

**CRAIOVA UNIVERSITY
FACULTY OF HORTICULTURE**

Ph.D. THESIS SUMMARY

**CONTRIBUTIONS TO SETTING THE *IN VITRO* MULTIPLICATION BIOTECHNOLOGY
FOR SOME HORTICULTURAL SPECIES – THE PEAR TREE (*PYRUS COMMUNIS* L.) AND
THE QUINCE TREE (*CYDONIA OBLONGA* MILL.)**

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INTRODUCTION

The necessity to modernize the planting material production technologies for the two species to satisfy the actual standards is also determined by many other considerations such as :

- * increasing the tree density within plantations and adopting intensive culture systems in which the selections of new father plants (predominantly vegetative), creating and introducing new cultivars together with the modernization of tree conduct and carving, let us see a new “era” in cultivating these two species, of course conditioned by the quick providing with bigger and bigger quantities of planting material.
- * contributions to a quick replacement of non-economical sorts from old plantations by way of introduction of a fast pace of new middle-little vigour pear cultivars with fast fruit-bearing that are compatible with the quince and have tolerance towards fire blight.

The research aim

Using the *in vitro* technology for producing the planting material we consider :

- * obtaining bigger planting material quantities
- * reducing the time in producing it compared to classical methods that has as effect a quicker introduction of the new species into culture
- * substituting some sequences of the classical technological flaw that records the biggest “losses” mainly the grafting. It’s about obtaining father plants through micropropagation *in vitro* and using them in situ according to classical technology and micropropagation cultivars of pear on own roots, without grafting (to eliminate the incompatibility with the quince tree).

Objectives of own research

In order to fulfill the research aims the following objectives were set :

- * to study thoroughly the anatomic-morphological aspects, the physiological and biochemical ones of the meristematic tissues of the pear tree and quince tree as theoretical basis for *in vitro* micromultiplication process
- * setting the conduct of some pear tree and quince tree sorts in the organogenesis process
- * setting the best time for gathering the biological material and drawing explants knowing that endogenetic agents (varying depending on season) play a predominant part in preserving the viability and plenipotence of explanted cells
- * determining the nutritive mediums specific to pear tree and quince tree cultivars within different stages of the micropropagation process
- * determining the best environment conditions for different stages within the micropropagation process
- * suggesting a new scheme of micropropagation pear tree and quince tree cultivars.

BIOLOGICAL MATERIAL AND WORKING METHODOLOGY

The biological material used

The cultivars and father plants we used are from the experimental fields of I.C.D.P.P. Pitesti-Maracineni (mother plantations); the tree ages varied from 5 to 15 years old (Tabel 1).

The biological material we used was made up of branches aged 1 year, 30-50 cm long; we drew explants out of them.

Pear and quince cultivars

Tabel 1

SPECIES	CULTIVAR
<i>Pyrus communis</i> L.	Argessis
	Monica
	Republica
<i>Cydonia oblonga</i> Mill.	Moldovenesti
	Aurii
	Aromate

Working methodology

The experiences were made between 2005-2007. A series of exploring experiences within each stage of the *in vitro* culture was necessary because of the complexity of the pursuing aspects and because of lacking information in the specific literature. We explored all 6 pear tree and quince tree cultivars taken for our research and we drew 10 buds from each of them 3 repetitive times to initiate *in vitro* multiplication.

The 4 culture media (basic) we studied are Murashige-Skoog (MS), Fossard (F), Lepoivre (L), Woody Medium (WPM).

▶ Nutritive media used to initiate the culture: basic media were supplemented with dextrose (40g/l), IBA (0.1ml/l), AG3 (1ml/l), Na Fe EDTA (3.2ml/l).

▶ Nutritive media used to multiply *in vitro*: basic media were supplemented with dextrose (40g/l), AG3 (1ml/L), BAP (10ml/L), ANA (2ml/L), Na Fe EDTA (3.2ml/L).

