Research Article

Optimization of Nutrient Media of in Vitro Propagation for Some Cherry Rootstocks (*Prunus Cerasus*)

Horticulture

Keywords: Murashige & Skoog, Quoirin & Lepoivre, buds, leafs, shoots.

Avdirrahman Gashi	**Faculty of Agricultural & Environment, Agricultural University of Tirana.**
Tokli Thomaj	**Faculty of Agricultural & Environment, Agricultural University of Tirana.**
Taulant Mitrushi	**Faculty of Agricultural & Environment, Agricultural University of Tirana.**
Edlira Kukali	**Faculty of Agricultural & Environment, Agricultural University of Tirana.**

Abstract

The most important species of which have origin many populations, land-races and local varieties: wild cherry, wild pears, wild almond, wild medlar, etc. This study was focused in many important rootstocks of P. avium x P canascens - Gisela 6, P.avium x P. pseudocerasus - Colt (nano), P. mahaleb x P.avium (Maxma), P. Mahaleb and cultivar Prunus avium L. (Cerasus avium L. var. sativa Ser., P. cerasus): Bigarreau Hatif. Are used two important media in the micro propagation of the horticultural species above, MS (Murashige &Skoog) and LP (Quoirin & Lepoivre). Nutrients of MS media have affected with sensible changes compare with LP media, in the explants proliferation for differentiations of the buds, shoots and leafs. Inoculation of explants in LP media, results with lower result on the qualitative indicators of the research. Average growing of the shoots on the MS media, resulted 10.1 mm while on the LP media was 6.0 mm. Average number of leafs 2.47 on the MS media, while on the media LP was 1.34 leaf. In the statistic analyses for the min and max, in all the cases it has predominance of the MS media. Intensity of the growing has cv (coefficient of variation) = 63% and on the MS media results 1.88 highest of LP media.

Introduction

Albania as a Mediterranean country and with appropriate eco clime for cultivation of the cherry shows high interest on production of the cherry sapling, *cv. Prunus mahaleb* rootstocks used for cherry production. [9] However, it has many disadvantages. *Mahaleb* has not strong growing on the media with problems, with high capacity of water maintaining etc likewise mahaleb plant is with short life [11]. Rootstock *Gisela 6* is one of the most used rootstocks in the nursery for sweet cherry and cherries and it is consistent against the phytopathogenic and land defects. [8] In Vitro Micro propagation is a functional and efficient method for the production of vegetal rootstocks [10]. But there is no unique media, for all horticultural species, rootstocks, varieties, because genetic characteristics, are different, but also, eco climes and agro-technical services, too[1]. Erbenova et al (2001) [7] reported a 50% increase of micro propagation rate on the dwarf rootstocks of sweet cherries in MS media culture containing 1.5mg l^{-1} BAP. [12] Ruzic, et al. (2000) [2] reported that the MS and 2 MS (double macro-salts) media culture containing 4.4 mμ BA, 0.5 mμ NAA and 0.3 mμ GA_3 are a suitable media culture for propagation of *Gisela 5*. [5]. In a study on micro propagation of *prunus avium*, the combination of 0.5 mg l^{-1} BAP and 0.05 mg l^{-1} TDZ are suitable for proliferation and a media culture containing 0.3 mg l^{-1} IBA is desirable for the rooting [4]. Carolina et al (2006) [6] cultured nodal section of *prunus serotina* in MS media culture supplement with 4.44 mμ BA, 0.49 mμ IBA and 0.29 mμ GA_3.[3]

Materials and Methods

Initial Explants

P. avium x P canascens - Gisela 6, P.avium x P. pseudocerasus - Colt (nano), P. mahaleb x P.avium (Maxma), P. Mahaleb and *Prunus avium L. (Cerasus avium L. var. sativa Ser., P. cerasus); Bigarreau Hatif* cultivar. Buds are sectioned with a size of over 3 mm by stripping off the outer layers. All nutrient media are autoclaved at 121^{o}C, at 1 atm pressure, for 20 min in order to ensure the conditions of aseptic. [2]

Stage I - proliferation and prolongation of explants

The buds were inoculated into two variants of media proliferation MS and LP (31 May 2015): - MS media with supplements of MS Vitamins (Murashige & Skoog, 1962)

- LP media with supplements of LP Vitamins (Quoirin & Lepoivre, 1977)

Are made measurements of the proliferation rate, the length of the shoots and the number of leaves per each inoculation field were performed. After about a month the developed buds were transferred to fresh media for further micro propagation.

