Fruit breeding ： pomegranate

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Many fruit trees have been whole-genome sequenced, and these genomic resources provide us with valuable resources of genes related to interesting fruit traits (e.g., fruit color, size and taste) and help to facilitate the breeding progress. Pomegranate (Punica granatum L.), one economically important fruit crop, has attracted much attention for its multiple colors, sweet and sour taste, soft seed and nutraceutical properties. In recent years, the phylogenesis of pomegranate has been revised which belongs to Lythraceae. So far, three published pomegranate genomes including ‘Taishanhong’,

‘Tunisia’ and ‘Dabenzi’ have been released on NCBI with open availability. This article analyzed and compared the assembly and annotation of three published pomegranate genomes. We also analyzed the evolution-development of anthocyanin biosynthesis and discussed pomegranate population genetics for soft seed breeding. These provided some references for horticultural crop breeding on the basis of genomic resources, especially pomegranate.

Keywords: pomegranate, Horticulture, Breeding, Agronomy,

1. Introduction

The growing interests regarding fruit quality and the sudden climate changes require breeders to accelerate the fruit breeding improvements with new efficient breeding approaches, both traditional and unconventional techniques [1][2][3]. Some popular fruit trees have been whole-genome sequenced, including grape[4], apple [5][6][7], peach [8][9], strawberry [10][11], pear [11], orange [12], banana [13], pineapple [14], kiwifruit [15][16][17], blueberry [18], durian [19], waxberry [20], walnut [21][22], mei [23], Chinese chestnut [24], apricot [25], pistachio [26], cherry [27], coconut [28] and pomegranate [29][30][31]. These genomic resources will help to explore the evolution-development of numerous key horticultural traits (such as the fruit color [32]), and facilitate the breeding progress through developing molecular markers from resequencing projects (e.g., GWAS analyses for apple fruit size[5]; QTL for watermelon taste [33]).

Pomegranate (Punica granatum L.) is an ancient crop with a long domestication history starting since 4000–3000 BC [34] and actually accounts for a total area of more than 6000 km^2 globally, especially with increasing cultivation in China, Iran, India, Turkey and America, and with the potential for marketing and mass consumption [29]. Hence, the biology of pomegranate has achieved considerable attention. Firstly, exciting advances in understanding the taxonomy of pomegranate have recently been revised by using whole-genomic analyses [29]. On the basis of morphological [35], molecular [36], genomic [29] evidences, pomegranate is widely accepted as being part of the Lythraceae rather than the Punicaceae family, and its well resolved phylogenetic position provides a key resource for plant phylogenomics. A second debated question is the origin of pomegranate, which was thought to be originated in Central Asia (Himalaya range) or from Mediterranean area [37]. It is adaptable to different climate conditions and has a wide range of global distribution [38]. The most popular attention is regarding its breeding, especially for fruit color, medicinal and pharmaceutical active compounds, and seed softness. Pomegranate possesses more than 500 globally-distributed varieties, and its morphological characteristics, quality and genotype are evaluated by morphological, biochemical and molecular markers providing some evidence for its genetic diversity [39]. It is known as a ‘super-fruit’ due to its high total phenolic content (e.g., around 1875 and 11,250 mg/L detected in whole pomegranate fruit [40]), especially for the abundant and unique punicalagin (128.02–146.61 mg/g in peel) [41] whose antioxidant activity is 3.11, 9.94 and 39.07 times more than blueberry, sweet orange and apple, respectively [42]. These active compounds have hypolipidemic, antiviral, anticancer, immunomodulation and improving metabolic syndrome function [43][44][45][46].

Molecular techniques can reduce the time and costs for genetic profiles screening. Although the screening is in infancy, the adoption of modern sequencing technologies is accelerating cultivar improvement.

2. Pomegranate Population Genetics for Soft Seed Breeding

Population genetic structure is considered as the amount and distribution of genetic variation within and between populations. The direct manifestation of genetic variation is mutation [47]. To elucidate the evolutionary processes acting on plant populations, we need to discuss levels and distributions of genetic variation, selection and adaptation and genetic structure within and between populations [48].

