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THE ROLE OF MOLECULAR MARKERS IN IDENTIFYING GENETIC DIVERSITY AMONG POMEGRANATE GENOTYPES

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Abstract

India is the largest producer of pomegranates in the world with a share of 50 % of world's pomegranate production producing finest varieties of pomegranate having soft seeds, very less acids and very attractive colour of the fruits and grains. Number of pomegranate genotypes with more or less phenotypic dissimilarities available in various regions of Maharashtra State has created a confusion about the real identity of theses genotypes. Especially, Bhagwa-like genotypes have urged an immediate need for either morphological, biochemical or molecular characterization of all such genotypes. DNA based molecular markers is the most advanced and most reliable technique among various characterization methods like molecular, morphological, biochemical characterization.

DNA based techniques such as ISSR and SSR markers are effective in assessing genetic diversity among the cultivars, because they provide unlimited potential markers to reveal differences at molecular level.

Keywords:

Molecular markers, Morphological markers, diversity, Pomegranate,

Introduction

Pomegranate (*Punica granatum* L.) is a member of a smallest botanical family i.e. Punicaceae. Punicaceae family consists of a single genus *Punica* L. and two species *Punica granatum* L. (Pomegranate) and *Punica protopunica Balf.* f. (*Socotria protopunica* Balf. f.). Punica granatum is indigenous to Iran and is a cultivated type. It is grown in almost all tropical and subtropical regions of the world. Whereas, Punica protopunica is native of the Islands of Socotra (Democratic Republic of Yemen). It is wild type and is found growing wild in the Socotra Island. There are two subspecies of species *granatum*; the *chlorocarpais* indigenous to Trans-Caucasus region and the *porphyrocarpa* is native of Central Asia.

World Scenario

Pomegranate is now widely cultivated in Mediterranean countries like Spain, Morocco, Egypt, Afghanistan and Baluchistan. It is also grown in Burma, India, China, Japan, California and Pakistan. India, Iran, China, USA and Turky are the major pomegranate producing countries.

India is the largest producer of pomegranates in the world with a share of 50 % of world's pomegranate production producing finest varieties of pomegranate having soft seeds, very less acids and very attractive colour of the fruits and grains. With adoption of different "bahars", India can supply pomegranates almost throughout the year.

Indian Scenario

There is a sizeable increase in acreage and production of pomegranate in India. The total area under pomegranate in India in 2012-13 was 113 thousand hectare and production was 745 thousand

metric tones with productivity of 6.6 metric tones / hectare. The total production of pomegranate is concentrated mainly in the Western Maharashtra, Karnataka, Andhra Pradesh, Gujarat and to a smaller extent in Rajasthan, Tamil Nadu and Himachal Pradesh.

In India, pomegranate is commercially cultivated in Maharashtra followed by Andhra Pradesh, Karnataka, Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Tamilnadu, Punjab and Haryana. In India in 2012-13, the production share of pomegranate was 0.9 % of all major fruits produced in India.

Maharashtra Scenario

Pomegranate is one of the important fruit crops commercially grown in Maharashtra. Maharashtra is the leading pomegranate producing state with production share of 54.8 % with about 69.00 % share of the total area under cultivation. In Maharashtra, in 2012-13, total area under pomegranate was 78 thousand hectares and production was 408 thousand metric tones with productivity of 5.2 metric tones / hectare. In Maharashtra, pomegranate is commercially cultivated in Solapur, Sangli, Nashik, Ahmednagar, Pune, Dhulia, Aurangabad, Satara, Osmanabad and Latur districts.

Objectives – Following are the objectives of this study.

1.To discuss about role of morphological markers in identifying genetic diversity among pomegranate genotypes.
2.To discuss about role of molecular markers in identifying genetic diversity among pomegranate genotypes

Research Methodology

This is descriptive study based on secondary data. Various Research Journals, Books, Websites & various reports related to the role of various markers in identifying genetic diversity among pomegranate genotypes and in improvement of pomegranate fruit crop were studied to draw the conclusions.

