Kashmir Journal of Science
https://kjs.org.pk **ISSN: 2958-7832**

Kashmir Journal of Science (2025), 4(1): 56-73

Research Paper

Somatic embryogenesis regulating shoot regeneration efficiency of Date palm (*Phoenix dactylifera* L.)

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ARTICLE INFO

Article history:

Received: 19 January 2025
Revised: 01 March 2025
Accepted: 01 March 2025
Available online: 17 March 2025

Keywords:
Date palm, Explant, Kinetin, Somatic Embryogenesis, Tissue culture

Abstract

The present study was conducted to estimate the specific potential of regenerated date palm by somatic embryogenesis of tissue culture technique. A total of thirty rooted offshoots were cultured on basal medium complemented with composition of 0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg / L of 6-BA and Kinetin. The observations on the duration of shoot initiation, total shoots number and shoots length were noted. Date palm (*Phoenix dactylifera* L.) culture redeveloped on MS basal medium without the addition of BA (6-benzylaminopurine) and kinetin (Kin) exhibited a significantly lower survival rate (72.50% $P < 0.05$) and no shoot formation was noticed throughout the experimental duration. The highest concentration (4.0 mg/L) resulted in the shortest time for shoot initiation (17.50 days), whereas lower concentrations (0.5 and 1.0 mg/L) exhibited extended durations (23.50 and 24.20 days, respectively). The maximum number of shoots per explant (5.36) was achieved with 4.0 mg/L BA + Kin, while the concentration of 2.0 mg/L produced an optimal number of shoots (3.30). This study highlights the significance of optimizing BA and Kin concentrations to enhance somatic embryogenesis shoot regeneration efficiency in date palm through tissue culture techniques.

Introduction

Date palm (*Phoenix dactylifera* L.), is an ancient growing tree in Egypt since primitive times, but its culture did not become necessarily until somewhat later than Iraq (Kamla *et al.*, 2009). Pakistan is one of the leading date palms growing country among the world's top date producer i.e. Saudi Arabia, Iran, Egypt, and U.A.E. with 10% share of worldwide production (Botes and Zaid, 2002).

It is well known that date palm is multiplied sexually through seeds and vegetatively by sucker (Al-Khayri et al., 2001). However, vegetatively proliferated plants accumulate various bacterial, fungal, viral as well as mycoplasmal diseases from the air, soil and insect-vectors, which resulted in deficiency of their productivity, whereas frequent droughts also increasingly threaten their productivity and survival (Abohatem et al., 2001). Furthermore, offshoot multiplication is time consuming process, and the mortality of suckers is very high. Propagation through seeds has many limitations as well, like seed dormancy, low rate of germination and progeny variation (Mazri, 2014; Al- Khayri et al., 2001).

To overcome these obstacles and fulfill the demand for planting material, it is essential to produce diseases free and drought tolerant date palm seedlings to confirm their resilience and adaptability in changing climatic conditions. Tissue culture techniques, including somatic embryogenesis and micro-propagation provide effective solutions for large-scale production of uniform and disease-free plantlets (Bekheet et al., 2001). These methods of propagation facilitate the development of date palms with desirable traits such as improved drought-tolerance, rapid growth rate and stress resistance, while maintaining genetic uniformity. The major aim of the proposed study was to increase the date palm survival and productivity in water-limited environments, probably supporting viable agricultural innovative practices that ultimately reduce the reliance on chemical inputs and conserve water resources as well.

Therefore, proposed study strengthens the regional food security by ensuring stable supply through high quality and disease-free dates seedling even under challenging environmental conditions. Moreover, tissue culture techniques also enable the conservation of elite cultivars and support the initiation of climate adapted cultivars to new environment. By addressing both current and future agricultural challenges, this study contributes to encouraging resilience to climate change while stimulating sustainable agriculture, environmental stewardship, and long-term economic stability in susceptible areas.

Materials and Methods

Collecting of explant materials

Explant material like offshoots or suckers (Figure 1) of date palm tree between the age of 3 to 4 Yrs. was removed from the mother tree, surrounding the region of Hyderabad and Mirpurkhas District, Sindh and brought to the Tissue Culture and Molecular Biology Laboratory of Biotechnology Department, Sindh Agriculture University, Tandojam.

MS- Basal medium

Formation of stock solution for micronutrients

The composition of each salt such as macronutrient, micronutrients, vitamin and iron based were measured and determined consequently and transferred to conical flask. Then double distilled water (700ml) was transferred into each conical flask, mixed to liquefy appropriately with the help of magnetic stirrer. Finally, the emulsion was moved to a volumetric flask (1L) and finalized to the final volume. The volumetric flask was categorized and transferred to the refrigerator for further use.

Figure 1. Offshoot/ sucker of date palm detached from the mother tree. The Suckers were carefully removed containing outer layers and emerging leaves at the top.

Formation of M.S. Basal medium

The 10ml of each stock solution macronutrient, micronutrients, vitamin and iron based, agar (7-8g), sucrose (30g) and activated charcoal (0.3g) were measured in conical flask and 800ml of sterilized distilled water was added. Emulsion was mixed and residual purified water was combined to end volume of total solution (1L). Media pH was calibrated (5.7) utilizing 1N sodium hydroxide and heated (5-8 mins.) till the solution converted into transparent. Media was shifted into bottles (100ml) and sterilized in autoclave and kept in refrigerator for further use.

