Response of Watermelon Plants Grafted onto Different Cucurbit Rootstocks to Sub-Optimal Growing Temperature

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ABSTRACT: This study was conducted to examine the relative tolerance of grafted watermelon to sub-optimal growing temperature conditions. Watermelon cv. Aswan scions were grafted onto different rootstocks, namely Giada, Shintoza, StrongToza, and Ferro. Grafted and un-grafted seedlings were grown in the field under low temperature during Jan.-Apr. to be compared to those growing under normal warm season conditions (Mar.-Jun.). Results indicated significant decrease in growth, yield, chlorophyll, and carotenoids in all grafted plants, in different degrees, depending on the graft combination. In this regards, plants of Aswan/Ferro recorded more than double the marketable yield of un-grafted ones under sub-optimal temperature, indicating the relative tolerance of Aswan/Ferro to cold stress. While, un-grafted plants exhibited the highest decline in all growth and yield parameters. Leaf proline content and the activities of anti-oxidant enzymes (SOD and CAT) were higher under cold stress than the control. It is concluded that grafted watermelon plants were relatively tolerant to sub-optimal temperature than un-grafted ones which could enable the production of out of season crop.

Keywords: *Citrullus lanatus,* grafting, rootstocks, cold stress, iso-enzymes.

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. And Nakai) is one of the most economically important vegetable crops in Egypt and is widely cultivated worldwide. World total production was 109.27 million tons, approximately 3.47 million hectares are planted yearly all over the world, with an average of 34.59 tons/ha (FAOSTAT, 2013). Egypt is ranked fifth in watermelon production in the world, with total production of 1.89 million tons from around 0.06 million hectares with an average of 34.49 tons/ha (FAOSTAT, 2013). Watermelon is a warm, dry climate crop with maximum and minimum temperatures for growth are about 35 and 18˚C respectively. The optimum soil temperature for root growth is in the range of 20 to 35˚C (Doorenbos and Kassam, 1979).

Grafting vegetable plants onto resistant rootstocks was recommended as an effective mean that may enable the control of soil-borne diseases, increasing tolerance of environmental stresses and increase fruit yield (Ioannou, 2001; Morra and Bilotto, 2006; Crinò *et al*., 2007; Rouphael*et al*., 2010 and Wimer *et al*., 2015). Earlier studies indicated that vigorous root system of the rootstock can powerfully absorb water and nutrients (Lee and Oda, 2003; Salehi-Mohammadi *et al*., 2009) when compared to un-grafted plants. Grafting is also related with obvious increases in fruit yield in many fruit-bearing vegetables such as watermelon, cucumber, melon, tomato, pepper and eggplant (Lee and Oda, 2003; Chung and Lee, 2007 and Mohamed *et al.*, 2014).

Bulder *et al.* (1991) tested three low temperatures tolerant rootstock genotypes grafted on cucumber, to determine the most appropriate cucumber rootstock at suboptimal root temperatures. Plants were grown at an air temperature of 20˚C/12˚C day/night and at fixed root temperatures of 20˚C, 16˚C or 12˚C. Although growth decreased at 12˚C for all genotypes, both *Sicyos angulatus* genotypes were more tolerant to low root temperature than *Cucurbita ficifolia*. Another study by Li *et al.* (2015) examined the response of grafted seedlings of cucumber to short exposure to low temperature (5˚C). Grafted plants onto rootstock figleaf gourd showed higher plant height and leaf area than un-grafted. Justus and Kubota (2010) evaluated seedlings of muskmelon (cv. Olympic Gold) grafted onto rootstock Tetsukabuto *(C. maxima* x *C. moschata*) under low temperature, and stored for a period of 2 or 4 weeks at temperature 9, 12, and 15˚C under photosynthetic photon flux 12 μmol m^{-2} s^{-1}. The results showed that un-grafted exhibited more damage than grafted plants when storing at 9˚C. Dry mass accumulation, post-storage growth and development were not affected by stored seedlings at 12˚C even stored for 4 weeks while grafted seedlings had a significant increase in dry mass and stem elongation at 15˚C for 4 weeks as compared with un-grafted seedlings. The rootstock (Tetsukabuto) had the best chilling tolerance and increased the storability of muskmelon seedlings.

Li *et al.* (2008) demonstrated that the activity of super oxide dismutase (SOD) and malondialdehyde contents were higher in both the grafted seedling roots and leaves of cucumber than self-rooted seedlings at both room temperature and chilling temperature.

Gao *et al.* (2009) studied the relative expression of Mn-SOD, Cu/Zn-SOD and CAT mRNAs and the changes of SOD, Mn-SOD, Cu/Zn-SOD and CAT activities in grafted and self-rooted cucumber leaves under low temperature stress, and their relations with the cold resistance of cucumber. The higher SOD activity regulated by the higher SOD mRNAs expression in grafted cucumber leaves might be the reason for grafted cucumber having a higher cold resistance to low temperature stress than self-rooted cucumber. The relative expression of CAT mRNA was slightly higher in functional leaves of grafted cucumber, while less difference was observed in CAT activity, comparing with self-rooted cucumber, which demonstrated that low temperature stress had minor

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effects on the activity of CAT in grafted cucumber leaves.

Li *et al.* (2015) evaluated cucumber seedlings grafted on three rootstocks genotypes under low temperature (5˚C). Grafted on figleaf had the higher proline contents and activities of SOD, CAT and ascorbate dehydrogenase (APX). The difference in rootstocks mediated tolerance improvement, were caused by the different increase of antioxidant defense system under low temperature and light. However, seedling was exposed to low temperature stress for short period (only two days in a growth room).

