ABSTRACT

Key words: hybridization seedless x seedless and pyrene x seedless; *in vitro* germination; *in vitro* regeneration; *ex vitro* acclimatization.

The thesis theme "***Research on the use of in vitro embryo culture in creating seedless vine varieties***,, represent a new start for the application of modern biotechnological methods to create new seedless varieties of vines with different directions of use and resistance to the adverse environmental conditions.

The experiments were carried out under the Research Station for Viticulture and Oenology Bujoru and Research Institute for Viticulture and Oenology Valea Călugărească during 2009-2012.

The thesis sums up a number of 178 pages and is divided in two parts and five chapters:

The first part presents the bibliographic study and covers general issues concerning the methods used to improve seedless vine varieties.

The second part presents an overview of the natural environment in which the research was conducted, the used material, the research method and the obtained results.

Chapter I *General considerations about the use of in vitro embryo cultures in the process of creating seedless vine varieties* (*Vitis vinifera L.*) contains an extensive documentary on the history, importance and used method to obtain seedless vine varieties.

The issue of using in vitro embryo culture in creating seedless varieties is structured in four subchapters, explaining the genetic formation mechanism and the amelioration methods used to create seedless vine varieties. Great advantages in the entire range of use of seedless grapes show that, in addition to using traditional raisin production, the fresh consumption is more hygienic. From these reasons, it is currently necessary to improve the resistance characteristics of seedless vine varieties to stress, thus to increase the production and the fresh consumption of grapes, given the growing preference for seedless grapes.

In subchapter 1.2 is defined the genetic mechanism of the seedless varieties formation.

The knowledge of seedless vine varieties formation under both aspects of the specific determined causes, and in terms of their lineage transmission effect plays an important role in the

amelioration of existing seedless varieties, and in the creation of new varieties seedless through hybridization and continuous selection.

Subchapter 1.3 presents methods for obtaining seedless vine varieties. The conventional methods used in genetic amelioration of seedless varieties of grapes are based on hybridization between seedless varieties used as female genitors and seedless varieties used as pollinators (male genitors). Using conventional breeding methods based on hybridization between seeds x seedless genotypes in ex vitro conditions leading to a wide variety of hybrids in F1 and a small percentage of individuals who have the desired characteristics, and by applying the selection to the obtained progeny results a low percentage of seedless plants.

Also the polyploidy plays an important role in the vine development. Induced polyploidy present great prospects for horticultural species who breed vegetative and at which the low fertility or lack of fertility of some types of polyploids is not a problem. These species sterility of polyploids may be advantageous because it leads to the formation of seedless fruits thereby increasing production quality. Another method of genetic improvement of the vine that was presented is the induced mutagenesis with physical and chemical mutagens, relatively new method compared to traditional used: hybridization and isolation of spontaneous somatic mutations. The last chapter is dedicated to the unconventional methods used in creating seedless varieties of grapevine, which is the theme of this research. By using embryo culture as embryo rescue technique and developing them in plants represents an effective method to improve seedless genotypes.

The efficacy of in vitro transfer technique of immature embryos greatly increased by the use of molecular markers for the identification and selection of the best genotypes in the hybridity process.

Currently, in order to counter the disadvantages related to the amelioration of seedless varieties through conventional methods the research is focused on deciphering the genetic determinism by developing biology molecular techniques of the seedless character. In this regard, the molecular markers can be used to identify the genes that control seedless character and thus speed up the process of introducing genes into a particular valuable seedless genotype.

Chapter II- The actual research stage of the vine embryo culture is divided in two subchapters that presents all major plant-breeding methods of seedless vines with accentuated

reference to the issue of the transferring *in vitro* process of the embryos and the involved factors in the process of obtaining hybrid plant**s.**

Chapter III is for the description of the purpose, objectives and the institutional framework where the research was carried out. This chapter contains information on the structure of the laboratory and of the ampelographic collections of RSDVV Bujoru and RIDVV Valea Calugareasca**.**

The research conducted in this thesis aimed to obtain sexual progeny, using as genitors seed and seedless varieties, ensuring the full embryo development of *in vitro* culture.

