



# Proceedings of the Final Conference of the PRIMA project “FIGGEN” - Valorising the diversity of the fig tree, an ancient fruit crop for sustainable Mediterranean agriculture

22<sup>nd</sup> March 2024

Aula Magna of Department of Agriculture,  
Food and Environment (University of Pisa).  
Via del Borghetto, 80 56124 Pisa (Italy)



CENTRO DE INVESTIGACIONES  
CIENTÍFICAS Y TECNOLÓGICAS  
DE EXTREMADURA



**CSIC**

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS













# FINAL CONFERENCE OF THE FIGGEN PROJECT

## Program Part I

**Chairmen Prof. Tommaso Giordani**

-  **09.00 - 09.15** Prof. Marcello Mele (Director of Department of Agriculture, Food and Environment) Opening-Welcome
-  **09.15 - 09.30** Prof. T. Giordani (University of Pisa) Presentation of the project “FIGGEN”
-  **09.30 - 09.45** Prof. M. López-Corrales (Extremadura Scientific and Technological Research Center - CICYTEX) The Spanish Fig Germplasm Bank and sustainable production systems in fig trees
-  **09.45 - 10.00** Dr. G. Usai (University of Pisa) The haplotype phased genome of fig (*Ficus carica* L.): a crucial resource for fig breeding
-  **10.00 - 10.15** Prof. A. Kuden (Çukurova University) Phenotyping analysis of potted fig plants exposed to drought and salt stress
-  **10.15 - 10.30** Dr. M.G. Domínguez Yagüe (Extremadura Scientific and Technological Research Center - CICYTEX) Phenotyping analyses on adult plants in three germplasm banks of the fig tree in the Mediterranean basin
-  **10.30 - 10.45** Dr. M. Castellacci (University of Pisa) Exploiting the genetic diversity of the fig tree to discover molecular markers associated to important traits
-  **10.45 - 11.00** Francisco Balas Torres (Head of production and R&D FIKI Europe) FIGGEN relevance for companies: an experience from Extremadura.

**11.00 - 11.30 Coffee-break**

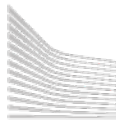
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## Program Part II

**Chairmen Prof. Tommaso Giordani**

-  **11.30 - 11.45** Prof. G. Baraket (Faculty of Sciences of Tunis - UTM) Overview on participatory assessment of the potential of fig genotypes in FIGGEN project.
-  **11.45 - 12.00** Dr. M. J. Serradilla Sánchez (Extremadura Scientific and Technological Research Center - CICYTEX) Management of innovative preharvest strategies to obtain high-quality standard and hygienic-sanitary figs for fresh and dry consumption “(INNOFIG)”
-  **12.00 - 12.15** Dr. S. Comlekcioglu (Çukurova University) Characterization of some fig genotypes selected from Mediterranean and Southeastern Anatolia
-  **12.15 - 12.30** Dr. P. Farina (University of Pisa) Twenty years after the first report of *Aclees taiwanensis* in Europe: how to protect fig orchards from this invasive alien threat?
-  **12.30 - 12.45** Dr. Fateh Aljane (Institute of Arid Regions - University of Gabès) Fig (*Ficus carica*) genetic resources in the oases of Kebili (South-West of Tunisia): richness and opportunity for valorization
-  **12.45 - 13.00** Dr. Olfa Saddoud Debbabi (National Genebank of Tunisia) Management of fig genetic resources in Tunisia

**13.00 - 13.30 Discussion and conclusions**

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The Final Conference will be organised as a hybrid event (both on-site and on-line). The participation is free, but the registration is mandatory.

The link to participate with Microsoft Teams platform is: [urly.it/3-p5-](https://urly.it/3-p5-)

For more info: [tommaso.giordani@unipi.it](mailto:tommaso.giordani@unipi.it)  
[www.figgen.eu](http://www.figgen.eu)

### **The FIGGEN project - Valorising the diversity of the fig tree, an ancient fruit crop for sustainable Mediterranean agriculture**

FIGGEN is a three-year project promoted by *PRIMA* (Partnership for Research and Innovation in the Mediterranean Area) programme supported by the European Union. The ambition is to make the fig tree one of the most suitable and profitable crop in the Mediterranean area in a climate change context. Among tree crops, the fig tree shows a good adaptation to dry, calcareous and saline environments, typical of different regions in the Mediterranean basin and the Middle East, where it has been cultivated for millennia. This crop has great potential for expansion thanks to valuable nutritional and nutraceutical characteristics and is particularly suitable for the application of sustainable agriculture based on biodiversity, such as mixed farming systems like agro-forestry.

Despite its importance, the fig tree has undergone low genetic improvement and most cultivation in the Mediterranean area is based on local cultivars that are currently highly threatened by genetic erosion due to various pests and diseases, abiotic stresses, intensive urbanization, monovarietal crops, migration from rural to urban areas. FIGGEN wants to contribute breeding efforts to address crop tolerance to multiple abiotic stresses, improving productivity, efficiency, and sustainability of agricultural farming systems.

Breeding depends on the collection; conservation and sharing of appropriate crop genetic resources among plant breeders and farmers. In this sense the project will create a participatory context involving main actors of the value chain following a transdisciplinary approach where socio-economic knowledge and recent scientific advances in assessing biodiversity will be combined with traditional knowledge of local private and public stakeholders.

FIGGEN aims to enhance the biodiversity of the fig tree and to select genotypes better adapted to environmental conditions coming from climate changes that can promote fig breeding and more sustainable fig production of the future.

