RESEARCHES REGARDING *IN VITRO* REGENERATION CAPACITY OF THE GRAPEVINE VARIETY

CERCETĂRI PRIVIND CAPACITATEA DE REGENERARE *IN VITRO* A UNOR SOIURI DE VIȚĂ DE VIE

DONICI Alina[1]
e-mail: donicialina@yahoo.com

Abstract. *Research has been conducted to determine the capacity in vitro multiplication of seedless grapevine varieties, defining the basic conditions for initiating, maintaining, proliferation regeneration and rooting explants. Biological material investigated was represented by two genotypes of grapes, Otilia and Călina, and biological material to initiation in vitro cultures was the apexes and meristems. A higher potential for regeneration was obtained from variety Călina. Viable plants were obtained by cultivating Murashige - Skoog medium (1962) supplemented with AIA and GA3.*
Key words: grapevine, in vitro regeneration, regeneration potential , explant, culture medium

Rezumat. *Cercetările au fost realizate în scopul determinării capacității de multiplicare in vitro a unor soiuri apirene de viță de vie , definirii condițiilor de bază pentru inițierea, menținerea – proliferarea, regenerarea, și înrădăcinarea explantelor. Materialul biologic investigat a fost reprezentat de două genotipuri de vița de vie, Otilia si Calina, iar materialul biologic pentru inițierea culturilor in vitro a fost reprezentat de apexuri și meristeme. Un potențial mai mare de regenerare s-a obținut la soiul Calina. Plantele viabile au fost obținute prin cultivarea pe mediu agarizat Murashige – Skoog (1962) suplimentat cu AIA si GA3.*
Cuvinte cheie: viță de vie, regenerare in vitro, potential de regenerare, explant, mediu de cultură

INTRODUCTION

Most of the studies carried out for a century in the field in a plant vitro culture have been used to clarify several aspects of regeneration, growth, organogenesis, embryogenesis systemic and , of tissue structures or cell types, depending on the composition of the culture medium and to the conditions of eco physiologic containers of culture and of the room for growth. In vitro cultures were found in a very short period of time much practical application in improving numerous species of crop, by default to vines, on multiplication genotypes valuable, propagation of plants-free, conservation of genetic resources and more.

Healthy plants obtained by regeneration in vitro, grown in the field, as a rule they're canned vigurosity and productive capacity. The method of multiplication on aseptic environments propagation by growing in vitro of

[1] Research and Development Station for Viticulture and Winemaking Bujoru, Romania

fragments derived from apices intense regenerative and meristems taken from the tip shoots, leads to the primordia caulinary in 20 -50 days of inoculation (Visoiu, et al., 2000). Axillary buds are under dominance apical meristem technique is used: that once deleted increase meristems underarms, from shoots apices.

Induction processes of regeneration in vitro culture and vegetative multiplication depend to a great measuring tool of knowledge of mechanisms of operation of caulinary meristems. The higher the level of organization of inocul is most simple, the more nutrient needs and the culture conditions are more demanding, and nutrient substrate must be more complex and contain a wide range of organic substances. In this context, it considers a good knowledge of the factors which sequentially, or during the entire culture, may influence triggering organogenesis or carrying out processes. Most of the times, the reactivity of different explants, has been linked with the potential regenerative native genotypes studied (Vişoiu et al., 2006).

MATERIAL AND METHODS

Biological material, as represented by apex and shoots axillary, has been taken from varieties *Vitis vinifera*. In the varieties of plant material and them Călina was harvested from the collection of ampelographic collection a SCDVV Bujoru. Sampling explants has been carried out in shoots harvested during the growing season. Explants used for initiating cultures in vitro have been disinfected with calcium hypochlorite ($CaCl_2O_2$ -6 %), in sterile conditions in fume cupboard with laminar, for five minutes to small apices and meristems. Initiating and multiplication Călina variety and they have been carried out on the culture medium Murashige and Skoog (M&S, 1962), supplemented with 1 mg/l benzilaminopurine (BAP) and 0,5 mg/l ß- indolacetic acid (IAA). As a source of carbon was used sucrose (20 g/l), and for solidification culture medium use has been made of agar-agar as a function of the amount of 6 g/l. Rooting shoots it has been on the Murashige and Skoog (M&S, 1962) to one-half, supplemented with 2 mg/l AIA,, in the presence of 10 g/l sucrose; pH of the media was adjusted to 5.7 -5.8, before autoclaving. Sterilization vessels and of the culture medium was made by autoclave at 120^0C (1 atm.), for 20 minutes. The inoculation operations and transfer to the medium fresh have been carried out in the spaces sterile. Culture medium inoculated was maintained under controlled conditions, the temperature 25 ± 10^0 C; for and lighting within the limits of 16 hours light. A periodic bird to small shots and bird fragment of callus on nutrient media fresh is mandatory to maintain the viability.

