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Full Length Research Paper

The efficacy of endogenous gibberellic acid for parthenocarpy in eggplant (*Solanum melongena* L.)

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Eggplants are generally grown by winter in Mediterranean areas. Therefore, growers prefer to use parthenocarpic fruit and plant growth regulators. This study determined the relationship between flower development and gibberellic acid (GA_3) levels in parthenocarpic and non-parthenocarpic eggplant (*Solanum melongena* L.) genotypes. A single crop was grown in an unheated greenhouse at the Bati Akdeniz Agricultural Research Institute, Antalya, Turkey, and samples were collected from November to March, GA_3 levels were measured with reverse phase high performance liquid chromatography at five different stages between small buds and small fruits. The results showed that there was no relationship between flower development and GA_3 levels in parthenocarpic and non-parthenocarpic eggplant genotypes.

Key words: HPLC, relation, cultivation, greenhouse, genotype, flower.

INTRODUCTION

Eggplant is a warm season crop (Romano and Leonardi, 1994) requiring high temperatures during growth and development compared with other *Solanaceous* crops. For good plant development and sufficient fruit setting, minimum day/night temperatures are 23 to 25/15°C (Abak and Guler, 1994).

Eggplant is cultivated as a single crop production in Mediterranean greenhouses, with seedlings planted in greenhouses in September and harvested by July (Ekiz and Boyaci, 2001). Generally, the temperature fluctuates during the eggplant growth period in Mediterranean greenhouses (Romano and Leonardi, 1994). A minimum night temperature of 4 to 8°C occurs between December and February and is the most critical period for eggplant cultivation. For economic reasons, growers only use heating devices when there is frost risk (Abak et al., 1995). In addition, insufficient light intensity and high humidity present significant difficulties in eggplant greenhouse cultivation.

The unfavorable environmental conditions mentioned fruit productivity and quality in eggplants grown under greenhouse conditions (Guler et al., 1995; Acciarri et al., 2002). Therefore, during the winter production of eggplant in unheated greenhouses in the Mediterranean area, fruit set is induced by using pollinators such as insects and bumble bees, or treating flowers with phytohormones (Donzella et al., 2000; Acciarri et al., 2002). Growers can make mistakes such as overdosing during phytohormone application, and these chemicals are expensive (Acciarri et al., 2002). Natural parthenocarpy, especially facultative parthenocarpy, negates pollination problems caused by unfavourable conditions like low temperature (Damidaux and Martinez, 1992). Parthenocarpic cultivars have regular fruiting and sufficient yield without exogenous auxin, and other chemical applications may overcome problems caused by unfavorable environmental conditions (Lipari et al., 1994). Parthenocarpy is controlled by a few genes with additive effects (Spena and Rotino, 2001). Some parthenocarpic eggplant varieties such as Talina and Galine have been improved and released for seed resources. However, these varieties do not have sufficient yield during the winter growth period, and growers therefore need to apply phytohormones (Acciarri et al., 2002). The tendency to parthenocarpy of eggplant is prefered, but its usage is limited by insufficient yield (Donzella et al., 2000). Transgenic parthenocarpic cultivars were developed (Rotino et al., 1997) that gave a high yield compared with the commercial facultative parthenocarpic

