EUROPEAN COOPERATION PROGRAM INTERREG VA GREECE - ITALY 2014/2020 - BEST PROJECT. PROCEDURE EX ART. 1 OF DLN 76 OF 16/07/2020 CONVERTED INTO LAW N. 120 OF 11/09/2020 AND PURSUANT TO ART. 95, PARAGRAPH 3 OF D.LGS. 50/2016 FOR THE ASSIGNMENT OF THE SERVICE OF "ANALYSIS OF AGROBIODIVERSITY AND STUDY OF VEGETABLE SPECIES GROWN AT RISK OF EXTINCTION IN THE AREA OF PILOT ACTION 1 OF THE BEST PROJECT AND RELATED ACTION PLAN". CUP: B38H19005670006 - CIG: 8730686601.

Report on the performance of the activities of the contractor of the LAG SEB scarl service referred to in point b) of art. 4 - TERMS FOR THE PERFORMANCE OF THE ACTIVITIES of the contract:

> **Descriptive documents containing the results of the genetic erosion / extinction assessment analysis, the definition of the in situ and ex situ conservation methodologies of the germplasm of the identified agricultural species and varieties at greatest risk of extinction, as well as the operational protocols of conservation and management pursuant to art. 1 point 2 lett. a) b)**

Text by Vincenzo Fucilli (project manager), Arcangelo Cirone, Alessandro Petrontino, Giacomo Maringelli , Cinzia Montemurro.

Summary

1.0 Materials and methods

1.1 Genetic erosion / extinction assessment analysis

For each of the identified species, a delinearerisk scenarios connected to evaluation elements which will be identified from time to time starting from internal and external characteristics of the analysis system. Specifically, thanks also to the setting of a SWOT analysis relating to the protection / conservation of the single agronomic species, the following are assessed: the intrinsic characteristics of the variety / cultivar analyzed, the characteristics of the most widespread territory, the capacity of the regional / local system to provide for their protection and conservation or even their productive exploitation, the evolution and trends of the market and the consumption preferences of local and niche products, the pressures deriving from urbanization and rationalization of production systems. The analysis thus contributes to assessing the risk of genetic erosion from different perspectives, giving a complete picture of the emergencies and the levers to be used in the formulation of the Action Plan.

RGV		VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
HERBACEOUS	1	Fava Viola		X	
HERBACEOUS	2	Grano duro San Pasquale			X
HERBACEOUS	3	Grano tenero Bianchetta	X	X	X
FORAGE	4	Trifoglio sotterraneo	X		
FORAGE	5	Trifoglio incarnato	X		
FRUIT	6	Fico Petrelli	X	X	
FRUIT	7	Fico Verdesca		X	X
FRUIT	8	Pero Gentile reale	X		X
FRUIT	9	Pero Recchia falsa	X	X	
FRUIT	10	Susino S. Anna Ovale		X	
ORTIVE	11	Carciofo Bianco di Taranto			X
ORTIVE	12	Cima di Cola	X		
ORTIVE	13	Fagiolino dall'occhio Occhiopinto	X		X
ORTIVE	14	Fagiolino pinto	X		
LIVES	15	Cigliola			X
LIVES	16	Notardomenico	X	X	
LIVES	17	Santa Teresa			X

L'aSWOT analysis si riferisce siafor the maintenance chand reintroduction of the selected varieties in the three project areas. Referring to some brief methodological hints relating to the realization of a SWOT analysis, we remind you that the identification of the elements of analysis follows the two principles of the sign (positive or negative) and of the position (internal or external) of the element considered. Therefore, we will consider strengths and opportunities as positive elements coming from inside and outside the analytics system respectively. On the contrary, we will consider the weaknesses and threats as negative elements coming respectively from inside and outside the analysis system.

The analysis system considered in this case has internal limits which can be identified in the characteristics of the varieties and in the capacity of the territory under consideration to host the type of crop in question. The external elements, on the other hand, can be traced back to aspects linked to the market, to the trends of

consumption, possible commercial relationships and climatic aspects or interaction with the urban fabric and the natural environment.

The realization of the SWOT is useful to identify both the plausibility of a possible project of recovery and maintenance of the varieties in question, and to identify the levers of valorisation which, depending on the origin, may be controllable or not. Recovery projects will therefore be identified with a prevalence of positive, negative (neutral in the case of equity) elements whose levers are controllable (internal to the system) or uncontrollable (majority of levers external to the system). This method of analysis contributes to forming an opinion on **the enhancement scenario of** the variety in question.

The second purpose of the SWOT is to organize and systematize the elements of knowledge of the individual varieties in order to determine the degree of risk of genetic erosion. The following sheet represents the format used to determine the **risk of genetic erosion or extinction** .

The risk factors were estimated starting from the elements of the SWOT, making ùa score equal to 3, medium 2 and low 1 correspond to higher levels of risk. The sum of the scores obtained determines the degree of risk which can therefore be **low** when overall it is less than or equal to 9, **medium** when it is between 9 and 18, **high** when it is greater than 18.

Risk factors Description	Level (degree) of risk
1. Number of growers	Greater than 30
	Between 10 and 30
	Less than 10
2. Average age of the growers	Less than 40 years old
	Between 40 and 70 years old
	Over 70 years old
3. Surfaces (% of regional area of the sector)	More than 1%
	Between 0.1 and 1%
	Less than 0.1% or smaller surfaces Isolated plants or crops in family gardens and vegetable gardens
4. Distribution of cultivated areas and farm types	Very different areas, with different agro-climatic characteristics
	Limited areas, with the same agro-climatic characteristics and the same cultivation techniques
	Same company / same range / single cultivation technique
5. Type of product market	Markets and / or producer cooperatives Main varieties in Geographical Indications (GI)
	Available in small surfaces locally. Secondary varieties in GI
	Self-consumption or for study purposes.
6. Role of varietal innovation	Absence of improved varieties competitive with the local one
	Persistence of the local variety only for self-consumption
	Quick replacement of local varieties with improved varieties
7. Trend new plants	Presence of new plants
	Absence of new plants
8. Presence of national Registers / Catalogs	Fruit: varieties present in the variety lists of the different regions and varieties registered in the
	Vine: vines registered in the regional register
	Horticultural and agricultural plants: varieties registered in the National Register of conservation varieties and / or without intrinsic value
	Vine: registration in the regional register in progress. Material available from few breeders and nurserymen
	Fruit: varieties not included in the variety lists and not registered in the National Variety Register
	Vine: vines not registered in the regional register
	Horticultural and agricultural plants: not registered in the National Register of Conservation Varieties and /
	No reproduction for off-company distribution
9. Ex situ conservation	Presence of collections replicated at least twice
	Presence of only one collection
	Absence of collections

1.2 Definition of in situ and ex situ conservation methodologies of the germplasm of the identified species and agricultural varieties at greatest risk of extinction

Guidelines for the conservation and characterization of plant, animal and microbial biodiversity of interest for agriculture (National Plan on biodiversity of agricultural interest-PNBA)

The guidelines developed by the PNBA define the concept of local variety, which derives from the English translation of landraces. Although several definitions of local variety have already been proposed, a local variety can be defined as: a crop that reproduces by seed or vegetatively which is a variable population, which is identifiable and usually has a local name. The crop has not been subject to "formal" genetic improvement, and is characterized by a specific adaptation to the environmental conditions of a cultivation area (tolerant to biotic and abiotic stresses of that area), and is closely associated with the uses , the knowledge, habits, dialects and recurrences of a population that develops and continues its cultivation ".
As reported in the PNBA, this definition is supplemented by those provided by the various Italian regional laws on the protection of indigenous genetic resources (in fact, local breeds and varieties), which, in summary, are referred to as species, races, varieties, cultivars. , populations, ecotypes and clones originating in a regional territory, or of external origin, as long as they have been introduced there for at least 50 years and traditionally integrated into the agriculture and breeding of that territory. Local varieties currently disappeared from the regional territory, but kept in botanical gardens, farms or research centers present in other regions, also fall within this scope.
It is quite evident that the local variety cannot and must not be disconnected from the territory of origin (bioterritory) understood, the latter, as a place in which it, thanks to the action of farmers, has shown its adaptation over time.

