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Molecular breeding for fruit quality and drought tolerance of date palm (*Phoenix dactylifera*)

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Date palm cultivation expanded very rapidly in Saudi Arabia during the last decade, and recently, date palm sector in Saudi Arabia is considered one of the largest date palm sectors in the world, as regarding total acreage, total number of palms, total production, and economical value. Due to the extremely great importance of this crop in Saudi Arabia, we should pay more attention to develop modern technologies regarding production under drought stress, fruit quality and marketing. Interest in phytochemical contents and antioxidant activity of fruits has been very high in recent years. Some researchers reported that some date fruit cultivars are very rich in phytochemical contents and antioxidant and their content of antioxidant exceed most of the other fruits. Accordingly, it is expected in the near future that the economical values of the date cultivars will depend mostly on their contents of antioxidants, and the higher the antioxidant contents in the fruits the higher the chance to export and market in Europe and foreign countries. This would necessitate the implement of modern technologies to identify different cultivars by using biotechnology for fingerprinting for the date palm cultivars and also determination of antioxidants contents and potency in the fruits as well as the relation to antioxidant potency with the genetic structure of the cultivars. In addition, development of necessary resources and genetic engineers aiming to develop more drought stress-tolerant cultivars of date palm

Keywords: Drought stress-Biotechnology- Molecular breeding – Fruit quality - Genetics transformation.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is a strict dioecious evergreen tree capable of living over 100 productive years. It is not only one of the oldest domesticated trees but also of socio-economic importance. The earliest cultivation of *P. dactylifera* was recorded in 3,700 BC in the area between the Euphrates and the Nile Rivers. It is thought to be native to the Arabian Peninsula regions, possibly originating from the south of Iraq. At a very early time, date palm was introduced by humans to northern India, North Africa, and southern Spain and it has a major economic role in the arid zones. Saudi Arabia, one of the most important countries for date palm cultivation, has >10% of the world's

date palm trees (14% of the total production) and nearly 340 out of ~2,000 varieties (Al-Maaslem, 1996). This review represents an up-to-date account of date palm progress in terms of genetics and breeding for drought stress and fruit quality, and underlines the challenges facing these two fields of research in date palm.

Breeding for fruit quality of date palm

Date varieties serve as a good source of natural antioxidants and could potentially be considered as a functional food or functional food ingredient. The antioxidant potential of dates from Saudi Arabia and Iran were reported by Al-Farsi et al. (2005) and Al-Juhaimi et al. (2020). Fresh date varieties were found to be considered as a source

of antioxidant (11687 – 20604 micro mol of torloxequiv/g), carotenoids (1.31- 3.03 mg/100g), phenolics (134-280 mg of ferulic acid equiv/100 g) (Al-Farsi et al. 2005). Significant correlation between antioxidant activity and phenolics in date palm fruits has been established by many investigators (Al-Juhaimi et al. (2020). Date fruits are an excellent source of phenolics and therefore possess an extremely high antioxidant capacity. Date fruits have been reported to contain various phenolics, such as protocatechuic, p. hydroxy benzoic, vanillic, syringic, caffeic, coumaric and ferulic (Al-Farsi et al. 2005), which contribute significantly to total antioxidant activity.

Flavonoids exist widely in the plant kingdom and are especially common in leaves, flowering tissues and fruits (Larson, 1988). Plant flavonoids are an important part of the diet because of their effects on human nutrition (Frankel, 1995). It is appearing that the flavonoids were the dominant phenolic compounds of the date palm fruits Al-Juhaimi et al. (2020). The antioxidant activity of dates may be due mainly the presence of water-soluble compounds with potent free radical-scavenging effects, such as phenolics and flavonoids (flavones, flavonols and flavanones) (Panche et al., 2016).

