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Effect of Brassinosteroids and Gibberellic acid on parthenocarpic fruit formation and fruit quality of Sugar Apple *Annona squamosa* L.

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ABSTRACT

Brassinosteroids and Gibberellic acid are essential for many developmental processes in plants. The present study consists of ten years old sugar apple, trees subjected to nine treatments viz., hand pollination (control), GA_3 (1000 mg /L), GA_3 (1500 mg /L), BRs (0.5 mg /L) BRs (1.0 mg /L), 1000 mg /L GA_3 + 0.5 mg /L BRs, 1000 mg /L GA_3 +1.0 mg /L BRs, 1500 mg /L GA_3+ 0.5 mg /L BRs and 1500 mg /L GA_3 +1.0 mg /L BRs sprayed at anthesis stage one time weekly for five weeks after anthesis. Results indicated that the highest concentration of GA_3 at 1500 or BRs at 1.0 mg /L and the combination between 1000 mg /L GA_3 + 0.5 mg /L BRs are the highest fruit set percentage, fruit retention, number of fruits/tree and yield. Concerning physical and chemical properties, data induced significantly higher value of fruit length, diameter, weight, pulp weight and peel weight, total sugars and lower acidity with foliar application of 1.0 mg /L BRs and 1000 mg /L GA_3 plus 0.5 mg /L BRs. Additional, BRs increased TSS of fruit. Moreover, fruit produced by GA_3 or BRs alone and application of both growth regulators more delayed than those from hand pollination, the use of GA_3 alone or combined with BRs in efficient for producing seedless fruits in sugar apple. Based on this research combined application of 1000 mg /L GA_3 plus 0.5 mg /L BRs had positive effect and therefore can be recommended for spray on sugar apple in order to obtain seedless fruit with high quality, so, it can be used as an alternative to hand pollination. Economic evaluation of each treatment results that the maximum net income from using the combined treatment of 1000 mg /L GA_3 + 0.5 mg /L BRs while the minimum value income from hand pollination treatment.

***Keywords*:** Sugar apple, Gibberellic acid, Brassinosteroids, fruit set, yield and fruit quality.

Introduction

Sugar apple (*Annona squamosa* L.), is one of the most scrumptious fruits from the family *Annonaceae* and the genus *Annona*, which comprises about 120 genera and more than 2000 species (Jalikop, 2011). The *Annonas* widely distributed throughout the subtropical and tropical regions of the world though, originated in tropical America. In Egypt, Annona fruiting area reached 990 Feddan in 2015, producing about 4168 tons according to (Economic Affairs Sector, Ministry of Agricultural, Cairo, 2016)**.** It's a semi-deciduous, tall, woody shrub or small tree with irregularly spreading branches. The greenish yellow flowers arise at in extra axillary position usually in clusters and rarely solitary. Six petals are in two whorls, the flowers are hermaphrodite, both female (carpels) and male (stamens) organs in the same flower. However, the female part matures before the male, which is known as protogynous dichogamy (Campbell and Philips, 1994).

The percentage of fruit setting is low which is previously reported could be due to several reasons such as, high and low humidity prevailing at the time of flowering, soil moisture stress, competition between vegetative and floral growth and dichogamy phenomenon, as well Those factors may result in fertilization failure of most or all ovules, producing poor fruit set and small fruits thus affecting fruit yield and quality (Pereira *et al*. 2011).

It is well known that hand pollination increases yield and fruit quality, thus it was used to overcome the phenomenon of dichogamy, however, it is extremely expensive and time consuming. Furthermore, the large number of seeds in the fruit make it unfavorable for consumption, this issue in order to solve the problem, application of some regulators may be used (Pereira *et al*., 2014).