Cultivated and wild species
Generally, in identifying germplasm conservation techniques, reference is made to two classes of genetic resources: wild and domesticated species. The former are best preserved in their natural habitats and within the plant communities to which they belong. In cases where these are in danger it is necessary to resort to specific forms of protection. This can occur in forest reserves, in protected areas, in special genetic reserves or ex situ, for example in germplasm banks.
All cultivated species, on the contrary, require active measures on the territory for their conservation. Ex situ conservation differs from in situ conservation because the plant material is stored in places other than those of origin. The ex situ can be a dynamic system if the populations of domesticated or wild species are kept in habitats where they are in any case exposed to selective pressure, while it is static in the event that recombination with external material is prevented and the genetic erosion of each minimized, as well as minimized accession is the selective pressure.
For many years ex situ conservation has been mainly adopted, keeping Plant Genetic Resources (RGV) in controlled environments far from the place of origin and subtracting them from their logical evolution over time and from the selective pressure of anthropogenic and environmental factors. Thus, the possibility that it was farmers, in their fields, who carried out this important function of

conservators of diversity of agricultural interest was neglected. In the rapid process of modernization, keeping the old traditional varieties, often not very productive, in cultivation was seen by younger farmers as a sort of tie that tied the rural community to a past from which it was trying to emancipate itself. In reality, it was then seen that much has been lost, but much has been preserved in situ thanks to the maintenance in cultivation of some old varieties for family self-consumption and within rural communities more oriented to tradition, in areas often marginal.

In summary, we recall the characteristics of the ex situ and in situ / on farm conservation systems. The first is conservation in special structures and with different means depending on the species. With the exception of the collection fields, it is a practically static system, at least during the conservation phase, even if the onset of variations or the loss of genetic diversity in the regeneration phase of the material in the field is possible, when the standards are not respected. In situ conservation is the conservation of ecosystems and natural habitats and the maintenance of populations and species, both wild and cultivated within them, or within the environments where they have evolved their distinctive characteristics. It is a dynamic conservation system: different populations continually adapt to biotic (including anthropogenic) and abiotic selective pressures. In situ conservation of cultivated species is generally defined as on farm.

The two systems - ex situ and in situ / on farm - must not be seen as alternatives, but as possible complementary actions to safeguard diversity. In fact, when it is not possible to carry out the in situ / on farm conservation of a certain genetic resource, at least the ex situ one guarantees its survival. In particular, however, it is believed that in situ / on farm conservation is better suited to local varieties, which have been selected and preserved for hundreds of years by farmers and are, in fact, a biological-cultural-territorial and non-biological "system". just a biological entity. Since in this case the farmer is the central figure of this system, he certainly represents the main actor of the conservation activity and it follows, therefore, that this centrality must be appropriately taken into consideration in all on farm conservation projects. . In some contexts it is appropriate to emphasize the conservation made by farmers and to support the initiatives present in the territory that operate in this sense, also to develop responsibility and awareness in the local holders of resources.

Ex situ conservation

Ultimately, ex situ conservation programs are not only complementary to in situ ones, but are sometimes the only ones that can be adopted in some situations. From a genetic point of view, ex situ conservation maintains a static genetic situation, while in situ conservation allows for evolution. Evolution means change in the wealth of genetic variants, but it is not known a priori whether it is increasing or decreasing. For small populations, the evolution generally goes towards a reduction of genetic diversity, which could culminate in the definitive extinction of the population. In this case, ex situ conservation is able to ensure the maintenance of a higher level of diversity than in situ. Furthermore, for species of agricultural and agri-food interest, where the intensity of the risk of erosion / extinction can drastically change, even in a very short time, ex situ conservation guarantees the maintenance of specific genotypes, populations, varieties, races, strains, etc. and / or their reintroduction into cultivation where they have been lost.

In summary, ex situ conservation becomes the mandatory conservation tool when:

- the populations are subjected to the highly impacting effects of anthropogenic activity, such as the replacement of local breeds and varieties with others alien to the territory (such as the introduction of modern varieties);
- changes in environmental or socio-economic conditions radically change the structure and vocation of a territory, with the abandonment of agriculture;
- the cultivation area of a given population is constantly decreasing for various reasons and there is a high risk of extinction.

To identify the most appropriate and effective conservation techniques, it is necessary to know well the biology of the species (especially the reproductive one) and the genetic structure of its populations and it can be carried out in different ways, synthetically grouped as follows:

- collections of plants in open field, in pots, in greenhouses;
- collections of seeds kept in seed banks or germplasm banks (very common mode);
- collections of propagation material, seedlings, tissues and more, kept in vitro or in cryopreservation.

All material stored ex situ should be managed in such a way as to minimize the risks in the event of natural disasters, technical problems, biological damage, socio-economic problems, etc. The protection procedures, therefore, must include continuous monitoring of the material and, in particular, the conservation of duplicates of the germplasm in different locations.
Furthermore, the management of ex situ populations must be careful to avoid any intervention that could undermine the genetic integrity and vitality of the material (reduction of genetic diversity, artificial selection, transmission of pathogens, uncontrolled hybridizations, etc.). Furthermore, particular attention must be paid to the collection of the minimum number of genotypes capable of guaranteeing the maximum diversity of the population, obviously in relation to logistical and financial limits.

In situ conservation

In situ / on farm conservation. This mode of conservation is dynamic, populations change continuously in response to the selective pressures to which they are subjected and from the pedo-climatic environment in which they are found, allowing the possibility of adaptation of species or populations and it is also possible a co-evolution between different living being. It follows, therefore, that it would be more appropriate to speak of "safeguarding" rather than "conservation", as the latter term has a connotation of static.
From this point of view, in situ / on farm conservation has a holistic approach to safeguarding the biodiversity of the agro-ecosystem, i.e. it tends to safeguard all living forms present in this situation, whether cultivated or spontaneous, but above all it does not neglect the maintenance, if not the strengthening, of the complex of relationships that develop between them. In this context, the conservation of local varieties fits well, which have been cultivated for a long time, with no temporal solution, in a certain locality and by a certain human community, so that they can be defined in the common jargon as "native", or "from always "cultivated there. With regard to the presence "always" in a given territory, a clarification is appropriate. For the annual species propagated by seed, fifty reproductive cycles (about 50 years) of continuous maintenance of a population in a certain area can

be considered sufficient time for a variety to develop those characteristics of adaptation and bond with the environment (also including the anthropic environment) such that it can be defined as "local". However, it should be noted that it is difficult to identify a precise and defined time frame, after which a variety can be considered "adapted" and therefore the 50-year threshold provided is to be considered entirely indicative. Furthermore, for some polyennial tree or shrub species, 50 years is insufficient to consider them adapted to a certain place and therefore “local”.

From the considerations on the times it follows that actions of reintroduction of local varieties in a territory or of development / selection of new populations starting from local varieties (actions which also contribute to maintaining diversity useful to man) should not be considered under the term of "On farm conservation". In fact, the "reintroduction" - a particularly topical topic - when it refers to populations preserved for decades ex situ, can lead to the cultivation of subjects lacking that adaptation to the physical, biological and cultural conditions of the reintroduction area that distinguishes the varieties locals. In other words, the moment of reintroduction triggers a new process of adaptation which, over time, will lead these populations to become real local varieties, different from the original ones.

It is also true that often the boundary between reintroduction and exchange of propagation material in an area (especially if this is large and with variable pedo-climatic conditions) is rather blurred. And it is equally true that evolving genetic material that is not perfectly adapted to a given environment can still be useful for conservation (eg shifting the frequencies of rare or poorly represented alleles in the original environment, etc.). Furthermore, reintroduction (even in contiguous areas) is sometimes necessary when the variety has completely disappeared from cultivation and it is not possible to reintroduce it in the same area due to changes in the environment or social fabric. In situ / on farm conservation must be carried out in such a way as to allow the local population / variety to maintain all the variability that distinguishes it and to remain in balance with the cultivation environment in which it has evolved its distinctive characteristics, in such a way that the latter are not lost. For this purpose it is particularly important to plan the production of the propagating material, which must take place in the area of origin and in conditions that avoid both mechanical pollution (pollution during sowing, harvesting, storage) and of genetic type.

The former are easier to control, the latter, on the other hand, can be more problematic and depend on the species (if autogamous or allogamous and in the latter case if the pollination is anemophilous or entomophilous), on the orographic conditions of the multiplication area , multiplied surfaces, climatic conditions, etc.

As for the development actions of new populations / varieties through crossing with other varieties or selection actions aimed at identifying, maintaining and propagating only some genotypes, it is obvious that these actions can distort the genetic constitution and therefore the characteristics of local varieties.

Genetic variability is the basis of all genetic improvement work and local varieties have been the raw material from which scientific research has begun since the beginning of the 20th century to produce improved or "modern" varieties and still many today varieties of vegetables and forage crops (grasses and legumes) are obtained by selection from local varieties. With this in mind, each selection process leads to a reduction in diversity with respect to the original material, because specific choices are made dictated by the objectives of the improvement program. Recently an interesting approach for the use of this variability in genetic improvement is offered by participatory plant breeding, the purpose of which remains - like classic breeding - to obtain improved varieties, but providing for the participation of farmers in the selection process and aiming at obtaining varieties with a broad genetic basis.