Venema *et al.* (2008) studied the effect of grafting tomato onto the cold-tolerant rootstock (*Solanum habrochaites*). The grafted and un-grafted plants were grown at different temperature of optimal (25˚C) and suboptimal (15˚C) air/root-zone temperatures. The relative growth rate of shoots increased in grafted plants at temperature 25/15 and 15/15˚C than un-grafted plants. At sub-optimal temperature, grafted plant had higher root mass ratios. The relative growth rate of un-grafted plant under suboptimal root-zone temperatures strongly reduced than grafted plants.

Recently, Riga *et al.*, (2016) tested grafted and un-grafted tomato Jack F_1 under low temperature conditions 12/18˚C night/day, and the vent opening temperatures were 20/22˚C night/day. The concentrations of serotonin contents in grafted rootstocks were significantly lower than in the un-grafted and self-grafted controls.

In general, watermelon is a warm season crop and is adversely affected by growing in sub-optimal cold temperature. Grafting onto resistance rootstocks can be suggested as an alternative mean to overcome such stress. However, It is still unclear why watermelon plants grafted on different rootstock usually show different response to low temperature. Therefore, the present study was conducted to examine the relative tolerance of grafted watermelon onto different rootstocks to cold stress and the mechanism of tolerance to such stress.

MATERIALS AND METHODS

This experiment was conducted under two conditions, cold (winter season) and normal growing season as control to study the relative tolerance of grafted watermelon to cold stress. The commercial watermelon (*C. lanatus*) cv. Aswan F_1 (Sakata Company, Japan) was used as scion and grafted onto hybrid rootstocks (*Cucurbita maxima* x *Cucurbita moschata*) Giada F_1 (Gi), Shintoza F_1 (Sh), Strong tosa F_1 (St), and Ferro F_1 (Fe), released by NunhemsZaden (The Netherlands), G.S.I Seeds (The Netherlands), Syngenta Seeds (The Netherlands) and Rijk-Zwaan, respectively. The seeds of the rootstocks were sown in the greenhouse (Tabarak Farm, Technogreen, Salhya, Ismailia, Egypt) 6-10 days later than the seeds of the scions to ensure similar stem diameters at the grafting time due to the differences in growth vigor. Seeds were sown in 216-cell styrophoam trays under greenhouse conditions. The trays were filled with soil mixture (peat moss and perlite mixes in 1:1 v/v). The environmental conditions for germination were 24-28˚C and 85-90% relative humidity. Splice graft method (one cotyledon graft) was used according to Hassell *et al.* (2008**)**. Hardening was performed as described by Hussien (2012).

Watermelon plants in normal growing condition were cultivated in the soil in 10 March 2015 until 20 of June 2015, while, those in the cold season were cultivated in 19 December 2015 until the 18 of April 2016. In each season, plants from each graft combination (5 plants) were arranged in a complete randomized design with three replicates. Statistical analysis was performed as factorial experiment (2 season x 5 graft combinations). The average temperatures in the area during the warm growing season were 29˚C as a maximum and 18.0˚C as a minimum. While, the average temperatures in the area during the cold growing season were 23˚C as a maximum and 13.0˚C as a minimum as shown in Table (1). Soil samples were taken from the farm before transplanting and analyzed at the Soil and Water Department, Faculty of Agriculture, Suez Canal University. The mechanical analysis of the soil at the experimental farm showed that the textural cultivar was loamy Sand (at depth 0-40 cm, clay was 12%, silt was 5% and sand was 83%). The chemical properties of soil analysis showed that the EC= 1.35 dSm^{-1}, pH= 7.74, cations were as follows: Ca^{2+}= 9.20 $meql^{-1}$, Mg^{2+}=2 $meql^{-1}$, Na^{+}= 1.60 $meql^{-1}$, K^{+}= 1.20 $meql^{-1}$, while anions were as follows: Cl^{-} = 2.40 $meql^{-1}$, HCO_3^{-} =3.40 $meql^{-1}$, SO_4^{2-} = 7.80 $meql^{-1}$, CO_3^{2-} = 0.40 $meql^{-1}$. The cultivation was carried out using a drip irrigation system, with 4 L/h drippers, spaced 50 cm along the irrigation tubes (16 mm). Irrigation tubes were allocated 2 m between each row in soli previously enriched with 30 m^3/feddan organic manure. The grafted and un-grafted plants were planted in the soil 1 m between plants at the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia. Fertigation regime took place two weeks after planting and terminated two weeks before fruit harvest. During this period, plants received amount of fertilization applied during watermelon growth as recommended by the Ministry of Agriculture, Egypt.

Measurements:

Vegetative growth: Vegetative growth was measured every two weeks, by counting number of leaves and branches and main stem length in four plants chosen randomly from each plot.

Leaves dry matter percentage (DM %) was measured by weighting 20 leaves (five leaves x four plants) and then dried in the oven at 70˚C for three days to determine dry weights. Relative water content (LRWC) was measured by taking the fourth leaf from the top of three plants per treatment and then weighted as fresh weight. The leaves were then rinsed in deionized water and blotted carefully with tissue paper and then weighted as fresh weight at full turgor. After that the leaves were put into a forced air oven at 70˚C for three days to determine their dry weights. According to Yamasaki and Dillenburg (1999), the LRWC was calculated using the following formula:

% LRWC = (fresh weight – dry weight) / (fresh weight at full turgor – dry weight) x 100

Fruit harvest and yield: Total number of fruits per plant, total yield of fruits (Kg/plant) and average fruit weight (Kg/fruit) were recorded after excluding un-marketable fruits.