To fulfill the purpose of research were pursued a number of objectives:

- The study of the possibilities to achieve embryo culture from intraspecific hybridization of seedless x seedless and seeds x seedless;

- Establish the optimal conditions which determine the rescue and the development of immature zygotic embryos *in vitro* conditions (inoculums period, the mediums culture composition, and the nature of the stimulating growth substances);

- The verification of the efficiency of the *in vitro* method compared with the conventional method for obtaining varieties in *ex vitro* conditions.

Also in this chapter are presented the participated genotypes in the hybridization (White Apiren, pink Ceauş, white Ceauş, and black Kiş miş) and the information regarding the techniques and used methods in the laboratory. Therefore are described working protocols used in making *in vitro* embryo culture to save immature embryos, germination, regeneration, rooting and acclimatization of hybrid plantlets and *ex vitro* conditions for obtaining hybrid saplings.

The last part of this chapter presents the statistical methods used for the processing of the experimental results. There are made specifications regarding the significance of the limit differences, the correlation and regression coefficients and graphic method through which were interpreted the experimental results.

Chapter IV – The results of the research about using in vitro embryo culture and the process of creating seedless vine varieties, presents results of the conducted research during doctoral studies. It is structured in five chapters and presents the obtained results for the *in vitro* and *ex vitro* culture.

Through the in vitro culture was possible to save the immature embryos on four hybrids combinations obtained by crossbreeding of seedless x seedless and seed x seedless at different times after pollination with the result of embryos growth and regeneration of plantlets.

As a result of crossbreeding between seedless x seedless (White Apiren X black Kis mis) seed x seedless (white Ceaus x black Kis mis, pink Ceauş x black Kis mis and Black Coarna x black Kis mis) were obtained a differential number for each carried combination of hybrid embryos. Berries were harvested at three different postanthesis dates 52-69-86 after anthesis. In total aseptic conditions have been excised embryos and inoculated on culture medium Murashige & Skoog basic, under controlled environment conditions 25 ± 2^0C and 85-95% humidity for 13 weeks, time needed to save the immature embryos.

The obtained number of embryos has differentiated according to the hybrid combination and the time of berries harvest. Thus, the hybrid combination white Apiren x black Kiş miş, the highest percentage of embryos was performed at 69 days after pollination (34.3%) and at an insignificant difference the obtained embryos at 52 days after pollination (33.5%).

It showed that at 86 days after pollination for hybrid combination of seedless x seedless was installed the abortion phenomenon of the embryo with a difference to the two previous post anthesis data 1.3-2.1%.

If interbreeding between seed x seedless percentage of the embryos is different from one period to another, showing that on average most of the embryos were obtained at 86 days after pollination with a percentage of 34.03%. Significant increases in the percentage of immature embryo rescue from seedless x seedless crosses at 52-69 days were recorded post anthesis culture medium M & S (1962) added with 2.0 mg / l indolyl butyric acid, 0.5 mg / l indolylaceticacid and 2.0 mg / l benzylaminopurine.

Embryos that developed normally were switched to culture medium Emershad & Ramming (1994).

Upon germination of the embryos and observations were made after 14 weeks of inoculation. Embryos which did not germinate were transferred to fresh culture medium Murashige & Skoog (1962) added with 2 mg / l benzylaminopurine (BAP) to continue the development.

Developing embryos on artificial culture medium through their germination was achieved at a rate of 47.33% according to hybrid combination and the used methodology of culture. In

vitro germination of zygotic embryos obtained from cross breeding of seedless x seedless and seed x seedless was significantly influenced by the basic medium Emershad x Ramming (1994), added with 1,0 mg / l gibberellic acid and 1.0 mg / indolyl acetic acid.

Research results regarding the expression of the regeneration capacity of explants of the four hybrid combinations showed that the differentiation and growth is possible only in the presence of a specific culture medium characterized by the concentrations of cytokinine that determines the stimulation of organogenesis processes.

