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Effect of Light Duration and Intensity on the Development of Embryogenic Callus of Date Palm *in-vitro*

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A B S T R A C T

The idea of this experiment was to study the impact of various light periods and levels on the in vitro growth and development of embryogenic callus created from date palm (Phoenix dactylifera L.) cv. Barhi. Seven treatments, each with five replicates, were employed in a completely randomized design (CRD). The treatments comprised three light intensities (0, 1000, 3000 Lux) and four light exposure periods (0, 8, 12, and 16 hours per day). The findings revealed that the continuous darkness treatment (A) had the highest mean values for fresh weight (1100.20 g), dry weight (220.04 g), and dry matter content (20%), far outperforming all other treatments. With a minimal browning incidence (10%) and no clear vitrification, it exhibited highest callus viability (100%). Growth traits were substantially reduced if the light period increased to 8 hours per day, with vitrification appearing up at 5% under 3000 Lux intensity. The adverse effects intensified at 12 hours daily, where fresh weight dropped to 354.8 g, browning increased to 35%, and vitrification reached 10%. The poorest outcomes were recorded for treatments F and G (16 hours daily), with fresh weight declining below 155 g, browning escalating to 50%, and vitrification reaching 20%. It is concluded that complete darkness is optimal for somatic embryo formation in date palm, and that light exposure, even briefly, suppresses growth and promotes physiological abnormalities due to oxidative stress.

Introduction

Date palm (*Phoenix dactylifera* L.) constitutes a fundamental fruit crop in arid and semi-arid zones, fulfilling crucial economic, environmental, and societal functions for local populations (Zaid and de Wet, 2002). Traditional propagation methods for date palm encounter multiple challenges, including slow growth rates, insufficient offshoot production from elite mother plants, and resultant plant variability (Mazri and Meziani, 2015). Consequently, adopting micropropagation techniques has become essential to satisfy the rising demand for superior date palm seedlings.

Achieving successful micropropagation in date palm relies heavily on the capability to generate high-quality

embryogenic callus, which subsequently yields numerous somatic embryos that develop into complete plantlets (Eke *et al.,* 2005). Factors impact the initiation and progression of embryogenic callus, including the hormonal composition of the culture medium, the genetic profile of the cultivar, and physical incubation parameters such as temperature and illumination (George *et al.,* 2008). Light is a major physical driver in plant tissue culture growth and development, effects processes like photosynthesis, organogenesis, and secondary metabolite accumulation (El-Dawayati *et al.,* 2020). Several investigations have verified that complete darkness provides optimal facts for creating an embryogenic callus in date palm, particularly in the early stages. Eke *et al.,* (2005) showed that culturing excised apical meristems from date palm offshoots on MS media under dark conditions allowed for callus production by the second subculture. Azad *et al.,* (2014) corroborated our results, noting that embryogenic callous development from young date palm leaves required 14 weeks of complete darkness, as shoot induction required 9 weeks of a 16/8 light/dark photoperiod.

Shehata *et al.,* (2017) found that explants left under complete darkness and low temperature (20±2°C) led to greater embryogenic callus stages and mean somatic embryo size. Explants made at high temps (27±2°C) and a high light intensity (3000 Lux) laid out low values throughout the experiment. Light effects go beyond growth inhibition to include qualitative changes in callus biochemical makeup. El-Dawayati *et al.,* (2020) assessed if light intensity impacts cellular characteristics and β-sitosterol accumulation in date palm callus cultures (cv. Hayani). The study found that dark incubation led in more differentiated somatic embryos, but light intensities of 14 and 42 µmol/m²/s lead to notable β-sitosterol accumulation in proliferating callus. Light has a negative impact on embryogenic callus development via a variety of mechanisms, the most important of which are the stimulation of phenolic compound production and oxidative enzyme activity, which increases tissue browning and oxidative darkening (Baharan *et al.,* 2015). Browning inhibits cellular proliferation and somatic embryo development. El-Din *et al.,* (2007) found that isozyme patterns exhibited discrete bands for peroxidase and polyphenol oxidase enzymes, with increased band clarity throughout senescence and seedling phases, which correlated with phenolic compound percentages.