Fruit characteristics:

a-Fruit rind thickness (cm).

b-Fruit circumference (cm): calculated by the following equation (C= π*d) where d is the diameter and the π is defined as the ratio of the circumference of the circle to its diameter. The numerical value of π is 3.14.

c-Soluble solids content (SSC) of the fruits: it was obtained using juice of fruit from the central endocarp with the use of a hand refractometer according to A.O.A.C. (1996).

Chemical analysis:

Lycopene content of fruits

Five to ten grams of the juice were extracted repeatedly with acetone in a pestle and mortar or a blender until the residue was colorless according to Ranganna (1977). The color was measured in a one cm cell at 503 nm in Spectrophotometer (model, Unico UV/Vis 2100.USA) using petroleum ether as blank using the following equation:

Mg of lycopene/100 g FW=

$$\frac{3.1206 \text{ X optical density (OD) of sample x volume made up x dilution x } 100}{1 \text{ x wt. of samples x } 1000}$$

Leaf chlorophyll contents:

According to the method of Lichtenthaler (1987), the amounts of photosynthetic pigments (Chlorophyll a, b, a+b and carotenoids) were determined.

Macro-nutrient determination:

Fifteen leaves were taken from 3 plants for all treatments. The leaves were dried at 70˚C for 48 hours and grounded. Half gram of the samples was digested by sulfuric acid and hydrogen peroxide according to Jackson (1967). After proper dilution of digested material, nitrogen was determined using modified Kheldahl method according to Jackson (1967). Phosphorus was determined using Spectrophotometer according to Black *et al.* (1965). Potassium was determined by using flame photometer according to Jackson (1967).

Proline:

Proline was estimated using the method described by Sadasivam and Manickam (1992). The fourth leaf from the top of three plants per treatment was taken. The toluene layer was separated and measured for red color intensity at 520 nm.

5. Enzymes:

Superoxide dismutase (SOD): Superoxide dismutase level was determined by using biodiagnostic kit No.SD2521 using the spectrophotometric (U\V Spectrophotometer spectronic 1201, Milton Roy, U.S.A) based on the method of Nishikimi *et al*. (1972). Color of the reduced dye was measured at 560 nm for 5 minute for control.

Catalase (CAT): Catalase activity was measured using biodiagnostic kit No.517 by spectrophotometric method described by Aebi (1984). The absorbance was measured at 510 nm.

Statistical analysis

Statistical analysis was performed with the aid of the SPSS 14 for Windows statistical package (IBM Corp., New York, USA). Data were evaluated by analysis of variance for the main effects and the means of values were compared by the Duncan Multiple Range Test (DMRT) at p=0.05.

Table 1: Monthly averages of air temperature, humidity, wind speed and evaporation (ET_o) during 2015 and 2016.

Months	Temperature Max.	Temperature Mini.	Humidity (%)	Wind speed (km/h)
Season 2015				
March	24	15	59.5	7
April	26	16	53.0	12
May	31	19	53.5	8
June	33	22	47.5	9
December	20	11	53.0	8
Season 2016				
January	17	9	60.5	16
February	23	9	59.0	6
March	26	16	39.5	11
April	32	19	54.0	27

Source: Central laboratory for Agricultural climate

RESULTS

Effect of sub-optimal growing temperature, graft combinations and their interactions on vegetative growth, marketable fruit yield and fruit characteristics:

Main effects of season: As tested over all graft combinations, all vegetative growth examined were significantly lower under cold vs. warm season, including number leaves per plant (ave. % decline = 29.4%), number of lateral branches (29.2%) and length of main stem (16.0%) as shown in Table (2). A decrease in leaf % DM and leaf relative water contents were also detected under cold season growth, compared to warm season, and the % DM was declined by 44.5%, whereas LRWC was decreased by 6.2% as shown in Table (3).

With regard to watermelon fruit yield components, results revealed reduction in fruit number by 24.3% and marketable fruit yield per feddan by 30.2% under cold season compared with warm season. However, mean fruit weight was not significantly different between plants grown in warm vs. cool season (Table 4). Other fruit characteristics, including rind thickness, fruit circumference and lycopene were also higher under warm season compared to cool season, but flesh SSC was not significantly different between the two growing conditions (Table 5and6). Reduction in fruit circumference and lycopene content was 11.4% and 10.5%, respectively, under cold season compared with warm season.

Main effect of graft combinations: Results of Table (2) indicated that grafting Aswan on the rootstock StrongToza or Ferro recorded the highest values of leaves number, number of lateral stems and length of main stem, while un-grafted Aswan plants had the lowest number of lateral stems and length of main stem.

Leaf %DM was not significantly different among the tested graft combinations, but LRWC% was significantly and little lower in control plants (un-grafted) vs. all graft combinations as shown in Table (3). Plants of Aswan/Ferro recorded significantly higher fruit number per plant and total marketable fruit yield per feddan with an average of 2.42 (fruit/plant) and 25.47 (ton/fed.). Aswan/Giada plants had the highest mean fruit weight with an average of 5.86 (Kg/fruit), as shown in Table (4). Aswan (un-grafted) plants recorded the lowest yield components under warm and cold seasons.

