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Effect of Foliar Spray with IAA and GA_3 on Production and Protein Synthesis of Two Summer Squash Hybrid Cultivars

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A FIELD experiment was carried out during 2015 and 2016 summer seasons at the Experimental Farm of the Vegetable Department and the Molecular Laboratory of Genetics Department, Faculty of Agriculture, Assiut University, Assiut, Egypt to study the effect of foliar spray by indoleacetic acid (IAA) and gibberellic acid (GA_3) on the production and protein synthesis of two F_1 summer squash hybrids (Rosina F_1 and Eskandrani F_1). The indoleacetic acid concentrations were 0,2.5,5 or 10 ppm and the gibberellicacid concentrations were 0,2.5,5 or 10 ppm. The results showed that IAA and GA_3 had a significant influence on the most studied traits in favor of 2.5 ppm IAA and 2.5 ppm GA_3. Furthermore, the obtained data indicated superiority of Rosina F_1 hybrid over Eskandani F_1 hybrid for all studied traits except sex ratio. Moreover, the different second order interactions had an inconsistent significant effect on the most studied traits. Rosina F_1 summer squash plants which were sprayed with 10 ppm IAA without GA_3 gave the highest mean values for total fruit yield/feddan. Spraying squash plants with GA_3 induced the synthesis of 8 new protein bands, while 10 new proteins were induced by IAA. The two F_1 hybrids of squash exhibited different protein patterns in response to thetreatments combinations of GA_3 and IAA. Eskandrani F_1 was highly sensitive to GA_3 and IAA treatments than Rosina F_1 which showed a reduction of several protein bands and low number of induced proteins.

Keywords: *Cucurbita pepo,* Indoleacetic acid, Gibberellic acid, Protein pattern.

Introduction

Summer squash (*Cucurbita pepo* L.) is considered one of the most important crop in Cucurbitaceae. It was used as a food for human, as well as in many medical purposes (Majeed and Mahmoud, 1988). Commonly, it exhibits more male flowers than female flowers so its yield comes low (Shafeek et al., 2016). Because of the previous reason, growth regulators may be used in farming to increase the total yield. Gibberellins and auxins are used to encourage the germination of seed, vegetative growth, flowering and fruiting in some vegetable crops (Abduljabbar and Ghurbat, 2010). Many obtainable reports show that high yield can be obtained by using growth regulators (Saleh and Abdul, 1980). Shawket (2005) found that use of low concentrations of IAA on summer squash plants at the stage of 3-4 true leaf led to increase fruit number, fruit yield/plant and total yield per unit area as compared to control plants. Moreover, plant growth increased by using high concentrations of gibberellins (Bora and Sarma 2006), while it inhibited by using a higher concentration of auxin (Hussain et al., 2010). Thus, only low doses of auxin are effective in growth upgrade (Vwioko & Longe 2009 and Rastogi et al., 2013). On the contrast, Rastogi et al. (2013) found that gibberellin alone at high dose showed some inhibitory effects and it was more effective at low levels.

Many investigators found significant differencesin fruityield and its components among the studied summer squash genotypes (Mohamed et al., 2003 and Sarhan et al., 2011). Also, Zanjan and Asli (2014) used growth regulators to enhance growth and crop production by enhancing vital physiological processes. This occurs by greater effect on the activation of growth regulator proteins which may be due to several cellular mechanisms related to the metabolism and growth (Adams et al.,1999). Treatment of plants with exogenous hormones

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rapidly alters gene expression and protein synthesis (Nemhauser et al., 2006 and Goda et al., 2008). Overall, hormone-responsive genes include those involved in hormone regulation, metabolism, signal transduction, transcription, protein synthesis, cell expansion and division. In addition, genes regulated by hormones are involved in hormone distribution and homeostasis as well as in negative transcriptional feedback. In *Arabidopsis*, hormone treatments for short periods (<1 h) changes the expression of ~10–300 genes, with roughly equal numbers of genes repressed and activated (Nemhauser et al., 2006, Goda et al., 2008 and Paponov et al., 2008). Several scientists had studied the changes in gene expression and protein synthesis in different plant species treated with GA_3 (Abel et al., 1994, Phillips & Huttly, 1994, Al-Rumaih et al., 2002, Reyes et al., 2006, Mekki, 2008 and Zanjan & Asli, 2014) and IAA (O'Neill and Scorr, 1987, Abel and Theologis, 1996, Ramos et al., 2001, Hagen & Guilfoyle, 2002 and Maraschin et al., 2009).

The objective of this study was to investigate the effect of IAA or/and GA_3 on both production and gene expression as revealed by soluble protein patterns in leaves of two F_1 summer squash hybrids (Rosina F_1 and Eskandrani F_1).

Materials and Methods

A field experiment was carried out during 2015 and 2016 summer seasons at the Experimental Farm of the Vegetable Department and the Molecular Laboratory of Genetics Department, Faculty of Agriculture, Assiut University, Assiut, Egypt to study the effect of foliar spray with indoleacetic acid (IAA) and gibberellic acid (GA_3), separately and in combinations, on the production and protein synthesis of two summer F_1 squash hybrids. The soil of the experimental site was clay with the properties as presented in Table 1(Page et al., 1982).

The experiment was laid out in a randomized complete block design (RCBD) using strip split plot arrangement with three replications. The indoleacetic acid concentrations (0, 2.5, 5 or 10 ppm) were arranged vertically, while the gibberellic acid concentrations (0, 2.5, 5 or 10 ppm) were arranged horizontally and the hybrids (Rosina F_1 and Eskandrani F_1) were arranged in the sub plots. The experiment unit area was 10.5 m^2 (3x3.5 m). Seeds of summer squash hybrids were sownin March 25th and 15th in the first and second seasons, respectively. The sowing was done in rows of 0.70 m apart in hills 40 cm distant. Plants were thinned to secure one plant per hill. The applications of indoleacetic acid (IAA) and gibberellic acid (GA_3) were done 30 and 32 days from planting, respectively. The control plants were sprayed with tap water. All other agricultural practices were done as recommended for summer squash production by the Egyptian Ministry of Agriculture (CAAE, 2012).

TABLE 1.The physical and chemical properties of the experimental farm soil.

Characteristic	Values	Characteristic	Values
pH(1:2.5)	8.01	**Clay %**	53.23
EC1:1 dSm^{-1}	1.35	**Silt %**	28.35
ECe dSm^{-1}	2.01	**Sand %**	18.42
Organic matter (OM)%	1.10	**Soil texture**	Clay
Soluble cations, (meq/kg soil)		**Bulk density, (g/cm^3)**	1.63
Ca^{2+}	10.00	**Field capacity, (F.C)%**	45.70
Mg^{2+}	4.00	**Wilting Point (W.P)%**	21.30
Na^+	4.70	**W. Saturation %**	67.30
K^+	1.30		
Soluble anions, (meq/100 g soil)			
Cl^-	5.70		
$HCO_3^-+CO_3^{2-}$	4.30		
SO_4^{2-}	10.00		
Total nitrogen (ppm)	13.00		
Available phosphorus (ppm)	10.20		
Available potassium (ppm)	312.00		

Growth and yield traits were recorded on 10 random guarded plants sample. Harvest was done at 3 days' intervals. These measurements were as follows:

Sex ratio (number of the female: male flowers), plant height, number of fruits/plant, average fruit weight, fruits weight/plant, total yield (weight of immature fruits in all harvests) and total soluble solids (TSS).

All collected data were subjected to analysis of variance (ANOVA) procedures using the SAS Statistical Software Package (v.9.2, 2008). Differences among means were compared by Revised Least Significant Differences test at 5% level of significant (Gomez and Gomez, 1984). The simple correlation coefficients (r) were calculated among the studied traits.

Electrophoresis for protein patterns

After one week from spraying with gibberellic acid, soluble proteins were extracted from squash leaves excised from H_1 (Rosina F_1) and H_2 (Eskandrani F_1) plants in each treatment of GA_3 and IAA. Proteins were extracted by grounding ~one gram fresh weight of each treatment in addition to their control in equal volumes of extraction buffer (10 ml 0.5 M Tris pH 6.8, 16 ml 10 % SDS and 30 ml Distilled water). The extracts were centrifuged for 30 minutes at 10000 rpm under cooling. Then, the samples were heated for 5 minutes at 100˚C. Fifty µl of each extraction was mixed with sample application buffer by (1:1) ratio. Electrophoresis for protein analysis were carried out on 12% SDS-PAGE (sodium dedocyl sulphate polyacrylamide gel) vertical slab using BIORAD Techware 1.5 mm according to the method described by Laemmli (1970). The gels were stained with Commassie Blue R and destained by the destaining solution (1acetic acid: 1methanol: 8water, by volume). The molecular weights of protein bands were estimated relative to protein marker consisted of 170, 125, 81, 62, 53, 43, and 32 kD using GS 365 electrophoresis data system program version 3.01 (Microsoft Windows @ version).