Shoot culture media

Formation of stock solution for Phytohormones 6-benzylaminopurine (BA) and Kinetin

BA and Kinetin (0.1g) were determined and added in beaker, liquefied by manipulating some droplets of sodium hydroxide. A slight amount of double purified water was added into beaker and assorted with stirrer. The emulsion was shifted to 100ml of conical flask and made the final volume up to the mark with double purified water. Stored the solution at $0^{\circ}C$ temperature for further experimentation.

Formation of shoot culture and somatic embryogenesis media

The basic constituents of M.S. salts were distributed into five conical flasks, individually containing 800 mL of double-distilled water. Stock solutions of BA (6-benzylaminopurine) and kinetin (Kin) were added to the flasks at specific concentrations (0.5, 1.0, 2.0, 3.0, and 4.0 mg/L). The solutions were thoroughly mixed using a magnetic stirrer, and the final volume was adjusted to 1L with double-distilled water. The pH of the medium was adjusted to 5.7 using hydrochloric acid, and the solution was heated until it became clear. The prepared medium was dispensed into 100 mL bottles, securely capped, and sterilized by autoclaving at 121°C and 15 psi for 20 minutes. The sterilized media were then stored for further use.

Formation of suckers or offshoot of date palm

Sucker or offshoot of date palm (Figure 2) was cut acropetally, and fibers, dead plant part, basic parts including roots and soil, along with upper mature parts were removed. The mid portion was kept for further handling.

Surface Sterilization of Explants

The suckers were thoroughly cleaned, and the outer layers were carefully removed to expose the lateral buds and shoot tip regions. The exposed areas were excised and immediately immersed in an antioxidant solution containing citric acid (150 mg/L) and ascorbic acid (100 mg/L) to prevent oxidation. The lateral buds and shoot tips underwent surface sterilization by soaking in 70% ethanol for 15 minutes, followed by immersion in 100% sodium hypochlorite solution with 2–3 drops of Tween 20 for 30 minutes. After sterilization, the tissues were rinsed three times with double-distilled water to remove any residual chemicals. The sterilized explants were then transferred to a laminar flow cabinet, where flamed instruments were used for further processing.

Figure 2. Preparation of offshoot (sucker) of date palm under laboratory conditions. The offshoot was carefully washed, trimmed and sterilized to promote shoot and root elongation. At this stage surface sterilization techniques were also applied and treated 10% of common

bleach to minimize the contamination and enhance successful establishment for further propagation.

Experimental process

The tissue culturing of date palm (Figure 3) accomplished by shoot tip culture process as shown by Hussain *et al.* (2001). The explants were grown on (MS, 1962) Basal medium. The 30 explants were cultured on Basal medium added with amount of (0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg / L) of B.A. + Kin through Completely Randomized Design (CRD). The observation on the survival rate, initiate shoots days, shoots number and shoots length noted. Additionally, the redeveloped shoots were cut and moved to rooting media (M.S. Basal + I.B.A) with 0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg / L quantities. The analysis on initiate roots days, roots number and roots length observed.

Statistical analysis

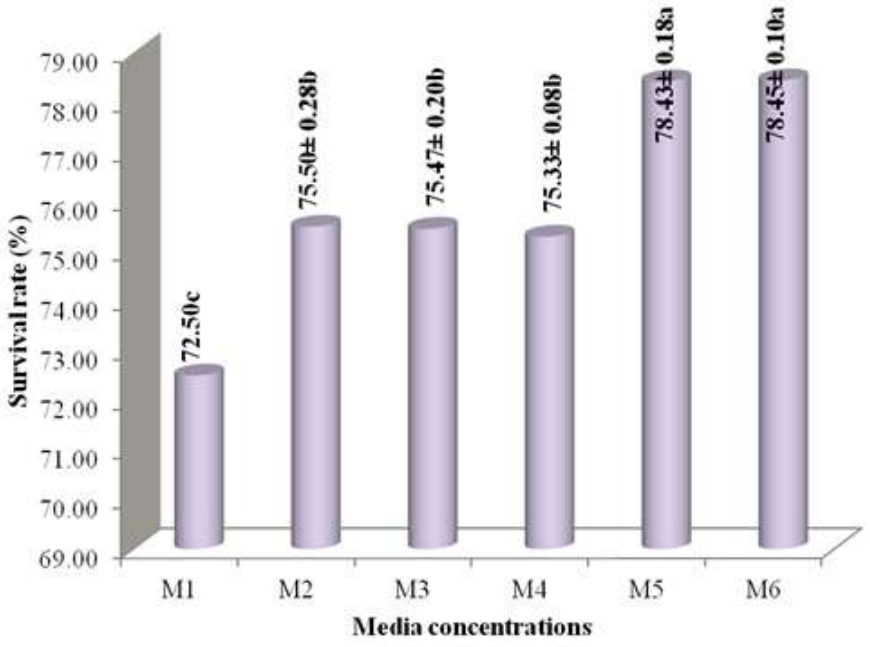
The achieved data were statistically analyzed by calculating consequently through the method of ANOVA. The treatment means were associated utilizing (LSD) at 5% Probability (Waller and Duncan, 1969). Entirely computational as well as statistical analysis were accomplished utilizing software student edition package 8.1.