[www.figgen.eu](http://www.figgen.eu)

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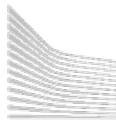




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## ORAL PRESENTATIONS

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## Presentation of the project “FIGGEN”

T. Giordani<sup>1</sup>

<sup>1</sup>Department of Agriculture, Food and Environment - University of Pisa - Italy

FIGGEN - "Valorising the diversity of the fig tree, an ancient fruit crop for sustainable Mediterranean agriculture" is a project of PRIMA, the Partnership for Research and Innovation in the Mediterranean Area and is promoted by EU.

It started in 2020 and ended in March 2024.

The consortium is composed by 5 units: the Department of Agriculture, Food and Environment of University of Pisa from Italy that is coordinator, the Centre For Scientific and Technological Research of Extremadura (CICYTEX) and the Institute for Mediterranean and Subtropical Horticulture "La Mayora" of the Spanish National Research Council (CSIC) from Spain, the Faculty of Science of the University of Tunis El Manar from Tunisia and the Department of Horticultura of Cukurova University from Turkey.

The objectives are:

- a) Exploring, valorising, and evaluating fig cultivars from available Spanish, Tunisian and Turkish fig collections:
  - analysing genetic characteristics using a genotyping by sequencing approach
  - analysing morphological and physiological traits of fig cultivars to select plants with characteristics wanted by stakeholders and most suitable to be cultivated in drought and salinity conditions (more adapted to climate change)
- b) Identifying genetic traits linked to yield, fruit quality and drought and/or salinity adaptation that can be exploited in future breeding programs
- c) Disseminating project's products and results to stakeholders.

To achieve these objectives the project is structured in 5 WorkPackages. Wp0 Project coordination and management, WP1 Participatory assessment of the potential of fig cultivars in which Living Lab with stakeholders have been organised where researchers and participants exchange knowledge for the evaluation and selection of fig cultivars, following a transdisciplinary approach. WP2 Fig tree valorisation, characterization and selection, includes genotyping and phenotyping analyses on 286 fig cultivars from Spanish, Turkish and Tunisian germplasm banks. A first product of these analyses is the identification of cultivars tolerant to drought and/or salinity conditions and with traits expected by stakeholders. In WP3 Genome Wide Association Studies (GWAS), data from genotyping and phenotyping analyses have been combined to identify single nucleotide polymorphisms (SNPs) associated with traits related to the fig production and quality. These

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specific genetic traits, after the end of the project, can be used for marker assisted selection and future breeding programs to develop new cultivars more adapted to climate change. Finally WP4 is related to Dissemination, Exploitation, Communication and Outreach activities and has the objective to maximize the impacts of the project.

The team of Agricultural and Food Economics of our Department carried out a desk analysis of the market of the fig, focusing in the Mediterranean region to understand the importance of fig cultivation and production, critical issues, and development opportunities. In addition, this team prepared guidelines with indications on Living Labs meetings organization and reporting.

In spring 2021 and 2023 first two Living Lab meetings were held in Spain, Tunisia, and Turkey. Among participants there were representatives of companies, farmers, plant growers, policy and administration, social associations, universities, research centres, consumer associations.

Following the selection of drought and/or salinity tolerant cultivars, a catalog with a description of selected cultivars has been shown to the public during the third Living lab meeting organized in 2024. The plants that best satisfy the expectations of the participants were made accessible to them for validation in field trials (in real production facilities and contexts).

As concerns genotyping analysis, the team of Agricultural Genetics of our department produced a high-quality reference genome sequence of the fig tree combining the last published genome sequence with the latest methodologies of chromosome conformation capture. This allowed a deep characterization of the genome, with the identification and annotation of about 34 thousand protein coding genes. This reference sequence is a prerequisite for genotyping analyses on 286 fig cultivars that have been carried out following a Whole Genome Sequencing approach.

Genotyping and phenotyping analyses showed a high genetic variability among genotypes, allowing identifying possible synonymous genotypes intra- and inter-germplasm banks.

Phenotyping analyses have been performed both on adult plants, considering a number of plant and fruit traits, and on propagated plants in pots. These plants were grown in drought and salinity condition allowing to select tolerant cultivars that could be more adapted to climate changes.

GWAS allowed identifying a number of SNPs associated to important fruit traits including fruit weight. These molecular markers, after validation, can be exploited for marker assisted selection and breeding programs.

With regards to communication and dissemination activities, all partners contributed to publication of press releases, to the creation of a project logo, social media accounts and a project website. We participated to national and international conferences where our project has been presented and project results have been reported. Finally, up to now, we published 8 scientific papers in open access peer reviewed journals.

**Keywords:** *Ficus carica*, project, genotyping, phenotyping, GWAS

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## Spanish germplasm bank and the different production systems developed in fig tree by CICYTEX

Margarita López Corrales<sup>1</sup>, Fernando Pérez Gragera<sup>1</sup>, M<sup>a</sup> Guadalupe Domínguez Yagüe<sup>1</sup>, Ana M<sup>a</sup> Montero de Espinosa<sup>1</sup>, Ana M<sup>a</sup> Fernández León<sup>1</sup>, Antonio Jesús Galán<sup>1</sup>

<sup>1</sup>Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX) - Spain

The National Fig Germplasm Bank is located at the Center for Scientific and Technological Research of Extremadura (CICYTEX) and includes about 300 different varieties. All these accessions were characterized at morphological level using the UPOV fig tree descriptor (TG265/1) and at molecular level using 9 SSR microsatellite markers. This trial field is arranged using a 5 x 5 m planting frame with 3 trees per variety and localized irrigation. One of the main tasks in this bank was identifying the core collection that includes 31 varieties. This core collection represents most of the fig diversity conserved in the genebank. It could serve as a basis for the exchange of plant material between researchers and breeders.

