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Gibberellins Contents in Leafy Fruitlets of *Satsuma mandarin* (*Citrus unshiu*, Marc.) in Relation to Fruit Quality

[1]Nasar Iqbal and [2]Ismail Karacali
[1]Hill Fruit Research Station, Sunny Bank, P. Code 47140, Murree, Pakistan
[2]Department of Horticulture, Faculty of Agriculture, Ege University, Izmir, Turkey

Abstract: Present studies were designed to find out the role of endogenous Gibberellin like compounds in production of low quality in leafy fruits of *Satsuma mandarin*. Gibberellin-like activity in the leafy and leafless fruitlets of *Satsuma mandarin* grafted on Troyer citrange, Sour orange and Trifoliate orange rootstock was tested with lettuce hypocotyl elongation test. Results showed that Gibberellin-like activity in the leafy fruitlets was considerably higher than the leafless fruitlets in Troyer citrange. Like wise Gibberellin-like activity in the leafy fruitlets of *Satsuma mandarin* grafted on Sour orange and Trifoliate orange was also higher than that of leafless fruitlets. Comparison of the leafy fruitlets indicated that maximum gibberellin like activities were noted in case of Trifoliate rootstock which was followed by Troyer citrange and Sour orange, respectively.

Key words: Leafy and leafless fruitlets, *Satsuma mandarin*, Gibberellin

INTRODUCTION

Plant growth regulators are also known to influence flowering, fruit size, peel growth and peel colour etc. It is, therefore, possible that endogenous Gibberellins may contribute to fruitlet growth, which becomes undesirable factor at some places in case of *Satsuma mandarin*. Endogenous Gibberellin like compounds and ABA were extracted simultaneously from leafy and leafless inflorescence of cultivars 'Shamouti' orange during first two months after flower opening. The concentration of GA like compounds in leafy and leafless born fruitlets reached a maximum 7 days after flowering and decreased gradually thereafter. Consistently, amounts and concentrations of ABA tended to be higher in leafless than in leafy. Fruitlets born on leafy inflorescence had higher GA levels than the adjacent young leaves at early stages of fruitlet development[1]. Hofman[2] monitored fruit growth and abscission as well as Gibberellins and ABA in fruitlets and subtending leaves of 'Valencia' orange from full bloom. Particularly from thirty days after full bloom, the fruitlets adjacent to young leaves (Leafy fruitlets) were greater than fruitlets adjacent to the mature leaves (Leafless fruitlets).

Appreciably more cytokinin and gibberellin like activities were found in both external (flavedo) and internal (albedo) peel tissues of rough than of smooth orange at a very young fruitlet stage when peel growth was at its peak (both cell division and elongation) and the rough fruit disorder was developing. At this stage no consistence differences could be detected in the levels of native Auxins or ABA like inhibitors. Higher cytokinin and gibberellin levels were also found in rough mature peel (November) when peel growth of rough peel resumed[3]. Wiltbank and Krezdorn[4] noted that changes in total amount of gibberellins per fruit appeared to affect the cell enlargement phase of fruit growth. Kauraoka *et al.*[5] applied *Satsuma mandarin* at the concentration of 100 ppm 25 and 35 days before harvest. Chlorophyll was more abundant in the peel of treated fruits than those of the untreated. GA-like substances in the peel decreased rapidly in untreated fruits in early September, then leveled off toward maturity. It increased more than forty times in the flavedo of treated fruits as compared with the control. Sugar content in the peel was depressed by GA_3 application. The certain physiological effects of Gibberellic Acid (GA_3) on the peel of citrus fruits may be attributed to GA^3 interaction with cellular membrane. Excised mesocarp tissues from Pummelo fruits was analyzed for electrolyte and K+ release overtime in varying concentrations of GA^3 electrolyte leakage and K+ efflux was significantly reduced (upto 30%)when tissue was incubated in the presence of GA_3. GA^3 improved the viability of mechanically isolated protoplast during 72 h of storage at 7°C, as shown by use of fluorscein diacetate[6]. Fruits born on leafy inflorescence usually have thick rough skin, (under suboptimal conditions) more puffy and unattractive appearance as the harvest season

Corresponding Author: Nasar Iqbal, Hill Fruit Research Station, Sunny Bank, P. Code 47140, Murree, Pakistan
E-mail: nasariqbal@yahoo.com

approaches. These fruits command lower market prices. Further more, they are also damaged in the process of harvesting, packing and transportation.

