

Responses of Annona Trees to Self or Cross Grafting: I. Vegetative growth

¹Zeina Sebaaly, ¹Salim Kattar, ¹Youssef Sassine and ²Linda Kfoury

¹Dept. Horticulture, Faculty of Agriculture and Veterinary, Lebanese University, Beirut, Lebanon

² Dept. Plant protection, Faculty of Agriculture and Veterinary, Lebanese University, Beirut, Lebanon

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. Grafted plants were transplanted in the field after one year from grafting. Different growth parameters were recorded such as number of leaves, number of lateral shoots, leaf area, fresh and dry weights of the leaves, length and diameter of the trunk as well as length and number of main and secondary roots of the rootstocks. Data showed that during nursery period, the graft combination of Ch/Sq did not show any promising growth and therefore was excluded from the study at that stage. Other graft combinations showed clear different growth responses with superiority in all measured parameters observed in Sq/Ch followed by Ch/Ch. All differences were significant and indicating to a clear interacting positive effect between the scion of *A. Squamosa* and *A. Cherimola* rootstock.

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

Introduction

Annona is a subtropical crop which is economically important in many countries of Africa and Asia as well as in South, North and Central America (SCUC, 2006). 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 human diet with a good supply of these nutrients (ICUC, 2002, Amoo *et al.*, 2008). 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 this 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 grafting of various *annona* species, the Lebanese farmers have some difficulties in selecting the scion-rootstock combination that is the most suitable to local climatic conditions.

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.

Materials 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. Splice grafting of the different combinations of *annona* species was carried out in 2011 using rootstocks of 18 months old and grafted plants were left to grow in the nursery. To note that the fourth cultivar

Corresponding Author: Zeina Sebaaly, Dept. Horticulture, Faculty of Agriculture and Veterinary, Lebanese University, Beirut, Lebanon.

(Ch/Sq) did not show any promising growth performance, therefore this cultivar was excluded during the nursery stage.

At Bebleye, the plantation site had a surface area of 80 hectares, where the three different graft 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 one, it was mixed with a “red” soil of sandy-loamy type. The “red” soil was added after digging holes of 1 m³ at the points where trees were planted. Results of chemical analysis of the orchards’ soil as well as the “red” soil that was added are shown in table 1 below.

Plants were distributed over three plots; each plot represented one of the three graft combinations (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 reach the goals of the research 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 the number of leaves, leaf area, fresh and dry weights of leaves, chemical composition of leaves, number of lateral shoots, internodes length, length and diameter of the trunk and fresh and dry weights of shoots. Leaf number was counted on 75 annona trees by the end of the season in 2011-2012 and on 90 trees over a period of 6 months (April - September) in the season 2012-2013. Ten plants of each variety of annona were chosen randomly. On each plant 30 leaves were measured to calculate leaf area in 2011-2012 season. Afterwards, leaf area was evaluated on a sample of 90 trees (30 trees of each cultivar), in April in 2012-2013 season by randomly choosing three lateral shoots on each plant, where the total number of leaves present on the upper and the lower side of shoots was considered in order to measure the related length and width. Consequently, leaf area was obtained by multiplying the length and the width of leaves. In September 2013, the sample of 90 annona plants, was removed from the field and moved to the laboratory where measurements of fresh and dry weights of leaves were carried out. The root system of plants was carefully washed to remove adherent soil particles then it was subjected to following measurements: number of primary and secondary roots, length and diameter of primary roots and fresh and dry weights of roots.

Chemical analysis took place on dry leaves to determine the total content of N, P, K, Ca, Mg, Fe, Zn, Cu and Mn.

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

Climate

In 2011, the lowest temperatures in Bebleye were recorded during the months of January and February while the highest ones were during the months of July and August. The mean minimum temperatures in the area ranged between 5.4°C and 23.8°C, while the mean maximum temperatures were between 14.4°C and 32.9°C. Meanwhile the annual average minimum and maximum relative humidity recorded 43.2 and 96.8. The annual

average precipitations reached 67.6 mm with the maximum amount recorded in January- February 2012. In the season 2012-2013, the mean minimum temperatures in this region ranged between 10.1°C in March and 26.9 °C in August, while the mean maximum temperatures were between 18.7°C in January and 33.4°C in August. Therefore, temperatures recorded during 2012-2013 were higher than those recorded during 2011-2012, therefore during the year 2012-2013, the climate was warmer than the previous year.

