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Effect of exogenous indole butyric acid on root formation and peroxidase and indole-3-acetic acid oxidase activities and phenolic contents in date Palm offshoots

Ahmed Qaddoury[1,]* and Mohamed Amssa[2]

[1]*Laboratoire de Biotechnologie et Physiologie Végétales, Université Cadi Ayyad, Faculté des Sciences et Techniques - Guéliz, B.P. 549, Marrakech, 40 000 Morocco*

[2]*Laboratoire de Biotechnologie et Physiologie Végétales, Université Mly Ismail, Faculté des Sciences, Meknes, Morocco*

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Abstract. Exogenous indole butyric acid had a significant positive effect on the rooting response of date Palm (*Phoenix dactylifera* L.) offshoots. IBA-treated offshoots rooted earlier and with a much higher frequency while untreated offshoots rooted poorly, later, and tended to produce fewer roots. The effect of exogenous application of auxin was also reflected in the metabolic changes in offshoots during the rooting process. Pox activity showed a minimum on the sixth day and a maximum on the twelfth day after the root-inducing treatment. Changes of IAA oxidase activity showed a parallel time curve while phenolic contents and auxin protector levels showed an inverse trend. These parameters changed slightly one week later in the untreated offshoots.

Keywords: IAA oxidase; IBA; Offshoots; *Phoenix dactylifera*; Phenolic compounds; Peroxidase; Rooting.

Abbreviations: HRP, horseradish peroxidase; **IBA,** indole butyric acid.

Introduction

The rooting property of auxins and their importance in plant propagation have been widely recognized for long time (Thiman and Went, 1934). Hence, the necessity of exogenous auxin application to induce root formation in cuttings has been reported in many species e.g. sequoia (Berthon et al., 1990), loblolly pine (Greenwood and Weir, 1994), juniper (Edson et al., 1996), almond (Caboni et al., 1997) and Eucalyptus (Fett-Neto et al., 2001). Currently, indole butyric acid (IBA) is the most widely used auxin to stimulate the rooting process in cuttings because of: 1) its high ability to promote root initiation (Weisman et al., 1988) and 2) its weak toxicity and great stability in comparison to naphthalene acetic acid and indole-3-acetic acid (Blazich, 1988; Hartmann et al., 1990). Though auxins are widely used in the induction of rooting, little is known about their specific action or their interactions with other endogenous compounds (Gaspar et al., 1997). Changes in enzyme activities which regulate different biochemical pathways e.g. protein, carbohydrates, nitrogen, and phenolics, during the rooting process in cutting have been investigated (Mato et al., 1988; Das et al., 1997; Druege et al., 2000). Several investigations have reported the involvement of peroxidase, IAA oxidase, and phenolics in the process of root formation in cuttings (Hahlbrock and Grisebach, 1979; Mosella et al., 1980; Vaughn and Duke, 1984; Mayer, 1987; Gonzalez et al., 1991; Caboni et al., 1997; Gaspar et al., 1997).

Date Palm multiplication by transplanting offshoots still remains the best and most common method of reconstituting a destroyed palm grove. Its accomplishment was however limited to offshoots that weigh 7 to 15 kg (Perreau-Leroy, 1958; Toutain, 1972; Saaïdi et al., 1979). In the last few decades, the rooting ability of the date palm offshoot has substantially improved using the misting system (Saaïdi, 1979; BenAbdelah, 1990; Qaddoury and Amssa, 2002). The present investigation was carried out to provide a more detailed insight into the relationship between the auxin (IBA) treatment and changes of peroxidase and IAA oxidase activities as well as endogenous phenolic contents and auxin protector levels in relation to rooting response, in the offshoot of the high quality Moroccan cultivar of date palm.

