JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THE EFFECT OF NUTRIENT MEDIA IN MICROPROPAGATION AND IN VITRO CONSERVATION OF WILD POPULATION OF MAHALEB CHERRY (*PRUNUS MAHALEB* L.)

*Valbona Sota**[1], Efigjeni Kongjika*[2]

Address(es):
[1]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Bul. Zog I, Nr. 25/1, Tel. +355 4 2229590, Albania.
[2]Academy of Sciences of Albania, Section of of Natural and Technical Sciences, Tel. +35542250368, Albania.

*Corresponding author: bona_sota@yahoo.com

ARTICLE INFO

Received 13. 3. 2014
Revised 6. 4. 2014
Accepted 30. 4. 2014
Published 1. 6. 2014

Regular article

OPEN ACCESS

ABSTRACT

Shoot tips of *Prunus mahaleb* L. isolated from wild populations of Zejmen (Lezhe), promising as rootstocks for sweet cherry cultivars, were submitted to *in vitro* culture to test if micropropagation could be used for their rapid production. This study was carried out to determine the optimal nutrient media for micropropagation and to develop a suitable protocol for mid-term storage of *Prunus mahaleb* L. germplasm. For micropropagation were tested three different basal media MS, WPM and LP, all the three combined with 0.3 mg l^{-1} BAP, 0.1 mg l^{-1} IBA, 0.3 mg l^{-1} GA_3. The highest shoot length (5.53 cm) was observed on explants cultured on MS media, whereas this parameter was reduced on explants cultured on WPM and LP media (4.63 and 2.10 respectively). During subculture stage, MS and WPM media didn't show statistical differences regarding to shoots number/explants and leaves number/explants. The rooting percentages of plantlets ranged from 10 to 90%, depending on NAA concentration in the rooting media. In order to find out a medium-term *in vitro* preservation protocol effect of reduced sucrose and MS salts concentrations and elimination of PGRs from nutrient media on a collection of 30 days old of *in vitro* wild mahaleb cherry nodal segments have been examined for different periods. The highest survival and regeneration percentage (respectively 93.36 % and 83.72 %) were found in cultures stored at ½ MS media without sucrose for the period of 3 months. The maximal time of conservation without subculture on reduced sucrose and MS salt (1/2MS) concentrations is up to 5 months and in basal MS media without PGRs is up to 3 months. Hence the shoot tips of *Prunus mahaleb* L. can be successfully stored *in vitro* for medium terms at ½ MS media without sucrose.

Keywords: *In vitro* culture, micropropagation, mid-term storage, MS, LP, WPM media, *Prunus mahaleb* L

