GENETIC DIVERSITY OF POTATO CYST NEMATODE USING INTERNAL TRANSCRIBED SPACER REGION (ITS) AND SIMPLE SEQUENCE REPEAT MARKERS

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ABSTRACT

Genetic diversity of potato cyst nematodes (Globodera spp) is important in potato breeding programmes worldwide. The objective of this study was to identify and determine the genetic diversity of potato cyst nematode (PCN) populations from Nyandarua, Nakuru and Meru Counties in Kenya. Twenty simple sequence repeats (SSR) markers, four of which were newly designed for this study were used to genotype the 88 PCN samples. Fragments of DNA were amplified using 434 bp primer (ITS5/PITSr3) specific for Globodera rostochiensis. From 20 SSR markers, four were polymorphic and specific for PCN. The expected heterozygosity (H) and Shannon’s index (I) ranged from 0.32 to 0.50 and from 0.50 to 0.69, respectively, across the 3 populations. As a result, the H and I values indicated a limited range of genetic diversity and heterozygote deficiency between the samples. The mean polymorphism information content (PIC) ranged from 0.344 to 0.365 indicating an intermediate polymorphism (PIC ≥ 0.25) across the populations. Furthermore, analysis of molecular variance (AMOVA) revealed that the highest genetic variance 96% (p<0.001) was due to intra-population variance. An unweighted pair group method with arithmetic mean (UPGMA) phylogenetic tree was constructed based on Nei’s genetic dissimilarity and the entire population was grouped into two major clusters and five sub-clusters. Principal coordinate analysis (PCoA) revealed that the variance accounted for by the first 3 principal coordinates were 27.73, 28.89 and 24.42, respectively. The Bayesian model-based population structure analysis assembled the populations into K=6 distinct genetic structures based on the highest ΔK=8.25. The fixation index (Fst) ranged from 0.02 to 0.05, indicating that the three G. rostochiensis populations had low genetic differentiation. The results from this study suggest that there was low genetic diversity among the PCN evaluated across the Counties.

Key words: Globodera rostochiensis, potato cyst nematodes, simple sequence repeats

INTRODUCTION

Population genetics has been used to investigate pest evolution, host-parasite interactions, and the development of effective and efficient control methods (Gautier et al., 2021). A pest genetic diversity is determined by the species, dispersal abilities, population size, and mode of reproduction (Alenda et al., 2014). This information aids in the determination of pest populations diversity as well as their ability to adapt to different environmental conditions and informs of possible management options (McDonald and Linde, 2002). Genetic diversity of plant parasitic nematode populations from various geographical areas may thus aid in predicting the efficacy and durability of some control strategies. Plant breeders and plant pathologists have also used species diversity to understand pathogens and develop new tolerant varieties (Boucher et al., 2013). Due to the economic significance of potato cyst nematode (PCN) in Kenya, identifying and determining genetic diversity is critical for developing appropriate management strategies.

Molecular methods have been used to identify PCN (Mwangi et al., 2021), the tobacco cyst nematode caused by G. tabacum (Alenda et al., 2014) and the cereal cyst nematode Heterodera avenae (Wang et al., 2017). The
deoxyribonucleic acid (DNA) based techniques are more accurate in differentiating the two species (*Globodera pallida* and *Globodera rostochiensis*). The validated molecular assays for *Globodera* identification are based on amplification of the ribosomal internal transcribed spacer region (ITS), or ITS ribosomal DNA (*ITS rDNA*) (Skantar et al., 2011).