2.0 Results

2.1 Genetic erosion / extinction assessment analysis

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
HERBACEOUS	1 Fava Viola		X	

Strengths	Weakness
✓ Appreciated for its organoleptic characteristics as it is more tender and flavorful than the most common commercial varieties. ✓ Vocation of the territory for the production of legumes / cereals and forage ✓ Interest of some young entrepreneurs in starting the activity linked to the spread of the short chain ✓ Supply chain companies capable of enhancing small quantities on niche segments ✓ Peasant agriculture, not intensive, traditional techniques	✓ Low productivity compared to modern commercial varieties ✓ Very low number of growers ✓ Agricultural area cultivated with the very low variety ✓ Persistence of the local variety only for self-consumption ✓ Poor vocation for the diversification of productions and poor integration between the productive sectors
Opportunities	**Threats**
✓ Diversified tourist offer (seaside, rural, cultural, naturalistic tourism) ✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Consolidated experiences of tourism and sustainable agriculture in line with the protection of agrobiodiversity ✓ Typical dishes based on legumes ✓ Development of short chain marketing forms ✓ Growing consumer interest in local and typical products ✓ Interest of the GDO for the procurement and / or enhancement of local products ✓ Growing demand for food products linked to the territories of origin	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Fruit and vegetable market with high fluctuations ✓ Wildlife damage ✓ Product characteristics not sufficient for marketing with large-scale distribution

SWOT scenario	**Recovery project with the possibility of success, however excessively influenced by external elements of analysis.**
Degree of risk of genetic erosion:	**22 - high risk**

RGV	VARIETY NAME		C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
HERBACEOUS	**2**	**Grano duro San Pasquale**			**X**

Strengths	Weakness
✓ Variety already locally widespread on the territory in the past in the Ionian arc. ✓ In the past it was highly appreciated for the production of pasta ✓ Data relating to the characteristics of whole flours already detected and known: proteins, yellow index, brown index, gluten index, carotenoids, polyphenols. ✓ Vocation of the territory for the production of cereals and fodder	✓ Variety of wheat with low productivity ✓ Currently it is cultivated and stored only in public bodies. ✓ Typical productions valued and little integrated with the tourist offer ✓ Aging of the agricultural entrepreneurial fabric little used to innovation and the recovery of ancient varieties.
Opportunities	**Threats**
✓ Growing consumer interest in local and typical products ✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Strong presence of structures and companies for the processing of agro-food products ✓ Presence of non-intensive peasant agriculture and traditional techniques.	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Abandonment of the countryside ✓ Wildlife damage

SWOT scenario	**Uncertain recovery project due to neutrality of the elements under analysis. The control of the valorisation levers can be difficult due to the large number of external ones.**
Degree of risk of genetic erosion:	**23 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
HERBACEOUS	**3 Grano tenero Bianchetta**	**X**	**X**	**X**

Strengths	**Weakness**
✓ Variety already locally widespread in the past throughout Puglia and therefore adaptable to the three areas of the project. ✓ Cultivation associated with the preparation of typical dishes (Grain of the dead, ciccecuotte , colva) ✓ Cultivation of Bianchetta also for use in the zootechnical field ✓ It adapts to cultivation both in the plains and in the hills, it happens well to durum wheat and tolerated the ringrani. ✓ Data relating to the characteristics of whole flours already detected and known: proteins, yellow index, brown index, gluten index, carotenoids, polyphenols. ✓ Vocation of the territory for the production of cereals and fodder	✓ Variety of wheat with low productivity. Currently it is cultivated and stored only in public bodies. ✓ Typical productions valued and little integrated with the tourist offer ✓ Aging of the agricultural entrepreneurial fabric little used to innovation and the recovery of ancient varieties. ✓ Data relating to the production characteristics detected and known only in part: components of production and resistance to physiopathies
Opportunities	**Threats**
✓ Growing consumer interest in local and typical products ✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Strong presence of structures and companies for the processing of agro-food products ✓ Presence of non-intensive peasant agriculture and traditional techniques.	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Abandonment of the countryside ✓ Wildlife damage

SWOT scenario	**Recovery project with a plausibly positive outcome. Enhancement consistently dependent on external elements of analysis.**
Degree of risk of genetic erosion:	**19 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
FORAGE	**4 Trifoglio sotterraneo**	**X**		

Strengths	**Weakness**
✓ Forage variety traditionally grown in Puglia ✓ Vocation of the territory for the production of legumes / cereals and forage ✓ Presence of three botanical varieties or subspecies with consequent greater amplitude of agro-climatic and pedological tolerance ✓ It also grows well where it is difficult to operate quickly with mechanical means ✓ The underground clover adapts to all types of soil and, depending on the pH and the degree of humidity, it is possible to choose the subspecies most suited to the particular edaphic situation. ✓ Strong and rapid growth ✓ Very dense and compact grass cover suitable for grazing ✓ Due to its precious characteristic of self- sowing , this subspecies, if well used, becomes perennial and can be very useful for the improvement of the turf of natural pastures and arable land.	✓ Strong geocarpism can make harvesting and grazing difficult ✓ Use for zootechnical purposes only
Opportunities	**Threats**
✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Presence of non-intensive peasant agriculture and traditional techniques.	✓ It faces competition from commercial fodder selected for animal feed ✓ Very low number of growers ✓ Climate changes causing extreme weather events ✓ Wildlife damage

SWOT scenario	**Recovery project with a plausibly positive outcome. A large number of internal elements of the analysis system can be leveraged.**
Degree of risk of genetic erosion:	**23 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
FORAGE	5 Trifoglio incarnato	X		

Strengths	Weakness
✓ Forage variety traditionally grown in Puglia ✓ Vocation of the territory for the production of legumes / cereals and forage ✓ adapted to the Mediterranean climate, ✓ interesting production on loose and dry soils. ✓ palatable and digestible zootechnical food as long as the harvest is carried out with flowering plants.	✓ Use for zootechnical purposes only ✓ Late harvests can cause problems for animals due to the numerous bristly hairs in the flower calyx
Opportunities	**Threats**
✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Presence of non-intensive peasant agriculture and traditional techniques.	✓ It faces competition from commercial fodder selected for animal feed ✓ Very low number of growers ✓ Climate changes causing extreme weather events ✓ Wildlife damage

SWOT scenario	**Recovery project with uncertain outcome. A large number of internal elements of the analysis system can be leveraged.**
Degree of risk of genetic erosion:	**24 - high risk**

RGV	VARIETY NAME		C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
FRUIT	6	Fico Petrelli	X	X	

Strengths	Weakness
✓ Known variety, characterized and preserved in 3 research institutes ✓ Particularly widespread in Puglia, specifically in the project areas, where it represents a territorial specificity. ✓ Due to the intravarietal variability it is also possible to find particularly early clones. ✓ Presence of established traditional techniques (inoleazione , caprification, attraction of pollinators with ripe fruit) ✓ Interest of some young entrepreneurs in starting the activity linked to the spread of the short supply chain of fresh products ✓ Supply chain companies capable of enhancing small quantities on niche segments ✓ Peasant agriculture, not intensive, traditional techniques ✓ High plant vigor ✓ High productivity ✓ Not particularly demanding plant ✓ Average ripening scale ✓ easy detachment of the fruit from the peduncle ✓ high juiciness and sweetness ✓ Resistant to drought and salty soils	✓ intravarietal variability makes univocal recognition difficult ✓ Very low number of growers ✓ Persistence of the local variety only for self-consumption ✓ Poor resistance to manipulation, especially of those supplied ✓ Local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Development of short chain marketing forms ✓ Growing consumer interest in local and typical products ✓ Interest of the GDO for the procurement and / or enhancement of local products ✓ Growing demand for food products linked to the territories of origin ✓ Diversified tourist offer in the area of possible cultivation (seaside, rural, cultural, naturalistic tourism)	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Fruit and vegetable market with high fluctuations ✓ Wildlife damage ✓ Product characteristics not sufficient for marketing with large-scale distribution ✓ Poor vocation for the diversification of productions and poor integration between the productive sectors

SWOT scenario	**Recovery project with a plausibly positive outcome, however there are many external elements that influence the success of the enhancement process.**

Degree of risk of genetic erosion:	**11 - medium risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
FRUIT	**7 Fico Verdesca**		**X**	**X**