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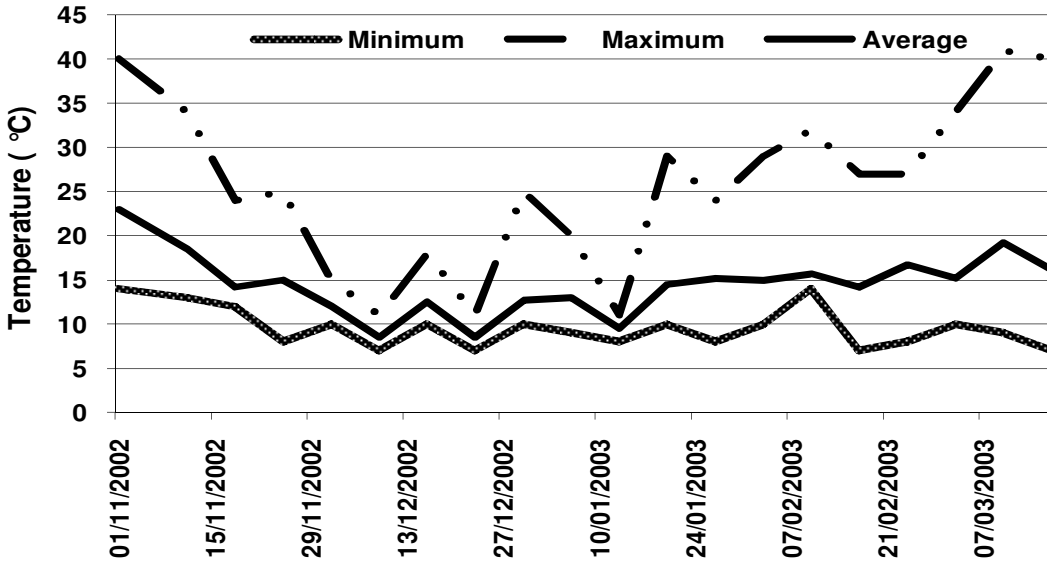

Figure 1. Daily minimum, maximum and average temperatures in greenhouse from November to March.

Talina variety used as a control in early spring production. There was no statistically significant difference in total yield between all tested hybrids (Acciarri et al., 2002). Therefore, there is a need for commercial natural facultative parthenocarpic eggplant cultivars, and further studies on the genes controlling natural parthenocarpy in eggplant.

The role of gibberellins was determined at parthenocarpic fruit development in tomato. The concentrations of GA_1 and GA_3 in ovaries before pollination were higher natural facultative parthenocarpic tomato than the other GA forms (Fos et al., 2001). Hence, auxin promoted GA biosynthesis in the unfertilized ovaries of tomato and both of the mechanisms stimulated fruit development (Phillips, 2004). Plant hormone biosynthesis is affected by environmental conditions (Reid et al., 2004).

HPLC is used in the analysis of plant hormones, and has higher sensitivity and separation speed compared to other chromatographic methods (MacDonald et al., 1981; Martin et al., 1981; Wurst et al., 1984; Hardin and Stutte, 1981; Jensen, 1982). This study determined gibberellic acid (GA_3) levels during flowering using HPLC. We also examined the relationship between flower development and parthenocarpic and non-parthenocarpic cultivars in eggplants cultivated in non-uniformly heated greenhouse conditions during the winter season (*Solanum melongena* L.).

MATERIALS AND METHODS

This study was carried out in a greenhouse at the Bati Akdeniz Agricultural Research Institute, Antalya-Turkey. Nine different eggplant genotypes were used: Parthenone F_1, Nahoma F_1, Waseshinkuro F_1, Karadaylak F_1, Cakıldak F_1, Faselis F_1, Halep Karasi, Topan 374, and a pure line BATEM 45. The parthenocarpic Parthenone F_1 hybrid was provided by the Research Institute of Vegetable Crops, Montanaso/Lombardo-Italy. Nahoma F_1 genotype was determined to have a tendency to facultative parthenocarpy in our previous work (Boyaci et al., 2009). The other six genotypes were non-parthenocarpic. Plants were planted in strips, 100 cm apart with a distance of 50 cm between rows and in-row plant spacing of 60 cm in September, 2002. The heating system was used only when necessary to maintain the temperature above 5°C. The averages, maximum, and minimum of the daily temperatures per month in the greenhouse are summarized in Figure 1.

Three replicate 10 g samples of the five developmental stages, as defined in Table 1, were collected once a month for five months from November to March. They were placed in plastic bags in ice and then stored at -20°C until laboratory analysis.