RESULTS AND DISCUSSIONS

The first days of inoculation, have been carried out observations of processes of growth and differentiation of explants inoculated. After the first week of the cultivation at the explants have been reported their first signs of reaction.

It has pursued regenerative capacity in-vitro cultivar depending on the components donor explants, their nature and culture medium used. This aspect has been pursued on a culture medium Murashige and Skoog (M&S, 1962), supplemented with 1 mg/l benzilaminopurine (BAP) and 0,5 mg/l b indolacetic acid (IAA) which has shown that explants originating in apices have been intensely .

Regenerative potential of the varieties has been carried out after 90 days of culture in vitro, at which time explants reaction has become positive, for the purposes of training resulting from play against as a result of stimulation at the level existing vegetative explants. A significant increase in the plants was carried out after a period of 140 days. The best results under the aspect regeneration, of the number of small plant and rysogenesis has been carried out at the explants harvested from apices at both varieties. It should be noted that the values of the indices multiplication was assessed as being in a report in proportion to the number ignorance isn't linked and can be fully justified in this experiment the influence and the nature explants genotyping. (fig. 1-2).

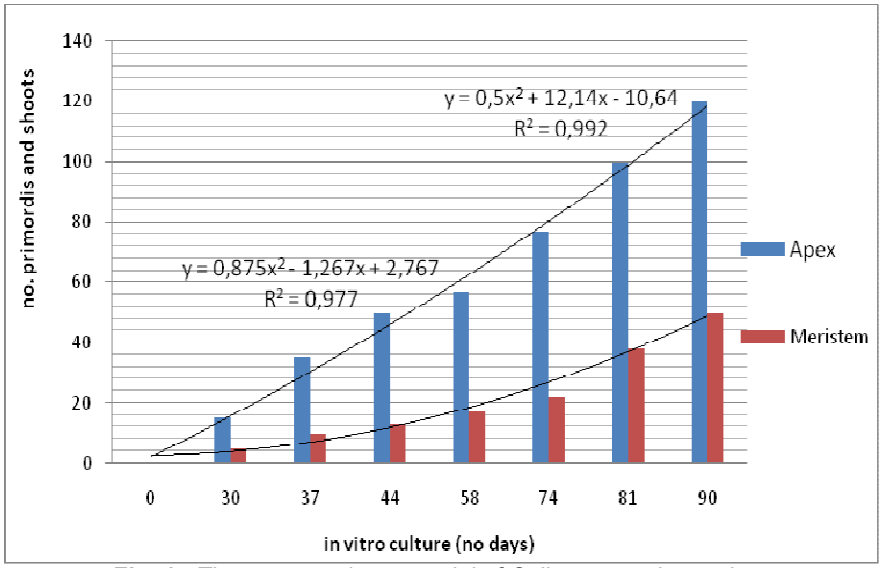

Fig. 1 - The regenerative potential of Călina grapevine variety

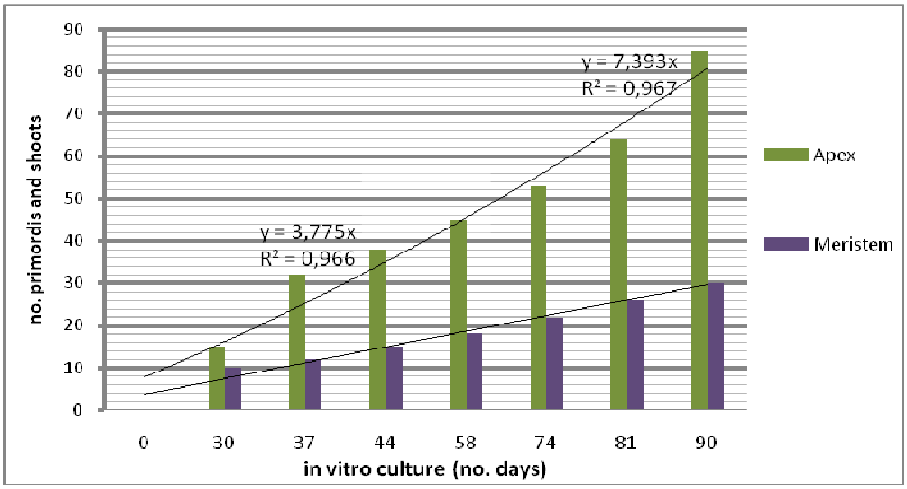

Fig. 2 - The regenerative potential of Otilia grapevine variety

For the function of rizogen small shoots detach regularly subculture, have been made comments during the sequence of taking root all over. Duration of the process of rysogenesis has been 50 days. So, based on the culture medium specific (1/2 M&S + 1 mg/l AIA), it is noted that small shoots coming from apices of 0.9 -3 cm have presented primordis root in proportion of progressed 30-45 %, after 15-20 days.

After 25- 27 days primordis root have developed in the 2- 2.5 cm to 60-70% of small shoot inoculate. Observations and tests carried out on biological material is inoculated on average rooting showed that the process of rysogenesis was completed after about 50 days when 75-85% of small shoots formed part of a well-developed system roots (fig. 3-4) , and shoots have been raised up to 4-7 cm.

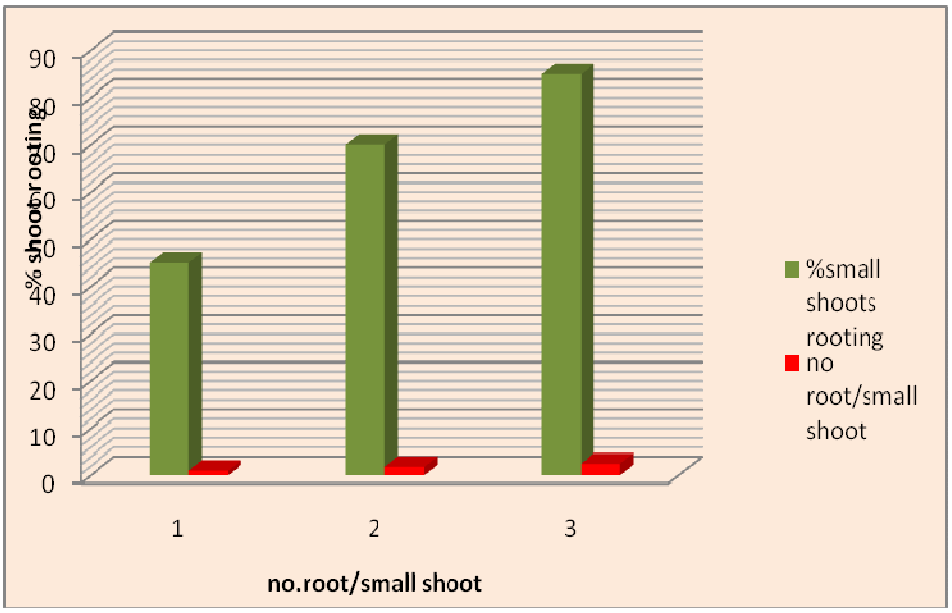

Fig 3 - Morphogenesis of Călina grapevine variety

In the case coming from meristems foliar, small shoots had a production low, have shown primordis root in a rate of 10-25 %, after 15-20 days. After 25- 27 days the root primordis have developed in radices of 1.5 - 1.8 cm to 35-45% of small shoots inoculating, and after 50 days only 50% of shoot formed part of a well-developed system roots.

