Ph.D. THESIS

Research on *in vitro* cultivation of potato (*Solanum tuberosum* L.) varieties rich in antioxidant compounds.

(SUMMARY OF Ph.D. THESIS)

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INTRODUCTION

Along with the population's tendency to consume more fruits and vegetables rich in antioxidants, Romanian farmers began to show interest in introducing into the culture some varieties of *Solanum tuberosum* L. with an increased content of anthocyanins in the tubers. In recent years, numerous varieties of potatoes with purple pulp (CPV) have started to be cultivated in Romania, but having no clear information yet, on their capacity for acclimatization and production in the climate conditions of our country.

The doctoral thesis, entitled "Research on *in vitro* cultivation of potato (*Solanum tuberosum* L.) varieties rich in antioxidant compounds" aims to carry out laboratory research aimed, among other things, at optimizing an *in vitro* growth and development protocol of some potato varieties with purple flesh. The research also involved the biochemical study of naturally occurring antioxidants, as well as transmission electron microscopy analysis of anthocyanin-producing tissues. In the end, the optimization of the *in vitro* micropropagation protocol can be the basis of a green technology, to consume microtubers or use them as planting material for agriculture.

STRUCTURE OF THE DOCTORAL THESIS

The doctoral thesis comprises a total of 150 pages and is structured into two parts:

- Current state of knowledge – four chapters, 48 pages (32%);
- Personal contribution – five chapters, 102 pages (68%).

During the research, 224 bibliographic references in the field were tracked and analysed, to report the results obtained most realistically manner and to emphasize the originality and the innovative potential of the doctoral thesis. The work contains a number of 22 tables, 47 figures which contain 20 individual graphs and 80 original photographs. From the total of figures and tables, 24 are provided from a statistical point of view (16 figures and 8 tables).

PURPOSE AND OBJECTIVES OF THE RESEARCH

The central aim of the PhD thesis was to study the purple-fleshed potato (PFP) to provide an *in vitro* culture protocol.

The main objectives of the research carried out in this doctoral thesis consisted in:

1. Determining the degree of knowledge and acceptance of PFP varieties among the population of Sibiu County, to raise awareness of the nutritional value of PFP consumption;
2. Optimizing a complete *in vitro* work protocol aimed at the *in vitro* growth and development of some CPV varieties;
3. Identification and biochemical determination of some bioactive compounds in *S. tuberosum* obtained through *in vitro* cultures and mother plant to verify the presence or absence of anthocyanins;
4. Analysis of ultrastructural elements by transmission electron microscopy (TEM) for *S. tuberosum* plantlets and microtubers from *in vitro* culture to cytologically analyse the tissues involved in the synthesis of anthocyanins.

PERSONAL CONTRIBUTION

The first part of the research sought **to apply a questionnaire** to identify the degree of knowledge and acceptance among the respondents of purple potato varieties. The

target group was made up of the residents of Sibiu County, Romania. Through this study, the level of knowledge about the existence of PFP varieties and their health benefits, together with the openness shown for the consumption of such varieties, was determined among the surveyed respondents. All collected responses (279) were confidential and anonymous, later being interpreted using the statistical program SPSS, version 13.

In **the second part** of the scientific research, the initiation, growth, and *in vitro* development of the potato varieties studied, namely: `Christian' (CH), `Blue Danube' (BD), `Salad Blue' (SB), `Purple Majesty' (PM), `Violet Negretin' (VN) and `Violet Queen' (VQ) was followed. In this context, the optimal variant for the plant material asepsis for *in vitro* inoculation was tested, resulting in a bifactorial experience of the 3x2 type, with 6 experimental variants, from the interaction of the 2 factors (treatment concentration and immersion duration). Determinations were made regarding the survival rate of the inoculum after three weeks from the time of inoculation, and the data obtained were processed in the Polifact statistical program. Techniques for *in vitro* PFP initiation and multiplication were then tested. For the initiation part, a monofactorial experience was used, of the 6x1 type (variety factor), thus resulting in 6 experimental variants. Determinations were made regarding the survival rate of inocula following initiation, and the data obtained were processed with the Polifact statistical program. For the multiplication part, different types of culture medium were tested, aiming at:

