Gibberellic Acid (GA$_3$), an Influential Growth Regulator for Physiological Disorder Control and Protracting the Harvesting Season of Sweet Orange

Rizwan Ullah[1,2*], Muhammad Sajid[2], Ghulam Nabi[3], Husain Ahmad[2,4], Abdur Rab[2], Faheem Ahmed Khan[5], Muhammad Shahab[2], Hizar Subthain[6], Shah Fahad[7] and Aziz Khan[7]

[1]*Key Laboratory of Horticultural Plant Biology (MOE), Huazhong Agricultural University, Wuhan 430070, China.*
[2]*Department of Horticulture, University of Agriculture Peshawar, Pakistan.*
[3]*Department of Horticulture, University of Agriculture (Amir Muhammad Khan Campus) Mardan, Pakistan.*
[4]*College of Horticulture, Northwest A and F University, yangling, shaanxi, China 712100*
[5]*Molecular Biotechnology, Laboratory of Triticeae crops HZAU Wuhan, China.*
[6]*National Key Laboratory of crop genetic improvement and centre of plant gene research, HZAU Wuhan 430070, China.*
[7]*National Key Laboratory of Crop Genetic Improvement, MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China.*

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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

---

Corresponding author: Email: rizwanhort@gmail.com;

different GA$_3$ treatments (10, 20 and 30ppm) were applied as a 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$_3$ treatments specially 30ppm 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; 30ppm GA$_3$ should be applied as a foliar spray to control fruit and shoot physiological disorders and to protract the harvesting season of sweet orange.

1. INTRODUCTION

Citrus is a group of fruits occupies a trenchant position in the 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 in curing 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 color 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 the fruit industry.

2. MATERIALS AND METHODS

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 the 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 a 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].

$$\text{a) 2.5g dissolved in 100 liters of water = 10ppm} \tag{1}$$

As per tree requirement 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 liters per 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=10ppm GA_3)

c) 0.075g×2= 0.15g   (0.15g/3L= 20ppm GA_3)

d) 0.075 g×3=0.225g   (0.225g/3L=30ppm GA_3)

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_3

2.3.1 Mode of Action

GA_3 acts as a plant growth regulator, owing to its physiological and morphological effects in extremely low concentrations. Translocated and predominantly influences the plant parts above the soil surface [22].

2.3.2 Structural and molecular formula

GA$_3$ (C$_{19}$H$_{22}$O$_6$)
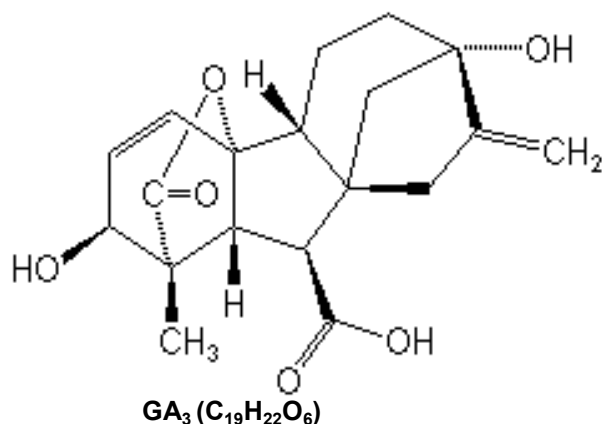
2.3.3 Important features

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 is biologically more active as compared to 20-carbon gibberellins due to the presence of hydroxyl groups on carbon 3 and 13, hence referred as dihydroxylation gibberellins. GA$_3$ is a dihydroxylated gibberellin [22,18].

2.4 Observations

Before the application of GA$_3$ as a 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:

2.5 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.

$$\%PDS = \frac{Number\ of\ abnormal\ shoots\ per\ tree}{Total\ shoots\ per\ tree} \times 100 \tag{2}$$

2.6 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.

$$\%PDF = \frac{Number\ of\ abnormal\ fruits\ per\ tree}{Total\ fruits\ per\ tree} \times 100 \tag{3}$$