Stages II – subculture

The buds were sub cultured in two variants of the media micro propagation:

- MS media with supplements of MS and combined with 0.7 mg l^{-1} BAP, auxins 0.01 mg l^{-1} of α-naphthalenecetic acid, ANA and 0.1 mg l^{-1} of GA3;

- LP media with supplements of LP vitamins (Quoirin & Lepoivre, 1977) and combined with 0.25 mg l^{-1} BAP; 0.6 mg l^{-1} AIB; 0.3 mg of l^{-1} GA3.

Culture Condition

After labeling with the date of culture, the name and type of the explants, all cultures were placed in the vegetative room with the controlled regimes (temperature 25^{o}C ± 2^{o}C, luminous intensity 2000 lux and photoperiod 16 hour lighting/24h). For each stage of development, cultures are held about 4 weeks.[5]

Statistical processing of data

- All experimental data were processed using Tucey-Kramer, Student's and Variance Analysis ($P < 0.05$) methods using the JMP 7.0 statistical program.

Results

- Stage of proliferation and prolongation

The effect of the media feeding on the number of leaves and the length of the shoots.

After 3-4 weeks of cultivation in each media, the formation of new buds is observed.

Measurements were made for the number of leaves and the stem length in each media nutrients for cultivars in this study. All of these parameters are highly influenced by the type of

media nutrient.For the rootstocks and cv. *Burlat* the types of genus *Prunus*, the inoculated explants on the media MS showed the best result for all parameters in the study compared to the results obtained from media cultivation LP (tables 1a, 1b, 1c, 1d , 1 e, figure 1 and 2). The following are provided tables with the metering data for each rootstock and cv. *Burlat* (Tables 1 a, b, c, d, e).

Table 1 a.- *P. avium x P canascens (Gisela 6)*

No.	4 days		12 days		19 days	
	No.leaves	Length of shoots cm	No.leaves	Length of shoots cm	No.leaves	Length of shoots cm
1	3	1.3	2	1.1	3	2.5
2	2	1.1	3	1.2	4	1.5
3	2	1.0	2	1.2	4	2.0
4	2	1.0	3	1.0	5	1.5
5	3	0.8	2	1.2	3	1.5
6	3	0.8	1	1.0	3	1.5
7	3	0.5	1	0.5	4	1.0
8	5	1.0	2	1.0	3	1.2
9	4	0.8	2	0.8	3	1.2
10	4	0.5	2	0.8	3	1.0

Table 1 b. *P.avium x P. pseudocerasus [Colt (nano)]*

No.	4 days		12 days		19 days	
	No.leaves	Length of shoots cm	No.leaves	Length of shoots cm	No.leaves	Length of shoots cm
1	1	1.2	2	2	3	2.4
2	2	1.1	3	1.5	4	1.8
3	2	1.0	2	1.3	4	1.6
4	2	1.0	3	1.0	5	1.3
5	3	0.8	2	1.2	3	1.5
6	2	0.8	3	1.0	7	1.3
7	1	0.5	4	0.5	6	1.0
8	1	1.0	3	1.0	3	1.2
9	1	0.8	4	0.8	4	1.2
10	2	0.5	4	0.8	4	1.0

