potatoes in Kenya and production trends over the past 10 years;
* assess the effects of bacterial wilt on yields of potatoes;
* seek information from producers on current management practices regarding potato cultivation, constraints to the expansion and

development of potato cultivation and methods of control of bacterial wilt; and
* investigate the need throughout East Africa for control of bacterial wilt.

The wider objective was to inform the future progress of Project R6629 'Biological control of bacterial wilt disease of potato in Kenya and Pakistan'.

Methodology

The socio-economic study was undertaken during October 1996 and involved discussion with appropriate parties in Kenya including representatives of:

* The Ministry of Agriculture, Livestock Development and Marketing (MALD&M);
* Kenya Agricultural Research Institute (KARI);
* International Potato Centre (CIP) Regional Office;
* The African Highland Initiative (AHI); and
* potato producers in Embu, Meru, Nakuru and Nyandarua Districts.

Attempts were made to contact a random sample of farmers. Although extension workers and officers of the National Potato Research Centre were keen to visit contact, progressive or commercial farmers, efforts were made to also contact smaller less commercial producers. In total, semi-structured interviews were conducted with 25 male and female potato producers.

4.3.2. RESEARCH WITH GMOS IN KENYA, RSA AND PAKISTAN

In Kenya and the RSA permission to assess the BCA under contained-use conditions has been pursued. In both countries a non-constituted biosafety assessment framework operates to advise the national plant protection service on the appropriateness and risk of GMO technologies. In Kenya and the RSA these advisory bodies request the submission of an application form that is analogous to the application form requested by Health and Safety Executive (HSE) of the UK. In Pakistan no such structures operate and hence assessment of the BCA (and consequently the SSPS) in this country was not progressed.

In anticipation of testing the BCA in Kenya, KARI counterparts Ms Miriam Optipa and Mr Kinyua Murimi[3] undertook 2 week and 3 month attachments with CABI UK Centre [Egham], respectively, and Mr Murimi a further 1 week attachment to ARC VOPI RSA. These attachments focused on familiarisation in contained-use working practices, BCA assessment methods and general bacteriology.

4.4. SEED PRODUCTION – IMPROVING SEED MANAGEMENT AND ESTABLISHING A 'WINDOW' FOR THE APPLICATION OF THE BCA

This research stemmed from the findings of the socio-economic study undertaken in 1996 (Barton et al., 1997) on potato production in Kenya by smallholder farmers that concluded: 1) the formal seed production system was inadequate to meet demand and 2) the current farmer practice of selecting under-sized seed from a ware-to-ware system was not optimal for yield and probably favoured the build-up of diseases, notably bacterial wilt and virus.

[3] This attachment received supplementary funding under NARP II

4.4.1. SEED SIZE AND WARE YIELD

This research tested the hypothesis that seed size affects yield. The assessments were undertaken at KARI NARL and through on-farm trials at Njabini, South Kinangop.

Assessment at KARI NARL:

This assessment comprised of 2 seed sources from Phase 1, SSPS seed and NPRC ware cultivations (see section 4.4.2), which were treated as factors. This represented a sub-objective to investigate seed health.

Experimental outline:

Potato variety	–	Tigoni and Roslin Tana
Treatment	–	Tuber seed size category <25, 25-35, 35-45, 45-55, >55 mm; SSPS and NPRC seed types
Experimental design	–	Single row of 10 tubers planted; randomised complete block design; 4 replicates
Data recorded	–	Tuber number within size classes <25, 25-35, 35-45, 45-55, >55 mm, and total weight
Statistical analysis	–	Hypothesis testing on differences between yields was undertaken by generalised linear model (Genstat); hypothesis testing on distribution of harvested tuber size classes was tested by split plot analysis where var.seed size.treatment = main plot and harvested tuber size class = split factor (Genstat)

Assessment at Njabini:

This assessment comprised a split factor treatment of chitted eye present or absent prior to planting. This represented a sub-objective to investigate a seed management practice on yield.

Experimental outline:

Potato variety	–	Tigoni and Roslin Tana
Treatment	–	Tuber seed size category <25-35, 35-55, >55 mm; with or without eye rubbing treatment prior to planting
Experimental design	–	3 rows of 13 tubers planted per treatment combination; split factor; randomised complete block design; 4 replicates (farms)
Data recorded	–	Tuber number within size classes<25, 25-35, 35-45, 45-55, >55 mm, and total weight
Statistical analysis	–	Hypothesis testing on differences between yields undertaken by split plot analysis where var.seedsize = main plot (Genstat) and treatment = split factor; hypothesis testing on distribution of harvested tuber size classes was tested by split-split plot analysis where var.seedsize = main plot, var.seedsize.treatment = split factor and harvested tuber size class = split, split factor (Genstat)

4.4.2. SMALL-SCALE (ON-FARM) SEED PRODUCTION SYSTEM (SSPS)

On-farm SSPS trials at Njabini

This research aimed to establish a 'window' for the BCA on-farm through improved/intensified on-farm seed management. The SSPS describes a flatbed cultivation planted at a high density (SSPS seed cultivation) that provides seed for the traditional ridge/furrow ware cultivation (SSPS ware cultivation) in the subsequent season. A schematic outline of the SSPS trial is presented Figure 1.

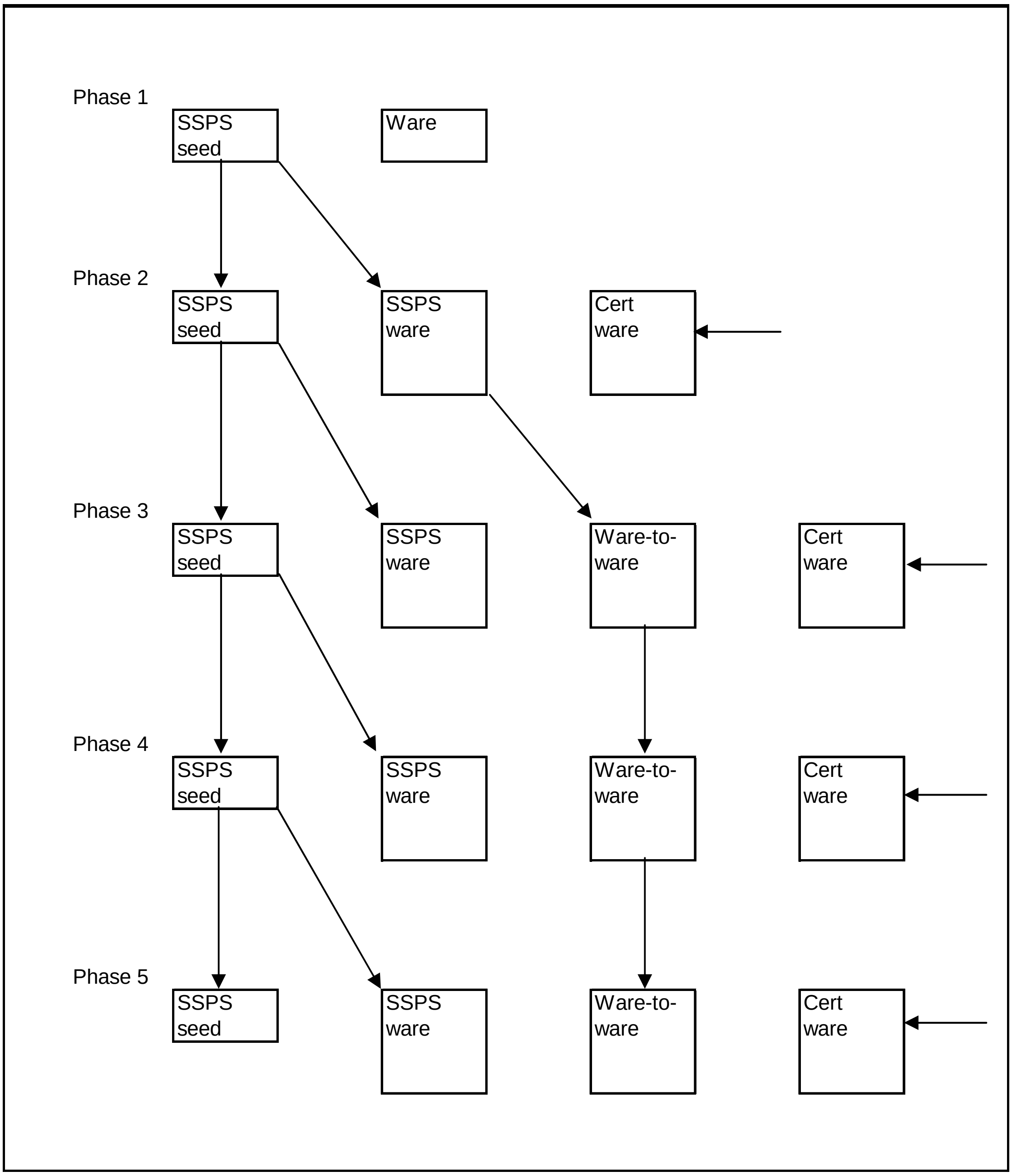

Figure 1: Schematic outline of seed flows under the SSPS trial

The following advantages to the SSPS were proposed:

1) The SSPS strengthens linkages between formal and informal seed production through reducing the initial requirement by a farmer for certified seed, thus

increasing the availability of certified seed amongst farmers and facilitating the diffusion of new potato cultivars.
2) Seed selection under the SSPS is optimal for yield and the maintenance of seed health.
3) The SSPS separates ware and seed production, and identifies a 'window' for intensive strategies for the management of disease and pest constraints that are precise and affordable to the farmer: these would include known beneficial practices of chemical applications and crop rotation, and be appropriate for application of the BCA.
4) The smallhold farmer is not asked to commit solely to seed production, thus avoiding financial risks associated with specialisation in a market without price guarantees.

Experimental outline:

The SSPS was initiated in October 1997 through on-farm trials at Njabini, South Kinangop (Nyandarua district). Under the trial, SSPS ware harvests are compared to ware yields under the 'normal' smallhold farmer ware-to-ware system, and with ware yields from NPRC seed (certified seed) acting as controls. SSPS and ware-to-ware systems are initiated from a common source of NPRC seed so direct comparisons can be made (see Figure 1). To date, 6 cycles of seed production, producing 4 ware harvests across all treatments (see Figure 2) have been recorded.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sept.	Oct.	Nov.	Dec.
1997										Phase 1		
1998	P1		Phase 2						Phase 3			
1999	P3		Phase 4						Phase 5			
2000	P5		Phase 6						Phase 7 ongoing			

Figure 2: Main potato seasons for Njabini and phases of SSPS field trail.

Njabini was selected for the on-farm assessments as this is an important potato-growing region that had been surveyed over the previous 3 years and had a known history of bacterial wilt infested and free farms. Furthermore, this region is served by a NPR sub-Centre that agreed to oversee the day-to-day management of the trials. Six smallholder farmers were invited to participate in the on-farm SSPS field trials (see Appendix A-i for more information). A new and a traditional potato variety were chosen, Tigoni and Roslin Tana, respectively, assessing the relative robustness of a new variety against a land-race adapted variety.

SSPS seed cultivation management: The salient features of the SSPS are:

i) The SSPS seed cultivation is planted each season on soil that was not under potato cultivation the previous season (see point vi).
ii) Seed spacing on the SSPS seed cultivation is at 20 x 20 cm under a flatbed cultivation (see Plate 3).
iii) No earthing-up is practised with the SSPS seed cultivation as the tubers are planted at a depth of 10-15 cm with the use of a dibber (see Plate 4).
iv) Zero disease and pest tolerance is aimed for on SSPS seed cultivation.

iv) Dehalming of the SSPS seed cultivation is undertaken 2 weeks earlier than for the ware cultivations.
v) Seed selection from the SSPS seed cultivation is targeted at medium-range sized seed (see Plate 4 & 5)[4].
vi) An area of land equal in size to the SSPS seed cultivation is managed as a fallow cultivation or planted to a non-solanaceous crop. This land is termed the SSPS setaside and is for the next season's SSPS seed cultivation.

Ware cultivation management: Ware production was undertaken according to standard practice: planted under a ridge/furrow cultivation at a spacing of 75 x 30 cm, respectively. Seed selection was in the 25-35 mm category for both varieties. No rotation was practised with the ware cultivations.

