LIFE
4FIR

NATURA 2000

LIFE4FIR – Project LIFE18 NAT/IT/000164

"Decisive in situ and ex situ conservation strategies to secure the critically endangered Sicilian fir, *Abies nebrodensis*"

Report on: 'Optimized protocols for the reproduction of *A. nebrodensis* trees by seed and grafting propagation'

Action A.1

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REPUBBLICA ITALIANA
REGIONE SICILIANA
ASSESSORATO REGIONALE
DELL'AGRICOLTURA, DELLO SVILUPPO RURALE
E DELLA PESCA MEDITERRANEA

Parco delle Madonie

UNIVERSITÀ
DEGLI STUDI
DI PALERMO

SAAF
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AGRARIE
ALIMENTARI
FORESTALI

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1. Introduction

The small number of individuals making up the current natural population of *Abies nebrodensis* and of sexually mature individuals determines a scarce availability of seeds which, together with the reduced germinability, represent a problem in the propagation of this important endemic species. For this reason, the activities of Action 1 were numerous and complex. The activities related to the reproduction by seed of *Abies nebrodensis* within the LIFE4FIR project were carried out at the 'Piano Noce' forest nursery, located in the municipality of Polizzi Generosa (in the Madonie Park) which represents one of the thirteen nursery centers of the 'Regional Department of Rural and Territorial Development' in Sicily. The choice of this nursery was based on obvious reasons, since it is located near the indigenous area of *A. nebrodensis* at an altitude of about 1,035 m a.s.l. (Fig. 1). The nursery has a total area of about 8 hectares, of which 5 hectares are dedicated to the propagation and breeding of postime. It is one of the largest nursery centers of the 'Regional Department' and up to ten years ago it ensured an average annual production of about 500,000 units. The seedlings produced are mainly raised in containers (8x8x20 cm phytocells) and, in most cases, we resort to the direct sowing of 2-3 seeds in phytocell and the subsequent selection of the best individual. The material at the end of the production cycle has a variable age between 8 and 24 months, with quality levels that are not always uniform. The cultivated species concern the Mediterranean pines (*Pinus halepensis, P. nigra* var. *calabrica, P. pinea, P. pinaster*), the common cypress (*Cupressus sempervirens*), some species of oaks (*Quercus ilex, Q. virgiliana, Q. petraea* subsp. *austrothirrenica*), chestnut (*Castanea sativa*), ash (*Fraxinus ornus*), Southern ash (*Fraxinus angustifolia*), sycamore maple (*Acer pseudoplatanus*), field maple (*Acer campestre*), etc. Among the reproduced shrubs are: *Spartium junceum, Crataegus monogyna, Genista demarcoi, Prunus spinosa, Cytisus scoparius*. The nursery has good quality irrigation water from local springs, warehouses for storing tools and nursery materials, a large room that can be used for conferences, offices, etc. The seed used is collected according to the protocols defined by regional and national legislation in the seed woods falling within the Madonie Park. In the nursery center of 'Piano Noce' there is also a small warehouse equipped with a mill for the preparation of the substrates, inside which the sowing operations of *Abies nebrodensis* were carried out in the winter period, and an old greenhouse structure which, despite having lost its original efficiency, it was nevertheless useful and functional to the activities of Action 1. In particular, after being sown, the containers were placed inside the greenhouse to encourage the germination of *Abies nebrodensis* seeds and the growth of young seedlings. Furthermore, the greenhouse was also functional to the activities of vegetative propagation by grafting, to stimulate the vegetative restart of the seedlings used as rootstocks and favor the formation of the scar callus at the point of union between the two bionts.

Fig. 1. View of the 'Piano Noce' nursery (*above*); postime growing (*below*)

2. Seed propagation in conifers

Sexual reproduction in gymnosperms is a process with different characteristics in different groups. This is a complex process, which in some species takes about two years to complete. In the fir, and in most of the other conifers, the reproductive systems are the cones (or strobili), always unisexual, generally carried by the same tree: in the male strobili differentiate the pollen grains, in the female ones the ovules containing the egg cells (female gametes). Pollen grains are produced in very large quantities, carried by the wind and captured by viscous substances produced by the eggs, then penetrate inside them. Fertilization does not take place immediately, in fact in this stage the meiotic division has not yet occurred in the ovary which leads to the formation of female spores (megaspores); only about a month after pollination, four megaspores are produced from a mother cell, one of which will give rise to the female gametophyte, the primary endosperm.