Results showed that the genotype of the rootstock did not significantly affect soluble solid content (SSC). Lycopene content was significantly affected by rootstock genotype. Watermelon grafted on Giada had higher in lycopene content than Aswan un-grafted plants (8.05 vs 7.10 mg/100 g FW), as shown in Table (6)

Effect of graft combinations x season interaction: No significant graft combination x season interaction was detected on number of leaves and branches per plant. The highest leaves number and branches number were found in the graft combination Aswan/StrongToza and Aswan/Ferro in the warm season, while the lowest was record in the cold season. Main stem lengths in all graft combinations declined under cold season, but in different degree (Table 2). All graft combinations were equal to the control (un-grafted plants) in leaf dry mater percentage (DM%) and percentage of leaf relative water content (LRWC) under warm season, and also were not significantly different in the cold season as shown in Table (3).

Additionally, all graft combinations recorded a decrease in fruit number under cold vs warm condition, in different degree with un-grafted plants recorded the highest decrease (Table 4). Mean fruit weight were equal among all graft combinations except the control plants under warm and cold conditions (Figure 1). Marketable Fruit yield of the graft combination Aswan/Ferro followed by Aswan/Giada recorded the highest marketable yield per feddan under warm season, while, the least marketable yield was found in un-grafted plants in the cold season. Total marketable fruit yield per feddan under cold stress recorded the highest increase percentage in Aswan/Ferro over un-grafted plants (20.03 vs. 8.49 ton/fed.) indicating that plants of Aswan/Ferro performed better than other graft combinations and had more than double marketable yield of the control under cold stress as shown in Table (4) and Fig. (2). Growing grafted watermelon in the cold season did not significantly affect fruit SSC since all graft combinations under cold stress recorded the same % SSC, rind thickness in all grafted plants, but fruit circumference was higher than the control. The fruit of control plants (Aswan) had more SSC under cold season by (5.2%), in addition to Aswan/Shintoza with increased SSC by 4.7%, as shown in Table (6). The highest decline in lycopene content (19.6%) was found in Aswan (un-grafted) than the other rootstocks compared with cold season. However, the lowest decline in lycopene was found in Aswan/Strong Toza (2.7%), as shown in Table (6).

Most growth and yield characters were decreased when grafted watermelon plants were grown under sub-optimal temperature (cold season). However, the % decline varied according to the rootstock used. In this respect, the lowest decreased were in the graft combination Aswan/Ferro for several examined parameters such as number of leaver per plant, number of branches per plant, length of main stem, mean fruit weight (in addition to Giada), and fruit circumference. The graft combination Aswan/Giada recorded the lowest % decline, as compared to other rootstock, in LRWC, and marketable yield. The graft combination Aswan/StrongToza also recorded the lowest % decline in fruit number per plant when grown under cold condition.

Table 2: Main effect of graft combinations, seasons and their interactions on some vegetative growth characters of watermelon plants

Scion/ Rootstock	Leaves number		Mean	Lateral stems number		Mean	Length of main stem (m)		Mean
	warm season	cold season		warm season	cold season		warm season	cold season	
A/Gi	302.00 bc	218.00 de	**260.00 C**	24.33 bc	17.66 cd	**21.00 B**	3.33 ab	2.39 de	**2.86 AB**
A/Sh	311.33 bc	211.66 de	**261.50 C**	24.00 bc	14.66 d	**19.33 BC**	2.93 a-d	2.46 cde	**2.69 B**
A/St	442.66 a	307.33 bc	**375.00 A**	32.66 a	23.00 bc	**27.83 A**	3.56 a	2.71 b-e	**3.14 A**
A/Fe	367.66 b	28300 c	**325.33 B**	27.66 ab	20.33 cd	**24.00 AB**	3.43 a	3.04 abc	**3.24 A**
A Control	251.33 cd	163.33 e	**207.33 D**	18.00 cd	14.00 d	**16.00 C**	2.10 e	2.30 de	**2.20 C**
Mean	**335.00 A**	**236.66 B**		**25.33 A**	**17.93 B**		**3.07 A**	**2.58 B**	
Significances									
Graft (G)	***			***			***		
Season (S)	***			***			***		
S*G	**ns**			**ns**			**ns**		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 3: Main effect of graft combinations, seasons and their interactions on leave dry matter percent (DM %) and leaf relative water content (LRWC) of watermelon plants.

Scion/Rootstock	Leave DM (%)		Mean	LRWC (%)		Mean
	warm season	cold season		warm season	cold season	
A/Gi	38.00 a	20.00 b	**29.00 A**	89.08 a	84.24 b	**86.66 A**
A/Sh	36.00 a	21.33 b	**28.66 A**	89.58 a	83.24 b	**86.41 A**
A/St	38.33 a	22.00 b	**30.16 A**	90.15 a	84.20 b	**87.18 A**
A/Fe	39.00 a	19.66 b	**29.33 A**	92.96 a	84.89 b	**88.93 A**
A Control	33.66 a	19.66 b	**26.66 A**	85.18 b	82.50 b	**83.84 B**
Mean	**37.00 A**	**20.53 B**		**89.39 A**	**83.81 B**	
			Significances			
Graft (G)	**Ns**			**		
Season (S)	***			***		
S*G	**Ns**			**Ns**		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 4: Main effect of graft combinations, seasons and their interactions on fruit number, mean fruit weight and marketable yield per feddan of watermelon plants

