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Genomic and biotechnological interventions for enhanced utilization of date palm (*Phoenix dactylifera* L.) germplasm

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Abstract

Date palm (*Phoenix dactylifera* L.) belongs to Arecaceae family and considered as the oldest domesticated plant on the globe. Genetically, date palm is strict dioecious evergreen tree which is being cultivated in arid and semi arid regions of the world as an important fruit tree, for providing staple food across the world. Nutritionally, the fruits of date palm are rich sources of vitamins, minerals and carbohydrates which also posses medicinal values. On the other hand, date palm is capable of surviving for longer periods even at extremely adverse environmental conditions with prolonged life span, which admires about its complex genetic makeup. In this context, date palm has tremendous scope to conduct the fundamental and scientific research for bio-prospecting of the stress tolerant genes, understanding the tolerance mechanism and their interactions with natural ecosystem. Furthermore, the developed genomic resources and biotechnological interventions so far in date palm could be useful to enhanced utilization of date palm germplasm for further crop improvement programme. However, to conduct a high through put research on improvement of date palm, it still needs more emphasis on genomic and biotechnological interventions for crop improvement. Therefore, a comprehensive report has been made to collect the information available on genomic and biotechnology in date palm carried out so far and their futuristic approach for improvement in date palm cultivation.

Key words: Arid zone, biotechnology, Date palm, germplasm, genomic

Introduction

Date palm (*Phoenix dactylifera* L.) is considered as the oldest domesticated plant on the globe (Mahmoudi *et al*., 2008). Genetically, date palm is dioecious in nature and belongs to Arecaceae family which posses very long life cycle with prolonged juvenility (El Hadrami *et al.,* 2011). The native place of date palm is unknown but the Arabian Peninsula regions including southern Iraq is considered its origin place (Wrigley, 1995). Date palm is widely cultivated in arid and semi arid regions as an important fruit trees, admires good economic return and considered as "tree of life" for providing staple food across the world (Nixon, 1951). Nutritionally, the fruits of date palm are rich sources of vitamins, minerals and carbohydrates (El Hadrami *et al.,* 2012), besides good medicinal value to cure the cardiovascular disorders (Alhaider *et al.,* 2017). Globally, date palm is cultivated on 1.4 million ha of land with production of 8.5 million tonnes (FAOSTAT, 2016). Iran, Saudi Arabia and Iraq together occupied more area and considered as major producer of date palm. Date palm can survive at very extreme environmental conditions including drought, high temperature and relatively high soil salinity levels (Yaish *et al.,* 2015; 2015). In this context, date palm has tremendous scope to conduct the fundamental and scientific research for mining of stress tolerant genes, understanding the tolerance mechanism and their interactions with natural ecosystem.

When compared to many other commercial fruit trees, relatively little investigations have been made in molecular genetics research of date palm, resulting serious constraint in development of basic information on genetics and genomic tools. However, in recent past, date palm has been subjected to some genomic studies. Consequently, date palm genome along with its mitochondrial and chloroplastic genome has been sequenced recently (Yang *et al.,* 2010; Fang *et al.,* 2012). As the advent of genome sequence initiatives, the size of diploid genome of date palm (2n=2x=36) has estimated between 550 Mb to 658 Mb long (Al-Dous *et al.,* 2011; Al-Mssallem *et al.,* 2013; Hazzouri *et al.,* 2015). Nowadays, the commonly published genome data and the availability of bioinformatics tools provide insights into the tools to identify molecular markers, development of genomic resources and mining of genes for genetic improvement in date palm (Hamwieh *et al.,* 2010; Zhao *et al.,* 2013). Furthermore, the developed genomic resources in date palm could be useful to efficiently assess the genetic diversity, to construct genetic linkage map, to use marker-assisted breeding and boost up the biotechnological research. However, the comprehensive report on genomic and biotechnological areas of date palm is very limited. Therefore, in the present paper, efforts have been made to collect the information available on genomic and biotechnology in date palm carried out so far and their futuristic approach for improvement in date palm cultivation.