Table 1 c - *P. mahaleb x P.avium (Maxma)*

No.	4 days		12 days		19 days	
	No. leaves	Length of shoots cm	No. leaves	Length of shoots cm	No. levaes	Length of shoots cm
1	1	1	3	1.2	4	1.6
2	2	1.1	2	1.3	3	1.6
3	1	1.1	3	1.3	3	1.6
4	1	1.0	2	1.1	4	1.3
5	3	0.9	3	1.1	3	1.5
6	2	0.7	3	1.0	4	1.3
7	1	0.6	2	0.9	3	1.4
8	2	0.7	2	1.0	3	1.2
9	1	0.5	3	0.8	3	1.0
10	2	0.4	3	0.8	4	1.0

Table 1 d - *P. Mahaleb*

No.	4 days		12 days		19 days	
	No. leaves	Length of shoots cm	No. leaves	Length of shoots cm	No.Leaves	Length of shoots cm
1	0	0	0	0	0	0
2	2	1.1	2	1.3	3	1.5
3	1	1.1	2	1.3	3	1.5
4	0	0	0	0	0	0
5	2	0.8	3	1.1	3	1.5
6	2	0.9	3	1.0	4	1.4
7	1	0.6	2	0.9	4	1.5
8	2	0.6	2	0.8	3	1.2
9	1	0.5	2	0.8	3	1.0
10	2	0.5	3	0.7	3	1.2

Table 1 e -*Prunus avium L. (Cerasus avium L. var. sativa Ser., P. cerasus); Bigarreau Hatif Burlat*

No.	4 days		12 days		19 days	
	No. leaves	Length of shoots cm	No. leaves	Length of shoots cm	No.leaves	Length of shoots cm
1	1	1	2	1.4	0	0
2	2	1.2	2	1.2	3	1.5
3	1	1.0	2	1.3	3	1.6
4	1	0.8	2	1.4	3	1.4
5	2	0.9	3	1.1	3	1.4
6	2	0.9	3	1.0	0	0
7	1	0.6	2	0.9	4	1.5
8	2	0.5	2	0.8	0	0
9	1	0.5	2	0.8	3	1.1
10	2	0.6	3	0.7	4	1.3

% of surviving 98 %

For the feature of increase the number of leaves:- for the feature of increase length of the shoots: As can be seen from the calculations, Cv1 <Cv2. This means that in the plants obtained from rootstocks (*Colt*, *maxma*, *Gisela* and *mahaleb* (May), the growth of the shoots in length is the feature that varies more.

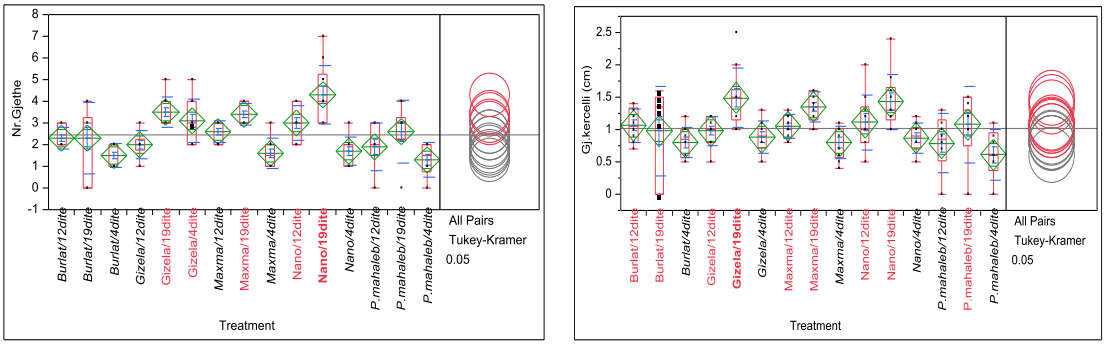

Figure 1. Oneway Analysis of Nr of leafs By Treatment Means and Std Deviations in vitro on the May month media MS
Figure 2. Variance Analysis, MS- Oneway Analysis of the Length og Shoots By Treatment in vitro on the Month May media MS Tukey-Kramer HSD q* 3.455, Alpha 0.05

