Study of the effect of different concentrations of indole-3-butyric acid (IBA) and season on the regrowth of date palms (*Phoenix dactylifera* L.) cultivar Aziza Bouzid

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Abstract : During its lifetime, and particularly before entering production, the date palm can produce several shoots at its base, up to thirty in total. In the absence of vitro plants of local varieties, these shoots remain the ideal method for regenerating plants that are consistent and faithful to the mother plant. Date growers adopt this technique in order to propagate the noble varieties of the Figuig oasis. Among them is the Aziza Bouzid cultivar, known for its difficulty in rhizogenesis. The objective of this work is to test the effect of the season and concentrations of 3-indolebutyric acid (IBA) on the rooting of shoots from this cultivar in order to identify the most favourable seasons for transplanting shoots. And the recommended IBA concentration. Three concentrations of IBA were applied: C1 (0 ppm), C2 (150 ppm) and C3 (300 ppm). The study showed that spring and summer are the most favourable seasons for transplanting suckers, with a root regeneration rate of up to 100%. The use of IBA significantly increased the regeneration rate, breaking records, particularly for the two seasons already mentioned.

Introduction:

The date palm is a productive tree species of the Arecaceae family, mainly cultivated in arid and semi-arid regions, particularly in the Middle East and North Africa [1, 2]. It is considered a strategic fruit species in oasis areas, which has been a fundamental element of the ecosystem. Propagation is generally carried out by suckers or biotechnological means to ensure the transmission of the characteristics of the mother plant and thus produce homogeneous, disease-free plants [3-5]. Propagation by seed is generally carried out in programs for the selection of new date palm varieties, due to their high heterogeneity, although in genetic improvement programs for this species, particularly in backcross programs [1].

Propagation by suckers is a traditional technique for propagating date palms. This technique has been used to propagate clones selected by date palm growers since ancient times. Suckers appear at the base of the trunk of female or male palms that are generally between 4 and 10 years old. [4] Among the disadvantages of this technique is the risk of disease transmission. [6] However, the most common problem with this technique is the low regeneration rate, which can be very low in some varieties. [7] As a result, date palm growers in Figuig are abandoning the transplantation of suckers from varieties that are difficult to propagate.

In this context, the use of plant growth and development hormones remains essential to ensure good plant development. These phytohormones are classified into several groups according to their specific function. In the case of suckers and the need for root regeneration, auxins remain the best choice for stimulating root formation by promoting cell division in plant tissues. [8]: These phytohormones are divided into three types: IBA (indole-3-butyric acid), effective for rooting cuttings, NAA (naphthaleneacetic acid), which stimulates rhizogenesis at high doses, and 2,4-D (2,4-dichlorophenoxyacetic acid) to induce callogenesis and somatic embryogenesis in culture media[9].

In order to protect the local palm tree heritage from deterioration, it is necessary to increase the success rate of vegetative propagation. To this end, we have attempted to study the effect of different concentrations of indole-3-butyric acid (IBA) and the season on the recovery of date palm (*Phoenix dactylifera* L.) cultivar Aziza Bouzid

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Materials and methods:

Plant material:

The suckers used in this study came from the Figuig palm grove, specifically from the Aziza cooperative 8 km from the city (Figure 1). These suckers were taken from several Aziza Bouzid palm trees at the beginning of each season (autumn, winter, spring, summer). The suckers were selected based on a length of between 90 and 140 cm and a circumference of 25 to 65 cm, with a weight ranging from 4.5 to 9.5 kg, to ensure that they had sufficient reserves to keep them alive and the ability to regenerate their own new roots. The latter were harvested using traditional weaning techniques and equipment, which involves cutting the canal connecting them to the mother plant, taking the utmost care to avoid damaging the harvested plants.
The weaning operation was followed by the trimming of the bunches. This involves keeping the fronds of each bunch the same length to make it easier to observe whether the operation has been successful or not. They are then rinsed with water to remove any soil that remains stuck to their bases. The bunches are then transported to the experimental station of the Faculty of Science in Oujda.