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Development of genomic resources

(A) Generation of EST database

Expressed sequence tags (ESTs) are one pass and partially generated end sequences of the cDNA libraries or transcripts. In other words, these are the bi-product of the cDNA/transcripts sequencing. Though, it is not very high throughput but considered as the first step of gene expression technologies. Now days, a huge amount of EST databases of various plants have been established in public domain. Case by case, they are used for designing the basis of identification of genes as well as development of molecular markers. Since date palm is having a lot of useful agro-economic traits, however, the initiatives of generation of EST database is very limited. Exceptionally, few reports are available in the literatures. In this context, Saidi *et al.* (2010) has generated 159 differentially expressed ESTs from date palm leaves affected by brittle leaf disease. Subsequently, a large collection of EST sequences were identified in date palm using *de novo* next-generation sequencing (Al-Dous *et al.,* 2011). They have generated 28,889 EST sequences and made available in public domain. Additionally, Al-Faifi *et al*. (2017) has also put efforts to generate a large scaled EST sequences. As a result, 6943 high- quality ESTs were generated from a normalized cDNA library of the date palm cv. Sukkari for deciphering the quality parameters and yield performance at field level. Further, the functional annotation of the ESTs showed that the majority of the ESTs are associated with binding (44%), catalytic (40%), transporter (5%), and structural molecular (5%) activities. It was also reported that some ESTs are categorized as stress/defense and fruit development related genes. These newly generated ESTs could significantly enhance date palm EST databases in the public domain and are available to scientists and researchers across the globe. This knowledge will facilitate the discovery of candidate genes that govern important developmental and agronomical traits in date palm. It will provide important resources for developing genetic tools, comparative genomics, and genome evolution among date palm cultivars.

(B) Transcriptome analysis and small RNA identification

Owing to survive under various biotic and abiotic stresses, make date palm as a potential genomic resource for identifying stress tolerant pathway genes. Transcriptome analysis during stressed conditions, though it is very limited in date palm, can became pivotal genomic tool for better understanding the tolerance mechanism behind these stresses in date palm. Few studies are available in this context. For example, Bourgis *et al*. (2011) deciphered the metabolic mechanisms for carbon partitioning using transcriptome analysis in date palm. The results of this investigation revealed that the carbon partitioning in the mesocarp has differed in date palm. Another report by Radwan *et al*. (2015) has recently put emphasis to characterize the salinity tolerance pathway in date palm through RNA-seq analysis. The study suggested that activation of *abscisic acid* signaling pathways through SNF1-related protein kinases 2. Further, key genes of sodium uptake and transport were shown down-regulated during salinity stress, suggested a potential mechanism for decelerating up-take and transport of salt solutes within plant tissues. Similarly, the genome-wide expression analysis of date palm leaf and root tissue during salinity stress revealed the differential expression of salinity stress responsive genes. The elevated levels of NaCl in salt affected tissues provide a foundation for functional characterization of salt stress-responsive genes in the date palm (Yaish *et al.,* 2017). The findings of these reports depicted new information on complex mechanism of date palm against salinity stress.

In the plants species including fruit trees, microRNA (miRNA) genes are known to be very highly conserved in nature (Sun, 2012). Similarly, the small RNAs were found to be conserved in date plam (Xiao *et al.,* 2013). Generally, the miRNA genes are involved in post-transcriptional regulation of gene expression. In the genomic era, numerous miRNA encoding genes were explored in the plant species and stated that the date palm is a potential crop to understand the stress tolerant mechanism. Whether the gene expression during stress conditions is regulated by small RNAs genes in date palm is still not well known?. However, date palm is exploited for involvement of small RNA genes in its gene expression during salinity stress (Yaish *et al.,* 2015) and fruit development stage (Xin *et al.,* 2015).

The Red Palm Weevil (RPW, *Rhynchophorus ferrugineus* Olivier) is a severe problem in date palm cultivation worldwide. In order to identify the differentially expressed genes against red palm weevil, the transcriptome analysis of date palm was carried out (Giovino *et al.,* 2015). In a study, higher transcript abundances of metabolic pathway genes and hormonal crosstalk belonging to auxin, jasmonate and salicylic acid (SA) pathways were observed. Apart from this, transcript analysis of mitochondrial genome was also revealed the functionality and co-regulation of mitochondrial genes in date palm (Fang *et al.,* 2012).

