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Research Application Summary

Blackberry germplasm diversity and its importance to cultivar development

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Abstract

The assessment of morphological and genetic diversity is an important component for cultivar development. It is a precursor to knowledge of the inheritance of key horticultural traits which is a basic requirement for cultivar development especially in minority crops like blackberry (*Rubus* L. sub-genus *Rubus*). Successful development of improved tree cultivar is dependent on variability among available genetic resources to act as a source of desirable genes. An increase in heterogeneity may increase the resistance against abiotic and biotic stresses. In addition, allelic variations could also be used to develop new combinations of improved plant cultivars in horticulture. This paper reviews three aspects (i) the significance of plant genetic diversity (PGD) on horticultural tree crops; (ii) postgenomic methods used in assessing plant genetic diversity in blackberry. This review is intended to provide information on the genetic and morphological diversity of fruit trees and other agriculturally important crops, germplasm management and possibilities of improving the crops.

Key words: Cultivar development, fruit trees, genetic diversity, germplasm management, *Rubus* L sub-genus *Rubus* Watson

Résumé

L'évaluation de la diversité morphologique et génétique est un élément important pour le développement des cultivars. Il est un précurseur à la connaissance de l'héritabilité des traits horticoles clés qui est une exigence de base pour le développement des cultivars en particulier dans les cultures minoritaires comme la mûre (*Rubus L.* sous-genre *Rubus*). Le succès du développement des cultivars améliorés des arbres dépend de la variabilité des ressources génétiques disponibles pour agir en tant que source de gènes souhaitables. Une augmentation de l'hétérogénéité peut augmenter la résistance contre les contraintes abiotiques et biotiques. En outre, des variations alléliques peuvent aussi être utilisées pour développer de nouvelles combinaisons de variétés végétales améliorées en horticulture. Cet article examine trois aspects: (i) l'importance de la diversité génétique végétale (PGD) sur les cultures d'arbres horticoles; (ii) les méthodes postgénomiques utilisées dans l'évaluation de la diversité génétique végétale dans la mûre. Cette revue vise à fournir des informations sur

la diversité génétique et morphologique des arbres fruitiers et autres cultures importantes en agriculture, la gestion du matériel génétique et des possibilités d'améliorer les cultures.

Mots clés: Le développement des cultivars, les arbres fruitiers, la diversité génétique, la gestion du matériel génétique, *Rubus* L sous-genre *Rubus* Watson

Introduction

Plant genetic diversity provides plant breeders with opportunity to develop new combinations of improved plant cultivars. Due to global warming, there is great need for improved plant cultivars in horticulture. Plant breeders therefore, are not only faced with well-known demands from farmers and consumers but also to develop crops that adapt to the ever changing climatic conditions. These demands are abiotic and biotic resistance that are breeder preferred and yield that is farmer preferred. The development of crops with the above mentioned characteristics is solely dependent on access to plant genetic resources and the availability of genetic variability in them. In fruit trees, this can be in form of in situ gene banks, seeds or clonal banks that must be maintained for immediate or future use. There are 84 wild species of blackberries in Kenya which belong in 24 genera (Chittaranjan, 2011). Worldwide, in addition to the wild blackberries, 15 species are in cultivation mainly in the USA (Clark *et al*., 2007).

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, especially in African populations, and with the current high deforestation rates being experienced. 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). For most of their history, they were fruits to be gathered from the wild. It was not until the mid to late 1800s that people started to select for better or, more typically in the early stages, novel characteristics in plants that were brought into cultivation (Clark *et al*., 2007). 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. More characters may be included in future particularly those relating to the nutritive aspects of each species, plant characteristics related to duration of production of the edible parts of the plant and attributes related to storability of the harvested part for consumption (Human and Rheeder, 2004).

