ADER 3.3.1: Research on the maintenance of the authenticity and health of propagating material for fruit planting, pre-basic, basic and certified biological category use by biotechnological and phytosanitary methods

- **COORDINATOR: Research Institute for Fruit Growing Pitești**
  Project manager: PhD. Catita PLOPA

- **Partner 1: Research Station for Fruit Growing Bistrița**
  Responsible project: PhD. Luminita ZAGRAI

- **Partner 2: Research Station for Fruit Growing Constanța**
  Responsible project: PhD. Cristina MOALE

- **Partner 3: Research Station for Fruit Growing Iași**
  Responsible project: PhD. Margareta CORNEANU

- **Partner 4 Research Station for Fruit Growing Voinești**
  Responsible project: PhD. Gheorghe PETRE

ADER 3.3.1 / 2015 - 2018
OVERALL PROJECT OBJECTIVE

Strengthening of the capacity to meet standards for the production and maintenance of fruit propagating material.

PROJECT SPECIFIC OBJECTIVES:

1. Progress and ensuring technical-scientific standards and their application to maintain the authenticity of fruit propagating material;

2. Progress and ensuring technical-scientific standards and their application to obtain healthy propagating material;

3. Development and operation of the system of production and maintenance of the propagating material from the PREBASIC, BASIC and CERTIFICAT biological category.

• Project budget: 1.061.622 lei
Results expected: Assured and maintaining fruit tree propagation material from Prebasic, Basic and Certificate biological category.

- The evaluation of the viral health status by visual observations; biological testing on woody indicators and herbaceous indicators recommended by OEPP standards; serological testing of new material and retesting of 1/3 of the material maintained in existing mother plantations of, fruit trees, small fruits and strawberry by DAS / TAS - ELISA serological methods and reconfirmation of results by the RT-PCT molecular method in each vegetation period 2016, 2017, 2018.
Biological test in field

Biological test using Luizet woody indicator for Olimp apricot cultivar-mother plant (Photo a) and using GF 305 woody indicator for Redhaven peach cultivar-mother plant (Photo b)
ELISA serological test

DAS-ELISA
Results expected: Implementation of plant protection standards and national legislation on the production of propagating material of higher biological categories.

Results obtained

Maintaining the cultural condition of existing biological material in mother plantations

THE EVALUATION OF THE PRESENCE OF VIRAL PATHOGENS: USE VIRAL TEST – BIOLOGICAL, SEROLOGICAL, AND MOLECULAR METHODS.

PREVENTION TREATMENTS PLAN, APPLIED TO WARNING FOR PENTRU FOR ELIMINATION OF PATHOGENS AND PESTS

HYGIENE MEASURES APPLIED TO PROPAGATION

- CULTURE SUBSTAT LEVEL;
- BIOLOGICAL MATERIAL LEVEL;
- WORK INSTRUMENTS LEVEL

ADER 3.3.1 / 2015 - 2018
Results expected: Efficient standardized protocols regarding viral testing, virus release of fruit tree material.

► In vitro proliferation protocols of Carpatin and Pescăruș plum cultivar.

► Introduction of infected biological material from Carpatin and Pescăruș plum variety in the virus free process for introduction into the certification process for obtaining PREBASIC plants;

► Introduction in propagation process of virus free plants obtained.
Carpatin and Piteștean cultivar- multiplication phase-growth room aspect.
## In Vitro Propagation - Parameters of Some Varieties

<table>
<thead>
<tr>
<th>Species:</th>
<th>Plum</th>
</tr>
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<tbody>
<tr>
<td><strong>Cultivars:</strong></td>
<td>Piteștean, Carpatin</td>
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<table>
<thead>
<tr>
<th>Parameters:</th>
<th></th>
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<tbody>
<tr>
<td><strong>- explant size:</strong></td>
<td>0.2-0.4 mm</td>
</tr>
<tr>
<td><strong>- differentiation culture media:</strong></td>
<td>LF (1977) + vit. Walkey (1972) + 0.01 mg/l aib+ 0.2 mg/l GA$_3$</td>
</tr>
<tr>
<td><strong>- multiplication culture media:</strong></td>
<td>LF (1977)+ vit. Walkey (1972) + 1 mg/l BAP + 0.1 mg/l GA$_3$ + 0.2 mg/l ANA</td>
</tr>
<tr>
<td><strong>- subcultures - optimal number:</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>- rate of multiplication:</strong></td>
<td>6/1 – shoots number/plants - Carpatin variety</td>
</tr>
<tr>
<td></td>
<td>5/1 - shoots number/plants - Pescăruș variety</td>
</tr>
<tr>
<td><strong>- rooting culture media:</strong></td>
<td>LF 1/2 (1962)+ vit. Walkey + 1.5 mg/l IBA + 0.01 mg/l GA$_3$</td>
</tr>
<tr>
<td><strong>- rooting of rate:</strong></td>
<td>70-80 %</td>
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Results expected: Methods and procedures for verifying the authenticity of the material obtained and the genetic stability of plants regenerated by biotechnological methods.

- Assessment of authenticity according to international descriptors and genetic stability by molecular PCR analysis-SSR on strawberry plants obtained by biotechnological methods and maintained biological material:
  - propagation material PREBASIC biological category obtained by *in vitro* cultures: Benton, Redgauntlet, Premial, Magic, Real variety;
  - material maintained in field: Benton, Redgauntlet, Premial, Magic, Real.
Results expected: Obtained of propagation material and maintained in biodepository for established new mother plantation for species with short exploitation period.