The number of regenerated shoots by simple direct organogenesis was between 40 and 59 depending on the hybrid shoots combination. The highest number of shoots was obtained with the combination of pink Ceauş x Kiş miş followed by white Ceauş x black Kiş miş.

From the point of the regeneration rate is highlighted the crossbreeding between white Ceauş x black Kiş miş hybrid combination with a regeneration rate of 73%, while the opposite cross breeding is highlighted white Apiren x black Kiş miş combination with a percentage of 57.14% regenerated shoots by direct organogenesis.

The percentage of the regenerative callus resulted from germination of the immature zygotic embryo at the age of 4 months was between 35.7% and 44.8%. In terms of the number of regenerated shoots via callus was obtained between 6.18 and 8.21 shoots / callus regenerating.

The most significant results were obtained from the hybrid combination of black Coarnă x black Kiş miş horns with the highest number of regenerated shoots/regenerative callus. Regarding the regeneration of multiple direct germination (polyembrions), significant results were obtained from the combination of seedless x seedless (white Apiren x black Kiş miş) respectively 18.9%. Other hybrid combinations have polyembrions regeneration rate between 1.04 and 7.73%.

Our research have highlighted that in in vitro conditions the elongation process of shoots according to the explants type revealed the superiority of the growth capacity of shoots derived from direct organogenesis and from polyembrions.

In the in vitro regeneration process the growth regulators also have an important role.

Our research showed a significant stimulation regarding the ability of regeneration at 1.5 mg/l BAP concentration, exerting a stimulating action of the shoot elongation process.

To induce the rooting to shoots was used the basic medium culture Murashige Skoog with different hormonal balance of AIA and ANA auxine. The role of the two auxine had a different

impact on rooting capacity. In the case of white Apiren x black Kiş miş hybrid combination on the medium culture added with indolyl acetic acid 1 mg/l has favored rooting capacity of 71.22% compared with the influence of naftilacetic acid 1 mg/l which favored rooting capacity regenerated shoots only of 65.11%.

In the case of hybrid combination of pink Ceauş x black Kiş miş the auxine influence has been reversed, in the sense that indolyl acetic acid favored a rooting capacity of 72.11%, and naphthyl acetic acid with only 65.67%.

The results of the research carried out at the stage of acclimatization, highlighted the importance of this stage in order to obtain progeny using the immature zygotic embryo culture in vitro method. Comparative analysis of results from acclimated hybrid progeny from the four combinations, indicate that they had a differentiate tolerance the stress factors.

Thus, it shows that the highest percentage concerning the acclimation class ranges between 40-70%, differentiated on each hybrid combination. Significant differences between acclimatization classes are noted. The combination of black Coarnă x black Kiş miş has the highest percentage of acclimated descendants between 51-60%, for pink Ceauş x black Kiş miş the percentage of acclimatization ranged between 61-70%, followed by the hybrid combinations white Ceauş x black Kiş miş and black Coarnă x black Kiş miş.

The worst results for the acclimatization process in terms of stress factors were obtained by the hybrid combination white Apiren x black Kiş miş. Analyzing plant phenotypic variability obtained from in vitro culture of immature embryos, in all genotypes analyzed was recorded an intermediate type behavior (adult and juvenile). Most of the analyzed plants presented similar characteristics with vine plants grown from seeds: the leaf disposition after a spiral filotaxye and tendrils absence.

For the creation of vine varieties was used in our experience also the conventional method. To obtain hybrids descendants was used mature seeds from three hybrid combinations of seed x seedless. Between the seedless x seedless forms, this method cannot be used for amelioration due the interruption of the evolution of the embryo development, so are formed only the rudiments of the seeds.

By analyzing the data related to the way to induce the ability to germinate in vitro and ex vitro was achieved differentiated. In vitro embryo germination was achieved on an average of 50.01% compared with embryos from mature seed germination sown in nutrient substrate, where

was obtained a germination average of 24.67%. The terms of plant acclimation to the solar conditions found no significant differences between the two ways to obtain hybrid *in vitro* and *ex vitro* plants.

At the end of the Thesis are presented general conclusions and studied references.