▶ Nutritive media used for rooting *in vitro*: macro-elements M&S (1/2), micro-elements M&S (1/2), vitamins L-S (1/1), indolilbutiric acid (0.5mg/l), giberelic acid (0.1mg/l), Na Fe EDTA (3.2ml/L), dextrose (40g/l).

▶ Nutritive sub-layers used to acclimatize to hothouse conditions the plants obtained *in vitro* are:

1. Perlite
2. Perlite + Red peat (1:1)
3. Perlite + Red peat (2:1)

Experimental versions for initializing stage of *in vitro* cultures

We studied the growing capacity of pear tree and quince tree explants function of the nutritive medium structure, genotype and vegetation stage of the biological material we drew explants from. The experience is three-factorial.

Experimental versions for multiplication stage

We studied the *in vitro* multiplication capacity of the pear tree and quince tree function of the nutritive medium structure, genotype and vegetation stage of the biological material we drew explants from. The experience is three-factorial.

Experimental versions for *in vitro* rooting stage

We studied the *in vitro* rooting capacity of the pear tree function of genotype and photoperiodism. The experience is bi-factorial. Photoperiodism (Factor B):

B. 1=Photoperiodism 16 hours for 35 days; B. 2= Photoperiodism 14 hours for 35 days; B. 3=Pre-treatment dark for the first 9 days + Photoperiodism 16 hours for 35 days; B. 4=Pre-treatment dark for the first 9 days + Photoperiodism 14 hours for 35 days.

Experimental versions for acclimatization stage

We studied the influence of the nutritive sub-layer on the acclimatization process of the pear tree and quince tree. The experience is bi-factorial.

The statistics data interpretation was done through Duncan test and graphic representation of correlations between experimental factors.

OBTAINED RESULTS

***In vitro* culture initializing stage**

The way the nutritive medium acts is generally similar in the case of each of the 3 pear tree and quince tree cultivars we studied. In nutritive medium B.1 (MS) we obtained the best results in pear tree and quince tree cultivars taken for research, the determining part being of the vitamin complex we used and of the hormonal balance achieved by giberelic acid 1mg/l and IBA 0.1mg/l.

The pear tree cultivar called Monica is far from the other two cultivars on the nutritive medium average by 55% grown explants, being excellent in nutritive medium B.2 (F) with 68% grown explants.

As for the quince tree cultivars, Moldovenesti are on top in nutritive medium with 65% grown explants, being excellent in nutritive medium B.4 (WPM) with 75% grown explants.

The pear tree and quince tree cultivars within our research behave differently during the growing explants stage for various times of drawing explants. For the medium effect of the two drawing times the pear tree called Monica is on top with 55% grown explants; as for the quince tree, Moldovenesti cultivar is on top with 65% grown explants.

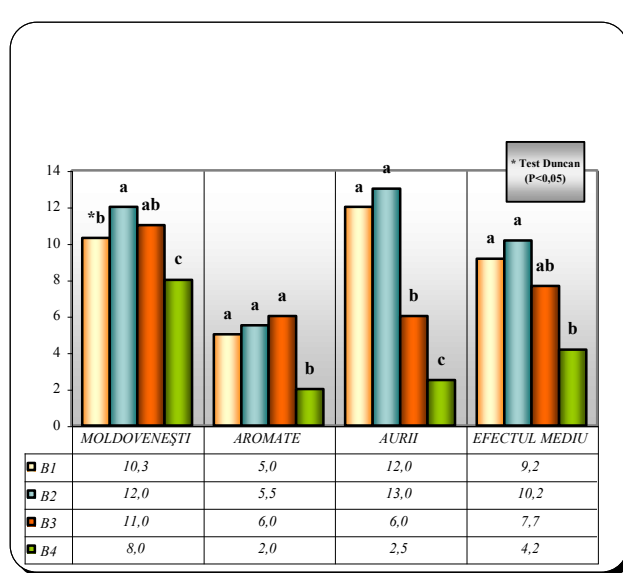
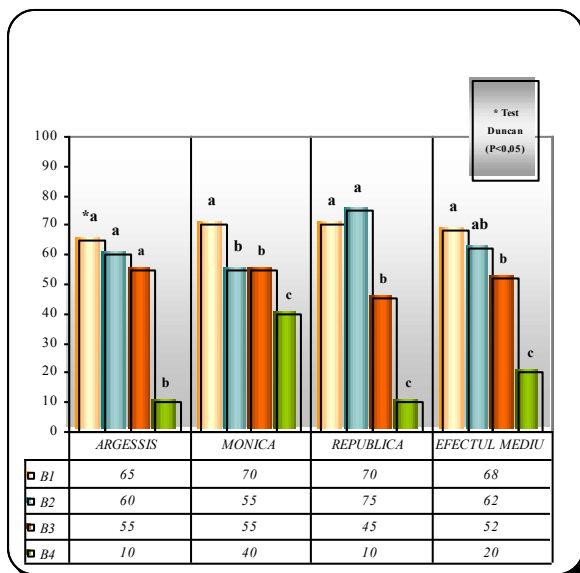
The best results in pear tree are for Monica cultivar, 60% grown explants when we used inocules drawn from branches in out of vegetative pause (February=C.2) to initiate the culture.