Results

Survival efficiency under various concentration of B.A and Kin

The results for the survival rate of date palm explants regenerated through somatic embryogenesis containing various composition of B.A and Kin showed that explants grown on M.S. without B.A. and Kin (M1) exhibited significantly inferior rate of survival 72.50% ($P < 0.05$) as associated to grown on M.S. containing different concentrations of B.A. and Kin (Figure 3). In order to reveal the further analysis, the survival rates of plants initiated on M.S. containing either 3 mg/L(M5) or 4 mg/L(M6) B.A. and Kin were significant ($P > 0.05$), and both concentrations showed remarkable higher rate of survival 78.43±1.18% and 78.45±0.10%, respectively compared to those initiated on M.S. with inferior composition of B.A. and Kin i.e., 2.0 mg/L (M4), 1.0 mg/L (M3), or 0.5 mg/L (M2). Notably, there were no significant differences ($P > 0.05$) were detected in the rate of survival of explants initiated on M.S. containing 0.5 mg/L, 1.0 mg/L, or 2.0 mg/L of both B.A. and Kin, with rate of survival 75.50±0.28%, 75.47±0.18%, and 75.33±0.8%, respectively. These findings are dependable as the previous studies of Aslam and Khan (2009) with slight modification in growth hormones

and culture condition of explants, the work of these researchers further reported that the higher concentrations of BA probably enhance the regeneration protentional of date palm shoots. Furthermore, the comparable results have also been reported in other tissue culture studies (Biroscikava et al., 2004; Junaid et al., 2007).



LSD (0.05) = 0.460, SE ± =0.211, CV% =0.34

M1	=	**MS-Basal + 0.0 mg/L each of BAP and Kinetin**
M2	=	**MS-Basal + 0.5 mg/L each of BAP and Kinetin**
M3	=	**MS-Basal + 1.0 mg/L each of BAP and Kinetin**
M4	=	**MS-Basal + 2.0 mg/L each of BAP and Kinetin**
M5	=	**MS-Basal + 3.0 mg/L each of BAP and Kinetin**
M6	=	**MS-Basal + 4.0 mg/L each of BAP and Kinetin**

Data are the average of 30 explants of date palm

Figure 3 Survival rate (%) of date palm shoots regenerated on M.S. Basal medium containing various concentration and composition of B.A. and Kin. The effect of these plant growth regulators on shoot growth and development were assessed to determine the optimal hormone composition for enhanced regeneration. Data analyzed are the average mean

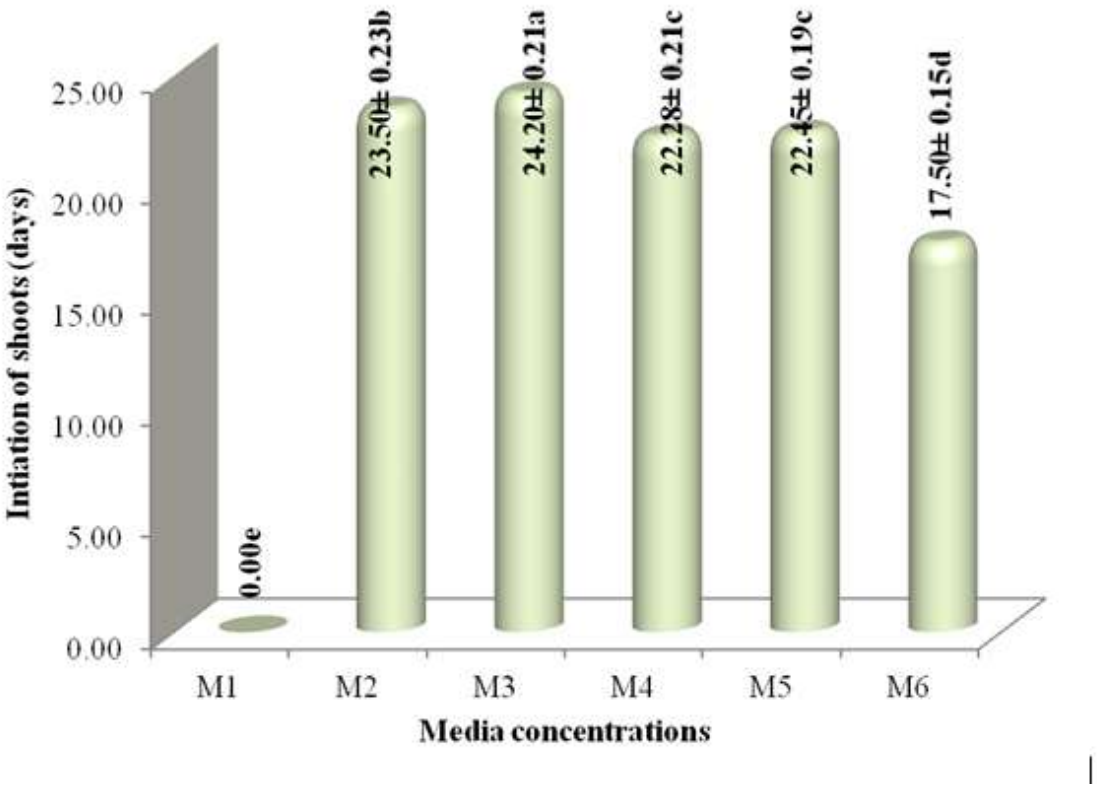
of three different replications. The alphabet on different columns denotes the significant differences at 5% level of probability.

