THE USE OF "IN VITRO" CULTURE AND TUBULAR CONTAINER SYSTEM TO PROPAGATE SELECTED GRAPEVINE PLANTS FOR SHERRY WINE PRODUCTION

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Abstract

The combination 161-49 Riparia x Berlandieri (rootstock) and the cultivar "Palomino Fino" is the most largely cultivated in the Jerez zone to produce Pale-Fine Sherry wine.

The clonal selection of these plants took place in collaboration with, P. Domecq, S.A. Four farms belonging and representative of Jerez-Xéres-Sherry origin denomination zone, were chosen. From 1968 to 1977, according to the production (Kg/plant) and must quality (pH, sugars, acidity, total amino-acids, polyphenols, malic, tartaric and citric acids) the 25 best plants of each farm were selected. Then, 10 scions/plant were grafted on 161-49, and planted together in homogeneous conditions, to continue the selection, having actually 12 selected clones. Cuttings from these clones, rootstock and cultivar, were propagated by tubular container system (Pat. nº 521533). From the very vigorous new shoots formed on the cuttings, explants (0.3-0.5 mm) were taken to be propagated in vitro. With this material, contaminated was clearly decreased. Different conditions and media were compared in the multiplication's stage, best was a modified Anderson medium. There were important troubles with the stage of transplanting, was difficult and expensive to adapt plants to outside conditions. Very good results were obtained rooting "in vivo" plantlets from "in vitro".

1. Introduction

In the "Jerez-Xéres-Sherry" Origen Denomination Zone, the famous Pale-Fine, "Oloroso", "Pedro Ximenez" and others, Sherry wines are produced.

Because the high content of active calcium in the soil is a limiting factor, the most largely used rootstocks are Riparia x Berlandieri 161-49; Chasselas x Berlandieri 41-B; Cabernet x Berlandieri 333 EM and Berlandieri x Resseguier 13-5 (García Gil, 1974). According to the Regulation Council rules the recommended varieties are "Palomino Fino", "Pedro Ximenez" and "Moscatel". Among these, the combination Riparia x Berlandieri (rootstock) with the cultivar "Palomino Fino" was used in this work.

Another factor affecting the grapevine production is the sanitary condition, viruses being important. Besides, harvest and fruit quality improvement could be obtained by a correct clonal selection of the best stumps.

For those reasons, the objectives of our work were:

(1) The selection of mother plants according to criteria of production. During the last stage of the selection process, the must quality was controlled to know the relationship between production and must quality.

(2) The propagation of the selected clones by methods guaranteeng the plant sanitary condition and the economical viability.

2. Materials and methods

The work was carried out in collaboration with "Pedro Domecq S.A." of Jerez de la Frontera (Spain).

For the clonal selection, four farms belonging to "P. Domecq S.A." and representative for "Jerez-Xéres-Sherry" Origen Denomination Zone were chosen. From a total population of $\approx 40\ 000$ plants ($\approx 10\ 000$ per farm) the 25 most productive plants per farm were selected by measuring the Kg of fruit per plant for the last 10 years. Scions from each plant were grafted on 161-49, 10 plant per clone were planted in as homogeneous conditions as possible, to have a plot of 250 plants. From 1977 on, the selection was continued and during the last 2 years, some factors of must quality (table 1) were determined (Catalina et al., 1982).

The explants for in vitro propagation were taken directly from the 12 final selected plants and from the very vigorous shoots formed on the propagating cuttings (from these plants) in tubular container (Pat. n° 521533) or mist system. Explants of about 0.5 mm were used, being sterilized with ethanol 70 % (15 seconds) and Na-hypochlorite 0.5 % during 30 minutes.

In the proliferation's stage, the purpose was to obtain 1 plantlet per explant. The media tested were of Chée and Pool (1982), Anderson (1980) and Anderson modified (modification 1, addition to Anderson's minerals of 200 mg/l of $(\text{NH}_4)_2\text{SO}_4$; modification 2, addition of 480 mg/l KNO_3 and modification 3 addition of 300 mg/l $(\text{NH}_4)_2\text{SO}_4$ and 500 mg/l $\text{Ca}(\text{NO}_3)_2$). In every case, vitamins (Galzy, 1972), glycine (2 mg/l), sucrose (30 g/l), 6-BAP (1 mg/l) and NAA (0.1 mg/l) were added to the medium.

When the purpose was to have a high number of propagules per explant, the Anderson modification 3 was used but eliminating the auxine. For the rooting stage BAP was eliminated. Agar 0.6 % was used as support and the final pH was always 5.8. 3 000 lux, $25 \pm 1^\circ\text{C}$ temperature and 16 h photoperiod were the growing conditions.

