

Report on the selection of parent plants and seed germination

C.1 – Development of disease-resistant olive varieties



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Action C.1: Development of disease-resistant olive varieties

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1. Summary

Since the beginning of the outbreak produced by *Xylella fastidiosa* in the South of Italy, different levels of resistance among local olive cultivars were observed. Recent studies have corroborated this variability; cultivars such as ‘Leccino’ and ‘Fs-17’ have shown high levels of resistance, while others such as ‘Ogliariola Salentina’ and ‘Cellina di Nardo’, have shown the opposite behaviour (Boscia et al., 2017).

This variability has been the foundation for the first olive breeding program to develop new cultivars resistant to *X. fastidiosa* that is currently being developed by the University of Cordoba (UCO) in the frame of this LIFE project.

The selection of the genitors (parents), the crossings and the evaluation and selection of the seedlings are being carried out during this project. To do so, the UCO team is developing the necessary protocols for the selection of the genitors and the evaluation and selection of the seedlings to fulfil the goals of this project.

2. Introduction

In order to develop the new varieties resistant to *Xylella fastidiosa* the UCO team has performed the following activities:

1. Selection of the olive cultivars acting as parents (genitors) in the breeding program. The cultivars were selected according to their resistance to *Xylella fastidiosa* (Boscia et al., 2017) and positive agronomical characteristics (Barranco et al., 2017). Among these latter, we selected cultivars with outstanding productivity, oil content and moderate-reduced vigor (Diez et al., 2016).

2. Directed crosses between the selected genitors that were carried out during the springs of 2017 and 2018 (Table 1; Figure 1A, B).
3. Fruit collection and seed germination. After collecting ~6000 fruits and germinating the seeds in controlled conditions (see attached protocol), more than 2500 seedlings (1080 and 1401 2017 and 2018, respectively) were obtained from the directed crosses (Table 1 and figure 1C, D).
4. Forced growth of the seedlings in controlled conditions (greenhouses, 24 hours of light) during at least 6 months (Figure 1E).
5. Selection of the seedlings according to their height (seedlings > 1 m height). The selected seedlings from 2017 crossings (501 genotypes) were planted in an experimental field (El Valenciano, Carmona, Seville) owned by Galpagro, to characterize their agronomical traits. The plantation followed a randomized block design, with 5 meters between rows and 2 meters between trees and the cultivars 'Picual', 'Frantoio', 'Arbequina' and 'Arbosana' as controls (Figure 1F).
6. Agreement between the University of Córdoba and the Italian company "Giovanni Presicce" of Scorrano (Lecce, main affected area by *Xylella fastidiosa* (CoDIRO) in Italy). This company own several olive orchards severely affected by the disease and agreed to establish an experimental field to carry out the evaluation of the selected genotypes in natural infection conditions. In June of 2019, and taking advantage of second meeting of LIFE-Resilience partners that was be held on Italy (Florence), we visited Lecce in order to select the experimental field location.

Here below, we describe in detail, the activities carried out to accomplish C1 action, taking into that during this project we have evaluated seedlings generated in 2017 and 2018.

3. Results

3.1. Seedlings from the directed crosses performed in 2017.

1. May of 2017. The following directed crosses were performed: 'Leccino' x 'Fs-17', 'Leccino' x 'Carolea', 'Oglariola Salentina' x 'Leccino' and 'Leccino' x 'Picual' following the UCO optimized protocol:

- a. Pollen from the selected male genitors was collected in different commercial olive orchards from Cordoba and Sevilla.
- b. The pollen was sieved and mixed with talcum powder in the laboratory.
- c. Trees from cultivars used as female genitors were bagged at the World Olive Germplasm Bank of Cordoba (WOGB).
- d. Pollination was performed by applying male pollen into the female bagged-branches.

2. June-October of 2017. Visits to the WOGB to monitor the set of the fruits.

3. September 15th to October 10th, 2017. Olive fruit harvest from the mother trees.

4. October 1st to 10th of 2017. Seed extraction and classification after removing the fruit flesh and the pit.

5. October 17th of 2017. Seed stratification and germination in controlled conditions (growth chamber) following an optimized protocol.