2.7 Days to Fruit Maturity (DFM)

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

2.8 Fruit Growth (mm)

Fruit growth was determined by randomly selected fruits on each tree. Their diameter was regularly measured on a weekly basis through Fruit Size Meter (Fig. 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.
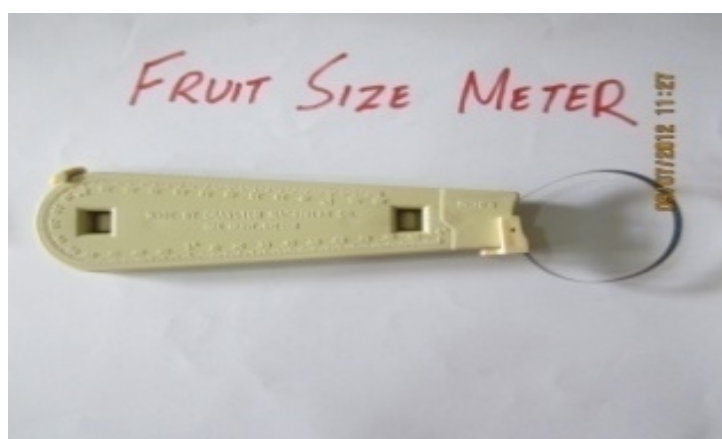

Fig. 1. Fruit size meter used to determine the fruit diameter regularly on weekly basis

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.

4. RESULTS AND DISCUSSION

3.1 Physiological Disorder in Shoots (PDS)

Data regarding PDS is graphically presented in Fig. 2. The data pertaining PDS shows that there were significant differences among different treatments and cultivars, 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_3), 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_3 and SO_2 [23]. Calcium binding proteins technically termed as Calcineurin 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 the cell membrane and facilitate the distribution of Ca^{2+} to rapidly growing tissues, consequently make it resistant towards tip burn disorder [15].
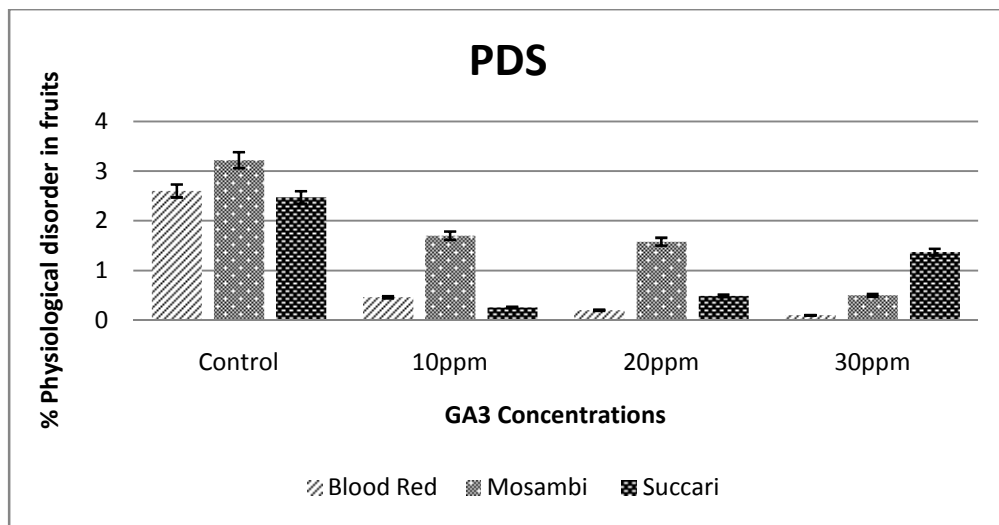

Fig. 2. Physiological disorder percentage in shoots as affected by different GA₃ treatments in sweet orange. (Graphical illustration based on grand means)

3.2 Physiological Disorder in Fruits (PDF)

The statistical analysis shows significant differences among different treatments and cultivars (Table 1). Figs. 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 GA₃ 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 the 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 were non-significant.