Poly Phenol Oxidase (PPO) is found to be encoded by a gene family. Recent molecular studies have shown that PPO gene family is differentially expressed in different organs, and at distinct developmental and physiological conditions. For example, seven PPO genes (PPOs A, A', B, C, D, E and F) were identified from tomato plants (Newmann et al. 1993). The expression of each member of gene family is differentially regulated in various vegetative and reproductive organs and in response to wounding in young leaves in tomato plants (Thipyapong and Steffens, 1997; Shahar et al. 1992; and Thipyapong et al., 1997). In potato plants, five distinct PPO cDNAs were isolated from developing tubers, and each gene was spatially and temporally expressed in a gene-specific manner in various tissues including tubers, roots, leaves, petioles and flowers (Hunt et al. 1993 and Thygesen et al. 1995). The multigene family of PPO was also identified from *Faba bean* leaves (Cary et al., 1992) pokeweed suspension culture (Joy et al., 1995), and apricot fruits (Chevalier et al., 1998). Constabel et al. (2000) have isolated a PPO cDNA clone (PtdPPO) from hybrid poplar and described it as a gene family. In contrast, Dry and Robinson (1994) reported the presence of only one PPO gene in grape berry. The browning reaction of date palm fruits reduces

economic and nutritional values of date palm fruits. This discoloration process is mediated by PPO activity, but information about the differential expression of date palm PPO gene family is limited. Therefore, information about genetic variability within and between genotypes of date palm is an important factor to study and to classify genotypes into different heterotic groups. The relatively narrow range of morphological traits and limited number of polymorphic isoenzyme systems are not adequate to discriminate all the genotypes of any given species. Furthermore, many phenotypic traits are developmentally regulated or influenced by the genotype by environment interaction. However, the advent of DNA-based genetic markers, such as restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) have become more efficient, reliable and useful (Caetano-Anolles et al., 1991, Nybon, 1994). Trifi et al. (2000) have applied RAPD for the discrimination among date palm cultivars at the genomic level. Also, inter simple sequence repeat Inter Simple Sequence Repeat (ISSR) are a new type of DNA markers which involve the use of microsatellite sequences directly in the polymerase chain reaction (PCR) for DNA amplification (Sanchez et al., 1996). Mapping approaches have not been widely applied in date palm. Hazzouri et al. (2019) conducted genome-wide association study (GWAS) on fruit-related traits in 145 date palm cultivars using moderate coverage whole genome Illumina sequencing reads mapped to an improved genome assembly. In date palm the decay of linkage disequilibrium is sufficiently fast that GWAS yielded candidate genes and probable causal mutations for fruit color and fruit sugar composition (Hazzouri et al. 2015; Flowers et al. 2019).

Molecular breeding for drought tolerance

There have been a very limited number of genome-wide studies on *P. dactylifera*. One is a recent report on a draft genome assembly based on data generated from the Illumina GAI sequencing platform. They estimated the genome size (658 Mb), assembled 58% of the genome (382 Mb) and predicted 25,059 genes (Al-Dous, et al., 2011). Another is a comparative transcriptomic study on mesocarps of both oil and date palms based on pyrosequencing data from the Roche GS FLX Titanium platform (Bourgis, et al., 2011).

Table 1: Omics studies of abiotic stress in date palm (Hazzouri et al. 2020)

Technology	Abiotic stress	Description	Date palm material
RNA-seq	Salinity	DGE ^a analysis of root tissue from 'DegletBeida' after salinity stress	Seedlings
RNA-seq	Salinity	DGE analysis of leaf and root from 'Khalas' after salinity stress	Seedlings
Small RNA-seq	Salinity	miRNA target assessment and DGE analysis of leaf and root from 'Khalas' after salinity stress	Seedlings
RNA-seq	ABA-treatment	Leaves were treated with ABA followed by DGE analysis between treatment and control	Seedlings
RNA-seq and methylomics	Salinity	Differential methylome and transcriptome analysis of 'Khalas' roots in response to salinity	Seedlings
Proteomics	Drought and salinity	Proteomic analysis of 18-month palms subjected to drought and salinity stress	Tissue culture
Metabolomics	Salinity and silicon treatments	Non-targeted metabolomics analysis on leaf and root tissues after treatments with salt and silicon.	Seedlings
RNAseq + Metabolomics	Mild heat, drought, and combined heat and drought	Transcriptomic and metabolomic analysis of <i>P. dactylifera</i> under mild heat, drought, and combined.	Seedlings

^aDGE, differential gene expression

Date palms are tropical plants of great economic interest belonging to the Arecaceae family. The date palm is adapted to arid and produces orthodox seeds that can be stored for about 15 years at room temperature. The oil palm is, however, adapted to humid tropical climates with semi-recalcitrant seeds that dehydrate after fruit drop and persist for 2-3 years. The interest in the study of these model plants lies in the fact that these species belong to the family but have contrasting responses towards their adaptation conditions of water stress and the ability to conserve their seeds.

