African Phytosanitary Journal http://www.africanphytosanitaryjournal.go.ke
ISSN 2617-1856 (Paper) DOI: 10.52855/HNVQ1482
Volume 4, Issue 1, 2024

Reaction of potato cultivars to Potato Cyst Nematodes (*Globodera rostochiensis* and *G. pallida*) under greenhouse conditions in Kenya

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Abstract

Several measures have been recommended in the control of potato cyst nematodes (PCN) with resistant potato cultivars being considered as the most practical and affordable for smallholder potato farmers in Kenya. However, the level of resistance in locally grown potato varieties is yet to be established. The aim of this study was to screen Kenyan potato cultivars against PCN under greenhouse conditions. Eleven potato cultivars namely Shangi, Dutch Robijn, Sherekea, Nyota, Roseline tana, Tigoni, Unica, Asante, Chulu, Kenya Mpya and Arka were screened with Desiree (susceptible variety), Manitou (resistant cultivar) as controls. For each potato cultivar, there were two sets of plants with the first set being inoculated with 50 cysts, while the second batch was nematode-free. The treatments were arranged in a completely randomized design with three replications. A scale of 1-9, with 9 indicating the highest level of resistance was used in the assessment of disease severity. Nematode infestation caused a reduction in root mass across the 11 cultivars from 20 to 100% compared to uninoculated controls. Reproductive index of PCN viable eggs across the 11 cultivars was <1 compared to control (Desiree). Potato cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya, and Roseline Tana had a severity score of 1-3 (>95%), hence were considered to be susceptible to PCN. The cultivars Sherekea and Nyota had a severity score of 4-6 (<25%) and hence were considered partially resistant to PCN. The findings of this study provides a basis of integrating partially resistant potato cultivars into PCN management in smallholder farms.

Keywords: Cyst viable eggs, reproductive index, resistance, susceptible, smallholder farmers, severity score.

Introduction

Potato (*Solanum tuberosum* L.) is the world's third most important non-cereal food crop after wheat and rice (FAO, 2013). In Kenya, potato is the second most important food crop after maize and is highly commercialized across its value chain. About 2–3 million tonnes of potatoes worth Ksh. 40 to 50 billion (US$ 345–431 million) are produced each year, engaging millions of Kenyans directly and indirectly (MoAL&F 2016). In the tropics, potato cultivation faces a number of challenges including lack of certified seeds, poor soil fertility and infestations by various pests and diseases (Gildemacher *et al.,* 2009; Muthoni *et al.*, 2013).

Potato Cyst Nematodes (*Globodera rostochiensis* and *G. pallida*) (PCN) cause 9% loss in potato yield worldwide (Turner and Subbotin, 2013). In Europe, PCN is the second most economically important pest of potato after late blight, with an estimated economic yield loss of £26 million annually and chemical nematicide use estimated at £20 million annually (Twining *et al.*, 2009; NSP, 2017). It has been established that yield losses associated with PCN varies due to environmental conditions, varieties grown and levels of nematode infestation with every 20 viable eggs/g of soil causing a loss of 2.75 t/ha in potato yield (EPPO, 2013).

Potato cyst nematodes are classified as restricted quarantine pests in over 100 countries in the world (EPPO, 2009). The pest status under subtropical and tropical conditions in Africa is yet to be established. In potato growing areas in Kenya, PCN have caused qualitative and quantitative losses, with infested plants having low root mass development, stunted growth and leaf chlorosis with a potential yield loss of up to 80% when the pest is not controlled (Mwangi *et al.*, 2015; Mburu *et al.*, 2018). In predicting yield losses, different modeling parameters focusing on the level of initial PCN population at the start of planting season, type of soil, nematicide use, rotation period and use of susceptible/resistant cultivars have been used (Trudgill *et al.,* 2014).

