

Effect of pollen parent on certain aspects of fruit development of Hillawi date palm (*Phoenix dactylifera* L.) in relation to levels of endogenous gibberellins

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Abstract. The effect of two pollen parents (Khikri Adi and Ghannami Akhdar) on certain fruit characteristics of Hillawi date palm (*Phoenix dactylifera* L.) was investigated in relation to changes in levels of endogenous gibberellins during fruit development. The results showed that, the pollen parent Khikri Adi caused a highly significant increase in fruit size, fresh weight of the whole fruit, pulp and seed in comparison with fruit produced by the pollen parent Ghannami Akhdar. The pollen parent had a significant effect on the levels of free gibberellins, with fruits produced by the pollen Khikri having the highest level, as compared with fruit produced by the pollen Ghannami Akhdar. The levels of endogenous gibberellins were low at fruit set (5-7 weeks from pollination) but rose to a peak value during maximum fruit growth rate (9-11 week from pollination) and then declined as the fruit advanced toward the stage of physiological maturity.

Key Words: Gibberellins, fruit growth, metaxenia, *Phoenix dactylifera* L.

Introduction. The date palm (*Phoenix dactylifera* L.) is a subtropical fruit tree, native to Iraq and other part of the Middle East. Due to its dioecious nature the female inflorescences are hand pollinated with pollen from males. Selection of pollinizer is important in date palm, as the pollen affects various aspect of fruit development, and the phenomenon is referred to as metaxenia (Nixon 1935; Osman et al 1974; Ream 1976). However, the physiological basis of metaxenia remains unknown, although it has been suggested first by Swingle (1928), later by Osman et al (1974) and Denney (1992), that metaxenia is due to endogenous hormone produced directly or indirectly by the pollen. However, such hypothesis has never been tested (Denney 1992).

Accordingly, the possibility was considered, that the reported effect of the pollen on the fruit of the date palm may be related to their effect on the production of endogenous hormones by the ovary to varying degrees during fruit development which subsequently affect fruit growth and development.

In this communication, the results of a study into the pollen effect on some aspects of fruit development of the date palm cv. Hillawi is presented in relation to changes in endogenous gibberellins 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 bag. After the spathes, cracked open, the female influences were hand pollinated on first of April, 2010. The pollen was taken from the following male clones:

- 1-Ghannami Akhdar
- 2-Khikri Adi.

Each three spathes 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 gibberellins. The freeze-dried samples were ground just before extraction.

Extraction of free gibberellins. The free gibberellins was extracted using the procedure previously described (Abbas et al 1995). Freeze-dried samples were extracted with 80% methanol for 48h, 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 50ml 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 gibberellins. The analyses of free gibberellins in fruit samples at various stages of development were performed by HPLC (Shimadzu, Osaka, Japan) by the injection of 20µl aliquots. A 4.6x250mm C-18 reversed phase column was used, packed with 5µm ODS (Fishers, USA). The mobile phase was 0.1M phosphoric acid : methanol, 60:40 (v:v), at pH 2.5. The flow rate was 1.0ml.min⁻¹. Isocratic elution procedure was used to obtain the effective separation of free gibberellins. Standard gibberellic acid (GA3) was prepared in different concentration (0.001-10µg.ml⁻¹) 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 205nm as described by Mac Millan (1983). 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 were determined on samples consisting of 30 fruit 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 fruit from each pollen type.

Statistical design and analysis. A completely randomize block design was used, with three replicates, each represented by three tree.

The experiment was factorial (7 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 (Snedecor & Cochran 1980). The block effect was significant at $p=0.05$.

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

Figure 2 shows the interaction between pollen parent and number of weeks from pollination on levels of free gibberellins. It is, obvious that on week 7, fruit produced by the pollen Khikri Adi had a significantly ($p=0.05$) higher levels of free gibberellins in comparison with fruit produced by Ghannami Akhdar pollen. However on week 9, fruits product by the pollen Ghannami Akhdar had a significantly higher of free gibberellins as

compared to fruits produced by the pollen Khikri Adi. On week 11, 13 and 15 from pollination, fruits produced by the Khikri Adi pollen had a significantly higher level of gibberellins in comparison with fruits produced by the pollen Ghannami Akhdar. Thereafter, there were no significant different in levels of free gibberellins as the fruits advanced toward the stage of physiological maturity (19 weeks from pollination).

