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CHANGES IN ZEATIN LEVELS OF DEVELOPPING FIG FRUIT (Ficus carica L. cv. Bursa Siyahı)

Nilda ERSOY[1] Şadiye GÖZLEKÇİ[2] Asuman KARADENİZ[3] Lami KAYNAK[2]

[1]Selcuk University, Faculty of Agriculture, Department of Horticulture, Konya/Turkey

[2]Akdeniz University, Faculty of Agriculture, Department of Horticulture, Antalya/Turkey

[3]Akdeniz University, Faculty of Science, Department of Biology, Antalya/Turkey

ABSTRACT

In this study, the levels of endogenous free-, bound- and total-zeatin (Z) has been examined in fig fruit (Ficus carica L. cv. Bursa Siyahı). The fruit harvested at different developmental stages were analysed for endogenous zeatin content. The index of maturity was stage 1 (green, small), stage 2 (green, big) and stage 3 (dark brown, harvest maturity). This work describes the identification of endogenous cytokinins through HPLC methodology in developing fig fruits.

At the end of the research, it has been showed that endogenous zeatin plant hormones, which has been synthesised, were both free and bound forms. Concentration of free, bound and total Z during the growing stages varied between 536-1707 ng.g^{-1}, 661-1932 ng.g^{-1} and 1208-2797 ng.g^{-1}, respectively. High concentrations of endogenous Z have been found in early stages of fig fruit (1). The large accumulation of endogenous Z in the first growth stage may have an important function in setting the development pattern of the "Bursa Siyahı" fig fruit.

Keywords: *Fig, Ficus carica L., Bursa Siyahı, zeatin, fruit development, High Performance Liquid Chromatography (HPLC)*

GELİŞEN İNCİR (Ficus carica L. Bursa Siyahı) MEYVELERİNİN İÇSEL ZEATİN DÜZEYLERİNDEKİ DEĞİŞİMLER

ÖZET

Bu çalışmada, incir meyvelerindeki (Ficus carica L. Bursa Siyahı çeşidi) içsel serbest-, bağlı- ve toplam-zeatin (Z) düzeyleri belirlenmiştir. Değişik meyve gelişim dönemlerinde hasat edilen meyvelerdeki içsel zeatin içerikleri bakımından analizler yapılmıştır. Meyvelerdeki olgunluk indeksleri dönem 1 (yeşil, küçük), dönem 2 (yeşil, büyük) ve dönem 3 (koyu kahverengi, hasat olgunluğunda) şeklinde olmuştur. Mevcut çalışmada, gelişen incir meyvelerindeki içsel zeatin düzeylerinin belirlenmesinde HPLC tekniği kullanılmıştır.

Çalışma sonucunda, incir meyvesinde zeatinin hem serbest hem de bağlı formda sentezlendiği belirlenmiştir. Gelişme dönemleri boyunca serbest, bağlı ve toplam-Z miktarları sırasıyla 536-1707 ng.g^{-1}, 661-1932 ng.g^{-1} ve 1208-2797 ng.g^{-1} aralıklarında değişim göstermiştir. En yüksek içsel zeatin düzeyi meyvelerin erken gelişim dönemlerinde (stage 1) belirlenmiştir. Bursa Siyahı incir meyvesinin ilk gelişim dönemlerinde yüksek düzeylerde Z birikimi bu hormonun meyve tutumunda etkin bir rol oynayabileceği sonucunu göstermiştir.

Anahtar Kelimeler: *İncir, Ficus carica L., Bursa Siyahı, zeatin, meyve gelişimi, Yüksek Performanslı Sıvı Kromatografisi (HPLC)*

INTRODUCTION

"Bursa Siyahı" fig (*Ficus carica* L.) is a parthenocarpic cultivar is very popular in Turkey due to its superior taste and size.

Plant hormones play an integral role in controlling the growth and development of plants (Ramirez et al., 1983, Salisburry et al., 1992). Phytohormones in plants are found in minute concentrations. For this reason, special technics are needed to measure them accurately. High performance liquid chromatography (HPLC) is a progressive analytical and preparative method which permits highly effective separation, isolation, identification and measurement of substances found in biological material in particular. The development of HPLC provides an accurate physicochemical method of detecting and separating cytokinins, gibberellins, indole acetic acid and abscisic acid (Baydar et al., 1998).

Hormonal control is a factor of prime importance among those influencing the setting of fruit and evidence points to the central role that cytokinins play in such a process. First analysis of developing fruits and seeds shows a high content of endogenous cytokinins. Furthermore, these hormones can induce setting and parthenocarpic development of fruit in several species when applied exogenously. A third kind of evidence shows that the probability for a fruit to set is directly related to the concentration of cytokinins translated from the root. However mechanisms of action of cytokinins on setting and development of fruits are far from clear and many gaps exist (Arnau et al., 1999).

