ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/309491385

# Genotyping of Kenyan Passiflora Edulis Flavicarpa Hybrid Accessions and their Parents using SSR Markers

**Article** · October 2016

DOI: 10.4172/2229-4473.1000181

CITATIONS
0

READS
13

**8 authors**, including:

Felix Matheri
Kenyatta University
**14** PUBLICATIONS **3** CITATIONS
SEE PROFILE

Maina Mwangi
Kenyatta University
**110** PUBLICATIONS **232** CITATIONS
SEE PROFILE

Mathew Piero Ngugi
Kenyatta University
**113** PUBLICATIONS **138** CITATIONS
SEE PROFILE

**Some of the authors of this publication are also working on these related projects:**

Antipyretic and antinociceptive properties of Harrisonia abyssinica Oliv and Landolphia buchananii(Hallier f.) Stapf in animal models View project

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: Mathew Piero Ngugi
Retrieved on: 31 October 2016

Matheri et al., Vegetos 2016, 29:4
DOI: 10.4172/2229-4473.1000181

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

# Genotyping of Kenyan *Passiflora Edulis Flavicarpa* Hybrid Accessions and their Parents using SSR Markers

**Felix Matheri[1*], Fred Teya[1], Festus Kioko[1], Amos Musyoki Mawia[1], Maina Mwangi[2], Duncan T Kirubi[2] Mathew Ngugi[1] and Steven Runo[1]**

## Abstract

The Passion fruit is a significant income earner for Kenya, ranking third after avocado and mango in terms of foreign exchange earnings. Increased research activities targeting variety improvement and development of new varieties have led to development of new passion fruit varieties by KALRO. These varieties are KPF-4, KPF-11 and KPF-12 which are hybrids of natural crosses of the coastal yellow varieties and purple variety. Despite these gains in breeding, there is little information on molecular variability of the hybrids as well as the parents. The present study aimed at evaluating the genetic variation of the hybrid and parent varieties using SSR markers. DNA was extracted using a modified CTAB protocol followed by PCR and analysis done using DARWIN 6 and GenAlex 6.502 software. The resulting dissimilarity matrix showed existing genetic variability among accessions within and among the varieties studied. Further, the results of principal component analysis and phylogenetic tree showed genetic homogeneity within the assigned populations. The findings of this study will supplement the existing body of knowledge in passion fruit breeding.

**Keywords**

Hybrids; Parents; SSR markers; Variability

## Introduction

The passion fruit is an allogamous species belonging to family *Passifloraceae* [1]. There are two main forms of domesticated passion fruit species; the yellow and purple forms. The purple form bears dark-purple; nearly black, rounded fruits of about 5 cm long, weighing 30-45 g. On the other hand, the yellow form bears deep yellow fruits, similar in shape to the purple form but higher length 6 cm (2.5 inches.). The weight of fruits of the yellow form is also comparatively higher than that of purple form; weighing 60-90 g and averages about 75 g [2,3]. The species of the family *Passifloraceae* are widely distributed in tropics and subtropical regions where they exist as wild or cultivated crops [4,5].

The yellow passion fruit has attracted great attention from fruit producers, due to its fast production cycle and high juice yield compared to other closely related fruit species [6]. On the other hand, the purple passion fruit has a favorable taste, due to its high sugar content compared to the yellow passion. However, compared to yellow passion fruit cultivars such as C5 and Brazil, the purple form is less tolerant to soil borne pathogens. The existence of contrasting superior traits of the two forms, there are concerted efforts to produce hybrids of the two forms, combining the superior traits from each of the parents. Such research activities were undertaken by the Kenya Agricultural and Livestock Research Organization (KALRO), for the development of three hybrids of the Coastal yellow varieties and the purple variety [7,8,9]. This conferred the hybrids varieties ability to tolerate *Fusarium,* a pathogen that affects the purple variety whereas the coastal yellow varieties are tolerant to it. Other superior traits passed on to the hybrids are high juice yield from the Coastal yellow variety and high sugar content from the Purple variety. The hybrids are; KPF-4, KPF-11 and KPF-12 which are yellow skinned varieties, despite sharing genetically from both Coastal yellow varieties and purple skinned varieties [10,11,12].

