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ORIGINAL ARTICLE

# Effect of $GA_3$ and 2,4-D foliar application on the anatomy of date palm (*Phoenix dactylifera* L.) seedling leaf

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2,4-D

**Abstract** Two concentrations (10-5M and 10-3M) of both GA3 and 2,4-D were used as foliar spray to evaluate the response of date palm (Phoenix dactylifera L.) cv. Khedri seedlings. They affected some of the anatomical characteristics of the first leaf emerging after the beginning of the spray. The high concentration of GA3 increased the size of the midrib and its vascular bundle numbers. Both low and high concentrations of 2,4-D inhibited the formation of the midrib. 2,4-D in both low and high concentrations decreased the number of vessels in both protoxylem and metaxylem and also decreased their diameters, where as GA3 in low and high concentrations have less effect on the number of vessels and its diameters. GA3 in high concentration increased the number of vascular bundles in 1mm long of the leaf blade, while 2,4-D in low and high concentrations decreased their numbers. 10-3M of 2,4-D increased the size and layers of special hypodermal cells.


## Introduction

Date palm (*Phoenix dactylifera* L., family Arecaceae) is one of the oldest cultivated trees in arid and semi-arid regions such as Saudi Arabia. It is valued mainly for its fruits (dates) as well as an ornamental tree. The dates are very rich in nutritive components, i.e. carbohydrate and contain different percentages of fat, minerals, protein, vitamins and a high percentage of dietary fiber (Fayadh and Al-Showiman, 1990; Al-Shahib and Marshall, 2003). Saudi Arabia is one of the major dates producing countries in the world and the estimated production of dates in 2006 was 986000 MT (Arab Agricultural Statistics Yearbook, 2009).

According to Fishel (2006) the plant growth regulators, such as auxins promote shoot elongation, thin tree fruit, and increase rooting and flower formation, gibberellins stimulate cell division and elongation; increase stalk length, and increase flower and fruit size, cytokinins stimulate cell division, bud initiation and root growth and prolong the storage life of flowers and vegetables, ethylene generators ripen and induce uniform ripening in fruit and vegetables.

The biological effects of applying plant growth regulators on plants have received much attention due to their important use in agriculture; in particular, the economical application of plant growth regulators on flowers and fruits (as parthenocar-

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pic, thinning, and elongating agents) and on shoot (as a controlling agent of plant height and lateral branching (Salisbury and Ross, 1985, 1992; Whiting, 2007). On the other hand, investigators used plant growth regulators in plant tissue culture, with auxin and cytokinin being the most widely used plant growth regulators (Slater et al., 2003).

Gibberellins were found to enhance the stem length in higher plants due to cell elongation in the internodes and to stimulate the cell division, and increase volume of individual cells (Stowe and Yamaki, 1959; Cleland, 1969; Stefanini et al., 2002). These effects lead to stem elongation, internode length, leaf area and growth of dry mass, although plant species might react to certain GAs (Kerbauy, 2004). In general, $GA_3$ increases xylem differentiation in vascular cambium, stem and leaf length, and xylem fibers, but it decreases the amount of leaves produced by the apical meristem, xylem vessels and xylem parenchyma (Ammar and Hadad, 1982). However, to the knowledge of the authors, no study has been encountered concerning the effects of 2,4-D and $GA_3$ foliar application on the changes in the anatomical structure of date palm leaf (*Phoenix dactylifera* L.) seedling. Therefore, aim of the present experiment was to study the effect of foliar spray of gibberellic acid ($GA_3$) and 2,4-Dichlorophenoxyacetic acid (2,4-D) on the leaf anatomy of date palm seedling.