Figure 4. Date Palm explants culture on nutrient media containing different concentrations of BA and Kin. Effects of these plant growth regulators were evaluated to optimize shoot induction, growth, and overall regeneration efficiency under in vitro conditions.

Initiation of shoots at various time intervals

When explants were grown on the M3 medium, the time required for shoot initiation was 24.20±0.21 days (Figure 4-5). In contrast, explants grown on the M6 medium exhibited the shortest time to shoot initiation taking only 17.50±0.15 days. Explants grown on the M4 and M5 media took approximately the same time to initiate shoots (22.28±0.12 and 22.45±0.19 days, respectively). On the other hand, explants grown on the M2 medium took 23.50±0.23 days, which was significantly longer compared to the other media. No growth was observed in

explants grown on the MS basal medium (in vitro). Additionally, no significant differences (P > 0.05) were observed in shoot initiation times across replicates on different media



LSD (0.05) = 0.515, SE ± = 0.236, CV% =1.58

M1	=	MS-Basal + 0.0 mg/L each of B.A. and Kin.
M2	=	MS-Basal + 0.5 mg/L each of B.A. and Kin.
M3	=	MS-Basal + 1.0 mg/L each of B.A. and Kin.
M4	=	MS-Basal + 2.0 mg/L each of B.A. and Kin
M5	=	MS-Basal + 3.0 mg/L each of B.A. and Kin.
M6	=	MS-Basal + 4.0 mg/L each of B.A. and Kin.

Figure 5. Shoot initiated (days) through somatic embryogenesis of date palm cultured on various compositions of B.A. and Kin.

The effect of these plant growth regulators on shoot growth and development were assessed to determine the optimal hormone composition for enhanced regeneration. Data analyzed were the average mean of three different replications. The alphabet on different columns denotes the significant differences at 5% level of probability

