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Academia de Științe Agricole și Silvicultură „Gheorghe Ionescu Sîrbi”  
INSTITUTUL NAȚIONAL DE CERCETARE-DEZVOLTARE  
PENTRU BIOTEHNOLOGII ÎN HORTICULTURĂ ȘTEFĂNEȘTI ARGES  
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## **SELF-ASSESSMENT REPORT**

***The National Research and Development Institute  
for Biotechnologies in Horticulture  
Ștefănești - Argeș***

**2011**

## SUMMARY

		Pag.
<b>1</b>	<b>Quantitative characteristics</b>	<b>3</b>
	<b>1 Identification data of INCD</b>	<b>3</b>
	1.1. Denomination	
	1.2. Establishing documents with the subsequent modifications	
	1.3. Registering number within the Register of potential contractors	
	1.4. General Manager	
	1.5. Address	
	1.6. Telephone, fax, webpage, e-mail:	
<b>2</b>	<b>General Information</b>	
	2.1. A short history	<b>4</b>
	2.2. Organization chart of INCD	<b>6</b>
	2.3. Specialty field of INCD	
	a. According to UNESCO classification	
	b. According to CAEN classification	
	2.4. Administrative structure diagram of the institution	
<b>3</b>	<b>General activity report of the institution</b>	<b>10</b>
	<b>A</b> Major Research Achievements	<b>11</b>
	<b>B.</b> Grapevine germplasm collection as starting plant material for the national system of producing planting material	<b>13</b>
	<b>C.</b> Recognition of research results at the national level	<b>13</b>
	<b>D.</b> Accredited laboratories	<b>14</b>
	<b>E.</b> Facilities	<b>15</b>
	<b>F.</b> Events organized by NRDIBH Stefanesti with international participation	<b>16</b>
	<b>G.</b> Publicity and information about research department results	<b>17</b>
	<b>H.</b> Training of personnel	<b>17</b>
	<b>I.</b> Looking to the future	<b>19</b>
<b>4</b>	<b>Activity report by team</b>	
	4.1. Genetics, Molecular Biology, Plant Breeding	<b>20</b>
	4.2. Biochemistry and Plant Physiology	
	4.3. Agrotechnology and Plant Protection	
	4.4. Applied Biotechnology	
<b>5</b>	Representative project	<b>29</b>
<b>6</b>	ANNEXES	<b>39</b>

## Quantitative characteristics

### 1. Identification data of INCD

#### 1.1. Denomination:

**NATIONAL RESEARCH - DEVELOPMENT INSTITUTE FOR BIOTECHNOLOGY IN HORTICULTURE – Stefanesti – Arges (NRDIBH)**

#### 1.2. Establishing documents with the subsequent modifications:

- Government Ordinance 78/2003 and the GD 2113/2004.
- Accredited to perform research-development activities financed by public funds in compliance with the Decision of ANCS no. 9634/14.04.2008

**Juridical statute:** Juridical person of common law

#### 1.3. Registering number within the Register of potential contractors: no. 101

**1.4. General Manager:** Eng. Tanasescu Constantin, Ph.D.

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## **2. Self-assessment report for the previous 4 years**

### **2.1. A short history**

The first denomination of our institution was "The Experimental Station for Horticulture and Viticulture of Arges County", which was founded in according to Order no. 498890/April 15, 1959. In a few years, a large range of specialized laboratories were established:

- the laboratories for agricultural technology, grapevine biology and fruit tree biology, in 1959;

- the laboratories for fruit tree technology, plant protection, production of grapevine planting material and winemaking, in 1960;

- the laboratory for plant and soil chemistry, and also the laboratory for soil improvement, in 1961.

Starting with 1960, this Experimental Station for Horticulture and Viticulture became a regional centre for scientific and technical development, engaged in extensive and sustained actions for grapevine and fruit tree growing. The major objective was to produce grapevine and fruit tree planting material, supplying annually the state and cooperative farms with more than 1.3 million grafted plants for establishing new vineyards and orchards. At the end of 1961 there were already established 508 ha of new vineyards and orchards.

After 1967, when The Research Institute for Viticulture and Enology Valea Calugareasca was founded, the Experimental Station for Horticulture and Viticulture Ștefănești-Argeș became one of the branches of this institute, dedicated to viticulture and wine production.

In 1969 the wine factory was put into operation, including its laboratories endowed with high performance equipments. This was a milestone for our research station and its further development. New directions of research were approached, such as: obtaining different types of wines; obtaining a range of new products derived from grapes, must and wine; studies on volatile compounds having a particular importance for the final products. Also, it was adopted a new management, with a scientific basis, in order to control the aging process and distillation of wines.

Since 1980, the Experimental Station for Horticulture and Viticulture Ștefanesti-Argeș became the Research and Production Station for Viticulture and Winemaking, as part of the network coordinated by the Research Institute for

Viticulture and Winemaking Valea Calugareasca. The following decade was a period of remarkable achievements in both research and production activities. The Stefanesti wines, produced by specific and own developed technologies in the wine factory, have won many awards in national and international wine competitions. Both young and old, the wines of *Sauvignon*, *Italian Riesling*, *Feteasca Alba*, *Feteasca Regala*, *Tamaioasa Romaneasca* and *Muscat Ottonel* are those that brought gold or silver medals at various competitions - Lubljana, Bratislava, Montpellier, Budapest, Montreal, etc. In our wine factory were also produced the valuable red wines of *Feteasca Neagra*, *Merlot*, and *Cabernet*, with a very pleasant taste, specific flavour and bouquet.

The quality of Stefanesti wines were guaranteed by a careful control during the winemaking process. Our experimental station was processing almost all the grape yields from Arges County and even neighbouring areas, and the obtained wines were characterized by specificity and distinctive flavour.

The year 1982 marked the beginning of a new stage of development for our research station. The activities were reorganized and new research objectives became priorities, especially in the fields of grapevine breeding and genetics, soil technologies, plant protection and plant physiology. In genetics, the efforts were oriented towards enriching and improving the grapevine assortment, breeding new varieties with resistance to the main diseases being a priority. In the field of wine-making, extensive microbiology studies were initiated in order to establish the specificity of yeast strains for Arges county vineyards. Also, there were improved the techniques for obtaining secondary products from wines, distillation processes, and methods of aging wines.

In 1987, the modern installations for rapid multiplication and virus elimination from valuable biological material started to work. The new Centre for grapevine breeding and propagation joined together the researchers working in the fields of Biotechnology, Genetics, Virology, and Plant nutrition. This was the starting point for a new and modern base in the production of grapevine planting material.

The National Institute of Research - Development for Biotechnologies in Horticulture (NIRDBH), was set up on the basis of Government Ordinance 78/2003 and the GD 2113/2004. The new Institute was established through reorganization of the former Research and Development Station for Viticulture and Enology. Now, is working under the administrative coordination of the Ministry of Agriculture, Forestry

and Rural Development, and also under scientific coordination of the **Academy of Agricultural and Forestry Sciences** "Gheorghe Ionescu - Sisesti".

## **2.2. Organization chart of NIRDBH is presented in ANNEX 1.**

**2.3. Specialty field of NIRDBH.** a) applied research in the field of biotechnology for horticulture, including (involving): *in vitro* clonal propagation, genetic improvement by classical methods and *in vitro* techniques, studies of microbiology and molecular biology, studies on the biology of the pathogens and pests aiming at controlling major diseases affecting vineyards, studies of ecology and protection of horticultural plants, studies on the physiology of horticultural plants. b) turning to account the horticultural biological material and their derived products (virus free planting material, wine and derived products) c) services for: specific grapevine virus detection, GMO detection and quantification, wine chemistry

### **a. According to UNESCO classification:**

2301 Analytical Chemistry; 2409 Genetics; 2415 Molecular Biology; 2417 Botany; 3108 Phytopathology; 3101 Agrochemistry; 3107 Horticulture; 3399 Other technological domain – Biotechnology

### **b. According to CNCS classification**

LS9 Applied life sciences and biotechnology: agricultural, animal, fishery, forestry and food sciences; biotechnology, chemical biology, genetic engineering, synthetic biology, industrial biosciences; environmental biotechnology and remediation

## **2.4. Administrative structure diagram of the institution**

### **(see the conclusive documents)**

At the moment, all the wage earners are full-time employees and the procedures for hiring were in accordance with Romanian legislation and also with Internal Regulation Policy. Researchers are free to undertake their own recruitment efforts when filling Post-Doctoral Fellowship, Research Manager, Research Associate or Student Employee positions.

Out of the total number of the people employed (76), human resources involved in research activities represent 36.5% (see Table 1.).

One ought to underline the fact that all personnel with higher education who are directly involved in research and development activities, are holders of a PhD, or

are PhD students (see the attached Personnel List). Their specialization is in concordance with the principal domains of activities of our institute: Plant biotechnology, Cell and molecular biology, Virology, Grapevine planting material, Genetics and breeding, Biochemistry, Viticulture.

Table 1. Present structure of employed personnel at N.I.R.D.B.H. Stefanesti

Number of employees	TOTAL	Age (years)		
		< 35	36 – 55	> 55
<b>Total:</b>	<b>76</b>	<b>9</b>	<b>55</b>	<b>12</b>
<b>A. Personnel employed on Research Department</b>	<b>27</b>	<b>6</b>	<b>17</b>	<b>4</b>
<b>with higher education diploma in research-development activity (CS, CS1, CS2, CS3)</b>	<b>17</b>	<b>6</b>	<b>8</b>	<b>3</b>
- certified with Ph D diploma;	10	-	7	3
- PhD students	7	6	1	-
- laboratory assistant – secondary school (AS)	7	-	7	-
- auxiliary personnel for Research Department	3	-	3	-
<b>B. Personnel employed on Development Department</b>	<b>38</b>	<b>1</b>	<b>30</b>	<b>7</b>
<b>with higher education diploma in research-development activity (CS2, CS3, IDT, IDTI, IDTII, IDTIII )</b>	-	-	-	-
- certified with Ph D diploma;	-	-	-	-
- PhD students	2	1	1	-
- technicians (TS)	4	-	3	1
<b>-auxiliary personnel for developmental and extension activities</b>	<b>32</b>	<b>-</b>	<b>26</b>	<b>6</b>
<b>C. Administrative personnel</b>	<b>11</b>	<b>2</b>	<b>8</b>	<b>1</b>
- with higher education	4	2	2	-
- auxiliary personnel	7	-	6	1

- the proportion between personnel involved in research activities versus personnel involved in development activities is 1/1.4 (27/338);

- in the Research department a proportion of 63% are PhD or PhD students. The number of researchers participating to the four research teams is in accordance with the volume of activities and relatively well balanced;

- in the Development department only 2 employees are holders of higher education diploma. The activities for producing grapevine planting material are coordinated by a researcher in a half part time;

- the administrative staff represents 14, 4% out of the total number of employees;

- the total number of people older than 55 from the total employees represents 15.8%.

Starting from 2004, the organizational chart of the institute was modified two times, in accordance to the new stages in its mission and organization and also in accordance to the national requirements. Ten research laboratories were designed thought at that moment as separate "spaces", without taking into consideration the human potential and research activities. When three of these laboratories were accredited, the organizational chart was modified to reflect the way in which these laboratories function under direct coordination of the general manager through Quality Management Committee. At the same moment, the former ten laboratories were reorganized, their research personell forming four team groups, in accordance with the priorities established for the research department.

