

## Genetic diversity of blackberry (Rubus subgenus **Rubus Watson) in Kenya using Simple Sequence Repeats (SSRs)**



# Capacity Bailding in Agriculture



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## Introduction

Blackberry has its centre of origin in Eurasia and Northern America and is widely present as wild types in Kenya (Clark et al., 2007). It is rich in antioxidants, flavonoids and phenolic compounds and is considered as anti-carcinogenic against oral, oesophageal and colon cancers (Overall et al., 2017)

#### **Plant Material /Collection of** germplasm

The blackberry samples taken were coded to reflect the county, district, division, subdivision, village and the collection number.

Genomic DNA Isolation and quantification was carried out from each dry young leaf using a modified CTAB protocol (Doyle and Doyle, 1990)

#### **Analysis of Molecular Variance (AMOVA)**

Table 3: Analysis of Molecular Variance (AMOVA) of the diversity of 90 blackberry accessions collected from selected counties in Kenya.

| Source of variation | DF | SS      | MS    | Estimated.<br>Variation. | Total Variation. % | P( <i>f</i> ) |
|---------------------|----|---------|-------|--------------------------|--------------------|---------------|
| Among accessions    | 6  | 39.059  | 6.510 | 0.198                    | 5                  | 0.008         |
| Within accessions   | 83 | 341.574 | 4.115 | 4.115                    | 95                 | 0.008         |
| Total               | 89 | 380.633 |       | 4.313                    | 100                |               |

Analysis of Genetic Diversity (GD) is essential for efficient breeding and improvement of its pomological traits and yield.

GD is vital for accrued benefits in genomics research, counteracting genetic erosion and understand evolutionary relationships

This leads to design of genetic conservation and breeding strategies (Jacob et al., 2017).

Simple Sequence Repeats (SSRs) were used to determine the genetic diversity of 90 blackberry accessions collected from 6 different counties in Kenya.

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#### **PCR Amplification and Microsatel**lites Analysis

11 out of 13 available blackberry SSR primer sets previously described by Castillo et al. (2010) were selected.

Primer *RhM031* was uninformative while *RiG001* failed to amplify any blackberry and hybrid accessions and was used to identify raspberry genotypes.

Alleles were scored as absent or present based on the size of the amplified product using a 100bp O'geneRuler ready to use DNA Ladder (Thermo Fisher Scientific Inc., USA).

#### **Data Analyses**

GenAlEx 6.5 (Peakall and Smouse, 2012) was used to cal-

culate genetic the diversity indices.

Table 1: Sequences, annealing temperatures and size of bands of sets of 13 primers used to screen 90 blackberry accessions collected from different regions in Kenya.

#### **Principal Coordinate Analysis (PCoA)**



Figure 1: Distribution of blackberry accessions by region of collection under principal component axes 1 and 2. BRG: Baringo; CV: Plant Introductions; KCO: Kericho; LC: Laikipia; NAK: Nakuru; NDI: Nandi; UG: Uasin Gishu

#### **Dendrogram generated by Jaccard's** similarity coefficients



### UDJECUVES

•To determine the diversity of genetic blackberries Kenya SSR markers. using

• To map blackberry germplasm occurance in kenya

It is aimed that the findings of this study will re-solve the taxonomic uncertainty of duplicate acces-sions in in-situ and ex-situ blackberry gene banks in Kenya and to document the extent of genetic diversity of the local blackberry for breeding purposes

## Materials and methods

Germplasm were collected from Nandi (0.1036° N, 35.1777° E) (14), Nakuru (0.3031° S, 36.0800° E) (26), Kericho (0.3689° S, 35.2863° E) (16), Uasin Gishu (0.5143° N, 35.2697° E) (7), Laikip-(0.3970° N, 37.1588° E) (6) and Baringo (0.4897° N, 35.7412° E) (21) totaling 90.