Discussion

In this Paper varietal scenario of pomegranate in context with marker technology including morphological and biochemical markers in pomegranate as well as DNA-based molecular markers like Randomly amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), Restriction Fragment Length Polymorphism(RFLP), Directed Amplification of Minisatellite DNA (DAMD), Sequence Related Amplified Polymorphism (SRAP), Random Amplified Microsatellite Polymorphism (RAMP), Inter-simple Sequence Repeat(ISSR), Simple Sequence Repeat (SSR), etc. markers in pomegranate are discussed as follows.

Variertal Scinario

A number of local types of pomegranate which are seedling progenies are cultivated in different growing regions. Most of them are known by names of places where they are popular. The varieties presently grown in India show distinct variation in fruit shape, colour, taste, colour of aril, rind thickness, etc. The varieties that are grown commercially include Ganesh, G-137 and Mridula. The cultivar such as Bhagwa and Phule Arakta variety have been recommended and released respectively for cultivation in the state. Among various cultivars grown as on today, Bhagwa is the leading cultivar of Maharashtra. This cultivar is known by various names viz., Shendri, Ashtagandha, Mastani, Jai Maharashtra and Red Diana in different districts of Maharashtra. This cultivar is a heavy yielder and possesses desirable characters like attractive glossy red fruit colour, dark red arils, free from blackening of arils, soft seeds, high TSS, tolerant to fruit cracking with good shelf life (15 to 20 days at room temperature).

Bhagwa cultivar is not only known by various names in Maharashtra but also expresses major variations in different phenotypic and morphological characters due to heterozygous nature of the crop and clonal variation.Tropical fruit species and so also pomegranate are mostly heterozygous due to high degree of outcrossing and require systematic morphological characterization backed by the molecular characterization to study the extent of variability and utilization of existing germplasm. Systematic characterization of physico-chemical characters of available germplasm provides the extent of genetic diversity in the fruits species and facilitate in identifying the superior genotypes with desired characters.

Marker technology

Morphological and biochemical markers are used on large scale for assessing genetic diversity in fruit crops but they show limited levels of detection of inter-varietal and intra-varietal polymorphisms on account of their environmental plasticity.

DNA based molecular markers provide a good and informative approach to estimate the genetic diversity and genetic relationships of horticultural plants. It is the most advanced and most reliable technique among various characterization methods like molecular, morphological, biochemical characterization. DNA based markers are abundant, highly polymorphic and independent of environment or tissue type. The isoenzyme markers are limiting due to low levels of polymorphisms. Consequently, DNA based techniques such as ISSR and SSR markers are effective in assessing genetic diversity among the cultivars, because they provide unlimited potential markers to reveal differences at molecular level.

Several morphological, biochemical and molecular markers have been extensively used in assessing diversity in pomegranate. Significant work on the genetic variability and relationships of pomegranate has been carried out in India using array of DNA-based molecular markers.Marker types that have been tested in pomegranate include RAPD, RFLP, AFLP, ISSR, DAMD and SSR. During recent years, these marker types were used to study genetic diversity and relatedness among pomegranate genotypes.

Morphological markers in pomegranate

Morphological markers are those traits that are scored visually, or those genetic markers whose inheritance can be followed with the naked eye. Morphological markers are generally used to identify appropriate genotypes for breeding and crop improvement programs. Bailey(1917) and Hodgson (1917) were the first to recognize the use of morphological characters and incorporate such important features as colour of rind, colour of petals and size of pomegranate tree in their description. Nath and Randhawa(1959) conducted detailed morphological studies in seven pomegranate varieties viz., Dholka, Double Flower, GBG -1(Ganesh), Japanese Dwarf, Khandhari, Muskat White and Patiala and concluded that no single character would be dependent upon to establish the identity of any pomegranate cultivar, but a combination of several characters was more useful.

Ferrara et al. (2011) differentiated eight pomegranate genotypes from the south of Italy by fruit weight, ?Brix, titratable acidity, as well as polyphenol, vitamin C and oil content. Some pomegranate morphological characters like fruit color and shape, tree size, shape and branching habit, can be affected by agro-ecosystems and mutations an therefore, emphasized the need for more robust molecular markers.

