

Original paper

ANALYSIS OF AFLP MARKERS AS POTENTIAL MARKERS FOR SCREENING RESISTANCE TO COMMON BEAN ROOTS ROT (*Pythium spp.*)

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ABSTRACT

Aims: To investigate AFLPs for suitability as potential markers for identification of sources of *Pythium* resistance in bean genotypes preferred by poor small holder farmers.

Ten common bean lines generated from a conventional breeding program with differential resistance to the pathogen *Pythium* were subjected to AFLP analysis.

Place and Duration of Study: Department of biological sciences Masinde Muliro University of Science and Technology, Kenya and Bangor University, North Wales, United Kingdom, between September 2010 and December 2012.

Methodology: We included 45 common bean accessions comprising samples (35 seed samples collected from farmers, market centers, as well as seed stockists and ten common bean lines generated from a conventional breeding program with differential resistance to the pathogen *Pythium* were subjected to AFLP analysis. 10 seed samples collected from KARI-Kakamega, Kenya. Genetic characterization using cluster and principal component analysis were conducted to determine segregating patterns of bean accessions in relation to tolerant and susceptible bean lines. using AFLP marker to determine the differences in genetic diversity patterns existing within the bean samples. Average genetic distances were calculated and similarity coefficients subjected to unweighted pair group method of arithmetic averages to generate dendrograms.

Results: Two informative AFLP primer combinations yielded 194 polymorphic loci. Genetic distance of bean samples from KARI Kakamega had 56 to 414 base pairs with a variability index of 0.63 to 0.90. Combined analysis of bean accessions from KARI breeding program and market class common bean samples revealed a variability index range of 0.62 to 0.90 with 56 to 420 base pairs. PCA contributed about 51.58% on the genetic variation. Cluster analysis of the 10 KARI-Kakamega bean lines revealed that resistant bean varieties were genetically different from the susceptible bean varieties. The dendrograms generated revealed four major sub-groups and with the exception of Alulu, a mildly resistant cultivar, that segregated alongside resistant cultivars, resistant varieties clustered differently from susceptible cultivars. However, screening with farmers' germplasm produced a dendrogram that revealed a mixture of distinct and relatively non-distinct categorization with regard to resistance. Only resistant cultivars AN1062, R2075, R719 and R1946 and susceptible cultivars GLP2 and GLP585 clustered together as expected. The others segregated randomly alongside the farmers' germplasm. Resistant varieties AND1062, R2075, R719, R1946 and SCAM80 were being more genetically related to marketable class of beans. R1946 R719, R2075 and AND1062 are closely related genetically compared to Mw001, KK15, Alulu and GLP2. Cluster analysis revealed that resistant bean varieties were genetically different from the susceptible bean varieties.

Conclusion: AFLP is a relatively informative, reliable technique that has a great potential of delineating susceptible and resistant *Pythium* root rot dry bean varieties, and can be used as a preliminary guide to carry out further analysis. It is notable that the AFLP markers used were not able to clearly distinguish all the cultivars comprehensively and should not be used alone in

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selection for traits which may be expressed in adult plants only such as, grain or fruit quality, male sterility, photoperiod sensitivity. DNA markers can easily target alleles that are difficult, expensive and/or time consuming to score phenotypically [24]. In addition, selection for desirable traits can be made on the basis of a single plant whereas this would not be possible by phenotypic selection. Poor heritability does not pose a problem if selection is based on marker information. For traits with complex inheritance every individual genetic component contributing to the trait can be selected separately. Also, multiple characters that would normally be epistatic can be maintained and ultimately fixed [25]. Molecular markers are essential in breeding as recessive genes can be maintained without the need for progeny tests in each generation, as homozygous and heterozygous plants can be distinguished with the aid of co-dominant markers [26]. [27], [28]. The paramount importance of marker assisted selection is achievement of breeding goals in a shorter time than is achieved through conventional breeding, by a precise assemblage of target traits with less unpremeditated crop loss [29].

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MAS can never replace phenotypic selection especially for disease resistance because a final testing of breeding lines is always obligatory, regardless of how tight a marker is linked to a gene or QTL [30]. It has been applied in the main agricultural crops, mainly wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare*), potato (*Solanum tuberosum*), maize (*Zea mays*), fruits and vegetables, particularly tomato (*Solanum lycopersicum*), complemented with some examples from rice (*Oryza sativa*) and soybean (*Glycine max*). Molecular markers have been used at CIMMYT (International Maize and Wheat Improvement Center) in applied wheat breeding to shows 45,000 MAS data points per year are generated between the two wheat growing seasons [31]. In their work, MAS is considered as a technology which will increasingly be adopted in breeding programs in the private and public sectors. According to CIMMYT, the success of utilization of markers in wheat breeding depends to a high degree on the understanding of biotechnologists and breeders working together. MAS in Barley have progressed further than in wheat, which is probably due to the simpler, diploid genome. In contrast to wheat, barley varieties have been released that are based on MAS [32]. Breeding for barley yellow mosaic virus resistance and rust resistance are a main focus of marker selection in Barley. The most important use of MAS in maize is backcrossing of transgenes into elite inbred lines [33], use of microsatellite markers for the conversion of normal maize lines into Quality Protein Maize (QPM), containing more lysine and tryptophan than the native lines [34], or the introgression of favourable QTL for earliness and grain yield between maize elite lines [35]. Marker-assisted resistance breeding in potato and marker-assisted introgression has been successful [36]. Although various markers have been developed [36], practical applications in breeding are still rare. Most marker applications are carried out in experimental populations of diploid potatoes [37], which impede the application of experimental results in practical breeding work. Bean germplasm lines have been improved through MAS too [38], [39], [40], [41], [42], [43], [44]. One white bean (*P. vulgaris* L.) variety that is resistant to bean golden yellow mosaic virus has been released successfully through MAS and carries QTLs for common bacterial blight resistance [45].

