Asian Journal of Chemistry Vol. 20, No. 5 (2008), 3786-3794

Some Endogenous Hormones in Loquat

NILDA ERSOY*, LAMI KAYNAK† and ABDULLAH KANKAYA‡
Department of Horticultural Science, Faculty of Agriculture
University of Selcuk, Konya, Turkey
E-mail: nersoy@selcuk.edu.tr

This work was carried out grown in an orchard of Citrus and Greenhouse Research Institute in Antalya. Fruit samples were taken from loquat trees (*Eriobotrya japonica* Lindl. cvs Gold Nugget and Akko XIII) on small, medium-size and ripe-fruit stages. Changes in the amounts of endogenous gibberellic acid (GA_3), indole-3-acetic acid (IAA), abscisic acid (ABA) and zeatin (Z) during these growth periods of the loquat. IAA, GA_3, ABA and Z were separated from plant extract and quantified by high performance liquid chromatography (HPLC) using a reversed-phase C_{18} column. Analysis of hormones was performed on a separate basis for seed and pericarp and levels of these hormones were found. According to the results, total-IAA was found high level in fruit flesh and seed samples in the small and mature loquat fruits. GA_3 was low level in mature fruit but it was high in the seed samples in the same period. ABA level was found maximum in the small fruit in the both of the fruit flesh and seeds. Level of zeatin was found maximum in the small fruit' s flesh and seeds.

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

INTRODUCTION

Determination of the changes in the internal hormone contents of the loquat fruit can be regarded as a step taken to understand the demands of this tissue. With the determination of the changes of this type of substances at different physiological periods in the loquat fruit, a basis will have been formed for subsequent studies. The loquat plant is one of the least studied plants in terms of hormone content and changes. We are of the opinion that hormone contents in the loquat fruit such as indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA) and zeatin (Z) have been studied for the first time by us to cover the entire vegetation period and significant information that will through light on the issue and compared with other plants[1-3].

†Department of Horticultural Science, Faculty of Agriculture,University of Akdeniz, Antalya, Turkey.

‡Department of Horticultural Science, Faculty of Agriculture, University of SDU, Isparta, Turkey.

Park and Park[4] found that when the fruit size increased, IAA did not change significantly while the amount of cytokinin increased. There is a limited number of studies concerning internal gibberellins levels in the loquat fruit. However, these studies usually concentrate on determination of the gibberellin levels in seeds. Kraft-Klaunzer and Mander[5] isolated 11 β-hydroxy gibberellin (GA_{84}), which is a new gibberellin, from the unripe loquat fruit seeds and determined the chemical structure of this substance through a synthesis of methyl esters. Yuda *et al.*[6,7] on the other hand, picked the unripe loquat fruit seeds from little fruit that formed 90 d after full bloom. They determined the gibberellins using gas-liquid chromatography/ mass spectroscopy (GLC/MS) techniques after a through cleaning with some chromatographic processes. In conclusion, he found GA_9, GA_{15}, GA_{27}, GA_{35} and GA_{50}. In addition to them, he found 6 substances similar to GA and two new GA's, *i.e.* GA_{80} (11 β-hydroxy-GA_7) and GA_{48} (11 β-hydroxy-GA_9).

Determination of the relationships between the internal hormone levels in different parts of the loquat fruit, which is an indispensable variety for both producers and consumers and the fruit behaviour is quite important. Instead of applying on a plant without knowing its hormone levels in its content and drawing conclusions on the basis of the reactions to it, by determining the levels and impact times, it will be easily ensured that subsequent studies succeed. It is necessary that the results that we obtained theoretically be adapted to practical studies that will be conducted later. It should be keep in mind that one of the goals of the studies on plant physiology is the understanding of plant growth metabolism and using it in increasing information generation[8].

