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REPORT OF THE SURVEY STUDIED ON SOMACLONAL VARIATIONS IN *IN-VITRO* PROPAGATED DATE PALM PLANTS

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ABSTRACT

In Vitro production using either the process of somatic embryo genesis or organogenesis has been established in recent years as a routine procedure in several commercial laboratories to produce large numbers of date palm plants at a competitive cost. The date palm micro propagation process, like other large-scale commercial plant production processes, carries number risks. Off type, i.e. non true to type and genetically not identical to the mother plant, may be among the resulting plants. This report was a survey studied in date palm orchard containing 2000 plants of two cultivars (Piarom and Mejdhoon) of five years old of *In Vitro* propagated date palm plants. Various somaclonal variations have been appeared. Different growth rate and differences in plant shape were appeared at high level in two cultivars. Dwarfism, morphological abnormality, chlorosis in one side of leaf, growth point bending, leaves rotating, leaflet chlorophyll losses (albinism or variegation), abnormality of growth point, leaf malformation, death of terminal growth point (hypoxanthic palm), high vigorous plants and plants with poor establishment were observed in two cultivars.

Keywords: *Date Palm, Tissue Culture, Soma Clonal Variation*

INTRODUCTION

Date palm (*Phoenix dactylifera*) can be propagated by seed, offshoot and via *In vitro* culture. The traditional method of date palm propagation is by offshoots. However, this method presents many disadvantages. Offshoots are produced in a limited number, the survival rate of offshoots is low, with high chances of spreading date palm diseases and pests, and the technique is difficult and laborious (Kunert *et al.*, 2003). The most recent method for date palm propagation is tissue culture which presents many advantages such as the propagation of healthy selected female cultivars, producing males with superior pollen, large scale economic affordability when large production is needed (Zaid and Al-kaabi, 2003). *In vitro* propagation should normally produce true-to-type plants. However, the appearance of plant off-type has been observed. This process is referred to as somaclonal variation. It occurs in plants as a result of tissue culture condition, and produces plants different from original plant (Kaeppeler *et al.*, 2000). The existence of genetic variability in plant tissue culture has been reported in many plant species including date palm. The abnormalities found include changes in morphology and structure, excessive vegetative growth, leaf variegation, dwarfism, leaf whitening, production of bastard offshoots, delayed flowering time, fertilization failure, formation of seedless fruits and higher susceptibility to diseases (Al-Mazroi *et al.*, 2007; Cullis, 1999; McCubin *et al.*, 2000; Zaid and Al-Kaabi, 2001; Zaid and Al-Kaabi, 2003). The *In vitro* production of date palm via somatic embryogenesis requires the application of relatively high concentrations of auxin-type plant growth regulators, such as 2,4-D or NAA, for process initiation (Bhaskaran and Smith, 1995; McCubin *et al.*, 2000). However, these auxins are known to be associated with genetic instability in plants, and have become a known cause of genetic variability in date palm (Al-Mazroi *et al.*, 2007; Cullis, 1999). Furthermore, variation in DNA methylation may be an important factor in initiating genetic variation (Cullis, 1992; Cullis and Kunert, 2000; Sala *et al.*, 2000). Zaid and Al-Kaabi (2003) was found that there were several morphological abnormalities in tissue cultured derived date palms, including abnormal leaves and inflorescences, dwarfing, leaf blanching, deformed offshoots, delayed flowering time, pollination failure and abnormal fruiting. Abnormal leaf shape and leaf bending were reported by Al-Ghamadi (1993) in different date cultivars produced using somatic embryogenesis.

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MATERIALS AND METHODS

A survey aimed at assessing the morphological abnormalities was performed during 2007-2008, and covered 2000 date palm plant plants (6 years old) cv. Medjool and Piaroom in Jahroom at I.R. Iran. The survey aimed to record the main abnormalities observed in cultivated date palms obtained via tissue culture and compared with date palms obtained from offshoots.

RESULTS AND DISCUSSION

Date Palm Morphology and Structure

Morphological abnormalities were found in cultivated two cultivars. It was included: abnormal leaf phyllotaxy; thin stem with weak, juvenile leaves; absence of an onion like base and plants with rolled leaves that observed up to 40% of plants. The occurrence of these abnormalities at rate of up to 63% in a date palm nursery containing 2000 hardened *In vitro* Medjool plants was observed in UAE (Al-Kaabi *et al.*, 2007). By contrast, a recently established date palm orchard in Namibia contained only 5% Medjool plants with abnormal characteristics (Zaid and Arias, 1999). Al-Mazroui, *et al.*, (2007) reported that the occurrence of abnormal morphology and structure in 5 cultivars of tissue cultured derived date palms in UAE was only very low and did not exceed 0.5%.

Dwarfism

Dwarf date palm plants are less than one meter after 5 to 6 years in the field in comparison to a normal plant of the same age with an average height of 4 meters was in two cultivars. The highest level of dwarfism (3%) was in Piaroom and lowest (0.5%) was in Medjool cultivars. Zaid and Al-Kaabi (2001) found that certain date palm cultivars such as Sukari, Barhee, Sultana and Oum Dahn exhibited abnormal dwarfing. Djerbi, (2001) reported that dwarfism frequency was affected by cultivar type. The causes of dwarfism in date palm are not known. A dwarf phenotype is also associated with black scorch disease. Black scorch, also called Medjnoon or Fool's disease is caused by the pathogen *Ceratocystis paradoxa* (Amira *et al.*, 2000). Black scorch-affected date palm trees can recover by chemical treatments but date palm plants derived from tissue culture are apparently more susceptible to this disease than offshoots and immediately after attack development of their meristems is restricted (Al-kaabi *et al.*, 2007).

Excessive Vegetative Growth

An abnormality found in the date orchard that surveyed was an excessive degree of vegetative growth. These plants were found to have broader leaves, compact growth and a different spine structure. Only 0.5% of Piaroom cultivar was observed with these abnormal characteristics. MacCubin *et al.*, (2001) found a much higher ratio of 1.4% in a survey carried out in South Africa on Medjool plants that had been produced by three separate tissue culture laboratories using somatic embryogenesis.

Leaf Blanching / Albinism

The blanching of leaves was observed only in Medjool variety and was due to partial or total loss of chlorophyll. Usually, 2 to 4 leaves were affected per tree. However, this abnormality was found to be rare in orchard (affecting 15 out of 200 plants surveyed) and is therefore of no great economic significance.

Deformed Offshoots

It is well known that date palm plants derived from tissue culture have a better growth habit and produce more uniform date palm orchards than those from offshoots (Smith, and Ansely, 1995). They also produce more primary and secondary offshoots. However, this fast growing habit and the abundance of offshoots accompanied by the appearance of abnormal offshoots and twisted inflorescences. The frequency of these abnormal offshoots was approximately 25% of plants. This deformed condition can be caused by an infestation with the date palm bud mite (*Makiella phoenicis* K.), or may be due to reduction in growth caused by an inequilibrium of endogenous growth regulators accumulated during *In vitro* propagation (Hajian, 2007).

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Figure 1: Dead of apical meristem



Figure 2: Multiple head



Figure 3: Differences in growth rate



Figure 4: Albinism

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Figure 5: Excessive vegetative growth



Figure 6: Rolled leaves



Figure 7: Leaf scorch



Figure 8: Abnormal leaves



Figure 9: Abnormal growth

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Figure 10: Dwarf Medjool (5 years old)



Figure 11: Unknown abnormality



Figure 12: Deformed offshoots

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