| rimers         | Primer sequence (5'-3' <u>)y</u>                 | SSR Motif | Ta (°C) | Expected size (bp) |
|----------------|--|-----------|---------|--------------------|
|                |  |           |         |                    |
| iM05F          | CGACACCGATCAGAGCTAATTC                           | (ATC)5    | 62      | 344-364            |
| iM01R          | ATAGTTGCATTGGCAGGCTTAT                           |           |         |                    |
| iM01F          | GAAACAGGTGGAAAGAAACCTG                           | (TG)6     | 59      | 181-201            |
| iM01R          | CATTGTGCTTATGATGGTTTCG                           | (1.2)     |         |                    |
| iM01F<br>iM01R | ATTCAAGAGCTTAACTGTGGGC<br>CAATATGCCATCCACAGAGAAA | (AG)12    | 59      | 140-196            |
| iM03F          | AGCAACCACCACCTCAACTAAT                           | (TG)7     | 51      | 227-335            |
| iM03R          | CTAGCAGAATCACCTGAGGCTT                           |           |         |                    |
| hM001F         | GGTTCGGATAGTTAATCCTCCC                           | (CA)7     | 51      | 229–282            |
| hM001R         | CCAACTGTTGTAAATGCAGGAA                           |           |         |                    |
| hM003F         | CCATCTCCAATTCAGTTCTTCC                           | (TG)10    | 50      | 173-264            |
| hM003R         | AGCAGAATCGGTTCTTACAAGC                           |           |         |                    |
| hM011F         | AAAGACAAGGCGTCCACAAC                             | (TC)18    | 56      | 252-346            |
| hM011R         | GGTTATGCTTTGATTAGGCTGG                           |           |         |                    |
| hM018F         | CACCAATTGTACACCCAACAAC                           | (CTT)6    | 54      | 363–381            |
| hM018R         | GATTGTGAGCTGGTGTTACCAA                           |           |         |                    |
| hM021F         | CAGTCCCTTATAGGATCCAACG                           | (TC)6     | 50      | 252-315            |
| hM021R         | GAACTCCACCATCTCCTCGTAG                           |           |         |                    |
| hM023F         | CGACAACGACAATTCTCACATT                           | (CAT)5    | 53      | 116-206            |
| hM023R         | GTTATCAAGCGATCCTGCAGTT                           |           |         |                    |
| hM031F         | CAACCTAATGACCAATGCAAGA                           | (CT)9     | 50      | 0, 391–433         |
| hM031R         | GCAGAATCCATTCTCTTGTTGA                           |           |         |                    |
| hM043F         | GGACACGGTTCTAACTATGGCT                           | (AC)6     | 56      | 332-386            |
| hM043R         | ATTGTCGCTCCAACGAAGATT                            |           |         |                    |
| iG001F         | TGTCCGATCCTTTTCTTTGG                             | (AT)6     | 55      | -                  |
| iG001R         | CGCTTCTTGATCCTTGACTTGT                           |           |         |                    |

## Results

**Diversity Indices of SSR Loci in Blackberry** 

#### Accessions

This study revealed moderate to significant differentiation (0.05> FST  $\geq$ 0.15) within the blackberry accessions (Table 2).

Figure 2: Dendrogram generated by Jaccard's similarity coefficients among 90 blackberry accessions. The accessions are depicted using the following colour codes: Red = Nakuru; Green= Kericho; Blue= Baringo; Yellow = Nandi; Black = Laikipia; Purple = Uasin Gishu and Orange = Plant Introductions (PIs).

Often, high HE values are observed when wild populations are grown in close proximity to cultivated populations, and this may explain the high HE values obtained in the cultivated types

## Conclusion

Findings from this research revealed that even with hybridizations and inbreeding depression, there is still a wide array of genes to be explored in breeding blackberry in Kenya.

The best markers for genotyping blackberry from this study were *RiM017*, *RhM043*, *RiM015* and *RhM001*.





Table 2. Estimates of genetic diversity of SSR loci used to screen 90 blackberry accessions sampled from 6 counties in Kenya.

| SSR<br>loci  | Range<br>of bps | A<br>E | I    | H<br>o | H<br>E | <b>F</b> <sub>IS</sub> | F<br>ST | М    | <b>P</b> I<br>C |
|--------------|-----------------|--------|------|--------|--------|------------------------|---------|------|-----------------|
| RhM01        | 252-346         | 1.86   | 0.65 | 0.31   | 0.46   | 0.314                  | 0.14    | 0.63 | 0.357           |
| RiM01        | 146–196         | 1.83   | 0.64 | 0.18   | 0.45   | 0.711                  | 0.05    | 0.32 | 0.729           |
| <b>RiM01</b> | 181-201         | 1.99   | 0.69 | 0.27   | 0.50   | 0.355                  | 0.17    | 0.38 | 0.611           |
| RhM04        | 332-386         | 1.99   | 0.69 | 0.60   | 0.50   | -0.048                 | 0.03    | 0.32 | 0.690           |
| RiM01        | 344–364         | 1.95   | 0.68 | 0.50   | 0.49   | -0.164                 | 0.04    | 0.26 | 0.753           |
| RhM00        | 229–282         | 1.98   | 0.69 | 0.63   | 0.50   | -0.359                 | 0.04    | 0.47 | 0.629           |
| RiM03        | 227-335         | 1.98   | 0.68 | 0.87   | 0.49   | -0.800                 | 0.01    | 0.57 | 0.493           |
| RhM01        | 363-381         | 1.96   | 0.68 | 0.55   | 0.49   | -0.635                 | 0.00    | 0.64 | 0.505           |
| RhM00        | 173-264         | 1.99   | 0.69 | 0.87   | 0.50   | -0.863                 | 0.00    | 0.76 | 0.366           |
| RhM02        | 252-315         | 1.51   | 0.52 | 0.43   | 0.34   | -0.379                 | 0.10    | 0.56 | 0.371           |
| RhM02        | 116-206         | 1.61   | 0.56 | 0.41   | 0.48   | -0.374                 | 0.01    | 0.51 | 0.375           |
| Mean         |                 | 1.88   | 0.65 | 0.54   | 0.46   | -0.234                 | 0.05    | 0.50 | 0.520           |
| S.E          |                 | 0.05   | 0.19 | 0.16   | 0.17   |                        |         |      |                 |

Effective number of alleles (AE), Shannon's diversity index (I), observed heterozygosity (HO), expected heterozygosity (HE), inbreeding coefficient (FIS), pairwise genetic distance between populations (FST), major allele frequency (M) and Polymorphic Information Content (PIC).

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