I. The effect of glycine on plantlets growth and development

II. The effect of vitamins on plantlets growth and development

III. The effect of active charcoal and chitosan on plantlets growth and development

The studying use of glycine assumed a bifactorial experiment of the 6x2 type, resulting in 12 experimental variants following the interaction of the 2 factors (variety and concentration of glycine added to the culture medium). Biometric determinations were made for the plantlets obtained from subcultures 2, 3 and 4, at an interval of 4 weeks from the moment of transfer, following the length of the shoots (cm), the number of internodes and the distance between internodes (cm). The obtained data were processed by Polifact statistical program. The vitamin study experiment was also based on a 6x2 bifactorial experience, with 12 experimental variants following the interaction of the 2 factors (variety and type of vitamins added to the culture medium), the same determinations being made as in the previous case. The experiment studying active charcoal and chitosan assumed a bifactorial experiment of the 6x4 type, with 24 experimental variants, following the interaction of the 2 factors (potato variety and type of vitamins). This time, the same determinations were made as in the previous cases.

Along with these experiments, optimal working protocols were established for the **initiation of callogenesis and the subculturing of the callus**. For the initiation part, a trifactorial experience was implemented, of the 4x5x2 type, with the factors A (potato variety), B (environment of culture) and C (photoperiod), and a randomized experience with 40 experimental variants was obtained. For the callus subculture, a bifactorial experiment, of the 4x5 type, was applied through the interaction of factors A (potato variety) and B (type of culture medium), resulting in 20 experimental variants. Four weeks after callus initiation, callus formation rate determinations were made, and

six weeks after inoculation of the third subculture, the following biometric parameters were determined: shoot regeneration rate (%), weight fresh (g) and the main callus characteristics such as color and texture. The obtained data were processed by Polifact statistical program.

The optimization of microtuberization techniques assumed the **induction of tuberization *in vitro*** by testing some work protocols, aiming at:

I. The influence of sucrose on the tuberization process *in vitro*

II. The influence of double-phase culture on the *in vitro* tuberization process

To analyse the influence of sucrose on the *in vitro* tuberization process, a bifactorial experiment, of the 6x4 type, was used, and following the interaction of the two factors (potato variety and sucrose concentration), 24 variants resulted. Biometric determinations were made on the rate of tuberization (%), the number of microtubers per plantlets, their weight (g) and diameter (mm). The collected data were processed using the Polifact statistical program. In the case of studying the influence of double-phase culture on the *in vitro* tuberization process, a monofactorial experiment of the 2x1 type (variety factor) was used, obtaining 2 experimental variants. Biometric determinations were carried out as in the previous case.

The third stage of research was devoted to research studies of the content of bioactive compounds of certain potato varieties. These analyses were carried out by spectrophotometry and HPLC (High Performance Liquid Chromatography) methods. Through these techniques, it was aimed to identify the presence of antioxidant compounds, both at the plantlet level from the *in vitro* cultures, and at the microtuber and tuber level.

To identify the place where these bioactive compounds accumulate, the analysis of the ultrastructural elements of the plantlets and microtubers obtained *in vitro* by applying the transmission electron microscopy (TEM) technique was used, analyses developed in **the last stage of the research**.

RESULTS AND DISCUSSION

1. Study regarding the knowledge and acceptance of purple-fleshed potato among the population

Regarding the degree of knowledge of *S. tuberosum* varieties with purple flesh, among the population, a percentage of 36.2% of the total surveyed respondents stated that they did not know that there are also potato varieties that have the flesh of the tuber completely colored in purple, and 69.90% of all respondents have never consumed such potato varieties, but a percentage of 65.20% would like to try to consume PFP tubers and only 1.80% of respondents stated that they are not willing to try to consume these varieties in any form.

2. *In vitro* initiation, growth, and development of studied potato variety

Asepsis. After testing the 6 asepsis treatments for the explants from shoots of the SB variety, the survival percentage of the inoculums was between 24.44% (T_1) and 79.99% (T_6). The expression of the contamination period varied between 2 and 6 days from the time of explant inoculation and depended on the disinfectant concentration used. The treatment with the best results used to achieve asepsis of the explants was the T_6 disinfection method, where a phytoinocula survival rate of 79.99% and very low contamination in the test tubes were obtained.