INTRODUCTION

Prunus mahaleb (syn. *Cerasus mahaleb* L., St. Lucie cherry, also occasionally Rock cherry or Mahaleb cherry) trees occur in thickets and open woodland on dry slopes; in Central Europe at altitudes up to 1700 m a.s.l. and in highlands at 1200-2000 m a.s.l. in Southern Europe **(Rushforth, 1999)**. It is drought resistant and can grow in very poor and calcareous soils, in full sun or partial shade **(Guitian, 1994)**.
The plant is cultivated for a spice, which is fragrant and has the taste of bitter almonds. It is used in small quantities to sharpen sweet foods **(El-Dakhakhny, 2006)**. The wood is hard, and is used in cabinet-making and for carving ornamental objects such as pipes and walking sticks. The bark, wood and seeds contain coumarin. They have anti-inflammatory, sedative and vasodilation effects **(Vedel *et al.*, 1960)**. Away from its native range, the species is grown as an ornamental tree for its strongly fragrant flowers, throughout temperate regions of the world.
The most common use is as rootstock for sweet or sour cherry cultivars. A number of cultivars have been selected **(Bean, 1976)**. In the first stage of the cherry rootstock breeding, the activity is focused on the collection of native mahaleb cherry (*Prunus mahaleb* L.) varieties **(Hrotkó, 2004; Hrotkó *et al.*, 2004)**. The common *Prunus mahaleb* L. rootstocks used for cherry production are becoming more unsatisfactory. Trees from grafted varieties are too large, fruit set is too late and harvest costs are too high **(Edin *et al.*, 1996)**.
Micropropagation is a suitable method for obtaining a large quantity of genetically homogeneous and healthy plant material which can be used for planting **(Kongjika *et al.*, 2002; Damiano *et al.*, 2008)**. The rapid *in vitro* multiplication of cloned plants is desirable to shorten crossing programs in fruit tree breeding **(Daorden *et al.*, 2004)**. *In vitro* culture is an effective method for ex situ conservation of plant genetic diversity, allowing rapid multiplication from very little plant material and with little impact on wild populations. For safe preservation, the in vitro slow growth storage method was developed and is considered an alternate solution for medium term storage of fruit germplasm **(Neveen *et al.*, 2008)**. The aim of medium term storage is to increase the interval period between subcultures by reducing growth. This might be achieved by the use of modified environmental conditions, modified culture medium, growth retardants, osmotic regulators and/or reduction of oxygen concentration **(Kameswara, 2004)**. Slow growth storage via *In vitro* cultures has been reported in many species **(Maqsood *et al.*, 2010)**.
The aim of this study is to determine the optimal nutrient media for micropropagation and to develop a suitable protocol for mid-term storage of *Prunus mahaleb* L. germplasm.

MATERIAL AND METHODS

Plant material: collection and disinfection

Cultures of *P. mahaleb* L. isolated from wild populations of Zejmen (Lezhe), were established from apical and lateral buds removed from adult field-grown trees. The plants were collected during February and March. Most often shoot tips and meristems are the explants of choice due to their genetic stability. Plant material used to introduce *P. mahaleb* L. cultivars is obtained by collecting the active explants between January and March, when buds were starting to swell from shoots in dormancy.
Active shoots were cut in two- or three-node sections. Two types of disinfection reagents were used: $HgCl_2$ and NaOCl. The stem sections were washed carefully with water and than were shaken for 5 min. in 70% ethanol, followed by 20 min. treatment with $HgCl_2$ 0.01% or NaOCl 0.3% and two drops of Tween 20. Finally stem sections were rinsed three times with sterile distilled water. Explants size is not as important for micropropagation as purposes as for obtaining disease-free plants. The buds were dissected up to 3 mm by removing the outer scales and showed no sign of contamination after over one year of continued culture.

Media composition for *in vitro* cultivation

Proliferation and subculture media: Three nutrient media were tested MS medium **(Murashige and Skoog, 1962)**, LP medium **(Quoirin and Lepoivre, 1977)** and WPM medium **(Lloyd and McCown, 1980)**, all the three combined with 0.3 mg.L^{-1} BAP; 0.1 mg.L^{-1} IBA; 0.3 mg.L^{-1} GA_3 and supplemented with 3% sucrose and solidified with 0.55% agar. The pH of the media was adjusted 5.7 – 5.8 before autoclaving. After a month, the developed buds were transferred to fresh media for further multiplication. Measurements of proliferation (%), length of the shoots and leaves number were taken.

Rooting media: When the explants derived from MS medium during proliferation and subculture stage reached 2 – 4 cm in length, they were transferred to rooting media. Three variants of rooting medium were analyzed:

- Rooting media I: ½ MS macronutrients, MS micronutrients, MS vitamins containing 0.1 mg.L^{-1} NAA;
- Rooting media II: ½ MS macronutrients, ½ MS micronutrients, MS vitamins with 0.1 mg.L^{-1} NAA;
- Rooting media III: MS macronutrients, ½ MS micronutrients, MS vitamins with 2 mg.L^{-1} NAA.

Rooting response was evaluated after 4 – 5 weeks of culture.