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In order to increase productivity and yield stability on farms, scientists need faster and proficient protocols to tag root rot resistance. Such procedures have the potential to facilitate development of common bean cultivars that are farmers need acceptable, well adapted, and marketable and disease resistant common bean cultivars. So far, the level of *Pythium* root rot resistance in the local farmers' bean germplasm remains unknown. The bean breeding program at Kenya Agricultural Research Institute (KARI) Kakamega station has over time developed several root rot resistant bean lines with albeit low farmers acceptability using classical crop improvement methods. This programme could be made more efficient by the development of MAS markers that tag root rot resistance. Since farmers germplasm may possess sources of resistance to this disease, there is need to develop faster seed trait identification processes and screen them for the traits in order to harness germplasm the potential. Because the KARI Kakamega bean breeding program relies on conventional techniques of selection characterized by introgression of desirable traits from exotic bean cultivars, incorporation of marker assisted techniques into the program will reduce duration taken in developing and releasing desirable bean lines. This will ultimately lead to the development of suitable *Pythium* root rot resistant germplasm for marketing purposes and for improved bean productivity in the region [13, 14]. This study therefore investigated the potential of AFLP markers to differentiate resistant and susceptible *Pythium* root rot phenotypes and explored the potential of AFLP markers and in enhance the identification and selection of *Pythium* resistant germplasm for probable utilization in bean improvement programs in the region. Knowledge about identified resistance sources of beans combined with information of the polymorphic levels that exists between the improved resistant bean lines and commercial varieties will reveal the level of diversity and contribute towards development of appropriate protocols with potential to decrease adoption rates and duration taken to release improved bean varieties in Eastern and Central Africa.

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2. MATERIALS AND METHODS

Plant materials: This study was based on a sample of 45 bean seeds obtained from Kenya Agricultural research institute (KARI Kakamega Station) and from a survey conducted in 2010 (Table 1; Table 2). The KARI common bean lines that had been developed in a conventional breeding program were used in developing a marker assisted protocol. Pathogenicity status of the KARI samples has been previously determined [14] [20] as described in table 1. The germplasm included 6 resistant one moderately resistant and 4 susceptible lines (Table 1). Thirty five marketable common

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122 bean samples obtained from a survey conducted in 2010 were used for screening purposes by determining the
123 comparative genetic diversity with the susceptible/resistant bean lines using AFLP markers (Table 2).
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125 **Leaf sample preparation, isolation of genomic DNA and primer selection:** Plant materials selected for the study were
126 grown in the green-house greenhouse sterilized soil filled in 20 cm plastic pots for three weeks. For each bean accession,
127 young fresh leaves about 5 - 6 leaf stage were harvested for DNA extraction using the Qiagen DNA extraction kit,
128 following the instructions of the manufacturer with minor modifications. 300gms of fresh tissue were used. 50 ng/ul of DNA
129 was used in subsequent AFLP analysis. A total of 5 primers (Table 3) previously used for screening the dry bean samples
130 and two universal primers were initially used to analyze the breeding lines collected from KARI the agricultural training
131 institute [14], [65]. Of these, three AFLPs primer combinations were selected due to reproducibility of polymorphic bands
132 and information content.
133

134 **Table 1. *Pythium* resistance status, growth habit and seed size characteristics of bean lines used in the study**

Serial no.	Variety	Reaction to <i>Pythium</i> spp.	Rate of disease development	Growth habit	Seed size
1	RWR- 719	Resistant (RR)	Very low	Determinate	Small
2	AND 1062	Resistant (RR)	Very low	Determinate	Large
3	RWR 1946	Resistant (RR)	Very low	Determinate	Medium
4	RWR 2075	Resistant (RR)	Very low	Determinate	Medium
5	KK 15	Resistant (RR)	Very low	Indeterminate	Medium
6	Scam 80	Resistant (RR)	Very low	Determinate	Medium
7	GLP 585	Susceptible (SS)	Fast	Indeterminate	Small
8	GLP 2	Susceptible (SS)	Fast	Determinate	Medium
9	Mwitmania	Susceptible (SS)	Fast	Determinate	Medium
10	Alulu	Moderately resistant (MR)	Moderate	Determinate	Large

137 Adapted from [14], [20]

138 **Table 2: Characterization of 35 bean accessions used in the genetic diversity study**

