Manipulation of splitting, sunburn and enhancing coloration of Wonderful pomegranates by preharvest foliar applications

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ABSTRACT

In order to manipulate splitting, sunburn and enhancing coloration of pomegranates. (*Punica granatum* L.) cultivar "Wonderful" was treated with Gibberelic acid (GA_3) at 20 ppm (w/ v), Calcium Chloride ($CaCl_2$) at 0.5 % (w/ v), ProTone (Active ingredient, Abscisic acid (ABA 10 %) at 100 ppm (v/ v), GA_3 plus $CaCl_2$, GA_3 followed by ProTone and GA_3 plus $CaCl_2$ followed by ProTone. The application of GA_3 and $CaCl_2$ treatments were sprayed twice during both years of study (in the second week of May and repeated 30 days after the first spray), in addition to each of the above treatments followed by ProTone at 100 ppm as a coloration treatment, which was sprayed at color initiation on 25, 28 August during 2014 and 2015 respectively. The results indicated that GA_3 treatments significantly increased shoot length, fruit length, fruit weight, aril weight and fruit acidity. While, reduced fruit splitting, fruit sunburn, light intensity and peel anthocyanin contents compared with the control treatment. Furthermore, application of 0.5 % $CaCl_2$ significantly increased fruit length, slightly fruit weight, aril weight and fruit acidity. While, reduced fruit splitting as compared with untreated fruits. ProTone treatments at 100 ppm had no effect on physical characteristics but increased peel anthocyanin contents, Total Soluble Solids (TSS), total sugar and reduced fruit acidity of pomegranate fruits. In general, the formulation containing GA_3 plus $CaCl_2$ followed by the application of ProTone at 100 ppm proved to be the best for improving vegetative growth, reducing splitting, sunburn and enhancing coloration of pomegranates.

Keywords: GA_3; Splitting; Vegetative Growth; Sunburn; $CaCl_2$; ABA.
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1. INTRODUCTION

Pomegranate are highly demanded by consumer and have necessary health benefits which are associated with high concentration of antioxidants, medicinal characteristics, vitamins c, sugars and important nutrients (Holland et al., 2009). Egyptian Pomegranate growers have been facing many production problems that impede them and represent a main obstacle towards obtaining high quality such as splitting, sunburn and a lack of adequate color intensity and uniformity. There are many attempts to reduce splitting and sunburn disorders, one known approach is to follow suitable orchard management. Another approach is to selected resistant cultivars (Abdollah Khadivi-Khub, 2015).

There is a lack of successful treatments that significantly reduced the incidence of fruit cracking and splitting. Moreover, pomegranate growers especially in warm regions suffer from the consequences of high temperature during the skin and aril colorations which adversely affect fruit marketability. Spray of reflective materials such as lime solutions affect the efficacy of photosynthetic capacity and need further application over time. Shading fruits by using paper coverage has been consuming time, cost and efforts. Large scale production of pomegranates still requires an effective-spray procedure to enhance fruit coloration and quality that is feasible under field conditions.

Application of GA_3 and $CaCl_2$ has been utilized to reduce preharvest disorders of splitting and sunburn but retards coloration or anthocyanin formation (Al-Hmadawi et al., 2011). However, the application of ABA resulted in an increase of anthocyanin. Thus, the applications of GA_3 plus $CaCl_2$ followed by ABA have the potential to achieve the desired skin coloration. GA_3 was found to delay ripening and senescence of fruits (Sher-Muhammad et al., 1996, a). Moreover, GA_3 application increased vegetative growth, leaf area and reduced light intensity (Jadhav et al., 2006 and Singh, 2008). High calcium content of fruit reduces disorders in addition to strengthen cell wall, also regulates some physiological processes that may directly affect the quality of fruit (Roy, 1995 and Valero, et al., 2002).