Extraction

Extraction was performed according to Durley et al. (1982) and Wurts et al. (1984) with some modifications. The samples were homogenized in cold 70% (v/v) methanol at room temperature and stored at 4°C overnight. The extracts were filtered through a Whatman No. 5 filter paper, the supernatants re-homogenized again in 70% (v/v) methanol, and the two extracts combined. The aqueous phase was adjusted to pH 8.5 with 0.1 M phosphate buffer and then partitioned three times with 3× ethyl acetate. The ethyl acetate phase was discarded. The aqueous phase was adjusted to pH 2.5 with 1 N HCl, and then partitioned three times with 3× diethyl ether. The aqueous phase was then discarded. The diethyl phase was filtered through anhydrous sodium sulphate to extract water, and then dried under vacuum at 40°C. The residue containing hormones was dissolved in 1 ml methanol and transferred to an Eppendorf tube.

Thin layer chromatography

Thin Layer Chromatography (TLC) was used to separate and purify the extracts dissolved in methanol. 100 µl extracts were spotted onto a 20 × 20 cm^2, 0.25 mm thick silica gel F_{254} TLC plate (Merc Plc, Darmstadt, Germany) with a Hamilton syringe. Standard GA_3

Table 1. Morphologic features of the samples collected for endogenous GA_3 analysis.

Sample stage	Morphological characteristic
I	Small bud stage (petals are not visible and sepals are tightly closed in buds)
II	Middle bud stage (sepals are open and petals are visible in buds)
III	Huge bud stage (petals are closed and colours are changed in petals)
IV	Flower stage (flowers are at anthesis stage)
V	Small fruit stage (flowers are just in fruit setting, sepals are dry but are not detached)

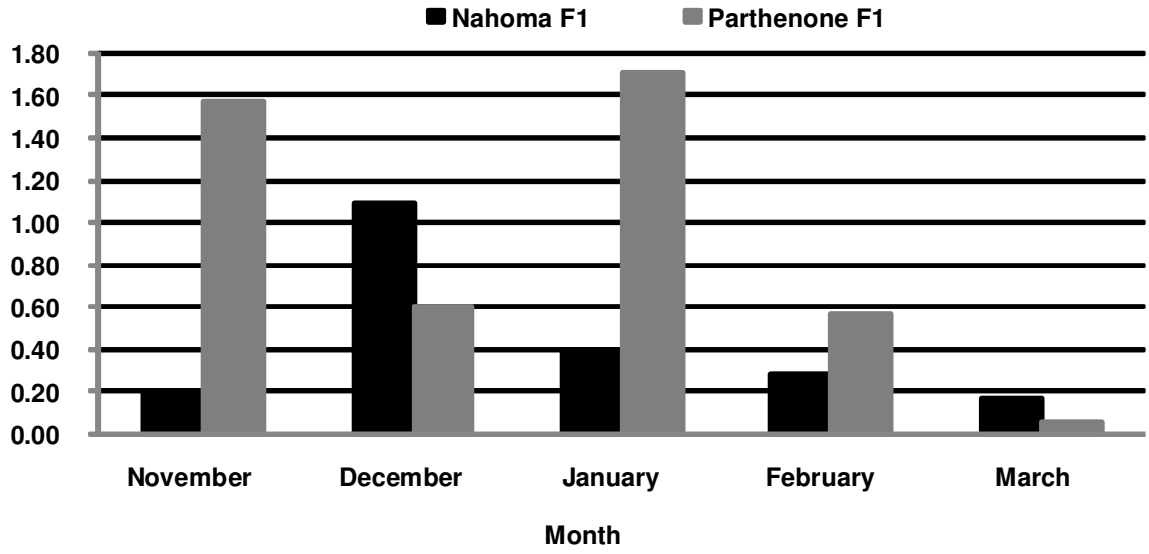

Figure 2. The content (µg.g^{-1}) of endogenous GA_3 in samples huge bud stage (III) of Parthenone F_1 and Nahoma F_1 genotypes from November to March.

was also spot-loaded in scored strips at both edges of the plates. The plates were placed in a TLC tank containing isopropyl alcohol: ammonia: water (21:2:2 v/v) as the solvent. After development, the relative fluidity (Rf) bands of GA_3 were detected under UV light at 254 nm. These bands were scraped off, dissolved in 1 ml HPLC grade methanol and filtered through a 0.45 micro pore filter, and then analyzed with HPLC.

