

Responses of Annona Trees to Self or Cross Grafting: II. Yield and quality

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ABSTRACT

Three years experiments were conducted in a private farm located in North Lebanon in order to investigate the responses of two *Annona* species namely *Annona squamosa* L. (Sq) and *Annona cherimola* Mill. (Ch) to self or cross grafting. Eighteen months old seedlings of both species were used in 2011 to be the rootstocks. Four different combinations of splice grafting were carried out as: Sq/Sq; Sq/Ch; Ch/Ch and Ch/Sq. During nursery, Ch/Sq grafted plants did not show any promising growth and it was excluded from the study at that stage. Grafted plants were transplanted in the field after one year from grafting. Flowering and fruit development and quality were monitored in order to assess the effect of grafting and rootstock species on such parameters. Data showed that cross grafted plants produced the highest fresh and dry weights compared to self grafted plants. Despite the fact that *Annona* trees usually do not yield before 4-5 years after planting, early flowering and fruiting could be recorded on some of the grafted trees. There were trends to have early flowering and fruit set in the Sq/Ch grafting and Ch/Ch higher than those recorded in Sq/Sq. Fruit analysis revealed that harvested fruits from Sq/Ch treatments had slightly lower fat content, higher dietary fibers and higher pulp percentage among all treatments. Other quality parameters such as sugar content were closer in Sq/Ch cultivars to the best known *Annona* variety *squamosa*.

Key words: *Annona squamosa* L., *Annona cherimola* Mill., grafting, yield, quality.

Introduction

Annona is one subtropical crop that has been successfully introduced in the last century to Lebanon being well adapted to Lebanese conditions (Chalak and Sabra, 2007). The limited local availability of *Annona* has resulted in a high dependency of the market on importation in order to meet the Lebanese demand. However, *Annona* is a promising crop that represents a potential for local and regional markets and it is expected to progressively increase its relative importance in the littoral zone for both local and export markets (Chalak and Sabra, 2007). Moreover, *Annona* production has a promising future since the demand for the fruit is showing a continuous increase locally, where it is considered as a high value product that forms an agreeable input to a healthy diet. Additionally, the fruit is used as a desert given its favorable taste highly appreciated by Lebanese consumers.

Several *Annona* species are widely cultivated worldwide for their edible fruits and their important nutritional value. Cherimoya, sugar apple, soursop (*Annona muricata*) and custard apple (*Annona reticulata*) are now the four widely distributed *Annona* species and can be found growing, cultivated and naturalized throughout the tropics (ICUC, 2002). *Annona* fruits are well-known for their content in vitamins A and C, minerals and carbohydrates; thus, they are considered an important part of a human diet with a good supply of these nutrients (Amoo *et al.*, 2008, ICUC, 2002). Other than their nutritional value, *Annona* fruits are valued for their very sweet creamy pulp (Pawar and Imran Hashmi, 2010). *Annona* fruits serve as sources of medicinal and industrial products. Thus, they are widely used in traditional medicine in Africa and recently some have been used for the production of modern medicines, for example in USA and India (SCUC, 2006). Despite these importance, *Annona* is still away of being a main fruit crop in Lebanon. Although the diversity of the different present agro-ecosystems in Lebanon which permits the cohabitation of subtropical crops, such as *Annona* (Chalak and Sabra, 2007), such crop is cultivated in scatter pattern there. This is mainly because of lack of research on the performance of *Annona* species under Lebanese environmental conditions. Also growers have no sufficient knowledge about propagation of *Annona*. Because of this weakness in their Know-how about the grafting of the various *Annona* species, the Lebanese farmers have some obstacles to select the best scion-rootstock combination suitable to the local climatic conditions. In addition, the lack of acquired information concerning the climatic requirements of the diverse *Annona* species creates some difficulties for the farmers to select the species the most adapted to the local conditions in order to obtain a better yield.

Therefore, this study was conducted in order to investigate the interacting effect of two species of *Annona* as a result of self-grafted or cross grafted plants.