Post-harvest seed treatment: Responsibility for the storage and chitting conditions of seed (SSPS seed and Ware-to-ware seed) for the next phase planting was left to the farmers. For this purpose each farmer was provided with a wooden crate for the SSPS seed only (see Plate 5); no provision was made for Ware-to-ware seed and this was left to the farmer to manage. In the early phases chitting was promoted for both seed lots by use of Rendite (see Appendix A-ii).

Phase 1 assessment of the on-farm small-scale seed production system

In phase 1 the potential for seed production under the SSPS seed cultivation and ware cultivation was compared. This comparison was not integral to the long-term outputs of the main field trial (see Figure 1), but was included to underlined the differences between the systems to the farmers. To allow direct comparisons to be made, it was necessary to impose identical management practice that deviated from the SSPS model (see Appendix A-ii).

Treatment combinations for Tigoni and R. Tana:

i) SSPS seed cultivations (3 x 3 m) using NPRC seed
ii) Ware cultivations (3 x 3 m) using NPRC seed

Phase 2 assessment of on-farm small-scale seed production system

Treatment combinations for Tigoni and R. Tana:

i) SSPS seed cultivations (4.5 x 2 m) using SSPS seed harvested from Phase 1
ii) Ware cultivations (4 x 4.5 m) using SSPS seed harvested from Phase 1
iii) Ware cultivations (4 x 4.5 m) using NPRC seed

Phase 3 assessment of on-farm small-scale seed-tuber production system

Treatment combinations for Tigoni and R. Tana:

i) SSPS seed cultivations (4.5 x 2 m) using SSPS seed harvested from Phase 2
ii) Ware cultivations (4 x 4.5 m) using SSPS seed harvested from Phase 2
iii) Ware cultivations (4 x 4.5 m) using ware-to-ware seed harvested from Phase 2
iv) Ware cultivations (4 x 4.5 m) using NPRC seed

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[4] Medium tuber size for Tigoni and Roslin Tana was within the size-range 35-55mm and 25-45mm, respectively. These size-ranges allow for differences in the shape character of the varieties and equate to tubers of comparable weight: Tigoni has a round shaped tuber, Roslin Tana a elongate shaped tuber.

Phase 4, 5 and 6 assessments of on-farm small-scale seed-tuber production system

Phase 3 realises the full experimental outline, with subsequent phases repeating these treatments.

Management aspects of SSPS: Detail on the operation of the field trials is given in Appendix A-ii.

Data collated: Data collated included potato emergence, disease and pest incidence, agronomic factors, chemical inputs, man operator hours and harvest data. Harvest data included total yield and a breakdown of tuber numbers into 5 tuber classes based on tuber diameter (<25, 25-35, 35-45, 45-55, >55 mm). From Phase 1 data only average tuber weights were recorded for each of the tuber size classes.

Storage pests and diseases were also monitored for both SSPS seed and Ware-to-ware seed. Latent infection of tubers by bacterial wilt was assessed with the use of a *Ralstonia solanacearum* specific ELISA Kit developed by CIP from Phase 4 onwards. The assessment method for ELISA was based on a sample of 5 x 10 sub-samples (= 50 tubers) per tuber class category under analysis. Thus a positive reaction indicated a minimum of 1 in 5 (20%) tubers infected per sub-sample.

Farmer perception and feedback was continual sought, and a Farmers Open Day was held (see below).

Statistical analysis: Hypothesis testing on factors (average seed weight, seed production and ware yields) at each Phase was undertaken by general linear regression (Gensat); to assess system affects over Phases, Phases were considered as a split factor; hypothesis testing of distribution of harvested tuber size classes between systems at each Phase was tested by split plot analysis where var.system = main plot and harvested tuber size class = split factor (Gensat); split-split plot analysis was used to assess the distribution of tuber size classes between systems over all Phases where var.system = main factor, var.system.phase = split factor and harvested tuber size class = split-split factor (Genstat).

4.4.3 OPTIMISATION OF SSPS SEED CULTIVATION SPACING AND INTERACTION BETWEEN VARIETIES

This researched aimed to establish the optimal spacing for the SSPS seed cultivation and made comparisons to ridge/furrow planting strategies. Varietal factors were also addressed for short (Romano), medium (Kerrs Pink) and long (Tigoni) stolon habit cultivars that were common varieties grown in Kenya. Included in this assessment was a comparison on mini-tuber productivity under a single spacing density. The trial was undertaken by the NPRC at Tigoni.

Experimental outline:

Potato variety	–	Tigoni, Romano and Kerrs Pink
Plant spacing	–	Flatbed 20 x 20 cm; 30 x 30 cm; Ridge/furrow 15 x 75 cm; 30 x 75 cm; minitubers tested at 30 x 75 cm only
Experimental design	–	$3m^2$ square cultivations; randomised complete block design; 3 replicates
Data recorded	–	Tuber number within size classes<25, 25-35, 35-45, 45-55, >55 mm, and total weight

Statistical analysis – Hypothesis testing of yield between the systems was undertaken by two-way ANOVAR in randomised blocks (Genstat); hypothesis testing of distribution of harvested tuber size classes between systems was tested by split plot analysis where var.spacing = main plot and harvested tuber size class = split factor (Gensat).

4.4.4. PROMOTION AND UPTAKE OF SSPS IN KENYA AND RSA.

Farmer field day – Acquiring baseline data and assessing farmer views on SSPS

A farmers open day was held at Njabini Farmer Training Centre, South Kinangop. (25th March 1999). Representation was invited from regional farmers, the MALD&M, KARI, CIP and NGOs (World Vision, KIOF).

A questionnaire of two sections (see Appendix A-iii) was conducted assessing a) baseline data on current farming practices and b) farmer perception of the SSPS. Section B was completed after an oral presentation and practical demonstration of the SSPS model.

Promotional material tested

Working with the Ministry of Agriculture and farmers of Njabini a range of promotional material was produced in support of project activities. This material targeted extension officers and farmers and aimed to heighten awareness on the management of bacterial wilt and on-farm seed production.

Assessment on the potential for SSPS in RSA

A 3 week survey of the potato growing regions of RSA KwaZulu-Natal and Eastern Province was undertaken from 16th Aug – 3rd Sept 1999. The primary objective was to establish links with sections of the ARC and NPPS, and NGOs with an interest in potato production by smallholder enterprises, and to assess demand for SSPS technologies.

4.5. MISCELLANEOUS ACTIVITIES

Assessment of bacterial wilt distribution in Kenya

This research aimed to establish the incidence and regional distribution of bacterial wilt within Kenya.

Survey outline: Ten farms from each potato growing region within Meru, Molo, and Njabini were selected at random. In each farm from a current potato stand a 10 x 10 m plot was selected at random. At mid-season the incidence of bacterial wilt was recorded within the 10 m^2 plot. Baseline data was also acquired on general farmer practices and farmer perceived constraints to potato production. The survey aimed to run over 3 years.

Screening for potato varieties for bacterial wilt tolerance

A range of traditional, NPRC recommended and breeding line varieties were obtained from NPRC. These seed were planted as a randomised complete block experiment at NARL on bacterial wilt infected land: a bacterial wilt infected field trial site was specifically prepared for this purpose. Assessments were conducted over 3 seasons. The assessment aimed to monitor field and latent infection levels of bacterial wilt amongst the varieties. This data would be complementary to the breeding programme of the NPRC which currently does not specifically evaluate bacterial wilt tolerance.

Solarisation of soil for control of bacterial wilt

These assessments were also conducted at NARL on the bacterial wilt infected field trial site. The assessment looked at incidence of bacterial wilt with and without solarisation. The assessment aimed to identify an intensive strategy for the control of bacterial wilt that could be applied to the SSPS setaside.

4.6. END OF PROJECT REVIEW

An 'End of project review' was held in Kenya 4th – 6th April 2000 at KARI NARL. Regional stakeholders were invited and asked to present their current research on potato production focusing on Kenya, Uganda and RSA. From this overview the demand and appropriateness of the SSPS and BCA technologies to smallholders was assessed and reported on by 3 DFID appointed reviewers.

5. RESEARCH OUTPUTS

5.1. BIOLOGICAL CONTROL AGENT (BCA) EFFICACY AND FORMULATION

5.1.1. EFFICACY OF BCA

BCA efficacy assessment undertaken at CABI UK Centre [Egham]

Disease progress curves are presented in Graph 1a, with a summary of data in Appendix C-i and statistical analysis in Appendix B.

Analysis of the data at mid- (56 days) and end-season (84 days) recorded significant differences between BCA +WT and WT treatments ($P<0.001$). No significant difference was apparent between these treatments at disease onset (21 days). It was anticipated that incidence of disease within a pot amongst the 3 plants was not neutral and this was allowed for in the analysis by using an estimated dispersion value.

BCA efficacy assessment undertaken at ARC VOPI

Disease progress curves are presented in Graph 1b, with a summary of data in Appendix C-ii and statistical analysis in Appendix B.

As observed in the assessments at CABI Bioscience UK Centre [Egham], application of the BCA (Ω5.1) resulted in the significant reductions ($P<0.001$) in the level of disease at mid- (56 days) and end-season (77 days). The *Pseudomonas resinovorans* BCA under assessment at ARC VOPI (BCA ARC) also recorded similar levels of protection, and no significant differences between the 2 BCAs were observed. Early observations on disease onset were not recorded.

Summary: These data support that the BCA Ω5.1 significantly reduced the incidence of disease by reducing the rate and number of plants infected, although the onset of disease appeared to be unaffected. How this disease reduction relates to yield was not addressed as within a pot experiment tuber yields are highly suppressed. It is also worth noting that under the contained-use conditions used incidence of disease was very high, approaching 100%. This would represent an extreme case under field conditions. An assessment to look at efficacy of the BCA in soils harbouring naturalised populations of bacterial wilt within pots was undertaken at ARC VOPI, however, loss of soil structure repeatedly resulted in poor tuber germination and plant health that nullified the assessment.

The data strongly points to the need to transfer the assessments to a field trial stage testing a) BCA efficacy against a background of naturalised *R. solanacearum* and other soil microbiota; and 2) the relationships between field incidence to total yield and latent tuber infection. Future research should also continue to include comparison with the *Pseudomonas resinovorans* BCA under investigation at ARC VOPI. Considering the potential legislative and consumer debate on implementing GMO technologies such as the Omega 5.1 mutant, the possible benefits of using a non-GMO BCA are significant.

5.1.2. PATHOGENICITY OF *R. SOLANACEARUM* BIOVAR 2A ISOLATES REPRESENTATIVE OF PERU, COLOMBIA AND OTHER COUNTRIES ON DIVERSE POTATO VARIETIES, AND IMPACT ON BCA EFFICACY

Pathogenicity assessment on *R. solanacearum* biovar 2a isolates representative of Peru, Colombia and other countries on *S. tuberosum* and *S. andigena* varieties

Analysis of these data did not reveal consistent significant host/isolate interactions and standard deviations were occasionally high. This was due to the use of tissue culture plants that tended to root weakly and the need for high treatment combinations that reduced scope for adequate replication. A more robust analysis of the data was apparent by combining data sets across potato varieties and isolates i.e. *S. tuberosum* vars against isolates from Peru, Colombian and other countries. Here again differences were not significant (tested at 19, 21, 23 and 25 days post inoculation). These data are presented in Graph 2, with a summary of the data in Appendix C-iii and statistical analysis in Appendix B. No value is placed on the statistically significant variety and isolate differences.

BCA efficacy assessment against *R. solanacearum* biovar 2a isolates representative of Peru and other countries (except Colombia)

The use of tissue culture plants and need for high treatment combinations again impacted on the robustness of the data sets obtained for the reasons stated above. Nevertheless, analysis of these data at mid (14 days) and end-season (25 days) revealed significant ($P<0.001$) reductions in disease due to the BCA application and no interaction between BCA and *R. solanacearum* isolate type. These data are presented in Graph 3, with a summary of the data in Appendix C-iv and statistical analysis in Appendix B.

