

Research Application Summary

Morphological and genetic diversity among blackberry accessions in Kenya

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Abstract

Horticultural crops' morphological and genetic diversity are important components for cultivar development and a pre-requisite for cultivar improvement. This study is being conducted to determine the morphological and genetic diversity among blackberry (*Rubus* L sub-genus *Rubus* Watson) accessions in Kenya and their relationship with introductions using SSR markers with the aim of improving the crop for local use. Thirteen Simple Sequence Repeat (SSR) markers will be used to assess the genetic diversity among twenty blackberry accessions which will be collected from the Genetic Resources Research Institute (GeRRI) – Muguga and the field. Field collections will be made in Kericho, Nanyuki, Bomet, Baringo, Molo, Mau-Narok, Naivasha and Limuru. The study will be conducted at Egerton University Agricultural Experimentation Station Field 7, Njoro and KALRO Molo in a Randomized Complete Block Design replicated three times. Morphological traits will be phenotypically characterized using RosBREEDII Blackberry Standardized Phenotyping protocol and, thereafter, subjected to multivariate analysis to determine eigenvalues. All data collected will be subjected to Analysis of Variance (ANOVA), principal component analysis (PCA) and cluster analysis. Their means will be compared by Tukeys test at $\alpha=0.05$. Genetic characterization will be based on Simple Sequence Repeat (SSR) markers analysis and genetic distances amongst all accessions will be based on Jaccard's variability index. Results of this study are expected to provide useful information on the diversity of blackberry germplasm available and the possibilities of improving this fruit in Kenya.

Key words: Blackberry, characterization, diversity, Kenya, SSR markers

Résumé

La diversité morphologique et génétique des cultures maraîchères sont des éléments importants pour le développement des cultivars et leur amélioration. Cette étude a été conduite pour évaluer la diversité morphologique et génétique entre les accessions de mûres (*Rubus* L sous-genre *Rubus* Watson) au Kenya et leur relation avec les introductions utilisant des marqueurs SSR avec pour but l'amélioration de la culture pour une utilisation locale. Treize marqueurs de séquence simple à répétition (SSR) seront utilisés pour évaluer la diversité génétique de vingt accessions de mûres qui seront obtenues à l'Institut de recherche sur les

ressources génétiques (GeRRI) - Muguga et sur le terrain. Les collections de terrain seront réalisées à Kericho, Nanyuki, Bomet, Baringo, Molo, Mau-Narok, Naivasha et Limuru. L'étude sera menée à la station d'expérimentation agricole de l'Université Egerton à la station 7, à Njoro et à KALRO Molo, suivant un dispositif de bloc aléatoire complet avec trois répétitions. Les caractères morphologiques seront phénotypiquement caractérisés à l'aide du protocole standard de phénotypage de RosBREEDII et, par la suite, soumis à une analyse multivariée pour déterminer les valeurs propres. Toutes les données collectées seront soumises à l'analyse de variance (ANOVA), à l'analyse en composantes principales (ACP) et à l'analyse typologique. Leurs moyennes seront comparées en utilisant le test de Tukeys à $p = 0,05$. La caractérisation génétique sera basée sur l'analyse des marqueurs de séquence simple à répétition (SSR) et les distances génétiques entre toutes les accessions seront calculées sur la base de l'indice de variabilité de Jaccard. Les résultats de cette étude devraient fournir des informations utiles sur la diversité du germoplasme de mûres disponible et les possibilités d'amélioration de ce fruit au Kenya.

Mots clés : mûres, caractérisation, diversité, Kenya, marqueurs SSR

Background

The evaluation of genetic diversity in crop species is a key resource in crop improvement (Mason *et al.*, 2015), and is a precursor to knowledge of the inheritance of key horticultural traits which is a basic requirement for cultivar development (Castro *et al.*, 2013) especially in minority crops like blackberry (*Rubus* L sub-genus *Rubus*). There are eight wild species of blackberries in Kenya representing 24 genera (Chittaranjan, 2011). Some attempts have been made to characterize genetic variation within and among blackberry populations in native and introduced regions (Ipek *et al.*, 2009; Miyashita *et al.*, 2015). However, there are still only a few detailed studies comparing the genetic diversity between native and non-native populations of blackberry, especially in African populations. With the current high deforestation rates being experienced in Africa, such useful germplasm is under threat of disappearance.

