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Review

A Review of Omics Technologies and Bioinformatics to Accelerate Improvement of Papaya Traits

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Abstract: Papaya (*Carica papaya*) is an economically important fruit crop that is mostly planted in tropical and subtropical regions. Major diseases of papaya, such as the papaya dieback disease (PDD), papaya ringspot virus (PRSV) disease, and papaya sticky disease (PSD), have caused large yield and economic losses in papaya-producing countries worldwide. Postharvest losses have also contributed to the decline in papaya production. Hence, there is an urgent need to secure the production of papaya for a growing world population. Integration of omics resources in crop breeding is anticipated in order to facilitate better-designed crops in the breeding programme. In papaya research, the application of omics and bioinformatics approaches are gradually increased and are underway. Hence, this review focuses on addressing omics technologies and bioinformatics that are used in papaya research. To date, four traits of the papaya have been studied using omics and bioinformatics approaches, which include its ripening process, abiotic stress, disease resistance, and fruit quality (i.e., sweetness, fruit shape, and fruit size). This review also highlights the potential of genetics and genomics data, as well as the systems biology approach that can be applied in a papaya-breeding programme in the near future.

Keywords: bioinformatics; comparative genomics; molecular markers; next-generation sequencing; omics; papaya

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1. Introduction

Papaya (*Carica papaya)* is one of the tropical fruits that is widely grown in tropical and subtropical countries such as Australia, Brazil, Malaysia, Thailand, and South America. In worldwide fruit production, the papaya is ranked in third place after mango and pineapple fruit production [1]. The papaya has a high nutritional content and medicinal value, which make it widely planted and globally popular. For instance, the fruit is rich in vitamin A, vitamin C, folate, potassium, and niacin, and its leaves, stems, and roots are suitable for alternative medicine [2].

Papaya has nine pairs of chromosomes and is a diploid. The papaya, which has a genome size of 372 Mb, belongs to the Brassicales order and Caricaceae family. Among the well-known papaya cultivars in Hawaii are Solo, Sunrise, SunUp, and Rainbow [3], while Eksotika and Sekaki are well-known cultivars in Malaysia [3]. To date, six papaya cultivars, namely Eksotika, Eksotika II, Sekaki, Three Pillars, Frangi, and Viorica have been registered with the Department of Agriculture, Malaysia [4]. Correspondingly, Japan also produces papaya cultivars, namely Ishigaki Sango and Ishigaki Wondrous [5]; in India, the well-known papaya cultivars are Co1 and Co2 [3].

As a perennial herb, the breeding cycle of the papaya takes about seven to nine months until a ripe fruit is produced. The breeding cycle of the papaya is divided into three stages that are represented by seed germination, flowering, and fruit setting to fruit harvesting (Figure 1) [6,7]. The papaya seed germinates within two to four weeks after sowing [8].

Then, after being transplanted, its vegetative state starts to grow into single-stemmed trees, each bearing a rosette of large, deeply lobed leaves at the apex about two to three months after transplantation [6–8]. The papaya plant produces its flowers at the age of three to six months, and produces ripe fruits at seven to nine months [6,7].

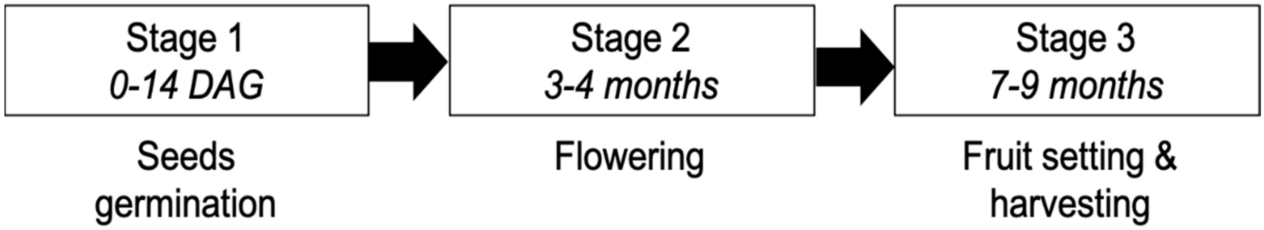

Figure 1. The breeding cycle of papaya. DAG denotes day after germination.

Papaya is cultivated in fertile and well-drained soils with a pH of 5.5 to 6.5 [8,9]. Nutrients for the papaya are essential in order to increase the productivity and quality of papaya production. Nitrogen (N) and phosphorus (P) are essential nutrients during the early growth of the papaya in order to ensure an optimum growth of foliage, trunks, and roots, as well as induce higher productivity [10]. Potassium (K) is needed at the papaya fruiting stage because it is essential for improving the fruit quality (i.e., sweetness and pulp thickness) [11,12]. Hence, these agronomic aspects are contributed to ensure that breeding of papaya is successful. The breeding of papaya has produced more papaya cultivars with desired agronomic traits such as high yield, fruit shape, fruit size, and sweetness. However, the papaya is easily exposed to pathogens and being infected by diseases, (i.e., the papaya dieback disease (PDD), papaya ringspot virus (PRSV) disease, and papaya sticky disease (PSD)), which have resulted in a decline in papaya production. In addition, high temperature and water stress in papaya plantation are being affected by climate change, which is a challenge to the papaya industry, especially for its production yield [13–15]. The high temperature (i.e., above 35 °C) causes flowers to drop and sex changes in female and hermaphrodite flowers [13]. Although high temperature (i.e., 28 °C) promotes the fast growth of papaya, low pollen viability and early maturation result in imperfect quality fruits and a low yield [13]. Interestingly, high temperature coupled with a higher moisture content produces higher total soluble solids (TSS) in papaya [13]. A sufficient amount of water is essential to papaya because it determines the fruit size and fruit quality [15]. For instance, in dry conditions, the fruits are smaller with a hard texture when ripe.