The best results in quince tree are for Moldovenesti cultivar, 73% grown explants when we used inocules drawn from branches in out of vegetative pause (February) to initiate the culture.

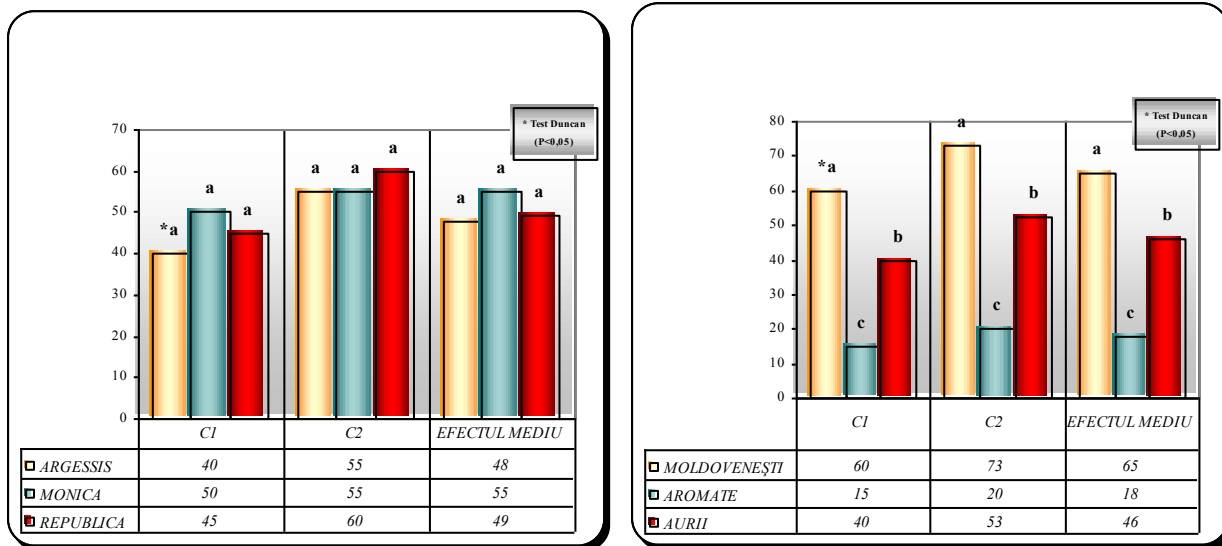
The best results were obtained from explants drawn within out of vegetation stage (February). Using these explants we obtained ranges as 60% grown explants with Republica cultivar and 55% with Monica and Argessis. As for quince trees we obtained 73% grown explants in Moldovenesti cultivar, 53% in Aurii cultivar and 20% in Aromate cultivar.

The nutritive medium influences the growing explants process for various times of drawing explants. Referring to average values of the two explants drawing times, the best results can be obtained in nutritive medium B.1 (MS), namely 68% grown explants for pear trees, respectively 53% grown explants for quince trees.