Materials and Methods

Plant Material

Young offshoots, less than 2 kg, were collected from the high quality Moroccan cultivar of date palm, Mejhoul (MJH), grown in the experimental station of the ORMVAT

*Corresponding author. Tel: (212) 61 25 96 96; Fax: (212) 44 43 31 70; E-mail: qaddoury@fstg-marrakech.ac.ma

(Office Régional de Mise en Valeur Agricole de Tafilallet). External leaves were removed, and the bottoms of the offshoots were dipped for 5 min in IBA solutions at 15 mM, 25 mM, 35 mM, 50 mM and 75 mM. Offshoots were then planted on peat and coarse sand (1/3: v/v) and kept in a mist greenhouse. There were five treatments (including control) with 30 offshoots each. ANOVA, using the Statistica program (StatSoft, 1995), was performed on a percentage of rooted offshoots and the root number per offshoot among the treatments. The significance was tested at the 5% level following the Newman-Keuls range test.

Subsequently, in a separate experiment, the best treatment (25 mM IBA) was subjected to biochemical analysis. Samples from the inner leaf of the IBA-treated and untreated offshoots of Mejhoul were collected every two days during the first month after the root-inducing treatment for biochemical analysis.

Enzyme Preparations and Assays

Tissues were ground in liquid nitrogen in a mortar and homogenized in 100 mM potassium-phosphate (pH 6.1). The homogenate was then centrifuged at 4°C for 15 min at 10,000 rpm, and the supernatant was taken off. The pellet was re-suspended in potassium-phosphate buffer and re-centrifuged under the same conditions as before, and the new supernatant was added to the first.

Peroxidase assay. Peroxidase activity was determined according to Baaziz (1989) by monitoring the formation of tetra-guaiacol at 470 nm. The reaction mixture contained 2.5 ml 50 mM tris-malate buffer (pH 6) containing 50 mM guaiacol, 88 mM H_2O_2, and 0.05 ml of extract. One unit of enzyme activity corresponded to 0.1 per min variation in absorbance at 470 nm, peroxidase activity was expressed on a fresh weight basis (unit g^{-1} f.w).

IAA oxidase assay. The reaction mixture consisted of 1/15 M potassium-phosphate buffer (pH 6) containing 100 µM $MnCl_2$, 50 µM p-coumaric acid, 320 µM IAA (Sigma Chemical), and 0.05 ml extract. Assays were conducted at 30°C for 1 h. The measurement of non-oxidized IAA was carried out with Salkowski reagent (2 ml) at 535 nm after 60 min at 30°C, and the remaining IAA was calculated after Pilet and Chollet (1970). IAA oxidase activity was expressed in µg IAA destroyed after 1 h per milligram fresh weight.

Assay of auxin protectors. The presence of enzyme inhibitors (auxin protectors) was detected in the partially purified enzyme extracts according to Mato and Vieitz (1986). Five ml of extract were brought to 70% saturation with ammonium sulfate and centrifuged at 27,000 *g* for 15 min at 4°C. The supernatant was discarded, and the precipitate was re-suspended in 5 ml of the same buffer. The reaction mixture was the same as used for IAA oxidase activity with addition of 3 µg/ml HRP (Sigma) (Stonier et al., 1979).

Phenolic content. Phenolic compounds were extracted from 0.2 g of fresh matter following Macheix's (1974) method, and their concentrations were estimated with the Folin Ciocalteu reagent at 750 nm. Concentrations were determined by reference to standards established with pure analytical grade chlorogenic acid and expressed on a fresh weight basis (µg.g^{-1}.f.w).

Results

Root formation in date palm offshoots was significantly improved by IBA treatment ($p < 0.001$) while 86.3% of rooting was induced by 25 mM IBA (the highest percentage among the treatments), only 55.9% of the untreated offshoots produced roots (Table 1). Furthermore, IBA treated offshoots produced significantly ($p < 0.001$) more roots per rooted offshoot. The maximum number of roots per offshoot (19.3) was observed for the treatment with 25 mM IBA while untreated offshoots produced fewer roots (6.1) per rooted offshoot (Table 1). Moreover, roots appeared by the 6^{th}~7^{th} week in the presence of IBA and after the 10^{th} week in untreated offshoots.

