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A review on date palm (*Phoenix dactylifera* L.) tissue cultured plants off-typeness in Iraqi laboratories

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Abstract

Date palm tree considered as one of the most important fruit tree in the Middle East countries, especially in the Arabian Peninsula. Date fruits are consumed fresh (Rutab) or after partial drying and storage (Tamar) during off-season. Iraq as a dates producer is among the top ten of the major dates producing zones in the world. Several commercial and government institutes, are working on tissue culture propagation of date palm cultivars. Date palm *in vitro* multiplication confronts different obstacles; among them the plant's off-typeness is considered the most important challenge. Plant's off-typeness is caused by somaclonal variations and lead to a severe abnormalities in micropropagated plants including dwarfism, leaf variegation, necrosis of midrib, single leaf chlorosis, albino, bastard offshoot formation, excessive vegetative growth, delayed flowering, failure of pollination, abnormal fruiting and sterilisation. This paper reviews all the potential factors responsible for plant's off- typeness; it's causes and their molecular detection in Iraqi laboratories.

Keywords: Auxins, ISSR, somaclonal variations, RAPD, tissue culture

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Introduction

Date palm (*Phoenix dactylifera* L.) is a member of the Arecaceae family. It is considered one of the most important trees of this family because of its economic and socioeconomic benefits. Date palm trees originated in Mesopotamia (Wrigley, 1995), 600 and more known cultivars of date palm are cultivated in Iraq, and before 1991, this country was in the top ten producer of dates with the largest forest of date palm trees in the world (Abass, 2013). Date palms are propagated by different methods, including sexual propagation by seeds, asexual propagation by offshoots and micropropagation by tissue culture technique (*in vitro* multiplication) (Aaouine 2003; Al-Khayri 2005, 2007; Abass *et al.*, 2016).

In Iraq, commercial and government institutes, are working on tissue culture propagation of date palm cultivars such as Sherafy, Al-Sayer, Hilawi, Khasab, Um Al-Dihin, Barhi, Kantar, Shwaythee, Breem, Alawaidy and Ashkar. The first date palm micropropagation attempt was done in the early 1980s by Mater (1983) at Basra University laboratories with the callus induction procedure, followed by Omar and Arif (1985) and Omar (1988).

One of the most important challenges of producing date palm through *in vitro* technique is the plant's off-typeness, which is the result of somaclonal variation, which includes phenotype abnormalities such as dwarfism, leaf variegation, necrosis of midrib, single leaf chlorosis, albino, bastard offshoot formation, excessive vegetative growth, delayed flowering, failure of pollination, abnormal fruiting and sterilisation (Arnholdt-Schmitt, 1995; Kaeppler, 2000; Al-Wasel, 2001, 2002).

Somaclonal Variation Types

There are different types of somaclonal variation, the first one is resulted from pre-existing genetic variation within the explants, while the other variation is induced during the tissue culture phase (Evans *et al*., 1984). Additionally, somaclonal variations could be divided into heritable (genetic) and epigenetic. Genetic variations are distinguished as a stable through the sexual cycle or repeated asexual propagation; on the other hand; epigenetic variation could be unstable even when asexually propagated. Epigenetic variation is also known as developmental variation, and includes persistent changes in phenotypes.

Causes of Genetic Instability

Hormonal Components

The tissue culture medium components are powerful inducers of variation in plants produced by tissue culture. The type of plant growth regulators and their concentrations have been linked to somaclonal variation along with the imbalance between auxin and cytokinin concentrations in the culture medium. The auxin 2,4-dichlorphenoxy acetic acid (2, 4-D) is known as the most potent auxin available for tissue culture. It is also known for its effect as a mutagen of callus cells (LoSchiavo 1989, Arnholdt-Schmitt 1995, Akasal *et al.* 2013). High 2,4-D concentrations induce 25% of somaclonal variations with evident changes in the leaf morphology and lead to poor flower pollination and low-quality dates (Fki *et al.* 2011).