ABA (ProTone, the commercial form) have the potential to increase the anthocyanin concentration and hasten maturation (Zhang and Dan, 2012). This could due to the stimulatory of ABA on phenylalanineammoniumlyase (PAL) activity or due to the increase in the expression of genes involved in the anthocyanin biosynthetic pathway in response to ABA. Generally, there is a close physiological association between increasing ABA, PAL activity and anthocyanin level (Boo et al., 1997).

Thus, the objectives of this research were to reduce splitting and sunburn disorders by preharvest application of GA_3 and $CaCl_2$ and investigate the possibility of using ABA as a coloring agent of pomegranate fruits. Moreover, provide pomegranate growers with feasible and applicable regime to follow in the field which reflects on quality and profitability.

2. MATERIALS AND METHODS

2.1. Plant material and Experimental design

The present study was conducted on “Wonderful” pomegranate cultivar (*Punica granatum* L.) on a commercial orchard at Elboustan region, Behira governorate, Egypt over the two seasons 2014 and 2015. Trees were four years old, spaced at 2× 4 m (2 m between trees and 4 m between rows). Twenty one uniform trees, free from various physiological and pathological disorders were selected for investigation. Soil texture was sandy and drip irrigation system was adopted. Treatments included:

1- Water as the control.
2- GA_3 at 20 ppm (w/ v).
3- $CaCl_2$ at 0.5 % (w/ v).
4- ProTone (Active ingredient, ABA 10 %) at 100 ppm (v/ v).
5- GA_3 plus $CaCl_2$.
6- GA_3 followed by ProTone.
7- GA_3 plus $CaCl_2$ followed by ProTone.

The application of GA_3 and $CaCl_2$ treatments were sprayed twice during both years of study (in the second week of May and repeated 30 days after the first spray), in addition to each of the above treatments followed by ProTone at 100 ppm as a coloration enhancer, which was sprayed at color initiation on 25, 28 August during 2014 and 2015, respectively. Trees were sprayed to the run off using a hand sprayer. The nonionic surfactant Tween 80 at 0.1% (v/v) was added to all treatments. Commercially ripe fresh fruits were picked on 25 September, 1 October during 2014 and 2015 respectively.

2.2. Shoot length

For measuring shoot length, eight uniform and healthy shoots were randomly selected all over the tree canopy in all directions. The length of each shoot was measured at harvest and expressed in centimeters.

2.3. Light intensity

Light intensity was detected as Foot Candle (F.C) in the central part of the canopy using Light Probe Meter apparatus in different treatments at harvest.

2.4. Fruit splitting % + Fruit sun burn %

At harvest time, the number of fruits per tree in each treatment was counted and also each fruit was visually rated depending on the severity of damage by either splitting or sunburn and calculated as a percentage relative to the total number of fruit on the tree.

For the measurement of physical and chemical parameters of pomegranate fruits, five fruits were randomly selected from each treatment.

2.5. Physical and chemical characteristics

Fruit length (cm), fruit diameter (cm) and peel thickness were measured using a Vernier caliper, fruit weight, peel weight and aril weight of five fruits were recorded and expressed as mean fruit weight in grams. On the other hand, chemical constituents were assessed as follow: TSS% was determined in pomegranate fruit juice using a hand refractometer, the acidity % and L-ascorbic acid (mg/ 100 ml) were determined according to (AOAC, 1985), TSS/Acidity ratio was calculated as a ratio between TSS (%) and acidity (%). Furthermore, peel anthocyanin content (mg/ 100 g) was assessed according to the method of Fuleki and Francis (1968), while total sugars were determined by using the phenol sulfuric acid method (Smith, 1956).

2.6. Statistical analysis

The experiment was laid out in randomized complete block design (RCBD). Each treatment was replicated thrice with single tree as one replication, thus twenty one trees were employed in this study. The data was statistically evaluated by analysis of variance procedures according to Snedecor and Cochran (1980) using SAS software (SAS, 2000). Least significant difference (LSD) test at 0.5 % level of probability was used for comparison of means.

