Original article

Indole-3-acetic acid concentration during fruit development in date palm (*Phoenix dactylifera* L. cv. Hillawi)

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Indole-3-acetic acid concentration during fruit development in date palm (*Phoenix dactylifera* L. cv. Hillawi).

Abstract — Introduction. Date palm fruit is an important fruit in Iraq, but no information is available on endogenous hormone levels during fruit development. The present work examines changes in indole-3-acetic acid (IAA) concentration during fruit development of the Hillawi cultivar which is the commercial cultivar in the Basrah area. **Materials and methods**. Fifteen year old date palm trees (*Phoenix dactylifera* L. cv. Hillawi) were hand-pollinated and fruit sampling started from pollination and continued till completely ripening stage was reached. After extraction, IAA levels were analyzed by high performance liquid chromatography (HPLC). **Results and discussion**. The IAA concentration was very high in non-pollinated flowers; it declined at the fruit set, rose again as fruit entered the rapid phase of growth (log phase), then declined as the fruit advanced toward the ripening phase. The high IAA content of the cv. Hillawi date palm female flower is probably responsible for the natural tendency of the female flower to set parthenocarpic fruit if it is not pollinated. Changes in the IAA concentration during the fruit development may reflect the role of this natural hormone in the control of the various fruit developmental stages. **Conclusion**. The natural auxin IAA is probably involved in the control of the various development stages of the cv. Hillawi date palm fruit. © Éditions scientifiques et médicales Elsevier SAS

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Teneur en acide indole-3-acétique pendant le développement de la datte (*Phoenix dactylifera* L. cv. Hillawi).

Résumé — Introduction. En Irak, le fruit du palmier dattier a une grande importance, mais aucune information n'est disponible sur les taux d'hormones endogènes présents dans le fruit pendant son développement. Les travaux présentés étudient l'évolution des taux d'acide d'indole-3-acétique (AIA) au cours du développement de la datte du cultivar Hillawi, cultivar commercialisé dans la région de Basrah. **Matériel et méthodes**. Des palmiers dattiers (*Phoenix dactylifera* L. cv. Hillawi) de 15 ans ont été pollinisés à la main et des échantillonnages de fruits ont été effectués du stade de la pollinisation à celui de complète maturation. Après extraction, les teneurs en AIA ont été évaluées par chromatographie liquide sous haute pression (CLHP). **Résultats et discussion**. La concentration d'IAI a été très élevée dans les fleurs non pollinisées ; elle a diminué lors de la formation du fruit, a augmenté quand le fruit est entré dans sa phase de croissance rapide, puis elle a régressé alors que le fruit entrait dans sa phase de maturation. La teneur élevée en AIA de la fleur femelle de palmier dattier Hillawi explique probablement la tendance habituelle de cette fleur à former un fruit parthénocarpique lorsqu'elle n'est pas pollinisée. Les changements de concentration en AIA dans le fruit pendant son développement pourraient refléter le rôle de cette hormone naturelle dans le contrôle des étapes du développement de divers fruits. **Conclusion**. L'AIA, auxine naturelle, est probablement impliquée dans le contrôle des diverses étapes de développement de la datte des palmiers Hillawi. © Éditions scientifiques et médicales Elsevier SAS

Irak / *Phoenix dactylifera* / fruit / croissance / substance de croissance végétale / AIA

1. introduction

The date palm *(Phoenix dactylifera* L.) is a subtropical fruit tree native of Iraq and other parts of the Middle East and it has been a staple food in those regions since first recorded history. The normal date palm fruit is a berry which results from the development of a single ovary [1]. Date fruit displays a single sigmoidal growth curve with an initial phase of slow growth rate mainly due to the cell division, followed by a period of rapid growth rate mainly due to the cell elongation, then the fruit enters into a steady phase of growth as it reaches the physiological maturity stage [1]. These stages of date fruit development presumably are under the control of various groups of endogenous plant hormones. However, to our knowledge, there is a very limited published work on the endogenous hormones role in fruit growth and development of the date palm [2, 3]. Accordingly, the present work was carried out to examine the pattern of changes and concentration of indole-3-acetic acid (IAA) during the date fruit development of the Hillawi cultivar which is the commercial cultivar in the Basrah area. Such information is of utmost importance to date fruit physiology.