2, 4-D at concentrations ranging from 50 mg/l to 150 mg/l have been used in the induction of date palm callus at the early stages of *in vitro* multiplication (Muhsen 2006; Al-Khalifa *et al.* 2009; Al-Meer and Jameel 2011). Omar *et al.* (1992) used 2, 4-D at 100 mg/l for *in vitro* propagation in a callus induction medium and for the embryonic callus proliferation of different cultivars, including Ashrasi, Barban, Bream, Khastawi, Khadrawy, Maktoom and Suada Zahidi by using different explants such as shoot tips, lateral buds, root tips leaf primordia, mantel meristem and bundle sheaths. In addition, high concentrations of 2, 4-D were used in the callus induction stage of date palm cultivars Askar, Barhi, Guntar, Halawy, Khadrawy, Sayer and Zahidi, which were propagated by using apical bud explants (Jasim 1999; Jasim and Saad 2001).

Abass *et al.* (2017) revealed that the high concentrations of 2, 4-D (100 mg/l) caused changes in the protein profile and loss of normal polypeptides (19 and 66 kDa); the appearance of new polypeptides were associated with produced calli at high 2, 4-D concentrations compared with control wild plants and plants treated with dicamba auxin. In addition, RAPD analysis of the produced calli showed that high-concentration 2, 4-D induced more polymorphic fragments in comparison with the wild type profile. The DNA profile found to be identical between 2,4-D at low concentration and mother plants. The abnormalities of cultured tissues and plants produced with 2, 4-D auxin at high concentrations are linked with genetic abnormalities, including DNA methylation and chromosomal aberrations, mutations and endoreduplication, thereby resulting in polyploidy and possibly sister chromatid exchange. Another result is the increase in the level of cell division of abnormal cells (Omar and Novak 1990; Bouman 2001; Ahmed 2004; Mohanty 2008).

Source of Date Palm Explants

The genetic stability (true-to-typeness) of date palm plantlets derived from tissue culture propagation largely depends on the source of explants; the type of plant tissues that are used in tissue culture course is linked to somaclonal variation (Kawiak *et al.* 2004; Chuang *et al.* 2009). In Iraq, different parts of the date palm tree have been used to propagate date palms *in vitro,* including the axillary buds of offshoots, leaf primordial, shoot tip meristems and inflorescence (Muhsen 2007; Khierallah 2007; Al-Khalifa *et al.* 2008; 2009 Hameed *et al.* 2012), in addition to the apical bud meristems or lateral buds with one or two layers of the sheath (Al-Najim 2009; Almeer and Yaseen 2010; Khierallah and Hussein 2013). Several studies showed that the explants from shoot tip meristems, leaf primordial and axillary buds decrease the possibility of genetic variation of the derived plants compared with differentiated tissues, such as roots and leaves, that produce more variants (Sahijram *et al.* 2003; Sharma *et al.* 2007). Notably, the age of donor plants (mother plants) affect the genome stability of plantlets produced via tissue culture technique. This effect could be attributed to the somaclonal variation that arose from somatic mutations already present in the tissues of the donor plant (Karp 1994; Saker *et al.* 2000; Al-Najim 2009). Then, the importance of donor plants is crucial in terms of genome uniformity and stability in the plants produced by tissue culture propagation.

Molecular Markers Used in Detecting of Genome Stability

The genetic fidelity of date palm clones produced by *in vitro* procedure is necessary for detecting any genetic changes in nuclear, chloroplast and mitochondrial genomes that resulted from somaclonal variation. For this purpose, several molecular approaches were used to indicate the genetic variations in regenerant date palm plants, such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) markers (Saker *et al.* 2000; Jubrael *et al.* 2005; Moghaieb 2011; Khierallah *et al.* 2011; Talaat *et al.* 2012; Hamza *et al.* 2012;, Khierallah *et al.* 2014; Aslam *et al.*; 2015, Abass *et al.* 2017).

