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Effect of grafting on anatomical and physiological characteristics on Nubian watermelon plant

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ABSTRACT

Two field experiments were carried out on Nubian watermelon (*Citrullus lanatus* var. *Colocynthoide*) during the summer season of 2009 and 2010 in the farm of Sakha Research Station in Kafr El-Sheikh Governorate. The main objective of the study was to compare between the four rootstocks: bottle gourd (*Lagenaria siceraria*), pumpkin (*Cucurbita moschata*), luffa (*Luffa cylindrical*) and fig leaf gourd (*Cucurbita ficifolia*) with non-grafted plants. The study included measurement of biochemical analysis of leaves and seeds, anatomical structure of the grafting union and successful grafting percentage. In general, grafting plants onto bottle gourd had higher nitrogen, phosphorus and potassium percentage, while the lowest values were obtained from grafting plants onto fig leaf gourd. Grafting onto different rootstocks significantly decreased oil content compared with non-grafted plants. On contrast, carbohydrate content was increased by grafting. Nubian watermelon grafted onto luffa had the highest protein content, while the lowest protein contents were obtained from grafting plants onto bottle gourd and from non-grafted plants. Grafting onto different rootstocks significantly increased area of both xylem and phloem compared with non-grafted plants. Also, grafting Nubian watermelon onto fig leaf gourd and bottle gourd had the highest successful grafting percentage.

Keywords: Nubian watermelon, grafting, rootstocks, carbohydrate, protein, oil, xylem and phloem.

INTRODUCTION

Nubian Watermelon is one species of the Cucurbitaceae family. It is an ancestor type of the cultivated watermelon **(Ziyada and Elhussien, 2008)**. Egypt is the fifth country worldwide in the production of cucurbits while China is the first **(FAO, 2008)**. Nubian Watermelon was cultivated from early times in Egypt, possibly from the Ancient Egypt **(Manniche, 1989)**. It is known as gurma watermelon **(El-Shabrawy and Hatem, 2008)**. Green parts of the plant are used as animal feeds, the seeds are used as snacks, and the residues are used as a source of heat energy for cooking **(Mariod *et al.* 2009)**. Nubian watermelon occupied about 174447 feddans in summer season of 2009, which yielded 101013 tons with an average yield of 0.58 ton/fed. according to statistical data of the Ministry of Agriculture of Egypt. Kafr El-Sheikh is one of the most important producing regions for such crop in Delta, since its percentage of the cultivated area and the productivity of Nubian watermelon reach more than the quarter **(statistical data of the Ministry of Agriculture, 2009)**.

Within the last years Nubian Watermelon has become an important crop able to export large quantities of gurma watermelon to the Arab countries **(El-Shabrawy and Hatem, 2008)**. Also **Ibrahim *et al.* (2002)** suggested that Nubian Watermelon can be used as a source of protein supplement to ruminant animals. Moreover, **Ziyada and Elhussien (2008)** cleared that Nubian Watermelon was investigated as a new source of vegetable oil.

Grafting has many benefits to plants grown in the open field or greenhouse; such as **Nie *et al.* (2010)** noticed that watermelon grafted seedlings leaves onto fig leaf gourd had significantly higher chlorophyll content. Also **Mounir (1965)** found that grafting watermelon onto *Cucurbita moschata*, var. Asli, had no significant effect on N, P and K percentage in the leaves. Moreover**, Hamed (2005)** studied the effect of grafting watermelon onto different rootstocks (*Lagenaria siceraria* 1 (bottle gourd), *Lagenaria siceraria* 2 (calabash gourd), *Cucurbita maxima, Cucurbita moschata, Cucurbita ficifolia* and *Cucurbita pepo*). He observed that the plants grafted onto *L. siceraria* 1 (bottle gourd), *C. maxima* or *C. moschata* rootstock produced fruits higher in their content of sugars (reducing, non-reducing and total) compared with those grafted onto the other rootstocks. Beside **Runqiu *et al.* (2003)** grafted watermelon cv. Zaojia onto bottle gourd, fig leaf gourd and three commercial rootstocks. They noticed that protein content in grafted watermelon was higher than non-grafted plants. Also **Mohammed *et al.* (2009)** found that roots of grafted watermelon onto *Lageneria siceria* and *Cucurbita pepo* showed significantly greater increase in total fatty acids percentage of total lipids, however, root of grafted watermelon onto *Cucurbita pepo* showed significantly greater increase quality of lipids.

