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The in Vitro Rooting of Pear Rootstock (*Pyrus Communis Pyraster*)

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***Abstract* – The purpose of this study is micropropagation with in vitro techniques of autochthonous wild pear (variety pyraster), determining the micropropagation protocol and rooting in the culture medium. For the cultivation of the pear rootstock were sampled two culture mediums: WPM (Lloyd & McCown, 1980) and universal MS medium (Murashige & Skoog, 1962). The first results were obtained in the shoot proliferation phase with BAP concentrated (0.35mg/l) after three subcultures of apical bud explants inoculated in growth medium. In the proliferation phase the WPM medium resulted with a mean number of shoots per plant (4) and shoots length (2cm), while the MS medium with mean number of shoots (5.1) and mean length of the shoots (2.6cm). The best results in rooting phase with IBA concentration (0.5 mg/l) were obtained in MS medium: rooting percentage 70%, mean number of roots (4.4) and mean length of the roots (1.8cm).**

***Keywords* – Pear Rootstock (*Prunus pyraster*), Rooting, Micropropagation, Proliferation, MS, WPM-medium.**

I. INTRODUCTION

Autochthonous wild pear, variety pyraster was cultivated in Albania for many years. Plants grafted on this rootstock have the ability to provide plants with a large and developed crown. They adapt well in dry and poor soils. Due to the deep and branched root system endure drought and soil conditions. Plants obtained from this rootstock are appropriate in hilly land with moderate slope. The crown grows in semi-spherical shape, 12 m in height and diameter 8-10m. These trees produce12-15 kv fruits and the average age reaches 50-60 years. Development and use of the pear rootstock is highly limited by the lack of effective and rapid proliferative systems. Rootstocks are difficult to propagate and cultivate as traditionally as with in vitro techniques. The improvement of in vitro micropropagation of several rootstocks will provide plant material to test in growth medium, with the aim to the commercial proliferation and to be used by growers. Rootstocks play an important role influenting to the production cycle, to fruit quality, diseases resistance, improvement of pest control methods, humidity adaptation and limestone soils. This study has in purpose defining an efficient protocol for in vitro micropropagation and rooting of autochthonous pear rootstock.

II. MATERIAL AND METHODS

Plant material of the pear rootstock was taken from the fruit crop collection of the Experimental Center, ATTC Shamogjin Vlore. Explants (apical and axillary buds) were collected from juvenille branches and were inoculated in April 2016. The study was conducted in the *in vitro* laboratory of ATTC Vlore. For each variant was experimented in 5 sterile jars with 10 explants each x 4 repetitions. The data was taken after three subcultures. Tissue culture passed these stages: inoculation, propagation, rooting and acclimatization. Apical and lateral buds explants were cut off without damaging the growing buds, making the final size about 1.0-1.5 cm in length. They were placed under running tap water, placed in ethyl alcohol 70% of 10 min, then were disinfected by using sodium hypochlorite (NaOCl) solution 10% with continuous agitation for 25 min. The sterilizing solution was decanted and the explants were given 3 rinses with distilled sterilized water. Explants after disinfection were cultured, placed in vertical position with their base immersed in culture medium MS (Murashige & Skoog, 1962) and VPM medium (Lloyd & McCown, 1980) containing GA_3 (0.1g/l). The explants at all stages are held in the vegetative room of plant growth in controlled optimal conditions T = 24 ± 1 °C, photoperiod 16 hours of light, radiant luminous intensity 3500 lux. For shoot proliferation, both apical and axillary buds, after a month inoculation, from established cultures were transferred in proliferation media culture WPM and MS.