***In vitro* initiation and multiplication.** In **the initiation process**, 2 methods of sampling plant material were tested: by stimulating the growth of potato buds by excising the "eyes" and placing them in Petri dishes with distilled water after their disinfection, and by using "fangs" of potatoes obtained from storage of tubers in the dark. The first method was not successful, so it was resorted to obtaining explants after sprouting the tubers by keeping them at room temperature, in the dark, for one week. The explants, consisting of potato sprouts, were successfully disinfected using the T_6 asepsis variant, and subsequently inoculated on MS62. Three days after inoculation, hypertrophic processes were observed in all explants. The highest success in the initiation process was recorded in the variety SB (87.50%), followed by the varieties VQ (82.50%), CH and BD (77.50%), VN (72.50%) and PM (70.00%). The success rate for this process was 77.91%. To **multiply the plant material**, it was continued with all the potato varieties studied, by making subcultures carried out on fresh culture medium, by sectioning the plantlets and taking microcuttings that contained 1-2 leaves, respectively 1 or 2 nodes, being organized 3 experiments.

I. The effect of glycine on plantlets growth and development

Glycine added to the culture medium contributed to obtaining longer shoots in CH, BD, SB, PM and VN cultivars, with significant statistical differences being recorded. Regarding the MS62+Gly culture medium, VN variety had a significant increase in shoot height (9.50 cm), followed by SB variety (9.00 cm), BD (8.40 cm), CH (7 .42 cm) and PM (5.70 cm). For the variety VQ, the MS62+Gly culture medium had the opposite effect compared to the other studied varieties, the shoot length was 4.27 cm, approximately twice as short as on the MS62 (Ct) culture medium, but the plantlets were more vigorous compared to those grown on the control culture medium, showing a more robust stem and well-formed deep green leaves. Regarding the analysis of the number of nodes and the internodal distance, the best results were obtained after growing plantlets on MS62 culture medium supplemented with glycine.

II. The effect of vitamins on plantlets growth and development

Each PFP variety was grown on two variants of culture medium, MS62 (control) and MS with the addition of Gamborg vitamins, MS+B_5. There were no significant differences between the two types of culture media for any of the cultivars studied, the average number of shoots per sample being between 1.07 and 2.03. Even in the case of shoot length, no significant differences were recorded, but significant positive differences can be observed between the length of plantlets grown on the MS62 medium (control), compared to those grown on the MS+B_5 medium. The biggest difference in plantlets growth on the two types of culture medium (3.32 cm) in the case of the SB variety, with a length of 7.99 cm on the MS62 culture medium, 4.67 cm on the second variety of culture medium, being followed by CH, PM, BD, VN and VQ variety.

III. The effect of active charcoal and chitosan on plantlets growth and development

In this experiment, 4 types of culture medium were used, as follows: MS62 (control), MS62+C, MS62+A and MS62+C+A. On MS62, the plantlets of all studied varieties showed the best development (6.17 cm–7.99 cm), followed by the medium variant MS62+A with similar values to the control (5.26 cm–7.78 cm). According to the results, the culture media to which chitosan and active charcoal were added or only chitosan, do not lend themselves to in vitro growth for the varieties studied, due to the manifestation of the phenomenon of stagnation in the growth of plantlets.

