Advanced studies on micropropagation of date palm.

By

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Thesis

Submitted in Partial Fulfillment
of the Requirements for the Degree of
DOCTOR OF PHILOSOPHY (Ph.D.)

In

Agricultural Science
"Horticulture, Pomology"

Department of Horticulture
Faculty of Agriculture
Menoufia University
Shebin El-Kom

2021

Supervision Committee

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Date: / / 2021

ACKNOWLEDGMENT

Firstly my unlimited thanks to ***Allah*** *who always help me and give me the strength and patience to achieve this research.*

Gratitude is due to ***Prof. Dr. Magdy Rabeh Mohamed Rabeh,*** *Prof. of Pomology, Hort. Dept., Fac. of Agric., Menoufia University, Shebin El-Kom.*

, for proposing the problem, keen supervision and valuable guidance, advice throughout the course of this study and for the constructive criticism during the preparation of the manuscript.

Moreover, deep gratitude and thanks for ***Prof. Dr. Ezz El din Gadalla Hussein,*** *Prof. of date palm, Director of Central Laboratory for Date Palm Research and Development (CLDPRD). Research Institute, ARG, Giza for helpful cooperation, keen supervision and guidance throughout the preparation of this manuscript.*

As I wishes to express his deep gratitude to ***Prof. Dr. Saher Anwar Ahmed,*** *Prof. of Pomology, Hort. Dept., Fac. of Agric. Menoufia University; Shebin El-Kom., for proposing the problem, keen supervision and valuable guidance.*

I wish to thank my colleagues in the Central Laboratory for Date palm Research and Development, Agricultural Research Center, Giza, Egypt, specially, for their valuable help.

Grateful appreciation is also extended to all staff members of Horticulture Department, Faculty of Agriculture, Menoufia University.

Great and special thanks to ***my father ,my Mother, brothers, my wife, my daughter*** *and* ***my son.*** *Great and special thanks are also extended to my dearest friends and all members of the of Fac of Agric., Menoufia University Shebin El.Kom, also my dearest friends and all members of the of Agricultural Research Center, Giza, Egypt.*

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V. SUMMARY

This work was carried out in the Horticulture Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt; and Biotechnology Department, Central Laboratory for Date Palm Research and Development (CLDPRD), Agricultural Research Center (ARC) Giza, Egypt; during 2015-2020.

This study aimed to micropropagate date palm cultivar Amri through direct organogenesis, improving the growth of plantlets and establish a protocol for photoautotrophic and photomixotrophic as a new micropropagation system in order to obtain highest success percentage and lowest the loss percentage in the acclimatized plantlets during the acclimatization stage, this was done by performing two main experimental groups *In-vitro* and *Ex-vitro*

First group – *In-vitro* propagation:

1. **Micropropagation via direct organogenesis.**

2. **Treatments with carbon nanotubes, *spirulina platensis* extract, Cu phosphite (Kafom - Cu) and their influence on growth and improvement of plantlets during elongation stage.**

3. **Rooting under influence of polyethylene glycol, carbon nanotubes, silicon dioxide and magnesium phosphite (Kafom – Mg).**

4. **Establish a protocol for photoautotrophic and photomixotrophic as a new micropropagation system for date palm during pre-acclimatization stage.**

5. **Performing anatomical studies, some chemicals analysis and determination the photosynthetic pigments for plantlets.**

Second group – *Ex-vitro* acclimatization experiments:

6. **Improving plantlet growth by using three different cultural systems**

during acclimatization stage.

7. Effect of alcohol sugars (sorbitol) and salicylic acid on plantlets growth in greenhouse.

First group – *In-vitro* propagation:

1- Micropropagation via direct organogenesis.

Sterilized explants were longitudinally divided and cultured on **Murashige and Skoog (1962)** medium supplemented with different concentration of auxins, cytokinins and activated charcoal to investigation of swelling degree, fresh weight, browning degree, callus formation percentages, root formation and shoot formation after 28 weeks.