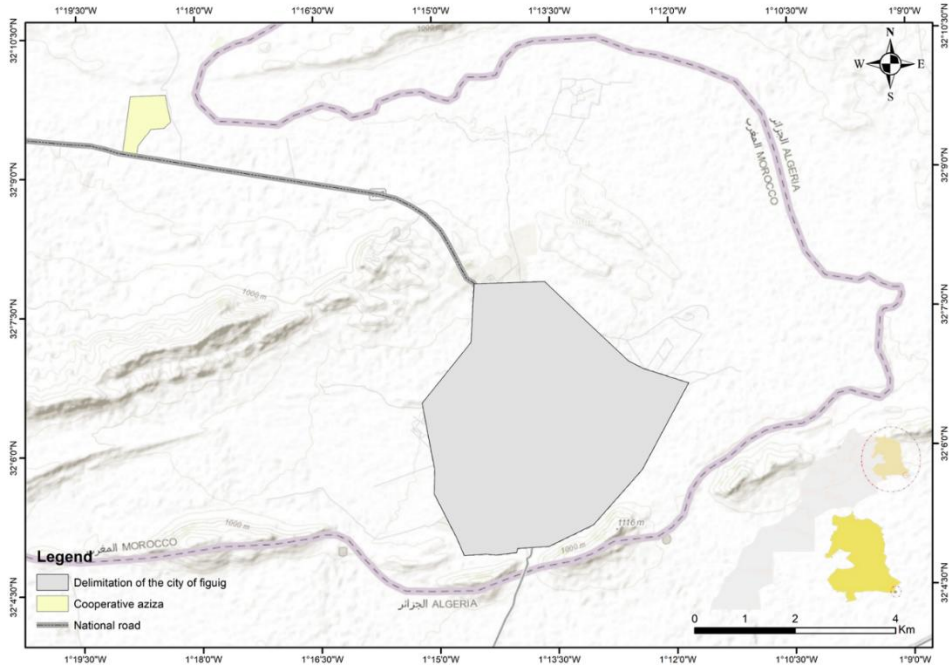

Fig. 1. Location map of the Aziza cooperative

Test conditions:

Three concentrations of IBA were used: C1 (0 ppm), C2 (150 ppm) and C3 (300 ppm). Given that 1 ppm is approximately equal to 1 mg/l, this translates to C1 (0 mg/l), C2 (150 mg/l) and C3 (300 mg/l). The hormonal treatment was carried out using a manual sprayer on the lower part of the cuttings. The cuttings were planted directly in 100 L plastic bags containing 25% sand and 75% peat (v/v). These bags were irrigated and placed in an experimental greenhouse at the Faculty of Sciences in Oujda. Maintenance consisted of regular watering once every two days in summer and once a week during the other seasons.

Method of collecting measured parameters:

Observations and measurements were made on the regeneration rate of suckers. This was defined as the ratio between the number of rooted suckers and the total number of suckers planted, according to the following formula:

$$Regeneration\ rate\ (\%) = 100 \times \frac{\text{Number of shoots that regrew}}{\text{Total number of shoots planted in each combination}}$$

Factors studied and experimental setup:

Climatic conditions play a very important role in plant growth and development, hence the idea of trying to determine the most favourable time for transplanting date palm suckers. The four seasons of the year were tested in this experiment. Rhizogenic substance: in our study, auxin (IBA) was used to determine its effect. we opted for two concentrations plus the control. The experimental design adopted was a split-plot with 3 replicates and 48 UEs, with the season factor allocated to the large plots and the small plots indicating the IBA concentration (Figure 2)