The institute has to meet the duties and responsibilities given by the Ministry of Agriculture and Rural Development and the Academy for Agriculture and Forestry Sciences, to preserve, to keep in repair the state buildings and to maintain the plantations and vineyards, without any financial support. This was the reason of maintaining a strong Development Department, which has the special duty to make capital out of fields and old patrimony.

### **Management structure**

Within the institute, the bodies with decisional power are: the Administration Council, the Directorate Committee and the General Director.

As consultative bodies are: the Scientific Council, the Juridical Office, the Quality Assurance Department, the Department of Audit and Financial Control, and the Public Relations and Mass-Media Department

As executive bodies are: the Research Department, the Economics Department and the Development Department

**The reference terms of the ADMINISTRATION COUNCIL** – according to GD 2113/2004, in our institute, this body has 9 members, nominated by the Minister of Agriculture and Rural Development for a mandate of 4 years, as follows:

- The General Manager of NRDIBH – President;
- 1 representative from the Ministry of Agriculture and Rural Development;
- 1 representative from the Ministry of Education, Research, Youth and Sport;
- 1 representative from the Ministry of Labour, Family and Social Protection;
- 1 representative from the Ministry of Public Finance – Local County General Directorate;
- 1 representative from **Academy of Agricultural and Forestry Sciences** "Gheorghe Ionescu - Sisesti";
- The President of the Scientific Council;
- 2 representatives of employees (as members of the union) from different departments

**The Directorate Committee is represented by:** General Manager, Scientific manager, Economic manager and one permanent guest of the trade-union from our institute.

The duties and tasks of each of these decisional bodies are stipulated in the establishing documents and in the Internal Regulation Policy, having as final aims:

- to state the strategy of the development programs for the NRDIBH;
- to settle the annual program for research and development activities;
- to prescribe the budget of income and expenses;
- to arrange the annual program for investments;
- to supervise and control the quality assurance system and the service activities;
- to verify and control all activities ongoing in research contracts and development area.

**The Scientific Council coordinate the research activities;** is organized according to GD 2113/2004, voted by all institute employees with university education and is composed by:

- President – who was elected by all members of the Scientific Council and is represented in our institute by the Scientific Manager;
- Vice-president - who was elected by all members of the Scientific Council;
- 5 members.

### **3. General activity report of the institution**

The NIRDBH mandate is to promote strategic, fundamental and applied research in the field of biotechnology for horticulture, including both *in vitro* clonal propagation and genetic improvement by *in vitro* techniques. NIRDBH is the only provider of grapevine virus-free planting material for establishing new vineyards, and also one of the most important producers of wines in Romania.

NRDIBH was successfully involved in setting up a collection of 250 grapevine cultivars, free of the main specific viruses. This valuable collection, with native and worldwide grapevine genotypes, represents the source of plant material for research activities promoted within the national programmes and also for production and propagation of planting material (scions and rootstocks) free of viruses and mycoplasmas. The main beneficiaries of this planting material obtained by applying the biotechnology of *in vitro* culture and thermotherapy and maintained in proper conditions, are the grapevine nurseries in the country and even from abroad. The provided planting material is fully guaranteed for cultivar authenticity point of view, and certified for its totally healthy status as well. This is also essential for allowing the export of planting material (scions and rootstocks), both from worldwide cultivated varieties and the highly valuable Romanian table and wine grapevine cultivars.

NIRDBH is charged with the national mandate for research in biotechnology for horticulture and is involved in developing a close interface between basic and applied research for biotechnology in horticulture, meeting both national and EU requirements for the development and transfer of biotechnologies and its products. Moreover, NIRDBH is responsible for development, transfer, and application of biotechnologies, including the enhancement of the knowledge, understanding and application of biological safety. Also, to the institute has been given the responsibility of the identification and detection of GMO and their derived foods. An additional goal is to share knowledge on all aspects of crop biotechnology with all stakeholders, including farmers, consumers, scientists, policy makers, and the mass media.

## A. Major Research Achievements

Table 2 provides an overview of the funding obtained in the period 2007-2011 by the research teams from projects won in national competitions. A few conclusions can be drawn from these tables:

- In terms of money, each year, a proportion of 60% was for salary costs, 16% for overhead charges and 34% for research activities costs;
- In the last 5 years, the total budget for research projects decreased nearly to 50%. This was the direct result of the cutback operations in the ongoing projects. This writing down of capital was applied as freezing the investments.
- Another reason of decreasing the budget for research activities was the lack of new project competitions.
- Although there is a marked tendency for funding to come from external sources, the present economic situation at the national level does not encourage the private companies to be involved in research project as co-financer.
- The research funds from the World Bank (450,000 €), was dedicated exclusively to establish the molecular biology laboratory and also to start the activities required for accredit the methods for detection of GMOs in plants and derived products

Table 2. Financial sources for research activity

Research Program	Year / lei / €				
	2007	2008	2009	2010	2011
<b>TOTAL (lei)</b>	<b>2.380.544</b>	<b>2.135.409</b>	<b>1.951.434</b>	<b>1.479.752</b>	<b>1.292.040</b>
Excellency (lei)	232.000	328.000	-	-	-
Partnership (lei)	43.300	335.000	750.034	438.032	80.000
Sectorial Programme (MAPDR) (lei)	423.004	374.409	107.239	172.122	129.440
NUCLEU Programme (ANCS) (lei)	1.681.740	1.098.000	1.094.161	869.598	1.082.600
MAKIS Project (€)	<b>132,379 €</b>	<b>76,261 €</b>	<b>132,379 €</b>	-	-

The main results from research activities performed within the projects are the following:

- ⇒ Establishing the *in vitro* propagation biotechnologies for horticultural species aiming to be used to an industrial scale;

⇒ Improved *in vitro* techniques of regeneration from somatic tissue for obtaining the genetic material as initial genotypes for genetic improvement of grapevine;

⇒ Efficient *in vitro* propagation methods for horticultural crops and *in vitro* tests for diagnosis the main viral diseases;

⇒ Setting up the grapevine *core* germplasm collection with *initial* and *base* planting material category (with over 250 grapevine varieties);

⇒ Establishing the laboratory of molecular biology and applying molecular analysis for germplasm characterization;

⇒ Obtaining / producing and approving of new varieties for table and high quality wine grapevines;

⇒ Elaboration and application of modern technologies for an ecological type of viticulture, aiming at improving the fertility parameters of the soil and increasing the economic efficiency by cutting down production costs;

⇒ Improving technologies used to obtain high quality sorts of wine, through the application of modern and new techniques;

⇒ Turning to better account the secondary winery products, and producing alcoholic drinks from must and wine.

Beside improved methods/technologies, the plant material obtained as results of the research projects and through accredited methods (Table 3) performed in certain laboratories, represented a supplementary income for the institute, brought by researchers.

Table 3. Revenues from contracts with national private entities, as results from research activities

Item	Products/services	No of contracts / type of beneficiaries
1	Physical-chemical analysis on soil samples	2/private farmers
2	Chemical analysis on wines and alcoholic drinks	25/ private wine producers
3	ELISA tests for virus detection on grapevines samples	12/ research units and private farmers
4	Qualitative and quantitative methods for GMO in plants and their derived products	23/state and private companies
5	Grapevine planting material <i>Initial</i> category- G1	8/ research units
6	ECO vegetables, grafted grapevines <i>Certificate</i> category, ornamental plants	5/ research units and private farmers

Total income from products and services (lei)

2007	2008	2009	2010	2011
41,780	32,408	55,253	64,037	42.145

## **B. Grapevine germplasm collection as starting plant material for the national system of producing planting material**

In the last five years, NRDIBH Stefanesti has gained prominence as **the only owner of a grapevine germplasm collection with “initial” category for Romania**, preserved in proper conditions and according to the Romanian and EU legislation. In this respects the Institute works in close liaison with the Research and Development Institute for Viticulture and Enology Valea Calugareasca and all its subordinated units (Research Stations from Iasi, Odobesti, Pietroasa, Bujoru, Murfatlar, Dragasani, Blaj, and Minis) as the curators and owners of grapevine varieties. The planting material obtained, produced or maintained in our institute was registered and transferred under the direct coordination of local authorities responsible for grapevine material.

Among the directly interested beneficiaries for this material are:

- all Research and Development Station for Viticulture in Romania, which are interested in cultivars conservation, sanitary control of planting material, and complete characterization of the new genotypes, these being essential requirements either for breeding research or production;
- private farmers and state enterprises for growing grapevine, who needs planting material guaranteed for authenticity, and certified for its healthy status;
- All the obtained results will be useful equally for the producers of planting material, seed producers, variety's patents owners, plant growers, food producers, and all categories of consumers.

## **C. Recognition of research results at the national level**

In the last 5 years some of our institute results (technologies, or products) were registered to the national authorities (SIVTR - The State Institute for Variety Testing and Registration and SOIT – The State Office for Inventions and Trademarks) and officially recognized (table 4).

The State Institute for Variety Testing and Registration (SIVTR) is the national authority in the field of the examination of new vegetal creations, in order to be registered in the Romanian Official Catalogue of Plant Varieties. The registration of the varieties from agricultural and vegetable species in the Official catalogue, allows their cultivation and marketing in Romania and EU member states. The State Office

for Inventions and Trademarks (SOIT) is the authority for granting the protection titles in the field of industrial property protection on the national territory.

Table 4. Breeding activities results approved by SIVTR

No.	Registered No / Year	Authors	Patent title
P1	1717/2007	<b>Popa Camelia</b> , Smaranda Gheorghe, Baditescu Margareta	AURIU DE STEFANESTI
P2	4419 /2009	<b>Popa Camelia, Radulescu Ion</b>	MUSCAT ´ADDA 22Şt.
P3	1698/ 2008	<b>Popa Camelia, Radulescu Ion</b>	FETEASCA NEAGRA 6 St
P4	1700/ 2008	<b>Popa Camelia, Radulescu Ion</b>	FETEASCA REGALA 72 St
P5	1699/ 2008	<b>Popa Camelia, Radulescu Ion</b>	FETEASCA ALBA 97 St
P6	1697/ 2008	<b>Popa Camelia, Radulescu Ion</b>	MUSCAT OTTONEL 16 St
P7	1701/ 2008	<b>Popa Camelia, Radulescu Ion</b>	PERLETTE 10 St
P8	4422 /2009	<b>Radulescu Ion, Popa Camelia, Onache Anca Petronela</b>	PINOT GRIS 14Şt.
P9	4421 /2009	<b>Radulescu Ion, Popa Camelia, Onache Anca Petronela</b>	MERLOT 202 St.
P10	4420 /2009	<b>Radulescu Ion, Popa Camelia, Onache Anca Petronela</b>	BURGUND MARE 86Şt.
P11	3317/2009	Oana Maria, Pedrumar Toader, <b>Radulescu Ion, Tita Ion</b> , Tetulea Raul	BURGUND MARE 63 Mn..
P12	3318/2009	Oana Maria, Pedrumar Toader, <b>Costescu Adriana</b> , Draghici Mircea	PINOT NOIR 33 Mn.

