CONTRIBUTIONS TO IN VITRO PROPAGATION TECHNOLOGY ENHANCEMENT AND VIRUS RELEASE OF SOME CULTIVARS FROM PRUNUS ARMENIACA L.

(SUMMARY)

INTRODUCTION

Cell and tissue culture is very useful for rapid propagation of cultivars, clones and valuable rootstocks, but also for regeneration of healthy plants, free of viruses and other pathogens with high biological value.

The necesity of apricot nursery matherial propagation modernisation is determined by reasons as follows:

- Even greather apricot nursery stoc quantities are needed as a consequence of the of the increasment of apricot orchard densities and shift to intensive growing systems;
- The rapid change of the perimate assortments and cultivars from the oldest apricot orchards (*Cea mai bună de Ungaria*), to replace the low productions and profits;
- Together with rapid propagation of the nursery material, the modern vegetal biotechnologies provide healthy virus free trees, which is very important for apricot, the second top fruit species after plum affected by viruses infections.

Together with rapid propagation of the nursery material, *in vitro* propagation techniques insure healthy viruses free fruit trees, with a longer and profitable exploitation period.

The diseases generated by viruses on top fruit trees lead to economical looses, especially by production reduction and by fruits quality depreciation.

At top fruit trees, the damages caused by viral diseases are amplified also due the fact that once infected with the viruses there are no curative methods of treatments under orchard conditions.

RESEARCH GOAL

The central goal of the researches was to improve the apricot micropropagation biotechnologies in order to:

- Increase of the production of apricot nursery material;
- Reduction of the time required for this material production, compared to the classical nursery methods, leading to rapid introduction of the new apricot cultivars and rootstocks assortments and reduction of the apricot orchards exploitation costs;
- Substitution of some technological sequences in the classical propagation technological flux, especially the ones with the highest looses risk as the sequence of grafting.
- The researches proposition is the micro-multiplication of the apricot cultivars and subsequent growing on their own roots, in order to avoid the incompatibility caused by grafting;
- Multiplication of some healthy material and / or viruses release in the propagation process of high biologically or economically valuable cultivars or clones but previous infected with viruses.

RESEARCH OBJECTIVES

In order to accomplish the research goal, the main research objectives were:

► Evaluation of some apricot cultivars behavior during the organogenesis process, determination of the optimum moment for biological material and meristems prelevation, knowing that the endogen factors (registering some variation according the season) and explants dimensions, play central roles in the preservation of viability and totipotence of explants cells in the micro-propagation process;

► Establishment of the most adequate nutritive media for many apricot cultivars, different steps of the micro-propagation process and also of the appropriate artificial *in vitro* (lab) and *in vivo* (glasshouse) conditions, necessary for apricot micro-propagation technology;

► Establishment of the biological material viral status, using modern methods: ELISA test and release of apricot biological material, free of main viruses by micro-propagation process;

► Elaboration of a micro-propagation scheme fore some valuable apricot cultivars.

BIOLOGICAL MATERIAL AND WORK METHODOLOGY

BIOLOGICAL MATHERIAL

The cultivars studied were preserved and grown in the experimental orchards (for grafting buds production and germplasm collections) owned by Research Institute for Fruit Growing Piteşti-Mărăcineni and Research Station for Fruit Growing Râmnicu-Vâlcea (Table 1) .

The apricot trees are 5 to 10 years old.

The biological matherial consisted in 1 year old twigs, 30-50 cm long, which served as source for explants prelevation.

The studied apricot cultivars

Table 1

Species	Cultivar
Prunus armeniaca L.	**DACIA**
	HARCOT
	VIORICA
	CARMELA
	FAVORIT
	RAREŞ
	MAMAIA
	COMANDOR
	OLIMP
	SULINA
	LITORAL
	CR 2 63
	NJA 19
	EXCELSIOR

METHODS USED TO DETECT THE MAIN VIRUSES ON APRICOTS

The researches were carried out during 2005-2010.

In order to detect the viral diseases, visual observation were carried out in May-August, on the trees belonging to the studied cultivars, and then serological test were done.

Aiming to a very precise detection and identification of the viruses, among all serological methods, the immunoenzymatic method ELISA (Clark and Adams, 1977) was chosen, DAS type (Double Antibody Sandwich), the one in which the specific antibodies are liked with an enzyme.

The biological material consisted in leaves gathered from every studied cultivar.

Among the 14 tested cultivars many cultivars were infected with one two or a combination of *PPV*, *PDV* and *PNRSV* as follow: Mamaia-*PDV*; Sulina-*PDV*; Litoral-*PPV*; Dacia-*PPV*; Viorica-*PNRSV*+*PPV*; Carmela-*PNRSV*; Favorit-*PNRSV*+*PDV*.

