Anatomical Study of Caluus Stages Intiation and its Development to Embryoiods in Date Palm *Phoenix dactylifera* L. Cultured *In Vitro*

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ABSTRACT

Stages of initial callus and embryiods of date palm *Phoenix dactylifera L.* were histological and chronological studied begging from the ٢nd weeks of culturing segment of shoot tip to ten months in MS medium containing ١٠٠mg/l ٢،٤-D with activated charcoal in the dark. The study reveled that the active meristematic cells were unequally distributed in the explant and concentrated in the shoot apical dome and leaf margin. These cells are the bases from which initial callus started its growth. Somatic proembryo appeared for the first time as a meristematic cells groups separated from each other by relatively thick walls after five months of culturing in continuos culture in the same medium .In progress months (٦-١٠) the small simple proembryos continued into advanced multicell embryos and underwent polarization. Also the embryos were observed with attendance for division and separation into smaller or budded to produce numerous other.

Key Words: callus, embryiods, meristematic cells, date palm.

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دراسة تشريحية لمراحل نشوء نسيج الكالس وتطوره إلى أجنة خضرية ونباتات كاملة من نخلة التمر (*Phoenix dactylifera* L.) المزروعة خارج الجسم الحي

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الملخص

أجريت دراسة تشريحية زمنية لمراحل نشوء نسيج الكالس الأولي وتطوره إلى كالس جنيني وأجنة خضرية وذلك ابتداء من الأسبوع الثاني من زراعة قطع البراعم الطرفية حتى نهاية الشهر العاشر في وسط غذائي نوع MS يحتوي على الأوكسين D-٢،٤ بتركيز ١٠٠ملغم/لتر مع مسحوق الفحم المنشط وفي الظلام. وقد أظهرت الدراسة أن الخلايا المرستيمية النشطة المنتشرة بصورة غير متساوية في النسيج الأصلي والمتمركزة في حدبة البرعم الطرفي وفي النهايات المحدبة من الوريقات البدائية في البرعم هي المنطلق الأساسي لنمو نسيج الكالس الأولي. ظهرت الأجنة الخضرية أول مرة بشكل مجاميع خلوية مرستيمية منفصلة عن بعضها بجدران سميكة نسبياً بعد مرور خمسة أشهر من الزراعة على الوسط الغذائي عالي الأوكسين. أما الأشهر المتقدمة من ٦ حتى نهاية ١٠ اشهر فبدأت الأجنة الخضرية الأولية بالتقدم إلي أجنة متعددة الخلايا، كما لوحظ ميل الأجنة إلى التجزؤ والانفصال والتبرعم لإنتاج أعداد أخرى من الأجنة.

الكلمات المفتاحية: كالس، أجنة خضرية، خلايا مرستيمية، النخيل.

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INTRODUCTION

The palm is something more than fruit which furnishes the principle means of existence to hundreds of thousands of people. To the Arab it is ascard institution with Sematic race since the down of history and consecrated by the prophet Muhammad both in his public and his private life (Popener, ١٩١٣).

Propagation of date palm *Phoenix dactylifera* through somatic embryogensis has been reported by Renolds &Murashige, (١٩٧٩); Tisserat, (١٩٧٩); Sharma *et al*, (١٩٨٦); Mater, (١٩٨٦); Omar, (١٩٨٨); Daguin & Letuze, (١٩٨٨); Calero, (١٩٨٩); Al-Utabi (١٩٩٨); Jassim, (١٩٩٩); Almusawi, (٢٠٠١).

The first histological study of embryogenic callus in date palm was mentioned by Tisserat & DeMeason, (١٩٨٠). They reported that the embryogenic callus composed of copact aggregates that are dispersed among friable tissue. Also the meristematic clusters prpbably derived from single cells were embedded in the callus and divided to form meristematic loci that also acquired polarity when transferred to low – auxin medium.

The objective of this investigation is to study the development of explant histologically from two weeks of culturing and till ten months.

MATERIALS and METHODS

Embryogenic callus was generated from shoot tip segments from ٢-٣ years old offshoot CV 'Hallawi' after ٦-٨ months on Murashigai and Skoog (MS) medium enrich with ١٠٠ mg/L ٢،٤- dichlorophenoxy acetic acid (٢،٤-D), and ٢mg/L of kinetin and benzyladenin in the dark.

The nutrient medium containing (MS) inorganic salts (Murashige & skoog ١٩٦٢). with the following materiles (in mg/l). Namely sucrose ٣٠ ٠٠٠, meso-inositol ١٠٠, thiamine –Hcl ٠,٤,$NaH_٢Po٢H_٢O$ ١٧٠, agar ٨٠٠٠. The P^H of medium was adjusted to ٥,٧±١. Medium then dispensed in to ١٠٠ml conical flasks at rate ٥٠ ml and ٢٠٠mm × ١٥٠mm tubes at rate ٢٥ml. The flasks and tubes were covered with spongy stoppers and, necks were covered with aluminum foil, and

sterilized by autoclaving for ١٥ minutes under ١,٥ kg /cm^{٣} at temperature of ١٢١^{o}C. At least ١٠ culture was initiated for each age of callus. The subculture of explant was achieved at every two months.

The callus were used in histological study were pass on medium in different period (٠,two week, ١ month to ١٠ months interval (three specimen are used for each age). The individual specimen of different ages are fixed with formalien, alcohol, acetic acid (FAA) The samples then dehydrated with ethanol –xylen series and then embedded in paraffin blocks. Paraffin sections that have been attached slides were deparaffinized before staining. The slides then stained with Fast green and Safranine stains refer to Johenson method's,(١٩٦٨).