Plantlets acclimatization: Rooted plantlets were transferred after 3 – 4 weeks on rooting media to a mixture of soil, peat and perlite (2:1:1) (v/v), in 7 cm diameter plastic pots and placed in a controlled growth chamber at 20ºC. The plantlets were covered with plastic bags in order to maintain high humidity by removing them periodically for some minutes day after day. The bags were removed completely after about four weeks.

***In vitro* chamber conditions**

The culture in the proliferation stage was grown in the growth chamber at temperature of 25° ± 2° C in a 16 h/8 h light/dark regime with cool, white fluorescent light of intensity 43.4 μmol m^{-2} s^{-1}.

For *in vitro* conservation, two different methods of minimal growth are tested:

- *Effect of reduced sucrose and MS salts concentrations:* The cultures are transferred onto ½ MS media without sucrose and supplemented with the same rate of plant regulators and agar as in the multiplication media. The incubation conditions are the same as in the multiplication stage.
- *Absence of phytohormones or growth regulators in the growth media:* The cultures are transferred onto MS media without growth regulators or phytohormones and supplemented with the same rate of other components as in the multiplication media. The incubation conditions are the same as in the multiplication stage.

The cultures are stored in these conditions for different periods (3, 4, 5 months) for each method tested. For each method are at least 15 shoots in each replication. Survival of the cultures is assessed on the basis of criteria as suggested by **Reed (1992)** as dead and brown shoots are considered as unsurvived while those with vigorous growth and having healthy leaves are considered survived.

Statistical analysis

All experiments were repeated at least twice. Data collections in experiment were subjected to analyses of variance and evaluated by computer using the statistical evaluation program JMP 7.0.

RESULTS

***In vitro* cultivation**

Explants disinfection: The explants isolated from developing buds, after the surface sterilization with $HgCl_2$ 0.01% for 20 min resulted in the highest percentage of developed explants (85%). Contamination rates were about 15% for primary explants and less than 2% for subcultures.
Sodium hypochlorite in the concentration 0.3% was not effective in disinfecting explants derived from field-grown adult trees. The contamination rates in this case were about 80%. Must be noted that the explants are isolated from the field trees with high contamination and it is required the disinfection with the most powerful reagents such as mercury chloride.

Proliferation and subculture stage: The shoots number per explants (SN) was affected by the type of media (Tab. 1, Fig. 1a, b). Explants cultured on MS and WPM media showed the highest shoot number/explants (3.00 and 2.80 respectively) compared to the explants cultured on LP media (1.50), after 4 weeks of culture. The results showed that the highest leaves number/explants (LN) (9.50) was recorded on explants cultured on MS media, value that wasn't statistically different from those cultured on WPM media (7.80), whereas leaves number/explants was smaller (4.20) on explants cultured on LP media. The mean shoot length (SL) was also affected by the type of media. The highest shoot length (5.53 cm) was observed on explants cultured on MS media, whereas this parameter was reduced on explants cultured on WPM and LP media (4.63 and 2.10 respectively).
Even in this stage, shoots number/explants, shoots length/explants and leaves number/explants were affected by the type of media. SN and LN values weren't statistically different between the explants cultured on MS and WPM media, whereas SL values were statistically different (Tab. 1).

Table 1 Shoot number per explants (SN), shoot length (SL) and leaves number per explants (LN) during proliferation and subculture stage of explants cultured onto MS, WPM and LP media, and rooting percentage on three different rooting media

Culture stage	Nutrient media	Shoot number (SN)	Shoot length (cm) (SL)	Leaf number (LN)
Proliferation	MS	3.00 ± 0.55 A	5.53 ± 0.16 A	9.50 ± 1.00 A
	WPM	2.80 ± 0.25 A	4.63 ± 0.12 B	7.80 ± 0.51 A
	LP	1.50 ± 0.22 B	2.10 ± 0.25 C	4.20 ± 0.53 B
Subculture	MS	3.80 ± 0.49 A	5.80 ± 0.31 A	13.90 ± 1.23 A
	WPM	4.00 ± 0.33 A	4.94 ± 0.15 B	11.50 ± 0.98 A
	LP	2.10 ± 0.27 B	4.30 ± 0.24 B	7.60 ± 0.56 B
		Rooting media I (%)	**Rooting media II (%)**	**Rooting media III (%)**
Rooting		90 ± 2.33	10 ± 1.92	30 ± 2.91

