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Yet another morphological abnormality in the tissue culture-derived date palms (*Phoenix dactylifera* L.)

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ABSTRACT

Somaclonal variations have been reported extensively in the tissue culture derived populations of date palms. Surveys conducted on the tissue culture derived populations of 'Nabtet-Saif' cultivar to enumerate the somaclonal variants, revealed that 29.12% of the tissue culture-derived populations of Nabtet-Saif have various degrees of abnormalities. A hitherto unreported abnormal phenotype with spear-head shaped trunk is described here. Perimeter measurement at every 50cm height showed that the abnormal trees have continuously declining girth towards the crown end while the normal trees have almost cylindrical trunk up to the crown area. The long phase of callus induction and somatic embryo formation with growth regulators might have generated genetic or epigenetic changes in the resulting plants.

KEYWORDS

Epigenetic;
Genetic;
Nabtet-saif;
Somaclonal variation;
Abnormal fruit setting.

INTRODUCTION

Genus *Phoenix* L. of approximately 17[20] species is distributed in the countries of the old world, from Canary Islands in the southern part of Mediterranean region, all over Africa, Saudi Arabia, Middle East, Iran, Iraq, Afghanistan, Pakistan, southern slopes of Himalayas, India, Malayan peninsula, Northern Sumatra and China. Most of the *Phoenix* species are well known for their ornamental value and edible fruits. Among them, date palms (*Phoenix dactylifera* L.) stands first in the productivity of agriculture economy of many desert countries. Being a key species, adapted to the harsh environmental conditions of arid zones date palms are regarded as one of the important components of biodiversity in the inhospitable areas of deserts. It is a highly out breeding dioecious species and even allows interspecific crosses[15] with allied species. This behavior of date palms has brought about thousands of genotypes which are familiar to the different parts of date growing countries. Demand and cost of date varieties is also variable from place to place and country to country. Large distribution of this species has resulted in nearly 3000[21] to 5000[5] cultivars popular to the date palm growers of the different regions of the world. A comprehensive enumerative study covering all date growing countries using standard diagnostic characters and modern biotechnology tools may eliminate ambiguity in the number of cultivars.

The genetic purity of each cultivar is maintained through off-shoot propagation and the limited availability of off-shoots per plant is regarded as the major constrain for the large scale cultivation of desired cultivars. Biotechnology has great potential of solving many prob-

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lems pertaining to agriculture, industry, environment and health which has direct relevance to sustainable development of desert countries. Since the beginning of domestication and cultivation of plants, human beings are looking for techniques that help to produce maximum number of individuals from the minimum number/quantity of propagules. Tissue culture is the ultimate solution for the mass multiplication of plants using minimum quantity of propagules. Some of the advantages of this technique are that heterozygous materials may be perpetuated without much alteration, easier and faster, dormancy problem eliminated and juvenile stage reduced. It is also a means for perpetuating clones that do not produce viable seeds or those that do not produce seeds at all.

Vegetative propagation by means of either offshoots or tissue culture should produce identical progenies genotypically and phenotypically true-to-their mother plants. However, due to somaclonal variations abnormal date palms that originated from tissue culture are generally detected. McCubbin et al.[14], reported abnormal leaves with wide leaflets, slow growth rate and development, variegated leaves, non flowering and low fruit setting in tissue culture derived date palms. Abnormal multi-carpel flowers and fruits with 6-7 carpals were also reported[3,6,8] from Israel, Jordan, Namibia and Saudi Arabia. A survey conducted by Hassanpour-Estahbanati and Hamidian[10] on the micro propagated date palms in Iran showed excessive vegetative growth, dwarfing, leaf bleaching, leaf malformation, single leaf chlorosis, twisted leaf, spreading lower leaves on the soil, necrosis on midrib, bastard off-shoot, abnormal terminal bud, twisted inflorescence, bending of whole plant etc. An extensive field survey conducted by Al- Kaabi et al.[1] in the tissue culture derived populations of date palms in United Arab Emirates also revealed many of these abnormalities in date palms. Abnormal fruit formation of date palms is a serious concern of fruit productivity in Saudi Arabia and for the last five years the authors have been trying various curative measures to control this menace. During one of these study trips an abnormal phenotype, which is not common in date palms was observed in the tissue culture-derived populations of 'Nabtet Saif' cultivar. This abnormality is hitherto unreported in date palms and hence this communication is necessitated.

Figure 1 : Colour variations during fruit ripening stages in the date palm cultivar 'Nabtet-Saif'

MATERIALS AND METHODS

The date palm cultivar 'Nabtet-Saif' is one of the most extensively cultivated date palms of Saudi Arabia. The characteristic colour variation (Figure 1(a-d)) of fruit makes this cultivar easily distinguishable from others and a brief description of the fruit is given to facilitate easy identification.