The origin of pomegranate is disputed. According to Levin [49], three mega-centers (primary, secondary and tertiary) and five macro-centers (Middle Eastern, Mediterranean, Eastern Asian, American and South African) are considered as the origin of pomegranate. Pomegranate has wild and cultivated species. It has been domesticated about 3500 BC in Western Asia [50]. Fluorescent-AFLP (amplified fragment length polymorphism) markers analysis was conducted for 85 pomegranates from six geographical populations located at Shandong, Anhui, Shaanxi, Henan, Yunnan, and Xinjiang Provinces in China. The results indicate that the diversity at a population level is lower than that at a species level, and genetic diversity between populations was significantly different [51]. Molecular variation analysis indicates that most genetic variation in 49 accessions of wild Indian pomegranate is within populations (54%) but not between populations, and gene flow in wild pomegranate accessions is lower than in Chinese pomegranate [51][52]. In Tunisia, the low diversity of pomegranate based on SRR (simple sequence repeats) markers analysis reveals its narrow genetic background due to its limited origins [53]. Sarkhosh, et al. [54]gathered 21 Iran soft-seeded pomegranates and low correlation was detected between fruit characteristics and RAPD (random amplified polymorphic DNA) markers [54].

The divergence between hard- and soft-seeded pomegranates is the embodiment of pomegranate germplasm resource diversity. Hence, it is important to explore the metabolic mechanism of influencing seed hardness in pomegranate. The softness of seeds is a desirable economic trait that enhances the consumptive qualities of fruits, but the complete soft-seeded pomegranate is restricted to a narrow ecological region [55]. In addition, study the formation of the soft seed is beneficial to elucidate the mechanism of lignin synthesis which contributes to plant growth and development and the build-up of resistance to biotic and abiotic stresses and plant evolution [56][57]. To breed a pomegranate which has the characteristic of soft seed is one of the breeding objectives. However, until then, deciphering the ecological and evolutionary forces that form the population structure of the soft-seeded pomegranate is a priority, and this will help us to understand the emergence of soft seed trait and benefit to crop breeding.

Seed hardness is related to cell wall biosynthesis [58]. By comparing protein expression profiles between two genotype pomegranates (‘Tunisia’ (soft seed) and ‘Sanbai’ (hard seed)) at 60 and 120 days after flowering, it was found that ‘Tunisia’ had lower lignin but higher cellulose biosynthesis [59]. Luo, et al. [60]