EXPERIMENTAL

In this study, extremely healthy Gold Nugget and Akko XIII loquat fruit varieties, which were grafted onto quince trees in a parcel planted in 1993 where cultural applications were periodically conducted in Citrus Fruit and Greenhouse Research and Application Institute were used as material. A total of 18 trees, *i.e.* 9 trees for each variety, were selected for the experiment. Every three trees were considered a replication. Loquat fruit samples of Gold Nugget and Akko XIII were included in the experiment when they were in small fruit (0.59 and 0.47 g, respectively), medium-size fruit (0.59 and 0.47 g, respectively) and ripe fruit (43.19 and 40.13 g, respectively) stages. The obtained samples were kept in deep-freezer (-18 ºC) until extraction and purification procedures.

The method used to determine free-, bound- and total indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA) and zeatin (Z), followed that of Ersoy[2,3]. The extraction and purification procedures are shown on Fig. 1.

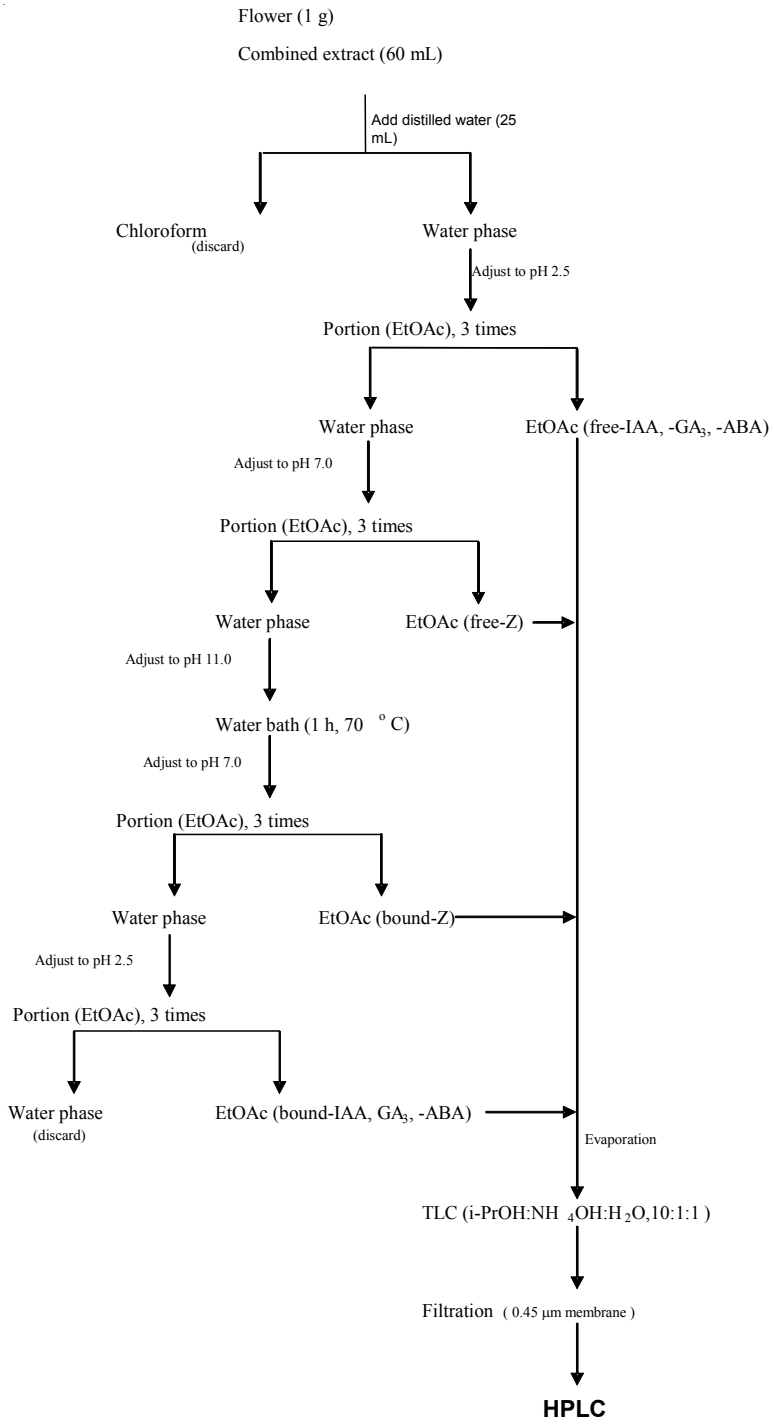

Fig. 1. Flow diagram outlining the extracts used in purification of IAA, GA_3, ABA and Z