The most results of this experiment are summarized as follows:

- Explants cultured on MS medium supplemented with 6 mg l^{-1} 2-4,D, 3 mg l^{-1} NAA, 3 mg l^{-1} IAA, 2 mg l^{-1} 2iP, 0.3 mg l^{-1} BA and 1.5 g l^{-1} AC (T2) recorded the highest swelling degree, fresh weight, callus formation percentages and root formation (3.67, 22.83 g, 23.33% and 23.33% respectively).
- Not shown browning degree by culture the explants on MS medium containing 0.6 mg l^{-1} NAA, 0.6 mg l^{-1} IAA, 0.1 mg l^{-1} 2iP, 0.15 mg l^{-1} BA and 0.1 g l^{-1} AC (T10)
- Explants cultured on MS medium supplemented with 0.2 mg l^{-1} 2-4,D, 0.12 mg l^{-1} NAA, 0.12 mg l^{-1} IAA, 0.1 mg l^{-1} 2iP, 0.15 mg l^{-1} BA and 0.5 g l^{-1} AC (T7) given the highest shoot formation percentage (43.33%).

2- Treatments with carbon nanotubes, *spirulina platensis* extract, Cu phosphite (Kafom - Cu) and their influence on growth and improvement of plantlets during elongation stage.

This experiment is studying the effect of different concentrations form Carbon Nanotubes, *Spirulina platensis* extract, Kafom - Cu. for getting optimal

elongation medium, for improving elongation, reducing contamination and growth of the plantlets.

The most results of this experiment are summarized as follows:

- The highest shoots length, number roots and roots length (14.13 cm, 8.10 and 4.9 cm respectively). Were obtained with elongation medium supplemented with SP at 20 mg/l.
- Shootlets culture on elongation medium supplemented with potassium phosphite as EDTA Cu (K-Cu) at 0.6 mg l^{-1} recorded the highest significant value the trunk thickness.
- The highest significant value was recorded for the growth vigor (4) with SP at 15 mg l^{-1}, CNTs at 0.4 mg l^{-1} and K-Cu at 0.6 mg l^{-1}.
- No contamination percentage was found using elongation medium contains K- Cu at various concentrations.

3- Rooting under influence of polyethylene glycol, carbon nanotubes, silicon dioxide and magnesium phosphite (Kafom – Mg).

This experiment is studying the effect of different concentrations of Polyethylene glycol (PEG), Carbon nanotubes (CNTs), silicon dioxide (SiO_2) and magnesium phosphite (Kafom – Mg) on vegetative growth parameters and root system of Amri cultivar date palm during rooting stage after 12 weeks.

The most results of this experiment are summarized as follows:

- The highest values shoots length, number main roots, main roots length, secondary roots length, degree leaves color and growth vigor (20 cm, 6, 7.77, 5.33 cm, 4 and 4 respectively) was obtained with rooting medium supplemented with K-Mg at 1.2 mg/l.
- The highest significant value was recorded for the trunk thickness and number of secondary roots (0.337 cm and 5 respectively), in addition to SiO_2 at a concentration of 20 mg l^{-1}**.**

- No contamination percentage was found using rooting medium contains K-mg at various concentrations.

4- Establish a protocol for photoautotrophic and photomixotrophic as a new micropropagation system for date palm during pre-acclimatization stage.

Effect of different ventilation rates of culture vessel (0, 1, 2, 3 and 4 holes) individually or in combination with different concentrations of sucrose (0, 15 and 30 g l^{-1}) on growth plantlets parameters during rooting and per-acclimatization under conditions enriched with CO_2 (photoautotrophic and photomixotrophic) or without enriched CO_2.

Experiment 1: Effect of sucrose concentrations, ventilation rates of culture vessels and combination on growth plantlets parameters during rooting and per-acclimatization without CO_2 enriched condition.

The most results of this experiment are summarized as follows:

- When rate of ventilation culture vessel was at 3 or 4 holes in combination with 30 g l^{-1} sucrose the highest values to rooting percentage and growth vigor (86.67 or 86.33% and 4 respectively) was observed.
- Interaction between ventilation rates of culture vessel and sucrose concentrations revealed that ventilation rate at 4 holes in combination with 15 g l^{-1} sucrose gave the highest survival percentage (76.67 %).
- Ventilation rate at 1 hole in combination with 30 g l^{-1} sucrose give highest value of shoot length (19 cm).

Experiment 2: Effect of sucrose concentrations, ventilation rates of culture vessels and combination on growth plantlets parameters during rooting and per-acclimatization under CO_2 at 500 ppm enriched condition.

Date palm plantlets possessed the best growing and development when incubated under CO_2 at 500 ppm enriched conditions with 4 holes ventilation rate of culture

vessels in combination with 15 g/l sucrose compare with all treatments. Survival percentage reached to 78 %.

Experiment 3: Effect of sucrose concentrations, ventilation rates of culture vessels and combination on growth plantlets parameters during rooting and per-acclimatization under CO_2 at 1000 ppm enriched condition.

