The Effect of Different levels and kinds of Cytokinins on Buds proliferation of Iraqian Date Palm Cultiver (Barhi) *In vitro*

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Abstract

The present study was conducted to determined the action of several levels of cytokinis (N^6-benzyladenine (BA), 6-furfurylaminopurine (kinetin), N^6-(Δ-isopentyladenine) (2,ip) on buds proliferation of *Phoenix dactylifera* L..3 ml explants of the heart of 3 years old offshoot were cultured on Murashige and Skoog medium (1962) containing 3 mg/L activated charcoal, 10 mg/L Naphthalene acetic acid proposed by (1) as a control. Other explants were cultured on this medium supplemented with 0.1, 0.5, 1 mg/L BA. 0.1, 0.5, 1, 2, 3, 4 mg/L Kinetin. 0.1, 0.5, 1 mg/L 2,ip.

Best number of buds proliferated has occurred on control medium containing 3 mg/L. 2ip.

Introduction

The date palm (*Phoenix dactyllifera* L. 2n = 36) is one of the important members of the palmae (Arecaceae) family. There are 11 other species in the genus Phoenix all of them are dioecious with male and female flowers produced in clusters on a seperat palm in the axils of leaves (2, 3).

Date palm is sub-tropical in origin and cultivation. The earliest records (3000 B.C.) suggested Mesopotamia (Iraq) as a possible site (4). Till the year 1980 there were 30 million date palm trees in Iraq declined to 9 million now (Agri. Ministry documents).

Iraqi farmers didn't propagate date palm from seeds due to the facts that approximately half of the progeny will be male and because date palm is heterozygous there will be much variation with the female progeny and desirable characteristics of the parent may be lost.

Rooted offshoots are preferred for conventional propagation because they produce true-to-type trees with fruit quality identical to that of the mother tree. However, there are many problems associated with this system.

The availability of offshoots is limited because the number produced by each palm trees is low and must remain attached to its parent tre for a long time (2-3 years) until an adequate root system develops. In addition to that the methods of excision are complicated and time consuming.

Micropropagation has the potential to provide very high multiplication rates of selected tree genotypes but the genetic stability of plantlets produced from callus is questionable (5).

Therefore the initiation of primary auxillary buds *In vitro* provides alternative methods for true-to-type large scale propagation of date palm (6). Hormones are chemical messengers in organisms (7). In plant a few of their effects are:- cell division, root growth, shoot production and flowering. The factors of increasing the mitosis (cell division) of shoot and buds are cytokines (8). They are adenine derivatives include kinetin, BA (which are synthetics cytokinin stimulate callus) and zeatin, naturally occurring cytokinin which is very costly and 2,ip an efficient hormone which include shoot and leaf growth and suppressing root formation.

So this investigation aims to make a comparative study on the effect of different levels of those cytokinins supplemented to medium proposed by (1) for primary auxillary buds proliferation of date palm *in vitro*.

Materials and Methods

- Plant materials

2-3 years old offshoot of Barhi cultivar were defoliated, the bases of leaves were removed sequencely till around 5 cm in diameter offshoots hearts were obtained. Few leaves which remained for protecting soft tissues from sterilization agent were cut up to 5 cm.

- Sterilization

Offshoot heart was surface sterilized for 15 minutes with 9% clorex (sodium hypochlorite NaOCl), and contained few drops of 20 by using vaccum apparatus connected with Bughner flask..

- Culturing

Leaves bases and hard hypocontaminated tissues were removed in sterilize condition on Laminar air flow cabinet. 3 ml explants were prepared by cutting the soft tissues which have mass of primary auxillary buds under leaflets and cultured them on the media.

- Nutrient Media

Control treatmentwhich is employed throughout this study is the medium proposed by (1) for the differentiation of shoot tips obtained from four to six months old seedling.

It contains M & S salt, $NaH_2PO4.2H_2O$ 170 mg/L, meso-inositol 100 mg/L, Thiamine HCl 0.4 mg/l, sucrose 3%, adenine sulfate $2H_2O$ 40 mg/L, 3 g and activated charcoal, NAA 10 mg/L.

The other treatments employed three kinds of cytokinins

- N^6-benzyladenine (BA).
- 6-furfuryl aminopurine (kinetin).
- N^6-(Δ^2-isopentyl) adenine (2,ip)

Supplemented to the control treatment medium in 0.1, 0.5, 1 mg/L levels for BA and kinetin and in 0.1, 0.5, 1, 2, 3, 4 mg/L levels for 2ip. pH of all media was adjusted to 5.7. Media were dispensed into culture vessels 120 x 120 mm and outocleved for 15 minutes at 121ºC.

- Culture conditions

All cultures are incubated in culture room at 29ºC with full darkness.

