

Metaxenic effect in date palm (*Phoenix dactylifera* L.) fruit in relation to level of endogenous auxins

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Abstract. The metaxenic effect in date palm fruit cv. Hillawi induced by pollinating with two types of pollen, namely Ghannami Akdhar and Khiri Adi, was investigated in relation to changes in the concentration of free IAA during fruit development. The results showed that the pollen type had a significant effect on the concentration of free IAA, with fruits produced by the Khikri Adi pollen having the highest concentration as compared to fruits produced by the Ghannami Akdhar pollen. The results also showed that the pollen type Khikri caused a significant increase in fruit size, fresh weight of the whole fruit, pulp and seed in comparison with fruits produced by the Ghannami Akhdar pollen.

Key Words: Khikri Adi pollen, Ghannami Akdhar pollen, pollinating, pollen, growth, IAA.

Introduction. The date palm (*Phoenix dactylifera* L.) is a subtropical fruit tree native to Iraq and other part of the middle East, and it has been a staple food in those regions since first recorded history. The date palm tree is dioecious, with male and female flowers borne on separate trees. In commercial plantation, female inflorescences are hand pollinated with selected pollens from males. That pollen from difference sources effects readily discernible characteristics of seeds and fruits in the period immediately following fertilization has been noted for well over century (Denney 1992). Such immediate or direct effects are termed 'Xenia' and have been described in many species (Denney 1992). These effects on tissue of purely maternal origin, rather than on parts resulting from syngamy have been described as 'Metaxenia' by Swingle (1928). Such effects include differences in the size, shape, developmental timing and chemical composition of the date fruit found as a result of fertilization by different pollens (Nixon 1935; Osman et al 1974; Swingle 1928). Swingle (1928) was precocious in suggesting a hormonal cause for metaxenic size effects in date palm, but he lacked techniques for testing his hypothesis. Denney (1992) in an article on xenia and metaxenia reformulated Swingle's hypothesis. He suggested that in date palm, differential metaxenic size effect can be ascribed to different concentrations in one or several of the three hormones, most closely associated with fruit growth: auxins cytokinin and/or gibberellic acid. He further suggested, that smaller fruit (seed + pericarp) will have lower levels of the hormone(s) and larger fruits will have higher levels of the hormone(s). However, Swingle's hypothesis, has never been tested (Denney 1992). Accordingly, the present work was undertaken to study the effect of two sources of pollen, namely Ghannami Akhdar and Khikri Adi, which are known to produce metaxenic effect in date palm fruit cv. Hillawi (Abbas 1995), on certain aspect of fruit development in relation to changes in IAA concentration during fruit development.

Material and Method

The experiment was carried out at the Department of Horticulture and Landscape Design, College of Agriculture, Basrah University, Iraq during the growing season of 2010. Nine female date palm trees cv. Hillawi were selected for uniformity on each tree, six spathes were covered with a paper bags. After the spathes, cracked open, the female

inflorescences were hand pollinated on April 1, 2010, by inserting three or four strands of male clones Ghannami Akhdar and Khkri Adi. Each three spathes on the female tree received one type of pollen referred to above. Thereafter, the spathes were bagged again to prevent contamination by foreign pollen from the atmosphere. The first sample of young fruit, were collected from the female trees five weeks after pollination, and thereafter sampling was done at weekly intervals till the fruit reached the stage of physiological maturity (19 weeks from pollination). 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.

Extraction of free IAA. The free IAA was extracted using the procedure previously described (Abbas et al 2000). Freeze-dried samples were extracted with 80 % methanol for 48h 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 lead 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).

HPLC quantitative determination of free IAA. The analyses of free IAA in fruit samples at various stages of development were performed by HPLC (Shimadzu, Osaka, Japan) by the injection of 20 µL aliquots. A 4.6 x 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 concentration in phosphoric acid : methanol, the relationship between area and concentration was linear over the range used. Peak areas, retention time and concentrations (based on an internal 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 1990). All measurements were replicated three times.