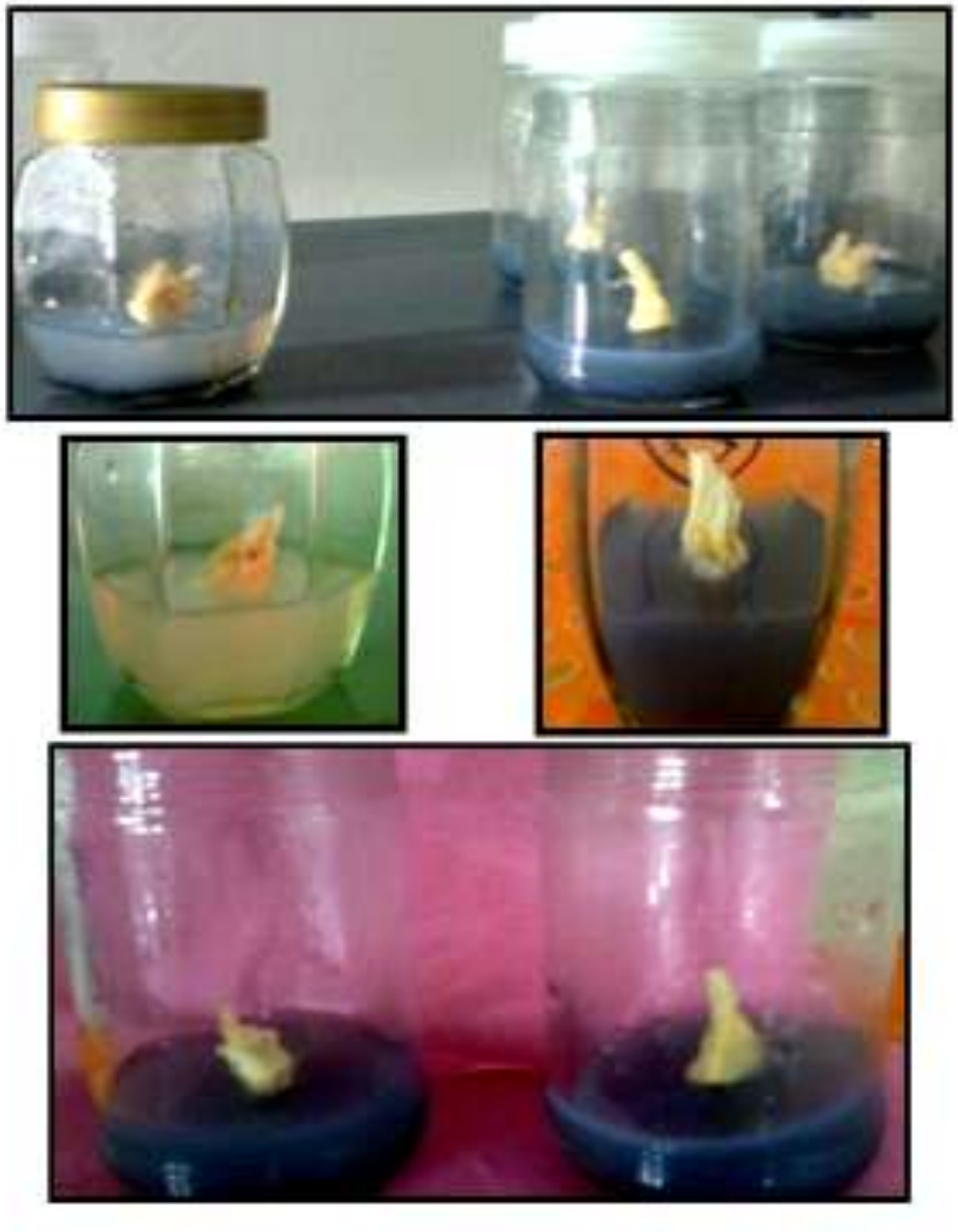


Figure 6. Induction of somatic embryogenesis of date Palm for shoot initiation. Explants were cultured under controlled conditions with specific growth regulators to stimulate embryogenic callus formation.

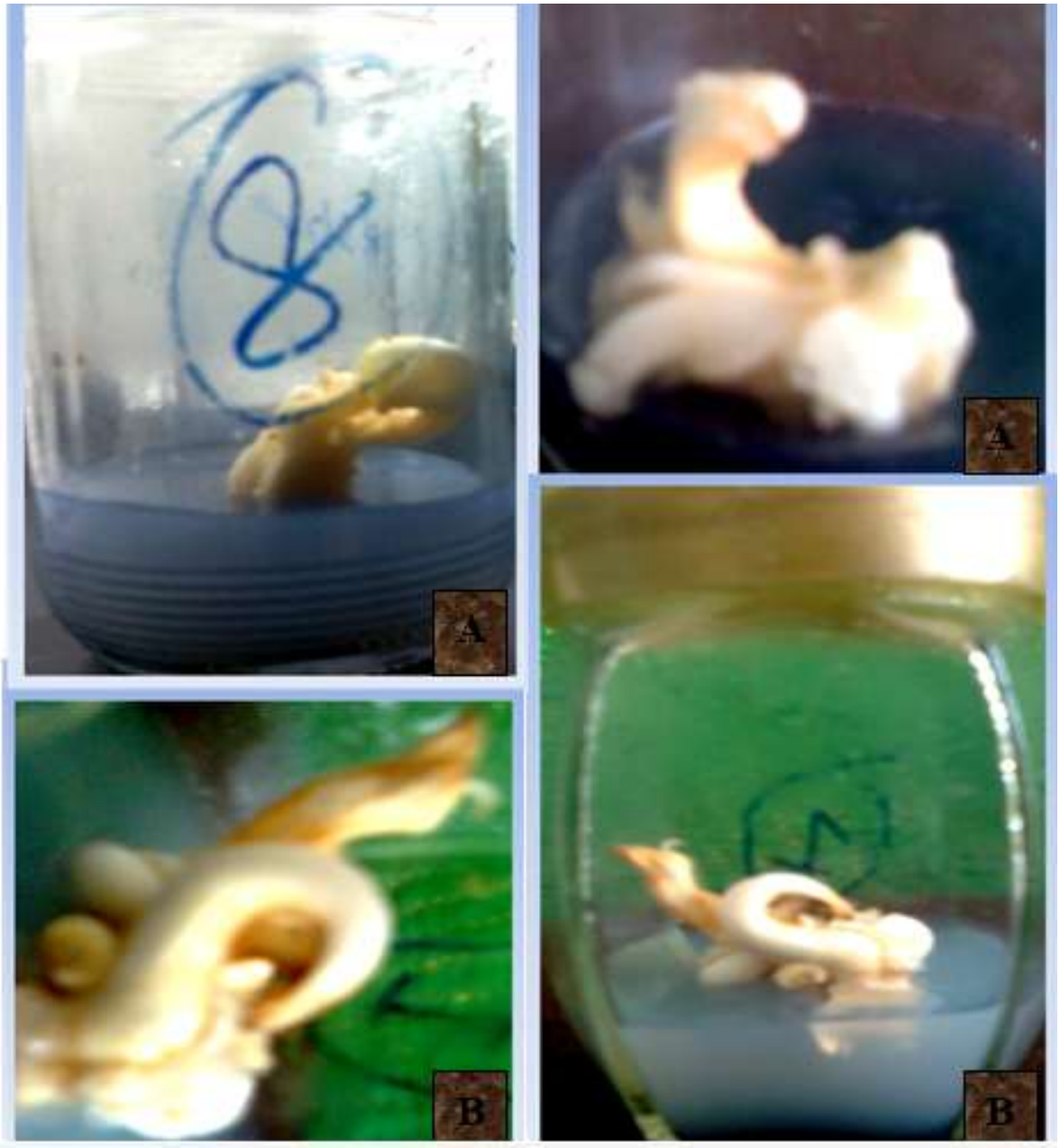


Figure 7. Date palm shoots initiated through technique of somatic embryogenesis under controlled condition. Explants were cultured on a specialized medium containing optimal concentrations of plant growth regulators to induce embryogenic callus formation. The controlled environment facilitated the transition from somatic embryos to healthy shoot development, ensuring efficient propagation and genetic stability of the regenerated plants**.**

