„IN VITRO" MICROPROPAGATION – QUICK AND EFFICIENT METHOD FOR SOME VALOROUS *PRUNUS CERASIFERA* ECOTYPES FROM THE SOUTH ROMANIAN AREA

ÎNMULȚIREA „IN VITRO" - METODĂ RAPIDĂ ŞI EFICIENTĂ PENTRU PROPAGAREA UNOR BIOTIPURI VALOROASE DE CORCODUŞ DIN SUDUL ROMÂNIEI

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Abstract *P. cerasifera (Ehrh.) is one of over 270 species of Prunus gender (Jucovski, 1952), and over some other authors is one of over 400 species (WGHROB – OECD, 2006). This species presents interest all over the world to preserve the genetic resources and to breed, cultivars and rootstocks of a great value. In this paper we have proposed to show up the Prunus cerasifera ecotypes in the South Romanian area and the micro propagation through "in vitro" cultures to establish a work method in the laboratory, to obtain quickly and efficiently of some south Romanian ecotypes and to preserve them in the National Prunus Gene Bank at S.C.D.P. Vâlcea. The evaluation of the material was done with IPGRI descriptors in agreement with ECP/GR PRUNUS WORKING GROUP, 1996. The micro propagation was done through meristems cultures during February and March. The used media were Murashige & Skoog for the starting and multiplication phases and Lepoivre for rooting with normal concentrations of micro- and macro elements and phytohormons as: Giberelic acid (GA3) 0,5 mg/l, Benzilaminopurine(6-BAP) – 1mg/l, Indolilbutiric acid (IBA) – 0,5-1 mg/l and mixtures of vitamins as: Murashige & Skoog and Liensmair in normal concentrations.*

Rezumat *P. cerasifera (Ehrh.) este una dintre cele peste 270 de specii ale genului Prunus (Jucovski, 1952), iar după alți autori ,dintre cele 400 specii ale acestui gen (WGHROB – OECD, 2006). Această specie prezintă interes la nivel mondial pentru conservarea resurselor genetice şi pentru ameliorarea de soiuri şi portaltoi de mare valoare biologică. Prin prezenta lucrare ne-am propus să punem în evidență câteva biotipuri aparținătoare la P. cerasifera în zona de Sud a României (Oltenia, Dobrogea) şi în acelaşi timp să încercam micropropagarea acestora prin culturi „in vitro" în vederea stabilirii unei metode de lucru rapide pentru obținerea celor mai reprezentative biotipuri şi conservarea lor în Banca Națională de Gene Prunus de la SCDP Vâlcea. Evaluarea materialului s-a făcut pe baza descriptorilor IPGRI – în conformitate cu ECP/GR PRUNUS WORKING GROUP, 1996. Microînmulțirea s-a realizat prin culturi de meristeme în cursul lunilor februarie – martie. Mediile de cultură utilizate, ca medii de bază au fost :Murashige & Skoog pentru inițiere şi multiplicare; Lepoivre pentru înrădăcinare, cu concentrații normale de macroelemente si microelemente, cu adaosuri de fitohormoni ca : Acid giberelic (GA3) – 0,5 mg/l; Benzilaminopurină (6-BAP) – 1 mg/l; Acid indolilbutiric (IBA) – 0,5-1 mg/l şi vitamine Murashige & Skoog şi Liensmair în concentrații normale.*

It has passed over 106 years since Haberlandt, discovered the assumption of totipotential of the vegetative cell, an assumption confirmed and developed in the same time with the discovery and the use of the plant growth regulators especially auxins and citochinines.

P. cerasifera (Ehrh.) is one of over 270 species of *Prunus* gender (Jucovski, 1952), and over some other authors is one of over 400 species (WGHROB – OECD, 2006). This species presents interest all over the world to preserve the genetic resources and to breed, cultivars and rootstocks of a great biological value. On Romanian territory *P. cerasifera* is present from the ancient times near by: P*runus domestica, P.insititia, P. spinosa, P.avium, P. cerasus, P.persica, P.armeniaca, etc.*

P.cerasifera is considered to be formed (Sonea 1957) of more varieties and ecotypes (P. *cerasifera var. pissardi Schn*., P. *cerasifera var. bliereiana Hort*., P. *cerasifera var. moserii*, P. *cerasifera var. woodiwood*., P. *cerasifera var. turcomanii* Pop., P. *cerasifera var. orientalis* Pop.).

The favourable ecological conditions from the South Romanian area and the high interest of the fruit growers for fruits, distilled drinks and also the use as rootstocks , go to the spread of this species and to form many populations formed of several ecotypes which present different morphological and biochemical characteristics.