Photo 1&2. Ready-labeled plants material and inoculation in autoclaved nutrients media in In Vitro's laboratory at the Department of Horticulture and Landscape Architecture Plants
Photo 3, 4, 5. Survives explants and leafs the first phase
Photo 6. No. Leafs and length of shoots on the fourth week

As seen in the above Dendrograma the number of leaves in the media MS is the feature that varies versus the average, resulting that the general average of 2,473 of Mean treatments. Leaf number variables were with Std. Dev 0.86. Following the Tukey-kramer variance test, visible changes were observed that corresponded to CV = 48.9%. The largest number of leaves has been verified and coincided with cv.*Gizella*, *Maxma*, and *Nan*o over 12 days.

Table 2 a.- *P. avium x P canascens* (*Gisela 6*) No leaves/ shoots length on the observation in the days in the media LP

No	4 days		12 days		19 days	
	No. leaves	Length of shoot cm	No. leaves	Length of shoot	No.leaves	Length of shoot
1	1	1.3	2	1.4	3	1.5
2	2	1.1	3	1.	3	1.3
3	1	1.0	2	1.2	3	1.4
4	2	1.0	2	1.1	2	1.4
5	1	0.6	2	1.2	3	1.5
6	0	0	0	0	0	0
7	1	0.5	1	0.7	2	1.0
8	0	0	0	0	0	0
9	2	0.5	2	0.8	3	1.2
10	1	0.4	2	0.8	3	1.0

Table 2 b. *P.avium x P. pseudocerasus [Colt (nano)]* No.leaves/ shoot length on the observation on the terrain LP

No.	4 days		12 days		19 days	
	No. leaves	Length of shoot	No. leaves	Length of shoot	No.leaves	Length of shoot
1	1	1.1	2	1,4	3	1.8
2	2	1.0	3	1.2	4	1.6
3	2	1.0	2	1.3	4	1.6
4	2	1.1	2	1.0	4	1.3
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0

Table 2 c. *P. mahaleb x P.avium (Maxma)* No.leaves / shoot length on the observation in days on the terrain LP

No.	4 days		12 days		19 days	
	No. leaves	Length of shoot	No. leaves	Length of shoot	No.leaves	Length of shoot
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	1	1.1	3	1.3	3	1.6
4	1	1.0	2	1.1	4	1.3
5	3	0.9	3	1.1	3	1.5
6	2	0.7	3	1.0	4	1.3
7	0	0	0	0	0	0
8	2	0.7	2	1.0	3	1.2
9	1	0.5	3	0.8	3	1.0
10	2	0.4	3	0.8	4	1.0

Table 2 d. *P. Mahaleb* No.leaves / shoot length on the observation in days on the terrain LP

No.	4 days		12 days		19 days	
	No. leaves	Length of shoot	No. leaves	Length of shoot	No.leaves	Length of shoot
1	0	0	0	0	0	0
2	2	1.1	2	1.3	3	1.4
3	1	1.1	2	1.3	3	1.5
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	2	0.6	2	0.6	3	1.1
9	1	0.5	2	0.8	3	1.0
10	2	0.5	2	0.7	3	1.2

Table 2 e. *Prunus avium L. (Cerasus avium L. var. sativa Ser., P. cerasus); Bigarreau Hatif Burlat* No.leaves / shoot length on the observation in days on the terrain LP

No.	4 days		12 days		19 days	
	No. leaves	Length of shoot	No. leaves	Length of shoot	No.leaves	Length of shoot
1	1	1	2	1.4	0	0
2	0	0	0	0	0	0
3	1	1.0	2	1.3	0	0
4	1	0.8	2	1.4	0	0
5	0	0	0	0	0	0
6	2	0.9	3	1.0	0	0
7	1	0.6	2	0.9	4	1.5
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	2	0.6	3	0.7	4	1.3

The MS analysis of Shoots differentiation in May, shown in the above-mentioned dendrogram, reflects the small variability among the experimental treatments. The average length on the MS media was 10.1 mm. They had the primacy to increase the addictions in 19 days. The increase was in correlation with the number of days. Shoots length variables had deviation Std.Dev 0.35. Conform Tukey-kramer the changes variance to CV = 34.6%. The proliferation of shoots cells and the increase in length is very important for the performance of the method. The growth has had an average intensity of 0.66 mm / days. This testifies to positive performance.