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Material and Methods

For three years study 2011-2014, two species of Annona plants were used namely *Annona cherimola* Mill. and *Annona squamosa* L. The two species were used as rootstocks and/or scions. Self-grafting or cross grafting using the two species resulted in four different grafted plants (cultivars) as follows: The first cultivar (Ch/Ch) was obtained by grafting *Annona cherimola* Mill. scion onto *Annona cherimola* Mill. rootstock. The second cultivar (Sq/Sq) was *Annona squamosa* L. scion grafted onto *Annona squamosa* L. rootstock. The third cultivar (Sq/Ch) consists of *Annona squamosa* L. scion grafted onto *Annona cherimola* Mill. rootstock. Finally the fourth cultivar (Ch/Sq) was obtained by grafting *Annona cherimola* Mill. scion onto *Annona squamosa* L. rootstock. Grafting of the different combinations of annona species was carried out in 2011 using rootstocks of 18 months old. Annona plants were propagated using the splice grafting. The grafted plants were left to grow in the nursery for one year. To note that the fourth cultivar (Ch/Sq) did not show any promising growth performance, therefore it was excluded during the nursery stage.

At Bebleye, the plantation site had a surface area of 80 hectares, where the three different grafting combinations of annona species were transplanted in August 2011. Before planting, soil was deeply tilled to a depth of 0.80 – 1.0 m. Since the analysis of soil samples taken from the field revealed that it is a calcareous-clay soil, it was mixed with a “red” soil of sandy-loamy type. The “red” soil was added after digging holes of 1.0 m³ at the points of plantation of annona trees. The chemical analysis of orchard’s soil and the “red” one that was added is shown in table (1).

Plants were distributed over three plots; each plot represented one of the three scion-rootstock combinations of annona (Sq/Sq; Ch/Ch; Sq/Ch) and contained 50 annona plants planted at a distance of 4 m, while the distance between rows was 4.5 m. In order to achieve the purpose of the study, 30 annona trees of each scion-rootstock combination were randomly selected from each plot and constituted the sample of the experimental work.

Parameters measured on the aboveground parts of plants were fresh and dry weights of shoots, number of flowers, percentage of fruit set and total number of fruits.

In April 2012, 75 annona trees were chosen randomly inside each plot: 25 trees belonging to each of the three cultivars (25 trees/cultivars) and were used to count the number of flowers and fruits on each plant. Meanwhile, this parameter was measured in May 2013 on 90 trees. After assessing fruit number of the chosen trees, fruit set percentage could be calculated. The quality of annona fruits was evaluated by testing the pH, sugar content, protein content, fiber content, fat content, moisture content, vitamin A, vitamin C and pulp percentage.

Experimental design was complete block with three replicates. Statistical analysis was carried out using ANOVA to determine the least significant difference (LSD) at an experimental error $\alpha < 5\%$. In the case of a failed normality distribution of the data (parameters), Tukey or Dunnett’s tests were used.

Table 1: Soil physical and chemical analysis (LARI, 2013)

	Obtained values		Recommended values
	“Red” soil	“White” soil	
Sand (%)	76.96	24	-
Silt (%)	6.0	38	-
Clay (%)	17.04	38	-
Organic matter (%)	0.39	3.1	2,5
pH	8.01	7.6	-
Electrical conductivity (mS.cm ⁻¹)	0.135	0.27	0,4
Total CaCO ₃ , lime (%)	0.8	88	-
Active CaCO ₃ , lime (%)	-	18	-
Available Nitrogen (Kg/Ha)	6.98	81	70
Olsen Phosphorus (ppm)	27.7	17	15
Available Potassium (ppm)	75.26	110	400
Available Sodium (ppm)	-	170	100
Exchangeable Magnesium(ppm)	478	154	250
Exchangeable Calcium (ppm)	2800	5948	3500
Available Iron (ppm)	0.127	3.9	10

Results and Discussion

The total fresh and dry weights of shoots (table 2) showed significantly higher values recorded on Sq/Ch cultivar followed by Sq/Sq then Ch/Ch. Rootstocks have been reported to affect the dry matter accumulation within the vegetative growth of grafted trees (Caruso *et al.*, 1997) and this has been reflected

through this study. The effect of the rootstock on vegetative growth parameters such as number of leaves and leaf area has been observed in this study and mentioned in Sebaaly *et al.* (2015) and resulted in a positive interactive effect in Sq/Ch combination. The observed positive effect especially on those two parameters must have been reflected on the total photoassimilate production resulting in higher dry matter accumulation. Considering that cherimoya is characterized with a large-sized root system (primary and secondary roots, (Sebaaly *et al.*, 2015) which means larger capability to uptake water and that fresh weight is a sum of dry matter and water content, it can be expected that Sq/Ch cultivar will have higher fresh weight.