Available information listed in the literature shows that endogenous cytokinis mostly occur during the early stages of fruit development (Ghosh et al., 1983; Hernandez-Minana et al., 1989; Palavan 1993; Lewis et al., 1996). Because this is a very complex and highly co-ordinated process, a traditional approach for analysing cytokinins on the understand the role of these hormones. In addition, cytokinins are group of plant hormones representing a wide array of natural compounds described so far. It falls from this that those cytokinins involved need to be identified by reliable methods and their endogenous levels accurately quantified throughout the developmental process (Arnau et al., 1999). Noting is known about the relationship between endogenous Z and fig fruit development. In this report, free, bound and total-Z in concentrations in the parthenocarpic fig fruit were measured, and the relationship between the zeatin and fruit development was examined.

MATERIAL AND METHODS

Material

Fruits were sampled at three different growth stages, having fresh weights of 2.41, 18.32 and 43.9 g respectively. Fruits weighing 43.9 g were at the mature dark brown stage and had attained full size. At each sampling, 10 to 15 fruits were harvested from the tree. First sample was taken on 15 July (fist stage), second was taken 15 August (second stage) and the last was taken 15 September (third stage). The fruit were weight (fresh weight) and the length and maximum width of each fruit determined using digital callipers. The dissected samples were placed in separate plastic bags and stored at – 18 ºC for Z analysis. Endogenous Z hormone analyses were repeated at least three times.

Methods

The method used to determine free and bound Z followed that on Ersoy 2004 and Ersoy et all (2008). The extraction and purification procedures are shown on Fig 1.

Each sample of whole fruit (1 g FW) was homogenised and combined extract (a mixture of methanol: chloroform:2 N ammonia, 12:5:3, 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)

Thin Layer Chromatography (TLC) was used for separation and purification of the extracts dissolved methanol. Plates were placed in TLC tank containing a mixture of isopropanol:amonia:bidistilled water (10:1:1, v:v:v). The relative fluidity (R_f) bands of hormones on the plates were studied by 254 nm UV lamp. The hormone extracts on the R_f bands were dissolved in grade methanol for use in HPLC analysis.

HPLC analysis

Apparatus

Analysis of Z was performed on a Cecil 1100 model equipped with UV detector and Cecil 1100 model pumps enabling the use of a concentration gradient of the mobile phase.

Chromatographic conditions

- Column:Supelcocil LC-18 (25cmx4.6mm and 5 µm)
- Column temperature: Room temperature (18-22 ºC)
- Mobile phase:70 % methanol:distilled water (70:30)
- Flow rate: 1.0 $ml.min^{-1}$
- Detector: UV, 254 nm
- Injection concentrate: 10 µl
- Total run time: 15 min

The amounts of Z were expressed as equivalent standard synthetic Z. Total Z was obtained as the sum of free and bound Z.

Statistical Analysis

Analysis of Variance was performed using the Statistical Analysis System (SAS Institute 1987).

RESULTS AND DISCUSSION

The changes in levels of free, bound and total Z during fruit development are shown in Table 1. HPLC analyses of free, bound and total Z levels are presented in Figure 2 and 3, respectively.

The data presented in Table 1 show that amounts of total Z decreased gradually till the end of the developmental period. Total-Z ranged from 1208 to 2797 $ng.g^{-1}$ fresh weight (FW) during the growing stages.

Stage 1, in July, was characterised by the presence of very high concentrations of free-Z, Stage 2, in August, was a time when the free-Z content of the fruit decreased sharply, in contrast in Stage 3, in September, free-Z concentration increased slightly but not important.

Concentration of bound-Z during the growing periods ranged from about 661 to 1932 $ng.g^{-1}$. Differences in bound-Z in fruit were marked, the maximum being reached in August (Stage 2) and the minimum in September (Stage 3, maturation period).

It is suggested that these three periods represent possible changes in both the mode of supply and action of Z in other parts of the tree.