Strengths	**Weakness**
✓ Known variety, characterized and preserved in 2 research institutes ✓ Particularly widespread in the provinces of Brindisi and Taranto, where it represents a territorial specificity. ✓ Interest of some young entrepreneurs in starting the activity linked to the spread of the short supply chain of fresh and processed products (dried figs, fig jam) ✓ Supply chain companies capable of enhancing small quantities on niche segments ✓ Peasant agriculture, not intensive, traditional techniques ✓ High vigor ✓ Easy detachment of the peduncle ✓ Variety with a sour and aromatic flavor, very pleasant, intense flavor and high juiciness and sweetness ✓ It has no particular agronomic needs. ✓ High resistance to manipulation.	✓ medium degree of ripening. ✓ Very low number of growers ✓ Few isolated individuals of the variety ✓ Persistence of the local variety only for self-consumption ✓ Poor resistance to manipulation, especially of those supplied ✓ Local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Development of short chain marketing forms ✓ Growing consumer interest in local and typical products ✓ Interest of the GDO for the procurement and /	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Fruit and vegetable market with high fluctuations ✓ Wildlife damage

or enhancement of local products ✓ Growing demand for food products linked to the territories of origin ✓ Diversified tourist offer in the area of possible cultivation (seaside, rural, cultural, naturalistic tourism)	✓ Product characteristics not sufficient for marketing with large-scale distribution ✓ Poor vocation for the diversification of productions and poor integration between the productive sectors

SWOT scenario	**Recovery project with a plausibly positive outcome, however there are many external elements that influence the success of the enhancement process.**
Degree of risk of genetic erosion:	**20 - high risk**

RGV		VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
FRUIT	8	Pero Gentile reale	X		X

Strengths	Weakness
✓ Known variety, characterized and preserved in a research institution ✓ Particularly widespread in Puglia. Especially in the Bari and Taranto areas. ✓ Interest of some young entrepreneurs in starting the activity linked to the spread of the short chain of fresh and processed products (juice, puree, jam) ✓ Supply chain companies capable of enhancing small quantities on niche segments ✓ Peasant agriculture, not intensive, traditional techniques ✓ Appreciable organoleptic characteristics ✓ Fair agronomic characteristics ✓ It adapts to all regional pedoclimatic environments, rustic variety, ✓ moderately resistant to scab. ✓ Good size,	✓ High productivity ✓ Very low number of growers ✓ Few isolated individuals of the variety ✓ Persistence of the local variety only for self-consumption and for the domestic market ✓ poor resistance to manipulation ✓ Local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Development of short chain marketing forms ✓ Growing consumer interest in local and typical products ✓ Interest of the GDO for the procurement and / or enhancement of local products ✓ Growing demand for food products linked to the territories of origin ✓ Diversified tourist offer in the area of possible cultivation (seaside, rural, cultural, naturalistic tourism)	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Fruit and vegetable market with high fluctuations ✓ Wildlife damage ✓ Product characteristics not sufficient for marketing with large-scale distribution ✓ Poor vocation for the diversification of productions and poor integration between the productive sectors

SWOT scenario	**Recovery project with a plausibly positive outcome, however there are many external elements that influence the success of the enhancement process.**
Degree of risk of genetic erosion:	**18 - medium risk**

RGV	VARIETY NAME		C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
FRUIT	9	Pero Recchia falsa	X	X	

Strengths	Weakness
✓ Known variety, characterized and preserved in 2 research institutes ✓ Particularly widespread in Puglia: especially in the Bari and Taranto areas. ✓ cited among the best summer pears of the Region because it ripens in a period in which there are no foreign varieties on the market ✓ very precocious ✓ described as an excellent table variety. ✓ High productivity and discrete agronomic characteristics ✓ It adapts to all regional pedoclimatic environments, rustic variety, moderately resistant to scab ✓ Very good flavor, sweet, with a slightly acidic aftertaste. ✓ Suitable for fresh consumption, but also for transformation into juice, puree, jam (very sugary, requires little sugar in the transformation) ✓ Interest of some young entrepreneurs in starting the activity linked to the spread of the short chain of fresh and processed products (puree, jam) ✓ Supply chain companies capable of enhancing small quantities on niche segments ✓ Peasant agriculture, not intensive, traditional techniques	✓ Medium vigor ✓ The high intravarietal variety makes univocal recognition difficult also due to the different cases of synonymy ✓ Very low number of growers ✓ Few isolated individuals of the variety ✓ Persistence of the local variety only for self-consumption and for the domestic market ✓ Local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather

✓ Development of short chain marketing forms ✓ Growing consumer interest in local and typical products ✓ Interest of the GDO for the procurement and / or enhancement of local products ✓ Growing demand for food products linked to the territories of origin ✓ Diversified tourist offer in the area of possible cultivation (seaside, rural, cultural, naturalistic tourism)	events ✓ Fruit and vegetable market with high fluctuations ✓ Wildlife damage ✓ Product characteristics not sufficient for marketing with large-scale distribution ✓ Poor vocation for the diversification of productions and poor integration between the productive sectors

SWOT scenario	**Recovery project with a probably positive outcome and notable external elements capable of influencing the evolution of the enhancement process.**
Degree of risk of genetic erosion:	**15 - medium risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
FRUIT	10 Susino S. Anna Ovale		X	

Strengths	Weakness
✓ Selected and stored in a public body ✓ Very ancient origin ✓ High productivity ✓ Supply chain companies capable of enhancing small quantities on niche segments ✓ Peasant agriculture, not intensive, traditional techniques ✓ It has no particular agronomic needs. ✓ Variety with a sour and aromatic flavor, very pleasant.	✓ Very low number of growers ✓ Few isolated individuals of the variety ✓ Persistence of the local variety only for self-consumption and for the domestic market ✓ Medium resistance to manipulation. ✓ Local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Interest of some young entrepreneurs in starting the activity linked to the spread of the short chain ✓ Supply chain companies capable of enhancing small quantities on niche segments ✓ Growing consumer interest in local and typical products ✓ Interest of the GDO for the procurement and / or enhancement of local products ✓ Growing demand for food products linked to the territories of origin ✓ Diversified tourist offer in the area of possible cultivation (seaside, rural, cultural, naturalistic tourism)	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Fruit and vegetable market with high fluctuations ✓ Wildlife damage ✓ Product characteristics not sufficient for marketing with large-scale distribution ✓ Poor vocation for the diversification of productions and poor integration between the productive sectors

SWOT scenario	**Recovery project with uncertain outcome, however there are many external elements that influence the success of the enhancement process.**
Degree of risk of genetic erosion:	**23 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
ORTIVE	**11 Carciofo Bianco di Taranto**			**X**

Strengths	**Weakness**
✓ Variety kept in 2 public bodies ✓ techniques and methods of cultivation, planting and multiplication of the artichoke, fertilization and genetic improvement already known and reported in the literature. ✓ Productivity greater than 3 years ✓ variety is hardly present in the Taranto area	✓ Ancient variety of artichoke grown only sporadically in the gardens of the province of Taranto ✓ Not remarkable for the content of antioxidant compounds compared to other local Apulian varieties. ✓ Typical productions valued and little integrated with the tourist offer ✓ Aging of the agricultural entrepreneurial fabric little used to innovation and the recovery of ancient varieties.
Opportunities	**Threats**
✓ Growing consumer interest in local and typical products ✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Strong presence of structures and companies for the processing of agro-food products ✓ Presence of non-intensive peasant agriculture and traditional techniques.	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Abandonment of the countryside ✓ Wildlife damage

SWOT scenario	**Recovery project with uncertain outcome, mainly dictated by internal weaknesses and a large number of external elements of uncertainty.**
Degree of risk of genetic erosion:	**25 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
ORTIVE	12 Cima di Cola	X		

Strengths	Weakness
✓ Variety preserved in a public institution ✓ Production associated with the preparation of typical dishes ✓ Vocation of the territory for the production of vegetables	✓ Aging of the agricultural entrepreneurial fabric little used to innovation and the recovery of ancient varieties. ✓ Organoleptic characteristics not always appreciated (intense smell during cooking and spongy consistency)
Opportunities	**Threats**
✓ Growing consumer interest in local and typical products ✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Strong presence of structures and companies for the processing of agro-food products ✓ Presence of non-intensive peasant agriculture and traditional techniques.	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Abandonment of the countryside ✓ Wildlife damage

SWOT scenario	**Recovery project with uncertain outcome with external elements that condition the outcome of the enhancement process.**
Degree of risk of genetic erosion:	**22 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
ORTIVE	13 Fagiolino dall'occhio Occhiopinto	X		X

Strengths	Weakness
✓ Variety kept in a public body. ✓ The pinto bean has been grown in Puglia since time immemorial and belongs to the agronomic tradition that adopts cultivation techniques consolidated over time and typical cultural references. ✓ Possible reintroduction in two project areas. ✓ Vocation of the territory for the production of vegetables ✓ it is highly appreciated in Puglia and is used in recipes linked to local tradition.	✓ Aging of the agricultural entrepreneurial fabric little used to innovation and the recovery of ancient varieties. ✓ The citations found do not report precise territorial references. ✓ Production is medium-low.
Opportunities	**Threats**
✓ Growing consumer interest in local and typical products ✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Strong presence of structures and companies for the processing of agro-food products ✓ Presence of non-intensive peasant agriculture and traditional techniques. ✓ Interest of the GDO for the procurement and / or enhancement of local products ✓ Growing demand for food products linked to the territories of origin	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Abandonment of the countryside ✓ Wildlife damage ✓ Product characteristics do not seem sufficient for marketing with large-scale distribution.