Flowering in *Abies nebrodensis* begins at the tree age of 20-25 years, and is very irregular between the years. The Madonie Fir is monoecious and the male and female reproductive systems are located in different parts on the same individual: the female ones are typically inserted on the foliage in the part between the median area and the apex; the male ones, on the other hand, are mainly located in the area between the basal and the median part and show a yellow color. The flowering period varies from the last ten days of April to the second ten days of May. The fully ripe seeds are mainly dispersed by the wind from the last decade of September to the second decade of October of the same year. The cones are erect, conical in shape (12-19 cm x 5-6.5 cm), resinous, ending in a slightly attenuated tip. The ovuliferous scales are reddish and tomentose and the bracts exert, deflected. They are initially green in color, and then become brownish-vinous; these are formed by the acuminate fan-shaped scales (Fig. 2) of woody consistency which, when ripe, flake off, detach and fall to the ground, while the central axis of the strobilus remains for a long time, even for several years, erected on the branch. The scales of the strobili vary in number from 150 to 200 and each scale carries two ovules which turn into fertile winged seeds that are dispersed by the wind. The seed, triangular in shape, is 6-9 mm long and has a yellow-brown color; it has a wing firmly attached to it that allows it, once released, to spin in the air. The strobili are harvested between the first and second ten days of October and are kept in the warehouse for a few weeks until they are completely disarticulated.

Fig. 2. Seeds and scales of A. nebrodensis fan-shaped on the cone.

2.1. General considerations

The Genus Abies constitutes a rather complex group in comparison to other Genera of the Pinaceae family. In fact, it includes 49 different species, distributed in the northern hemisphere, with great morphological variability (Farjon and Rushforth, 1989), for which the taxonomy of the Genus has been controversial for a long time. *Abies nebrodensis* (Lojac.) Mattei (Madonie Fir), an endemic forest entity in Sicily, represents one of the most important examples of relict species, not only for Italy but for Europe and the Mediterranean basin. It is part of that group of vicariant species of *Abies alba* Mill. which in different eras have evolved on the edge of the Mediterranean basin. The natural population consists of very few individuals (30) limited to a small area such as the Madonie Mountains, in Sicily. This drastic reduction seems to be due to the anthropogenic pressure which occurred in this area during the last century (Morandini, 1969; Raimondo et al., 1990; Morandini at al., 1994).

2.2. Propagation of *Abies nebrodensis* by seed

As part of Action C2 pof LIFE4FIR, in 2020, intense work was carried out which led to the isolation and subsequent manual fertilization of 488 female strobiles belonging to 24 different individuals of *Abies nebrodensis* which, in that year, recorded an abundant fruiting. After the continuous monitoring of the summer period, the collection of manually pollinated cones was started in the first week of October. After collection, the strobili of the different individuals were placed in their respective perforated boxes in order to favor their drying and consequent disarticulation (Fig. 3, *left*); the different batches of semen have been labeled and kept separate from each other, so as not to lose their origin. After a few days, the cones were manually disjointed and after eliminating the bracts, the cleaned seeds (Fig. 3, *right*) were stored in breathable paper bags, always appropriately labeled so as not to lose their identity. Overall, 4,904 grams of artificial pollination seed were obtained, equal to about 100,000 Madonie Fir seeds. The paper bags with the clean seeds were placed in a cool and dry place, awaiting sowing (they were not placed in the refrigerator to avoid secondary dormancy phenomena).


Fig. 3. Female cones in the drying phase (*left*); clean seeds of A. nebrodensis (*right*).

The sowing activity was started in the second ten days of December 2020. In order to facilitate future handling operations and, above all, to prevent any phenomena of "spiraling" of the root system, sowing was carried out in alveolar forest containers, with 35 alveoli having dimensions 6.1 x 5.8 cm, in the part upper, and 3.4 x 3.8 cm, at the bottom, and a depth of 16 cm. The use of this type of container has increased the efficiency of the spaces in the nursery, considering that it allows to raise 240 seedlings per square meter (Fig. 4).

Fig. 4. Sowing of *A.nebrodensis* in the plateaus (*left*); plateau prepared in the nursery (*right*).

Furthermore, this type of container avoids transplant stresses, and this factor assumes considerable importance as the higher mortality of *Abies nebrodensis* seedlings occurs precisely at transplantation with losses of over 90% (Raimondo and Schicchi, 2005). The "pull" extraction action, carried out in the past, was avoided in consideration of the fact that it is a Gymnosperm, in which in the first year of life only the main taproot and a few secondary roots develop. Therefore, during the extraction of the young seedling from the container, both the tip of the taproot and the secondary roots were damaged, as they often remained attached to the earthen bread. Sowing in the alveoli eliminated these problems as, at the time of transplantation, the complex seedling-bread of earth remains intact. Another strong point of sowing in alveolar containers is that, thanks to the microclimate that is formed in the alveolus, they favor germination. Finally, the reduced volume of the cells allows to sow a large number of seeds using small quantities of substrate. In this regard, in order to favor germination and maximize the growth of the postime, sowing was carried out using a technical-professional substrate, easily available on the market, whose standardized characteristics are in line with the eco-physiological needs of the species. In the past, in fact, the sowing of Madonie Fir at the ‘Vivaio di Piano Noce’ was done using agricultural land of various origins, whose chemical-physical and microbiological characteristics changed from time to time and could not even be suitable for the needs of the species, resulting in stunted growth of seedlings. Factors such as pH, salinity, porosity, adequate water retention capacity, good drainage, structure, the presence of organic matter and nutrients are the necessary conditions for the seedling to develop at its best. The growing substrate is of fundamental importance in container cultivation, and it is therefore essential to know its characteristics and parameters. It also needs to be readily available on the market and at affordable prices. Taking into account the eco-physiological needs of *Abies nebrodensis*, after careful market research, the “Complete” soil from the Vigorplant Co. was chosen as the substrate for sowing, with a pH between 6 and 6.5, salinity between 0.30 and 0.40 dS/m and with the following composition:

