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Enhancement of Acclimatization of in Vitro Propagated Date Palm (*Phoenix dactylifera* L.) Plantlets, Barhi Cultivar, Using Paclobutrazol and Sorbitol

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Abstract

The study was carried out in the period 2024 to 2025 in the Plant Tissue Culture Laboratory, which is part of the Date Palm Research Center, University of Basra, to investigate the effect of using two concentrations of the growth regulator paclobutrazol (PBZ) (2 and 4 mg/L) and two concentrations of the sugar alcohol sorbitol (Sor) (5 and 10 g/L) via foliar application, and a control treatment where only distilled water was used to spray, on improving the acclimatization of Barhi date palm (*Phoenix dactylifera* L.) plantlets. Findings from this research revealed that paclobutrazol and sorbitol significantly affected the acclimatization success rate. This was supported by an increase in acclimatization success rate to 90% with paclobutrazol at 4 mg/L. Additionally, the concentration enhanced fresh and dry weight, carbohydrate, and proline content in the leaves relative to the control treatment. The results also indicated that the application of (Sor) at 10g/L to the plantlets resulted in higher level of chlorophyll in comparison with the control (untreated) plantlets.

Keywords: acclimatization; Date palm; PBZ; Sor; stress; tissue culture.

1. Introduction

Phoenix dactylifera L. is a male/female (unisexual) dioecious tree. It is peculiar for palm species because it can give birth to offshoots. It is common throughout most of the world, ranging from 10° – 30° North and as far South as 20°. Its capacity to withstand extreme environmental conditions like high temperatures, dry winds and salinity of soil makes it remarkable (Ibrahim, 2013).

Sexual reproduction has not been deemed an appropriate way of commercial reproduction of date palms. Its use is largely limited to breeding initiatives, mainly because it cannot ensure the genetic fidelity of offspring to the parent plant. Moreover, in sexual propagation, about half of the descendants are male. On the other hand, offshoot propagation has been internationally acclaimed as the best option for vegetative reproduction of date palms, enabling the procurement of desirable cultivars (Al-Bakr, 1972). A notable decline in date palm populations has been observed in Iraq, especially in the city of Basrah. This degradation can be attributed to a combination of factors, especially prolonged wars, agricultural neglect, and harsh environmental stressors, which have collectively had a deleterious effect on the national economy (Al-Yasiri, 2018). In addition, several obstacles and challenges are associated with offshoot propagation, including the limited number of offshoots the mother palm produces during its initial stages of development (Al-Qatrani et al., 2023).

As a result, scientific research has focused on the use of tissue culture technology to produce numerous plantlets, including date palm apical meristem culture on synthetic nutrient media, which has been successful (Muhsen, 2004). Plant cell and tissue culture is a science that focuses on the *In vitro* culture of plant cells, tissues, and organs using specific techniques. These methods include the acquisition of plant parts to be used as culture (explants), their proper handling and sterilization, and the selection of appropriate culturing methods, nutrient media, and environments (Al-Miyahi, 2023). Many growth regulators have so far been used in different plant species, including not only growth promoters such as auxins, cytokinins, and gibberellins, but also growth inhibitors such as abscisic acid, ethylene, and paclobutrazol. These antagonistic inhibitors have effects that counteract those of growth promoters, inhibiting cell division and elongation. They are all used with different concentrations (Jakubekova et al., 2011).

Considering the dearth of studies on the impacts of paclobutrazol and sorbitol on date palm *in vitro* culture followed by acclimatization, the current experiment, the first of its kind, aimed to determine the effects of these growth retardants on the acclimatization of date palm plantlets.

2. Materials and Methods

The current research was conducted to determine the impact of foliar applications of Paclobutrazol and Sorbitol on the acclimatization success of tissue-cultured date palm plantlets.

This study was carried out to estimate the optimal concentration of the growth inhibitor PBZ and the sugar alcohol Sor, applied as a foliar spray to plantlets, to assess the success of acclimatization of treated plantlets. It used Sor and PBZ, and the following treatments were used.

- PBZ at 2 mg/L
- PBZ at 4 mg/L
- Sorbitol at 5 g/L
- Sorbitol at 10 g/L
- Control treatment (distilled water only)

2.1. Plantlets source

The date palm plantlets (Barhi cultivar), homogeneous in length, number of leaves, and vigor of growth, and ready to be acclimatized, were selected. Culturing floral explants on MS medium resulted in these plantlets (Muhsen et al., 2023). The culture vessels were emptied into the culture plantlets and washed thoroughly under running tap water to remove any traces of the culture medium. To avoid fungal infection, the

plantlets were dipped in a solution containing 1 g/L Cuprosate for 15 minutes. The plantlets were then potted in a growth medium consisting of peat moss mixed with perlite in a ratio of 3:1. This medium was sterilized in the autoclave at 121 °C and 1.05 bar steam pressure for 20 minutes. When sterilization was complete, the growth medium was removed, cooled, and the plantlets were then planted in it.