THE METHODS USED IN RESEARCHES REGARDING PRODUCTION OF VIRUS FREE PLANTS ON SOME APRICOTS CULTIVARS USING *IN VITRO* CULTURE TECHNIQUES

The biological material consisted in one year old twigs, 30-50 cm long, used for explants prelevation.

Due to the complexity of followed aspects and due to the lack of information in the scientific literature referring to the research objectives, a series of orientation experiments were needed for each of in vitro culture phases.

The orientation experiments were carried on all 14 apricot cultivars included in the study, a number of 10 buds in three replicates being prelevated from each cultivar in order to initiate the in vitro cultivation.

Four growing media were studied: Murashige-Skoog (MS), Fossard (F), Lepoivre (L), Woody Plant Medium (WPM).

The nutritive media used for in vitro culture initiation were supplemented with: dextrose (40 g/l), IBA (0,1 mg/l), GA_3 (0,1mg /l) and Na Fe EDTA (3,2 mg/l).

The nutritive media used for in vitro multiplication were supplemented with: dextrose (40 g/l), GA_3 (0,1mg/l), BAP (1 mg/l), ANA (0,2 mg/l), and Na Fe EDTA (3,2 mg/l).

The nutritive media used for in vitro rooting of the resulted plants the B.2 variant - the basal media Fossard (F) (because in the previous steps of the in vitro propagation this media variant gave the best results).

This media variant was supplemented with indolil butyric acid (IBA) and indoliacetic acid (IAA) in different concentrations, Na Fe EDTA (3,2 mg/l), and dextrose (40 g/l).

THE EXPERIMENTAL VARIANTES USED FOR *IN VITRO* CULTURES INITIATION

During the initiation phase was investigated the apricot explants growing capacity according to nutritive media composition, genotype, explants dimensions, and vegetation stage of the biological material used as source of explants. The experiment is a three-factorial one.

THE EXPERIMENTAL VARIANTES USED FOR *IN VITRO* CULTURES MULTIPLICATION PHASE

In vitro multiplication capacity of the apricot was investigated according to the nutritive media composition, genotype, explants dimensions. The experiment is a three-factorial one.

THE EXPERIMENTAL VARIANTES USED FOR *IN VITRO* CULTURES ROOTING PHASE

There were established the growing conditions which permit the highest and reproducible rooting degree but also formation of a high quality root system, including as much as possible primary roots with homogenous growth and as few as possible wound callus at the base of the apricot micro-shoots.

THE STATISTICAL INTERPRETATION OF THE EXPERIMENTAL DATA

The statistical interpretation of the experimental data was done using both Duncan test and graphical representation of the correlations between experimented factors.

THE OBTAINED RESULTS

THE *IN VITRO* CULTURE INITIATION PHASE

On apricot, the in vitro culture initiation phase consist in study of the explants growing capacity according with growing media composition, genotype, the explants dimensions and vegetation phase of the biological material used as explants source.

Among the 126 variants, the best results were obtained in 12 variants as follow: V.6, V.15, V.27, V.30, V.36, V.39, V.48, V.54, V.57, V.69, V.90, V.114, where the apricot cultivars registered 100% grown explants, regardless the used growing media.

For the meristematic explants obtained from the 14 apricot cultivars, statistical interpretation of the experimental data performed with Duncan test reveal that differentiation rates superior to 50% were obtained with the cultivars Sulina (54,4%), Carmela (52,2%), N.J.A. 19 (51,1%), Favorit and Mamaia (50,0%) on all three growing media.

A constant behavior manifested the cultivars Carmela, Sulina, folowed by N.J.A. 19, Rareş and Dacia. Stataistical analyses of the explant number variation (%) according to growing media reveal not very high diferences generated by the growing media, the highest growing rates (47,4%), being obtained with Lepoivre growing media.

Because one of the research objectives was the production of virus free initial plants, three explants dimensions 0,1 mm (C.1.); 0,1 – 0,3 mm (C.2.) şi 0,3 – 0,5 mm (C.3.) were experimented.

The Duncan statistical analyses carried on regarding the explant dimensions, the average explants initiation were positive (C.3.) or distinct signifificant (C.2.), comparative with C.1.

Data assessment using Duncan test shows that, for apricot plants regenerated from 0,3-0,5 mm explants the viability percentage was 83,8%, in comparation with 18,3% when 0,1 mm were used.

It can be concluded that, when *in vitro* culture is initiated starting with virus free plants and the goal is regeneration and multiplication of biological material, can be used explants of 0,3 mm or higher. In this situation the differentiation process can reach even 100%.

When a genotype or cultivar must be released of one or more viruses, for the initiation phase use of the meristematic doms up to 0,1 mm must be used.