In Romania were done many researches over this species regarding the genetic and breading aspects, growth and fruiting processes spread in different areas, etc. *(Bordeianu, 1956; Sonea, 1957; Duțu, Parnia şi Botu, 1987; Botu, 1987; Giorgota, 2005, Botu I., Achim Ghe, Preda S., Giorgota A.)*.

In this paper we have proposed to show up some *Prunus cerasifera* ecotypes from the South Romanian area (Oltenia, Dobrogea) and in the same time to try the micro propagation through "in vitro" cultures to establish a work method to obtain quickly and efficiently of some south Romanian ecotypes and to preserve them in the National Prunus Gene Bank at S.C.D.P. Vâlcea.

MATERIAL AND METHODS

The researches were done during the period 2002-2007. The identify and the collecting of the biological material was done in the south Romanian area and included areas from Oltenia and Dobrogea regions.

The evaluation of the material was done with IPGRI descriptors in agreement with ECP/GR PRUNUS WORKING GROUP, 1996.

The micro propagation was done through meristems cultures during February and March. The used media were *Murashige & Skoog* for the starting and multiplication phases and *Lepoivre* for rooting with normal concentrations of micro- and macro elements and phytohormons as: Giberelic acid (GA3) 0,5 mg/l, Benzilaminopurine (6-BAP) – 1mg/l, Indolilbutiric acid (IBA) – 0,5-1 mg/l and mixtures of vitamins as: *Murashige & Skoog and Liensmair* in normal concentrations.

In the growth room, the little plants were maintained at an photoperiod of 16 h light and a temperature of 23-24 ° C and 8h dark and a temperature of 18-20 ° C.

The "in vivo" acclimatization was realised in perlite and than the plants were moved in vases with mixture of peat, soil and sand (1/1/1) and than were planted directly in the field.

Were identify, evaluated and propagated through classic methods a number of 120 ecotypes *of Prunus cerasifera* which were introduced in S.C.D.P. Vâlcea "Prunus collection" as clones. Of these a number of 11 were introduced in the micro propagation technology.

RESULTS AND DISCUSSIONS

In the micro propagation process were used meristems of *P. cerasifera* from the buds.The explants were formed from the meristematicã bud (the dom) and one or two small leaves.

The branch small parts had the length of 2 cm, each one with one bud, were sterilized in ethylic alcohol 70% for 1 minute, then in calcium hypochlorite 6 % for 5 minutes, followed by 3 washes with distilled, sterile water.

The entire sterilization was done after the moment when the branches were washed with water and dish drops.

THE *Prunus cerasifera* ECOTYPES IN THE MULTIPLICATION PHASE

The used media as we said were Murashige & Skoog and Lepoivre with phytohormons adding , vitamins, sucrose and a gelling agent as agar (Table nr. 1).

For the multiplication media were used vitamins as *Murashige & Skoog* and for the *Lepoivre* media was used *Linsmair* vitamin and for both media was used BAP in different concentrations 1-1,5 mg/l, GA3 1 mg/l, and for rooting was used IBA 0,5-1 mg/l. For both media were used agar (7g/l), and sucrose (20-40 g/l).

After the effected researches the results showed up that the Murashige & Skoog media is good for the starting and multiplication phases and the Lepoivre media is good for rooting phase, this media assure to obtain over 66 % rooted plants which survived till the acclimatization phase. (Table no. 2 and Fig. 1.)

Table 1.

The composition of the used culture media

Media Murashige & Skoog (MS)		***Media Lepoivre (L)***	
Substance	***The quantity for 1000 ml***	***Substance***	***The quantity for 1000 ml***
KNO3	*1900 mg*	*NH4NO3*	*400 mg*
NH4NO3	*1650 mg*	*KNO3*	*1800 mg*
MgSO4 7H2O	*370 mg*	*MgSO4 7H2O*	*360 mg*
CaCl2 2H2O	*440 mg*	*KH2PO4*	*270 mg*
KH2PO4	*170 mg*	*CaCl2 2H2O*	*1200 mg*
MnSO4 4H2O	*22,3 mg*	*MnSO4 4H2O*	*1,000 mg*
ZnSO4 7H2O	*8,6 mg*	*ZnSO4 7H2O*	*8,600 mg*
H3BO3	*6,2 mg*	*H3BO3*	*6,200 mg*
CuSO4 5H2O	*0,025 mg*	*CuSO4 5H2O*	*0,025 mg*
Na2MoO4 2H2O	*0,250 mg*	*Na2MoO4 2H2O*	*0,250 mg*
CoCl2 6H2O	*0,025 mg*	*CoCl2 6H2O*	*0,025 mg*
KI	*0,830 mg*	*KI*	*0,080 mg*
FeSO4 7H2O	*27,9 mg*	*FeSO4 7H2O*	*27,8 mg*
Na2 EDTA	*37,5 mg*	*Na2 EDTA*	*37,02 mg*