Another line of research of this group focuses on the study of the agronomic and quality performance of fig tree varieties in different sustainable training systems adapted to both fresh and dry fig consumption. These coordinated projects are carried out with the post-harvest group of the Technological Institute of Extremadura (INTAEX) and the University of Extremadura. Thus, to produce fresh figs and dry figs, different varieties have been evaluated, formed in low vase and intensive frames (5 x 4 m) with 3-4 feet/tree and light pruning that allows illumination in the interior zone of the tree, avoiding sunburns on the main branches. This type of plantation requires localized irrigation and an average irrigation rate close to 3500 m<sup>3</sup> /ha, producing between 15 and 30 t/ha in the seventh green depending on the variety. To improve both fig lighting and manual harvesting and, to optimize cultivation work, horizontal trellis training system for fresh consumption has been studied, obtaining higher average yields, fruit weight and soluble solids content. The plantation height is limited to a maximum of two meters, making manual harvesting suitable from the ground. On the other hand, north-south orientation of the planting lines is fundamental, as well as painting tree trunks to avoid sunburn, since fig wood is soft.

Agronomic performance of the Calabacita variety for dried consumption markets was also studied under super-intensive training, using a planting frame of 5x2 m and localized irrigation. In addition, a system of nets suspended about 50 cm above the ground was implemented to facilitate harvesting and improve hygienic and sanitary quality of the figs. Locating the irrigation line in the center of the planting lanes is fundamental to avoid humidity in the area where the figs are drying. Ninth year's dried figs yields exceed 10t/ha with a humidity of less than 26%, in accordance with the production standard.

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These plantations have a higher cost, so trellis training system is currently being studied in the Calabacita variety for dry consumption even with a frame of 4x 3.5m. In this case, the plantation can be formed using four lines of wires at different heights, since dried figs fall to the ground. In the harvesting months, white anti-weed nets are placed to improve the hygienic-sanitary quality of the product, where figs can be harvested using vacuum cleaners.

**Keywords:** germplasm bank, fig tree, production systems.





## The haplotype phased genome of fig (*Ficus carica* L.): a crucial resource for fig breeding

Usai G.<sup>1</sup>, Vangelisti A.<sup>1</sup>, Castellacci M.<sup>1</sup>, Simoni S.<sup>1</sup>, Mascagni F.<sup>1</sup>, Natali L.<sup>1</sup>, Cavallini A.<sup>1</sup>, Giordani T.<sup>1</sup>

<sup>1</sup>Department of Agriculture, Food and Environment, University of Pisa – Italy

Access to top-notch genome sequences is crucial for employing advanced crop breeding methods. This is particularly true for fruit trees such as the fig tree (*Ficus carica* L.), which maintain genetic diversity through clonal propagation. Although the fig tree shows promise for commercial growth because of its nutritional value and ability to thrive in harsh conditions, high-quality genomic data for this species has only recently become accessible.

This research introduces a phased assembly of the fig tree genome using single-molecule, real-time sequencing, and chromosome conformation capture techniques. By combining around 55x coverage of HiC reads with the current genomic assembly, we produced two pseudo-haplotypes, each composed of 538 sequences, covering approximately 98% of the estimated 356 Mb fig genome. Moreover, we allocated 400 out of the 538 sequences (roughly 96% of both pseudo-haplotypes) to the 13 chromosomes, leading to the development of version 2.0 of the fig genome.

Through a combination of methods, including RNA-seq data analysis, protein alignment, and *de novo* prediction, we identified approximately 33,954 and 33,379 protein-coding genes per pseudo-haplotype. Functional annotation was provided for roughly 82% of these genes. Additionally, synteny analysis revealed approximately 20,441 allelic gene pairs, constituting about 65% of the total genes, which together compose the genetic map of the fig tree.

Structural variation analysis within the identified synteny blocks revealed 832,619 SNPs, 996,026 INDELs ranging from 1 to 50 bases, and 308 large structural variations longer than 50 bases, resulting in 1,828,953 variations. The intragenomic diversity was estimated to be around 5.2 variations per kilobase.

The positions of the 20,441 allelic gene pairs were correlated with structural genomic variations across various regions, including promoter regions, 5'-UTR, CDS, intronic regions, and 3'-UTR. Notably, CDS showed a greater abundance of SNPs within these regions compared to INDELs (55.8%), whereas the other regions consistently exhibited a higher frequency of INDELs relative to SNPs. Specifically, the 5'UTRs displayed the highest prevalence of INDELs over SNPs (61%).

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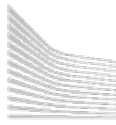


Functional annotation was performed to assess the impact of genomic variations on allelic gene pairs. Analysis of variant annotation identified 349,284 genomic variants across five paired regions, including 189,677 SNPs (54.30%) and 159,607 INDELs (45.70%). Non-synonymous mutations were the most prevalent coding variants, followed by synonymous mutations and splice region and intron variants. Among the 15 most common categories of coding variants, six notably impacted protein encoding.

The fig genome sequence is currently being utilized to evaluate the genetic diversity of fig varieties from Spanish, Tunisian, and Turkish collections using genotyping by sequencing within the PRIMA project FIGGEN (Partnership for Research and Innovation in the Mediterranean Area). The aim is to conduct genome-wide association studies (GWAS) to pinpoint genes or molecular markers linked to fruit quality traits and adaptation to adverse environmental conditions influenced by climate change, ultimately contributing to the enhancement of fig species through genetic improvement.

**Keywords:** phased genome assembly, genome annotation, genetic variability, fig tree





## Phenotyping analysis of potted fig plants exposed to drought and salt stress

Ayzin Kuden<sup>1</sup>

<sup>1</sup>Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana - Turkey

One of the aims of the FIGGEN project is the selection of genotypes tolerant to drought and salt conditions and with traits of plant and fruit expected by stakeholders. For this reason, two experiments of drought and salt treatments on propagated plants grown in pots were set in Spain (CICYTEX), Tunisia (UTM), and Turkey (CU).