**D. An important objective was to establish and accredit laboratories with specific activities (Table 5)**

These three laboratories obtained accreditation after:

- Renewing or reorganizing the laboratory areas;
- Acquisition or improvement the laboratories equipment;
- Attending training courses for specific methods and procedures;
- Passing the standard requirements for accreditation.

The employers working in these laboratories have responsibilities to perform specific analyses for different clients, such as:

- grapevine planting material producers;

- vineyards farmers;
- wines and alcoholic drinks producers and traders;
- farmers cultivating / or seed producers soy and maize possible GMOs;
- private farmers, or state research units having field trials
- growers / or farmers with conventional and organic crops.

Table 5. Accredited laboratories from NRDIBH Stefanesti

Item	Laboratory name	Type of test / materials	Certificate no.	Financial support
1	Virology Laboratory	Serological tests by ELISA technique / leaf, petiole, phloem tissue	LI 590/17.12.2007	Accreditation through Infrac 182 Project Maintenance - NRDIBH
2	Wine Chemistry Laboratory	Chemical analysis – gravimetric, volumetric and spectrophotometric methods / wine, ethylic alcohol and alcoholic drinks	LI 614/14.02.2008	Accreditation through Infrac 174 Maintenance - NRDIBH
3	GMO detection, identification and quantification Laboratory	Qualitative detection of GMO in plant material (soy and maize) and their derived products / seeds, plants, flour, groats	LI 883/21.06.2010	Accreditation through MAKIS project Maintenance - NRDIBH

The same personnel working in the accredited laboratories are responsible to perform research activities and fulfil the objectives in different research projects.

### E. Facilities

The majority of the facilities and equipment used to perform all research activities were acquisitioned and functionally maintained with capital from research projects. In the last three years it was not possible to improve the endowment due to cutting of the financial support from research projects dedicated for new acquisition.

The existing equipments are adequate to a certain level of studies, reflected in the present achievements (Annex 2). All or most of the equipment are in working order, calibrated or verified by institutions in charge for this and keeping records are used to know their status of working. The main investments with new and performed equipments are presented in the attached documents (Infrastructures functioning at the date of submission – document 4)

The endowment and facilities of NRDIBH Stefanesti are currently used to complete activities in the following domains: Virology, Wine Chemistry, Molecular Biology, Plant Breeding, Plant Physiology, Biotechnology, Agrotechnology and Plant Protection.

Other relevant issues are:

- All researchers have their own desks and computers, rapid access to all major programmes and the internet;
- Most researchers have their own room;
- Some researchers share a room with a PhD student;
- Services for computers and ICT connections are secure by a private company through service contract;
- We have internet access to almost all the important journals and publications in the fields of interest for our institute.

#### **F. Events organized by NRDIBH Stefanesti with international participation:**

##### **Conferences**

1. "Genetic Modified Plant Crops in Romania and the National Biosafety Network", 16 November, 2007.
2. "Plant Biotechnologies – Present and Perspectives. The Cultivation of Genetically Modified Plants in Romania and National Biosafety Framework", 18-19 February, 2010

##### **Workshops**

1. "Theoretical and practical Course for virology tests on grapevine planting material – ELISA and PCR methods" – in collaboration with the Ministry of Agriculture, Forestry and Rural Development, and Territorial Inspectorates for Seed and Planting Material Quality Control, 27-31 August, 2007.
2. "DualChip®GMO Kit V2.0-A multiplex GM screening method" – in collaboration with Eppendorf Biochip Systems, 22 January, 2009.
3. "Values and Principles in national and European politics regarding genetically modified crops" – in collaboration with the University of Pitesti and the Biotechnology Commission within Academy of Agricultural and Forestry Sciences, 5 May, 2011.

Annually, at the beginning of the year, the Scientific Council analyzes and approves an internal "Program of scientific events in relatedness with local and central authorities responsible for horticultural activities". In common meetings our researchers, the representatives of different agencies and all specialists involved in certain activities, share knowledge and experience.

#### **G. Publicity and information about research department results**

Between 2007 and 2011, the NRDIBH Stefanesti has a permanently or regularly presence within local TV /radio programs, or local newspapers.

The results obtained from research programmes were presented as:

- Articles in national journals covered by Thomson Reuters, such as "Romanian Biotechnological Letters" and "Notulae Botanicae Horti Agrobotanici";
- Production of books and edited volumes published by national publishers;
- Keynote presentations and organised workshops and meetings at national and international level;
- Participation to national and international events with the results obtained in our institute;
- Continue sending information to potential clients

Although were no publications in journals with relative article influence score, the level of the dissemination of research results outside the scientific community through written publications, but also by presentations and oral participation in debates, is adequate and in concordance with present human potential.

#### **H. Training of personnel**

The responsibilities of all personnel are defined and recorded in job descriptions together with their qualifications and competence defined in education and training records. To maintain adequate levels of competence, the institute bestowed attention on the qualifications of staff, and to both internal and external training given to personnel. The Institute has been offered all support (financing or encouraging) for the employers' participation to different teaching programmes (Table 6) at Graduate, Post-Graduate and Doctorate level in order to develop trained personnel able to meet challenges at national and international requirements.

Table 6. Information regarding the training activities

Place	Course - Title	Period	No. of trained persons	Financial supports
Italy, Universita degli Studi di Udine, Udine	Theoretical and practical course in task: Grapevine germplasm characterization by molecular markers	19-30 March 2007	2	MAKIS Project
RENAR	General Requirements for SR EN ISO/CEI 17025: 2005	18-20 April 2007	1	NRDIBH Stefanesti
Switzerland, Rotkreuz	The PCR Training Course for ABI 7900. Basic Real Time PCR Training Course	23-24 January 2008	1	MAKIS Project
TUBITAK Marmara Research Centre, Gebze Kocaeli, Turkey	Training Course on "The Analysis of Food and Feed Samples for the Presence of Genetically Modified Organisms"	12-16 April, 2010	1	Joint Research Centre European Commission – Molecular Biology&Genomics
Stefanesti-Argeș, EURO Consulting	SR EN ISO/CEI 17025: 2005 applied in accredited laboratories General Requirements, and Method validation	25-30 July 2010	2	NRDIBH Stefanesti
FIATEST Bucharest	Course for auditors formation in quality management systems: SR EN ISO/CEI 17025: 2005 and SR EN ISO/CEI 19011/2003	27 Sept. - 01 Oct. 2010	1	NRDIBH Stefanesti
FIATEST Bucharest	Course Inter-laboratory comparisons	07-09.2011	1	NRDIBH Stefanesti
FIATEST Bucharest	Measurement Uncertainty in testing laboratories	14-16 Sept. 2011	1	NRDIBH Stefanesti

In the past four years, the number of the employed personnel holding a PhD title increased, 2 researchers defended their doctoral theses in the domains of Biochemistry and Virology, as following:

Bejan Carmen - "CONTRIBUTIONS REGARDING THE OPTIMIZATION OF EXPLOITATION REGIME OF SPRAYING IRRIGATING INSTALLATIONS ENDOWED WITH DRUM AND HOSE" - University of Agricultural Science and Veterinary Medicine Bucharest, 2010

Guta Ionela Catalina - "ALTERNATIVE METHODS FOR OBTAINING VIRUS-FREE GRAPEVINE PROPAGATING MATERIAL" - University of Agricultural Sciences and Veterinary Medicine Bucharest, 2010

Now, seven of our employers are PhD students and are working on their theses in the following domains: viticulture, plant protection, plant biotechnology, molecular biology, plant breeding, wine chemistry.

An important task of the Human Resources Office, collaborating with the heads of the departments and depending of available funds, was to support the

training of researchers and specialized staff in the fields of activity which are specific to our Institute, through:

→ attending and participation of young people to theoretical and practical training courses of in the field of biotechnologies and research project management;

→ participation to scientific events (national and international symposia and conferences, meetings) in the fields of interest for our institute (plant biotechnologies, industrial biotechnologies, molecular genetics, genetics of populations, physiology and plants protection);

→ activities for proficiency raising of the research staff, through study grants, training courses in other similar institutions, both in Romania and abroad;

→ supporting the researchers for affiliation as members in national and international scientific societies;

→ organizing a complex system entirely computerized to scientific documentation, including the access to the international databases by Internet.

### **I.Looking to the future**

Taking off the currently financial problems, the teams of researchers joint their efforts and participated to the last research program competition (November 2011) with six proposals. The objectives of these proposals followed the general aims to obtain new knowledge, applicable to farmers' needs that eventually will result in new or improved products, processes, or services. Moreover, with these project was enlarged the range of approached subjects (plant species analysed and methods applied) and beside research units and universities, were involved private companies, as the main beneficiaries of the project results.

**Lists containing the publications and patents, ongoing projects and major equipments and infrastructures are presented in conclusive documents (according to structure to be set by ANCS)**

## Activity report by team

### 4.1. Genetics, Molecular Biology, Plant Breeding

Breeding activities in the NRDIBH was focused mainly on grapevine. Using various crossing and selection methods, and starting from the available populations or germplasm collections, were identified individuals with valuable and highly heritable features as parental material, which allowed obtention of a series of new cultivars. Also, a wide diversity of approaches have been developed in the last years for improving important horticultural traits in grapevine, tailored to the crop species and breeding objectives, as follows:

- Every year, morphological/phenotypical data were analysed and recorded according to OIV descriptors of the local varieties;
- Specific activities of crosses and selection of the most valuable individuals with valuable/improved features for: a) resistance to biotic stresses (bacteria, fungi, insects, pests); b) resistance to abiotic stresses (low temperature, frost, drought); c) higher yielding potential; d) size of grapes, commercial aspect, or special flavours;
- The selected genotypes were analyzed for their features stability in pilot vineyards and after that recommended for multiplication;
- Evaluation of the Romanian grapevine genetic resources by molecular methods (RAPDs and microsatellite markers) aiming to and providing useful information about the genome of each genotype preserved within the NRDIBH germplasm collection, or to verify genetic similarities or dissimilarities / stability or instability when using certain micropropagation systems;
- The guaranty of authenticity for grapevine genotypes from core collection enforced the use of molecular markers for testing the genetic stability and integrity of genetic resources. In the same time, were initiated research activities aiming at identifying duplicates in collection and eliminating redundant material (to maintain only as much as is necessary).

These objectives were carried out by a team of four researchers (two of them are PhD students) in close collaboration with researchers from the other groups, such as “Plant Protection” and “Applied Biotechnology” groups. The morphological and ampelographical characteristics of hybrid plants, expressed in field conditions, represented essential criteria for choosing of highly valuable genotypes, possessing

the trait of seedlessness, high yielding, and enhanced resistance to specific diseases. This plant material was used in the following breeding stages, mainly in back-crosses, to obtain stability of these new features/traits and maintain the polygenic characteristics of productivity and quality.

The most important results were obtained through research projects won by national competition, and were presented in articles, books, or conferences. Based on comparative studies in the ampelographic collection, the following cultivars were recommended for new local vineyards: *Argessis*, *Iantarnai Muscat*, *Augusta*, *Golden Stefanesti*, *Palava*. Other 10 valuable hybrid progeny elites having a good chance to become new varieties are now under evaluation.