Scion /Rootstock	Fruit number (no./plant)		Mean	Average fruit weight (kg/fruit)		Mean	Marketable yield (ton/fed.)		Mean
	warm season	cold season		warm season	cold season		warm season	cold season	
A/Gi	2.13 ab	1.83 ab	**1.98 AB**	5.90 a	5.82 a	**5.86 A**	25.22 ab	19.79 bc	**22.51 AB**
A/Sh	2.44 a	1.71 ab	**2.08 AB**	4.61 a	4.32 a	**4.46 AB**	22.28 b	14.84 cd	**18.56 BC**
A/St	1.89 ab	1.82 ab	**1.85 AB**	5.53 a	4.58 a	**5.06 AB**	20.20 bc	15.25 c	**17.72 C**
A/Fe	2.91 a	1.94 ab	**2.42 A**	5.50 a	5.42 a	**5.46 AB**	30.90 a	20.03 bc	**25.47 A**
A Control	1.73 ab	1.11 b	**1.42 B**	4.23 a	3.82 a	**4.02 B**	13.82 cd	08.49 d	**11.15 D**
Mean	**2.22 A**	**1.68 B**		**5.15 A**	**4.79 A**		**22.48 A**	**15.68 B**	
				Significances					
Graft (G)	**ns**			**ns**			***		
Season (S)	*			**ns**			***		
S*G	**ns**			**ns**			**Ns**		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 5: Main effect of graft, seasons and their interactions on fruit rind thickness and fruit circumference

Scion/Rootstock	Rind thickness (cm)		Mean	Fruit circumference (cm)		Mean
	warm season	cold season		warm season	cold season	
A/Gi	1.30 a	0.92 b	**1.11 A**	74.83 a	66.74 b	**70.78 A**
A/Sh	1.30 a	0.88 b	**1.09 A**	72.57 a	63.49 b	**68.03 A**
A/St	1.54 a	0.93 b	**1.23 A**	73.86 a	63.32 b	**68.59 A**
A/Fe	1.30 a	0.85 b	**1.08 A**	74.18 a	66.32 b	**70.25 A**
A Control	1.30 a	0.71 b	**1.00 A**	63.54 b	58.12 c	**60.83 B**
Mean	**1.34 A**	**0.86 B**		**71.79 A**	**63.60 B**	
			Significances			
Graft (G)	**Ns**			***		
Season (S)	***			***		
S*G	**Ns**			**Ns**		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 6: Main effect of graft combinations, seasons and their interactions on soluble solid content (SSC) and lycopene content of watermelon fruits

Scion/Rootstock	SSC (%)		Mean	Lycopene (mg/100 g FW)		Mean
	warm season	cold season		warm season	cold season	
A/Gi	11.27 ab	11.00 ab	**11.13 A**	8.54 a	7.56 c	**8.05 A**
A/Sh	10.38 ab	10.87 ab	**10.62 A**	7.00 d	6.39 e	**6.69 D**
A/St	11.32 ab	10.98 ab	**11.15 A**	7.86 bc	7.65 bc	**7.75 B**
A/Fe	11.70 a	10.82 ab	**11.26 A**	7.94 b	7.18 d	**7.56 B**
A Control	10.17 b	10.70 ab	**10.43 A**	7.87 bc	6.33 e	**7.10 C**
Mean	**10.97 A**	**10.87 A**		**7.84 A**	**7.02 B**	
			Significance			
Graft (G)	**Ns**			***		
Season (S)	**Ns**			***		
S*G	**Ns**			***		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Effect of sub-optimal growing temperature, graft combinations and their interactions on biochemical and nutrient analysis

Main effect of season: Data indicated that growing watermelon in the cold season under sub-optimal temperature had resulted in significant decline in photosynthetic pigment contents under cold stress (47.6% for Chl a, 49.7% for Chl b, 51.3% for total Chl, and 40.1 % for carotenoids (Table 7). Analysis of data for Nitrogen (N), Phosphorus (P) Potassium (K) contents in leaves of grafted watermelon revealed no change in N% and K%, but % P was higher under sub optimal growing temperature (Table 8). However, growing watermelon under sub-optimal temperature had resulted in significant increase in proline (38%), SOD (32.6%) and CAT (24.9%) in the leaves compared to those growing under normal warm season condition (Table 9).

Main effect of graft combinations: Chlorophyll contents were the highest in the graft combination Aswan/Ferro (Chl a, b, and total), in addition to the graft combination Aswan/Giada for Chl. b,while, control plants recorded the lowest Chl.a and total Chlorophyll. Carotenoids content were equal among the different graft combinations, except the non-grafted plants which recorded the lowest carotenoids in their leaves (Table 7). Nitrogen and potassium were the lowest in un-grafted compared to all grafted plants, while P percentage was not significantly different among all grafting treatments as shown in Table (8).

Among the different graft combinations, proline was the highest in Aswan/Strong Toza with an average of 199.27 (µg/g FW) and the lowest was found in un-grafted plants with an average of 55.55 (µg/g FW). SOD was the highest in Aswan/Giada, Aswan/Shintoza and Aswan/Ferro with no significant difference among them, and the lowest in the control (un-grafted plants). CAT activity was higher in Aswan/Strong Toza and Aswan/Ferro and the lowest in the control plants, as shown in Table (9).