THE *IN VITRO* CULTURE MULTILICATION PHASE

The *in vitro* multiplication phase can be considered the most important step of the micropropagation process, the one which determine number of the plants obtained at the end.

Knowledge of the multiplication rate is useful to establish the subcultures number, according the plantlets number needed to be produced in a defined period of time.

Among the 126 studied variants a number of 14 had a multiplication rates of 10 and 12 shoots per explants, as follows :

- 3 variants with multipliction rate 12 (V.9, V.27, V. 90);
- 2 variants with multipliction rate 11 (V.72, V81);
- 9 variants with multipliction rate 10 (V.6, V.36, V.45, V.54, V.63, V.78, V.87, V. 99, V.126);

It must be noticed that for all apricots cultivars, higher multiplication rates were obtained in the variantes where the explant dimensions ranged between 0,3-0,5 mm (3-6 foliar primordia).

THE *IN VITRO* CULTURE ROOTING PHASE

From the statistical interpretation of the cultivar influence on the multiplication phase, on can observe that for the average of the growing media, the highest multiplication rate (shoots/explant) was registered with the cultivar Viorica (7,8) followed by the cultivar Dacia (7,6) and the cultivars Olimp, Sulina and Excelsior (7,3).

The results obtained regarding the growing media influence on the explants multiplication rates reveal that Fossard media provided the best *in vitro* growing conditions 7,9 shoots/explants, comparative with Murashige&Skoog on which the multiplication rate average was 5,4 shoots/explants.

The analysis of the results regarding the influence of the explants dimensions on the multiplication rate evidenced that the best multiplication rate was on average 8,5 shoots/explants when the meristems used for in vitro culture initiation had the dimensions 0,3-0,5 mm.

As regard the influence of the explants dimensions on the shoots number/explants, can be noticed that the highest number of shoots/explants was obtained when 0,3-0,5 mm explants were used. At this dimensions, the explants had a very good regeneration capacity (8,5 shoots/plantlet).

When meristematic doms of 0,1 mm were used, a number of 4,8 shoots/plantlet were obtained, but in this situation the plants release from viruses is more efficient.

On woody species, the *in vitro* rhizogenesis is frequently the most difficult one, especially for *Prunus* species.

The obtained micro-cuttings were realized immediately after the multiplication phase.

By assessment of the rooting degree for all 14 apricot cultivars taken into the study it was concluded that the best results were obtained when the following variants were used:

- *V3* (BM with macro- and microelements reduced at 1/2 + IBA 1 mg/l + 8 days obscurity and 32 days photoperiod of 16 hours),
- *V5* (BM with macro- and microelements reduced at 1/2, supplemented with IBA 1,5 mg/l, the plants being maintained 8 days in darkness, and then passed on BM with macro- and microelements reduced at 1/2, but without auxines and with a photoperiod of 16 hours), when the rooting rate ranged between 75 - 93%.

The rooting of the obtained micro-cuttings can be done immediately after multiplication phase.

Regarding to the influence of the cultivar, it was concluded that, the best results on in vitro rooting phase of the apricot were obtained at the cultivars: Comandor (93,0%), Favorit (92%), Dacia and Favorit (91%) and Viorica (90%).

As regard the *mean number of roots per plant*, the best results were obtained with the cultivar Comandor (4,9), followed by the cultivar Viorica (4,7) and Rares (4,5)

THE DEGREE OF RELEASE FROM VIRUSES AT SOME APRICOT CULTIVARS MICRO-CUTTINGS GROWN *IN VITRO*

At the variants establishment, in the micro-propagation process, fro this objective accomplishment, the explants dimensions were taken into the account, knowing that the degree of release from viruses is proportionally inverse with the explants dimensions (Deogratias J.M.,1987).

With the increase of the subcultures number, the viral infection degree decrease regardless to the explants dimensions used for *in vitro* culture initiation, although this phenomena is not identical for each cultivar and virus.

As smaller the explants are (at about 0,1 mm), keeping as much as possible the regeneration capacity, the virus release process is more efficient, compared with the situation when fro the regeneration are used larger explants (0.3-0.5 mm).

The possibility to remove the viral infections exist even for explants of 0,1-0,3 and 0,3-0,5 mm but the chances are much more lower.

The percentage of the plantlets infected with *PPV* virus decreased with the subcultures number increase and at the end of the rooting phase virus free plantlets were obtained when small size explants of 0,1 mm were detached from the three apricot cultivars (Comandor, Dacia, Favorit) and medium size explants of 0,1-0,3 mm were detached from the cultivar Viorica.

For large size explants of 0,3-0,5 mm the percentage of infected plants droped down to 23,3-26,6% face to 33.3-70,0% registered after the first subculture.

The reduction of the infected plantlets percentage was noticed both in the case of *PPV* and *PDV*, depending on the subcultures number and the explant size used for this purpose.