To examine genetic diversity and monitor the spread of plant parasitic nematodes, researchers have utilized *ITS rDNA* and simple sequence repeats (SSRs) have been utilized (Boucher et al., 2013). Microsatellite markers are short tandem DNA repeats with 2-8 motif (Handayani et al., 2020). The SSR markers are highly reproducible, codominant, abundant and evenly distributed in the nematode genome, they are either coding or noncoding regions (Omondi et al., 2016). Moreover, SSR markers were shown to be highly transferable between species or closely related genera, allowing discrimination between closely related species in nematodes (Bornet et al., 2002; Kostova, 2021). Therefore SSR markers are one of the reliable PCR based markers that have been used successfully to determine genetic diversity of populations (Handayani et al., 2020). An earlier study in Europe (Plantard et al., 2008) demonstrated the successful use of microsatellite markers to examine the genetic diversity of *Globodera pallida* populations. In Peru, SSRs were used to reveal phylogeographical structure and the allelic diversity of *G. pallida* populations (Picard et al., 2004). Boucher et al. (2013) employed microsatellite markers to assess the genetic diversity and phylo-geographical history of both *G. pallida* and *G. rostochiensis* populations in Canada.

The objective of this study was to identifying the PCN species present in Kenya and evaluate the genetic diversity among the genus *Globodera* from PCN populations collected from Meru, Nakuru and Nyandarua Counties using SSR markers.

**MATERIALS AND METHODS**

**Source of potato cyst nematode populations**

The PCN populations used in this study were obtained from potato-producing Counties in Kenya of Nyandarua, Meru and Nakuru Counties.

**Soil sampling**

The soil samples were taken from Upper highland 3 (UH3), Upper highland 2 (UH2) and Lower highland 4 (LH4) agro-ecological zones. Ten farms were chosen at random from each of the 3 AEZs in each County. Ninety soil samples were collected in total and each farm had a random sampling of 10 sub-samples collected from the potato rhizosphere at a depth of 30 cm. Each sub-sample weighed about 40 g and were composited into approximately 400 g in 1 kg paper bags thereafter transported in cooler boxes to the laboratory and kept at an ambient temperature of 20 - 25 °C.

**Cyst extraction**

The composite of each soil sample was thoroughly mixed and cyst were extracted using the Fenwick Can method (Fenwick, 1940) from approximately 300 g of soil. The cysts were examined and isolated under a binocular microscope (EPPO, 2013). Three cysts containing eggs and juveniles were picked from each of the samples for molecular analysis.

**Molecular identification of the species**

**DNA isolation**

Two second stage juveniles (J2s) were handpicked individually under a stereo microscope from the cysts used during morphometric characterization and put in 1.5 ml Eppendorf tubes in which 20 μl 10X lysis buffer was added. The nematodes were ground with a pestle and 5 μL of Proteinase K (20 μg μL\(^{-1}\)) was added to each tube. Genomic DNA was isolated from each sample following the manufacturer’s instructions, using the ISOLATE II Genomic DNA kit (Bioline). They were incubated for 1 hour at 60°C and 10 mins at 94°C before being briefly cooled on ice and centrifuged at 11,000 X g to remove residues. Approximately 5 μl of supernatant from each sample was collected and kept at 20°C.

**Quality of DNA**

Genomic DNA was visualized in 1.5 % agarose gels using the gel electrophoresis method based on a modified protocol of Lee et al. (2012). Approximately 1.5% agarose gel was prepared in Tris Acetate Ethylenediaminetetraacetic acid (EDTA) (TAE) (1% w/v) buffer. The hot agarose mixture was allowed to cool under running water to about 50°C.
before adding 5 µl of 1% Gel red. The warm solution was then poured into the gel tray in which a 16 comb was inserted to form 16 wells. The gel was allowed to solidify for 30 mins. Thereafter, the gel was placed in a buffer tank containing Tris Acetate Ethylenediaminetetraacetic acid (EDTA) (TAE) (1% w/v). Approximately 6 µl of loading solution was added to 5 µl of genomic DNA sample and 2 µl loading dye (bromophenol blue) was loaded to the wells of the gel. BIOLINE hyper ladder 100 bp was used. Electrophoresis was done at 100 V, 100 W for 40 mins. At the end of electrophoresis, gel was visualised under gel documentation system under UV light. The quantity and quality of DNA were assessed by visual comparison of the sharpness and intensities of the DNA bands.