Results and Discussion

Vegetative growth and yield traits

Sex ratio

Sex ratio was significantly affected by indoleacetic acid and hybrids in both seasons, while it was reacted significantly with GA_3 in the second season only (Table 2). Both the first and second order interactions did not reach a significant level in both seasons.

Summer squash plants sprayed with IAA showed a significant increase for sex ratio as compared to untreated plants (without IAA). Foliar spray with 2.5 ppm IAA (I_1) recorded the highest mean values of sex ratio which were 1.19 and 1.16 in the first and second season, respectively. Similar results were reported by Abduljabbar and Ghurbat (2010) who stated that IAA increased the female flowers number and reduced the number of male flowers and thus increased the sex ratio value. Further similar results were found by Manacini (1999) and Jalal (2000) in squash and cucumber (*Cucumis sativus*) plants.

Here too, squash plants which were sprayed with 2.5 ppm gibberellic acid (G_1) registered the maximum mean value (1.10) in the second season. These results are in agreement with those obtained by Ashrafuzzaman et al. (2009) and Pervin & Rahman (2010) who reported that different concentrations of IAA and GA3 gave higher sex ratio than the control. On the other hand, Byari (2008) found that sex ratio increase with IAA was higher than GA_3 or the mixture of them.

Furthermore, the Eskandrani F_1 hybrid surpassed the Rosina F_1 in this respect and produced a sex ratio of 1.18 and 1.14 in the first and second season, respectively. This may be due to the genetic differences between the two studied summer squash genotypes and their interaction with the environmental factors which were suitable for the Eskandrani F_1 hybrid than the other one.

Plant height

Exhibited data in Table 3 show that IAA had a highly significant effect on squash plant height in the second season only. Application of 2.5 ppm IAA as foliar spray to summer squash plants gave the tallest plants which were 43.63 cm in the second season. Otherwise, higher IAA concentrations produced the shortest summer squash plants (38.16 and 39.16 cm). These results are in a good way with Weaver (1972) who found that low concentrations of auxin encourage the plant growth, but high concentrations reduced it and the optimum concentration depends on the types of plant and tissue.

Data in Table 3 reveal that the plant height of summer squash was significantly increased by gibberellic acid foliar application in the first season only. Summer squash plants which were sprayed with 2.5 ppm produced the highest mean value of plant height (41.29 cm) in the first season. Moreover, the same gibberellic acid concentration produced the maximum mean value of plant height (40.58 cm) in the second season, but the differences between this concentration and other studied GA_3 concentrations did not reach a significant level. On the other hand, foliar spray with GA_3 at high concentrations (5 or 10 ppm) showed tendency towards reduction of plant height. These results are in agreement with Byari (2008) who found that plant height was increased by using growth regulators IAA, GA_3 and the mixture of them than the control.

Data presented in Table 3 also denote that the studied summer squash hybrids varied significantly in plant height in both seasons. Rosina summer squash hybrid surpassed Eskandrani F_1 hybrid in plant height in both seasons. The values of plant height increased for Rosina F_1 as compared to Eskandrani F_1 and reached about 27.69 and 26.20 % in the first and second season, respectively. This may be due to the genetic makeup of different studied hybrids and their interaction with the environmental factors.

Here too, the illustrated data in Table 3 point out that the all first order interactions involved in this respect had a significant effect on summer squash plant height in both seasons except, the interaction between GA_3 and hybrid cultivars in the first season only. In addition, the second order interaction had a highly significant effect on plant height in the second season only. The tallest plants (49.60 cm) in the second season were obtained from Rosina F_1 hybrid which was sprayed with 10 ppm IAA and without GA_3.

Number of fruits/plant

Presented data in Table 4 reveal that IAA had a highly significant effect on the number of fruits/plant in both seasons. The highest mean values (12.24 and 11.41 fruits in the first and second season, respectively) were obtained from summer squash plants sprayed with IAA at 2.5 ppm. This is logic since the same IAA concentration produced the maximum values of sex ratio and consequently produced the highest number of fruits/plant. The increase in number of fruits per plant due to IAA treatments of the present study agrees with the findings of Choudhury & Babel (1969) and Rahman et al. (1992) on bottle gourd.

The data show that the studied GA_3 concentrations did not significantly affect the number of fruits/plant in both seasons. However, the fruit number/plant was affected significantly ($P \leq 0.01$) by the studied summer squash hybrids in the two growing seasons. Rosina F_1 hybrid surpassed Eskandrani F_1 hybrid in this respect and produced the maximum mean values which were 11.59 and 11.27 fruits in the first and second season, respectively. This may be due to an elevated fruit set percentage.

Regarding the interactions effect in this respect, data presented in Table 4 reveal that all involved interactions effect in this respect had an insignificant effect on number of fruits/plant except the first order interaction between IAA and each of GA_3 and hybrid cultivars in the second season only.

Average fruit weight

Illustrated data in Table 5 denote that the studied IAA concentration had a highly significant effect ($P \leq 0.01$) on average fruit weight in both seasons. Application of IAA as foliar spray to summer squash at low concentration (2.5 ppm) produced the highest values of fruit weight which were 105.15 and 102.99 g in the first and second season, respectively. This trend could be explained by the highest plant growth occur by the IAA concentration of 2.5 ppm which reflect high photosynthesis metabolism to fruit.

Concerning, the GA_3 effect on squash average fruit weight, data presented in Table 5 reveal that GA_3 affected fruit weight significantly in the second season only. Thus, squash plants which were sprayed with 2.5 ppm GA_3 produced the heaviest fruit weight which was 93.86 g. These results are in the same line with Byari (2008) who reported that average fruit weight higher than control by spraying IAA, GA_3 and their mixture.

Data exhibited in Table 5 clear that the tested summer squash hybrids varied significantly in this respect in both seasons. Rosina F_1 hybrid produced the maximum mean values of average fruit weight (99.22 and 96.72 g) in the first and second season, respectively. Here too, recorded data in Table 5 show that all first and second order interactions involved in this respect had a highly significant influence on squash fruit weight in the second season only. The highest mean value (111.67 g) in the second season was obtained from Rosina F_1 squash hybrid which was sprayed with 10 ppm IAA under the control treatment of GA_3.

Fruit weight per plant

Data presented in Table 6 point out that the tested IAA concentrations had a significant and a highly significant effect on the fruit weight/plant in the first and second season, respectively. Summer squash plants which treated with 2.5 ppm IAA gave the maximum mean values of fruit weight/plant which were 1.49 and 1.42 kg in the first and second season, respectively. This is to be expected since the same concentration produced the highest fruit number/plant and average fruit weight traits as mentioned above. Furthermore, the effect of GA_3 in this respect was significant in the second season only. Thus, the highest mean value (1.28 kg) was obtained from summer squash plants treated with 2.5 ppm GA_3 in the second season. This is logic since the same trend was observed regarding to average fruit weight.

Sowing of Rosina F_1 summer squash hybrid out yielded the maximum mean values of fruit weight/plant in the two growing seasons. Meanwhile, Rosina F_1 hybrid produced 1.41 and 1.35 kg in the first and second season, respectively. The amount of increase due to sowing Rosina F_1 hybrid in fruit weight/plant reached about 18.49 and 22.73% as compared to Eskandrani F_1 hybrid in the first and second season, respectively. In addition, all first order interaction in this respect had a significant influence on fruit weight/plant in both seasons except, the first order interaction between GA_3 and hybrid cultivars in the first season. Also, the second order interaction had a highly significant effect in this respect in the second season only, while the effect in the first season was not significant. Thus, the highest mean value (1.61kg) was obtained from Rosina F_1 hybrid which was treated with 10 ppm IAA under control GA_3 in the second season. This is logic since the same trend was obtained from the same interaction with regard to fruit number/plant and average fruit weight.

Total yield

Total yield was affected significantly by studied IAA concentrations in both seasons (Table 7). The highest mean values of total yield (16.57 and 16.08 tons/feddan in the first and second seasons, respectively) were obtained from summer squash plants which were treated with 2.5 ppm IAA. This is logic since the same concentration produced the maximum values of fruit weight/plant. These results are in harmony with other researchers (Sitaram et al., 1988 in cucumber, Das and Das, 1995 in pumpkin, Gedam et al.,1998 in bitter gourd, Balaraj, 1999 in chilli and Rafeekher et al., 2002 in cucumber) who reported that auxins cause physiological changes in plants mostly sex ratio, improved fruit set, increased growth of fruits, source-sink relation and increased yield.