Plant growth

Total leaf numbers in the season of (2011-2012) and during the period of six months in (2012-2013) (table 2) were significantly different among the three different grafting combinations of annona. One exception was only observed during the month of April, where “cherimoya” scion grafted onto “cherimoya” rootstock and “sugar apple” scion grafted onto “sugar apple” rootstock did not present any statistically significant difference regarding the leaf number, however, they differed from the cultivar (Sq/Ch). The combination of both species (cultivar Sq/Ch) had the highest mean leaf number in the first season and during the six months of the second season, and the lowest value was observed for the self-grafted “sugar apple” (cultivar Sq/Sq). In general, the total number of leaves produced on a graft varies among different scion-rootstock combinations showing the effect of the interaction between the rootstock and the scion on leaf production (Heekenda *et al.*, 2009). Since cherimoya tree is more vigorous than sugar apple tree (Orwa *et al.*, 2009) and the scion growth is determined by the rootstock due to the significant interaction between scion and rootstock (Heekenda *et al.*, 2009), our results reflected the positive influence of the “cherimoya” rootstock on the growth of the “sugar apple” scion.

Table 2. Total Number of leaves of seasons 2011-2012 and 2012-2013 (number of samples were 9 and 90 plants for 2011-2012 and 2012-2013 seasons respectively)

Cultivar	2011-2012	2012-2013					
		April	May	Jun	Jul	Aug	Sep
Ch/Ch	23.32 ± 7.4 ^c	65.7±12.93 ^b	82.33±11.85 ^b	98.4±8.05 ^b	118.26±8.72 ^b	165.7±22.78 ^b	215.23±23.99 ^b
Sq/Sq	42.00 ± 7.2 ^b	54.6±8.85 ^b	67.00±9.3 ^c	80.03±8.36 ^c	93.97±7.65 ^c	135.6±14.57 ^c	182.63±23.84 ^c
Sq/Ch	76.28 ± 19.1 ^a	93.9±8.67 ^a	107.1±10.09 ^a	125.1±10.47 ^a	153.13±15.08 ^a	210.2±14.68 ^a	302.27±33.89 ^a

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

The mean leaf area (table 3) was significantly different among the three annona cultivars. According to Orwa *et al.* (2009) and Nakasone and Paull (1998), leaves of *Annona squamosa* L. are usually smaller in length and width than those of *Annona cherimola* Mill., therefore, the leaf area is smaller as well. Our results confirmed what was stated, since grafting “sugar apple” scion onto “sugar apple” rootstock has resulted in the development of leaves having a smaller leaf area as compared to those obtained by grafting “cherimoya” scion onto “cherimoya” rootstock. However, during the season of 2011-2012, the mean leaf area of the cultivar Sq/Ch was bigger than the mean leaf area of the variety Sq/Sq but it was smaller than the one of the variety Ch/Ch. This may be due to the smaller number of the considered leaf sample (only 30 leaves per cultivar). On the other hand, in the season of 2012-2013, the mean leaf area of the cultivar (Sq/Ch) was significantly higher than the one of the self-grafted cultivars (Ch/Ch) and (Sq/Sq). The effect of the cherimoya rootstock in annona trees belonging to the combination (Sq/Ch) was therefore sensed through the higher leaf area of assessed plants.

Table 3: Calculated leaf area (cm²) of seasons 1 and 2 (number of samples was 30 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

Cultivar	2011-2012	2012-2013
	X ±O	X ±O
Ch/Ch	56.8±10.92 ^a	97.59 ± 26.27 ^b
Sq/Sq	49.66±8.07 ^c	63.92 ± 29.79 ^c
Sq/Ch	52.35±10.47 ^b	103.67 ± 47.19 ^a

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

There was a significant difference in fresh and dry weights of leaves among the different grafting combinations of annona (table 4). The highest value of mean fresh weight was recorded for the cultivar (Sq/Ch). The two self-grafted "cultivar" (Ch/Ch) and (Sq/Sq) reached almost similar mean values of fresh weight, which were lower than the value of the cultivar (Sq/Ch).

On the other hand, the mean dry weight of leaves of the cultivar (Sq/Ch) had also the highest value among the three cultivars. A lower value was reached for the cultivar (Sq/Sq) and the lowest value was for the cultivar (Ch/Ch). Since the accumulation of dry matter in leaves was higher when both annona cultivars (cherimoya and sugar apple) were combined than when the two cultivars were self-grafted, consequently, the

interaction between the “sugar apple” scion and the “cherimoya” rootstock has promoted the adaptation of the annona trees to the local climate and resulted in a greater accumulation of dry matter in plant leaves.