Determination of certain fruit characteristic. Fruits of the trees used in the present work were harvested at the ripening phase (Rutab stage) and were subjected to the following determinations:

- a. Fruit length and diameter. Fruit length and diameter were determined on sample consisting of 30 fruits from each type of pollen.
- b. Fresh weight of the fruit. Average weight of the whole fruit, pulp and seed was determined the basis of 30 fruits.

Statistical design and analysis. A completely randomized block design was used, with three replicates, each represented by three trees. The experiment was factorial (6 sampling dates x 2 pollen type x 3 replicates). The results were subjected to the analysis of variance, and revised LSD ($p = 0.05$) was used to compare mean values. The block effect was significant at $p = 0.05$.

Results and Discussion. Figure 1 shows the main effect of pollen type on levels of free IAA in the pericarp of date palm fruit cv. Hillawi. It is clear that the pollen type had a significant ($p = 0.05$) effect on level of free IAA, with fruits produced by the Khikri Adi pollen having the highest level in comparison with fruits produced by Ghannami Akhdar pollen.

Figure 2 shows the interaction between pollen type and the number of weeks from pollination on the levels of free IAA. It is clear, that on weeks 5, 7 and 10, fruit produced by the Khikri Adi pollen had a significantly ($p = 0.05$) higher levels of IAA in comparison with fruit, produced by Ghannami Akhdar pollen. However, on weeks 13, 15 and 17,

there were no significant difference in levels of free IAA between fruits produced by the two types of pollen.

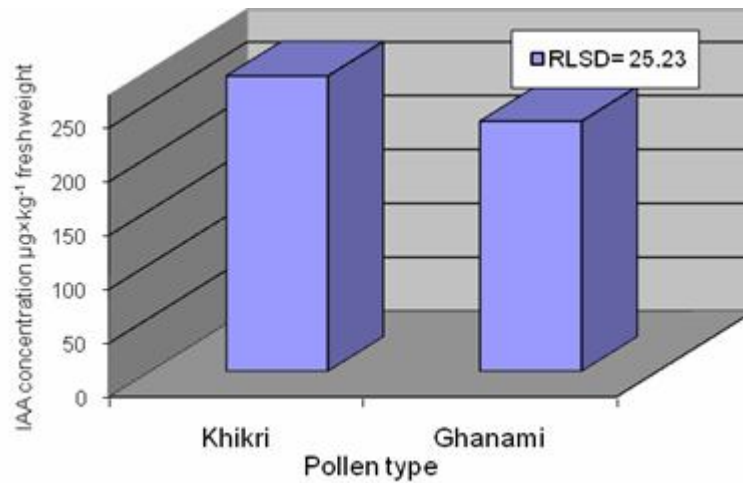


Figure 1. The main effect of pollen type on IAA concentration during growth and development of date palm fruit cv. Hillawi.