found that several miRNA-mRNA pairs regulated seed hardness by altering cell wall structure, and mdm-miR164e- and mdm-miR172b-targets included *WRKY*, *MYC* and *NAC1* mainly involving brassinosteroid biosynthesis, cell division and lignin biosynthesis. Luo, et al. [31] summarized that genomic variations and selective genes are two critical factors resulting in the divergence between hard- and soft-seed pomegranates. Twenty-six pomegranate varieties were collected and re-sequenced, and the neighbor-joining tree, population structure, PCA (principal components analysis), and LD (linkage disequilibrium) analyses all indicate support for the clustering of pomegranate clade according to seed hardness. Numerous candidate *MYB*, *WRKY*, *AP2-like*, *MYC* and *NAC* genes with different proportions of SNPs (single nucleotide polymorphisms) and InDels (insertion-deletion) in pomegranate and hawthorns were found to play roles in regulating seed hardness. These transcription factors are involved in brassinosteroid, cell division, lignin, cellulose flavonoid and xyloglucan biosynthesis [31][61][62]. Zarei, et al. found *COMT* exhibited higher expression in soft-seed pomegranate, and *CCR* and *CAD* showed higher expression in hard-seed pomegranate. In the soft-seed variety of hawthorn, four *NAC* and twelve *MYB* TF are all significantly down-regulated [61]. A battery of Secondary Wall-associated NAC Domain Protein (SND1) regulated transcription factors such as *SND2*, *SND3*, *MYB46*, *MYB103*, *MYB85*, *MYB52*, *MYB54*, *MYB42*, *MYB43*, *MYB20*, and *KNAT7* in *Arabidopsis* has been demonstrated to be involved in the process of secondary wall formation and *MYB46* is able to activate the entire secondary wall biosynthesis program [63][64]. Xia, et al. [65] characterized the role of a NAC transcription factor (*PgSND1-like*) involved in the regulation of seed hardness in pomegranate. They found that *PgSND1-like* gene from ‘Tunisia’ and ‘Sanbai’ had a single base replacement at the 166-bp position of CDS (coding sequence). Overexpression of *PgSND1-like* (‘Sanbai’) transgenic *Arabidopsis* could enhance lignin content, while *PgSND1-like* (‘Tunisia’) in *Arabidopsis* exhibited no phenotypic differences compared with wild type. They speculated that *PgSND1-like* in ‘Tunisia’ and ‘Sanbai’ may regulate lignin biosynthesis and seed hardness in pomegranate. The adaptation process of a population following a rapid environmental change or the colonization of a new niche has been reported in two ways—either through new beneficial mutations or through using alleles of the standing genetic variation [66]. The study of phenotypic evolution reveals that strong selection and rapid heritable trait changes in nature are common [67][68]. All soft-seed pomegranate varieties seem to prefer a tropical Asian climate and hard-seed

pomegranates have a stronger ability to endure cold [31]. Examples are that some genes such as *PgL0044640* and *PgL0314990* are enriched in the forkhead box O (FoxO) signaling pathway and *PgL0044700* is involved in the mitogen-activated protein kinase (MAPK) signaling pathway in the hard-seeded population of pomegranate (Table 3) [31]. Besides, in Drosophila melanogaster and tomato, both FoxO and MAPK are involved in cold response [68][69].

Table 3. Genes involving the regulation of seed hardness in pomegranate.

Gene	Function	Reference
PgSND1-like	**The overexpression of the *NAC* transcription factor PgSND1-like enhanced lignin concentration in transgenic plants compared with wild-type *Arabidopsis*.**	**[65]**
SUC6	***SUC6*, one sucrose transport protein, which was more highly expressed at 60 days after flowering than 120 days after flowering in 'Tunisia' and 'Sanbai'.**	**[60]**
SUC8-like	***SUC8-like* was important for controlling seed development and was down-regulated significantly in soft-seeded pomegranate 'Tunisia' compared to hard-seeded pomegranate 'Sanbai'.**	**[31][60]**
PgL0044640	**These two genes were enriched in the FoxO signaling pathway indicated in the hard-seeded population by KEGG analysis.**	**[31][68][69]**
PgL0314990		**[31][68][69]**
PgL0044700	**This gene was enriched in the MAPK signaling pathway in the hard-seeded population by KEGG analysis.**	**[31][68][69]**

3. Conclusions

Some genomic resources have revealed many metabolic pathways and offer molecular markers for agronomics traits. Many genes in the anthocyanin biosynthesis pathway have been identified, cloned, and their functions partially verified function. We reconstructed a maximum likelihood tree with 27 *MYB* genes related to anthocyanin synthesis. These *MYB* members were divided into the typical three major branches and evolved diverse functions. It was speculated that *MYBs* evolved new functions, due to the natural development of producing different colors to attract birds or other animals to spread seeds. The complexity of regulation of lignin synthesis is the embodiment of soft- and hard-seeded pomegranate germplasm resource diversity. Hence, it is of important significance to study the formation of the soft seed trait. The soft and hard degree of seed in pomegranate is influenced by environmental factors and genetic background. Because of the complexity of metabolic synthesis, additional research will be continued. Besides, many more excellent traits of pomegranate germplasm resources should be explored and exploited to breed pomegranate with various colors and soft seed.

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