Advances in DNA based technology have allowed the integration of the existing genetic information in the selection of superior genotypes as well as evaluation of heritability of favorable traits from parents to their hybrids. Therefore, based on the knowledge of the level of genetic variation in a population and the greatest genetic distance between the plants, the genetic gains can be quantified through techniques such as SSR-PCR [13,14].

## Materials and Methods

A stratified randomized design was used for collection of plant material for this study. Young leaves were individually collected from vigorous healthy, growing vines from plants belonging to the KPF 4, KPF 11, KPF 12, Brazil, and purple varieties with replicates per variety being assigned to populations based on the respective variety. The samples were dried in silica gel granules and transported to Kenyatta University Plant Transformation Laboratory where they were kept at -80°C awaiting DNA extraction.

### DNA extraction

DNA was extracted using a modified cetyl trimethyl ammonium bromide (CTAB) protocol [15]. A 1% agarose gel was used to estimate genomic DNA concentrations and quality by comparing the fluorescent signal from DNA stained with Sybr green dye. Formation of distinct and bright bands was used as an indication of a quality DNA.

### Primer selection

Twelve sets of SSR primers of the PE series (*Passiflora edulis Sims*), were used (Table 1) [16].

### Polymerase chain reaction (PCR)

PCR amplification was done in a thermocycler Applied Biosystems (E2720 thermo-cycler). Each reaction contained 22 mM KCl; 20 mM Tris-HCl (pH 8.9 at 25°C); 1.8 mM $MgCl_2$; 22 mM $NH_4Cl$; 0.05% Tween® 20; 5%glycerol; 0.06% IGEPAL® CA 360; 0.2 mM of each dNTPs; 0.5 µM of each primer; 2 µg (1 µg/µl) DNA

***Corresponding author:** Matheri Felix, Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, P.O. Box: 43844-00100, Nairobi, Kenya, Tel: 871090 Ext. 3711; E-mail: felmat06@yahoo.com

**Received:** September 02, 2016 **Accepted:** October 13, 2016 **Published:** October 18, 2016

SciTechnol International Publisher of Science, Technology and Medicine

All articles published in Vegetos: International Journal of Plant Research are the property of SciTechnol, and is protected by copyright laws. Copyright © 2016, SciTechnol, All Rights Reserved.

**Table 1:** SSR loci, primer sequence, motif, expected allele size (bp) of the primers.

| Locus | Forward Primer | Reverse Primer | Repeat Motiff | Estimated bp |
|---|---|---|---|---|
| PE74 | ccctcttatcaatagcgttgg | gcacgagcacgagtatttatt | $(ATCACA)_5$ | 215 |
| PE38 | gatcggtcctcggttagac | agtcacacagcatgagaaatc | $(TG)_8$ | 215 |
| PE58 | gcaatttcaccatcttctgct | ccacggtcatggatgttc | $(AC)_{11}$ | 243 |
| PE11 | gcataagttgtcggtcttgg | cctcgaacctctatcatcca | $(GT)_{11}$ | 178 |
| PE04 | atgcttttggaaatccgttt | tgctcatgcaaagtcactgg | $(TG)_9$ | 235 |
| PE24 | tcaaactgaactcgtaaagg | gtgctgggagactgatgtt | $(CA)_{15}$ | 294 |
| PE66 | ccatagtcccaacaagcatc | gctgtggaccctaactcagtc | $(AC)_9$ | 165 |
| PE90 | tcaggaagattgcatgttagt | ctgggttttgtttatgttgc | $(AGC)_5$ | 245 |
| PE18 | ccgtgaaccaaccatttctc | ttgcagcacaaacaagtcaa | $(TG)_9$ | 220 |
| PE20 | aggatcaccatagaaaaccat | gttaggttggcattgctctct | $(AAAC)_4$ | 242 |
| PE42 | gtcacttcattcttcctttcc | ttagcccactcaaacacaa | $(GT)_8$ | 216 |
| PE75 | cacaatcggtgggaaagata | gtagttttgggcagtttgc | $(TG)_{17}$ | 178 |

template and 25 units/ml of One Taq DNA Polymerase to make a final volume of 25 µl.