Here too, the tested GA_3 concentrations did not affect significantly the total yield in both seasons. Meanwhile, the data show that the studied summer squash hybrids had a highly significant effect on total yield in both seasons. Rosina F_1 hybrid surpassed Eskandrani F_1 hybrid in total yield. The percentage of increment reached 9.17 and 11.15 tons/feddan in the first and second season, respectively. This is logic since the same hybrid gave the highest fruit weight/plant and consequently produced the highest total yield.

Moreover, the presented data in Table 7 denote that the first order interaction between IAA and GA_3 had a significant influence on total yield in both seasons. Sprayed summer squash plants with 2.5 ppm of both indoleacetic acid and gibberellic acid produced the maximum total yield which was 17.35 and 17.02 tons/feddan in the first and second season, respectively. These results are in the same line with those detected by Akter & Rehman (2010) and Ghani et al. (2013) who reported that a significant effect of growth regulators was found on plant yield and fruit characteristics in cucurbitaceous crops.

Also, the first order interaction between IAA and hybrids were significant and highly significant in the first and second seasons, respectively. The highest mean values of total yield mean values (17.22 and 16.92 tons/feddan) in the first and second seasons, respectively, were obtained from Rosina F1 hybrid sprayed with 2.5 ppm IAA. Concerning the second order interaction, data exhibited in Table 7 show that the second order interaction had a highly significant effect on total yield/feddan in the second season only. The maximum total yield in the second season (18.35 tons/feddan) was obtained from Rosina F_1 hybrid which was sprayed with 10 ppm IAA under the control treatment of gibberellic acid.

Total soluble solids (TSS)

Data presented in Table 8 reveal that the IAA, GA_3, hybrid cultivars and their interactions did not have a significant effect on total soluble solids in both seasons, except the influence of IAA in the second season, and hybrid cultivars in the first one as well as the interaction between IAA and hybrid cultivars. Summer squash plants which were treated with 2.5 ppm IAA produced 3.46 ºBrix in the second season. Also, Rosina hybrid had a high amount of TSS as compared to Eskandrani one (3.51ºBrix) in the first season. Furthermore, the Rosina F_1 plants which were treated with IAA at 2.5 ppm had a TSS of 3.67º Brix in the first season.

The correlation coefficient

Data recorded in Table 9 show that the total yield was correlated positively and highly significantly with all studied traits in both seasons. High correlation coefficient (r) value in the second season (0.742) was obtained from the correlation between average fruit weight and total yield followed by the correlation between fruit weight/plant and total yield (r =0.667). Closely similar results were obtained by Mohamed et al. (2003) who found a positive and significant correlation between sex ratio and total fruit yield and a negative correlation between sex ratio and plant height.

Effect of GA_3 and IAA on protein patterns

Auxins, e.g. IAA, and GA_3 play an important role in regulating many aspects of plant growth and development. This occurs as changes in the expression of specific gene products (Theologis, 1986, Phillips & Huttly, 1994 and Hagen, 1995). The cellular responses to IAA and GA_3 included stimulation of the expression of numerous genes and simultaneously reduce protein synthesis from other genes. Several of these auxin- and GA_3 responsive genes have been identified, and the functions of the proteins they encode have been studied and determined (Abel et al., 1994 and Phillips & Huttly, 1994). In the present study, the effect of IAA and GA_3 on protein synthesis as revealed by soluble protein patterns in leaves of two F_1 squash hybrid cvs (Rosina F_1 and Eskandrani F_1) was studied.

Electrophoretic analysis of protein profiles of squash leaves was determined after spraying with three levels of IAA and GA_3 separately and in combinations, in comparison with control treatment. In addition to variations in band intensity, the results showed large variations in the number of polypeptide bands among the two studied hybrids in different treatments (Tables 10-13, Fig. 1-4).

Effect of GA_3 on protein patterns

Electrophretic analysis of soluble proteins revealed that spraying squash plants of H_1 (Rosina F_1 cv.) with GA_3 [(G_1 , 2.5 ppm), (G_2 , 5 ppm), (G_3, 10 ppm)] induced the synthesis of ten newly protein bands as compared with the control treatment (Table 10 and Fig.1, Lanes 1-4). Three proteins at molecular weights 20.1, 61.7 and 81.7 KD, four proteins at 41.4, 81.7, 85.1 and 173.4 KD, and three proteins at 61.7, 81.7 and 173.4 KD were newly induced in squash leaves by G_1, G_2 and G_3 treatments, respectively.

Protein expression in H_2 (Eskandrani F_1 cv) was highly sensitive to GA treatment than H_1 (Rosina F_1) and this may interpret the higher yield of Rosina F_1 in which seven proteins were induced and simultaneously other four proteins were reduced or inhibited by GA treatments (Table 12 and Fig. 3, Lanes 17-20). In this instance, 2 proteins (81.7 and 85.1 KD), two bands (41.4 and 81.7 KD) and 3 bands (17.6, 41.4 and 81.7 KD) were induced by G_1, G_2 and G_3 treatments, respectively. In contrast, 3 proteins (53.4, 58.6 and 173.4 KD) and one band at 61.7 KD were inhibited by G_2 and G_3 treatments, respectively.

Effect of IAA on protein patterns

Results in Tables 10 & 11 and in Fig. 1&2, Lanes 1, 5, 9 & 13 show that spraying squash plants of H_1 (Rosina F_1) with low level of IAA (I_1) induced 6 new protein bands at 20.1, 49.8, 61.7, 81.7, 85.1 and 173.4 KD. While, only two new proteins at 17.6 and 85.1 KD were induced by I_2 treatment. The maximum induction of protein synthesis was observed by I_3 treatment where eight proteins at 20.1, 38.8, 41.4, 61.7, 74.4, 81.7, 85.1 and 173.4 KD were newly expressed.

Spraying hybrid 2 (Eskandrani F_1) with low level of IAA (I_1) induced the synthesis of 17.6, 41.4 and 81.7 KD proteins and reduced the expression of 53.4 and 173.4 KD protein bands in comparison to the non-treated plants (Tables 12 & 13 and Fig. 3 & 4, Lanes 17, 21, 25 and 29). Meanwhile, no quantitative differences were found between the control and I_2 treated plants in their protein profiles. However, high level of IAA (I_3) induced only one band at 81.7 KD as compared to the control treatment.

Effect of IAA and GA_3 combinations on protein patterns

Low level of IAA (I_1 = 2.5 ppm) in combination with GA_3

Squash plants of H_1 (Rosina F_1) treated with low level of IAA (I_1 = 2.5 ppm) in combination with G_1 (2.5 ppm) or G_2 (5 ppm) displayed similar patterns of 16 protein bands of which 7 bands (20.1, 49.8, 61.7, 74.4, 81.7, 85.1 and 173.4 KD) were new as compared with the control treatment (Table 10 and Fig.1, Lanes 1, 6-8). While only five of these new proteins (49.8, 74.4, 81.7, 85.1 and 173.4 KD) were induced by the low level of IAA (I_1) in combination with the high level of GA_3 (G_3=10 ppm).

In H_2 (Eskandrani F_1), treatment with I_1(2.5 ppm) in combination with G_1(2.5 ppm) or G_2 (5ppm) revealed three common new proteins at MW 20.1, 23.2 and 41.4 KD as compared with the non-treated plants (Table 12 and Fig.3, Lanes 17, 22-24). In addition, extra new band at 81.7 KD were expressed under I_1(2.5 ppm)/G_1(2.5 ppm) treatment. While two protein bands (23.2 and 81.7 KD) were induced under I_1(2.5 ppm)/G_3(10ppm) treatment, as compared to the control plants. One band at 173.4 KD was reduced by I_1(2.5 ppm)/ G_1(2.5ppm) and I_1(2.5ppm)/G_2(5ppm) treatments.

Moderate level of IAA (I_2) in combination with GA_3

Similar protein profiles of 14 bands were detected in squash leaves of H_1 (Rosina F_1) after treatment with moderate level of IAA (I_2) in combination with the three levels of GA_3 (Tables 10 & 11 and Fig.1 & 2, Lanes 1, 10-12). In these profiles, five protein bands at 41.4, 61.7, 81.7, 150.6 and 173.4 KD were newly expressed as compared to the non-treated control plants.

Protein profiles of squash H_2 (Eskandrani F_1) revealed the induction of 41.4 KD band and the reduction of three proteins at 58.6, 68.4 and 173.4 KD after treatment with I_2/G_1 as compared with the control (Tables 12 & 13 and Fig.3 & 4, Lanes 17, 26-28). While H_2 treatment (Eskandrani F_1) with I_2/G_2 induced one band at 81.7 KD and reduced other band at 173.4 KD. One band at 81.7 KD was newly expressed while two bands at 53.4 and 58.6 KD were inhibited when H_2 (Eskandrani F_1) plants treated with I_2/G_3 as compared with the control treatment.