The various members of the genus have had a multitude of uses throughout human history as documented in archaeological studies, as well as in art and herbals (Hummer and Janick, 2007; Hummer, 2010). Wild relatives and landraces are the best source for increasing diversity in the improved exotic introductions that are expected to be high yielders but less adapted to local conditions (Hajjar and Hodgkin, 2007). Characters that show diversity within each species are commonly used in the characterization process. Attributes of the edible part of the plant such as leaf shape, length, persistence and total foliage cover are used in many crops (Chweya, 1997). For those crops whose fruit is the edible part, attributes such as fruit size, texture, colour, length and weight are used. Characterization of these collections is therefore, crucial, as it can identify blackberry germplasm diversity with well adapted important agronomic traits that can be availed to farmers for cultivation (Ipek *et al.*, 2009) and also further our understanding of the processes underlying the demographic establishment and evolutionary adaptation following invasion (Alice and Campbell, 1999).

Literature summary

Blackberry (*Rubus* L sub-genus *Rubus* Watson) is a perennial plant with biennial canes. The cultivated types have trailing to erect growth habits with canes up to 5 meters tall (Clark *et al.*, 2007). Blackberries were first mentioned in 370 B.C. by the Greek writer Theophrastus who reported that the plant was used in hedges to keep out invading forces 2000 years ago (Jennings, 1988). They were domesticated in Europe by the seventeenth century and in North America during the nineteenth century (Jennings, 1988).

The basic chromosome number of *Rubus* is seven although there is substantial variation in ploidy levels in wild and cultivated genotypes (Castillo, 2010). Presently, only four diverse groups of blackberries have been domesticated (Clark *et al.*, 2007). These include the European blackberries derived from a group of diploid and polyploid species erect blackberries and trailing dewberries domesticated from diploid and tetraploid species, and the trailing blackberries domesticated only from polyploidy species from Western America. Hybrids of *Rubus allegheniensis* Porter × *Rubus frondosus* played an important role in the domestication of the crop (Hedrick, 1925). The discovery and development of intersectional hybrid most likely between a pistillate *Rubus ursinus* selection ‘Aughinbaugh’ and ‘Red Antwerp’ was a crowning moment in blackberry breeding. This eventually led to the first release of a blackberry cultivar from a breeding program (Logan, 1955). It was later established that ‘loganberry’ was in fact an allohexaploid derived from a reduced gamete of an octoploid *Rubus ursinus* and an unreduced gamete of diploid *Rubus idaeus* (Jennings, 1981). Other interspecific polyploidy hybrids were selected in the late 1800s and early 1900s including ‘Laxtonberry’ and ‘Boysenberry’ (Clark *et al.*, 2007). The first public blackberry breeding program was started in Texas Agricultural Experiment Station with emphasis on developing blackberries with low chilling requirement whilst adapted to warmer climates (Darrow, 1937).

Blackberry varieties are often classified according to their cane architecture into three types: erect, semi-erect, and trailing (Strik, 1992). Erect-caned cultivars include the thornless; ‘Arapho’, ‘Triple Crown’, ‘Ouachita’ and ‘Navaho’; and the thornless ‘Cherokee’, ‘Cheyenne’, ‘Choctaw’, ‘Kiowa’ and ‘Prime Arkansas 45’. Semi-erect cultivars include ‘Chester Thornless’, ‘Thornfree’, ‘Loch Ness’, and ‘Hull’. Trailing cultivars include ‘Marion’, ‘Silvan’, and ‘Thornless Evergreen’ and the blackberry-raspberry hybrids ‘Boysen’ and ‘Logan’ (Clark *et al.*, 2007). There are also primocane-fruiting cultivars ‘Prime-Jan’ and ‘Prime-Jim’, ‘Prime Arkansas Traveler’ and ‘Prime Arkansas 45’ that are erect, thorny types (Clark, 2010). In addition, there is the ‘Prime Arkansas freedom’. They are fourth in the Prime Arkansas series, are thornless and grow vigorously (Clark and Finn, 2011). The erect blackberry varieties produce primocanes from buds at the base of floricanes at the crown or from buds on roots, while trailing and semi-erect types only produce new primocanes from buds on the crown (Strik *et al.*, 2007).