► Strawberry mother plantation PREBASIC biological category, varieties: Premial 3,000 plants, Magic 1,500 plants Red Gauntlet 800 plants, Benton 800 plants and Real 1,500 plants/2016, 2017, 2018;

► Strawberry mother plantation BASIC biological category, varieties: Premial 18,000 plants, Magic 17,000 plants, Red Gauntlet 2,000 plants, Benton 1,500 plants, Real 1,500 plants/2016, 2017, 2018;

► Raspberry mother plantation PREBASIC biological category, varieties: Opal 100 plants and Ruvi 100 plants;

► Blackberry mother plantation PREBASIC biological category, varieties: Darrow 100 plants and Thornfree 100 plants.
Results expected: Transfer of the results by supplying propagating and planting material to officially registered producers for the large scale production of propagating and planting CERTIFICATION material.

Results obtained

- Delivery to the producers CERTIFICATE biological category branches of apple: Florina, Idared, Goldspur, Luca, Ionathan, Romus 3, Idared cultivars; grafting branches in the plum: Anna Spath, Tuleu Gras, Stanley, and Centenary; grafting branches of the pear: Williams, Curé, Beure Bosc; grafting branches of sour cherry: Schattenemorelle variety; cherry branches of the: Van and Germersorf cultivars;

- Delivery to the producers BASIC biological category: MM 106- apple roostock;

- Delivery to the producers –blackberry BASIC category, Thornfree cultivar;

- Delivery to the producers–strawberry propagation material Certificate biological category, Premial cultivar;
Results expected: Dissemination results

► "Guide regarding the pathogens with economically importance for fruit propagating material";

► "Cultivars of fruit trees and small fruits admitted to propagation in Romania in 2018".
Results expected: Dissemination results at scientific events

- The resistance of apricot genotypes to the attack of certain pathogens in Romania, 3rd International Symposium on Horticulture in Europe Chania-Grecia, 17 – 21 October 2016, (currently being published in Acta Horticulturae);
- The analysis of the viral status for the PPV, PDV and PNSRV viruses in peach tree and nectarine tree mother plants, Basic biological category at RSFG Constanța, IX International Peach Symposium” Bucharest, 2- 6 July 2017, (currently being published in Acta Horticulturae);
- Viral status of plum mother plantations in three important centers from Romania, (currently being published in Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact and Applied Sciences);
- Plum germplasm resources and breeding in Romania, (currently being published in Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact and Applied Sciences);
► Plum pox virus elimination from some plum cultivars by in vitro culture, FRUIT GROWING RESEARCH, vol. XXXII, ISSN 2286 – 0304;

► Evaluation of the viral status of the Base and Certificate category mother plantations of the main stone species in the Argeș area, FRUIT GROWING RESEARCH, vol. XXXIII, ISSN 2286 – 0304;

► Evaluation of the viral status for main viruses, to apricot mother plants, maintained at RSFG Constanța, FRUIT GROWING RESEARCH, vol XXXIV, ISSN 2286 – 0304;

► Preservation of propagating material of superior biological categories in plum and cherry species at RSFG Bistrita - ADER 331/2015 results. BULETIN DOCUMENTAR INFORMATIV HORTICOL nr. 38: 8-9.
Results expected: Dissemination results workshops, meetings with producers, farmers, etc.

Results obtained

► 29.11.2016, Meeting on the theme "Application of the new European legislation on propagating and planting material from January 1, 2017; PNDR - sub-measure 4.1a, opportunity for the modernization of the fruit-tree nurseries in Romania", event organized by the Academy of Agricultural and Forestry Sciences" Gheorghe Ionescu Sisesti ", Association of Seeders and Seeders Producers and Traders in Romania and Research Institute for Fruit Growing Pitesti. Specialists from the organizing institutions and representatives of MADR, ITCSMS, AFIR, National Phytosanitary Authority, ISTIS, private farmers, fruit-growing research centers, universities, AGERPRES, etc;

► 17.03.2017, Workshop "Tree research in support of farmers – Control of the diseases and pests in fruit trees" organized by Partner 2 - RSFG Constanța and Agro Romania Summit. Participants: Constanta County Agricultural Department, professors from Ovidius University in Constanta, ITCSMS Constanta, private farmers and fruit growers from Tulcea, Ialomita, Brăila, as well as fruit trees in the area.

ADER 3.3.1 / 2015 - 2018
Results expected: Dissemination results workshops, meetings with producers, farmers, etc.

► 16.07.2018, within the Program of Scientific Activity and Technological Transfer, took place the event "Promotion of apricot and peach varieties. Exhibition with tasting " organized by RSFG Constanța in partnership with SUMMIT AGRO Romania. Participants: Research Institute for Fruit Growing Pitesti, representatives of Constanta County Agricultural Direction, professors from Ovidius University in Constanta and Poarta Albă Agricultural College, private farmers / fruit growers from Tulcea, Ialomița, Braila counties, as well as fruit trees from area;

► 06.06.2018, Workshop on "Projects under ADER Program 2015-2018 in progress at RSFG Iasi". Participants: professor and students from the University of Agricultural Sciences and Veterinary Medicine Iasi, amateur farmers and amateurs from Iasi County.

ADER 3.3.1 / 2015 - 2018
The process of producing and maintaining fruit propagation material is a continuous activity.

In order to meet the requirements of current legislation, it is necessary to financial support of the authentic and healthy material maintenance centers, as well as the financial support of the viral testing activity and producing the fruit propagation and planting material from Prebasic, Basic, and Certificate mother plants, under the new legislation ORD. 784/2016.