HPLC analysis

The GA_3 contents were analyzed with a Varian Model 9050 (Walnut Creek, CA, USA) equipped with a variable wavelength UV detector and auto-sampler. A nucleosil C18 (4.6 × 150 mm i.d.) column was used for separation and determination. The column was eluted with 30% (v/v) methanol (adjusted to pH 3.0 with 0.1 M H_3PO_4). GA_3 was detected at 208 nm wavelength in the UV detector at a flow rate of 1 ml.min^{-1}. The concentrations of GA_3 (µg.g^{-1} fresh weight) in the samples were automatically calculated from peak area software using authentic standards (Sigma Chemical, St. Louis, MO, USA).

RESULTS

The endogenous GA_3 levels were determined by HPLC analysis of samples. Endogenous GA_3 levels varied in time for both genotypes and the floral developmental stages. The parthenocarpic Parthenone F_1 genotype had its highest endogenous GA_3 level (1.71 µg.g^{-1}) at the huge bud stage (III) in January, and its lowest endogenous GA_3 level (0.01 µg.g^{-1}) in March. While the endogenous GA_3 level at the huge bud stage of Nahoma F_1 genotype increased in December, there was a regular decrease in the other months (Figure 2).

All non-parthenocarpic genotypes, except Topan 374 and BATEM 45, had very late generative phases and samples were not collected in November. The highest GA_3 levels (2.97 and 2.79 µg.g^{-1}) were for Faselis F_1 in February and Karadaylak F_1 in January, respectively. GA_3 levels of non-parthenocarpic genotypes were higher than those of parthenocarpic genotypes (Figure 3).

GA_3 content was examined at the small bud, huge bud, and small fruit stage of Nahoma F_1 and Waseshinkuro F_1 samples collected from November to March. During this time, GA_3 content declined in the Nahoma F_1 (Figure 4) and increased in Waseshinkuro F_1 (Figure 5).

Also, it was detached GA_3 content from initiating bud to small fruit stage in non-parthenocarpic Halep Karasi and parthenocarpic Parthenone F_1 genotypes in Fabruary and March. It was observed that the GA_3 content was high in III-V stage of Halep Karasi genotype in comparison with