Management of potato cyst nematodes through crop rotation is challenging since they are known to survive in soil for a long time with viable eggs surviving for decades in the absence of suitable host. Crop rotation must therefore be integrated with other methods to lower PCN population density in the soil (Christoforou *et al.,* 2014). Use of trap cropping has been shown to lower the population density of PCN by inducing hatching without the formation of new cysts (Mimee *et al.,* 2015). This method requires an adequate understanding of the nematode's life cycle. Tolerant traits of plant varieties are an ideal strategy to reduce yield losses resulting from pests and diseases (Peng and Moens, 2002; Cook and Starr, 2006). Among all the methods used to lower PCN population density, the use of resistant varieties has proved to be most successful in managing plant-parasitic nematodes in various parts of the world (Cook and Starr, 2006).

Plant resistance to plant-parasitic nematodes is an important aspect in management of PCN given the concerns over environmental hazards caused by continuous use of chemical pesticides (Peng and Moens, 2002). Over 1,200 wild potato species from the Commonwealth Potato Collection gene bank have been screened for resistance to PCN and the first PCN resistance locus (*H1*) from *S. tuberosum* ssp. *andigena*, offering complete resistance to *G. rostochiensis* pathotypes Ro1 and Ro4 in potatoes has been identified (Whitworth *et al.,* 2018). Growing of resistant potato cultivars on farms with high PCN population densities has led to the realization of yield benefits with a significant reduction in nematode population densities (Lane and Trudgill, 1999). Subsequent studies have shown that population densities of *G. rostochiensis* can be reduced by up to 95% for each season that a resistant cultivar is grown depending on initial inoculum density, whereas when susceptible cultivars are grown in an infested field, the nematode population densities increase by 2–35 times (Brodie 1996). This study was therefore conducted to determine the reaction of Kenyan potato cultivars to PCN under greenhouse conditions.

Materials and methods

Experimental site and cultivar selection

A greenhouse experiment was conducted from March-May and June-August 2021 for seasons one and two respectively, at the University of

Nairobi-Upper Kabete campus (1° 15´S and 36° 44´E) at an elevation of 1,820 m above sea level. Eleven potato cultivars commonly grown by Kenyan farmers; Dutch Robijn, Shangi, Roslin Tana, Tigoni, Asante, Sherekea, Nyota, Kenya Mpya, Unica, Chulu and Arka were selected for the study. Potato cultivars Desiree (susceptible) and Manitou (resistant) were included as controls. Certified potato tubers were obtained from Kenya Agricultural and Livestock Research Organization – Tigoni.

Preparation of PCN inoculum

Potato cyst nematode inoculum was extracted from naturally infested fields in Nyandarua county. The Fenwick can method (Fenwick, 1940), was used to extract PCN cysts from infested soil. Viability of eggs within the cysts was verified by soaking a random sample of 10 cysts per soil sample in 0.001% Nile blue stain (*v/v*) for 48 hours (Faggian *et al.*, 2012). Dead eggs or juveniles stained blue while the live ones did not stain after opening the cyst. Samples with at least 50% of either live eggs or juveniles were used as sources of inoculum. Each eppendorf tube was used to conserve 50 cysts until needed.

Set-up of greenhouse experiment

Each of the eleven chitted potato cultivars in two batches (inoculated and nematode-free) was sown in three plastic pots containing 400g of soil and sand (steam-sterilized at 180°C for 30 minutes) mixed at a ratio of 3:1 (*v/v*). This was replicated three times. Two weeks later, 50 cysts of PCN having about 100 eggs/ cyst were introduced into each pot as described by Whitworth *et al.*, 2018, while the controls pots were nematode-free (non-inoculated). Treatments were arranged in a completely randomized design. Plants were watered at intervals of four days.

Data collection

Data on root mass, cyst count and egg viability from each potato cultivar were collected 70 days after planting. Final population (P_f) densities of cyst and egg viability per 3 cysts of each treatment were evaluated, following cyst extraction, picking and counting under a dissecting microscope at 40x magnification.