2. materials and methods

The experiment took place at the Department of Horticulture and Date Palms, College of Agriculture, Basrah University, Basrah, Iraq. It was carried out during the 1994 growing season with 15-year old uniform female trees of the cv. Hillawi date palm. After the spathes cracked open, the female inflorescence was hand-pollinated on March 4, 1994, by inserting three or four strands of male flowers from the Ghannami Akhdar male clone. For fruit sampling, five trees were selected. Sampling, done at 2-week intervals, started from pollination and continued till the completely ripening stage was reached. Each sample consisted of 10 g whole fruit fresh weight, plus 10 g non-pollinated flowers. The samples were frozen immediately and freeze-dried. The freeze-dried samples were used for determination of free IAA. The freeze-dried samples were ground just before extraction.

2.1. extraction of free IAA

The free IAA was extracted using an already published method [4–6]: freeze-dried samples were extracted with 80 % methanol for 48 h at 4 °C in darkness; during that time the alcohol was changed twice. The methanolic extracts were combined and evaporated to an aqueous phase at 35–40 °C under vacuum. The aqueous phase was fitted to 50 ml with deionized water. Then, basic acetate was used to clean the extract. After readjusting the aqueous phase to pH 2.5 with 2N HCl, the acid fractions were partitioned three times with freshly distilled diethyl ether. The separated ether fractions were evaporated to a small volume convenient for further purification by high performance liquid chromatography (HPLC).

2.2. HPLC quantitative determination of free IAA

The analyses of free IAA in fruit samples at various development stages were performed by HPLC (Shimadzu, Osaka, Japan) by the injection of 20 µl aliquots. A 4.6 × 250 mm C-18 reversed phase column was used, packed with 5 µm ODS (Fishers, USA). The mobile phase was 0.1 M phosphoric acid:methanol, 60:40 (v:v), at pH 2.5. The flow rate was 1.0 ml·min^{-1}. Isocratic elution procedure was used to obtain the effective separation of free IAA. Standard IAA was prepared in different concentrations in phosphoric acid:methanol; the relationship between area and concentration was linear over the range used. Peak areas, retention times and concentrations (based on an international standard) were calculated with a CR-4A computing integrator. The detection procedure was done by UV absorption at 280 nm, as described by Saimoto et al [7]. All measurements were replicated four times.

2.3. statistical design and analysis

A completely randomized block design was used, with four blocks, each repre-

sented by one tree. The results were subjected to the analysis of variance, and revised LSD ($p = 0.05$) was used to compare any two values on the figure. The block effect was insignificant at $p = 0.05$.

3. results and discussion

The female date palm flowers were very rich in endogenous IAA (*figure 1*). Such high levels of IAA in the cv. Hillawi female flowers is probably responsible for the natural tendency of the date palm trees to set parthenocarpic fruit if the flowers are not pollinated [1]. However, such seedless fruit will never reach a fully ripe stage, and recent work in our laboratory had shown that the failure of ripening of the parthenocarpic cv. Hillawi date fruit is due to the absence of the phenomenon of the climacteric rise in respiration [8]; in contrast, the normal (pollinated) fruit with seed shows a typical climacteric rise in respiration [3].

The concentration of IAA in the whole fruit declined two weeks after pollination (at the fruit set) (*figure 1*) probably due to the utilization of IAA in the process of cell division known to occur at this stage of fruit development [9].

Four weeks after pollination, the level of IAA rose again (*figure 1*); that is probably due to embryo development, a known source of endogenous auxins [10]. The concentration of the IAA auxin remained at high levels up to the eighth week after pollination. Such levels of IAA are probably related to its involvement in the process of cell elongation known to occur at this stage of fruit development. Then, IAA concentration continued to decline as the fruit advanced in development and reached its lowest value in fully ripe fruit, 18 weeks after pollination. Work in our laboratory had also shown that there was a marked increase in gibberellins levels in cv. Hillawi date palm fruit during the maximum fruit growth rate, when auxin levels decreased to its lowest values. The pattern of changes in the concentration of IAA during the cv. Hillawi date palm fruit development is completely different from that reported for other fruit species showing a sigmoidal growth curve such as for strawberry fruit. In such fruit, the concentration of free IAA was minimal at anthesis, but the levels increased after pollination and reached a peak value after 10 d, then declined rapidly [11]. The response of date palm fruit to exogenous treatment with plant growth regulators could be probably explained by the fruit IAA concentration during development. Thus, it bas been widely reported that treatment of date palm fruit with exogenous auxins such as NAA (naphthalene acetic acid), two weeks after pollination, lead to a heavy fruit drop [9]. This is probably due to the fact that date fruit contains, at this stage, high IAA levels, and that exogenous treatment with auxins increases auxin levels in the fruit to supra-optimal concentration with a subsequent release of ethylene and, hence, fruit abscission. Also, it has been reported that spraying cv. Hillawi date palm fruit with the auxin NAA at 50 ppm, one month after pollination, was very effective in increasing fruit size and fruit fresh weight, but spraying with this auxin at later fruit development stages had no such effect [12]. This would probably suggest that high IAA levels at an early fruit growth stage are critical for subsequent growth and development.