Note: *Values represent mean ± standard error. Means followed by the same letter within the column do not differ significantly ($P \leq 0.05$) according to a Tukey's Honestly Significant Difference test*

Rooting and acclimatization stage: Rooting induction appears very difficult, especially regarding to trees species. For this reason, three nutrient rooting media containing different concentrations of auxin, α-naphthalene acetic acid, NAA and macro- and micronutrients, presented in the universal medium MS were compared for the explants derived from MS medium during proliferation and subculture stage. After 3 weeks of the culture on rooting medium, rhizogenesis was observed. The explants reacted differently in three types of rooting media (Fig. 1c, d, e). The mineral and NAA concentration of the culture media affect rooting percentage and roots characteristics.
Best results were observed in explants cultured on I rooting media where the percentage of rooting appeared to be too high (90% ± 2.33). The two other media, II rooting media and III rooting media showed lower rooting percentage, respectively 10% ± 1.92 and 30% ± 2.91 (Tab. 1). At the ultimate case (rooting media III), higher concentrations of NAA tended to induce callus formation on the proximal end of shoots. In this case, the number of roots was high, but those had an abnormal look being two short and thick (Fig. 1e).
Acclimatization was affected directly by rooting conditions. Survival was best when plantlets were transferred to pots after a short period of root emergence on rooting media (Fig. 1f). Maintenance on rooting media gave longer roots but resulted in poor survival. Acclimated plantlets are able to pass in the permanent place in the green-house.

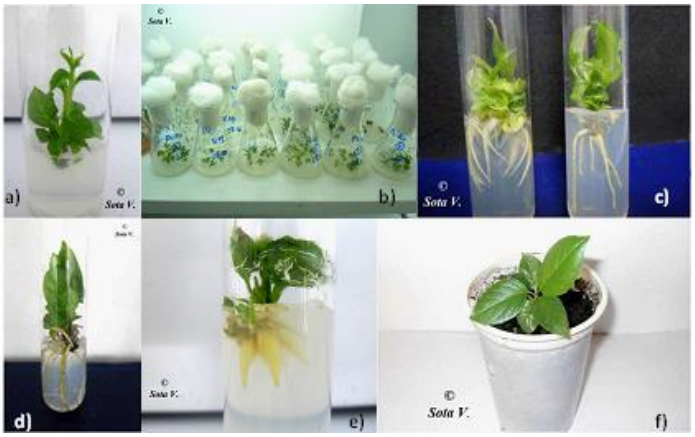


Figure 1 Micropropagation of wild mahaleb cherry shoot tips **a, b)** Development of explants during proliferation and subculture stage **c, d, e)** Rooted explants in three different rooting media, respectively I rooting media, II rooting media, III rooting media **f)** Acclimated plant

Germplasm mid-term storage

In Table 2 and Graphics 1, 2 are presented and analyzed the results of survival percentage and regeneration percentage for both methods of conservation tested in three different periods (3, 4 and 5 months). The regeneration percentage is calculated after transferring the survived shoots in the nutrient media used for micropropagation purposes.

