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Novel Genic Markers for Genetic Improvement of Pomegranate

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Abstract

The generation of marker resources and development of genetic maps with breeding applications is crucial for the genetic improvement of pomegranates. Next-generation sequencing (NGS) technologies have helped to generate huge genomic resources in pomegranate that incudes genome, transcriptome of coding and noncoding RNAs sequencing. These findings have provided direct access to a huge number of gene models and their families of pomegranate. Based on gene information we could develop novel gene-based markers resources like micro-RNA based simple sequence repeats (miRNA-SSRs), potential intron polymorphism (PIP) and insertion deletions (InDels) markers for applied research in pomegranate. Since, gene-based markers would show functional differences at the trait level directly. Breeders can thus bypass linkage drag by choosing the segregating progenies of various crossings during breeding directly at the gene level. Therefore, we assume that these markers would be an invaluable resource for future trait mapping and gene discovery applications to enable gene editing in pomegranates. There by significantly hasten pomegranate genetic improvement and lead to the development of elite cultivars with significant export potential.

Keywords: Pomegranate, Gene, Markers, Genome, Breeding

Introduction

The discovery of new genes and SNP markers, identification of gene families, study of evolution, creation of transcriptome maps, identification of metabolic pathways, and other applications were the immense promise of next-generation sequencing (NGS) technologies in plant genome research (Blanca et al., 2012). The generation of marker resources and development of genetic maps with breeding applications is crucial for the genetic improvement of pomegranate. Consequently, the creation of gene-based DNA marker systems in

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pomegranate may substantially aid in genomics assisted genetic advancement in the future (Patil et al., 2020a, c).

Through international efforts on pomegranate genome sequencing, at present four genomes are publically accessible on NCBI, the largest of which is "Bhagawa" at 342 Mb, followed by "Dabenzi" at 328 Mb, "Tunisia" at 320 Mb, and "Taishanhong" at 274 Mb. In order to create useful gene-based markers resources like single nucleotide polymorphisms (SNPs), insertion deletions (InDels), and simple sequence repeat markers, these findings have provided direct access to a huge number of gene models and their families. Furthermore, the development of gene based functional DNA markers in pomegranates, such as EST-SSRs, EST-SNPs (Ono et al., 2011; Ophir et al., 2014), and miRNA-SSRs (Patil et al., 2020b), has been made possible by the availability of transcriptome sequencing of coding and noncoding RNAs. The high throughput genetic analysis needed for pomegranate genetic improvement may be aided by these marker resources. However, lesser polymorphism potential and the requirement for specialized, expensive platforms for marker genotyping are the drawbacks that prevent the widespread use of these gene-based DNA markers.

Harel-Beja et al. (2015) previously shown usefulness of SNP markers for identifying 25 QTLs for pomegranate fruit quality attributes. Trainin et al., (2021) also conducted a fine mapping of the candidate gene, anthocyanidin reductase (*ANR*) with a point mutation that causes the black peel colour in pomegranates. This allowed for the development of an SNP-based functional marker for fruit colour. Deploying gene-based DNA markers is therefore more crucial to improving pomegranate breeding efficiency. This call for the urgent development of effective gene-based marker systems that are widely distributed throughout the genome and that can show polymorphism on basic genotyping platforms (Badoni et al., 2016). Because gene-based markers have remained relatively stable over evolutionary time across genera, they serve as the foundation for comparative genome research in pomegranates.

Micro-RNA based markers

In many organisms, including plants, short RNAs (sRNAs) are recognized as the primary genetic and epigenetic regulators. These have the ability to alter DNA, modify histone methylations, and change the amount of coding (mRNA) or non-coding RNAs, which in turn controls how traits are regulated. MicroRNAs (miRNAs) and small interfering RNAs (siRNAs) are the two main regulatory sRNAs identified in plants. MiRNAs predominantly engage in post

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transcriptional regulation, while siRNAs largely involve transcriptional control (Chen et al., 2018).

The NCBI database now has more genomic information due to the sequencing of the pomegranate genome and its coding and non-coding RNAs (http://www.ncbi.nlm.nih.gov). Due to the fact that polymorphisms in MIR genes encoding miRNAs are known to change the expression, specificity, and/or targeting ability of miRNAs, this can ultimately change the expression of many phenotypic traits. This sequence information has aided in the characterization of the MIR genes that encode miRNAs and has contributed to the development of the first set of large-scale genome-wide miRNA-SSRs. In total, 1054 miRNA-SSRs specific to seedling to fruit developmental stages (Patil et al., 2020b) and 132 miRNA-SSRs specific to seed hardness traits (Patil et al., 2022a) have been designed. In rice, for example, salt sensitive (trait specific) miRNA-SSRs have been discovered by previous researchers (Mondal and Ganie, 2013; Ganie and Mondal, 2015), who have linked them to gene expression and salt tolerant phenotype. The pomegranate miRNA-SSRs may therefore be useful for discovering master miRNAs that control different genes for fruit quality attributes. Eventually, these miRNAs may be used in genome editing programmes to develop pomegranates varieties with desired fruits (Patil et al., 2020b).