Each sample of whole fruit (1 g fresh weight) was homogenized and combined 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. Then stored at -18 ºC for 2 weeks and then filtered.

Thin layer chromatography (TLC) was used for separation and purification of the dissolved methanol. Plates were placed in TLC tank containing a mixture of isopropanol:ammonia:bidistilled 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 for use in HPLC analysis.

HPLC analysis

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

Chromatographic conditions: Column: Supelcocil LC-18 (25 cmx 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}; Dedector: UV, 280 nm for IAA, 208 nm for GA3, 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 aquivalent 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[9].

RESULTS AND DISCUSSION

Free-, dependent - and total-IAA (indol-3-acetic acid), -GA_3 (gibberellic acid), -ABA (abscisic acid), -Z (zeatin) analyses were conducted on samples collected in three periods (small-green, medium-size-green and ripe-orange) of the Gold Nugget and Akko XIII loquat fruit varieties and the seasonal changes were determined.

The hormone levels in the samples were calculated as ng g^{-1} fresh weight (FW) by using linear regression equations formed with standards belonging to each hormone and making use of the right equations. Internal hormone levels of the samples were expressed as equivalent to standard synthetic hormones.

Indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA) and zeatin (Z) levels in the flesh and seeds of the fruit of the Gold Nugget and Akko XIII varieties are given in Table-1. As will be understood from

the data given in Table-1, some differences emerged concerning IAA, GA_3, ABA and Z hormone levels in the fruit flesh and seeds of the loquat fruit taken at different times and these differences were found to be statistically significant ($p \leq 0.05$).

Total-IAA was found in high levels for both varieties in small unripe fruit and ripe fruit periods just as in the free-IAA levels. In seeds, on the other hand, total-IAA was found to be high and at the same levels in unripe and ripe Gold Nugget loquat fruit variety, just as in the free-IAA levels but lower in medium-sized unripe fruit. In the Akko XIII loquat fruit variety, no change was observed in different periods in terms of total-IAA levels (Table-1).

It has been proven in many researches that auxins exist profusely in plants in free and dependent (to like glucose, amino acid and myoinositol compounds) forms[10]. This study also found that IAA, which is an internal regulator of growth, exits in both free and dependent forms in the fruit of both loquat varieties.

According to the experimental findings, when all samples are considered, maximum IAA was determined to be 493.15, 439.45 ng g^{-1}, respectively in the flesh of the ripe Gold Nugget and Akko XIII loquat fruit varieties. Indeed, Wurst *et al.*[11] reported that auxins, which are of indol type growth substances, existed in very small concentrations in plants and microorganisms. In studies conducted with GC-MS in the 1970's, it was found that IAA concentration in most plant tissues varied between 1-10.000 ng/g[12].

Park and Park[4] argue that IAA does not change during the fruit growth period. Our data, however, showed that IAA levels increased in parallel to the fruit growth and were in maximum level in the flesh of ripe fruit.

According to the results obtained, whereas IAA is at a rather low level in medium-sized fruit of both varieties, it reached a maximum level in small-unripe and ripe fruit. As a matter of fact, it is emphasized that auxins increase the development of conduction clusters together with gibberellins and thus accelerate assimilant transmission to the fruit. A fast growth phase occurs in the loquat fruit together with fruit behaviour. Fruit weight increases rapidly. Fruit that have reached a certain size undergo a stagnant period. In other words, fruit growth slows down. Thus, it has been assumed that IAA was at a low level and it might have a connection with this. Respiration increases and enzyme activity and water intake rise during fruit formation. These phenomena are also thought to have occurred to the impact of oxins. The increase in substance flux on the axis of stem is controlled by auxins as well as cytokinins[13]. The fact that oxins are in maximum levels in ripe fruit may stem from the fact that IAA internal hormone is probably transmitted to the fruit during this period.