Biochemical markers in pomegranate

The first biochemical molecular markers used were the protein based markers. One of the earliest pro¬tein based markers to be used was Isozyme. Jalikop and Kumar (1998) described a monogenic marker (designated R) suitable for estimating the extent of natural cross-pollination, since it enables heterozygous progenies to be visually distinguished from homozygous recessive progenies at the seedling stage. The dominant gene (present in cv. 'Ganesh') confers red and the recessive gene (cv. 'Kabul Yellow') yellow pigmentation of the petiole base, leaf margin, flower bud and fruit rind.

Mars and Marrakchi (1999) used fruit size and color and juice characteristics to discriminate 30 Tunisian pomegranate accessions based on principal component analysis (PCA). PCA and cluster analysis showed a considerable phenotypic and genetic diversity in the local pomegranate germplasm. Gadaleta et al. (2012) applied morphological characterization ona set of 15 pomegranate accessions collected from different areas of Apulia region.

DNA-based molecular markers

The se¬quence of nucleotides in DNA of an individual is unique and thus determines its identity. The ultimate difference between individuals lies in the nucleotide sequence of their DNA. The detection of such differences employing different molecular biological techniques led to the devel¬opment of DNA markers. Molecular markers have made it easier to characterize germplasms and identify genotypes with desirable traits in breeding programs. Marker types that have been tested in pomegranate (Punica granatum L.) include RAPD, RFLP, AFLP, ISSR, DAMD and SSR.

Randomly amplified polymorphic DNA (RAPD) markers in pomegranate

RAPD analysis is the most extensively used technique to determine the genetic diversity among cultivars and genotypes of pomegranate. RAPD molecular markers have been used to understand and

unravel the diversity and systematics of pomegranate by different workers from time to time. RAPD markers were employed to determine the genetic diversity amongst 24 Iranian pomegranate genotypes (Zamani et al 2007). Jambhale et al.(2007) successfully applied RAPD markers to distinguish four popular pomegranate cultivars viz; Ganesh, G-137, Mridula and Phule Bhagwa in Maharashtra State. In another study on 25 pomegranate cultivars from China using RAPD markers, carried by Yang et al. (2007), complexity was mentioned in assessing genetic background of pomegranate germplasm resources in Yun-nan province in China

Similarly, Hasnaoui *et al.*(2010a), Noormohammadi *et al.* (2010), Ercisli *et al.*(2011), Zhang *et al.*(2012), Mahajan *et al.* (2013), Singh *et al.* (2013), Zamani *et al.* (2013), Orhan *et al.*(2014) studied genetic diversity in pomegranate germplasms using RAPD markers.

Amplified fragment length polymorphism (AFLP) markers in pomegranate

AFLP markers are based on a combination of RFLP and PCR techniques and have been used for species identification, assessing genetic diversity and genetic mapping.

Earlier, Yuan *et al.* (2007)studied eighty-five pomegranate (*Punica granatum* L.) cultivars from six geographical populations located at Shandong, Anhui, Shaanxi, Henan, Yunnan, and Xinjiang Provinces for its population genetic diversity by means of fluorescent-AFLP markers. Awamleh et al.(2009) used Amplified Fragment Length Polymorphism (AFLP) technique to assess the genetic variability among pomegranate landraces. Malfa *et al.* (2010) and Moslemi et al. (2010) used AFLP markers to estimate intra-specific genetic diversity among pomegranate genotypes.

Restriction Fragment Length Polymorphism (RFLP) markers in pomegranate

RFLP analysis has been exploited for construction of linkage maps and assessment of genetic diversity in pomegranate.

Melgarejo *et al.* (2009) evaluated a genetic method to identify pomegranate cultivars. The procedure was based on the Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) techniques. Ten pomegranate accessions were evaluated. The results proved the appropriateness of the PCR–RFLP technique for identifying pomegranate cultivars. All evaluated cultivars were differentiated according to their genetic profiles.

Directed Amplification of Minisatellite DNA (DAMD) markers in pomegranate

DAMD is a robust and simple PCR-based methodology that uses mini satellites to assess polymorphism.