Present studies were designed to ascertain that endogenous Gibberellin like compounds have some kind of role in production of low quality in leafy fruits and this will be helpful in controlling this disorder.

MATERIALS AND METHODS

Extraction and purification of GA-like substances: For extraction and purification of GA-like substances 15 fruits born on leafy and leafless inflorescence were collected from each of the three rootstocks viz., Troyer citrange, Sour orange and Trifoliate orange. Fruits were picked early in the morning and brought to the laboratory. Twenty grams sample was prepared by cutting the strips along the fruit length in order to obtain homogenous sample. Sample was put in the pestle after cutting it into small pieces and after adding 40 mL of cold methanol maceration was done. Material was shifted to flask after 3-4 min of maceration and added 1-2 crystals of BHT (2,6-tert.butyl-4-methly-phenol) to avoid oxidation. The flasks were covered with aluminum foil and stored in the dark for 24 h at room temperature. Samples were taken out and filtered. The residual substrate was washed twice with 20 mL of methanol. The combined extract was dried at 35°C by evaporation with low pressure vacuum rotary evaporator. After evaporation, funnels were washed with 50 mL distilled water in order to remove the extracted material. Sample may be filtered if they have some kind of precipitation. Then pH of the supernatants was adjusted to about 3 (2.5-3). The supernatant was then taken to separation funnel and partitioned three times against 40 mL diethyl ether. The whole diethyl ether phase (120 mL) was again evaporated at 35°C with vacuum rotary evaporator and after evaporation residual material was taken with 2 mL methanol and stored in refrigerator. Thin layer chromatographs were prepared by spreading the silica-jel on the glass plate (20x20 cm) and after drying were marked at 2 cm on both ends of chromatographs[7]. After that, 25 μL of sample was streaked on chromatograph with the help of 25 μL syringe. Total of 225 μL of sample was used. For use as a marker 1000 ppm GA^3 was prepared and 25 μL was applied at one point along with constant drying with dryer. The running solvent was iso-propanol, ammonia and water (80:10:10). For detecting the movement of the marker 0.5% potassium per magnate ($KMnO_4$) solution was sprayed. The upper and lower height to which GA_3 marker moved was noted for each chromatograph. Chromatographs were then dried and after dividing into 10 equal Rf zones, each Rf zone was scrapped separately to a test tube.

Qualitative analysis of GA-like substances by biological test: Five milli liter methanol was added to each test tube and left over for 24 h at 0°C. The extract was then filtered and added to petri dishes lined with single filter paper. The methanol was allowed to evaporate and 5 mL of distilled water was added to each of the petri dish. Petries were then stored in dark for 24 h for realizing the movement of active material into the solvent. For qualitative analysis of gibberellins, Lettuce hypocotyls development biological test as purposed by Frankland and Wareing[8] was used. At the same time, Lettuce seeds were made to germinate (about 36 h at 22°C in darkness), ten uniform size germinated seeds were selected and put in to every petri dish. Beside this, five petries having distilled water and 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} GA_3 solution were prepared in the same way. All the prepared petri dishes were put under 1500 lux continuous light at 24°C. The hypocotyls length of lettuce plants was the measured separately after 24 h. By taking average hypocotyl length in distilled water as base, a chromatogram was drawn with average of each Rf zone and Effective Biological Level (EBL) was controlled with Tukey[9] test.