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In this concern, GA_3 application is currently widely used and it has been standardized especially for sugar apple fruit. The first report on the use of GA_3 in custard apple was published by Saavedra (1979), who revealed the effects of GA_3 as a plant growth regulators, which include an enhancement of fruit length and diameter as well as production of seedless fruits in cherimoya. Likewise, (Santos *et al*., 2016) showed that, 1500 ppm of GA_3 produces high physical and chemical properties of sugar apple and efficient in producing seedless fruits. According to Pereira *et al*. (2014) application of GA_3 on "Gefner" custard apple (*Annona cherimola* x *Annona squamosa*) results in good pulp proportion, excellent soluble solids and mainly seedless fruit. Although the effect of exogenously applied gibberellins have been featured in numerous studies, nothing up to date has include the effects of brassinosteroids on sugar apple fruit set and growth.

Brassinosteroids a new group of polyhydroxyl steroids which have been recognized as a class of phytohormones. These were the first explored when Mitchell *et al*. (1970) observed that cell division and cell elongation were improved by the treatment of organic extracts from the pollen of rape plant (*Brassica napus* L.). Brassinolide, 24-epibrassinolide and 28-homobrassinolide are three biologically active brassinosteroids which isolated by Michael *et al*. (1979). Brassinosteroids play prominent roles in many developmental processes including the increase of cell elongation, pollen tube growth, flowering, senescence, abscission and maturation Swamy and Rao, (2008). According to Mussig (2005) brassinosteroids can regulate and integrates different processes necessary for growth via interactions with phytohormones in a synergistic way. The growth increased by brassinosteroid has been related to promote photosynthesis accumulation in fruits or increase in RNA and DNA content, protein synthesis and polymerase activity (Bajguz and Hayat, 2009). The yield induced by brassinosteroids has been related to improvement the efficiency of photosynthesis process of the sprayed trees. The application of BRs with submicromolar concentrations stimulate various physiological and biochemical responses in various system, from simple cells to whole plants (Mandava *et al*., 1981; Clouse and Sasse, 1998; Sasse, 2003). Moreover, Gomes *et al*. (2006) showed that sprays of brassinosteroid in yellow passion fruits increased number of fruit per plant. Furthermore, the application of BRs to unpollinated flowers in cucumber produced seedless fruit similar to those of the pollinated ones (Fu *et al*., 2008).

Therefore the present study was conducted to evaluate the effect of gibberellic acid and brassinosteroids on induces parthenocarpy and increases fruit set, yield and quality of sugar apple (*Annona squamosa* L.).

Materials and Methods:

The experiment was conducted during the two successive seasons 2017 and 2018 on ten years old sugar apple trees (*Annona Squamosa* L.). The trees were spaced at 4 x 5 m in sandy soil at Al-Salmia orchard in El-Nubaria region (Km 80 Alex-Cairo desert road) El-Beheira Governorate, Egypt. Trees as uniform as possible in growth and appearance were randomly selected for this study. Selected trees were subjected to the same cultivar practice.

Twenty seven trees were subjected to nine application treatments with three replicates. The treatments were: T1, hand pollination (Control), T2: gibberellic acid (GA_3) at 1000 mg /L , T3: GA_3 at 1500 mg /L, T4: Brassinolide (BRs) at 0.5 mg /L , T5: BRs at 1.0 mg /L, T6: 1000 mg /L GA_3 +0.5 mg /L BRs, T7: 1000 mg /L GA_3+ 1.0 mg /L BRs, T8: 1500 mg /L GA_3+ 0.5 mg /L BRs, T9: 1500 mg /L GA_3+ 1.0 mg /L BRs. Hand pollination was performed by using pollen grains from sugar apple (*Annona squamosa* L.) which was collected from stamens of flowers in the male stage and deposited to the stigmatic surfaces at the base of the slightly open petals of the female stage flowers (Pereira *et al*., 2011). Hand pollination was installed on the same time and the trees were sprayed with plant growth regulator during 2017 and 2018 seasons respectively, at anthesis stage or functionally pistillate flowers on stage (pre female to female), and repeated one time weekly five week after anthesis. The flower was probably tagged and plant growth regulators was applied by using spray bottle.