In this paper, we summarize the results of two years' phenotyping on propagated plants subjected to drought (in 2021) and salinity (in 2022) treatments. In both experiments, the number of genotypes were 52 in Spain, 116 in Turkey and 110 in Tunisia.

**In drought experiments**, propagated 18-month-old plants were used. From these, leaf temperature, the chlorophyll content (SPAD), relative water content (RWC) and growth-related traits were collected. A standard curve for the quantification of chlorophyll content from SPAD values was used following a published procedure. Leaf temperature is an indirect parameter related to water status, linked to transpiration process: plants are water stressed if temperature increases due to transpiration reduction. Growth parameters such as plant height and stem diameter, the total number of leaves were measured at the beginning and at the end of the experiment.

At the beginning of experiments water requirement was calculated. To do this, we saturated two plant pots of 8 genotypes with water, and once the drainage was finished (approximately 10 minutes), the pots were weighed (PS) and weighed again the following day. The difference between the two successive daily weightings allows them to quantify the daily water requirement. Finally, the average of 10-15 plants was calculated and assumed as water requirement. Control plants were irrigated based on water requirement while drought treated plants were watered with 70% of water requirement. The experiments lasted 50 days.

**In salinity experiments**, propagated 30-month-old plants in 7 liters volume pots with a substrate of peat (of good quality) and perlite (3:1 ratio) were used. At the end of March, the pots were fertilized with about 10g/pot of NPK 15-15-15 fertilizer. At the beginning of experiment water requirement was calculated as described above. Salt treated plants were watered with a 100 mM NaCl solution. The experiment lasted about 60 days.

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After data processing, we identified drought and salt tolerant genotypes that were described in a catalog.

### **Definitive Catalog of Spain**

#### **List of drought tolerant genotypes**

1. Argelina, 2. Botanda, 3. Clon 300, 4. Coll Dama Negre, 5. Conadria, 6. Cordobis, 7. Desconocida 1, 8. King A, 9. Negra Cabezuela, 10. Panacheé, 11. Smyrna, 12. Tofoles

#### **List of salt tolerant genotypes**

1. Zuliverdeja, 2. La Casta, 3. Calabacita, 4. Napolinata Negra, 5. Cordobis, 6. Burjassot V, 7. Alacantina Negra, 8. Granito, 9. Smyrna, 10. San Antonio

#### **List of drought and salt tolerant genotypes**

1. Cordobis, 2. Smyrna

### **Definitive Catalog of Turkey**

#### **List of drought tolerant genotypes**

1. 1008 -Yeşilguz, 2. 404-Kis Hayri, 3. 216-Siyah Incir, 4. 1005-Seker Inciri, 5. 102-Sarilop, 6. 1004-Kusadasi Bardakci, 7. 709-Kizil Mor, 8. Abbas, 9. 334-Fetike, 10. 342-Kızıl Yemiş, 11. 344 -Güzlük Mor, 12. 227-Yediveren, 13. Taşlık, 14. 528-Kara İncir 2, 15. 317-Aş1, 16. Barbaros, 17. 514-Deniz İnciri, 18. 253-Sultan Selim, 19. 335-Lop, 20. 1115-Gökçe, 21. Siyah Orak 1

#### **List of salt tolerant genotypes**

1. 342-(07İ05) Kızıl Yemiş, 2. 514 Deniz İnciri, 3. 540-Kilis-1, 4. 534-Sarı, 5. Barbaros, 6. 344 Güzlük Mor, 7. 251-Dereköy, 8. 224-Beyaz, 9. 343 (33İ01) Gök, 10. 1001-Göklop, 11. Şeytan1, 12. Kod-3 Banana, 13. 216 Siyah İncir, 14. 335-Lop, 15. 1115-Gökçe, 16. 1002-Bardacık, 17. 1010-Karayaprak, 18. 1113-Beyaz, 19. 322-Beyaz Seyhan, 20. 528-Kara İncir 2, 21. Kış Hayrı

#### **List of drought and salt tolerant genotypes**

1. 216-Siyah, 2. 344-Güzlük Mor, 3. 528-Kara İncir 2, 4. Abbas, 5. Barbaros, 6. 514-Deniz İnciri, 7. 404-Kış Hayrı, 8. 342-Kızıl Yemiş, 9. 335-Lop, 10. 1115-Gökçe

### **Definitive Catalog of Tunisia**

#### **List of drought tolerant genotypes**

1. Tounsi, 2. Harchi, 3. Sekni, 4. Zargui, 5. Bayadhi, 6. Bither Abyadh, 7. Dorghami, 8. Rogabi, 9. Minouri, 10. Zidi Kesra, 11. Tchich Assal, 12. Bargoug, 13. Soltani, 14. Tchich w Assal, 15. Marsaoui, 16. Ghabri, 17. Bither Gafsa, 18. Assal Boudchich, 19. Khili, 20. Bouslames,



21. Khartoumi<sup>2</sup>, 22. Jebali, 23. Hafer Bghal, 24. Bith Bghal, 25. Besbessi, 26. Wahchi, 27. Kahli<sup>1</sup>, 28. Jrani, 29. Assafri, 30. Zidi<sup>2</sup>, 31. Dhokkar Matmata, 32. Bayoudhi (cherih)