Summary: These assessments explored possible interactions between potato spp., *R. solanacearum* isolate of world-wide origins and efficacy of the BCA. The finding of no significant interaction between these factors infers that the efficacy of the BCA is not obviously constrained to variety/country locations and therefore should have broad application in potato regions affected by *R. solanacerum* biovar 2a. This is a very significant finding.

5.1.3. CARRIER MATRIX FORMULATION OF BCA

Half-life of BCA in various matrix carriers

Enumeration of the BCA from the 4 matrixes was achieved for t = 0, 1, 2 and 5 weeks. These data are presented in Graph 4a. A full data set was not made available and therefore estimates on variability are not presented.

From these data it is indicated that the perlite-based matrix is the most suitable carrier in terms of survival and longevity of the BCA. Ideally the assessment should be repeated over a longer timeframe.

Half-life of BCA on potato surface

Enumeration of the BCA using 2 perlite-based application methods and a water application was achieved for t = 0, 3, 5, 8 and 13 days. These data are presented in Graph 4b. A full data set was not made available and therefore estimates on variability are not presented.

From these data it is indicated that dusting the perlite-based matrix onto wetted tuber surfaces is the optimal way of applying the BCA.

Summary: This research identified a near-to-the-market perlite-based matrix as a suitable carrier for the BCA. The matrix is in development by ARC for rhizobial inoculum products and potentially presents an option for the commercial production of the BCA.

5.2. EPIDEMIOLOGY OF BCA AND *R. SOLANACEARUM* WT POPULATIONS IN SOIL, AND INTERACTION WITH POTATO AND ROTATION CROPS

Survival of BCA and *R. solanacearum* WT populations in soil, and interaction with potato

The control assessment (T1) recorded no indigenous bacterial isolates with natural resistance to kanamycin or rifampacin, whereas numerous isolations were evident on T2 – T4. Confirmation of the specificity of the selective media to *Ralstonia solanacearum* WT and BCA was undertaken by the positive identification of a random sample of 50 isolates taken across T2 – T4 using GC fatty acid analysis and the MIDI identification system. Using this approach a minimum detection level of approx. 10^3 cfu cm^{-3} of soil was achieved.

The results of the enumeration assessments are summarized in Graph 5 with a summary of the data presented in Appendix C-v and statistical analysis in Appendix B. From these data, there is a significant trend that the pathogenic WT population was maintained in soils through infecting potato, a susceptible host, and declined under fallow conditions, whereas the non-pathogenic BCA population declined equally in the presence or absence of potato. These observations were supported by ANOVAR analysis of the WT and BCA populations at days 56 and 113 (Treatment effect = $P<0.05$ for WT and $P>0.05$ for BCA). ANOVAR analysis also revealed no significant difference in the BCA and WT populations densities between T2 and T4, and T3 and T4, respectively, suggesting that there was no interaction between these populations.

No bacterial isolates were obtained on SMSA containing kanamycin and rifampacin under T4 as may arise through recombination of WT and BCA populations (genetic transfer events).

Interaction of BCA and *R. solanacearum* WT with rotation crops

The data presented compares the mean value of BCA and WT bacterial populations associated with rhizosphere soil of 5 plants taken from a single pot over three sampling dates. These 5 plants represent pseudo-replicates as variation between pot is not allowed for and thus a full statistical analysis was not undertaken. The sampling dates aimed to assess changes over a growing season.

GC fatty acid analysis using the MIDI identification system on 50 randomly selected colonies isolated on SMSA supplemented with either kanamycin or rifampacin, confirmed that the selective media were specific to the target bacterium. Enumeration data is presented in Graph 6a & b, with a summary of the data presented in Appendix C-vi.

Observation on these data clearly indicated potato and tomato as hosts of *R. solanacearum*. In this context, reference to the BCA population acted as a good internal control, with ratio index values (BCA/WT populations) obtained for tomato and potato substantially greater than the soil (fallow) control (see Graph 6b) notably at mid-season. The ratio index for pea also exceeded that of the fallow control at 9 weeks, whereas no interaction was evident with maize. It was thought possible that under the pot conditions of this experiment *R. solanacearum* biovar 2a was weakly pathogenic to pea.

Analysis of the BCA/WT ratio index alone does not give a measure of total populations. From the total cfu g^{-1} data it is apparent that the pathogenic interaction with potato and tomato resulted in a 10 - 100 fold increase in *R. solanacearum* cells when compared to fallow and that non-*solanaceous* crops also supported the WT population (see Graph 6a). This observation was mirrored with the BCA populations, inclusive of *solanaceous* crops.

Summary: These assessments showed that the BCA population did not interact with *solanaceous* crops to the same extent as the WT population, yet it appears that all the crops assessed stimulated both BCA and WT populations to levels above that supported under fallow. From this result it can be inferred that BCA populations will decline more rapidly than WT populations under cropping systems with a *solanaceous* component, and that a fallow break represents the most effective way to reduce both BCA and WT populations.

The assessment to observe genetic transfer of the omega cassette to the pathogenic population did not recover any recombinant bacterial isolates. This would indicate that either transfer of the omega cassette did not occur or that the event occurred at a frequency that was too low to detect using the methods described here. It is estimated that the method employed tested per agar plate approx. 10^4 BCA and WT cfu (at t = 0) and 0.00002% of the total volume of soil in a microcosm.

There is a strong need to validate these results under field conditions and to extend the range of rotation crops assessed.

5.3. Assessment of uptake needs for BCA in Kenya, Republic of South Africa (RSA) and Pakistan

5.3.1. Socio economic review on the need and implementation of the BCA

The summary and recommendations from this report are abstracted below. The full report is presented in the Appendix A-v.

1. SUMMARY AND RECOMMENDED ACTION

1. Potatoes are an important food and cash crop in Kenya. The area cultivated during 1995 was estimated to be 96,000 hectares, nearly 4 times the area cultivated in 1965 (27,000 ha). Yields are low at between 7-10 tonnes/ha and increases in output are the result of an expansion in area rather than in yields per unit area. Production is confined to the highland areas of Central, Eastern and Rift Valley Provinces.

2. Potatoes are grown by smallholders, both men and women, and often play a dual role as cash and subsistence crops. Subdivision of holdings with each generation is reported to be creating difficulties for farmers as it becomes impossible to rotate potato crops while continuing to meet the subsistence and cash needs of their families.

3. Marketing presents few problems for cultivators, particularly in the major producing areas, although farmers have little market information or control over the price they receive for their produce. Seasonal price variations can be large (KSh400 to KSh1,000 per 120kg bag), although few farmers have access to, or knowledge of, storage technology to take advantage of these differences.

4. Most farmers appear to attempt some form of rotation, although where 75% of the farm is planted to potatoes at each season (e.g. Meru District), a break of sufficient length to manage pests and diseases must be impossible to achieve. As a result yields are low, despite the use of fertilisers and fungicides. One cause of low yields is the presence of bacterial wilt. All farmers questioned during this brief survey reported having wilt on their farms. The extent and economic consequences of the disease are impossible to quantify on a local or national scale. However, farmer cultural practices are certain to contribute to a spread of the disease in the future. For example, volunteer plants are rarely rogued and disposal of infected plants and tubers is inadequate, many are simply thrown on the headland and must be a potential source of future infection.

5. An analysis of available data suggests that potatoes are potentially one of the most profitable annual crops grown by farmers in the highlands of Kenya. Despite the potential profitability credit has not been made available for potato cultivation partly because of the risk of crop failure as a consequence of disease attacks. Late blight can be effectively controlled with fungicides but there is no control available for bacterial wilt.

6. One of the major constraints farmers face is a shortage of certified or clean seed for potato production. Certified seed is available only in small quantities and farmers rely on home saved or neighbours' tubers for planting material. Farmers reported that they only select tubers from disease-free fields, or seek seed from neighbours that they have observed 'growing well' in the field. Those farmers forced to sell their entire crop at harvest to meet their cash needs, often have to rely on the market for seed for the next season. They often have no assurance that the seed is not infected, other than by physical examination, and are at risk of introducing bacterial wilt and other diseases to their farms.

7. The size of the tuber used for seed is also of concern. These tubers are often very small and farmers may be selecting for disease or for poor genetic characteristics.

8. Having established that a shortage of clean seed is a major constraint to potato cultivation and that bacterial wilt is a growing problem for farmers, it is safe to conclude that a biological control for bacterial wilt would play an important role in reducing the incidence of the disease, thereby leading to increased yields and assisting in the production of clean seed. Assuming the biocontrol agent was available in the form of a seed dressing it could be used on all seed at planting time. Alternatively, depending upon the price of the biocontrol preparation it could be targeted at:

* those plots being bulked up for seed by commercial seed producers and farmers; and
* those parts of the farm known to have soil borne *Ralstonia solanacearum*.

Much will depend upon the price of the biocontrol, the labour costs associated with its application (likely to be minimal if in the form of a powder) and the incidence of bacterial wilt and its effect on yield.

9. With regard to the potential value of a biocontrol agent for bacterial wilt the project should:

* proceed as rapidly as is practical to testing of the agent on farmers fields;
* seek to coordinate activities with the African Highlands Initiative (AHI), which has an interest in potato cultivation and IPM, and is shortly to begin diagnostic activities in Embu District (see Appendix 3);
* seek to collaborate with KARI and AHI on surveys, over three years to identify the incidence of bacterial wilt and the economic consequences of the disease in the major producing Districts in Kenya (AHI to provide data on the regional implications). The survey should seek to establish:

  - regional and seasonal differences in the occurrence of the disease;
  - the effects of altitude on disease outbreaks; and
  - farmer's strategies for managing bacterial wilt (IPM) and their efficacy.

10. Monitoring of 10 farmer's fields over a three year (6 seasons) period in all the major potato growing districts will provide evidence of the geographical spread of the disease in Kenya, its economic importance and those areas which should be targeted for assistance with information on IPM, biocontrol and seed production strategies.

11. AHI have agreed in principal to the drafting of a formal collaboration between their project and this project as they plan their second phase early in 1997. This provides Project ZA00885 with a opportunity to extend outputs beyond Kenya to the East African Region. The AHI has already undertaken survey and experimental work in Uganda. Ethiopia and Tanzania are also reported to suffer from bacterial wilt in potatoes (Lemaga, 1996) (personal communication Nancy Kaaya, HRTI, Tengeru, Arusha, Tanzania).

12. It proved premature to attempt to investigate the likely future demand for a biocontrol agent as the prevalence and geographical distribution of the disease has not been firmly established. When more data is available and a product is available for field testing it will be appropriate to undertake further work to establish the social and economic constraints to the adoption of the technology, the training needs of extension workers and farmers and the likely impact on the yields and incomes of potato producers.

5.3.2. RESEARCH WITH GMOS IN KENYA, RSA AND PAKISTAN

Kenya: The submission of an application to allow testing of the BCA in Kenya has taken longer than expected due to the need to implement the appropriate assessing/evaluating committees and processes in Kenya. These delays have prevented the intended in-country assessment of the BCA in Kenya over the duration of the project. The current status is that an application to allow contained-use testing of the BCA in Kenya has been submitted by KARI to the National Biosafety Committee (NBC), a body within the National Council of Science and Technology (NCST). At the request of the NBC the same application has also been submitted to the KARI Biotechnology Committee (KARI BC) for internal assessment. In April 1999 the KARI BC recommended approval of the application to the NBC. The NBC has subsequently reviewed the application and requested minor clarification on a few points only. These are in the process of being addressed, but are not expected to represent a significant delay in attaining NBC approval. Evidence that the NBC is now effectively processing applications can be shown by the recent approval of research in Kenya on GMO sweetpotato.

The application submitted to the NCB and KARI BC is presented in the Appendix A-vi.

Activities of the project have aimed to anticipate the introduction of the BCA into Kenya for testing. Three study attachments by KARI counterparts to strengthen the capability of KARI to undertake research on the BCA and to work to GMO contained use working practices have taken place.

1) Ms Miriam Otipa spent a 2 week attachment to CABI Bioscience UK Centre [Egham] (Jan 1997). Instruction was given in general bacteriology, species identification and GMO quarantine procedures. It had been the intention that Ms Otipa participate in a 2 year MSc programme[5], however, obligations beyond the scope of the project prevented her from fulfilling this role.