TABLE-1
LEVELS OF FREE-, BOUND- AND TOTAL-IAA, GA_3, ABA AND Z IN THE FRUIT FLESH AND SEED ON DIFFERENT FRUIT DEVELOPMENTAL STAGES IN GOLD NUGGET AND AKKO XIII LOQUAT CULTIVARS

Variety	Part of fruit	Stage of fruit	Aquavalent levels of IAA (ng/g FW)			Aquavalent levels of GA_3 (ng/g FW)			Aquavalent levels of ABA (ng/g FW)			Aquavalent levels of Z (ng/g FW)		
			Forms of IAA			Forms of GA_3			Forms of ABA			Forms of Z		
			Free	Bound	Total	Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
Gold Nugget	Flesh	Small	314.6b*	15.8a	330.4b	763.30a*	218.87b	949.65a	41.6c	974.2a	1015.8a	1876.5a	994.8a	2871.3a
		Medium	0.0c	16.4a	16.4c	519.85b	391.15a	911.00a	139.2b	31.35c	170.55c	848.8b	884.3a	1733.1b
		Mature	477.2a	15.95a	493.15a	210.90c	263.10ab	474.00b	470.0a	325.45b	795.45b	1231.8ab	1545.4b	2777.2a
	Seed	Small	210.05*	15.95b	226.0a	188.15b*	224.20b	425.85b	899.85a	31.05b	912.9a	2881.1a	883.9b	3764.9a
		Medium	102.35b	17.6a	119.95b	164.30b	298.30b	462.33b	225.2c	59.47b	288.0c	1996.7b	995.2ab	2991.8b
		Mature	238.95a	17.8b	256.75a	332.40a	411.30a	743.70a	587.3b	94.45a	681.75b	1101.1ab	1304.5a	2405.5b
Akko XIII	Flesh	Small	319.85a	15.35b	335.2a	350.30a	215.10ab	565.40a	41.9c	1019.4a	1061.3a	1783.3a	1248b	3031.5a
		Medium	0.0b	16.2a	16.2b	423.25a	200.40b	623.65a	115.95b	11.0c	127.0b	1029.96b	1240.6b	2270.5b
		Mature	424.05a	15.46b	439.45a	227.77b	257.05a	484.82b	589.1a	318.3b	907.4a	1569.0a	1864a	3433.0a
	Seed	Small	256.8a	15.85b	272.65a	192.40b	264.30b	456.70b	574.35a	50.85a	625.2a	2568.2a	1014.4b	3580.5a
		Medium	267.8a	17.45ab	285.25a	166.95b	249.15b	416.10b	180.65b	49.1a	229.75b	1878.3b	1313.1b	3191.3a
		Mature	272.2a	17.9a	290.1a	337.50a	364.25a	701.75a	690.15a	124.7a	808.2a	492.3c	2471.9a	2964 b

Mean separation within columns by Duncan' s multiple range test ($p < 0.05$).

While fruit in the total-GA_3 Gold Nugget variety were small and medium-sized, they were statistically in the same group and in high amounts and it was found that total-GA_3 (474 ng/g) began to decrease with fruit ripening. In the Akko XIII loquat fruit variety, on the other hand, it was found that total-GA_3 was in high amounts (565.4 and 623.65 ng/g) in small and medium-sized fruit just as in free-GA_3, but when the fruit ripened, they were statistically in lower amounts (470.40 ng/g) (Table-1).

743.7 and 701.75 ng/g total-GA_3, respectively were found in maximum levels in the ripe fruit of the Gold Nugget and Akko XIII loquat fruit varieties. In small and medium-sized fruit, on the other hand, it was discovered that there was statistically no change in either variety and it was relatively low (Table-1).