**Modified multiplex Polymerase Chain Reaction (PCR)**

In this study, each of the 88 samples was subjected to a modified multiplex polymerase chain reaction (PCR) protocol to distinguish the species (G. pallida and G. rostochiensis) present in soil samples (EPPO, 2017). The ribosomal internal transcribed spacer (ITS) region was used in the multiplex PCR reactions (White, 1990). The \( \text{PITSr3} \) (5’ AGCGCAGACATGCGCGCAA-3’) primer specific for detection of only \( G. \text{rostochiensis} \) and \( \text{PITSp4} \) (5’-ACAACAGCAATCGTCGAG-3’) marker specific for detect only \( G. \text{pallida} \) in combination with the forward universal marker \( \text{ITS5} \)- (5’ CGTACCAAGGTAGCTGTA-3’) were amplified (White, 1990; Bulman and Marshall, 1997). Each PCR reaction contained a total of 25 µl reaction volume, having 0.5 µl \( \text{Taq} \) polymerase, 8 µl 50 X DNA reaction buffer, 0.5 µl of each primer (forward and reverse) and 8 µl of DNA template 6.5 µl nuclease free water. The reactions were carried out in an Applied Biosystems \text{ProFlex} \) thermocycler that was programmed. The PCR cycling conditions were: denaturation of DNA at 94°C for 3 min., 94°C for 30 sec, primer annealing at 55°C for 30 sec 40 cycles of extension at 72°C for 30 sec each, followed by 5 min at 72°C.

**Agarose gel electrophoresis**

To analyze each of the 88 samples, 0.5 µl DNA loading buffer was added to 10 µl of amplicons of each sample and mixed. Electrophoresis was conducted using 1.5 % agarose gel stained with 1X \text{GelRed}. A standard DNA marker 100bp (Biolabs) was used as a standard to determine the size of the bands on each gel. Electrophoresis was performed in 1 x TAE buffer at 100V, 100 W for 40 mins. The \text{Gel Doc EZ} \) system was used to visualise the gel under ultra violet light. A 100 bp ladder was used to estimate the sizes of the successful amplicons. Samples yielding a PCR product of 265 bp amplicon were considered \( G. \text{pallida} \) whereas samples giving a PCR product of 434 bp amplicon were considered \( G. \text{rostochiensis} \) (Bulman and Marshall, 1997). Samples that produced no PCR product or produced a PCR product beyond these ranges were thought to belong to species other than \( G. \text{pallida} \) and \( G. \text{rostochiensis} \).