The interaction of factors nutritive medium X explants drawing time (B x C) shows that in nutritive medium B.1 (MS) for drawing time in out of vegetative pause (February), we obtain the best results, 80% grown explants comparing to 57% grown explants for the researched pear tree cultivars and respectively 60% grown explants comparing to 47% grown explants for the researched quince tree cultivars, in the same nutritive medium but used to initialize *in vitro* culture with explants drawn in the stage of starting vegetative pause (November=C.1). The explants drawing time has a great influence on each experienced medium. The best results for the pear tree cultivars we studied, of 80% grown explants, were obtained from the inocules drawn in out of vegetation pause (February) for the nutritive medium B.1 (MS). The best results for the quince tree cultivars we studied, of 60% grown explants, were obtained from the inocules drawn in of vegetation pause (February) for the nutritive medium B.1 (MS).



Grown explants (%) function of nutritive mediums for pear and quince cultivars



Grown explants (%) function of cultivars for different prelevation moments

Our research results regarding the *in vitro* multiplication stage of pear tree and quince tree

The highest values in multiplication rate were recorded for the pear tree, all cultivars namely 12.0 offshoot/explant; as for the quince tree we recorded 14.2 offshoot/explant.

The highest multiplication rate was 12 offshoot/explant for Moldovenesti quince tree cultivar and 6 offshoot/explant for Aromate quince tree cultivar.

The best results for Argessis cultivar have as nutritive media B.2 (F) and B.3 (L), for Monica cultivar B.1 (MS), B.2 (F) and B.4 (WPM) and for Republica cultivar B.1 (MS) and B.2 (F).

The best results for quince trees are with Auriu cultivar in nutritive medium B.2 (F).

It is obvious again that the nutritive medium as abiotic factor within multiplication process is very important.

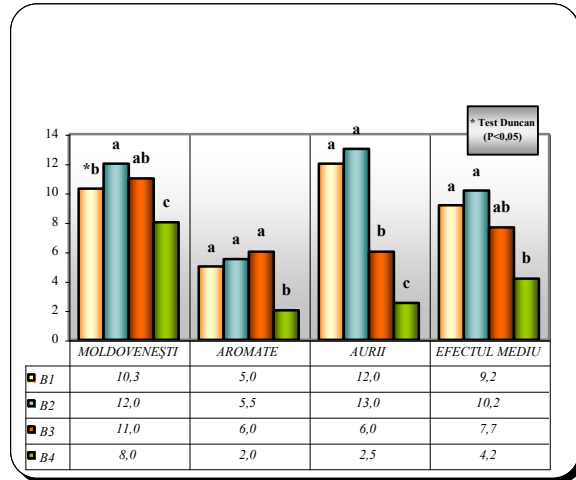
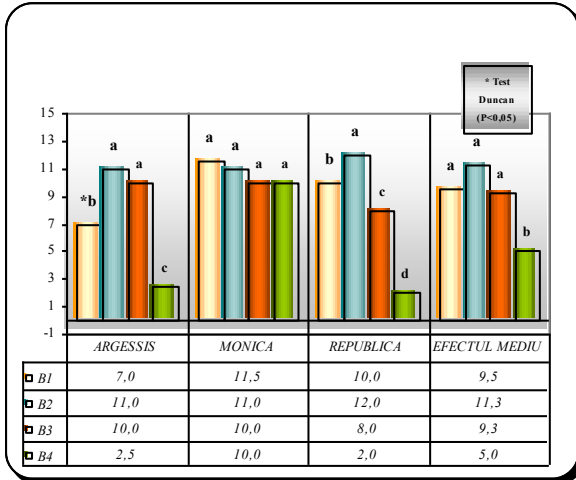
The best results in multiplying rate were obtained in out of vegetative pause (February=C.2).

Taking into account our results, we can say that the cultivar is the most important biotic factor within *in vitro* multiplication process of cultivars. Within the same nutritive medium conditions, the 3 studied cultivars behaved differently ending in different levels of multiplication rate. The cultivar influence was also obvious while interacting with the explants drawing time.