Clear understanding the germplasm diversity and relationships among germplasm is critical to its improvement, especially the high yielding introductions (Lewers *et al*., 2008). Wild relatives of blackberries are crucial reservoirs of natural diversity, often possessing abiotic stress tolerance, disease resistance, and other characteristics that are absent or inadequate

in breeding material with narrow genetic base (Clark *et al*., 2007). 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). In addition, previous germplasm collectors searched only for characteristics based on phenotypic expression such as objective descriptions of tree and fruit characteristics discriminating against undesirable traits in the process (Marinoni *et al*., 2003). This preference for specific traits based on phenotypic descriptions led to the discarding of potentially important and advantageous germplasm (Castillo *et al.*, 2010). As such, classifying germplasm collections based solely on phenotyping protocols may not provide an accurate indication of genetic diversity (Menkir *et al*., 1997). In addition, characterization of germplasm aims to preserve useful genetic diversity for later introgression back into crop cultivars and for targeted breeding attempts in crop improvement. Characterization of germplasm can also reveal cases of species misclassification, providing useful genetic diversity information and confirming genome composition of the crop (Mason *et al*., 2015). With this in perspective, the combined use of both morphological and molecular markers in breeding is preferable because it provides useful complementary information. Morphological marker-assisted selection has been used by blackberry breeders for primocane-fruiting trait, implying that molecular marker-assisted selection has the potential for adoption (Lewers *et al*., 2008). Characterizing individuals and cultivars within blackberry germplasm collections is important to give insight on the evolutionary history of the crop in Kenya, as well as help breeders narrow the search for new alleles at loci of interest. This will assist in the identification of marker alleles from candidate genes that can then be introduced into new varieties along with their associated desirable traits.

Characterization of these collections is therefore, crucial, to 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). Artificial crossing and selection are usually done to improve on fruit characteristics to achieve specific uses (Human and Rheeder, 2004). Characteristics related to plant architecture, phenology, fruit quality, pest resistance and environmental adaptation are among the traits identified in wild blackberry that might be introgressed into cultivated germplasm (Finn and Clark, 2011). In addition, information on interspecific hybridization can be employed to provide high potential germplasm for commercial production (Mason *et al*., 2015).

Genetics of blackberry

The basic chromosome number of *Rubus* is seven although there is substantial variation in ploidy levels in wild and cultivated genotypes (Meng and Finn, 2002). This ranges from 2n=2x=14 to 2n=18x=126 including odd-ploids and aneuploids (Meng and Finn, 1999). Presently, only four diverse groups of blackberries have been domesticated (Clark *et al*., 2007); the European blackberries were derived from a group of diploid and polyploid species

(2n=4x=28, 2n=6x=42 and 2n=8x=56); erect blackberries and trailing dewberries domesticated from diploid and tetraploid species and trailing blackberries domesticated only from polyploidy species from Western America, predominantly *Rubus ursinus* (2n=8x=56, 2n=12x=84). Hybrids of *Rubus allegheniensis* Porter × *Rubus frondonsas* Bigelow 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).

Assessment of genetic diversity in Blackberry. The assessment of genetic diversity within and among plant populations can be done by using various techniques: morphological, biochemical characterization/evaluation (allozyme) and DNA (or molecular) marker analysis. Markers can exhibit similar modes of inheritance, as we observe for any other traits that is, dominant/recessive or codominant. If the genetic pattern of homozygotes can be distinguished from that of heterozygotes, then a marker is said to be codominant. Generally codominant markers are more informative than the dominant markers.

Morphological diversity. The characterization of germplasm have traditionally used morphological descriptors which consist of phenotypic traits like flower color and growth habit (Fajardo *et al*., 2002). This method of classifying germplasm is the oldest and is considered as an initial step in classifying germplasm (Hedrick, 2005). Morphological markers are straightforward, easy, cheap technique for plant identification and characterization (Li *et al*., 2009). Variables of interest to the plant breeder are usually visually monitored and noted. This is because they are easily detectable plant characteristics like form and structure. However, there exists errors in scoring which may be attributed to environmental effects and, hence, scoring of some morphological descriptors tends to be subjective in nature (Fajardo *et al*., 2002; Marinoni *et al*., 2003; Li *et al.,* 2009).