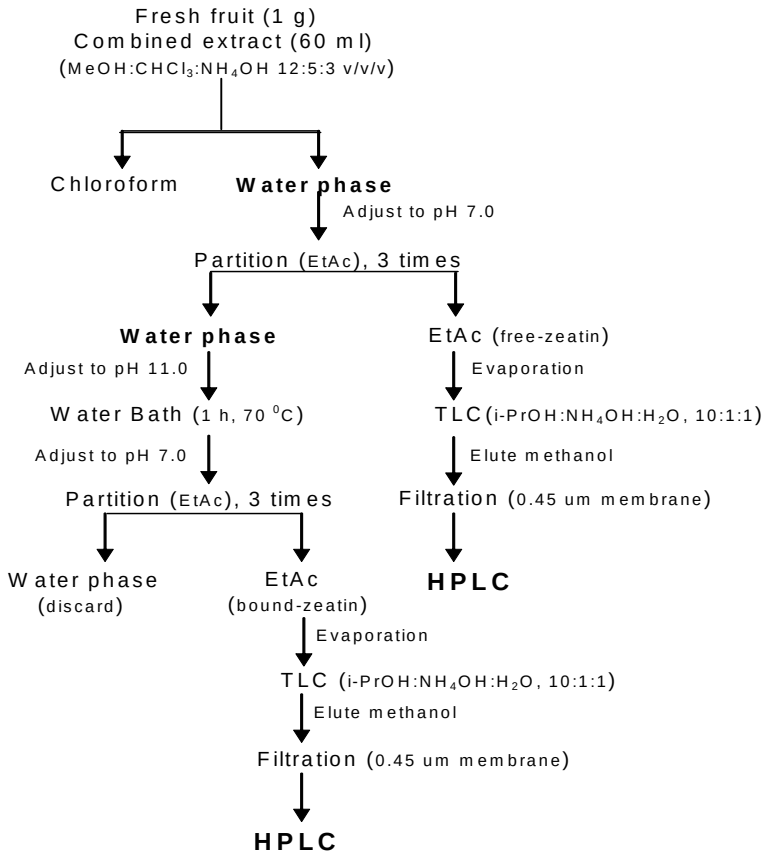

Figure 1. Flow diagram outlining the extracts used in purification of zeatin

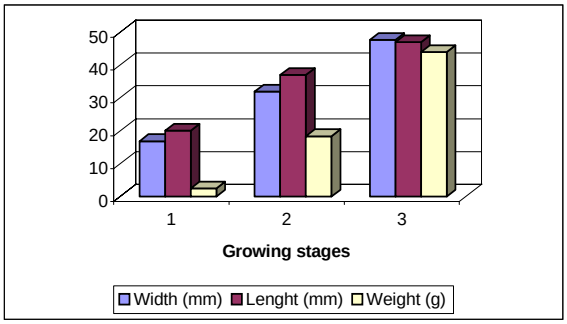

Figure 2. Growth parameters of developing fig fruit

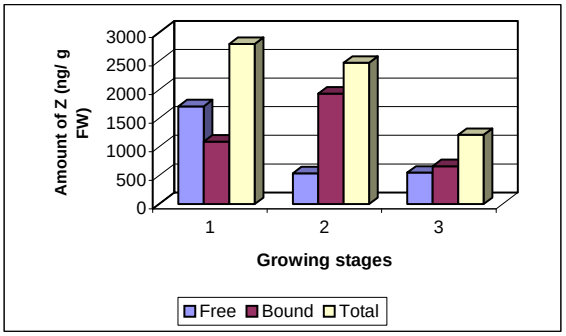

Figure 3. Changes in the levels of free-, bound- and total-zeatin in developing fig fruit

The large accumulation of Z in the first growth stage may have an important function in setting the development pattern of the fruit. High concentrations of endogenous Z have also been found during early stages of fruit in Aegle marmelos (Gosh et al., 1983), citrus (Hernandez-Minana et al., 1989), kiwifruit (Lewis et al., 1996) and peach (Arnau et al., 1999). Similarly, the changes in the levels of endogenous growth regulators, and their effects on the fruit development of *R. roxburghii* were investigated. The results showed that the contents of gibberellic acid (GA), IAA and zeatin riboside (ZR) in the flower receptacle of the young fruit were initially high then decreased with maturation (WeiGuo et al., 2004). In another research conducted by Chen (1983), the cytokinin activity has been isolated and identified from extracts of immature mango (*Mangifera indica* L.) fruits. According to the results about this review (Chen 1983), the structures of zeatin, zeatin riboside, and N(6)-(Delta(2)-isopentenyl) adenine riboside were confirmed on the basis of their chromatographic behavior and mass spectra of trimethylsilyl derivatives. Both trans and cis isomers of zeatin and zeatin riboside were also identified by the retention times of high performance liquid chromatography. In addition, an unidentified compound appeared to be a cytokinin glucoside. The concentration of cytokinins in the pa-

nicle and pulp of mango reached a maximum 5 to 10 days after full bloom and decreased rapidly thereafter. The quantity of cytokinins in pulp per fruit increased from the 10th day after full bloom, the maximum being attained around the 50th day after full bloom. Some plant hormones important effect in the fruit set. Auxins, cytokinins and gibberellins also play a role in growth of the young fruit (Sarivastava, 2002).

Table 1. Amounts of free, bound and total zeatin in developing fig fruit[1].

Growing Stages	EQUVALENTS AMOUNTS OF ZEATIN (ng.g^{-1} FW)		
	Forms of zeatin		
	Free	Bound	Total
I	1707.07 ± 20.32 a	1090,3 ± 14.32 a	2796,97 ± 16.83 a
II	536,9 ± 7.18 b	1932 ± 59.42 b	2468.90 ± 66.27 b
III	548.27 ± 18.85 b	661.17 ± 15.80 c	1208,8 ± 22.17 c

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

Zeatin content in the young fig *(Ficus carica L. cv. Bursa Siyahı)* fruit was found very high level and this plant hormone is very important fig fruit set.

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