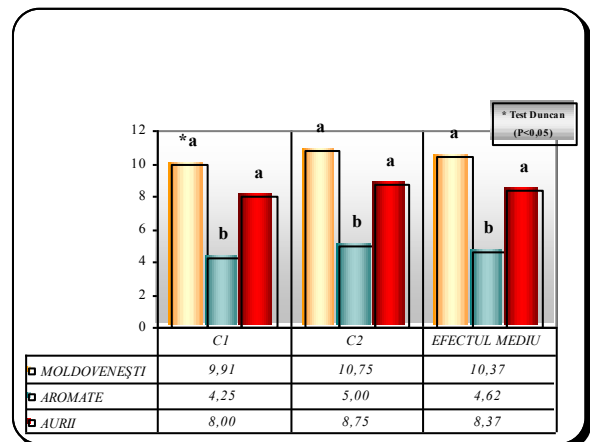
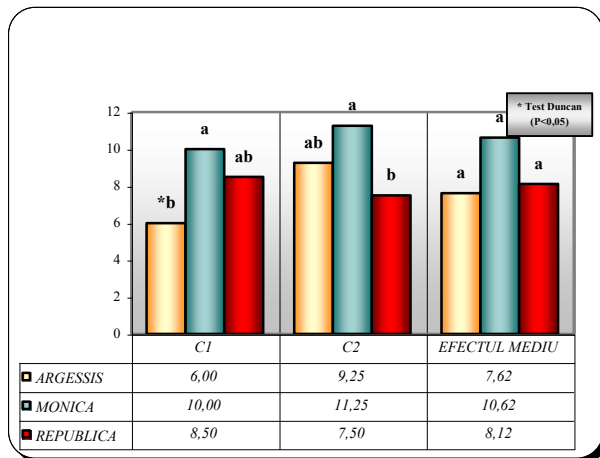
The differences due to the cultivar kind range from simple to double for the same level of the explants drawing time. This way the pear tree, Monica cultivar records a multiplication rate of 11.25 offshoot/explant when out of the vegetative pause (February) and 10 offshoot/explant when starting vegetative pause (November) dislike Argessis cultivar whose multiplication rate was 9.25 respectively 6 offshoot/explant.

The quince, Moldovenesti cultivar recorded a multiplication rate of 10.75 offshoot/explant when out of the vegetative pause (February) and 9.91 offshoot/explant when starting vegetative pause (November) dislike Aromate cultivar whose multiplying rate was 5 respectively 4.25 offshoot/explant.

The vitrification process showed up and was very severe between 20-80% in nutritive medium B.3 (Lepoivre) for the pear tree. The quince tree suffered 5-70% of vitrification especially in nutritive medium B.3 (L). A quince tree characteristic is the vitrification tendency in multiplying stage never mind the nutritive medium. This means that the species is the determinant factor in causing vitrification.



In vitro multiplication rate (number of offshoot/explant) function of nutritive mediums for pear and quince cultivars



In vitro multiplication rate (number of offshoot/explant) function of cultivar for different prelevation moments

Our research results regarding the *in vitro* rooting stage of pear tree and quince tree

The best results for the pear tree out of the 12 experimental variants were obtained for Monica cultivar having a rooting degree of 74.0%.

The best results for the quince tree were obtained with variant V.8 (86.0%) for Aurii cultivar.

The best results were obtained with the variants undertaking dark pre-treatment for 9 days.

Our results confirm that auxins play an essential role in inducing and maintaining the rhysogenesis process.