(C) Development of molecular markers

Recently, use of gene-targeting molecular marker approaches to study biodiversity and genetic variations in various plant species has increased the attention of researchers to develop their interest in date palm, especially to carry out phylogenetic studies using these novel marker systems. Thus, numerous molecular marker systems have been developed and extensively used for detecting variability in the germplasms of date palm (Palliyarakkal *et al.,* 2011; Atia *et al.,* 2017; Zhao *et al.,* 2017). Molecular markers are good indicators of genetic distances among the germplasm including land races, cultivars, etc., because DNA-based markers are neutral in the face of selection. In the earlier stage, the employment of multidisciplinary molecular marker has been described in date palm. Such markers are mostly randomly distributed rather than sequence specific in the genome. Recent trends in plant research is towards the use of gene-targeted rather than random DNA markers as inexpensive and speedier estimation of genome sequence lately offer enormous potential for the development of such gene-based markers (Andersen *et al.,* 2003). Gene-based markers are more useful in mapping of quantitative trait loci (QTL), molecular breeding, and gene cloning. The Expressed Sequence Tags (EST)-SSRs are also referred to as genic SSRs. With the advent and availability of genomic resources such as ESTs and genome sequences, a large number of genic markers have been developed in date palm. Zhao *et al*. (2013) has identified and characterized gene-based molecular markers (EST-SSRs) in date palm. They have utilized a large collection of EST sequences which were generated from *de novo* assembly of the date palm genome. EST-derived SSRs form a valuable genetic marker type, a class of functional markers as a putative function, in mapping candidate genes. Distribution of genic SSRs on the genetic map will show the distribution of genes in the genome. Thus, EST-SSRs have been widely used to construct high-density linkage maps in recent years (Chen *et al.,* 2008; Durand *et al.,* 2010; Ramchiary *et al.,* 2011). Some EST-SSRs associated with phenotype are useful in marker assisted breeding programs (Qi *et al.,* 2010; Zhang *et al.,* 2011). Another important feature of the genic SSR markers is that the unlike genomic SSRs, they are transferable among related species and genera (Varshney *et al.,* 2005). Additional to genic markers, a cDNA start codon-targeted (cDNA-SCoT) marker has been also used for the study of gene expression during salinity stress in date palm (Al-Qurainy *et al.,* 2017). A high degree of variability has been determined by using these markers among the germplasm of date palm under salinity and drought stresses.

(D) Identification of sex linked genomic resources

Initially, sexual propagation was followed in date palm multiplication. However, this method cannot be adopted for commercial propagation of the cultivars of interest in a true-to-type manner due to some genetic constraints. In fact, date palm is a dioecious plant, and sex of the seedlings can be determined only at the time of first flowering which takes 4-5 years (Kharb *et al.,* 2017). Thus, the sex determination at seedling stage is one of the major problems in date palm cultivation worldwide. On the other hand, female plants of date palm are of economic importance as they bear the fruits. Therefore, sex identification at an early stage is highly desirable not only for breeding programmes but also for establishment of commercial orchard. However, the lack of molecular markers in date palm restricts the application of molecular breeding.

At the earlier stages of the research, an extra-heterochromatin region of chromosome was reported on the both arms of the male chromosomes which was considered as sex determinant (Siljak-Yakovlev *et al*., 1996). The technology was applied to differentiate the gender in date palm. Subsequently, date palm gender was differentiating using Fluorescence *In-Situ* Hybridization (FISH) technology by Atia *et al*. (2017). The above mentioned technologies was not succeeded substantially because it required an extensive cytological tasks which is labor intensive, time consuming and need some sophisticated instruments and procedures. Whereas, DNA-based markers are very useful and easy for determination of sex at early stages of the plants. Historically, RFLPs and RAPD markers were effectively used for discriminating the sex-specific trait in date palm (Abdallah *et al.,* 2000; Trifi *et al.,* 2000). However, the utilized RFLP and RAPD markers were not seemed to be reliable because of their cumbersome detection techniques and reproducibility. Therefore, there is a huge gap to determinate the gender prospective in date palm, thereby continuous research efforts are going on in these aspects. It is, therefore, emphasized that the gender specific molecular markers either from male or female plant can solve this problem. In this context, male-specific sequence-characterized amplified region (SCAR) markers to identify sex in date palm at the seedling stage have been reported (Kharb *et al.,* 2017). Amplification of genomic DNA isolated from male and female plants using the SCAR primers results in an amplicon of 406 bp in both female and male samples and a unique amplicon of 354 bp only in male samples. Based on this amplification pattern, the sex of date palm seedlings can be predicted. Similarly, a large number of sex-linked markers have been reported (Table 1) and applied to differentiate the male and female plants at seedling stage.