The effect of IBA was also reflected in peroxidase and IAA oxidase activities as well as endogenous phenolic contents and auxin protector levels analysed during root formation, in the treated and untreated offshoots. Peroxidase activity decreased during the first 6 days after the application of the root inducing treatment, steeply increased and reached a peak on the twelfth day, and then decreased again (Figure 1a). IAA oxidase activity showed a parallel trend with a minimum on the fourth day and a maximum on the fourteenth day (Figure 1b). In the untreated offshoot, peroxidase and IAA oxidase activities followed the same variations but at a lower level and one week later (Figure 1a, b). Phenolic contents steeply increased immediately after IBA treatment, reached a peak on day 6, and then sharply decreased in the IBA-treated offshoots. In the control, phenolics gently increased over the first two weeks and then decreased.

In the IBA-treated offshoots rooting was accompanied by an increase in IAA protection until day 6, after which protector levels declined (Figure 2a). In the untreated offshoots, auxin protection decreased during the first 8 days, then steeply increased and reached a peak on the fourteenth day, and then decreased again and remained low (Figure 2b).

Table 1. Percent rooting and number of roots per rooted offshoots in relation to IBA treatment, in young offshoots of date Palm transplanted in mist greenhouse. Mean ± SE (n = 30).

IBA (mM)	Rooting percent	Number of roots per rooted offshoot
0	55.9 ± 2.3	6.1 ± 0.3
15	68.8 ± 2.4	8.1 ± 0.6
25	86.3 ± 3.6	19.3 ± 0.6
35	76.6 ± 2.9	13.85 ± 0.7
50	70.5 ± 4.3	13.1 ± 0.3
75	45.4 ± 3.1	9.5 ± 0.6

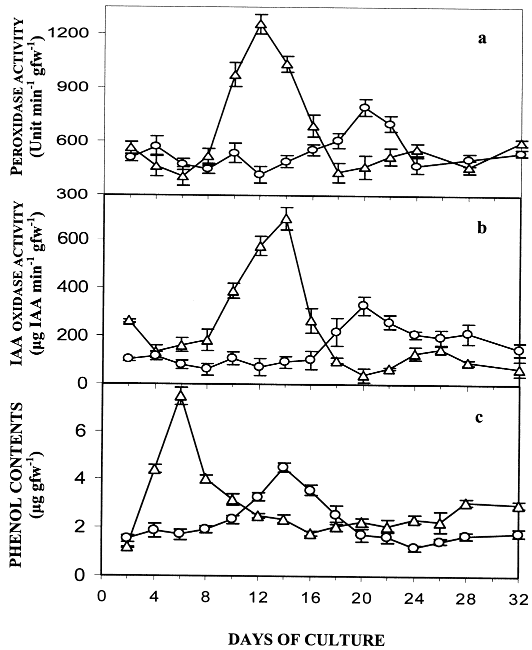

Figure 1. Peroxidase (a) and IAA oxidase (b) activities and phenolic contents (c), determined during the first month prior to root appearance, in IBA-treated (triangles) and untreated (rounds) offshoots of date Palm cultured in mist greenhouse. Means ± SE (n = 30).

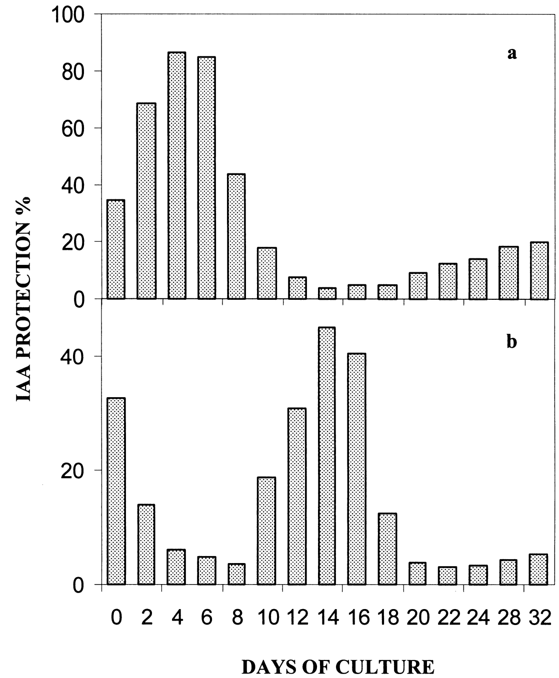

Figure 2. Auxin protection activity of partially purified enzyme extracts from the inner leaves of young offshoots of date Palm during rooting. Data in % inhibition of HRP (IAA oxidase) activity.