As a result, this study aims to evaluate the effects of varying light durations (0, 8, 12, 16 hours daily) and intensities (1000 and 3000 Lux) on date palm embryogenic callus growth and development in vitro, assessing physical parameters (fresh weight, dry weight, dry matter percentage, water content) as well as qualitative characteristics (embryo count, callus viability, browning, and vitrification).

The objective is identifying optimal light conditions for producing high-quality embryogenic callus.

Materials and Methods

Experiment Location and Date

The Plant Tissue Culture Laboratory, Department of Palms, Basra Agriculture Directorate, performed this study in 2025.

Plant Material

A four-year-old date palm offshoot of the Barhi cultivar weighing up to 15 kg was used. The offshoot was chosen with caution so it was free of disease and bug.

Dissection and Sterilization of the Apical Meristem

Dissection proceeded with a gradual removal of outer leaves and surrounding fibers until the apical meristem was seen. Surface sterilization in the excised apical meristem was achieved by soaking it with running water to remove any adhering soil and debris.

Sterilization employed commercial sodium hypochlorite at 15% concentration for 20 minutes, supplemented with 3-4 drops of Tween20 as a spreading agent to enhance sterilization efficacy. Following sterilization, the plant material was rinsed three times with sterile distilled water, then preserved in an antioxidant solution comprising citric acid at 150 mg/L and ascorbic acid at 100 mg/L until planting.

Callus Induction

The plant material, after removal from antioxidant solution and rinsing with sterile distilled water, was sectioned into four pieces and cultured on nutrient medium containing MS salts at 4.4 g/L, sucrose at 40 g/L, NAA at 6 mg/L, 2ip at 3 mg/L, and agar at 7 g/L. The pH was adjusted to 5.7 prior to sterilization. Medium

sterilization was accomplished using an autoclave at 121°C for 20 minutes. Cultured explants were incubated in a dark growth room maintained at 27°C, with subculturing repeated on identical medium composition every 4 weeks until callus initiation occurred.

Callus Multiplication

Following callus acquisition, multiplication was achieved by transferring to similar nutrient medium containing reduced plant growth regulator concentrations (NAA at 3 mg/L and 2ip at 1 mg/L). The multiplication process continued under identical conditions with subculturing onto fresh multiplication medium every 4 weeks for four passages until sufficient callus quantities were obtained.

Experimental Treatments

The experiment incorporated two factors:

1. **Light Duration:** Four levels (0, 8, 12, 16 hours daily), regulated using timers to adjust exposure periods for each treatment.
2. **Light Intensity:** Three levels (0, 1000, 3000 Lux).

Seven treatment combinations were established as detailed in Table 1.

Callus Preparation for the Experiment

Prior to treatment initiation, callus was transferred to plant growth regulator-free nutrient medium composed of MS salts at 4.4 g/L, agar at 7 g/L, glutamine, adenine sulfate, myo-inositol, and sodium phosphate, with pH adjusted to 5.7. This phase extended for a single two-week subculture to eliminate residual effects of plant growth regulators from preceding passages.

Experiment Implementation

Callus was inoculated at 100 mg per glass tube containing 20 mL of culture medium, with ten replicates allocated per treatment. Replicates were positioned in designated areas corresponding to specified light durations and intensities.

Treatments continued for two subcultures (8 weeks) using identical culture medium components, with subculturing performed 4 weeks after experiment commencement.

Studied Traits

Upon completion of the two treatment subcultures, the following characteristics were assessed:

1. **Fresh Weight (FW):** Callus masses were weighed using a sensitive balance (PHOENIX AB-224).
2. **Dry Weight (DW):** Callus samples were dried in an oven at 70°C until constant weight achieved, then weighed using the sensitive balance.
3. **Dry Matter Percentage (DM%):** Computed according to the formula: DM% = (Dry Weight / Fresh Weight) × 100
4. **Water Content Percentage (WC%):** Computed according to the formula: WC% = (Fresh Weight - Dry Weight) / Fresh Weight × 100
5. **Number of Embryos:** The mean number of somatic embryos formed per treatment was determined.
6. **Callus Activity:** Determined as the percentage of active, healthy replicates relative to total replicates.
7. **Browning Percentage:** Determined as the percentage of replicates exhibiting browning relative to total replicates.
8. **Vitrification Percentage:** Determined as the percentage of replicates displaying vitrification symptoms relative to total replicates.