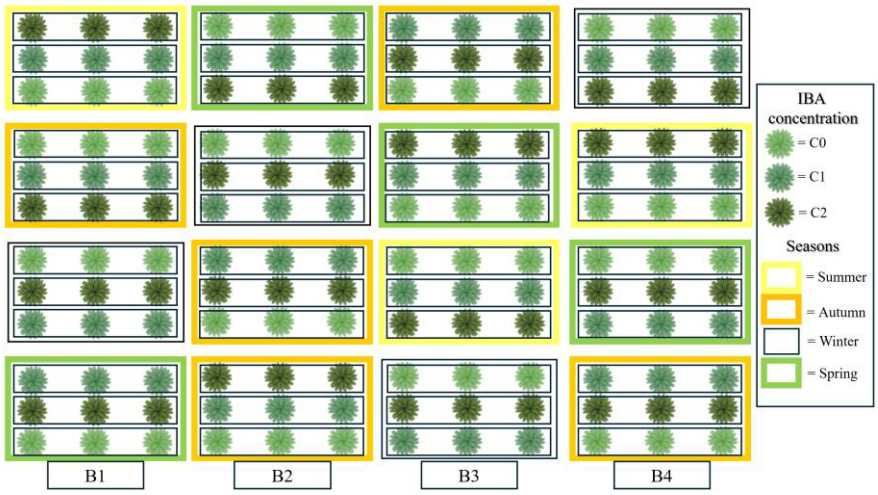

Fig. 2. The experimental design used (split-plot design)

Statistical analyses:

The values of the different parameters were expressed as mean ± standard deviation (x ± S.D). Statistical analysis was performed using a three-factor analysis of variance (ANOVA) with IBM SPSS Statistics version 21 software, using a multiple comparison based on Tukey's test; p = 0.05.

Results and analysis:

The results were collected one year after transplanting the shoots, calculating the regeneration rate using the above formula.

Autumn season:

Figure 3 shows the root regeneration rate of Aziza cultivars during the autumn season. Shoots that did not undergo any treatment (C0) had a root regeneration rate of 50%. This value was evaluated by applying IBA, reaching 59% for the 150-ppm dose (C1) and 60% for 300 ppm (C2).

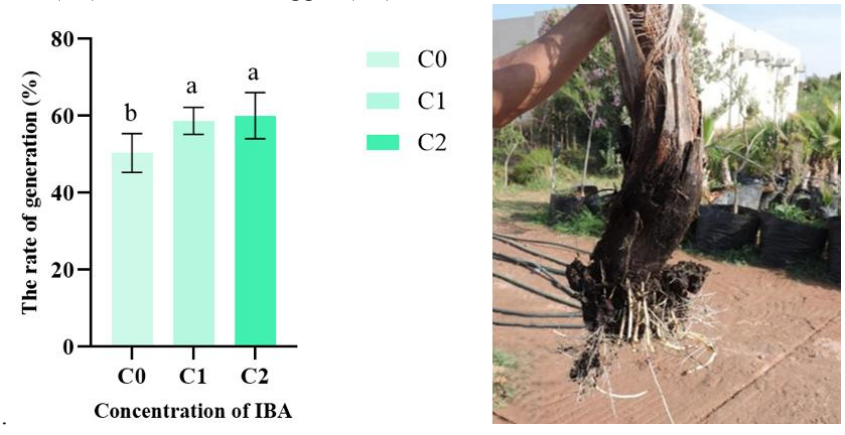

Fig. 3. Root regeneration rate for the autumn season and image illustrating this regeneration

The results of the statistical study (0.05) separated the three treatments into two groups, showing that IBA has a significant effect on the rooting of shoots of this cultivar.

Given that climatic conditions are a limiting factor in plant growth and development, they remain a significantly important element to mention, due to the study of seasonal effects. In autumn, temperatures generally vary between 15 and 32°C with moderate rainfall, particularly in October and November. Given that the ideal temperature for the vegetative growth of date palms is between 25 and 35°C [2], these temperatures are almost the same in autumn, which further promotes root regeneration for both the control group and the treated groups. The same study was conducted in 2019 by Chafi el al., using AIA as the hormone treatment [10]. The autumn season did not yield positive results, even with the control group.

