

## **Original Research Article**

### **Gibberellic acid (GA<sub>3</sub>), an influential growth regulator for physiological disorder control and protracting the harvesting season of sweet orange**

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#### **ABSTRACT**

Citrus particularly sweet orange occupies an exalted position in fruit industry. Quality sweet orange production is affected by various factors including fruit physiological disorders and harvesting season which contribute towards consumer preference. This study was designed to find out an economically feasible solution to these problems. Three different GA<sub>3</sub> treatments (10, 20 and 30 ppm) were applied as foliar spray to three different sweet orange cultivars (Blood Red, Mosambi and Succari) at full bloom stage to investigate its influence on physiological disorder in fruits as well as in shoots, fruit growth and fruit maturity delay. The obtained results revealed that all GA<sub>3</sub> treatments specially 30 ppm significantly controlled fruit as well as shoots physiological disorders and was also found efficient in case of fruit maturity delay as compared to untreated trees. Finally we were able to conclude that; 30 ppm GA<sub>3</sub> should be applied as foliar spray to control fruit and shoot physiological disorders and to protract the harvesting season of sweet orange.

**Key words:** Foliar spray, Full bloom, Gibberellic Acid, Maturity delay, Physiological disorder, Sweet orange cultivars.

#### **1. INTRODUCTION**

Citrus is a group of fruits occupies a trenchant position in fruit industry all over the world. In Pakistan, citrus was grown on an area of 194.5 thousands hectares with an average production of 1982.2 metric tonnes, which ranks it first among fruits grown in country [1]. Likewise in United States of America the on tree crop value was estimated up to 1.35 billion USD during 2011-12 which generated roughly 76,000 full and part time jobs [2]. The benefit-cost ratio of sweet orange is high as compared to lemon and mandarin [3]. Sweet orange is also the most important fruit through nutritional as well medicinal point of view. Several extracts from the peel of sweet orange have been reported which helps

incurring of many diseases, like Naringin extracted from its peel has a therapeutic effect for Alzheimer type in humans [4], while the peel of citrus also proved helpful in insulin stimulation [5]. Several aromatic compounds extracted from sweet oranges have been used in various food products for colour and taste development [6]. The demand of sweet oranges among consumers is increasing day by day, to fulfill this need, good quality fruit production as well as high production per unit area is required. A huge amount of research work has been conducted on these two aspects, many cultural practices have been recommended in this regard like proper application of nutrients and irrigation [7], managing row to row and plant to plant distance, root stock selection [8] as well as the application of various growth regulators [9,10]. To be more specific, in sweet oranges the nutritional and water imbalance produce severe fruit disorders like fruit splitting, puffing, creasing and water marks etc. [11], which decreases the fruit quality and ultimately the consumer's preference. Gibberellic acid has been reported as an effective treatment in controlling bunch fading disorder in date palm [12], double fruit and fruit splitting disorder in peach [13], fruit creasing in Washington Navel sweet orange [14] and tipburn disorder in the leaves of several vegetables [15]. However in another study [16] Naphthalene Acetic Acid (NAA) mentioned as an effective treatment for improvement in physiochemical properties of date palm. With relation to break seed dormancy GA binds to a receptor and  $Ca^{2+}$ , activates the protein calmodulin. The complex in turn binds to DNA producing an enzyme to stimulate growth in embryo [17]. The bioactive gibberellins (GAs) are ( $GA_1$ ,  $GA_3$ ,  $GA_4$  and  $GA_7$ ) among which  $GA_3$  is the most biologically active form of gibberellic acid [18]. In concern with fruit maturity delay,  $GA_3$  has been reported as an influential treatment in date palm, protracting its harvesting season [19,20]. With respect to these desirable and important impacts of growth regulators, the present research study has been designed with objectives; (1) to control fruit physiological disorder and (2) to prolong the harvesting season of sweet orange through the foliar application of  $GA_3$ , which are highly desirable in fruit industry.