RESULTS AND DISCUSSION

Gibberellin-like activity in the leafy and leafless fruitlets of *Satsuma mandarin* grafted on Troyer citrange rootstock tested with lettuce hypocotyl elongation test are presented in Fig. 1 and 2. It is evident from the figure that gibberellin-like activity in the leafy fruitlets was considerably higher than the leafless fruitlets. Like wise gibberellin-like activity in the leafy fruitlets of *Satsuma mandarin* grafted on sour orange and Trifoliate orange (Fig. 3-6) was also higher than that of leafless fruitlets. Comparison of the leafy fruitlets indicated that maximum gibberellin like activities were noted in case of Trifoliate stock which was followed by Troyer citrange and Sour orange, respectively. Comparatively higher gibberellins activities were found in LY fruitlets than that of LS ones in all three rootstocks (Fig. 1-3). Gibberellins are source of sink strength, as application of GA like compounds generally improve the size in citrus fruits. So, the higher growth of LY fruits (especially the peel) may be due to higher gibberellin activity. In another study, it was noted that GA concentration in the mature leaves subtending leafy inflorescence were consistently higher than those leaves subtending leafless or in the leafy young leaves themselves. No such differences were observed for ABA

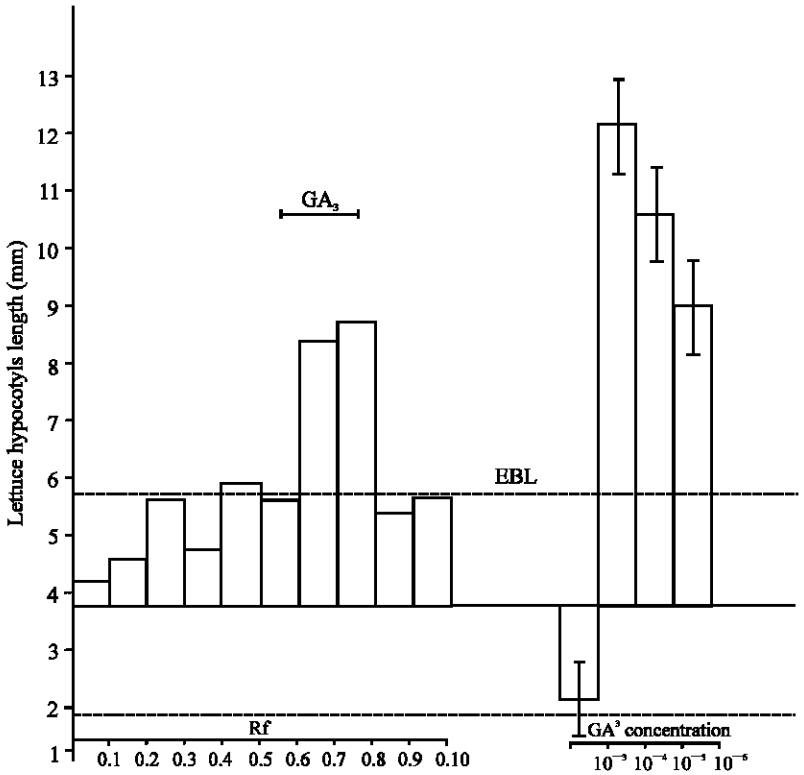

Fig. 1: Activity of GA-like compounds in leafy fruitlets of *Satsuma mandarin* grafted on Troyer citrange