High level of IAA (I_3) in combination with GA_3

Squash plants of H_1 (Rosina F_1) treated with high level of IAA (I_3) in combination with GA_3 displayed different patterns of protein profiles (Tables 10 &11 and Fig.1 & 2, Lanes 1, 14-16). The newly expressed proteins were 5 (20.1, 49.8, 81.7, 85.1 and 173.4 KD) in the treatment I_3/G_1, 6 (41.4, 49.8, 81.7, 85.1, 150.6 and 173.4 KD) in I_3/G_2, and only 3 (17.6, 49.8 and 85.1 KD) in the high levels of IAA and GA_3 (I_3/G_3).

No new protein bands were detected when H_2 (Eskandrani F_1) plants treated with I_3/G_1 or I_3/ G_2 while, two bands (58.6 and 61.7 KD) on the treatment I_3/G_1 and three (53.4, 58.6 and 61.7 KD) in I_3/G_2 were reduced as compared with the control treatment (Tables 12 & 13 and Fig.3 & 4, Lanes 17, 30-32). In the high levels of IAA and GA_3 (I_3/G_3), only one band at 81.7 KD was newly expressed while no missing bands were observed.

The above results showed that combination treatments of IAA/GA_3 (I/G) had different variations in protein patterns for the two hybrids of squash. In H_1(Rosina F_1), 7 proteins (20.1, 49.8, 61.7, 74.4, 81.7, 85.1 and 173.4 KD), 5 bands (41.4, 61.7, 81.7, 150.6 and 173.4 KD) and 8 proteins (17.6, 20.1, 41.4, 49.8, 81.7, 85.1, 150.6 and 173.4 KD) were induced by I_1/G, I_2/G and I_3/G combinations, respectively. While H_2 (Eskandrani F_1) was highly sensitive to I/G combinations and showed the induction of 4 bands (20.1, 23.2, 41.4 and 81.7 KD), 2 bands (41.4 and 81.7 KD) and one band (81.7 KD) by I_1/G, I_2/G and I_3/G combinations, respectively. The H_2 (Eskandrani F_1) also revealed the reduction of 4 bands (53.4, 58.6, 68.4 and 173.4 KD) and 3 bands (53.4, 58.6 and 61.7 KD) by I_2/G and I_3/G treatments, respectively.

Among all tested treatments, the combinations I_1/G_1 in H_1 (Rosina F_1) and H_2 (Eskandrani F_1), and I_1/G_2 in H_1(Rosina F_1) showed the best effect on protein expression enhancement where they induced the highest numbers of protein bands in the two treated hybrids. These results were substantiated before as squash plants treated with these combinations of I/G produced the highest values of all studied traits including total yield. However, I_1/0 and I_3/G_2 also showed high enhancement of protein synthesis in H_1(Rosina F_1). In H_2 (Eskandrani F_1), the treatments 0/G_1, I_1/0 and I_1/G_2 showed moderate enhancement of protein synthesis.

In the present study spraying of squash plants by GA_3 induced the synthesis of 8 new protein bands (17.6, 20.1, 41.4, 53.4, 61.7, 81.7, 85.1 and 173.4 KD). Four out of these proteins (17.6, 41.4, 81.7 and 85.1 KD) were commonly induced in both treated hybrids. While the other 4 bands (20.1, 53.4, 61.7 and 173.4 KD) were newly expressed in H_1(Rosina F_1) only. Meanwhile, it should be noted that in addition to the induction of new proteins in squash leaves by GA_3, some proteins were not expressed, degraded, and/or decreased to very low level which could not be detected by electrophoresis. In this instance, H_2 (Eskandrani F_1) showed the missing of 4 bands (53.4, 58.6, 61.7, 173.4 KD) by GA_3 treatment. Similar results were also obtained by Reyes et al. (2006) and Mekki (2008). The early study of Jacobsen and Knox (1974) showed that GA_3-induced 12 proteins in barley leaves ranged from 15.5 to 81.0 KD, while only two new proteins were detected in aleurone layers. However, Adams et al. (1999) observed that the most changes in protein pattern included those of MW between 20KD and 60KD following a treatment of cereal grains with GA_3. Meershad and Raming (1994) found that GA_3 treatment in different plant species has shown an increase in the RNA polymerase and DNA polymerase synthesis, including the synthesis of proteins. Al-Rumaih et al. (2002) reported that gibberellin may increase protein levels in root and aerial organs of cowpea by decreasing the activity of enzymes involved in the catabolism like ribonuclease which lead to increase protein synthesis. Mekki (2008) found that only one protein band with MW 128.97 KD was newly synthesized with the use 62.5, 125 and 250 ppm GA_3 while two bands (95.1 & 12.3 kD) were synthesized with 62.5 ppm GA_3., and also found that three bands at 53.98, 47.30 and 30.60 KD disappeared with the use of 250 ppm GA_3 and one 44.22 kD band with the use of 62.5 and 250 ppm GA_3. The external GA_3 application also increased protein content in tobacco root and leaves and induced the expression and density of protein bands in transgenic and non-transgenic plants (Zanjan and Asli, 2014).

The IAA auxin (indole-3-acetic acid) regulates plant development by inducing rapid cellular responses and changes in gene expression. Maraschin et al. (2009) and Ramos et al. (2001) reported that auxin promotes the degradation of the transcriptional repressors Aux/IAA, thereby allowing the ARFs (auxin response factors) to activate the transcription of auxin-responsive genes which enhanced protein synthesis. The present study revealed that 10 new protein bands (17.6, 20.1, 38.8, 41.4, 49.8, 61.7, 74.4, 81.7, 85.1 and 173.4 KD) were induced after treatment of squash plants with IAA. The three proteins 17.6, 41.4 and 81.7 KD were induced in both hybrids while the other proteins were newly expressed in H_1(Rosina F_1) only. The reduction of bands was observed in H_2 (Eskandrani F_1) only where two bands (53.4, 173.4 KD) were missing. O'Neill and Scorr (1987) found that treatment of carrot suspension culture cells with IAA induced two low peptides with MW of 27 and 43 KD. Abel & Theologis (1996) and Hagen & Guilfoyle (2002) reported that the Aux/IAA genes are a large gene family, rapidly induced by exogenous IAA treatment and encode 25- to 35-kD proteins. Van Huizen et al. (1996) studied the effect of Cl-IAA and GA on *in vivo* protein synthesis during pea fruit growth. They found that the most reproducible polypeptide changes were between molecular weights of 20 and 60 KD. Reed (2001) suggests that Aux/IAA proteins can mediate light responses. Also, the results indicated that exogenous IAA and GA_3 changes gene expression to induce several proteins involved in various developmental processes in squash plants. Similar conclusion was also obtained by O'Neill and Scorr (1987), Abel et al.(1994), Ramos et al. (2001), Hagen and Guilfoyle (2002), Reyes et al. (2006), Mekki (2008), Maraschin et al. (2009) and Zanjan & Asli (2014).

Conclusion

From the obtained results, it could be recommended to grow summer squash Rosina F_1 hybrid cv. and use foliar spray with 10 ppm indoleacetic acid to maximize the total yield under conditions similar to those of the present study.

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TABLE 2. Sex ratio of two F_1 squash hybrid cvs. as affected by foliar applications of indoleacetic acid (IAA) and gibberellic acid (GA_3).