Additional limitations of morphological-marker assisted traits include phenological changes in plant phenotypes depending on the growth stage, insufficient variation and the length of time required for appearance of informative traits particularly in tree crops (Castillo, 2010). Currently, there is no list of accepted morphological descriptors for blackberry (Finn, personal communication). A standardized phenotyping protocol for blackberry is also currently being developed with the aim of standardizing blackberry phenotyping for the purpose of identifying horticulturally important quantitative trait loci (QTLs). Accurate identification, therefore, becomes difficult in the process, lowering the reliability of morphological markers for germplasm characterization (Finn *et al*., 2010).

Genetic diversity. The assessment of genetic diversity is an important aspect of plant breeding if there is to be improvement by selection (Odongo *et al*., 2015) as it provides a platform for stratified sampling structure available and breeding populations. Molecular

markers are generally superior to morphological, pedigree, heterosis, and biochemical data and are preferred for evaluation of genetic diversity of genotypes (Melchinger *et al*, 1991). This genetic relatedness of cultivars is commonly is measured by genetic distance (GD) or genetic similarity (GS = 1 - GD), both of which imply that there are either differences or similarities at the genetic level. Random Fragment Length Polymorphism-based GDs have been used in evaluating the genetic diversity of maize in bred lines and in determining their hybrid performances (Benchimol *et al*., 2000).

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 a minor crop in the world. In spite of this, there are some molecular techniques available for blackberry. These include biochemical markers, amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), 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), estimate levels of agamospermy (Kraft *et al*., 1996), conduct phylogeny and diversity studies (Alice *et al*., 1997) and determine similarity/ dissimilarity among cultivars (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).

Use of molecular markers in assessing genetic diversity in Blackberry. Biochemical markers were introduced in the 1960s and involve protein and enzyme electrophoresis. These markers are useful in analysis of genetic diversity as they reveal differences between seed storage proteins or enzymes encoded by different alleles at one (allozymes) or more gene loci (isozymes) (Rao, 2004). The enzymes are differently charged variants that are separable by electrophoresis. Visualization is achieved by supplying the bands with the substrates and co-factors and observing the formation of protein products encoded by different alleles/genes and provide co-dominant markers (Castillo *et al*., 2010). Allozymic polymorphism has been used to ascertain genetic diversity in almost all major crops and in identification of cultivars (Veasey *et al*., 2002). However, the level of isozyme variation is too low for cultivar identification and hence, some *Rubus* cultivars remain undistinguishable (Cousineau and Donnelly, 1992). This is a major constraint and a limitation in using isoenzyme analysis for fingerprinting mostly because of lack of or low level of variation in many cultivars and species.

Restriction Fragment Length Polymorphism (RFLP) is the first DNA-based marker developed (Bostein *et al*., 1980) and resulted from differences in the sequences of nucleotides in different plants. This technique is based on the restriction enzymes that reveal the pattern difference between DNA fragment sizes in individual organisms (Semagn *et al*., 2006). DNA fragments are transferred by Southern blotting to a nitrocellulose or nylon membranes that are generally hybridized to a radioactively-labeled DNA probe. These markers require no sequence information, are co-dominant and analysis of band profiles is easy to score.

This marker has been found to be effective in identifying *Rubus* cultivars (Waugh *et al*., 1990) and demonstrating genetic variability among the selected taxa (Nybom *et al*., 1992). This shows the ability of RFLP to reveal genetic differences among closely related *Rubus*' species or taxa. The disadvantages of RFLPs include the requirement of high quantity and quality of DNA and for radioactive labeling of specific probe libraries.

Random Amplified Polymorphic DNA (RAPD) is the most commonly used PCR-based markers (Williams *et al*., 1990). This is due to the simplicity and low cost of agarose gel electrophoresis. The RAPD protocol usually uses an oligonucleotide that is 10 bp long at constant annealing temperature, in a PCR reaction to amplify many copies of random genomic DNA sequences simultaneously. In *Rubus* RAPD markers have been used in identification of raspberry cultivars (Graham *et al*., 1997), establishing the genetic relationships (Weber, 2003). The approximation of the relatedness in pedigree analysis of RAPD data using cluster analysis can overestimate or underestimate percentage relationships. This results in uncertainty of the relationship showed by pedigree analysis. RAPD markers also have limitations like the irreproducibility of banding patterns preventing comparisons to be made between studies (Nybom, 2004).