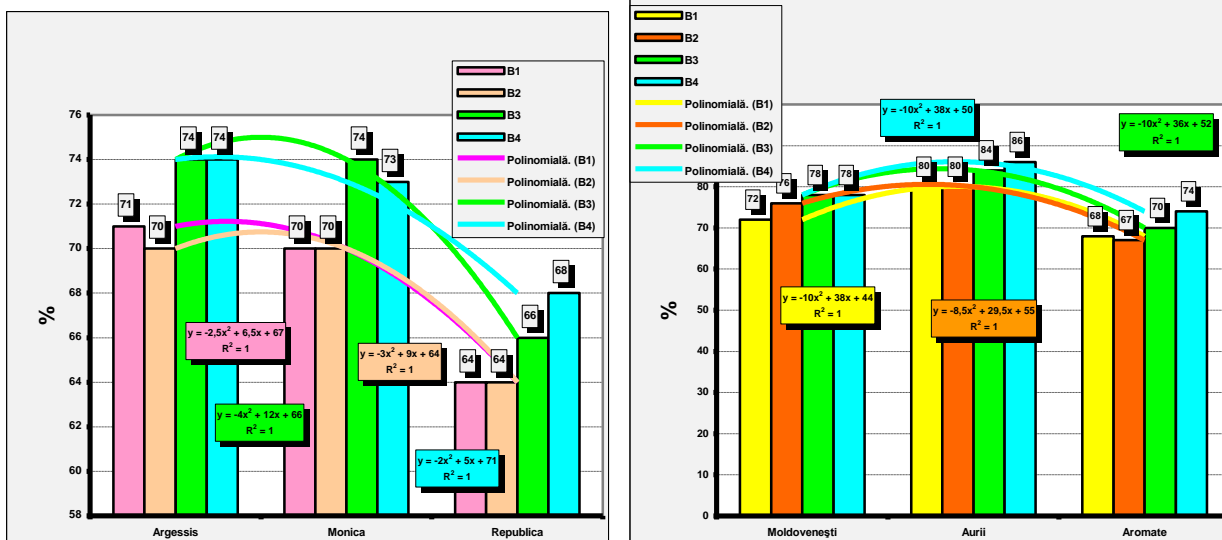
Analysing the interaction cultivar x photoperiodism, it confirms that the potential rhysogene exists in the genetic dowry of each cultivar, is a strong genetic characteristic and produces effects irrespectively the photoperiodism level.

Analysing the rooting degree determined by experimental photoperiodism levels, we notice that the influence of photoperiodism is limited for the researched cultivars.

The highest average root number of plants obtained *in vitro* is recorded both for pear cultivars (2.4-3.7) and for quince (2.4 - 5.5) studied in variants undergoing 14 hour photoperiodism after dark pre-treatment for 9 days.

My opinion is that differences between cultivars, from the point of view of average root number per plant, is the expression of the rhysogenic capacity of any cultivar.

The average biggest root length (1.8 – 3.5 cm) per plant was also recorded for the plants obtained through variants dark pre-treated for 9 days and my opinion is that differences between cultivars at this biometric parameter is due to the genetic character of cultivars.



Correlations between photoperiodism and cultivar during *in vitro* rooting vitropplants

Our research results regarding the acclimatization stage of the pear tree and quince tree

The acclimatization stage is known as the most difficult sequence of micropagation biotechnology of plants through *in vitro* cultures. The best results obtained within the acclimatization process of vitropplants belonging to pear cultivars are quantified from 79.0 – 70.0% acclimatized plants.

The quince cultivars showed superior acclimatization degrees such as 82.0 – 80.0% for the best variants.