Accession Code	Local name	Colour and Seed size	Longitude	Latitude
<i>Mw01</i>	Mwitmania	Pinto and medium	00.26589°	037.75072°
<i>Ro02</i>	Rose coco	Calima and medium	00.26836°	037.66205°
<i>GA03</i>	Gasele	White and medium to large	00.31809°	037.66199°
<i>Mw04</i>	Mwitmania	Pinto bean and medium	00.33229°	037.64431°
<i>Gas05</i>	Gasele	White and medium to large	00.04799°	037.65575°
<i>Can06</i>	Canadian wonder	Kidney red and medium to large	00.12636°	037.56236°
<i>Wai07</i>	Wairimu	Red haricot small	00.56743°	037.49013°
<i>Can08</i>	Canadian wonder	Kidney red and medium to large	00.60266°	037.51808°
<i>Rmw09</i>	Red Mwitmania	Pinto and medium	00.49228°	037.46354°
<i>Roc10</i>	Rose coco	Rose coco; Variegated, purple on cream, medium sized, globular.	00.40642°	037.62504°
<i>Mw11</i>	Mwitmania	Pinto and medium.	00.39233°	037.60790°
<i>Rmw12</i>	Red Mwitmania	Pinto and medium.	00.38413°	037.58708°
<i>Ny13</i>	Nyayo	Calima and medium	01.56349°	037.25222°
<i>Kb14</i>	KATB1	Yellow and medium oblong.	01.56406°	037.25496°
<i>Kik15</i>	Kikuyu	Rose coco; Variegated, red on cream, medium sized, globular.	01.53483°	037.26243°
<i>Roc16</i>	Rose coco	Grey calima and medium	00.12736°	034.73612°
<i>KKf17</i>	KK15	Black and medium oblong	00.05426°	034.74751°
<i>Rh18</i>	Red Haricot	Red haricot small	00.06007°	034.72225°
<i>Sugo19</i>	Sugar 1	Surgar and Medium size oblong.	00.12375°	034.78494°

<i>KKt20</i>	KK 20	Rose coco; variegated with large red flecks on cream, medium to large sized, oblong.	00.11862°	034.80309°
<i>Rosc021</i>	Rose coco	Rose coco; Variegated, purple on cream, medium sized, globular.	00.28283°	034.72213°
<i>Rosc022</i>	Rose coco	Rose coco; Variegated, purple on cream, medium sized, globular.	00.28405°	034.70447°
<i>Sugf23</i>	Sugar 5		00.28305°	034.72014°
<i>Wai24</i>	Wairimu	Red haricot; small to medium sized, oblong.	00.29542°	034.54762°
<i>Okuo25</i>	Okuodho	Canadian wonder; Purple- black, medium to large sized, oblong	00.29649°	034.54858°
<i>Kik26</i>	Kikuyu	Rose coco; Variegated, red on cream, medium sized, globular	00.29649°	034.54858°
<i>Rai27</i>	Raila	Rose coco; variegated with large red flecks on cream, medium sized and oblong.	00.56354°	034.57097°
<i>Katv28</i>	Kat. Variety	Canadian wonder; Purple- black, medium to large sized, oblong	00.25069°	034.75063°
<i>Gi3029</i>	Gasele	Canadian wonder; white to light grey, medium to large sized, oblong	00.24770°	034.75199°
<i>Okuo30</i>	Okuodho	Canadian wonder; Purple- black, medium to large sized, oblong	00.33535°	034.47865°
<i>Mwitmania</i>	Mwitmania	Mwitmania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	Nil	Nil
<i>Mwezi moja</i>	APC	Mwezi moja; many fine purple spots, medium to large sized, oblong.	Nil	Nil
<i>Mwitmania</i>	Mwitmania	Mwitmania; resembles Pinto bean, variegated, green – grey on cream, small to medium sized globular.	Nil	Nil
<i>Raco4C</i>	Rose coco	Rose coco; Variegated, purple on cream, medium sized, globular.		
<i>Gac03C</i>	White haricot	Canadian wonder; white to light grey, medium to large sized, oblong	Nil	Nil

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Table 3._Sequence of AFLP primers used for evaluation of improved bean types from KARI- Kakamega

250 The generated binary data transformed into spreadsheets and subjected to population diversity analysis. Nei's genetic
251 distances were determined. Data scoring and eCluster analysis were performed using simple matching coefficients. The
252 similarity coefficients was subjected to unweighted pair group method of arithmetic averages (UPGMA) and a dendrogram
253 was generated using with the help of GenAlEx software and NTSYS- PC software version 2.1. (Execter software, New
254 York, USA; [49] Rohlf, 2000). Principal component analysis was performed on the dataset. Since According to Rohlf, 2000
255 the the first three components of the multidimensional data set explains the variation in the observed relationships among
256 the population under a study [49], the. These first two Eigen values were plotted to show the degree of similarity among
257 the entries.

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259 3. RESULTS

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261 Polymorphism Genetic distance among the 45 dry bean samples by AFLP analysis:

262 AFLP profiles of the *Pythium* related samples were generated using two AFLP primer combinations (E-TC/M-CAT, E-
263 ACA/M-CTT) and produced a total of 194 polymorphic loci. The size of the AFLP generated fragments by KARI
264 Kakamega dry bean samples ranged between 56 and 414 base pairs and 56- 420bp for the 35 dry bean market samples
265 respectively. Dry bean samples from KARI kakamega revealed a genetic distance of 0.62 to 0.90 (Figure 1). Genetic
266 distance of dry bean samples from farmers and Kari Kakamega samples analyzed together revealed a genetic distance of
267 0.63 – 0.90 (figure 12). *KK15* had a distance matrix of 0.71, close to the distance generated from *SCAM80* and *Alulu* of
268 0.77. There is close genetic relationship between *KK15* and the sub cluster of *SCAM80* and *Alulu* compared to the
269 susceptible sub cluster of *GLP585* and *GLP2* with the distance 0.79. A sub cluster comprising of four resistant varieties
270 (*AND1062*, *R2075*, *R719* and *R1946*) had a distance matrix 0.86. The genetic similarity within these samples is closer
271 compared to the other samples used in the group. *Mw001* was most dissimilar to the rest of the samples used in this
272 analysis with a distance matrix of 0.62, indicating a wide genetic dissimilarity with the rest of the bean samples analyzed.

