Changes in Endogenous Hormones in Fruit during Growth and Development of Date Palm Fruits

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Abstract: The length and diameter of pollinated and un-pollinated fruits of two date cultivars, piarom and shahani, were measured. Concentration of endogenous hormones in pulp during different stages of fruit growth and development were also determined by spectrophotometric techniques. The results indicated that a relatively rapid increase of fruits length occurred until 15 weeks after pollination thereafter, growth rate was moderate. In two cultivars, auxin and zeatin content, similar to gibberellins (GA) were increased gradually during the early fruit growth stage and the highest value was detected at 12 and 16 weeks after pollination, respectively. Changes in ABA levels were closely associated with ripening. In conclusion, data on un-pollinated fruits show the same pattern of growth, though at a different rate un-pollinated fruits contained lower hormones levels than pollinated fruits in both cultivars. Comparing hormonal changes pattern curve in the fruits of this cultivars, it is seen that there are a little differences among the cultivars.

Abbreviations: GA - gibberellic acid • IAA - Auxin • TLC - Thin-layer chromatography • ABA - Abscisic acid; 

Key words: Abscisic acid • Auxin • Cytokinin, Gibberellin • Phoenix dactylifera L • Ripening.

INTRODUCTION

The early studies showed that reproductive development was positively correlated with some biochemical substances. This was mainly endogenous hormones. Plant hormones play a significant role in the processes that lead to mature fruit and viable mature seed [1]. They would produce different effect under different climate condition and cultivars. In general, normal fruit growth requires the presence of developing seeds [2-4]. From previous work on fruit development during the last 30 years, it is well established that all classes of plant hormones, auxins, GAs, cytokinins, inhibitors (for example, ABA) and ethylene play an important role in fruit development [1]. A proper balance of these factors is required for optimal fruit development.

Date palm is one of the major agricultural crops of the Near East region, where about 90% of the world dates production take place [5]. In many countries in this region, the date palm plays an important economical and sociological role. Iran is considered as one of the largest producers of date with production about 1000,000 tons which is about 15% of total world production. About 400 varieties are grown in Iran, among these with good taste and sweetness is piarom and shahani. Color of piarom is dark brown and skin of it is thin. The piarom date has a kind of sugar that suitable for diabetic people. These varieties are the most important variety of semi-dry and fresh date in Iran respectively. Growth and development of date palm fruit involves several external and internal changes. These changes are often classified on the basis of change in color and chemical composition of the fruit, as four distinct stage of fruit development, known as kimiri, khalal, rutab and tamr. These terms are used to represent the immature green, the mature full coloured, the soft brown semi-ripe and the hard raisin like fully rip stage of development, respectively. At the kimiri stage, there is a rapid increase in size, weight and reducing sugars. At the khalal stage, sucrose content increase and moisture content begins to decrease. The tannins will also precipitate and also their astringency. At the third stage (rutab), there is a decrease in weight due to moisture loss
and inversion of sucrose into invert sugar and browning of the skin and softening of the texture. When the dates left on the palm, they will turn into tamr at which they have the least amount of moisture and tannin and are self-preserving. As date mature, their texture become soft which is an associated with progressive changes in the fruit fiber. Dates are commonly consumed as fresh, short shelf life fruit at the khalal and rutab stage, with little or no processing. Tamr stage is characterized by its good storability. The flesh of a fully ripe (tamr) date consists of two-thirds sugar and one-quarter water, the rest being mainly minerals, cellulose, ash and vitamins.

There are several reports that show changes of hormones in grape [6] guava [7] pear [8] during fruit development. Many studies have also been carried out on date such as date past and physical and chemical compositional characteristic [9,10]. However, data on plant growth regulators during fruit development are still scarce. The aim of this experiment was to clarify the role of plant hormones in the fruit growth development, from pollination of the ovary through ripening in pollinated and un-pollinated piarom and shahani fruits.

MATERIALS AND METHODS

Two local varieties (piarom, shahani) were used in this study. These varieties are well-known commercial samples grown in Iran. The experiment was carried out in 12 years old commercial date palm orchards located in agricultural research center of Jahrom (28°57N, 53° 57E°). This location represents the major production areas in this region and has subtropical climate condition with hot dry summers and mild winter and full bloom occurred April 24-30. The studied were performed by collecting the sample directly from tree, 4 weeks interval (in different stage development), around tree spikelets of pollinated and non-pollinated tree cut from a selection of bunches chosen randomly from tree growing at the same location fresh fruit of uniform size, free of physical damage and injury from insects and fungal infection, were brought to the laboratory on the day of harvesting and frozen with liquid nitrogen for 2-3 minutes then immediately stored in a freezer at-70°C for subsequent analysis. The longitudinal length and transverse diameter of 30 fruit were measured using a digital caliper.