Once established the basic assortment, the activity of selection and improvement has been geared towards creating new clones and new varieties of high yielding potential and possessing better qualities. Following a long time work and after careful selection, 14 new clones have been obtained and approved by the national authorities: Pinot noir 3 St, Sauvignon 111St., Cabernet Sauvignon 131 St., Feteasca regala 7St., Feteasca alba 97St., Feteasca neagra 6St., Muscat Ottonel 16St., Aligote 63St., Chardonnay 15St., Perlette 10 St., Muscat d'Adda 22St., Burgund mare 86St., Merlot 202St., Pinot Gris 14St.

Beside clonal selection, reciprocal crosses using seeded and seedless varieties were performed. In the recent years were obtained, analysed, registered, approved and patented two new table varieties, named *Argessis* and *Golden Stefanesti*, respectively.

In the last two years, beside the morphological aspects, molecular markers for genetic characterization of the accessions have been used. The RAPDs and SSR markers were applied to evaluate the genetic variability of grapevine assortment from the NRDIBH collection, and also to genetically characterize the most valuable genotypes under investigation.

In the last five years, the researchers forming the team involved in this work published over 30 articles/papers, and presented their results in many national and international conferences. Experience, large amount of data available, and the possibility to analyze the plant material at the molecular level were the main reasons to apply with two proposals at the projects competition on November 2011. Thinking to the future, the Genetics, Molecular Biology and Plant Breeding group is committed

to continue the activities towards exploiting the genetic and horticultural value of the new grapevine genotypes, and also to establish new targets, such as:

- Genetic diversity characterization of Romanian cultivars by molecular markers Identification, collection preservation (*in vitro* and *ex situ*) and genetic analysis of *Vitis vinifera subsp. sylvestris* existing in wilderness
- Inventory of *Vitis* genetic resources in Romania - Recording registered values of the OIV descriptors and their download into the European *Vitis* Database
- Applying the molecular analysis to other species (*Pyrus*, *Malus*, *Rosa*, *Tulipa*, *Syringa*, etc)

#### **4.2. Biochemistry and Plant Physiology**

This group was (and still is) involved in research projects aiming at integration of grapevine physiological aspects with those of yield potential and wine quality. Some aspects of vine plants physiology were studied, such as: canopy and root system dynamics, grape development and their nutrient composition, the interaction between short-time culture in pots versus controlled ambient factors and long-time plantation in the field versus uncontrolled environmental factors. These aspects are important for the establishment of practically applicable principles to improve grape and wine quality.

The activities carried out for reaching this objectives were focused on the following aspects:

1. Applied biotechnological procedures for controlling the submersible fermentation of grain subproducts under the action of edible and medicinal mushrooms. As results, were improved the methods for producing and selecting edible and medical macromycetes strain of *Ganoderma lucidum*, *Grifola fondosa*, *Pleurotus sp.* and *Lentinus edodes*. The fungal biomasses were evaluated by biochemical analyses for their nutritional qualities based on nutrition and toxicity tests;
2. Polyphenols induced synthesis involved in the defense mechanisms of grapevine plants to biotic stress. The main purpose of this project was using the aluminum chloride, as elicitor agent under *in vitro* and *in vivo* cultivation, to stimulate the biosynthesis of polyphenols phytoalexines

(stilbens) in different *V. vinifera* genotypes in order to improve their tolerance to diseases.

Continuing the previous studies for wine making, the research were focused on developing methodologies (chromatographic, spectral and sensorial) to assist in the analysis of the chemical composition of wine. In parallel, specific methods were used for identifying and quantifying different useful flavour compounds and undesired products.

The laboratory for wine chemistry has been performing research activities and also has responsibilities for wine quality control on processing. Working together the breeders, the results obtained from micro-vinification are essential for approval the new grapevine varieties for wine production. The physical characteristics of the wines and identification of certain components are of great importance for improving wine-making methods.

As in any accredited laboratory, validated methods are used for state and private enterprises. The offered services for wine, must, alcoholic drinks, liquors and plum brandy are analysed for alcohol concentration, total dry extract content, total and volatile acidity, free and total sulphur dioxide, reduced sugar, iron content, methanol, esters, aldehydes, furfural, copper, lead and other toxic compounds.

The results obtained by this group over the last five years have been presented in various scientific meetings, and published in papers, including a PhD thesis (successfully defended in 2009). Most part of this information was essential for the physico-chemical analysis of the soil and plant samples, and highly useful for the groups in “Agrotechnology and Plant Protection” and “Applied Biotechnologies”.

### **4.3. Agrotechnology and Plant Protection**

This group is formed by specialists on ecology, technology and plant protection. In the last five years were finalized 7 research projects, from which 1 as the main coordinator and 2 with responsibilities in the name of institute. At this time, the researchers from this group are involved in 4 research projects (see the on-line information).

A. In the domain of ecology, the group works to apply some principles and implement them in own results, such as:

- Elaboration, substantiation and applying new concepts (biology maintenance, traceability and retraceability, amplified cumulative effect, genetic space, physical space, image space, ethics in business) for microproduction activities;
- Risk evaluation for the production of grapevine planting material;
- Contribution to correct definitions used in Certification scheme for the multiplication of grapevine planting material – to separate the specific activities of breeders and horticulture crop producers.

The main results achieved through the above mentioned research projects were:

- Selection of two valuable tomato genotypes and submission of the documentation to obtain the approval for releasing as new varieties;
- Improvement of the technologies for obtaining the horticultural planting material dedicated to ecological crops;
- Official certification of ecological products (planting material, seeds and fruits) – the recognition is under direct supervision of Austria bio Garantie Company;
- Improvements of different techniques for: modelling and preparing the field for plantation, increasing the efficiency of photosynthesis process in plants;
- Elaboration of 12 improved technologies.

B. In the domain of technology for establishing and maintenance the horticultural crops, the research themes have the following objectives:

- Eco-biological restoration of physical and nutritional state of soils intended to be used for replanting vineyards;
- Elaboration of alternative technologies for reducing the negative impact on the soil properties and also for decreasing the infection pressure of pathogens causing cryptogamic diseases;
- Production of grapevine virus-free planting material in protected spaces (dedicated greenhouses for G0, G1 and G2 categories)

In the last year, as a necessity at the national level, a new research subject was approached, regarding *Agrobacterium sp*, a dangerous pathogen affecting Romanian vineyards. Various strains of this soil bacteria were already isolated and identified on culture media.

The main results from these projects are the following:

- Rehabilitation of 4430 m<sup>2</sup> of nucleus-isolation (green)house for multiplication and production of *initial* (G0 and G1) grapevine planting material. A double protection of plants – to pathogen infection and to mechanical transmitted diseases, is assured;
- A supplementary source of G1 plants (1200 rooted plants grown in individual pots) – for canes and buds for grafting
- Establishing 0.9 ha with the most useful rootstocks varieties and clones (Base category);
- Establishing 1.6 Ha of SO4-4 rootstock clone (Certificate category);
- Establishing 0.7 Ha of four different grapevine clones (Base category);
- Establishing 1.94 ha of *Mother plantation* - Base category, with the most important Romanian grapevine cultivars. The whole amount of grafting material was sent to France, grafted at ENTAV and verified for sanitary status and genetic authenticity.

C. In the domain of virology the main objectives planned to be achieved within the projects were:

- *in vivo and in vitro* comparative studies of virus infected grapevines and healthy plants;
- Monitoring of grapevine viruses/virus diseases/virus-like diseases in vineyards established with autochthonous cultivars;
- Studies on grapevine viruses elimination by electrotherapy and *in vitro* chemotherapy, comparatively to the classical methods of heat treatment and/or *in vitro* culture;
- An increased effectiveness of the virus detection and elimination methods used for the protection of grapevine germplasm.

The relevant results from these activities are:

- Establishing a collection of virus infected grapevines, included in an international network of grapevine virus collections;

- Accreditation of the methods for detection of the most important and harmful viruses for grapevine;
- Obtaining a patent for virus elimination in plants by electro-therapy;
- Specific services for state research stations and private farmers.
- participation to the “**Proficiency tests for virus detection methods**” together with VCR Rauscedo (Italy), IAMB Bari (Italy), Mendel University Brno (Czech Republic), Analyse- und Diagnoselabor DLR Rheinpfalz (Germany).

The group working in the domain of **Agrotechnology and Plant Protection** elaborated, published and presented to national and international scientific meetings a number of 30 papers. Most of these were the result of joint and collaborative activities with researchers from other research institutions and universities.

Is important to underline the participation to the last competition from November 2011 with two proposals, proving the commitment to approach new targets, such as:

- comparative study of ampelographic and technological features on grapevine clones and cultivars in two different areas (belonging to the institute and respectively to a private company);
- the elaboration of a functional technological model for reducing the period between the moment of releasing new varieties and the moment of reaching commercial yield;
- an interdisciplinary approach in plant virology and recovery of virus-free plants for two different species: grapevine and potato;
- validation of new technologies for virus detection and identification in grapevine and potato.

#### **4.4. Applied Biotechnology**

This group has the main responsibility to apply biotechnology methods aiming to:

- obtaining and maintaining grapevine planting material of high biological value (G0 initial material) in long- and medium-time tissue cultures;
- establishment of *in vitro* propagation technologies for dendrological species difficult or impossible to multiply by conventional methods;
- *in vitro* induction of bioactive compounds involved in the defence response to biotic stress in grapevine genotypes;

- obtaining and medium- and long-term preservation of gametophyte and sporophyte of different pteridophytes species from restricted areas, which are under threat or near extinction.

Results obtained through research projects:

- *in vitro* propagation of ornamental plants, cultivars free of viruses and difficult to multiply by conventional methods: rose (*Rosa* sp.), gardenia (*Gardenia jasminoides*), gypsophila (*Gypsophila paniculata*), drosera (*Drosera rotundifolia*), lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*), redwood (*Sequoia sempervirens*), strawberry (*Fragaria* sp.), artichoke (*Cynara scolimus*), violet (*Saintpaulia ionantha*), gloxinia (*Gloxinia hybrida*), lisianthus (*Eustoma grandiflora*), petunia (*Petunia* sp.), chrysanthemum (*Chrysanthemum* sp.), blackberry (*Rubus nigra*), magnolia (*Magnolia soulangiana*), *Albizzia julibrissin*, *Asimina triloba* and *Ginkgo biloba*;
- improved methods for *in vitro* plant regeneration of virus-free plants (thermotherapy and/or *in vitro* culture, chemo-therapy);
- optimizing the methods for *in vitro* multiplication and preservation in grapevine cultivars, of high biological category;
- establishing the grapevine germplasm core collection of 250 genotypes (table grapes, wine grapes, rootstocks from the autochthonous and world assortment). The over 5000 plants are maintained under strict safety conditions according to the national and international legislation as G0 planting material, or Initial planting material. All these plants were obtained starting from the canes sent by the owners of each genotype – breeders working in research stations belonging to the network under coordination of the Research and Development Institute for Viticulture and Oenology Valea Calugareasca;
- improved method for *in vitro* micro-grafting aiming to: a) test the grafting compatibility between scion and rootstock, especially for the new grapevine cultivars; b) as fast diagnostic method (2-3 months) of virus and virus-like diseases (corky bars, vine necrosis and leaf-roll);
- procedure of *in vitro* induction of polyphenols (stilbene compounds) synthesis with aluminium chloride as elicitor. The aim was to identify grapevine cultivars for red wines having higher potential of polyphenols biosynthesis as response to certain fungi infection (i.e. *Botrytis cinerea* and

*Plasmopara viticola*). This procedure will be also applied for testing the most important Romanian grapevine cultivars for white wines;

- establishing the methods for *in vitro* regeneration, propagation and preservation of gametophytes and sporophytes belonging to 7 different species of ferns from the protected area of Valsan Valley. The acclimated plants were planted in a protected area to create an *ex situ* collection of pteridophytes.