Initiation of callogenesis and callus subculturing. It was tested to obtain the callus culture at PFP, on five variants of culture media with different concentrations of phytoregulators of growth (PGR) and/or sources of organic nitrogen (glycine). The biological material was represented by tissue fragments from tubers, sterilized with the T_6 treatment. The explant proliferated approximately three weeks after culture initiation. Both for the callus rate and the regeneration frequency for the callogenesis period, the best results were obtained under light conditions with a photoperiod of 16 hours of light and 8 hours of darkness, for all environmental variants tested. The highest frequency of callus was recorded on the C_5 culture medium, containing ANA 5.00 mg/l, GA_3 1.00 mg/l, and TDZ 1.00 mg/l, to which were also added, 15.00 mg/l of glycine. Under light conditions, the callus proliferated generating new plantlets on all the medium variants tested, except for variant C_1, which had 2.50 mg/l 2,4-D in its composition. And this time, as in the case of callus frequency, the best results were also obtained on the C_5 variant, as follows: BD 74.07%, SB 73.07%, CH 70.00%, VN 65 .38%, PM 63.33% and VQ 60.00%. The best results regarding the proliferation and fresh weight of the callus were visible, both on the C_1 culture medium variant with a content of 2,4-D 2.50 mg/l, and on the C_5 variant, having in its composition ANA 5.00 mg/l, GA_3 1.00 mg/l, TDZ 1.00 mg/l, to which glycine 15.00 mg/l was also added. The callus with the highest weight was grown on the C_1 culture medium, belonging to the CH variety (2.06 g), while the lowest callus weight value was recorded on the C_2 culture medium for the PM variety (0.42 g). The predominant color of the cultivated callus was green, except for the callus cultivated on the C_5 culture medium, where the purple color was highlighted in 4 of the 6 potato varieties studied. The texture of the callus was different, varying especially according to the culture medium, from soft to friable and rough.

***In vitro* tuberization.** In the first part of this experiment, the microtuberization process was tested for four variants of culture medium (MS62) supplemented with different concentrations of sucrose (6, 8, 10 and 12%). It continued in the second part, with the study of the behavior of the double-layer culture medium of the CH and SB varieties.

I. The influence of sucrose on the tuberization process *in vitro*

The culture medium with a concentration of 8% sucrose (MT_2) showed the best results, obtaining the following tuberization rates: 37.78% SB variety, 36.67% VN variety, 35.56% CH variety, 33.33% VQ variety, 31.11% BD variety, 27.78% PM variety. The lowest percentage *in vitro* tuberization was obtained using the culture medium with a concentration of 120 g/l sucrose (MT_4), where the tuberization rate was below 7% in the case of all varieties studied. The best results in obtaining microtubers per plant were recorded on MT_2. On this type of culture medium, the most productive variety was SB (3.67), followed by VN (3.00), CH (2.33), VQ (2.33), PM (2.00) and BD (1.67). For MT_1 and MT_4 culture medium variants, all six varieties generated only one microtuber per plantlet. In the variants of MT_2 and MT_3 culture media, the weight of microtubers had a similar value for all studied varieties, being also superior to the weight of microtubers obtained following cultivation on MT_1 and MT_4. For example, in the SB variety, the average weight of the microtubers obtained on the MT_2 culture medium was 1.11 g, while in the culture on the MT_4 medium variant, the average weight of the microtubers of the same variety was significantly reduced, reaching only the value of 0.36 g. Also, in the case of measuring the diameter of microtubers, similar

results were obtained for culture media MT_2 and MT_3, respectively MT_1 and MT_4, but with statistically significant differences. The largest diameter of microtubers was recorded on MT_2 medium variant for SB cultivar with 8.70 mm, followed by CH (8.27 mm), BD (8.03 mm) and PM (7.77 mm) on the same culture medium. The smallest diameter was in the microtubers obtained on MT_4 for the variety VQ (4.07 mm) followed by the varieties VN (4.17 mm), PM (4.23 mm) and BD (4.27 mm), on the same medium of culture.

II. The influence of double-phase culture on the *in vitro* tuberization process

For the CH variety, an average of 10.33 microtubers per culture dish was obtained, a significantly reduced value, compared to the SB variety, where the average obtained was 20.67 microtubers per culture dish. The double-phase culture medium favored the obtaining of microtubers for the SB variety. The SB variety recorded an average of microtubers of 1.08 g, while the CH variety had an average of 0.92 g, a statistically insignificant difference. Even in the case of the length of the microtubers, no statistically significant differences were recorded, even though the SB variety had an average length of 93 mm, and CH only 63 mm. In terms of diameter, the SB variety showed a higher value (9.00 mm) compared to CH (3.67 mm).