## **2. MATERIALS AND METHODS**

### **3.**

#### **2.1 Experimental site, design and plant material.**

The experiment was carried out at Agricultural Research Institute (ARI) Tarnab, Peshawar-Pakistan, in year 2012. The experimental design used throughout the study was; Randomized Complete Block Design (RCBD) with two Factors and three replications. Different gibberellic acid concentrations (0, 10, 20 and 30ppm indicated as  $G_0$ ,  $G_1$ ,  $G_2$  and  $G_3$ ) were considered as (Factor A), while different sweet orange cultivars (Blood Red, Mosambi and Succari indicated as  $C_1$ ,  $C_2$  and  $C_3$ ) were taken as (Factor B). Thirty six trees of three different sweet orange cultivars (mentioned above) each having twelve trees were tagged. The tagging was based on the physical and micro environmental similarity among trees. Four different concentrations of  $GA_3$  including control (mentioned above) were sprayed at full bloom stage of sweet orange cultivars and their results were compared with control treatment. Each treatment was replicated three times.

#### **2.2 Foliar application of growth regulator**

$GA_3$  with product name (ProGibb SG) produced and merchandised by Sumitomo chemicals Australia [21], containing the active ingredient ( $GA_3$ ) 400g/kg was used. The chemical's applicable concentrations were prepared and pH was adjusted according to the user's guide [21]. We carried out the spray procedure as prescribed in the company manual [21] except the spray timing and schedule. We applied  $GA_3$  as single spray at full

bloom stage in the entire fruiting season. In order to prepare 10, 20 and 30ppm aqueous solutions, weight measurements were determined applying the following procedure [21].

a) 2.5g dissolved in 100 liters of water = 10 ppm (1)

As per treerequirement was found prior the actual spray by spraying distilled water to find the actual volume of water sufficient for each tree. Therefore weight calculations were made for 3 litersper tree according to the following procedure.

b) No. of grams dissolved in 3L of water =  $\frac{2.5g \times 3L}{100L} = 0.075g$  (0.075g/ 3L=10 ppm GA<sub>3</sub>)

c). 0.075g×2= 0.15g (0.15g/3L= 20ppm GA<sub>3</sub>)

d). 0.075 g×3=0.225g (0.225g/3L=30ppm GA<sub>3</sub>)

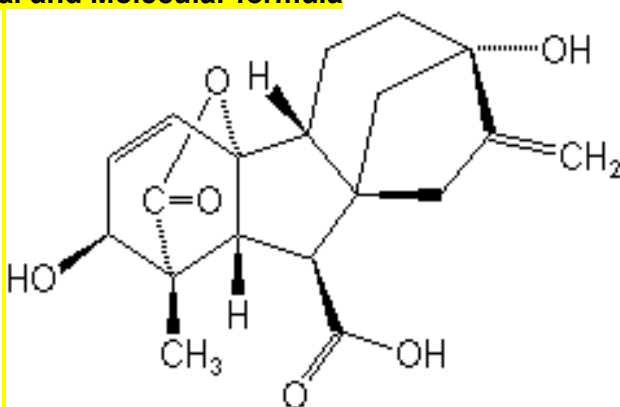
Solutions prepared based on above mentioned calculations, were then applied as foliar spray on full bloom stage of sweet orange.

## 2.3 Mode of Action, Molecular Structure, formula and features of GA<sub>3</sub>

### 2.3.1 Mode of action

GA<sub>3</sub> acts as a plant growth regulator, owing to its physiological and morphological effects in extremely low concentrations. Translocated. Predominantly influences, at most the plant parts above the soil surface [22].

### 2.3.2 Structural and Molecular formula



GA<sub>3</sub> (C<sub>19</sub>H<sub>22</sub>O<sub>6</sub>)

### 2.3.3 Important features

Gibberellins are tetracyclic diterpene. There are two categories according to the presence of carbon number either 19 or 20. Gibberellins having 19-carbons, lost carbon-20 and consequently forms a five-member lactone bridge that produces a link between carbon 4 and 10. In general the 19-carbon form of gibberellins are biologically more active as compared to 20-carbon gibberellins due to the presence of hydroxyl groups on carbon 3 and 13, hence referred as dihydroxylated gibberellins. GA<sub>3</sub> is a dihydroxylated gibberellin [22, 18].