Two of the researchers from this team are PhD students, and their results will be included in their PhD thesis, entitled "Studies for establishing the biotechnologies of *in vitro* propagation in species of the *Albizzia* genus" and "The expression on *in vitro* systems of morphogenetic potential in species of *Magnolia* genus", respectively.

The group working in the domain of **Applied Biotechnology** elaborated, published and presented 29 papers at national and international scientific meetins. All these research articles were the result of joint and collaborative activities with researchers from other groups, from other institutions and universities.

Thinking ahead, as a necessity to enlarge the range of applied techniques and approached subjects, the group applied with a research proposal to the last project competition. The fungal diversity of *Aspergillus sp.*, *Penicillium sp.* and *Botrytis sp.* in Romanian vineyards will be analyzed for the first time. In this respect, the new targets for this group are:

- establishing an *in vitro* collection of moulds isolated from certain vineyards;
- morphological and molecular characterization of *Aspergillus* and *Penicillium* isolates;
- identification of the isolates responsible for mycotoxins and volatile molecules production in wines.

## **5. A representative project for NRDIBH Stefanesti-Arges**

### **Development of high quality, authentic planting materials for rehabilitation of the national vineyards in Romania**

#### **Short introduction**

Grapevine is one of the major horticultural crops in Romania and wines and grapes production represent important profitable agro-industries. Vine-growing has been an old tradition especially for rural population since ancient times. Although in Romania vineyards produce grapes of unsurpassed quantity and quality with autochthonous genotypes, these cultivars are known and appreciated only in our regions. Also, the new breeders' creation are planted only in local areas and are commercialized only on national market. All these grapevine varieties in order to be accepted as new cultivar or as multiplication material have been identified by physical features (their leaves and fruit) and characterized with ampelographic and biochemical parameters. But all those traits are not stable, but highly vary depending the environmental conditions where the grapevines are planted.

One of the most challenging tasks for our country is to replace the old vineyards with grafted-plants from authentic and certificated grapevine cultivars. Replacing almost all the old vineyards with new planting material (pure wine and table grape cultivars) within the next one or two decades is a national strategy for developing Romanian viticulture. Both the ancient and new creations of grapevine varieties from Romania could be particularly valuable as gene resources for planting material producers, for wine-maker, or breeders. This is the reason to implement and put into force the legal framework for producing and commercialization the grapevine planting material (Law 266/2002 and Order 1267/2005). According to these documents, the *Core collection* on grapevine germplasm has a central role (see Annex 3).

The National Research and Development Institute for Biotechnology in Horticulture was given by establishing document the responsibility to establish the national collection of grapevine genetic resources for the benefit of present and future generation. Starting with 1988, were initiated research activities for sanitary selection and virus elimination according to the certification scheme applied in other European countries, in parallel with tissue culture procedures (see Annex 4). Since that time, the program for obtaining virus-free grapevine plants was constantly developed due to the increasing number of cultivars and clones needed to be available as healthy material. The plant material within our collection is the result of

standard operating procedures currently applied, including thermotherapy and/or tissue culture with periodically tests for sanitary selection and grapevine virus presence diagnostics. Thus, is guaranteed the germplasm resources not only for authenticity (trueness to type), but also for its phytosanitary status.

The grapevine cultivars have to be tested annually for detection any virus infection by using ELISA tests. Only infected cultivars and clones are subjected to virus elimination through thermotherapy and / or *in vitro* meristem, apex, or axillary bud culture, and routinely checked during *in vitro* culture and acclimatization phases. The healthy plants are transferred into greenhouse for nuclear stock (core collection) under a severe regime for avoiding any virus infection. These plants are considered as *initial* planting material and represent the source for scion and rootstocks in establishing mother plantations with *base* material. So far, the institute assured optimal conditions for production and distribution grapevine planting material of superior biological categories, according to the in force European legislation, but only for the *initial* material and quantitatively to a reduced scale.

The development of profitable and sustainable grapevine production involves capacity to produce the planting material in the best sanitary condition for nurseries and in quantities as much as are necessary to replant 110,000 ha with authenticated wine cultivars and 16,000 ha with authenticated table grape cultivars.

#### **Objective and expected outcome:**

The responsibility to establish a germplasm collection involves a whole range of activities, including applied research and valorisation of the research results, oriented towards producing and delivering the *initial* high quality grapevine planting material, in accordance to EU regulations.

The main beneficiaries of the planting material from the *initial* and *base* categories, obtained by applying the biotechnology of *in vitro* culture and thermotherapy, are the grapevine nurseries in the country and even from abroad. The provided planting material shall be fully guaranteed from the cultivar authenticity point of view, and certified for its totally healthy status as well. This is also essential for allowing the export of planting material (scions and rootstocks), both from worldwide cultivated varieties and the highly valuable Romanian table and wine grapevine cultivars.

The project will assist towards improving the efficiency and effectiveness of the current delivery network in order to ensure the widespread availability of low-cost virus-free planting materials to farmers, for establishing new vineyards.

The final beneficiaries will be both grape and wine producers, since the use of authentic and certified planting material within Romanian vineyards is crucial for meeting the European standards related to agricultural and food products.

The overall aim of this activity within the proposed project is to develop the capacity to supply the Romanian grapevine nurseries for *base* and *certificate* categories with sufficient propagation material. In this context, the subcomponents of the main activity will be focused on:

- promoting the use of the best available plant material obtained by Plant Certification Scheme for grapevine
- assuring the optimal condition for thermotherapy treatment, *in vitro* multiplication and develop the methodology for multiplication and growing of certified material
- making available the clonal material in the best sanitary condition
- assuring the required quantities of acclimatized plants, or canes for the beneficiaries
- at the end of the project, the planting material, obtained, maintained and delivered by the institute, will have to be characterized as: (1) well-documented plant material; (2) guaranteed for the authenticity of cultivars.

### Description of activities

Phase title	1 Establishing the assortment of grapevine cultivars necessary for obtaining the planting material - <i>Initial</i> category (new genotypes) in accordance with establishment guideline for future plantings				
Involved teams	E1	E2	E3	E4	Breeders
Start month	1 <sup>er</sup> year - month 1 ; 2 <sup>d</sup> year - month 13; 3 <sup>th</sup> year - month 25				
End month	1 <sup>er</sup> year - month 10; 2 <sup>d</sup> year - month 22; 3 <sup>th</sup> year - month 34				
Activities					
A1.1 Reception and registration of breeder's material - E1,E2, E3,E4; A1.2. Virology tests to breeder's material – E3; A1.3. DNA extraction, checking the quantity and quality of extracted DNA, maintain the DNA samples in freezer.- E1; A1.4. Evaluation the quality of breeder's material (the degree of canes maturation) – E2; A1.5. Healthy plant material is multiplied rapidly (one bud woody cuttings) - E4; A1.6. The infected plant material is used to initiate <i>in vitro</i> cultures from meristematic tissues for sanitation - E4; A1.7. Transfer the healthy material obtained by vegetative multiplication (one bud cuttings) in the G0 depository greenhouse – E4;					
Deliverables					
Act for guaranty of authenticity (AGA) from breeder/maintainer					

Activity reports and analysis bulletins for quality and sanitary status of breeder's grapevine material;

Activity reports for evaluation of the multiplication capacity by on bud cuttings of the material of breeder

Phase title	2 Obtaining the grapevine <i>Initial</i> propagating material (G0)				
Involved teams	E1		E3	E4	
Start month	1 <sup>er</sup> year - month 1 ; 2 <sup>d</sup> year - month 13; 3 <sup>th</sup> year - month 25				
End month	1 <sup>er</sup> year - month 10; 2 <sup>d</sup> year - month 22; 3 <sup>th</sup> year - month 34				
Activities	<p><b>A.2.1.</b> <i>In vitro</i> regeneration, multiplication and rooting of healthy / virus infected biological material submitted to virus elimination technology - E4</p> <p><b>A.2.2.</b> Serological retesting of grapevine biological material during <i>in vitro</i> culture - E3</p> <p><b>A.2.3.</b> Checking the genetic fidelity of plant material during <i>in vitro</i> propagation / versus extracted DNA from breeders' material – E1</p>				
Deliverables	<p>Activity reports - evaluation of regeneration capacity during <i>in vitro</i> cultures for breeder's material (healthy and submitted to virus elimination technology);</p> <p>Analysis bulletins - evaluation of sanitary status to plant material during virus elimination technology;</p>				

Phase title	3. Obtaining the batches of <i>Initial</i> propagating material G0				
Involved teams			E3	E4	
Start month	2 <sup>d</sup> year - month 13; 3 <sup>th</sup> year - month 25				
End month	2 <sup>d</sup> year - month 22; 3 <sup>th</sup> year - month 34				
Activities	<p><b>A.3.1.</b> Acclimatization and fortification of biological material obtained by <i>in vitro</i> culture – E4</p> <p><b>A.3.2.</b> Serological retesting of recovered grapevine biological material, before planting in the greenhouse - E3</p>				
Deliverables	<p>Activity report (evaluation the capacity of accommodation to <i>ex vitro</i> environment and fortification of healthy / recovered material obtained by <i>in vitro</i> culture);</p> <p>Analysis bulletin on sanitary status of grapevine material submitted to virus elimination technology</p>				

Phase title	4. Enriching the grapevine germplasm resources with <i>Initial</i> propagating material G0 (new genotypes)				
Involved teams	E1	E2	E3	E4	
Start month	2 <sup>d</sup> year - month 13; 3 <sup>th</sup> year - month 25				
End month	2 <sup>d</sup> year - month 22; 3 <sup>th</sup> year - month 34				
Activities					

<b>A.4.1.</b> Plant the obtained plants in the depository greenhouse – E4
<b>A.4.2.</b> Monitoring the growth and development processes in the first year after transferring in the depository greenhouse (G0) – E4 , E2
<b>A.4.3.</b> Two molecular markers systems RAPD (random amplified polymorphic DNA) and SSR (simple sequence repeats) are employed for identification, genetic diversity and stability analysis of autochthonous Romanian grapevine varieties – E1
Deliverables
Activity report (on growth and development of vines in the first year after transferring in the G0 depository greenhouse)

