Optimization of the *in vitro* propagation methodology of selected clones of soursop (*Annona muricata* L.) and evaluation of the compatibility of different scion and rootstock combinations for *in vitro* micrografting

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Background:

Between 1996 and 2001, we developed a methodology for the *in vitro* clonal propagation of elite selections of soursop (or guanábano in Spanish, *Annona muricata* L; Royero *et al.*, 1998), a fruit tree that is native to tropical America. The developed methodology allows a rapid clonal multiplication of selected trees and the production of disease-free plants. The plants produced through *in vitro* micrografting have been found to develop normal and healthy growing trees that produce the first fruits 15 months after planting (MAP) in the field.

The developed micrografting technology fulfills all the requirements for ridding planting materials of diseases. However before it can be applied for large-scale propagation, some critical steps in the propagation process have to be optimized.

In 2002 several steps involved in the propagation were reevaluated and media modifications were tested. Furthermore, the compatibility of scions from different selected clones of soursop micrografted over different rootstocks from the same and/or other related annonaceus species were studied at the laboratory and greenhouse levels.

Methodology:

The general methodology for *in vitro* propagation of soursop through micrografting has been described in previous reports (Royero *et al.* 1998 and 1999).The composition of the media and solutions used in the whole process is presented in the tables 1 and 2.

Table 1. Composition of the culture media previously used and of the new improved media (in bold) for the propagation process of soursop through micrografting

Use	Medium denomination	Salts	Sucrose	B5 Vitamins	Casein-Hydrol.	BAP	GA_3	PVP	Agar[1]
		g/L	g/L	g/L	g/L	mg/L	mg/L	g/L	g/L
Culture of shoot pieces for axillary bud induction	M-I	WPM[2]	20			1			4.8
	RO-BAP 1	MS[3] 1/2	20	0.112	0.2	1			4.2
Culture of shoot pieces for axillary bud elongation	M-II	WPM	20			0.2			4.8
	RO-BAP 0.2	MS 1/2	20	0.112	0.2	0.2			4.2
Seed germination	M-III	B5[4]	20				0.5		4.8
	RO 1/2 GA3	MS 1/4	10	0.056	0.2		1		4.2
Micrograft culture and development	M-IV	WPM	20	0.112					4.8
	RO 1/2	MS 1/4	10	0.056					4.2
Micrografting solution	M-V	WPM	20					1	
	T3	WPM	20		0.2	0.2		1	

1 From Duchefa, Netherlands
2 Lloyd y McCown 1981, purchased from Duchefa, Netherlands
3 Murashige and Skoog 1962, purchased from Duchefa, Netherlands
4 Gamborg et al. 1968, purchased from Duchefa, Netherlands

Table 2 . Composition of the different antioxidant treatments evaluated during micrografting of combinations of scions of the clone Rosa with rootstocks of Rosa, *A. montana* and *A. glabra*.

Treatment	Basal medium	Antioxidant (g/l)	Additional compounds	Growth regulator mg/l
T0	Half concentrated MS[2] Salts	PVP[3] 1	-	-
T1	WPM[1] Salts	PVP 1	Sucrose 2%	-
T2	WPM Salts	PVP 1	Casein hydrolysate 200 mg/l Sucrose 2%	-
T3	WPM Salts	PVP 1	Casein hydrolysate 200 mg/l Sucrose 2%	BAP 0.2

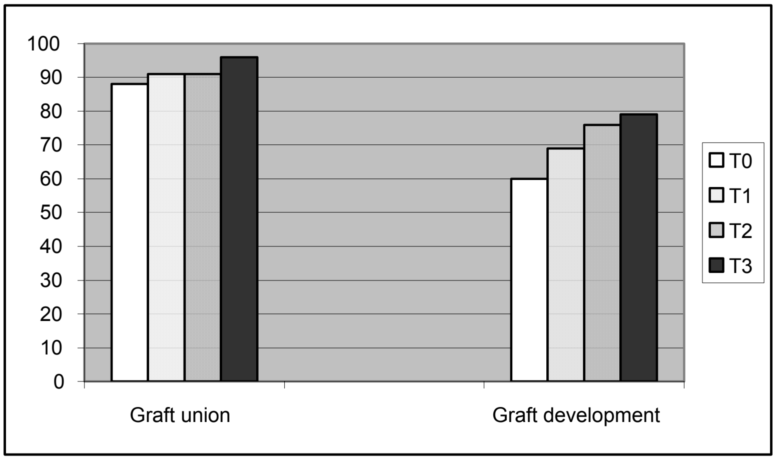
1 Lloyd and McCown, 1981
2 Murashige and Skoog, 1962
PVP = Polyvinylpyrrolidone