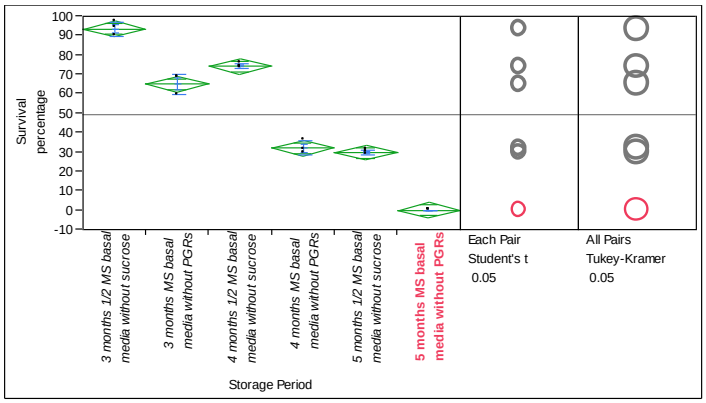
From the obtained data result that the highest survival and regeneration percentage is found in cultures stored in ½ MS media without sucrose (respectively 93.36% and 83.72%) for the period of 3 months. The maximal time of conservation without subculture on reduced sucrose and MS salt (1/2MS) concentrations is up to 5 months and in basal MS media without PGRs is up to 3 months.

With increase in storage period, survival rate as well as regeneration is reduced significantly. Also are observed significant statistical differences in survival and regeneration rates of shoots for each method tested (Graph. 1).

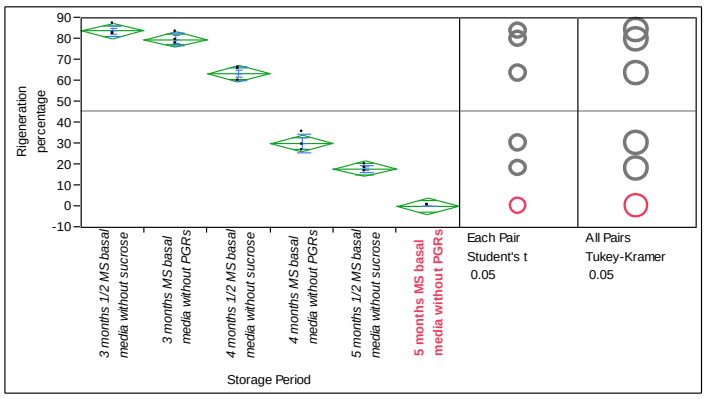
Table 2 Survival and regeneration percentage of wild mahaleb cherry explants conservated with different methods of minimal growth, for different periods

***Prunus mahaleb* L.**	**Survival percentage**		
	3 months	**4 months**	**5 months**
½ MS media without sucrose	93.36± 2.14	74.10 ± 0.73	29.6 ± 0.77
Basal MS media (without PGRs)	65.00 ± 3.00	32.00 ± 2.08	0
	Regeneration percentage		
	3 months	**4 months**	**5 months**
½ MS media without sucrose	83.72 ± 1.51	63.31 ± 1.87	17.80 ± 0.86
Basal MS media (without PGRs)	79.65 ± 1.76	30.02 ± 2.64	0

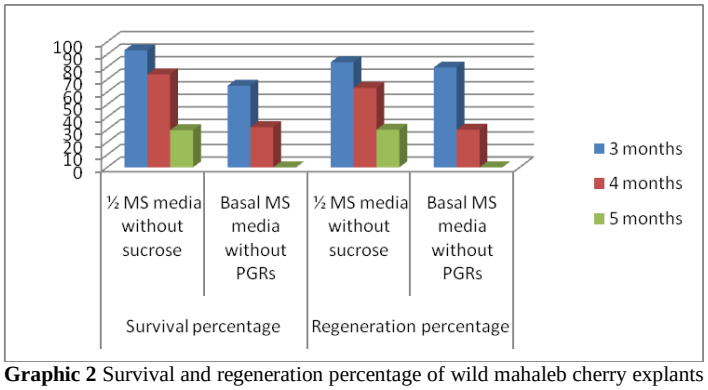
Note: *Values represent mean ± standard error.*



Graphic 1 Oneway analysis of survival and regeneration percentage by storage period



Graphic 1 - continue Oneway analysis of survival and regeneration percentage by storage period