- 21% BALTIC PEAT, 0-10 mm grain size, which gives softness to the substrate, avoiding water stagnation and gradually regulating the supply of nutrients;
- 37% IRISH PEAT, grain size 0-20 mm, which guarantees oxygenation to the roots and keeps the substrate structure stable over time;
- SLOW RELEASE FERTILIZER based on nitrogen, phosphorus and potassium which gradually releases its nutritional elements to the plants;
- 13% VOLCANIC PUMICE, grain size 3-8 mm, which guarantees excellent drainage;
- 29% SUPERFINE PEAT, grain size 0-3 mm.

In order to further improve the drainage and fertility of the aforementioned substrate, agri-perlite with a grain size of 2-5 mm was added in the proportion of 12%, as well as a ternary fertilizer 11-22-16 + microelements in the proportion of 2% (Fig. 5, *left*).

The sowing activity of *Abies nebrodensis* at the regional nursery of ‘Piano Noce’ was carried out in the period between 15/12/2020 and 22/12/2020. After filling the trays with the chosen substrate, a light wetting was carried out and the seeds were then placed, burying them slightly (about 1 cm deep). Due to the well-known poor germinability of *Abies nebrodensis* seeds, 5 seeds were placed in each alveolus. Subsequently, each plateau, as a precaution, was marked with two labels and a second wetting was carried out (Fig. 5, *centre* & *right*).

Overall, n. 242 containers (plateaux) were seeded, equal to n. 8,451 sown alveoli, with a total of 42,255 seeds placed to germinate. As previously mentioned, the seed used is that obtained from controlled artificial pollination activity (Action C2); the crossings made were the following:

1♀ x 13♂; 13♀ x 7♂; 19♀ x 1♂; 11♀ x 1♂; 6♀ x 2♂; 15♀ x 23♂; 7♀ x 17♂, 2♀ x 1♂, 12♀ x 24♂, 14♀ x 10♂, 24♀ x 14♂, 16♀ x 7♂, 10♀ x 27♂, 8♀ x 16♂, 15♀ x 24♂, 17♀ x 24♂, 18♀ x 24♂, 22♀ x 24♂, 21♀ x 23♂, 23♀ x 13♂, 25♀ x 13♂, 27♀ x 1♂, 29♀ x 24♂.

Fig. 5. Addition of agri-perlite to the substrate (*left*); sowing of *Abies nedrodensis* (*centre*); labeling (*right*).

After having finished sowing, the trays were transported to the greenhouse of the nursery, placed inside it (Fig. 6, *left*) and irrigated on a weekly basis. In the first week of March 2020, the seeds began to germinate. Monitoring activities were carried out on a weekly basis (Fig. 6, *centre* & *right*).

Fig. 6. Placing the plateaus at the 'Piano Noce' nursery greenhouse (*left*); monitoring and surveys on *Abies nebrodensis* seedlings (*centre*); *Abies nebrodensis* seedlings (*right*).

At the beginning of the summer, when the germination phase could be considered completed, special surveys were carried out for the collection of data relating to the germination of the seeds of the aforementioned crosses; Table 1 shows the germination data.

As regards fertirrigation, only products of biological origin were used, with the ability to be easily absorbed by the roots (Pimpini, 2004. The products taken into consideration are:

- liquid biological fertilizer based on microelements - *Lixor micro*;

- liquid organic biological fertilizer based on extracts of algae, aminoacids and calcium - *Lysodin calcium* - *express*;
- liquid organic biological fertilizer based on peptites and nitrogen - *Lysofert*;
- liquid fertilizer – *Foxter*.

For the transplant phase, in order to improve the basic substrate both in terms of organic substance and slow release nutrients, two types of pelleted fertilizers were taken into consideration, namely: TOP NPK 7-5-14 of the CIFO, and Guanito of Italpollina Co. having NPK title 6-15-2, with total CaO at 10% and magnesium oxide (MgO) at 2%.

Tab. 1. Crossing among the trees and relative germinability of seeds.