The characterization of germplasm has traditionally used morphological descriptors such as flower color and growth habit (Fajardo *et al.*, 2002). This method of classifying germplasm is the oldest and is considered an initial step in classifying germplasm (Hedrick, 2005).

Morphological markers are straightforward, easy and cheap to use technique for plant identification and characterization (Li *et al.*, 2009). The limitations of morphological-marker assisted traits however include phenological changes in plants depending on the growth stage, insufficient variation and the length of time required for appearance of informative traits particularly in tree crops (Castillo, 2010).

In the Rosaceae, an array of molecular marker techniques have been developed. However, these molecular techniques have not been pursued as vigorously in blackberry as it is the case with raspberry. This may be because the crop is still considered minor in the world. In spite of this, there are some molecular techniques available for blackberry. These include biochemical markers, amplified fragment length polymorphisms, restriction fragment length polymorphisms, random amplified polymorphic DNA (RAPDs) and simple sequence repeats (SSRs). These techniques have been used in the differentiation of blackberry genotypes (Stafne *et al.*, 2003), estimation of levels of agamospermy (Kraft *et al.*, 1996), conducting phylogeny and diversity studies (Waugh *et al.*, 1990; Alice *et al.*, 1997) and determining similarity/dissimilarity among cultivars (Kraft and Nybom, 1995; Stafne *et al.*, 2003, Stafne and Clark, 2004). Phylogenetic insights in *Rubus* have also been studied using *in situ* hybridization techniques (ISH) - Genomic *in situ* hybridization (GISH) and fluorescence *in situ* hybridization (FISH) with an objective of determining clues to infer the role of *R. parvifolius* allegedly plays in speciation and polyploidization of the genus (Yan *et al.*, 2015). In this study, subgenera *Idaeobatus* and *Malachobatus* were evaluated.

Study description

This study will utilize SSR markers in molecular characterization and RosBREEDII Blackberry Standardized Phenotyping protocol for morphological characterization. SSRs are highly polymorphic PCR-based markers and are found in coding and non-coding regions (Russell *et al.*, 1997) and are occasionally transcribed, hence, may be identified in expressed sequence tags (ESTs). SSRs have many advantages which include requiring small amount of starting DNA, are multi allelic, co-dominant, high reproducibility, easily detected by PCR, relatively abundant and has extensive genome coverage (Powell *et al.*, 1996). Genotyping using SSRs is reproducible between labs and, therefore, provides a platform for collaborative research. Since SSRs are highly reproducible and easily detected, they can distinguish between closely related crops that have narrow genetic bases like blackberry.

Research application

The analysis of genetic relationships among and within crop species is an important component of crop improvement and a pre-requisite to any viable plant breeding program. Traditionally, germplasm characterization has been based on morphological descriptors (Fajardo *et al.*, 2002) coupled with reactions to pest, diseases and other stresses existing within germplasm collections. Such phenotypic traits, however, tend to vary according to environment (Marinoni *et al.*, 2003; Lewers *et al.*, 2008) and are most useful for traits that are controlled by only a small number of genes (Brown-Guedira *et al.*, 2000). This preference for specific traits based on phenotypic descriptions led to the discarding of potentially important and

advantageous germplasm (Castillo, 2010). As such, classifying germplasm collections based solely on phenotyping protocols may not provide an accurate indication of genetic diversity (Menkir *et al.*, 1997). Characterization of germplasm is important in understanding processes underlying early demographic establishment and evolutionary adaptation which show levels of intrapopulation diversity and population differentiation of native and introduced counterparts. This is due to the fact that the genetic characteristics of populations can profoundly impact their range expansion capacity. Problems associated with species identity and misclassification within germplasm collections are also sorted.

The use of morphological markers alone is limiting in differentiating varieties and cultivars. Therefore, genotyping individuals and cultivars within blackberry germplasm collections can be used to provide insight on the evolutionary history of the crop in Kenya, as well as to help breeders narrow the search for new alleles at loci of interest and, subsequently, assist in the identification of marker alleles from candidate genes that can then be introduced into new varieties along with their associated desirable traits.

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