### List of salt tolerant genotypes

1. Souwadi, 2. Zidi<sup>6</sup>, 3. Ghabri, 4. Boukhobza, 5. Wahchi, 6. Kkadhari, 7. Dhokkar Matmata, 8. Zidi Kesra, 9. Kahli<sup>1</sup>, 10. Dchich Assal Asfar, 11. Baghali<sup>3</sup>, 12. Marchini, 13. Safouri, 14. Minouri, 15. Tchichi w Assal, 16. Zidi Sahel, 17. Lawi (karmous Mbargat), 18. Bettenjene, 19. Soudi khechine, 20. Bither<sup>2</sup>, 21. Dorghami, 22. Sawoudi<sup>3</sup>, 23. Croussi, 24. Mazouzi, 25. Baydha, 26. Sawoudi Matmata, 27. Khedhri Sahel, 28. Khili, 29. Dhokkar kesra, 30. Bou Abda, 31. Temri Sfax, 32. Bouslames, 33. Zergui, 34. Mlouki<sup>1</sup>, 35. Wedlani, 36. Bither Sahel, 37. Safri Gares, 38. Sekni

### List of drought and salt tolerant genotypes

1. Dorghami, 2. Tchich w Assal, 3. Sekni, 4. Minouri, 5. Kahli<sup>1</sup>, 6. Wahchi, 7. Ghabri, 8. Bouslames, 9. Zidi Kesra, 10. Dhokkar Matmata, 11. Khili

**Keywords:** phenotyping, drought, salinity, genotypes



## Phenotyping analyses on adult plants in three germplasm banks of the fig tree in the Mediterranean basin.

M.G. Domínguez Yagüe<sup>1</sup>

<sup>1</sup>Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX) - Spain

Within the FIGGEN PRIMA Project, phenotypic analyses of 280 genotypes of *Ficus Carica L.* across Spain (CICYTEX), Tunisia (Université de Tunis Manar) and Turkey (University of Kurucova) have been performed providing information about diversity and features of this species. In Spain, 52 genotypes have been studied, 50 female, 1 male and one ancestor of *Ficus Carica L.*, *Ficus Palmata*. 116 genotypes have been evaluated in Turkey, 105 female, 11 male. 110 genotypes were evaluated in Tunisia, 104 female and 6 male.

This phenotypic work includes the characterisation of 26 plant and fruit traits, qualitative, quantitative, and pomological traits. This task has been performed following the TG265/1 UPOV guideline for Distinctness, Uniformity and Stability (DUS) exam in *Ficus Carica L.* Regarding plant traits, reproduction, productive type, harvesting date, growth habit and vigour of the referred 280 genotypes were determined during two years. In these ordinal traits, scores were used for statistical analysis. Qualitative traits such as predominant type of leaf, and qualitative fruit traits as attachment of stalk to stem, fruit shape, fruit size, fruit ostiole size, fruit stalk length were also determined. The ground colour, over-colour of skin and colour of the pulp were determined according to Royal Horticultural Society colour chart. Cracking of skin was evaluated by visual observation and easy of peeling was carried out removing the skin from the neck to the ostiole. The scratch resistance of skin directly related with firmness was measured with a TA-XT Texture Analyser applying a force to produce a 6% deformation by a 70 mm aluminium plate. Regarding the quantitative traits, the length and width were determined with a digital caliper. The total soluble solid content (TSS), expressed as °Brix, was determined with a RM40 Mettler Toledo digital refractometer. The titratable acidity (TA) expressed as citric acid g / 100 g fresh weight (FW), was determined with a T50 Mettler Toledo automatic titrator. The maturation index (MI) was calculated as the ratio between TSS (°Brix) and TA, which is positively correlated with consumer acceptability. Concerning the statistical analysis, the means, median, range, standard errors, and coefficient of variation for the quantitative traits were calculated over the two years of phenotyping for the three collections of fig genotypes. The estimated marginal means were used to calculate all the possible correlation coefficients among the traits recorded during 2021 and 2022. PCA was performed in order to determine the importance of the different traits in the explanation of the multivariate polymorphism in fig

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genotypes and then a cluster analysis was carried out to measure the hierarchical similarity among genotypes.

The PCA carried out with Spanish, Turkish and Tunisian genotypes generated biplots with highly variable at phenotypic level with no clear clustering distinguishing genotypes. A hierarchical clustering analysis with all the traits was performed, all the genotypes were grouped in different clusters based on Euclidean distance representing the relevant traits for stake holders.

This work has provided substantial information for the stakeholders in relation with most important traits such as weight, size, total soluble solid and titratable acidity as well as some qualitative traits such as reproduction, harvesting date, juiciness, attachment of stalk to stem. This phenotypic analysis supplied an inestimable vision about diversity and relevant traits in relation with breeding programs.

**Keywords:** phenotyping, fruit traits, *Ficus carica* L.



## Exploiting the genetic diversity of the fig tree to discover molecular markers associated to important traits

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Identifying and characterizing variants implicated in desirable traits holds a potential for advancing plant breeding efforts. Despite the promising commercial prospects of the fig tree (*Ficus carica* L.) owing to the nutritional richness of its fruits and the plant's good adaptability to harsh conditions, elucidating the genetic foundations of these traits remains a critical challenge. To date, there has been a lack of in-depth studies on the genetic basis of these important traits. In this sense, this research aims at valorizing and characterizing the fig tree biodiversity in the Mediterranean basin, and discovering important traits associated with genes.

To assess genetic variability and perform Genome-Wide Association Studies (GWAS) within the FIGGEN project of the PRIMA action, 286 fig varieties underwent genotyping using our reference fig genome assembly. In detail 61 varieties were located from Spain, 110 from Tunisia and 115 from Turkey.

Through the whole genome sequencing, and variant calling pipeline we initially identified about 15 million single nucleotide polymorphisms (SNPs). After stringent quality control filters, 1.371.111 biallelic SNPs were identified. Population structure analysis revealed three distinct clusters corresponding to the geographical origin of genotypes, supported by principal component analysis (PCA), and phylogenetic tree construction. Interestingly, certain genotypes showed genetic relatedness across different clusters and countries, indicating potential synonymies or cryptic relatedness.