The success of grafting union was introduced by **Tiedemann (1989)** who reported that phloem development in the graft union resulted in different numbers of connecting sieve tubes in each individual graft, but the average number of scion tube connections in cucumis on cucurbita was much lower than in Cucumis on Cucumis.

Hence, the purpose of this investigation was to study the effect of different rootstocks on anatomical and physiological characteristics of Nubian watermelon plant in Kafr El-Sheikh Governorate, Egypt..

Materials and Methods

Two field experiment were carried out in the Experimental Farm of Sakha Research Station in Kafr El-Sheikh Governorate, Egypt during the summer season of 2009 and 2010 by using cut grafting method according to **Lee, 1994** and **Oda, 1995** for study the effect of grafting Nubian watermelon by using grafting technique onto four rootstocks [bottle gourd (*Lagenaria siceraria*), pumpkin (*Cucurbita moschata*), luffa (*Luffa cylindrical*) and fig leaf gourd (*Cucurbita ficifolia*)] comparison with non-grafted plants (control) to improve Nubian watermelon production.

The steps of this method were conducted timely as follows:

Day (1) Rootstock seeds were sown into seedling foam trays.

Day (7) Nubian watermelon seeds were sown into seedling foam trays.

Day (17-21): Grafting was performed as rootstock and scion seedlings were taken off from the seedling trays. The apical meristem of the rootstock is removed and a hole about 2 mm in diameter is made at the rootstock hypocotyl with a razor blade. A wedge-shaped hypocotyl of the scion as then firmly inserted into the hole of the rootstock. To avoid wilting of the graft, at the same day and a day later, a plastic covers with a net screen were spread on the grafted plants to give more humidity and shade.

Day (21-25): The plants were exposed to sunlight.

Day (32-33): The usual nursing method was applied.

The different steps of cut grafting method are shown in Plate 1.

Plate 1: Cut grafting method.

The properties of soil are shown in Table 1. The experimental plot contained 10 meters length and 2 meters width, and transplanted on one side of the ridge. The recommended N, P and K were added according to the recommendation of ministry of Agriculture. The other cultural practices were done similarly as practiced by the local growers. The design used was randomized complete blocks design with 4 replicates in both seasons; Data were tested by analysis of variance (**Little and Hill, 1972**).

Data recorded:

- **Biochemical analysis of leaves:**

1. Chlorophyll content (SPAD): It was estimated in the fifth leaf from growing tip after two months from transplanting by using chlorophyll meter Model-SPAD. 502 Minolta. Co., Japan. **(Yadva, 1986)**.

2. Mineral contents (N, P and K %):

After two months from transplanting the fifth leaf from the plant growing tip were dried at 70^{o}C then ground in a Willy mill. The dry material was wet digested with sulphuric acid-percholoric mixture as described by **Allen *et al.* 1974**. Total N, P and K content were determined according to the following methods.

2-1. Total nitrogen (%): was determined in the digestion product according to **Naguib (1969)**.

2-2. Total phosphorus (%): was determined colourimetrically by using a spectrophotometer at 660 nm **(Jackson, 1967)**.

2-3. Total potassium (%): was determined using a flame photometer **(Jackson, 1967)**.

- **Biochemical analysis of seeds:**

Seed-kernels were ground then dry in an oven at 70 ^{o}C till constant weight. Oil concentration, carbohydrate and protein content were estimated according to the following methods:

1. Oil concentration (%) was determined as described by the **A. O. A. C. (1984)** method using petrolium ether (60-80 ^{o}C) as a solvent and Soxhlet apparatus.

Carbohydrate and protein contents were estimated after taking off oil from seed-kernels as follow methods:

2. Estimation of carbohydrate (%): was determined by colourimetric method at 490 µm according to **Dubois *et. al.* (1956).**

3. Estimation of total soluble protein (mg/g):

The total soluble protein content was estimated quantitatively in the borate buffer extract using the method described by **Bradford (1976).**

Anatomical structure of the grafting union:

Plant samples of 10 mm in length from the grafting unit were collected from grafted Nubian watermelon onto different rootstocks and Nubian watermelon plants without grafting (Control) and prepared for microscopic examination **(Ghamrawy and Zaher, 1953)**. The successful grafting percentage, phloem area and xylem area will be determined.

