Journal of Crop and Weed, 17(3): 158-168 (2021) ISSN- O : 2349 9400 ; P : 0974 6315

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Review article

Characterization of cashew: A review with reference to morphological, biochemical and molecular descriptors

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Received : 20.09.2021 ; Revised : 29.10.2021 ; Accepted : 12.12.2021

DOI: https://doi.org/10.22271/09746315.2021.v17.i3.1505

ABSTRACT

Anacardium occidentale L. belonging to the family Anacardiaceae, order Sapindales, is a fruit-cum-nut crop of world importance. In this article, we have reviewed scientific characterization using several DNA markers in addition to morphological and biochemical markers which are being utilized for molecular characterization of cashew, an economically important fruit. Cashew being a socio-economic tree nut and its increasing market demand across the globe inculcate breeders to adopt new methodologies using molecular tools to select superior cultivars which will not only be exclusively beneficial for higher cashew production with respect to its quality but also it will reduce nut wastage due to biotic and abiotic limitations, ultimately to meet increasing demands globally. To overcome limitations in the morphological variations being affected by environment, molecular techniques are adopted since recent past in characterization of cashew genotypes

Keywords: Anacardium occidentale L., characterization, molecular markers, morphological markers

Cashew tree (*Anacardium occidentale* L.) belonging to the family Anacardiaceae predominantly is a cross pollinated plant with entomophilous pollination (Nambiar and Pillai, 1985). Being a tropical evergreen tree produces two main products: seed and cashew apple. Cashew tree has the ability to grow higher than 14m; however, the dwarf varieties may grow up to 6m. The dwarf varieties have been found to be greater yielder thus more profitable (Ohler, 1979; Masawe *et al.,* 1999; Aliyu, 2006). Cashew nut is eaten raw and used in recipes. Being native to Brazil, it was introduced into India by the Portuguese between 1563 and 1578 for afforestation and soil conservation (Rao *et al.,* 1999). India is the largest area holder of this crop in the globe which includes Maharashtra (1.86 lakh ha) being the leading producer of cashew nut followed by Andhra Pradesh (1.85 lakh ha), Odissa (1.82 lakh ha), Karnataka (1.26 lakh ha) and Kerala (0.87 lakh ha). India, the first country in the world exploited international trade in cashew kernels in the early 20th Century along with characterization, variability/ genetic diversity as well as relationship among accessions determine its proper utilization in the breeding programmes (Aliyu, 2005; Chipojola *et al.,* 2009). There are fewer chromosome studies in spite of a commodity crop of sub-Sahara Africa, Asia and South America. Cashew plant has been described as a polymorphic species with chromosome numbers ranging between *2n*=24, and *2n*=42 (Aliyu *et al.,* 2007). Karyotypes being mostly symmetric mainly composed of metacentric pairs and several submetacentrics (Aliyu, 2007). Classification and knowledge of genetic diversity have significant role in the in conservation of plant genetic resources (Adoukonou- Sagbadja *et al.,* 2007). Coefficients of variation confirming agro morphological traits can be used in discriminating germplasms (Mzena *et al.,* 2018). Phenotypic variations have been reported largely in the coastal regions of India (Rao *et al.,* 1999). Morphological characters showed high degree of variation among cultivars as evident from the previous researches [Samal *et al.* (2003b), Dasmohapatra *et al.* (2014), Sika *et al.* (2015) Gbohaïda *et al.* (2015), Jena *et al*. (2016), Mzena *et al*. (2018)]. Moreover, phenotypic characters does not necessarily a reliable descriptor, consequently use of morphological traits are not always informative method to evaluate distances and relatedness (Samal *et al.,* 2003a). Direct selection of nut weight as a single character would be impractical and insignificant in yield improvement strategies (Aliyu, 2005). Theoretically, among several markers which are available such as Inter Simple Sequence Repeats (ISSR) (Archak *et al*., 2003), Amplified Fragment Length Polymorphism (AFLP) and Microsatellites (SSR) (Croxford *et al*., 2006) have been found useful in cashew for its characterization. The present review will synthesis already published literature on characterization of cashew germplasm to explore the potential of different methods of characterization with special reference to molecular characterization.