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277 Cluster analysis

278 The AFLP marker used to delineate the KARI-Kakamega bean lines was able to distinguish the cultivars clearly. A
279 neighbor-joining dendrogram based on Nei's genetic distances [50] clustered the KARI-Kakamega bean lines into four sub
280 groups in which all except *Mw001* that was susceptible segregated together (Fig. 1). The major cluster segregated into
281 three subgroups in which susceptible and tolerant accessions grouped together with the exception of *Alulu* a mildly
282 resistant line that clustered alongside resistant lines in Cluster 1. Cluster 2 formed two subgroups, Cluster 2a and 2b, all
283 comprising of resistant accessions. Cluster 3 comprised of *GLP585* and *GLP2* which were all susceptible. Cluster 3 and 4
284 were located closely and thereby formed the susceptible group while cluster 1 and 2 formed the resistant grouping.

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286 Since the first three components of the multidimensional data set explains the variation in the observed relationships
287 among the population under a study [49], the first two Eigen values were plotted to show the degree of similarity among
288 the entries (Table 4). Principal coordinates analysis clearly a major split between the KARI-Kakamega populations into
289 distinct clusters. Principal component analysis based on allele frequencies among the groups across mapped AFLP
290 markers clustered the bean lines into four groups (Fig. 2). As observed, 27.25% of the variation could be attributed to the
291 first two principal components, 13.02% to the second and 11.31% to the third. Group 1 was comprised of accessions
292 belonging exclusively to resistant group, group 2a and 2b to also resistant cultivars and group 3 and 4 to susceptible
293 cultivars. The first three Eigen values of the ten dry bean lines from KARI Kakamega using NTSYS-PC version, revealed
294 51.59% similarity (Table 4). As observed in the dendrogram (Fig. 1), the lines clustered out into 4 groups that clearly
295 delineated susceptible and tolerant lines. *R1946*, *R719*, *R2075* and *AND1062* are closely related genetically compared to
296 *Mw001*, *KK15*, *Alulu* and *GLP2*. The clustering revealed how diversely related market class Kenyan dry beans are in
297 relation to the bean samples developed from the breeding program at KARI- Kakamega. Principal component analysis of
298 the *Pythium* samples (Figure 2) revealed a closer clustering of the resistant varieties *AND1062*, *R1946* and *R719* and
299 susceptible varieties *GLP2*, *GLP585* clustered together.

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302 On screening the entire bean population using the AFLP markers, the dendrogram produced did not clearly depict the
303 differences as observed in the KARI-Kakamega bean lines. A similarity analysis made on all the bean samples used in
304 this study including the *Pythium* related germplasm was performed to validate the genetic relatedness that existed within
305 the KARI – Kakamega bean samples that had already been evaluated and approved in terms of with their reaction to
306 *Pythium* species together with bean samples present in the market and with farmers (Figure 3-2). The degree of similarity
307 within the samples products from a conventional breeding exercise at KARI- Kakamega breeding station generated a
308 dendrogram with 84 clusters; cluster 1 to cluster 4 (Figure 31). *Mw001*, a susceptible variety to *Pythium* again segregated
309 differently from the rest of the bean population as earlier observed (Fig. 1) was found totally different from the rest of the 9
310 bean samples. Susceptible lines *GLP2* and *GLP585* grouped together in cluster 4 alongside *GA03*. There was distinct

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clustering between the resistant varieties and susceptible varieties in cluster 12 and cluster 43; resistant and susceptible varieties respectively. Cluster 1, 2 and 3 can be said to comprise of *Pythium root rot* tolerant accessions. Cluster 4 comprised of only susceptible accessions. Cluster 5 comprised accessions that segregated alongside *Alulu*, a mildly resistant line and thus may be portrayed to also be mildly resistant. Cluster 7 comprised of accessions that clustered together with *KK15* which is a tolerant line. Thus, cClustering results for the 45 dry bean samples (35 bean samples from farmers and 10 bean samples from KARI kakamega) revealed 8 clusters, with the resistant varieties clustering together with some local seed samples from farmers in cluster 1. *AND1062*, *R2075*, *R719* and *R1946* which are resistant varieties clustered with bean samples from Eastern province (Meru and Embu population). Several resistant varieties clustered in cluster 5 and 7, with susceptible varieties being found in cluster 4 and 8 (Figure 32).

The first three Eigen values of the ten dry bean samples from KARI Kakamega using NTSYS-Pc version, revealed 51.59% similarity (Table 4). *R1946*, *R719*, *R2075* and *AND1062* are closely related genetically compared to *Mw001*, *KK15*, *Alulu* and *GLP2*.

