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Pomegranate Genome and Its Prospects for Breeding Elite Pomegranate Varieties

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Abstract

The availability of complete genome sequence for pomegranate represents the very important genomic resource for downstream breeding applications. The genome sequence will fast tract the breeding new varieties by helping in identifying the key genes governing different traits. Genome sequence of pomegranate has been already explored to develop genome wide informative markers and development of physical maps for breeding applications. Apart from this, genome sequence has served as a reference sequence to cross validated the differentially expressed genes identified through transcriptome analysis. These experiments have resulted in identification of many putative candidate genes viz., genes for integument development for soft and hard seeded types, punicalagin biosynthesis, ellagitannin biosynthesis, colour formation in peels and arils and ovule development processes. Identification of allelic variations in these candidate genes will helps in development of gene based markers to transfer of these traits to another cultivar through marker-assisted selection or by targeted editing of these candidate genes to breed new improved varieties. Genome sequence information would empower the development of better varieties with high export potential by accelerating the genomics assisted translational breeding in pomegranate.

Keywords: Pomegranate,Breeding. Genome.Transcriptome

Introduction

Pomegranate (*Punica granatum* L.) is a high-value, nutritionally rich, and export-oriented crop that ensures high returns on investment to growers across the world. Globally, India stands first in pomegranate cultivation with area 2.62 lakh ha and production of 27.91 lakh tonnes. India is the world leader in pomegranate production with a 50 per cent contribution to global production. Being a high-value crop, until now, due to the lack of

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resistant pomegranate varieties, there has been a very high dependence on chemical pesticides for managing biotic stresses. Pomegranate supports the livelihood security of an estimated 2.5 lakh farm families mostly in climatically and edaphically-challenged regions. The breeding efforts aimed at improving pomegranate through hybridization and selection from natural genetic variants has led to the development and release of few improved varieties in India (Jalikop et al., 2005). India exports an abysmal 2-3 per cent of its indigenous production, which is far below its real potential.

Therefore, there is scope for deploying and implementing modern genomic tools in routine breeding applications to develop new pomegranate varieties with exportable grade fruits. Towards this end, sequencing and decoding the genetic material through genome sequencing efforts will unlock the several genetic mysteries such as identifying particular genes responsible for sweetness, seed softness or colour of the fruit, those responsible for disease and pest resistance, and those for the enlargement of the fruit size in pomegranate. This will helps pomegranate framers in long way in enhancing their income and thereby improving lives. The availability of pomegranate genome sequence will also open up incredible avenues for vastly improving yield, growing much better and safer varieties for human nutritional needs, and all this at a much faster rate. Domestic consumers' standpoint, they would get new varieties with high-quality pomegranates for further fulfilling their health and nutritional needs. Overall, the export potential of pomegranate fruits can be increased at International markets through genomics assisted fruit quality breeding.

Genome sequencing

Genome sequences would provide a valuable resource for the dissection of many biological and biochemical traits for accelerating breeding new varieties in pomegranate. Currently, four genome sequences are there in the public domain, of which two genomes cv. Dabenzi (Qin et al., 2017) and cv. Taishanhong (Yuan et al., 2018) are considered as draft level genomeassemblies developed using second generation sequencing technologies, and Tunisia genome (Luo et al., 2020) as a high quality assembly that involved third generation sequencing technology. Most recently, in a path-breaking development in horticultural science at the National Research Centre for Pomegranates (NRCP), Solapur, under the Indian Council for Agriculture Research (ICAR), we report reference/finished quality genome sequencing of the most popular indigenous commercial pomegranate variety 'Bhagawa'

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(Roopa et al., 2023). Here we combined four commercially available second and third sequencing technologies i.e. Illumina, PacBio, 10X Genomics, and BioNano Genomics to assemble and cover 346.08 Mb (of estimated ~352.53Mb, 98.17% of Bhagawa genome)in 342 scaffolds with an average N50 of 16.12 Mb (Patil et al., 2021b).This assembly represented a very highly quality genome sequence with highcontinuity for downstream genomicsapplications. These sequencing efforts lead to discovery of overall 29,229 to 33,594 protein coding genes in the pomegranate genome. In the Bhagawa genome we report 29,435 gene models, of which 1,573 genes characterized as resistant (R) genes having 32 functional domains and 314 as amicro-RNA coding genes that represented 26 different micro-RNA families (Roopa et al., 2023). The availability of reference-quality genome assembly of most important commercial ruling Indian variety 'Bhagawa' represents the most awaited and publicly accessible genomic resource. Thisgenome information in future willhelp in pomegranate genetic improvement programmes through genomics-assisted trait mapping, breeding and genome editing applications to develop new improved varieties for Indian farmers with high exportable grade fruits.

Genome sequence for Genomics assisted breeding of new varieties

Identification and exploitation of genetic variation is the basis of plant breeding. Traditional selection based on phenotype is tedious and time consuming (Thottathil et al., 2016). Therefore, so far a very few number of pomegranate varieties have been developed and released in India through classical breeding i.e. hybridization and selections. The reference genome of pomegranate will helps to shorten this selection processof superior plants by the breeders and fast-track the recommendation of genetic material for commercial cropping. For this genome wide characterization and development of marker resources from the genome sequence is pre-requisite. These markers not only helpful in identifying genes of desirable traits, but also helps to understand influence of the environment on structuring of plant genetic diversity. Although molecular techniques can reduce the time and costs for genetic profiling the screening is in infancy, the adoption of modern sequencing technologies could accelerate the cultivar improvement. The genome sequence will also reveal the presence of genes of reproductive incompatibility making it easier to identify which individuals cannot breed with each other. This helps breeders in the selection of female individuals to be used in breeding programs.

