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Levels of Some Endogenous Plant Hormones During the Flowering Period in Loquat (*Eriobotrya japonica* Lindl.)

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Changes in the amount of endogenous phytohormones were determined in loquat flowers. Samples were chosen among flowers in the beginning bloom stage, full bloom stage and the end of the flowering period. Among these samples, amounts of free-, bound- and total-forms of some endogenous plant hormones (indole-3-acetic acid, gibberellic acid, abscisic acid, zeatin) were separated from plant extract and quantified by high performance liquid chromatography using a reversed-phase C_{18} column. According to the results, indole-3-acetic acid level did not change during the flowering period in Gold Nugget cultivar, but it was observed to be high in Akko XIII cultivar in the initiation of flowering period. Level of gibberellic acid in the flowers decreased from initiation of flowering period till the end of it. During flowering period abscisic acid level dramatically increased and reached its maximum, which indicates that abscisic acid might play an active role in flowering process. Zeatin was observed in high levels in both the initiation and the end of flowering period. Its level, however, was determined to be minimum at the full bloom stage.

Key Words: Loquat, *Eriobotrya japonica* Lindl., Indole-3-acetic acid, Gibberellic acid, Abscisic acid, Zeatin, HPLC.

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl., Rosaceae, Maloideae) is a subtropical evergreen fruit tree that blooms at fall, develops its fruits during winter and ripens them at early spring. Fruit species that flower in autumn and have flowers and fruits during winter months are quite rare. One of this type of fruit species is loquat. Determination of endogenous plant growth regulator levels is very important in this cultivar due to its physiological differences. Endogenous and exogenous plant growth substances have been correlated with both the promotive and inhibitory aspects of flowering in many plants. Some research studies can be found concerning the distribution of these substances during the flowering period of other species, whereas little information is available for loquats.

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Chen[1] investigated that changes in cytokinins and gibberellins in xylem sap of lychee (*Litchi chinenesis* Sonn. Cv. Heh yeh) trees at leaf expansion and dormant bud (when apical leaves are dropped) stages, 30 d before flower bud formation, during flower bud formation and the full bloom of grafted field-grown lychee trees. In this experiment, high level of gibberellin was found in the xylem sap at the stage of leaf expansion. A constant level of indole-3-acetic acid (IAA) was maintained through the five growth stages. Abscisic acid (ABA) increased dramatically 30 d before flower bud formation. Total cytokinin content increased in the xylem sap, reaching a maximum during flower bud formation and full bloom. Gibberellin content in the xylem sap was at a low level 30 d before flower bud formation and through this stage. On the other hand, changes in gibberellin and cytokinin activities were investigated at the stages of leaf differentiation, mature green leaves, early flower bud formation (7 d after formation) and full bloom of 3-year-old mangos (*Mangifera indica* L.) in pot culture by Chen[2]. In this research, experiments demonstrated a dramatical increase in ABA during early flower bud formation. Cytokinins are important factors in the regulation of flower bud initiation and development in certain fruit trees. A strong role influence on flower bud differentiation in apple has been published. Chen[2] found that total cytokinin-like activity increased in the xylem sap, reaching a maximum level at full bloom. Chen[1] interpreted this as an indication that the increase in endogenous cytokinin levels during flower bud differentiation might be correlative, but it was not the cause of flower bud initiation. In olive[3] and in mango[2], Pal and Ram[4] found that high gibberellic acid (GA_3) level exhibited an inhibitory effect on floral formation during induction and initiation periods. On the other hand, Ülger *et al.*[3] proposed that the high concentrations of GA_3, ABA and certain cytokinin levels might have a positive effect on flower formation in olive during those periods. Baydar and Ülger[5] confirmed the correlation between low levels of GA_3, as well as high levels of ABA and the initiation of flowering. IAA reached its maximum level during bud formation, indicating that IAA might play an active role in the differentiation of bud formation. Changes in the amount of endogenous phytohormones in flower buds located in different parts of the plant indicate that ABA in particular plays an important role in formation of flowering order in safflower.

If endogenous hormon levels during the flowering period of loquat is accurately illuminated, application of exogenous hormon levels during growing periods for plant could be easily found[6]. The aim of the present study was to obtain information on changes in the amount of natural hormones in loquat during the flowering process.