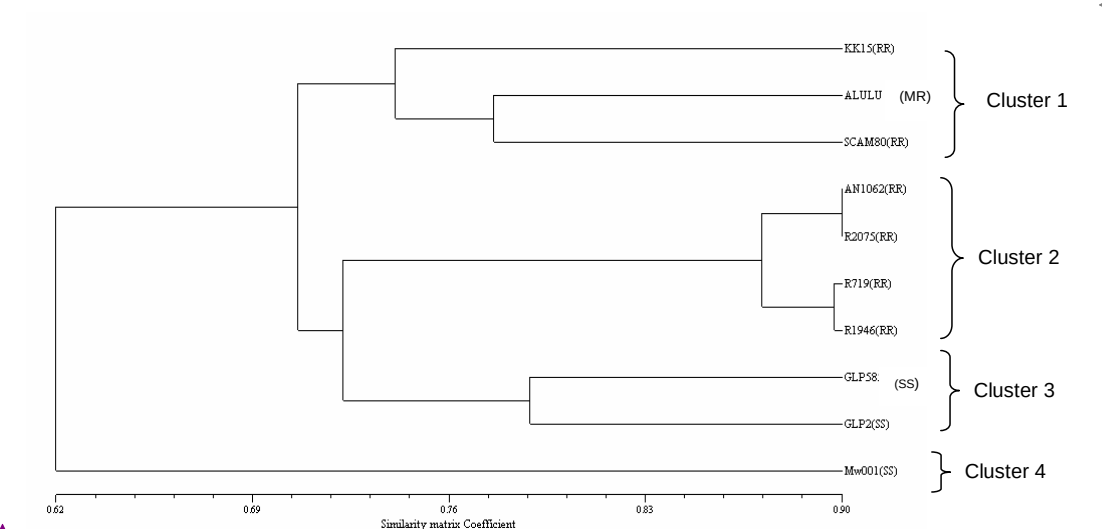


Figure 1: A Neighbor-joining dendrogram of 10 KARI-Kakamega bean lines based on genomic DNA fingerprinting with 194 polymorphic bands generated from two primer combinations of AFLP. The dendograms were drawn based on Nei's (1978) genetic similarity distances using the GenAEx and NYSYS-PC version 2 software.

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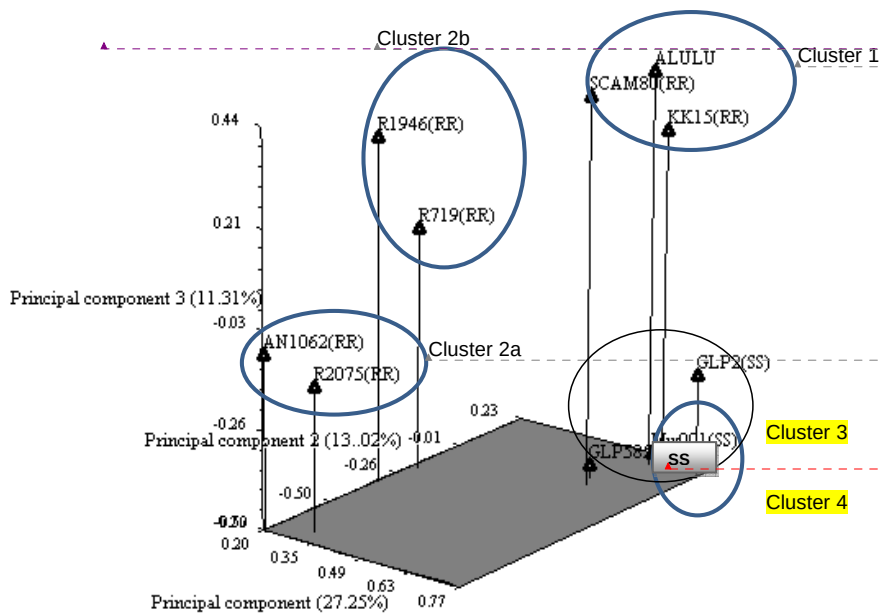


Figure 2: Principal component analysis of 10 breeding bean lines developed in a conventional breeding program based on Nei's genetic distance. The first, second and third principal coordinates are indicated 10 common dry bean lines developed by the KARI Kakamega conventional breeding program on *Pythium* root rot resistance. The PCA describes 51.58% of the variation in the observed relationships existing in the bean samples.

Table 4. Description of genetic variation percentage in the observed relationship among 10 improved bean lines used in the study

Eigen Value	Percent (%)	Cumulative
2.72	27.25	27.25
1.30	13.02	40.27
1.13	11.31	51.59
1.09	10.98	62.58
0.89	8.99	71.57
0.74	7.40	79.04

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The clustering revealed how diversely related market class Kenyan dry beans are in relation to the bean samples developed from the breeding program at KARI Kakamega. Principal component analysis of the *Pythium* samples (Figure 3) revealed a closer clustering of the resistant varieties AND1062, R1946 and R719 and susceptible varieties GLP2, GLP585 clustered together.

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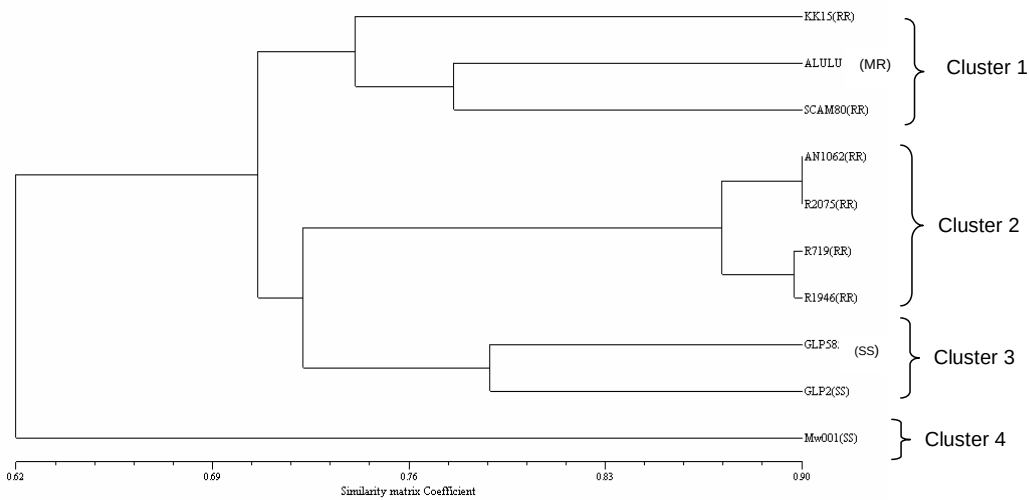