Seed (1st number is the ID of the mother tree, 2nd is the father tree)	**% germination**	**Seed** (1st number is the ID of the mother tree, 2nd is the father tree)	**% germination**
1♀ x 13♂	19.43%	**16♀ x 7♂**	16.57%
2♀ x 1♂	16.86%	**17♀ x 24♂**	1.43%
6♀ x 2♂	9.26%	**18♀ x 24♂**	9.80%
7♀ x 17♂	35.09%	**19♀ x 1♂**	13.00%
8♀ x 16♂	0.00%	**21♀ x 23♂**	12.37%
10♀ x 27♂	15.31%	**22♀ x 24♂**	14.21%
11♀ x 1♂	43.8%	**23♀ x 13♂**	15.37%
12♀ x 24♂	5.03%	**24♀ x 14♂**	13.37%
13♀ x 17♂	15.18%	**25♀ x 13♂**	9.37%
14♀ x 10♂	13.37%	**27♀ x 1♂**	14.37%
15♀ x 23♂	27.00%	**29♀ x 24♂**	9.52%

3. Vegetative propagation in conifers

Vegetative reproduction is the capacity of a woody plant to replicate itself as a genetically identical, but physically separate, plant. Such replicates may remain physically connected to the parent plant but the connection is not obligate. Differently, seed propagation is not considered a vegetative form of propagation and the genetic material within a seed is a mix of the DNA from the plant bearing the seed and the DNA from the plant that pollinated the embryo that becomes the seed. Therefore when the seed germinates, it creates a plant that may be similar to its parents, but will not be genetically identical.

Vegetative propagation allow to get a superior genetic gain in a shorter time, in comparison to seed propagation (Fig. 7).

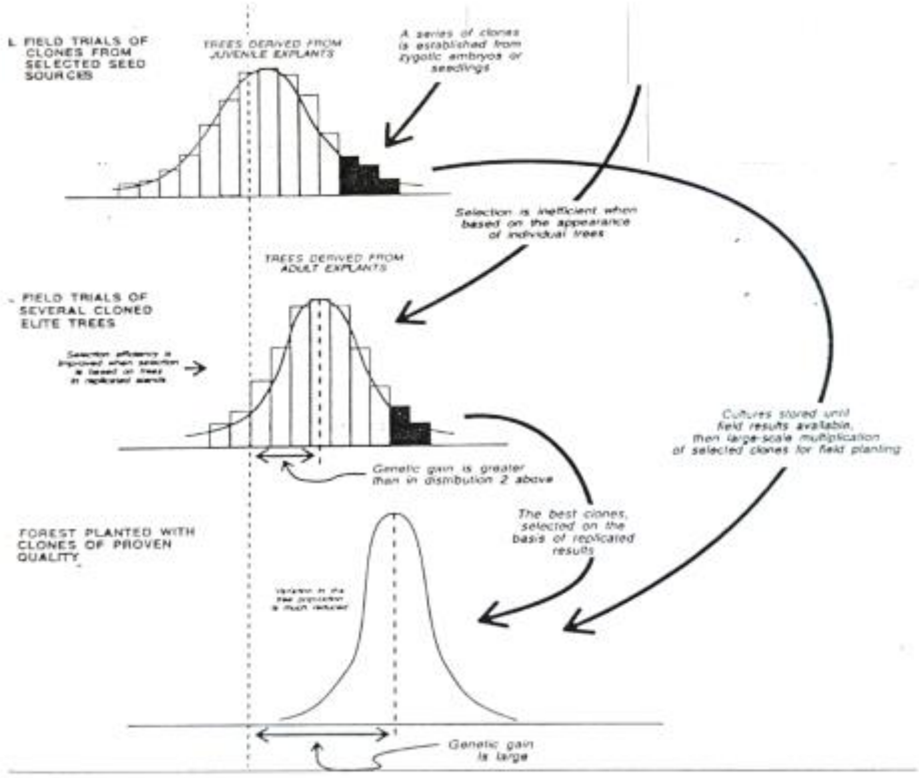

Fig. 7. A native forest consists of numerous different genotypes which show considerable variation in size and timber characteristic; only few best individuals (elite trees) are selected (top). These trees are then propagated by cutting or grafting, and established together in an orchard so that they may cross pollinate. Mass tree plantings are then made from seed gathered from the orchard (centre). The seedlings will again produce trees of varying size, but their average yield is expected to be greater than the average yield of the population (bottom) (George, 1993).

Cutting and grafting propagation are vegetative forms of propagation. In other words, a vegetative portion of the parent plant is removed and in some way induced to produce its own roots (as in a cutting) or attached to an existing root system (as in grafting). This vegetative portion of the parent plant retains the exact same characteristics of the parent plant and is simply growing on a new set of roots. With this division of propagation techniques in mind, if a new plant exactly like the original is desired, then a vegetative technique is the procedure that must be selected. If diversity is desired, then seed propagation is appropriate.

Vegetative propagation techniques are used to produce plants that are genetically identical to the parent plant, although certain epigenetic effects may cause the resulting offspring not to develop in a true-to-type manner. Some species can be propagated by simple layering and air layering but these techniques are not normally used commercially. Hence, cutting and grafting propagation are the most common approaches to conifer vegetative propagation. More recently there have been also significant advancements in the development of micropropagation techniques for conifers. However, while this technology is now reaching the stage of commercial application in many fruit and crop species, it is still far to become a common practice in conifers.