2) Mr Kinyua Murimi spent a 3 month attachment to CABI Bioscience UK Centre [Egham] (May – July 1997[6]). Extended instruction was given in general bacteriology and species identification. In addition, Mr Murimi initiated, monitored and recorded all aspects of a BCA assessment and assisted in the development of methods to assess longevity and impact of the BCA in a soil environment. His report is presented in Appendix A-vii.

3) Mr Kinyua Murimi spent a 1 week attachment at ARC VOPI (Oct 1999) for induction in the GMO contained-use working protocols operational at the institute.

Culminating from these attachments, pathogenicity protocols and working protocols have been transferred to KARI NARL, facilitating an eventual BCA testing programme in Kenya.

Pakistan: At the time of implementing this phase of the project (1998), Pakistan did not have a well advance national GMO policy. This had been shown by experiences in Kenya to be critical for implementing GMO assessments in-country and thus based on this knowledge it was agreed not to pursue the development of a BCA strategy for bacterial wilt control in Pakistan.

Republic of South Africa: Delays in implementing the BCA technology in Kenya led to discussion being held with ARC VOPI. From these discussion it was evident that RSA had a highly efficient GMO biosafety policy and that the assessment of the BCA in RSA represented an expeditious option for promoting the efficacy and wider acceptance of the BCA technology. Accordingly, an application for contained-use assessment of the BCA was submitted by ARC VOPI in early 1998 to SAGENE. Approval was obtained in July 1998.

The contents of the application for testing the BCA submitted to the SAGENE was analogous to that submitted to NBC in Kenya (see Appendix A-vi).

Summary: This activity has constituted a substantial undertaking, involving close collaboration with, and monitoring of, national biosafety activities. The drive of KARI to progress biotechnology in Kenya has been central to formulating and implementing the application processes and must be commended. The KARI BC and NBC have acted throughout with well measured caution, and whilst these procedures have taken more time than anticipated and led to a delay in project outputs, the due processes have been necessary. In this context, the BCA has played a much wider role in terms of demonstrated demand for an operational national biosfety procedure. From between 1995 – 2000 it was one of only 2 such applications submitted in Kenya. Additional applications are now being presented to the NBC, to date exclusively by KARI.

[5] The MSc programme was to receive supplementary funding under DFID NARP II
[6] Additional funding was provided under DFID NARP II to allow for an extended attachment

5.4. SEED PRODUCTION – IMPROVING SEED MANAGEMENT AND ESTABLISHING A 'WINDOW' FOR THE APPLICATION OF THE BCA

5.4.1. SEED SIZE AND WARE YIELD

ASSESSMENT AT KARI NARL:

These data are presented in Graph 7a, with data summarised in Appendix C-vii and statistical analysis in Appendix B. Analysis of these data showed a significant positive effect between seed size and yield, with the smaller seed size category (<25 mm) significantly ($P<0.001$) yielding less than the recommended seed size category for planting (35-45 mm). No difference between the seed sources was apparent.

ASSESSMENT AT NJABINI:

These data are presented in Graph 7b, with data summarised in Appendix C-vii and statistical analysis in Appendix B. As with the data obtained at NARL, statistical analysis showed a significant positive effect between seed size and yield, with the smaller seed size category (<25-35 mm) significantly ($P<0.001$) yielding less than the other two larger seed size categories. The treatment of eye rubbing was also shown to negatively affecting yield ($P<0.05$).

Summary: This research confirmed that the farmer practice of planting small seed (<25-35 mm) contributed significantly to reduced yields. Data on eye number, shoot number, emergence and plant vigour (days till canopy closure) were not taken so it is not possible to apportion the basis for this. Previous studies would suggest that resilience to environmental factors, especially drought, conferred by the larger reserves held by large seed tubers is critical (Steyn, 1997). This clearly demonstrates the potential negative consequences of planting small seed on yield. These data also suggest that the distribution of tuber size classes at harvest was also affected by the size of the seed planted, however, no convincing trend is apparent across varieties and the 2 similar trials (see Graph 7c & d).

5.4.2. SMALL-SCALE (ON-FARM) SEED PRODUCTION SYSTEM (SSPS)

On-farm SSPS trials at Njabini

Management of field trial: No significant difficulties were experienced with the field trial evaluation of Tigoni on any of the 6 farms with a full set of treatments maintained[7]. However, for Rosilin Tana, only 4 of the 6 farms have maintained successfully the full trial[8]. Six cycles of production have been completed. Additional detail is presented in Appendix A-ii.

Harvest data: A summary of these data from the trials is presented in Appendix C-viii, with statistical analysis in Appendix B. Statistical analysis and graphic presentations are based on data from Phases 3 – 6, unless stated otherwise, as at this stage the trial realised its full scale of operation.

Key parameters include:

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[7] SSPS ware was not recorded in Phase 3 for Tigoni due to loss of seed to cows, but this did not impact on subsequent phases.

[8] Farmer intervention and loss of seed to cows ended full comparisons on Farms 1 and 5, respectively for Roslin Tana.

Seed production: Assessment of the impact of SSPS seed cultivation on seed production, seed size distributions and average seed size are made on SSPS seed and SSPS ware data sets as these systems share a common source of seed that allow direct comparisons to be made.

Analysis of total tuber numbers under SSPS seed and SSPS ware recorded significant differences between System (P<0.001), Phases (= seasonal factors; P<0.01) and Replicates (= Farms; P<0.001). Varietal differences and interactions with Phases were not apparent. In terms of the total variability of the data System, Phase and Farm accounted for 27.8%, 0.5% and 4.6%, respectively. This indicates that the major impact on tuber production was the System. The impact of the SSPS seed cultivation would appear to operate through the suppression of large tubers, skewing tuber production towards tubers of a seed size (Graph 8a; Cl.Syst interaction P<0.001; 14.1% of data variation). This was also measured by average tuber weight that decreased by approx. 30% for both varieties (System P<0.001) under SSPS seed cultivation. Additional interactions were evident between Class and Variety, System, Phase, Variety.System and System.Phase. Cl.Var and Cl.Var.Syst interactions are indicative of the distinct Class distribution profile of tubers of Tigoni and Roslin Tana as defined by size, whereas interactions with Phase indicate seasonal effects on the distribution of tuber sizes at harvest.

Seed production was measured per tuber planted (Seed Index) and per unit area of land (Land Index) (see Graph 8b & c). From these data it is clear that the SSPS seed cultivation significantly (System P<0.001 and 82% of the total variation) increased the Land Index, but at the expense of the Seed Index. A further facet of the data was that variability between farms was more pronounced in the SPSS ware system than the SSPS seed cultivation (see Graph 8d). Analysis of Land and Seed Indexes under SSPS ware, Ware-to-ware and NPRC showed no significant difference (Statistical analysis not presented).

Effects of management can be eliminated for the SSPS seed cultivations as these were undertaken by the NPRC to ensure conformity; and no apparent differences in ware management practices were evident between the farmers.

Ware Production: The SSPS model predicts no significant differences between the systems in the early Phases, but a general trend towards greater productivity from the SSPS ware cultivations over time, reflecting the maintained health of the SSPS seed compared to the Ware-to-ware seed. Statistical evidence in support of the hypothesis would be seen in a significant interaction between System and Phase. NPRC seed represents the external positive control and was expected to give the optimal yield at each Phase.

Comparisons of ware production from the 3 seed sources (SSPS ware, Ware-to-ware, NPRC ware) are presented in Graph 8e, f and g. An overview of the data supports the hypothesis in part as SSPS ware out-yielded Ware-to-ware for both varieties in Phases 5 (P<0.05) and 6, and with higher average yield over the 4 seasons. These trends were not shown to be statistically significant, but were close to the 0.05 probability level. Deviating from the model was the yield of the NPRC cultivation that was below that expected and highly erratic: this was attributed to differences in the sprouting condition at planting of the NPRC seed compared to on-farm seed which was not always optimal for the growing season. Statistical analysis also revealed significant differences (P<0.001) between Farm, Phase and Variety. In accounting for total variability in the data Farm, Variety, System and Phase explained 57.3%, 8.8%, 0.7% and 41.6%, respectively (ADDS to more than 100%). This is in strong contrast to the Land Index data where the System was the major factor. The variation between Farms in ware yield is shown in Graph 8g.

Projection of seed input: Projected inputs based on averaged production values for land and seed required to produce sufficient seed to maintain a 1 ha closed system[9] ware cultivation are presented in Table 1. Ware projections are based on the Ware-to-ware data and assume the retention of seed between 25 – 55 mm. Areas for SSPS seed cultivation do not include SSPS setaside.

Land and seed inputs under SSPS seed and Ware-to-ware systems are calculated as below:

SSPS seed cultivation – land input (Y)

$$Y = A*\rho^1/\rho^2(SI^1-1)+2\rho^1$$

SSPS seed cultivation – seed input

$$= Y*\rho^2$$

Ware-to-ware cultivation – land input (Z)

$$Z = A*\rho^1/SI^2$$

Ware-to-ware cultivation – seed input

$$= Z*\rho^1$$

Where A = Field size to be maintained
ρ^1 = Ware cultivation density
ρ^2 = SSPS seed cultivation density
SI^1 = Seed index per tuber planted under SSPS seed cultivation
SI^2 = Seed index per tuber planted under Ware-to-ware cultivation

Agronomic constraints: No significant varietal interactions were recorded between SSPS seed cultivation and Ware-to-ware cultivation, with both varieties showing a similar increase in the total number of seed and decrease in the average tuber weight under the SSPS seed spacing. Significant varietal differences were apparent with regards to yield, with Tigoni being the more productive variety (see Graph 8e and f).

Pest and disease constraints: The incidence of pre- and post harvest diseases and pests recorded over the duration of the trial has been low (see Appendix A-iii for summary). Bacterial wilt has only been observed in Farm 1 and mainly in association with the Tigoni stands. Development of bacterial wilt on Farm 1 over Phases has been recorded in all Tigoni and R. Tana seed lots by ELISA. These data are summarised in Appendix B-ix and presented in Table 2 for the SSPS seed, SSPS ware and Ware-to-ware cultivations of Tigoni.

Man hour inputs to planting SSPS seed and ware cultivations: During planting of Phase 3 an assessment on the investment in human time to plant the 2 systems was undertaken. It was observed that the SSPS seed and ware cultivations for each field site took on average 2 hours to plant, with an equal division of labour. This equates to a rate

[9] Closed system indicates that seed production = seed requirements for next seasons planting.

of 3.7 and 3.9 tubers planted per minute, respectively and demonstrates that the human input to planting is roughly equal. With improved familiarity in the SSPS system, planting efficacy may well favour the SSPS seed cultivation as in this analysis considerable time was invested in the preparation of the land for the SSPS seed cultivation which should be unnecessary when the SSPS setaside cultivation is managed correctly.

Management of SSPS setaside: The value of SSPS setaside as a rotation practice has not been acknowledged by farmers and researchers alike, and has been poorly maintained throughout the trials with the exception of Farm 6. In general this land has been planted again to potato (farmer seed), or when left fallow, managed without effective roguing of volunteer potato. Consequently it has been necessary on most occasion to source new land for the SSPS seed cultivation. This represents a significant departure from the SSPS model.

Summary

The data demonstrates the high productivity of the SSPS seed cultivation for seed production per unit area of land (Land Index) at the premium of increased seed usage (Seed Index). These data when extrapolated to estimate land and seed inputs to maintain a 1ha field cultivation and when allowing for SSPS setaside indicate that the SSPS seed cultivation has an approximately equal land requirement to the Ware-to ware cultivation and a 2-3 times higher seed input (Table 1). Thus these data need to be viewed in the context of prioritised needs on land and certified seed availability. The socio-report of Barton et al., 1997 established that both of these parameters were limiting; certified seed was insufficient to meet demand; land availability was insufficient to allow effective crop rotation.

This represents a very empirical analysis based on seed selection within the size range 25-55 mm and does not give weight to studies on farmer practices that have consistently shown that farmers give insufficient attention to the need for optimal seed selection and crop rotation under a ware-to-ware system. The merit of optimal seed selection has been shown in section 5.4.1., and the comparisons on ware production under SSPS and Ware-to-ware systems in this trial over 4 seasons appear to be mirroring these results. Thus value has to be placed on the improved concept of seed production that the SSPS system promotes. In this context the low disease pressure experienced by the trial was, from an experimental point of view, disappointing as under a greater disease pressure the merits of the SSPS system would have been expected to be more evident.