Genome-wide association study (GWAS) identified significant marker-trait associations for fruit traits including fruit weight, fruit length, fruit width, total soluble solid content, titratable acidity, and firmness.

Notably, a strong association peak on chromosome 2 showed five SNPs within a *Probable flavin-containing monooxygenase 1* gene (*FMO1*), suggesting its potential role in determining fruit

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weight, as previously observed in tomato. These preliminary findings contribute to understanding the genetic basis of important agronomic traits in figs and provide insights for future breeding programs. Further annotation and validation of SNPs are underway to achieve a better understanding of the genetic basis of these important traits.

**Keywords:** whole genome sequencing, genetic variability, population structure, gwas, fig tree





## FIGGEN relevance for companies: an experience from Extremadura

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The cultivation of the fig tree is of great socioeconomic importance in Extremadura (Southwestern Spain). FIKI EUROPA SL is a company dedicated to the cultivation and marketing of figs in different formats. We consider that there are three challenge areas in fig production and marketing: the challenges of the agri-food chain, those of climate change, and regulatory challenges.

Among the challenges of the agri-food chain, it is worth highlighting the short shelf life of figs, since they are highly perishable fruits and push the whole logistics chain (producer, transport, wholesaler, retailer, consumer) to be very fast and very efficient in order to avoid losses. It is also needed to adjust ripening dates to the best sales dates, as well as the shape and colour of the fruits, which may vary from one market to another.

The challenges derived from the environment and more specifically from the climate change scenario are especially complicated. Our production area is located in the province of Badajoz, characterized by very hot and very dry summers that coincide with the fig harvest season. When spring or fall is drier than normal, drought problems often manifest. In last decades, a general rise in temperatures has been observed, as well as a decrease in rainfall. This makes us fear more and more frequent episodes of drought at harvest time with the consequent effects such as anomalies in the reproductive cycles of plants, severe drought, frost, lack of water for irrigation, soil degradation and the appearance of new pests. and diseases.

Regarding regulatory challenges, but also socioeconomic, all producers in Europe must be prepared for the new EU Green Deal regulatory framework, which will affect all areas of activity of agricultural producers, and which are summarized in the search for sustainability, energy efficiency and zero waste in order to mitigate the effects of climate change in the coming decades. In addition, a high level of food safety, traceability and certifications will be required. In some areas of Europe we face a very significant lack of manpower. Finally, the cultivation of the fig tree suffers from a general shortage of registered inputs to manage orchards.

The challenges that emanate from these three areas gives us an idea of how the perfect fig should look like: extended shelf life, high yield, parthenocarpic, nice colour, fitting shape, no cavity, thight ostiole, drying ability, juiciness, aroma, sweetness, salinity tolerance, drought resistance, pest resistance, disease resistanc, low input requirements, suited to new cultivation systems, adaptation

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to new harvesting methodologies. Therefore, new, more and better adapted varieties are needed. To achieve these objectives, breeding is the most appropriate tool. The FIGGEN project has addressed some of these challenges by identifying genotypes that meet these criteria, thus being an aid to producers and breeders for the future. There is still a lot of work to be done, but initiatives like FIGGEN have a positive impact on the private sector.

**Keywords :** FIKI EUROPA SL, climate change, harvesting, shelf-life

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## Overview on participatory assessment of the potential of fig genotypes in FIGGEN project

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FIGGEN impacts on valorization and conservation of biodiversity analyzing genotypes of fig germplasm including neglected or under-utilized cultivars from three countries that are the main producers of figs in the Mediterranean region. The introduction in agricultural systems of new fig cultivars better adapted to drought/salt conditions will help fig sustainable production of the future, contributing to implement biodiversity-based agriculture, more resilient to climate uncertainties, and more sustainable, producing beneficial effects in terms of conservation of natural resources including soil and water conservation, poor soil valorization and, consequently, better ecosystem services. These changes in the broader socio-technical environment, require an adaptation of agroecological conditions, new agricultural practices, labour reorganization, and may imply a reconfiguration of the relationship in the value chain. So users' participation is needed to anticipate the potential benefits, costs, barriers to adoption, and related risks. Thus the project created a participatory context through the organization of three Living Lab (LL) in Spain, Tunisia and Turkey, involving main actors of the value chain following a transdisciplinary approach. We adopted the Delphi method during three LLs. The multistep process was adopted to perform this method and was implemented through two sequential rating rounds.

The first LL aimed to identify the most important traits that a selected cultivar should have. The experiment of drought treatment performed the first year allowed to the development of the draft catalog of drought tolerant fig cultivars. This catalog was shown to stakeholders during the second LL meeting. Based on the characteristics of the fruits and cultivars, the most important cultivars were selected. In the second year of the work, also an experiment of salt stress was performed and a final set of cultivars tolerant to drought and salinity was obtained and a definitive catalog with their characteristics was developed. The Tunisian catalog includes 32 drought tolerant, 38 salt tolerant genotypes and 11 genotypes that resulted tolerant both to drought and salt conditions. The Spanish catalog includes 12 drought tolerant and 10 salt tolerant genotypes. The Turkish catalog includes 21 drought tolerant, 21 salt tolerant and 10 genotypes tolerant both to drought and to salt conditions. A third LL meeting was organized with stakeholders to evaluate these selected cultivars and to gather further information on their potential and on the problems and opportunities for introducing them in the value chain. The genotypes chosen were made available to the users for evaluation in field trials.