These differences among different treatments and cultivars might be due to the influence of GA₃ 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_3 and NAA sprays in June and July to Washington Navel Sweet orange reduced the incidence of fruit creasing up to 25 and 14% respectively [14] and bunch fading disorder in Date palm [12].
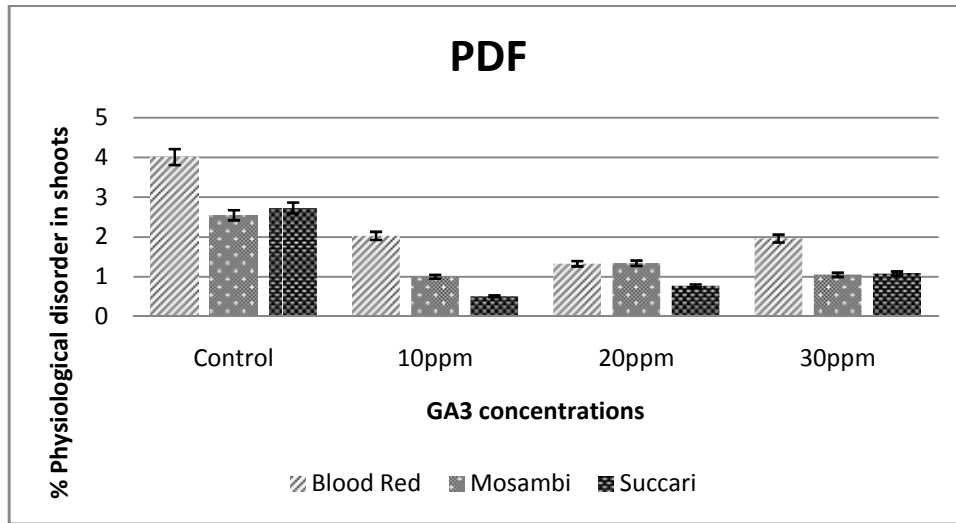

Fig. 3. Physiological disorder percentage in fruits as affected by different GA_3 treatments in sweet orange. (Graphical illustration based on grand means)

Fig. 4. Intensity of fruit splitting disorder increasing with passage of time and fruit size

3.3 Days to Fruit Maturity (DFM)

The data regarding DFM is presented in Fig. 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 30ppm treated trees closely followed by 10ppm (196.78) and 20ppm (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 Mosambi sweet orange.

Almost all GA_3 treatments showed a delaying effect in terms of fruit maturity; it might be due to the influence of GA_3 on chemical compositions of sweet orange fruits. It might also be due

to varietal differences with respect to the number of days required to attain fruit maturity by different cultivars. GA_3 reduces chlorophyll depletion and carotenoid accumulation, and hence causes color break delay in fruit's peel [27]. Similarly GA_3 application results fruit ripening delay in Strawberry, which is a highly perishable fruit crop [28].

Table 1. PDS, PDF and DFM as affected by different GA_3 treatments in sweet orange cultivars

GA_3 (Conc.)	PDF	PDS	DFM
G_0	2.7633[a]	3.0989[a]	187.22[c]
G_1	0.8178[b]	1.1789[b]	196.78[b]
G_2	0.7589[b]	1.1489[b]	196.78[b]
G_3	0.9567[b]	1.3667[b]	197.89[a]
Significance	*	*	*
LSD (Treatments)	0.6890	0.5967	1.0456
Cultivars			
C_1	1.7608[a]	2.3350[a]	195.75[a]
C_2	1.0650[b]	1.4858[b]	192.50[b]
C_3	1.1467[b]	1.2742[b]	195.75[a]
Significance	*	*	*
LSD (Cultivars)	0.5967	0.5168	0.9055
Interaction			
$G_0 \times C_1$	2.5967[ab]	4.0100[a]	188.67[d]
$G_0 \times C_2$	3.2233[a]	2.5533[b]	183.67[e]
$G_0 \times C_3$	2.4700[abc]	2.7333[b]	189.33[d]
$G_1 \times C_1$	0.4633[ef]	2.0333[bc]	199[a]
$G_1 \times C_2$	1.7333[bcd]	0.9967[de]	194.33[c]
$G_1 \times C_3$	0.2567[f]	0.5067[e]	197[b]
$G_2 \times C_1$	0.2033[f]	1.3333[cde]	197[b]
$G_2 \times C_2$	1.5800[bcde]	1.3400[cde]	197[b]
$G_2 \times C_3$	0.4933[ef]	0.7733[e]	198.33[ab]
$G_3 \times C_1$	0.9967[def]	1.9633[bcd]	198.33[ab]
$G_3 \times C_2$	0.5067[ef]	1.0533[cde]	195[c]
$G_3 \times C_3$	1.3667[cdef]	1.0833[cde]	198.33[ab]
Significance	NS	NS	*
LSD (Interaction)	-	-	1.8111