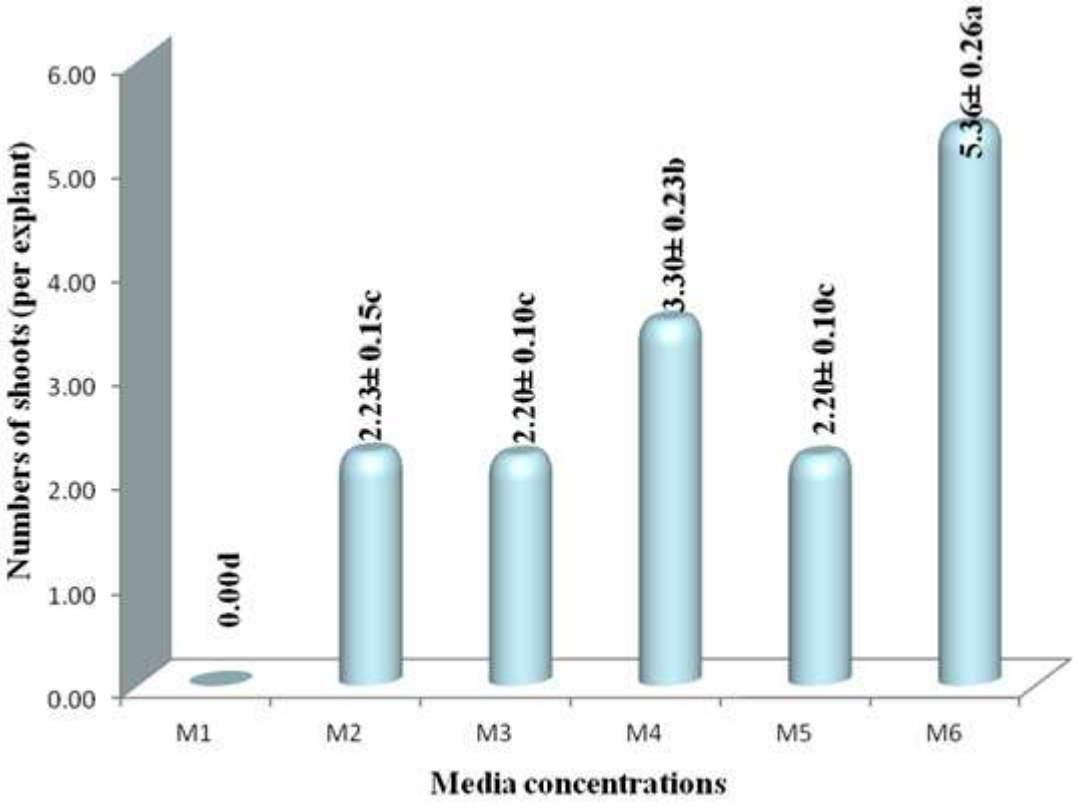
Total number of shoots

Effect of medium added with dissimilar concentrations of B.A+ Kin was examined in the existing study. There was no any regenerated date palm shoot noted on M.S. Basal medium (invitro). Nevertheless, M2, M3 and M4 media noted that alike (P>0.05) shoots numeral per

explant of date palm i.e. 2.23±0.15, 2.20±0.10 and 2.20±0.10, correspondingly. Although, with maximum amount of B.A + Kin in M.S. Basal medium considerably improved the shoots numeral (i.e. 3.30±0.23 and 5.36±0.26, correspondingly) per explant. Recently the highest shoots number (5.36) detected in M.S. Basal medium accompanied with 4 mg / L B.A. + Kin. These outcomes are erratic with the research study of El-sharbasy *et al.* (2001), who reported that the maximum shoots numeral of date palm Sewi cultivars on M.S. medium added with 4 mg / L B.A. They also detected the highest shoots number in Zaghloul cultivars of date palm on M.S. medium accompanied with 1 mg / L 2ip. In alternative studies Aslam and Khan (2009) informed the peak shoot regeneration occurrence was (85%) and shoot number developed per explant (5.6) on M.S medium appended with 7.4µM B.A. Conversely, explants of date palm cultured on M.S. Basal medium accompanied with 2mg / L B.A. + Kin displayed finest shoots number (3.30). Related products have been perceived by shah *et al.* (2006) on explants that cultured on M.S. medium appended with diverse amounts of B.A. (0.25 to 2.0 mg / L) or Kin (0.25 to 2.0 mg / L) and N.A.A. (0.1 to 0.5 mg / L) added with the B.A. (0.25-5.0 mg / L). Hussam et al. (2007) identified the most effective combination of plant hormones for shoot regeneration from the shoot tips of date palm (Phoenix dactylifera L.) var. Marktoon on MS basal medium supplemented with 4.0 mg/L 2ip, 2.0 mg/L BA, and 1.0 mg/L NAA, which resulted in the highest number of shoots. In the present study, media supplemented with 0.5 mg/L, 1.0 mg/L, and 2.0 mg/L BA and Kin produced similar ($P > 0.05$) shoot numbers per explant, with values of 2.23, 2.20, and 2.20, respectively. These findings align with the results of Gaber and Abd-Alla (2010), the shoot optimal initiation of date palm on 0.5 mg/L NAA was detected. Furthermore, the research also indicated that M.S. containing 1.0 mg/L BA addition to 2ip, and NAA , resulted the finest results for development of specific shoot.