Figure 1: A Neighbor joining dendrogram based on Nei's (1978) genetic similarity distances based on AFLP-PCR results for 10 approved bean varieties

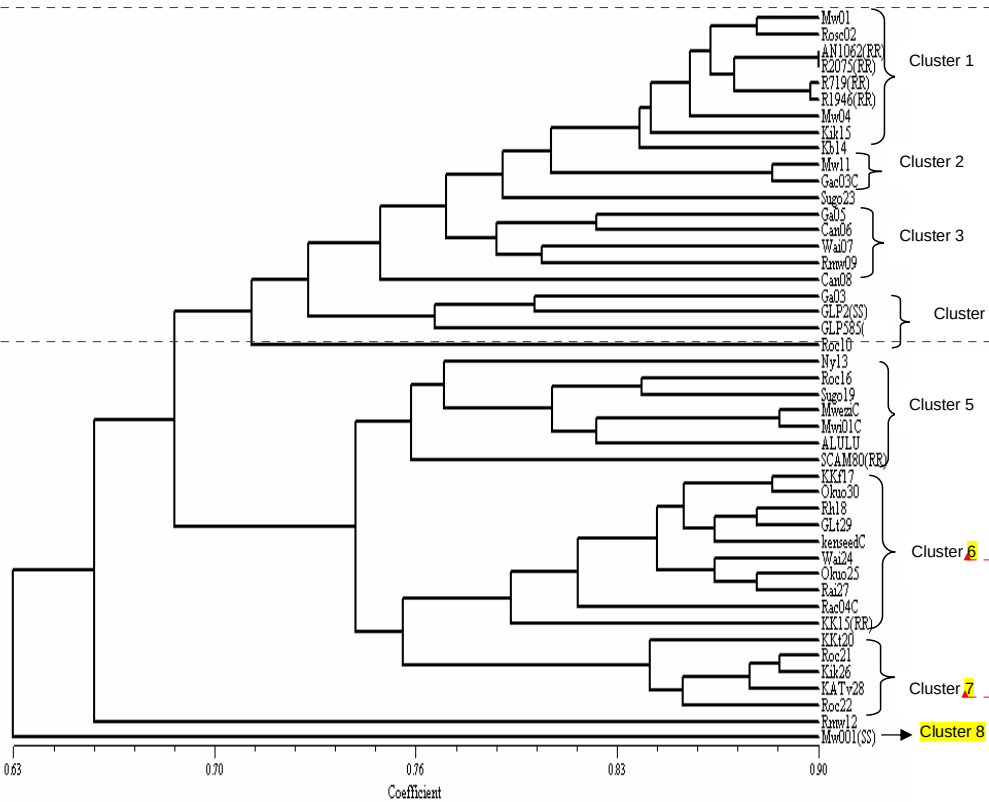
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Fig. 3. AFLP clustering dendrogram generated from matrices of genetic distances obtained by the complement of the similarity coefficients of 45 bean samples of *P. vulgaris* including *Pythium* resistant/susceptible accessions from KARI – Kakamega.

4. DISCUSSION

The AFLP markers used in this study were able to clearly delineate the KARI-Kakamega bean lines into susceptible and tolerant groups with a relatively high degree of precision. Mw001 a susceptible cultivar is definitely very different from all the assessed accessions. Because AN1062, R2075, R719 and R1946 were tightly linked together, they could be the best placed tolerant accessions for use in screening other accessions for tolerance in comparison to KK15 and SCAM80. However it should be noted that Alulu, a mildly resistant bean line segregated together with the resistant lines SCAM80 and KK15. This therefore suggests that it may be closely associated with the resistant lines or the AFLP marker used was not tightly linked to the allele for resistance. That the association may have had to do with unrelated gene loci. From these results it is clear that the AFLP markers used were able to segregate the KARI-Kakamega bean lines with regard to root rot resistance. But as a result of the inability to clearly distinguish Alulu from the other tolerant lines, there is need to use another protocol to test the reproducibility of the results before it can clearly be stated that the markers were proficient.

Principal components analysis is one of the most important methods of ordination analysis. It constructs a new set of orthogonal coordinate axes such that the projections of points onto them have maximum variance. While defined in terms of variances and co variances, PCA is usually applied to standardized data since the results are sensitive to the often arbitrary choices of units of measurement used in a study. Cluster analysis involves performing various types of agglomerative cluster analysis of some type of similarity or dissimilarity matrix. From the bean samples analyzed, a data matrix was computed using the distance coefficients among the columns of the standardized data matrix. The distance matrix is clustered using the single-link clustering method to form of a phenogram. The samples developed from a conventional breeding exercise were subjected to analysis to yield a distance matrix ranging from 0.62 to 0.89.