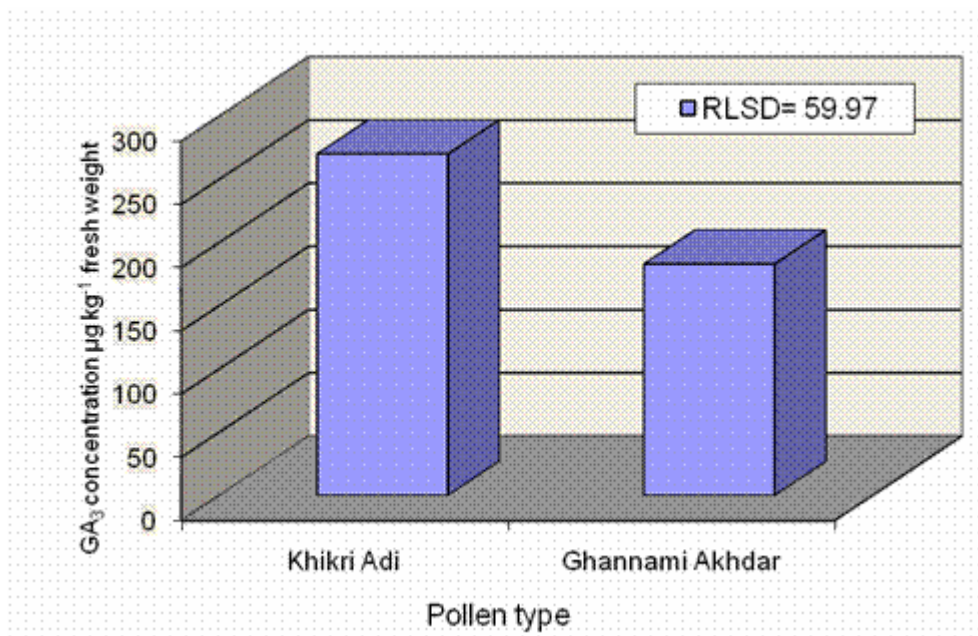


Figure 1. The main effect of pollen parent on levels of free gibberellins in the pericarp of date palm fruit, cv. Hillawi.

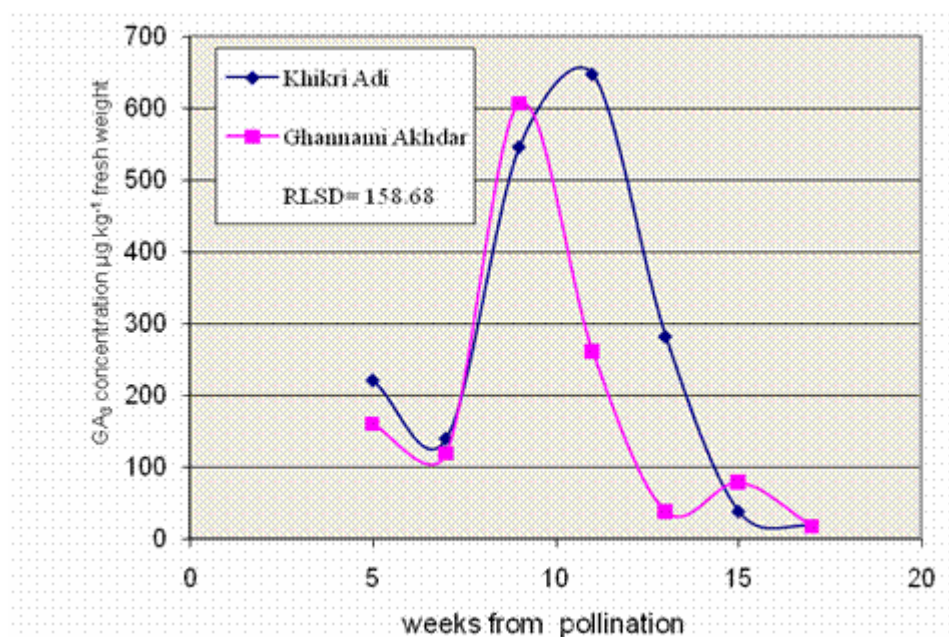


Figure 2. Interaction between pollen parent and number of weeks from pollination on levels of free gibberellins during growth and development of date palm fruit, cv. Hillawi.

Tables 1 and 2 shows the effect of pollen parent on fruit size, and fresh weight of whole fruit, pulp and seed. It is obvious, that there are significant differences ($p=0.05$) between fruits produced by the two pollen parents, with fruits produced by the pollen Khikri Adi having the highest size, and fresh weight, in comparison with fruits produced by the pollen parent Ghannami Akhdar.

Table 1

Effect of two pollen parent on fresh weight of whole fruit, pulp and seed of the date palm cv. 'Hillawi' in Ruitab stage each value is mean of 3 replicates

<i>Pollen Source</i>	<i>Fruit Weight (g)</i>	<i>Pulp Weight (g)</i>	<i>Seed Weight (g)</i>
Khikri Adi	6.65	5.52	1.12
Ghanami Akhdar	6.17	5.08	1.08
L.S.D. at 5%	0.283	0.342	0.071

Table 2

Effect of two pollen parent on fruit size, of the date palm cv. 'Hillawi' in Ruitab stage each value is mean of 3 replicates

<i>Pollen Source</i>	<i>Fruit Volume (cc)</i>	<i>Length (mm)</i>	<i>Diameter (mm)</i>
Khikri Adi	6.61	34.13	18.45
Ghanami Akhdar	6.11	32.15	17.34
L.S.D. at 5%	0.218	0.713	0.449

Similar results were obtained by other worker using different types of pollen (Nixon 1935; Al-Delaimy & Ali 1969; Osman et al 1974; Ream 1976). It is clear, from the data presented in Figure 1 and 2, that the process of pollination of the female flowers of the date palm tree cv. Hillawi with two types of pollen parents produced fruits, which differed significantly in the levels of endogenous gibberellins during growth and development, such difference are probably responsible for some manifestation of the phenomenon of metaxenia found in the present work (Table 1 and 2) and reported by many workers over the years (Denney 1992).