Measurements:

Fruit set and Fruit retention

Number of fruits after fruit set and the remained fruits before harvest were counted, then the fruit set and fruit retention percentages were calculated according to the equations:

$$\text{Fruit set (\%)} = \frac{\text{Average number of developed fruitlets}}{\text{Average number of flower}} \times 100$$

$$\text{Fruit retention (\%)} = \frac{\text{Average number of retained fruit at harvest}}{\text{Average number of developed fruitlets}} \times 100$$

Tree yield

At harvest, the total number of fruits/ tree were counted and weighed, then the yield as kg/tree was calculated.

Days to fruit harvesting

The days from flowering to harvesting time (separation of the carpels green, yellowish color, intercarpelar tissue and light green peel) (Kavati, 1992) were recorded.

Fruit Quality

Physical characteristics

Fruit were randomly selected and collected during September in both seasons to staminate some physical characteristics. Average fruit weight (g), fruit length (cm), fruit diameter (cm), pulp weight (g), peel weight (g) and number of seeds per fruit (g) were recorded.

Chemical characteristics

Pulp samples was extracted from fruits and the total soluble solids percent (TSS %) was recorded by using a hand refractometer. Fruit acidity was determined according to (A.O.A.C., 2000) by titration with 0.1 N sodium hydroxide using phenolphthalein as an indicator and expressed as gluconic acid percentage. Also, fruit total sugars percent were determined by using the phenol sulfuric acid method outlined by Malik and Singh (1980).

Economic evaluation

Economic evaluation of sugar apple (*Annona Squamosa* L.) production was estimated according to Radinovic *et al*. (2004). The cost of production and yield price was calculated depending on farm`s owner information as follows:
- Total return (EGP / feddan) = Total yield (kg /feddan) × (price / (Kg).
- Growth regulators (g/feddan) cost = Total quantity × (price / unit).
- Operation cost (Laborers) = number of labor × (price/labor).
- Net income = Total return – (Growth regulators cost + operation cost)

Statistical analysis

The obtained data were subjected to analysis of variance in randomized complete block design (RCBD) according to Snedecor and Cochran (1980). The mean were compared by using the method of new least significant differences (New L.S.D) described by (Waller and Duncan, 1969).

Results and discussion

Fruit set and fruit retention

The results given in Table (1) represented the effect of spraying GA_3 and BRs on fruit set and fruit retention percentage and the data showed that, application of GA_3 at 1500 mg /L, BRs at 1.0 mg /L, GA_3 1000 mg /L combined with 0.5 mg /L BRs and hand pollination gave the highest percentage of fruit set but differences between them were not big enough to be significant as compared with other treatments in the first season. Meanwhile, the combination treatment between GA_3 at 1000 mg /L plus BRs at 0.5 mg /L was the most effective on increasing fruit set in the second season.

Concerning the fruit retention percentage, data in the same table displayed clearly that, the response fruit retention % was followed the same trend previously detected with above mentioned parameter, i.e., (fruit set %). However, the treatments with GA_3 at 1500 mg /L, GA_3 at 1000 mg /L combined with BRs at 0.5 mg /L and hand pollination (control) had ability to significantly increase fruit retention relative to the other treatments in both seasons.

In this respect, it can be observed that the application of GA_3 at 1500 mg /L or the participation of both growth regulators (GA_3 at 1000 mg /L and 0.5 mg /L BRs) is essential to improve fruit set and retention in sugar apple. So, we can be used as an alternative to hand pollination. Similar behaviour was observed with "Gefner" custard apple flowers treated with GA_3 at 1500 mg /L presented an 80 % fruit set (Santos *et al*., 2016). Furthermore, Pino (2008) found that application of gibberellin alone or mixture with cytokinin provided up to an 84% fruit set of cherimoya (*Annona cherimola* Mill.).

Total number of fruit per tree

There was significant differences in respect of total number of fruits per tree affected by GA_3 and BRs treatments in sugar apple, as can be seen from the data presented in Table (1). 1000 mg /L GA_3 +0.5 mg /L BRs recorded Maximum number of fruits per tree. However, highest concentration of GA_3 1500 mg /L, BRs (1.0 mg /L) give the highest number in the second seasons.