## 2.4 Observations

Before the application of GA<sub>3</sub> as foliar spray four branches (one in each direction) were selected. The selection was made on the basis of branch size (having approximately same length and diameter) and vigor. The following attributes were determined as:

### Physiological disorder percentage in shoots (PDS)

Those shoots which have the symptoms of nutrients deficiency like yellowing of old or new leaves, malformed or chlorotic leaves etc. which mainly involves environmental factors were carefully counted [22]. The Physiological disorder percentage was then determined through the following formula.

$$\%PDS = \frac{\text{Number of abnormal shoots per tree}}{\text{Total shoots per tree}} \times 100 \quad (2)$$

#### Physiological disorder percentage in fruits (PDF)

Physiological disorder in fruits like fruit splitting, puffing, creasing, water marks and peel pitting etc. was found by counting the abnormal fruits as well as total fruits per tree [22]. The percent physiological disorder in fruits was calculated using following formula.

$$\%PDF = \frac{\text{Number of abnormal fruits per tree}}{\text{Total fruits per tree}} \times 100 \quad (3)$$

#### Days to fruit maturity (DFM)

The number of days was counted from first day of fruit set to the initiation of fruit maturity indices (colour, taste and aroma), their mean was taken and presented graphically.

#### Fruit growth (mm)

Fruit growth was determined by randomly selected fruits on each tree. Their diameter was regularly measured on weekly basis through Fruit Size Meter (Figure 1), made by (Cranston machinery co. oak grove, Oregon, Australia), their means were taken and then presented graphically to study the trend of fruit growth throughout the cell elongation and expansion stage.



**Figure 1:** Fruit size meter used to determine the fruit diameter regularly on weekly basis.

## 2.4 Statistical analysis

The collected data for all parameters was analyzed through (Statistix8.1) statistical software and then **least significant difference (LSD)** was computed for those parameters which were statistically significant, the graphical illustrations were done through MS excel.

## 3. RESULTS AND DISCUSSION

### 3.1 Physiological disorder in shoots (PDS)

Data regarding PDS is graphically presented in Figure 4. The graphical illustration shows that there were significant differences among different treatments and cultivars regarding

PDS however the interactive effect was non- significant (Table 1). According to the mean values a higher percentage (3.1) of PDS was observed in trees which were kept control while low intensity of PDS was observed in treated trees that were (1.15, 1.18 and 1.46) in 20, 10 and 30ppm respectively. Among different Cultivars the maximum value (2.33%) was observed in Blood Red while the Succari (1.27%) and Mosambi (1.48%) showed minimum counts of PDS.

These differences among different treatments and cultivars might be due the physiological response to the application of growth regulator (GA<sub>3</sub>) however there might also be some genetic and environmental factors responsible for these differences. In previous studies certain vitamins were also used as foliar spray, dusting or drench application to overcome nutrients deficiency by strengthening the plants to absorb more nutrients from soil and those treatments caused several desirable changes within plant body, like stimulation of root formation and flower induction under non-inductive conditions as well as protecting plants from several air pollutants i.e. O<sub>3</sub> and SO<sub>2</sub> [23]. Calcium binding proteins technically termed as Calceineurin B-like (CBL) proteins is up-regulated in the aleurone layer by GA. This layer plays a vital role in the proper development of seed in rice and barley [24]. Further CBL represent a unique family of calcium sensors in plant cell, sensing the calcium signals elucidated by a variety of abiotic stresses [25]. Plants growing in stress free environment have more physiologically active gibberellins which tend to increase the permeability of cell membrane and facilitate the distribution of Ca<sup>2+</sup> to rapidly growing tissues, consequently make it resistant towards tipburn disorder [15].