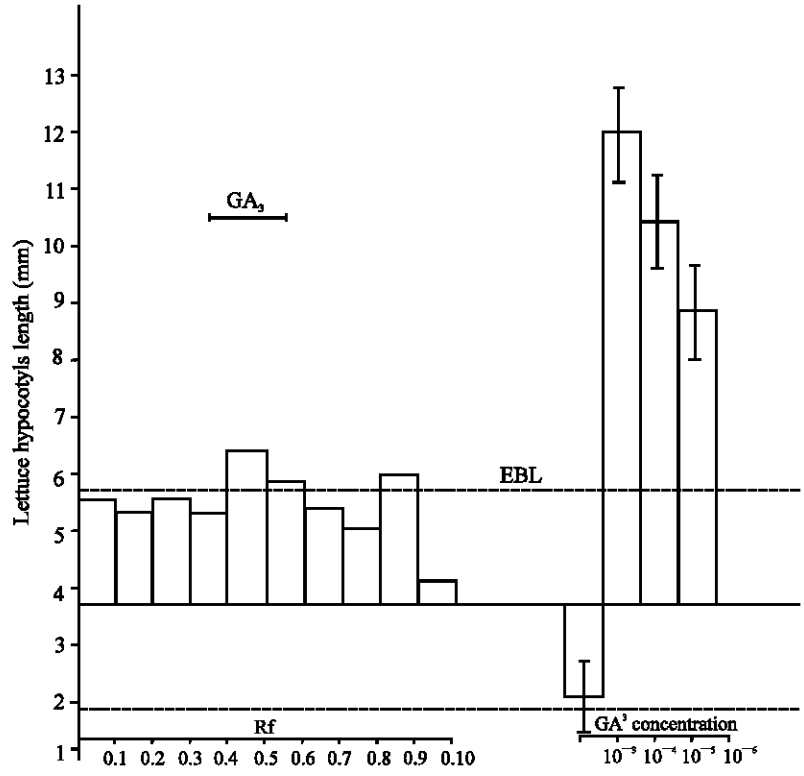

Fig. 2: Activity of GA-like compounds in leafless fruitlets of *Satsuma mandarin* grafted on Troyer citrange

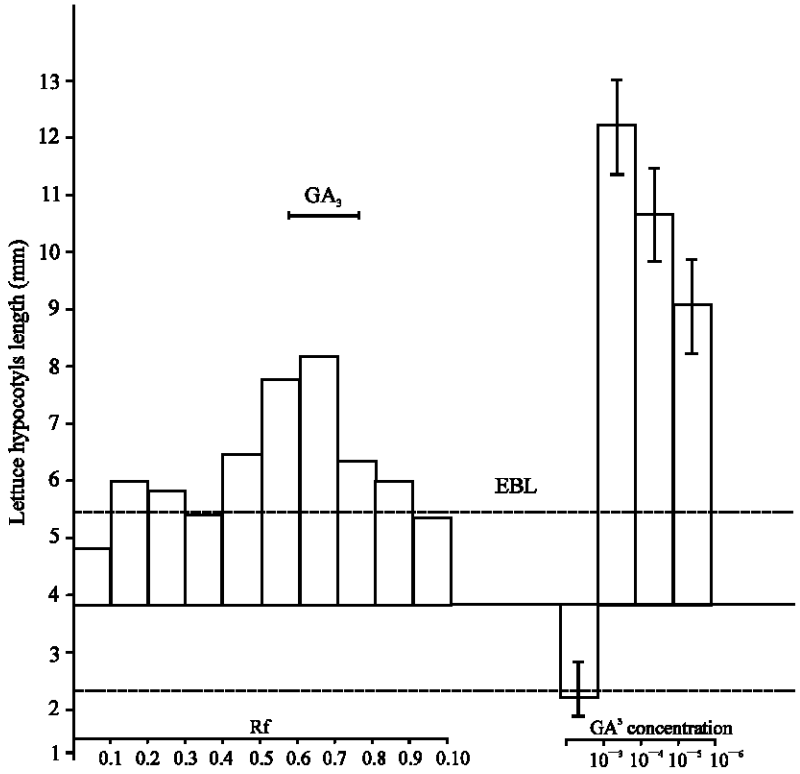

Fig. 3 Activity of GA-like compounds in leafy fruitlets of *Satsuma mandarin* grafted on Sour orange