Narzary*et al.* (2009) described the use of DAMD and RAPD methods that generate the profiles, to study genetic diversity in wild genotypes of the *Punica granatu*m in India. Forty nine accessions representing two regions of Western Himalayas were analysed. The results indicated that DAMD (97.08%) revealed more polymorphism in comparison to RAPD (93.72%). The results showed that these methods are sufficiently informative to unravel the genetic variations in wild pomegranates in Western Himalayas. They further described the use of RAPD and DAMD markers in unraveling the genetic diversity in wild pomegranate of India. The study revealed that DAMD (97.08%) showed more polymorphism in comparison to RAPD (93.72%), i.e., DAMD was more powerful than RAPD and ISSR in assessment of genetic diversity in pomegranates.

Sequence Related Amplified Polymorphism(SRAP) markers in pomegranate

Zhang et al. (2008) applied sequence related amplified polymorphism (SRAP) to analyze 23 Chinese pomegranate genotypes (*Punica spp.*) using 7 pairs of primers. Soleimani *et al.* (2012) assessed the genetic diversity of 63 cultivated, wild, and ornamental pomegranate genotypes from five different geographical regions of Iran by using sequence-related amplified polymorphism (SRAP).

Random Amplified Microsatellite Polymorphism (RAMP) markers in pomegranate

Zhao *et al.* (2013) examined genetic relationships among 46 pomegranate genotypes collected from 7 provinces in China by using random amplified microsatellite polymorphism (RAMP) technique.

Inter-simple Sequence Repeat(ISSR) markers in pomegranate

ISSR and SSR are the microsatellite markers that are successfully used for assessing genetic diversity, pedigree analysis and cultivar identification.

Narzary *et al.* (2010) described genetic diversity across natural populations of Indian pomegranate based on inter-simple sequence repeat (ISSR) markers. Various studies have been carried out to study genetical diversity in pomegranate using molecular markers such as inter simple sequence repeats (ISSR) (Narzary *et al.*, 2010 ; Bedaf *et al.*, 2011 ; Zhao *et al.*, 2011 ; Noormohammadi

et al., 2012 and Ismail *et al.*, 2014).

Narzaryet al. (2010) described genetic diversity across natural populations of Indian pomegranate based on inter-simple sequence repeat (ISSR) markers. Bedafet al. (2011) evaluated 24 Iranian pomegranate cultivars using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. Zhao et al. (2011) used ISSR markers to analyze the genetic relationship of 47 pomegranate cultivars.

Simple Sequence Repeat (SSR) markers in pomegranate

Koohi et al.(2005) evaluated the level of polymorphism in pomegranate cultivars using SSR markers. Curro *et al.* (2010) assessed the level of genetic diversity among cultivars and wild genotypes using nine Simple Sequence Repeat (SSR) markers. Hasnaoui et al. (2010b) reported the development of 11 microsatellite markers (SSR) for *Punica granatum* L. to evaluate on a set of 27 pomegranate accessions sampled in Tunisia. Alamuti *et al.* (2012) evaluated the genetic background of 738 pomegranate (*Punica granatum* L.) by using a set of twelve simple sequence repeat markers from 23 provinces of Iran. Hasnaoui *et al.* (2012) in their study reported the development of 4 new polymorphic SSR markers they used in addition to 11 SSRs to investigate molecular diversity of 33 pomegranate ecotypes. Jbir *et al.* (2012) used specific microsatellites (SSRs) markers to characterize a set of 32 Tunisian pomegranate (*Punica granatum* L.) cultivars. Parvaresh *et al.* (2012) used microsatellite markers to study the genetic diversity and phylogenetic analysis among 75 pomegranate genotypes from Iran, Japan, Turkmenistan, Russia, Italy and USA. Raina *et al.* (2013) assessed genetic diversity in pomegranate genotypes using 47 SSR markers.

CONCLUSION

There is a sizable increase in the area, production and productivity of pomegranate in India. India is the largest producer of pomegranates in the world with a share of 50 % of world's pomegranate production producing finest varieties of pomegranate having soft seeds, very less acids and very attractive colour of the fruits and grains. A number of local types of pomegranate which are seedling progenies are cultivated in different growing regions. Most of them are known by names of places where they are popular. Due to high export potential for pomegranate varieties like Bhagwa, there is an urgent need to identify the diversity or similarity among the pomegranate varieties available all around. Systematic characterization either morphological, biochemical or molecular characterization of available germplasm is necessary to know the extent of genetic diversity and facilitate in identifying the superior genotypes with desired characters.

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