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Levels of Internal Indole-3-acetic Acid and Abscisic Acid in Different Physiological Growth Periods in The Leaves of Loquat (*Eriobotrya japonica* Lindl.) Fruit

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The loquat fruit variety, which blooms in fall and bears flowers and fruit in winter, differs from other varieties in terms of periods of physiological growth. Plants produce the nutrients they need with their leaves. Hence, it is important to determine the levels of internal growth regulating substances found in leaves of the loquat fruit in different growth periods. The present study aims to determine internal indole-3-acetic acid (IAA) and abscisic acid (ABA) levels by taking samples from the leaves of the Gold Nugget and Akko XIII loquat fruit varieties in different physiological growth periods. According to the results, the total-IAA level increased two-fold in the first year in May, when fruit begin to ripen, in comparison to August, when morphological differentiation occur, an one-fold in the second year. The total-IAA level in the Akko XIII loquat fruit variety increased one-fold in May in comparison to August in the first year and 4-fold in the second year. The total-ABA level in the Gold Nugget loquat fruit variety decreased three-fold in May in comparison to August in the first year and four and a half-fold in the second year. On the other hand, the total-ABA level in the Ako XIII loquat fruit variety decreased nearly three-fold in May, when fruit begin to ripen, in comparison to August, when morphological differentiation occurred and 3-fold in the second year.

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

INTRODUCTION

The loquat fruit is a treelet or a tree from the *Pomoideae* subfamily of the *Rosaceae* family of the *Rosales* team. The origin of the loquat fruit, which is a subtropical fruit variety, is in China, Japan and Northern India. It is believed to have been introduced in Turkey from Algeria and the Lebanon[1].

Although the loquat fruit farming in Turkey is more common in the Mediterranean region, it is nevertheless grown in the Aegean and the Black Sea regions[2]. Its total production stood at 6500 tons in 1985 but rose to 11500 tons in 2001[3].

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Leaves are one of the bloodlines of the plant. They are no difference from a genuine chemical plant or a laboratory. They are the most indispensable part for the survival of plants and they are respiratory organs that inhale and exhale, perspire and feed the plant. They perform photosynthesis, a vital phenomenon, thereby forming the plant's source of nutrients. Hence, they assume significant roles in blooming, fruit behaviour, fruit development and other physiological phenomena. The hormones generated by the plant are used to make such extraordinary events to happen[4].

Fruit varieties of temperate climate blossom in spring after short day conditions of winter are left behind. The number of the types of fruit that blossom in Fall and bear fruit and flowers in winter is next to nothing. One of these fruit varieties is the loquat fruit. Because of this physiological difference, it would be beneficial to investigate internal hormone levels. As a matter of fact, Wurst *et al.*[5], reported that the IAA levels which plants contain are affected by many factors (age of the plant, length of the day, position of light, drought *etc.*) and that these factors might cause changes of up to 10 to 50 %.

The existence or non-existence of hormones, which play an important part in both generative and vegetative development of plants and which naturally exist within the plant, their type, their effect and if they exist, their amount must be known for sure. It is known that growth regulators inherent in plants undergo periodical changes in terms of type and amount. Internal growth regulators may be at different levels for species, varieties and even types. Different limbs of the plant also yield different results in terms of internal growth regulators. If the changes of the inherent growth regulators in different periods are determined, it will be easier to determine the limits of the dosage to be administered to the plant in cases of external hormone additions[6]. The present study determined the changes that occurred in IAA and ABA in the leaves of the Gold Nugget and Akko XIII loquat fruit varieties in different physiological periods. The absence of such a study in the relevant literature adds to the value of the present study.

EXPERIMENTAL

In this study, Gold Nugget and Akko XIII loquat fruit varieties that were grafted on the quince rootstock in the loquat fruit garden established in 1993 at Kayaburnu Station of the Western Mediterranean Agricultural Research Institute were used as research material. Eighteen trees, *i.e.*, 9 for each variety, were selected for the study. Every 3 trees were considered a replication. Leaf samples were taken from trees in different physiological periods [morphological differentiation (August), beginning bloom stage (November), fruit set (February) and fruit maturation (May)]. The samples collected from the experimental plot were kept in a deep freezer (-18 °C).

Method: IAA and ABA analyses of the samples were conducted according to Ersoy *et al.*[7]. SAS packet program was used in statistical analyses. The experiment was performed in 3 replications, each replication including 3 tress and designed according to 'Random Plots' experimental design. 'Duncan multiple comparison' test was used in comparison of averages[8]. Indole-3-acetic acid and abscisic acid levels of the samples were calculated as μg g^{-1} depending on lineer regression relationships obtained from standard synthetic-IAA and -ABA areas prepared in various concentrations.