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Morphological characterization

Phenotypic characters does not necessarily a reliable descriptor, consequently use of morphological traits are not always informative method to evaluate distances and relatedness (Samal *et al*., 2003). However, scanty reports are available on for qualitative and quantitative traits to assess mapping study of cashew (Archak *et al*., 2003; Samal *et al*., 2004; Aliyu and Awopetu, 2007). The selection of desirable genotype (s) from the existing variations and its use in the specific breeding programme is an important way to increase productivity. Variability or genetic diversity determines the success of a crop improvement programme to a great extent. In India, enhancement, evaluation and maintenance of genetic diversity of cashew emphasised genetic improvement. Cashew breeding is mostly based on traditional methods of selection of useful traits, which is based on morphological attributes, such as, yield performance, nut size, sex ratio, nut weight and length of panicle (Swamy *et al.,* 1998; Mneney *et al.,* 2001). Positive phenotypic correlation between nut yield and various primary characters such as nuts per tree, nuts per panicle and number of hermaphrodite flowers per panicle has been reported by Aliyu (2005). Similar results were obtained by Rao (1974) and Murthy *et al.* (1984) which clearly signify that for cashew nut yield improvement these highly correlated characters can be selected to increase the nut yield (Pedro De Azevedo *et al.,* 1998). Other traits such as canopy diameter, number of kernels, and plant height were found to be positively correlated with yield and number of nuts (Mzena *et al.,* 2018). However, yield per tree found to be negatively correlated with nut width Vikram *et al*. (2016). For clonal propagation, traits such as high plant height and canopy size (intensive branching patterns) are preferable and nut weight should be ranged from 4.5g to 8g as evident from many reports [Aliyu and Awopetu (2007), Mzena *et al.* (2018), Olubode *et al.* (2018) and Ibukun (2020)]. The details of these studies have been given in Table 1. Yield per tree showed positive correlation with number of panicles per meter^2 and shelling per cent (Vikram *et al.,* 2016). High yielding genotypes and better nut quality may result into potential strategy for breeders Mzena *et al.* (2018). Selection of a single character such as nut weight would be impractical for breeding as nut weight and nut yield are insignificantly associated (Aliyu, 2005). Two more traits kernel weight and number of kernel revealed negative correlation with yield and nut weight (Geradina *et al.,* 2018). Significant and diverse research works ~~been~~ around the world revealed the importance of coefficients of variation of cashew germplasm to generate better availability of cashew cultivars in Malawi (Chipojola *et al*., 2009), Benin (Sika *et al.,* 2015), India (Jena *et al.,* 2016 ; Samal *et al.,* 2003 and Dasmohapatra *et al.,* 2013), Nigeria (Aliyu, 2005). In one study by Costa *et al*. (2020) revealed that coefficients of variation are dispersed highly with variables such as lamina width, index and length. Higher phenotypic coefficients of variation has been recorded for plant height, crown size, number of panicles per meter square, number of nuts per panicle, apple volume, apple weight and yield per tree Vikram *et al.* (2016) similar results was shown by Sika *et al.* (2015) with largest variations more than 40% which indicates that quantitative variables are more important than qualitative variables displaying lesser diversity. Principal component analysis showed more than 90% total variation is explained together by quantitative traits such as ratio of kernel to shell, shell weight, out turn percent, flower sex ratio and apple length Chipojola *et al.* (2009). Sika *et al.* (2015) suggested that the flower sex ratio is a good selection criterion of cashew trees as it influences nuts per panicle (Archak *et al.,* 2003). Biometric characterization is evolving as an important tool in evaluating genetic variability among similar species in relation with environmental factors and morphological traits (Goudel *et al.,* 2013; Zuffo *et al*., 2016; Costa *et al.,* 2016; Menegatti *et al.,* 2017; Santos *et al.,* 2018; Zuffo *et al.,* 2019). PCA *vis-a-vis* Biplot is also confirmed as a powerful tool to examine and discriminate profile of genotypes (Geradina *et al.,* 2018).