EXPERIMENTAL

Experiments were conducted on "Gold Nugget" and "Akko XIII" loquat trees growing on Quince C (*Cydonia vulgaris* L.) rootstock. Trees were planted in 1993 at 5 m × 5 m spacing at the West Mediterranean Research Institute near Serik, Antalya Turkey. Irrigation and fertilization programs were in accord with commercial practice. For each of the cultivar, nine typical plant samples were selected from the orchard. Flower samples from 9 trees were taken at three stages of development in Gold Nugget and Akko XIII cultivars as follows: 1) initially of flower inflorescence (November 11-November 26), 2) flowering (December 11-December 21) and 3) end of the flowering (25 December, 10 January) periods. Samples were placed in deepfreeze (-18 °C) for extraction and purification processes.

To determine free-, bound- and total-IAA, -GA_3, -ABA, zeatin (Z), the method of Ersoy[7] was followed. The extraction and purification procedures are shown in Fig. 1.

Each sample of whole fruit (1 g FW) was homogenized and combined with an extract (a mixture of methanol:chloroform:2 N ammonia, 12:3:3, (v:v:v) containing butylated hydroxytoluene (BHT) at 100 mg L^{-1} as an antioxidant. Samples were then stored at -18 ºC for 2 weeks and filtered later on.

Thin layer chromatography (TLC) was used for separation and purification of dissolved methanol. Plates were placed in TLC tank containing a mixture of isopropanol:ammonia:double distilled water (10:1:1, v:v:v). The relative fluidity (R_f) bands of IAA, GA_3, ABA, Z on the plates were studied by 280, 208, 265, 254 nm UV lamp, respectively. The hormone extracts on the R_f bands were dissolved in grade methanol to be used in HPLC analysis.

Analysis of endogenous plant hormones was performed on a Shimadzu CR-7A plus model equipped with UV detector and CR-7A 10AV-VP model pumps enabling the use of concentration gradient of the mobile phase.

Column: Supelcocil LC-18 (25 cm × 4.6 mm and 5 µm); Column temperature: Room temperature (18-22 ºC); Mobile phase: 35 % methanol (in 1 % acetic acid) for IAA, 30 % methanol (pH:3) for GA_3, 55 % methanol (in 0.1 M acetic acid) for ABA, 70 % methanol for Z; Flow rate: 1.0 mL min^{-1}; Detector: UV, 280 nm for IAA, 208 nm for GA_3, 265 nm for ABA, 254 nm for Z; Injection concentrate: 10 µL; Total run time: 0.5 h.

The amounts of endogenous hormones were expressed as equivalent standard synthetic hormones. Total-endogenous hormones were obtained as the sum of free- and bound-hormones.

Statistical analysis: Analysis of Varience was performed using the statistical analysis system (SAS Institute, 1987).