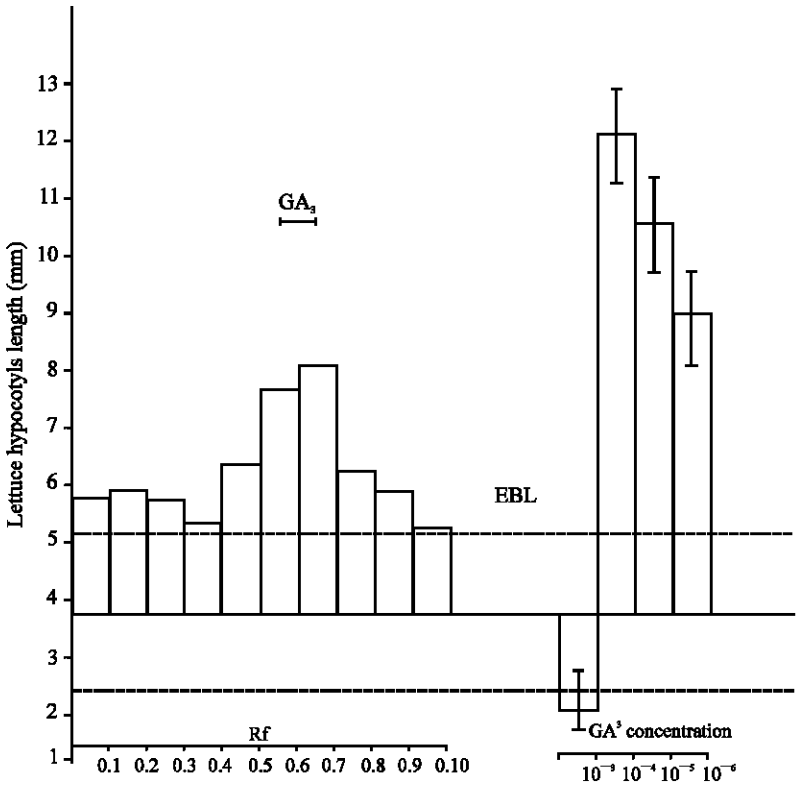

Fig. 4: Activity of GA-like compounds in leafless fruitlets of *Satsuma mandarin* grafted on Sour orange

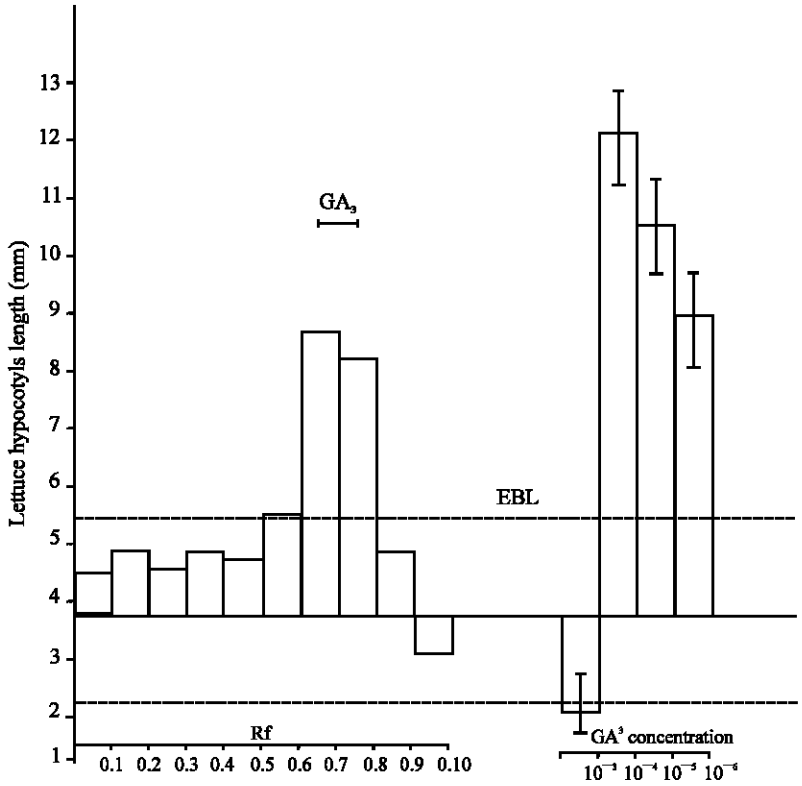

Fig. 5: Activity of GA-like compounds in leafless fruitlets of *Satsuma mandarin* grafted on Trifoliate orange