6. November 15th of 2017. Forced growth of the seedlings in growth chamber (24 hours of light and 24°C).

7. January 16th of 2018. Seedlings transplant to 1.5 L pots and transfer to the greenhouse to continue applying the forced growth protocol.

8. 04/12/2018. Plantation of the selected 501 seedlings in an experimental field (El Valenciano, Carmona, Seville) owned by Galpagro, to characterize their agronomical traits. The plantation followed a randomized block design, with 5 meters between rows

and 2 meters between trees and the cultivars 'Picual', 'Frantoio', 'Arbequina' and 'Arbosana' as controls (Table 1 and figure 1).

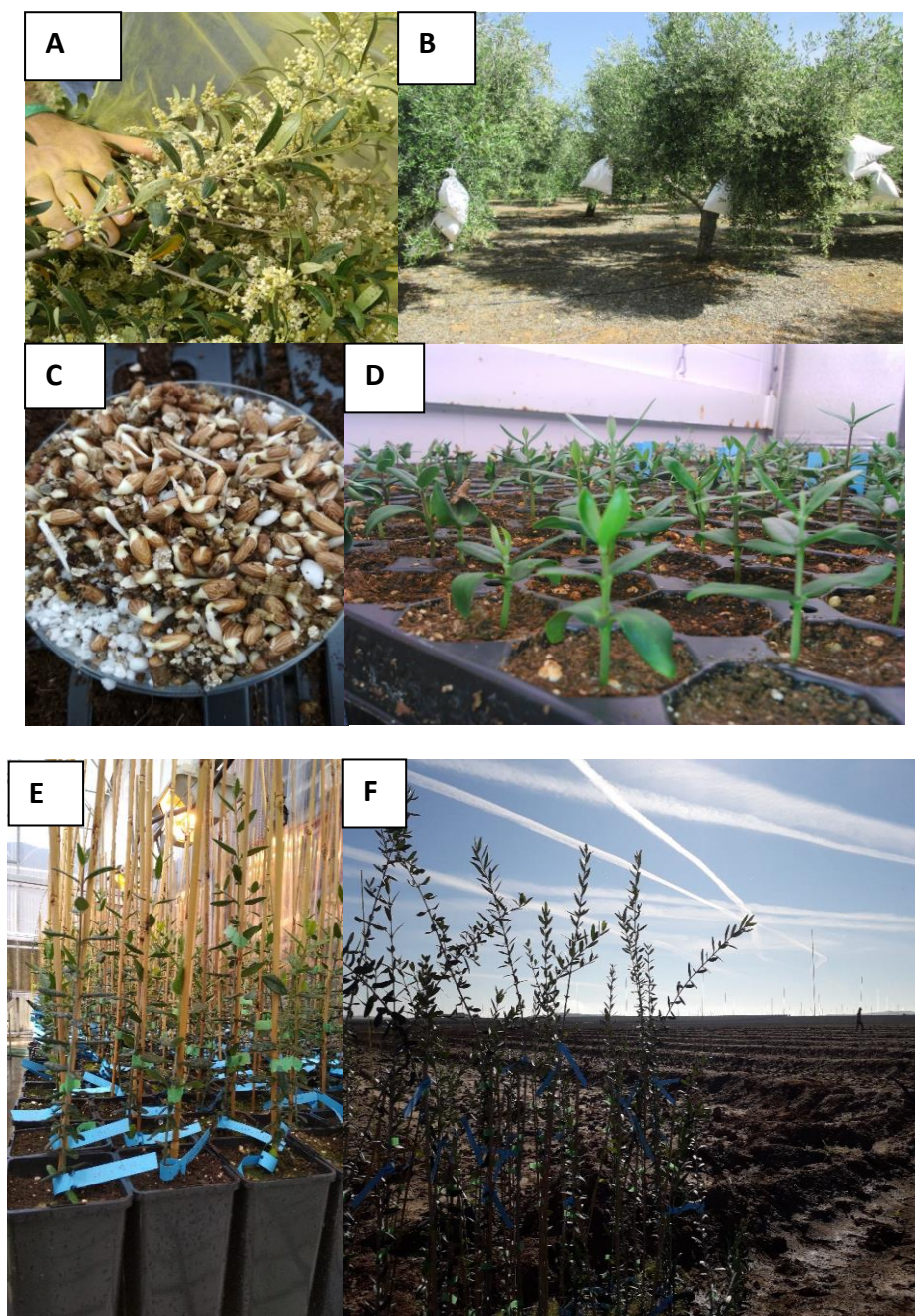


Figure 1. Activities to accomplish the goals proposed in Action C1. A. Collection of pollen from the cultivars used as male genitors; B. Cultivars used as female genitors with bagged branches during the directed crosses; C. Seed germination; D. Seedlings in the growth chamber ready to be transplanted; E. Seedlings following a forced growth protocol; F. Plantation of the selected genotypes to the field “El Valenciano” in Sevilla.

3.2. *Seedlings from the directed crosses performed in 2018.*

1. May, 2017. The following directed crosses were performed: 'Leccino' x 'Arbequina', 'Leccino' x 'Arbosana', 'Fs-17' x 'Arbequina', 'Fs-17' x 'Arbosana' and 'Fs-17' x 'Picual' following UCO optimized protocol:

- a. Pollen from the selected male genitors was collected in different commercial olive orchards from Cordoba and Sevilla.
- b. The pollen was sieved and mixed with talcum powder in the laboratory.
- c. Trees from cultivars used as female genitor were bagged at the World Olive Germplasm Bank of Cordoba (WOGB).
- d. Pollination was performed by applying male pollen into the female bagged-branches.