**Table-1:** PDF, PDS and DFM as affected by different GA<sub>3</sub> treatments in sweet orange cultivars.

| GA <sub>3</sub> (Conc.)        | PDF                   | PDS                   | DFM                 |
|--------------------------------|-----------------------|-----------------------|---------------------|
| G <sub>0</sub>                 | 2.7633 <sup>a</sup>   | 3.0989 <sup>a</sup>   | 187.22 <sup>c</sup> |
| G <sub>1</sub>                 | 0.8178 <sup>b</sup>   | 1.1789 <sup>b</sup>   | 196.78 <sup>b</sup> |
| G <sub>2</sub>                 | 0.7589 <sup>b</sup>   | 1.1489 <sup>b</sup>   | 196.78 <sup>b</sup> |
| G <sub>3</sub>                 | 0.9567 <sup>b</sup>   | 1.3667 <sup>b</sup>   | 197.89 <sup>a</sup> |
| Significance                   | *                     | *                     | *                   |
| LSD (Treatments)               | 0.6890                | 0.5967                | 1.0456              |
| Cultivars                      |                       |                       |                     |
| C <sub>1</sub>                 | 1.7608 <sup>a</sup>   | 2.3350 <sup>a</sup>   | 195.75 <sup>a</sup> |
| C <sub>2</sub>                 | 1.0650 <sup>b</sup>   | 1.4858 <sup>b</sup>   | 192.50 <sup>b</sup> |
| C <sub>3</sub>                 | 1.1467 <sup>b</sup>   | 1.2742 <sup>b</sup>   | 195.75 <sup>a</sup> |
| Significance                   | *                     | *                     | *                   |
| LSD (Cultivars)                | 0.5967                | 0.5168                | 0.9055              |
| Interaction                    |                       |                       |                     |
| G <sub>0</sub> ×C <sub>1</sub> | 2.5967 <sup>ab</sup>  | 4.0100 <sup>a</sup>   | 188.67 <sup>d</sup> |
| G <sub>0</sub> ×C <sub>2</sub> | 3.2233 <sup>a</sup>   | 2.5533 <sup>b</sup>   | 183.67 <sup>e</sup> |
| G <sub>0</sub> ×C <sub>3</sub> | 2.4700 <sup>abc</sup> | 2.7333 <sup>b</sup>   | 189.33 <sup>d</sup> |
| G <sub>1</sub> ×C <sub>1</sub> | 0.4633 <sup>ef</sup>  | 2.0333 <sup>bc</sup>  | 199 <sup>a</sup>    |
| G <sub>1</sub> ×C <sub>2</sub> | 1.7333 <sup>bcd</sup> | 0.9967 <sup>de</sup>  | 194.33 <sup>c</sup> |
| G <sub>1</sub> ×C <sub>3</sub> | 0.2567 <sup>f</sup>   | 0.5067 <sup>e</sup>   | 197 <sup>b</sup>    |
| G <sub>2</sub> ×C <sub>1</sub> | 0.2033 <sup>f</sup>   | 1.3333 <sup>cde</sup> | 197 <sup>b</sup>    |
| G <sub>2</sub> ×C <sub>2</sub> | 1.5800 <sup>bcd</sup> | 1.3400 <sup>cde</sup> | 197 <sup>b</sup>    |

|                    |                        |                       |                      |
|--------------------|------------------------|-----------------------|----------------------|
| $G_2 \times C_3$   | 0.4933 <sup>ef</sup>   | 0.7733 <sup>e</sup>   | 198.33 <sup>ab</sup> |
| $G_3 \times C_1$   | 0.9967 <sup>def</sup>  | 1.9633 <sup>bed</sup> | 198.33 <sup>ab</sup> |
| $G_3 \times C_2$   | 0.5067 <sup>ef</sup>   | 1.0533 <sup>cde</sup> | 195 <sup>c</sup>     |
| $G_3 \times C_3$   | 1.3667 <sup>cdef</sup> | 1.0833 <sup>cde</sup> | 198.33 <sup>ab</sup> |
| Significance       | NS                     | NS                    | *                    |
| LSD ( Interaction) | -                      | -                     | 1.8111               |

\*significance at  $\alpha=0.05$ , NS= Non significance. Values having same alphabets have no significant differences among each other, while different letters indicate significant differences. Statistical analysis computed on replicated means.