Pollinated and un-pollinated endocarp fruits at different stage of development were used as experimental materials. Extraction, purification and quantitative determination of free and bound IAA, GA3, ABA and zeatin in both cultivars were done, with minor modifications, according to the methods of Ergun [11]. The procedure is particularly useful for the study more than one hormone simultaneously in a given sample.

Either one gram fresh weight of each fruit sample was taken and combined with 60 ml of methanol: chloroform: 2N ammonium hydroxide (12:5:3 v/v/v). IAA, GA, ABA and zeatin extraction assays were done. Combined extract was treated with 25 ml of distilled water. The chloroform phase was discarded. Water-methanol phase was evaporated. The water phase was adjusted to the extract pH value of 2.5 or 7 or 11 with 1 N HCl or 1 N NaOH respectively and 15 ml ethyl acetate was added at each of three steps. This procedure provided the isolation of free-form IAA, GA, ABA and zeatin from the extraction solvent. After an incubation period of 1 hour at70°C, the same procedure was used for the isolation of bound-form IAA, GA, ABA and zeatin from the extraction solvent. Evaporation of ethyl acetate was performed at 45°C using a rote-evaporator system. Thin-layer chromatography (TLC) was done using 20×20 cm, 0.25 mm thick silica gel GF254 (Merck Chemicals, Germany). TLC separated IAA, GA, ABA and zeatin were isolated from the glass plaques according to the standard synthetic IAA, GA, ABA and zeatin Rf values. IAA, GA, ABA and zeatin were dissolved with 2 ml of methanol for filtration and separation from silica plate. spectroscopy assay was done using 222 nm wave lengths for IAA, 254 nm for GA, 263 nm for ABA and 269 nm for zeatin and for all standard synthetic IAA, GA, ABA and zeatin isolated samples. Total IAA,GA, ABA and zeatin was then obtained as the sum of free and bound IAA, GA, ABA and zeatin. The amounts of IAA, GA, ABA and zeatin in the fruits samples were expressed as standard synthetic IAA, GA, ABA and zeatin equivalent.

Statistical analysis was performed using SPSS for windows statistical software (SPSS Inc. USA) for ± standard error and mean of each value.

RESULTS

In two cultivars at the first stage of growth, fruit increase in length and diameter and mainly in length from the middle or the end of the kimri stage (Figs 1and 2). The pollinated fruit grow faster and reached its largest diameter and length on the 16th week. The growth of the seeded fruit was greater than that of the ones. In spite of its initial equal growth rate, the seedless fruit at maturity were less size rather than pollinated fruit.
Fig. 1: (A,B) changes in length and diameter of pollinated and un-pollinated shahani fruits during fruit growth and development.
Fig. 2B
Fig. 2: (A,B) changes in length and diameter of pollinated and un-pollinated piarom fruits during fruit growth and development.

Fig. 3A

Fig. 3B
Fig. 3: (A-D) changes in endogenous hormones in fruit during growth and development of shahani date palm.

Fig. 3C

Fig. 3D

Fig. 4A
Fig. 4: (A-D) changes in endogenous hormones in fruit growth and development of piarom date palm.
The changes in IAA contents of the shahani and piarom fruits are presented in Figs. A3 and A4. In piarom, IAA content was increased gradually during the early stage of fruit growth and the highest value was detected at 12 weeks after pollination. Then start to reduction in IAA content during 4 week, a second increase but at lower magnitude was obtained in the Piarom at 20 week after pollination .subsequently, the amount of IAA dropped again and reached their minimum level at the time of ripening. The similar curve was detected in un-pollinated piarom fruits expect reduction in IAA content was later. In pollinated and un-pollinated fruit of shahani similar to piarom, IAA was increased at early stage and then decreased gradually with slight increase in the late stage.

The changes in GA contents of the shahani and piarom are presented in Fig. B.3 and B4. In shahani fruit GA levels were increased gradually and reached the peak in 20 week after pollination, then decrease before ripening with slight rise was shown at about ripening. GA was increased gradually to 12 and then it was shown a slight decrease during un-pollinated fruit development.

As for shahani, in pollinated piarom fruit GA levels were increased gradually and peaked in 16 week after pollination. Un-pollinated piarom fruit developed with a relatively paralleled concentration curve of GA in pollinated fruit but peaked at 20 weeks, after pollination. In tow cultivars un-pollinated fruit contained lower Gibberellin levels than seed fruit during all fruit development.

In both cultivars Zeatin was increased gradually and peaked 10 weeks after pollination, then shifted to decrease to ripening. In piarom a second peak was also observed at the before ripening. Un-pollinated fruit of these varieties also show similar pattern but in lower level. In shahani un-pollinated fruit also zeatin curve was increased gradually and peaked 12 week after pollination, then sharply decrease and the last stage of growth, slight increase (Fig C 3 an d4).

ABA content in the shahani and piarom are presented in Fig D3 and D4. In shahani seeded fruit, ABA content was increased gradually during first stage of fruit growth and peaked 20 weeks after pollination and then decreased. In un-pollinated ABA curve was relatively high during first month but following with rise during the ripening.