Table 1: Some physical and chemical properties of the experimental soils during 2009 & 2010 seasons**.**

Soil analysis	Seasons	
	2009	2010
Mechanical analysis:		
Clay (%)	53.21	49.17
Silt (%)	25.14	26.11
Sand (%)	21.65	24.72
Texture	Clay	Clay
Chemical analysis:		
pH (1:2.5 soil: water suspension)	8.05	8.2
EC dS m^{-1} (soil paste extract)	2.1	2.4
Organic matter	1.70	1.6
Available N mg Kg^{-1} (1 M Kcl extracts)	36	28
Available P mg Kg^{-1} (0.5 NaHCo$_3$ extracts)	6.1	5.8
Available K mg Kg^{-1} (ammonium acetate extracts)	280	214

RESULTS And Discussion

Biochemical analysis of leaves:

1. Chlorophyll content:

Data in Table 2 cleared that Nubian watermelon plants grafted onto fig leaf gourd had the higher chlorophyll content followed by those grafted onto pumpkin in both seasons, while the lowest values were obtained from non-grafted plants. The differences were significant in both seasons.

These results may be refer to that grafting influences absorption and translocation of magnesium (Ruiz et al. 1997), also the grafted plants contain more Mg in their leaves than the non-grafted ones (Masuda and Gomi, 1984 and Arai et al. 1995) and that may interpret their higher chlorophyll content.

2. Total nitrogen (%):

Data presented in Table 2 demonstrate that Nubian watermelon plants which were grafted onto bottle gourd had higher nitrogen percentage values. It was followed by those grafted onto pumpkin, while the lowest values were obtained from grafted Nubian watermelon onto fig leaf gourd. However the differences were significant in the first season only.

3. Total phosphorus (%):

Data of Table 2 indicated that plant grafted onto bottle gourd rootstock had significantly higher phosphorus content compared to the other treatments in both seasons. It was followed by those grafted onto pumpkin and luffa and non-grafted plants. While plants grafted onto fig leaf gourd had the lowest phosphorus content. The differences were significant in both seasons.

4. Total potassium (%):

Data in Table 2 indicate that, in first season, the highest potassium content was obtained from grafted Nubian watermelon onto bottle gourd, pumpkin and non-grafted plants, while the lowest potassium content obtained from grafted plants onto fig leaf gourd and luffa. While in second season, the highest potassium content was obtained from grafting plants onto bottle gourd. It was followed by those grafted onto pumpkin, and the lowest potassium content from grafted plants onto fig leaf gourd. The differences were significant in both seasons.

The higher values of mineral contents obtained from grafting plants onto bottle gourd and pumpkin may be due to that the rootstock may surpass Nubian watermelon in size of the root system, then a significant amount of xylem sap could be translocate by the rootstock; it is known to contain fairly high concentrations of minerals, organic substances and plant hormones (Masuda and Gomi, 1982 and Lee, 1994).

Table 2: Effect of grafting Nubian watermelon onto different rootstocks on total chlorophyll, nitrogen, phosphorus and potassium percentage of leaves during 2009 & 2010 seasons.

Rootstocks	Total chlorophyll		Total N%		P %		K %	
	2009	2010	2009	2010	2009	2010	2009	2010
Control	47.09 d	42.47 c	3.56 b	3.43	0.17 b	0.15 b	2.37 a	2.21 bc
Bottle gourd	50.13 c	44.80 b	3.92 a	4.10	0.19 a	0.17 a	2.49 a	2.38 a
Pumpkin	52.16 b	45.13 b	3.72 ab	3.65	0.18 b	0.16 b	2.40 a	2.24 ab
Luffa	48.96 c	42.65 c	3.62 b	3.47	0.17 b	0.15 b	2.02 b	2.14 bc
Fig leaf gourd	53.66 a	48.80 a	3.28 c	3.12	0.14 c	0.12 c	1.97 b	2.08 c
F. test	*	*	*	N.S.	*	**	**	*

Values marked with the same alphabetical letter (s) are not significantly different, using Duncan's test at 0.05 levels.

Biochemical seed analysis:

1. Oil (%):

Table 3 indicated that the oil contents of grafted plants onto different rootstocks were decreased as comparing with non-grafted plants in both seasons. The differences were significant in both seasons.

2. Carbohydrate (%):

Data of Table 3 showed that the highest carbohydrate contents were obtained from grafting Nubian watermelon onto pumpkin. It was followed by those grafted onto bottle gourd. While, the lowest values were obtained from non-grafted plants and from grafting plants onto fig leaf gourd. The differences were significant in both seasons.