After 4 weeks of incubation, a number of initiated buds was counted and completely Randomized design (C.R.D.) was used for 12 treatments with 3 replicates for each.

- Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the means were separated with a least significant difference (LSD) at 5% significant level (9).

Fresh callus which appears for treatments have 1 mg/L kinetin or BA was weighed for 2 treatments with 11 replicates for each. The date were subjected to analysis of t-test (10).

The results showed that 2,ip at 3 mg/L level, significantly have increased the number of buds proliferation compared to all other treatments.

Addition of BA in more than 0.5 mg/L level inhibited buds proliferation from 4 to 2.3 buds and addition of kinetin at the same level inhibited buds proliferation from 2.6 to 1.3. However, BA has significantly increased buds proliferation more than kinetin.

2,ip has improved buds proliferation to 6.6 buds when it was included in nutrient media in the level of 3 mg/L.

The results showed that the level of 1 mg/L for both BA and kinetin has stimulated callus formation ,but t-test showed that kinetin significantly gave callus fresh weight more than BA as shown in the table below.

Growth regulators	replicates	Degree of freedom	Mean callus fresh weight in mg	Sum of square
Kinetin	11	10	9.0	2.52
BA	11	10	5.7	13.56
		Sum 20	Difference=3.3	Sum= 16.08=$\sum X^2$

Calculated t= 8.639

Tabulated t (0.05 probability) 2.228

t-test shows high significant differences for kinetin.

Discussion

Micropropagation which used isolated meristems and shoot tips is often periformed to maintain the genetic identify of the parent clone (11, 12, 13). The present investigation has shown that buds can be proliferated from date palm excised offshoot heart comprising mass of primary auxillary buds under leaflets.

Surface sterilization of explants has been noted to be a paramount problem in palm tissue culture (14). In our study, it was found that 9% of NaOCl treatment with vaccum were necessary to obtain contaminate-free cultures.

Prolific 3 ml explants were obtained from the heart of 3 years old of (Barhi) offshoot.

Since the majority of plant tissue culture are heterotrophic, it is necessary to supply them with a carbon source, usually in the form of sugar, macro and micronutrient elements, amoni acids, B vitamins and plant growth regulators.

The presence of plant growth regulators in general is essential to promot growth, but the nature and concentrations required varies from species to species.

Plant growth regulators are the critical media components in determining the development pathway of the explant.

There is some considerable difficulty in predicting the effects of plant growth regulators, This is because of great difference in culture response between species, cultivars and even plants from the same cultivar grown under different conditions.

Cytokinins promote cell division. Naturally occurring cytokinins are a large group of structurally related (they are purine derivatives) compounds. Two of the naturally occurring cytokinins, have some use in plant tissue culture media. There are zeatin and 2,ip (isopentyl

adenine), Zeatin is not widespread as it is expensive and relatively unstable. The synthetic analogues, kinetin and BA (Benzylaminopurine) are therefore used more frequently.

Among the cytokinins (BA, kinetin and 2,ip) tested, 2,ip particularly the 3 mg mg/L level, was found superior over both , BA and kinetin in accelerating buds proliferation.

Also, the present investigation has shown that BA or kinetin stimulate white and yellow-white colored friable callus at 1 mg/L level if supplemented to medium contain 10 mg/L NAA. It indicates that they are fair enough to stimulate callus which we have to avoid passing through this stage due to genetics stability problems.

Callus culture concerned the initiation and continued proliferation of undifferentiated parenchyma cells from parent tissue on clearly defined media. We didn't propagate date palm through callus due to the genetic variation among plantlets have been produced which from. This variation included loss or addition of chromosomes, gene mutation or combination of these factors (14).

Probably BA and kinetin stimulate callus because they are synthetic growth regular but 2,ip is a natural occurring one.

Our finding coincides with other investigator statements which confirms that 2,ip induces shoot and leaf growth but BA stimulates a production of adventitious shoot and callus. Kinetin stimulates shoot and leaf growth at lower concentrations and callus proliferation at higher concentrations (15, 16).

So the importance of this investigation is that, this specific medium manipulations can be used to direct the development of date palm in culture and can be used as a protocol to initiate primary axillary buds under leaflet of the heart of date palm shoot *in vitro*.