Graphic 2 Survival and regeneration percentage of wild mahaleb cherry explants conservated with different methods of minimal growth

DISCUSSION

Better results obtained in *in vitro* culture of *Prunus mahaleb* explants in MS medium comparing to LP and WPM ones could be related to different composition of these media. The major differences in macronutrients among these three basal media are in ammonium and nitrate ion concentrations and total ion concentration. Full-strength MS medium has higher values of ammonium and nitrate, while WPM and LP are low ammonium mediums. MS medium has even potassium nitrate as a major nitrogen source, while LP and WPM medium have calcium nitrate. Beside this, some micronutrients are present only in the MS medium. Effectiveness of MS media in micropropagation of *Prunus* sp. is also reported from other authors **(Sedlák *et al.*, 2008; Shatnawi *et al.*, 2007; Ružić *et al.*, 2008; Gurel *et al.*, 1998; Namli *et al.*, 2011; Marino *et al.*, 1989; Muna *et al.*, 1999).** Meanwhile, from other studies resulted that LP basal media gives better results **(Lamrioui *et al.*, 2009; Hasan *et al.*, 2010)** and others report WPM basal media more effective **(Yao *et al.*, 2011; Harada *et al.*, 1996; Liu *et al.* 2010)**.

Positive effect of lower doses of one of the auxin, "inductor" of rhizogenesis, α-naphthaleneacetic acid, NAA, is reported in the studies of some authors on the *in vitro* rooting of apple plantlets **(Nemeth, 1981; Monter, 1992)**. Inside a species in the level of different varieties is demonstrated that the use of higher concentration of auxins (2-3 mg l^{-1}) favors the development of callus and limits root formation. As result, the use of the lower doses than 0.5 mg l^{-1} is recommended.

Effectiveness for minimal growth conservation reducing MS salt concentration in basal media without sucrose is also reported from other authors during conservation of *Vitis* sp. **(George, 1996)** *Pyrus* sp. **(Ahmed *et al.*, 2009; Moriguchi *et al.*, 1989)**, *Coffea* sp. **(Desbrunais *et al.*, 1992)** etc.

Plant growth could be reduced even if PGRs concentration in nutrient media is under optimal levels **(Gunning *et al.*, 1985)**. Reducing PGRs concentration resulted effective in conservation of *Fragaria* sp. **(Jungnickel, 1988)**, meantime eleminating them from nutrient media is reported as an optimal storage method for this specie **(Reed *et al.*, 1995)**. This storage method is also reported for conservation of *Ramonda sp.* **(Kongjika *et al.*, 1998)**.

CONCLUSION

The most optimal nutrient medium is considered Murashige & Skoog medium (MS) supplemented with MS vitamins and combined with 0.3 mg l^{-1} BAP; 0.1 mg l^{-1} IBA; 0.3 mg l^{-1} GA_3, which favors the buds development in the first stage of *in vitro* culture.

Comparing the data of the response of the explants in three different proliferation media is observed the difference in shoots number, leaves number and shoot

length parameter. During subcultures was observed not only the production of a considerable number of plantlets, but even increase in length of secondary and tertiary adventitious shoots in the explants multiplicated on MS medium.

Best results on rooting percentage were observed in explants cultured on I rooting medium containing ½ MS macronutrients, MS micronutrients, MS vitamins supplemented with 0.1 mg l^{-1} NAA.

Conservation via reduction of MS salt concentration and sucrose elimination from nutrient media resulted effective for mid-term storage periods up to 5 months. With increase in storage period, survival rate as well as regeneration is reduced significantly.

REFERENCES

AHMED, M., ANJUM, M.A. 2009. *In vitro* storage of some pear genotypes with the minimal growth technique. *Turkish Journal of Agriculture and Forestry*, 34, 25–32.

BEAN, W.J. 1976. Trees and Shrubs Hardy in the British Isles. 664 p. ISBN 0-7195-2427.