3. RESULTS

3.1. Effect of various applied treatments on shoot length and light intensity of "Wonderful" pomegranate trees

Shoot length of "Wonderful" pomegranate trees as influenced by various treatments was reported in Table 1. The data indicated that during 2014 and 2015 seasons, maximum shoot length was recorded in trees sprayed with GA_3 alone at 20 ppm or in combination with other treatments. However, the difference in shoot length between GA_3 alone was significant as when compared with other GA_3-included treatments except GA_3 when followed by ProTone in the first season. Whereas, minimum shoot length was recorded in ProTone at 100 ppm especially in the second season. Changes in light intensity as influenced by various applied treatments were also reported in Table 1. The data proved that GA_3 treatment caused a significant reduction in light intensity when compared with the control and other individual treatments in both seasons. However, both $CaCl_2$ and ProTone treatments had the highest light intensity inside the tree canopy as compared with GA_3 treatments and were similar to the control.

Table 1: Effect of foliar application of GA_3, $CaCl_2$ and ProTone on shoot length and light intensity of "Wonderful" pomegranate trees during 2014 and 2015 seasons.

Treatments	Shoot length (cm)		Light intensity (Foot/ Candle)	
	2014	2015	2014	2015
Control	52 d	57 c	35.67 a	36.67 a
GA_3 at 20 ppm	78 a	80.67 a	21.67 c	22.33 c
$CaCl_2$ at 0.5 %	58 c	54 c	33.33 a	35.33 a
ProTone at 100 ppm	54 cd	52.33 d	34.67 a	33.67 a
GA_3 plus $CaCl_2$	70.33 b	70 b	24.67 bc	23.67 bc
GA_3 followed by ProTone	74.67 ab	72.33 b	21.33 c	21.33 c
GA_3 plus $CaCl_2$ followed by ProTone	70 b	73 b	26 b	26.33 b

Means, within columns, with the same letter are not significantly different using least significant difference (LSD) at $P \leq 0.05$ levels.

3.2. Effect of various applied preharvest treatments on splitting, sunburn and peel anthocyanin contents of "Wonderful" pomegranate fruit during 2014 and 2015 seasons

The present data in Table 2 showed that foliar application with GA_3 and $CaCl_2$ significantly reduced the percentage of splitting fruits in Wonderful pomegranate cultivar in both seasons as compared with the control. The minimum splitting values were found by using GA_3 and $CaCl_2$ individually. On the other hand, the highest splitting value was observed by ProTone and control fruits. However, when GA_3 preceded the application of ProTone, there was a significant reduction of fruit splitting when compared with the application of ProTone alone.

The data in Table 2 indicated that, all GA_3 treatments whether applied individually or incorporated with $CaCl_2$ or followed by ProTone at 100 ppm reduced the percentage of sunburned-fruits in both seasons as compared with the control. Furthermore, $CaCl_2$-treated fruits had insignificant change in fruit sunburn as compared with the control especially in the first season but tended to reduce sunburn in the second season. Similar trend of results was obtained with ProTone in terms of its influence on sunburned fruits when compared with the control.

The changes in "Wonderful" pomegranate fruit skin color were also reported in Table 2. The data illustrated that there was a trend of reduced anthocyanin content by the application of GA_3 alone or when incorporated with $CaCl_2$. However, ProTone-treated fruits had significantly higher anthocyanin content in the skin of pomegranates when compared with those treated with GA_3, $CaCl_2$ or the control in both seasons. Such trend stayed true even when GA_3 or GA_3 plus $CaCl_2$ preceded the application of ProTone. On the other hand, the application of GA_3 or GA_3 plus $CaCl_2$ followed by the application of ProTone in both cases resulted in greater anthocyanin to each others but much higher anthocyanin than that obtained in the control or individual treatment.

Table 2: Effect of foliar sprays with GA_3, $CaCl_2$ and ProTone on splitting, sunburn and peel anthocyanin contents of "Wonderful" pomegranate fruit during 2014 and 2015 seasons.