3. Identification and determination of bioactive compounds of *Solanum tuberosum* L.

Spectrophotometric analyzes were carried out to compare the amounts of anthocyanins and polyphenols in the tubers and microtubers of the SB and CH cultivars. The amount of anthocyanins determined for the 4 samples is generally quite low. In the case of microtubers of the SB variety (23,839 mg/100 g s.u.), respectively of CH (16,387 mg/100 g s.u.), the anthocyanin content is significantly higher, compared to the amount determined in the composition of the tubers of the same varieties (7,877 mg/100 g s.u. SB, respectively 4.774 mg/100 g s.u. CH). Regarding the total content of polyphenols, it is observed that both microtubers and tubers contain appreciable amounts, the highest value is obtained for the TSB sample (301.6709 mg GAE/100 g s.u.) and the lowest value for TCH (230.7533 mg GAE/100 g d.u.).

In 2 of the 4 varieties with purple flesh, an intense purple coloration was visually identified at the level of the *in vitro* stem, and the identification and quantification of the amount of anthocyanins at the *in vitro* level on the plantlets were resorted to, by HPLC analysis for the SB and VN varieties. 19 phenolic compounds belonging to the subclasses of anthocyanins, benzoic and hydroxycinnamic acids, to which flavones are also added, were identified. The anthocyanidins identified in the two cultivars were peonidin and pelargonidin. From a quantitative point of view, the VN variety is significantly richer in phenolic compounds (1,304.361 μg/g) than the SB variety (842.853 μg/g). If we refer strictly to the anthocyanin content, the SB variety contains a higher amount of anthocyanins (5.72%), while the VN variety has an anthocyanin content of 4.52% of the total phenolic compounds identified.

4. Analysis of ultrastructural elements for anthocyanin-producing tissues

In the **microtubers** of the SB variety, the presence of cells with a large vacuole, active tonoplast with fine electron-dense deposits, due to the precisely expressed sinuosity, of a rough, well-represented endoplasmic reticulum can be noted. Added to these are the presence of well-developed amyloplasts, as well as spherical inclusions such as protrusions. In the microtubers of the CH variety, in the cytoplasm of their parenchyma

cells, the presence of amyloplasts in contiguity with peroxisomes containing rectangular protein bodies can be distinguished, demonstrating a high synthetic activity. In the **tubers` sprouts** of the CH variety, agglomerations of electron-dense materials, mitochondria and profiles of the smooth endoplasmic reticulum are observed at the cytosol level. In tuber sprouts from the SB variety, the presence of metabolically active cells is observed due to the tonoplast membrane which is lined with fine electron-dense material that is also found inside it and is accompanied by the abundant presence of well-formed amyloplasts. Spherical inclusions of the type of protrusions with fine electron-dense material can also be found in their immediate vicinity. The protrusion-type secretory vesicles and the electron-dense material at the vacuole are arranged in contiguity, suggesting the transport mechanism of anthocyanins to the vacuole. These are often accompanied by flattened vesicles of the Golgi Complex and many mitochondria. The presence of proplastids with thylakoid formations in formation is also observed, accompanied by secretion vesicles of the type of protrusions and electron-dense materials, electron-dense deposits arranged at the level of the cell wall in contiguity with the metabolically active plasmalemma and with the tonoplast, also, metabolically active. The sections made through the **plantlet stem** of the CH variety rarely showed electron-dense deposits in the cells, in the vacuoles, and in the cytoplasm plastids and corpuscles (mitochondria) can be identified. On the other hand, in the sections performed on the stem of the plantlets of the SB variety, it was possible to observe, in the cytoplasm, the presence of plastids containing corpuscular, vesicular formations. The ***in vitro* root** from the CH variety can generally be considered as not colored. On the other hand, in the zone of formation of secondary radicles in the SB variety, one can distinguish - in the junction zone - cells naturally colored in red, an appearance due to the presence of anthocyanins. In the case of roots from the CH variety, the presence of rough endoplasmic reticulum, protein crystals of the peroxisome type, as well as spherical inclusions of the protrusion type can be noted at the level of the highly active plasmalemma. Added to these are the presence of electron-dense formations at the level of the protrusions adjacent to profiles of the endoplasmic reticulum and a highly active plasmalemma. Active young cells in which dense REG profiles, mitochondria and a highly metabolically active membrane can be seen. The presence of electron-dense material deposited on the lower face of the tonoplast is also noted. In the roots of the SB variety, the presence of young cells with proplastids that already accumulate starch inclusions between the thylakoid membranes still immature to granna structures is noted. Also here, mature cells are observed, showing a large vacuole, a fine metabolically active cytoplasm, containing peroxisomes, mitochondria, profiles of the rough endoplasmic reticulum, as well as fine deposits of electron-dense material on the inner face of the tonoplast.