THE *Prunus cerasifera* ECOTYPES IN THE MULTIPLICATION AND ROOTING PHASES

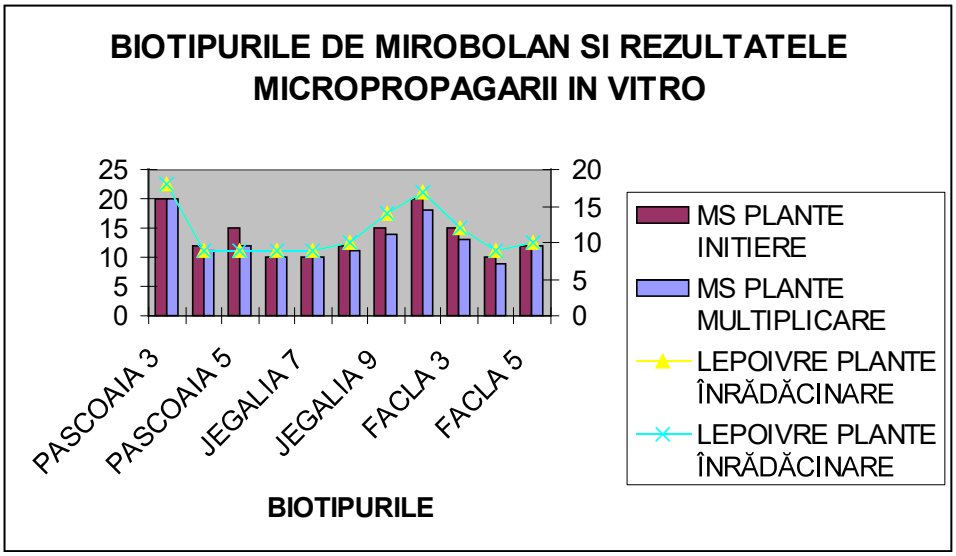

Fig. 1. The *Prunus cerasifera* ecotypes and the results of "in vitro" micro propagation

Table 2

The *Prunus cerasifera* ecotypes and the results of "in vitro" micro propagation

No	BIOTYPE	THE CULTURE MEDIA			ACLIMATIZED PLANTS
		MS STARTING PLANTS	MS MULTIPLICATION PLANTS	LEPOIVRE ROOTED PLANTS	
1	PASCOAIA 3	20	20	18	18
2	PASCOAIA 4	12	11	9	9
3	PASCOAIA 5	15	12	9	9
4	JEGALIA 6	10	10	9	9
5	JEGALIA 7	10	10	9	9
6	JEGALIA 8	12	11	10	10
7	JEGALIA 9	15	14	14	14
8	JEGALIA 10	20	18	17	17
9	FACLA 3	15	13	12	12
10	FACLA 4	10	9	9	9
11	FACLA 5	12	12	10	10

CONCLUSIONS

- The results obtained in the behaviour of some *Prunus cerasifera* ecotypes both in aseptic and natural conditions of life, prove the possibility of realising the ontogenesis and the propagation of *Prunus cerasifera* through tissues cultures "in vitro", very important method for the assurance of the rootstocks necessary for the propagation of the species of *Prunus* gender in our country.

REFERENCES

1. **Botu I., 1978** – *Studiul agrobiologic al unor biotipuri autohtone de prun din zona subcarpatică a Olteniei şi comportarea lor ca portaltoi pentru prun şi cais*. Teză de doctorat, Universitatea din Craiova.
2. **Botu I. ş.a., 2001** – *The Genetic Diversity of Prunus Genus into Sub Carpathian Area of Oltenia – Romania and Value for Breeding*. Acta Horticulturae no. 577, Plovdiv – Bulgaria.
3. **Botu M., Hagima I, 1998** – *Cercetări privind diversitatea genetică în cadrul genului Prunus cu ajutorul polimorfismului izoenzimelor peroxidazei (PRX*). Analele Univ. Craiova, vol. II (XXXVIII).
4. **Ermin G.V., 1978 –** *Genetic potential of species Prunus cerasifera Ehrh. and its use in breeding.* Acta Horticulturae no.74, Bordeaux, France.
5. **Sonea V., 1957 –** *Mirobolanu*l. Edit. Acad. a României, Bucureşti.