Table 1. Culture media WPM e MS composition

Culture Medium	VPM (Lloyd & McCown, 1980)	MS (Murashige & Skoog, 1962)
Macroelements	mg l^{-1}	mg l^{-1}
K_2SO_4	990	---
$CaCl_2.2H_2O$	96	440
$MgSO_4$ 7 H_2O	370	370
NH_4NO_3	400	1650
KH_2PO_4	170	170
$Ca(NO_3)_2.4$ H_2O	556	---
KNO_3	---	1900
Micrroelements		
$MnSO_4$ .H_2O	22.3	16.9
$CuSO_4$.$5H_2O$	0.25	0.025
$CoCl_2$ $6H_2O$	---	0.025
$ZnSO_4.7H_2O$	---	8.6
H_3BO_3	6.2	6.20
$Na_2MoO_4.2H_2O$	0.39	0.25
KI	---	0.83
Chelator		
$Na_2EDTA.2H_2O$	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8
Vitamins	mg/l	
Inositiol	100	100
Ac.nikotinik	0.5	0.5
Tiamine	1	0.1
Pirodiksina	0.5	0.5
Glicine	2	2
Hormons		
BAP	0.35	0.35
IBA	0.5	0.5

Biometric indicators in proliferation and rooting:
- number of shoots and length (cm) shoots per explant
- number of roots per explant and root length

Statistical Analysis

Data processing was performed with Analysis of Variance ($P < 0.05$) Anova test. Data are presented as mean accompanied by standard deviation and standard error. Comparison of mean is made with the Tukey-Kramer test. Statistical Analyses were made with JMP version 7.0.

III. Results and Discussion

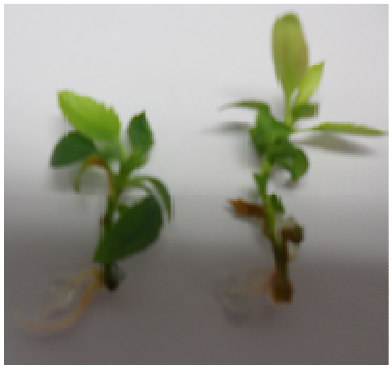
3.1 Proliferation Phase

After 40 days in the inoculation culture medium MS and WPM explants were transferred in proliferation medium. Proliferation percentage was higher (83%) on MS medium, as compared to WPM medium resulting with proliferation percentage (55%). Explants proliferation stage was studied after three subcultures which lasted 20 days each. On the proliferation phase was not observed contamination of pear explants. In subculture phase was observed formation of young shoots with medium green leaves (Fig 1(a) (b)).

Best results in proliferation phase[12] the parameters in study, even statistically verified, were obtained in MS medium: number of shoots (5.1) and shoot length (2.6 cm),as compared with WPM medium: number of shoots (4) and shoot length (2cm) (Table 1 and 2). This successful growth in MS medium is due to higher concentration of N_2 (nitrogen 1471 mg l^{-1}) and K (potassium 781 mg l^{-1}). [10][13][9] have reported successful micropropagation of *Prunus avium* in MS medium. High number of shoots is due to cytokine BAP, which stimulates their development on the stem basis [1] [6].

(a) Shoots proliferation media MS

(b) Shoots in subculture MS



(c) Rooting in media WPM

(d) Rooting in media MS

(e) Rooting in media MS

(f) Acclimatized plants

Fig 1. Pear shoot in in vitro culture stages, propagation, rooting and acclimatization.(a,b,c,d,e,f)

3.1.1 Statistical Analysis (Proliferation Phase)

Table 1. The analyses of the univariable variance in relation to the number of shoots

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Variants	1	19.098143	19.0981	24.6515	<.0001*
Error	56	43.384615	0.7747		
C. Total	57	62.482759			

Graphic 1. The diagram of boxplots (variances, standard deviation and the mean) for the number of shoots per medium