2. June to October, 2018. Visits to the WOGB to monitor the set of the fruits.

3. September 25th to October 15th, 2018. Olive fruit harvest from the mother trees.

4. October 1st to 15th, 2018. Seed extraction and classification after removing the fruit flesh and the pit.

5. October 15th, 2018. Seed stratification and germination in controlled conditions (growth chamber) following an optimized protocol.

6. November 16th, 2018. Forced growth of the seedling in growth chamber (24 hours of light and temperature 24°C).

7. December 15th to 21st, 2018. Seedling having more than 2 pairs of leaf were transplant to 1.5 L pots and transfer to the greenhouse to continue the forced growth protocol.

8. December 21st, 2018 to Plantation. In greenhouse, daily monitoring of pruning, place a stake, irrigation and treatments to force the change of phase in the different genotypes and to be able to evaluate the plants in field in adult stage.

Table 1. Directed crosses between the selected olive cultivars and the fruits, seeds and seedlings generated from them in 2017 and 2018.

Crossings 2017	Harvested fruits	Planted seeds	Selected seedlings
'Leccino' x 'Fs-17'	500	200	94
'Leccino' Open pollination	550	220	90
'Leccino' x 'Carolea'	550	220	86
'Fs-17' Open pollination	550	220	97
'Oglariola salentina' x 'Leccino'	175	70	41
'Leccino' x 'Picual'	375	150	93
	2700	1080	501
Crossings 2018			
'Leccino' x 'Arbequina'	735	294	114
'Leccino' x 'Arbosana'	1212	485	297
'Fs-17' x 'Arbequina'	960	384	168
'Fs-17' x 'Arbosana'	100	40	24
'Fs-17' x 'Picual'	495	198	38
	3502	1401	641
Total	6202	2481	1142

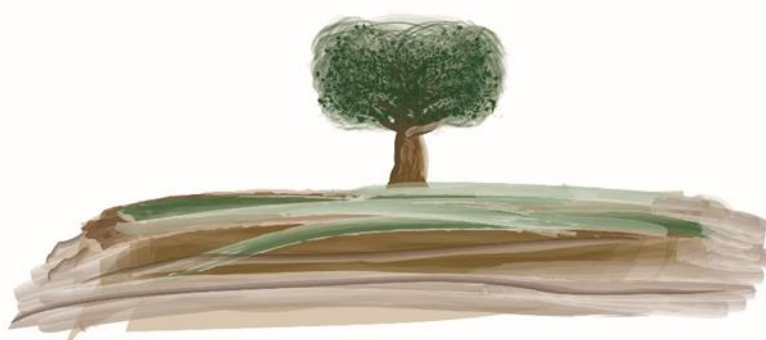
4. References.

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