Seasons		2015					2016				
Hybrid cvs (H)	IAA(I) / GA_3(G)	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean
H_1 Rosina F_1	G_0 (control)	0.87	1.13	0.91	1.30	**1.05**	0.85	1.12	0.89	1.20	**1.02**
	G_1 (2.5 ppm)	0.97	1.22	1.05	1.12	**1.09**	0.94	1.15	1.03	1.09	**1.05**
	G_2 (5 ppm)	1.03	1.17	1.09	1.12	**1.10**	0.99	1.13	1.05	1.10	**1.07**
	G_3 (10 ppm)	0.97	1.03	1.10	0.99	**1.02**	0.93	1.01	1.07	0.97	**1.00**
Mean		**0.96**	**1.14**	**1.04**	**1.13**	**1.06**	**0.93**	**1.10**	**1.01**	**1.09**	**1.04**
H_2 Eskandrani F_1	G_0 (control)	1.06	1.19	1.17	1.20	**1.15**	1.01	1.14	1.13	1.15	**1.11**
	G_1 (2.5 ppm)	1.20	1.29	1.13	1.12	**1.19**	1.18	1.25	1.09	1.08	**1.15**
	G_2 (5 ppm)	1.15	1.26	1.15	1.10	**1.17**	1.10	1.24	1.12	1.04	**1.13**
	G_3 (10 ppm)	1.21	1.25	1.14	1.19	**1.20**	1.19	1.20	1.09	1.14	**1.16**
Mean		**1.16**	**1.25**	**1.15**	**1.15**	**1.18**	**1.12**	**1.21**	**1.11**	**1.10**	**1.14**
General Mean		**1.06**	**1.19**	**1.09**	**1.14**	**1.12**	**1.03**	**1.16**	**1.06**	**1.10**	**1.08**
I x G	G_0 (control)	0.96	1.16	1.04	1.25	**1.10**	0.93	1.13	1.01	1.17	**1.06**
	G_1 (2.5 ppm)	1.08	1.25	1.09	1.12	**1.14**	1.06	1.20	1.06	1.09	**1.10**
	G_2 (5 ppm)	1.09	1.21	1.12	1.11	**1.13**	1.05	1.18	1.08	1.07	**1.09**
	G_3 (10 ppm)	1.09	1.14	1.12	1.09	**1.11**	1.06	1.10	1.08	1.05	**1.07**
F test and revised LSD 0.05		**F value**			**LSD**		**F value**			**LSD**	
Indoleacetic acid (I)		**			**0.03**		**			**0.05**	
Gibberellic acid (G)		**N.S**			---		*			**0.02**	
Hybrid cultivars (H)		**			**0.05**		**			**0.04**	
I X G		**N.S**			---		**N.S**			---	
I x H		**N.S**			---		**N.S**			---	
G x H		**N.S**			---		**N.S**			---	
I x G x H		**N.S**			---		**N.S**			---	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively.

TABLE 3. Plant height (cm) of two F_1 squash hybrid cvs. as affected by foliar applications of indoleacetic acid (IAA) and gibberellic acid (GA_3).

Seasons		2015					2016				
Hybrid cvs (H)	IAA (I) / GA_3 (G)	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean
H_1 Rosina F_1	G_0 (control)	40.78	47.34	42.61	51.75	**45.62**	39.82	46.00	41.26	49.60	**44.17**
	G_1 (2.5 ppm)	43.30	49.84	44.34	46.10	**45.89**	42.25	48.20	43.40	45.20	**44.76**
	G_2 (5 ppm)	43.79	48.74	45.57	46.23	**46.08**	43.13	47.34	44.11	45.57	**45.04**
	G_3 (10 ppm)	43.25	44.00	45.95	43.16	**44.09**	41.75	43.34	44.50	42.71	**43.08**
Mean		**42.78**	**47.48**	**44.62**	**46.81**	**45.42**	**41.74**	**46.22**	**43.32**	**45.77**	**44.26**
H_2 Eskandrani F_1	G_0 (control)	26.89	36.50	35.32	37.10	**33.95**	26.43	35.90	34.19	36.32	**33.21**
	G_1 (2.5 ppm)	38.05	47.16	31.39	30.15	**36.69**	37.50	46.86	31.20	30.00	**36.39**
	G_2 (5 ppm)	33.11	42.10	34.99	28.55	**34.69**	32.98	41.40	34.14	28.10	**34.16**
	G_3 (10 ppm)	38.75	40.29	32.72	36.00	**36.94**	37.83	40.00	32.50	35.80	**36.53**
Mean		**34.20**	**41.51**	**33.61**	**32.95**	**35.57**	**33.69**	**41.04**	**33.01**	**32.56**	**35.07**
General Mean		**38.49**	**44.49**	**39.11**	**39.88**	**40.49**	**37.71**	**43.63**	**38.16**	**39.16**	**39.67**
I x G	G_0 (control)	33.83	41.92	38.96	44.42	**39.78**	33.12	40.95	37.72	42.96	**38.69**
	G_1 (2.5 ppm)	40.68	48.50	37.86	38.12	**41.29**	39.88	47.53	37.30	37.60	**40.58**
	G_2 (5 ppm)	38.45	45.42	40.28	37.39	**40.38**	38.06	44.37	39.13	36.84	**39.60**
	G_3 (10 ppm)	41.00	42.14	39.34	39.58	**40.51**	39.79	41.67	38.50	39.26	**39.80**
F test and revised LSD 0.05		**F value**			**LSD**		**F value**			**LSD**	
Indoleacetic acid (I)		**NS**			**---**		**			**1.52**	
Gibberellic acid (G)		*			**1.09**		**NS**			**---**	
Hybrid cultivars (H)		**			**1.78**		**			**0.67**	
I X G		**			**6.71**		**			**1.99**	
I x H		*			**8.67**		**			**1.39**	
G x H		**N.S**			**---**		**			**1.46**	
I x G x H		**N.S**			**---**		**			**2.98**	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively.

TABLE 4. Number of fruits per plant of two F_1 squash hybrid cvs. as affected by foliar applications of indoleacetic acid (IAA) and gibberellic acid (GA_3).

Seasons		2015					2016				
Hybrid cvs (H)	IAA (I) / GA_3 (G)	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean
H_1 Rosina F_1	G_0 (control)	9.94	12.70	10.10	13.00	**11.44**	9.20	12.12	10.01	12.90	11.06
	G_1 (2.5 ppm)	10.32	12.97	11.60	12.52	**11.85**	10.27	12.50	11.35	11.98	11.53
	G_2 (5 ppm)	11.00	12.80	11.63	12.67	**12.03**	10.78	12.30	11.50	12.00	11.65
	G_3 (10 ppm)	10.25	11.03	12.36	10.65	**11.07**	10.15	11.00	11.70	10.50	10.84
Mean		**10.38**	**12.37**	**11.42**	**12.21**	**11.59**	**10.10**	**11.98**	**11.14**	**11.85**	**11.27**
H_2 Eskandrani F_1	G_0 (control)	7.62	10.23	9.86	10.30	**9.50**	7.13	9.90	9.72	10.22	9.24
	G_1 (2.5 ppm)	10.73	13.24	8.94	8.93	**10.46**	10.29	11.26	8.39	8.29	9.56
	G_2 (5 ppm)	9.67	12.70	9.82	8.61	**10.20**	9.48	11.17	9.70	7.29	9.41
	G_3 (10 ppm)	10.73	12.29	9.49	10.00	**10.63**	10.61	11.00	8.48	9.88	9.99
Mean		**9.69**	**12.11**	**9.53**	**9.46**	**10.20**	**9.38**	**10.83**	**9.07**	**8.92**	**9.55**
General Mean		**10.03**	**12.24**	**10.47**	**10.83**	**10.89**	**9.74**	**11.41**	**10.11**	**10.38**	**10.41**
I x G	G_0 (control)	8.78	11.47	9.98	11.65	**10.47**	8.17	11.01	9.87	11.56	**10.15**
	G_1 (2.5 ppm)	10.53	13.10	10.27	10.73	**11.16**	10.28	11.88	9.87	10.14	**10.54**
	G_2 (5 ppm)	10.33	12.75	10.72	10.64	**11.11**	10.13	11.73	10.60	9.65	**10.53**
	G_3 (10 ppm)	10.49	11.66	10.92	10.33	**10.85**	10.38	11.00	10.09	10.19	**10.42**
F test and revised LSD 0.05		**F value**			**LSD**		**F value**			**LSD**	
Indoleacetic acid (I)		**			**1.19**		**			**0.85**	
Gibberellic acid (G)		**N.S**			---		**N.S**			---	
Hybrid cultivars (H)		**			**0.71**		**			**0.48**	
I X G		**N.S**			---		*			**1.87**	
I x H		**N.S**			---		*			**1.16**	
G x H		**N.S**			---		**N.S**			---	
I x G x H		**N.S**			---		**N.S**			---	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively.

TABLE 5. Average fruit weight (g) of two F_1 squash hybrid cvs. as affected by foliar applications of indoleacetic acid (IAA) and gibberellic acid (GA_3).