One major way to overcome the constraints due to climate change and papaya diseases is by breeding for new and improved papaya that has been enhanced with desired traits, such as resistance to disease, resistance to abiotic stress, delayed ripening, and sweetness. Recent trends in crop improvements have shown the integration of omics approaches (i.e., genomics, transcriptomics, proteomics, and metabolomics) and bioinformatics in breeding programmes [16–19]. The use of omics and bioinformatic approaches in crop breeding helps to obtain a holistic understanding of the genetic and genomic bases of the crop, as well as to understand the molecular interaction among genes, proteins, and metabolites, especially regarding complex traits. Thus, the integration of omics and bioinformatic approaches in crop-breeding programmes is anticipated to facilitate the development of climate-resilient crop varieties and efficient germplasm screening, as well as to accelerate the rate of the genetic gain in a crop [18,19].

To date, papaya-breeding programmes have focused on improving yield and quality, resistance to abiotic stress condition, resistance to disease, as well as delayed ripening. Remarkable success has been achieved in the genetic engineering of papaya: the first transgenic papaya cultivar SunUp developed resistance to papaya ring spot virus (PRSV) disease [20]. In 2008, the first papaya genome, from the cultivar SunUp, was sequenced using whole-genome shotgun Sanger sequencing [21]. The papaya cultivar SunUp is a transgenic papaya and was the first papaya genome sequenced [21]. The SunUp genome

sequence was annotated, and yielded 27,793 protein-coding transcripts. To date, SunUp has been used as a reference genome for various comparative genomics analyses of the papaya genome. Previous efforts have exploited the genome sequences, molecular markers, and physical and genetic maps for improvement of papaya traits [22–25]. The domestication and genetics of the papaya have also been discussed [26]; while Dhekney et al. [8] and Palei et al. [27] have summarised the use of biotechnology tools and the progress of research that has been conducted on papaya, they have not covered all the omics and bioinformatic approaches that have been used in the improvement of papaya traits.

Hence, this review paper aims to examine the recent progress of the improvement of papaya traits using the omics and bioinformatic approaches, and their application in breeding for new and improved papaya cultivars (Figure 2). Each omics approach is shown in Figure 2, outlining how most of the generated molecular or omics data are analysed and visualised using bioinformatics approaches. The integration of systems biology with the analysed omics data analysis will cater to the identification of molecular markers, as well as candidate genes, proteins, and metabolites, for application in papaya-breeding programmes. In this review paper, the future perspective of using omics approaches in improving the desired papaya traits are also discussed.

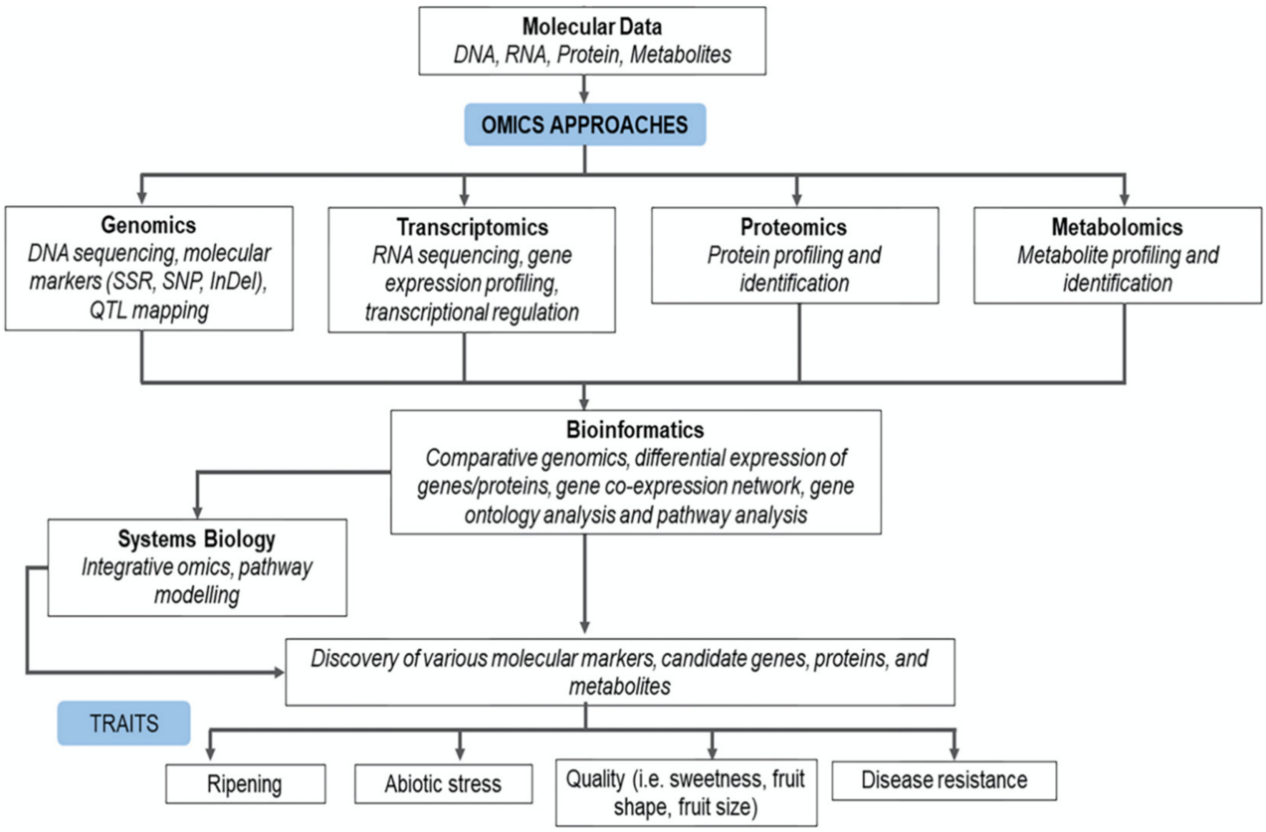

Figure 2. A summary of the omics approaches that are used in the improvement of papaya traits. Four traits have been studied using omics and bioinformatic approaches, including ripening, abiotic stress, disease resistance, and fruit quality.