From the statistical analyses of these data it was evident that Farm and Phase contributed significantly to the variability of the data, reflecting abiotic soil and seasonal factors, respectively. This was particularly true in the case of ware yields (Farm = 57.3%; Phase = 41.6% of data variability (Ware analysis)), whereas SSPS seed cultivations appeared less variable, presumably because the high density planting (System) imposed an additional yield limiting factor that overrode other factors (System = 82% of data variability (Land Index analysis)). An analysis of the soils of the 6 farms was undertaken that confirmed that the farms of highest yield (Farms 5 & 6) were the most fertile and of the most suitable pH, whereas the lowest yielding farms were characterised by poor fertility and excessive acidity. Soil analysis data are presented in Appendix B-x.

As noted above the disease and pest status across all Systems has been generally low with only Farm 1 experiencing any notable diseases. The detailed study on bacterial wilt infection levels on Farm 1 provided some indication that latent and field infestation levels can be correlated, however, the incidence of bacterial wilt was too low to draw robust inferences on the relative bacterial wilt management aspects of the potato crop systems.

SSPS setaside has been poorly understood by the farmers and national researchers Greater emphasis need to be instilled as to the importance of the SSPS setaside which is a unique and critical component of the SSPS system.

A final consideration is how representative is Njabini potato production of other potato regions of Kenya, sub-Sahara Africa and world-wide. In the context of Kenya, other potato regions tend to experience greater pressures on land utilisation and thus more pronounced pest and disease constraints. It is therefore vital that the SSPS system is validated under other agroclimatic zones. This is, in part, being addressed under a PRAPACE funded initiative in the Meru area of Kenya and a DFID funded initiative in Bolivia.

5.4.3 OPTIMISATION OF SSPS SEED CULTIVATION SPACING AND INTERACTION BETWEEN VARIETIES

These data are presented in Graph 9a, b & c, with a summary of the data presented in Appendix C-xi and statistical analysis in Appendix B. In the statistical analysis the data on mini tubers was excluded.

From this single experiment it was apparent that major difference in seed productivity per unit area of land (Land Index) were apparent between the planting systems ($P<0.001$). These observations mirrored that observed in the SSPS field trials, with greater seed production achieved at 20 x 20 cm than at 30 x 75 cm spacing and with the skewing of tuber production at the high planting density towards the smaller tuber (Sp.Cl $P<0.001$). However these results also indicated that 20 x 20 cm is not an optimal spacing density for seed production, and that greater productivity can be achieved at a 30 x 30 cm spacing. The data from planting mini-tubers at 30 x 75 cm spacing strongly suggests that for seed production this is not the correct cultivation.

Summary: The superiority of the 30x30 cm spacing for seed production is a highly significant result as it indicates that greater seed production can be achieved per unit area of land at a reduced investment in seed input than currently demonstrated. Using these data land and seed inputs at a planting density of 20 x 20 cm, 30 x 30 cm and 30 x 75 cm are estimated in Table 3 for Tigoni. By comparison of these estimated land and seed input values with the analogous data sets presented in Table 1 it is evident that the values at 20 x 20 and 30 x 75 are of a similar order of magnitude. This gives a degree of confidence that the optimisation experiment undertaken on-station at NPRC has yielded meaningful data. Accordingly, the impact of the 30 x 30 cm spacing on land utilisation and seed inputs is substantial and strongly suggests marked benefits over other spacing/cultivation systems in terms of both land and seed inputs. Additional on-farm assessments need to be undertaken to substantiate these very promising preliminary data.

5.4.4. PROMOTION AND UPTAKE OF SSPS IN KENYA AND RSA.

Farmer field day – Acquiring baseline data and assessing farmer views on SSPS

The Farmer Field Day was well attended with a registration of 72 persons, comprising 35 regional farmers and representatives from KARI (Embu, NARL and NPRC), MALD&M, KIOF and the Farmer Field Centre (S. Kinangop). Visual estimates on counts of farmers present exceeded this number indicating that not all farmers attending registered.

Merit of questionnaire: The questionnaire comprised of 2 sections: Section 1 aimed to attain baseline data on the cropping systems of S. Kinangop; and Section 2 gauged farmer opinion of the SSPS. Section 1 was completed before induction in the SSPS.

The attendant farmers were divided into 4 groups comprising 15-20 persons, and an elected extensionist was given responsibility for facilitating the form-filling process. Section 2 was completed as a single group, co-ordinated through discussion led by Mr Wachira and Mr Murimi. A total of 23 questionnaires were returned that exhibited varying degrees of completion. Aspects of these data are summarised in Table 4a and b and Appendix C-xii.

The salient points from the baseline questionnaire would appear that farming is the primary source of livelihood for these families and that potato is the major crop. Inputs to farmer systems (agrochemical, quality seed etc.) are low, and this was reflected in poor yields. Effective crop rotation practices were also not evident due to the prominence placed on potato. Disease and pests were commonly quoted as a major constraint, notably cutworm, aphids and bacterial wilt, but of greater concern were constraints on marketing. Access to markets, the role of commercial middlemen and market prices were the main points mentioned. Road links between Njabini and the main Nairobi – Kisumo highway are currently being improved and this is likely to have a significant impact on Njabini.

Feedback from the farmers during the Farmer Field Day was generally positive. It was apparent that the farmers had gained a good awareness of the SSPS and its relative merits. Of the disadvantages of the SSPS cited, the main theme related to time/labour inputs that were perceived as greater and more appropriate for the male member of the household. These opinions had also been voiced by the field trial workers, although field observation on time inputs for planting did not support this view. The increased physical input needed in preparation of the holes for the SSPS seed cultivation was apparent, and this was therefore generally more suited to the male. This apparent imbalance was, however, countered by the activity of seed planting in the SSPS seed cultivation holes that was more suited to the female.

Promotional material tested

Promotional materials developed in support of the SSPS and its aim to alleviate bacterial wilt are presented in Plates 4, 5, 7 and 8. The aids to seed selection and planting (seed grader and dibber) were developed in tandem with working experience gained during the trials. Some of the trial farmers had further modified the design of the dibber using local materials. Approximately 150 calendars, 100 bacterial wilt bulletins, 100 seed graders and 20 dibbers were released.

These material were always readily accepted by farmers, researchers and extensionist, however, a measurable impact was difficult to gauge. Within the Njabini district it was apparent at the Farmers Field Day that the majority of farmers were unaware of the trials being undertaken. This was surprising when considering that the trials had been operational for 2 years and had a very visible presence at planting and harvest. Additional consideration needs to be given towards effective methods for the widescale promotion of the SSPS.

Assessment on the potential for SSPS in RSA

Potato cropping in RSA: Agriculture by smallholder communities in the RSA has seen a decline over past years in preference for employment within the cities and towns. This trend has been exacerbated by affirmative action policies, particularly amongst male family members. It is now a national priority to stimulate agriculture amongst rural communities. Small-scale semi-commercial enterprises are seen as the direction for agriculture building. It is recognised that a primary constraint to development relates to the fact that the vast majority of land (90%) is communally managed through tribal chiefs.

This removes an individual's incentive to be a progressive farmer. A land reform act (tenure for land reform) will to address this point in the near future, although it is recognised that the acceptance of change will be hard to achieve.

Potato production in RSA operates mainly on a large-scale commercial basis that relies on high inputs of certified seed-tubers, chemicals and irrigation. KwaZulu Natal and Eastern Province are significant seed and ware potato producing areas, respectively. Analogous to Potato Great Britain, commercial potato production operates as an industry through Potato South Africa (PSA) that is supported through a producer levy scheme. PSA manages a rigorous certification scheme for seed and funds a number of research programmes.

Certification of seed involves 3 field and 1 post-harvest inspection. Varying degrees of disease and pest tolerance are allowed, depending on the organism. For example, bacterial wilt is regarded as a zero tolerance pathogen and is screened for using ELISA technology. The monitoring for BW is very robust, and states a 99% chance of detecting 0.1% infection (1 infected tuber/1000 tubers). This is a higher standard than in Europe. The costs of these inspections are borne by the farmer and passed on to the ware producer. It is estimated that the purchase of certified seed accounts for about 25% of the total financial inputs (pers. comm. Mr. Chris Dwen, a leading certified seed producer (*Super Spud*)). Commercial outlets of certified seed appropriate for purchase by the smallholder are available, however, the cost of such seed is even greater than with bulk purchases and smallholders in remote areas often do not have access to this seed due to poor transport facilities.

Research on potato in RSA has focused on the commercial producer. Varietal improvement schemes have shown notable successes with numerous improved varieties. More recently, the ARC VOPI has been involved in extending varietal awareness to smallholders. Such research is taking into account the very different agricultural opportunities for smallholders, particularly the lack of irrigation and low soil fertility. One such scheme involves the NGO, Africa Co-operative Action Trust (ACAT), that is working in KwaZulu Natal region. It was unfortunate that an invitation to meet with this organisation was not accepted. Nevertheless, it was evident that the ARC was actively redressing any imbalance in the focus of their research between small and large-scale operator. The strengthening of linkages between the ARC, the National Department of Agricultural (NDA) and a plethora of NGOs that operate in RSA is a current priority. Three smallholder based rural development initiatives were visited:

Siyathuthuka Sobantu Agricultural Cooperative, KwaZulu-Natal: Recent smallholder initiate schemes have focused on the development of co-operatives, such as the Siyathuthuka Sobantu Agricultural Co-operative. This is an umbrella organisation encompassing 3 co-operatives on town council land about the Pietermaritzburg area that was initiated in 1998. These co-operatives are overseen by the Institute of Natural Resources (INR), an outreach institute of the University of Natal, that provides training to the farmers. These farmers are predominately women that have no background in agriculture. Each co-operative has about 10-15 members (households) and occupies about 1 ha of fenced (8 foot razor blade fencing) land. A motivation (for the town council) for the scheme was to prevent unstructured housing development along flood plain land. In this context these sites are very atypical of RSA: 1) the majority of rural areas do not have access to irrigation and 2) Town council land that is available for agriculture is not common.

Indonsa Farmers Cooperative, KwaZulu-Natal: The Indonsa Farmers Co-operative is located on tribal land within the Lasikiski area. This is rainfed land that had been made available for farming use as a co-operative through negotiations with the tribal chief.

This co-operative also benefits from linkages with INR and had been running for 3 years. Previous cropping has established potato, maize and beans as the primary crops. Water availability probably represents the main constraint on the range and productivity of crops cultivated.

Trail sites about East London, Eastern Province: Discussions were held with Mr. Stapies Stapelburg (ARC, Western Cape) with whom ARC activities with smallholders in Easter Province rest. He described a new initiative led by the NDA to encourage potato production by smallholders about the East London area. Potato was selected as a priority crop for the province, along with wheat and vegetables. The initiative aims to diffuse new potato varieties amongst smallholders and educate farmers in appropriate management methods. The ARC provides backstopping expertise. This is a 4-year project and, as in KwaZulu-Natal, has a strong commercial angle.

Role of SSPS in RSA: Some of the findings of this visit were surprising, not least the need to ‘sell’ agriculture to rural communities. In this context perhaps the main factor counting against the uptake of the SSPS falls outside what a project can address; namely, the reform of land tenure. This factor aside, the visit clearly established that potato was, or was seen as, a significant crop for smallholders, and that although certified seed was available it was prohibitively expensive and often not available locally. These factors identify with the main attributes of the SSPS as an on-farm method of maximising a scarce and expensive resource (certified seed), and would be compatible with commercial enterprises run through community driven co-operatives. Discussions held, both with the farmers and the project leaders of the co-operatives, were highly supportive of the SSPS idea, and an invitation was extended to assess the scheme at some of the sites visited. Whilst it is hard to envisage that the future for smallholder practices is within such highly secure compounds as witnessed in Siyathuthuka Sobantu Co-operative, these set-ups do provide an ideal window for the testing of the SSPS. The recent initiation of these projects is also timely. Dr John Barrett (DFID Senior Natural Resources Advisor to Southern Africa) was also highly supportive of the scheme and underlined the need for commercialisation of farming for smallholders. He requested to be kept abreast of developments and to reciprocate information on DFID’s role in agricultural development in RSA, as this becomes more clearly defined through pending RSA governmental reforms.