### Analysis of the PCR products

The PCR products were then separated on 3% agarose gels to enable visualization of polymorphism. The PCR products were prepared by adding 5 µl loading buffer (0.05% Bromophenol blue, 1 mM EDTA, 10% Ficoll, 20% sucrose and 5 M urea) and 5 µl of Sybr® green dye to 5 µl of each sample. One well was left on one side of the gel and was loaded with 5 µl of 100 bp DNA ladder (Invitrogen®). The gels were then run at 80V for one and a half hours.

### Data analysis

Each DNA fragment was treated independently with presence being reported as 1 while absence was reported as 0. Analysis of variability of the accessions was then done using a dissimilarity matrix and a dendrogram was then constructed using neighbor joining tool of DARWIN® version 6. Viewing of the phylogenetic tree was done using Treeview® software. Analysis of molecular variance (AMOVA) was carried out to determine variation within and among the different populations while PCoA based on Nei's genetic distance [17] was used to visualize the genetic relationship among the accessions using GenAlex® version 6.502 software [18].

## Results

Of the 12 SSR markers, 1 marker (PE 20) was found to be monomorphic and showed only one allele (uniform band size of 300 bp) among all the accessions and as such could not be used for the variability studies.

Results of Analysis of Molecular Variance (AMOVA) indicated 57% variation among population and 43% variation within populations, P<0.001 (Table 2).

The results of Principle coordinate analysis (PCoA) are as shown in Figure 1.

The first two principle axes accounted for 31.79% and 24.5% variation respectively. All accessions within each population were located on the same coordinate axis. Some of the accessions within a population were placed on the same graphical location on the scatter plot. Such combinations included accessions; KRP-2, KRP-3 and KRP-4; all which belonged to the purple passion fruit variety.

### Clustering of *Passiflora edulis* accessions using neighbor joining

The phylogenetic tree revealed that there was genetic relatedness among the accessions within the different populations based on the 11 polymorphic SSR markers. The accessions were clustered into three major clusters; I, II and III. The dendrogram also revealed that the farthest phylogenetic distance was that between KRC-2 (a Coastal accession) and KRP-1 (Purple accession) (Figure 2).

A dissimilarity matrix targeting shared SSR alleles among the selected hybrid and parent *Passiflora edulis* accessions was computed using DARWIN version 6 [19] was presented in Appendix 1 .The pairwise genetic dissimilarity presented showed that the dissimilarity values ranged from 0% (KRP-2 vs KRP-4) to 90% (KR11-1 *vs* KR12-4).

## Discussion

The evaluation of genetic variability is important in germplasm characterization and breeding. The existing genetic variation among the studied accessions a value for inter-population variation such as that on the *Passiflora* genus (7%) [21]. On the other hand, the Intra-population variation (43%) was much lower than the findings of previous studies [20,21].

The neighbor joining tree showed great divergence among the accessions under study, indicating the underlying genetic variability among the varieties to which the accessions under study belong. Furthermore there was genetic homogeneity within the assigned populations, where all accessions from a specific population were clustering closely. Clustering of accessions belonging to the hybrid varieties KPF-4 (KR4-1, KR4-2, KR4-3 and KR4-4) and KPF-11 (KR11-1, KR11-2, KR11-3, and KR11-4) together with accessions of one of the parent varieties (Purple) in main cluster I is an indication of their closer relatedness compared to other varieties. This close genetic relation may have been through shared alleles among the respective varieties. These alleles can be traced from the purple variety which is a parent variety of the two hybrids (KPF-4 and KPF-11). The closeness may also be due to the use of loci in preserved regions of the genome which may have contributed to this close grouping [22].