Just as in woody angiosperms, there is considerable variation between conifer species in their ease of vegetative propagation. For example, cuttings taken from most *Chamaecyparis*, *Juniperus* and *Cupressus* species are relatively easy to root, whilst other species, such as many of the pines and several spruce species, are difficult or impossible to propagate by cuttings and selected genotypes have to be reproduced by grafting. In many conifers, the rooting capacity of cuttings is greatly affected by maturation change. The ability of cuttings to root is associated with the juvenile, non-reproductive phase of growth, rooting capacity declines to nothing after trees have become mature. This loss of rooting capacity is a major obstacle in tree improvement programmes, as it prevents the simple mass cloning of mature trees selected for desirable traits. The conifer tree is however characterized by a gradient of youth that affects the different shoots that compose it, depending on the position in which they are found (Fig. 8).

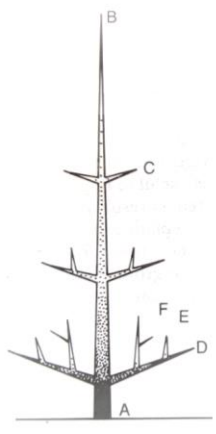

Fig. 8. Juvenile-mature gradient in conifer seedling trees. The gradient in juvenile state is A>F>E>D>C>B and produces material that, from A to B, is more responding to vegetative propagation (Hartmann et al., 1990).

Even if rooting is successful, shoot growth in rooted cuttings taken from side shoots often exhibit plagiotropic (horizontal) growth for varying lengths of time after rooting. Indeed, the first major study of this phenomenon was carried out on a conifer, namely *Araucaria araucana*, where horizontal forms can persist for 50 years or more. Clearly, the persistence of positional effects in vegetative offspring is a disadvantage in tree improvement programmes for forest trees, but the phenomenon is exploited in the production of ornamental cultivars that have horizontal or prostrate habits.

3.1. Cutting propagation of conifers

Propagation of many conifers by cuttings can be done with semi-hardwood cuttings. This term refers to the fact that the cuttings are no longer actively growing, but have not yet gone fully dormant for the winter. Semi-hardwood cuttings are good for *Chamaecyparis*, *Thuja*, and *Taxus* selections, while *Junipers* may best be done by hardwood, fully dormant cuttings in December and generally require a greenhouse with some way to heat the soil. Semi-hardwood cuttings are collected in September or early October and stuck in a sand based media in a cold frame, greenhouse or even just a pot covered with a plastic bag and placed in a cool location. The cuttings will develop roots during the fall and following spring. A 50/50 mix of sand and peat moss or sand and perlite makes a good rooting media to stick cuttings in. If this is not available, potting mix can be utilized, but it needs to be not over-moistened as it can rot the cuttings. The media should be placed in a pot or tray, and should be moist but not wet.

An example of cutting propagation of Thuja occidentalis is showed in Fig. 9. In general, collect strong, healthy, 10-15 cm long (a dwarf plant may only provide cuttings that are 2.5-5 cm long) cuttings from the current season growth. The cuttings should be prepared by removing the lower 1/3 of the needles and/or branches from the stem and make a fresh cut on the basal end of the cutting. On most conifers, a thin slice or wound on one side of the cutting is applied. The wound should be about 2-3 cm long, and extend from the basal end upward. This wound will encourage stronger root development on conifers. The bottom 2 cm of the cutting is then dipped in a rooting hormone powder, gel or liquid solution. Rooting hormones are readily available online or at agriculture shops and markets. The cuttings are then sticked in the prepared moist medium, and the medium firmed around the cuttings with the operator’s thumb and forefinger. A hole is often dibbled with a pencil or nail to make insertion of the cutting easier and prevent removing of the hormone. The base of the cutting is positioned above the bottom 1/3 of the medium in the container, as the bottom 1/3 contains more water than air and can inhibit rooting and promote rot. After sticking, the cuttings are watered in and covered with something clear to maintain high humidity. Cuttings kept in a greenhouse or cold frame may need to be shaded until winter to prevent overheating during the fall. If a cold frame or greenhouse is not available, the cuttings can be placed in a bright but shaded location that is protected from the elements. Cuttings should be inspected often during the fall. The media should not dry out, and watered when necessary. Any dead or diseased foliage and cuttings, as soon as they appear, should be removed. Cuttings are then periodically checked during the winter, while the media are kept moist. Some cuttings may root by winter, but many will root the following spring. As the weather warms up, the cuttings are checked to avoid they get overly warm. Once rooting has begun in the spring, the cuttings are ventilated as necessary to acclimate them to lower humidity. However, it is important that the cuttings are not ventilated until they have established roots. They will desiccate if the humidity is too low and they don't have any roots to draw up moisture. When the cuttings begin to root and grow, and all danger of frost is gone, they are placed in a shaded area until they are ready to be planted. Watering them with a mild liquid fertilizer solution will encourage further root and top growth.