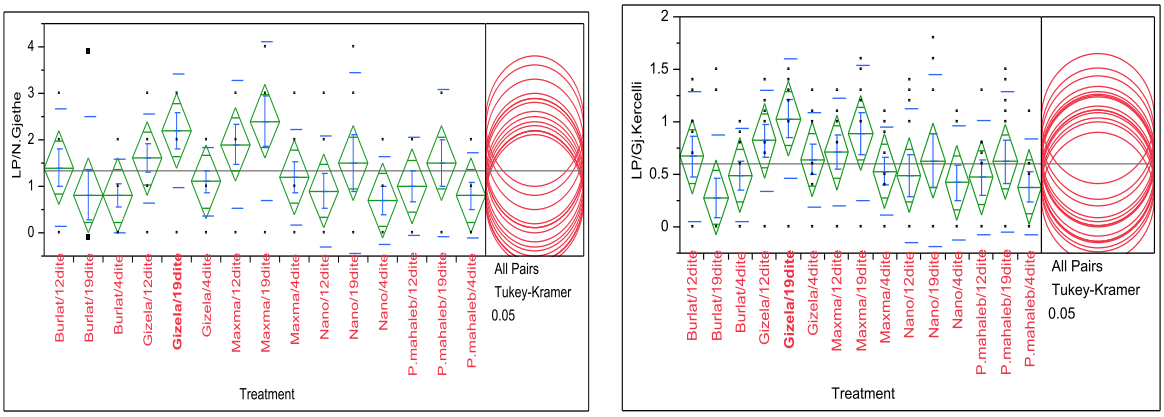

Figure 3. Variance Analysis, Oneway Analysis of LP/No Leaves By Treatment Means and Std Deviations in vitro
Figure 4 . Variance Analysis, Oneway Analysis of LP/Length of shoots By Treatment Means and Std Deviations in vitro
Oneway Analysis of LP/N Leaves By Treatment

The Dendrograma representative of the variance analysis tested with Tukey-kramer has verified variability variance for p = 0.05. The above Dendrograma reflects the average growth rate of the variance, with LP and in three different times. As seen through the variance test, the treatments do not have any real changes. The main data of the variance analysis of the number of LP leaves are probable because the factual F (1.7296) has greater than the theoretical F Prob> F <.0001 *. lsd. q * 3.455, Alpha 0.05. The MS type of nutrient media has affected sensitive changes against the reaction of explants to the differentiation of buds, shoots and leaves. While cultivation on nutrients media LP results with lower response some varieties are distinguished by the number and size of the best on both media. The average growth of the shoots in MS is 10.1mm while in LP 6.0mm. Thus, the daily growth intensity was 0.53mm/days (MS), and 0.3mm / day (LP). The average number of leaves of 2.47 leaves (MS), while the LP plot 1.34 leaves while the physiological growth intensity was 0.13 µg / day (MS) and 0.07 µg / day (LP). In Charts 2 and 3, in all cases there is predominance of the MS media. While in the chart below, the growth intensity has cv = 63% and in the MS field results 1.88 times greater than the LP media.