In piarom ABA was slight high at early stage of fruit development, then decreased suddenly. After 4 weeks, it started to rising. In un-pollinated fruits of piarom the ABA levels changing during first stage of development then it peaked suddenly near ripening, after declined.

**DISCUSSION**

In general there is a little study on date palm fruit growth and development. Shabana et al. 1981, reported that at the end of the "hababauk" stage (refers to the earliest stages of development, about 5 weeks from spathes crack) and at the beginning of the kimri stage a higher rate growth of fruit lets was observed for 3-4 weeks [12]. This period is followed by a lower rate of growth at the later kimri stage as also found by Reuveni, [13], study the pericarp and seed development in fertilized and unfertilized date fruit. He divided it into six stages. Almost of our results are similar to him. A little difference about time, there might be difference between cultivars, growing conditions and pollen source. When investigating changes in IAA level in the un-pollinated and seeded in two cultivars during development, similar pattern were found between them, only small differences, however, were observed between them at the later of the stage of fruit development. Either of them had higher level of IAA in early stage of fruit development and peaked at 15 weeks after pollination. This may be related to the rapid growth phase of the fruit for enhancing cell division or enlargement [14]. Similarly, Reuveni [13] reported that progressive enlargement of the carpel is evident during (7-11) weeks after pollination. The basipetal meristem is very active and the newly formed cells are enlarging very fast. Comparing IAA change pattern curve in the both cultivars it is seen that IAA concentration was higher in fruits of piarom than shahani. In un-pollinated fruits content of IAA was lower than seeded fruit in two varieties. The low IAA level in seedless fruits may be due to a lack of seed, a site for IAA synthesis. Similarly, Coombe [15], Nitch et al. [16] and Farmahan and Pandey [1] found higher auxin content in seeded berries than in seedless berries of grape.

Data presented here clearly indicate that endogenous gibberellins levels are high during the middle stage of fruit development, in both cultivars. In fact, the peak of GA activity occurred around 25 weeks after pollination at a time when rapid morphological changes take place. Also, Reuveni [13] showed that during (18-22) weeks after pollination, mesocarp meristems cell division cease completely and the growth of the pericarp is mainly by cell expansion, specially at the middle and the bottom of fruit, therefore it has been suggested that gibberellins was one of the important active substance to promote cell enlargement of fruit. This result support that the assumption that GA is necessary for cell expansion [17]. So as IAA the peak GA was higher in seeded fruit than in seedless ones.
The present results in this study also showed that in both cultivars, un-pollinated and seeded fruit, ABA peaks presented before fruit ripening. Other studies Baydar and Harmankaya [6] and Zhang et al. [18] indicated that the highest amount of ABA was found just before the time of ripeness in grapes. They suggested that ABA may be a factor as a ripening-promoting in grape; therefore, endogenous ABA must be accumulated to a certain level simultaneously with inactivation of endogenous auxin before ripening initiates. In shahani fruits, the peak of ABA for the un-pollinated ones was higher than seeded ones. Our results were similar to those obtained by Baydar and Harmankaya [6]. There are also seedless grape had higher ABA at the late stage of berry development.

In two cultivars zeatin level was relatively low in the early stage of fruit development then peaked at 20th week after pollination after this declined. Our result confirmation study of Reuveni [13], who reported that in the mesocarp meristems cell division occur mainly 14th week and during this period the fruit is growing very fast. In un-pollinated piarom fruits, the curve was similar to seeded fruit but in shahani fruit had a little difference, the peak was later at 25th week after pollination. Although, in both cultivars un-pollinated fruits has lower zeatin content than seeded fruit, the low cytokinin contents of seedless fruit to those compared with seeded ones seems to indicate that cytokinin may originate from the seeds. This evidence suggest that, similar to IAA, zeatin could accelerate division of cell. Negar and Rao [7] indicated that in guava, high cytokinin content at early stage of fruit development point to a role in cell division.

CONCLUSION

Based on these results, it can be concluded that there is positive relationship between the levels of hormones and development degree of seed. In the pollinated fruits of both cultivars the activity of these factors increased during fruit development and in the un-pollinated fruits this was slower. Also, different variety can show different changes pattern during fruit growth. It might be possible that IAA and GA both play roles in promoting cell division and elongation. In our study, the activity of GA, IAA, zeatin were quit high during the early stage in pollinated fruits. In general, it has been certain that fruit development is not linearly graphed as a function of any given endogenous hormone because hormones do not act alone, nor do they have a single function in fruit. Thus, the balance of concentration among all the endogenous hormones in fruit appears to promote its development more properly. The level of inhibitors was highest at the time of late stage of fruit growth in both cultivars studied. As the fruit approach maturity, the promoters decline, the inhibitor is the only growth regulator detectable and the rate of fruit growth slows.

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