CONCLUSIONS

1. **Study regarding the knowledge and acceptance of purple-fleshed potato among the population**

The obtained results highlight the openness of the respondents towards the acceptance of new varieties of potatoes in their diet, and more than that, in their view, purple potatoes are assimilated into a "novel food" product.

2. ***In vitro* initiation, growth, and development of studied potato variety**

Starting from the plant material **asepsis**, the best results were obtained following the application of the treatment: 1 minute immersion in 70% ethanol, 20 minutes immersion in Domestos® solution 20% (v/v) and repeated washing with sterile water, under continuous stirring under a laminar flow hood under sterile conditions. For the **initiation of the PFP** culture, *S. tuberosum* shoots inoculated on the MS62 medium variant were successfully used, ensuring constant growth, following which excellent results were obtained in the ***in vitro*** **multiplication** process on the MS62 culture medium variant with the addition of glycine 15 mg/l. Thus, much more vigorous plants with very well-developed leaves were obtained, compared to the plants obtained after cultivation on the other varieties of culture medium. In the case of **callogenesis initiation and callus subculturing**, the best results regarding callus proliferation were obtained under light conditions with a photoperiod of 16 hours light and 8 hours dark, with an irradiance capacity of 100-112 µmol/ m2/s. The highest frequency of callus in the callogenesis initiation stage was recorded on the MS62 culture medium variant with the addition of ANA 5.00 mg/l, GA_3 1.00 mg/l, TDZ 1.00 mg/l and glycine 15 mg/ l. For callus multiplication, excellent results were obtained on the MS62 culture medium variant with 2,4-D 2.50 mg/l. On the MS62 culture medium variant with the addition of ANA 5.00 mg/l, GA_3 1.00 mg/l, TDZ 1.00 mg/l and glycine 15 mg/l, the callus texture became softer, with the appearance of purple colorings, so it can be stated that glycine stimulated the production of anthocyanins in the callus. The best results were obtained for the SB variety, followed by CH. The presented results showed that **microtubers** can be obtained from the varieties of *S. tuberosum*, taken in the study, on the culture medium supplemented with 8% sucrose, obtaining excellent results, especially for CH and SB. These results are similar to those obtained for classic potato varieties. It has been proven that the double-phase culture method lends itself to the process of obtaining microtubers, obtaining in a relatively short period, a significant amount of microtubers.

3. Identification and determination of bioactive compounds of *Solanum tuberosum* L.

Following the investigation of the content of bioactive compounds with a polyphenolic structure, the obtained results show that for both the SB and CH varieties, the microtubers have a higher concentration of anthocyanins, compared to the tubers. From the plant material of the SB and VN varieties, 5 biochemical compounds belonging to the subclass of anthocyanins were identified, of which 4 belong to the peonidin group and one is part of the pelargonidin group, following HPLC analyses. It has been demonstrated that anthocyanins accumulate in plant material grown *in vitro*. It appears that at the *in vitro* stem level, the VN variety is richer in both phenolic compounds and anthocyanins compared to the SB variety.