3. Soluble protein content of seed kernel:

Data in Table 3 cleared that Nubian watermelon grafted onto luffa had the highest protein content, followed by those grafted onto pumpkin and fig leaf gourd, while the lowest protein contents were obtained from grafting plants onto bottle gourd and from non-grafted plants. The differences were significant in the second season only.

It is clear from the above mentioned data that plants grafted onto most different rootstocks had higher values of carbohydrates and soluble protein content. The results were in agreement with Balaz (1982) and Buitelaar (1987) for carbohydrate on watermelon and with Yu-mei et al. (2006) and Gu et al. (2008) for protein on cucumber. This could be attributed to vigorous root system of rootstocks and higher ability of mineral uptake of grafted plants. On the other hand, oils content was lowered by grafting as there is negative relationship between oil and protein content (Attia, 1980). Also the carbohydrate content was increased in concomitant with decreasing the oils. It may be attributed to degradation of oils content which was converted to carbohydrates.

Anatomical structure of the grafting union:

The compatibility between stocks and scion was considered as a major step to set forward successful or failure of grafting. Also the present study was performed to study the possibility of success and failure of grafting Nubian watermelon on different rootstocks as followed:

1. Differentiation of connecting vascular tissues (Xylem and Phloem).

2. Successful grafting percentage.

1. Differentiation of connecting vascular tissues (Xylem and Phloem):

1.1. Xylem area:

The presented data in Table 4 showed that the largest xylem area was obtained from grafting Nubian watermelon onto luffa and bottle gourd (28.37 and 25.686 respectively) followed by those grafted onto fig leaf gourd (20.44) and pumpkin (20.11), while the lowest values were obtained from non-grafting plants (14.76). The differences were significant.

Table 3: Effect of grafting Nubian watermelon onto different rootstocks on some metabolic activities (oil, carbohydrate and protein) of seed kernels during 2009 & 2010 seasons.

Rootstocks	Oil %		Carbohydrate %		Protein mg/g d. w.	
	2009	2010	2009	2010	2010	2009
Control	54.58 a	56.20 a	7.15 c	7.17 c	0.37	0.38 c
Bottle gourd	52.99 c	53.13 c	7.37 ab	7.45 ab	0.36	0.37 c
Pumpkin	52.97 c	53.83 bc	7.50 a	7.59 a	0.38	0.42 b
Luffa	53.65 b	53.88 b	7.28 bc	7.33 bc	0.40	0.45 a
Fig leaf gourd	53.67 b	54.24 b	7.20 c	7.25 c	0.38	0.42 b
F. test	**	*	*	*	N.S	*

Values marked with the same alphabetical letter (s) are not significantly different, using Duncan's test at 0.05 levels.

1.2. Phloem area:

The data in Table 4 showed that the grafting Nubian watermelon significantly increased phloem area comparing with control (without grafting).

The largest phloem area was obtained from grafting Nubian watermelon onto bottle gourd, pumpkin and luffa, where there were not significantly different between them, which followed by those grafted onto fig leaf. The differences were significant.

The results may be due to that callus tissues in both scions and stocks were connected together (as shown in plate II) and began to differentiate into xylem and phloem elements (Hamed, 2005) on watermelon. It may refer to that grafting watermelon on stocks caused a special growth in vascular bundles of the stock laying near the scion; such bundles were characterized by the presence of internal cambium which provided the bundles with internal secondary tissues of phloem and xylem. All these tissues were formed on the side of the primary xylem opposite to the secondary one produced from the outer intra vascular cambium (Mounir, 1965).

2. Successful grafting percentage:

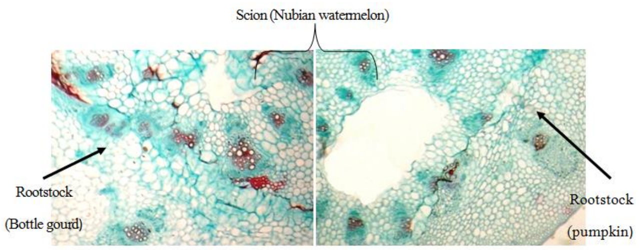
The results reported in Table 4 indicated clearly that the highest successful grafting percentage was obtained from grafting plants onto bottle gourd followed by those grafted onto fig leaf gourd, while the lowest values were obtained from grafting plants onto pumpkin rootstocks. The differences were significant in both seasons.