Phase title	5. Evaluation the quality of grapevine <i>Initial</i> propagating material G0 belonging to the new genotypes from depository greenhouse				
Involved teams	E1	E2	E3	E4	
Start month	3 <sup>th</sup> year - month 25; 4 <sup>th</sup> year - month 37				
End month	3 <sup>th</sup> year - month 34; 4 <sup>th</sup> year - month 46				
Activities	<b>A.5.1.</b> Virology retesting of plants in the second year of culture – E3; <b>A.5.2.</b> Growth capacity evaluation of plants in the depository greenhouse (G0)- E4, E2; <b>A.5.3.</b> Comparative studies on the main morphological features - the <i>Initial</i> plant material G0 and breeder documents (OIV descriptors) – E4; <b>A.5.4.</b> Molecular markers used for testing genetic stability of plant material from core collection and identifying duplicates genotypes – E1				
Deliverables	Analysis bulletins (for sanitary status of grapevine material submitted to virus elimination technology) Activity report (on growth and development of plants in the second year from the transfer in G0 depository greenhouse)				

Phase title	6. Maintaining and revaluation the grapevine <i>initial</i> propagating material (G0, G1, G2) used for establishing the <i>Basic</i> mother nursery vineyards				
Involved teams	E1	E2	E3	E4	
Start month	4 <sup>th</sup> year - month 37				
End month	4 <sup>th</sup> year - month 46				
Activities	<b>A.7.1.</b> <i>Initial</i> material G0 pre-multiplication (vegetative multiplication – woody cuttings of 1-2 buds), for obtaining the G1 and G2 <i>Initial</i> material, required for establishing the <i>Basic</i> mother plantation -E 3, E2 <b>A.7.2.</b> Plant growth and development evaluation in the depository greenhouse (G0)- E4, E2 <b>A.7.3.</b> Comparative studies between the main morphological characters of Initial material G0 and breeder's material (OIV descriptors) – E4; <b>A.7.4.</b> Comparative studies on the genetic steady between G0 Initial and breeder's material - E1				
Deliverables	Activity reports on: - evolution of rooted plants in the pre-multiplication plant material, - growth and development of vines in the 3rd year after plantation in the G0 depository greenhouse;				

- morphological characters of G0 Initial material;  
 - delivery the G1 and G2 *Initial* material to the maintainers (Document of quality and conformity for the beneficiary released in the base of documents for certification issued from TISPMQC)

Phase title	7. Establishing the <i>Base</i> mother plantations at maintainer (breeders, owner of mother plant)				
Involved teams			E3		
Start month	4 <sup>th</sup> year - month 37				
End month	4 <sup>th</sup> year - month 46				
Activities	Performing specific field activities for establishing and maintaining the <i>mother</i> nursery plantation providing the scions and rootstocks canes – E3				
Deliverables	Activity report Obtaining the grapevine propagating material as canes and one-buds cuttings starting with the first year after plantation				

### Expected S/T results

- An efficient implementation of this activity, as a major component of the network for producing grapevine planting material at the national level, will guaranty the economic effectiveness of high quality grapevine planting material delivery;
- The capacity of producing planting material it is expected to increase to an annually production of 1.1 million canes (branches with 10 buds) and, as a consequence, the actual price for delivered planting material will decreases to about a fourth;
- The intended direct beneficiary for planting material from the *initial category*, obtained by applying the technology for virus-free plant, are the grapevine nurseries in the country and even from abroad. By using such planting material, they have full guaranty of establishing vineyards at the standards required by European Union, as well as the opportunity to export planting material (scions and rootstocks) of superior quality and certificated as free of any viruses and pathogens;
- At the end of the project, the number of grapevine genotypes preserved in the core collection will increased from 170 (of the present day) to at least 300 cv.
- The Core collection established based on qualitative - quantitative characteristics and on genetic variation using molecular markers will be registered in the European data base.

- Finally, beside a high quality planting material delivered to their owners, will be created a functional national network for data system or web server available for all farmers or wine producers.

### **Expected impacts**

- At the end of the project, it is expected that all obtained information will be very useful for a complete view of the Romanian grapevine gene resources, which are well adapted to various climate, are planted on a large area of vineyards and express characteristic features (morphological aspects, certain flavor of grape or wine, resistance to pest, diseases and abiotic stress).

- This gene resource should be made known at international level and properly used as genetic material in genetic improvement, research activities, or as planting material for table grapes or wine production.

- The obtaining of initial planting material, its' preservation and producing the *base* planting material involved complex teams, large number of researchers with scientific competences (senior researchers as well as young researchers; postdoctoral researchers, doctoral students, master students and laboratory technicians).

- The vineyards established with healthy planting material are economically more efficient, have long term exploitation (25-30 years) and ensure sustainable yields.

- The reduced number of treatments with pesticides applied over a year required on a vineyard with certified and guaranteed planting material has a favourable effect to reduction of soil and plant pollution

### **Viability and risks of the project**

- The aim of this activity is to establish a grapevine core collection recognized at the European level. A complete characterization of Romanian grapevine cultivars by using OIV descriptors combined with molecular methods represent a certain guarantee for a comprehensive assessment, identification, characterization and preservation of grapevine diversity.

- The safety conditions for preservation offered by our present endowment and the possibilities to perform genetic characterization to these grapevine genotypes belonging of Romanian cultivars are very useful for all research stations for viticulture in our country, which are the owners of these genotypes and the main

beneficiary of cultivars available as planting material. Also, the grapevine growers and wine makers will have the guaranty for the authenticity of the varieties they have been planted.

- One major risk is that in Romania there is no legal framework for germplasm collections, or gene bank activities. All related activities for collecting plant material, establishing a gene bank collection, its' maintenance in and capitalization are carried out with research units financial supports, partially through research projects earned in national competitions. Without a government financial support will be difficult to continue these activities in grapevine germplasm collection in the same way.

### **Revaluation the results and potential beneficiaries**

The grapevine planting material from core collection represents the source of scions and rootstocks for establishing plantations with *base* material and could be available for the main beneficiaries. In agreement with them, the planting material will be multiplied by two procedures:

1. *in vitro* multiplication, for *initial* planting material (4-5 plants each genotype individual potted from greenhouse core collection or from breeder' material at their request). For this activity the institute has the capacity to multiply 20 genotypes / year and to obtain *in vitro* rooted plants and acclimatized plants. O part of this material is re-planted for maintenance in corer collection, and the greatest part is delivered to beneficiaries (if they fulfilled condition to preserve safely the *pre-base* plant material). For the new and valuable creations, in order to be authorized and registered, plant material is tested by ELISA for viruses and virus-like diseases listed in Law 266/2002 and Order 244/ 2002. The procedure enforced by European legislation is applied for infected plants involving thermotherapy/chemotherapy/electrotherapy, *in vitro* culture, repeated ELISA tests, acclimatization and delivery of planting material.

All these activities impose a good coordination between technological flux of *in vitro* propagation and planting material demands. Therefore, will be necessary to modernize the actually endowment for *in vitro* multiplication, and to ensure proper condition for acclimatization phase, when are registered the highest percentage of losses (30-40%).

2. one / two bud cuttings multiplication, starting from core collection plants of three years old, for *base* grapevine planting material. By this procedure, it will be possible to obtain 300 cuttings of 1 bud / cultivar / year, and 660 canes / cultivars / year.

Implementation of this project will allow to be created *the first core collection of grapevine planting material of high genetic value, free of viruses / virus diseases, and completely documented from genetic point of view*. This will represent the only reliable and competitive source of grapevine cultivars for the establishment of new vineyards producing high quality grapes. Among the directly interested beneficiaries for this material are:

- all Research and Development Station for Viticulture in Romania, which are interested in cultivars conservation, sanitary control of planting material, and complete characterization of the new genotypes, these being essential requirements either for breeding research or production. Equally important, they are the owners of the grapevine nurseries and therefore the only producers and providers of planting material for the surrounding regions;
- private farmers and state enterprises for growing grapevine, which needs planting material guaranteed for authenticity, and certified for its healthy status.

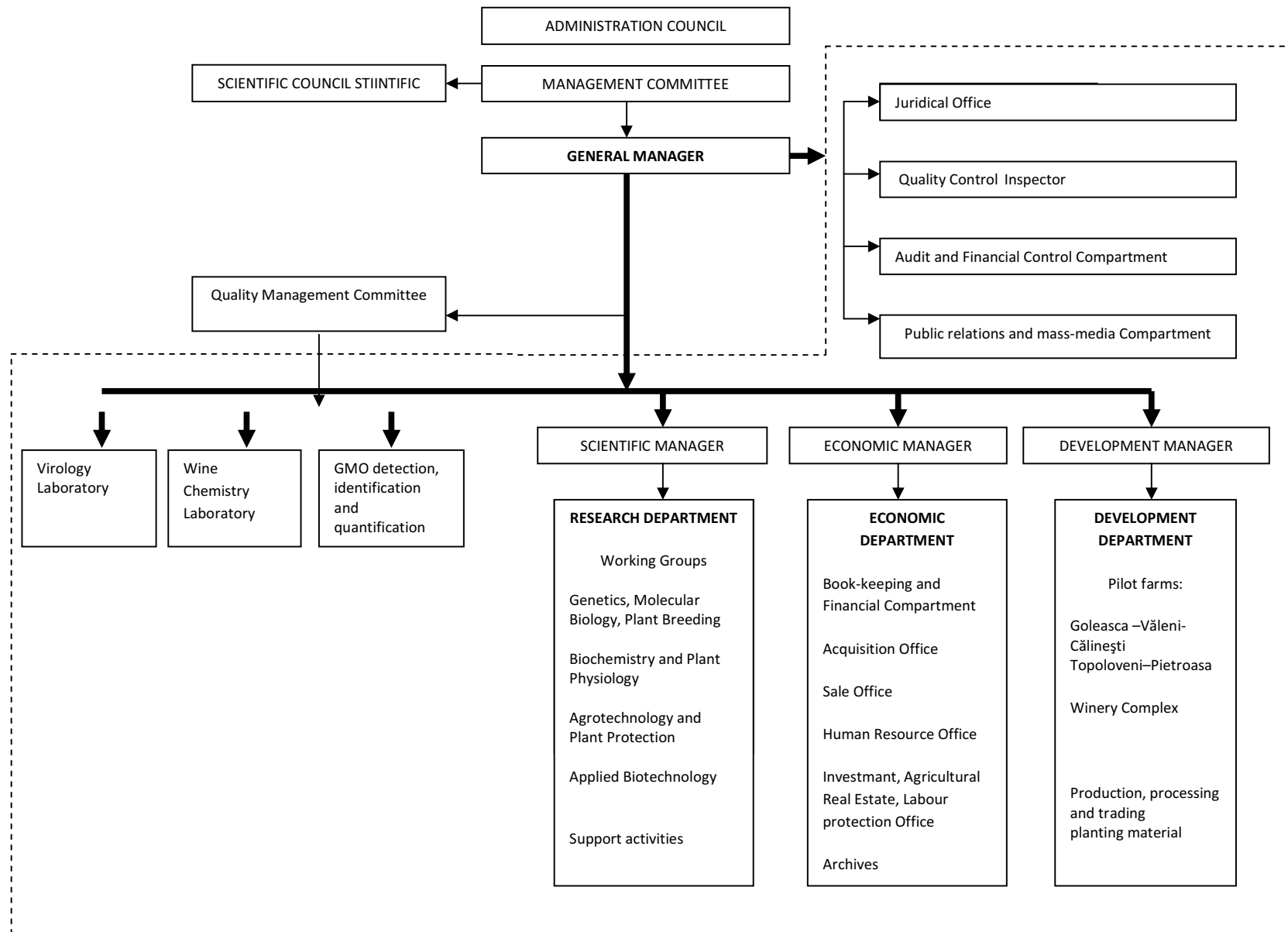
The main benefits for NRDIBH, possible to be obtained by this proposed project

Item	Actual situation	With project implementation
Grapevine germplasm core collection	250 cvs.	400 cvs. + wild <i>Vitis</i> sp.
Cultivar identification	Morphological with OIV descriptors	With genetic characterization
Cultivar registration to the International Grapevine Genome database	No	Yes
NRDIBH as deliverer of virus-free grapevine planting material	Known in Romania	Known in Europe
Number of <i>in vitro</i> derived and acclimatized plants/ cultivar/year	300	600
Number of canes/cultivar /year	200	500
Estimated value (lei / year) for delivered planting material (acclimatized plant, canes, buds for grafting)	10,000 lei / year	25,000 lei / year
The possibility to use the molecular techniques for plant genetic analysis to different crops	Yes without sequencing	Yes with sequencing
Scientific papers published in journals with non-zero relative Article Influence Score	No	5

### **Dissemination of results**

The expected results in this project will be disseminated by publishing the papers in national and international journals, book, and participation to national and international manifestations, workshops. Scientific results will be capitalized by research team members and will be also presented to the potential beneficiaries in meetings organized towards result and knowledge dissemination (trials, round tables, trainings, etc.). Much prominence is given for open dialogue and exchange of information (legislation, data record, methods and results analysis) with national and European authorities with responsibilities in grapevine collection conservation and revaluation.





