South Western Journal of Horticulture, Biology and Environment
P-Issn: 2067- 9874, E-Issn: 2068-7958

Vol.5 , No.1, 2014
pp. 11-18

ELIMINATION OF *PNRSV* FROM SCHATTENMORELLE SOUR CHERRY CULTIVAR USING *IN VITRO* TECHNIQUE

Catita PLOPA

Research Institute for Fruit Growing Piteşti-Mărăcineni, Romania
Corresponding author, C. Plopa, E-mail:catitasarpe@hotmail.com

ABSTRACT. *The research was carried out to obtain viruses Prunus necrotic ringspot virus (PNRSV) free plants to Schattenmorelle sour cherry cultivar by means of meristem culture. The results obtained -using the MS, QL and LF basal medium added 0.2 mg/l GA_3; 0.03 mg/l IBA; 0.1 mg/l BAP - were good especially on MS formula in regeneration phase. One important factor for regeneration degree was the meristems size: 0.7-0.8 mm (a) and 0.2-0.3 mm (b). The highest multiplication rate of 5.2 plants/explants after 5 subcultures was obtained on MS culture media. After rooting phase developed on MS and ½ MS culture media, the results obtained showed that optimal was ½ MS culture media. ELISA test was used to identify whether they were infected by viruses; it showed that the viruses were 70% eliminated when using explant b and 35% when started in vitro culture with explant a.*

KEYWORDS*: Schattenmorelle, virus, in vitro.*

INTRODUCTION

Schattenmorelle' sour cherry variety is a kind of base in Europe with huge demand in the market with fruit and other clones from this variety. Safe yields are limited because, in case of infection with *PNRSV*, losses of 'Schattenmorelle' variety are very high and can reach up to 76-93% (Kegler et al.1972). Other researches (Ramsdell et al.1992) indicate that the loss of production can reach up to 54.5% for *PNRSV* infection associated with *Tomato ringspot virus* to 'Motmorency' sour cherry variety. *Virus free* plants

obtained from infected material with different viruses were studied that used simultaneously on several factors: temperature and size of explant, thermotherapy with chemotherapy etc. Limited or partial knowledge of some of these parameters can lead to incomplete elimination of the pathogen, even if the applied treatment is actually able of blocking the activity of viral replication (Luvisi et al.2011). Obtaining *virus free* plant for plum infected with *PNRSV* was tested by combining various factors: *in vitro* thermotherapy for Earliblue variety (Dziedzic 2008) or thermotherapy associated with in vitro chemotherapy for Empress and Early Rivers varieties (Cieśliński 2007). Results obtained release a rate of 75% virus free plant for Empress variety. Although in the literature data are found about *in vitro* culture - tissue culture and aspects of obtaining *virus free* plants for different species and varieties, but due to complex reaction specificity variety-virus, they do not have universal applicability. The combination in culture media of some compound to excessive concentrations used for purposes of chemotherapy can induce phytotoxicity for some species/variety (Jakab-Ilyefalvi et al. 2012), or inhibiting the growth of explants (Vescan et al. 2011). However, it is essential to optimize the factors influencing that. The main influencing factors are represented by variety, explant type, virus and culture medium (Isac et al. 2005, Calinescu et al. 2009). Led by certain specific parameters, *in vitro* culture without other association methods can ensure production of healthy plants. The major purpose of this experiment was established using *in vitro* method for production of *PNRSV* free biological material of Schattenmorelle cultivar.

MATERIALS AND METHODS

Biological material was represented by 'Schattenmorelle' sour cherry variety infected with *PNRSV*. The diagnostic was established using DAS-ELISA test (Clark et al. 1977) and TAS-ELISA test (Cambra et al.1994). The branches collected from infected plants were cut in small cuttings of 1 node. The disinfection of cuttings consisted on washing them in 3% commercial detergent solution and after this step the cuttings were washed

in running tap water. Sterilization was started in ethylic alcohol 96 vol % for 10 minutes. Next step in sterilization flux was immersion in 6 % (w/v) $CaClO_2$ for 15-20 minutes, followed by three rinses in sterile water for 10 minutes each in laminar hood. The meristems excised from buds were classified in two sizes: a = 0.7-0.8 mm and b = 0.2-0.3 mm.