Graphical analysis of the two main coordinates revealed genetic dissimilarity among the various assigned populations. The analysis also confirmed closeness of accessions belonging to the same population, indicating that they indeed belong to the same homogenous variety. The closeness of some accessions on the scatter plot indicates that they shared a closer ancestry compared to other accessions in the population and other populations. For example the graphical closeness of accessions; KRP-2, KRP-3 and KRP-4 on the scatter plot indicate their much closer genetic ancestry compared to KRP-1 which belonged to the same variety (purple).

From the results of the dissimilarity matrix, the high dissimilarity observed between KR12-4 and KR11-1, accessions from KPF-12 and KPF-11 respectively, is an indication that both accessions are potential progenitors, useful in intraspecific interbreeding [23]. This

**Table 2:** Analysis of molecular variance (AMOVA),Degrees of freedom (df), sum of squares (SS), mean of square (MS), % variation and P-values.

| Source | df | SS | MS | Est. Var. | % |
|---|---|---|---|---|---|
| Among Pops | 4 | 29.600 | 7.400 | 1.554 | 57% |
| Within Pops | 15 | 17.750 | 1.183 | 1.183 | 43% |
| Total | 19 | 47.350 | - | 2.738 | 100% |

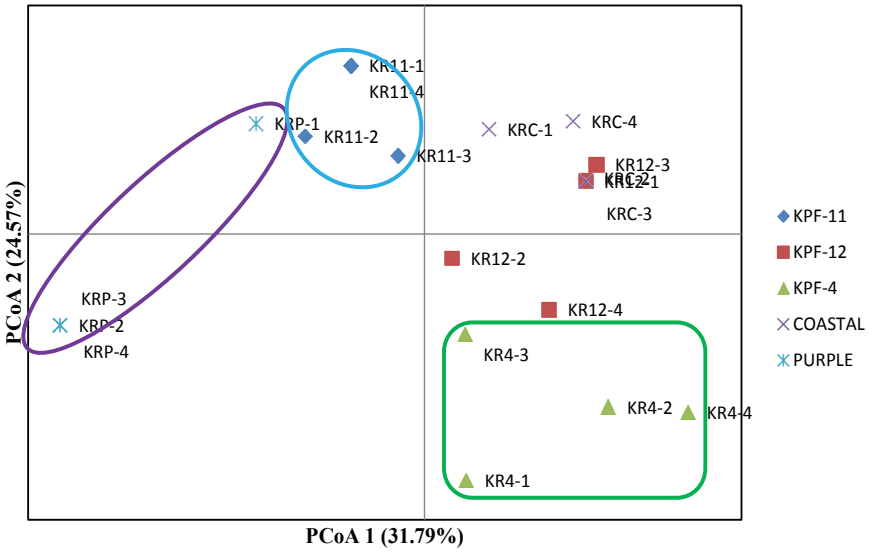


**Figure 1:** Principle coordinate analysis of the *P.edulis* accessions based on 11 SSR markers.

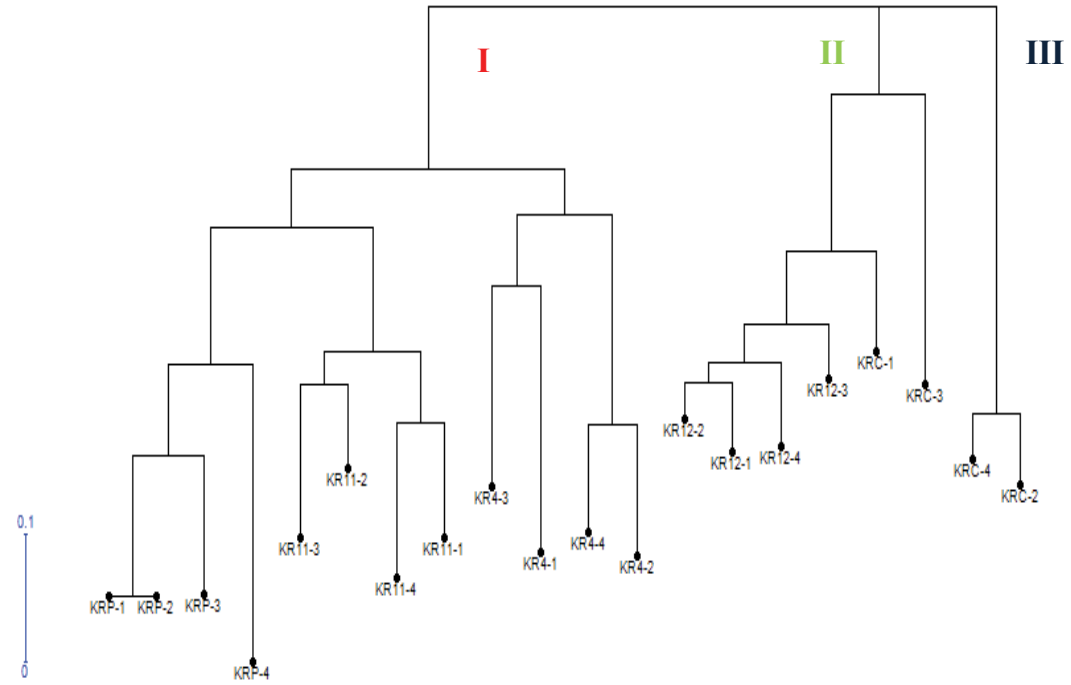