Table 4. Fresh and dry weights of annona leaves (g) of season 2012-2013 (number of samples was 90 plants)

Cultivar	Fresh weight	Dry weight
	X ± O	
Ch/Ch	13.39 ± 6.28 ^b	5.85 ± 2.33 ^c
Sq/Sq	25.93 ± 9.8 ^b	12.33 ± 5.23 ^b
Sq/Ch	130.89 ± 25.62 ^a	46.22 ± 9.01 ^a

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

Furthermore, the combination between “sugar apple” and “cherimoya” has helped the “sugar apple” scion to develop leaves that are richer in some nutritive elements (P, K, Fe, Cu and Mn) compared to when it was grafted on its own rootstock. Therefore, grafting “sugar apple” scion onto “cherimoya” rootstock has influenced the capacity of the scion to assimilate nutrients from soil, thus, to form leaves with a richer chemical composition (table 5a&b).

Table 5a: Chemical composition of annona leaves of season 2011-2012 (number of samples was 9 plants)

Cultivar	Macro-elements (%)			Microelements (ppm)					
	N	P	K	Fe	Cu	Mn	Zn	Ca	Mg
Ch/Ch	2.85	0.23	1.53	83	2.5	19.135	14.965	2.65	0.2
Sq/Sq	1.48	0.26	1.575	152.45	1.87	26.75	28.39	1.78	0.125
Sq/Ch	2.38	0.17	1.035	149.75	9.6	26.3	20.38	1.51	0.185

Table 5b. Chemical composition of annona leaves of season 2012-2013 (number of samples was 90 plants)

Cultivar	Macro-elements (%)			Micro-elements (ppm)					
	N	P	K	Fe	Cu	Mn	Zn	Ca	Mg
Ch/Ch	3.68	0.3	1.59	303.5	11.4	18.2	44.74	27.3	3.1
Sq/Sq	2.28	0.21	1.05	177	8	12	45.7	29	4
Sq/Ch	2.23	0.25	1.28	191	33	29	27	23.4	2.3

The mean number of lateral shoots (table 6), length of internodes (table 7) and length of trunk (table 8) of the cultivar (Ch/Ch) and the one of the combination (Sq/Ch) were significantly higher than the mean number of those of the cultivar (Sq/Sq). Since rootstocks markedly affect scion shoots growth and number, which is an important indicator of vigor differences among rootstocks (Weibel *et al.*, 2003), therefore, the use of the “cherimoya” rootstock was successful in terms of enhancing the vigor and the vegetative growth of the “sugar apple” tree by increasing the number of its lateral shoots. The sugar apple tree is smaller in height than the cherimoya tree (Nakasone and Paull, 1998). Thereby, grafting “sugar apple” scion onto “cherimoya” rootstock has enhanced the vegetative growth of the scion and resulted in the development of annona trees that are longer than those of self-grafted cultivars of “cherimoya” and “sugar apple”.

Table 6. Number of lateral shoots of season 2012-2013 (number of samples was 90 plants)

Cultivar	X ± O
Ch/Ch	10.87 ± 2.08 ^a
Sq/Sq	7.9 ± 1.65 ^b
Sq/Ch	12.03 ± 1.40 ^a

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

Table 7: Internodes length (cm) for seasons 2011-2012 and 2012-2013 (number of samples were 30 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

Cultivar	2011-2012	2012-2013
	X ± O	
Ch/Ch	2.13 ± 0.92 ^b	3.58 ± 1.12 ^a
Sq/Sq	1.57 ± 0.3 ^c	2.87 ± 1.26 ^b
Sq/Ch	2.72 ± 1.04 ^a	3.54 ± 1.12 ^a

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

Table 8: Length of the trunk (cm) for seasons 2011-2012 and 2012-2013 (number of samples were 30 and 90 plants for 2011-2012 and 2012-2013 seasons respectively)

	2011-2012	2012-2013
Cultivar	X ± O	X ± O
Ch/Ch	90.72 ± 13.55 ^a	158.83 ± 29.01 ^a
Sq/Sq	26.76 ± 6.3 ^b	115.98 ± 25.71 ^b
Sq/Ch	83.88 ± 9.53 ^a	167.54 ± 22.79 ^a

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

The trunk diameter (table 9) showed also a superiority of the cultivar Sq/Ch compared to the other two cultivars. The differences were significant and the lowest value was recorded with self grafted cultivar Ch/Ch. According to Weibel *et al.* (2003), a reduction in the trunk cross-section area growth is the result of a certain decrease in water status caused by high temperatures. Moreover, *Annona cherimola* Mill. is less tolerable to high temperatures than *Annona squamosa* L. (Nakasone and Paull, 1998; Pinto *et al.*, 2005). However, since the cultivar (Ch/Ch) developed a longer but thinner trunk than the cultivar (Sq/Sq), the performance of the tree could be explained by the fact that during the year 2012-2013, cherimoya trees were slightly negatively affected by climate which was in general warmer than the climate of the previous year, which resulted in a trunk diameter lower than what was expected. However, this negative effect of high temperatures on the trunk growth of “cherimoya” (Ch/Ch) was compensated by the availability of water provided through irrigation during the growing season, therefore, the trunk increased in length but did not significantly developed in width.