Results:

Evaluation of different antioxidant solutions used during the process of *in vitro* micrografting

Cut explants of woody species cultivated *in vitro*, are characterized by the exudation of phenolic compounds that oxidize in contact with the air, forming a dark precipitation. This phenomenon is known as phenolization or oxidation and when it is severe, can cause the explants to die. Soursop as a woody tree is not an exception, and tissue phenolization greatly affect the success of micrografting. We have in the past years tested the effect of different antioxidants in reducing phenolization during the micrografting process, and found polyvinylpyrrolidone (PVP) as being the best. In 2002 we investigated the effect of different modifications to the micrografting solution used on the efficiency of graft union, and in supporting growth of the micrografted scion. The results are shown in fig. 1. Compared to the antioxidant solution used before, rates of graft union and development of the scion after micrografting were improved by using micrografting solutions that in addition to the antioxidant were supplemented with WPM salts, sucrose and casein hydrolysate. But the best results regarding the scion development were obtained when the antioxidant solutions were supplemented additionally with the growth regulator BAP. This growth regulator or the combination of it with the other components of the solution also promoted a fast growth of the scion allowing the plantlets to be transferred to the greenhouse in less than 6 weeks after micrografting.

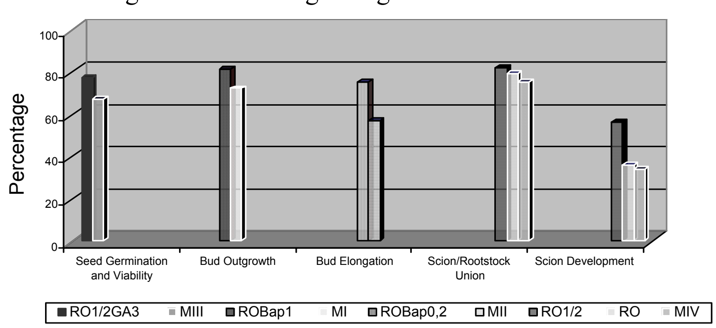
Figure 1. Evaluation of the effect of different antioxidant-solutions used during micrografting, on the graft union and on the development of the micrografted bud. Buds of the clone Rosa were micrografted on rootstocks of Rosa, A. montana and A. glabra. The composition of the antioxidant solution is explained on table 2.



Evaluation of the culture media used

The *in vitro* phase of the propagation methodology of soursop through micrografting consists of many step: (1) seed germination *in vitro* (for rootstock production); (2) induction of growth of axillary buds, (3) elongation of axillary buds (for scion production), (4) culture of micrografts and (5) culture of mother micrografts (*in vitro* micrografts used as source of buds for the production of new micrografts). Each of these steps required a different culture medium of a different composition (Table 1). In 2002 these culture media were revised and their preparation simplified by using only one source of a ready to use salt mixture as the basal medium. The newly developed media can be prepared easier and faster. Also by using them an improvement on the success rate of every step involved in the propagation process was achieved (Fig. 2) , allowing the performance of the propagation with more efficiency than before. The most important modification of the culture media is the use of the MS-salt mixture in half or one-quarter concentration, instead of the complete WPM salts.

Fig.2 Comparison of the overall success rate achieved in the different steps of the propagation of soursop through *in vitro* micrografting with the old and the new culture media.