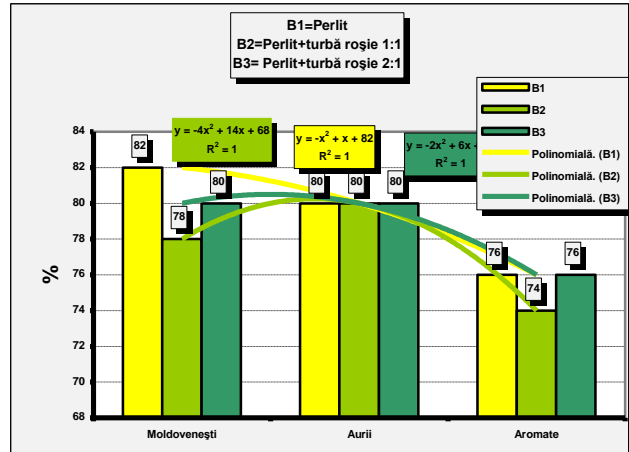
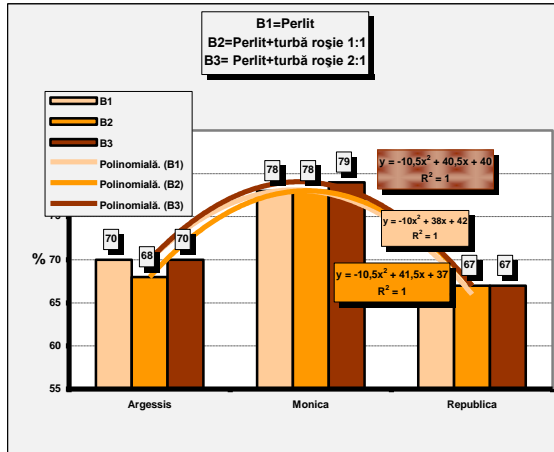
Pear tree cultivars showed the highest acclimatization degree on nutritive mixture B.3 which contains perlite and red peat in proportion 2:1. The red peat assures an optimal humidity in the culture sub-layer.

Quince tree cultivars showed the highest acclimatization degree on nutritive mixture B.1 which contains just perlite, this way rising the production cost.

The nutritive mixture has a very little influence for the pear cultivars in our research.

The quince tree cultivars behaved differently during the acclimatization stage, but their influence on acclimatization degree is smaller comparing to the one of nutritive mixtures.

The coming out of the first little leaves *ex vitro* marked the acclimatization end. The acclimatization lasted for 20 days.



Correlations between of nutritive mixture and cultivars during acclimatization stage

CONCLUSIONS

► The research done and the results obtained make local pear and quince trees multiplying possible in order to satisfy the market needs.

► The main parameters set for each technological stage are :

a) for initiating the culture and growing the plants

* The nutritive medium B.1 (Murashige-Skoog), including a vitamin complex of 5 vitamins and fitohormones IBA (0.1ml/l), AG3 (1ml/l), proved the best both for the pear and quince tree cultivars we studied

* Talking about the pear trees, we can say that Monica followed by Republica and Argenssis cultivars behaved the best

* Talking about the quince trees, we can say that Moldovenesti followed by Aurii and Aromate cultivars behaved the best

* The inocules drawn from branches in out vegetative pause (February) had the most grown explants.

b) for *in vitro* multiplication

* The nutritive medium B.2 (Fossard), rich in vitamins, containing giberelic acid 1 ml/L + naftilacetic acid 0.2 mg/l + benzilaminopurina 0.1 mg/l, was the most efficient both for the pear and quince tree cultivars we studied. That's the confirmation concerning the great importance of the nutritive medium in multiplication process.

* Monica cultivar had the highest multiplication rate, followed by republica and argenssis

* Moldovenesti quince cultivar recorded the highest multiplication rate, followed by Aurii and Aromate

* The very precious results in the multiplication rate were obtained within out of vegetative pause (February).

* A particular characteristic of the quince tree is the vitrification tendency within multiplication stage, especially on nutritive medium B.3 (Lepoivre). The pear tree cultivars also vitrified on nutritive medium B.3.

- c) for rooting *in vitro*
- * The potentially highest rhysogene was recorded at Argenssis cultivar, followed by Monica and Republica
 - * The potentially highest rhysogene was recorded at Aurii, followed by Moldovenesti and Aromate quince tree cultivars
 - * The 14-hour photoperiodism preceded by a 9-day dark treatment determined the best rooting capacity.
- d) for acclimatizing plants
- * The best acclimatization results for pear tree cultivars were obtained on nutritive mixture out of red peat + perlite, in proportion 1:2
 - * The best results for quince tree cultivar were obtained on the nutritive sub-layer containing only perlite
 - * Monica cultivar showed the highest acclimatization capacity, followed by Argenssis and Republica
 - * Moldovenesti and Aurii quince tree cultivars had the best results tightly followed by Aromate cultivar.

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