Molecular markers provide better data to evaluate genetic distance and degree of morphological traits to study divergence at phenotypic level Hamrick and Godt (1990). Phenotypic characters are not mandatory correlated with genotypic classification and genetic events such as gene mutations (Bachmann, 1992). Thus, most of the researchers agree that morphological descriptors are less informative. However, while studying genetic relationships among Indian cashew verities Samal *et al*. (2003) revealed that both morphological and molecular marker (RAPD) are relatively useful for assessment of cashew variety in plant improvement and purity assessment certificate programmes. Dasmohapatra *et al.* (2013) suggested that the morphological traits and data from ISSR banding can be useful to assess diversity. Characterization solely based on morphometric descriptors could be misleading as these are highly influenced by uncontrolled environmental factors (Rao *et al.,* 2010and Costa *et al.,* 2015). More focus should be given for mapping of cashew varieties across the globe to conserve cashew trees with higher yield, reduce selection pressure and discard unproductive trees. Morphological characteristics still remains extremely useful in selection; however the efficiency may be reduced by environmental effects or developmental stages on measured traits and are often faced with low penetrance and heritability.

Table 1: Morphological descriptor in cashew

S.No.	Traits studied	Key findings	Ref
1.	36 quantitative characters and 33 qualitative characters	Morphologically four distinct diverse were grouped in accordance with geographical, functional and agronomic habitat.	Aliyu *et al.,* 2007
2.	Elucidation of phenotypic discrimination between 9 local selections and 3 hybrids	Segregated cultivars at 0.53 at Jaccard's similarity coefficient	Ram C. Jena *et al.,* 2016
3.	Characterization from selfing F1 using yield, number and weight of nuts, number and weight of kernels, percentage out-turn, plant height and canopy diameter.	CV 11.6 % PH to 42.7% yield insignificant correlation between yield and percentage outturn	Mzena *et al.,* 2018
4.	Evaluation of physical characteristics such as mass, height and diameter at the equator of cashew apples of Benin	North-East of Benin red morphotypes apples have the highest values for the mass of 94.78 ± 18.28 g,, height of 73.69 ± 9.93 mm and diameter at the equator of 48.52 ± 2.97 mm respectively	Gbohaïda, 2015
5	Characterization of eight morphological descriptors: no of laterals/m^2, no of flowering laterals/m^2, length of penicle, yield, shelling% , net weight, no of nuts per penicle and sex ratio	Similarity coefficient with 70% similarity Genotypes divided into four clusters	Samal *et al*., 2003
6	genetic diversity among 40 accessions of cashew from Liwonde, Nkope, Kaputu and Chikwawa were quantitatively and qualitatively characterized	Two principal components accounted for total variation of 70.1%. Traits such as nut and apple (weight, shell weight, apple length and ratio of kernel to shell) evaluated and account for variation.	Chipojola *et al.,* 2009
7	Assessed genetic diversity of cashew varieties in India	Observed wide variation in shelling percentage, nut yield, plant height, trunk girth and nut weight as well as in apple weight , fruit colour, fruit quality, nut yield, nut weight and shelling percentage	Dasmohapatra *et al.,* 2014
8	59 cashew genotypes were selected to find out relation-ships between nine agronomic traits and cashew nut yield (nut and floral).	Nuts per panicle, number of nuts per tree and number of hermaphrodite flowers per panicle (r = 0.844, r = 0.988and r = 0.863) respectively. Phenotypic correlation positively correlated with nut yield	Aliyu 2005
9	91 individuals morpholo-gically studied	Morphological diversity showed standard deviation <30% Nut and kernel showed 15% deviation	Archak *et al.,* 2009

Table 2: Physio-biochemical characterization in cashew

S.No	Aim	Findings	Ref
1	Characterized alkyl phenols in different cashew products in order to study content of anacardic acids, cardanols and cardols and cashew nut shell liquid (CNSL)	353.6 g/kg of major alkyl phenols and anacardic acid detected in CNSL, 6.1 g/kg of cashew fibre and lowest 0.65 g/kg detected in cashew nut (roasted).	Trevisan *et al.,* 2006
2	Identification and characterization of major allergens in cashew nut by N-terminal amino acid sequencing and technique of IgE immunoblots	IgE-binding antigens (dominant antigen) in its reduced preparation included peptides in the range of 31"35 kD Major food allergens hensforth found to be legumin-group proteins and 2S albumins	Teuber *et al.,* 2002
3	Characterization of major globulin in cashew	Among total amino acids hydrophobic (36.4), uncharged polar (19.88), acidic (25.3), and basic amino acids (18.4%) were accounted. First and second limiting amino acids in the purified globulin are sulfur amino acids and threonine. Pepsin was the most efficient in hydrolyzing the globulin *in vitro*.	Sathe *et al.,* 1997
4	Cashew nut protein characterization by solubalization and electrophoresis	Minimum solubility at pH 5.0 of cashew nut proteins and highly soluble in 0.1 M NaOH. Electrophoretically, cashew nut protein composition dominated by a single multimeric protein.	Sathe *et al.,* 1994
5	Purified cashew allergens ana 01, ana 02 and ana 03	Purification by SDS-PAGE, glycoprotein staining, protein identification and Western blotting resulted ana 01 to be gycoprotein	Reitsma *et al.,* 2016
6	Studied physico-chemical changes in two cashew apple juice composition from Yamoussoukro	Protein content found to be from 0.51 to 0.53 g/100 g and amino acids in order of size are leucine, cysteine and asparagines	Marc *et al.,* 2012