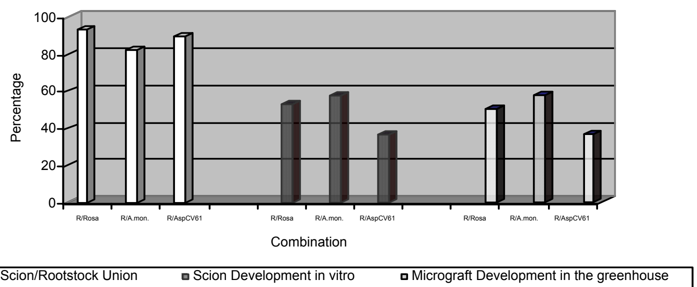


Use of rootstocks of other *Annona* species for the production of more vigorous, disease resistant and widely adapted soursop trees

The use of rootstocks of different genotypes or species from that of the scion for the production of vigorous, widely adapted or disease resistant plants is a common practice in fruit tree propagation. In soursop this avenue has been largely under-exploited.

We are investigating both at the laboratory and greenhouse levels, the compatibility of scions of different selected clones of soursop and different rootstocks of the same species and of the related species *A. montana* and *A. glabra*. The results of the micrografting experiments of rootstocks of these species with scions of the clone Rosa are presented in the figure 3. Regarding micrograft union (measured 15 days after micrografting) no significant differences were found between the different rootstocks. However regarding the development *in vitro* and in the greenhouse of the micrografted buds, significant differences were found. Surprisingly, it was the combination Rosa/A. montana, and not the micrografts of the scion of Rosa over its own rootstocks that was the combination that has shown the highest frequency of development. This combination is also the one that shows the highest growth rate in the greenhouse (data not shown).

Fig.3 Percentage of union and development *in vitro* and in the greenhouse of scions from the clone Rosa of soursop micrografted over rootstocks of Rosa, *A. montana*, and *A. glabra*.



Total number of plants produced from different combinations of scion and rootstocks

In 2002, a total of 1871 micrografted plants were produced from different combinations of scion and rootstocks (Table 3). All of these will be planted in the field for further evaluation of their agronomic performance under different agroecological conditions.

Conclusions:

With the newly evaluated media and solutions, an improvement on the success rate of every step of the propagation process was achieved

The survival of the micrografted plants in the greenhouse depends largely on the development achieved by them in the *in vitro* phase. With the combination Rosa/A. montana V54 the highest survival rate so far in the greenhouse, 68.4%, has been achieved.

The *in vitro* micrografting propagation methodology could be applied to 3 different soursop clones (all the clones evaluated) and 5 different rootstocks from the same and other annonaceus species (also all the rootstocks evaluated).

Efficiency of development of micrografted plantlets *in vitro* should be improved in order to apply the technology for massive propagation of soursop clones.

Table 3. Overall number of micrografts made, and micrograft development efficiency with different combinations of soursop scions and rootstocks of soursop or related annonaceus species between June

2001 and May 2002. Cristina, Elita, Rosa and Francia are selected clones of soursop (*Annona muricata* L.).

Scion/Rootstock Combination	Total of micrografts made	Micrografts showing graft union	Micrografts showing bud development	% of micrograft development
Rosa/*A. montana* V54	38	29	26	68.4
Elita/Rosa	260	207	157	60.4
Rosa/Rosa	289	268	174	60.2
Rosa/Elita	911	835	542	59.5
Cristina/Rosa	310	245	182	58.7
Francia/Cristina	178	150	99	55.6
Rosa/*A. glabra* CV61	282	258	154	54.6
Rosa/Cristina	100	90	54	54.0
Elita/Cristina	512	434	264	51.6
Cristina/Elita	273	234	136	49.8
Rosa/*A. montana* V51	124	93	55	44.3
Cristina/Cristina	100	39	28	28.0
TOTAL	**3377**	**2882**	**1871**	**55.4**

Future plans:

- To improve the methodology of production of rootstocks *in vitro*, which is the most limiting step in the process of scaling up of the propagation methodology.
- To include more elite selected clones and more rootstocks in the propagation and evaluation process.

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