In Iraq, the RAPD-PCR protocol has been used to detect the genetic fidelity of date palm clones propagated by tissue culture, several RAPD primers used; Ali *et al.* (2007) used 30 different sets of primers with Barhi cultivar and their mother plants, and their results revealed the genetic instability of produced plantlets, OPC.16, OPG.O8 and OPN.16. RAPD primers showed polymorphic bands compared with mother plants. In another study, the examination of 25 universal RAPD primers was applied to the genomic DNA of date palm plantlets derived from tissue culture (Barhi and Maktoom cultivars), and three primers (OPD.01, OPB.07 and OPC.08) revealed the polymorphism of tissue cultured plantlets compared with donor plants (Khierallah *et al.* 2007). Abass *et al.* (2017) showed that genome instability of cultured date palm tissue of Hilawii cultivar induced by high concentration of 2, 4-D (100 mg/l) can be detected in the early stages of callus induction using five different RAPD-PCR primers. Four primers showed the high genetic instability of callus produced under high 2, 4-D concentrations compared with donor plants. Low concentration of 2, 4-D and dicamba auxin did not induce any change in the DNA profile compared with mother plants (Table 1).

Table 1. RAPD primers and their sequences used in date palm genetic analysis in Iraq.

RAPD code	Sequence 5'……...3'	Reference
OPC.16	CACACTCCAG	Ali *et al.* (2007)
OPG.O8	TCACGTCCAC	
OPN.16	AAGCGACCTG	
OPD.01	ACCGCGAAGG	Khierallah *et al.* (2007)
OPB.07	GGTGACGCAG	
OPC.08	TGGACCGGTG	
OP-D20	ACCCGGTCAC	Alansari *et al.* (2014)
OP-F10	GGAAGCTTGG	
OP-C02	GTGAGGCGTC	

OP-M06	CTGGGCAACT	
OPAR.3	GTGAGGCGCA	Abass *et al.* (2017)
OPAR.8	GTGAATGCGG	
OP.640	CGTGGGGCCT	
OP.650	AGTATGCAGC	

Amplified fragment length polymorphism (AFLP), which was introduced by Vos *et al.* (1995), has been used in the detection of genetic fidelity of different plant cultures and the polymorphisms among date palm cultivars. In Iraq, the results of Jubrael *et al.* (2005) showed a high level of polymorphisms among 18 different date palm cultivars using five primer combinations. In addition, AFLP marker analysis of date palm male cultivars showed the efficiency of this molecular marker in the genetic discrimination of five cultivars using six AFLP primer combinations, namely, P11-aacg/M88-tgc, P104-aagc/M95-aaaa, P74-ggt/M95-aaaa, P11-aacg/M95-aaaa, P293-ggt/M62-tgc and P101-aacg/M95-aaaa (Khierallah *et al.* 2011a).

Table 2 . Some SSR primers and their sequences used in date palm genetic analysis in Iraq.

ISSR code	**Sequence** **5'…….....…….3'**	**Reference**
PDCAT 14	ACAGAGAGGTGGAGTTTTCGGATT	Khierallah *et al.* (2011b)
mPdClR050	CTGCCATTTCTTCTGAC	
mPdClR010	ACCCCGGACGTGAGGTG	
PDCAT5	GGCCCGTCCTTGGATTAGAG	
mPdClR032	CAAACGGCGATGGGATTAC	
mPdClR010	ACCCCGGACGTGAGGTG	Kareem *et al.* (2017)
mPdClR015	AGCTGGCTCCTCCCTTCTT	
mPdClR016	AGCGGGAAATGAAAAGGTAT	
PDCAT4	TAACGAGTCCACACAC	
PDCAT5	GGCCCGTCCTTGGATTAGAG	

