Research Article

Nutrient Media Optimization for Date Palm Micropropagation (*Phoenix dactylifera* L.)

Ghulam Sughra Mangrio¹, Altaf Ahmed Simair^{*2}, Shumaila Tabassum³, Bharat kumar¹, Nadia Mangrio⁴

1 Department of Biotechnology, Sindh Agricultural University, Tandojam 70060, Sindh

2 Department of Botany, Government college university, Hyderabad, 71000, Sindh

3 Department of Plant Breeding and Genetics, Sindh Agriculture University Tandojam, 70060, Sindh

4 Sugarcane Research Institute, Agriculture Research Centre, Tandojam, 70060, Sindh

*Corresponding author: altafsimair@gcu.edu.pk

Abstract:

An experimental study was conducted to optimise nutritional media for date palm (*Phoenix dactylifera* L.) micropropagation by employing a shoot tip as an explant on basic Murashige and Skoog (MS) medium with varied Auxin (IAA, NAA) and Cytokinin (Kinetin and 2ip) concentrations for shoot and root multiplication. The findings of date palm regeneration on basal MS medium (Control) without Kinetin and IAA indicated a decreased survival rate (5.0%) and no root or shoot up to the end of the experiment. When the basal MS medium having different Kinetin and IAA concentrations showed an increased rate of survival, i.e. basal MS +0.5 mg/L of Kinetin and 0.0 IAA (8.0%), MS basal+1.0 mg/L of Kinetin and 0.5 IAA (6.6) and basal MS +2.0 mg/L of Kinetin and 1.5 IAA (7.0) and basal MS contains 1.5 mg/L of Kinetin and 1.0 IAA resulted in highest survival rate (8.8%). However, the highest number of shoots (2.2) and a maximum length of shoots (8.8 cm) were recorded on basal MS medium containing 2.0 mg/L of Kinetin and 1.5 IAA. Furthermore, basal MS media with 1.0 NAA and 1.5 mg/L of 2ip yielded the highest number of roots (3.34) and measured the maximum length of roots (3.56 cm).

Keywords: Tissue culture, MS basal medium, Date palm

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1. Introduction

The introduction should briefly place the study in a broad context and highlight why it is important. The date palm (*Phoenix dactylifera* L.) is a member of the Arecaceae family with a height of around 20 metres and the ability to live for up to 80 years. Date palm has been cultivated since 4000 B.C. [1] and It is estimated that there are over 3000 varieties. According to the fruit moisture content, there are three types of cultivars i.e. dry, semi-dry and soft [2].

Pakistan is amongst the top sixth major producer of dates and its cultivation is successfully practised in arid regions [3-6]. The date palm has an economically important value due to delicious fruit which contains nutritive and energy providing properties [7]. Other than the carbohydrates dates contain proteins, minerals, vitamins and fatty tissues. Referable to the presence of high carbohydrate contents (70-80%), dates are hence a rapid energy source [8].

Date palm grows in tropical and arid areas where irrigation water is easily available and in the coastal regions. These trees grow slowly but last for many decades. Date palm plants are heterozygous, so in the new plants, many desirable characteristics of parents will be missed and being observed much variation within the offspring. For the commercial production or true to type propagation, sexual propagation is unsuitable for date palms due to their dioecious and heterozygous nature of seedlings [9]. The fruits produced by the female plants will be inconsistent and of lesser quality [10].

Tissue culturing technology may be more true to type than other methods of plant propagation, and it can be done in a short amount of time and area [11-13]. Tissue culture technology is currently and widely employed as a biological tool for date palm mass propagation, disease removal, and clonal propagation, as well as for a variety of other horticulture crops. Date palm lateral buds and apical shoot tips are employed as explants for successful plant regeneration [9]. Until twenty years ago regarding the inducing and sustaining the good callus a little achievement was accomplished. Even so, some workers analyzed that growth regulators cannot affect morphogenetic responses, but interact with the media components like nitrogen, sucrose, etc. [14-16].

Auxin and cytokinin-mediated morphogenesis is the subject of numerous written publications involving various plant tissues and organs [17]. For a successful program in improving date palm cultivation, one should go with a study and the valuation of the cultivars grown. Hence, by keeping the above-noted points in view, the present research was extended out to set up a protocol for optimization of date palm media through the usage of different growth regulators.