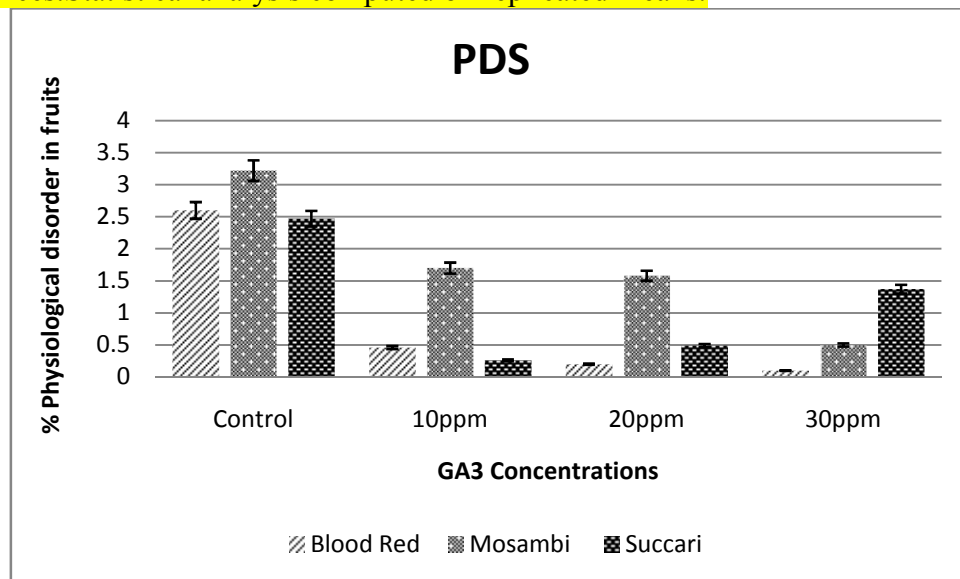


**Figure 2:** Physiological disorder percentage in shoots as affected by different  $GA_3$  treatments in sweet orange. (graphical illustration based on grand means)

### 3.2 Physiological disorder in fruits (PDF)

The statistical analysis shows significant differences among different treatments and cultivars (Table-1). Figure 4 and 3 represent the severity of fruit disorders and its response to different levels of gibberellic acid respectively. According to the data presented, different  $GA_3$  treatments significantly influenced PDF in all tested sweet orange cultivars. With reference to the data given, a higher percentage (2.76) of PDF was observed in untreated trees which was significantly higher than (0.96, 0.82 and 0.76%) recorded in 30, 10 and 20ppm respectively. The inter varietal differences were also significant, with a highest value (1.76%) of PDF observed in cv. Blood Red followed by cv. Succari (1.15%) and cv. Mosambi (1.07%). While the interaction of different treatments and cultivars was non-significant.

These differences among different treatments and cultivars might be due to the influence of  $GA_3$  by preventing calcium deficiency directly or indirectly as stated earlier [25, 26], which can lead to fruit splitting, however there might be some other environmental reasons for fruit disorders as in sweet oranges the nutritional and water imbalance produce severe fruit disorders like fruit splitting, puffing, creasing and water marks etc. [11]. The intensity of double fruit and fruit splitting disorder in peach was successfully controlled through the application of gibberellic acid and nitrogen to the trees with no water

stress [13]. GA<sub>3</sub> and NAA sprays in June and July to Washington Navel Sweet orange reduced the incidence of fruit creasing upto 25 and 14% respectively [14] and bunch fading disorder in Date palm [12].

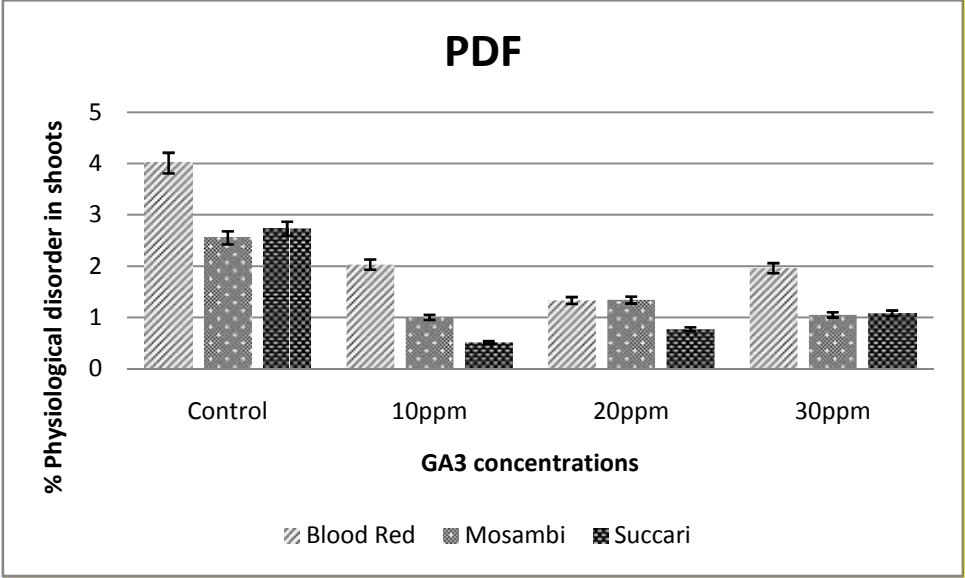


**Figure 3:** Physiological disorder percentage in fruits as affected by different GA<sub>3</sub> treatments in sweet orange.(graphical illustration based on grand means)



**Figure 4:** Intensity of fruit splitting disorder increasing with passage of time and fruit size.

### 3.3Days to fruit maturity (DFM)

The data regarding DFM is presented in Figure 5. There were highly significant differences among different treatments and cultivars regarding DFM as well as the interaction of both factors was also significant at (p=0.05)level of significance(Table-1). Accordingly,more number of days to fruit maturity (197.89) was observed in 30 ppm treated trees closely followed by 10 ppm (196.78) and 20 ppm (196.78) which have non-significant difference with each other, while less number of days (187.22) was taken by trees which were kept control. Likewise, maximum number of days to fruit maturity (195.75) was recorded for cv. Blood Red and Succari and minimum number of days (192.50) was taken by Mosambisweet orange.