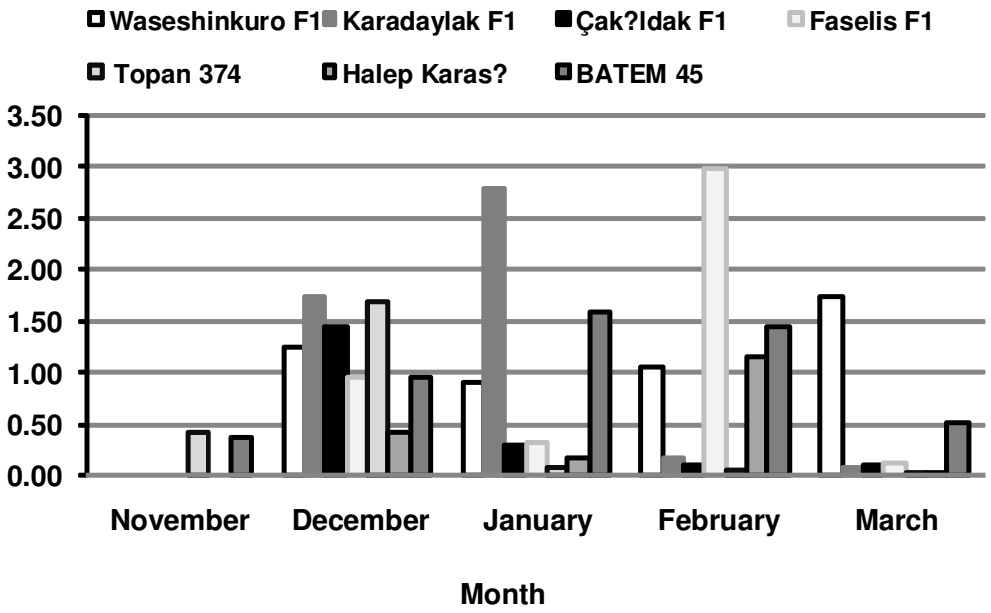

Figure 3. The content (µg.g^{-1}) of endogenous GA_3 in Huge bud stage (III) of Waseshinkuro F_1, Karadaylak F_1, Cakildak F_1, Faselis F_1, Halep karasi, Topan 374, BATEM 45 genotypes from November to March.

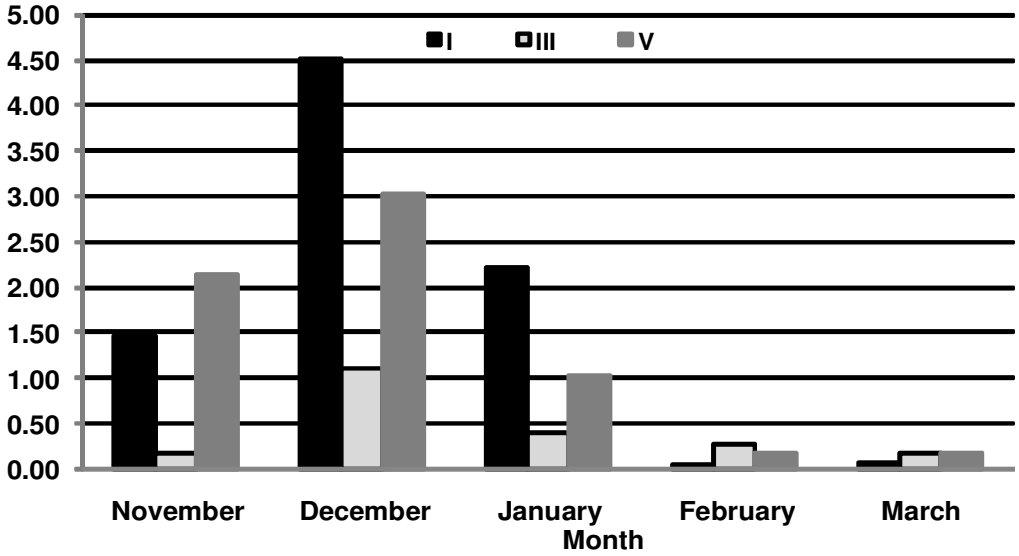

Figure 4. The content (µg.g^{-1}) of endogenous GA_3 in Small bud stage (I), Huge bud stage (III) and Small fruit stage (V) of Nahoma F_1 genotype from November to March.

stage I and II in February (Figure 6). However, GA_3 content declined in stage III-V in March (Figure 7). Also, GA_3 level in Halep Karasi was higher than Parthenone F_1 genotype.

DISCUSSION

Fruit set can be induced by GA treatment of an unpollinated ovary (Blazquez and Leon, 2006). Auxins and gibberellins are considered as the key elements in parthenocarpic fruit development of those tomato lines (Gorguet et al., 2005). Nothmann and Koller (1975a, b) reported an increase in endogenous gibberellin levels in flowers that have non-germinable pollen in winter. In our study, there was no observing of linear increase endogenous gibberellin (GA_3) and different bud stage. GA_3 levels in all genotypes were varied. The value of