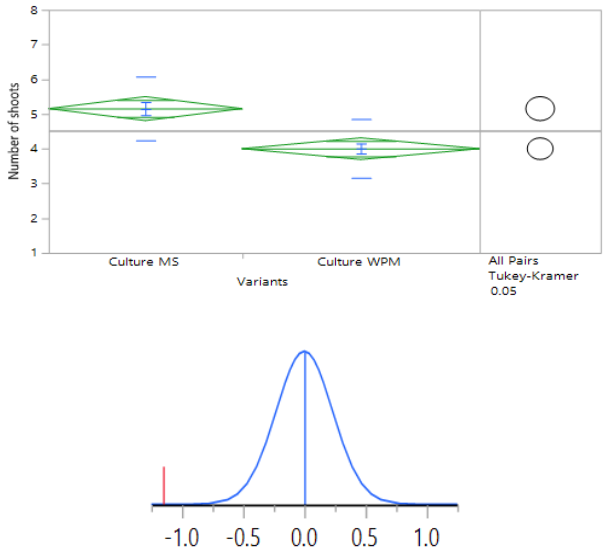
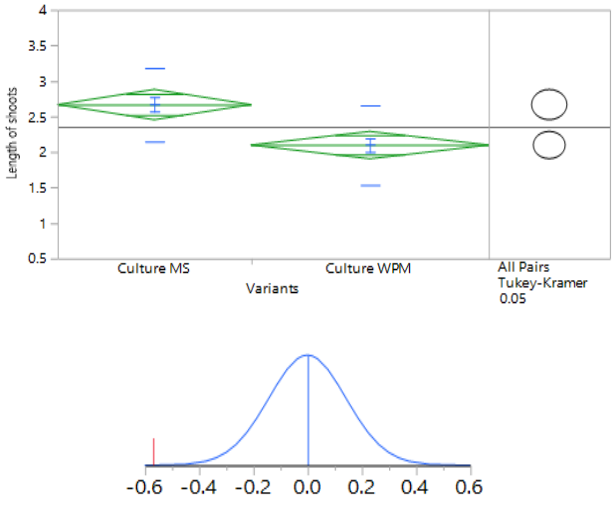


Table 2. The analyses of the univariable variance in relation to the length of shoots

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Variants	1	4.662207	4.66221	15.7787	0.0002*
Error	56	16.546615	0.29548		
C. Total	57	21.208822			

Graphic 2. The diagram of boxplots (variances, standard deviation and the mean) for the length of shoots per medium



Based in the table of Variance Analysis (P <0.05) since the F value is much higher than the theoretical value (Prob> F) then there is a statistical difference for the compared data of number of shoots and shoot length in proliferation phase on MS and WPM medium, shows that we have statistically proven differences by Tukey-Kramer test.

3.2 Rooting Phase

After proliferation phase was passed to rooting phase using rooting medium respectively to the two protocols of proliferation mediums (WPM and DKW), in the presence of the hormone IBA(0.5mg l^{-1}) which induces root formation, the creation of a root system helping rhizogenezis [2]. Higher rooting index resulted in MS medium (70%), number of roots (4.4) and roots length (1.8 cm), as compared with WPM medium, number of roots (2.6) and roots length (0.7cm). Mediums differences are statistically verified (Table 3,4). MS medium [7], is the most appropriate, in which plants grow with big leaves, intense green color (Figure 1 (c) (d)). Role of AIB auxinin inducing rhizogenezis is reported by several authors in rooting of many species [13] [5] [11] [3].

3.2.1 Statistical Analysis (Rooting phase)

Table 3. The analyses of the univariable variance in relation to the number of roots

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Variant	1	69.13636	69.1364	26.0259	<.0001*
Error	86	228.45455	2.6564		
C. Total	87	297.59091			

Graphic 3. The diagram of boxplots (variances, standard deviation and the mean) for the number of roots per medium