Intron Length Markers (ILM)

Introns are the gene sequence components and are abundant in the majority of eukaryotic genomes. Because there was less purifying selection pressure during evolution, these introns have remained less varied and less conserved than coding areas. According to Badoni et al. (2016), introns can serve as highly polymorphic genetic markers. These markers have been shown to exhibit more plant intra-species variability than other types of markers, while being based on genic regions (Muthamilarasan et al., 2014). Due to its unique properties, including as direct depiction of variation within certain genes and subspecies, intron length polymorphism (ILP) is becoming more and more popular. Its advantages over SSR markers are especially noteworthy (Wang et al., 2006). As that of SSR markers cross-species amplification became possible for ILP primers that are designed to conserved flanking exons to amplify introns. Therefore based on intron position predictions across species, Yang et al. (2007) created a database of potential intron polymorphism (PIP) markers. Using this data base, PIP markers have been developed in many plant species and not yet in pomegranate. Given

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their enormous advantages in terms of subspecies specificity, neutrality (no phenotypic effect), and the capacity to perform test variation within genes, PIP markers could be employed in conjunction with SSR markers to establish genetic diversity (Huang et al., 2013).

The whole genome sequencing of numerous crops has provided an increasing amount of information on structurally and functionally annotated genes, which is a valuable resource for the creation of ILP markers across the entire genome. In comparison to other DNA marker systems, there are still very few studies on the development of ILP markers in fruit trees. Genome-wide markers (SSR, ILP, and PIP) were previously reported by Xia et al. (2017) using data from 16 sequenced tree species. Despite the availability of whole genome sequences for four pomegranate genotypes, no research on ILP markers has been published yet. In light of this, we have recently designed 8,812 potential intron polymorphism (PIP) markers that are unique to 3,445 (13.40%) gene models that are distributed across 8 Tunisian chromosomes. Also, we demonstrated their potential utility for genetic analysis by studying genetic diversity among 31 pomegranate genotypes (Patil et al., 2022b). These markers would be an invaluable resource for research on genetic variation, finding functional genes, and genomics-assisted pomegranate breeding.

Insertion/Deletion (InDeL) Markers

The genetic structural alterations that are extensively distributed across the genomes of plants are known to be mostly caused by insertion-deletions, or InDels. Many of the desired intrinsic genetic features of SSR and SNP markers are present in InDels, including co-dominance, abundance, and random distribution throughout the genome (Lv et al., 2016; Pan et al., 2021). InDel markers have emerged as substitutes for SSR and SNP markers (Garcia-Lor et al. 2013). Recently, there has been increased interest in PCR-based InDels due to their potential to identify polymorphism (Vasemagi et al. 2010). Especially when it comes to trait mapping and genotyping, it is crucial to remember that InDels offer more potential than SSRs. A number studies have effectively finished the in-depth examination of important gene families implicated in development and growth in pomegranate *viz*., sucrose synthase (SUS), sucrose transporter (SUT), and exported transporter (SWEET), as well as gene families for transcription factors like three-amino-acid-loop-extension (TALEs), YABBY, auxin responsive factors (ARFs), and basic leucine zipper (bZIP). *In silico* examination of these gene families through multiple sequence alignment across four pomegranate genomes led to the

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designing of 245 unique InDel markers that targeted 140 genes involved in growth and development. Further, the immediate utility of these markers for genetic analysis was proved through genetic diversity study in 16 pomegranate genotypes (Un published). InDel marker developed here may prove to be very useful for future trait mapping and gene discovery applications to enable gene editing in pomegranates.

Conclusion

The deployment of gene-based markers would significantly hasten pomegranate genetic improvement and lead to the development of elite cultivars with significant export potential. Gene-based markers would show functional differences at the trait level directly. Breeders can thus bypass linkage drag by choosing the segregating progenies of various crossings during breeding directly at the gene level. The development of gene-based marker resources in pomegranates may definitely help to identify their allelic variability for prospective genome editing applications to breed superior varieties with improved traits.

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