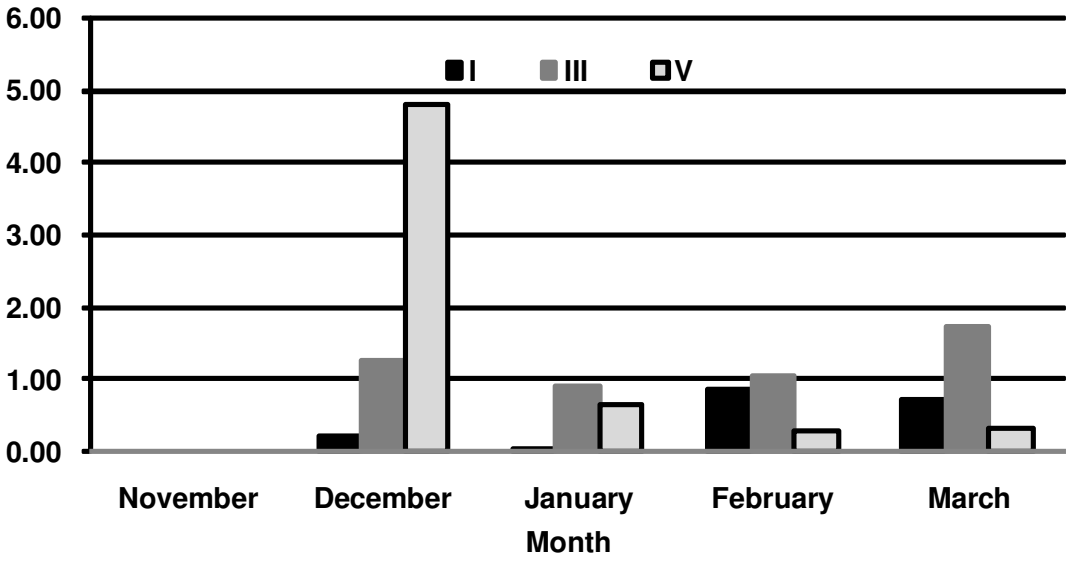

Figure 5. The content (µg.g^{-1}) of endogenous GA_3 in Small bud stage (I), Huge bud stage (III) and Small fruit stage (V) of Waseshinkuro $F_1$from November to March.

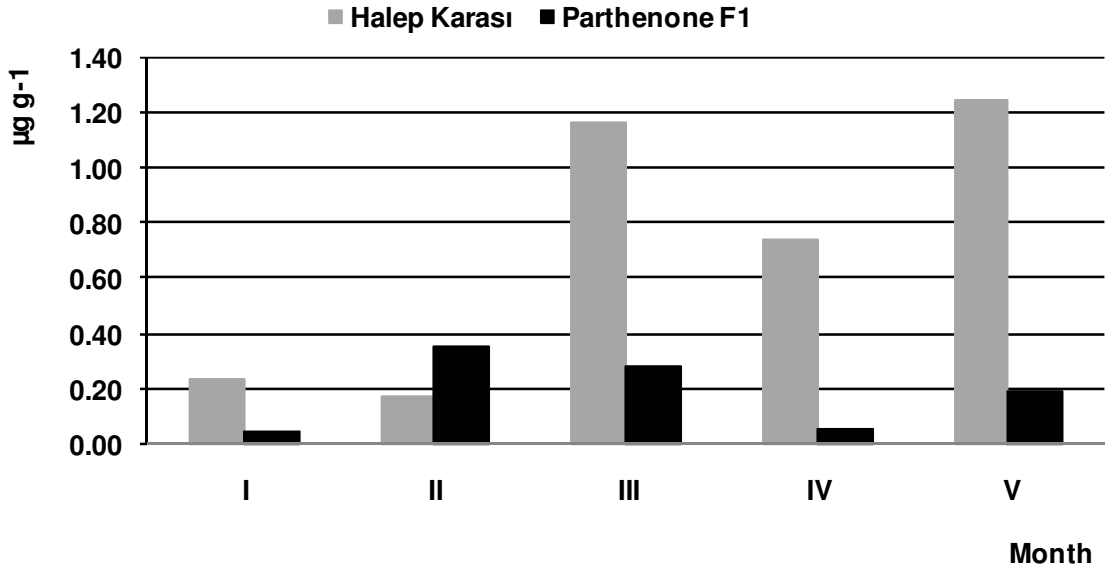

Figure 6. The content (µg.g^{-1}) of endogenous GA_3 in Halep karasi and Parthenone F_1 genotypes samples according to sample stage in February.