RESULTS AND DISCUSSION

Analyses of IAA and ABA were conducted on leaf samples collected 4 times a year from fruit bearing shoots of the Gold Nugget and Akko XIII loquat fruit varieties for a period of 2 years and seasonal changes were determined.

IAA and ABA levels: Total -IAA and -ABA amounts in present data state the total of free and bound -IAA and ABA values. IAA and ABA amounts in the samples were calculated as fresh weight μg g^{-1} using correct equations obtained from linear regression relationships formed with standards. Internal IAA and ABA amounts in the samples were stated as equal to standard synthetic-IAA and ABA.

As can be inferred from the data obtained, some changes occurred in terms of IAA hormone levels in the loquat fruit leaves collected at different periods and these differences were considered statistically significant.

TABLE-1
FREE-, BOUND- AND TOTAL-IAA AND -ABA LEVELS IN GOLD NUGGET AND AKKO XIII LOQUAT VARIETIES LEAVES IN TWO YEARS

Cultivars	Stages	IAA levels (μg/g fresh weight)			Stages	ABA levels (μg/g fresh weight)		
		Free	Bound	Total		Free	Bound	Total
Gold Nugget	Aug. 99	0 c*	0.08 a	0.08 b	Aug. 99	0.28 a*	0.00 a	0.28 a
	Nov. 99	0.03 b	0.08 a	0.11 b	Nov. 99	0.09 b	0.03 a	0.08 b
	Feb. 00	0.03 b	0.06 b	0.09 b	Feb. 00	0.00 c	0.02 a	0.02 b
	May 00	0.10 a	0.07 ab	0.17 a	May 00	0.00 c	0.09 a	0.09 b
	Aug. 00	0.02 c	0.03 bc	0.05 b	Aug. 00	0.31 a	0.00 b	0.31 a
	Nov. 00	0.12 b	0.05 a	0.17 a	Nov. 00	0.01 b	0.26 a	0.27 a
	Feb. 01	0.16 a	0.02 c	0.17 a	Feb. 01	0.00 b	0.03 b	0.03 b
	May 01	0.18 a	0.04 ab	0.22 a	May 01	0.00 b	0.07 b	0.07 b
Akko XIII	Aug. 99	0.03 b	0.04 a	0.07 a	Aug. 99	0.31 a	0.00 b	0.31 a
	Nov. 99	0.07 a	0.02 b	0.09 a	Nov. 99	0.15 b	0.00 b	0.15 b
	Feb. 00	0.06 a	0.03 ab	0.09 a	Feb. 00	0.00 c	0.01 b	0.01 c
	May 00	0.07 a	0.03 ab	0.09 a	May 00	0.00 c	0.12 a	0.12 b
	Aug. 00	0.03 b	0.01 b	0.05 b	Aug. 00	0.06 b	0.14 a	0.20 a
	Nov. 00	0.16 a	0.04 a	0.20 a	Nov. 00	0.16 a	0.07 b	0.23 a
	Feb. 01	0.16 a	0.03 a	0.19 a	Feb. 01	0.00 c	0.03 b	0.03 b
	May 01	0.19 a	0 b	0.19 a	May 01	0.00 c	0.06 b	0.06 b

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

Free-IAA and -ABA levels: While there existed no free-IAA in the Gold Nugget loquat fruit variety as of August 1999, it increased slightly in November 1999 and reached a maximum level in May 2000 after a steady progress in February 2000. In the second year, the free IAA amount, which was at a minimum level in

August 2000, made a steady progress until May 2001, but the values obtained between February 2001 and May 2001 were in the same group.

In the Akko XIII loquat fruit variety, free-IAA was at the same level in the months of August in both years, *i.e.* at a minimum level, but after a rise in November remained constant in February and May. However, the values obtained in these three months were at higher levels in the second year.

According to the first year's data of the Gold Nugget loquat fruit variety, while free-ABA level was at the highest in August, it fell considerably in November, when first blooming occurred and it could not be found in February and May. In the second year, free-ABA was obtained only in August, when morphological differentiation occurred in November, when first blooming took place. Found in high amounts in August, free-ABA decreased almost to zero in November with sharp fall. No ABA in free form was found in February and May as in the first year. No significant difference was found among the values obtained in the months of February, May and November as a result of the statistical analysis conducted.