Table 3: Molecular descriptors in cashew

S.No.	Type of marker(S)	Purpose of use	Key findings	Ref
1	RAPD	the biofield energy treatment to the treated farms, biophoton emission study, and DNA fingerprinting using RAPD method to determine the epidemiologicalrelatedness and genetic characteristics	100% (true polymorphism) detected with primers RPL 13A and RPL 19 A inV4 cashew variety after treatment with of biofield energy	Trivedi 2015
2	RAPD	To elucidate genetic diversity among 3 hybrids, 12 promising Indian cashew nut cultivars and 9 local selections	polymorphism at an average of 79.187% was generated	Jena *et al.,* 2015
3	AFLP	AFLP markers were used to genetically diversify 91 individuals	354 indentified polymorphic loci	Archak *et al.,* 2009

Contd.

Table 3 Contd.

S.No.	Type of marker(S)	Purpose of use	Key findings	Ref
4	RAPD	Analysis using forty 10-mer primers to distinguish 20 cashew varieties from Orissa, India	80 distinct DNA fragments (0.2 to 3.0 Kb) amplifiedULLAL-3 and H-1608 showed (87%) similarity which was the highest	Samal *et al.,* 2004
5	RAPD, SSR and Isozymes	To assess genetic diversity of ten accessions of cashew of 54%	151 bands obtained with 123 polymorphic bands with an average genetic similarity	Thimmappaiah *et al.,* 2009
6	RAPD	to study diversity and elucidate population of 90 cashew accessions from the National CashewGene Bank	Ward's method, squared Euclidean distance conformed moderate to high diversity of Indian cashew collections the core collection represents same diversity as that of the entire population.	Dhanaraj *et al*., 2002
7	RAPD, ISSR	Molecular profiling of 24 selections and 11 hybrids using five RAPD and four ISSR primers for repeatability and maximum discrimination	94 markers generated discriminating all varieties by 20.8 x 10^{-11}Generation of 38 bands of which 78.9% were found to be polymorphic using ISSR	Archak *et al.,* 2003
8	SSR	Assessment of genetic diversityof cashew genotypes from regions of Benin, West Africa by simple sequence repeat markers	146 polymorphic bands produced which showed low genetic diversity with Shannon index of 0.04 that are relatively importantfor an imported species	Sika *et al.,* 2015
9	SNP	Study aimed to develop SNPs	High quality SNPs of 57.6% transition and 42.4% trans version was determined	Mzena *et al.,* 2018
10	SSAP	This study aimed to isolate cashew genome consisting two LTR Sequences (Tao1 and Tao2) to develop SSAP marker	SSAP found to be Highly poly morphic markers while comparing to AFLP	Syed *et al.,* 2005
11	RAPD, AFLP and ISSR	for detecting variation and for comparing efficiency in cashew germplasm using 19 cashew genotypes with random primers, AFLP and ISSR	maximum discrimination efficiency was shown by AFLP similarity matrices was lower but comparatively significant ($r = 0.63$; $p < 0.005$) between random primers and ISSR	Archak *et al.,* 2003
12	RAPD AND ISSR	Identified and analyzed unique subset of cashew collection at NCFGP, Puttur	RAPD and ISSR generated 40 and 56 polymorphic bands, respectively. Combined marker analysis generated 91.8% polymorphic bands	Thimmappaiah *et al*., 2015
13	protein-isozyme marker	Assessment of cashew genotypes using protein-isozyme marker	recorded 187 protein-isozyme bands among cashew accessions, with an average of 3.2 bands per accession	Aliyu *et al.,* 2006
14	SSR	Genetic analysis using micro satellites of cashew cashew tree	Great variability observed in cashew and a commonlyused dwarf clone	Buso *et al.,* 2011
15	RAPD, SSR and ISSR	from F_2 population and germplasm BSA was carried out in order to associate molecular markers with nut weight and plant height	Polymorphism found between bulks	Shobha *et al.,* 2011