**Genetic diversity of PCN using SSR markers**

**Polymorphism and primer design**

Twenty SSR markers were used to evaluate the genetic diversity of 88 PCN samples. Sixteen SSR markers (Gr50, Gr67, Gr75, Gr85, Gr90, Gr91, Gr96, Gr82, Gr70, Gr79, Gr94, Gp109, Gp116, Gp118, Gp126 and GP135) were chosen based on their high polymorphic information content (PIC) (Boucher et al., 2013) (Table I). Four new primers (GRM1 and GRM2 specific for \( G. \text{rostochiensis} \), GPM3 and GPM1 specific for \( G. \text{pallida} \) both forward and reverse) were designed for this study (Table I). To design these markers, \( G. \text{rostochiensis} \) and \( G. \text{pallida} \) genome were downloaded from Genebank (www.ncbi.nlm.nih.gov/dbEST). The SSR locator software (http://micosatellite.org/ssr.php) was then used to select the SSR markers. Primer 5.0 software (www.premierbiosoft.com) was used to design the SSR marker basing on primer size 18-20 oligonucleotide length below 250 bp and melting temperature between 50 - 63°C (optimum 60°C). The SSR markers were then synthesised by Macrogen (South Korea). Thirteen of SSR markers were specific for \( G. \text{rostochiensis} \) and seven of them belonged to \( G. \text{pallida} \). All the 20 SSR markers were screened for polymorphism using four random samples from each County. Primers with polymorphism were selected for largescale PCR amplification and agarose gel electrophoresis using 88 PCN populations.
<table>
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<tr>
<th>No</th>
<th>Primer ID</th>
<th>Species</th>
<th>Primer forward sequence (5’–3’)</th>
<th>Primer reverse sequence (5’–3’)</th>
<th>Motif</th>
<th>Size (bp)</th>
<th>Annealing temp° C</th>
<th>Polymorphic markers</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>GRM1</td>
<td>G. rostochiensis</td>
<td>TTCCTTCAGACAGTTGTCAGAG</td>
<td>GAGAGA GAGGAGAGAAAGC</td>
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<td>155</td>
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<td>2</td>
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<td>G. rostochiensis</td>
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<td>ATCTGCACAAACACACAGAG</td>
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<td>G. pallida</td>
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<td>333</td>
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<td>GPM2</td>
<td>G. pallida</td>
<td>TTTAGGCGAACATCCTTCTGTG</td>
<td>CATTGGGTAACACATCCTGGA</td>
<td>(TG)4</td>
<td>289</td>
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<td>G. pallida</td>
<td>TCTCGCAGAAGAAACGAAAAGGAA</td>
<td>TAAAGACGGGAAAGACGGGGGA</td>
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<td>G. pallida</td>
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<td>18</td>
<td>Gp118</td>
<td>G. pallida</td>
<td>ACCGTGAAGAACATCGTTCC</td>
<td>TCGTTCGCCCTTCCTGTAACCT</td>
<td>(TCCG)4</td>
<td>133</td>
<td>52</td>
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<tr>
<td>19</td>
<td>Gp126</td>
<td>G. pallida</td>
<td>GTATTGTGGCGGAGATGGAAT</td>
<td>GTACTGTATGATGCGGGCT</td>
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<td>192</td>
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<td>20</td>
<td>GP135</td>
<td>G. pallida</td>
<td>GCGAAATGACGCGTCTAGT</td>
<td>ATTACATTGCCCCAAATCGGA</td>
<td>(GA)7</td>
<td>146</td>
<td>54</td>
<td>No</td>
</tr>
</tbody>
</table>

Selected or not selected for PCR amplification of all 88 PCN population, bp; base pairs
Polymerase Chain Reaction (PCR)

The PCR reactions were carried out in a 25 μl reaction volume; 0.5 μl Taq polymerase, 8 μl 50X DNA reaction buffer, 0.5 μl of SSR primer pair and 8 μl of DNA template and 6.5 μl nuclease free water. Each test included controls without nematodes. The reactions were performed in a programmable temperatures under the following regimes: 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 sec each, annealing (temperature of each SSR primer is indicated as Ta in Table I for 30 sec) and extension at 72°C for 30 sec and with a final elongation at 72°C for 5 min, followed by a final extension at 60°C for 30 min. To validate the 20 SSR markers, screening was carried out using agarose gel electrophoresis using four samples from each County.

Agarose gel electrophoresis

To analyse each of the 88 samples, 10 μl of PCR product of each sample was mixed with 0.5 μl DNA loading buffer and was separated using 1.5% agarose stained with 1X Gel red prepared according to Lee et al. (2012). A standard DNA marker 100bp (Biolabs) was also run on each gel. Electrophoresis was performed in 1 x TAE buffer at 100V, 100W for 40 mins. Using the Gel Doc EZ system, the gel was visualized under ultra violet light (Bio-Rad).