## I. The important equipment purchase through research project in currently used

Equipment	Characteristics (wearing %)
1	2
<b>Equipments with less 25% exploitation</b>	
Thermotherapy chamber type KTLK-3.000	2 pieces - Multifunctional chamber with double system to control the humidity and temperature. Supplied with UV and IR lamps. Capacity 3,000 l; (wearing 82%).
Thermotherapy chamber type KTLK-1.600	Multifunctional chamber with double system to control the humidity and temperature. Capacity 1,600 l; (wearing 82%).
Laboratory washing machine with high temperature and Chlorine wash, type Miele Professional	General utility for laboratory; stainless steel; 4 washing programmes; parameters adjustable: program, temperature, time for each step, time for chemical reactions; system for self-diagnosis; 2 peristaltic pumps to dose the washing and neutral reagents; supplied with cold water; (wearing 10%).
Stereo microscope, types MC5A and Docuval	2 pieces - Research microscope with zoom; variable magnification using a continuous zoom control; three dimensional optic resolution; adjustable interpupillary distances from 55 to 75 mm; rotating head of 360°; triple illumination system: incident light (episcopacy), transmitted light, incident light incorporates a condenser lens; wearing 10%.
<b>Equipments with 25% - 50% exploitation</b>	
Mixer for processed products Model Grindomix GM200	Used for fast grinding different kind of plant material (fresh, dry, seeds, leaves, fodder) in order to obtain powder for DNA, RNA, proteins or enzymes extractions; (wearing 10%).
Spectrometer UV-VIS, type SPECORD M40 VEB Carl Zeiss JENA	With double beam, dual monochromator for UV range, for measurement in a spectra range of 185-900 nm. Digital display, accuracy of wave number 3 cm <sup>-1</sup> (0.25 nm) for 11000 cm <sup>-1</sup> , 10 cm <sup>-1</sup> (0.03 nm) for 54000 cm <sup>-1</sup> ; (wearing 60%)
Spectrometer VIS, type Spekol 11	Used in analytical chemistry for the quantitative determination of different analytics, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Determination is usually carried out in solutions. Spectra domains 320-900 nm; (wearing 60%)
<b>Equipments with 50% - 75% exploitation</b>	
Autoclave type SystecV-75	Useful to kill potential GMO-material, infected products, gels with infected DNA or RNA; complete destruction of all microorganisms including the most resistant bacteria or spores; to autoclave the instruments, vessels and media; outside and inside containers of stainless steel; (wearing 10%).
Autoclave type Kirana	To sterilize the vessels, the instruments, solutions and media with hot air (120°C); automatic adjustment of the pressure; (wearing 90%).
Centrifuge with cooling system type Andreas Hettich Mikro 22 R	2 pieces - Designed to separate from mixtures compounds with different densities; control panel for programmable programmes; temperature in the refrigerated centrifuges controllable within a range of -20°C to +40°C; 2 different rotors; (wearing 10%).
Small centrifuge for Eppendorf tubes, type Bio-rad 16 K	Designed to separate from mixtures different compounds depending of their density; control panel for programmable programmes, program memory; (wearing 10%).

1	2
Transiluminator UV, Bio-rad DGI DOC	Multitasking workstations which offer a ultra-violet source for the analysis of fluorescently stained DNA, RNA, and Protein electrophoresis gels. These also offer space to place tube racks, cutting tools or waste agarose gel, ideal when there is a need to cut bands. Model 21 x 26cm, dual intensity for analytical and preparatory work and 302 nm midrange wavelength; (wearing 10%)
Centrifuge for 96/384 well-plate, type Andreas Hettich Rotanta 460R	Designed to homogenize mixtures distributed in special plates; benchtop centrifuge; control panel for programmable programmes, program memory; (wearing 10%).
Water-bath 65C type R. Espinar BAD-02	Water bath with thermostat is used for biological sample (in different step of purification) to be treated by high temperature; (wearing 10%).
Spectrophotometer for measure DNA content or DNA/RNA Analysing Spectrophotometer, type Thermo Spectronic BioMate 5	Designed for molecular biology laboratories, with programs for measurements of RNA, DNA, ssDNA, primers (oligo-nucleotides), proteins, cell cultures, wavelength automatically selected and measured, results displayed on the screen and a printout, timed and dated is transferred on an internal/external printer, standard software for: A260/280 Ratio, A260/280 with background correction 320 nm, direct UV method for protein at 280 nm, ssDNA concentration, dsDNA concentration, RNA concentration, A230/A260 Ratio; (wearing 10%).
UV/vis spectrophotometer, type BioPhotometer plus	Used in molecular biology, biochemistry and cell biology provides instant, out-of-the-box access to: measurement of DNA, RNA and protein concentration; incorporation rate of fluorescent molecules (550 nm/650 nm); enzymatic assays; optical density of cells (OD 600); (wearing 10%).
Electrophoresis equipment + Power supply type Maxigel Eco	4 pieces; used to separate nucleic acids in agarose gel; consists of three primary components: the electrophoresis apparatus, the external gel casting system and the blot transfer system; 3 different dimension of the gel; (wearing 10%).
Real-time PCR machine, type ABI PRISM® 7900HT	Integrated system designed to perform both real-time PCR (polymerase chain reaction) and post-PCR (end-point) analysis. The instrument can be used with 96- and 384-well plate format. The instrument is used for specialized applications with specific software that collects and analyzes the fluorescence data for the probes, for absolute quantification of DNA structures, or allelic discrimination/SNP (Single Nucleotide Polymorphism) detection; (wearing 10%).
Termocycler with 96-wells, PCR System, type Techne TC 512	Automated instrument specifically designed for the amplification of nucleic acids using the Polymerase Chain Reaction (PCR) process, internal Memory: minimum 100 complete PCR methods consisting of pre-PCR holds, PCR cycling conditions and post-PCR holds. Variable up and down ramp speeds, auto extend/decrement for both times and temperatures. Auto restart function allows for power outages and safe continuation of a PCR experiment after resumption of power. Variable up to 5°C/sec–heating/cooling rate of the sample block, temperature Range: 4.0–99.9°C; (wearing 15%).
Ice maker, type Ziegra ZBE30-10-WI	Offers quick cooling to exactly 0°C; no freeze on the skin, no bruises, type ice obtained: fine and flake ice, cabinet and storage stainless steel; (wearing 10%).