Biochemical Characterization

During recent past, a good number of researches have been published on biochemical characterization of cashew. Cashew apple juice is rich in sugar (Azevado and Rodrigues, 2000); organic acids and vitamin C (Akinwale, 2000); potassium (Nagaraja, 2009 and Bhakyaraj *et al.,* 2012) which is essential for preventing bone mineralisation (Tucker *et al.,* 1999 and Hunter *et al.,*, 2001). Phytochemicals are often associated with heath benefits and antioxidant properties (Siebenhandl *et al.,* 2007). Many phytochemicals viz., phenolics, flavonoids, tannins (NNMDA, 2006) and anthraquinones were reported as phytonutrients of cashew apple juice by (Daramola, 2013 and Sivagurunathan *et al.,* 2010) The studies of physiochemical aspects of cashew apple juice reported the presence of vitamin C, total sugars, glucose, fructose and sucrose, organic acids, citric acid leads, tartaric acid, acetic acid, oxalic acid and fumaric acid (Marc *et al.,* 2012; Talasila *et al.,* 2011). The cashew apple juice qualitatively is as same as orange juice (Ihekoronye and Ngoddy, 1985). Fermented cashew apple juice is not only suitable for growth of *Saccharomyces cerevisiae* but can be directly used for ethanol production in good acceptance (Mohanthy *et al.,* 2006; Sivagurunathan *et al.,* 2010 and Srinivasarao *et al.,* 2013). Having highly acidic, cashew apple juice is used as a potential substrate for lactic acid production Silveira *et al.,* (2012). Cashew being commercially important crop even its by-product has tremendous importance in agro-based industries as well as automobile industries. The by-products of cashew nut shell liquid are enormously used in chemical paint and cement industries. CNSL (Cashew nut shell liquid) a natural phenol have attracted researchers in areas of antimutagenic activity (Cavalcante *et al.,* 2003), anti-tumour activity (Itokawa *et al.,* 1987; Itokawa *et al.,* 1989; Kubo *et al.,* 1993), antimicrobial activity (Himejima and Kubo, 1991; Kubo *et al.,* 1993; Muroi and Kubo, 1993), Trevisan *et al.,* (2006) characterized alkyl phenols in different cashew products in order to study content of anacardic acids, cardanols and cardols and cashew nut shell liquid (CNSL) eventually reported CNSL to be potent scavenger is in agreement with other reports like Itokawa *et al., (*1987); Kubo *et al.,* (1993); Cavalcante *et al.,* (2003). CNSL and cashew resins are also used as a pesticide and insect repellents against termites in timber, and the bark gum is repellent to insects (DUKE, 1983). Details have been presented in Table 2. Thereby Cashew nut shell liquid is a renewable by-product of the cashew industry. **Discarded cashew nuts** are used to feed livestock as these are unfit for human consumption and **Cashew nut testa** are the red skins that are manually or mechanically removed in the final step of preparing cashew nuts however, these skins may contain pieces of broken kernels and can be used as feed (Donkoh *et al.,* 2012). To intensify renewable alternative sources cashew by-products can be a potential raw material to meet sustainability. Prehydrolysis of Cashew bagasse biomass is a potential raw material for bioethanol production, 9.29% moisture content of dry bagasse reported by Lima *et al.,* 2012 ~~this result is in contrast with Uchoa *et al.,* 2008~~. Cashew apples were collected from different areas by different researchers for enhancement of agricultural resources and the reduction of post-harvest losses through the evaluation of physical (mass, height and diameter at the equator) and physicochemical characteristics (juices were extracted to evaluate pH, the level of soluble solids (brix), density, acidity and carbohydrates) of different morphotypes of cashew apples with the aim to maximize the usage of by-products in agro-based industries as juice, jams, candies and animal feed (Morton, 1987; Akinwale, 2000; ITDG, 2002; Campos *et al*., 2002; Attri, 2009; Dedehou *et al*., 2015) and in pharmaceutical industries.