SWOT scenario	**Recovery project with a generally positive outcome with external and internal elements capable of influencing the outcome of the enhancement process.**
Degree of risk of genetic erosion:	**19 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
ORTIVE	**14 Fagiolino pinto**	**X**		

Strengths	Weakness
✓ Variety kept in a public body. ✓ Vocation of the territory for the production of vegetables ✓ present in Puglia since ancient times. ✓ Once widespread in the province of Bari and probably also in other Apulian provinces. ✓ Used in traditional regional culinary preparations. ✓ belongs to the agronomic tradition that adopts cultivation techniques consolidated over time and typical cultural references ✓ very rustic plant	✓ Aging of the agricultural entrepreneurial fabric little used to innovation and the recovery of ancient varieties. ✓ Today very rare, it is grown almost exclusively in small plots of land or in family gardens. ✓ The citations relating to the variety do not give precise territorial references.
Opportunities	**Threats**
✓ Growing consumer interest in local and typical products ✓ Good propensity of young people to work in the agricultural and artisanal sectors. ✓ Strong presence of structures and companies for the processing of agro-food products ✓ Presence of non-intensive peasant agriculture and traditional techniques. ✓ Interest of the GDO for the procurement and / or enhancement of local products ✓ Growing demand for food products linked to the territories of origin	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Abandonment of the countryside ✓ Wildlife damage ✓ Product characteristics do not seem sufficient for marketing with large-scale distribution.

SWOT scenario	**Recovery project with a plausibly positive outcome, however the elements outside the analysis system have a high consistency therefore capable of influencing the outcome of the enhancement process.**
Degree of risk of genetic erosion:	**19 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
LIVES	15 Cigliola			X

Strengths	Weakness
✓ Preserved at a research institution ✓ characterized by early phenological phases, starting from bud break; ✓ the phases of flowering, veraison and finally of ripening take place in an early period. ✓ Productivity is regular and constant, fertility is good. ✓ it lends itself very well as a base for table wines ✓ Wine with an olfactory profile characterized by moderate intensity especially for floral and herbaceous notes ✓ good alcohol content and structure are accompanied by an excellent balance and gustatory persistence	✓ Various cases of synonymy in the different regional areas make the name of the variety little known ✓ Winemaking on a farm scale not yet tested. ✓ local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Growing consumer interest in local and typical products ✓ Growing demand for products linked to the territories of origin	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Wildlife damage

SWOT scenario	**Recovery project with a plausibly positive outcome with the possible involvement of positive internal levers to influence the success of the enhancement**
Degree of risk of genetic erosion:	**19 - high risk**

RGV	VARIETY NAME		C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
LIVES	16	Notardomenico	X	X	

Strengths	Weakness
✓ Preserved at a research institution ✓ Wine variety tested with micro vinification at company level. ✓ Possible reintroduction in two areas ✓ Present in the production disciplinary of the Ostuni DOC. ✓ Known technological characteristics: medium sugar content of the must; average total acidity of the must; low pH of the must; ✓ high production of grapes per m^2 ✓ High fertility, both basal and distal, and productivity. ✓ it lends itself very well to obtaining a fine rosé wine ✓ if vinified in red it has good aromatic complexity with a prevalence of notes of ripe fruit	✓ Present since ancient times, with always rather modest surfaces ✓ Poor specialization of production: widespread in old mixed vineyards ✓ often mixed with other white, red and black varieties therefore the varietal characters are not particularly recognizable today. ✓ High synonymy: present with other denominations in different wine-growing areas of the Puglia region ✓ Wine with weak structure therefore not suitable for aging ✓ local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Growing consumer interest in local and typical products ✓ Growing demand for products linked to the territories of origin	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Wildlife damage

SWOT scenario	**Recovery project with a generally positive outcome with negative elements that can be overcome thanks to the enhancement levers inside the analysis system.**
Degree of risk of genetic erosion:	**19 - high risk**

RGV	VARIETY NAME	C. RIPAGNOLA	DUNE COSTIERE	MAR PICCOLO
LIVES	17 Santa Teresa			X

Strengths	Weakness
✓ Preserved at a research institution ✓ Variety that in the past "accompanied" the primitivo in the vineyards of the Taranto area. ✓ high vigor of the shoot ✓ high production of grapes per m^2 ✓ Technological characteristics of the must already known: low sugar content of the must; average total acidity of the must; medium pH value of the must ✓ Good fertility, both basal and distal, and productivity ✓ With a fairly low alcohol content, it reveals a good content of total acidity: the right balance between the acid taste and a fair fullness of the body	✓ ripening of the grapes, quite late therefore increases the risk of fog and excess water etc. ✓ slightly poor wine structure ✓ local nurseries not yet ready to produce plant material of local varieties
Opportunities	**Threats**
✓ Connection of manufacturing companies with tourism activities in an area where the sector is developed and relevant ✓ Growing consumer interest in local and typical products ✓ Growing demand for products linked to the territories of origin	✓ Advanced age of entrepreneurs in the sector and insufficient interest of young people ✓ Climate changes causing extreme weather events ✓ Wildlife damage

SWOT scenario	**Recovery project with a probably positive outcome with numerous elements on which to leverage for enhancement.**
Degree of risk of genetic erosion:	**20 - high risk**

2 .2 Storage protocols

2.2.1 Ex situ seed conservation protocol for herbaceous plants

1. **Ventilate and sift** the samples to remove foreign seeds, plant fragments, soil and other coarse impurities.

2. The lots or samples collected in the field, before sending to the bank, must be kept in a cool, dry and shady place. In particular:

- Avoid leaving the germplasm in the car or in any other place where there are high temperatures. Exposure to high temperatures and direct radiation can, in fact, damage it or compromise access.
- Always keep the ventilation around the germplasm high ; use only and exclusively paper bags or cotton bags, able to guarantee correct transpiration.
- Always check the correct closure of the bags and bags in order to avoid the loss and / or contamination of the germplasm collected.
- Close the envelopes preferably with pins or staples; if you use adhesive tape, take care to apply it only to the outside of the wrapper. In the case of very small seeds it may in fact happen that when the bag is opened these adhere to the glue and become unusable.
- Under no circumstances should you freeze the germplasm before delivering it to the bank.

3. Verification of the phytosanitary status of the collected material: it is necessary to document the presence of pathologies and the health status of the population where the sampling is being carried out, as well as any treatments to which the germplasm has been subjected (e.g. fumigations, fungicide or insecticide pretreatments). The pathologies present in the moved germplasm should always be noted, as well as the diseases present in the sampling region. It is also important to indicate whether the plants are healthy in an area known or historically known as prone to infestations. Before any shipment of the material for scientific and conservation purposes, make sure that it is not necessary to attach a phytosanitary certificate; Community regulations currently allow the free circulation of germplasm within all the territories of the European Union, while they provide for certifications of origin and phytosanitary documentation for third countries.

4. Quarantine: Before the collected material is introduced into the bank's premises, a period of quarantine, variable over time, should be respected, during which the germplasm is stored in an external environment and isolated from the bank's structures. This procedure allows to evaluate the phytosanitary state of the collected material and in particular to ascertain the possible presence of mycosis and phytophagous or harmful parasites. In fact, it is not rare to find, even in perfectly clean and treated accessions, material that presents damage or harmful organisms capable of compromising the germplasm .

5. Initial tests aimed at evaluating the incoming batches: if the batch allows it, a series of tests can be performed (germination, vitality, vigor, internal humidity calculation, calculation of the number and initial weight of the seeds, etc.) to have useful data in order to plan the access destination, the number of test replicas and the number of seeds per replicate, as well as to monitor the productivity of the population. The results of each test are recorded in a specific form that will be attached to all documentation relating to the access.

6. Quantification of accession and germplasm analysis : seeds are counted by comparing the average weight of a seed to the total weight of clean seeds. There are several image analysis systems that allow the measurement of the weight and number of seeds of a sample without this being counted in advance, but to do this the lot must not have impurities . At the same time, a series of observations on the germplasm (integuments, endosperm, cotyledons, embryo, etc.) must be carried out under the microscope, stereoscope or negativoscope in order to identify anomalies or highlight peculiar characters of the taxonomic unit analyzed. The internal moisture content of the seeds (mc%) is also determined, which is essential for identifying the times and methods of dehydration for subsequent storage.