ABA/(IAA + GA + ZR) in the fruitlets could be used to select the parthenogenic lines, as reported by Zhang et al. (2009). In our study, the GA_3 content of parthenocarpic genotypes could not be used to distinguish the genotypes. Fos et al. (2001) showed that mutations may induce natural facultative parthenocarpic capacity in tomatoes by increasing the concentration of GA_1 and GA_3 in the ovaries before pollination. According to our findings, there was no correlation between parthenocarpy and GA_3 levels in eggplant.

Conclusion

Features such as parthenocarpy managed under the environmental impact have independent mechanisms. In breeding, physiological mechanisms of these features are less advantageous than other methods.

This study was conducted to determine the relationship between flower development and GA_3 levels in parthenocarpic and non-parthenocarpic eggplant (*Solanum melongena* L.) genotypes. We found no correlation

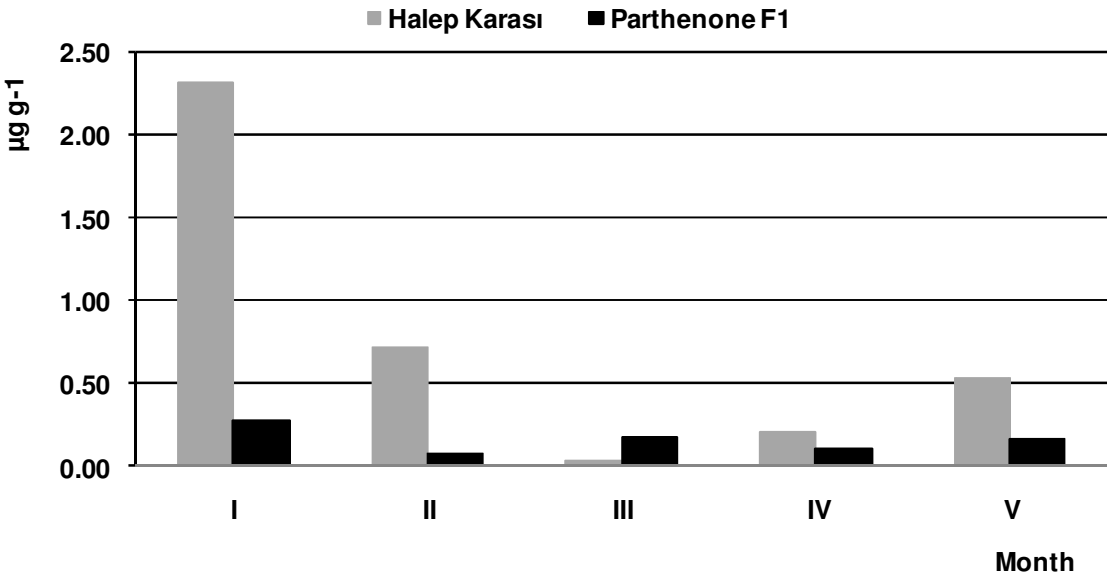

Figure 7. The content (µg.g^{-1}) of endogenous GA_3 in Halep karasi and Parthenone F_1 genotypes samples according to sample stage in March.

between endogenous GA_3 levels and parthenocarpic fruit setting. However, other endogenous GAs could be analyzed to reveal a correlation with other GA types.

Physiological features such as parthenocarpy which emerged under environmental conditions are having independent mechanisms. The physiological mechanisms are still not fully understood. Physiological mechanisms of these features are thus less advantageous for selection in breeding than other approach like biotechnology.

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