Table 9. Diameter of the trunk (cm) of season 2012-2013 (number of samples was 90 plants)

Cultivar	X ± O
Ch/Ch	1.31 ± 0.27 ^c
Sq/Sq	1.74 ± 0.27 ^b
Sq/Ch	2.43 ± 0.204 ^a

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

The number of primary roots (table 10) did not show significant difference among the treatments although there was a tendency for self grafted cultivar of Sq/Sq to have higher number of primary roots. Differences were significant in terms of length (table 11) and diameter (table 12) of primary roots where cross grafted cultivar Sq/Ch showed the longest primary roots followed by self grafted cultivar of Sq/Sq. Number of secondary roots (table 13) showed also superiority in cultivar of Sq/Ch compared to the other two cultivars. Heekenda *et al.* (2009) affirmed that scion cultivar grafted on its own rootstock is not the best combination. In fact, the self-grafted cultivar have developed a lower root biomass compared to the one obtained by crossing cultivar.

Table 10: Number of primary roots of seasons 2011-2012 and 2012-2013 (number of samples was 9 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

	2011-2012	2012-2013
Cultivar	X ± O	X ± O
A/A	19.67 ± 3.51	36.37 ± 11.35
B/B	22.67 ± 4.5	42.62 ± 19.93
B/A	20.00 ± 5.2	32.97 ± 12.19

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

Table 11: Length of primary roots (cm) of seasons 2011-2012 and 2012-2013 (number of samples was 9 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

	2011-2012	2012-2013
Cultivar	X ± O	X ± O
Ch/Ch	19.95 ± 13.3 ^a	12.38 ± 9.52 ^c
Sq/Sq	10.17 ± 9.22 ^b	13.51 ± 11.42 ^b
Sq/Ch	19.7 ± 13.55 ^a	17.82 ± 13.24 ^a

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

Table 12: Diameter of primary roots (mm) of seasons 2011-2012 and 2012-2013 (number of samples was 9 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

	2011-2012	2012-2013
Cultivar	X ± O	X ± O
Ch/Ch	1.31 ± 1.42 ^b	1.63 ± 1.69 ^c
Sq/Sq	1.34 ± 1.28 ^b	1.73 ± 1.65 ^b
Sq/Ch	2.41 ± 2.11 ^a	3.17 ± 2.95 ^a

Within the same column, values followed by different letters are significantly different ($P < 0.05$)

Table 13: Number of secondary roots of seasons 2011-2012 and 2012-2013 (number of samples was 9 and 90 plants for 2011-2012 and 2012-2013 seasons respectively).

	2011-2012	2012-2013
Cultivar	X ± O	X ± O
Ch/Ch	11.10 ± 7.75 ^a	9.857 ± 9.39 ^b
Sq/Sq	6.56 ± 4.81 ^b	7.88 ± 8.38 ^c
Sq/Ch	10.75 ± 9.51 ^a	12.21 ± 9.84 ^a

Within the same column, values followed by different letters are significantly different ($P < 0.05$).

Total fresh and dry weight (table 14) of roots showed the same tendency observed for all parameters where the cultivar Sq/Ch showed the highest values of fresh and dry weight of roots as compared to the other two cultivars. Since, the “sugar apple” tree is the most drought tolerant (Nakasone and Paull, 1998), it was evident that the cultivar (Sq/Sq) showed a better adaptation to the surrounding climatic conditions, thus, a higher accumulation of dry matter than the cultivar (Ch/Ch).

Since the grafting combination (Sq/Ch) has resulted in the development of leaves, shoots and roots with a greater dry matter accumulation, it could be assumed that grafting “sugar apple” scion onto “cherimoya” rootstock has increased the resistance of the “sugar apple” scion to water deficiencies, and has led to a better adaptation of trees to local climatic conditions.

Table 14: Fresh and dry weights of roots (g) of season 2012-2013 (number of samples was 90 plants for 2012-2013 seasons respectively).

Cultivar	X ± O	X ± O
Ch/Ch	42.39 ± 14.34 ^b	22.32 ± 8.77 ^c
Sq/Sq	63.02 ± 27.94 ^b	35.14 ± 16.07 ^b
Sq/Ch	178.48 ± 39.78 ^a	93.07 ± 26.45 ^a

Conclusion

It can be concluded that choosing the right scion and rootstock combination can result in better growth because of their positive interaction which reflects on different growth aspects. Grafting sugar apple scion onto Cherimoya rootstock gave the best results compared to the self grafted two species and improved the adaptation of the annona trees to the Lebanese environmental conditions.

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