The increases in number of fruits per tree may be due to that both of growth regulators positively affected fruit set, fruit retention, cell division, cell elongation, cell enlargement and ultimately, leads to better growth of fruit. The above results are in agreement with Patel *et al*. (2010) on custard apple, Roghabadi and Pakkish (2014) on sweet cheery and Chaudhari *et al*. (2016) on custard apple. In addition, Khripach *et al.* (2000) suggested that brassinosteroids confer resistance to plants against biotic and abiotic stresses which may result in improve the physiological processes from opening of flower to fertilization and fruit set. Furthermore, Bansidharrao, (2016) noted that spraying of 1.5 mg /L brassinolide at anthesis in sapota tree increased the number of fruits per tree.

Fruit yield

The data regarding fruit yield as affected by GA_3 and BRs treatments as presented in Table (1) the data clearly indicated that maximum yield was observed in combined treatment of 1000 mg /L GA3+0.5 mg/LBRs which was significantly superior over control in the both seasons, also, treatment with GA_3 1500 mg /L and 1.0 mg /L BRs application gave the highest fruit yield in the second season. The increased in yield under GA_3 and BRs treatments was associated with increase the number of fruit, more fruit set, fruit retention and increased fruit growth, these findings are close conformity with Lal *et al*. (2013) in guava. The yield increase in the sugar apple may be related to improvement in the assimilation efficiency of photosynthetic carbon of the sprayed plants (Gomes *et al*., 2006) and the movement of metabolites and nutrients into developing fruits (Agusti *et al*., 1994).

Days to fruit harvesting

The data in Table (2) revealed that, number of days required for flowering to harvesting was influenced by application GA_3 and BRs. However, all treatments with GA_3, BRs and the combination between them resulted in significantly more delayed fruit harvest with hand pollination on up to 16-17 days. Moreover, GA_3 at 1000 mg /L combined with BRs (0.5 mg /L or 1.0 mg /L) was significantly superior over the rest of treatments as compared with control (HP) and the other treatments in both seasons. It might be due to the presence of many seeds and the possibility of the embryos to produce high level of natural hormones in the fruits arising from hand pollination (Pereira *et al*., 2014).

Table 1: Effect of gibberellic acid and Brassinolide on fruit set, retention%, total number of fruits/ tree and yield of sugar apple fruits during 2017 and 2018 seasons.

Treatments	Fruit set (%)		Fruit retention (%)		Total number of fruit/Tree		Yield (Kg/Tree)	
	2017	2018	2017	2018	2017	2018	2017	2018
T1: Hand pollination (Control)	78.33	80.33	82.67	82.67	100.0	101.65	23.28	23.80
T2: GA_3 at 1000 mg /L	66.00	67.67	68.33	70.33	93.30	97.60	20.80	21.76
T3: GA_3 at 1500 mg /L	79.33	82.67	82.33	81.67	106.30	110.70	24.58	25.95
T4: BRs at 0.5 mg /L	74.00	75.67	76.67	76.33	100.37	101.90	23.56	24.02
T5: BRs at 1.0 mg /L	79.33	80.33	79.00	80.00	106.65	109.50	25.31	25.80
T6: GA_3 at 1000 mg /L + BRs at 0.5 mg /L	80.00	83.67	82.67	81.67	111.34	110.55	26.53	25.78
T7: GA_3 at 1000 mg /L + BRs at 1.0 mg /L	73.67	80.67	75.33	75.00	102.13	108.80	23.71	25.60
T8: GA_3 at 1500 mg /L + BRs at 0.5 mg /L	71.00	77.33	72.67	75.33	98.60	105.80	22.86	24.71
T9: GA_3 at 1500 mg /L + BRs at 1.0 mg /L	74.67	80.00	76.67	77.33	102.32	108.51	24.11	25.57
New LSD at $_{0.05}$	2.41	2.26	1.92	1.87	1.31	1.37	0.34	0.26