In this sense, when large size explants of 0,3-0,5 mm were used, after the first subculture the percentage of infected plantlets was 60,0% to fall down at 40% after the rooting phase, but using small size explants of 0,1 mm, after the first subculture the percentage of infected plantlets at Sulina cultivar was only 3,3%.

In the case of *PNRSV*, when large size explants of 0,3-0,5 mm were used for cultures initiation, all the plantlets obtained were released viruses after the fourth subculture. After the tests carried out at the finish of the rooting phase, it was noticed a decrease of the infected plantlets (16,6-23,3%) compared to the results of the test done after the first subculture (30,0-40,0%).

Under the researches conditions, the plants released from viruses was more efficient for *PNRSV* than for *PPV* and *PDV* viruses.

CONCLUSIONS

For cultures initiation and explants growing phases of the apricot cultivars:

► In the initiation phase the best results were obtained on basal medium B.2. (Lepoivre) supplemented with IBA (0,1 mg/l); GA_3 (0,1 mg/l).

The cultivars Sulina, Carmela, NJA 19, Favorit and Mamaia registered differentiation percentages higher than 50,0%.

► If the biological material is free of pathogens like viruses, the best results are obtained when large size explants of 0,3-0,5 mm are used. When the biological material is infected with one or more viruses and the final aim is to release the plants of this kind of organisms it is recommended to use meristematic domes of 0,1 mm.

For *in vitro* multiplication phase of the apricot cultivars:

► On the nutritive media B.3. (Fossard) supplemented with: dextrose (40 g/l); GA3 (0,1 mg/L), BAP (1 mg/L), ANA (0,2 mg/L), the highest multiplication rates were achieved.

► The cultivar Viorica registered the best mean multiplication rate followed at a small difference by the cultivars Dacia, Olimp, Sulina, Excelsior, Carmela and Rareş.

► For in vitro multiplication the best results are obtained were obtained when large size explants of 0,3-0,5 mm are used (C.3.).

For *in vitro* rooting phase of the apricot cultivars:

► The best results regarding the rooting percentage (more than 75%) for the 14 apricot cultivars were obtained with the variants:

-***V3*** (BM with macro- and microelements reduced at 1/2 + IBA 1 mg/l + 8 days in obscurity and 32 days photoperiod of 16 hours);

-***V5*** (BM with macro- and microelements reduces at 1/2, supplemented wit IBA 1,5 mg/l, the plantlets being kept 8 days in the dark and then passed on BM cu macro- şi microelemente reduces at 1/2, but without auxines and with a photoperiod of 16 hours).

► The cultivar Comandor registered the best rooting percentage followed at a very small difference by the cultivars: Litoral, Favorit, Dacia and Viorica.

► Analysing the mean roots number per plant for each cultivar and on the eight work variants, it was noticed that the highest mean roots number per plant (3.-,0-4,0) was registered also in the ***V3*** and ***V5*** variants.

Apricot cultivars release from the key viruses

►As the explants dimensions are smaller (keeping as much as possible their regeneration capacity 0,1 mm), more efficient is the release from viruses, in the comparation with the situation when large size explants of 0,3-0,5 mm are used.

► The possibility to eliminate the viruses infections is feasible using small size explants of 0,1 mm and even medium size explants of 0,3 - 0,5 mm, but the chances of viruses remove are much more lower.

► Whit the increase of the subcultures number the viruses infection degree is decreasing, regardless the size of the explants used for *in vitro* cultures initiation even this tendency is not identical for every cultivar or virus.
► Under the presented research conditions the apricot plants release from viruses was more efficient for PNRSV compared to PPV and PDV.

RECOMMENDATIONS

► The use of the growing media established by the researches presented in this work for apricot multiplication and *in vitro* study of some other aspects related to the studied cultivars and their extension at higher scale on some other apricot cultivars.
► Release of plants from key viruses to obtain valuable biologic material useful in the cultivar breeding and apricot assortment improvement.
► Rapid propagation of the new apricot cultivars, to assess them as regard their qualities.
► The apricot micropropagation is like an industry, the activity is running in well organized and controlled spaces, protected against the riscks generated by climatic factors, is not polluting the environment and is a highly intensive activity (on 1 m² of space 5.000-10.000 plants can be obrained).
► Introduction into mass production of high valuable planting material in order to establish orchard with initial apricot trees, in measure to provide grafting stocks, free of viruses which diminish both the production and fruits quality.
► Life extension of the bearing apricot orchards, using high quality biological material obtained by *in vitro* cultures technologies.
► Significant reduction of the time needed for apricot cultivars propagation, at 1 year by *in vitro* cultures technologies application face to 3-5 years, when the classical propagation technology is used.

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