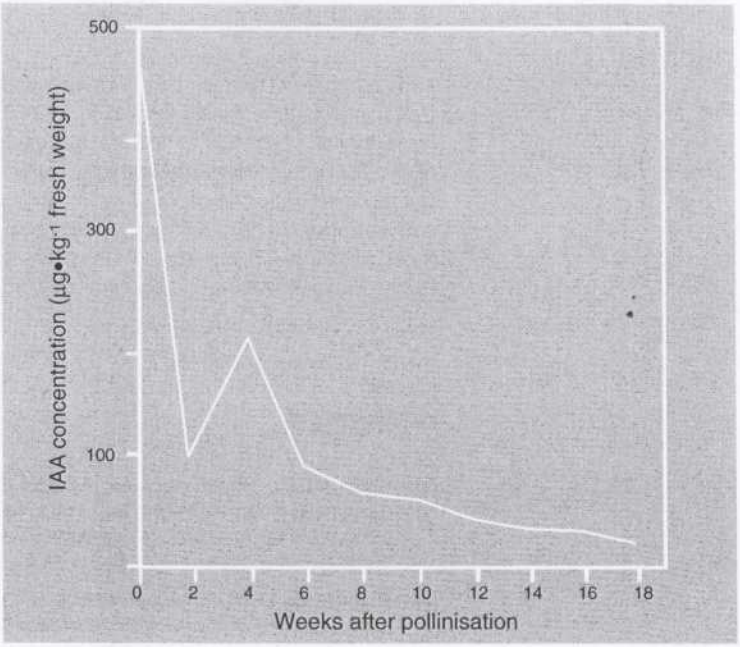

Figure 1.
Changes in the IAA concentration during date palm cv. Hillawi fruit growth and development. Each point is the mean of four replicates (bar: LSD, $p = 0.05$).

4. conclusion

The female flower of the cv. Hillawi date palm, which was found very rich in IAA, could probably explain the natural tendency of the flowers to set parthenocarpic fruits if not pollinated. Furthermore, changes in the concentration of IAA during fruit development probably reflects the involvement of IAA in various fruit developmental stages.

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Contenido de ácido indol-3-acético durante el desarrollo del dátil (*Phoenix dactylifera* L. cv. Hillawi).

Resumen — Introducción. El dátil tiene una gran importancia en Irak, pero no se dispone de ninguna información sobre los niveles de hormonas endógenas presentes en el fruto durante su desarrollo. Los trabajos que se presentan estudian la evolución de los niveles de ácido indol-3-acético (AIA) durante el desarrollo del dátil del cultivar Hillawi, que se comercializa en la región de Basrah. **Material y métodos**. Se polinizaron palmeras datileras (*Phoenix dactylifera* L. cv. Hillawi) manualmente y se efectuaron tomas de muestras de frutos desde la fase de polinización hasta la de maduración completa. Tras extracción, se evaluaron los contenidos en AIA mediante cromatografía líquida de alta resolución (HPLC). **Resultados y discusión**. La concentración de AIA fue muy alta en las flores no polinizadas; disminuyó durante la formación del fruto, aumentó cuando el fruto entró en la fase de crecimiento rápido y volvió a disminuir cuando el fruto entraba en la fase de maduración. El alto contenido de AIA de la flor macho de la palmera datilera Hillawi explica probablemente la predisposición de esta flor para formar un fruto partenocárpico cuando no ha sido polinizada. Los cambios de concentración de AIA en el fruto durante su desarrollo podrían ser el reflejo de la función de esta hormona natural para el control de las etapas del desarrollo de diferentes frutos. **Conclusión**. El AIA, auxina natural, se halla probablemente implicado en el control de las diferentes fases de desarrollo del dátil de las datileras Hillawi. © Éditions scientifiques et médicales Elsevier SAS

Irak / *Phoenix dactylifera* / fruto / crecimiento / sustancias de crecimiento vegetal / AIA