Fruit growth

Data presented in Table (2) revealed that GA_3 and BRs showed significant effect of fruit length and diameter. Hand pollination (control) and 1000 mg /L GA_3 combined with 0.5 mg /L BRs showed significantly more length of fruit over rest of all treatments .As for fruit diameter, hight concentration of BRs (1 mg /L) recorded the highest fruit diameter as compared with tested treatments in the both seasons. While, hand pollination (control) and 1000 mg /L GA_3 plus 0.5 mg /L produced the superior fruit diameter in the second season. In general, BRs increased the fruit dimension comparably better than GA_3 with two concentration (Engin *et al*., 2015).

The data of this study indicated that fruit derived from treatments with hand pollination showed higher fruit dimension, this might be due to the supply hormones produced by embryo and seed which control the role and maintance of cell division (Varga and Brainsma, 1986). Furthermore, applying brassinosteroid alone or with GA_3 resulted in the longest and widest fruit but application the same GA_3 alone were not effective on fruit dimension. This might suggest that GA_3 and BRs seemed to increase cell elongation and cell division which was mentioned in Yokota (1997) and Engin *et al.* (2015). In addition, Gregory and Mandava (1982) and Steber and Mc Court (2001) reported that exogenous application of GA_3 and BRs can act on each other synergistically and induced increased fruit length and diameter. Additionally, the growth induced by brassinosteroid has been related to increase in RNA and DNA content polymerase activity and protein synthesis (Kalinich *et al*., 1985).

Table 2: Effect of gibberellic acid and Brassinolide on days to fruit harvest, fruit length and diameter of sugar apple fruits during 2017 and 2018 seasons.

Treatments	Days to fruit harvest		Fruit length (cm)		Fruit diameter (cm)	
	2017	2018	2017	2018	2017	2018
T1: Hand pollination (Control)	108.00	110.33	**9.37**	8.87	8.17	8.40
T2: GA3 at 1000 mg /L	124.67	123.33	7.77	6.63	7.30	7.83
T3: GA3 at 1500 mg /L	123.33	124.33	7.90	7.80	7.30	7.50
T4: BRs at 0.5 mg /L	113.67	118.00	8.16	8.07	7.73	7.70
T5: BRs at 1.0 mg /L	119.67	120.00	8.23	8.17	8.60	8.03
T6: GA3 at 1000 mg /L + BRs at 0.5 mg /L	126.33	126.33	9.27	8.67	8.10	8.37
T7: GA3 at 1000 mg /L + BRs at 1.0 mg /L	127.00	126.00	9.12	8.70	7.93	7.20
T8: GA3 at 1500 mg /L + BRs at 0.5 mg /L	122.67	123.67	8.63	8.20	8.23	8.17
T9: GA3 at 1500 mg /L + BRs at 1.0 mg /L	125.00	124.67	8.43	7.97	8.00	8.07
New LSD at 0.05	4.56	3.82	0.36	047	0.23	0.37

Fruit weight

In concerning with influences of GA_3 and BRs spraying in fruit weight of sugar apple, data in Table (3) revealed that there was a big tendency for fruits to be heavier when exposed to BRs alone or combined with GA_3. High concentration of BRs (1.0 mg /L) significantly increased the fruit weight. However, all combination treatment with GA_3 and BRS and treatment with hand pollination recorded the next best treatments both seasons. Possibly, thus was due to BRs and GA_3 which acted through cell elongation (Fujioka, 1997) and mobilized metabolites to the fruits that may have improved fruit size (Bhatia and Kaur, 1997). In this respect, Kappel and MacDonald (2002), Wang *et al.* (2004) and Roghabadi and Pakkish (2014), reported that brassinolide increased fruit weight of orange. However, Engin *et al*. (2016) showed that combined application of GA_3 and brassinosteroid increased fruit weight in sweet cherries fruits. Furthermore, BRs affected fruit weight more than GA_3 did. In addition, Eid *et al*. (2016) revealed that foliar application of Milagro (0.2% Brassinolide) increased fruit weight of avocado tree cv.Fuerte. Also, Bhat *et al*. (2011) observed that brassinosteroid 0.4 mg /L combined with CPPU (Cytofex) give the highest bunch and berry weight due to the increased assimilation efficiency of photosynthetic carbon, however, brassinosteroid stimulate greater Co_2 assimilation besides increased cell division by CPPU (Cytofex).