Also, special Torpedo pots were used for transplanting the plantlets. The substrate was then firmly compacted around the roots to prevent aeration that could cause root rot and plantlet mortality. Plantlets were then irrigated with a nutrient solution containing 1/4-strength MS solution, supplemented with 0.5 g/L Cuprosate fungicide. After irrigation, the plantlets were transferred to an acclimatization chamber and thoroughly sprayed with the experimental treatments. They were then sprayed with agricultural nylon and left to rest for 10 days; the humidity was retained within the agricultural nylon. Controlled conditions characterize the acclimatization chamber: a temperature of 28 ± 2 °C, light intensity between 1000-10000 lux during a 16-hour period of light and darkness, respectively, followed by humidity above 75%. The cover was removed slowly after 10 days. During that time, the plantlets relied on the humidity supplied by the agricultural nylon cover. After two months, the cover was completely removed. During this time period, the plantlets were sprayed with fungicide once a week and irrigated with a nutrient solution about once a week, consisting of 1/4-strength MS salts supplemented with distilled water as needed. Each treatment contained 10 plantlets, each a replicate.

2.2. Characteristics for Investigation

- Number of adapted plants = Percentage of adapted plants/ Percentage of adapted plants $\times$ 100.
- The chlorophyll content of leaf tissue was determined in the laboratories of the Date Palm Research Center using the method described by (Porra, 2002).
- The total soluble carbohydrates in the leaves, resulting from hardening and acclimatization processes, were quantified at the Date Palm Research Center. This determination was conducted based on fresh weight, utilizing the method described by (Watanabe et al., 2000).
- The proline content of plant tissue was determined in the laboratories of the Palm Research Center following the method of (Bates et al., 1973).

2.3. Statistical Analysis

The design used was the simplest which is Completely Randomized Design (C.R.D.). The Revised least significant differences test (R.L.S.D) was used for the significant difference between the means at 0.05 level of significance. There were 10 replicates for each treatment (Al-Rawi and Khalaf Allah, 2000). The results were analyzed statistically using Genstat v. (2007) software package.

3. Results and Discussion

3.1. Leaf Total Soluble Carbohydrate Content

The experimental treatment significantly affected the total soluble carbohydrates of the leaves as shown in the figure 1, which was much higher than the control. In particular, the total soluble carbohydrates (TSC) content of leaves was significantly higher in the 4 mg/L paclobutrazol treatment at 18.35 mg.g⁻¹ FW and the highest among all the treatments. This was followed by the 10 g/L sorbitol treatment which produced total soluble carbohydrates of 15.72 mg/g fresh weight. In contrast, the control treatment had the lowest amount of carbohydrates (9.95 mg/g fresh weight).

This can be explained by the ability of PBZ to inhibit gibberellin biosynthesis causing reduced vegetative growth and an increase in the photosynthetic products in the leaves. Increased photosynthetic efficiency and decreased carbohydrate consumption during growth results in higher accumulation of carbohydrates in plant tissues due to PBZ (Kishore et al., 2015; Tesfahun, 2018). Also, the increase in S.C. observed in the leaves could be due to increased chlorophyll in the leaves, which results in higher photosynthetic efficiency (Tekalign & Hammes, 2005).

Sorbitol acts as a carbon source and also as an osmotic agent and thus it positively affects the water balance of the cell and consequently enhances biosynthetic processes (Jain et al., 2010). The results of the current study are similar to de Sousa Oliveira et al. (2022) who found the most total soluble carbohydrates in the leaves of 'Manka' cultivars (Hosui and Santa Maria) treated with PBZ. In addition, the present investigation is in agreement with Hashim (2011) who found that after using PBZ, the amount of carbohydrates in Sidr trees of the cultivar Tuffahi significantly increased.

3.2. Leaf Free and Accumulated Proline Content

The proline concentration in leaf tissues markedly increased when plantlets were treated with paclobutrazol (PBZ) and sorbitol (Sor) as shown in the statistical analysis presented in Figure 1. Specifically, a 4 mg/L application of PBZ had the highest mean leaf tissue proline content of 19.930 µg g⁻¹ FW. This was much higher than the mean leaf tissue proline

content recorded in the control treatment which was 11.197 µg g⁻¹ fresh weight. The treatment with sorbitol 10 g/L resulted in 17.23 µg g⁻¹ fresh weight, in contrast.