Data analysis

Data on root mass were compared using *t*-test. Potato cyst nematodes and egg reproduction index was expressed as RI=Pf /Pi (Van Den Berg and Rossing, 2005). Susceptibility rating of the eleven potato cultivars to PCN were determined by calculating, PF (final cyst population of the test variety)/ PF (final cyst population of standard susceptible control variety i.e. Desiree) × 100, while severity score was divided into 9 susceptibility groups i.e. 1 (>100), 2 (50.1–100%), 3 (25.1–50%), 4 (15.1–25%), 5 (10.1–15%), 6 (5.1–10%), 7 (3.1–5%), 8 (1.1–3%), 9 (≤1%), with 1-3 being susceptible, 4-6 partially resistant and 7-9 resistant (EPPO, 2006). Data on cyst and egg counts were normalized using square root transformation before ANOVA was performed (Gomez, 1984). Thereafter, ANOVA was done using GenStat (15th edition) and means of cysts and egg counts in potato cultivars were compared and separated using Tukey's least significant difference (LSD) test ($p<0.05$).

Results

Effect of PCN infections on root mass of different potato cultivars

Potato cyst nematode infestation caused a decrease in root mass of the 11 potato cultivars compared to their controls 70 days after planting. There was a significant difference ($p \leq 0.05$) in root mass of nematode-infested plants compared to non-inoculated controls (Table 1). Cultivar Arka had significantly reduced root mass between inoculated and non-inoculated treatment and was comparable to Desiree (susceptible cultivar). Amongst the 11 cultivars tested, Arka, Tigoni, and Unica had a significant reduction in root mass by >100%. Cultivars Sherekea and Chulu had the least reduced root mass by 2.5 and 3.3 % respectively. Root mass reduction ranged from 20% to 75% in other potato cultivars (Table 1)

Table 1. Effect of PCN infection on root mass of different potato cultivars

Potato cultivar	Season one				Season two			
	Inoculated	Non-inoculated	*p*-value	% Decrease in root mass from uninfected	Inoculated	Non-inoculated	*p*-value	%Decrease in root mass from uninfected
Shangi	6.5	10.6	0.1	62	38.2	67.1	0.9	75
Dutch Robijn	7.2	13.4	0.2	87	51.0	80.4	0.3	58
Sherekea	4.6	4.8	0.9	2	31.6	32.7	0.9	3
Desiree	2.7	4.1	0.05*	48	19.5	32.4	0.5	66
Nyota	3.6	4.6	0.4	28	20.4	23.1	0.6	14
Roseline Tana	4.2	5.4	0.14	28	20.9	23.9	0.3	14
Tigoni	5.2	12.0	0.06	132	16.6	37.4	0.09	125
Unica	6.7	14.2	0.16	111	11.8	22.9	0.8	93
Asante	3.7	6.3	0.24	71	24.4	39.1	0.1	60
Chulu	10.5	11.1	0.82	6	39.7	40.0	0.2	0.6
Kenya Mpya	3.4	4.5	0.08	34	83.2	98.0	0.5	18
Arka	1.7	4.1	0.04*	142	6.1	17.2	0.7	183
Manitou	4.1	5.6	0.3	35	21.9	25.7	0.6	17
SE	2.9	5.3			25.0	32.2		
L. S. D	5.1	9.5			44.5	57.3		
CV%	111.6	131.7			153.7	145.5		

Means of root mass in inoculated and non-inoculated plants across the eleven potato cultivars are significantly different *($p \leq 0.05$)
CV = Coefficient of Variation, LSD = Least Significant Difference, SE = Standard Error

Population density of PCN on different potato cultivars

The final PCN population density was significantly different ($p \leq 0.05$) among the cultivars in both seasons one and two. The final cyst population in cultivars Manitou (resistant) and Nyota had significantly lowered by 14-67% compared to controls, while an increase by up to 300% in cultivars Sherekea, Shangi, Tigoni, Dutch Robijn, Chulu, Asante, Unica, Arka, Kenya Mpya and Roseline Tana was observed compared to controls. The lowest PCN reproductive index of RI=0.6 and RI=0.53 were observed in cultivars Nyota and Manitou, followed by Sherekea with the reproductive index of RI=1.18 compared to Desiree (Table 2). Cultivars (Shangi, Tigoni, Dutch Robijn, Chulu, Asante, Unica, Arka, Kenya Mpya, Roseline Tana) had PCN with reproductive index of >1. The severity score of cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya, and Roseline Tana was 1-3 and were hence considered susceptible to PCN. Sherekea and Nyota had a severity score of 4-6 and were hence considered partially resistant to PCN (Table 3).