Pulp weight and peel weight

The results concerning to pulp weight of fruit in sugar apple as affected by GA_3 and BRs are presented in Table (3). The maximum pulp weight of fruits was noted in treatments of 1.0 mg /L BRs and 1500 mg /L GA_3 which were significantly superior over control and followed by 1500 mg /L GA_3 combined with 1.0 mg /L BRs. On the other hand, all treatment with GA_3 or BRs significantly increased the weight of peel as compared with control. The increase in fruit pulp weight may be due to stimulate cell division and increased volume in the newly divided cells (Metraux, 1988) or may be enhance uptake of water and accumulation of sugar and other food reserves in a greater amount as well as increased volume of intracellular spaces in pulp of fruit due to GA_3. Moreover, higher pulp content may be due to higher accumulation and translocation of extra metabolite from other parts of the tree towards developing fruits (Barkule *et al*., 2018). The results are in agreement with findings showed by Bhoye (2010), Patel *et al*. (2010) on custard apple, Debnath *et al*. (2011) on Phalsa, Mulagund *at al*. (2015) on banana and Chaudhari *et al*. (2016) on custard apple.

Number of seed per fruit

Data in Table (3) showed that hand pollination (control) recorded the highest number of seeds per fruit significantly, during investigated seasons. However, all treatments with gibberellin alone or combined with brassinosteroid showed the lowest number of seeds. Furthermore, the decrease in number of seeds per fruit might be due to parthenocarpic effect of gibberellic acid in seeded fruit.

Similar effects were observed by Pereira *et al.* (2014) who found use 1000 mg /L GA_3 alone or with growth regulator resulted high rate of parthenocarpic fruit in the "Gefner" custard apple. In this respect, Santos *et al.* (2016) indicated that three applications of GA_3 with 1500 and 1000 mg/L are efficient for producing seedless fruits in custard apple. Similarly, Hartmann and Anvari (1986) also found GA_3 treated plum fruits with no seeds or with aborted seeds. Fu *et al*. (2008) reported that the application of (24-epibrassinolid, EBR) induced parthenocarpic growth in cucumber.

Total Soluble Solid

The results in Table (4) showed, the positive influences of GA_3 and BRs on total soluble solid (TSS) content of sugar apple. The treatment (BRs 0.5 mg /L) recorded the higher TSS content than other treatments in the first season but it was found statistically at par with treatment (BRs 0.5 or 1.0 mg /L) and control in the second season. Two concentration of GA_3 had the least amount of total soluble solids in both seasons as compared with control and the other treatments. According to Pereira *et al*. (2014) the commercial standards of TSS ranging from 20:32°Brix which proves trees achieved high volume and excellent quality of all fruits produced. In this respect Dhananjay (2017) on custard apple revealed that the increase in TSS might be due to the rapid metabolic transformation of pectin and starch into soluble compounds and quick translocation of sugars from the leaves to fruits. The upper values of soluble solids induced by brassinosteroid may be due to mobilization of metabolites from source to sink and also conversion of starch and acids into sugars which the major part of soluble solids (Barkule *et al*., 2018). Moreover, Gomes *et al*. (2006) indicated that brassinosteroid increased 1°Brix in the total soluble solid content in the Passion fruit than the control. These results also agreement with Hong Bo *et al*. (2013) who indicated that applied 1ppm of brassinolide recorded highest TSS in "Baiyulong" pitaya fruits.