An important physiological mechanism in plants for coping with various stresses is the accumulation of proline in leaves. Besides its ameliorating role in oxidative stress-induced damage, Proline also serves as an osmoprotectant, keeping membranes intact and protein structure, which are essential for life. Proline increases in plants grown with paclobutrazol and sorbitol is thought to result from ability to activate stress tolerance mechanisms. In particular, paclobutrazol increases the effectiveness of the antioxidant system. (Srivastava et al., 2010; Basu et al., 2016). In addition, sorbitol is found to control Osmotic potential of the cells resulting in higher accumulation of this amino acid (Jain et al., 2010). These results agree with the results of (Kiwani et al., 2025) who applied PBZ to rough lemon seedlings via the leaves. Furthermore, the findings are consistent with the previous studies of (Alkhateeb et al., 2024) on foliar application of PBZ to leaves of *Oryza sativa* L.

3.3. The chlorophyll content in leaves

The data presented in Fig. 1 show the influence of the treatments studied on total amount of chlorophyll in leaves. The results showed that there were statistically significant differences between the different treatments and the control treatment, which had the lowest mean chlorophyll content of 1.9 mg/g fresh weight. In contrast, sorbitol applied as foliar (10 g/L) treatment yielded the highest total chlorophyll content, 3.483 mg/g, which was significantly higher than the other treatments. The 4 mg/L paclobutrazol treatment was next most effective with a total chlorophyll content of 3.077 mg/g.

The better results of the above-mentioned treatments regarding the total chlorophyll content might be due to the marked improvement of the photosynthetic efficiency in the different treatments. This is also due to the ability of paclobutrazol to prevent the degradation of chlorophylls by inhibition of enzymes that break down chlorophylls and its ability to increase chloroplast biogenesis (Soumya et al., 2017). In addition, sorbitol could be used to prevent the chlorophyll pigments from being harmful due to stress, thus maintaining their stability and improving their concentration in the foliage (Kocaman, 2024). The observed effects may also be related to the importance of sorbitol in the translocation of macro- and micronutrients, including less mobile ones like calcium and boron, in the phloem vasculature to the leaves, stems, branches and roots. This helps them to move around freely inside the plant. This mechanism will then improve, activate and support the necessary physiological and

biochemical functions, which play a key role in photosynthesis, protoplasmic synthesis and respiration (Aydın & Sevine, 2006). The results of this study agree with the results of (Pacyga et al., 2024) who showed that foliar application of sorbitol for cabbage seedlings induced higher photosynthetic pigments including chlorophyll. These findings are also supported by the results obtained by (Li et al., 2020) where high concentrations of sorbitol were found to inhibit the production of chlorophyll in corn seedlings. On the other hand, low levels significantly stimulated its biosynthesis and accumulation.

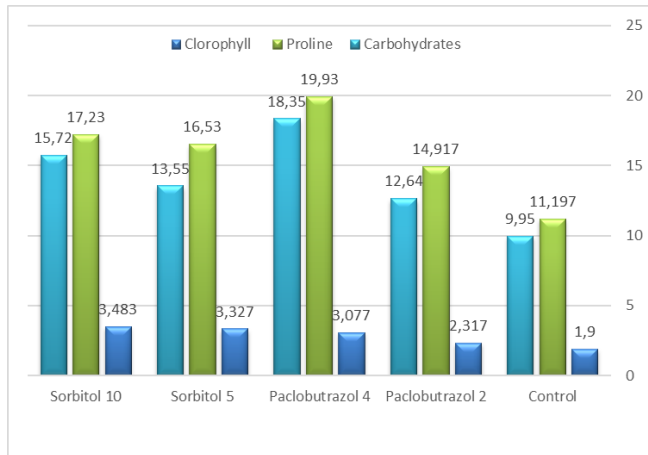


Fig. 1. Effect of paclobutrazol and sorbitol spray on the total soluble carbohydrate content in leaves (mg/g fresh weight), the free and accumulated proline content in leaves (μg/g fresh weight), and the chlorophyll content in leaves (mg/g fresh weight).

3.4. The Influence of Paclobutrazol and Sorbitol on Fresh and Dry Weight, and Acclimatization Success

Compared with the Sorbitol, the results shown in Figure 2 suggest that the fresh weight of leaf tissue was significantly affected after foliar application of PBZ. The mean fresh weight was the highest for the 4 mg/L Paclobutrazol treatment (5.2050 mg) and significantly higher than the 2 mg/L Paclobutrazol treatment (4.4063 mg). The 10 g/L Sorbitol treatment however, yielded a fresh weight of 4.7317 mg while the control treatment yielded 4.4550 mg.