Treatments	Fruit splitting (%)		Fruit sunburn (%)		Anthocyanin (mg/ 100 g)	
	2014	2015	2014	2015	2014	2015
Control	10.27 a	12.59 a	22.2 a	25.9 a	12.92 c	12.8 c
GA_3 at 20 ppm	1.41 b	0.853 bc	10.49 b	9.22 cd	12.28 cd	11.87 de
$CaCl_2$ at 0.5 %	0.953 b	0 c	21.77 a	21.47 b	12.51cd	12.64 cd
ProTone at 100 ppm	10.34 a	12.48 a	23.45 a	22.08 b	21.53 a	22.23 a
GA_3 plus $CaCl_2$	0.547 b	0 c	8.82 b	8.88 d	11.63 d	11.64 e
GA_3 followed by ProTone	0.777 b	1.66 b	11.19 b	10.11 c	18.68 b	19.13 b
GA_3 plus $CaCl_2$ followed by ProTone	0 b	0 c	8.79 b	9.34 cd	17.64 b	18.26 b

Means within columns with the same letter are not significantly different using least significant difference (LSD) at $P \leq 0.05$ levels.

3.3. Effect of various applied preharvest treatments on some physical characteristics of "Wonderful" pomegranate fruits during 2014 and 2015 seasons

The data in Table 3 showed that there was a significant increase in fruit length obtained with GA_3 treatment compared with the control. Furthermore, $CaCl_2$ treatment results in further improvement in fruit length as compared with the control. Meanwhile, fruit length was increased by GA_3- contained treatments. On the other hand, ProTone treatment had no significant influence on pomegranate fruit length as compared with the control.

Fruit diameter data (Table 3) proved that GA_3 alone or in addition to $CaCl_2$ or when followed by ProTone treatment caused a significant increase in fruit diameter in a consistent manner in both seasons. On the other hand, Fruit diameter did not significantly change by the application of either $CaCl_2$ or ProTone in both seasons relative to the control. There were no significant differences between various used treatments on fruit peel thickness (Table 3).

The average fruit weight was increased with treatments that contained GA_3. Meanwhile, such treatments resulted in similar fruit weight. Furthermore, GA_3 and calcium caused an increase in peel weight and aril weight in both seasons as compared with control fruits or ProTone-treated fruits. ProTone, individually, did not result in a significant alteration on pomegranate physical characteristics as compared with the control (Table 3).

Table 3: Effect of foliar application of GA_3, $CaCl_2$ and ProTone on some physical characteristics of "Wonderful" pomegranate fruit during 2014 and 2015 seasons.

Treatments	Fruit length (cm)		Fruit diameter (cm)		Peel thickness (cm)		Fruit weight (g)*		Peel weight (g)*		Aril weight (g)*	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	8.18c	8.38d	9.24c	9.3c	0.67a	0.7a	420.43bc	453.43bc	242.06b	249.85bc	178.37b	203.55b
GA_3 at 20 ppm	9.35a	9.45a	10.41a	10.44a	0.64a	0.73a	528.8a	561.76a	304.5a	308.68a	224.27a	253a
$CaCl_2$ at 0.5 %	8.88b	8.72c	9.37c	9.36c	0.66a	0.72a	487.96ab	520.56ab	263.56ab	286.27ab	224a	234.29ab
ProTone at 100 ppm	8.18c	8.28d	9.22c	9.3c	0.63a	0.71a	411.9c	444.9c	241b	241.43c	177.48b	203.44b
GA_3 plus $CaCl_2$	9.22ab	9.25b	9.49b	9.99b	0.65a	0.69a	513.3a	546.3a	271.47b	298.13a	241.83a	248.08a
GA_3 followed by ProTone	9.36a	9.48a	10.44a	10.58a	0.66a	0.72a	512.12a	545.12a	303.18a	294.46a	216.03a	250.61a
GA_3 plus $CaCl_2$ followed by ProTone	9.25a	9.24b	10.07ab	9.97b	0.65a	0.71a	501.31a	534.31a	296.17a	288.61a	205.14ab	245.61a

Means, within columns, with the same letter are not significantly different using least significant difference (LSD) at $P \leq 0.05$ levels.
* mean of five fruits.