A thorough knowledge of the processes that determine desiccation tolerance is essential control protocols for conservation of the species. The tissues can tolerate the loss of almost all of their intra- and extracellular water has developed adaptations to protect cell components damage associated with dehydration. Several mechanisms probably acted together have been described (Esterbauer et al., 1991). Among these mechanisms are the elimination of free radicals, slowing the metabolism and accumulation of sugars and proteins (Esterbauer et al., 1991). For example, the LEA (Late Embryogenesis Abundant) proteins usually accumulated during seed maturation and in the vegetative tissues exposed to water, salt and cold stress. These proteins play key roles in maintaining cellular components and protect the protein structure during dehydration (Young and Woodside, 2001). LEA proteins are predominantly composed of hydrophilic amino acids ordered in repeated sequences. Based on sequence similarities, LEA proteins were divided into five groups (Ebadi et al., 2001). Group 1

proteins belong to Em (early methionine-labeled protein), and have the motif "Small Hydrophilic Plant Seed Protein" (Ashok and Ali, 1999). Group 2 proteins correspond to dehydrins (dehydration-induced proteins) and have the Y motifs, S, K (Aruoma, 1994). The dehydrins play a role of surfactant and would prevent coagulation of macromolecules, thus contributing to the maintenance of membrane integrity (Cheeseman and Slater, 1993). A recent study by Hazzouri et al. (2020) has adopted metabolomics or proteomic approaches to characterize the salinity response (Table 1). Al-Khateeb et al. (2019) simulated osmotic stress in micropropagated date palm plantlets by adding mannitol to the culture medium. In addition, Yaish (2015) indicated that the production of proline is a common response to various abiotic stresses and its differential accumulation cannot be used as molecular markers in date palm breeding programs aimed at improving drought.

Genetic transformation

The global population growth rate is alarming and the situation demands to enhance food production to feed increasing population by developing new tools for plant breeders. Genetic engineering would assist in reducing time scale in developing new cultivars; only when precisely single trait genes to be expressed without altering the remaining genetic makeup. A large number of plant species have subsequently been genetically transformed, primarily using two different strategies for DNA delivery into totipotent cells; T-DNA delivery with *Agrobacterium tumefaciens* (Horsch et al., 1984) and direct gene transfer with particle bombardment. For date palm,

Agrobacterium-mediated transformation has been tried using GUS (β -glucuronidase) gene as a reporter gene, but no conclusive report is available on the expression of economically important genes in date palm. Saker et al. (2009) reported the first report on successful infection of date palm embryogenic callus with *Agrobacterium*. They evaluated the factors influencing transient expression of the GUS gene, and proposed that higher bacterial density (OD600 1-1.5) and prolonged infection (2 hrs) gave the highest percentage of GUS-expressing calli. Alternatively, direct gene transfer in date palm cells was optimized by particle bombardment method (Saker et al., 2006; 2007).

CONCLUSION

Understanding the genetic diversity and cultivar classification for date palm will help authorities in planting high quality fruit producing and genetically diverse cultivars. Overall, it appears that date palm reacts to drought conditions in a similar manner to other plants. The hallmarks of drought stress were visible in the transcriptomics data such as ABA signaling. Since the plants were exposed to the stress for several days before harvesting. The successful high resolution mapping of these traits demonstrated the viability of combined NGS-based sequencing and standard structured association mapping in date palm and produced markers for commercially important fruit traits that could in principle be incorporated in future marker assisted selection programs.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SMA designed and performed the experiments and also wrote the manuscript.

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