The results presented in Figure 2 showed that application of paclobutrazol and sorbitol to plantlets had a significant effect on the dry weight of the leaves. Paclobutrazol produced the greatest mean leaf dry weight (2.3273 mg) which was significantly greater than all other treatments. On the other hand, the dry weight of the control treatment was least (1.4840) mg while the sorbitol treatment with 10 g/L gave dry weight of (2.1050) mg.

This effect may be due to the ability of paclobutrazol to inhibit the formation of gibberellin-like substances that in turn blocks cell expansion and elongation. This, combined with a decrease in water content of cells, leads to an increase in dry matter content of the cells at the expense of water content. (Chaney, 2005) This is similar to the results obtained by (Kiwan et al., 2025) when they used PBZ with rough lemon seedlings. In addition, the present results agree with those of (Alkhateeb et al., 2024) who applied PBZ to the rice plants.

The results shown in the figure 2 also revealed that spraying with PBZ and Sor had an effect on the success rate of acclimatization. The treatment with paclobutrazol at 4mg/L gave significant results with 90%. The control treatment, however, had the lowest acclimatization success rate, at 20%. The sorbitol treatment at 10 g/L produced an acclimatisation rate of 80% compared with this.

The results showed that application of growth inhibitors was effective in improving plant physiological characteristics through proline accumulation, increased carbohydrate level and maintenance of the chlorophyll. This, in turn, has a positive effect on growth efficiency and the resistance to environmental conditions (Sabagh et al., 2021). Besides, the success of acclimatization could be explained by the stress state created by the application of growth inhibitors which in turn led to high ABA levels in leaf tissues. This hormone plays a key role in minimizing transpiration during acclimatization, reducing relative water loss from the leaves of plantlets when they are moved to ex vitro conditions, even in cases of abnormal stomatal activity (Rai et al., 2011).

These findings are consistent with (Rabeh et al., 2021) study where they found that foliar application of sorbitol to tissue-cultured date palm plantlets resulted in higher survival. This study also corroborates with (Sidky et al., 2009) study on the use of paclobutrazol in date palm culture media and higher percentage of surviving plantlets.

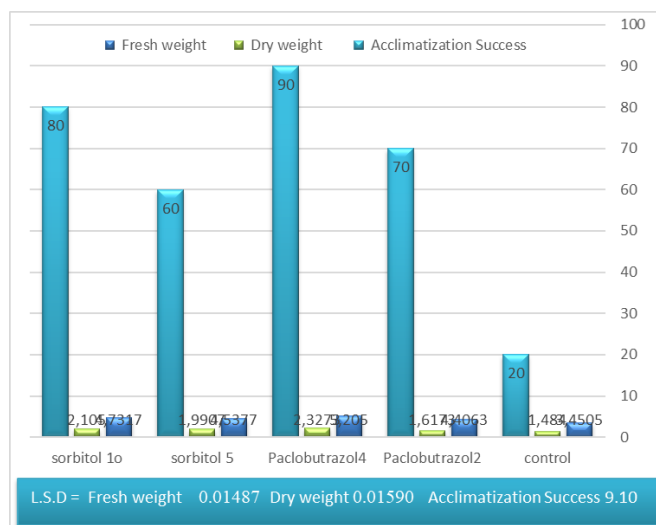


Fig. 2. illustrates the effect of foliar application of paclobutrazol and sorbitol on the mean fresh and dry weight of leaves (mg) and the percentage of acclimatization success.

4. Conclusions

Based on the results of the current study, the foliar application of paclobutrazol (4 mg/L) during acclimatization stage of tissue-cultured date palm plantlets increased the acclimatization rate, as well as the amounts of carbohydrates, proline and, fresh and dry weight and the survival rate of plantlets after completing the acclimatization stage. The study concluded that spraying plantlets with Sorbitol 10 g/L increased the growth parameters and traits to be studied, such as the acclimatization rate, leaf content of carbohydrates, proline, and chlorophyll, as well as fresh and dry weight. The use of sorbitol at a concentration of 5 g/L also improved leaf chlorophyll content. Therefore, the study recommends conducting further studies on other imaginal varieties and other plant species, using other concentrations of these substances, or using other inhibitory substances.

Conflict of Interest

Author(s) has no conflict of interest to disclose.

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