7. Dehydration of seeds by at least one of the following methods: ▪ Forced air dehydration

Drying cabinets or cells: T 10-20 ° C

NB: hot drying (> 40 ° C) and drying in the sun are strongly discouraged as there is the danger of overexposing the seeds to high temperatures after reaching the desired level of humidity. Also, large seeds can crack or break off the embryo if dehydrated at a high temperature or too quickly.

- Sealed containers and desiccants (e.g. silica gel / lithium chloride)
- Desiccant incubators
- Dehydration cells
- Do not release substances that can alter the physiology of the semen
- Resist the attack of pathogens and saprophytes (paper in none of its forms is therefore suitable)
- Prevent the passage of humidity and gas

It is recommended to dehydrate the seeds, balancing them in an environment with 15-20% RH (relative humidity) and a temperature of 15 ° C, provided with sufficient air circulation to avoid the formation of micro-zones with different parameters. The ultimate goal is to reach a water content of the seed between 3.5 and 6.5%, depending on the oil content of the seeds (plus oil plus water), if the subsequent storage is foreseen at temperatures below 0 ° C.

8. Packaging of samples for storage. The containers used for storage must have the following characteristics: It is advisable to use triple layer bags (PVP-aluminum-PVP), steel cans (of the type used for food preserves, glass jars with airtight lids.

9. Storing the samples. The methods of conservation vary depending on whether it occurs in: ▪ Medium-short period (exchange of material with the outside world, experimentation and research, periodic regeneration, etc.): it is advisable to keep about 10 bags of material for each sample. The cell must be kept at a temperature close to 0 ° C and RH of about 35% (to avoid dripping or frosting due to condensation of external humidity). Envelopes should only be opened for the above needs. This type of conservation guarantees the vitality of the seeds for a period of more than 10 years.

Long term: temperatures around -20 ° C. Under these conditions the seeds should theoretically have a *lifespan* (lifespan) higher than the same samples stored at 0 ° C by a factor of around 4, i.e. they should remain very viable for times of about 40 years or more.

The relevant **information** relating to the sample must be reported on **the label** on each container containing seeds, in order to facilitate the activity of the operators. In addition, a **database** of information relating to the sample must be maintained . Each sample must be identified by a number (**accession number**) that uniquely identifies it. All information relating to this sample should refer to this number.

10. Management of samples in storage: a **periodic regeneration of the samples in the field is necessary** , due to loss of vitality of the seed, to the impoverishment of the sample for reasons of exchange or research activities, to the too long storage period compared to forecast models, or any another reason that endangers the integrity of the sample itself.

Through a sampling program, it is necessary to periodically monitor the vitality of the collections by carrying out periodic random screening. Precautions should be taken in order to minimize intrinsic effects of this process which could alter the genetic integrity of the material.

A representative number of individuals (about 400-500) of the genetic variability present in the sample must be multiplied.

No special precautions are necessary for self-game species

- For allogamous species, appropriate isolation measures must be put in place to avoid unwanted gene flow
- For plants with entomophilic pollination, tunnels or insulators can be arranged built with metal or wooden structures and non-woven fabric or anti-aphid nets, to prevent pollinators from other plots from spreading. It may be necessary to insert pollinators in the isolators to promote pollination. It is necessary to install the isolators when the first flowers are still in bud and to remove them only after the end of flowering
- For plants with anemophilic pollination it is necessary to prepare a field scheme in which the parcels of allogamous plants are sufficiently spaced to avoid cross-fertilization. The distance varies from species to species and from other factors such as the presence of natural barriers, such as windbreak hedges, the habit of the species that stand between the plots, etc.

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GUIDELINES for the conservation and characterization of plant biodiversity of interest to agriculture - National plan on biodiversity of agricultural interest. 2013. Annex 4.1. ISBN 978-88-8145-261-3

Puglia region ex situ conservation guidelines (draft)

2. 2.2 Protocols for the conservation of tree species

The sanitary selection is carried out in adult orchards, with field observations repeated several times throughout the year in conjunction with the periods of maximum symptomatological manifestation of the individual diseases and for several vegetative cycles. The visual investigation is always followed by a diagnostic investigation to verify the real health status of the selected plants.

The ascertainment of the sanitary status can be carried out by means of immunoenzymatic assays (ELISA), mechanical transmission on differential herbaceous hosts, transmission by grafting on indicator plants, with molecular diagnosis techniques. Healthcare selection in health improvement programs is always accompanied by rehabilitation.

The rehabilitation consists of a series of techniques used alone or in combination with each other that applied to diseased plants are able to free them in whole or in part from the infection and thus obtain, by explantation and propagation of only healthy tissues (buds and / or meristematic apexes) virus-free individuals.

For the main fruit species including stone fruit, citrus fruits and vines, the most widely used techniques with which valid results have been obtained are thermotherapy, in vitro cultivation of meristematic apexes and micrografting alone or in combination with each other.

Thermotherapy

Thermotherapy is successfully applied in the rehabilitation of material infected with viruses, phytoplasmas and xylem bacteria. The use of heat is the oldest, simpler and above all more practical therapeutic method than the other practices used.

Thermotherapy consists in exposing the infected plant material to sources of humid or dry heat, for a determined time sufficient to guarantee the therapeutic purpose sought without irreversibly compromising the vital functions of the plant.

Currently, thermotherapy is carried out using hot air at a temperature of 35-38 ° C. Both plants in vegetative activity and dormant organs (cuttings, bulbs, tubers, rhizomes) can be subjected to thermotherapeutic treatment . Infected material is subjected to high temperatures for extended periods of time (over 4 weeks).

Most phytoviruses , excluding viroids, replicate with difficulty at temperatures above 32 ° C and freeze at 38 ° C.

At high temperatures it seems that there is a block or slowdown in viral replication; moreover, at high temperatures the senescence processes would increase, which seems to disadvantage viral synthesis. All this determines a lower migration capacity of the virus in the plant and in particular a reduction of the invasion capacity of the actively growing tissues which consequently are virus free. Therefore, the rehabilitation following the heat treatment mostly affects the vegetative apexes and the buds. These are removed from the mother plant and propagated separately (grafted on healthy rootstocks, micropropagated or rooted individually) originating new individuals, probably virus-free. The rehabilitation, therefore, mainly affects the vegetative apexes and the buds even if there are examples of total rehabilitation;

The treatment is easy to implement as it does not require particularly complex structures. It is performed in warm rooms equipped with fluorescent lamps that guarantee a brightness of around 5000 lux, a photoperiod of 16 hours of light and 8 dark. Optimal humidity is easily maintained through the water used for irrigation. The temperature is kept constant throughout the treatment period.

The effectiveness of thermotherapy can be increased by alternating the temperature between 30 and 40 ° C and with a variation in temperature between the aerial part and the basal part. The intrinsic characteristics of the viral species involved in the infection will also affect the success of the treatment

(degree of thermal inactivation) and those of the host (survival limit of the different varieties at high temperatures).

As regards the sensitivity of the species, it should be considered that the pome fruit tolerate the heat better than the stone fruit and that among these the cherry tree is particularly sensitive; on the contrary, most of the vine varieties tolerate high temperatures well. In some species, heat resistance can be increased with preconditioning achieved through gradual exposure to increasing temperatures.

This technique finds considerable use in combination with other recovery techniques such as the in vitro culture of apical meristems and the micrograft , of which it greatly enhances the effectiveness.

In vitro culture of meristematic apices

The in vitro culture of meristematic apexes is a technique that involves the aseptic culture of explants of meristematic tissue (0.2-0.6mm) taken from apical and / or axillary buds. The possibility of obtaining virus-free plants seems to be due to a series of factors:

- in the meristem the antagonism between normal cellular metabolism and viral infection processes would be enhanced
- in some virus-host combinations there would be a block in the migration of viral components into meristematic cells
- disorders that occur in cells after dissection.

The in vitro culture is characterized by fundamental phases that characterize a standard cycle:

Phase 0 (mother plant breeding): the mother plants are prepared to be subjected to the removal of the shoots from which to take the apexes. The plants, on which the diagnostic tests have been carried out, are usually grown in greenhouses in conditions of T 24 ° C considered optimal for growth.

Phase 1 (sterilization and installation of explants): involves sterilization of the sprouts with 10% sodium hypochlorite for 20 min , washing with sterile distilled water and removal of the meristematic apex. The sprout taken with sterile forceps is dried on sterile filter paper and the bud is cleaned with the aid of the stereomicroscope . The approximately 0.4 mm sized explant is placed on a suitable culture medium and stored in the growth chamber.

Phase 2 (multiplication): after about 30-40 days the explant is transferred to a renewed culture medium and subjected to multiplication.

Phase 3 (rooting): the rooting of the explant can be carried out in vivo or in vitro on a suitable culture substrate containing auxins.