Fruits ovate or elliptic, base flat or very slightly incurved, apex rounded, $\pm 3.65 \times \pm 2.85$ cm. On maturity lemon yellow, turns reddish brown from the tip on ripening with basal portions remained light orange. A fully ripened fruit is reddish brown, with broadly wrinkled skin. Flesh is very smooth and fibrous, constituting ca.70% of the fruit-diameter. Fruit cap covering ca.25% of the fruit-base, engulfed by the fruit-base. Seeds elliptic-oblong, acute at apex, light brown below the germ pore and dark brown above, $\pm 2.0 \times \pm 0.9$ cm. Germ pore represented by a small projection in the middle. Ventral groove narrow throughout the length, edges wrinkled, extending towards the tip.

Almost a decade before, a large plantation of 'Nabtet-saif' cultivar was raised on a farm near Al-Kharj, Saudi Arabia by a private entrepreneur using tissue culture derived plantlets through somatic embryogenesis. During the fruiting stage these plants showed various abnormalities in the vegetative and bearing structures as described by the earlier workers. Among these variations one particular phenotype was specially noted

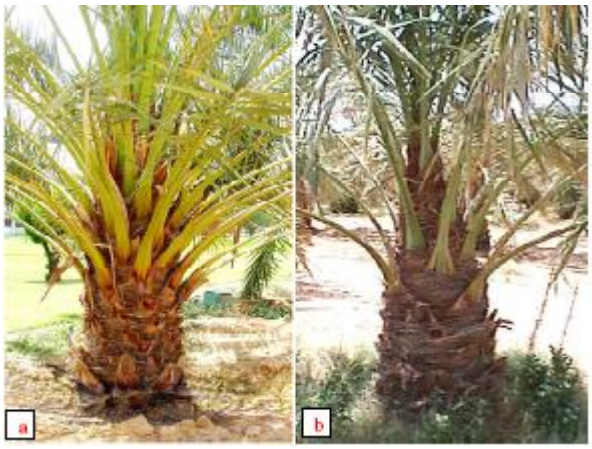

Figure 2:(a) Normal palm with many leaves and cylindrical trunk; (b) Spear-head shaped abnormal palm with few lax leaves

Figure 3: Close-up view of the crown area of an abnormal tree with split leaf bases and compact fibers

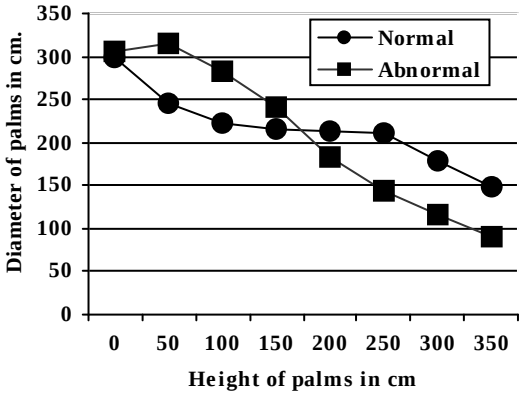

Figure 4: Growth pattern of normal and abnormal date palm

as hitherto unreported with an overall spear-head shaped trunk rather than the normal cylindrical stem. An extensive survey was conducted in three blocks within a large population of 'Nabtet-Saif' cultivar. Five

Figure 5: Close-up view of an abnormal and a normal fruit bunch

trees each of this particular phenotype and normal phenotypes were selected for measuring their height and diameter. Using a measuring tape, diameter from the base up to the crown end was measured at every 50cms interval. Similar measurements were taken from the normal trees also. Morpho variations were carefully recorded between normal and abnormal trees.

RESULTS AND DISCUSSION

Field surveys revealed that 29.12% of the tissue culture-derived populations of Nabtet-Saif have various degrees of abnormalities including 'spear syndrome' constituting major share. These abnormal phenotypes have a large base and continuously decreasing girth towards the growing end provides a typical spear-head shape to the plant. In all the abnormal trees crown ends measured less than one meter perimeter whereas in all the normal trees it was 140cm or more. Normal trees have a very distinct crown area distinguishable from the cylindrical trunk (Figure 2a) whereas in the abnormal trees crown area is hardly distinguishable from the main trunk with continuously diminishing perimeter (Figure 2b). Figure 3 shows that in the abnormal trees perimeter are continuously decreasing from the base up to the crown end at every 50 cm heights while in the normal trees distinct trunk showed almost uniform perimeter giving a cylindrical shape. Only in the crown area, normal trees showed declining perimeter. Leaf production per year is lesser than the normal trees which gives a lax crown to the plant. Due to the abnormal lateral expansion of the trunk most of the mature leaf bases are seen vertically split (Figure 4). Fibers attached to