According to the experimental results, when the whole of the fruit was considered (fruit flesh + seed) in the loquat fruit variety, endogenous GA_3 decreased in both varieties with ripening. In this period when fruit ripening occurs in the loquat fruit, spring shoots also develop at the same time. We conducted the studies on leaves in this period that internal GA_3 had reached maximum levels. Therefore, the internal GA_3 produced in the leaves is carried to the fruit in far lower amounts than in other periods and development of shoots is supported. In this context, Ramirez *et al.*[14] studied gibberellin contents of the shoots of apples, plums, apricots and peaches and discovered that gibberellins were generated in the apical section of the shoots and therefore they were effective in the vegetative growth fruit trees. They also found that as GA level increased, so did growth of the shoots. Lavee and Paskal[15] reported that GA_3 encouraged vegetative growth in young olive trees and concluded that GA_3 decreased flower but differentiation without significantly affecting vegetative development.

As is known, gibberellins enable growth in height by increasing cell growth and division just as auxins do. It might be possible that plants bring GA_3 levels on leaves to high levels in May, when growth of spring shoots occur in loquat fruit leaves in order to support growth of shoots. All of the stimulating substances participate in vegetative growth (auxin, gibberellin, cytokinin). Of these, especially gibberellins play a significant role in the development of vegetative elements as they are influential in the longitudinal growth of cells[16,17]. It is assumed that in the phenomenon of the storage of assimilants in the growing fruit, gibberellins, besides auxins, increase assimilant transportation to the fruit by enhancing the development of conduction clusters[13]. Indeed, internal GA_3 levels were found quite high in the loquat fruit throughout fruit growth.

Total-ABA amount in the Gold Nugget loquat fruit variety was in maximum level (1015.8 ng/g) in the small fruit period but it regressed to a minimum level (170.55 ng/g) when the fruit were in medium size. In the Akko XIII

loquat fruit variety, total-ABA, which was at a maximum level (1061.3 ng/g) when the fruit were small, reduced to a minimum level (127 ng/g) when in medium-sized fruit. However, it was found that there was an increase again with ripening (Table-1).

While total-ABA was at a maximum level in the seeds of small fruit in the Gold Nugget loquat variety (912.9 ng/g), it decreased to a minimum (288 ng/g) when the fruit reached medium-size, but it began to increase again when the fruit ripened. Total-ABA was found at a minimum level (229.75 ng/g) in the seeds of the medium-sized fruit of the Akko XIII loquat variety but at a maximum level (808,2 ng/g) with ripening (Table-1).

ABA, which was at a maximum level in the month of August, when the development of shoots came almost to a halt in both loquat fruit varieties and morphological separation occurred in the buds, demonstrated a steady decrease in paralel to blooming, fruit growth and shortening of days and fell to indeterminable levels in February, when there were small fruit in trees. However, an increase was observed in ABA level in May, when fruit began to ripen.

In present study, it is found that there were higher amounts of ABA in small fruit. Likewise, in his study on apples, Soejima *et al.*[18] found more ABA in the younger parts of the fruit.

Total-Z level in the Gold Nugget variety was at a maximum level (2871.3 ng/g) when fruit were small and unripe. It displayed a decrease in the flesh of medium-sized fruit but showed a steady state during the ripening process. In the Akko XIII loquat fruit variety, just as in the free form, maximum levels and values of the same group were obtained in small-unripe and ripe fruit. In the medium-sized fruit period, on the other hand, lower levels of total-Z were determined (Table-1).

In the Gold Nugget loquat fruit variety, total-Z was determined in a maximum level (3764.9 ng/g) when the fruit were small but in later periods lower levels that were statistically in the same group were obtained. It was found in a maximum level (3582.5 ng/g) in the Akko XIII loquat fruit variety just as in the Gold Nugget variety. This value decreased to the level of 3191.3 ng/g in medium-sized fruit and was found to be 2964 ng/g in ripe fruit (Table-1).