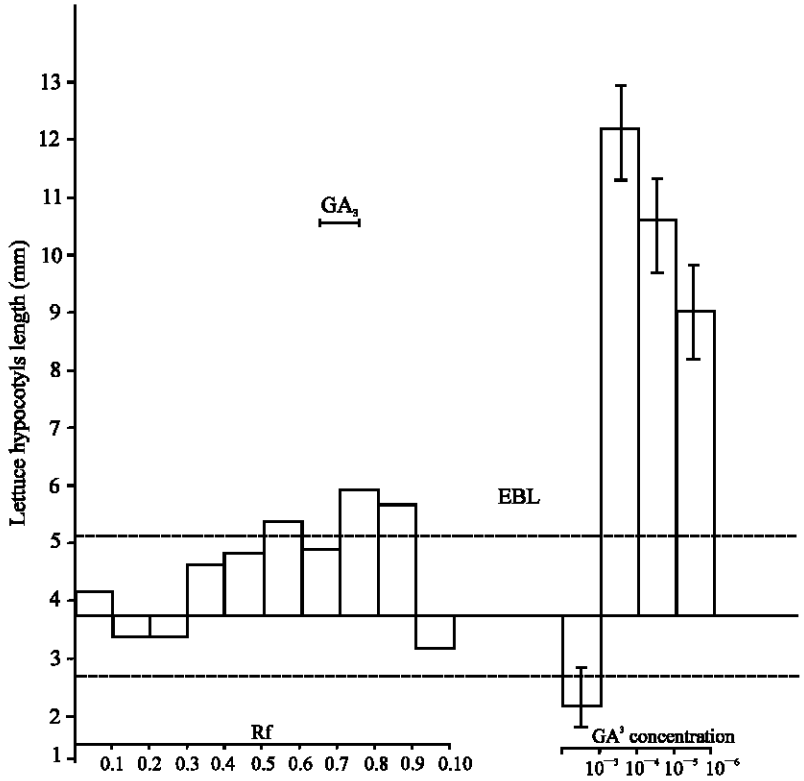

Fig. 6: Activity of GA-like compounds in leafless fruitlets of *Satsuma mandarin* grafted on Trifoliate orange

in mature or young leaves. During early fruitlets development, until seven days after flowering, ABA concentrations were significantly higher in leafless than in the leafy born fruitlets[1]. So besides GA concentrations of fruitlets, GA contents of mature leaves subtending leafy inflorescence and ABA concentration in the fruitlets have significant role in the development of rough fruit condition in *Satsuma mandarin*. Our results are also substantiated by another study in which higher cytokinin and gibberellins activity was observed in both flavedo and albedo peel tissues of rough than of smooth orange at very young fruitlet stage[3]. The other dimension of physiological impetus provided by GA_3 like compounds might be that amount of gibberellins per fruit. Contents of Gibberellins per fruit appeared to affect the cell enlargement phase of fruit growth. The first significant increase in total Gibberellins per fruit occurred four weeks after petal fall while the first significant increase in mean weight occurred five weeks after petal fall. Mean sample volume and total gibberellins per fruit showed an identical relationship. Thus at a time when young fruit growth was primarily due to cell enlargement was being initiated, total Gibberellins per fruit increased significantly one week before the first significant increase in the fruit weight or volume[4].

So based on the information incurred it can be concluded that gibberellins like compounds have played role in the development of low quality fruits in *Satsuma mandarin* under suboptimal growing conditions.

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