**Keywords:** Living Lab, drought tolerance, salt tolerance, stakeholders.

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## Management of innovative preharvest strategies to obtain high quality standard and hygienic sanitary figs for fresh and dry consumption “(INNOFIG)”

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INNOFIG, entitled "Implementation of super-intensive systems and management of innovative preharvest strategies to obtain figs of high standard and hygienic-sanitary quality for both fresh and dry consumption", is a project coordinated with the University of Extremadura and funded by the Spanish State Research Agency. The main objective of this project is to obtain figs of the 'Calabacita' variety of high nutritional, bioactive and hygienic-sanitary quality, both for fresh and dry consumption, through the application of super-intensive systems and other innovative strategies based on preharvest biostimulants. During the first two years of the project, oxalic acid,  $\gamma$ -aminobutyric acid (GABA) and melatonin were used as elicitors or biostimulants. These molecules were applied between the end of phase II and the beginning of phase III of fruit development when the fruit is considered to have reached physiological maturity. Foliar treatments with oxalic acid (1 and 2 mM), GABA (10 and 50 mM) and melatonin (0.1 and 0.5 mM) were applied at dusk; once most of the figs had reached this stage of ripening for 3 applications, one per week. Figs treated with Tween 20, a surfactant used to facilitate the absorption of these molecules, were used as a control. After harvesting, figs were stored at 1 °C with 90 % relative humidity for up to 10 days under ordinary atmosphere conditions to see the impact of preharvest treatments on the postharvest life of fresh figs. First preliminary results showed that fresh figs treated with these molecules at the preharvest stage maintained a higher quality during postharvest storage, highlighting figs treated with 2 mM oxalic acid and 10 mM GABA for enhancing the enzymatic and non-enzymatic antioxidant systems of fruit and, therefore, extending the shelf life of 'Calabacita' figs in optimal conditions for consumption and with better sensory attributes than control figs.

**Keywords:** *Ficus carica*, fruit quality, elicitor, storage, total phenols, antioxidant enzymes

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## Characterization of Some Fig Genotypes Selected from Mediterranean and Southeastern Anatolia

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This study was carried out at the Cukurova University. Twenty two fig genotypes, selected from the Mediterranean and South East Anatolia regions were experimented. Morphological and pomological characteristics of genotypes were evaluated under same environmental conditions for the fresh fruit consumption. In the morphological characteristics, tree habitus, strength, branching, lateral shoot development, annual shoot length and thickness, tendency of branching, leaf shape, number of leaf per shoot, leaf length, leaf lobes, leaf stalk length and thickness of the genotypes were investigated for three years.

Leaf length of the genotypes changed between 13.6 (Dolap inciri and Yerli Siyah) and 20.40 cm (Sarığöllü); leaf width differed between 12.90 (Dolap inciri) and 20.60 cm (Mut). The lobes of the leaves changed between 2.6 (Güz inciri) and 5.0 (Özmen, Keten Gömleği, Kuyruklu) considering the mean values. Leaf petiole length changed between 5.5 cm (Dolap inciri) and 9.2 cm (Mut), while leaf petiole width was between 3.2 mm (Dolap inciri) and 5.4 mm (Mut). Number of leaves per annual shoots changed between 6.7 (Güzlük) and 10.5 (Kızılcık). The highest annual shoot length was observed in Hacı Abi with 31.01 cm, while the lowest value was obtained from Dolap inciri with 12.48 cm. Finally annual shoot thickness changed between 7.18 mm (Siyah incir) and 19.26 mm (Özmen).

Concerning pomological characteristics, the highest mean value for fruit weight was 60.59 g (Özmen). The highest fruit width was observed in Kuyruklu (49.97 mm), whereas, for fruit length the highest value was obtained from Siyah incir (48.61 mm).

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Ostiole width values were changed between 2.50 mm (Güzlük) and 4.32 mm (Karaverden). The highest pulp length (35.77 mm) was obtained from Özmen. No neck was observed in 10 genotypes while the others had necks and the length of the necks changed between 4.8 mm (Hacı Abi) and 9 mm (Güzlük). Skin thickness values were below 1 mm, except Siyah incir fig genotype which was about 1.16 mm. TSS ratio of the genotypes changed between 18.7% (Akçakale) and 28.2% (Erken yazlık).

Fruit juice pH were found between 4.53 (Akçakale) and 5.65 (Hacı Abi), titratable acid ratio was found between 0.18% (Hacı Abi) and 0.48% (Erkek incir). Fruit flesh color changed from white to light yellow, while fruit skin color was different in each genotype. These results were in accordance with published data, confirming the high variability among the 22 genotypes analyzed.

**Keywords:** *Ficus carica*, fig descriptor, fruit traits, morphological traits





## Twenty years after the first report of *Aclees taiwanensis* in Europe: how to protect fig orchards from this invasive alien threat?

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*Ficus carica* L. (Moraceae), the common fig, is part of the Mediterranean culture as a key crop, staple food, and characteristic element of the landscape. The tree has good adaptability to dry, calcareous, and saline soils, low requirements for its maintenance, and used to be threatened only by a limited number of insect pests that hardly compromised its production and survival. But something has changed in the last 20 years.

Indeed, in 2005, *Aclees taiwanensis* Kono (Coleoptera: Curculionidae) was detected for the first time in the Pistoia province of Tuscany, Italy. This invasive alien weevil is native to Taiwan and was accidentally introduced in Europe through the trade of *Ficus* bonsai trees. Currently, its presence is confirmed in eleven Italian regions and two French departments. *A. taiwanensis* is an oligophagous pest that feeds and develops on various plants belonging to the genus *Ficus*, widely grown and traded for their fruits or as ornamental species. Adult weevils feed on buds, leaves, and ripening infructescences, but the lethal threat is represented by the xylophagous larvae that dig feeding galleries inside the wood of trunks and surface roots, interrupting the sap flow and bringing the trees to death in a few years.