DAMIANO, C., ARIAS PADRO, M.D., FRATTARELLI, A. 2008. Propagation and establishment *in vitro* of myrtle (*Myrtus communis* L.), pomegranate (*Punica granatum* L.) and mulberry (*Morus alba* L.), *Propagation of Ornamental Plants*, 8(1), 3-8.

DAORDEN, M.E., MARÍN, J.A., ARBELOA, A. 2004. Stratification Temperature affects the *in vitro* Germination of Immature *Prunus* Embryos, *ISHS Acta Horticultura*, 658(2), 135-140.

DESBRUNAIS, A.B., NOIROT, M., CHAIRRIER, A. 1992. Slow growth in vitro conservation of coffee. *Plant cell tissue and tissue culture*, 31, 105–110.

EDIN, M., GARCIN, A., LICHOU, J., JOURDAIN, J.M., 1996. Influence of dwarfing cherry rootstocks on fruit production. *ISHS Acta Horticultura* (Proceedings of International Cherry Symposium), 410, 507–510.

EL-DAKHAKHNY, M. 2006. Some coumarin constituents of *Prunus mahaleb* L. fruit kernels. *Journal of Pharmacology Sciiences*, 59(4), 551-553.

GEORGE, E.F. 1996. Plant Propagation by Tissue Culture. Part 2, In Practice, 799 p. ISBN 0-9509325-5-8.

GUITIAN, J. 1994. Selective Fruit Abortion in *Prunus mahaleb* (Rosaceae). *American Journal of Botany*, 81(12), 1555-1558.

GUNNING, J., LAGERSTEDT, H.B. 1985. Long term storage techniques for *in vitro* plant germplasm. (Proceeding of the International Plant Propagation Society), pp. 199-205.

GUREL, S., GULSEN, Y. 1998. The effects of IBA and BAP on *in vitro* shoot production of almond (*Amygdalus communis* L.). *Turkish Journal of Botany*, 22, 375-379.

HASAN, S.Z.U., AHMAD, T., HAFIZ, I.A., HUSSAIN, A. 2010. Direct plant regeneration from leaves of *Prunus* rootstock Gf-677 (*Prunus Amygdalus* X *P. Persica*). *Pakistan Journal of Bot*any, 42(6), 3817-3830.

HROTKÓ, K. 2004. Cherry Rootstock Breeding at the Department of Fruit Science, Budapest. *ISHS Acta Horticultura* 658(2), 491-495.

HROTKÓ, K., MAGYAR, L. 2004. Mahaleb Rootstocks from the Department of Fruit Science, Budapest. *ISHS Acta Horticultura*, 658(2), 497-500.

JUNGNICKEL, F. 1988. Strawberries (*Fragaria* spp. and Hybrids). In: Bajaj, Y.P.S. (ed.): Biotechnology in Agriculture and Forestry, 6, 38-103.

KAMESWARA, N.R. 2004. Plant genetic resources: Advancing conservation and use through biotechnology. *African Journal of Biotechnology*, 3(2), 136–145.

KONGJIKA, E., ÇAUSHI, E., JUNGNICKEL, F., MULLAJ, A., DINGA, L. 1998. Të dhëna paraprake për përhapjen, mikroshumimin dhe konservimin 'in vitro' të bimës së rrallë ballkanike *Ramonda serbica* Panc. *Punime të Institutit të Kërkimeve Biologjike*, 11, 81-91.

KONGJIKA, E., ZEKAJ, ZH., ÇAUSHI, E., STAMO, I. 2002. Bioteknologjia e bimëve – Kulturat "in vitro", Akademia e Shkencave, 336 p. ISBN 99927-783-6-9.

LAMRIOUI, A.M., LOUERGUIOUI, A., ABOUSALIM, A. 2009. Effect of the medium culture on the microcutting of material resulting from adult cuttings of wild cherry trees (*Prunus avium* L.) and of *in vitro* germination. *European Journal of Scientific Research*, 25(2), 345-352.