Table 2. Cyst numbers and reproductive index (RI) of PCN in different potato cultivars

Potato cultivars	Season one			Season two		
	Final cyst count (Pf)/pot	RI (Pf/Pi)	% Change in cyst count from initial population (Pi)	Final cyst count (Pf)/pot	RI (Pf/Pi)	% Change in cyst count from initial population (Pi)
Desiree	241.0e	4.82e	382	170.7e	3.41e	241
Shangi	172.7cde	3.45cde	245	122.3de	2.45de	145
Tigoni	200.0de	4.00de	300	98.0cd	1.96cd	96
Dutch Robijn	158.3bcd	3.17bcd	216	87.7cd	1.75cd	75
Chulu	151.7bcd	3.03bcd	203	85.0cd	1.70cd	70
Asante	109.0abc	2.18abc	118	93.0cd	1.86cd	86
Unica	95.3abc	1.91abc	91	70.7bcd	1.41bcd	41
Arka	107.7abc	2.15abc	115	66.0abc	1.32abc	32
Kenya Mpya	95.3abc	1.91abc	91	64.0abc	1.28abc	28
Roseline Tana	69.0ab	1.38ab	38	53.3abc	1.07abc	6
Manitou	34.7a	0.69a	-31	18.7ab	0.37ab	-63
Sherekea	70.7ab	1.41ab	41	47.0abc	0.94abc	-6
Nyota	43.0a	0.86a	-14	16.3a	0.33a	-67
S.E.M	34.5	0.69		18.0	0.36	
L. S. D	100.2	2.00		15.3	0.31	
CV (%)	50.1	50.1		40.8	40.8	

Means in the same column with a different letter(s) are significantly different ($p \leq 0.05$). CV (%) = Coefficient of Variation, LSD=Least Significant Difference, Initial Population (Pi) = 50 cysts, reproductive index (RI = Pf/Pi).

Table 3. Susceptibility rating of potato cultivars to PCN

Potato cultivars	Season one		Season two	
	Severity score	% Susceptibility score	Severity score	% Susceptibility score
Desiree	2	100	2	100
Shangi	2	72	2	72
Tigoni	2	57	2	83
Dutch Robijn	2	51	2	66
Chulu	2	50	2	63
Asante	2	54	3	45
Unica	3	41	3	40
Arka	3	39	3	45
Kenya Mpya	3	37	3	40
Roseline Tana	3	31	3	29
Manito	4	11	4	14
Sherekea	4	25	4	24
Nyota	4	10	4	18

Severity score; 1-3 susceptible, 4-6 partially resistant and 7-9 resistant.

Reproductive index of PCN viable eggs on different potato cultivars

There was no significant difference ($p \leq 0.05$) in viable eggs across the varieties tested in seasons one and two. All the 11 cultivars had a reproductive index of less than 1 compared to Desiree which was the susceptible control (Table 4).