Besides development of molecular markers for deciphering sex-linked traits, some gene based technology has been developed for sex determination in date palm. Recently, a sex-linked gene *SRY1* was cloned and validated in date palm for differentiating male and female plants (El-Din Solliman *et al.,* 2017). Actually, the *SRY1* gene is male chromosome specific; therefore, it amplified only in male seedlings rather than in female ones. The technology was very efficient to identify male plants in a population at seedling stage (EL-Din Solliman *et al.,* 2017).

Genome sequencing

The sequencing of genome is a fundamental basis for understanding the genetic phenomena of the complex traits of

Table 1. List of sex linked molecular markers developed in date palm.

S. No.	Date palm species	Type of markers	References
1	*Phoenix dactylifera*	RFLP and RAPD	Abdallah *et al*. 2000; Trifi *et al*. 2000
2	*Phoenix dactylifera*	SSR	Maryam *et al.,* 2016
3	*Phoenix dactylifera*	RAPD, SCAR, and SSR	Awan *et al.,* 2017
4	*Phoenix dactylifera*	SCAR	Kharb *et al.,* 2017
5	*Phoenix dactylifera*	RAPD	Ageez *et al.,* 2011
6	*Phoenix dactylifera*	RAPD	Al-Khalifah *et al.,* 2017
7	*Phoenix dactylifera*	ISSR	Al-Ameri *et al.,* 2016
8	*Phoenix dactylifera*	SCAR	Dhawan *et al.,* 2013
9	*Phoenix dactylifera*	SSR	Al-Faifi *et al.,* 2017
10	*Phoenix dactylifera*	SSR and SNP	Mokhtar *et al.,* 2016

a plant. In this context, the genome-wide studies of date palm have been carried out to fulfill the gape for conducting high throughput research in date palm. In recent genomic era, date palm has been subjected to intensive research on genome sequencing. Historically, the first report on nuclear genome sequence of date palm was published in 2011 (Al-Dous *et al.,* 2011). Unfortunately, it covered only ~60% of the genome which represent ~380 Mb (25,059 genes) out of the 658 Mb estimated genome size. However, as a result, they recognized more than 3.5 million polymorphic sites among the nine investigated varieties of date palm (Al-Mssallem *et al.,* 2013). Subsequently, Al-Mssallem *et al*. (2013) has reported the second nuclear genome assembly which is about 605.4 Mb and covers 90% of the date palm genome. To refine the sequencing in terms of complete genome with maximum coverage, a recent report on a whole genome assembly was reported by Hazzouri *et al*. (2015). In this study, they have sequenced 62 cultivars at whole genome level and identified the SNP markers to decipher the population structure in date palm which revealed the geographical domestication of the date palm. Regarding extracellular component of the genome, Yang *et al*. (2010) has reported first on complete chloroplast genome sequence of date palm. Additionally, mitochondrial genome was also assembled by Fang *et al*. (2012) with an approximate length of about 715,001 bp. In nut shell, date palm genome analysis has provided a detailed view on genome-wide structural parameters of genes, histories of genome/gene duplications, genetic diversities of cultivar resources and functional genes in key functional categories. These genomic resources of date palm have high scientific value to generate the complete set of genomic resources, understanding the genome to develop biotechnological interventions.

Genetic transformation and genetic engineering

The sustainable production of date palm is hampered by various challenges including loss of gene pool and climate change. The adverse climatic conditions are the major concern in date palm cultivation and due to the climatic changes, several new diseases and insect-pests have emerged (Saker, 2011). A lot of techniques such as *in-vitro* propagation, tissues culture, somaclonal variation, breeding efforts have been adopted to improve the date palm to overcome the yield and quality reducing factors. However, the efforts carried out were not gained the signified success in these aspects.