3.4. Effect of various applied preharvest treatments on some chemical characteristics of "Wonderful" pomegranate fruits during 2014 and 2015 seasons

The data in Table 4 provided evidences that there was a significant reduction in fruit TSS in response to GA_3 and GA_3 plus $CaCl_2$ treatments in both seasons as compared with the control in a consistent manner. Moreover, the highest percentage of TSS in "Wonderful" pomegranate fruits was found with ProTone treatment in both seasons. Furthermore, when the application of GA_3 and $CaCl_2$ were followed by ProTone, a significant increased was also obtained in TSS relative to the sole application of either GA_3 or $CaCl_2$ or their combination in both seasons. Similar trend of results was obtained with TSS/ acidity ratio and total sugars (Table 4).

The change in fruit acidity of "Wonderful" pomegranates was reported in Table 4. The data revealed that there was a trend of increased fruit acidity by the application of GA_3 alone or in combination with $CaCl_2$. However, a remarkable reduction in pomegranates acidity occurred by the application of ProTone when compared with those treated with either GA_3 or $CaCl_2$ but similar to the control in both seasons. Such trend stayed true even when GA_3 or $CaCl_2$ preceded the application of ProTone.

Data of Vitamin C was also reported in Table 4. The data indicated that both GA3 and GA3 plus $CaCl_2$-treated fruits were equally effective in their influence on Vitamin C when compared with the control. Moreover, the application of ProTone alone caused a significant increase Vitamin C content at harvest as compared with control fruits. This was also the case when ProTone followed the application of GA_3 or $CaCl_2$, since all used combinations had significant change in Vitamin C content as compared with control fruits.

Table 4: Effect of foliar application of GA_3, $CaCl_2$ and ProTone on some chemical characteristics of "Wonderful" pomegranate fruit during 2014 and 2015 seasons.

Treatments	TSS (%)		Acidity (%)		TSS/ acidity (Ratio)		Total sugar (%)		L-ascorbic acid (mg/ 100 ml)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	15.43b	15.4b	1.1c	1.09d	14.08b	14.16b	10.43b	10.4b	17.33c	17.55c
GA_3 at 20 ppm	15.03c	15.03c	1.44a	1.42a	10.43d	10.55d	10.03c	10.03c	17.33c	16.68d
$CaCl_2$ at 0.5 %	15.53b	15.43b	1.24b	1.25c	12.58c	12.39c	10.53b	10.43b	16.68d	16.25e
ProTone at 100 ppm	16.17a	16.33a	1.03d	1.08d	15.66a	15.13a	11.17a	11.33a	19.28a	19.28a
GA_3 plus $CaCl_2$	14.8c	15c	1.45a	1.42a	10.24d	10.56d	9.8c	10c	16.47d	16.9d
GA_3 followed by ProTone	15.63b	15.47b	1.29b	1.27bc	12.14c	12.17c	10.63b	10.47b	18.42b	18.2b
GA_3 plus $CaCl_2$ followed by ProTone	15.63b	15.53b	1.26b	1.29b	12.38c	12.1c	10.63b	10.53b	18.2b	18.42b

Means, within columns, with the same letter are not significantly different using least significant difference (LSD) at $P \leq 0.05$ levels.

4- DISCUSSION

The role of exogenous applied of gibberelic acid (GA_3) in increasing shoot length is previously reported (Sharma and Ananda, 2004; Jadhav et al., 2006; Singh, 2008). Moreover, Dev (2002) working on "Flemish Beauty" pear cultivar reported an increase in vegetative growth by spraying GA_3 at 20 or 30 ppm. The reason of such increase reported by GA_3 was attributed to increased cell division, cell elongation, enhanced photosynthesis process, increased protein content, increased chlorophyll content, increased carbohydrates biosynthesis and finally increased GA_3 activities (Ferguson *et al.*, 1999). On the other hand, GA_3 reduced the light intensity in the central part of the canopy due to increase vegetative growth which reflects on the quality and quantity of light reached to this part.