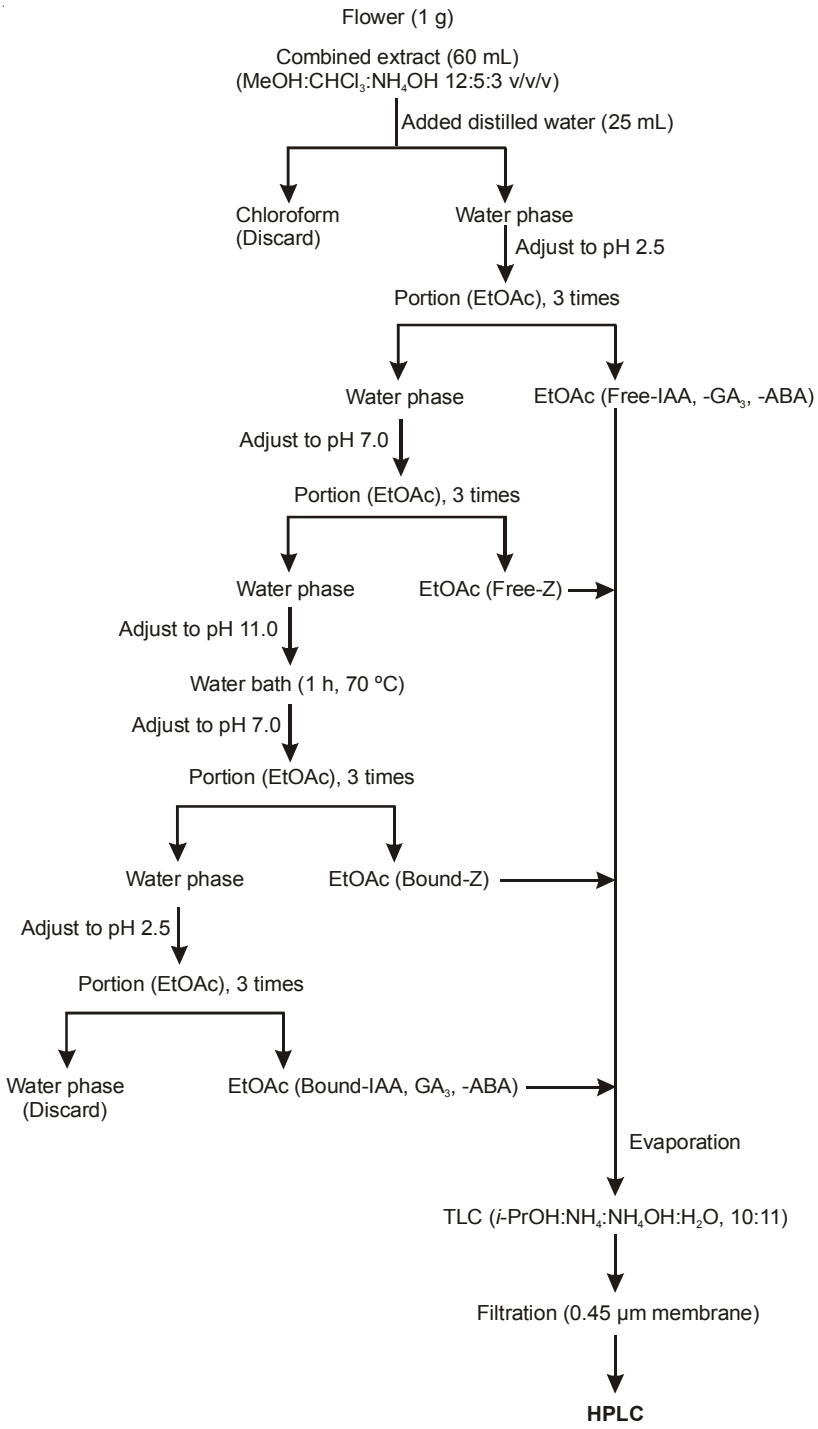

Fig. 1. Flow diagram outlining the extracts used in purification of IAA, GA_3, ABA and Z

RESULTS AND DISCUSSION

Changes in free- and bound-IAA, -GA_3, -ABA and -Z activities were investigated at the beginning bloom stage, full bloom stage and the end of the flowering period in Gold Nugget and Akko XIII loquat cultivars. Total-IAA, -GA_3, -ABA and -Z were then obtained as the sum of free and bound IAA, GA_3, ABA and Z. Amounts of free, bound and total-IAA, -GA_3, -ABA and -Z are presented in Table-1. The differences among hormone levels listed in the table are statistically important ($p \leq 0.05$).

The amounts of free, bound and total-IAA,-GA_3, ABA and -Z were expressed as equivalent standard synthetic IAA, GA_3, ABA and Z. Values are means ± SE of 3 replicas.

Free, bound and total-IAA contents were found to be 485.3, 165.35 and 650.65 ng/g at the beginning bloom stage, 255.4, 144.3 and 399.7 ng/g at the full bloom stage and 108.1, 197.05 and 305.15 ng/g at the end of the bloom period, respectively in Gold Nugget loquat cultivar. In Akko XIII loquat cultivar, these data were found as 442.7, 334.3 and 734.25 ng/g, at the beginning bloom stage, 170, 136.1 and 342.25 ng/g at the full bloom stage, 128.6, 213.65 and 306.1 ng/g at the end of the bloom period respectively (Table-1).