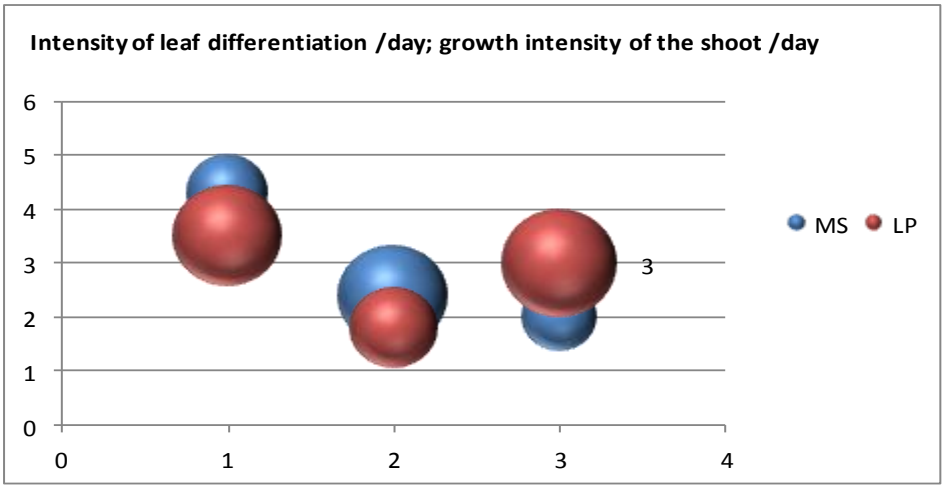

Figure 5. Intensity of differentiation of leaves and shoots length on the days observation

Conclusions

• The MS type of nutrients media has affected sensitive changes against the LP in the reaction of the explants to the differentiation of buds, shoots and leaves.
• Cultivation on LP nutrients media results with fewer responses to some rootstocks that are distinguished by the best number and size on both Medias.
• The average growth of the shoots in MS is 10.1mm while in LP 6.0mm.
• Daily growth intensity was 0.53mm / days (MS), and 0.3mm / day (LP).
• The average number of leaves 2.47 leaves (MS), while on LP 1.34 leaves
• The physiological growth intensity was 0.13 g / day (MS), and 0.07 g / day (LP).

References

Aitken-Christie J., Kozai T., Takayama S, (1995). Automation in plant tissue culture General introduction and overview. In: Automation and environmental control in plant tissue culture (eds. Aitken-Christie J., Kozai T., Smith M. L.). Kluwer Academic Publishers, Dordrecht, the Netheland.

M.Akita, K.Negishi, A.Kitano, M.Wasaki, Y.Ohta, T.Kuriu, and T.Takii, . (2006), Acta Horticulture, 579-584.

G.M.Bonga, P.V.Adkares; (2004). In vitro culture of trees, 1st edition. Trans. by E.Bagheri, et al., Ferdowsi University Press, Mashhad, pp.96-104.

A.Carolina, M.Paula, C.Pijut, H.Michler, Horticulture Science. 2006, 41(1), 193-201.

Murashige T., Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum 15(3): 473-497.

E. Kukali, E. Kongjika, (2011). Journal of Environmental Protection and Ecology , ISSN 1311-5065, Book 1, factor Impact 0.33; The Importance of genetic material 'In Vitro', rizogenic ability of grape

Nacheva L., Gercheva P. (2009). Micropropagation of 'Gisela 5' (cherry dwarf rootstock): The effect of the type and the concentration of the carbohydrates in the nutrient medium. Acta Horticulturae 825: 261-267. DOI: 10.17660/ActaHortic.2009.825.41.

Pierik R.L.M. (1997). In vitro culture of higher plants. Springer Science and Business Media, Springer, Dordrecht, the Netherlands. DOI: 10.1007/978-94- 011-5750-6.

Quoirin M., Lepoivre P. (1977). Edude de milieu adaptes aux cultures in vitro de prunus. Acta Horticulturae 78: 437-442 (in French).

Sœbø A., Krekling T., Appelgren M. (1995). Light quality effects photosynthesis and leaf anatomy of birch plantlets in vitro. Plant Cell, Tissue and Organ Culture 41, 177 – 185.

Solarova J., Souckova D., Ullmann J., Pospisilova J., (1996). In vitro culture: environmental conditions and plantlet growth as effected by vessel and stopper type. HortScience 23(2), 51 –58.