Effect of graft combinations x season interactions: Chlorophyll and carotenoids contents were decreased under cold stress and the percentage of decrease varied among the different graft combinations. In this regards, the highest decrease in Chla (54.7%), Chl b (59.5%), were recorded in Aswan/Ferro while the lowest decrease in Chl b was in Aswan/Strong Toza (26%). Total Chlorophyll was the highest in Aswan/Ferro and Aswan/Shintoza during warm season with an average of 286.28 and 274.41(mg/100 g FW), respectively. The highest content of carotenoids was recorded in all graft combinations, as compared to the control, under warm season. Leaf of Aswan/Strong Toza was the least affected by growth under cold stress, recording only 31% reduction in carotenoids (Table 7).

The graft combinations x season interaction for the leaf N, P and K contents were significant. The highest N concentrations were recorded in Aswan/Shintoza and Aswan/Ferro under warm season with an average of 51.46 and 50.80 (mg/g^{-1}), respectively. The highest P and K % were recorded in Aswan/Ferro under cold stress with an average of 4.26 and 16.06 (mg/g^{-1}), respectively, as shown in Table (8).

Under sub-optimal growing temperature, all graft combinations showed increase in proline content, but in different degree, depending on the rootstock genotype. In this respect, the increase in proline was only 11.9% in Aswan/Ferro, while it was 73.6% in Aswan/Strong Toza under cold stress and Aswan/Strong Toza leaves had the highest proline content in the cold season as shown in Table (9) and Fig. (3). SOD activity followed the same trend line as proline; it was increased in all graft combinations under sub-optimal temperature, but in different degree. SOD recorded the highest increase (71.3%) in Aswan/StrongToza leaves, while Aswan/Giada recorded the lowest increases in SOD (11%) as shown in Figure 4. The highest recorded SOD activity was found in Aswan / Ferro under cold stress. CAT activity was also higher under cold stress compared to warm season in all graft combinations, and the highest was recorded in Aswan/Giada (44.81U/g FW) and Aswan/StrongToza (44.50 U/g FW), while un-grafted plants had the lowest (42.65 U/g FW) in CAT under cold stress. The lowest increase in CAT activity was recorded in Aswan/Ferro (11%) under sub-optimal growing temperature as shown in Table (9).

DISCUSSION

Watermelon, as a warm season crop, usually grow well in a range of air/or soil temperature of 20-35°C. Under the conditions of this experiment, grafted and un-grafted plants were grown in the field during the normal (warm) cultivated season (Mar. - Jun., 2015), as compared to cultivation during the cool season (Nov. - Feb., 2016). Results demonstrated large decline in vegetative growth (16-29%), %DM (44.5%) and LRWC (6.2%), as well as number of male (20.7%) and female (38%) flowers under low temperature season. Marketable yield attributes were also adversely affected by low temperature. The decrease in fruit number per plant, marketable fruit yield and mean fruit weight were 24%, 30% and 7%, respectively.

Examination of nutrients and photosynthetic pigment contents showed declines in P, but not in N and K whereas Chl a, Chl b and total Chl were significantly decreased by about 50% and carotenoids by about 40% in leaf sample after exposure to low temperature stress, as compared to growth in normal warm season.

Growth under sub-optimal temperature conditions (<20˚C) may leads to slowing of metabolism, solidification of cell membrane, and loss of membrane function (Jewell *et al*., 2010). In accordance with our results, low temperature was reported to reduce leaf expansion and growth (Smith and Stitt, 2007), and restrict the reproductive development (Kumar *et al*., 2010), impairment of photosynthesis (Nayyar *et al.*, 2005) and decreased Chlorophyll contents (Li *et al*., 2008).

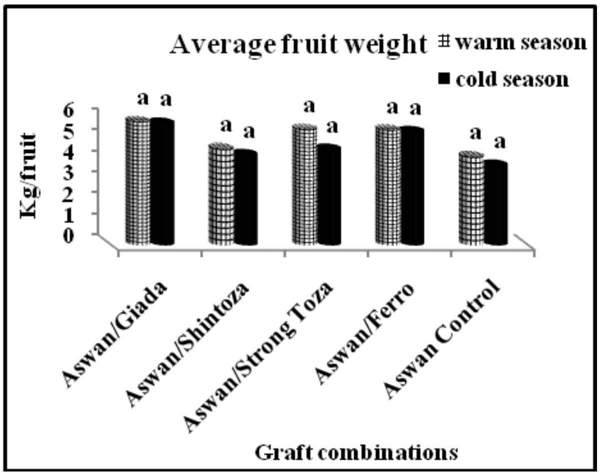

Fig. (1): The interaction effect between seasons and graft combinations on average fruit yield

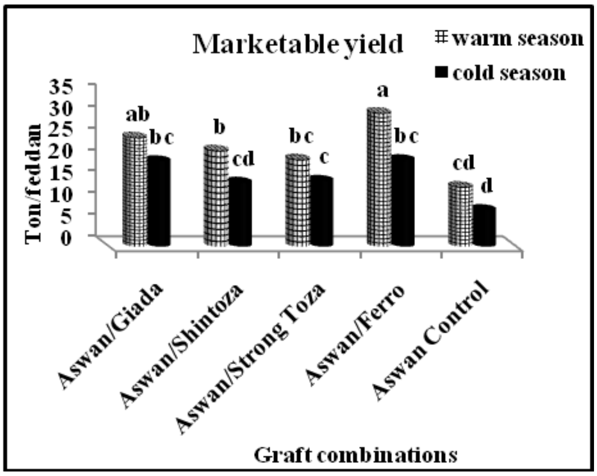

Fig. (2): The interaction effect between seasons and graft combinations on marketable yield