*significance at α=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

3.4 Supplementary Information

3.5 Fruit Growth (mm)

A continuous fruit growth recorded on weekly bases is presented in Fig. 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_3 applications. The graphical presentation of fruit growth showed non-significant differences because the bars representing different concentrations followed almost same pattern for fruit growth.
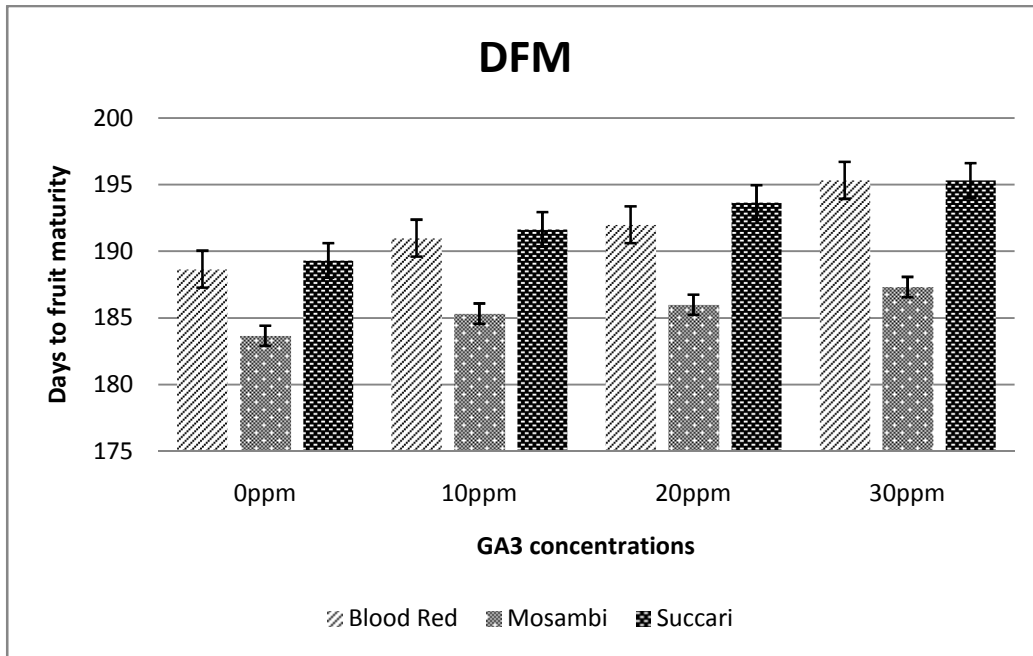
Fig. 5. Graphical illustration for DFM, as influenced by different GA₃ application as foliar spray. (Graphical illustration based on grand means)
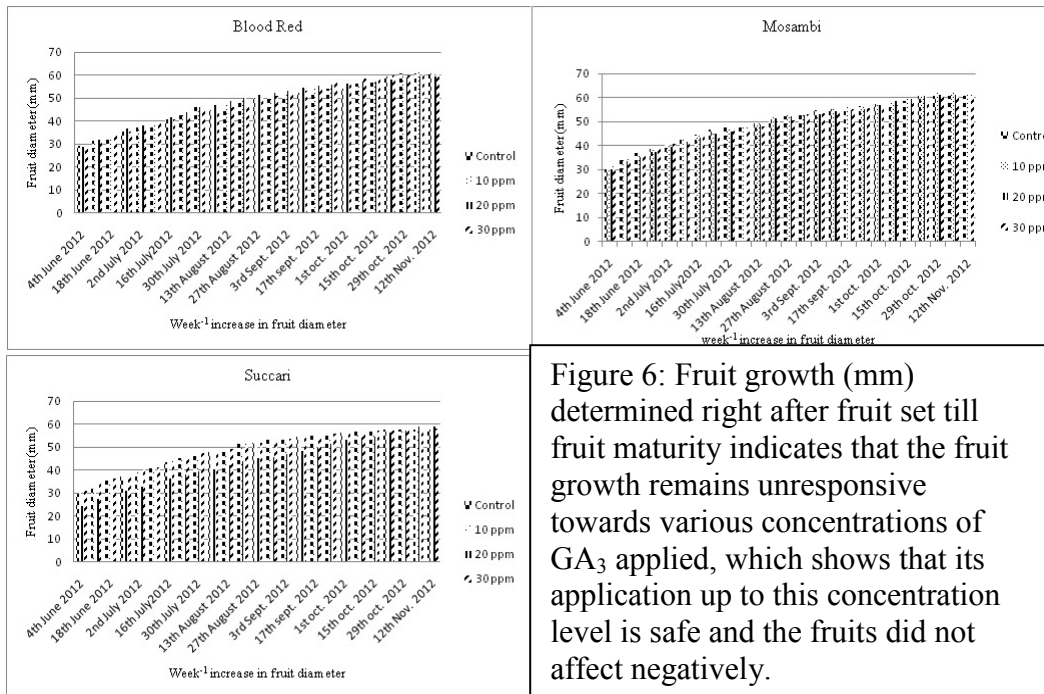