2. The Role of Bioinformatics in Analysing Omics Data

Bioinformatics is an interdisciplinary field of various research backgrounds that comprises biology, computer science, mathematics, and statistics in order to extract, analyse, integrate, and visualise the biological data that are generated from omics platform technologies [28]. Several bioinformatics tools and biological databases have been developed and are accessible to other researchers in similar fields [19,28]. The development of useful bioinformatics tools is also important for crop breeders in order to inform them of the target selection of traits effortlessly [19,28]. The continuous effort in building and managing the

integrated databases would contribute to the translational research in which multiomics data analysis has become an integral part of systems biology.

Comparative Genomics Analysis of Carica papaya

The completion of the SunUp papaya reference genome sequence provides the opportunity for researchers to perform a comparative genomics analysis of the papaya genome. Comparative genomics, which is also known as comparative genome-wide analysis, is an area of study in which the structure and function of genomes from different species or varieties are compared [29]. Using computational approaches, the comparison of genomes and their contents among the cultivars or species enable researchers to identify sequence features (i.e., genes and proteins) that are conserved among the species [30].

Such examples include the comparative genome-wide analysis of the papaya genome in order to identify, annotate, and classify the genes into several gene and transcription-factor families that are associated with abiotic stress [31], disease resistance [24,32], ripening [33,34], and flower development [35]. The identification and annotation of the selected gene families in the papaya genome have also provided information on their gene structure, and phylogenetic tree relationships in relation to orthologs or paralogs from other species. Consequently, the potential genes and transcription factors were validated to further investigate their expression patterns under different conditions [31–35].

The papaya is susceptible to multiple pathogens, such as *Erwinia mallotivora* [36], ringspot virus [37], and papaya meleira virus (PMeV) [38]. Hence, in order to understand the basis of papaya resistance and susceptibility, the method is to perform a comparative genome-wide analysis of the papaya genome. The genome-wide analysis of papaya sequences has revealed that the papaya has fewer (0.2%) disease-resistance genes (i.e., nucleotide binding site (NBS)-containing *R* genes) than *Arabidopsis thaliana* (0.68%) and *O. sativa* (1.38%), which makes conventional breeding for resistance difficult [24]. Similarly, Praza-Echeverria et al. [32] investigated the potential of *NPR1*, a pathogenesis-related gene, in papaya resistance against a pathogen by comparing the *NPR1* in *Arabidopsis* and tomato genome sequences. This could lead to the application of the identified gene in genetic engineering for crop improvement.

The understanding of the papaya ripening process is important in order to reduce the occurrence of postharvest losses in the papaya industry. To improve the ripening trait in papaya, it is crucial to identify the genes that are responsible for the ripening process. Coupled with bioinformatics analysis, Liu et al. [33] identified 14 potential SQUAMOSA protein-binding protein-like (SPL) genes, whereas Xu et al. [34] investigated 18 potential auxin/indole-3-acetic acid (Aux/IAA) genes in the papaya ripening process. The involvement of SPL and Aux/IAA gene families in the papaya ripening process is not well known. Hence, these efforts provide an opportunity to understand the roles of auxin-responsive genes and SPL in the papaya ripening process.

Using the genome sequence of the papaya, the basic helix–loop–helix (bHLH) transcription factors that were associated with abiotic stress were identified [31]. A total of 73 candidate genes from the bHLH family were detected using comparative genome-wide analysis. The quantitative real time PCT (qRT-PCR) experiment also revealed the role of candidate bHLHs that might be responsible for abiotic stress responses (i.e., salt, drought, and cold stresses) in papaya. Using a similar approach, Liu et al. [35] identified 11 potential genes in the auxin response factor (ARF) transcription factor family, and their role in papaya flower development.

In summary, a comparative genomics analysis of papaya can be initiated by retrieving the papaya genome sequence from public databases. To date, five databases can be used to retrieve the papaya genome sequence; namely, the NCBI (https://ftp.ncbi.nlm.nih.gov/genomes/all/annotation_releases/3649/100/GCF_000150535.2_Papaya1.0/ accessed on 22 June 2021) [39], PlantGDB [40], Phytozome [41], EnsemblPlants [42], and PLAZA [43]. HMMER [44] and BLAST [45] were developed to perform similarity and homolog sequence analyses. InterProScan [46], Pfam [47], and SMART [48] tools were designed to identify

the gene family, motif sequence, and domain association, respectively. PLACE [49] and PlantCARE [50] are tools that are used for the analysis of cis-regulatory elements and promoter identification of the genes of interest. The Gene Structure Display Server (GSDS) [51] annotates gene structures; i.e., exon, intron, 3′UTR, and 5′UTR. To construct a phylogenetic tree, multiple sequence alignment must be performed using the gene sequences containing the domain or motif of interest as input data. ClustalX [52] and MAFFT [53] are multiple sequence alignment programmes that are used for aligning the gene sequences, and MEGA is a tool that is used for constructing a phylogenetic tree [54].

Figure 3 summarises the workflow of the comparative genomics analysis of the papaya gene families using bioinformatic approaches that are related to disease resistance, ripening, flower development, and abiotic stress.