Table 7: Main effect of graft combinations, seasons and their interactions on photosynthetic pigment contents of watermelon leaves

Scion/Rootstock	Chlorophyll a (mg/100 g FW)		Mean	Chlorophyll b (mg/100 g FW)		Mean
	warm season	**cold season**		**warm season**	**cold season**	
A/Gi	167.40 b	84.33 e	**125.86 B**	74.39 ab	34.96 ef	**54.68 A**
A/Sh	148.76 cd	84.89 e	**116.82 BC**	55.25 cd	30.48 ef	**42.87 B**
A/St	164.36 bc	89.58 e	**126.97 B**	59.45 c	43.60 de	**51.53 AB**
A/Fe	198.27 a	89.91 e	**144.09 A**	85.90 a	34.75 ef	**60.32 A**
A Control	135.27 d	78.16 e	**107.71 C**	62.86 bc	26.23 f	**44.54 B**
Mean	**162.81 A**	**85.37 B**		**67.57 A**	**34.01 B**	
			Significances			
Graft combination (G)	***			**		
Season (S)	***			***		
S*G	**			**		
Scion/Rootstock	**Total Chlorophyll (mg/100 g FW)**		**Mean**	**Carotenoids (mg/100 g FW)**		**Mean**
	warm season	**cold season**		**warm season**	**cold season**	
A/Gi	241.74 b	119.3 de	**180.50 B**	295.64 a	160.71 cd	**228.17 A**
A/Sh	274.41 a	115.4 de	**194.88AB**	265.20 a	157.83 cd	**211.51 A**
A/St	223.77 b	133.15 d	**178.46 B**	264.42 a	180.56 c	**222.49 A**
A/Fe	286.28 a	124.6 de	**205.46 A**	287.56 a	169.89 c	**228.72 A**
A Control	198.13 c	104.36 e	**151.24 C**	228.45 b	134.06 d	**181.26 B**
Mean	**244.86 A**	**119.35 B**		**268.25 A**	**160.61 B**	
			Significances			
Graft combination (G)	***			**		
Season (S)	***			***		
S*G	***			ns		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Differences among the different graft combinations were also detected in their tolerance to low temperature stress, and the grafted plants had always better performance than un-grafted ones. Although all graft combinations showed reduction in growth and yield under cold stress, in different degrees, depending on the rootstock used. In this respect, the lowest decreases in vegetative growth were found in plants of Aswan/Ferro and Aswan/Giada. Plants of Aswan/Giada were relatively tolerant to cold stress, based on marketable fruit yield, and Aswan/Strong Toza based on fruit number per plant. These observed differences among watermelon plants tolerance to cold stress upon grafting onto different rootstocks were also reported previously on cucumber (Bulder *et al*., 1991; Ahn *et al*., 1999 and Li *et al*., 2008) and tomato (Venema *et al*., 2008).

To shed more light on the mechanism of tolerance to cold stress in grafted watermelon plants, the accumulation of compatible solute (proline) and the activity of antioxidant enzymes (SOD and CAT) were detected in plants grown under normal warm season vs. cold season conditions. Results indicated increase in proline contents (38%), SOD (32.6%) and CAT (24.9%) under cold vs. warm season conditions. These increases in proline and antioxidant enzyme activities are in agreement with the finding of Li *et al*., (2008) and Li *et al*., (2015).

High activity of SOD was proposed as the key factor of grafted cucumber having higher tolerance to low temperature stress than un-grafted plants (Gao *et al*., 2009). Therefore, the increase in proline content and antioxidant enzyme activities under sub-optimal growing conditions is a possible mechanism for grafted watermelon plants to withstand growth under such conditions.

Table 8: Main effect of graft combinations, seasons and their interactions on N, P and K concentrations of watermelon leaves

Scion/Rootstock	N (mg/g^{-1})		Mean	P (mg/g^{-1})		Mean	K (mg/g^{-1})		Mean
	warm season	cold season		warm season	cold season		warm season	cold season	
A/Gi	45.60 ab	46.30 ab	**45.95 A**	2.23 de	3.81 ab	**3.02 A**	10.87 bc	12.46 ab	**11.67 AB**
A/Sh	51.46 a	43.03 b	**47.25 A**	1.66 ef	3.61 abc	**2.63 A**	07.94 c	12.70 ab	**10.32 AB**
A/St	48.53 ab	45.80 ab	**47.16 A**	2.71 cd	3.62 abc	**3.16 A**	13.59 ab	10.53 bc	**12.06 AB**
A/Fe	50.80 a	44.90 ab	**47.85 A**	1.26 f	4.26 a	**2.76 A**	10.46 bc	15.06 a	**12.76 A**
A Control	23.43 c	42.83 b	**33.13 B**	2.77 cd	3.20 bcd	**2.98 A**	11.50 abc	08.36 c	**09.93 B**
Mean	**43.96 A**	**44.57 A**		**2.12 B**	**3.70 A**		**10.87 A**	**11.82 A**	
				Significances					
Graft (G)	***			ns			ns		
Season (S)	ns			***			ns		
S*G	***			**			**		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 9: Main effect of graft combinations, seasons and their interactions on proline content, superoxide dismutase (SOD) and catalase (CAT) activities of watermelon leaves