Culture media for *in vitro* initiation, multiplication and rooting phase, basal media included Lee Fossard (1977, LF) macro and micro elements and vitamins as well as Quoirin-Lepoivre (1977, QL) macro and micro elements with vitamins according to Linsmaier-Skoog (1965, LS) and Murashige & Skoog (1962, MS) macro and micro elements and vitamins. For rooting phase using only MS in two variants: V1=MS and V2 = ½ MS (macro and microelements). The hormonal balance was represented by giberelic acid (GA_3), indole-3-butyric acid (IBA), 6-benzylaminopurine (BAP), ά-naphthyl acetic acid (NAA):

- for initiation phase: 0.2 mg/lGA_3; 0.03 mg/l IBA; 0.1 mg/l BAP;
- for multiplication phase: 1.2 mg/l BAP; 0.2 mg/l NAA;
- for rooting phase: 0.2 mg/lGA_3; 1mg/l IBA.

All culture media contained 0.7% plant agar, 3% sucrose and or iron accessibility this element was added in culture media in $Na_2FeEDTA$ in 32 mg/l quantity.

Established of *in vitro* cultures for all phases were in growth chamber were conditions set to 21-23^{0}C and a photoperiod of 16 hours at light intensity of 2500 lx provided by cool white fluorescent tubes and 8 hours dark. After 4-5 week the plants were transferred on fresh culture medium with compounds specifically for each phase.

The data were analyzed for significance ($P<0.050$ by ANOVA (analysis of variance) with the mean separation by Duncan ′s Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Regeneration ability of meristem from 'Schattenmorelle' sour cherry cultivar

was good on all 3 basal media (Figure1 and 2); regenerated plants were observed in a short period of time. Maximum regeneration was recorded on MS medium (66.6-85.0%) and lowest on LF medium (50.0-66.6%) and QL medium (40-50%). With regard to the size of explants between two sizes of meristems (a and b), differences were observed (Figure 1 and 2). The a tip had a higher percentage of responding explants (50-85 %) compared with b tip (40-66.6 %). Similar method *in vitro* culture Manganaris et al. (2003), and use explants with size 0.8-1.3 mm obtained on WPM culture media 2-5% regeneration explants for nectarine infected with *PNRSV* and 38% explants regeneration from meristem size 1.3-2.0 mm. The effects of size of inoculated shoot tip, method of inoculation, medium and genotype on shoot tip culture of sweet cherry was low, 8.3 % to 23.7 %, while the rate of shoots forming from tips of actively growing shoots was higher, 27.3 % to 37.5 % (Hongyan et al. 2003), for biological material infected with *PNRSV*.

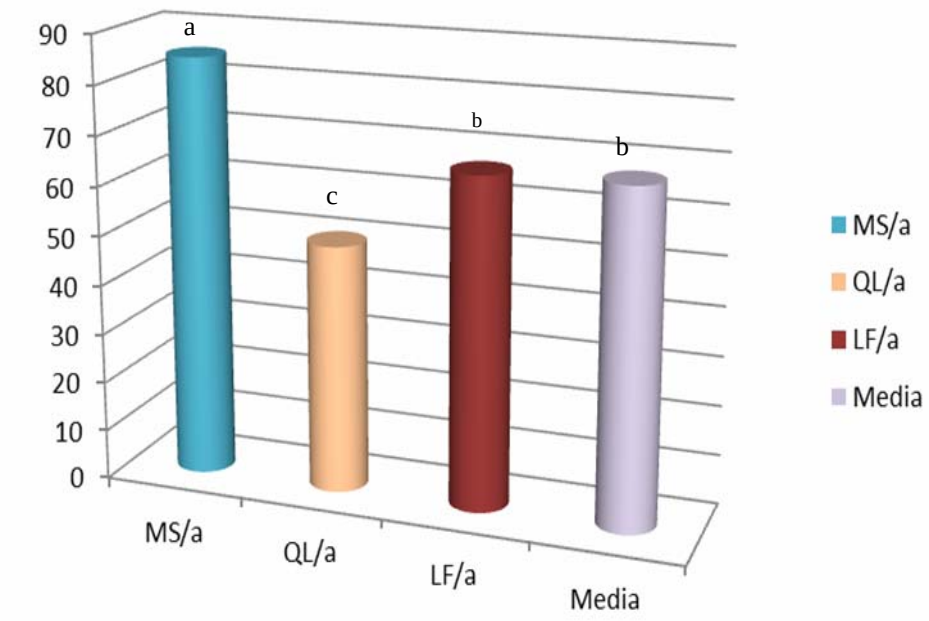


Figure 1. Regeneration ability of Schattenmorelle cultivar *a tip* explants depend on culture media (Duncan test <0,05)

