Clonal Micropropagation of Main Grape and Rootstock Varieties of Turkish Viticulture for Obtaining Virus-Free Basic Nursery Stocks

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Keywords: grapevine, virus, meristem culture, basic material

Abstract

The main purpose of this project is establishing the mother blocks with 22 clones of eight grapevine cultivars ('Sultani', 'Kalecik Karasi', 'Cabernet Sauvignon', 'Merlot', 'Shiraz', 'Mourvedrè', 'Chardonnay', 'Sauvignon Blanc'), and five clones of five rootstocks ('110 R', '1103 P', '140 Ru', 'Kober 5 BB', '41 B'), which are widely used in different viticultural areas of Turkey. Plant material was tested for eight grape viruses (GFLV, GLRaV- 1, 2, 3, 6, GVA, ArMV, GFkV, SLRSV, RpRSV, ToRSV-chi) using DAS-ELISA. Virus-infected clones were in vitro meristem cultured and sub-cultured to eliminate the viruses in MS media and re-tested for the infectious viruses. All previously infected clones were found to be free after in vitro meristem culture. Finally, all clones were mass-propagated by in vitro shoot-tip culture and acclimatized under mist, then transplanted into the plastic containers as a single-node cutting source of the basic nursery stocks.

INTRODUCTION

Viruses and virus-like diseases are the main causal agents of yield and quality losses related with growth declines in major viticultural areas of Turkey. Major viruses are GFLV, rugose wood complex and asteroid mosaic in Aegean (Erdiller, 1982; Azeri et al., 1998), GFLV, ArMV, GVM, GVN in Marmara-Thrace (Nogay et al., 1995; Gürsoy et al., 1997), GFLV, GLRV, GVA in Eastern Mediterranean, Southeast and Centraleast (Özaslan, 1998; Cigsar and Yilmaz, 1998), GFLV, GLRV, TBRV in central-north and central-south (Akbas and Erdiller, 1998; Celik et al., 2000) regions. Although these diseases can be detected by biological indexing, serology (ELISA), electronmicroscopy, DS-RNA analysis, PAGE, molecular methods, and PCR-based techniques, ELISA is still the best for detecting the nepoviruses, closteroviruses, and GFkV as a sensitive, rapid, and quite cheap method (Walter et al., 1994). An efficient sanitary selection is a prerequisite in grapevine certification to produce planting materials in basic or certified categories valid for international exchange. In vitro culture of shoot-tip meristems is widely used as a routine technique for elimination of these diseases from the clones of grape and rootstock varieties, mostly combined with thermotherapy at 35-38°C or in vitro micro grafting (Jako, 1989; Gürsoy, 1991; Celik et al., 2000).

The aim of this study was to obtain virus-free plants from widely used clones of scion and rootstock cultivars of Turkish viticulture, to establish the mother blocks as the source of propagating materials that will be used to produce basic nursery stocks.

MATERIALS AND METHODS

Experiments of the present study were conducted between 2002-2006.

Plant Material

Grape and rootstock varieties and their clones are in Table 1.

Detection of Viruses

All clones with the exception of 'Sultani' types were pre-tested for the presence of Grapevine fanleaf nepovirus (GFLV), Grapevine leafroll closterovirus (GLRaV-1, 2, 3, 6), Arabis mosaic nepovirus (ArMV), Raspberry ringspot nepovirus (RpRSV), Tomato ringspot nepovirus (ToRSV-chi), Strawberry latent ringspot nepovirus (SLRSV), Grapevine closterovirus A (GVA), and Grapevine fleck virus (GFkV) with DAS-ELISA (Clark and Adams, 1977; Martelli, 1993) using the bark shavings of dormant canes collected in mid-autumn (Borgo, 1990). 'Sultani' clones (types) were pre-tested only for the most widespread viruses (GFLV, GLRaV-1, 2, 3, 6, ArMV, GFkV).

Virus-infected clones were re-tested for the infectious viruses using DAS-ELISA after in vitro meristem-tip culture.