Scion/ Rootstock	Proline (µg/g FW)		Mean	SOD (U/g FW)		Mean	CAT (U/g FW)		Mean
	warm season	cold season		warm season	cold season		warm season	cold season	
A/Gi	079.22 f	107.50 d	**093.36 C**	3735.23 d	4169.98 b	**3952.60 A**	34.59 f	44.81 a	**39.70 B**
A/Sh	151.00 c	192.61 b	**171.80 B**	3629.98 d	3877.99 cd	**3753.98 A**	34.24 f	43.70 abc	**38.97 B**
A/St	145.66 c	252.88 a	**199.27 A**	2409.99 f	4127.28 bc	**3268.63 B**	37.89 e	44.50 ab	**41.19 A**
A/Fe	090.71 e	101.50 d	**096.10 C**	3059.98 e	4476.09 a	**3768.03 A**	39.13 d	43.36 bc	**41.25 A**
A Control	050.95 g	060.16 g	**055.55 D**	2039.99 g	3077.00 e	**2558.49 C**	29.50 g	42.65 c	**36.07 C**
Mean	**103.51 B**	**142.93 A**		**2975.03 B**	**3945.67 A**		**35.07 B**	**43.80 A**	
				Significances					
Graft (G)	***			***			***		
Season (S)	***			***			***		
S*G	***			***			***		

Values followed by the same letter within a column or row are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

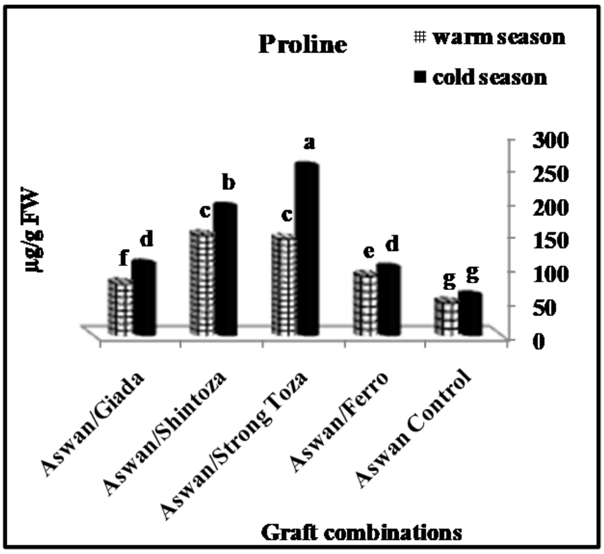

Fig. (3): Effect of the interaction between seasons and graft combinations on proline content of watermelon leaves.

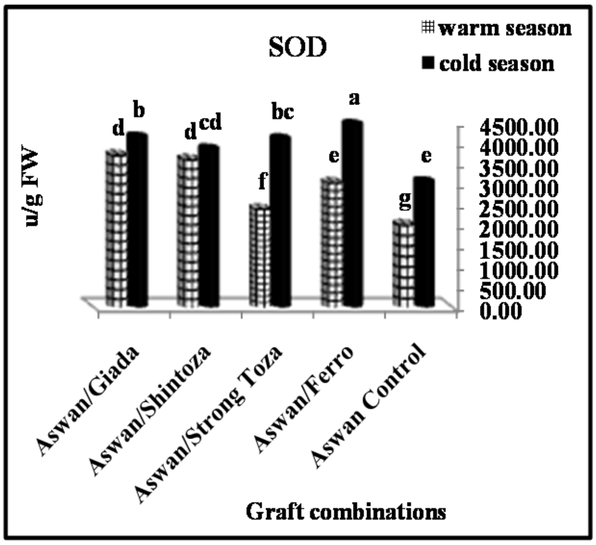

Fig. (4): Effect of the interaction between seasons and graft combinations on SOD activity in watermelon leaves.

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إستجابة نباتات البطيخ المطعومة على أصول مختلفة لحرارة النمو الأقل من المثلى

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أجريت هذه الدراسة لإختبار التحمل النسبى للبطيخ لظروف النمو تحت حرارة أقل من المثالية حيث تم إستخدام الصنف أسوان وتطعيمه على أصول مختلفة مثل جيادا ، شنتوزا ، سترونج توزا ، فيرو . وقد تم زراعة النباتات المطعومة مع نباتات الكونترول الغير مطعومة فى الحقل تحت ظروف حرارة منخفظة خلال الفترة يناير – إبريل ومقارنتها مع تلك المنزرعة تحت ظروف الموسم الدافئ خلال الفترة مارس – يونيو. أوضحت النتائج إنخفاضا معنويا فى النمووالمحصول ومحتوى الكلوروفيل والكاروتينات فى كل النباتات المطعومة بنسب مختلفة حسب الأصول المستخدمة حيث سجلت نباتات الصنف أسوان المطعومة على الأصل فيرو محصولا أكبر من ضعف المحصول الناتج من النباتات غير المطعومة تحت ظروف حرارة أقل من المثلى مما يؤكد التحمل النسبى للنباتات المطعومة (أسوان / فيرو) لإجهاد البرودة بينما أظهرت النباتات الغير مطعومة أكثر الإنخفاضات فى مقاييس النمو والمحصول. وكان محتوى الأوراق من البرولين ونشاط الإنزيمات المضادة للأكسدة (كتاليز وسوبر أوكسيد ميوتاز) عاليا تحت ظروف البرودة مقارنة بظروف الزراعة الدافئة. ويستخلص أن نباتات البطيخ المطعوم كانت أكثر تحملا لظروف النمو تحت حرارة أقل من المثلى مقارنة بالغير مطعومة مما يساهم فى إمكانية الحصول على ثمار البطيخ فى غير موسمه.