Data analysis

For each PCN sample, the SSR amplified bands were scored as 1/0 (presence/absence). Only bands that were reproducible and polymorphic were included in the study. Based on the alleles identified in the samples, genetic diversity parameters were computed using GenAlEx version 6.5 (Banks and Peakall, 2012). The polymorphic information content (PIC) and allele frequency were calculated using power maker software version 3.25. The PIC was calculated using Nei’s statistic (Nei, 1973) as follows:

$$PIC=1-\sum(p_i^2),$$

where $p_i$ is the frequency of the $i^{th}$ pattern for microsatellite marker $i$ and is summed across $n$ patterns.

Analysis of molecular variance (AMOVA) was computed using GenAlEx software version 6.5. The principal coordinate analysis (PCoA) was utilised to provide graphical representation of genetic relationship of the PCN population studied. Dendrograms were generated using an unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on the matrix of Nei’s genetic distance with POPGENE version. 1.31. Genetic distances were calculated as described by Nei and Li (1979) based on the likelihood that the amplified fragment from one genotype will be present in another genotype. The Bayesian model clustering analysis was used to estimate the most likely number of groups/populations ($K$) by STRUCTURE version 2.3.2 software, this model allowed admixture of populations (Pritchard and Stephens, 2000). The results were then imported to STRUCTURE HARVESTER software to test the proper $K$ by using Inp value (Earl, 2012).

RESULTS

Population identification using molecular techniques

DNA quantity and quality assessment

Based on the agarose gel electrophoresis, the quantity and quality of DNA for most samples were good (Figure 1).

Identification of PCN species

The polymerase chain reaction (PCR) amplicons were observed at 434 bp in all the cyst isolates from Nyandarua, Nakuru and Meru Counties. This indicates that the species detected was *G. rostochiensis* in all the samples except the ones that did not amplify (Figure 2). Primer (ITS5/PITSr3) specific for *G. rostochiensis* amplified 82.1% of the samples from Nakuru County, 86.6% of the samples from Meru county and 90% of the samples from Nyandarua county. However, there was no amplification on 15.5% of DNA samples.

Genetic diversity of PCN populations using microsatellite markers

Marker design and polymorphism

Among 13 out of the 20 loci produced amplicons and among these, 9 appeared to be monomorphic. Only loci Gr67, Gr79, Gr90 and GRM2 were polymorphic (Table I) and among these polymorphic loci markers, locus GRM2 was designed for this study. In this study, 35% of the markers failed to produce amplifications and were therefore excluded from the study.
Figure 1. The quality of potato cyst nematode DNA extracted. (Lane M-100 bp molecular marker, lane 1- a sample from Nyandarua, lane 2- a sample from Nakuru, lane 3- a sample from Meru, lane NC- Negative control).

Figure 2. Multiplex PCR using primers ITS5, PITSR3 and PITSP4; Lane M-100 bp Molecular marker, Lane 1-3 samples from Meru (UH3, UH2, LH), Lane 4-6 samples from Nakuru (samples UH3, UH2, LH4), Lane 7-12 samples from Nyandarua (2 samples in UH3, 2 samples in UH2, 2 samples in LH4), Lane 8 Absent, Lane NC-negative control.

Genetic analysis of SSR markers and G. rostochiensis populations

This study showed that 4 alleles displayed variations with 2 alleles observed per locus. The highest allele frequency of 0.8 and the lowest allele frequency of 0.2 were observed on locus Gr67 in samples collected from Nyandarua (Figure 3).

This study demonstrated that there was genetic variation among the samples analysed (Figure 3 and Table II). The observed allelic (At) variation across the 3 Counties ranged from 1.471 to 2.00, while the expected heterozygosity (He) and Shannon’s index (I) across the samples varied from 0.325-0.500 and 0.500-0.693, respectively. The mean expected heterozygosity for all the populations ranged from 0.436-0.482 (Table II). There was no significant genetic difference among the samples for the He and I, thus indicating a narrow range genetic diversity. All the loci exhibited significantly high $F_{IS}$ values of 0.109 for Gr67, 0.131 for Gr79, 0.217 for Gr90 and 0.144 for GRM2. The PIC values of the locus for all the PCN populations were 0.344 for Gr67, 0.340 for...
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Gr79, 0.364 for Gr90 and 0.359 for GRM2 (Table II). All the four loci demonstrated an intermediate polymorphism (PIC ≥ 0.25) on samples collected from the 3 counties (Table II) (Botstein et al., 1980).