2. Materials and Methods

Plant material like offshoots/suckers of date palm (CV. Aseel) aged 3-4 years was detached from the mother tree brought to the laboratory from the area of Khairpur and Hyderabad, districts of Sindh. For the root culture of date palm, several concentrations 0.0, 0.5, 1.0, 1.5 and 2.00 mg/L of NAA whereas, 0.0, 0.5, 1.0, 1.5 and 2.0 mg/L concentrations of 2ip were added with the basal MS media and the pH was adjusted to 5.7. Whereas, M1=MS-Basal (Control), M2=MS-Basal+0.0 IAA and 0.5 Kin, M3=MS-Basal+0.5 IAA and 1.0 Kin, M4=MS-Basal+1.0 IAA and 1.5 Kin and M5=MS-Basal+1.5 IAA and 2.0 Kin. Media were then transferred and correctly plugged in bottles (100 mL). They were autoclaved for 20 minutes at 121°C below 15 psi. Offshoots/suckers were cleaned gently and the outermost layer was removed to let out the lateral bud region and shoot tip. The outermost exposed part was immersed in an antioxidant solution comprising (150 mg/L citric acid and 100 mg/L ascorbic acids) right away. Shoot tips and lateral buds were sterilized in 70%

ethanol for 15 minutes before being immersed in 100% sodium hypochlorite solution with 2-3 drops of Tween-20 for 30 minutes and washed three times in sterilized distilled water. Additionally, the explants were handled in a laminar airflow cabinet. The gathered data were statistically examined using the analysis of variance technique (ANOVA). The Least Significant Difference (LSD) was used to compare the treatment means at a 1% level of Probability. The student edition software package 8.1 was used for all computational and statistical analyses.

3. Results

3.1 Survival rate (%)

The date palm explant cultivations on MS media were found to be significantly lower about explant products having various IAA and Kinetin concentrations compared to those on MS medium without the addition of Kinetin and IAA (M1) (Fig. 1). The highest survival rates of 8.8 and 8.0 were noted in M2 and M4. It is of interest that M3 showed a less survival rate (6.6 %) instead to other media, i.e. M1, M2, and M4 (5.0, 8.0, and 8.8 % respectively). Results further revealed that the survival rate of explants cultured on M2 and M4 were higher (8.0 and 8.8 %, respectively) than that cultured on the M3 and M5 (6.6 and 7.0 %), respectively.

Pooled S Pooled St Dev = 0.6325

Figure-1. Survival rate (%) of date palm regenerated on MS medium supplemented with different concentrations of Kinetin.

3.2 Number of shoots

There was a zero regenerated shoot of a date palm cultured on basal MS medium (control). However, M2 and M3 media showed more number of shoots per explants of date palm i.e. 2.06, 2.08 respectively. Whereas, an increase in concentrations of Kinetin and IAA in basal MS medium, increased the number of shoots in M4 and M5 with 2.12 and 2.22 per explants, respectively (Fig. 2). Approximately all media have taken 20-27 days to initiate shoots except basal MS medium (Control).

Pooled St Dev = 0.0938

Figure-2. Number of shoots (per explants) of date palm regenerated on MS medium supplemented with different concentrations of Kinetin.

3.3 Length of shoots (cm)

Explants on alternative media such as M4 (3.60 cm), M3 (3.36 cm), and M2 (2.7 cm) had much shorter shoots (Fig. 3). Date palm explants cultivated in MS-basal media (control) did not die, but no new shoots appeared.

Pooled St Dev = 0.2587

Figure-3. Length of shoots (per explants) of date palm regenerated on MS-medium supplemented with different concentrations of Kinetin an

3.4 Number of roots

The MS basal medium (control) displaced zeroes regenerated root of date palm, however, M3 and M5 media showed a more or less similar number of roots per date palm explants i.e. 3.12 and 3.2 respectively. It was further noted that approximately all media have taken 15 to 23 days to initiate roots except MS basal medium. M2 medium showed less number of roots (2.5) as compared to M3 (3.12) and M5 media (3.2). Whereas, shoots of date palm cultured in M4 medium exposed significantly the highest number of roots (3.34) amongst the other media (Fig. 4).

Pooled St Dev = 0.0707

Figure-4. Number of roots (per explants) of date palm regenerated on MS medium supplemented with different concentrations of NAA.

3.5 Length of roots (cm)

On the basal MS medium, no roots were found (control). Root length is zero at the same time. The root lengths of M2 (2.15 cm) and M3 (2.7 cm) were nearly identical. Shoots grown on M5 medium, on the other hand, had larger roots than those grown on M2 or M3. In addition, of all the media tested, the M4 medium had the longest roots, measuring 3.56 cm. (Fig. 5).