Zeatin reached maximum levels during the small fruit period. Substances that are effective in the regulation of growth by increasing cell division are cytochinins. They are synthesized in all meristematic tissues and especially in root ends. It was found to be effective in the transmission of nutrients and in metabolism. It was further argued that it prevented the aging and shedding of flowers, fruit and leaves and stopped taking root[19].

Kaynak *et al.*[20] studied changes in zeatin in the fig fruit that occurred due to growth and found that small fruit contained high amounts of zeatin.

In conclusion, it is believed that indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA) and zeatin (Z), which are internal vegetative hormones, have significant roles in the growth and ripening of loquat fruit and if the levels of these internal hormones, which were determined with the present study, are taken into consideration, they could form a basis for further studies.

ACKNOWLEDGEMENT

Thanks to TUBITAK for financial support.

REFERENCES

1. N. Ersoy and L. Kaynak, IV National Horticultural Plants Kongres, 8-12 Eylül, Akdeniz Üniversitesi Bahçe Bitkileri Anabilim Dali, pp. 157-158 (2003).
2. N. Ersoy, Ph.D. Thesis, Akdeniz University, p. 93 (2004).
3. N. Ersoy, L. Kaynak and A. Kankaya, *Asian J. Chem.*, **20**, 571 (2008).
4. Y.S. Park and H.S. Park, *J. Korean Soc. Horticult. Sci.*, **36**, 522 (1995).
5. P. Kraft-Klaunzer and L.N. Mander, *Phyt. Chem.*, **31**, 2519 (1992).
6. E. Yuda, T. Yokota, M. Nonaka, Y. Fukushima, S. Nakagawa, N. Katsura, Y. Ota, N. Murofushi and N. Takahashi, Proceedings of the Plant Growth Regulator Society of America, pp. 167-173 (1987).
7. E. Yuda, S. Nakagawa, N. Mourfushi, T. Yokota, N. Takahashi, M. Koshioka, Y. Murakami, D. Pearce, R.P. Pharis, G.L. Patrick, L.N. Mander and P. Kraft-Klaunzer, *Biosci. Biotechnol. Biochem.*, **56**, 17 (1992).
8. N. Ersoy and L. Kaynak, *J. Akdeniz Univ. Agric. Fac.*, **11**, 51 (1998).
9. SAS Institute, SAS® User's Guide, Release 6.03 Edition, Cary, North Caroline, SAS Institute Inc. (1987).
10. N.P. Ünsal, Plant Growth Substances, Istanbul University Pres and Film Center, Istanbul, p. 357 (1993).
11. M. Wurst, Z. Prikryl and V. Vancura, *J. Chromatogr.*, **284**, 499 (1984).
12. S. Ülger, Ph.D. Thesis, Akdeniz University, p. 204 (1997).
13. M. Seçer, *Derim*, **6**, 109 (1989).
14. H. Ramirez, A. Rumayor and J.N. Estrada, *Acta Horticult.*, **134**, 179 (1983).
15. S. Lavee and A. Paskal, *Adv. Hort. Sci.*, **7**, 83 (1993).
16. I. Çimen, *Derim*, **5**, 134 (1988).
17. N. Ersoy and M. Paksoy, *J. Biol. Sci.*, **9**, 30 (2006).
18. J. Soejima, M. Watanabe and T. Moriguchi, *J. Japan Soc. Hort. Sci.*, **58**, 819 (1990).
19. M.N. Westwood, Hormones and Growth Regulators, Temparate Zone Pomology: Physiology and Culture, Timber Press, Inc. 9999 S.W. Wilshire, Suite 124, Portland, Oregon, p. 97225 (1993).
20. L. Kaynak, N. Ersoy, A. Karadeniz and S. Gözlekçi, The Second Symposium on Fig, Badajoz (Espana), 07-11 May, Acta Horticulturae, 2001 (Represented).

(*Received*: 25 September 2007; *Accepted*: 6 February 2008) AJC-6313