The PCN sample populations from Nakuru had the highest heterozygosity of 0.481, whereas Meru PCN samples from Meru showed the least heterozygosity of 0.396. The mean number of alleles in the PCN populations from the 3 Counties were 2.00 (Figure 4). The private and locally common alleles were not detected in any population.

The AMOVA results showed significant contribution (p<0.001) to the overall genetic variation. The results revealed that 96% of genetic variance came from within population and 4% of genetic variation came from among the populations. Therefore, the genetic variance within the population was higher than between the populations (Table III).

![Figure 3. Allele frequency by locus for the 3 potato cyst nematode populations obtained from Nakuru, Nyandarua and Meru Counties](image)

<table>
<thead>
<tr>
<th>County</th>
<th>NS</th>
<th>Na</th>
<th>Ar</th>
<th>I</th>
<th>He</th>
<th>Gr67</th>
<th>Gr79</th>
<th>Gr90</th>
<th>GRM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nakuru</td>
<td>28.0</td>
<td>2.0</td>
<td>1.990</td>
<td>0.691</td>
<td>0.497</td>
<td>0.683</td>
<td>0.652</td>
<td>0.670</td>
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</tr>
<tr>
<td>Nyandarua</td>
<td>30.0</td>
<td>2.0</td>
<td>1.471</td>
<td>0.500</td>
<td>0.320</td>
<td>0.580</td>
<td>0.684</td>
<td>0.693</td>
<td>0.500</td>
</tr>
<tr>
<td>Meru</td>
<td>30.0</td>
<td>2.0</td>
<td>1.800</td>
<td>0.637</td>
<td>0.444</td>
<td>0.580</td>
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<td>0.543</td>
<td>0.358</td>
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<tr>
<td>For all populations</td>
<td>88.0</td>
<td>88.0</td>
<td>88.0</td>
<td>88.0</td>
<td>0.444</td>
<td>0.436</td>
<td>0.482</td>
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</tbody>
</table>

**TABLE II- GENETIC DIVERSITY INDICES FOR EACH LOCI AND PCN POPULATIONS COLLECTED FROM NAKURU, NYANDARUA AND MERU COUNTIES**

NS: number of samples; Na: alleles number, Ar: allelic richness; He: expected heterozygosity; I: Shannon information index; PPL- the percentage of polymorphic loci, PIC: polymorphism information content FIS: deviation from random mating.
Figure 4. Allelic patterns across potato cyst nematode populations from Nakuru, Nyandarua and Meru Counties; Na: number of observed alleles; Na Freq.-Allele’s frequency; Ne- effective number of alleles, I: Shannon information index, No. private alleles: Number of private alleles, No. LComm Alleles: Number of local common alleles.

<table>
<thead>
<tr>
<th>TABLE III- ANALYSIS OF MOLECULAR VARIANCE FOR POTATO CYST NEMATODE (PCN) SAMPLES FROM NYANDARUA, NAKURU AND MERU, COUNTIES</th>
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</thead>
<tbody>
<tr>
<td>Source</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Among populations</td>
</tr>
<tr>
<td>Within populations</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Pairwise genetic differentiations

The fixation index (Fst) ranged from 0.021 to 0.048, indicating little genetic differentiation (Fst < 0.05) among the three *G. rostochiensis* populations (Table IV). The Fst between Nyandarua and Meru (0.048) was higher than to between Nyandarua and Nakuru (0.041) and Nakuru and Meru (0.021).