Seasons		2015					2016				
Hybrid cvs (H)	IAA(I) / GA_3(G)	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean
H_1 Rosina F_1	G_0 (control)	75.33	108.11	82.33	116.11	**95.47**	72.50	106.05	81.00	111.67	**92.81**
	G_1 (2.5 ppm)	95.00	110.00	101.11	104.45	**102.64**	90.00	108.67	98.33	103.33	**100.08**
	G_2 (5 ppm)	97.78	109.44	101.94	105.00	**103.54**	95.00	107.33	100.02	104.05	**101.60**
	G_3 (10 ppm)	84.44	98.00	102.78	95.66	**95.22**	82.00	96.33	100.30	91.00	**92.41**
Mean		**88.14**	**106.39**	**97.04**	**105.31**	**99.22**	**84.88**	**104.60**	**94.91**	**102.51**	**96.72**
H_2 Eskandrani F_1	G_0 (control)	66.67	91.11	85.56	94.45	**84.45**	65.00	87.67	82.50	90.73	**81.48**
	G_1 (2.5 ppm)	97.78	111.67	74.44	71.11	**88.75**	95.84	110.18	73.67	70.85	**87.63**
	G_2 (5 ppm)	80.89	107.78	84.89	68.33	**85.47**	78.33	105.20	81.67	62.51	**81.93**
	G_3 (10 ppm)	99.45	105.00	79.80	87.00	**92.81**	97.63	102.51	76.67	85.09	**90.47**
Mean		**86.20**	**103.89**	**81.17**	**80.22**	**87.87**	**84.20**	**101.39**	**78.63**	**77.29**	**85.38**
General Mean		**87.17**	**105.14**	**89.11**	**92.76**	**93.54**	**84.54**	**102.99**	**86.77**	**89.90**	**91.05**
I x G	G_0 (control)	70.99	99.61	83.94	105.28	**89.96**	68.75	96.86	81.75	101.20	**87.14**
	G_1 (2.5 ppm)	96.39	110.84	87.78	87.78	**95.70**	92.92	109.43	85.99	87.09	**93.86**
	G_2 (5 ppm)	89.34	108.61	93.42	86.67	**94.51**	86.67	106.27	90.85	83.28	**91.76**
	G_3 (10 ppm)	91.95	101.50	91.29	91.33	**94.02**	89.82	99.42	88.49	88.03	**91.44**
F test and revised LSD 0.05			**F value**		**LSD**			**F value**		**LSD**	
Indoleacetic acid (I)			**		**7.81**			**		**2.79**	
Gibberellic acid (G)			**N.S**		---			**		**1.95**	
Hybrid cultivars (H)			**		**2.13**			**		**1.14**	
I X G			**N.S**		---			**		**3.22**	
I x H			**N.S**		---			**		**2.88**	
G x H			**N.S**		---			**		**2.28**	
I x G x H			**N.S**		---			**		**4.65**	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively.

TABLE 6. Fruit weight per plant (kg) of two F_1 squash hybrid cvs. as affected by foliar applications of indoleacetic acid (IAA) and gibberellic acid (GA_3).

Seasons		2015					2016				
Hybrid cvs (H)	IAA(I) / GA_3(G)	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean
H_1 Rosina F_1	G_0 (control)	1.08	1.59	1.13	1.67	**1.37**	1.01	1.54	1.09	1.61	**1.31**
	G_1 (2.5 ppm)	1.22	1.62	1.42	1.58	**1.46**	1.15	1.59	1.38	1.49	**1.41**
	G_2 (5 ppm)	1.25	1.61	1.53	1.59	**1.50**	1.19	1.57	1.42	1.52	**1.43**
	G_3 (10 ppm)	1.21	1.30	1.56	1.24	**1.33**	1.11	1.25	1.45	1.17	**1.24**
Mean		**1.19**	**1.53**	**1.41**	**1.52**	**1.41**	**1.11**	**1.49**	**1.34**	**1.45**	**1.35**
H_2 Eskandrani F_1	G_0 (control)	0.78	1.26	1.14	1.33	**1.13**	0.76	1.12	1.05	1.19	**1.03**
	G_1 (2.5 ppm)	1.40	1.59	0.98	0.88	**1.21**	1.29	1.49	0.96	0.86	**1.15**
	G_2 (5 ppm)	1.05	1.51	1.09	0.86	**1.13**	0.98	1.48	1.00	0.83	**1.07**
	G_3 (10 ppm)	1.44	1.46	1.05	1.17	**1.28**	1.30	1.32	0.98	1.07	**1.17**
Mean		**1.17**	**1.46**	**1.06**	**1.06**	**1.19**	**1.08**	**1.35**	**0.99**	**0.99**	**1.10**
General Mean		**1.18**	**1.49**	**1.24**	**1.29**	**1.30**	**1.09**	**1.42**	**1.17**	**1.22**	**1.23**
I x G	G_0 (control)	0.93	1.42	1.14	1.49	**1.25**	0.88	1.33	1.08	1.40	**1.17**
	G_1 (2.5 ppm)	1.31	1.61	1.19	1.23	**1.34**	1.22	1.55	1.17	1.18	**1.28**
	G_2 (5 ppm)	1.15	1.56	1.31	1.23	**1.31**	1.09	1.52	1.21	1.18	**1.25**
	G_3 (10 ppm)	1.32	1.38	1.30	1.21	**1.30**	1.21	1.28	1.21	1.12	**1.20**
F test and revised LSD 0.05		**F value**			**LSD**		**F value**			**LSD**	
Indoleacetic acid (I)		*			**0.21**		**			**0.59**	
Gibberellic acid (G)		**N.S**			**---**		*			**0.07**	
Hybrid cultivars (H)		**			**0.10**		**			**0.05**	
I X G		*			**0.39**		**			**0.16**	
I x H		**			**0.21**		**			**0.11**	
G x H		**N.S**			**---**		**			**0.12**	
I x G x H		**N.S**			**---**		**			**0.25**	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively.

TABLE 7. Total yield (ton/feddan) of two F_1 squash hybrid cvs. as affected by foliar applications of indoleacetic acid (IAA) and gibberellic acid (GA_3).

Seasons		2015					2016				
Hybrid cvs (H)	IAA(I) / GA_3 (G)	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean
H_1 Rosina F_1	G_0 (control)	9.96	17.64	13.52	18.88	**15.00**	9.79	17.02	13.40	18.35	**14.64**
	G_1 (2.5 ppm)	14.28	18.35	15.01	16.09	**15.93**	14.14	18.15	14.87	15.95	**15.78**
	G_2 (5 ppm)	14.85	17.87	15.14	16.74	**16.15**	14.56	17.76	15.06	16.35	**15.93**
	G_3 (10 ppm)	13.86	15.01	15.99	14.53	**14.85**	13.67	14.73	15.73	14.39	**14.63**
Mean		**13.24**	**17.22**	**14.91**	**16.56**	**15.48**	**13.04**	**16.92**	**14.77**	**16.26**	**15.25**
H_2 Eskandrani F_1	G_0 (control)	9.58	15.16	14.96	15.24	**13.73**	9.55	14.60	14.20	14.72	**13.26**
	G_1 (2.5 ppm)	15.54	16.35	12.36	12.08	**14.08**	14.80	15.88	12.18	11.85	**13.68**
	G_2 (5 ppm)	13.90	16.29	14.16	11.50	**13.96**	13.73	15.52	13.97	11.41	**13.66**
	G_3 (10 ppm)	15.80	15.94	12.88	15.10	**14.93**	14.96	14.99	12.65	14.50	**14.28**
Mean		**13.70**	**15.93**	**13.59**	**13.48**	**14.18**	**13.26**	**15.25**	**13.25**	**13.12**	**13.72**
General Mean		**13.47**	**16.57**	**14.25**	**15.02**	**14.83**	**13.15**	**16.08**	**14.01**	**14.69**	**14.48**
I x G	G_0 (control)	9.77	16.40	14.24	17.06	**14.37**	9.67	15.81	13.80	16.54	**13.95**
	G_1 (2.5 ppm)	14.91	17.35	13.68	14.09	**15.01**	14.47	17.02	13.53	13.90	**14.73**
	G_2 (5 ppm)	14.38	17.08	14.65	14.12	**15.06**	14.15	16.64	14.52	13.88	**14.79**
	G_3 (10 ppm)	14.83	15.47	14.43	14.82	**14.89**	14.32	14.86	14.19	14.45	**14.45**
F test and revised LSD 0.05		**F value**			**LSD**		**F value**			**LSD**	
Indoleacetic acid (I)		**			**1.48**		**			**1.01**	
Gibberellic acid (G)		**N.S**			---		**N.S**			---	
Hybrid cultivars (H)		**			**0.81**		**			**0.48**	
I X G		**			**2.54**		**			**1.46**	
I x H		*			**3.72**		**			**1.06**	
G x H		**N.S**			---		**N.S**			---	
I x G x H		**N.S**			---		*			**2.71**	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively.

TABLE 8. Total soluble solids (ºBrix) of two F_1 squash hybrid cvs. as affected by foliar applications of indoleacetic acid (IAA) and gibberellic acid (GA_3).