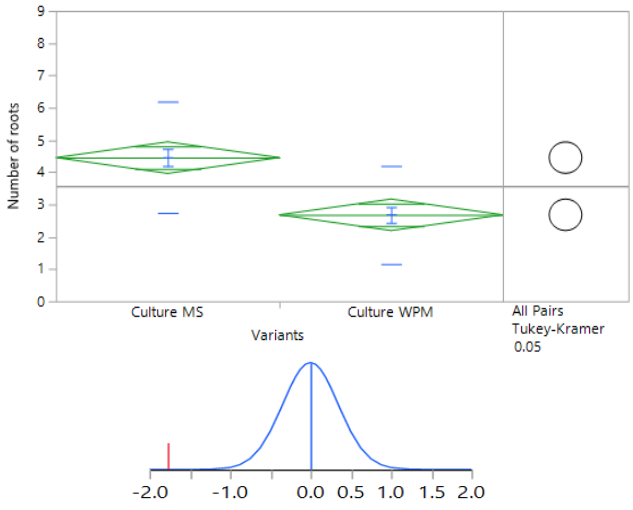
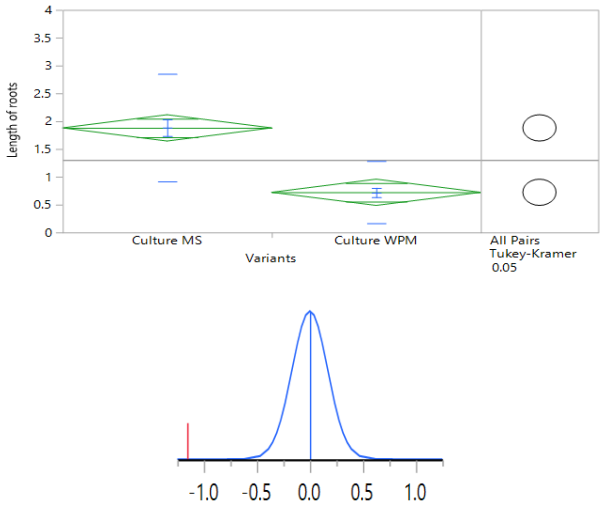


Table 4. The analyses of the univariable variance in relation to the length of roots

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Variant	1	29.475737	29.4757	47.4770	<.0001*
Error	86	53.392470	0.6208		
C. Total	87	82.868208			

Graphic 4. The diagram of boxplots (variances, standard deviation and the mean) for the length of roots per medium



3.3 Acclimatization Phase

In vitro shoots of pear rootstock were transferred in vases with peat and perlite in the nursery, in vivo autotrophic conditions for a month. Percentage of acclimatization resulted 80% and then plants were transferred in big vases under shade. (Fig.1 (f)).

IV. Conclusion

In vitro culture of pear rootstock *(Pyrus Pyraster)* is an efficient method for cultivation and successfully in our country. After analyzing the biometric parameters, results showed that buds, as organized structures possess strong regenerating potential in micropropagation of pear rootstock. The best growth medium in micropropagation (proliferation phase) resulted MS (Murashige & Skoog, 1962), macro-micro MS, vitamin MS, BAP 0.35 mg l^{-1}, IBA 0.5 mg l^{-1}, GA_3 0.1 mg l^{-1}. In subculture phase was observed high micropropagation index in MS medium, in the number of shoots obtained. In rooting phase the universal MS medium (Murashige & Skoog, 1962) gave the best results in numerical and morphological aspect reflected in statistically verified differences.

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Author's Profile

Elektra Papakosta (Spahiu) was born in Vlore, Albania on 27/10/1970. She graduated in Biology-Chemistry in the Faculty of Natural Sciences in University of Tirana in 1993. Then she continued the Master of Sciences in Botany in the Faculty of Natural Sciences in UT in 2006. After she finished her PhD in Plant Biotechnology in the Faculty of Agriculture and Environment in the Agricultural University of Tirana in 2015.

She works as a BIOLOGIST RESEARCHER since 2000 and onward in Agricultural Technology Transfer Center in Vlore, Albania. In the past she was a PART-TIME LECTURER. Some of her numerous articles are:

Elektra Spahiu & al. Callus induction and adventitious shoot regeneration from different explants of rootstocks GF-677 (Prunus amygdalus x P. persica). Albanian Journal of Agricultural Sciences (AJAS), 2015, Volume/issue:14(1)

Elektra Spahiu & al. The effects of the salts concentration on the in vitro micropropagation of GF-677 peach rootstock (peach x almond)". Albanian Journal of Natural and Technical Sciences (AJNTS), 2012, Vol 32 (1): 107-116.

Elektra Spahiu & al. The sanitation of Olive Tree Varietes from viral Infections Using the In Vitro Thecnique. Journal of International Environmental Application & Science (2012), Volume/issue:7(5): 901-906.

As her current research interest is the sanitation of autochthonous grape vine cultivars from viral infections using the *in vitro* techniques.