**Figure 2:** A Neighbor Joining tree showing the genetic relationships among the *Passiflora edulis* accessions based on the 11 polymorphic SSR markers under study.

implies that the two accessions are candidates for furtherance of genetic variability which is important for passion fruit breeding. The analysis of intraspecific variability among the four accessions in each population reveals genetic dissimilarity among plants within the same assigned population, confirming that their origin is by crosses and not as clones. However the lack of dissimilarity between combination KRP-2 vs KRP-4 can be interpreted to mean that they are biological replicates and could therefore be clones rather than products of crosses. The findings of 0% dissimilarity of the combination KRP-2 vs KRP-4 corroborates the findings of the phylogenetic tree which indicated that the two accessions were closely associated and are replicates on the tree.

## Conclusion

The findings of the current study are consistent with those of other studies that the wide genetic variability observed among the varieties under study is a characteristic of the genus *Passiflora*. Understanding of genetic variability is therefore a fundamental aspect for the conservation and maintenance of genetic resources in passion fruit breeding programs [1,24]. Studies of genetic variability also help

in understanding of kinship between genotypes and identifying the most suitable parents to obtain higher genetic gains in segregating populations [25,26].

### Acknowledgements

The authors wish to thank the Kenya Agricultural Productivity and Agribusiness Program (KAPAP) for funding this research work. We also wish to thank the Kenya Agricultural and Livestock Research Organization (KALRO) for providing plant material.