Similar decrease in pomegranate splitting by GA_3 and calcium sprays was recorded (Singh et al. 1993; Al- Dulaimi, 1999; AL- Rawi, 1999). In addition, spraying of GA_3 at 40 ppm minimized the incidence of cracking on the young pomegranate fruits (Yilmaz and Özguven, 2006). Furthermore, Reducing fruit splitting which results through spraying the GA_3 was ascribed to the increase the leaf area, total chlorophyll, vegetative growth and thus enhanced the partitioning of carbohydrates into fruits leading to increased content of pectic materials (Stern, 2008). In addition, gibberelic acid could resulted in increasing cell wall strength or elasticity (Byers, 1990). Moreover, exogenously applied calcium gave positive role in increasing of calcium pictate which reflected on greater ligation and in strength of the cell walls, which makes it more to resistant to pectin hydrolyses (Roy, 1995). In addition, calcium plays a role in reducing the permeability of cell membranes resulting in obstruction ethylene and enzyme transport that increase the hydrolysis of the cell walls and pectin links (Carl et al., 1991). Therefore, a significant decrease in fruit sunburn was obtained by GA_3 spray was due to decreased light intensity in the central part of pomegranate canopy which strongly ascribed to the reduction in harmful radiation reaching the fruit surface and modified the internal microclimate. Moreover, increased vegetative growth reflected on reducing the pomegranates exposure to direct sunlight. In addition, improved rind qualities of fruit under GA_3 application (Coggins et al., 1971).

On the other hand, ABA treatment promoted highly color formation of skin and enhanced some important qualities for Hanguang apple. ABA treatment was favorable for accumulation of anthocyanin content and reduction of chlorophyll content, and also increased PAL activity. They showed that ABA treatment played a role on altering content and balance among hormones during maturation of the fruits. ABA treatment increased ABA content while decreased GA_3 content in the fruits (Li et al., 2004). Since anthocyanin content in pomegranate is a main quality attribute, the preference of one of these effective treatments on anthocyanins would depend upon the other quality parameters such as fruit splitting, sunburn and other physical and chemical characteristics)

Applying GA_3 to “Wonderful” pomegranate fruit trees improved fruit quality attributes (fruit weight, fruit diameter and fruit length) by enhancing formation and translocation of carbohydrates into the fruit (Richard, 2006; Zhang et al., 2007). Moreover, Gibberellins promote growth by increasing plasticity of the cell wall followed the hydrolysis of starch to sugar which reduces the water potential in the cell, resulting in the entry of water into the cell then causing elongation (Richard, 2006).

The non-significant effect of calcium on fruit weight could be due to the involvement of calcium in the cell wall and plasma membrane integrity, rather than loading carbohydrates in fruit tissue. Also, the non-specific pattern of the impact of ProTone on physical characteristics could be due to the application of ProTone after the completion of fruit growth and no additional effect was obtained. The decrease in fruit TSS, fruit acidity and total sugar of pomegranates, found in this study, caused by preharvest application of GA_3 agreed with the findings of others such as (Lee-ChongSuk et al., 2000 a, b; Hussein *et al*., 2001, b; Kappel and MacDonald, 2002; Usenik et al., 2004). They all reported that, GA_3 tended to reduce the concentration of sucrose and glucose. Meanwhile, ABA treatment increased TSS and reduced acidity (Reynolds et al., 2013).

5. CONCLUSION

From this study, it could be concluded that foliar application of GA_3 alone or in combination with $CaCl_2$ resulted in an increase in vegetative growth, enhanced physical characteristics of wonderful pomegranate fruit trees as compared to control but retards the formation of anthocyanin in peel of pomegranate fruits. On the other hand, the application of ProTone preceded by the formulation containing GA_3 plus $CaCl_2$ proved to be the best for improving vegetative growth, reducing splitting and sunburn while enhancing coloration of pomegranates.

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