## Materials and methods

Seeds of date palm (*Phoenix dactylifera* L.) c.v. Khedri were washed and soaked in double distilled water (DDW) with aeration for four days. Three selected seeds were sown in a pot containing field soil-peat moss mixture (1:1). After sowing, pots were kept in the greenhouse (temperature 30 °C and relative humidity 60%). After three months of sowing of plant, each seedling was transferred into a larger pot. Seedlings were irrigated with DDW two times a week, except every other two weeks with one nutrient solution (for composition of IX solution, see Al-Whaibi and Al-Ackhal, 1985). Seedlings were divided into five groups. Each group contained six seedlings. Control was only sprayed with IX nutrient solution and treatments were sprayed with IX solution containing $10^{-5}$ M or $10^{-3}$ M of either 2,4-D or $GA_3$. All spraying solutions contained four drops of Tween 20 to reduce surface tension. Seedlings were sprayed weekly at 10:00 a.m., until at least the fourth leaf emerged. Seedlings were then harvested and divided into shoots and roots. The first leaf emerging after the beginning of spray for each treatment was chosen. Segments (2 cm) of leaf blade were taken at the leaf base (3 cm from the blade base) middle and apex (3 cm from the blade apex). Each leaf segment was divided into 5 mm pieces and immediately fixed in FAA (Formalin: Acetic acid: Alcohol), dehydrated in ethanol, embedded in paraffin, sectioned at 10–20 $\mu$m thick using a rotary microtome, stained with safranin and light green and then mounted in Canada balsam. Sections were examined and photographed using Novel 002002 microscope with USB pc Camera 301 + . Cell wall thickness was determined according to Doaigey et al., 1989. This procedure was repeated for the same length in both the middle and near the base of the leaf. Vessel diameter was also recorded at both middle and near the base of the leaf. *Statistical analysis*: The data were analyzed statistically with SPSS-12 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan's multiple – range test at $p < 0.05\%$ level.

## Results

The internal characteristics of the first leaf emerged after the beginning of spray for each treatment (near leaf base, major and minor veins) were summarized and compared with those of the control. The observed features of this leaf in transverse section were described sequentially and in brief starting with the upper epidermis proceeding through to the mesophyll, vascular bundles and lower epidermis.

### *Upper epidermis*

Foliar application of $GA_3$ enhanced thickness of the cuticle as compared to the control and 2,4-D (Fig. 3 (13–16)). The effect of low and high concentrations of $GA_3$ and 2,4-D foliar spray was not found significant on epidermal cells, ovate to tabular (irregular shapes); except over the multilayer hypodermis where cells were palisade-like forming a zip-like shape (Bulliform cells) with thin cell walls. They were not affected either by low or high concentrations of $GA_3$ and 2,4-D treatments (Fig. 3 (13–16)).

### *Hypodermis*

One or 2(5) layers of parenchymatous cells with thin cellulosic walls, were devoid of chloroplasts in control, the multilayer hypodermal cells under the bulliform cells became slightly large in cell size and limited to 1–3 layers by the effect of low concentrations of $GA_3$ and 2,4D treatments (Fig. 3(14,16)). These cells were also affected by the high concentration of 2,4-D treatment as they became larger in size and increased in layers (up to six layers) (Fig. 3(15)). However, they were not affected by the high concentration of $GA_3$ treatments (Fig. 3(17)).

### *Fiber strands*

Small, many cells with lignified cell walls, were present under upper and lower epidermises. They were not obviously affected by low and high concentrations of $GA_3$ and 2,4-D treatments, except the lumen of the cell became slightly wide at high concentration of $GA_3$ and 2,4-D foliar spray. Some of the outer cells of fiber strands contained silica bodies which were not affected by either low or high concentrations of $GA_3$ and 2,4-D treatments (Figs. 1 and 3(1–6,18)).

### *Vascular bundles*

Two types, (a) large vascular bundles (VBs) surrounded with one layer of bundle sheath having cells devoid of chloroplasts and some of them contain silica bodies (Fig. 2(7,12)). Protoxylem with narrow vessels, metaxylem with wide vessels, 8–20 vessels in each vascular bundle were found in control plants (Fig. 2(7). However, they became less in the number of vessels (5–13 vessels) in plants that received low and high concentrations of 2,4-D treatment. An increase in number (10–24 vessels) was found at the high concentration of $GA_3 \times 10^{-3}$ (Table 1, Fig. 2(8–11)). Foliar application of $GA_3$ and 2,4-D had a significant change in the number of VBs (Table 1). A number of large vascular bundles were present in all the areas (top, middle, and base) of the leaf. Plants treated with higher dose of $GA_3$ exhibited greater number of VBs at all areas of leaf.