5.5. MISCELLANEOUS ACTIVITIES

Assessment of bacterial wilt distribution in Kenya

Constraints on time and transportation prevented this activity being accomplished. Some preliminary data was acquired in year 1 only (data not presented)

Screening for potato varieties for bacterial wilt tolerance

Constraints on water availability at KARI NARL prevented the realisation of meaningful data on all three of these assessments (data not presented).

Solarisation of soil for control of bacterial wilt

As experienced with the varietal screening constraints, water availability at KARI NARL prevented the realisation of meaningful data on this assessment (data not presented).

5.6. END OF PROJECT REVIEW

The review comprised 1 day of formal oral presentations, 1 day of discussion about specific potato production themes and a final day visiting the Njabini field trial site. The content of the review is presented in Appendix A-viii, along with short summaries on the presentations made by the invited speakers. A total of 39 participants attended (listed in Appendix A-viii).

Based on the information and views expressed from this review and independent discussion with other persons, an independent review team compiled a report on the merits of project R6629. This report in its entirety is presented below.

END OF PROJECT REVIEW

PROJECT R6629: BACTERIAL WILT OF POTATOES

REPORT TO THE CROP PROTECTION PROGRAMME MANAGED BY NATURAL RESOURCES INTERNATIONAL

UNDER CONTRACT TO THE UK DEPARTMENT FOR INTERNATIONAL DEVELOPMENT

Malcolm Blackie, Fred Wangati, and Peter Mills

MAY, 2000

POTATO PRODUCTION IN KENYA

Production and consumption characteristics

Potatoes are a major food and cash crop in Kenya, with that country being the major producer in eastern and central Africa. The area under the crop has grown steadily since 1965 (27000 hectares) to 96000 hectares in 1995. Potato yields are typically underestimated but CIP data indicate that they vary between 5 and 30 tonnes per hectare, with the mode being in the range 8-12 tonnes. Potatoes are grown as a cash crop but home consumption in producing areas is an important contribution to local food security. The crop is mainly eaten in the urban areas and in the rural areas where the crop is grown. It is not an important food staple in the remaining rural areas.

Consumer demand in Kenya is some 23 kg/person/year, exceeded only by Madagascar at 37kg/person/year. Uganda, Rwanda, and Eritrea all consume around 16-19kg/person/year. In the towns, potatoes are purchased for home consumption and for processing. There has been a continuing growth in the demand for potatoes by processors – who include small entrepreneurs producing chips for sale in markets, at roadsides, and in small cafes. There is a small industrial processing capacity and some potential interest in frozen French fries. Crisps, like chips, are cooked and packaged by small scale businesses, although there are trends towards improved quality and a uniform product. The best potatoes and potato products are high quality but the availability of suitable raw material is variable, which acts as a disincentive to development in processing.

Most growers are smallholders living in the densely populated, intensively cultivated highland region (between 1800 and 2700 metres above sea level). Data on the characteristics of potato growers are few but the information available suggests that most producers are small farmers working farms of between 0.25 and 5 hectares. There are a few larger farms but the bulk of marketed production is by smallholders. In the potato growing areas, the bimodal rainfall seasons are of sufficient length each to allow two crops of potatoes to be grown in any year, as compared to a single crop of the alternative staple of maize.

Input use

Fungicides are used (mainly to control late blight) and there is some fertiliser use. But both inputs are used well below their optima. Seed supply is problematic. Many farmers either use as seed that proportion of the crop that is neither sold nor eaten, or buy small tubers of unknown origin in the market. Market traders often grade potatoes into small and large tubers, with the small tubers being offered as planting material. Many of the smaller tubers used for planting come from diseased plants and contribute directly to yield reductions in the subsequent crop. The availability of adequate, reliable, and attractively priced potato seed is essential to improving the productivity of Kenyan potato producers.

Many producers find themselves in a vicious cycle of poverty driven by low crop yields. Low yields can be attributed to:

- continuous cultivation and build-up of diseases,
- declining fertility,

- a shortage of clean seed and high yielding planting material.

Pests and diseases

Particularly on the smaller farms, low yields often mean that land is continually put under potatoes and adequate crop rotation is rare. This makes breaking the build up of potato pests and diseases difficult under current farmer practice. Potato diseases, particularly late blight, potato viruses and bacterial wilt (*Ralstonia solanacearum*), are a problem in many seasons and for many farmers.

Late blight, followed by bacterial wilt, are the two most important diseases of potato production in the East African Highlands. There are good sources of late blight resistance and CIP is collaborating with national programmes in east and central Africa to increase the availability of late blight resistant varieties. By contrast, breeding for bacterial wilt resistance has been unsuccessful and control relies on crop hygiene and rotation. Losses from bacterial wilt have proved difficult to determine but the pathogen is a problem of increasing seriousness to many farmers. In Ethiopia it is reported to have spread to altitudes higher than 3000 metres. In Uganda it is said to account for a reduction in total yield of 30%.

Continuous cropping of potatoes on small farms, combined with a shortage of clean seed are providing the ideal environment for the spread of the disease. In the worst case, this disease build up could threaten the sustainability of potato production in the near future. On-farm work reported by the African Highlands Initiative indicates that bacterial wilt can be eliminated from potato seed farms by a combination of clean seed, good rotation, and strict sanitation. On farm ware losses from the disease can be reduced significantly with clean seed and realistically achievable improved management practices.

Unusually, late blight and bacterial wilt occur together in Kenya; in other regions, late blight occurs in different areas from bacterial wilt. From Table 1 it can be seen that a third of the area planted is to a traditional variety of unknown origin which is vulnerable to both major diseases. An increasing number of late blight resistant materials are being steadily released by KARI through collaboration with CIP and other agencies and some new materials have been imported from Germany and Holland.

TABLE 1: MAJOR POTATO VARIETIES IN KENYA, 1999

VARIETY	PERCENT OF AREA PLANTED	DISEASE RESISTANCE/ TOLERANCE	DISEASE VULNERABILITY
Nyayo	30		Late blight, bacterial wilt
Kerr's Pink	9		Late blight, virus
Desiree	8		
Mukori	8	Late blight	
Ngure	7	Late blight	
Tigoni	3	Late blight	Bacterial wilt
Tana Kimande	3		
Others	32		
TOTAL	**100**		

Source: CIP

Marketing and pricing

Potato marketing is informal with most of the crop being sold at harvest or even before. Traders come with transport to the producing areas and buy up the crop for sale in the urban markets. Often traders will actually harvest the crop themselves. Kenya has a bimodal rainy season and potatoes can be grown in both seasons. There is sufficient spread of harvesting dates within seasons that traders can move from district to district buying up the crop as it matures. This tends to buffer the price rise after harvest in any one district and discourages farmers from storing the crop for higher prices later in the season. In addition, consumer preference is heavily biased towards freshly dug potatoes.

The main cause of price variation – which can be substantial from season to season – is due to supply fluctuations. There are two main (and often interrelated) causes for changes in supply – rainfall and disease. There is a high and unpredictable variability in rainfall amounts both within and between seasons. Poor, or poorly distributed, rainfall results in poor harvests. Unusually wet seasons provide an ideal environment particularly for late blight, again reducing yields. Improved disease management can play an important part in stabilising yields (and thus prices) and providing a conducive environment for farmers to invest in productivity raising inputs such as improved seed, fertiliser, and fungicide.

The crop is sold in bags rather than by weight. The fact that the traders harvest the crop enables them to manipulate to an extent the bag size – which, in some districts, has pushed farm gate prices (per tonne) particularly low in some seasons. The producing areas are mainly close to the capital, Nairobi, but poor roads, communications, and transport links make it difficult for growers to explore marketing options.

Constraints and opportunities

The potato industry needs:

- higher yields to improve farm incomes and to release land for crop rotation,
- wider distribution of improved varieties, and,
- an effective seed production system

Farmers in the east African highland region have shown a clear demand for clean, high quality seed of varieties which are recognised by the market and therefore easy to sell. Rates of adoption of acceptable varieties have been extremely high. Consumers tend to be quite conservative which does act as a drag somewhat on the adoption of new varieties. However, the reality is that very few farmers in Kenya have access to improved potato seed.

Potato breeding is, at present, entirely in the public sector. The Kenya Agricultural Research Institute (KARI) has its potato breeding capacity sited at the National Potato Research Centre at Tigoni. NPRC-Tigoni produces a small amount of pre-basic seed each year which is sold on to farmers for multiplication. NPRC-Tigoni has irrigation, storage and cultivation equipment but insufficient land for large scale multiplication. Sub-centres at Meru, South Kinangop, and Molo could produce more basic seed if necessary infrastructural investment was made. CIP data show that seed multiplication and certification in the public sector has been uniformly unsuccessful in subSaharan Africa. This model was followed in earlier years with seed bulking up being undertaken by the parastatal Agricultural Development Corporation (ADC). ADC largely gave up potato seed production although there are plans to revive some capacity in the future.

Estimates suggest that probably, at best, some 5% of Kenyan farmers are able to purchase clean seed in any one year. There are efforts ongoing to improve this situation. ASARECA, through its Technology Transfer Programme, is supporting a farmer based seed multiplication project. CIP, World Vision, Plan International, and KARI have a collaborative programme to multiply the varieties Tigoni and Asante. USAID has a programme to support the contracting of farmers to multiply basic seed. Infrastructural and other support for foundation, pre-basic, and basic seed production within KARI is being provided by the World Bank and USAID. The model recommended by CIP – basic seed production with the NARS linked to farmer based seed multiplication – forms the core of Kenya’s potato variety dissemination strategy. However, the reality is that, even with this strategy fully implemented, the demand for clean seed is likely to exceed supply by a substantial amount. No firm estimates of potential potato seed supply in the immediate future were available but even an optimistic estimate of some 30% of farmers being able to purchase foundation seed in any one year leaves a large part of the market unfulfilled.

This points to two key needs of Kenyan potato producers:

- Seed plot and other mechanisms to help ware producers maintain the quality of their own seed more effectively, and,
- good monitoring of bacterial wilt and viruses in seed distribution systems.

The mechanics of providing most farmers with fresh certified seed each year are problematic and almost certainly too costly in the present circumstances. Under Kenya law, the Kenya Plant Hygiene Inspection Service (KEPHIS) has the mandate (and responsibility) for certifying any plant materials sold as seed in the country. KEPHIS regulations conform to international norms under which widespread smallholder production of certified potato seed is unlikely. However these regulations do not preclude farmers from maintaining or building up their supplies of clean planting material for their own use or the local trading of potatoes between farmers as improved planting material (but not as certified seed).

EVALUATION OF TECHNICAL OUTPUTS

The project is based on the experience that, in most situations, no effective control methods exist for bacterial wilt disease of potatoes. Breeding for resistance and the use of bactericides have apparently had a modest impact on disease incidence. Field sanitation and crop rotation, as noted above, have been difficult for smallholders to practice effectively under current conditions.

Research carried out at CABI Bioscience has, however, produced non-pathogenic mutants for the race 3 of *R. solanacearum*. These mutants seem to be adapted to cooler climates and the most commonly occurring *R. solanacearum* bacteria in potato growing regions of Kenya. The selected mutants have demonstrated significant levels of protection against the onset of the disease under laboratory conditions. The objectives of the original project memorandum dated March 1996 were therefore:

- to establish the agricultural potential of biological control of bacterial wilt of potato in Kenya; and,

- to establish the feasibility of incorporating the biocontrol agent (BCA) developed at CABI Bioscience as a component of an IPM strategy affordable to the small hold farmer within peri-urban regions of Kenya.

A third objective was added late in 1997 to evaluate establishment of small on-farm seed production plots (SSPS plots) as a mechanism for the multiplication of improved planting material. This was to provide a potential entry point for BCA and other IPM technologies in potato production systems in Kenya.