Although IAA level reached its maximum at the beginning of the flowering period, this level decreased at the full bloom stage and the level was stationary at the end of the flowering period in both cultivars. Similarly, Chen[1] worked about lichi (*Lichi sinensis* L.) trees at five different growth periods [(i) leaf expansion, (ii) bud rest, (iii) 30 d before flower bud formation, (iv) flower bud formation, (v) full bloom] in order to observe changes of IAA, GA and ABA levels. Consequently, Chen[1] found IAA levels to be stationary at these five periods.

Auxins were proved to be widely in free and bound (glucose, amino acid and myoinositol, *etc.*) forms in plants by several investigations[8]. This research also agrees that IAA is found in both free and bound forms in the flowers of the two loquat cultivars.

According to the experimental results with whole samples taken into consideration, maximum IAA levels in Gold Nugget and Akko XIII cultivars were found to be 650.65 and 734.25 ng/g, respectively. Wurst *et al.*[9] notified that auxins, which are indole-type plant growth regulators, were found in plant and microorganisms at too low concentrations. Ulger[3] estimated IAA concentration to vary in between 1-10,000 ng/g through his studies performed on many plants with GC-MS.

Endogenous IAA levels were found to be maximum level at the beginning of the flowering period in both cultivars. Similar results were obtained by Baydar and Ülger[5] in Safflower (*Carthamus tinctorius* L.), Capiello and Kling[10] in pecan (*Carya illinoinensis* L.) trees.

In Gold Nugget loquat cultivar, free-, bound- and total-GA_3 levels were found to be 1745.20, 319.95 and 2065.15 ng/g at the beginning of the bloom stage; 169.40, 994.95, 1164.35 ng/g at the full bloom stage; 505.10, 203.35 and 708.45 ng/g at the end of the flowering period, respectively. Data determined in Akko XIII loquat cultivar were 171.1, 1318.3, 1492 ng/g at the beginning bloom stage, 661.05, 555.7 and 1216.7 ng/g at the full bloom stage; 662.95, 196.6 and 859.6 ng/g at the end of the flowering period respectively (Table-1).

Although GA_3 level, as in free and bound forms, was in maximum level (2065.15 ng/g) at the beginning of the flowering period in Gold Nugget cultivar, this level decreased towards the end of the flowering period and reached its minimum (708.45 ng/g) at the end. In Akko XIII loquat cultivar, total-GA_3 had its maximum level (1492 ng/g) at the beginning of the flowering period and minimum level (859.6 ng/g) at the end and the level observed at the full bloom stage was in between the 2 levels (Table-1).

Endogenous GA_3 level in experimental loquat cultivars was observed to be high in November when the flowering initiates. However, Chen[2] found that flower bud formation in mango depends on the low GA_3 level in xylem exudates. Chen conducted this research in xylem exudates. It is estimated that endogenous GA_3 found in xylem exudates of loquat is transferred to flowers which are the generative organs, causing an increase of GA_3 level in these tissues.

GAs have been shown to induce flowering in most plants, but GA application on an operational basis has been worked out only for a few species. GAs have been applied effectively by topical treatment, by stem or branch injections and by foliar sprays. The timing and concentration of applied chemicals are critical and vary from species to species. Another plant growth regulator (PGR), naphthalene-acetic acid, is synergistic with GAs in some species. Adjunct cultural treatments are often used in combination with treatment with GAs. The success of treatment with GAs is determined to some extent upon the stage of development of the treated plants. The endogenous PGRs that regulate flowering are not completely known, but it appears that GA, cytokinin and abscisic acid levels might all be changed by treatments which induce flowering. Recently improved techniques for measuring PGRs would stimulate research on endogenous PGRs that play a major role in stimulating flowering in trees.

Free, bound and total-ABA contents were found to be 534.83, 3000.26 and 3535.09 ng/g at the beginning bloom stage, 85067.04, 16208.20, 101275.24 ng/g at the full bloom stage and 38576.19, 9773.69 and 48349.88 ng/g at the bloom weathering period in Gold Nugget loquat cultivar. Corresponding data for Akko XIII loquat cultivar were 603.49, 4485.31 and 5088.8 ng/g at the beginning bloom stage, 40451.21, 5388.72 and 45839.93 ng/g at the full bloom stage 18283.81, 5674.52 and 23958.33 ng/g at the bloom weathering period (Table-1).