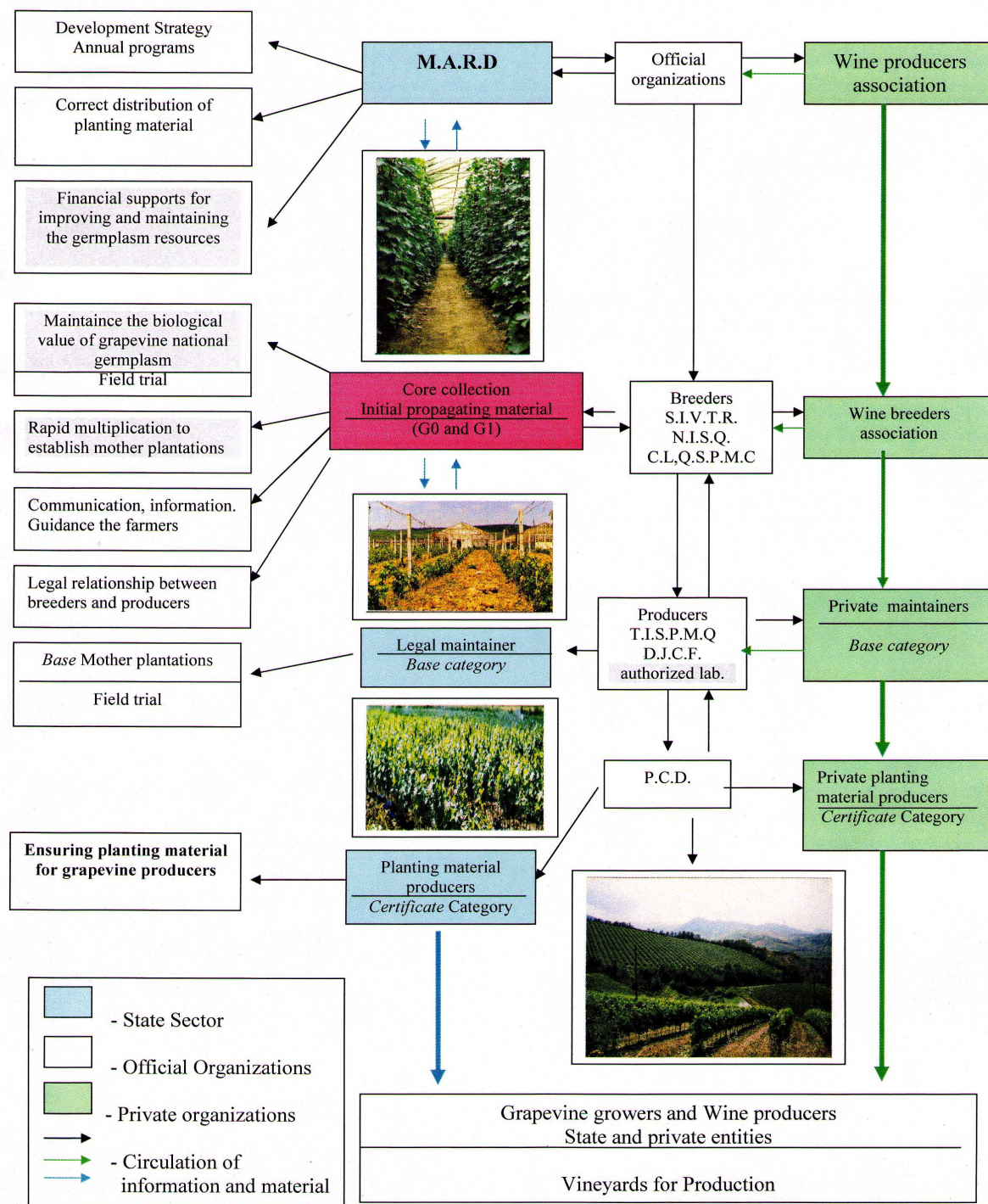
1	2
Balance 0-5 kg, type Kern&Sohn EG 4200	3 pieces - Designed for precise detection of weights in any kind of laboratory; laboratory balance to establish the weight of samples for/under research analysis; with adjusting program for quick setting of the balance's accuracy; test weight included; display for piece counting to weight; standard printer connected with an optional data interface; wearing 10%.
Precision Balance 0-400 g, type Kern&Sohn PGB 510	5 pieces - Designed for precise detection of weights in any kind of laboratory reagents, or components, d=0,1mg (100%), capacity 5 mg - 300 g, resolution 0.1 mg; wearing 10%.
Thermostat, type MLM LP122	3 pieces - A control system which regulates the temperature of a system; switching heating or cooling devices on or off, or regulating the flow of a heat transfer fluid as needed, to maintain the correct temperature. With sensors to control the heating or cooling temperatures between 4 to 120°C; wearing 70%.
Elementar Analyzer, type Gerhardt varioMACRO CHNS	Used to detect the content of C, N, H and S by dry combustion in samples of soil and plant materials; (wearing 10%)
Flame photometer Sherwood Sci LTD, type 420 Dual Channel	Measures Alkali and Alkaline Earth metals Sodium (Na), Potassium (K), Lithium (Li), Calcium (Ca), Barium (Ba), Caesium (Cs), Rubidium (Rb) and Strontium ( Sr) by means of a low temperature flame using propane, butane or Natural gas in soil and plant materials; (wearing 10%)
Shaking Incubator, Progen sci. type GFL 3032	Operate with comprehensive software programmed enables independent PC control and data analysis of up to 64 laboratory appliances. Specialized in gentle mixing as well as vigorous shaking, used for applications that require exactly reproducible orbital motions and temperatures of up to 70°C. Capacity 46 liters set up the temperature, light and timing during shaking the samples; (wearing 10%)
<b>Equipments with more than 75% exploitation</b>	
Freezer, type Sanyo NOF – U52V	Necessary for long term storage of organic substances, DNA, plant samples, DNA samples, enzymes, solutions; (wearing 10%).
Water purification system type Ultra Clear TWF UV plus	Recommended to produce ultra-pure water from any potable water supply, for laboratory use: molecular biology, cell tissue culture, molecular analysis (DNA, RNA). The system guarantees the bacteria elimination in a proportion of 99% with 1 or 2 UV lamps, obtaining of water with un-detected trace of chemical elements, RN-ase, DN-ase, DNA, or RNA; fully automatic; (wearing 10%)
Fume hood, type Talassi MA 90	Extraction hood with double extraction system is essential equipment in molecular labs due to its property to absorb dangerous and inflammable vapours and clear the hood and the down drawer; fireproofed; (wearing 10%).
Safety cabinet with UV light, type Aquaria Flow Active	Provides personnel, environmental and product protection in biological laboratories; with microprocessor system and alphanumeric display providing following data: exhaust air flow, laminar flow air velocity, cabinet temperature, UV lamp, HEPA filters last change date, efficiency 99.99% for particle, accessible for replacement; (wearing 10%).
Laminar air flow type BL 1200	9 pieces - these cabins have been especially designed for working in sterile conditions; the equipments ensure the absence of contamination in the product or samples during handling, without requiring protection for the operator or the environment; wearing 60%.

1	2
Water Distiller, type Daihan Lab tech LWD-3004	2 pieces - Capacity 6 l distillate water / hour; provided with quartz resistance for double distilled water, Power Consumption 220/230 V. AC supply single phase 50 Hz.; wearing 90%.
Refrigerators	15 pieces, different types, capacities 200-1200 l; duplex system for 10 <sup>0</sup> C to - 8 <sup>0</sup> C, and -20 to - 32 <sup>0</sup> C; programmed temperatures; wearing 20-90%.
Laminar air flow type BL 1200	9 pieces - these cabins have been especially designed for working in sterile conditions; the equipments ensure the absence of contamination in the product or samples during handling, without requiring protection for the operator or the environment; wearing 60%.
Ovens	3 pieces, different capacities 50-100 l. Suitable for all drying and sterilisation tasks. With temperature-accurate and highly efficient; temperature range 40-220 <sup>0</sup> C ± 0, 5-1 <sup>0</sup> C, Electronically-controlled APT.line™ preheating chamber technology with natural convection; digital temperature setting; wearing 70%.
ELISA Plate Reader, type Bio-rad PR 3100	The reader uses a grating monochromator to select the exact wavelength in a sample. With a wavelength range from 190 to 1000nm; (wearing 10%)
ELISA Plate Washer, type Bio-rad LP 35	Designed to control the procedure of washing experimental samples arranged in plate-based formats. Users load a plate and select a program; improves the speed and accuracy of many different washing procedures, and is particularly useful for Enzyme-Linked Immunosorbent Assays (ELISAs). Microplate washers are also employed to wash cell cultures, protein arrays; (wearing 85%)
ELISA Plate Incubator, type Bio-rad STAT FAX 2200	Superior temperature control and efficient orbital shaking dramatically increase the sensitivity of EIA assays, as well as reducing incubation times. The detection limit of an HBsAg assay, for example, can be increased by a factor of two from 0.8 U/ml to 0.42 U/ml, simply by incubating at a constant 37 <sup>0</sup> C; (wearing 10%)

## II. Greenhouses for research purposes

Type	Purpose	Degree of exploitation
Grapevine Core Collection	Maintenance of the National grapevine germplasm collection – G0 (177 accessions - breeders' material)	80%
Grapevine pre-multiplication planting material	Maintenance and pre-multiplication of G1 Planting material	80%
Grapevine depositor for clones and new varieties unapproved	Maintenance of breeding / improvement purposes of new grapevine genotypes under investigation	80%
Multiplication of other horticultural crops	Ornamental species, medicinal species, grapevine	80%
Ecological crops	Ecological technologies for horticultural species (vegetables)	90%

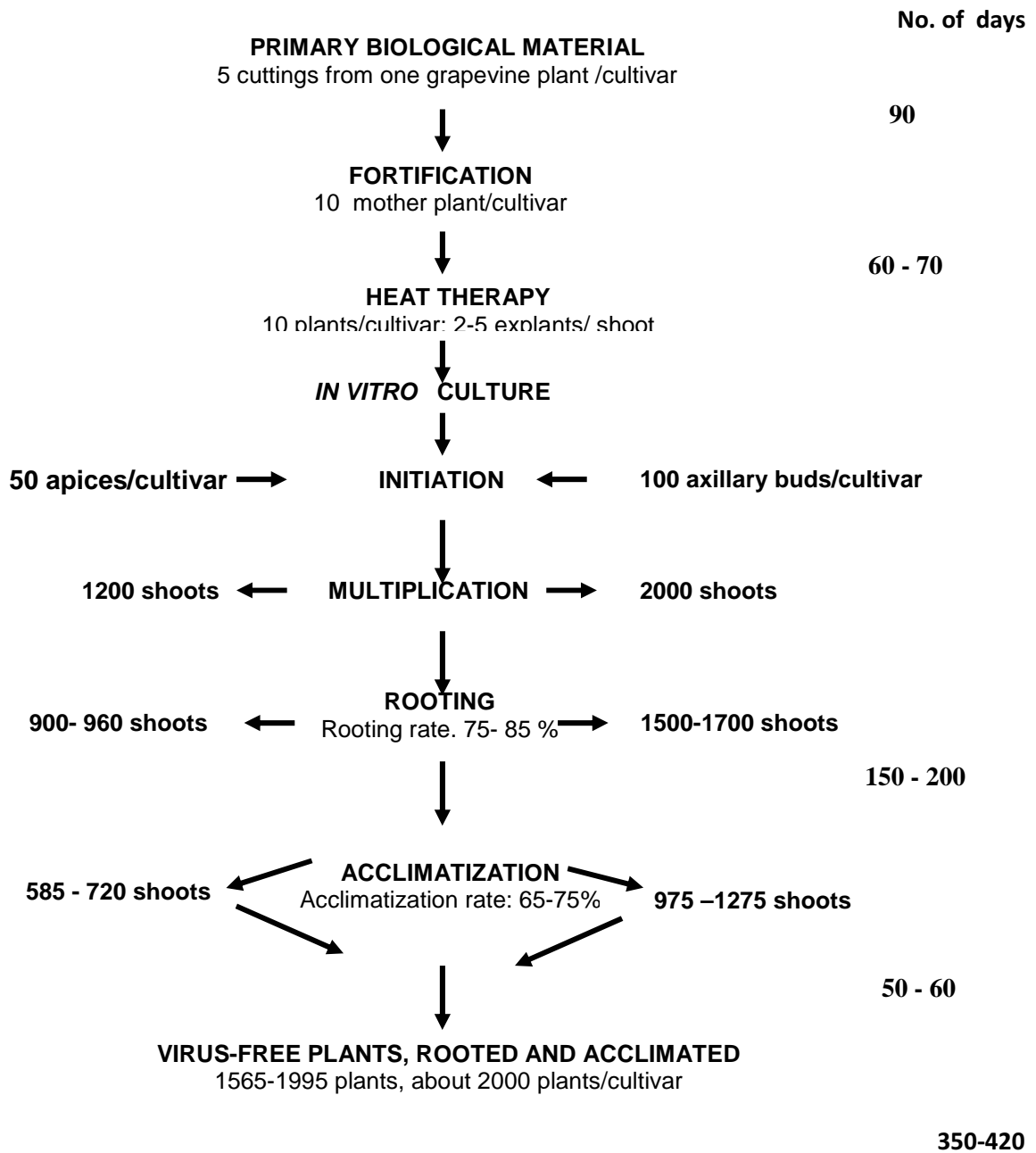
## THE CORE COLLECTION ROLE IN THE NATIONAL CERTIFICATION NETWORK for GRAPEVINE PLANTING MATERIAL IN ROMANIA



Annex 3

MARD – MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT  
 C.A.D- COUNTY AGRICULTURAL DIRECTIONS  
 T.I.S.P.M.Q - TERRITORIAL INSPECTORATES FOR SEEDS AND PLANTING MATERIAL QUALITY  
 N.I.S.Q. - NATIONAL INSPECTION OF SEEDS' QUALITY  
 S.I.V.T.R. - STATE INSTITUTE FOR VARIETY TESTING AND REGISTRATION  
 P.C.D. - PHYTOSANITARY COUNTY DIRECTIONS  
 C.L.Q.S.P.M.C - CENTRAL LABORATORY FOR QUALITY SEEDS AND PLANTING MATERIAL CONTROL

**OBTAINING THE VIRUS-FREE AND *IN VITRO* PROPAGATED GRAPEVINE MATERIAL**



**ESTIMATED PRICE – 5, 3 EURO/ ACCLIMATED PLANT**