As it is difficult to promptly identify the attacks and reach larvae and pupae that live protected inside the plants, all the management strategies attempted so far focused on the adults, but none of the efforts made proved to be decisive. Therefore, to implement more targeted control strategies, it is fundamental to know and respect the insect's biology.

Under laboratory conditions, using fig twigs for the rearing of the species, females lay about 120 eggs per year (mainly from April to June) and 70% of these hatch. The embryonic development lasts about 10 days, and then larvae develop through five ages in almost 80 days, and adults emerge from the pupae after more than 20 days. All in all, the complete life cycle from egg to adult takes about 110 days. When rearing the species still under laboratory conditions but using an artificial agarised medium containing fig wood sawdust, the larval and pupal developmental times are shorter

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(respectively, around 40 and 12 days). In Tuscan fig orchards, the population peaks occur in late April, mid-June, and at the end of October.

At present, the research is focused on the identification of sexual and/or aggregation pheromones emitted by the two sexes to activate funnel and fabric traps for the mass trapping of the weevil in the field, as well as on the use of other potentially attractive molecules such as monoterpenes and volatile organic compounds from host plants. Still at the orchard level, the application of a biostimulant to reinforce the natural defences of fig trees is under evaluation.

**Keywords:** *Ficus carica*, *Aclees taiwanensis*, pheromones



## Fig (*Ficus carica* L.) genetic resources in the oases of Kebili (South-West of Tunisia): varietal richness and opportunity for valorisation

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In Tunisia, oases constitute the most important reserves of plant genetic resources. More than 300 varieties of date palm, 25 fruit trees species and 30 vegetables and feed species are growing in these regions. The changes of irrigation frequency, fertilization types, phytosanitary treatments methods and climate changes (rise of temperatures and decrease in rainfall) threatens the diversity of date palm, fruit trees, *etc.*

This work studied the prospecting and inventory of fig (*Ficus carica* L.) genetic resources in Kébili oases (South-west of Tunisia) using a field survey; an inventory, identification and characterization of caprifig (male fig) and fig varieties were done.

The result showed that the oases of Kebili contained more than 12 fruit species; fig trees have an important place, representing more than 40 % of the number of fruit trees inventoried. The assessment of the fruit trees agrobiodiversity of the Kebili oases through the Simpson and Shannon-Weaver index shows a dominance of fig trees in many oases ('Nouiel' and 'Telmine') that threatens the sustainability of this diversity. Despite of the importance of caprifig (pollination) in the production of good quality fig fruit, the cultivation of the caprifig tree is often marginalized. In total, 18 and 30 local varieties for caprifig and fig, were inventoried, respectively.

The analysis of variance of the data obtained showed highly significant differences between all the varieties studied. A considerable morphological diversity for the following parameters: precocity, percentage of pollen viability, blastophages richness, fruit shape, external color, fruit weight, fruit length, neck length, ostiole diameter and total soluble solid were detected.

The viability rate of pollen varieties is between 12% and 97% and fruit fig has shown that the sugar level varies between 15 and 30 °Brix. The principal component analysis (PCA) revealed that the first two axes absorb more than 70 % and 82 % of the total variability for caprifig and fig, respectively. Several groups of varieties were distinguished according to morphological criteria and various cases of homonyms and synonyms were revealed.

In conclusion, fig local diversity could become a basis for genetic improvement and breeding for this species and provides different valorization ways. Furthermore, some male varieties such as

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'Bethri' and 'Limi' are identified as potential candidates for the pollination of this species and can be exploited in *in vivo* pollination studies in order to select the most efficient pollinators.

**Keywords:** Tunisian oases, caprifig, fig, prospecting, varieties, morphological characterization.

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## Management of fig genetic resources in Tunisia

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Fig tree (*Ficus carica* L.) is an old cultivated fruit tree in Tunisia that has been considered as secondary center of diversification. It is cultivated from north to south of Tunisia and is represented by more than 100 cultivars. In addition, traditional knowledge relying on cultivation, caprification and fruit collecting have been conserved through generations. Fig tree is adapted to several climate conditions. Many studies have been interested to the characterization of this germplasm using morphological traits relying to the tree, leaves, and fruit according to descriptors of Bioversity International.

Molecular markers such as ISSR, RAPD, AFLP and SSR have been used in order to identify and fingerprint cultivars, to resolve problems of homonymy and synonymy and to study genetic diversity of accessions collected. SSR markers have been usually the most suitable markers for this purpose. Accessions identified have been propagated by grafting in the case of fig in order to be *ex situ* conserved.

Several collections have been established in order to conserve this species *ex situ*. Recently, National Genebank of Tunisia (NGBT) has settled a national fruit tree field genebank in Takelsa in North Est in 2014 according to international genebanks standards. This national fig collection needs to be enriched with fig cultivars grouping the major part of Tunisian fig diversity. Collecting missions in several regions have been undertaken with collaboration of researchers, farmers, and NGOs. NGBT has coordinated with national stakeholders to establish a strategy for the management and the conservation of fig genetic resources. It has supported existing collections belonging to different institutions. It has also created a safety duplicate fig collection in the field genebank in Takelsa. It is also an active partner in actions of valorization and use of this species. It is involved in actions of implementation of labels for fig cultivars such as PDO (Product of Denomination of Origin) and PGI (Product of Geographical Indications). It has organized several actions for building capacities and for strengthening awareness to conserve fruit trees genetic resources. Documentation of fig accessions has been conducted in order to register all the data for each accessions.

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In addition, *on farm* conservation of genetic resources in specific agro-ecosystem where cultivation of fig is traditionally developed were established in collaboration of several stakeholders at regional level. Fig products are in high demand and are a source of incomes for farmers and local communities.

**Key words:** *Ficus carica* L., genebank, genetic resources, Tunisia.