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Development of marker resources

With recent advanced made through high throughput sequencing in pomegranate has resulted in accumulation of colossal amount genomic information in the form of genome, transcriptome and non-coding transcriptome (sRNA) sequences (Patil et al., 2021b). A pomegranate genome sequence has formed the referencefor development of novel informative markers. For instance, Ravishankar et al., (2015) reported development of first set of 171 SSRs through partial genome sequencing of Ganesh variety. However, with availability of complete genome sequences in pomegranate, we first time report development of 2856 hypervarible SSRs based on draft genome Dabenzi(Patil et al.,2020a) and subsequently we report development of highly polymorphic chromosome specific 3,839 HvSSRTs by exploring Tunisia genome. However, these markers are selectively neutral in nature, as they are located in non-coding and non-regulatory regions. When such markers are used for marker assisted selection, there will be chances of false positives, due to genetic recombination. Therefore, gene based functional nucleotide polymorphism are more powerful and reliable and more advantageous than neutral markers, as there is no recombination between the marker and the gene of interest (Salgotra et al., 2014). Therefore, by integrating information from transcriptome of coding and non-coding, and genome assemblies, we first time report development of large-scale novel DNA markers viz. 1054 miRNA-SSRs and 8000 potential intron polymorphism (PIP) markers for future genetic mapping work in pomegranate (Patil et al., 2020b, Patil et al., 2022b).

Structural variations (SVs) for marker designing

The SVs in the genome represent the major source of genetic diversity, the comparison of ‘Bhagawa’ genome with the ‘Taishanhong’ and ‘Dabenzi’ genomes for six different classes of SVs i.e. Insertion-deletion, repeat expansion and contraction, and tandem expansion and contraction, revealed 5.7 Mb and 9.2 Mb SVs for ‘Taishanhong’ and ‘Dabenzi’, respectively, with ‘Bhagawa’ as a reference (Roopa et al., 2023). Among the SVs, insertions and deletions contributed much greater to total variations in the analyzed genomes suggesting these are the important resources for designing of genome-wide InDeL markers in pomegranate.

Development of physical maps

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Genome sequence information's not only helps in designing of genome-wide informative markers, but also helps in development of marker based physical maps. Towards this end we report development of 906highly variable SSR based chromosome specific physical maps using Tunisia genome (Patil et al., 2021a). Apart from this we also report development of 80 miRNA-SSRs marker based physical map in relation to seed hardness traits (Patil et al.,2022a). More recently, 1,233 PIP markers based physical maps showing exact positions on the Tunisia chromosomes (Patil et al., 2022b). The information's of physical positions of these markers helps breeder in precise selection of markers covering entire genome during markers assisted selection and introgression to breed new improved pomegranate varieties.

Identification of candidate genes for breeding new varieties

A complete and precise knowledge of the genome sequence, expression and functions of the genes has to be obtained before translating them into application through breeding. The main challenge is that the functions of many genes identified by genome sequencing remain unknown and the genetic control of the majority of agronomic traits has yet to be determined. In this direction deciphering of the pomegranate genome followed by bioinformatics analysis has resulted in identification of 29,435 gene models in Bhagawa, 29,229 (Dabenzi), 30,903 (Taishanhong) and 33,594 (Tunisia) genes respectively. Also helped in identification of many putative candidate genes for Integument development gene -*INNER NO OUTER* (*INO*) for fleshy outer layer, putative genes for soft- and hard- seeded types and punicalagin biosynthesis (Qin et al., 2017), ellagitannin biosynthesis, colour formation in peels and arils, ovule development processes(Yuan et al., 2018) and selective loci containing *SUC8- like, SUC6, FoxO* and *MAPK* for hard- and soft- seeded types (Luo et al., 2020).

Apart from this information obtained from transcriptome sequencing, along with their expression profiles will help to identify the key genes governing different traits. Therefore, currently in pomegranate total five transcriptome assemblies have been reported that are specific to fruit peel (Ono et al., 2011), fruit developmental stages (Ophir et al., 2014), seed hardness (Xue et al., 2017), flowering (Chen et al., 2017) and bacterial blight challenged tissues (Singh et al., 2020). These studies resulted in identification of many tissue specific

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differentially expressed genes, which have been deployed to validate on reference genome sequences to identify many candidate genes which are mentioned above. These data will also helpin identification of allelic variations in the candidate genes that are controlling important agronomic traits. This intern helps to transfer of these traits to another cultivar or species by genetic modification or by introgression into cultivar by marker-assisted selection (Edwards and Batley, 2010).

Genome or gene editing to develop elite pomegranate lines

The availability of complete genome assembly will serves as the important reference sequence for precise gene editing efforts in pomegranate. With the availability of information on trait specific superior candidate genes with desired haplotypes and regeneration protocols, now it is possible to go for genome editing tool for targeted modification of key genes to develop new improved varieties. In this directionsto prove the proof of concept for gene editing in pomegranate,Chang et al. (2019) first time targeted editing of two UDP-dependent glycosyltransferases (UGTs), *Pg*UGT84A23 and *Pg*UGT84A24, that resulted in unique accumulation of gallic acid 3-*O*- and 4-*O*-glucosides (galloylglucose ethers) CRISPR/Cas9-edited lines (i.e., *ugt84a23 ugt84a24*) as compared to control. This technology is very much essential in tree species were the juvenile period is long and conventional or molecular breeding takes more time.

Conclusion

The utilization of genome sequence information for genetic improvement of pomegranate would empower the development of better varieties with high export potential. Although, the reference genomes have been obtained in this crop, massive re-sequencing and gene expression studies are most essential to identify the key genes responsible for a desired trait and to find its allele variability for future genome editing applications to breed new improved varieties.

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