LLOYD, G., MCCOWN, B. 1980. Commercially feasible micropropagation of mountain laurel (*Kamlia latifolia*). *International Plant Propagation Society*, Comb. Proc. 30, 421–427.

MAQSOOD, A., MUHAMAD, A.A., ASAD, H.S., ABDUL, A. 2010. In vitro preservation of *Pyrus* germplasm with minimal growth using different temperature regimes. *Pakistan Journal of Botany*, 42(3), 1639–1650.

MARINO, G., MAGNAINI, E., BATTISTINI, S., RIGHETTI, B. 1989. Effect of hormone and main carbon energy sources on *in vitro* propagation of apricot (*Prunus armeniaca* L.) cvs. San Castreses and Portici. *Acta Horticulturae*, 293, 355-362.

MONTER, A.V. 1992. Micropropagation des fruitiers. In: Rosell C.H., Villalobos A.V.M. *Fondements théoriques et pratiques de la culture des tissus végétaux*, FAO, 145-153.

MORIGUCHI, T., KOZAKI, S., YAMAKI, S., SANADA, T. 1990. Low temperature storage of pear shoots *in vitro*. *Bull. Fruit Tree Res. Stat*, 17, 11-18.

MUNA, A.S., AHMAD, A.K., MAHMOUD, K., ABDUL-RAHMAN, K. 1999. *In vitro* propagation of a semi-dwarfing cherry rootstock. *Plant Cell, Tissue and Organ Culture*, 59, 203–208.

MURASHIGE, T., SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plantarum*, 15, 473-497.

NAMLI, S., ISIKALAN, Ç., AKBAS, F., BASARAN, D. 2011. Improved in vitro rooting of almond (*Amygdalus communis*) cultivar 'Nonpareil'. *Plant Omics Journal*, 4(1), 14-18.

NEMETH, G. 1981. Adventitious root induction by substituted 2-chloro-3-phenyl-propionitriles in apple rootstocks cultured *in vitro*. *Scientia Horticulturae*, 14, 253-259.

NEVEEN, A.H., BEKHEET, S.A. 2008. Mid-term storage and genetic stability of Strawberry tissue cultures. *Research Journal of Agriculture and Biological Sciences*, 4(5), 505–511.

QUOIRIN, M., LEPOIVRE, P. 1977. Etudes de milieux adaptés aux cultures *in vitro* de *Prunus*. *Acta Hort*., 78, 437 – 442.

REED, B.M. 1992. Cold storage of strawberries *in vitro*: A comparison of three storage systems. *Fruit Var. Journal*, 46, 93-102.

REED, B.M., HUMMER K.E. 1995. Conservation of Germplasm of Strawberry (*Fragaria* Species). In: Biotechnology in Agriculture and Forestry, 32, 354-374.

RUSHFORTH, K. 1999. Trees of Britain and Europe. Collins, 1336 p. ISBN 0-00-220013-9.

RUŽIĆ, V., VUJOVIĆ, T. I. 2008. The effects of cytokinin types and their concentration on *in vitro* multiplication of sweet cherry cv. Lapins (*Prunus avium* L.). *Hort. Sci.* (Prague), 35(1), 12–21.

SEDLÁK, J., PAPRŠTEIN, F. 2008. *In vitro* shoot proliferation of sweet cherry cultivars Karešova and Rivan. *Hort. Sci.* (Prague), 35(3), 95–98.

SHATNAWI, M.A., SHIBLI, R., QRUNFLEH, I., BATAEINEH, K., OBEIDAT, M. 2007. *In vitro* propagation and cryopreservation of *Prunus avium* using vitrification and encapsulation dehydration methods. *Journal of Food, Agriculture & Environment*, 5(2), 204-208.

VEDEL, H., LANGE, J. 1960. *Trees and Bushes in Wood and Hedgerow*. London, 224 p. ISBN 978-0416617801.