Swingle (1928) was the first, who suggested that metaxenia in date palm is due a hormone action by the embryo or endosperm. The results obtained in the present work provide a strong support to Swingle's hypothesis. To our knowledge, this is the first report in the literature which shows the effect of pollen parent on gibberellins levels in the fruits of the date palm.

The physiological role of free gibberellins extracted from the development fruits of the date palm cv. Hillawi in the present work is twofold:

- a. Affecting fruit growth via their effect on the process of cell division and cell elongation.
- b. Affecting fruit growth, through their effects on the mobilization of assimilates toward the fruit in a process known as the hormone-directed transport (Waeing & Phillips 1981).

Thus, differences in fruit size and fruit weight (Table 1 and 2) between fruit produced by the two pollens are probably related to differenced in levels of free gibberellins, which would influence the processes in a and b referred to above.

Figure 3 shows the effect of stage of fruit development on levels, of free gibberellins in the pericarp. It is clear, that gibberellins levels are low up to the 7th week from pollination. Such low levels of gibberellins at this stage of fruit development are probability due to their use in the process of fruit set, which is known to occur at this stage of fruit growth (Abbas et al 2000). The levels of endogenous gibberellins in the fruits increased to peak value during week 9 and 11 from pollination. Such increase in gibberellins levels are coincided with maximum fruit growth rate, which is known to occur at this stage of fruit growth (Abbas et al 2000). The level of endogenous gibberellins then

declined as the fruit advanced toward the stage of physiological maturity. Such results, clearly indicate, that gibberellins are growth promoters and are involved in the control of cell elongation during maximum fruit growth and have no role to play in the process of fruit maturity (Gillapsy et al 1993).

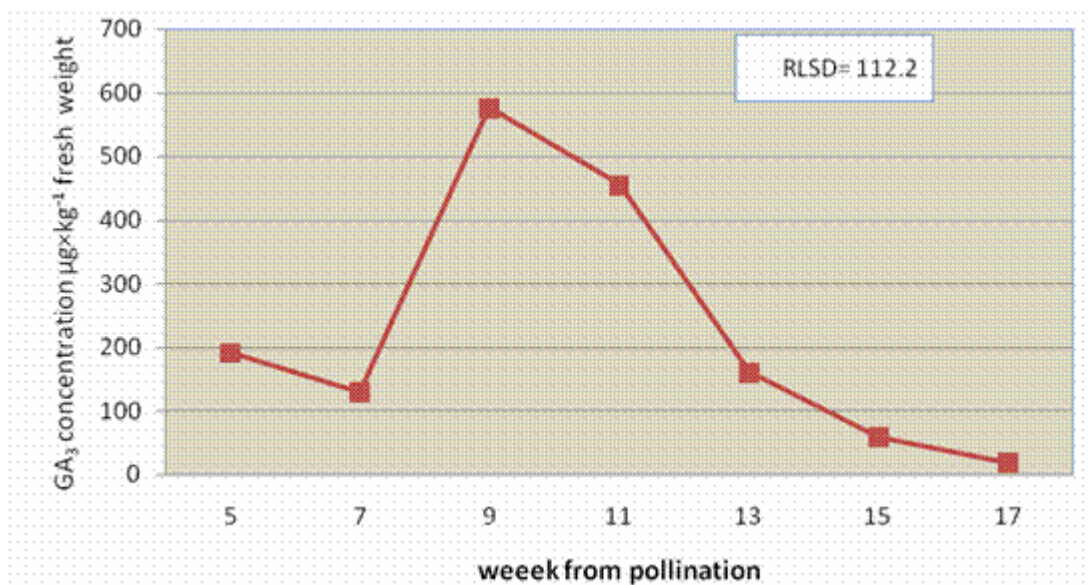


Figure 3. Change in the levels of free gibberellins during growth and development of date palm fruit, cv. Hillawi.

Conclusions. In conclusion, the results obtained in the present work suggest, the differences in the levels of free gibberellins among date fruits produced by pollinating the female flowers with different roles are probably responsible for some manifestation of the phenomenon of metaxenia. Furthermore, change in levels of gibberellins probably reflects the involvement of these natural hormones in various stages of date fruit development.

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Received: 10 July. Accepted: 07 August, Published online: 10 September 2012.

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How to cite this article:

Abbas M. F., Abdulwahid A. H., Abass K. I., 2012 Effect of pollen parent on certain aspects of fruit development of Hillawi date palm (*Phoenix dactylifera* L.) in relation to levels of endogenous gibberellins. AAB Bioflux 4(2):42-47.