The reduction in TSS of the fruit treated with GA_3 may be explained by direct effect of cell expansion promoted by GA_3 and increased xylogucan endotransglycosylase hydroxylas (XTH) which facilitate entry of expansions in the cell wall and preventing the development of wall pressure which enabling water to enter the cell, thereby decreasing the concentration of total soluble solid in the cytoplasm (Taiz and Zeiger, 2010).

Table 3: Effect of gibberellic acid and brassinolide on fruit weight, pulp weight, peel weight and number of seed /fruit of sugar apple fruits during 2017 and 2018 seasons.

Treatments	Fruit Weight (g)		Pulp weight (g)		Peel weight (g)		Number of seed / fruit	
	2017	2018	2017	2018	2017	2018	2017	2018
T1: Hand pollination (Control)	232.91	234.13	161.16	161.40	52.55	53.13	64.00	65.33
T2: GA_3 at 1000 mg /L	222.96	222.96	165.30	164.20	56.26	57.76	3.66	3.33
T3: GA_3 at 1500 mg /L	231.25	232.30	172.80	171.80	61.60	60.16	2.00	2.33
T4: BRs at 0.5 mg /L	234.73	235.50	170.90	172.16	62.13	62.06	5.66	4.80
T5: BRs at 1.0 mg /L	239.56	238.57	174.60	173.40	63.16	63.40	5.00	5.55
T6: GA_3 at 1000 mg /L + BRs at 0.5 mg /L	235.66	233.19	172.10	170.86	62.40	61.33	3.67	3.33
T7: GA_3 at 1000 mg /L + BRs at 1.0 mg /L	232.16	233.86	169.93	170.86	61.33	62.00	3.00	3.33
T8: GA_3 at 1500 mg /L + BRs at 0.5 mg /L	234.06	233.50	170.26	169.90	63.00	62.80	2.66	2.67
T9: GA_3 at 1500 mg /L + BRs at 1.0 mg /L	235.80	235.63	171.03	171.50	63.40	63.33	3.33	3.33
New LSD at 0.05	3.044	2.82	2.17	1.84	2.15	1.15	2.26	1.87

Acidity

The data pertaining to acidity percentage of sugar apple fruits as affected by GA_3 and BRs treatment are presented in Table (4). Minimum acidity was observed in treatment of BRs 0.5 mg/L which was significantly superior over control in both season, followed by the treatments with 1.0 mg/L BRs and control. The results are in agreement with the findings reported by Nguyen and Yen (2013) on wax apple, Engin *et al.* (2015) on sweet cherry, İşçi and Gokbayrak (2015) and Thapliyal *et al*. (2016) on pear.

The reason for decrease in titratable acidity maybe due to the metabolic changes with fast conversation of organic acids into sugars and their derivatives by reactions involving reversal of glycolytic pathway or be used in respiration Barkule *et al*. (2018).

Total sugars

Results is presented in Table (4) showed response of GA_3 and BRs on total sugars content of sugar apple fruits. Data showed that the application of BRs alone (0.5 or 1.0 mg/L) recorded the highest total sugars content and which was found significantly superior all over the treatments in the second season but statistically at par with the treatment (1000 mg /L GA_3 +0.5 mg/L BRs) in the first season.

Similary, Wang *et al.* (2004) indicated that brassinosteroid increased sugars content of orange. Synergistic interaction between GA_3 and BRs was also reported by Padashetti *et al*. (2010) in Arka Neelamani and Thompson seedless by application of 50 ppm GA_3 + 1ppm BRs at fruit set resulted in increased sugars content.

The increase in sugars content with the application of GA_3 might have been due to the increase in the capacity of fruits to draw more carbohydrates through increased auxin content due to the rapid metabolic transformation of soluble compounds (Singh *et al*, 1993). BRs is involved in increasing

ABA content which activates sugar metabolic pathway (Symons *et al*., 2006). Therefore, application of BRs increases the sugars in fruits.

Table 4: Effect of gibberellic acid and brassinolide on TSS, acidity and total sugars content of sugar apple fruits during 2017 and 2018 seasons.