Statistical Design and Data Analysis

The experiment used a completely randomized design (CRD) with seven treatments and five replicates each. The data was studied using SPSS software, with mean comparisons performed using the Least Significant Difference (LSD) test at a probability level of 0.05.

Results and Discussion

Effect of Light on Fresh Weight and Dry Weight

The data in Table 2 show significant differences between treatments in terms of mean fresh and dry weight. The continuous darkness treatment (A) outperformed all other treatments, had the highest mean fresh weight (1100.20 g) and the highest mean dry weight (220.04 g). Extending the light duration to 8 hours a day led to significant reductions in fresh and dry weights, with treatment B recording 898 g and 152.66 g, respectively, and treatment C recording 816 g and 122.40 g. The decline was noticeable when the light duration increased to 12 hours a day, with treatment D recording 354.8 g

and 49.67 g and treatment E recording 342.4 g and 44.51 g, respectively. Treatments F and G (16 hours daily) had the lowest values, in treatment F at 154.8 g and 19.35 g, and treatment G at 142.4 g and 17.09 g. The Least Significant Difference (LSD) value was 18.15 g for fresh weight and 4.25 g for dry weight.

Effect of Light on Dry Matter Percentage and Water Content

Table 2 results indicate that the maximum dry matter percentage occurred in the darkness treatment (A) at 20%, subsequently declining progressively with increasing light duration and intensity to reach 12% in treatment G. Conversely, water content increased gradually from 80% in treatment A to 88% in treatment G. The LSD value was 0.98% for dry matter percentage and 0.98% for water content.

Effect of Light on Number of Embryos and Callus Activity

The darkness treatment (A) exhibited the highest somatic embryo count, reaching 12 embryos, together with maximal callus activity at 100% (Table 2). Embryo numbers decreased to 8 in treatment B and 7 in treatment C, with callus activity declining to 80% and 70% respectively. The reduction in embryo numbers continued, reaching only 5-6 embryos in high-light treatments (D, E, F, G), accompanied by callus activity decreasing to 55-50%. The LSD value was 1.32 for embryo number and 8.54% for callus activity.

Effect of Light on Browning and Vitrification

The minimum browning percentage was recorded in the darkness treatment (A) at 10% (Table 2). Browning percentage increased progressively with increasing light duration and intensity, reaching 20% in B, 30% in C and D, 35% in E, 40% in F, with the maximum percentage occurring in G at 50%.

Vitrification appeared exclusively in light-exposed treatments, with treatment C recording 5%, treatments D and E recording 10%, treatment F recording 20%, and treatment G recording 10%, whereas treatments A and B remained vitrification-free.

The LSD value was 6.21% for browning and 2.15% for vitrification.

First: Effect of Light on Fresh Weight, Dry Weight, and Dry Matter Percentage

The findings show the continuous darkness treatment (A) beats the other treatments in terms of mean fresh and dry weights, with progressive reductions in these values tied to increased light duration and intensity.

This phenomenon can be interpreted physiologically the darkness directing cellular metabolic activities toward division and proliferation rather than differentiation and elongation, so it's consistent with Shehata *et al.,* (2017), who saw peak embryogenic callus formation under continuous darkness.

Moreover, light exposure stimulates phenolic compound production and oxidative enzyme activity, resulting in cellular growth inhibition (Baharan *et al.,* 2015). El-Dawayati *et al.,* (2020) demonstrated that dark incubation enhanced callus size and globularization compared to light incubation, explaining the pronounced fresh weight reduction in high-light treatments.