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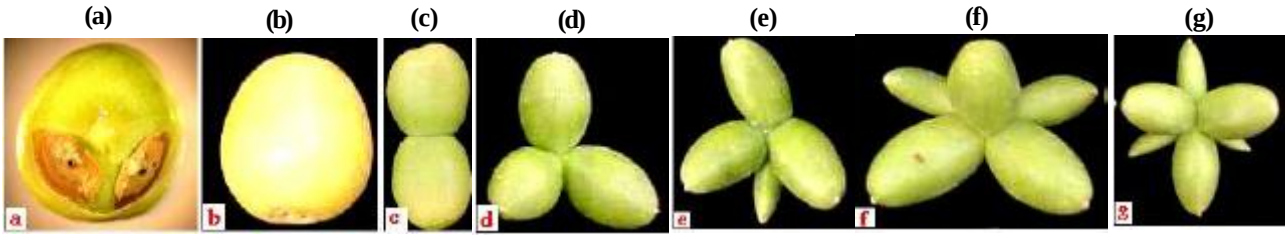

Figure 6 : Normal and abnormal fruit development in date palm

the leaf base are thick and prominently reticulated than the normal trees. Fruit production was also found to be very low and whatever fruits produced were abnormal multi carpellary ones (Figure 5).

Female date flowers are tricarpellary, apocarpous with three to six staminodes. Upon pollination two carpals degenerate (Figure 6a) and the remaining one carpel develops in to a normal fruit (Figure 6b). In abnormal trees instead of degenerating the other one or two carpals also develop simultaneously in to an abnormal fruit (Figures 6(c, d)). In some other cases along with the carpellary fruitlets staminodes also develop in to false fruits (Figures 6(e-g)). In almost all cases these fruits fail to mature and go for premature shedding, which results in severe economic loss to the farmers.

Unlike most monocots, palm stems can grow in girth by an increased number of parenchyma cells and vascular bundles. In most monocots the shoot apical meristem is normal but the leaf primordia are inserted in close order with virtually no separating internodes. A broad sub-apical meristem that exhibits periclinal cell division underlies the leaf primordia and is called primary thickening meristem. It adds layers of cells towards the base of the stem thus contributing to its height. Cell enlargement at the periphery of the primary thickening meristem also causes the stem to increase in diameter close to the apex contributing an almost cylindrical shape to the palm stem. In some plants as in abnormal phenotypes the primary thickening meristem is continuous around the flanks of the stems and extends to its base. This extension of primary thickening meristem is called secondary thickening meristem, which normally undergoes periclinal divisions adding more cells towards the center of the stem. Vascular bundles and parenchyma differentiate within the derivatives of the secondary thickening meristem and add to the diameter of the stem as they enlarge and provide a stem with wide base and narrow apex. As is evidenced from the distantly placed leaves in abnormal trees the primary thickening meristem adds layers of cells towards the base of the tree contributing height while the secondary thickening meristem through periclinal divisions adds cells towards the center of the palm giving a spear-head shape to this phenotype.

Various explanations have been put forwarded to explain the somaclonal variations in the tissue culture derived plants. Kaeppler et al.[12] attributed this phenomenon to genetic and epigenetic alterations generated during the in vitro process. Molecular genetic analysis of abnormal trees using RAPD[2,17] and isoenzyme profiles[4] were not able to detect somaclonal variations expressed during juvenile and mature stages. AFLP analysis conducted by Gurevich et al.[9] between normal and abnormal 'Berhee' cultivar also failed to detect any DNA marker for the abnormal phenotypes. Corley et al.[7] reported some abnormal phenotypes in the tissue culture derived populations of oil palms (*Elaeis guineensis* Jacq.) with very low level of fruit setting and supernumerary carpel development. Altered DNA methylation pattern generated during the tissue culture stages has been attributed to these abnormal phenotypes[11,13]. Al-Kaabi et al.[1] detected some reproducible molecular variations in the AFLP analysis of 'Khlass' plantlets developed through embryogenesis and organogenesis. Since the leaf samples for analysis were taken from the tissue culture derived plantlets it is uncertain to say what type of morpho variations are going to be produced by these molecular variants. Riseman and Chennareddy[16] investigated the effect of growth regulator regimen on *Exacum* L. in tissue culture and found that different regimes caused genotypic variation in the form of varied ploidy levels. The trend of somaclonal variants that differ with respect to height, plant form and number of floral or stem structures has been discussed with regard to two species of *Picea* Link[19]. Skirvin and Janick[18] observed clonal variants origi-

nated from callus tissue differed from original ex-plants with changes in plant and organ size, leaf and floral morphology and other characters such as pigmentation. Until recently micro propagation of date palm cultivars was carried out through somatic embryogenesis that originated from callus tissues induced by the higher concentration of auxins and cytokinins. The long phase of callus induction and somatic embryo formation with growth regulators might have generated genetic and epigenetic changes in the resulting plants. Since many variations have been recorded by a number of authors in tissue culture derived date palms, causing serious economic loss to the growers a prior validation of the trueness-to-type using modern biotechnology tools is very much essential before releasing the plantlets to the growers.

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