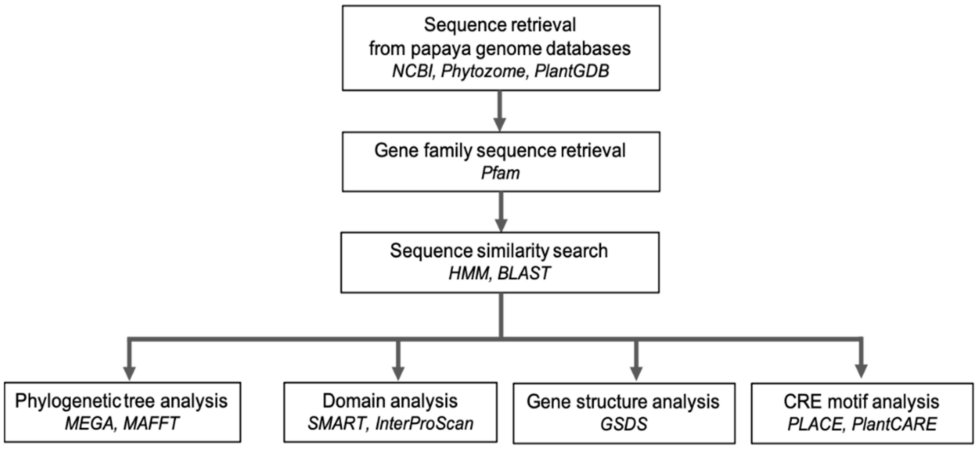

Figure 3. Workflow for comparative genomics analysis of the papaya gene families.

3. Application of Omics Technologies in *Carica papaya*

The suffix 'omics' can be described as the screening of the whole molecular data from a living organism for as genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics). As seen by the expanding number of publications over the years, these omics methods, as well as systems and synthetic biology, are becoming increasingly popular [55]. As compared to other model fruit crops such as the tomato and grape, the multiomics study of papaya is still in its infancy. However, various omics data of papaya have been generated from each omics platform, which can lead to the understanding of the complex phenotypic variations that can facilitate papaya breeding strategies in the improvement of papaya traits (Table 1) [56].

3.1. Genomics and Molecular Markers

Advancements in next-generation sequencing technology (NGS) have resulted in the different types of sequencing platforms (i.e., Illumina, PacBio, and Oxford Nanopore Technologies), which produce high-quality sequences, including longer sequence reads and fewer sequence error rates [56]. In addition, NGS has offered an affordable cost, facilitating the sequencing of the genomes of various plant species and cultivars [57]. For instance, the whole-genome resequencing of the papaya cultivars Eksotika and Sekaki from Malaysia [58], as well as the Sunset cultivar from Hawaii [59], have been carried out using the Illumina platform. The whole genome resequencing of papaya cultivars has led to the identification of a large number of variants that have been annotated into coding genes, where the identified SNPs could be developed as molecular markers in the application of a marker-assisted selection of papaya breeding.

The large volumes of data that have been generated using next-generation sequencing technology have increased the efficiency of the development of molecular markers, such as simple sequence repeat (SSR) and single nucleotide polymorphism (SNP). SSR and SNP are types of molecular markers that are widely used to improve the agronomic traits of fruit

trees [5]. Molecular markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD), are often limited by low reproducibility, laborious techniques, and time-consuming processes [60]; using SSR and SNP will greatly expedite the detection by using a high-throughput genotyping platform.

Previous efforts have identified 116,43 SSRs in the SunUp genome sequence [61]. In addition, 73 SSRs were found in 25 genes that were related to fruit ripening. A similar study focused on the development of high polymorphic SSR from the whole-genome resequencing of Sunrise Solo (Hawaiian cultivar) and RB2 (Australian cultivar) [62]. The developed SSR markers can be used to differentiate the elite cultivars, and can be used in papaya breeding selection.

Genomics variants such as SNP and insertion–deletion (InDel) are highly abundant in plant genomes [63]. Previous efforts have discovered putative SNPs in the genome-wide analysis of papaya genome resequencing [20,21,48]. Zainal-Abidin et al. [58] sequenced the genome of the papaya cultivars Eksotika and Sekaki in order to identify and annotate SNPs. The identified SNPs will be useful for genotyping to develop a papaya SNP panel. Similarly, the whole-genome resequencing of papaya cultivars such as SunUp (transgenic) and SunSet (nontransgenic) has identified SNPs, InDels, and structural variations [59]. These variants' data have been used to perform a comparative genomics analysis between transgenic and nontransgenic papaya. Recently, Bohry et al. [64] carried out genome-wide identification of SNP and InDels between the two parental lines of UC10 hybrids, the Formosa elite lines Sekati and JS-12, using the Illumina MiSeq (Illumina Inc., San Diego, CA, USA) platform. Interestingly, the putative variants that were located in the ripening-related genes (RRGs) were suggested to be validated through functional analysis and the genotyping platform. Subsequently, these candidate SNPs that are associated with RRGs can be applied in diversity and genetic-mapping studies, as well as for application in marker-assisted selection (MAS) of papaya.

Recent efforts have been conducted to identify the structural variations (SVs) in the genomes of 25 wild and 42 cultivated papaya [65]. The SVs (i.e., insertions, deletions, inversions, transposable elements, and copy number variations) contain relatively long DNA changes as compared to SNPs and InDels [63,65]. These SV data have been used to unravel the effect of SVs on the papaya phenotype and its adaptation during the domestication process [65]. Detailed GO enrichment between the wild and cultivated papaya has identified genes that are artificially selected during papaya domestication. Environmental adaptability, sexual reproduction, and essential traits such as pistil development, embryonic development, flowering duration, crop yield, pedicel elongation, defence response, and disease response are all influenced by these genes. This study would facilitate the understanding of the genes that are involved during the process of papaya domestication, and provide potential SV data to develop molecular markers in papaya breeding programmes.

The establishment of a bioinformatics pipeline in discovering large genomic variants (i.e., SNP, InDel) has made it possible to unravel the genomic variants in the papaya genome from the various cultivars. The identified and annotated SNPs in disease-resistance and ripening-related genes could be applied in the MAS of the papaya as a tool to aid the selection process in papaya germplasm, as well as in the study of its genetic diversity.