On screening the whole bean population using the AFLP markers earlier used on the KARI-Kakamega lines, it was evident that although some accessions were clearly segregating alongside either tolerant or susceptible lines as could therefore be identified as so. Thus the AFLP markers used clearly delineated those accessions that clustered out in Clusters 1, 2 and 3 and can be identified as being tolerant. However, some accessions especially those in cluster 7 could not be identified clearly as being tolerant or susceptible. This necessitates a need to explore other AFLP markers or to screen the population with a tried and tested protocol before it can be conclusively be employed as credible marker. Thus the AFLP markers used should not be used alone but in combination with other markers.

AFLP markers have the potential to resolve genetic differences at the level of 'DNA fingerprints' for individual identification and parentage analysis. In the ideal case, a few primer combinations will suffice to generate an adequate number of polymorphic markers. The key feature of AFLP-PCR is its capacity for the simultaneous screening of many different DNA regions that are distributed randomly throughout the genome. To achieve high reliability of the screen, genomic DNA is prepared in an ingenious, but technically straightforward. The large number of percentage (over 50%) on the component analysis indicates this was a successful analysis [5146]. We observed considerable genetic diversity within the marketable common beans of Kenya and resistant and susceptible bean samples conventionally developed from KARI Kakamega. Bean root rot caused by several *Pythium* species has been a quandary on beans (*Phaseolus vulgaris* L.) increasing in importance recently in East and Central Africa as total crop loss when susceptible dry bean varieties are used is evident [3].

Previous studies to monitor *Pythium* root rot disease development have been conducted in a field and screen house setting [5217]. Authors uprooted seedlings and scored lesion development using the CIAT scale of 1-9 [5319]. This formed the basis of conventionally producing the ten common bean samples used in this study. In their experiments, RWR719, MLB 4989A and AND1062 were controls and received disease interaction phenotype score of either 1, 2 or 3 denoting resistance to *Pythium ultimum* while GLP 2 was susceptible (8-9 score) under same conditions [5217].

Molecular characterization of 35 different *Pythium* strains affecting the Ugandan common bean was done using RFLP marker, to demonstrate the wide diversity that exists among different bean plants affected by *Pythium* root rot [5419]. Difficulties in interpreting the relatedness among the isolates were experienced because of wide intra specific variation that exists within the *Pythium* species. To significantly reduce the effects of this disease on the bean crop, identification of sources of resistance to bean genotypes preferred by resource poor small holder farmers is imperative. Resistant

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486 varieties have been identified in Kenya but they were not of preferred market classes [14]. Farmers are reluctant to absorb
487 the new release of resistant bean varieties due to presence of undesirable characteristics. For this reason, farmers prefer
488 to hold on to their preferred from KARI institutions. The farmers save the seed of bean varieties that possess preferred
489 traits such as high yielding, adaptation to local growing conditions, vigorous, early maturing and posing good culinary
490 qualities. Farmers indicate that saving their own seed is economical and there is assurance of seed growth once
491 replanted.

492 Subsequent work of characterization has been done of susceptible common bean varieties from Africa [5529] in different
493 seed sizes, such as the Kenyan varieties GLP 2 and GLP 585 which are respectively large seeds (Andean gene pool) and
494 small seeds (Mesoamerican gene pool), the Ugandan variety CAL 96 (Calima) with large seeds (Andean gene pool) and
495 the Rwandan variety Urugezi with an intermediate seed size (Mesoamerican gene pool). All the resistant common bean
496 varieties have been characterized into different sizes and are advanced lines from an international breeding nursery run
497 by CIAT (Cali, Colombia). This include the small-seeded variety RWR 719 from the Mesoamerican gene pool, the
498 intermediate-seeded varieties *MLB 49-89A* and *SCAM 80-CM/15*, both from the Mesoamerican gene pool, and the large-
499 seeded varieties AND 1055 and AND1062, both from the Andean gene pool [3].

500 *Pythium* root rot resistance is controlled by a single dominant gene [2456]. Experiments in earlier studies involved
501 investigating the genetic relationship between *Fusarium* and *Pythium* resistance inheritances in a resistant bean line *RWR*
502 *719*. There is an association of PYAA19₈₀₀ with *Pythium ultimum* resistance in *RWR 719*, *MLB 49-89A* and *AND-1062*,
503 located 1.5cM from the resistance gene [5722]. These common bean varieties are possible sources of resistance to
504 *Pythium* root rot disease to market acceptable common beans in Kenya. The most effective way of managing bean root
505 rot for small scale farmers is exploiting the host plant resistance [58, 5923,24]. *KK15* had a distance matrix of 0.71, close
506 to the distance generated from *SCAM80* and *AluluLULU* of 0.77. There is close genetic relationship between *KK15* and
507 the sub cluster of *SCAM80* and *AluluLULU* compared to the susceptible sub cluster of GLP585 and GLP2 with the
508 distance 0.79. A sub cluster comprising of four resistant varieties (*AND1062*, *R2075*, *R719* and *R1946*) had a distance
509 matrix 0.86. The genetic similarity within these samples is closer compared to the other common bean samples used in
510 the group. Major crosses can be conducted to transfer resistance from resistant varieties to the market acceptable
511 varieties.

512 Resistance to *Fusarium solani* is complex and is conditioned by two or more genes [6025, 261, 6227]. *Pythium ultimum*
513 resistance is controlled by a single dominant gene, marked by a dominant SCAR marker-PYAA19₈₀₀ [3, 14, - 6227]. The
514 presence of joint resistance to both *Fusarium* and *Pythium* species in root rots have been observed in several resistance
515 sources [4, 9, 2863]. Some market class samples are closely related genetically to the *Pythium* related samples
516 developed in a conventional breeding exercise at KARI- Kakamega. In a further breeding exercise, these bean samples
517 can be used as susceptible parents in efforts to obtain crosses for transfer of the resistant genes in improvement
518 programs.