Table 2: Fresh and dry weights of shoots of seasons 1 and 2 (number of samples was 75 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

Cultivar	2011-2012		2012-2013	
	Fresh weight (g)		Dry weight (g)	
Ch/Ch	51.41 ± 24.26	51.44 ± 16.24 ^c	42.2 ± 2.01	26.62 ± 10.46 ^c
Sq/Sq	42.95 ± 6.82	88.96 ± 25.03 ^b	33.68 ± 4.43	48.11 ± 14.32 ^b
Sq/Ch	87.91 ± 3.14	337.62 ± 40.94 ^a	66.14 ± 3.71	173.12 ± 45.19 ^a

Within the same column, values with the same letters are not significantly different ($P < 0.05$)

The mean number of flowers is shown in table 3. In 2011-2012 season, the mean number of flowers of the cultivar (Sq/Ch) differed significantly from the other two cultivars of annona (Ch/Ch and Sq/Sq) and had the highest value in 2011-2012. A lower value was reached for the variety (Ch/Ch), and the lowest value was for the variety (Sq/Sq). Meanwhile in the second season (2012-2013) investigated plants developed a reduced number of flowers and the previous significant difference could not be found although there was a close trend to the first season results. While Pinto *et al.* (2005) reported more abundant flowers in sugar apple than cherimoya, this difference was not so evident regarding our results. Our findings could be explained by the fact that during the year 2011, the winter season was long and cold where rainfall was high; temperatures decreased to reach a minimum of 5.4°C in February. Since the sugar apple tree is tropical and is less tolerant to cold temperatures than the cherimoya, and being the most drought-tolerant annona species, it grows but produces poorly in high rainfall regimes (Nakasone and Paull, 1998). Consequently, it could be assumed that the cultivar Sq/Sq did not adapt well to the fluctuations of temperatures and rainfall at Bebleye, and thus, produced a higher number of leaves (Sebaaly *et al.*, 2015) but a lower number of flowers. Nevertheless, the results we reached clearly reflect that the grafting combination formed by “Squamosa” scion and the “Cherimoya” rootstock has enhanced the flowering capacity of the scion. In fact, cherimoya is more adapted to cold temperatures than the sugar apple and the use of cherimoya as a rootstock could have enhanced the adaptation of the sugar apple to colder temperatures and thus its flowering capacity (1.68 for Sq/Ch and 0.32 for Sq/Sq). Meanwhile, in 2012-2013 season, if ANOVA test had to be applied, it would indicate that only the mean number of flowers of the cultivar (Ch/Ch) is close to the mean number of the cultivar (Sq/Ch) and both are different from the mean number of flowers of the cultivar (Sq/Sq). Thus, it could be considered that grafting “sugar apple” scion onto “cherimoya” rootstock has the best flowering capacity compared to the cultivars (Ch/Ch) and (Sq/Sq). The results of the first season supported this hypothesis.

Table 3: Number of flowers in seasons 1 and 2 (number of samples was 75 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

Cultivar	2011-2012	2012-2013
	X ± O	
Ch/Ch	0.56 ± 0.58 ^b	2.13 ± 1.28
Sq/Sq	0.32 ± 0.48 ^b	1.67 ± 1.09
Sq/Ch	1.68 ± 1.03 ^a	3.00 ± 1.10

Within the same column, values followed by the same letters are not significantly different ($P < 0.05$)

Although annona plants start to bear fruits when 3 to 4 years old (Pinto *et al.*, 2005), some fruits have developed on a small number of trees at the field in 2012-2013 season. Therefore, the number of fruits was considered and evaluated (table 4).

Table 4. Number of fruits of season 2012-2013 (number of samples was 90 plants for 2012-2013 season)

Cultivar	X ± O
Ch/Ch	0.37 ± 0.56
Sq/Sq	1.1 ± 0.76
Sq/Ch	1.86 ± 1.09

There was no significant difference among the three grafted annona species regarding the mean number of fruits. However, based on the results, it was noticed that the mean number of flowers and fruits of the cultivar