Seasons		2015					2016				
Hybrid cvs (H)	IAA(I) / GA_3(G)	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean	I_0 (control)	I_1 (2.5 ppm)	I_2 (5 ppm)	I_3 (10 ppm)	Mean
H_1 Rosina F_1	G_0 (control)	3.11	3.71	3.21	3.88	**3.48**	3.05	3.42	3.15	3.67	**3.32**
	G_1 (2.5 ppm)	3.25	3.83	3.59	3.63	**3.58**	3.17	3.59	3.28	3.33	**3.34**
	G_2 (5 ppm)	3.38	3.73	3.63	3.67	**3.60**	3.22	3.50	3.28	3.39	**3.35**
	G_3 (10 ppm)	3.25	3.42	3.63	3.34	**3.41**	3.17	3.28	3.28	3.20	**3.23**
Mean		**3.25**	**3.67**	**3.52**	**3.63**	**3.51**	**3.15**	**3.45**	**3.25**	**3.40**	**3.31**
H_2 Eskandrani F_1	G_0 (control)	3.10	3.39	3.33	3.39	**3.30**	3.07	3.33	3.30	3.34	**3.26**
	G_1 (2.5 ppm)	3.39	3.66	3.16	3.11	**3.33**	3.39	3.62	3.13	3.10	**3.31**
	G_2 (5 ppm)	3.28	3.50	3.33	3.11	**3.31**	3.25	3.50	3.28	3.09	**3.28**
	G_3 (10 ppm)	3.44	3.50	3.17	3.33	**3.36**	3.42	3.45	3.15	3.30	**3.33**
Mean		**3.30**	**3.51**	**3.25**	**3.24**	**3.33**	**3.28**	**3.48**	**3.22**	**3.21**	**3.29**
General Mean		**3.28**	**3.59**	**3.38**	**4.43**	**3.42**	**3.22**	**3.46**	**3.23**	**3.30**	**3.30**
I x G	G_0 (control)	3.11	3.55	3.27	3.63	**3.39**	3.06	3.38	3.23	3.50	**3.29**
	G_1 (2.5 ppm)	3.32	3.75	3.38	3.37	**3.45**	3.28	3.61	3.20	3.22	**3.33**
	G_2 (5 ppm)	3.33	3.62	3.48	3.39	**3.45**	3.24	3.50	3.28	3.24	**3.31**
	G_3 (10 ppm)	3.35	3.46	3.39	3.34	**3.38**	3.29	3.37	3.21	3.25	**3.28**
F test and revised LSD 0.05		**F value**			**LSD**		**F value**			**LSD**	
Indoleacetic acid (I)		**NS**			**---**		**			**0.13**	
Gibberellic acid (G)		**N.S**			**---**		**N.S**			**---**	
Hybrid cultivars (H)		**			**0.10**		**N.S**			**---**	
I X G		**N.S**			**---**		**N.S**			**---**	
I x H		*			**0.49**		**N.S**			**---**	
G x H		**N.S**			**---**		**N.S**			**---**	
I x G x H		**N.S**			**---**		**N.S**			**---**	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively.

TABLE 9. Correlation coefficient for all studied traits of two F_1 squash hybrid cvs. in 2015 (upper) diagonal and 2016 (lower) diagonal (n=96).

Seasons	2015							
2016	**Characters**	**Sex ratio**	**Plant height**	**Average fruit weight**	**Fruit number per plant**	**Fruit weight per plant**	**Total yield**	**Total soluble solids**
	Sex ratio	-----	-0.022	0.186**	0.376**	0.128	0.304**	0.182*
	Plant height	0.029	-----	0.485**	0.546**	0.591**	0.569**	0.555**
	Average fruit weight	0.284**	0.835**	-----	0.383**	0.386**	0.432**	0.460**
	Fruit number per plant	0.153	0.685**	0.757**	-----	0.514**	0.562**	0.364**
	Fruit weight per plant	0.214*	0.793**	0.866**	0.663**	-----	0.505**	0.519**
	Total yield	0.298**	0.684**	0.742**	0.659**	0.667**	-----	0.464**
	Total soluble solids	0.222*	0.381**	0.568**	0.397**	0.488**	0.518**	-----

NS, * and ** mean non-significant and significant at 0.05 and 0.01 probability level, respectively.

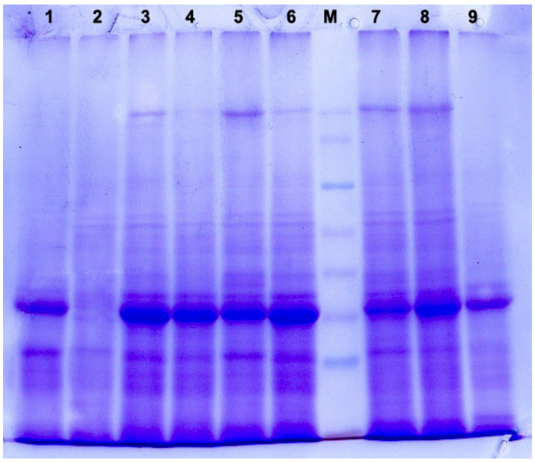

Fig. 1. Electrophoretic patterns of protein profiles detected in Rosina F_1 leaves after treatments with GA_3 (G) and IAA (I) in comparison to the control treatment (0.0).

Lane 1: (0.0), Lane 2: (0, G_1), Lane 3: (0, G_2), Lane 4: (0, G_3), Lane 5: (I1, 0), Lane 6: (I1, G_1), Lane 7: (I1, G_2), Lane 8: (I1, G_3), Lane 9: (I2, 0), Lane M: Marker.

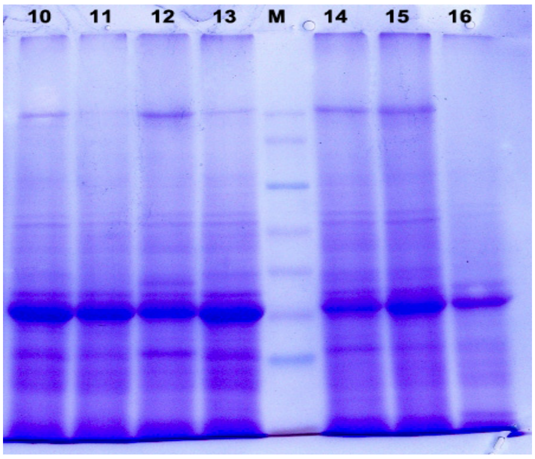

Fig. 2. Electrophoretic patterns of protein profiles detected in Rosina F_1 leaves after treatments with GA_3 (G) and IAA (I).

Lane 10: (I2, G_1), Lane 11: (I2, G2), Lane 12: (I2, G_3), Lane 13: (I3, 0) Lane 14: (I3, G_1), Lane 15: (I3, G_2), Lane 16: (I3, G_3), Lane M: Marker.

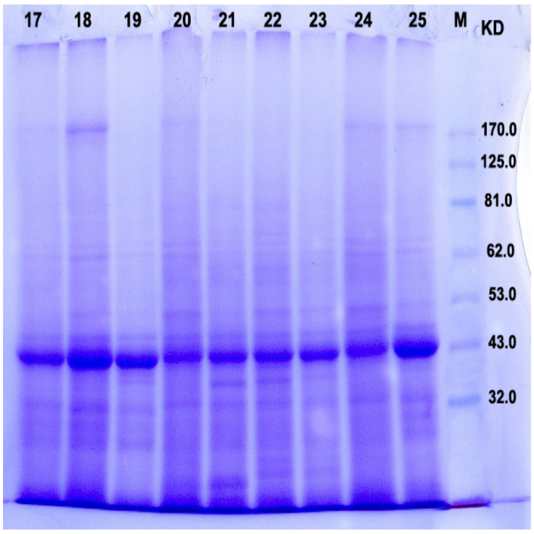

Fig. 3. Electrophoretic patterns of protein profiles detected in Eskandrani F_1 leaves after treatments with GA_3 (G) and IAA (I) in comparison to the control treatment (0.0).

Lane 17: (0.0), Lane 18: (0, G_1), Lane 19: (0, G_2), Lane 20: (0, G_3), Lane 21: (I1, 0), Lane 22: (I1, G_1), Lane 23: (I1, G_2), Lane 24: (I1, G_3), Lane 25: (I2, 0), Lane M: Marker.

†GA_3 [(G_1, 2.5 ppm)- (G_2, 5 ppm) - ($G_{3,}$ 10 ppm)]

‡IAA [(I_1, 2.5 ppm)- (I_2, 5 ppm) - (I_3, 10 ppm)]

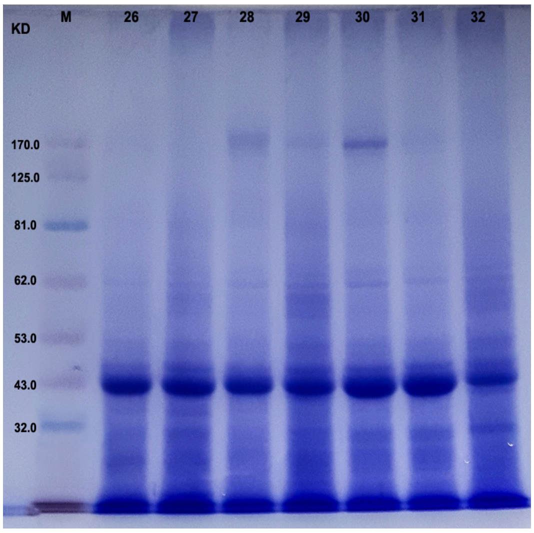

Fig. 4. Electrophoretic patterns of protein profiles detected in Eskandrani F_1 leaves after treatments with GA_3 (G) and IAA (I).