### References

1. Bruckner CH, Suassuna TMF, Rego MM, Nunes ES (2005) Auto-incompatibilidade do maracujá: implicações no melhoramento genético. Embrapa Cerrados: *Planaltina, Brazil*, pp. 475–506.
2. Knight RJ, Sauls JW (2005) The passion fruit. University of Florida IFAS Extension HS60.
3. HCDA. (2011) 2010 Horticulture Validated Report. Nairobi: HCDA.
4. Vanderplank J (2000) Passion Flowers. MIT Press, Cambridge, UK.
5. Ulmer T, MacDougal J (2004) *Passiflora*: Passionflowers of the world. Timber Press, Portland, USA.
6. Zeraik ML, Pereira CAM, Zuin VG, Yariwake JH (2010) "Passion Fruit: A Functional Food?" Revista Brasileira de Farmacognosia, 20: 459-471.
7. Fushimi N, Gitonga L (2001) Cultivation of Passion fruits in Kenya: Technical Bulletin. Nairobi: PSDA.
8. Erbaugh M, Sally M, Matt K, Kovach J, Dan T, et al. (2010) Regional IPM Programs in East Africa: Kenya, Tanzania and Uganda. Annual Report.
9. Hortinews (2015) http://www.hortinews.co.ke/article.php?id=356 accessed on March 26, 2015.
10. MOA (Ministry of Agriculture) (2003) Fruits and Vegetables Technical Handbook (2nd edit.). Nairobi-Kenya: Agricultural Information Centre: 146-150.
11. Morton J (1987) Passion fruit. In: Fruits of warm climates. Miami, USA: 320-328.
12. Wangungu C (2012) Etiology, Epidemiology and Management of Dieback Disease of Passion Fruit (Passiflora Spp) in Central and Eastern Regions, Kenya. MSc Thesis, Kenyatta University.
13. Townsend MS, Henning JA (2005) Potential heterotic groups in hop as determined by AFLP analysis. Crop Sci 45: 1901-1907.
14. Reis VR, Alexandre PV, Eder JO, Marcelo G, Moraes S (2011) Phenotypic and molecular selection of yellow passion fruit progenies in the second cycle of recurrent selection. Brazilian Society of Plant Breeding, 12: 17-24.
15. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15.
16. Oliveira EJ, Pádua JG, Zucchi MI, Camargo LEA, Fungaro MHP et al. (2005) Development and characterization of microsatellite markers from the yellow passion fruit (*Passiflora edulis f. flavicarpa*). Mol Eco Notes 5: 331-333.
17. Nei M (1972) Genetic distance between populations. Ameri Nat 106: 283-292.
18. Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539.
19. Perrier X, Flori A, Bonnot F (2003) Data analysis methods. Enfield, Science Publishers. Montpellier: 43-76.
20. Ansari SA, Narayanan C, Wali SA, Kumar R, Shukla N, et al. (2012) ISSR markers for analysis of molecular diversity and genetic structure of Indian teak ( *Tectona grandis L.f.*) populations. Ann. Res 55: 11-23.
21. Cerqueira-Silva CBM, Santos ESL, Jesus ON, Vieira JGP, Mori GM, et al. (2014) Molecular Genetic Variability of Commercial and Wild Accessions of Passion Fruit (*Passiflora* spp.) Targeting *ex Situ* Conservation and Breeding. Int J Mol Sci 15: 22933-22959.
22. Paiva CL, Viana AP, Santos EA, Freitas JCO, Silva, et al. (2014) Genetic variability assessment in the genus Passiflora by SSR markers. Chil Jour of Agri Res 74: 355-360.
23. Sousa AGR, Souza MM, Melo CAF, Sodré GA (2015) ISSR markers in wild species of Passiflora L. (Passifloraceae) as a tool for taxon selection in ornamental breeding. Genet Mol Res, 14: 18534-18545.
24. Costa JL, Jesus ON, Alvarenga G, Oliveira F, Oliveira EJ (2012) Effect of selection on genetic variability in yellow passion fruit. Crop Breed Appl Biot 12: 253-260.
25. Viana A, Souza M, Araujo I, Correa R, Ahnert D (2010) Genetic diversity in Passiflora species determined by morphological and molecular characteristics. J Bio Plnt 54: 535-538.
26. Ganga RMD, Ruggiero C, Lemos EGM, Grili GVG, Gonçalves MM, et al. (2009) Diversidade genética em maracujazeiro-amarelo utilizando marcadores moleculares fAFLP. Rev Bras Frut 26: 494-498.

## Author Affiliations

Top

[1]*Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya*

[2]*Department of Agricultural Science and Technology, School of Agriculture and Enterprise Development, Kenya*

**Submit your next manuscript and get advantages of SciTechnol submissions**

- 80 Journals
- 21 Day rapid review process
- 3000 Editorial team
- 5 Million readers
- More than 5000 facebook fans
- Quality and quick review processing through Editorial Manager System

Submit your next manuscript at ● www.scitechnol.com/submission