Winter season:

Figure 4 shows that the control group (C0) in this study showed 41% root regeneration, a value that decreased compared to the autumn results, but the treated group still maintained the same results as in the autumn season, 59% in both treatments (C1 and C2). The results of the statistical study (0.05) separated the three treatments into two groups, showing that IBA has a significant effect on the rooting of shoots of this cultivar.

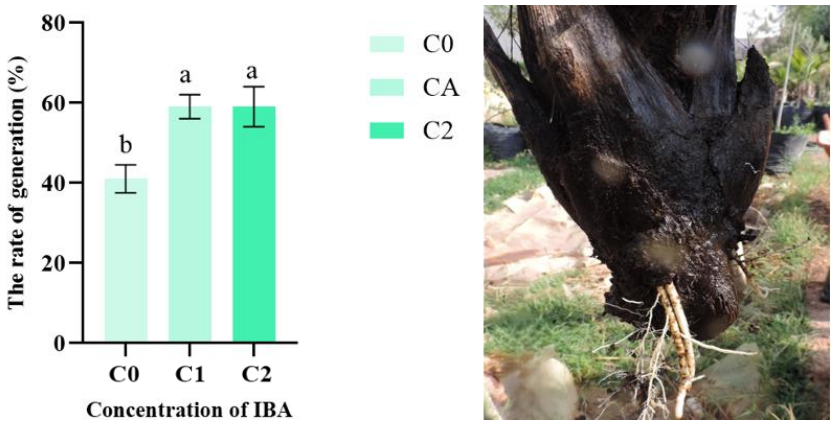

Fig. 4. Root regeneration rate for the winter season and image illustrating this regeneration

The average temperature during the winter season generally varies between 9 and 15°C, with more frequent rainfall that can reach up to 120 mm. However, a temperature of 10°C is considered to be the physiological limit for the development and growth of date palms [2].

This temperature risks blocking the plant's metabolites, which completely halts its activities [2]. In this temperature context, we would like to point out that our trials are conducted in a plastic tunnel, which serves to more or less reduce the intensity of the season's climate on the plants.

Spring season:

Figure 5 shows that 66% of the shoots regenerated their roots in the control group (C0), which is a higher value than in the two previous seasons. Under these conditions, IBA clearly demonstrated its effect in the two treated groups, reaching 90% for (C1) and 100% for (C2). The results of the statistical study (0.05) separated the three

treatments into three significantly different groups, which explains that the higher the IAB dose, the higher the regeneration rate.

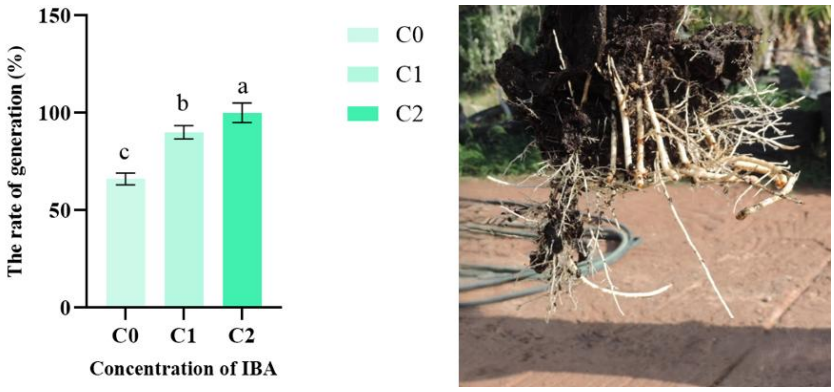

Fig. 5. Root regeneration rate for the spring season and image illustrating this regeneration

The climate in the city of Oujda during this season is cooler than in other seasons, with temperatures generally between 18 and 25°C and rainfall of up to 50 mm. During this season, date palms transition from a phase of slow growth to a phase of active growth and development. This metabolic awakening generally leads to good growth, which may explain our results. Other studies have shown 8% root regeneration in the control group, rising to 38% with a treatment of 150 ppm of AIA [10]. These results, obtained in a tunnel in the town of Figuig compared to Oujda, can be explained by the different climatic conditions in the two towns, particularly in terms of humidity and temperature.