Figure 6: Fruit growth (mm) determined right after fruit set till fruit maturity indicates that the fruit growth remains unresponsive towards various concentrations of GA₃ applied, which shows that its application up to this concentration level is safe and the fruits did not affect negatively.

Fig. 6. Response of fruit growth towards different GA₃ concentrations applied as a foliar spray in the period from fruit set till fruit maturity

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_3 and almost same environmental conditions. The application of (20 mg L^{-1}) GA_3 delayed fruit maturity 5-8 days only for the late-maturing genotypes, but did not affect early fruit maturing genotypes, while GA_3 application to Strawberry significantly increased fruit weight and size as compared to untreated plants [29]. Gene ontogeny of plum fruit revealed that GA_1 and GA_4 are very important for fruit growth and development. Further several genes have been believed to be involved in GA signaling prior fruit development [30].

4. CONCLUSION

The results revealed that 30ppm GA_3 application is the best treatment to control fruit and shoot physiological disorder along with prolonging the harvesting season of sweet orange. All tested cultivars responded positively towards the foliar application of GA_3 without any malformation in their fruit growth. Therefore, it could be recommended that 30 ppm GA_3 should be applied at full bloom stage of sweet orange in order to get these desirable effects under similar environmental conditions.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Agricultural Statistics of Pakistan, Government of Pakistan, Statistics Division, Pak Bureau of Statistics. 2010-11;89:1-2.
2. Florida Agricultural Statistics Services, Citrus statistics. Florida citrus mutual. Orange Street, Lakeland USA; 2011-12.
3. Ozkan B, Handan A, Feyza K. Energy requirement and economic analysis of citrus production in Turkey. Energy conservation and management. 2004;45:1821-1830.
4. Anil K, Samrita D, Atish P. Protective effect of Naringin, a Citrus Flavonoid, against colchicine-induced cognitive dysfunction and oxidative damage in Rats. J Med Food. 2010;13:976-984.
5. Parmar HS, Kar A. Medicinal values of fruit peels from *Citrus sinensis*, *Punicagranatum* and *Musa paradisiaca* with respect to alternations in tissue peroxidation and serum concentration of glucose, insulin and thyroid hormones. J Med Food. 2008;11:376-381.
6. Kafkas E, Ercisli S, Kemal KN, Baydar K, Yilmaz H. Chemical composition of Blood Red varieties from Turkey. A comparative study. 2009;5:329-385.
7. Alva AK, Paramasivam S, Graham WD, Wheaton A. Best nitrogen and irrigation management practices for citrus in sandy soil. Water Air Soil poll. 2003;143:139-154.