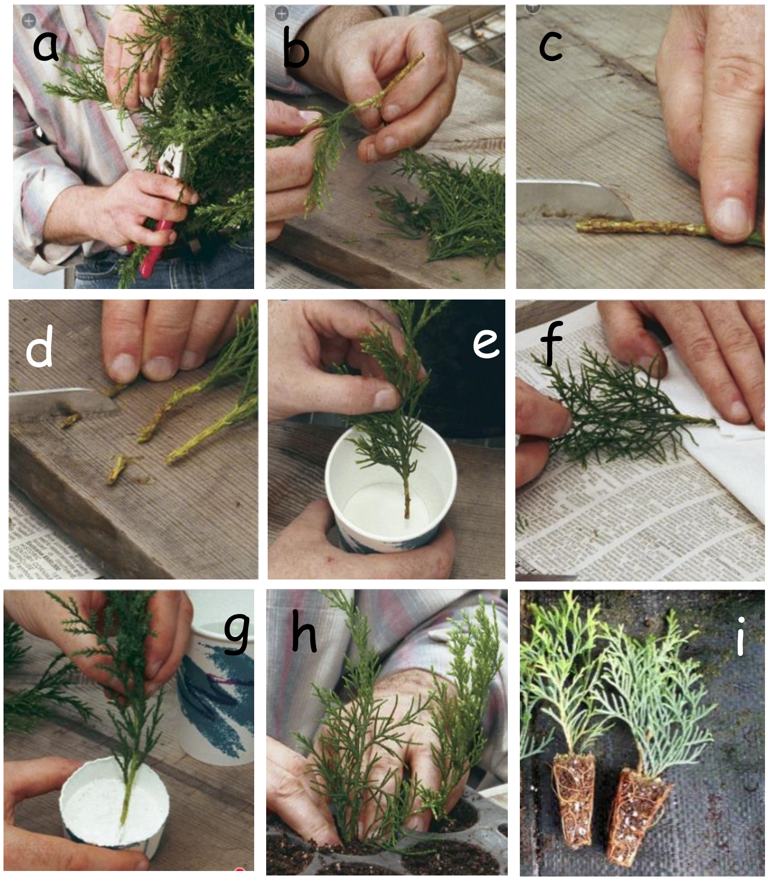

Fig. 9. The procedure of cutting propagation in conifers. a, cuttings are collected by taking the apical parts of shoots (10-15 cm); b, needles are stripped by the basal 1/3 part; c, a couple of cuts are made to allow, further, a better contact with the rooting hormone; d, an additional angled cut is made at the base; e, the basal part of cutting is wetted and, after, (f) partially dried; g, the treatment with a rooting powder, generally containing indole-butyrric acid (IBA); h, cuttings are then potted in a sand-peat moss compost; i, examples of rooted cuttings.

Propagation by cuttings was tried in *Abies nebrodensis* in the frame of a previous project with poor results. The rhizogenic potential of the Mdonie Fir cuttings proved to be very limited and inadequate to obtain a sufficient number of plants from each grafting campaign. For this reason, the technique was not used for producing clonal trees of *A. nebrodensis.*

3.2. In vitro propagation of conifers by somatic embryogenesis

Somatic embryogenesis is a very effective in vitro culture technique for the reproduction of large numbers of plants. It consists in the induction of an embryogenic callus, starting from different organs and tissues cultured in vitro, capable of proliferating and producing somatic embryos, genetically identical to the genotype of the mother plant. In conifers, the organ of excellence for obtaining embryogenic callus is the immature embryo. With this technique, efficient propagation protocols have been developed for numerous species of conifers (Fig. 10). In LIFE4FIR, this technique was investigated for the first time in *Abies nebrodensis* and will be used to implement the cryobank established inside the MAN (Museum of *Abies nebrodensis*). However, in terms of propagation efficiency, the technique has not proved efficient with the various *A. nebrodensis* genotypes, and is not totally reliable in terms of clonal propagation. Hence, it was decided not to use for the propagation of the trees of Madonie Fir.

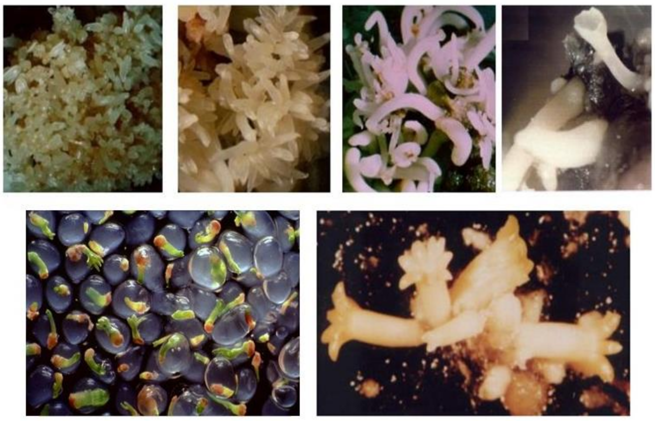

Fig. 11. Examples of embryogenic callus lines of different conifers. *Bottom-left,* somatic embryos are included in a matrix of Ca-alginate (artificial seeds).