In the Akko XIII loquat fruit variety, on the other hand, the free-ABA, which was remarkably high in the first year in the August period, when morphological differentiation occurred, was halved in November, when first blooming took place. Free-ABA was not found in other periods. According to the data of the second year, low free-ABA level in August increased in November. In the months of February and May, on the other hand, when fruit growth occurred, no free-ABA was found as in the first year.

Bound-IAA and -ABA levels: In the Gold Nugget loquat fruit variety, bound-IAA level was found at the same level in the months of August and November. Subsequent to a slight decrease in February, no considerable variation was observed in May. In the second year, on the other hand, bound-IAA level was measured to be 0.03, 0.05, 0.02, 0.04 μg g^{-1}, respectively in the months of August-November-February and May.

While bound-IAA level in the Akko XIII loquat fruit variety was 0.04 μg g^{-1} in August in the first year, it decreased to as low as 0.02 μg g^{-1} in November but remained constant in the months of February and May with a slight increase. In the second year, on the other hand, no bound-IAA was encountered in May and the value of 0.01 μg g^{-1} obtained in August was in the same group as in May. Higher values were obtained in the months of November-February, which were in the same group.

No bound-ABA was encountered in August in the first year in the Gold Nugget loquat fruit variety and it was in ignorable levels in other months. In the second year, on the other hand, considerably high level of bound-ABA was determined in the month of November, but it was not encountered in the other months.

In the Akko XIII loquat fruit variety, no bound-ABA was found in the months of August-November and February, but in May bound-ABA at a level of 0.12 μg g^{-1} was determined. In the second year, on the other hand, 0.14 μg g^{-1} bound-ABA was found in August whereas lower levels of bound-ABA were encountered in the other months. As a result of the statistical analysis conducted, it was found that these values obtained in the months of November-February and May were in the same group.

Total-IAA and -ABA levels: No statistically significant change was observed in the months of August-November and February in the first year in the Gold Nugget loquat fruit variety, but maximum levels of total-IAA were obtained in May. The amount of total-IAA in the month of August in the second year, which was at a minimum level, increased in November but displayed no change in the months of February and May. The total-IAA level increased 2-fold in the first year in May, when fruit begin to ripen, in comparison to August, when morphological differentiation occur, an one-fold in the second year.

In the Akko XIII loquat fruit variety, the total-IAA level did not change in the first year. In the second year, the total-IAA, which was at a minimum level in August, increased in November but remained constant in February and May. The total-IAA level in the Akko XIII loquat fruit variety increased one-fold in May in comparison to August in the first year and 4-fold in the second year.

Whereas there was 0.28 µg g^{-1} total-ABA in August 1999 in the first year in the Gold Nugget loquat fruit variety, it was found in much less amount in other months and the values obtained in those months were statistically in the same group. In the second year, the total-ABA, which was at high levels in the months of August and November, fell to rather low levels in February and May. The total-ABA level in the Gold Nugget loquat fruit variety decreased three-fold in May in comparison to August in the first year and four and a half-fold in the second year.

In the Akko XIII loquat fruit variety, on the other hand, the total-ABA was at a maximum level in August in the first year, but it was halved in November and fell to a minimum level in February. However, it displayed another increase in May with a value of 0.12 µg g^{-1}. In the second year, while the total-ABA was at a maximum level in the months of August and November, it decreased to remarkably low levels in February and May. The total-ABA level decreased nearly three-fold in May, when fruit begin to ripen, in comparison to August, when morphological differentiation occurred and three-fold in the second year.

It has been proven by many studies that auxins exist commonly in plants in free and bound (on compounds such as glucose, amino acid and myoinocitol) forms[9]. The present study also determined that IAA, which is an internal growth regulator, existed in leaves in both free and bound forms in both loquat fruit varieties.

Experimental findings found that Gold Nugget loquat fruit variety contained 0.22 µg g^{-1} IAA in its leaves at the most whereas Akko XIII variety contained 0.20 µg g^{-1} IAA. Wurst *et al.*[5], reported that, auxins, which are indol type growth substances, exist in very limited concentrations in plants and microorganisms. It was found in studies conducted using GC-MS in the 1970's that IAA concentration varied between 1 to 10.000 ng g^{-1} in most plant tissues[10].

In the present study, the amount of IAA, which is generally at a minimum level in both loquat fruit varieties in August, increased steadily and reached maximum levels in May of the next developmental year. Likewise, Okuda[11] found that IAA, which was at a low level in Aoshima and Miyagawa orange varieties prior to December, began to increase in March before new shoots came out. Chen[12], reported a lack of change in IAA levels in the litchi fruit variety in samples collected in 5 different periods (1-leaf development, 2-bud resting, 3-30 d before flower formatin, 4-formation of flower bud, 5-full bloom). The present study also found that there was no change in terms of total-IAA in the samples collected from the Akko XIII loquat fruit variety in the first year.