8. Kang SS, Chao KS, Son DS, Song JH, Kim YK, Jeong et al. Field performance test of 'Baeyun No. I' selected rootstock against some physiological disorders in pear fruit. Agris Records. 2009;3:26.
9. Saleem BA, Malik AU, Pervez MA, Khan AS. Growth regulators application affects vegetative and reproductive behavior of 'Blood red' Sweet orange. Pak J Bot. 2008;40:2115-2125.
10. Ahmed W, Tahir FM, Rajwana IA, Raza SA, Asad HU. Comparative evaluation of plant growth regulators for preventing premature fruit drop and improving fruit quality parameters in 'Dusehri' Mango. J Fruit Sci. 2012;12:372-389.
11. Bower JP. The physiological control of citrus creasing. ActaHortic. 2004;632:111-115.
12. Rooshan, Vahid I, Mahmood O, Ghulam RR, Ismaeil. The effect of some plant growth regulators on Date palm bunch fading disorder in (*Mozafti and Kabkab*) cultivars. Agris Records. 2009;2006:29.
13. Engin H, Sen F, Pamuk G, Gokbayrak Z. Research on the physiological disorders and fruit quality of cardinal peach. New Zeal J Hort Sci. 2010;38:39-46.
14. Greenberg H, Holtzman S, Egozi Y, Giladi B, Oren Y, Kaplan I. Effect of NAA and GA(3) on fruit size and the incidence of creasing of 'Washington' Navel Sweet orange. ActaHortic. 2010;884:273-279.
15. Saure MC. Causes of the tipburn disorder in leaves of vegetables. Scihortic-Amsterdam. 1998;76:131-147.
16. Hesami A, Abdi G. Effect of some growth regulators on physiochemical characteristics of Date palm (*phoenix dactylifera* L. cv. Kabkab) fruit. J Agric Envir Sci. 2010;7:277-282.
17. Editor's summary: Gibberellins' light touch. Nature. 2008;451:7177.
18. Yamaguchi S. Gibberellin metabolism and its regulation. Annual Review of Plant Biology. 2008;59:225–251.
19. El-Kassas SE. Effect of somegrowthregulatorsontheyield and fruitquality of Zaghloul date palm. 3rd symposiumon date palm, date palmresearch centre, King Faisal UnivSaudi Arabia. 1993;179-186.
20. Hussein MA, EL-Agamy SZ, Amin KIA, Galal S. Physiological studies for prolonging harvest dates of Samany dates under Assint Governorate conditions. A- Effect of GA_3 on fruit thinning.3rd symposiumon date palm, date palmresearch centre, King Faisal UnivSaudi Arabia. 1993;423-433.
21. Sumitomo chemicals Australia. Online accessed may 06 2014. Available: http://www.sumitomo-chem.com.au/sites/default/files/pdf/labels/progibb-sg_label.pdf.
22. James DM. Gibberellin, in AccessScience @ McGraw-Hill. Accessed 06 May 2014. Available: http://www.accessscience.com.ezproxy.torontopubliclibrary.ca.
23. Avidan B, Klein I. Physiological disorders in loquat (Eriobotrya japonica Lindl.). In Proceeding of the first international Symposium on loquat. Valencia*, Zaragoza,* Spain: ciheam-IAMZ Press. 2003;87-95.
24. Guardiola JL, Barries MT, Albert C, Garcia LA. Effect of exogenous growth regulators on fruit development in *Citrus unshiu*. Ann Bot-London. 1993;71:169-176.
25. Miwa M, Sato S, Ohara H, Yahata S, Matsui H. Effects of GA_3 and CPPU application on growth and quality of seedless fruit in triploid loquat (*Eriobotrya japonica*). Agris Records. 2006;2:157-164.
26. El-Sharkawy I, Sharif S, El-Kayal W, Mahboob A, Abu BK, Ravindran et al. Characterization of gibberellins signaling elements during plum fruit ontogeny defines the essentiality of gibberellin in fruit development. Plant Mol Bio. 2013;l84:399-413.
27. Hwang YS, Bethke PC, Cheong YH, Chang HS, Zhu T, Jones RL. A gibberellin-regulated calcineurin B in rice localizes to the tonoplast and is implicated in vacuole function. Plant physiology. 2005;138(3):1347-1358.

28. Oh SI, Park J, Yoon S, Kim Y, Park, S, Ryu M, Kim KN. The Arabidopsis calcium sensor calcineurin B-like 3 inhibits the 5'-methylthioadenosine nucleosidase in a calcium-dependent manner. Plant physiology. 2008;148(4):1883-1896.
29. Oertli JJ. Exogenous application of vitamins as regulators of growth and development of plants, a review. J Plant Nutr Soil Sc. 2007;150:375-391.
30. Alos E, Cercos M, Rodrigo MJ, Zacarias L, Talon M. Regulation of color break in citrus fruits, changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. J Agr Food Chem. 2006;54:488-95.