Phase 4 (acclimatization): is the most delicate phase in which the material passes from optimal conditions of temperature, humidity and light obtained in vitro to poorer conditions in the greenhouse. Therefore, in this phase it is very important to follow the seedlings by gradually decreasing the humidity and subjecting them to preventive treatments against rotting agents.

Particular attention in this technique deserves the culture medium as the explant will draw from it

all the nutrients for its survival and growth, in addition to the hormonal substances that determine and guide the different phases of morphogenesis and development.

The recovery success rate of this technique depends on various factors, such as size of the explant, type of virus, genotype of the host. The smaller the explant, the greater the probability of obtaining remediation but at the same time it is more difficult to regenerate

the explant itself. The in vitro culture of meristematic explants should not be confused with micropropagation, for which larger explants (1-10 mm) are generally taken which, not only do not allow to restore the plants, but, on the contrary, determine a rapid multiplication and spread of infected plants.

Micrograft

A further application to in vitro culture that has been successful mainly for the restoration of citrus fruits is micrografting . The main application of the micrograft is in the remediation of viral diseases and similar viruses.

This technique consists in sterile grafting a 0.4-1.5 mm explant into a healthy rootstock in vitro. It differs from the culture of meristems in that the explant once cut is not placed directly on the culture substrate. The stages are the same as for the culture of meristematic apexes.

An example of large-scale application is the micro- grafting of citrus fruits. It can be briefly described as follows: sterilization of the seeds (eg rootstocks of trifoliate orange or its hybrids) is carried out with 0.5% sodium hypochlorite for 10 minutes and washing with sterile distilled water. Settlement of the same on a sterile culture medium and incubation in the dark for 15 days. The micrograft takes place with apexes taken from previously sterilized shoots. With the aid of a stereomicroscope , the apex is cut and placed on the pollarded rootstock. The grafted seedling installed on a sterile culture medium is stored in the growth chamber at 24 ° C, light intensity of 1000 lux and a photoperiod of 16 light and 8 dark hours.

Although this technique is more complex, it has advantages due to the absence of contact between the explant and the culture medium which guarantees a reduction in the risks of genetic instability, greater speed in obtaining seedlings and overcoming problems of youth .

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2.2.3 In vitro preservation and cryopreservation protocol

The genetic resources of horticultural and fruit species are generally conserved (ex situ) as seeds, as plants in conservation fields, and in vitro conservation is added to these.

Regarding the in vitro conservation of germplasm, several techniques have been developed:

- in vitro culture of organs or parts of plants (uses the principle of micropropagation)
- techniques based on slow growth , where the plant material is kept in the short and medium term, such as plant tissues or seedlings on a gelled culture substrate;
- cryopreservation, by which the plant accessions, after being suitably treated, are stored at low or ultra -low temperatures (immersion in liquid nitrogen) (Bacchetta et Al., 2008).

In vitro culture (micropropagation)

For the conservation of plant genetic resources, the most widely used in vitro culture technique involves the use of pre-formed buds in mother plants (Bacchetta et Al., 2008).

The techniques that exploit indirect organogenesis and embryogenesis passing from the callus stage, i.e. from undifferentiated tissue, are not applicable for the conservation of the germplasm as they can lead to the onset of possible mutations and, therefore, a genetic non-correspondence of the stored material.

The micropropagation process can be divided into different phases:

Phase 0: selection of mother plants from which to take the explants to be stabilized in aseptic culture.

Mother plants must have the typical characteristics of the species to be propagated and must be free of evident signs of plant diseases. In the case of species at risk of extinction, if seeds are available, it is advisable to germinate them in vitro in order to obtain sterile seedlings to be used later for the removal of explants.

Phase 1: this phase is characterized by the passage of the live material to the vitro of the material to be preserved; provides for the sterilization of explants in order to make them suitable for in vitro cultivation.

After a short incubation period, explants showing symptoms of contamination are discarded. Stage I will be satisfactory if an adequate number of uncontaminated explants can be obtained (Bacchetta et Al., 2008).

Phase 2: multiplication phase. The objective of this phase is to obtain propagation material to be used in the subsequent phases to obtain whole plants. Multiplication is achieved by adjusting the ratio of growth factors in the culture medium. Normally, means are used with the ratio between cytokinins and auxins shifted in favor of the former which are responsible for the elimination of the apical dominance and, therefore, for the development of lateral shoots (Bacchetta et Al., 2008). Some of the propagules produced during this phase can be used as a basis for further multiplication cycles (subcultures) which allow the number of plants obtained to be increased exponentially (Bacchetta et Al., 2008).

Phase 3: elongation and rooting phase. The propagation material obtained from stage 2 is usually of reduced size for this reason it is necessary to provide for its elongation and its rooting. Elongation and rooting are obtained by increasing the auxin content in the culture medium (Bacchetta et Al., 2008).

Phase 4: is a critical phase which consists in the passage of the plants obtained in vitro in ex vitro conditions. If the transfer to the external environment is not carried out with caution, there may be a considerable loss of the propagated material due to its reduced ability to regulate transpiration, to carry out photosynthesis and to absorb nutrients from the soil.

Conservation under controlled growth conditions (slow growth)

Some crops need special conditions to reduce their growth rate and the number of transfers from one growth medium to another (excessive growth rate and the need for transfer are among the disadvantages of in vitro propagation). The slow growth technique is an important tool for medium-term conservation.

The reduction of the growth rate can be obtained by physical or chemical treatments or by integrating the two types of treatment (Hawkes , 2000; Shibli et Al., 2006, Paunescu , 2009).

With regard to physical factors, the growth of explants can be reduced by intervening on the thermal and light conditions of the cultivation environment. The storage temperature can be reduced to levels between 0 and 5 ° C for cold-resistant species, while for tropical species, sensitive to this factor, the reduction can be made up to levels of 15-20 ° C. Growth can also be affected by reducing the brightness or number of hours of light in the growing environment to, in some cases, complete darkness. Normally the relative humidity must be maintained between 40 and 50% (Hawkes , 2000; Shibli et Al., 2006, Paunescu , 2009).

Growth can be chemically reduced by intervening on the presence of essential factors in the medium, such as sugars, a source of carbon for plants grown in vitro. Another chemical technique involves the administration of growth retardants, such as abscisic acid, or substances that act osmotically on the growth of seedlings, including mannitol, sorbitol or sucrose, if administered in excessive concentrations. The application of osmotically active substances reduces the osmotic potential of the cells and the cellular distension capacity; this stress reduces the growth rate of explants (Hawkes , 2000; Shibli et Al., 2006).

Another approach to conservation in slow growth conditions involves the reduction of available oxygen through cultivation in a controlled atmosphere or treatment with oils that reduce the gaseous exchange capacity of the seedlings (Paunescu , 2009).

An example of a protocol to be adopted can be the following (approach adopted by the Umbria Region):

- storage of the micropropagated material for 3, 6, 9 and 12 months in a climatic cabinet (phytotron) set at 4.3 ± 0.3 ° C and with a photoperiod of 12 hours of light.
- At the end of each of these cold storage periods, the explants are transferred to a climatic cell (at 23 ° C) for a normal proliferation cycle, and then reinserted into the phytotron for a new cold storage period equal to the previous one.
- This cycle is repeated as long as the explants prove capable not only of withstanding low temperatures but also of resuming development once they are restored to normal growth conditions.

Cryopreservation

For longer storage times it is advisable to completely block the development of the seedlings by resorting to the cryopreservation technique (Hawkes , 2000).

Cryopreservation allows the conservation of living material (cells and tissues) at ultra-low temperatures , normally in liquid nitrogen at -196 ° C, for several years, maintaining the ability of the material to resume normal biological activities upon thawing (Day *et Al.* , 2008). During cryopreservation the various physiological processes are blocked due to the ultra-low temperatures and , consequently, the cells can maintain their characteristics unaltered (Shibli *et Al.* , 2006).

The research carried out in recent years has made it possible to develop the conservation of different types of plant material, despite this, the explants most used for cryopreservation are the vegetative apexes with a length between 1 and 3 mm; they are preferred as they are characterized by adaptability to ultra-low temperatures and high genetic stability, a guarantee not provided if cell or callus cultures are preserved. Furthermore, the vegetative apexes are characterized by the presence of a high number of cells in active division which allow a prompt resumption of growth after thawing (Shibli *et Al.* , 2006).

The fundamental principle of cryopreservation is to avoid the formation of intracellular ice which could irreparably damage the cells. This is accomplished by dehydrating the cells to a level that prevents the nucleation of ice crystals and the solidification of intracellular water into a glassy state. The formation of intracellular ice can be avoided by using two approaches:

- Cryopreservation with extracellular ice formation or **controlled rate cooling** ;
- Cryopreservation based on **vitrification** in the absence of extracellular ice (Day et Al., 2008; Gonzalez-Arnao, 2008).