SSR, which is also known as short tandem repeat (STR), microsatellite and simple sequence length polymorphism (SSLP), was used in date palm genomic studies that widely include genetic diversity studies. In Iraq, Khierallah *et al.* (2011b) analysed the genetic

diversity of 30 different date palm cultivars using 33 SSR primer pairs. A total of 22 primers showed polymorphic bands patterns. The highest heterozygosity values were reported using the set of SSR markers PDCAT 14, mPdClR050, mPdClR010, PDCAT 5 and mPdClR032. In a recent study conducted by Kareem *et al.* (2017), the usefulness of five different sets of ISSR primers in the discrimination of 65 different date palm cultivars was revealed (Table 2). ISSR markers provide a good solution for the detection of genetic fidelity in tissue-cultured date palm plants, in addition to date palm cultivar discrimination using a low number of ISSR primers. In Iraq, SSR markers were used successfully for cultivar characterisation. Khierallah *et al.* (2014) used 15 different ISSR primers to differentiate 17 date palm cultivars. Furthermore, Kareem *et al.* (2018) showed the efficiency of 10 ISSR primers in the discrimination of 24 date palm cultivars. The ISSR-PCR technique has been used to detect genome stability of date palm offshoots of Barhi cultivar derived from tissue culture (Abass *et al.* 2018, Table 3).

Table 3. Some ISSR primers and their sequences used in date palm genetic analysis in Iraq.

ISSR code	Sequence 5'…….....…….3'	Reference
ISSR 06	GAG AGA GAG AGA GAGAC	Kareem *et al.* (2016)
UBC824	TCT CTC TCT CTC TCT CG	
UBC823	TCT CTC TCT CTC TCT CC	
UBC826	ACA CAC ACA CAC ACA C	
UBC835	AGA GAG AGA GAG AGA CYA	
UBC840	GAG AGA GAG AGA GAGAYT	
UBC841	GAG AGA GAG AGA GAGAYC	
UBC842	GAG AGA GAG AGA GAGAYG	
UBC888	CAC ACA CAC ACA CA	
UBC889	ACA CAC ACA CAC AC	
815	CTC TCT CTC TCT CTC TG	Abass *et al.* (2018)
818	CAC ACA CAC ACA CAC AG	
822	TCT CTC TCT CTC TCT CA	
834	AGA GAG AGA GAG AGA G (CT) T	

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الشذوذ المظهري في نخيل التمر .Phoenix dactylifera L المكثر عن طريق زراعة الانسجة في المختبرات العراقية

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جامعة البصرة/العراق

الخلاصة

تعد نخلة التمر من اشجار الفاكهة المهمة التي تزرع في بلدان الشرق الاوسط، سيما في شبة جزيرة العرب. وتستهلك ثمار نخلة التمر طرية (رطب)كما قد تستهلك شبة جافة كما في التمر خلال نهاية الموسم. يعد العراق من البلدان العشرة الاولى غي انتاج وتصدير انواع مختلفة من التمور في العالم، وتوجد العديد من المؤسسات الحكومية والخاصة التي تعمل على اكثار اصناف متنوعة من نخيل التمر ومن بينها زراعة الانسجة او ما يعرف بالاكثار الدقيق والذي يواجه بذاته عديد من التحديات ومن بينها عدم التطابق الوراثس للنخيل المكثر نسيجياً مع النخيل الام. يتسبب عد التطابق الوراثي بين المكثر نسيجياً والنخيل المصدر الام عن ظاهرة Somaclonal variation وتختلف اعراضها مثل تقزم النخيل المكثر وتغير لون الورقه والتنخر في العرق الوسطي واصفرار الاوراق والنموات المشوهة وتاخر التزهير وفشل عملية التلقيح وعقم الثمار. يتناول استعراض المراجع هذا العوامل المسببة للتغايرات الوراثية وانواعها وطرق الكشف عنها سيما في المراحل الاولية من اكثار نخيل التمر سيما بالطرق الجزيئية.

الكلمات المفتاحية: الاوكسينات، التغايرات الوراثية، زراعة الانسجة، نخيل التمر