4. Analysis of ultrastructural elements for anthocyanin-producing tissues

The analyzed cells are generally young, with an obvious nucleus, active plasma membrane, with obvious sinuosity. In addition, at the level of microtubers, the presence of groups of amyloplasts in contiguity with spherical inclusions of the type of protrusions, often associated with spherical formations present at the level of amyloplasts and with starch granules in different stages of development, can be noted. These are less obvious in shoots. Mitochondria generally associate in clusters near amyloplasts, the rough endoplasmic reticulum, and the nucleus. This obvious association supports the hypothesis of the manifestation of a high cellular energy

demand, essential for biosynthetic processes. The tonoplast of the vacuole is generally lined with fine electron-dense material on the internal face and most of the time, only in the case of purple varieties, it is contiguous with the protrusions at the level of the amyloplasts, also suggesting the transfer of anthocyanins to the vacuole, as well as organized for their storage. The presence of the Golgian Complex completes the metabolic picture of these extremely active cells, to which we also associate the presence of paramural bodies as membrane structures eminently involved in the localization of enzymes with specific roles, necessary for cell development.

Cells originating from purple potato cultivars frequently contain spherical peroxisome-type formations that include protein crystals rectangular in shape and permanently associated with rough endoplasmic reticulum profiles, most often located near the vacuole. The problem of cytological localization of secretion products in general is more difficult, because in the process of preparation of biological materials, they are degraded or lost in sample washing operations. From this point of view, currently, electron microscopy techniques are associated with the use of molecular markers that can precisely identify both the place of synthesis and the route of their distribution at the cellular level.

RECOMMENDATIONS

Based on the results obtained from the research carried out in this doctoral thesis, the following is recommended:

1. Implementation of the *in vitro* cultivation protocol of PFP varieties starting with:
   - taking explants from buds generated at the level of "eyes" sprouted on adult tubers of *S. tuberosum* from varieties with purple flesh;
   - asepsis of plant material for 1 minute immersed in 70% ethanol, followed by immersion for 20 minutes in Domestos® solution 20% (v/v) and consecutive washing with sterile water under continuous agitation, until the complete elimination of the solution of sterilization, under the hood with sterile laminar flow;
   - inoculation of explants on MS62 culture medium to initiate *in vitro* culture to achieve clonal micropropagation;
   - multiplication of vitro-cultivated plant material on the MS62 culture medium variant with the addition of 15 mg/l glycine;
   - inducing tuberization in vitro by implementing double-layer culture;
   - harvesting and storing the obtained microtubers until the time of their consumption or use.
2. The addition of glycine in the culture media aimed at obtaining callus, to stimulate the obtaining of purple callus with hard texture;
3. Obtaining microtubers on an industrial scale to future material for planting or consumption;
4. Cultivation and consumption of the SB variety due to its adaptability to in vitro conditions and also due to the increased content of active principles;
5. The use of the data obtained in this thesis, in obtaining some Romanian varieties of PFP, by breeders, given the fact that there is an increased interest in these potato varieties on the market.
6. Continuation of studies on PFP, by deepening studies aimed at the biochemical composition of plant material.

7. Continuation of electron microscopy studies with the support of molecular markers for strict localization of anthocyanins at the cellular level.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The study carried out shows a profound character of interdisciplinarity and trans-disciplinarity starting from the deepening of fundamental research studies towards applied research. In the doctoral thesis, the fundamental knowledge of biology, plant physiology, biochemistry, genetics, plant biotechnology, optical and electronic microscopy, pharmaceutical and food potential of the studied species was accessed.
As elements of originality, the following can be scored:

- the approach model regarding the initiation and successful *in vitro* multiplication of the 6 varieties of PFP;
- the study of the effect of chitosan and glycine added in the composition of the culture medium, for the growth of PFP plantlets *in vitro*;
- the experimental model, in double-phase, for microtuberization of PFP varieties;
- analysis of ultrastructural elements for anthocyanin-producing tissues of PFP varieties.

The innovative character of the thesis consists, first, in the optimization of a protocol for vegetative propagation of PFP, which is different from white-fleshed potato varieties. Also, the type of study approach, regarding the *in vitro* cultivation of PFP varieties, starting with the initiation process and ending with obtaining microtubers, can be considered another innovative element of the present work due to the association with the methods of morphometric analysis and cellular ultrastructure.
The doctoral thesis is at the laboratory level, and the transfer of knowledge to the pilot level can be easily achieved, following the models already offered by the potato culture, which is why these results can be implemented directly.

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