<table>
<thead>
<tr>
<th>TABLE IV- GENETIC DIFFERENTIATION (FST) AMONG Globodera rostochiensis SPECIMENS OBTAINED FROM NAKURU, NYANDARUA AND MERU COUNTIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Nakuru</td>
</tr>
<tr>
<td>Nyandarua</td>
</tr>
<tr>
<td>Meru</td>
</tr>
</tbody>
</table>

Genetic relationship of *G. rostochiensis* populations

The 88 PCN samples formed major clusters 1 and 2 (Figure 5). Cluster 1 had 23 PCN samples falling into sub-structures 1.1 and 1.2. While cluster 2 had a high number of PCN samples being categorized into sub-groups 2.1, 2.2 and 2.3. The result showed that Sample No. 77 from Meru was categorized in its own sub-structure 2. This could be due to unique genetic evolution (Figure 5). Each cluster was composed of different populations emanating from Nyandarua, Nakuru and Meru counties.

Genetic structure of PCN samples from Nyandarua, Nakuru and Meru

The principal coordinate analysis PCoA analysis classified the PCN populations into three groups (Figure 6). The samples did not cluster according to counties for example, all the 3 clusters had samples from all the 3 counties.
According to PCoA, out of the 88 PCN samples, 16 unique genotypes were identified. In this study, population I, II, III accounted for 27.73%, 28.89%, and 24.42%, respectively (Figure 6 and Table V). The variation of all the 88 samples due to the first three axes accounted for 81.05%. In this study, the Eigen values of 9.943, 9.543, 8.404 and 6.520 were observed for the 4 axis, respectively. All the axes showed Eigen value >1 indicating that they are highly informative. The Fst between Nyandarua and Meru (0.048) was higher compared to between Nyandarua and Nakuru (0.041) and Nakuru and Meru (0.021).

![UPGMA dendrogram of 88 PCN samples belonging to G. rostochiensis](image)

**Figure 5.** UPGMA dendrogram of 88 PCN samples belonging to *G. rostochiensis*; Sample 1-28 were samples from Nakuru county, Samples 29-58 were samples from Nyandarua County, and Samples 59-88 were samples from Meru County

**TABLE IV- EIGEN VALUES AND TOTAL VARIATION OF SIX PRINCIPAL COMPONENTS FOR 88 PCN SAMPLES**

<table>
<thead>
<tr>
<th>Axis</th>
<th>Eigen value</th>
<th>% of variance</th>
<th>Cumulative % of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.943</td>
<td>28.89</td>
<td>28.89</td>
</tr>
<tr>
<td>2</td>
<td>9.543</td>
<td>27.73</td>
<td>56.63</td>
</tr>
<tr>
<td>3</td>
<td>8.404</td>
<td>24.2</td>
<td>81.05</td>
</tr>
<tr>
<td>4</td>
<td>6.520</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Based on the highest K-value obtained by the structure harvester (K=8.25), the population structure analysis revealed six distinct genetic structures (K=6) (Figure 7 and 8). The bar-plot model of clustering revealed that all the populations evaluated were admixtures. Each of the individual populations was denoted by 6 distinct group of colours red, green, blue, yellow, pink and blue. Individuals from the various populations shown above are separated by vertical bars. All the 88 PCN were assigned to 6 genetic clusters.