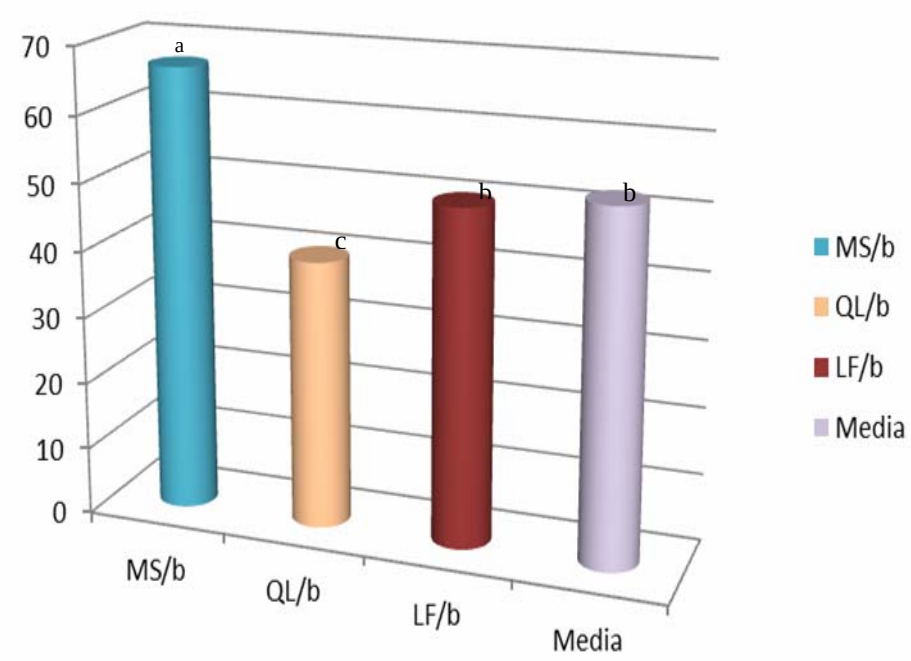
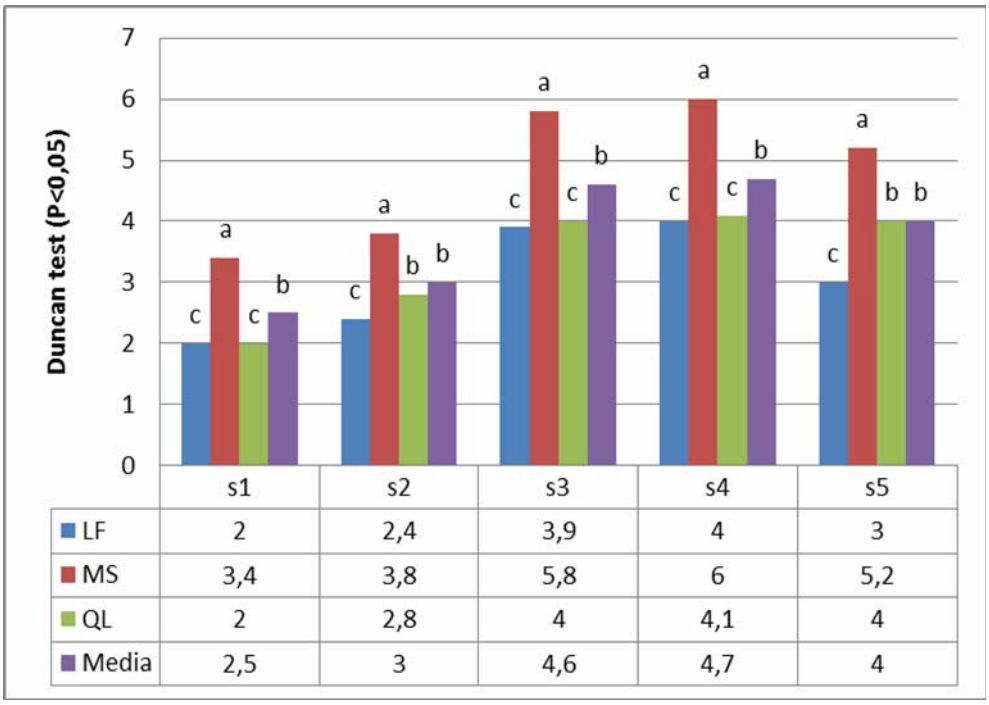


Figure 2. Regeneration ability of Schattenmorelle cultivar *b tip* explants depending on culture media (Duncan test <0,05)

Multiplication rate depended on basal medium and number of subcultures (Figure 3). The best multiplication medium for 'Schattenmorelle' cultivar was MS resulting in 3.4 and 6 shoots/explant. No significant differences were recorded between multiplication rate on QL and LF medium was lower, respectively 2-4.1 and 2-4 shoots/explant. There were no differences between variants to size of meristems from initiation phase, in multiplication rate, all shoots obtained a simillary proliferation reported to this factor.