Amplified Fragment Length Polymorphism (AFLP) is based on the amplification of subsets of genomic restriction fragments using PCR (Vos *et al*., 1995). The first step of the AFLP protocol involves digestion of the DNA with two restriction enzymes, a rare cutter like *Eco*RI and a frequent cutter like *Mse*I. Polymorphisms are revealed after separating the amplified DNA fragments by electrophoresis on a sequencing gel, and visualized by silver staining, radioactive or fluorescent detection. A large number of bands are generated that facilitates the detection of polymorphisms. AFLP reveals a high level of polymorphism, has a high diversity index and can analyse a large number of bands (Russell *et al*., 1997). AFLP has been used in *Rubus* to demonstrate sexual recombination (Kollmann *et al*., 2000) and to evaluate genetic diversity (Amsellem *et al*., 2001). AFLP markers are cost efficient, easy to use, require a small amount of DNA. The information generated is replicable, is of high quantity and resolution in comparison to other standard molecular markers (Mueller and Wolfenbarger, 1999). The technique also permits the detection of restriction fragments and can generate fingerprints of any DNA regardless of origin and complexity. It also has a broad taxonomic scope and can be developed in any organism with DNA without prior knowledge of the organism's genomic make-up.

Simple sequence repeat markers are repeats of short nucleotide sequences, usually one to six base pairs in length that vary in number (Rafalski *et al*., 1996). 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). Reproducibility of SSR markers between laboratories as primer sequences is also easy and, therefore, provides a platform for collaborative research due to available common language. Since SSRs are highly reproducible and easily detected, they can distinguish between closely related crops that

have a narrow genetic bases like blackberry. In addition, the possibility of adding new data to an existing database, even when developed in a different laboratory has been a major advantage (Sehic *et al*, 2012). This however, is dependent on use of common SSR markers and suitable standardization procedures which have not been very successful in fruit tree characterisation studies as most research work develop their own SSR markers or choose various sets of SSRs from the literature.

SSR markers have become particularly useful in assessment of genetic diversity (Amsellem *et al*., 2001). SSR markers have also been used in fingerprinting and ecological-genetic studies (Li *et al*., 2009), marker assisted selection and genetic linkage mapping studies (Stafne *et al*., 2005). Microsatellite markers for blackberry were recently developed from an expressed sequence tag library of 'Merton Thornless' (Lewers *et al*., 2008). Eight SSRs have been isolated from the invasive weed *R. alceifolius* Poir. (Amsellem *et al*., 2001) and in red raspberry (Graham *et al*., 2002; Graham *et al*., 2004). Primers for SSR loci in blackberry have been published (Castillo *et al*., 2010).

Conclusion

An all-inclusive approach is vital to increase diversity in agriculture. Plant breeders should closely co-operate with farmers to see which crops best fit into current farming systems. Collaboration with other researchers and other stakeholders is also important to definitively identify germplasm and improve it with farmers and consumers in mind. As elucidated in this review, different methods are available for the use of plant genetic resources in crop improvement. The choice mainly depends on the crop, the trait(s) of interest, availability of molecular markers, the chosen time-frame and on the available finances. A combination of advanced, molecular techniques with classical and farmer-participatory breeding methods will most likely achieve the desired impact. With is in perspective, characterization and evaluation of core collections; increased genetic enhancement and base-broadening efforts; development and commercialization of underutilized species; development of new markets for local varieties and promoting public awareness of the value of plant genetic resources for food and agriculture is imperative. Therefore, all stakeholders viz, farmers, breeders, agronomists, and donors are key to the implementation of an all-inclusive method to enhance plant genetic diversity in one way or the other.

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