Meristem Culture

Shoot tip meristems (0.2-0.5 mm in size with two-leaf primordia) of virus-infected clones were in vitro cultured in solid MS (Murashige and Skoog, 1962) medium for establishment (I), shoot proliferation (II), and rooting (III) stages supplemented with 0.5 mg/L GA_3 and 2.5 mg/L BAP in stage I, 2.0 mg/L BAP and 0.5 mg/L IBA in stage II, 1.0 or 2.0 mg/L IBA and 0.0 or 0.5 mg/L BAP in stage III, to get virus-free stocks in a growth chamber at 16 h/3000 lux illumination, and 25°C temperature regimes. Subcultures at stage II were performed in some genotypes to obtain more auxiliary shoots. Rooted microplants were transplanted into a perlite+peatmoss (1:1) in small-size pots (8 cm in diam.) in stage IV, after a two-week acclimatization under high humidity, transferred into a perlit+peatmoss (1:2) mix in medium-size pots (16 cm in diam.) in stage V (Fig. 1) (Celik et al., 2000).

Healthy clones were directly in vitro mass-propagated using shoot tips (1.0-2.0 mm in size) with the same procedure as in meristem culture.

RESULTS AND DISCUSSION

Data on pre-tested 16 clones of seven grapevine cultivars and five clones of five rootstocks for eight virus diseases showed that only four clones were found to be infected ('Kalecik Karasi' cl. 4 with ArMV, 'Kalecik Karasi' cl. 16 and cl. 23 with GLRaV-3, and '140 Ru' cl.101 with GLRaV-2). All other clones including 'Sultani' types were healthy.

Virus-infected clones were found to be free from the infectious virus diseases following in vitro meristem culture, as witnessed by the results of re-test. Then, 25 virus-free stock plants for each of the 27 selected clones relevant to the Turkish viticulture (Gürsoy et al., 1997, Celik et al., 2000) were obtained by meristem and shoot-tip culture and maintained in the screenhouse as a mother block. As a final output of this study, we will produce virus-free nursery plants from these clones as basic category, and make them available to the commercial nurseries to establish their source blocks as the source of healthy propagating materials that are necessary to produce certified nursery plants in EC Standards (Martelli, 1992; Walter et al., 1994).

ACKNOWLEDGEMENTS

We are thankful to Biotechnology Institute of Ankara University for their support of this project (Project No: BAP-72), Sunfidan A.S., and Manisa Viticultural Research Institute for their aids to supply vegetal materials, and also Nesrin Karaca for her technical assistance.

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Tables

Table 1. Grapevine genotypes used as plant materials of the project

Grape cultivars	Clones	Origin
Cabernet Sauvignon (RW)	337	French
Kalecik Karasi (RW)	4, 10, 11, 12, 13, 15, 16, 19, 21, 23	Turkish
Merlot (RW)	181	French
Mourvedrè (RW)	450	French
Sirah (RW)	747	French
Chardonnay (WW)	132	French
Sauvignon Blanc (WW)	297	French
Sultani (WT-WR)	S5(T), S6(T), S4(T-R), T3(R),Y3(R), S1(R)	Turkish
Rootstock cultivars		
Berl. x Rup. 1103 Paulsen	113	French
Berl. x Rup. 110 Richter	140	French
Berl. x Rup. 140 Ruggeri	101	French
Berl. x Rip. Kober 5BB	114	French
Chas. x Berl. 41 B M.G.	172	French

RW: Red Wine, WW: White Wine, WT: White Table, WR: White Raisin, T: Table, R: Raisin

Figures

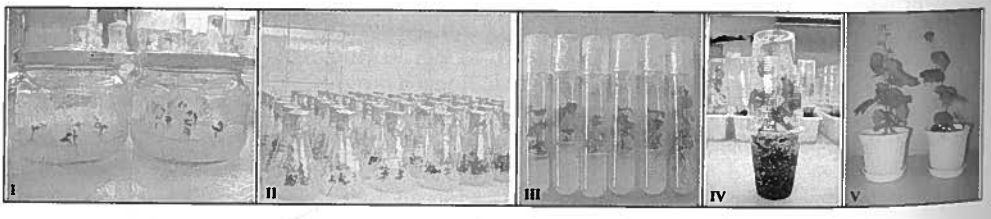

Fig. 1. In vitro meristem cultured plants at different growth stages.