Length of shoots

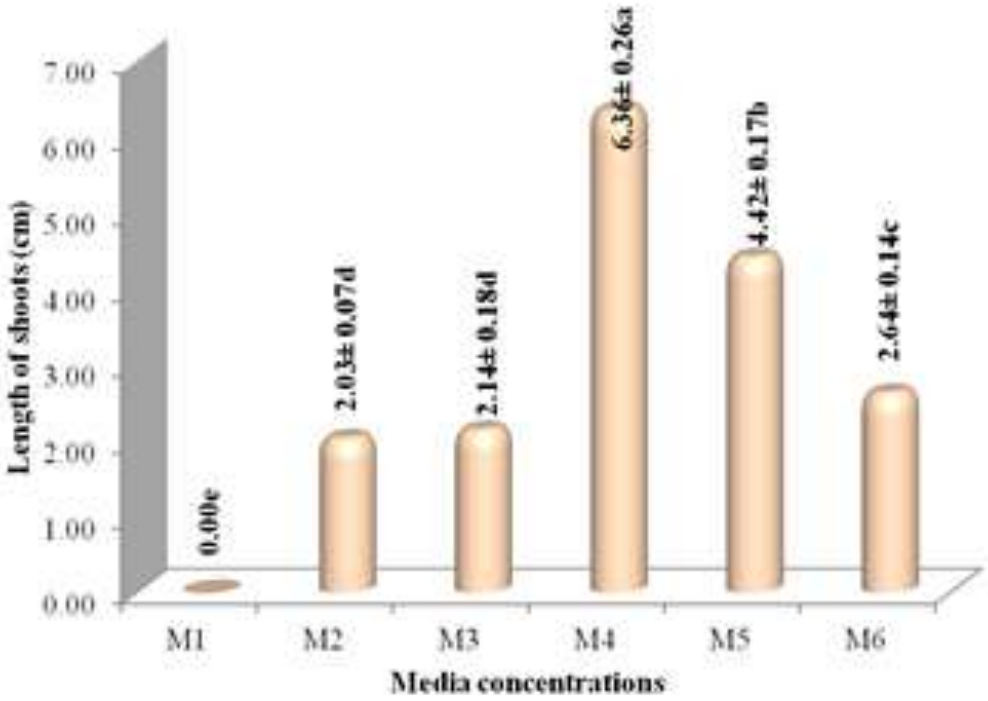
The results for shoot length of explants grown on M.S. containing varying concentrations of BA and Kin are presented in Figure 4. The longest shoots were observed on the M4 medium (6.36 ± 0.26 cm), followed by explants grown on other media: M5 (4.42 ± 0.17 cm), M6 (2.64 ± 0.14 cm), M3 (2.14 ± 0.18 cm), and M2 (2.03 ± 0.07 cm) (Plates 4.5, 4.6, 4.8, and 4.9). Although explants grown on the MS basal medium (control) did not die, no shoot formation was observed throughout the experiment. Additionally, no significant differences were found between replicates in the current study.



LSD (0.05) =0.5077, SE ± = 0.2330, CV% = 11.05

M1	=	MS-Basal + 0.0 mg/L each of B.A. and Kin.
M2	=	MS-Basal + 0.5 mg/L each of B.A. and Kin.
M3	=	MS-Basal + 1.0 mg/L each of B.A. and Kin.
M4	=	MS-Basal + 2.0 mg/L each of B.A. and Kin
M5	=	MS-Basal + 3.0 mg/L each of B.A. and Kin.
M6	=	MS-Basal + 4.0 mg/L each of B.A. and Kin.

Figure 8. shoots in number of (per explant) of date palm regenerated through somatic embryogenesis various composition of B.A. and Kin. The effect of these plant growth regulators on shoot growth and development were assessed to determine the optimal hormone composition for enhanced regeneration. Data analyzed were the average mean of three different replications. The alphabet on different columns denotes the significant differences at 5% level of probability



LSD (0.05) = 0.493, S.E ± = 0.226, CV% = 9.45

M1	=	MS-Basal + 0.0 mg/L each of B.A. and Kin.
M2	=	MS-Basal + 0.5 mg/L each of B.A. and Kin.
M3	=	MS-Basal + 1.0 mg/L each of B.A. and Kin.
M4	=	MS-Basal + 2.0 mg/L each of B.A. and Kin
M5	=	MS-Basal + 3.0 mg/L each of B.A. and Kin.
M6	=	MS-Basal + 4.0 mg/L each of B.A. and Kin.