Achievements in related programmes: AHI, CIP and PRAPACE

The objective of the African Highlands Initiative (AHI), a regional programme under ASARECA, is to provide mechanism for testing an integrated approach to improvement and sustainability of the natural resource base in the high potential highlands of Eastern Africa. The AHI has identified improved seed as an entry point for improved land management practices. Opportunity for collaboration in extending trials of the SSPS technology therefore exists at AHI benchmark site at Embu in Kenya and at other sites in Uganda, Tanzania and Ethiopia.

The International Potato Centre, CIP, has regional programmes working in collaboration with national agricultural research systems in East Africa. CIP also coordinates and provides technical backstopping to PRAPACE, a regional potato research network under ASARECA. In these programmes, the focus is evaluation of potato germplasm for yield, quality, resistance to diseases and adaptation to local conditions, evaluation of agronomic practices, and production of foundation (pre-basic) seed. No sources of resistance to bacterial wilt have been found and there is apparently confusion on existence of disease tolerance. Regional programmes supported by CIP and PRAPACE have therefore emphasised integrated disease management research which has shown promise to eliminate bacterial wilt disease from seed farms with a combination of clean seed, good rotation and strict sanitation. For example, trials show lowest incidence of bacterial wilt and highest yield to be associated with good soil fertility. On-farm work with AHI has also shown that farm-level losses can be reduced significantly with clean seed and better crop management

Achievements within the project

Indigenous populations of *R. solanacearum* affecting potato in Kenya were characterised leading to observation of clear isolate types. Inoculation of potato plants under controlled conditions with the non-pathogenic omega mutants derived from the isolate types resulted in 30% reduction in the number of plants which appeared to suffer a yield loss from the disease. In the course of these studies exchange visits facilitated exposure of three KARI scientists to the processes and biosafety aspects of bacterial characterisation, development of mutants, and inoculation techniques. Two KARI staff (Kinyua Murimi and Miriam Otipa) have received instruction through this project.

CIP has developed an ELISA kit for the detection of latent infection of *R. solanacearum* in tubers. This kit has been verified in Kenya by project staff and potentially could provide an additional mechanism for checking on seed quality. At this stage, however, the ELISA kit remains a research tool which could possibly be used by qualified seed inspectors. Further work would be needed to make it suitable for wider use. Work has also been undertaken on the detecting and culturing of the pathogen in selective media. The opportunities for field verification of the presence or absence of the pathogen have been significantly improved as a result of the project.

The second objective of testing the effectiveness of the BCA under Kenyan conditions could not be implemented due to delays in phytosanitary and biosafety approval to import the BCA into Kenya. The BCA was however tested in South Africa in collaboration with the Agricultural Research Council. Levels of protection near 50%have been recorded from the South African studies. These are similar to those obtained with sterile soil in the UK. These data need to be verified under proper field conditions as experience from elsewhere suggests that effective protection levels drop markedly in such situations. Testing of the BCA in indigenous soils harbouring natural populations of bacterial wilt in pots failed due to the difficulty of growing potatoes in this way. This experience provides to emphasise the need for a controlled field release of the BCA as an essential component of the testing process.

A socio-economic study on smallholder potato production in Kenya was undertaken as part of the project. This results confirmed the underlying assumptions of the initial project. Smallholders are unable to obtain certified seed, in part due to inadequate supply. This situation encouraged retention of undersized and diseased tubers as seed. It also verified bacterial wilt and potato blight as primary constraints to potato production.

The third project objective (to evaluate an on-farm small scale seed production system (SSPS)) was initiated with six smallholder farmers in a major potato growing area (Njabini) in South Kinangop. The key innovation under this system is the separation of seed from ware production on farm. Under current practice, as noted previously, seed, at best, is often reject ware tubers. Even if a farmer purchased clean seed, much of the benefit from the seed was lost after the first season. By separating out a defined seed plot and using this plot to maintain clean seed of known origin, farmers could gain greater benefit from any investment they made in improved seed.

The system requires the farmer to select a small plot in a part of the farm that has not been planted with potato for at least three seasons. Clean certified seed tubers are then planted at high density on carefully prepared narrow seedbed. The high planting density, combined with early dehaulming, is expected to induce a larger number of tubers with higher proportion of seed-size tubers. The seed plot should therefore provide enough disease free seed tubers for the farmer to plant on the ware potato fields. Farmers select from the seed plot tubers of the optimum seed size (25-55mm depending on the variety) for planting the next season. Importantly, an area of land needs to be set aside each year (and either left fallow or planted to a non-solanaceous crop) so as to provide clean land for the next season's seed plot.

On-farm trials carried out with two popular potato varieties over five seasons show a larger number of tubers and a significantly higher proportion of seed size tubers is produced in the high planting density small seed plots than at the normal planting density (planting for a sixth season is underway). The data indicate that there may be increased yields under the SSPS system but the evidence is inconclusive at present. More detailed analysis may produce clearer results but a longer period of testing is almost certainly going to be needed to verify the benefits adequately.

Funding for the SSPS trials is in place for the current rainy season. However, unless fast arrangements are made for the continuation of the work, the very real momentum that has been achieved is in danger of becoming lost. Funds for the continuation of the SSPS trials need to be in place well ahead of the next rainy season in September, 2000.

Evaluation

The application for importation of BCA into Kenya was delayed by the slow development of reviewing system and protocols. KARI management has made considerable effort to address these issues and a streamlined system is now in place. The original BCA application was the first made under Kenya's new biosafety regulations and, inevitably, became part of the learning process on both sides. The necessary procedures should be completed in the next month or so. An application to evaluate genetically modified sweet potato is currently being reviewed and proceeding smoothly through the evaluation process. Importantly, this last exercise also includes dummy field trials implemented collaboratively by KARI ahead of actual on farm trials of GMO sweet potato. These trials provide a training opportunity for all concerned as well as an additional mechanism for ensuring that all safety aspects are adequately planned for in advance.

Although the statistical significance of SSPS on farm trial results is affected by the large coefficient of variation introduced by using different farms as replicates, the trials have demonstrated the value of the small seed plot technique as a means of on-farm production of relatively clean potato seed. Farmers exposed to the system appear interested although there is little evidence that the technology is being taken up by farmers not directly working with project staff. During a farmers' open day at one of the participating farms, the farmers participating in the trials expressed their confidence on the SSPS technology, citing improved yield of ware potato and almost total elimination of bacterial wilt disease on their farms. Some of the farmers have also benefited by being able to sell surplus planting material to their neighbours at a premium price. Such farmers see potential for eventually entering the market as producers of improved planting material (and, possibly, in the longer term of certified seed) and are encouraging other farmers to try out the technique on their farms.

UPTAKE PATHWAYS

Scale seed production system

Multiplication of disease free potato planting material is a major challenge and a potential area for collaboration with CIP, PRAPACE and AHI in Kenya and other countries of the region. The project has taken steps in this direction by sponsoring a training visit on ELISA techniques for screening potato seed tubers for latent bacterial wilt infection. Eight KARI staff and 3 scientists associated with CIP programmes in Kenya and Uganda benefited from this training. Successful introduction of BCA on bacterial wilt disease and the addition of SSPS to the range of integrated disease management (IDM) technologies will provide incentive for greater collaboration with these programmes.

Francis Alacho, of Africare in Uganda, plans to introduce SSPS into Africare's ongoing seed improvement and storage programme in southwest Uganda. There is an obvious opportunity to involve the Uganda National Agricultural Research Organisation (NARO) in follow up work on SSPS with technical backup from experienced KARI and CPP researchers. The Agricultural Research Council (ARC) in South Africa, who are already collaborating in the BCA work and who have also started some SSPS activity, also provide an opportunity to test the system in a wider context. PRAPACE are testing the system in Kenya at Meru and in Uganda. This provides an opportunity for the wider evaluation of the system as part of a follow up activity.

Within Kenya, KARI has a clear interest in promoting the uptake of improved varieties and has revised its incentive system for plant breeders to encourage them to become actively involved in ensuring their varieties reach as many farmers as possible. World Bank funding is in place to support this activity and KARI management are committed to getting improved seed out to farmers. A further phase of the project will likely benefit from this revised emphasis within KARI. Discussions with senior staff at KARI indicated a clear commitment to become actively involved in the technology transfer process. There is an immediate problem of ensuring continuity for the coming season. Discussions need to be held with KARI policy makers to ensure that KARI is able to commit the necessary funds to support this work in future seasons.

Collaboration between the CPP supported researchers and KARI staff is good. Locally, there is good interaction with extension staff which provides a sound base for expanding the work on a wider scale. The farmers' open day showed a high degree of teamwork between researchers, extension workers, and farmers. There is an evident commitment by local extension staff to the work but it was not possible to verify what financial and staff resources the Ministry of Agriculture might commit to this work in the future. This needs to be established by project staff in the immediate future.

However, the results from the first four years of on-farm SSPS trial work do not show unequivocal and substantial benefits from the system. This is unsurprising. The farmers are operating at low levels of productivity and yields are constrained by the absence of inputs other than seed – almost certainly fungicides and fertiliser. Data from CIP and PRAPACE show good response of potatoes to fertiliser, even at low levels of application. An effort to introduce modest fertiliser and fungicide use, combined with SSPS could transform the economic attractiveness of the activity.

This would require building linkages with an organisation with field experience in providing inputs to smallholders in eastern Africa. The number of such agencies is limited for a variety of reasons. Some donors perceive a conflict between trading and charitable work. Concerns in the developed world regarding modern agricultural inputs makes fund raising difficult for agencies involved in promoting such inputs. Field workers with the requisite business experience are difficult to find and retain. In Uganda, Sasakawa Global 2000 has shown an interest in starting a programme with potato producers and has the requisite experience. In western Kenya, SCODP (Sustainable Community Orientated Development Programme) have been highly successful in expanding fertiliser use amongst smallholders and are looking for opportunities to develop their model with different commodities and in different regions.

BCA testing

The prospects for field testing of the BCA in Kenya and South Africa are good. The preliminary laboratory testing has been carried out and the results look promising. Facilities already exist in South Africa for field testing. In Kenya, under existing law, KEPHIS have the responsibility for approving the local laboratory testing facilities. KEPHIS has approved the National Agricultural Research Laboratory (NARL) in Nairobi for the sweet potato GMO trial work. Some refurbishment is needed for bacterial work but no major construction. A second phase of the project including the BCA activity is entirely feasible. However, it is essential from the KARI perspective that appropriate CABI scientists be actively involved in all key stages of field implementation so as to be able to provide 'hands on' training and assistance to their Kenyan collaborators. The methodology adopted by Monsanto for their sweet potato work with a two year 'dummy run' of on-farm trials ahead of the release of the materials from NARL is recommended and could easily be accommodated within a second phase.

The South African BCA does not involve a genetically modified organism and a routine application, under existing plant hygiene regulations, could be made to KEPHIS for its importation. The South Africans are willing to collaborate in making their BCA available.

RECOMMENDATIONS

The key to effective reduction of bacterial wilt constraint to potato production is an integrated disease management strategy involving good management practices (crop rotation, field sanitation and plant nutrition), and availability of disease free seed at prices the farmers can afford. While management practices can be improved through appropriate farmer training and market incentives, availability of disease free seed of the large number of varieties suited to various ecozones and end uses poses a major problem. Potato research is regularly producing new varieties and small amounts of basic seed. However, formal seed potato multiplication at a scale that could meet demand is virtually non-existent in the region. Where such seed is available, it becomes too expensive for small scale farmers due to cost of storage and transportation. Any technologies that would facilitate on-farm production of clean seed potatoes up to certification level would therefore go a long way in meeting the needs of small holder farmers in terms of increasing crop yields and profitability of potato production.

The Team therefore recommends that:

- The on-farm small seed plot system (SSPS) should be tested more rigorously and in more environments in Kenya as a potential entry point for integrated disease management and improved potato production. The collaboration with PRAPACE at its sites in Kenya and Uganda should be continued. Tests could be extended to Uganda and South Africa in collaboration with NARO and AFRICARE (Uganda) and ARC (South Africa). There is some urgency in this if the work is to be continued without a disruptive break. Funding for planting the next series of trials will be needed well in advance of the next growing season in September.

- Assuming the application for importation of BCA in Kenya will be approved shortly, the BCA already developed in UK should be tested under controlled conditions in Kenya and biosafety requirements for its use under field conditions established. Linkages with South Africa should be continued with a view to evaluating in Kenya the BCA developed in South Africa.