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The Results

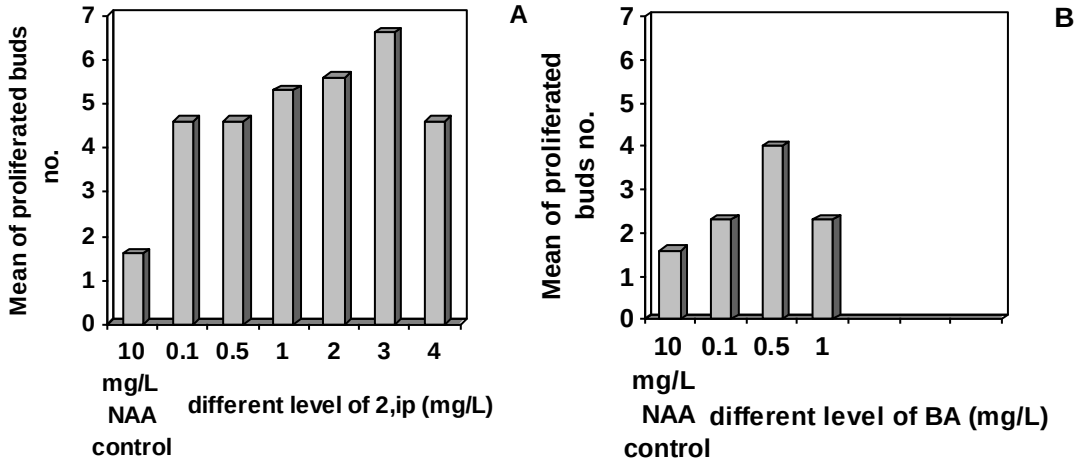

Fig. (1) (A) Effect of (2,ip with 10 mg/L NAA supplemented to modified M & S medium) on buds proliferation.
(B) Effect of (BA with 10 mg/L NAA supplemented to modified M & S medium) on buds proliferation.

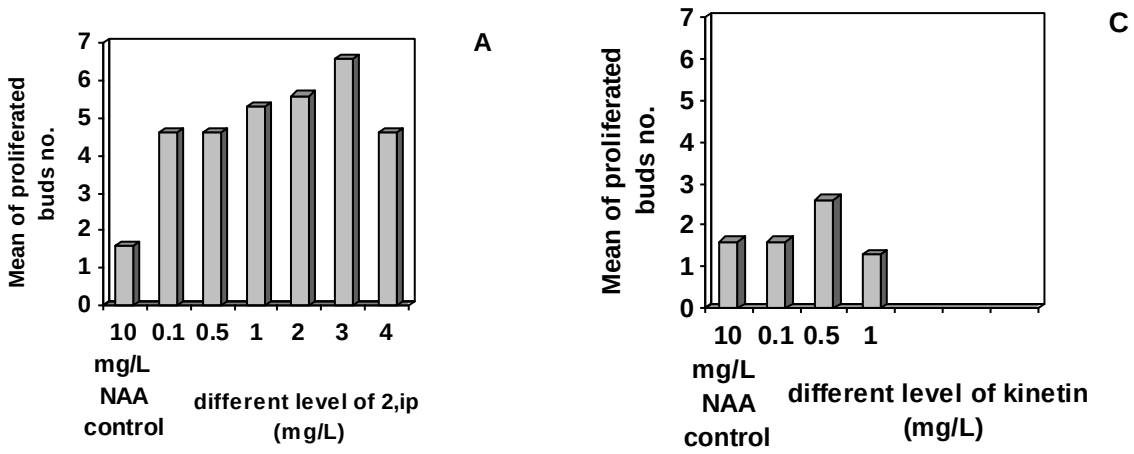

Fig. (2) (A) Effect of (2,ip with 10 mg/L NAA supplemented to modified M & S medium) on buds proliferation.
(C) Effect of (kinetin with 10 mg/L NAA supplemented to modified M & S medium) on buds proliferation.

تأثير مستويات وأنواع مختلفة من السايتوكينينات في تفتح براعم الصنف العراقي برحي من نخلة التمر خارج الجسم الحي

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الخلاصة

أجريت هذه الدراسة لتحديد فعل مستويات مختلفة من السايتوكينينات (BA, Kinetin, 2,ip) على تفتح براعم نخلة التمر (Phoenix dactylifera L.). نماذج بحجم ٣ ملم^{٣} من قلب فسيلة بعمر ٣ سنوات زرعت على وسط معاملة السيطرة الحاوي على وسط Murshige وSkooge (١٩٦٢) والمجهز بـ ٣ غم/لتر فحم منشط و١٠ ملغم/لتر NAA والمقترحة من Tisserat و Zaid (١٩٨١). ونماذج أخرى زرعت على هذا الوسط مجهز بـ ٠,١، ٠,٥، ١ ملغم/لتر BA و٠,١، ٠,٥، ١ ملغم/لتر Kinetin و٠,١، ٠,٥، ١، ٢، ٣، ٤ ملغم/لتر 2,ip. أعلى عدد من البراعم المتفتحة حصلت على وسط السيطرة المجهز بـ ٣ ملغم/لتر 2ip.