Another aspect that was noted was that a part of cultures have had an indirect regeneration through the formation of his gag. The callus diameter had sizes between 0.4 -1.8 cm, for a consistency slightly soft, green in color. Its capacity to regenerate the calus has been insignificant

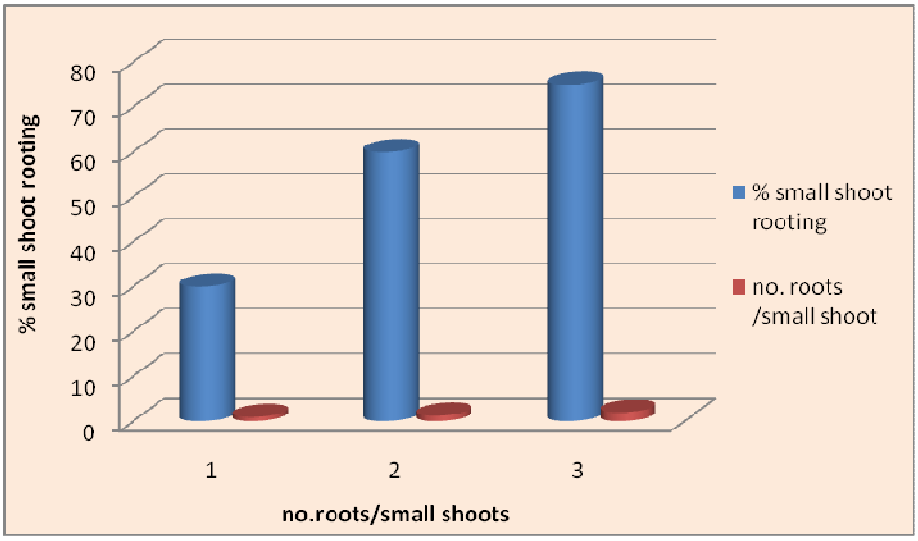

Fig. 4 - Morphogenesis of Otilia grapevine variety

CONCLUSION

1. Results of the research on the expression capacity of the two types of explants, varieties and they Călina, depend on the origin of explants;

2. A significant increase in the rate of multiplication has been registered after approximately 140 days of cultivation in vitro;

3. Type of meristem influenced significantly the plant neoformation rooted. Developments in the best have had the apical meristem;

4. The process of rysogenesis to biological material is inoculated on average nursery has been completed after approximately 50 days when 80-85% of small shoot formed part of a well-developed system roots (roots main ramifications secondary and tertiary), and shoots were height until 4-7 cm;

5. The difference of potentials of organogenesis of the two types of explants can be attributed to cellular metabolism of apices more active.

Acknowledgements: *Research has been supported by financial Sector Understanding ADER Plan Contract no. 2.2.6 /2011 " technology system for the production of wine of the propagation material free of viruses in protected spaces"*

REFERENCES

1. Heloire M.C., Fournioux J.C., Ozial L., Bessis S.R., 1997 – *An improved procedure for the propagation in vitro of grapevine (Vitis vinifera cv. Pinot noir) using axillary bud microcuttings.* Plant cell. Tissue and Organ Culture 49, p. 223 – 225.

2. Visoiu Emilia, Teodorescu Al., Bejan Carmen, Buciumeanu Elena Cocuța, Zaharia F., **2000** - *Contributii la perfectionarea biotehnologiei de înmultire in vitro a vitei de*

vie. Lucrari stiintifice, U.S.A.M.V. "Ion Ionescu de la Brad" Iasi, Anul XXXXIII – vol.1 (43), Seria Horticultura, Ed."Ion Ionescu de la Brad" Iasi. 2000. pp. 572-582

3. Vişoiu Emilia, Teodorescu A., Buciumeanu Elena Cocuța, Popescu C.F., Tița I., Bejan Carmen, Popa Camelia, Stănescu G., Contoman Maria, 2006 – *Perfecționarea biotehnologiilor de devirozare şi înmulțire in vitro a viței de vie în scopul creşterii valorii biologice a materialului săditor viticol la standardele internaționale.* Analele ICVV Valea Călugărească vol. XVIII, p. 43 – 52