MORPHOLOGICAL AND ANATOMICAL ASPECTS OF A CLEFT MICROGRAFTING OF GRAPE EXPLANTS IN VITRO

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Abstract

In vitro adaptation of the traditional field cleft grafting of grape plants was studied.

Apexes (measuring 0.3 mm) of the 110 Richter, 161-49 Couderc and 41B Millardet rootstocks and of the Palomino fino, Pedro Ximénez and Zalema cultivars were cultured in vitro. The rootstock plants obtained in vitro were divided into 3-4 explants without buds, and their tops were cut with a scalpel, producing a small cleft where an explant with buds from the cultivars was inserted. The set (graft) was again cultured in vitro.

Evolution of the graft-junction was followed by observation of correlative samples in optical and electron microscopes, finding that healing of the callus occurred during the first six days and the vascular junction between the 8th and 12th after grafting.

Most of grafted plants (60%) survived and a good multiplication factor was produced by the possibility of obtaining 3-4 explants per plant.

1. Introduction

By law, to avoid Phylloxera infections, grape cultivars must be grafted on tolerant rootstocks. Graftings normally used in fields present no especial troubles except sometimes they transmit virus infections.

Grape shoot-tip grafting in vitro (Bass et al., 1976, Ayuso and Peña-Iglesias 1978, 1980; Englebrech and Schewedtfeger, 1979) is used as a system to control virus proliferation. As alternative we present an in vitro adaptation of the in field cleft grafting considering also the tissues evolution in the junction zone between scion and rootstock.

2. Material and methods

Apexes (measuring 0.3 mm) of the 110 Richter, 161-49 Couderc and 41B Millardet rootstocks and of the Palomino fino, Pedro Ximénez and Zalema cultivars were cultured in vitro. The rootstock plants obtained in vitro, were divided into 3-4 explants without buds (internode) and their tops

were cut with a scalpel, producing a small longitudinal cleft. In the cleft, a small scion with bud from the in vitro cultivars was inserted (figure 1). The set was again cultured in vitro. A total of 342 graftings were made.

The plant material was previously sterilized with ethanol 70% (1 min) and sodium hypochloride 30% (6% of active chloride, 10 min), and washed with sterilized water. The nutritive medium was the previously used by Troncoso et al. (1990) (table 1) and the incubation conditions were 25°C of temperature, $30 \mu\text{E m}^{-2} \text{s}^{-1}$ of light intensity and 16h of light photoperiod.

The evolution of the in vitro graftings was followed by direct observation, without any previous preparation, of samples of the grafting area in scanning electron microscope (SEM) and by observation in optical microscope of prepared longitudinal sections (10 μ) of the same samples. In this case, the samples were dehydrated with the serie butyl-alcohol, infiltrated and embebed with parafine, sectioned with a rotary microtome, stained with safranin (Jensen, 1962) and mounted with Eukitt.

For both optical and electron microscope observations, the plant material was sampled the 3rd, 4th, 6th, 8th, 12th, 16th and 20th days after grafting.

After 30 days of in vitro culture the grafted plants were transplanted into 330 ml pots, containing sterilized peat. During the first days after transplanting the pots were enclosed with polyethylene bags to minimize moisture loss.

Some of the in vitro grafted plants were transplanted into especial little containers (minirhizotrons) to follow the evolution of the radicular system (rootstock) and aerial part (scion).

3. Results and Discussion

Due to non significant differences in the behaviour of the different combinations rootstock-scion, their results are discussed together.

During the first days after grafting there was only a mechanical contact between rootstock and scion (figure 1). This was the most critical phase of the process, with the highest number of unsuccessful graftings, due to contaminations (10%) and detaching (30%).

Since the 6th day after grafting a healing of callus began (figure 2), becoming larger and stronger in few days. After this initial biological union, there were no new losses of grafted plants.

The first connections between the vascular tissues of rootstock and scion were observed from the 8th day after grafting and over the 12th day a complete junction was reached (figure 3).

Between the 20th and 30th days the grafting union was completed. The scion had grown forming expanded leaves and the rootstock showed a developing adventitious radicular system being the in vitro plants ready to be transplanted.

After transplanting to greenhouse conditions, the grafted plants showed a survival of 60% similar to those normally obtained with non grafted in vitro cultured grape plants.

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Table 1 - Composition of the basal nutritive medium.

Chemical compound	mM	Chemical compound	μ M
KNO ₃	7.91	MnSO ₄ H ₂ O	5.00
Ca(NO ₃) ₂ 4H ₂ O	1.27	H ₃ BO ₃	100.00
NH ₄ NO ₃	10.00	ZnSO ₄ 7H ₂ O	30.00
KH ₂ PO ₄	1.25	Na ₂ MoO ₄ 2H ₂ O	1.03
MgSO ₄ 7H ₂ O	1.50	CuSO ₄ 5H ₂ O	0.10
FeSO ₄ 7H ₂ O	0.09	CoCl ₂ 6H ₂ O	0.10
Na ₂ EDTA	0.10	Thiamine	2.96
Sucrose	87.60	6-BAP	4.65
M-inositol	0.55	IBA	2.46

Figure 1 - Insertion of the scion in the rootstock.

Figure 2 - Healing and junction of callus between scion and rootstock. 6th day after grafting (Scanning electron microscope).

Figure 3 - Vascular tissues junction (——→) between scion (S) and rootstock (R). 12th day after grafting (Optical microscope).