The genetic map is useful for dissecting the genetic components of complex traits [66]. The first genetic map of papaya was developed between Sunrise Solo x Line UH536, which comprised 61 RAPD markers and was distributed in 11 linkage groups (LG) [22]. Then, Deputy et al. [67] developed the genetic map of Kapoho x SunUp that comprised 1498 AFLP in 12 LG; while Chen et al. [23] developed the genetic map of AU9 x SunUp, which comprised 706 SSR in 12 LGs. Blas et al. identified 14 QTL controlling fruit size and shape of papaya [68]. However, progress in the development of papaya QTL has been limited by a lack of genetic and genomic information. Genome-wide identification of SNPs in the papaya genome sequence has allowed the development of a high-density genetic map. This approach has been applied for unravelling the fruit quality of papaya, which has been

developed in the F2 population from the RB2 x Sunrise Solo cross using the genotyping-by-sequencing approach (GBS) [69]. The highlights include the candidate genes that are related to the fruit quality traits, such as fruit size, fruit shape, sweetness, length, and firmness. These QTL data that are associated with the genes, and which are related to the fruit quality traits, can be used as candidates for gene exploration in the selection of SNPs and InDels using bioinformatics analysis [64]. Notably, candidate genes with associated SNP markers represent a valuable resource for the future of strategic selective breeding of elite papaya cultivars.

Although the genome sequences of papaya cultivars have been determined, no work has been carried out on the pangenome analysis of *C. papaya*. Pangenome analysis enables us to capture the entire set of genes from papaya cultivars, as well as to overcome the limitations of relying on a single reference genome [70,71]. The identification of SVs can represent pangenome analysis that would enable us to capture the entire set of genes from papaya cultivars, as well as to overcome the limitations of relying on a single reference genome [70,71]. In addition, more new candidate genes (i.e., genes for disease resistance and ripening) or gene pools can be identified from the wild germplasm and molecular markers, and these can be developed to screen for resistant varieties in the field [70,71].

3.2. Transcriptomics

The transcriptome is defined as the whole set of transcripts in a cell and the quantification of its specific developmental stage or physiological condition [72]. Hence, transcriptome plays a role in estimating the expression of genes, as well as in deciphering the regulation of genes in tissues and organs. Unravelling the transcriptome of tissues and organs of the species that are of interest can be carried out using RNA sequencing technology (RNA-seq), which has been shown to be highly reproducible and enables the simultaneous study of expressed gene samples [73–79]. These RNA-seq features have made RNA-seq experiments widely used in most transcriptome studies, including the transcriptome analysis of papaya plants.

Previous studies on papaya transcriptomes have focused on the analysis of several papaya traits, such as drought effects [73], fruit quality [74,75], sex determination [76,77], and disease mechanisms [78,79] (Table 1). The molecular response of papaya plants can be observed by analysing the tissue-specific differentially expressed genes (DEGs) using a gene-enrichment analysis. Gene-enrichment analysis using AgriGO [80] or ShinyGO [81] provides information on the biological processes that are regulated in response to the desired traits in papaya.

A transcriptome study of the delayed sticky disease symptoms in papaya has revealed the involvement of stress-responses genes in tolerance mechanisms at the pre-flowering stage [79]. In addition, the authors found that the salicylic-activated genes (i.e., *PR1, PR2, PR5*, WRKY) contributed to the delayed symptoms, while the activation of candidate genes such as *NPR1*, UDP-glucuronosyltransferase (UGT), and ethylene limit salicylic acid allowed the PSD symptoms to develop. The nutrient transporter gene family (i.e., nitrate, ammonium, potassium, sodium, phosphate, and sulfate) was the upregulated gene in the host during the infection, and had been shown to act as sensors for plant immunity [78,79].

Shen et al. [75] used RNA-seq technology to elucidate the fruit-colouration process in the ripening condition of papaya. A total of 13 candidate genes, including beta-carotene hydroxylase (CHYB), carotene ε-monooxygenase (LUT1), violaxanthin de-epoxidase (VDE), phytoene synthases (PSY1, PSY2), phytoene desaturases (PDS1, PDS2), zeta-carotene desaturae (ZDS), lycopene cyclases (CYCB, LCYB1, LCYB2, LCYE), and zeaxanthin epoxidase (ZEP), were detected in the papaya fruit transcriptome, which showed that these genes were involved in the carotenoid biosynthetic pathway.

A transcriptome study of the papaya male flower and male-to-hermaphrodite sex-reversal flower demonstrated the involvement of 1756 differentially expressed genes in sex determination [76]. Of these, four papaya homologous genes, including three PIN1 and one PIN3, were found to be upregulated in the male-to-hermaphrodite sexual-reversal flowers.

In addition, the authors found the phytohormone-biosynthesis and signal-transduction pathways in the male-to-hermaphrodite sex-reversal flowers. Similarly, Zhou et al. [77] used RNA-seq technology to investigate the underlying mechanism in DNA methylation contribution to the male and female flowers. Dissecting the molecular mechanism that regulates sex expression in the papaya provides valuable resources that facilitate an understanding of sexual differentiation in the papaya.

In the papaya disease-resistance trait, the transcriptomes of SunUp and Sunset leaves were sequenced to investigate the expression changes in both of the papaya cultivars, and were compared to the regulated genes in the resistant and susceptible varieties [78]. This comparative transcriptomic analysis found that there were few disease-resistance and hormone-related genes in SunUp, indicating the PRSV resistance in SunUp transgenic papaya. The finding of this study also provided evidence that genetically modified papaya is not harmful.

Using transcriptome data, gene clusters can be observed by performing a gene coexpression network analysis (Figure 4). This analysis identifies gene–gene interaction, regulation of biological processes, and molecular functions that occur within a cluster of genes. In a transcriptome study of papaya leaves, sap, and roots that were under mild and severe drought, several transcription factors (i.e., WRKY, MYB, bHLH) that were commonly linked to abiotic stress conditions were identified [73]. These transcription factors can be potential regulators in the leaves and roots of papaya plants under drought-stress conditions.