3.3. Grafting propagation of conifers: the protocol optimized for *Abies nebrodensis*

Propagation by cuttings and micropropagation are the most efficient techniques of vegetative reproduction. On the other hand, many species of conifers do not respond to these techniques, producing limited results, not sufficient to guarantee a number of plants suitable for use in reforestation. In all these cases (and the *Abies nebrodensis* is one of them) it is necessary to resort to the propagation by grafting. The pot grafting technique that will be described here and that has been used in this Project is called "veneer-side grafting" (Fig. 11), by far the most used in the nurseries for the grafting propagation of conifer species.

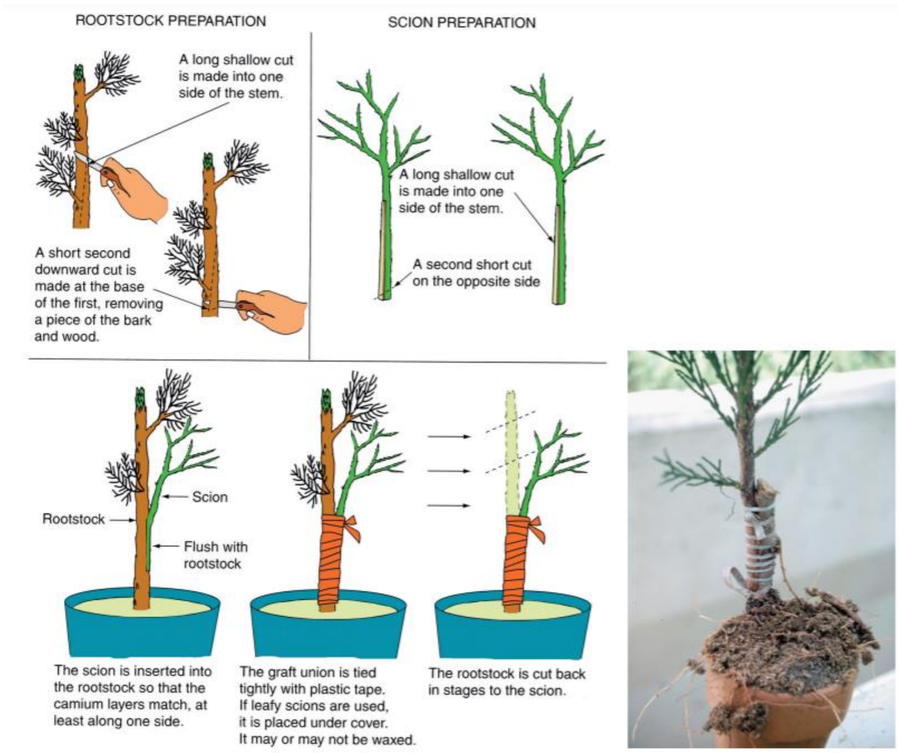

Fig. 11. The “veneer-side graft”, a typical grafting technique used in conifers.

The specific procedure of “veneer-side graft” followed for the grafting propagation of *Abies nebrodensis* is summarized here:

- two weeks before the grafting, rootstocks (seedling of *A. nebrodensis*) were moved into a greenhouse to force vegetative activity and root growth. The potting media were moisted, avoiding to be too wet. The lower 7-10 cm of the seedling stems were kept clean by removing any branches, needles and dirt within this area;
- scions were collected at the beginning of April 2020, i.e. as soon as the climatic conditions have allowed it: in fact, the plants are located at a height of 1,400-1,700 meters a.s.l.. and the presence of abundant snow did not allow to arrive on site earlier. However, at the time of collection the plants still presented closed buds;
- three days after scions collection, grafting was performed. Rootstocks, scions and equipment was assembled at a comfortable work station in the ‘Vivaio Piano Noce’. A team of 6 persons (expert grafters, plus helpers) was organized; the grafting knife were prepared extremely sharp and clean;
- the collected scions were terminal shoots, mainly taken from the lower 1/3 of the tree (Fig. 12, a). The scions for grafting (10-15 cm long) were prepared by removing any needles in the lower 1/2 of the length (Fig. 12, b);

- a straight, blemish and wound free section in the lower 4 inches of the rootstock stem was selected to make the first cut. All cuts on the scion and rootstock were made in one, smooth motion. This yielded the best surface for mating the scion to the rootstock. The first cut was made in a downward direction to create a small flap on the stem of the rootstock. The width of this cut was as close to the width of your scions as possible, while still penetrating the bark of the rootstock;
- a downward cut on the scion was the made with a one angled cut at the end of the scion to create a flap. The length of the cut was equal to the length of the cut made on the rootstock;
- the scion was then inserted into the "pocket", created in the basal cut part of the rootstock; the side of the scion aligned with the cut surface of the rootstock. When the grafting was done properly, the scion remained perfectly inserted in the rootstock pocket, with a perfect alignment of the cut surfaces (Fig. 12, c-d);
- the scions were then tied with a rubber strip to tighten the graft; the wrapped area of the strip started and end above and below the cuts. Te grafted area was then covered with aluminium foil in order to limit drying (Fig. 12, e);
- as conifers require high humidity while the scion is healing, the grafted plants were covered with a clear plastic bag (Fig. 1, f).