The fact that internal IAA increased during the budding and initial blooming periods, as was demonstrated by our study, was determined by Baydar and Ülger[13] in aspir and by Cappiello and Kling[14] in pican.

According to Cappiello and Kling[14], Alden had found that IAA activity increased in *Pinus silvestris* during the development of shoots and reached a maximum level in June. In *Cornus cericea*, on the other hand, IAA levels increased actively when shoots began to lengthen. The present study also found that IAA was in maximum levels in May, when shoots were in active spring developmental stage.

Park and Park[15] maintains that IAA does not change during fruit growth. However, substantial increases were observed in all varieties where free and total-IAA levels were investigated from November, when trees bloomed, to May, when fruit ripened. A substantial change was not observed only in the bound-IAA, which may have resulted from the experimental material. For, while present study observes changes of IAA in leaves, Park and Park[15] refers to changes in fruit.

Existing in maximum levels in both loquat fruit varieties in August, when growth of shoots almost stop and morphological differentiation occurs in buds, ABA demonstrated a steady decrease due to blooming, fruit growth and shortening of days and fell to undetectable levels in February, when there are small fruit in trees. In May, on the other hand, when fruit began to ripen, a slight increase was observed in ABA level.

Present study found higher total-ABA levels in young leaf samples collected from the loquat trees. Likewise, Soejima *et al.*[16] determined more ABA in young parts of the plant in his study conducted on apples.

ABA was found in relatively higher levels in the present study in late summer and early fall when loquat trees bloom. In the same vein, Baydar and Ülger[13] determined a close relationship between high levels of ABA in aspir and stimulation of blooming. Cappiello and Kling[14] found that ABA increased during the bud bursting period in the pican nut and *Cornus cericea*. Likewise, Chen[17] found that ABA was in subtstantially high amounts in the early flower formation period in the samples of shoots' ends from three mango plants grown in vases. Chen[12] also found that ABA levels increased significantly in litchis 30 d before bud formation and bud formation and full blooming periods and that increase in ABA slowed down development of shoots.

According to the results obtained, IAA levels, which were quite low in the loquat tree leaves during the blooming and fruit bearing periods, reached a maximum in May, when shoots were undergoing their spring growth and fruit began to ripen. In this context, it has been emphasized that auxins increased development of conduction clusters together with gibberellins thereby accelerating assimilant transmission to fruit. Respiration increases during fruit formation and enzyme activity and water absorption rise. These phenomena are supposed to occur due to the effect of auxins. The increase in the movement of substance on the axis of stem is under the influence of auxins besides cytochinins[18]. Auxins were found in maximum levels in loquat tree leaves when fruit began to ripen. Therefore, internal IAA hormone may be transmitted to the fruit during this period. Moreover, according to experimental findings, a maximum of 0.22 µg g^{-1} IAA existed in the Gold Nugget loquat fruit variety whereas 0.19 µg g^{-1} IAA existed in the Akko XIII variety. Indole-3-acetic acid levels were rather low (the highest level was found in the Gold Nugget variety as 0.22 µg g^{-1} in May 2001) in loquat tree leaves in comparison other hormones studied.

Trees which shed their leaves in winter need a certain period of cooling (resting) in order to blossom in spring. Resting period is regulated by growth-preventing substances. Although auxins are stimulants, they act as preventers here, because NAA applications have delayed blooming time in spring. The IAA levels in the loquat fruit leaves were at a minimum in fall, when blooming takes place, in comparison to other periods.

ABA, which was at a maximum level in both loquat fruit varieties in August when morphological differentiation occurred, demonstrated a steady decrease in accordance with growth and development and became hardly detectable in February, when there were small fruit on trees. In May, on the other hand, when fruit began to ripen, a slight increase was observed in the amount of ABA.

Abscisic acid (ABA), which was at a low level in respect of GA_3 and Z like IAA, demonstrated its highest figures in August, when morphological diferentiation took place. ABA is at a maximum in this month and varies between 2-3 µg g^{-1}. ABA levels displayed a constant decrease depending on growth and development. Absiscic acid is a hormone that acts counter to gibberellic acid. Exterior application of ABA in the period of morphological differentiation in years when blooming will be ample may prevent formation of flower budding. As a result, since the tree will not form many flowers, it gets less tired and hence more regular harvest becomes possible. The results well-planned in theory can not always be applied in practice. Therefore, feasibility of ABA application must be investigated so that the practical results of this application, which seems possible in theory, can be put forth in practice.

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