(Sq/Ch) showed a tendency to be higher than those of the other two varieties. This difference was not so well reflected by the statistical test due to the high standard deviation values compared to the mean values. Thereby, the obtained results concerning the number of flowers and fruits could not lead to final assumptions regarding the effect of grafting on the flowering and fruiting capacity, since the annona trees were still only three years-old. Besides being young, the behavior of annona trees would have been affected by climatic conditions. During the year 2012-2013, the climate was characterized by slightly higher temperatures and lower rainfall amounts than the previous year. Since the sugar apple is more tolerant to high temperatures than the cherimoya, it showed a better fruit set (as average, 1.67 flowers of the cultivar (Sq/Sq) gave approximately 1 fruit, while an average of 2 flowers of the cultivar (Ch/Ch) did not give even 1 fruit). Also, the fruit set of the cultivar (Sq/Sq) was almost similar to the one of the cultivar (Sq/Ch) (3 flowers of the cultivar Sq/Ch yielded about 2 fruits or even less). In other terms, if the percentage of fruit set was to be considered, the three annona cultivars (Ch/Ch), (Sq/Sq) and (Sq/Ch) would have reached respectively the following values: 17.37%; 65.86% and 62%. So, the two cultivars (Sq/Sq) and (Sq/Ch) resulted approximately in a close percentage of fruit set with the highest one for self-grafted “sugar apple” (Sq/Sq). However, the self-grafted “cherimoya” (Ch/Ch) had a very lower fruit set than the other two cultivars.

Fruit quality

Several parameters were tested in order to evaluate the quality of fruits produced by the three different annona cultivars (table 5). Fruits of the cultivar (Ch/Ch) are slightly more acidic than those of the two varieties (Sq/Sq) and (Sq/Ch).

Since the “sugar apple” is considered the sweetest of the annona fruits (Pareek *et al.*, 2011), it was evident that fruits produced by the cultivar (Sq/Sq) had the highest percentage of sugars among the three others (Ch/Ch: 7.1%; Sq/Sq: 13.3% and Sq/Ch: 10.7%). The cultivar (Sq/Ch) gave fruits with higher sugar content than those of the variety (Ch/Ch).

Moreover, the “cherimoya” fruits had the highest protein percentage among the commercially important annonas (Pinto *et al.*, 2005). Our results confirmed what was stated, since fruits of the cultivar (Ch/Ch) had a higher protein content compared to the two cultivars (Sq/Sq) and (Sq/Ch). Furthermore, the grafting combination between the “sugar apple” scion and the “cherimoya” rootstock has resulted in the production of fruits with a higher percentage of dietary fibers compared to those produced by the self-grafted cultivars (Ch/Ch) and (Sq/Sq). The “cherimoya” fruit was richer in fibers than the “sugar apple” fruit, thus, grafting “sugar apple” scion onto the ‘cherimoya’ rootstock has increased as well the fiber content in annona fruits.

In addition, fruits of (Sq/Ch) and (Sq/Sq) had the same fat content, which was lower than the one of the (Ch/Ch). Also, fruits belonging to the three annona cultivars did not present any clear difference in their vitamin A content, and fruits of (Sq/Sq) were the richest in vitamin C among the three types of fruits. Furthermore, fruits of (Sq/Ch) represented the higher pulp percentage compared to those of (Ch/Ch) and (Sq/Sq), which had approximately a very similar percentage. Moreover, the highest moisture content was recorded for “cherimoya” fruits, followed by the cultivar (Sq/Ch), and the lowest value was obtained for the “sugar apple” fruits (Sq/Sq). Thereby, grafting “sugar apple” scion onto “cherimoya” rootstock has resulted in the production of fruits with chemical and physical characteristics closer to those of “sugar apple” fruits more than “cherimoya” fruits. It could be considered as well that this grafting has improved the quality of annona fruits, in terms of dietary fibers content, water content and pulp percentage.

Table 5. Fruit quality

Parameters	Cultivar		
	Ch/Ch	Sq/Sq	Sq/Ch
pH	4.5	5.5	5.5
Total sugars (%)	7.1	13.3	10.7
Proteins (%)	2.3	1.6	1.3
Total dietary fibers (%)	3.9	3.5	4
Fat content (%)	0.2	0.1	0.1
Vitamin A (mg) (in 100 mg)	<0.1	<0.1	<0.1
Vitamin C (mg) (in 100 mg)	10.7	13.5	8.3
Pulp percentage (%)	59.8	60.2	63.3
Moisture content (%)	80.3	73.2	76.6

Conclusion

Grafting “sugar apple” scion on “cherimoya” rootstock has improved the growth of both scion and rootstock resulting in a better overall growth and a tendency for better flowering and fruit set (although it is only an indicating result and further yield monitoring must be done). Moreover, fruit quality of that combination of grafts has improved in terms of pulp percentage, water content and dietary fibers while keeping other important quality characters close to the best known variety squamosa.

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