The results showed that the kind of the stock had a pronounced effect on the percentage of successful grafting. These results may be attributed to the fact that, the callus was rapidly formed in both tissues of the stock and scion and filled the space between them (Hamed, 2005). These results are in accordance with those reported by Bekhradi et al. (2009). The failure of grafting was mainly due to some physiological repulse between Nubian watermelon and stock, as it appeared through the formation of an impermeable layer of suberin, which in turn, obstructed the connection between tissues as shown in plate III.

Table 4: Effect of grafting Nubian watermelon onto different rootstocks on area of vascular tissues at joined region and successful grafting percentage.

Rootstocks	Area of vascular tissues (μ)		Successful grafting (%)	
	Xylem	Phloem	2009	2010
Control	14.76 c	8.21 c	-	-
Bottle gourd	25.69 a	18.33 a	97.73 a	96.50 a
Pumpkin	20.11 b	18.02 a	88.24 d	85.00 d
Luffa	28.37 a	18.44 a	90.00 c	89.00 c
Fig leaf gourd	20.44 b	14.31 b	93.00 b	94.50 b
F. test	**	**	**	**

Values marked with the same alphabetical letter (s) are not significantly different, using Duncan's test at 0.05 levels.



Nubian watermelon grafted onto bottle gourd Nubian watermelon grafted onto pumpkin.

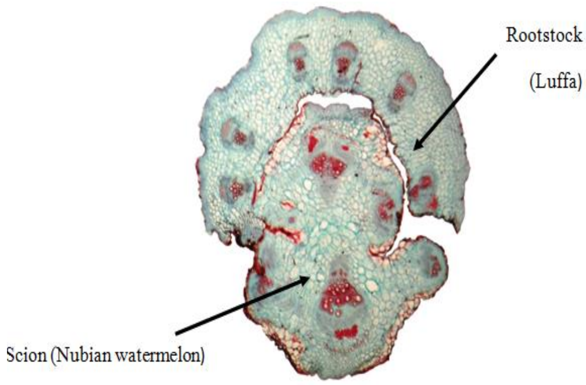


Plate III: Cross-sections of the grafting union after two weeks from grafting Nubian watermelon onto luffa (x1).

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الملخص العربى

تأثير التطعيم على خصائص التركيب التشريحى والفسيولوجى لنبات بطيخ اللب

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أجريت تجربتين على بطيخ اللب فى مزرعة محطة بحوث سخا بمحافظة كفر الشيخ خلال الموسمين الصيفيين ٢٠٠٩ و ٢٠١٠ م. وقد كان الهدف الرئيسى هوالمقارنة بين النباتات المطعمة على أربعة أصول مختلفة (اليقطين و القرع العسلى و اللوف و الفيسفوليا) و النباتات الغير المطعمة بهدف زيادة الإنتاج باستخدام تقنية التطعيم.

شملت الدراسة قياس التحليل الكيميائى للأوراق و التحليل الكيميائى للبذور و التركيب التشريحى لمنطقة التطعيم و نسبة نجاح التطعيم.

اوضح تطعيم نبات بطيخ اللب على مختلف الاصول زيادة محتوى الكلوروفيل خاصة التطعيم على الفيسفوليا مقارنة بالنباتات الغير مطعمة .

ادى تطعيم النباتات على اليقطين الى زيادة نسبة النيتروجين و الفسفور و البوتاسيوم و بينماحدث تناقص للقييم من تطعيم النباتات على الفيسفوليا. كانت هناك اختلافات معنوية كبيرة فى معظم الحالات.

تناقص محتوى البذرة من الزيوت فى كل الاصول المستخدمة فى التطعيم خاصا اليقطين و القرع العسلى. و قد سجل تطعيم النباتات اعلى محتوى للكربوهيدرات خاصة المطعمة على اليقطين و القرع العسلى. وجد ان المحتوى الكلى للبروتين قد ازداد بالتطعيم بالموسم الاول عدا التطعيم على اليقطين. لوحظ من التركيب التشريحى ان التطعيم على الاصول المختلفة خاصا اللوف و اليقطين ادى لزيادة ملحوظة لمساحة الخشب و اللحاء مقارنة بالنباتات الغير مطعمة. و قد وجد ان التطعيم على الفيسفوليا و اليقطين اظهراعلى نسبة نجاح مقارنة بباقى الاصول خلال الموسمين.