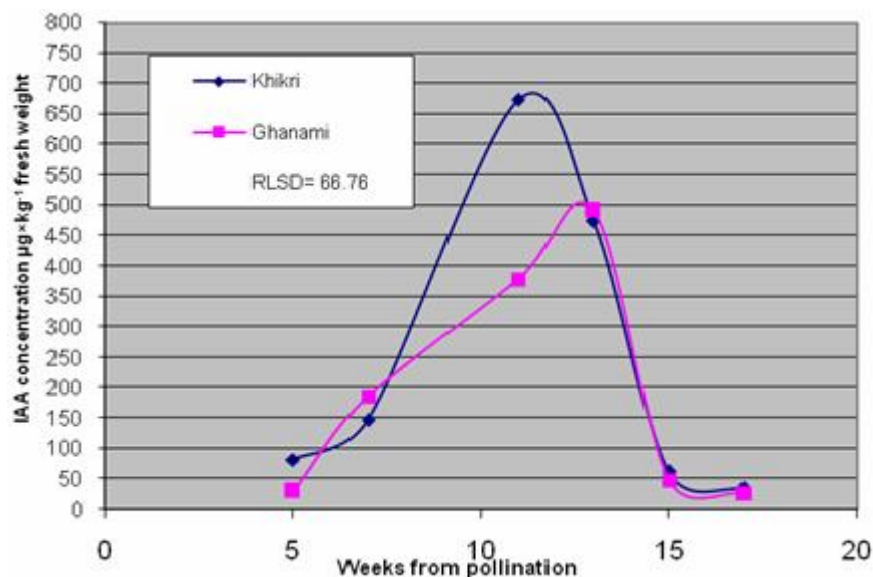


Figure 2. Interaction between pollen type and number of weeks from pollination on concentration of IAA during growth and development of date palm fruit cv. Hillawi. Each point is the mean 3 replicates.

Tables 1 & 2 shows the effect of pollen type on fruit size and fresh weight of the whole fruit, pulp and seed. It is clear, that there are significant differences ($p = 0.05$) among fruits produced by the two types of pollen, with fruits produced by the pollen Khikri having the highest size and fresh weight, in comparison with fruit produced by the type of pollen Ghanami Akhdar. Similar results were obtained by other researchers using different types of pollen (Khalifa et al 1980; Nixon 1935; Osman et al 1974; Ream 1976; Shafaat & Shabana 1980; Swingle 1928). It is obvious from figure 1 & 2 that the process of pollination of the female flower of the date palm cv. Hillawi with two types of pollen produced fruits, which differed significantly in the level of endogenous auxins during growth and development. Such differences are probably responsible for some of the manifestation of the phenomenon of metaxenia found in the present work (Table 1 & 2) and reported by many authors over the years (Al-Delaimy & Ali 1969; Denney 1992;

Nixon 1935; Osman et al 1974). Swingle (1928) was the first who suggested that metaxenia is due to the influence of endogenous hormones production by the embryo or the endosperm.

Table 1

Effect of two pollen source on fresh weight of whole fruit, pulp and seed of the date palm cv. Hillawi at Rutab stage

<i>Pollen source</i>	<i>Fruit weight (g)</i>	<i>Pulp weight (g)</i>	<i>Seed weight (g)</i>
Khikri Adi	6.67	5.57	1.11
Ghanami Akhdar	6.01	4.93	1.08
L.S.D. at 5 %	0.09	0.12	0.06

Each value is mean of 3 replicates.

Table 2

Effect of two pollen source on fruit size, of the date palm cv. Hillawi at Rutab stage

<i>Pollen source</i>	<i>Fruit volume (cc)</i>	<i>Length (mm)</i>	<i>Diameter (mm)</i>
Khikri Adi	6.44	32.90	17.29
Ghanami Akhdar	6.05	31.92	17.20
L.S.D. at 5%	0.32	0.06	0.07

Each value is mean of 3 replicates.

The results obtained in the present work provide a strong support to Swingle's hypothesis. The physiological role of free IAA extracted from the developing date fruits in the present work are twofold:

- a. Affecting fruit growth via their effect on the process of cell division and cell elongation.
- b. Affecting fruit growth, through their effect on the mobilization of assimilates toward the fruit in a process known as the hormone-directed transport (Wareing & Phillips 1981). Thus, differences in fruit size and fruit weight (Tables 1 & 2) between fruit produced by the two pollens are probably related to differences in the levels of free IAA, which would influence the processes in "a" and "b" referred to above.

Conclusions. In conclusion, the results obtained in the present work suggest, that difference in levels of free IAA among date fruit produced by pollinating the female flowers with different pollens are probably responsible for some manifestation of the phenomena of metaxenia. Furthermore, work in our laboratory on the phenomena of metaxenia have shown, that different pollens affect the levels of cytokinin and gibberellins, and the results of such work will be reported soon.

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