The availability of conclusive reports on the expression of economically important transgenes in date palm is very limited so far. The technology towards successful transformation proved to be a more difficult one in date palm. Therefore, the introduction of foreign genes or gene of interest into date palm using genetic engineering is a complex task. However, availability of some successful transformation protocols (Aslam *et al.,* 2015) and genomic resources (Al-Khayri *et al.,* 2015) have created the ways for development of transgenic date palm. The successful infection of embryogenic callus of date palm with *Agrobacterium*, led to the development of the genetic transformation method (Saker *et al.,* 2009). Subsequently, few efforts on successful genetic transformation in date palm have been carried out in recent past (Mousavi *et al.,* 2014; Hassan, 2013; Allam *et al.,* 2017; Solliman *et al.,* 2017). In these reports, the transformation was mainly carried out by using particle bombardment method. The particle bombardment method has several limitations and thus not much reliable method. To overcome the drawbacks of this transformation method, Aslam *et al.* (2015) has made an *Agrobacterium* mediated transformation in date palm. Though, it requires some tissue culture practices to regenerates the transgenic plants which are bit laborious in nature and required technical skills. These efforts can be the fundamental basis for developing protocols of genetic transformation and expressing the agro-economical transgenes in date palm.

In a very recent report, endotoxin *cry3Aa* gene has been transformed into date palm against the coleopteran insect (Badr-Elden *et al.,* 2017). It was found that one transgenic embryogenic callus for both Medjool and Khalas showed a single copy of gene integration. Thus, the report signifies the successful genetic transformation of date palm plant. In a another study, a construct harboring a cholesterol oxidase gene, which renders plants resistance to insect attack, was also introduced into embryogenic date palm callus using particle bombardment system. The tree is a target host for several biotic and abiotic stresses; hence it is necessary to focus on its *genetic transformation* and genetic engineering to overcome these problems in future. However, the transgenic date palm is very far for its releasing as commercial cultivation.

Future prospective

Though, genomic developments in date palm have been flourished in recent past. A sufficient knowledge and scientific informations are gathered by various groups. However, to conduct a high through put research on improvement of date palm, it still needs more emphasis on genomic and biotechnological interventions for crop improvement point of view. The genome editing techniques such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the contemporary clustered regularly interspaced short palindromic repeats (CRISPR) along with CRISPR-associated protein 9 (Cas9) have established their hierarchy in editing plant genomes. It has been successfully applied, either transiently or through stable transformation, for precise genome editing and knockout mutations in the citrus plants (Jia and Wang, 2014), populous (Fan *et al.,* 2015; Zhou *et al.,* 2015), and apple (Nishitani *et al.,* 2016).

Nevertheless, no GE tool has been exploited in date palm genome engineering. The date palm has ability to grow under extremely adverse climatic conditions such as drought, heat, and relatively high levels of salinity. It is imperative that the nature of the existing salt-adaptation mechanism be understood in order to develop future date palm varieties that can tolerate excessive soil salinity. The application of a full range of OMICS technologies, coupled with reverse genetics approaches, aimed toward understanding the stress-adaption mechanism in the date palm. Information generated by these analyses should highlight transcriptional and post-transcriptional modifications controlling the stress-adaptation mechanisms. As an extremophile with a natural tolerance to a wide range of stresses, the date palm may represent potential and novel genetic resources for understanding the mechanisms of stress tolerance.

Previously, biotechnological approaches, such as plant tissue culture, marker-assisted breeding and DNA finger printing, have been used in date palm genomics but failed to bring a significant improvement. Additionally, conventional breeding programmes in date palm are not cost effective as it need three backcrosses, thus usually takes long time (30 years) for breeding. For the sustainability of date palm, employment of new techniques in date palm breeding programs is highly needed to develop tolerant varieties and enrich the existing germplasm. The genome editing of date palm against various biotic and abiotic stresses can be the way forward to overcome these problems. The modified genome of date palm can regulate the defensive pathway accordingly to create resistance against various pests and diseases. Further, deciphering of genomic information in date palm would help in understanding the role of various genes involved in sex determination, enzymatic reactions controlling fruit ripening, fruit sweetness and other fruit quality parameters. In order to determine the universal efficacy of CRISPR/Cas9, extensive investigation in date palm is also necessary.

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