Figure 9. Length of shoots (cm) of date palm regenerated through somatic embryogenesis on various composition of B.A. and Kin. The effect of these plant growth regulators on shoot growth and development were assessed to determine the optimal hormone composition for enhanced regeneration. Data analyzed were the average mean of three different replications. The alphabet on different columns denotes the significant differences at 5% level of probability

Discussion

Tissue culture procedures are convenient for date palm due to its nature of dioecious, which puts constraint on the seed multiplication for the development of plant. Alternatively, the palm commonly have no branches just apical meristem present. It grew limited suckers at initial stage of life time, thus low offshoots numbers. Subsequently, meristems number are accessible

as basis of explants from solitary palm is generally low. The *invitro* rejuvenation of date palm effectively utilized to attain supreme explant numeral in numerous states, even though enhancements in protocols are still in progress and proliferation problems.

B.A. and Kin. proficiency regulating the shoot regeneration of date palm

In recent studies, date palm regenerated on M.S. (control) lacking B.A. and Kin (M1) showed meaningfully ($P<0.05$) minimum existence rate (72.50%) and not showed shoots/roots up to the end of experiments. Whereas, M.S. added with various amount of B.A.+ Kin showed increasing rate of survival i.e. 0.5 mg / L (75.50%), 1.0 mg / L (75.47%), 2.0 mg / L (75.33%), 3.0 mg / L (73.43%) and 4.0 mg / L (78.45%). These consequences are completely sustained by the searching of Aslam and Khan (2009) who testified that with aggregate quantities of B.A.P and Kin also amplified regeneration rate in date palm. Analogous properties were also distinguished in numerous other tissue cultures (Biroscikava *et al.,* 2004; Junaid *et al.,* 2007). It has been perceived that M.S. Basal medium supplemented with highest B.A. + Kin concentration (4.0 mg / L) taken slightest time (17.50 days) to shoots start. Whereas, augmented with B.A.+ Kin in lower amount of 0.5 and/or 1.0 mg / L, correspondingly the shoots have taken more time (23.50 and 24.20 days). Accordingly, no shoots formed on M.S. (invitro), El-Sharabasy *et al.* (2001) parallelly presented the outcomes reported through experiments resulted uppermost percent of initiation shoot in Zaghloul and Sewi cultivars, whereas M.S. complemented with 2ip alone and B.A. + 2 mg /L 2ip, respectively both media were designed showed uppermost shoot and root length of any plants. This could be because of the cytokinin responsible for shoot enhancement and proliferation while auxin promoted the root development. The studies further explored that together two cytokinin combined at once may accelerate the shoot growth and development of date palm. Although in further studies the researcher stated the maximum percent of formation shoot of Zaghloul and Sewi relicates from M.S. enhanced with 4mg / L B.A. together with 4 mg / L 2ip, correspondingly.

In present study, the highest number of shoots (5.36) was significantly observed on M.S. containing 4 mg/L of BA and Kin. El-Sharbasy et al. (2001), reported the work in contrast with these findings, who reported the maximum and highest number of shoots in Sewi cultivars of date palm on media containing basal M.S. salts composited with 4 mg/L of B.A. the researcher also demonstrated that highest number of shoots in cultivars Zaghloul of date palm on M.S. composited with 2ip. Additionally, the highest (85%) regeneration protentional efficiency of shoot per explant (5.6) was reported by Aslam and Khan (2009) on solid M.S.

composited 7.4mg BA. In the present study, shoots cultured on M.S. composited with 2 mg/L B.A. and Kin. resulted the optimal number of shoots (3.30). Similar outcomes were reported by Shah et al. (2006), who observed date palm explants cultured on MS medium supplemented with varying concentrations of BA (0.25 to 2.0 mg/L), Kin (0.25 to 2.0 mg/L), and NAA (0.1 to 0.5 mg/L) in combination with BA (0.25 to 5.0 mg/L). Hussam et al. (2007) identified the best combination of plant growth regulators for shoot regeneration from the shoot tips of Phoenix dactylifera L. var. Marktoon on MS basal medium supplemented with 4.0 mg/L 2ip, 2 mg/L BA, and 1.0 mg/L NAA, which resulted in the optimum number of shoots. Furthermore, in the current study, explants grown on media supplemented with 0.5 mg/L, 1.0 mg/L, and 2.0 mg/L BA + Kin produced similar ($P > 0.05$) shoot numbers per explant, with values of 2.23, 2.20, and 2.20, respectively. These results are consistent with those of Gaber and Abd-Alla (2010), who reported the best shoot regeneration rate in date palm on MS medium supplemented with 0.5 mg/L NAA. Another study by them showed that MS basal medium supplemented with 1.0 mg/L BA, 1.0 mg/L 2ip, and 0.1 mg/L NAA resulted in the best shoot elongation.

Conclusion

Present study concludes that irrespective the date palm explants grown on M.S. Basal medium (invitro) without the addition of growth hormones did not show any sign of shoots up to end of experiment. Whereas, the B.A.+ Kin at the quantity of 4.0 mg / L taken time slightest to shoot start and provided ominously maximum existence rate with extraordinarily developed shoots numeral. Conversely, shoots length developed at 2.0 mg / L B.A.+ Kin concentration. Present study, further concludes that growth regulator concentrations significantly modulates the induction of shoots and proliferation rate of date palm. This optimized method offers a valuable substance for improving the shoot regeneration and somatic embryogenesis efficiency in date palm, markedly under climate-resilient procedures. Forthcoming scientific research can leverage these findings to enhance somatic embryogenesis and regeneration in date palm.

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