RESULT and DISCUSSION

Explant (٠ time)

The transverse section (Fig-١ A & B) of shoot tip (Explant ٠ time) reveled that the apical dome was positioned in the shoot apex and surrounded with leaf primordial. Also the section show the base of biggest leaf wrapped the smaller one. On the other hand the active meristematic cells, (small in size, dense in cytoplasm and big nucleus) are unequally distributed, concentrated in apical dome and leaf margin. However the semimeristematic cells positioned below the apical dome and epidermis of leaf primordial. These findings are similar to those reported by Tomlinson, (١٩٤٠).

Formation of initial callus

After tow weeks of culturing the meristematic cells were emerge from leaf margin and form protrusion (Fig-٢). The cells of protrusion are meristematic and very active. However, these cells are precursor of formation of initial callus. On the other hand the explant was increased in size. This studies was at variance with histological study of coconut palm, therefore the primary callus formation resulted from mitotic division of perivascular cells. (Morel *et al,* ١٩٩٢)

Fig.١. Transverse section of shoot tip before culture on medium
A. Apical dome (ap) with leaf primordial (١p).
B. Asection magnified from (A) reveled the merastimatic (mc) and non-merastimatic cells (nmc) in shoot tip.

Fig.٢. Protrusion (p) emerge from leaf margin

After one to three months of culturing in the same medium the initial callus started its growth and appeared surrounding the explant (Fig-٣). On the other hand the friable callus were formed from the wounded surface of the explant. The cells of this callus are friable, elongated and unmeristematic. Also many tannin cells are discarded in this callus (Fig-٤). However at these ages the phenomena of unorganized and unsynchronized callus growth was also increased to cause obliteration in the identity of the original explant. Cells in the periphery of the callus protrusions also maintained their meristematic characteristics. On the other hand the most cells in the subepidermal and internal regions of protrusion differentiated to large nonmeristematic paranchymatus (Fig-٥). Also in the age three months there are aggregate or separation from meristematic cells in the peripheral and subperipheral region of calluses (Fig-٦). These aggregates are meristematic centers and from this centers the embryogenic callus were formed.

Fig.٣. The aggregate of callus (c) growth appeared surrounding the explant.

Four months later of culturing on the same medium the boundaries surrounding the independent meristematic centers became more obvious and separated from adjacent tissue by thick walls (Fig-٧). Also the number of these centers were increased. However, most of the meristematic centers occupied the peripheral and subperipheral regions of the compact callus masses. These results are coincided with Williams and Maheswarian, (١٩٨١), suggestion that the indirect embryogensis requires redetermination of differentiated cells, callus proliferation and the development of embryogenically determined state .For these induced embryogenically determined cells, growth regulaters are required not only for re-entry mitosis but also for determination of the embryogenic state.

Fig.٤. Formation of friable callus (f.c) from wounded surface of the explant. The tannin cells (tc) are scattered with callus.

Fig.٥. A section of callus growth magnified from (fig.٣) reveled the meristematic cells (mc) in the peripheral of callus protrusion and nono-meristemation (nmc) cells in the sub-epidermal and interior regions of callus.

Fig.٦. The meristematic centers (mce) separation from meristematic cells on peripheral and sub-peripheral callus protrusions.

Fig.٧. The boundaries (b) of meristematic centers (mce) from nigbouring tissue.

Formation of Embryogenic Callus

Five months later for continuous culture, growth of the compact calomeristematic masses continued so callus acquired nodular rough appearance. Also, at these stages, somatic proembryoids appeared form of meristematic groups separated from each other by thick walls (Fig –٨ A& B). In contrast, the emberyoids are consisting of different numbers of cells. However this stages of callus growth represent the beginning of conversion to embryogenic callus. Even though, the identity of original explant was lost in this age. With callus achieving six months of age in the same medium, the small simple proembryos continued to develop in to advance multicell embryo. Also this embryo are underwent polarization by accumulating cells in one end and non-meristematic in the other end of embryo (Fig-٩). Eventually the bipolar structure becomes apparent for the necked eye and easy to separate from each other's.

Fig.٨. A.. Somatic proembryos (spe) separated from each others by relative thick walls. B. A proembryos magnified from (A).

When the embryogenic callus reached seven to ten months in propagation medium, the numbers of embryoids of free nodules greatly increased, and concentrated in the peripheral regions of the callus clumps (Fig-١٠). On the other hand, in these stages many of embryoids were observed the attendance for division and separation or budded to produce numerous others.

Fig.٩. Embryo underwent polarization.

g.١٠. Somatic embryo separation and budded to produce a numerous others.

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These findings are similar to these reported by DeMeason and Tisserat, (١٩٨٠) and Mater, (١٩٨٨). They found that in date palm, meristematic clusters are probably derived from single cells were embedded in the callus and derived to form a meristematic loci. Also Steward *et al*. (١٩٦٤) suggest that the embryo originated from single

cells that isolation of cell from its adjacent tissue is necessary for it to develop into an embryo. Bhojwani and Bhatanger, (١٩٧٨) were supported this concept by *invivo* situation were the zygote, before starting embryogenic development, becomes isolated from the surrounding cells by cytoplasm continuities (Bhojwani and Razan, ١٩٨٥). Also the secondary embryogensis was induced in somatic embryo in coconut palms (Morel *et al*, ١٩٩٢).

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