Aftercare of the grafts, the grafted plants were moved back to the greenhouse, after cutting the top part of the rootstock. The media in the pots were periodically moisted, avoiding dripping wet. Particular attention was reserved to avoid that the medium dryid out, as this is a critical time for the healing of the grafts. The plants needed plenty of light, but direct, intense sunlight was avoided. After about 4-5 weeks, the scion the plastic covering was removed, and another 1/3 of the rootstock was cut, just over the scion insertion. By mid to late summer, the rubber strip was removed from the union and the grafted plants moved outside, in a shaded area of the 'Piano Noce' nursery (Fig. 13, *left*). This was important to prevent possible girdling of the trunk by the strip.

Depending on the plant material available from each genotype to be used as a graft, a variable number of grafts was made for each of the 30 residual *Abies nebrodensis trees*. In total, 440 grafts were made. At the survey carried out 4 months after the grafting, more than 50% of the grafts resulted well healed (Fig. 13, *right*), a result of absolute excellence for a species like the Madonie Fir. It should be underlined that, in the next vegetative season, when the grafted trees will have a prompt vegetative restart, it will be necessary to evaluate and confirm this obtained data. In Grafted trees will need to be staked for the first couple of years to establish a straight trunk.

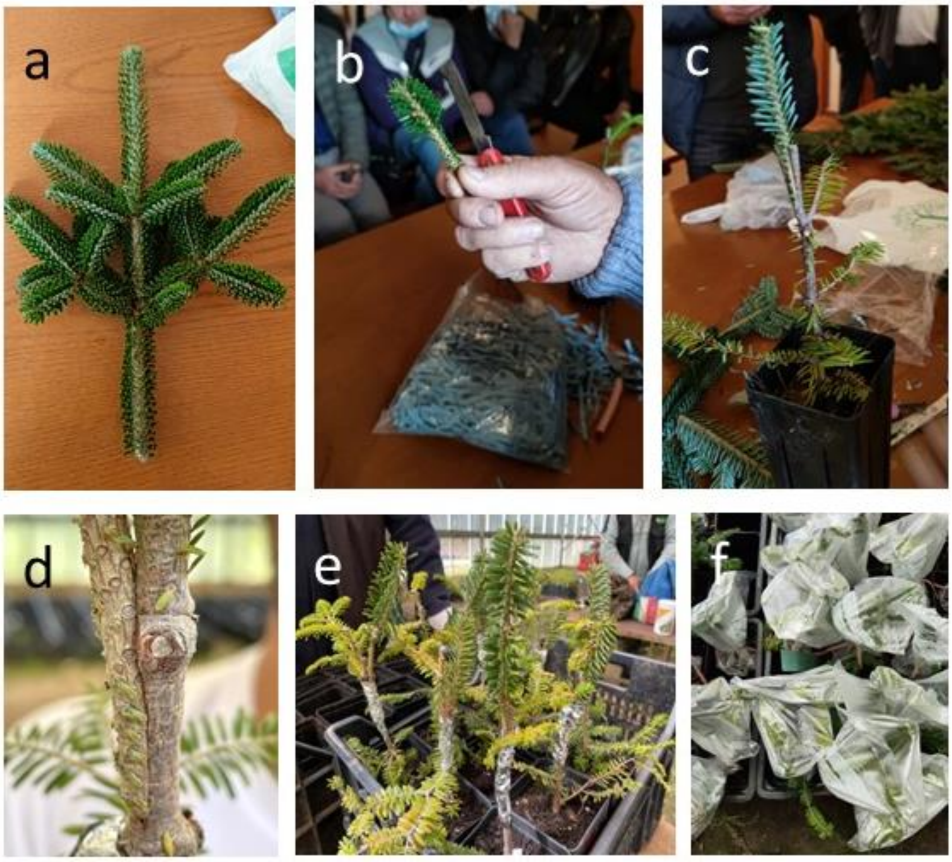

Fig. 12. The grafting of *Abies nebrodensis*. a, a collected scion; b, the scion preparetion: the needle stripping; c, a performed graft before tying with rubber strips; d, particular of the perfect conjunction rootstock-scion; e, the grafted plants after tying and covering with aluminium foil; f, the final covering of grafted plants with plastic.

Fig. 13. *Abies nebrodensis* grafted plants, moved in late summer in a shaded area of the 'Vivaio Piano Noce' (*left*); example of a well healed graft (*right*).

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Produced on October 10th, 2022