Summer season:

This season is characterised by higher temperatures, ranging from 30 to 45°C, and low to dry rainfall. During this season, the results are 88% with the control group (C0). On the other hand, the treatment did affect root regeneration in the two treated groups, reaching 91% for (C1 and C2) (Figure 6).
The results of the statistical study (0.05) kept the three treatments in a single group (A), showing that the difference between them is not significantly representative.

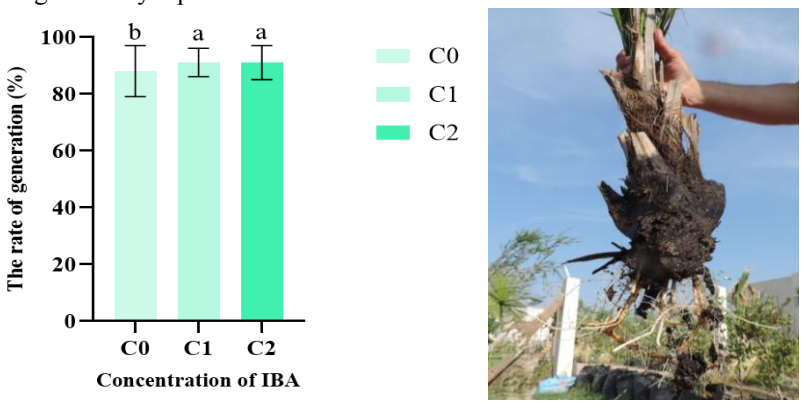

Fig. 6. Root regeneration rate for the summer season with image illustrating this regeneration

As 10°C is a low temperature that causes physiological blockage in date palms, 45°C is also considered a physiological heat limit. Plastic tunnels with their opening system, which create a kind of air current inside, lower the temperature compared to the air temperature in the field. This effect results in a cooler temperature, giving the shoots more favourable conditions for root regeneration.
Several studies have examined the effect of IBA on root regeneration, particularly on the Najda cultivar, indicating that this treatment resulted in good rooting of the shoots, accompanied by good leaf growth [11]. Others have studied the effect of branch size and indole-3-butyric acid on the rooting of date palms. The study shows that a significant increase in IBA concentration to 3,000 ppm increased both the fresh weight and dry weight of large and small diameter roots respectively [12]. This study highlights the most favourable seasons for transplanting

unrooted cuttings. According to this study, root regeneration is most effective in spring and summer, but less effective in autumn and winter. Statistically speaking, the results generally show no significant difference between the two doses of IBA treatment (C1 and C2), except in the spring season, when increasing the dose increases the rate of root regeneration. However, suckers not treated with IBA are still in a statistically inferior group compared to the others. At the end of this work, we can make a few recommendations: proper weaning of date palm suckers, like all plants, ensures a 50% chance of successful regrowth. Mastering the weaning angle and how to handle the suckers is half the battle. Proper grooming of suckers after weaning and proper washing and maintenance always keep our suckers in good condition, bearing in mind that extreme conditions weaken our suckers, which creates complications for recovery. Suckers must be transported under controlled conditions in terms of humidity to keep water in the suckers and ambient temperature. If the suckers are exposed to cold or hot air currents, they may be damaged. Finally, the best time for transplanting is spring and summer, with treatments containing varying doses of IBA.

Conclusion:

At the end of this work, the difficulties encountered with the Aziza Bouzid variety were observed during regeneration by shoots with different hormone concentrations. The use of AIB significantly increased the regeneration rate, breaking records, particularly for the spring and summer seasons.
It is also worth noting the effect of temperature on shoot regrowth, even without hormonal control treatment (summer and spring), which yields interesting results. However, the combination of seasons and hormones allows for maximum regrowth. These results are of great importance for crop cultivation in the Figuig oasis, particularly for noble varieties, whose traditional regrowth remains very low.

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