Discussion

There were clearly differences in rooting response between the IBA-treated and untreated offshoots of date palm. In the presence of auxin, offshoots rooted earlier and with a much higher frequency. Untreated offshoots rooted poorly, later, and tended to produce fewer roots. Our data showed that rooting response in date Palm can be substantially improved by applying exogenous IBA, with results like that already observed in sequoia (Berthon et al., 1990), loblolly pine (Greenwood and Weir, 1994), almond (Caboni et al., 1997), and Eucalyptus (Fett-Neto et al., 2001).

Changes of peroxidase and IAA oxidase activities as well as phenolic contents and auxin protector levels were analyzed during root formation, in offshoots treated with and without IBA. In IBA-treated offshoots, root formation was characterized by a clear decline in IAA oxidase activity on the fourth day and a marked increase on day 14. Peroxidase activity underwent the same time-course curve while phenolics changed inversely. These parameters remained almost unchanged with a slight increase after one week in the untreated offshoots. Data also show that IBA treatment induced a rise in the endogenous auxin protector level, which changed sharply and earlier in the IBA-treated offshoots than in the untreated ones. The great differences in rooting response and enzyme activities between IBA-treated and untreated offshoots is good evidence of IBA's efficiency in promoting rooting in date Palm, a result also found in many other species (Greenwood and Weir, 1994; Caboni et al., 1997; Fett-Neto et al., 2001). Exogenous IBA may induce changes in enzyme activities (peroxidase and IAA oxidase) and in their effectors contents (phenolics) allowing the establishment of the favourable endogenous hormone balance. It is well known that applied auxins induce modifications in their own metabolism, mostly by conjugation, and in other hormones such us cytokinins (Ribnicky et al., 1996; Gaspar et al., 1997). The metabolism of the exogenously provided auxin, especially its combination with phenolic compounds, has been considered in relation to the promotion of adventitious rooting (Haissig, 1974; Weisman et al., 1988). The fall in IAA-oxidase activity and the rise in the endogenous auxin protector level indicate that auxin is necessary for rooting induction, confirming previous results (Quoirin et al., 1974; Vazquez and Mato, 1991; Liu et al., 1996). While the period of higher enzyme activities and weak auxin protector level probably correlated to the early events of the initiation phase, it may be indicative of the requirement for a reduction of endogenous auxin level to bring about favorable hormonal balance (Berthon et al., 1990; Moncousin, 1991; Blakesley et al., 1991; Tsala et al., 1996; Gaspar et al., 1997). It is therefore desirable to measure changes of the endogenous IAA level in relation to kinetical changes of IAA-oxidase and auxin protector levels during the course of rooting, and this forms part of our research currently in progress.

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外加 indole butyric acid 對 date Palm (*Phoenix dactylifera* L.) 切莖之發根和過氧化酵素及 indole-3-acetic acid 氧化酵素活性和酚類化合物含量之影響

Ahmed Qaddoury[1] and Mohamed Amssa[2]

[1]Laboratoire de Biotechnologie et Physiologie Végétales,
Université Cadi Ayyad, Faculté des Sciences et Techniques - Guéliz, B.P 549,
Marrakech, 40 000 Morocco
[2]Laboratoire de Biotechnologie et Physiologie Végétales,
Université Mly Ismail, Faculté des Sciences, Meknes, Morocco

外接 indole butyric acid（IBA）對 date Palm 切莖之發根情形具明顯的正效果。和控制組對照，經 IBA 處理後，切莖發根較早、較頻、較多。外加之 IBA（乃植物生長素 auxin 之一形式）之效果除了外觀性狀如發根之外，也反映在發根過程之代謝改變。過氧化酵素活性在 IBA 處理後第 6 天最低而第 12 天時最高。IAA 氧化酵素之活性變化也有相似之時間曲線，而酚類含量及 auxin保護成份之水位顯示相反之時間變化。控制組則一星期後才有微小的變化。

關鍵詞：IAA氧化酵素；切莖：*Phoenix dactylifera*；酚類化合物；過氧化酵素；發根。