Pooled St Dev = 0.1364

Figure-5. Length of roots (cm) of date palm regenerated on MS-medium supplemented with different concentrations of NAA and 2ip.

Discussion

Micro-propagation is the most effective and rapidly multiplying process by which plant material can be produced into a large number of plants progeny, in a well-defined tissue culture medium under aseptic conditions. Due to the short and limited number of offshoots produced by the date palm, rapid propagation is impossible because in the date palm life span the production of offshoots is limited to a certain period. Date palm contains one apical meristem and does not produce branches and in its early life duration it can only produce a few suckers, therefore several meristems and the number of offshoots are the accessible sources of explants, is generally very low from a single palm.

In the present study, the date palm was cultured on basal MS medium (control) without the addition of Kinetin+IAA (M1) showed a low survival rate (5.0) and did not show shoots/roots up to the end of the experiments. However, basal MS medium supplemented

with IAA + Kinetin showed an increased rate of survival, such as M2 (8.0), M3 (6.6) and M4 (8.8). [18] found that increasing Kinetin and IAA concentrations improved the rate of date palm regeneration. Many additional plant tissue culture experiments have shown similar results [19, 20].

The MS media, which contains 1.5 mg/L IAA and 2.0 mg/L Kinetin, produced the most shoots (2.2), whereas the control medium yielded no shoots or roots. According to [21], media with 0.20 Kinetin+0.10 2ip+ 0.10 IAA + 0.10 BAP + 0.10 NOA + 0.10 mg/L of NAA produced the most shoots. [22] found that on MS medium containing 100 mg/L 2,4-D+3 mg/L 2ip and 0.10 mg/L NAA+3 mg/L 2ip, respectively, Sewi and Zaghloul cultivars developed the highest percentage of shoots. [23], reported that the date palm explants which were cultured on MS media containing different Kinetin (0.25-2.0 mg/L) and BAP (0.25-2.0 mg/L) concentrations in combinations produced maximum shoots. [24], reported that the best combination of plant growth regulators directly from shoot tips of date palm in var. On basal MS media containing 4.0 mg/L 2ip, 2mg/L BAP and 1.0 mg/L of NAA, showed the optimum number of shoots. By increasing the concentration of hormones, the height of shoots was also decreased.

In the case of several roots, basal MS media containing basal MS + 1.0 2ip and 1.5 mg/L of NAA, resulted in the highest number of roots (3.8). Our findings showed resemblance with [25] results, who reported that increasing the concentration of NAA showed a significant effect in culture media for root formation of palm explants, regarding several roots and root length. Generally, with raising in concentration from 0.0 to 0.1, 0.3, 0.5 and 1.5 mg/L also showed slowly increase in the number of roots and root length, till at 1.5 mg/L of NAA gave the highest mean for a number of roots (2.50 roots) with (3.00 cm) of highest length. In the present study, Basal-MS+1.5 2ip and 2.0 mg/L NAA decreased in several roots. Similarly, [25], found that increasing the NAA concentration from 1.5 mg/L to 4.5 mg/L resulted in a significant reduction in the number of roots as well as root length. According to [24], adding NAA to the culture medium increases root formation, with the 0.5 mg/L concentration exhibiting the excellent and ideal percentage (90 percent) of rooting and average root length (5.4 cm). Similar findings were observed by [25], who discovered that increasing NAA from 1.5 mg/L to 4.5 mg/L resulted in a significant decrease in root length as well as in specific roots. [24], discovered that adding NAA to the culture medium boosted root development, with an optimum concentration of 0.5 mg/L (90 percent) of rooting and average root length (5.4 cm). No, raising the auxin concentration caused the root length to shrink. There was no reduction in root length when auxin concentration was increased.

The enhancing effect of NAA at 1.5 mg/L on palm explants rooting shows that this is an optimum concentration for promoting the cell division of the cambium and the formation of roots. In the present study, basal MS+1.5 2ip and 2.0 mg/L NAA showed decreases in

length of roots (3.23 cm). [25], noted similar results that by increasing in the NAA concentration at the highest level (4.5 mg/L) showed the reduction in the number of roots (4.22) and also in root length (4.55 cm), instead of to results obtained from 1.5 mg/L of NAA.

Conclusion

The conclusion of this investigation demonstrated a considerably greater shooting time (3.8 cm) in the basal MS medium containing 2.0 mg/L of Kinetin 1.5 mg/L IAA per explant compared to other medium supplemented concentrations. The baseline MS comprising 1.0 Zip, 1.5 mg/L NAA was remarkably longer (3.5 cm).

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