519 Eight major clusters were obtained with the cluster analysis majority of the resistant bean samples from KARI clustering
520 with bean varieties from Eastern province (Meru and Embu population) and Western province (Kakamega region) (Figure
521 2). In the first cluster four resistant varieties clustered among market classes (*AND1062*, *R2075*, *R719* and *R1946*). The
522 fifth and seventh cluster had two resistant varieties *SCAM 80* and *KK15*. *KK15* is a recent bean line that is resistant to
523 *Pythium* root rot, is high yielding and fast maturing. Most farmers find the colour of the seed coat unacceptable, therefore
524 as much as it is a wonder seed that is being taken up by small scale farmers to counteract poverty and improve food
525 availability, further crossing should be done to break the linkage between the gene responsible for resistance and seed
526 coat colour, to increase the acceptance rate of this new improved variety among the bean farmers in Kenya. However,
527 since the clustering produced mismatching, it is possible that there may be linkage to the pathogenicity of the bean
528 germplasm. There is need to determine the pathogenicity status of the 35 bean samples and comparisons made with the
529 molecular markers' findings of this study.

530 Efforts to integrate *Pythium* root rot resistant gene into Rwandan susceptible varieties have been successful after carrying
531 out crosses between three local susceptible varieties and two known sources of resistance to *Pythium* root rot, *R719* and
532 *AND1062* [6429]. The Rwandan bean profile of resistance to *Pythium* found in the progenies from back-crosses between
533 (*RWR 1668*, *R617-97A* and *Urugenzi*, as recipient parents) and the selected sources of resistance (*RWR 719* and *AND*
534 *1062*, as donor parents) is achievable. At each back cross generation A SCAR marker PYAA 19₈₀₀ has been used and
535 found to be linked tightly to the gene responsible for resistance in *R719* and *AND1062*. This is a feasible exercise which
536 can be experimented in Kenyan bean breeding institutions to help improve resistance of market acceptable beans to
537 attacks by root rots. However, needs further work with SCAR marker PYAA₈₀₀ and propagation to enhance the
538 transference of the resistant gene to acceptable bean samples of market class in Kenya.

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Knowledge about identified resistance sources of beans combined with information of the polymorphic levels that exists between the improved resistant bean lines and commercial varieties will reveal the level of diversity and contribute towards development of appropriate protocols with potential to decrease adoption rates and duration taken to release improved bean varieties in Eastern and Central Africa. Effective bean root rot management calls for the use of integrated approaches with a strong component of resistant varieties. To design resistant gene development in bean varieties ideal by resource poor small holder farmers, long lasting strategies are dependent on the identification of useful genes and a good understanding of the host – pathogen interaction. The use of resistant cultivars is the most viable option for controlling bean root rot, particularly for small-scale growers to solve this quagmire in the agricultural sector of bean breeding. To increase the productivity and yield stability of the dry bean of Kenya, there is need to come up with varieties that are well adapted to the changing climate, fit consumers' trait preferences and are resistant to root rots. Knowledge about identified resistance sources and mode of inheritance of resistant genes is needed to develop more stable resistant bean cultivars to be adapted to regions of Eastern and Central Africa where bean root rot is an increasing problem.

In view of the lack of public access to primers like SCAR marker PYAA₈₀₀ with proven capability to tag resistant and susceptible varieties, there is an need to redesign the primers used in this study with an aim to increasingly target the resistant gene. However, since there are primers already designed in similar studies in Rwanda and Colombia, it is desirable that the same population be subjected to screening using such markers and comparisons with this study made.

Fig. 2. AFLP clustering dendrogram of 45 bean samples of *P. vulgaris* with *Pythium* related samples from KARI — Kakamega samples loci based on simple matching coefficient similarity matrix.

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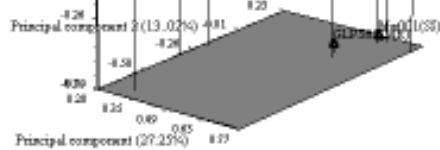


Fig. 3. Principal component analysis describing relationship between 10 bean samples developed from a KARI - Kakamega conventional breeding exercise on *Pythium* root rot resistant varieties.

5. CONCLUSION

This study has revealed the genetic relationships between and within bean population from the study areas by use of AFLP marker. It is a preliminary work to be advanced towards developing a protocol for a breeding exercise for marketable *Pythium* resistant common bean. It has yielded useful information on the level of polymorphism and genetic relatedness between common bean varieties developed from KARI Kakamega through a convectional breeding program and bean varieties of market classes of Kenya. However, it did not conclusively segregate resistant and susceptible bean lines to distinction and should be used cautiously. Designing our own primers to target the resistant gene could take a long duration of time, which wasn't sufficient within the time frame available to carry out this study. There are primers already designed in similar studies in Rwanda and Colombia, but lack of public access to these primers designed made the complete of the study of developing a protocol to tag resistant and susceptible varieties unfeasible. Therefore AFLP is a potential marker which can be used to help breed for *Pythium* resistance in market class of beans thus relieve the small scale farmer of food insecurity and increasing poverty.

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7. COMPETING INTERESTS

"Authors have declared that no competing interests exist."

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