For transplanting and acclimatization of the rooted plantlets the agar was washed away and the plantlets transplanted into a sterilized mix (peat - sand 1:2) in small pots. The plantlet were protected from desiccation in shaded high-humidity conditions with centrifugal humidifiers. Because no good results were obtained with this system,

non rooted plantlets (from in vitro) one node cuttings and 2-3 nodes cuttings (from aseptically cultured plants obtained from in vitro plantlets) were compared to root outside. In these cases, the tubular container (Troncoso, 1984) and mist (comparing perlite and synthetical formol-phenol resine as substrate) system were compared.

3. Results and discussion

At the final stage of the plant selection process, 12 plants with a production of over 5 Kg fruit/plant and year (average of 10 years) under homogeneous cultural conditions were selected and used as mother plants. Some determinations of the must quality from fruits of these plants are shown in table 1 (average of 2 years). Non significant differences exist with the average of the plants in the sherry zone (Catalina, pers. comm.) and with the most productive plants do the must quality does not decrease.

A very significant reduction of the degree of contamination in the test tubes was found when explants originated from cuttings in tubular container or mist propagation system (3 % contamination) instead of field plants (31 % contamination).

In the table 2 the results of the explants activity (variety and rootstock average) in relation to the nutritive media when the purpose was to have one plantlet per explant are shown. All the media gave high percent of explant activity but the best were the modification 2 and 3 of Anderson's.

When the purpose was to have a high number of propagules per explant (table 3) the factor of multiplication was $\approx$ 13 plantlets per explant, with both variety and rootstock.

An average of 73 % rooted plantlets inside the test tube, was obtained. This result has only an indicative character because important troubles upon transplanting were found, (over 95 %) of the plants did not survive. For that, "in vivo" rooting of plantlets and cuttings were considered (table 4).

When non rooted plantlets from in vitro, were used as propagation material, the best results were obtained with a normal mist propagation system because of the better insertion of the plantlet in the substrate. With one or more node cuttings all the propagation systems used gave very high per cent of rooting, but the tubular container system was the most economical. In all the cases the percent of survival of transplanted plants was very high.

4. References

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Table 1 - Control of must quality. Average of 12 selected clones (2 years)

Production ml/Kg of fruit		pH	Total sugars g/l	Reducing sugars g/l	Non red. sugars g/l	Glucose g/l	Fructose g/l			
578		4.23	259.3	201.4	60.8	110.7	111.9			
Total acidity meq/l	Malic acid g/l	Tartaric acid g/l	Citric acid ml/l	Total amino-acids g/l	Total polyphenols g/l					
9.2	0.61	3.15	74.8	1.08	0.48					
N mg/l	P mg/l	K mg/l	Ca mg/l	Mg mg/l	Na mg/l	S mg/l	Mn mg/l	Zn mg/l	Fe mg/l	Cu mg/l
500	94.4	1.653	89	50.7	5.9	79.8	0.43	0.41	1.29	0.28

Table 2 - Influence of the nutritive media on the propagules formation

Medium	N° explants	Plantlets (%)	Callus (%)	Total (%)
Chée-Pool	100	58	15	73
Anderson	280	58	25	83
Modif. 1 And.	100	55	16	71
Modif. 2 And.	100	77	23	100
Modif. 3 And.	170	78	16	94

Table 3 - Plantlets formation per explant

Medium	Plant	N° explants	N° plantlets	Plantlets/explant
Modif. 3 And.	Variety	100	1316	13.16
Modif. 3 And.	Rootstock	100	1315	13.15

Table 4 - Rooting of plantlets and cuttings in mist and tubular container systems (average of variety and rootstock)

Propagation system	Substrate	Plant material	Initial number	Rooting (%)
Mist	Perlite	Plantlet	150	83.3
Mist	Perlite	1 node cutting (1)	150	80.0
		(2)	150	96.6
Mist	Perlite	2-3 node cutting (1)	150	94.6
		(2)	150	98.6
Mist	Synt. resine	Plantlet	150	38.0
Mist	Synt. resine	1 node cutting (1)	150	83.3
		(2)	150	80.0
Mist	Synt. resine	2-3 node cutting (1)	150	93.0
		(2)	150	68.0
Tub. contain.	Perlite	Plantlet	150	45.3
Tub. contain.	Perlite	1 node cutting (1)	150	70.0
		(2)	150	96.0
Tub. contain.	Perlite	2-3 node cutting (1)	150	96.0
		(2)	150	97.3

(1) hardwood cuttings

(2) herbaceous cuttings