**DISCUSSION**

This study revealed that *G. rostochiensis* was present in all of the soil samples, while *G. pallida* was absent. The absence of *G. pallida* in the three counties could be as a result of their preference to low temperature below 10°C which favours reproduction unlike *G. rostochiensis* which reproduces well in temperatures ranging from 11.5°C to 32.5°C (Kaczmarek et al., 2012; Jones et al., 2017). To explore intra-population genetic diversity among the *G. rostochiensis* populations in Kenya microsatellite markers were used. The SSRs markers used in this study demonstrated that they were polymorphic among the PCN populations obtained from the three counties. The *F*<sub>is</sub> value revealed that the 3 *G. rostochiensis* populations from Nyandarua, Nakuru and Meru were probably similar. Additionally, the genetic differentiation pairwise matrix showed insignificant differentiation (*F*<sub>st</sub> < 0.05) in all the populations. This could be within the scope of this study in addition to the polyandry mode of reproduction and human activities (Picard et al., 2004). According to Mwangi (2019), farming practices such as recycling of seeds, use of farm implements and footwear without disinfection and transportation of seed with soil could accelerate spread of PCN resulting in gene flow.*G. rostochiensis* across the locations in Kenya. The result of this investigation showed that every population exhibited significantly high heterozygote deficiency therefore deviating from Hardy-Weinberg equilibrium. It is hypothesised that the deficiency in the populations is as a result of assortative mating and limited dispersal of J2s to short distance. J2s of *Heterodera* spp. and *Globodera* spp. are mobile therefore dispersal is very low (Wang et al., 2017). Given the low dispersal of *G. rostochiensis* there is a high likelihood of individuals mating with one another, and as a result, the population becomes either siblings or half-siblings (Picard et al., 2004). Furthermore, the assortative mode of reproduction is common in organisms characterized with...
limited dispersal and would result in reduction in genetic variability (Picard et al., 2004). Heterozygote deficiency has been found in cysts forming nematodes *Heterodera schachtii* (Plantard and Porte 2008), *G. tabacum* (Alenda et al., 2014), *Globodera pallida* (Picard et al., 2004), *H. avenae* (Wang et al., 2015).

In this study, the analysis of molecular variance (AMOVA) showed that variation within the population accounted for the majority of the diversity across all the counties. These findings suggest that the Kenyan PCN population could be from one origin. This could be as a result of spread of PCN across the counties through uncertified seeds. The PCoA analysis of genetic diversity structure showed that, PCN populations were in 3 genetic clusters. Moreover, the samples from the three counties were clustered and intermixed within the three populations in the PCoA analysis. The mixing of populations from different counties could be as a result of gradual migration of PCN populations, primarily resulting from agricultural practices that may have contributed to passive dispersal such as transfer of planting material among the counties (Alenda et al., 2014).

The phylogenetic tree in this study confirmed the intermixing of populations from the test regions of collection based on *Nei’s* genetic dissimilarity. Despite their different origins, the three populations have a close genetic relationship, suggesting that they may have evolved together. In the phylogeny tree, samples that were placed closer together suggest that they were genetically similar, whereas samples that were placed further apart indicated that they were genetically dissimilar despite being from the same region. Natural mutation and natural selection within the population may have contributed to this genetic variance. This was also reported by Mwangi (2019) who observed that Kenyan PCN populations formed single cluster when compared with samples from Germany.

The current genetic structuring observed in the present study revealed there was gene flow between the populations, this was shown by the intermixing of the PCN populations. The relationships between population structures with geographical origin were indistinct. This could be as a result of close proximity of the regions. Furthermore, cyst nematodes practice polyandry fertilization, genetic diversity within a single cyst having hundreds of individual’s eggs may occur (Mimee et al., 2015). In Western Europe, genetic variability of *G. pallida* occurs in a single plant, a field and on an entire region (Plantard et al., 2008).
Generally, this study revealed that gene flow occurred not only within counties but also between counties. Therefore, potato cyst nematode should be classified as a pest with a high capacity of overcoming the resistance because of the polyandry reproduction and high mutations which allows gene variability and those species with mutant genes may have selection/survival advantage (McDonald and Linde, 2002).

**CONCLUSION**

The study revealed that there was low genetic diversity among the PCN evaluated across the counties. It is therefore important to avoid introduction of PCN from other clades since they would require additional resources to control and would enhance high adaptive potential of the current populations. To limit spread, measures such as use of certified seeds cleaning farm implements and footwear should be promoted.

**ACKNOWLEDGEMENT**

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Genetic Diversity of Potato Cyst Nematode Using Internal Transcribed Spacer Region (ITS) and Simple Sequence Repeat Markers