Molecular characterization

Cashew, being highly heterozygous and cross-pollinated crop exhibit number of variation for different agro-morphological characteristics explaining lower morphological taxonomic descriptors. To overcome such limitations in the morphological variations being affected by environment, molecular techniques are being adopted (Mullis and Faloona 1987; Arnheim *et al.* 1990). RAPD analysis differentiates larger numbers of unique nucleotide sequences in nuclear genome (Whitkus *et al.,* 1974) and is the cheapest and reliable markers used to estimate molecular diversity (Mneney *et al*., 2001, Dhanaraj *et al*., 2002 and Samal *et al*., 2003b). Despite questions about its reproducibility, its utility in diversity analysis, mapping, and genotype identification has been exploited in many plant species (Weising *et al.* 1995; Harris, 1999; Hemanth and Vasanthaiah, 2009; Ishtiaq *et al.,* 2008; Jena *et al.,* 2010b; Archak *et al.,* 2003; Ravishankar *et al.,* 2000) and also used in Tanzanian cultivars (Mneney *et al.,* 2001) and Indian cultivars (Dhanaraj *et al.,* 2002; Samal *et al.,* 2003a) of cashew (Table 3). RAPD endowed precisely for differentiating cashew cultivars when compared with morphological descriptors (Jena *et al.,* 2016). For managing germplasm more efficiently a core collection has been identified based on the study which represents the same diversity as the entire population using RAPD (Dhanaraj *et al.,* 2002).

ISSR markers are efficient, robust, highly polymorphic, co-dominant, multi-allelic and highly reproducible in nature. ISSR markers have been used

for genetic analysis of many crops (Gonzalez *et al*., 2005; Qiao *et al*., 2006; Manimekalai and Nagarajan, 2006; Gajera *et al*., 2011). Archak *et al*. (2003) analyzed superior cashew germplasm utilizing ISSR and AFLP. Researchers across the world characterized cashew cultivars effectively (Dasmohapatra *et al.,* 2013; Thimmappaiah *et al.,* 2009; Lemos *et al.,* 2021). Eventually ISSR markers have been used to characterize gene bank accessions (Blair *et al.,* 1999; Charters and Wilkinson, 2000), as well as to identify closely related cultivars (Fang and Roose, 1997).

Though SSR markers are considerably efficient, robust, multi-allelic, co-dominant, highly polymorphic and reproducible, only limited number of SSR markers has been reported in cashew (Croxford *et al*., 2006). SSR markers exhibited higher polymorphism of 83.3% (Thimmappaiah *et al.,* 2009). Microsatellite markers were found to be absolute polymorphic in cashew (Sika *et al.,* 2015).

Quantitative trait locus- an overview

Cavalcanti and Wilkinson (2007) provided initial platform and represented identification of QTLs linked with economically important traits in cashew to develop genetic maps. dos Santos *et al.,* 2010 identified candidate QTLs in cashew apples for physical traits. They also detected QTLs in cashew apple to map cashew population based on physiochemical characters. Mzena *et al.,* (2018) firstly revealed SNPs linkage map to efficiently study breeding program and identification of QTL through marker assisted selection.

DISCUSSION

Cashew tree (*Anacardium occidentale* L.) belonging to the family Anacardiaceae is an important commercial crop of global importance. It is mostly cross pollinated plant with entomophilous pollination. India, the first country in the world exploited international trade in cashew kernels in the early part of 20th Century. The characterization, variability/ genetic diversity as well as relationship among accessions determine its proper utilization in the breeding programmes. The present day breeding for the improvement of the cashew aims to enhance the quality cashew nut particularly the commercially important traits like nut size and its biochemical properties. Both traditional and molecular breeding approaches were exploited for cashew breeding as evident from the present reviews of previously published literature. Cashew breeding is mostly based on traditional methods of selection of useful traits, which is based on morphological and biochemical attributes which are not much reliable and sometimes misleading. Molecular markers are considerably efficient, robust, multiple allelic, co-dominant, highly polymorphic and reproducible. RAPD and ISSR endowed precisely for differentiating cashew cultivars when compared with morphological descriptors. The molecular markers like RAPD and ISSR have been exploited more in comparison to more reproducible SSR and AFLP markers. To accelerate conventional breeding QTLs that can be co-segregated with different useful traits have to be exploited to introgress polygenic traits in desired background of Cashew genotypes.

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