Treatments	TSS (%)		Acidity (%)		Total sugars (%)	
	2017	2018	2017	2018	2017	2018
T1: Hand pollination (Control)	28.00	28.40	0.213	0.223	20.77	21.33
T2: GA_3 at 1000 mg /L	27.53	27.00	0.227	0.270	18.92	19.52
T3: GA_3 at 1500 mg /L	27.23	26.33	0.263	0.250	19.05	19.29
T4: BRs at 0.5 mg /L	29.17	28.70	0.203	0.206	21.70	22.10
T5: BRs at 1.0 mg /L	28.10	28.60	0.223	0.220	21.87	21.93
T6: GA_3 at 1000 mg /L + BRs at 0.5 mg /L	27.80	27.70	0.230	0.220	21.70	20.23
T7: GA_3 at 1000 mg /L + BRs at 1.0 mg /L	27.75	27.53	0.233	0.233	19.65	20.08
T8: GA_3 at 1500 mg /L + BRs at 0.5 mg /L	27.63	27.13	0.276	0.240	19.48	20.97
T9: GA_3 at 1500 mg /L + BRs at 1.0 mg /L	27.60	27.07	0.290	0.233	19.35	17.70
New LSD at 0.05	0.50	0.84	0.006	0.021	0.75	0.63

Economic evaluation

Data in respect of economics evaluation of each treatments ,presented in Table 5 revealed that, the maximum net income resulted from using the combined treatment of 1000 mg /L GA_3 +0.5 mg /L BRs in both seasons. On the contrary, the lowest value of net income resulted from hand pollination treatment. Thus, it is observed that hand pollination was not beneficial in terms of monetary returns. So, we can recommend with applying 1000 mg /L GA_3 +0.5 mg /L BRs to get the highest economic return.

Table 5: Economic evaluation as influenced by Brassinosteroids and Gibberellic acid on sugar apple fruits "*Annona squamosa* L.".

Treatments	Quantity of growth regulators (g/L)		Growth regulators (gm/feddan) cost	Operation cost (Laborers)	Total cost (Growth regulators + labors) (EGP / feddan)	Yield (Kg /feddan)	Total return (EGP / feddan)	Net income (EGP / feddan)
	GA_3	BRs						
T1: Hand pollination (Control)	--	--	--	6000	6000	4943.4	74151.0	68151.0
T2: GA_3 at 1000 mg /L	1	-----	3024	1000	4024	4468.8	98313.6	94289.6
T3: GA_3 at 1500 mg /L	1.5	-----	4536	1000	5536	5306.7	116747.4	111211.4
T4: BRs at 0.5 mg /L	---	0.5	3150	1000	4150	4995.9	109909.8	105759.8
T5: BRs at 1.0 mg /L	---	1	6300	1000	7300	5366.5	118041.0	110741.0
T6: GA_3 at 1000 mg /L + BRs at 0.5 mg /L	1	0.5	6174	1000	7174	5492.5	120836.1	113662.1
T7: GA_3 at 1000 mg /L + BRs at 1.0 mg /L	1	1	9324	1000	10324	5177.5	113906.1	103582.1
T8: GA_3 at 1500 mg /L + BRs at 0.5 mg /L	1.5	0.5	7686	1000	8686	4994.8	109886.7	101200.7
T9: GA_3 at 1500 mg /L + BRs at 1.0 mg /L	1.5	1	10836	1000	11836	5216.4	114760.8	102924.8

Conclusion

On the basis of the results obtained in the present investigation it is indicated that, the use of GA_3 promoted the production of seedless fruit and increased fruit set and fruit retention but smaller, later and less quality than those arising from brassinolide and hand pollination. BRs could cross-talk with other hormones such as GA_3, so, it has observed synergistic effect between these bio-regulators, indicating that the best treatment was 1000 mg /L GA_3 plus 0.5 mg /L BRs, it had the most effective result producing seedless fruit with high physical and chemical parameters.

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