Lane 26: (I2, G_1), Lane 27: (I2, G_2), Lane 28: (I2, G_3), Lane 29: (I3, 0), Lane 30: (I3, G_1), Lane 31: (I3, G_2), Lane 32: (I3, G_3), Lane M: Marker.

TABLE 10. Molecular weights of protein bands detected in Rosina F_1 leaves after treatments with GA_3 (G) and IAA (I) in comparison to the control treatment (0.0).

MW	1	2	3	4	5	6	7	8	9
	0, 0	0, G_1	0, G_2	0, G_3	I_1, 0	I_1, G_1	I_1, G_2	I_1, G_3	I_2, 0
173.4			+	+	+	+	+	+	
85.1			+		+	+	+	+	+
81.7		+	+	+	+	+	+	+	
74.4						+	+	+	
68.4	+	+	+	+	+	+	+	+	+
64.8	+	+	+	+	+	+	+	+	+
61.7		+		+	+	+	+		
58.6	+	+	+	+	+	+	+	+	+
53.4	+	+	+	+	+	+	+	+	+
49.8					+	+	+	+	
46.2	+	+	+	+	+	+	+	+	+
44.3	+	+	+	+	+	+	+	+	+
41.4			+						
32.8	+	+	+	+	+	+	+	+	+
29.2	+	+	+	+	+	+	+	+	+
26.5	+	+	+	+	+	+	+	+	+
20.1		+			+	+	+		
17.6									+
No.	9	12	13	12	15	16	16	14	11
Induced		3	4	3	6	7	7	5	2
Reduced		-	-	-	-	-	-	-	-

† GA_3 [(G_1, 2.5 ppm)- (G_2, 5 ppm) - (G_3, 10 ppm)]
‡ IAA [(I_1, 2.5 ppm)- (I_2, 5 ppm) - (I_3, 10 ppm)]/

TABLE 11. Molecular weights of protein bands detected in Rosina F_1 leaves after treatments with GA_3 (G) and IAA (I).

MW	10	11	12	13	14	15	16
	I_2, G_1	I_2, G_2	I_2, G_3	I_3, 0	I_3, G_1	I_3, G_2	I_3, G_3
173.4	+	+	+	+	+	+	
150.6	+	+	+			+	
85.1				+	+	+	+
81.7	+	+	+	+	+	+	
74.4				+			
68.4	+	+	+	+	+	+	+
64.8	+	+	+	+	+	+	+
61.7	+	+	+	+			
58.6	+	+	+	+	+	+	+
53.4	+	+	+	+	+	+	+
49.8					+	+	+
46.2	+	+	+	+	+	+	+
44.3	+	+	+	+	+	+	+
41.4	+	+	+	+		+	
38.8				+			
32.8	+	+	+	+	+	+	+
29.2	+	+	+	+	+	+	+
26.5	+	+	+	+	+	+	+
20.1				+	+		
17.6							+
No.	14	14	14	17	14	15	12
Induced	5	5	5	8	5	6	3
Reduced	-	-	-	-	-	-	-

† GA_3 [(G_1, 2.5 ppm)- (G_2, 5 ppm) - (G_3, 10 ppm)]
‡ IAA [(I_1, 2.5 ppm)- (I_2, 5 ppm) - (I_3, 10 ppm)]

TABLE 12. Molecular weights of protein bands detected in Eskandrani F_1 leaves after treatments with GA_3 (G) and IAA (I) in comparison to the control treatment (0.0).

MW	17	18	19	20	21	22	23	24	25
	0, 0	0, G_1	0, G_2	0, G_3	I_1, 0	I_1, G_1	I_1, G_2	I_1, G_3	I_2, 0
173.4	+	+		+				+	+
85.1		+							
81.7		+	+	+	+	+		+	
68.4	+	+	+	+	+	+	+	+	+
64.8	+	+	+	+	+	+	+	+	+
61.7	+	+	+		+	+	+	+	+
58.6	+	+		+	+	+	+	+	+
53.4	+	+		+		+	+	+	+
46.2	+	+	+	+	+	+	+	+	+
44.3	+	+	+	+	+	+	+	+	+
41.4			+	+	+	+	+		
32.8	+	+	+	+	+	+	+	+	+
29.2	+	+	+	+	+	+	+	+	+
26.5	+	+	+	+	+	+	+	+	+
23.2						+	+	+	
20.1						+	+		
17.6				+	+				
No.	11	13	10	13	12	14	13	13	11
Induced		2	2	3	3	4	3	2	-
Reduced		-	3	1	2	1	1	-	-

† GA_3 [(G_1, 2.5 ppm)- (G_2, 5 ppm) - (G_3, 10 ppm)]
‡ IAA [(I_1, 2.5 ppm)- (I_2, 5 ppm) - (I_3, 10 ppm)]

TABLE 13. Molecular weights of protein bands detected in Eskandrani F_1 leaves after treatments with GA_3 (G) and IAA (I).

MW	26	27	28	29	30	31	32
	I_2, G_1	I_2, G_2	I_2, G_3	I_3, 0	I_3, G_1	I_3, G_2	I_3, G_3
173.4			+	+	+	+	+
150.6							
81.7		+	+	+			+
68.4		+	+	+	+	+	+
64.8	+	+	+	+	+	+	+
61.7	+	+	+	+			+
58.6		+		+			+
53.4	+	+		+	+		+
46.2	+	+	+	+	+	+	+
44.3	+	+	+	+	+	+	+
41.4	+						
32.8	+	+	+	+	+	+	+
29.2	+	+	+	+	+	+	+
26.5	+	+	+	+	+	+	+
No.	9	11	10	12	9	8	12
Induced	1	1	1	1	-	-	1
Reduced	3	1	2	-	2	3	-

† GA_3 [(G_1, 2.5 ppm)- (G_2, 5 ppm) - (G_3, 10 ppm)]
‡ IAA [(I_1, 2.5 ppm)- (I_2, 5 ppm) - (I_3, 10 ppm)]

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تأثير الرش الورقي بكل من إندول حمض الخليك وحمض الجبريليك على إنتاجية وبناء البروتين في صنفين هجينين من الكوسة

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أجرى هذا البحث خلال مواسم صيف ٢٠١٥ و٢٠١٦ في المزرعة البحثية بقسم الخضر ومعمل الوراثة الجزيئية بقسم الوراثة بكلية الزراعة – جامعة أسيوط لدراسة تأثير الرش الورقى بكل من إندول حمض الخليك وحمض الجبريليك على إنتاجية وبناء البروتين لصنفين هجينين من الجيل الاول من الكوسة (هجيني روزينا والاسكندرانى). وكانت تركيزات إندول حمض الخليك هى (صفر، ٢,٥، ٥ أو ١٠ جزء في المليون) وكانت تركيزات حمض الجبريليك (صفر، ٢,٥، ٥ أو١٠ جزء في المليون). أشارت النتائج الى ان كل من التركيزات محل الدراسة من إندول حمض الخليك وحمض الجبريليك كان لها تأثيرا معنويا على معظم الصفات المدروسة لصالح ٢,٥ جزء في المليون في كل من إندول حمض الخليك وحمض الجبريليك والذي أعطى اعلى متوسطات قيم لكل الصفات المدروسة في موسمي الدراسة. كما تشير النتائج الى تفوق الهجين روزينا على الهجين الاسكندرانى في كل الصفات محل الدراسة ماعدا صفة النسبة الجنسية. كما تشير النتائج أيضا الى التاثير المعنوى لمختلف تفاعلات الدرجة الثانية على معظم الصفات محل الدراسة حيث أعطت نباتات الكوسة روزينا والمعامل بتركيز ١٠ جزء في المليون من إندول حمض الخليك بدون حمض الجبريليك أعلى متوسطات قيم لصفة المحصول للفدان. أدى رش نباتات الكوسة بواسطة GA_3 إلى حث بناء تمانية بروتينات جديدة، في حين أن عشرة بروتينات جديدة تم استحداثها بواسطة IAA. كما اظهر هجينى الكوسة نماذج مختلفة من طرز البروتينات عند المعاملة بتوليفات مشتركه من GA_3 ، IAA . وكان الهجين الاسكنرانى أكثر حساسية من الهجين روزينا لمعاملات GA_3 ، IAA حيث اظهر غياب لعدد من الحزم البروتينية وانخفاض عدد البروتينات المستحدثة.