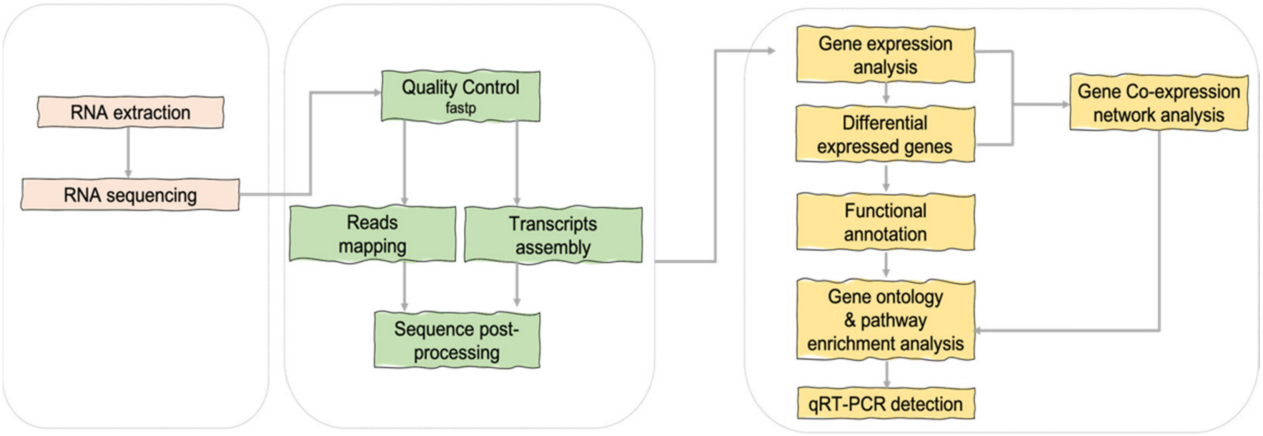

Figure 4. Schematic representation of the papaya RNA-seq analysis using a bioinformatics approach. The potential genes and transcription factor can be validated using qRT-PCR detection, which can be applied in precision breeding through gene editing or marker-assisted selection (MAS).

3.3. Proteomics

The two-dimensional differential gel electrophoresis (2D-DIGE), isobaric-tags for relative and absolute quantification (iTRAQ), and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/TOF MS) platforms have been widely used to estimate the expressed and abundance of proteins in plants, animals, and humans [82]. In papaya, the use of a proteomics platform has focused on the ripening process [83,84] and disease mechanism [85,86] (Table 1).

The use of a proteomics platform to study the underlying mechanism in papaya fruit ripening was first reported in 2011. Nogueira et al. [83] performed a comparative proteomic analysis of climacteric and preclimacteric papaya cultivars using the 2D-DIGE platform. Several proteins that were closely related to metabolic changes (i.e., cell wall, ethylene biosynthesis, climacteric respiratory burst, stress response, and chromoplast differentiation) in the ripening papaya were found, suggesting that these candidate proteins might be involved in the fruit-ripening process. The 2D-DIGE protein analysis, which was performed in order to identify the differentially expressed proteins during papaya ripening, also suggested the role of 1-methylcyclopropene (1-MCP) in affecting the fruit-ripening

process [83]. In addition, several expressed proteins were associated with sugar and cell-wall metabolism, signalling, defence and stress responses, folding and protein stability, and ion channels. Proteins related to pectinesterases, SODs, and the dienelactone hydrolase family deserve further attention, as these proteins might be involved in the ripening processes [84]. In a proteomic study that was applied to different ripening stages in papaya, differential accumulated proteins (DAPs) were quantified using HPLC fractionation and LC-MS/MS [85]. Interestingly, unsaturated fatty acids (i.e., methyl palmitoleate and methyl alpha-octadecatrienoic) were increased during the ripening, indicating that they might be associated with volatile formation in papaya fruit.

Rodrigues et al. [86] used 2-DE and DIGE to identify and distinguish proteins that had accumulated during sticky disease infection, which was caused by the papaya meleira virus (PeMV). A proteomics study of the compatible reaction of *C. papaya* cv. Eksotika in response to *E. mallotivora* attack was carried out using an iTRAQ mass spectrometry analysis, and showed differentially expressed proteins that were related to metabolic processes, defence response, and response to stress [87]. Similarly, iTRAQ mass spectrometry was used to quantify the effector proteins in *E. mallotivora*, and suggested the type III secretion system (T3SS) as an important protein that contributed to the bacterial pathogenicity and virulance [88]. A high-throughput proteomic study using an LC-MS/MS-based label-free proteomics approach was used to assess the protein expression between PMeV-infected preflowering *C. papaya* and control plants [89]. It was suggested that the increased modulation of photosynthesis, the 26S proteasome, and cell-wall remodelling-associated proteins were involved in the initiation of papaya plant immunity.

In general, identifying differentially expressed proteins provides valuable resources for selecting essential proteins in improving *C. papaya* agronomic traits (i.e., disease resistance and ripening). To encourage reproducibility of proteomics data, the MS-based proteomics data can be uploaded to a public database such as the ProteomeXchange Consortium [90]. This effort adds additional value of the data in efforts towards the improvement of papaya traits.

3.4. Metabolomics

Metabolomics is the study of small molecules such as metabolites, substrates, and metabolic production in an organ, tissue, or cell [5]. Metabolomics has been used to identify and measure differentially expressed metabolites and to gain an insight into biochemical composition under different environmental conditions [91]. Papaya is rich in secondary metabolites that serve as a source of nutrients for human health. High-performance liquid chromatography or gas chromatography tandem mass spectrometry (LC/GC-MS) and liquid chromatography-quadrupole time-of-flight (LC-QTOF) have been widely used in studies of plant metabolomics [92]. Determining the standard chemical compounds of metabolites and their MS spectrum data is essential in a metabolomic-profiling analysis.