Almost all GA<sub>3</sub> treatments showed a delaying effect in terms of fruit maturity, it might be due to the influence of GA<sub>3</sub> on chemical compositions of sweet orange fruits. It might also be due to varietal differences with respect to number of days required to attain fruit

maturity by different cultivars. GA<sub>3</sub> reduces chlorophyll depletion and carotenoid accumulation, and hence causes colour break delay in fruit's peel [27]. Similarly GA<sub>3</sub> application results fruit ripening delay in Strawberry which is a highly perishable fruit crop [28].

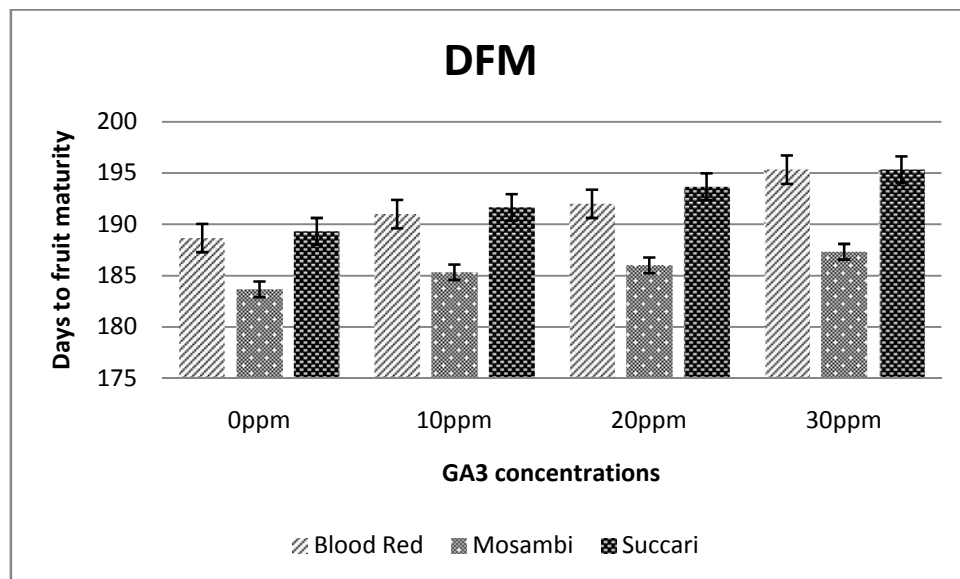


**Figure 5:** Graphical illustration for DFM as influenced by different GA<sub>3</sub> application as foliar spray. (graphical illustration based on grand means)

### Supplementary information

#### 3.4 Fruit growth (mm)

A continuous fruit growth recorded on weekly bases is presented in Figure 6. Accordingly, cultivar Blood Red and Succari showed very minor increase in fruit diameter for treated trees as compared to untreated (control) trees while Mosambi remained unresponsive to GA<sub>3</sub> applications. The graphical presentation of fruit growth showed non-significant differences because the bars representing different concentrations followed almost same pattern for fruit growth.

All sweet orange cultivars started cell expansion in early June and attained maximum average fruit diameter (60mm with little fluctuation) in early November. The main reason for following same fruit growth pattern might be due to the fact that all cultivars belong to the same species, received same concentrations of GA<sub>3</sub> and almost same environmental conditions. The application of (20 mg L<sup>-1</sup>) GA<sub>3</sub> delayed fruit maturity 5-8 days only for the late-maturing genotypes, but did not affect early fruit maturing genotypes, while GA<sub>3</sub> application to Strawberry significantly increased fruit weight and size as compared to untreated plants [29]. Gene ontology of plum fruit revealed that GA<sub>1</sub> and GA<sub>4</sub> are very important for fruit growth and development. Further several genes have been believed to be involved in GA signaling prior fruit development [30].