Of particular importance is the application of an adequate **cryoprotective strategy** (Day et Al., 2008). The **cryoprotective solutes** they are additives that, when administered to cells before their freezing, allow an increase in their post-thawing survival (Fuller, 2004). The cryoprotective substances generally used are (Benson, 2008):

- Glycerol
- dimethyl sulfoxide (DMSO)
- methanol
- low molecular weight glycols
- oligosaccharides
- amino acids (proline)
- low molecular weight polymers (PEG1000)
- high molecular weight polymers (PEG6000, PVP)

Cryopreservation by Controlled rate cooling : it is based on the controlled cooling of the plant material immersed in a cryoprotective solution up to a temperature of about -40 ° C (Day et Al., 2008; Gonzalez-Arnao et Al., 2008). Controlled cooling is implemented through the use of programmable cooling rate freezers (Benson, 2008). The most adopted cooling rate is 0.5-1 ° C min-1 until reaching -40 ° C (Gonzalez-Arnao et Al., 2008). Upon reaching -40 ° C, the explants can be immersed directly in liquid nitrogen or be kept at this temperature for 30-45 minutes (hold) before immersion (Benson , 2008).

Also in the thawing phase it is important that the formation of intracellular ice is hindered; this is done by heating the samples in a water bath at 40 ° C for a few minutes. The high thawing speed means that the time available is not sufficient for the aggregation of water molecules into ice crystals (Gonzalez-Arnao et Al., 2008).

Cryopreservation based on vitrification in the absence of extracellular ice: Vitrification is a physical process that occurs at the so-called glass transition temperature, consisting in the solidification of a liquid without crystallization, in the form of amorphous glass (Benson, 2008). This state maintains the arrangement of the molecules typical of the liquid state but has physical and mechanical characteristics typical of the solid one (Benson, 2008; Gonzalez-Arnao et Al., 2008). Given the high viscosity of the glassy state, all chemical reactions that require diffusion in aqueous medium are blocked, allowing the stability conditions to be maintained for long time intervals (Benson, 2008; Gonzalez-Arnao et Al., 2008). This state, thanks to the absence of an organized structure, is much less harmful to the cells than that of ice and, consequently, allows an increase in the post-thawing recovery rate (Benson, 2008).

The glassy state is metastable, i.e. able to return more or less easily to the liquid state and / or to give rise to crystallization in the event that the thermal conditions are changed therefore, even in the thawing phase, it is necessary to adopt measures to prevent the formation of intracellular ice crystals large enough to cause structural damage to the cryopreserved material, which is also characterized by greater delicacy due to the increased cellular rigidity. This risk is usually avoided by resorting to rapid heating. (Benson, 2008; Day et al., 2008).

When the cryopreservation protocol is optimized, all or most of the meristematic structures remain intact, allowing the direct resumption of growth without going through the formation of undifferentiated callus; this constitutes an advantage in terms of genetic stability of the conserved material (Gonzalez-Arnao et Al., 2008). A significant advantage of vitrification without the formation of extracellular crystals is that it does not require controlled cooling and the related technologies, consequently this technique can also be applied in non-specialized laboratories (Day et Al., 2008).

The cryopreservation technique by vitrification is based on the dehydration of the cells through the application of vitrifying solutions , i.e. extremely concentrated solutions (7-8 M) of cryoprotectants that allow the passage of water present in the tissues in the glassy state (Sakai et Al. , 2008).

A vitrification protocol consists of the following phases:

1. preconditioning , with the aim of increasing the resistance of the explants to the subsequent phases of cryopreservation. Feasible treatments are the cultivation of mother plants at low temperatures, dissection of the explants after a certain period of cultivation and in a certain physiological state (Gonzalez-Arnao and Engelmann , 2006; Sakai and Engelmann , 2007).
2. Preculture of explants after their dissection. The pre -culture of the explants on a solid medium enriched in sucrose with a concentration of 0.3 to 0.7 M for one or two days allows to significantly increase their survival at the end of cryopreservation. During the pre -culture there is a notable increase in the sucrose content in the tissues and the accumulation of endogenous cryoprotectants such as sugars and alcohol, which allow to increase the stability of the membranes during the subsequent intense dehydration (Sakai and Engelmann , 2007).
3. Osmoprotection by treatment with loading solution. This phase reduces the toxicity due to destabilization of the cell membranes that could occur in the case of direct exposure to the vitrifying solution . Furthermore, exposure to the loading solution allows to increase the concentration of solutes present in the cytoplasmic solution so that it undergoes vitrification when immersed in liquid nitrogen. Highly concentrated solutions based on glycerol (1-2.5 M) and sucrose (0.4-0.7M) are normally used for loading (Kim et Al., 2009a).
4. Dehydration by means of a vitrifying solution. Vitrifying solutions are solutions that can be slowly cooled below the transition temperature of the glassy state without any appreciable production of ice crystals. They therefore allow to obtain the production of the intracellular amorphous glassy state during freezing and its maintenance during heating. Vitrification solutions consist of a mixture of penetrating and non-penetrating elements. Penetrating additives are able to quickly enter cells, increasing the concentration of solutes and viscosity and protecting against osmotic stress that can occur during dehydration. Most used penetrating elements are DMSO, Ethylene glycol (EG) and glycerol. As mentioned, non-penetrating elements are also administered, consisting mainly of sugars, having the function of dehydrating the explants and constituting an osmotic buffer (Kim et Al., 2009b). The most

commonly used vitrifying solutions are the so-called plant vitrification solution 2 (PVS 2), consisting of 30% (weight / volume) glycerol, 15% (weight / volume) DMSO, 15% (weight / volume) EG and 13.7% (weight / volume) sucrose, and the PVS 3, consisting of 50% (weight / volume) glycerol and 50% (weight / volume) sucrose (Sakai et Al., 2008). The treatment time must be suitably calibrated according to the species and the type and size of the explants used (Sakai and Engelmann , 2007). Immersion times in vitrifying solution can be reduced by treating at 0 ° C instead of room temperature, as this will reduce the diffusion rate of the penetrating elements through the wall (Sakai et Al., 2008).

5. Freezing carried out by placing the explants in cryotubes filled with 0.5 ml of PVS and immersing them directly in liquid nitrogen; in this way a cooling rate of about 200 ° C / min is achieved (Sakai and Engelmann , 2007; Sakai et Al., 2008).
6. Heating. To avoid damage from devitrification and crystallization it must be implemented quickly. This is achieved by immersing the samples in a water bath at 37-40 ° C. By means of this procedure it is possible to obtain a heating rate of about 250 ° C / min, sufficiently high to avoid the formation of intracellular ice crystals (Sakai and Engelmann , 2007).
7. Dilution carried out by means of the so-called unloading solution, a solution enriched in sucrose, and has the aim of eliminating cryoprotective elements that are potentially lethal for cells (Day et Al., 2008).
8. After these phases it is possible to complete the cryopreservation protocol by culturing the defrosted explants, which should be kept in dark conditions for a few days in order to avoid damage from photo- oxidative stress .

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2.3 Summary results of the risk and conservation analysis for the selected varieties

Manageable enhancement path and high priority	POSITIVE SWOT SCENARIO HIGH DEGREE OF RISK

Genetic resource	In vivo conservation	In vitro conservation / germplasm banks
Fava Viola	Test fields	Germplasm banks
Grano tenero Bianchetta	Test fields	Germplasm banks
Trifoglio sotterraneo	Test fields	Germplasm banks
Fico Verdesca	Collection fields	Cryopreservation of meristematic apexes
Fagiolino dall'occhio Occhiopinto	Test fields	Germplasm banks
Fagiolino pinto	Test fields	Germplasm banks
Vite Cigliola	Collection fields	Cryopreservation of meristematic apexes
Vite Notardomenico	Collection fields	Cryopreservation of meristematic apexes
Vite Santa Teresa	Collection fields	Cryopreservation of meristematic apexes

Uncertain development path and high priority	UNCERTAIN SWOT SCENARIO HIGH DEGREE OF RISK

Genetic resource	In vivo conservation	In vitro conservation / germplasm banks
Grano duro San Pasquale	Test fields	Germplasm banks
Trifoglio incarnato	Test fields	Germplasm banks
Susino S. Anna Ovale	Collection fields	Cryopreservation of meristematic apexes
Carciofo Bianco di Taranto	Test fields	Germplasm banks
Cima di Cola	Test fields	Germplasm banks

Manageable enhancement path and medium priority	POSITIVE SWOT SCENARIO MEDIUM DEGREE OF RISK

Genetic resource	In vivo conservation	In vitro conservation / germplasm banks
Fico Petrelli	Collection fields	Cryopreservation of meristematic apexes
Pero Gentile reale	Collection fields	Cryopreservation of meristematic apexes
Pero Recchia falsa	Collection fields	Cryopreservation of meristematic apexes