Table 4. Reproductive index of PCN viable eggs on different potato cultivars

Potato cultivars	Season one		Season two	
	Final egg count (Pf)/cyst	RI (Pf/Pi)	Final egg count (Pf)/cyst	RI (Pf/Pi)
Chulu	35.0 a	0.35 a	55.0 abc	0.55
Shangi	90.7 ab	0.9 ab	98.7 cd	0.99
Unica	68.7 ab	0.69 ab	74.0 abc	0.74
Nyota	24.7 a	0.25 a	89.0 bcd	0.89
Dutch Robijn	30.3 a	0.30 a	34.3 ab	0.34
Kenya Mpya	64.7 ab	0.65 ab	89.0 bcd	0.89
Tigoni	48.3 ab	0.48 ab	28.7 a	0.29
Sherekea	32.3 a	0.32 a	84.3 abc	0.84
Roseline Tana	30.0 a	0.30 a	48.3 abc	0.48
Manitou	52.3 ab	0.52 ab	67.7 abc	0.68
Desiree	114.3 b	1.14 b	139.7 d	1.4
Arka	70.0 ab	0.70 ab	84.7 abc	0.85
Asante	84.7 ab	0.85 ab	69.7 abc	0.7
S.E.M	24.38	0.24	19.51	2.0
L. S. D	70.89	0.71	56.71	0.57
CV (%)	63.9	63.9	45.6	45.6

Means in the same column with a different letter(s) are significantly different ($p \leq 0.05$). CV (%) = Coefficient of Variation, LSD = Least Significant Difference, Reproductive index (RI) = Pf/Pi, Pi- initial population (100 viable eggs).

Discussion

Potato cyst nematodes had a significant effect on root mass of different potato cultivars during their growth. Potato cultivar Arka had significantly lower root mass followed by Tigoni and Unica amongst the eleven potato cultivars. PCN J2 penetrated and developed in the root systems of potato cultivars Arka, Tigoni and Unica forming feeding tubes that limited the absorption of water and nutrients (Urek *et al.* 2008; Sudha *et al.* 2017). Cultivars Chulu and Sherekea had higher root density due to ability to produce extra roots when infested with PCN hence increasing their efficiency in nutrient and water uptake (Mei *et al.*, 2015). These findings are reflected by Thorpe *et al.*, (2014) who noted that host-nematode defense response to resistance and susceptible potato cultivars in the root system at early stage are affected by PCN effectors, which are important in syncytia formation, hence suppressing defense response in susceptible cultivars, unlike resistant cultivars.

There was a significant difference in final PCN population density among the cultivars in seasons one and two.

Difference in final cyst population on susceptible potato cultivars is dependent on different PCN species and pathotypes, as same pathotypes have different population densities across susceptible cultivars (Sudha *et al.*, 2016). On the other hand, increase in the number of cysts and viable eggs in susceptible potato varieties was because of their sensitivity towards PCN (Urek *et al.*, 2008, Hajji-Hedfi, 2017, Mezerket *et al.*, 2018). Decrease in number of cysts and viable eggs in resistant potato cultivars Nyota and Sherekea was due to presence of H1 resistance gene which prevents the nematode from molting to adult stage (Simko *et al.,* 2007).

Severity score of potato cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya, and Roseline Tana showed that they were susceptible to PCN, while potato cultivars Sherekea and Nyota were partially resistant/tolerant to PCN. Resistance of potato cultivars to *G. rostochiensis* is attributed to several genes, which confer partial (Gro1.2, Gro1.3, Gro1.4, Grp1) or complete (H1, Gro1, GroVI) resistance (Finkers-Tomczak *et al.* 2011). The H1 gene confers resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis,* which inhibits the multiplication of PCN juveniles to develop into females (Simko *et al.,* 2007, Finkers-Tomczak *et al.*, 2011). These findings corroborate with Sudha *et al.*, (2016), who showed that cultivar Kufri Swarna was resistant to PCN pathotype Ro1, 4, Pa2, 3 Pa2/3 but susceptible when tested with pathotype Ro5. Nematode reproduction levels on plant tissues are used to measure resistance while damage levels are used to quantify tolerance (Bethke *et al.*, 2017).

Conclusion

From this study, potato cultivars Sherekea and Nyota are partially resistant to PCN and have the potential to reduce PCN under greenhouse conditions. However further studies need to be conducted to assess reactions of locally available potato cultivars in controlling PCN under different field conditions in Kenya.

Acknowledgment

The Kenya Climate Smart Agriculture Project (KCSAP) funded this work through the Kenya Agricultural and

Livestock Research Organization (KALRO).

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