Cultured plantlets of date palm ventilation rates at 4 holes of culture vessel under CO_2 enriched at 1000 ppm and sucrose concentration at 15 g l^{-1} sucrose gave the highest survival percentage (79.83 %). While maximized results growth vigor recorded by using sucrose at 30 g l^{-1} with ventilation rate at 4 holes of culture vessel under CO_2 enriched at 1000 ppm.

Experiment 4: Effect of sucrose concentrations, ventilation rates of culture vessels and combination on growth plantlets parameters during rooting and per-acclimatization under CO_2 at 1500 ppm enriched condition.

- When ventilation rate of culture vessels were at 3 holes in combination with 30 g l^{-1} sucrose the highest value to rooting percentage (97.13 %) was observed.
- Interaction between ventilation rates of culture vessel and sucrose concentrations revealed that ventilation rate at 4 holes in combination with 15 g l^{-1} sucrose gave the highest survival percentage (85.33 %) and growth vigor (3.67)

5. Performing anatomical studies, some chemicals analysis and determination the photosynthetic pigments for plantlets.

The best and lowest survival percentage treatments were selected in the greenhouse after being treatment in vitro with different concentrations of sucrose, ventilation rates and under traditional culture and CO_2 enriched conditions (photomixtrophic or photoautotrophic).

Chemicals analysis and determination the photosynthetic pigments:

cultivation the plantlets on medium containing 15 g sucrose and in vessels with ventilation rates at 4 holes under condition CO_2 enriched at 1500 ppm gave the highest significant values of amino acids , phenols, reduced sugar and total sugar 8.284, 134.721, 12.194 and 9.786 mg/g respectively. Regard to, chlorophyll a+b, ventilation rate at 4 holes under CO_2 enriched at 1500 ppm in combination with 15 g l^{-1} sucrose gave the highest chlorophyll a+b content (41.769).

Second group – *Ex-vitro* acclimatization experiments:

6. Improving plantlet growth by using three different cultural systems during acclimatization stage.

This experiment studied the effect of three different cultural systems on survival percentage, plant length, number of leaves and estimation photosynthetic pigments in greenhouse.

The use of both the hydroponic (first culture system) and the traditional systems, with the addition of Barbary-Plante (third culture system) during the acclimatization stage gave highest values of survival percentage, plant length, number of leaves and chlorophyll a+b content compared to the second culture system (traditional) cultivation systems, where the lowest values were recorded.

7. Effect of alcohol sugars (sorbitol) and salicylic acid on plantlets growth in greenhouse.

This experiment was conducted on plants growing third culture system to investigate the effect of foliar spray and watered three concentrations of Sorbitol (0, 15, 30 and 45 g l^{-1}) and salicylic acid at concentrations (0, 20, 40 and 60 mg l^{-1}) on some vegetative growth parameters such as survival percentage, leaf width and growth vigor.

Treatment plantlets with spraying and irrigation with sorbitol at 30 g l^{-1} plus

salicylic acid at 40 mg l^{-1} giving the highest values of survival percentage, leaf width and growth vigor (91%, 2.73 cm, 4 respectively) compared to other treatments**.**

Recommendation:

1. To obtain the highest and greatest percentage of shoots formation s, cultivation of shoot tip on MS medium supplemented with 0.2 mg l^{-1} 2-4,D, 0.12 mg l^{-1} NAA, 0.12 mg l^{-1} IAA, 0.1 mg l^{-1} 2iP, 0.15 mg l^{-1} BA and 0.5 g l^{-1} AC

2. To get the highest shoots length and growth vigor or increase trunk thickness of the plantlets should be planted on medium containing 3/4 MS salts strength plus 20 mg l^{-1} SP or 0.6 mg l^{-1} K-Cu.

3. To increase shoots length, number main roots, secondary roots length, degree leaves color and growth vigor during the rooting stage, using rooting medium containing 3/4 MS salt strength added 1.2 mg l^{-1} K-Mg.

4. To establish a protocol for photomixotrophic micropropagation use vintilation rate at 4 holes of culture vessel + 15 g l^{-1} sucrose added rooting medium under CO_2 at 1500 ppm enriched condition give you the highest growth vigor and survival percentage.
5. To obtain a higher percentage value of survival during acclimation stage, cultivation plantlets in mixture of Peat moss with Perlite (1: 1 v / v) plus 3 g/pot Barbary-Plante with foliar spray and watered by sorbitol at 30 gl-1 plus salicylic acid at 40 mg l^{-1}.