Table.1 Light treatments employed in the experiment

Treatment	Light Duration (hours/day)	Light Intensity (Lux)
A (Control)	0	0
B	8	1000
C	8	3000
D	12	1000
E	12	3000
F	16	1000
G	16	3000

Table.2 Mean values and standard deviations of studied characteristics for light duration and intensity effects on date palm embryogenic callus

Treatment	Light Duration (h/day)	Light Intensity (Lux)	Fresh Weight (g)	Dry Weight (g)	Dry Matter %	Water Content %	Embryo Number	Activity %	Browning %	Vitrification %
A	0	0	1100.20 ± 21.3	220.04 ± 4.3	20.0 ± 0.0	80.0 ± 0.0	12	100	10	0
B	8	1000	898.0 ± 5.7	152.66 ± 0.97	17.0 ± 0.0	83.0 ± 0.0	8	80	20	0
C	8	3000	816.0 ± 18.2	122.40 ± 2.7	15.0 ± 0.0	85.0 ± 0.0	7	70	30	5
D	12	1000	354.8 ± 3.8	49.67 ± 0.53	14.0 ± 0.0	86.0 ± 0.0	7	55	30	10
E	12	3000	342.4 ± 2.3	44.51 ± 0.30	13.0 ± 0.0	87.0 ± 0.0	6	50	35	10
F	16	1000	154.8 ± 3.7	19.35 ± 0.46	12.5 ± 0.0	87.5 ± 0.0	5	50	40	20
G	16	3000	142.4 ± 2.3	17.09 ± 0.27	12.0 ± 0.0	88.0 ± 0.0	6	50	50	10
LSD (0.05)			18.15	4.25	0.98	0.98	1.32	8.54	6.21	2.15

Second: Effect of Light on Browning and Vitrification

Maximum browning percentage occurred in treatment G (50%) under the highest light duration and intensity combination (16 h/3000 Lux), whereas minimum percentage appeared in darkness treatment A (10%). Browning in date palm tissue cultures associates with hydroxycinnamic acid derivative accumulation. El-Hadrami *et al.,* (2004) isolated two novel phenolic compounds from date palm callus, observing greater accumulation in browning-susceptible cultivars. El-Din *et al.,* (2007) further demonstrated that isozyme patterns revealed distinct peroxidase and polyphenol oxidase bands, with enhanced band clarity during senescence stages correlating with phenolic compound percentages.

Regarding vitrification, this phenomenon manifested exclusively in light-exposed treatments, with maximum value in treatment F (20%) at 16 hours light duration and 1000 Lux intensity.

Vitrification is a physiological disorder involving defective cell wall formation and excessive water uptake, it is considered a stress response to unfavorable culture conditions (Abohatem *et al.,* 2011).

Third: Effect of Light on Number of Embryos and Callus Activity

Somatic embryo formation decreased from 12 in darkness treatment (A) to merely 5-6 embryos in high-light treatments (F and G), while callus activity declined from 100% in A to 50% in high-light treatments. This reduction attributes to light stimulating expression of specific genes directing cells toward elongation pathways and terminal differentiation rather than embryogenic pathways (Von Arnim and Deng, 1996). A recent preprint (2022) demonstrated that somatic embryo germination in darkness played a crucial role in subsequent plantlet conversion, with dark-germinated embryos exhibiting continuous growth and elongated stem formation, whereas light-germinated embryos displayed inhibited longitudinal growth.

Fourth: Relationship between Light Intensity and Oxidative Stress

The substantial differences among treatments can be elucidated through oxidative stress concepts. Light, particularly at elevated intensities, stimulates reactive oxygen species (ROS) production in plant cells, leading to unsaturated fatty acid oxidation in cellular membranes and damage to proteins and nucleic acids. El-Dawayati *et al.,* (2020) documented marked β-sitosterol accumulation in proliferating callus exposed to light intensities of 14 and 42 µmol/m²/s, representing an important sterol compound contributing to cell membrane stability and environmental stress resistance.

Fifth: Differences between Light Intensities of 1000 and 3000 Lux

Results indicated that 3000 Lux light intensity proved more detrimental than 1000 Lux across equivalent time periods, with higher intensity treatments recording lower fresh and dry weights and increased browning.

These results agree with those of Kintzios *et al.,* (2001) regarding the effects of light intensity on somatic embryogenesis expression, and they echo Kong *et al.,* (2016), that show that higher light intensity affects cellular structural properties and secondary compound accumulation.

Author Contributions

Ahmed Zaer Resan: Investigation, formal analysis, writing—original draft. Aqeel Hadi Abdulwahid: Validation, methodology, writing—reviewing. Jamal Abdul Redha AL-Rabea'a:—Formal analysis, writing—review and editing. Khudhair M. Al-Kanany: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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