After 5 subcultures 50% (108 shoots) from biological material obtained were tested TAS/DAS-ELISA. The shoots that remained were transferred for rooting on two culture media variants V_1 and V_2. *In vitro* rooting of 'Schattenmorelle' cultivar was most successful when using ½ MS (Figure 4), resulting in 75% plant rooted shoots and 30 % rooted shoots when using MS integral (Figure 4).



	s1	s2	s3	s4	s5
LF	2	2,4	3,9	4	3
MS	3,4	3,8	5,8	6	5,2
QL	2	2,8	4	4,1	4
Media	2,5	3	4,6	4,7	4

Figure 3. Multiplication rate of Schattenmorelle variety in relationship with different basal culture media.

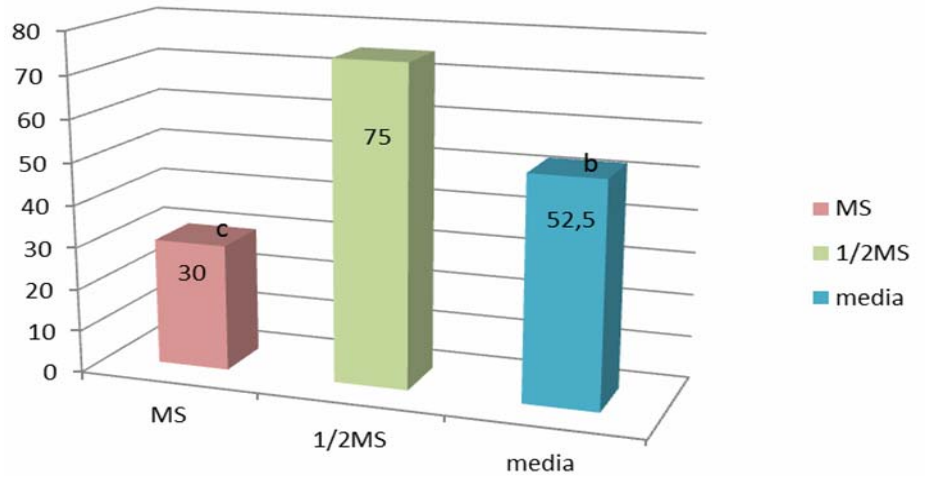


Figure 4. Rooting results obtained on two variants culture media

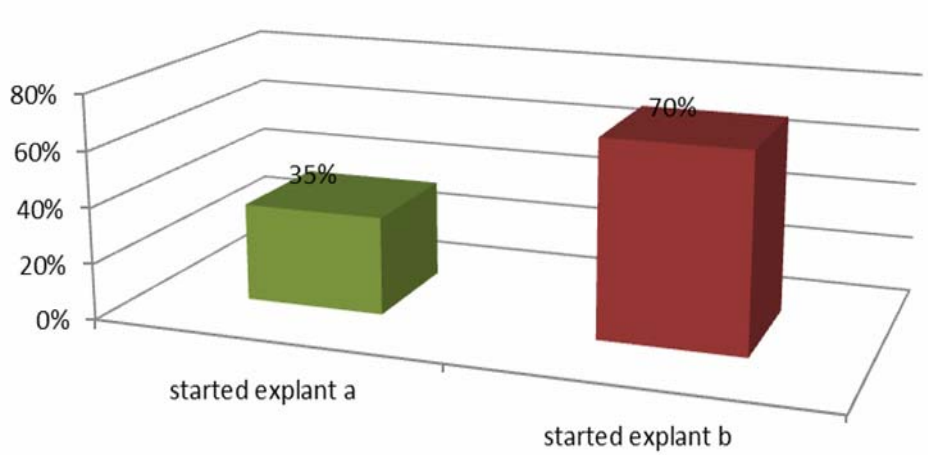


Figure 5. The effect of influence of *in vitro* culture and explant size in obtained *virus free* plant from Schattenemorelle variety.

Regarding the elimination of *PNRSV*, ELISA serological tests revealed that shoots provided from meristem type a, are only 35% virus free, while shoots provided from meristem type b, are 70% virus free (Figure 5). Comparing data obtained by Manganaris et al (2003) with other association virus-species when the presence of *PNRSV*, 81% were found to be virus-free. Hongyan et al (2003) proved that *PNRSV* could not be eliminated effectively from sweet cherry plantlets *in vitro* by culture of minor shoot tip from some sweet cherry variety.

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