Previous studies performed a comparative analysis of metabolites among papaya cultivars [93,94] (Table 1). The identification of metabolites among these papaya cultivars is important in order to identify the cultivar with the essential metabolites that are associated with quality traits (i.e., sweetness and ripening) that subsequently are to be applied in papaya breeding programmes. For example, carotenoids, tocopherols, and glucosinolates are highly abundant in papaya, and all of them are sources of antioxidants [95,96]. The identification of unique metabolites in a papaya cultivar has highlighted the importance of the chemical marker in authenticating papaya-based food products [94].

Chilling injury is a part of the constraint in maintaining the freshness of papaya, especially for exported papaya. A study by Wu et al. [97] identified different metabolite profiles in the papaya peel at a temperature of 4 °C. The metabolites were associated with aroma traits, such as organic acid, amino acids, hexanal, carbonic acid, pentadecyl propyl ester, and methyl geranate, in papaya peel [97]. The elucidation of the metabolite profile that involves chilling stress at 4 °C can be applied to regulate the storage temperature of the papaya to prevent chilling injury, and to extend its storage period.

Apart from the fruit, studies of metabolite profiling in other parts of the papaya, including its leaves and seeds, have been conducted to detect the metabolites' phytomedicinal properties [98,99]. Papaya leaves are rich in metabolites (i.e., phenolics, flavonoid, saponins, and tocopherol) that have potential antimicrobial, anticancer, antioxidant, and pesticide properties [100]. Hence, papaya leaves have been widely used in the pharmacological industry for drug development.

Metabolomics that has been combined with other high-throughput omics technologies, such as transcriptomics and proteomics, is referred to as integrated metabolomics, which is sometimes used in studies that are aimed at understanding metabolism as the phenotype of genome function [92]. However, little is known on the integration between metabolomics and transcriptomics to understand the mechanism during the fruit-ripening process. It has been reported that metabolites (i.e., flavonoids, terpenoids, organic acids, phenolic acids, and alkaloids) are closely related to the ripening disorder of fruits [101–103]. The elucidation of these potential metabolites during papaya-ripening processes will provide valuable information for developing a strategy for postharvest storage and improving the fruit quality.

Table 1. A summary of the recent omics and bioinformatics approaches that are used in the improvement of papaya traits.

Type of Omics Platform	Traits/Conditions	Descriptions	Approach	Reference
Genomics	-	Whole-genome sequences of papaya cultivar SunUp. Development of first papaya reference genome sequences.	Whole-genome shotgun Sanger sequencing	[2]
	-	Whole-genome resequencing of papaya cultivars Eksotika and Sekaki to identify putative SNPs. The identified SNPs between Eksotika and Sekaki located in genes of interest could be suggested for validation using a genotyping platform.	Whole-genome resequencing using Illumina HiSeq2000 (Illumina Inc., CA, USA) and bioinformatic analysis	[58]
	-	Whole-genome resequencing of papaya cultivar SunUp (transgenic) and Sunset (nontransgenic) to identify SNPs and InDels, and used in comparing transgenic and nontransgenic papaya. The identified SNPs and InDels that were located in high-impact genes could be applied in marker-assisted PRSV disease-resistance breeding in papaya.	Whole-genome resequencing using Illumina HiSeq2000 (Illumina Inc., CA, USA) and bioinformatic analysis	[59]
	-	Whole-genome resequencing of wild-type and cultivated papaya to detect structural variations in papaya, and used in understanding the process of papaya domestication.	Whole-genome resequencing using Illumina HiSeq2500 (Illumina Inc., CA, USA) and bioinformatic analysis	[65]
	Ripening	Gene-based SSR marker development focusing on genes related to fruit ripening.	Bioinformatics and genotyping	[61]
		Polymorphic SSR marker development for marker-assisted breeding in papaya.	Whole-genome resequencing using Illumina HiSeq4000 (Illumina Inc., Foster City, CA, USA), bioinformatics, and genotyping	[62]
		Genome-wide identification of SNPs and InDels using whole-genome resequencing of two papaya cultivars, namely Sekati and JS-12. The SNPs that were located in RRGs are potential SNPs to be converted in PCR markers, and could be applied in papaya genetic mapping and diversity studies, as well as marker-assisted selection.	Whole-genome resequencing using Illumina Miseq (Illumina Inc., Foster City, CA, USA)	[64]