TABLE-1
FREE-, BOUND- AND TOTAL-IAA, -GA_3, -ABA AND -Z LEVELS OF GOLD NUGGET AND AKKO XIII LOQUAT VARIETIES DURING DIFFERENT FLOWERING PERIODS

Varities	Plant hormones	Forms of hormones	Stages of flowering		
			Initiation of flowering	Flowering	End of flowering
Gold Nugget	IAA	Free	485.3 a*	255.4 b	108.1 c
		Bound	165.35 ab	144.3 b	197.05 a
		Total	**650.65 a**	**399.7 b**	**305.15 b**
	GA_3	Free	1745.20 b	169.40 a*	505.10 c
		Bound	319.95 b	994.95 a	203.35 c
		Total	**2065.15 a**	**1164.35 b**	**708.45 c**
	ABA	Free	534.83 c	85067.04 a	38576.19 b
		Bound	3000.26 c	16208.20 a	9773.69 b
		Total	**3535.09 c**	**101275.24 a**	**48349.88 b**
	Z	Free	1500 a	485 b	1550 a
		Bound	375 a	105 b	570 a
		Total	**1875 a**	**590 b**	**2120 a**
Akko XIII	IAA	Free	442.7 a	170 b	128,6 b
		Bound	334.3 a	136.1 b	213.65 ab
		Total	**734.25 a**	**342.25 b**	**306.10 b**
	GA_3	Free	171.10 b	661.05 a	662.95 a
		Bound	1318.30 a	555.70 b	196.60 c
		Total	**1492.00 a**	**1216.70 ab**	**859.60 b**
	ABA	Free	603.49 c	40451.21 a	18283.81 b
		Bound	4485.31 a	5388.72 a	5674.52 a
		Total	**5088.8 c**	**45839.93 a**	**23958.33 b**
	Z	Free	1645 a	275 c	835 b
		Bound	425 b	415 b	1025 a
		Total	**2070 a**	**690 b**	**1860 a**

Data are shown the means ± SE of three replications means in each line with a common letter are not significantly different ($p < 0.05$) based on Duncan test.

ABA level showed the same pattern in both cultivars. Nonetheless, because of the high level in free and bound forms in Gold Nugget, data obtained from this cultivar were at a much higher scale when compared to Akko XIII cultivar.

In this research, ABA level was determined to be considerably high during the flowering process in loquat trees. Similarly, Baydar and Ülger[5] discovered relationship between high ABA level and stimulus of flowering in safflower. Cappiello and Kling[10] determined increase of the ABA level at the bud burst period in pecan and Cornus cericea. Chen[2] stated

ABA level to be high in the shoot-tip of mango (*Mangifera indica* L.) plants at the early bud formation stage. Chen[1] also found out that ABA level increased significantly 30 d before bud formation, during flower bud formation and full bloom stages in lichi and the increase of ABA level caused a decrease in shoot growth.

Free-, bound- and total-Z levels were found to be 1500, 375 and 1875 ng/g at the beginning of the flowering period; 485, 105 and 590 ng/g at the full bloom stage; 1550, 570 and 2120 ng/g at the end of the flowering period in Gold Nugget cultivar. Free-, bound- and total-Z were found to be 1645, 425 and 2070 ng/g at the beginning of the flowering period, 275, 415 and 690 ng/g at the full bloom stage and 835, 1025 and 1860 ng/g at the end of the flowering period in Akko XIII loquat cultivar, respectively (Table-1).

Total-Z was determined to be maximum and statistically in the same group, at the beginning of the flowering period. However, this level decreased to its minimum (590 ng/g) at the full bloom stage.

Chen[2] determined that activity of cytoninin-like compounds increased in xylem exudates at the early flower bud formation period in 3 mango young trees grown in pots and that maximum activity level was at the full bloom satge. This research indicates that flowers involve high cytokinin content at the beginning of the flowering process.

ABA, which is one of the endogenous plant growth regulators and known to be an inhibitor, was concluded to have a direct effect on loquat tree's flowering process, whereas high levels of endogenous plant hormones that were experimentally determined at the flowering period in loquat tree, displayed the significance of these hormones in the flowering process.

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