- The project should be split into separate but interacting components dealing with the SSPS and BCA elements. The BCA proposal can wait upon formal approval from the Kenyan authorities while the important verification of the SSPS system through KARI and collaboration with PRAPACE can continue uninterrupted.

- More training should be provided and systems established for incorporation of various types of BCA as components of integrated disease management strategies in seed potato production.

- A new DFID/CABI/KARI bacterial wilt project should be initiated as soon as possible to provide continuity in testing and validation of the SSPS technology and to provide technical backstopping for introduction and testing of the efficacy of BCAs for control of bacterial wilt.

- Dialogue started with suitable NGOs with experience in developing smallholder farm input supplies to make available on a wider scale an appropriate potato production package incorporating clean seed and modest amounts of other essential production inputs.

FUNDING

CPP should consider continuing its support for the SSPS work. KARI and CABI Bioscience should actively seek out partners for the extension of the system, combined with additional inputs as appropriate, from bilateral sources in the countries participating in the next phase of the work. The CIP technology transfer funds are a potential source of support. The Technology Transfer Project is a regional programme under ASARECA (Association for Strengthening Agriculture Research in East and Central Africa). The programme is at present funded by USAID and is hosted and coordinated by CIP at their offices in Nairobi. The project provides funding on a competitive basis on all aspects of agricultural technologies, the aim being to facilitate pilot testing of application of known technologies under local conditions. The project is included in the proposed funding of regional research under ASARECA by EU under EDF8 hopefully by late this year. KARI or any NGO or University group in the region can apply individually or as a team for funding to continue and complete the SSPS trials.

Gatsby Foundation and the Agriculture Biotechnology Support Project in the US should be approached for linked funding to support the BCA research.

OUTSIDE CONSULTATIONS AND CONTACTS

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6. CONTRIBUTION OF OUTPUTS TO PURPOSE

Contribution of outputs towards DFID's developmental goal

The research activities of this project:

1) validated the BCA developed under R5310 against bacterial wilt under CU conditions, but no field testing was undertaken;
2) provided evidence that the BCA strategy would have application outside of Kenya where potato was affected by *R. solanacearum* biovar 2a;
3) provided evidence that the BCA was environmentally benign; and
4) modelled an on-farm smallscale seed production system (SSPS) that identified a target site for the application of the BCA, maximised land utilisation for seed production, promoted crop rotation of land used for seed production and increased availability/affordability of certified seed, thus strengthening linkages between formal and informal seed lines.

Implementation of these technologies will result in:

1) reduced on-farm incidence of bacterial wilt;
2) improved access by farmers to good quality (certified) seed;
3) improved maintenance of on-farm seed health;
4) flush-out of degenerate on-farm seed;
5) increased yield and quality of ware potato yield; and
6) associated benefit to smallholders in terms of financial return, food security and alleviation of poverty.

Identified promotion pathways to target institutes and beneficiaries

The primary outputs of R6629, namely the BCA and the SSPS, are critically poised for uptake by the target institutes and beneficiaries.

BCA: KARI and ARC VOPI have expressed considerable interest in developing the BCA in-country. These organisations have led on the application processes to allow testing of the BCA in-country, and have the capability to undertake the assessments with appropriate backstopping inputs from CABI Bioscience. KARI and ARC VOPI are therefore the natural target institutes for field testing of the BCA in-country and additional impact assessments.

SSPS: KARI NARL has been the lead institute in validating the system, however, implementation of the system would more logically fall under the mandate of KARI NPRC. Interest in the system has been also expressed in RSA (ARC VOPI and INR) and Uganda (NARO and AFRICARE). In addition the SSPS links with certification of seed and hence target institutes would also include KEPHIS (Kenya) and Potato South Africa (RSA).

For both technologies promotion pathways to farmers (users) has yet to be positively defined. The role of potato growers associations (PGA's) is the current favoured mechanism, and this is based on positive experience of PGA's developed by AFRICARE in Uganda and INR in RSA.

The SSPS is also being trialed by KARI in Meru under PRAPACE funding. PRAPACE operates as a network for dissemination of potato related technologies throughout sub-Sahara Africa. This network is very closely linked to CIP, and continued effective dialogue and research with PRAPACE and CIP will synergise uptake of research outputs.

What further follow-up action/research is necessary to promote the findings of the work to achieve their development benefit?

BCA: Testing (validation and environmental impact) the BCA in Kenyan soils was an objective of the current project, however, the processes involved in gaining permission to undertake this research were under-estimated and no assessments have taken place in Kenya. Nevertheless, applications for contained-use and field trial testing of the BCA are with the respective national councils on biosafety of Kenya and RSA for approval, and a favourable decision on these applications is deemed likely by early 2001. It is probable that testing of the BCA in Kenya and RSA will be possible in 2001.

A minimum of 3-5 years would be needed to validate fully the BCA and its environmental impact. The research should ideally be conducted by a post-doctorate scientist or through a Ph.D. programme where rigorous monitoring would ensure the highest standards are achieved. A dual country testing of the BCA in Kenya and RSA would maximise the likelihood of wide-scale adoption of the BCA.

The need to control bacterial wilt within potato cropping systems of Kenya, RSA and many other sub-Sahara African counties is not refuted, however, no study has been conducted on the perception by potato farmers and consumers of the BCA and its genetically modified constitution. A pre-requisite to the wide-scale adoption of the BCA would be farmer and consumer acceptance of GMO technologies for farming, and thus a market study towards this end in Kenya, RSA and other sub-Sahara African countries is essential. Fulfilment of these activities would need to engage the public and media by nature of the publicity surrounding the use of GMO's for farming.

Subsequent to the successful testing of the BCA and market studies to show farmer/consumer acceptance of GMOs in farming, the establishment of micro-enterprise(s) for the manufacture and marketing of the BCA would be needed. Rhizobial inoculants are produced in RSA by private enterprises and these may represent an expedient option for BCA production.

SSPS: In addition to the SSPS trial reported on in this study, analogous research has been initiated in Meru, Kenya as mentioned above. The validation of the SSPS under different agro-climates with associated disease pressures represents a vital next step for the wide-scale implementation of the system. Accordingly, additional validation trials in Uganda and RSA are required. The data presented herein also indicates that additional research is required to optimise the seed spacing for the SSPS seed cultivation.

Further studies are also needed to define implementation strategies of the SSPS within farmer communities. The role and functioning of community-structured potato grower associations need to be assessed as a mechanism of managing seed.

Wide-scale adoption of the SSPS is dependent on the availability of an initial clean seed source. Research is needed to model clean seed inputs assuming wide-scale adoption of the SSPS, the extent to which current certified seed sources meet this demand in terms of output and quality assurances, and the inputs required to bridge any identified shortfalls.

These necessary next-steps, knowledge gaps and promotion of uptake pathways identified above are being more precisely defined under a current DFID RNRKS CPP 1 year short project (R7858) that will culminate in Terms of Reference for a open-call on a locally led SSPS and BCA technology promotion/support project, working in an integrated crop management system (sustainable livelihoods support framework). The agreed Terms of Reference will be referred to international donors (DFID; Gatsby; Rockerfellow; ABSP) inviting expressions of interest.

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8. DISSEMINATIONS

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9. TABLES, PLATES & GRAPH

Variety	Area of land (m^2)		Seed inputs	
	SSPS seed-tuber cultivation	Ware cultivation	SSPS seed-tuber cultivation	Ware cultivation
Tigoni	895 SE90	2219 SE520	22367 SE2241	9874 SE2314
R. Tana	1228 SE304	2445 SE344	30703 SE7595	11221 SE1287

Table 1: Land and seed inputs to maintain a 1 ha closed system cultivation of potato using the SSPS and Ware-to ware model.

Phase	Test	% infection SSPS seed cultivation	% infection SSPS ware cultivation	% infection Ware-to-ware
Phase 1	Field observation	1.4	2.1	0
	ELISA results	Not tested	Not tested	Not tested
Phase 2	Field observation	6.8	1.3	0
	ELISA results	Not tested	Not tested	Not tested
Phase 3	Field observation	2.3	0	0
	ELISA results	Not tested	Not tested	Not tested
Phase 4	Field observation	2.3	0	0
	ELISA results	3.6	3.9	0
Phase 5	Field observation	1.4	0	0
	ELISA results	0.5	1.4	0.5
Phase 6	Field observation	0	0	0
	ELISA results	1.2	2.7	0

Table 2: Field observed and ELISA detected bacterial wilt infection levels in Tigoni SSPS seed, SSPS ware and Ware-to-ware cultivations of Farm 1. Note % infection for SSPS seed cultivation indicates disease status of seed planted in subsequent phase for SSPS seed and SSPS ware cultivations .

	SSPS flatbed cultivation				Ridge/furrow cultivation	
Variety	20 x 20 cm spacing		30 x 30 cm spacing		30 x 75 cm spacing	
	Land input (m^2)	Seed input	Land input (m^2)	Seed input	Land input (m^2)	Seed input
Tigoni	602	15054	169	1872	1067	4749

Table 3: Estimate on land and seed inputs to maintain a 1 ha closed system cultivation of potato under 3 planting strategies.

Item	Questionnaire analysis	Comment
Farmer holding	5.2 ac (SE1.0)	Mix of tenant and freehold
Main source of income	Farming	crops & livestock
Main crops	Potato Maize Peas Cabbage Nappier grass Carrot Kale Plants Oats Fruit	22 (1.1)[10] (times scored by farmer) 12 (3.2) 11 (3.5) 20 (2.7) 5 (2.6) 12 (3.5) 1 (5) 7 (3) 2 (4) 1 (4)
Potato yields	38.2 bags/ac (SE5.2)	
Seed sources	Home Neighbour NPRG Market	19 (times scored by farmer) 5 5 3
Area to potato	1.3 ac (SE0.1)	
Varieties planted	Kimandi Tigoni Roslin Tana Asante Molo Kimandi Dhamana Nyayd	20 (times scored by farmers) 7 4 2 1 1 1
Fertilizer use	0 application 1 2 3	0 (times scored by farmers) 23 0 0
Fungicide use	0 application 1 2 3 4	0 (times scored by farmers) 6 6 7 2

[10] Values in parenthesis indicates average farmer rating: 1 = high, 5 = low

Item	Questionnaire analysis	Comment
Insecticide use	0 application 1 2 3 4	14 (times scored by farmers) 3 4 1 1
Herbicide use	0 application 1 2	18 (times scored by farmers) 5 0
Use of middlemen	Trader Self marketing	21 (times scored by farmers) 2
Current av. value (debe) Max/min value stated	254 Ksh (SE 12) 600/50 Ksh	
Sources of credit	None	
Changes in yield (5 yrs)	Decrease Increase Varies	16 (times scored by farmers) 5 2
Major marketing constraints	Road links to markets Market price variation Vechicles Traders	21 (times scored by farmers) 13 1 4
Major production constraints	Disease, Weather, Inputs, Seed, Soil Labour costs	14 (times scored by farmer) 10 8 5 2 1
Major pest constraints	Bacterial wilt Cutworm Blight Aphids Insects Disease	5 (times scored by farmers) 19 5 9 1 2

Table 4a: Summary of baseline data obtained during Njabini Farmers Field Day 1999

Item	Questionnaire analysis	Comment
Had you heard of the trials	Yes No	3 (times scored by farmers) 15
Do you ever plant chats	Yes No	6 (times scored by farmers) 16
Advantages see in SSPS	Seed multiplication Seed quality High yields Improved financial return Better land utilisation	4 (times scored by farmers) 7 4 1 1 1
Disadvantages of SSPS	Time inputs Increased disease weak stems none	6 (times scored by farmers) 1 1 2
Factor affecting adoption	Prod. of small seed Labour Lack of knowledge Tradition None	1 (times scored by farmers) 7 9 1 1
Gender preference	None male female child yes	10 (times scored by farmers) 6 0 0 3
General comments	Seed multiplication Easy to spray Economic Good land use Good management Control of bacterial wilt more advice/promotion	1 (times scored by farmers) 2 3 3 3 1 3

Table 4b: Summary of SSPS feedback during Njabini farmers Field Day 1999