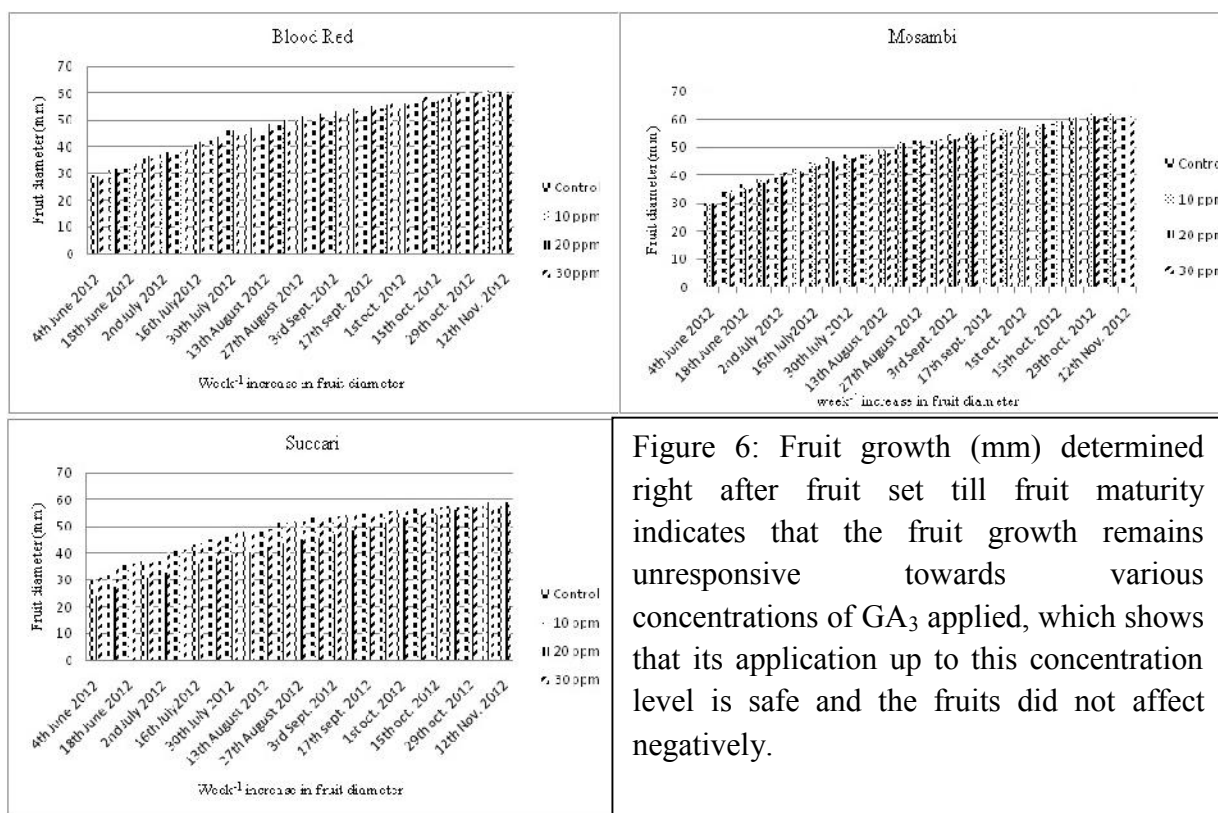


Figure 6: Fruit growth (mm) determined right after fruit set till fruit maturity indicates that the fruit growth remains unresponsive towards various concentrations of GA<sub>3</sub> applied, which shows that its application up to this concentration level is safe and the fruits did not affect negatively.

**Figure 6:** Response of fruit growth towards different GA<sub>3</sub> concentrations applied as foliar spray in the period from fruit set till fruit maturity.

#### 4. Conclusion

The results revealed that 30 ppm GA<sub>3</sub> application is the best treatment to control fruit and shoot physiological disorder along with prolonging the harvesting season of sweet orange. All tested cultivars positively responded towards the foliar application of GA<sub>3</sub> without any malformation in their fruit growth. Therefore it could be recommended that 30 ppm GA<sub>3</sub> should be applied at full bloom stage of sweet orange in order to get these desirable effects under similar environmental conditions.

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RIZWAN ULLAH

#### Author's contributions

We followed the "sequence-determines-credit" approach (SDC) for author's contribution [27]. However all the authors read and approved the final manuscript.

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