Table 1. *Cont.*

Type of Omics Platform	Traits/Conditions	Descriptions	Approach	Reference
	Abiotic stress	Genome-wide analysis of basic helix–loop–helix (bHLH) transcription factors. Candidate bHLH genes that might be responsible for abiotic stress.	Comparative genomics and quantitative real-time PCR (qRT-PCR)	[31]
	Disease resistance	Genome-wide analysis of NBS resistance gene family. Candidate resistance (*R*) genes potentially responsible for disease-resistance mechanism.	Comparative genomics and quantitative real-time PCR (qRT-PCR)	[24]
	Disease resistance	Genome-wide analysis of NPR1 family. Candidate pathogenesis-related genes that might be responsible for a disease-resistance mechanism.	Comparative genomics and quantitative real-time PCR (qRT-PCR)	[32]
	Ripening	Genome-wide analysis of SQUAMOSA promoter binding protein-like gene family in papaya. Candidate ripening- and development-related genes.	Comparative genomics and quantitative real-time PCR (qRT-PCR)	[33]
	Ripening	Genome-wide analysis of Aux/IAA gene family. Candidate ripening-related genes in papaya.	Comparative genomics and quantitative real-time PCR (qRT-PCR)	[34]
	Flower development	Genome-wide analysis of auxin response factor (ARF) family genes related to flower and fruit development in papaya. Candidate genes related to flower and fruit development.	Comparative genomics and Quantitative real-time PCR (qRT-PCR)	[35]
Transcriptomics	Drought tolerance	Coexpression network analysis to identify genes and transcription factors related to abiotic stress.	Transcriptome sequencing using Illumina NextSeq500 (Illumina Inc., Foster City, CA, USA) and coexpression network analysis	[73]
	Ripening mechanism	Identification of potential regulatory genes during papaya ripening underlying 1-MCP treatment.	Transcriptome sequencing using Hiseq Xten (Illumina Inc., Foster City, CA, USA)	[74]
	Fruit colouration	Identification of potential TF regulating the carotenoid biosynthetic pathway.	Transcriptome sequencing using Illumina HiSeq2500 (Illumina Inc., Foster City, CA, USA)	[75]
	Sex determination	Differential expressed genes in sex determination of papaya, in male-to-hermaphrodite and male flowers.	Transcriptome sequencing using Illumina HiSeq2500 (Illumina Inc., Foster City, CA, USA)	[76]
	Disease resistance	Identification of disease-resistance genes in PRSV-resistant and susceptible cultivars.	Transcriptome sequencing using Illumina HiSeq2500 (Illumina Inc., Foster City, CA, USA)	[78]
	Disease resistance	Identification of stress-response genes and nutrient upregulated genes in tolerance mechanism of papaya sticky disease.	Transcriptome sequencing using Illumina HiSeq2000 (Illumina Inc., Foster City, CA, USA)	[79]
Proteomics	Ripening mechanism	Comparative proteomic analysis of climacteric and preclimacteric papaya cultivars.	2-DGE and LC-MS/MS	[83]

Table 1. *Cont.*

Type of Omics Platform	Traits/Conditions	Descriptions	Approach	Reference
	Ripening mechanism	Differentially expressed proteins during papaya ripening.	2-DGE and QTRAP hybrid tandem mass spectrometer	[84]
	Ripening mechanism	Differentially accumulated proteins (DAPs) during papaya ripening.	HPLC and LC-MS/MS	[85]
	Disease mechanism	Identification of differentially expressed proteins in healthy and PMev disease leaf samples in the Golden cultivar. Metabolism-related proteins were downregulated, and stress-responsive proteins were upregulated.	MALDI-TOF-MS/MS and DIGE/LC-IonTrap-MS/MS	[86]
	Disease mechanism	Differentially expressed proteins of compatible reaction between Eksotika papaya and *E. mallotivora*	iTRAQ mass spectrometry	[87]
	Disease mechanism	Protein expression between PMeV-infected preflowering *C. papaya* and control plants	LC-MS/MS-based label-free proteomics	[88]
Metabolomics	Fruit ripening	Comparative analysis of metabolite profiling between Eksotika and Sekaki cultivars.	GC-MS	[93]
	Fruit ripening	Profiling analysis of bioactive and volatile compounds in two papaya cultivars, namely Sel-42 and Tainung.	HPLC-ESI-MS/MS	[94]
	Fruit ripening	Comparative profiling of carotenoids and volatile in yellow and red flashed between Sui huang and Sui hong cultivars.	HPLC-ApCI-MS	[95]
	Fruit ripening	Identification of genes and metabolites regulating fruit ripening and softening in papaya cultivar Suiyou-2.	Transcriptome sequencing using Illumina Hiseq Xten (Illumina Inc., Foster City, CA, USA) and metabolomics profiling using HPLC-ESI-MS/MS	[103]
	Chilling injury	Elucidating of primary metabolites and volatile changes in papaya peel in response to chilling stress.	GC-MS /MS	[97]
	Bioactive properties	Metabolite profiling in papaya leaves.	UPLC-ESI-MS and GC-MS/MS	[98–100]

4. Future Perspective

The progress of the improvement of papaya traits has been limited due to the lack of genetic and genomic information on papaya. The outcome of recent omics studies of papaya plants suggests that there is potential for using these valuable genetic and genomic resources as a breeding tool to improve the desired traits in papaya. Incorporating omics data in papaya breeding programmes with a focus on abiotic stress, disease resistance, delayed ripening, and sweetness offers a promising strategy for developing high-quality traits in papaya cultivars without compromising their yields or agronomic traits. A previous study in Mexico conducted a network analysis of the interaction between viruses in papaya orchards. This viral metagenomics study, which was coupled with a network analysis, could contribute to the understanding of the host–pathogen interactions, which would cater to the management strategies against PRSV and non-PRSV symptoms in papaya [104]. The potential of computational approaches in understanding these biological systems has been employed in crop improvement. The computational models that were constructed integrate genome and phenome information, which led to new experimental strategies in improving crop production [105].

In papaya, pangenome analysis has not received much attention. Using whole-genome resequencing of papaya cultivars from diverse germplasms enables researchers to perform pangenome analyses, which would facilitate the identification of core and variable genes in

various papaya genomes. This effort has been performed in soy bean [106] and *B. rapa* [107]. A further area of interest is to screen for favourable alleles of diverse resistance genes sourced from the wild germplasm of papaya.

Another direction in papaya-trait improvement is employing the genome-editing approach. Functional analysis of candidate genes in the papaya–pathogen system can be performed using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9(Cas9) system. The CRISPR-Cas9 system was successfully applied in banana [108], apple [109], and kiwifruit [110].

5. Conclusions

The use of omics data plays a role as an advanced breeding tool that will enable the faster and more accurate selection of key consumer-driven traits. Integration of various high-throughput omics platforms may accelerate the research on papaya crop improvement. In addition, the application of computational approaches is key in revealing and filling data gaps, which will be valuable in the designing of new experimentation and measurement strategies that would result in enhanced papaya quality, as well as the ability of it to be sustained under various environmental conditions.

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