UNIVERSIDAD DE CÓRDOBA

GOBIERNO DE ESPAÑA · MINISTERIO PARA LA TRANSICIÓN ECOLÓGICA Y EL RETO DEMOGRÁFICO

Tragsa GrupoTragsa

GOBIERNO DE ESPAÑA · MINISTERIO DE UNIVERSIDADES

Responses of *Quercus ilex* seedlings to combined, drought and *Phytophthora cinnamomi*, stresses: a metabolomic analysis.

Marta Tienda-Parrilla, Cristina López-Hidalgo, Rocío Valderrama-Fernández, María Dolores Rey, Jesús V. Jorrín-Novo

Department of Biochemistry and Molecular Biology

Agroforestry and Plant Biochemistry, Proteomics and Systems Biology

University of Cordoba, Spain

SESSION 3. GLOBAL CHANGE, VULNERABILITY AND ADAPTIVE MANAGEMENT OF FORESTED LANDSCAPES – HOW TO MANAGE INCREASING PRESSURES AND THREATS ABOVE THE CURRENT RESILIENCE TIPPING POINTS

Holm oak decline — How to manage? — Molecular Biology Approach

What scenario is the *queen* of the Mediterranean Basin facing?

God save the queen! How and why the dominant evergreen species of the Mediterranean Basin is declining?

Francesca Alderotti* and Erika Verdiani

Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Sesto Fiorentino, Florence 50019, Italy
*Corresponding author's e-mail address: francesca.alderotti@unifi.it

Morpho-anatomical, biochemical, genetic and physiological traits

Until now, considered the **best adapted and most drought tolerant** species within *Quercus* genus

Dominate the Mediterranean Basin

Healthy individual of holm oak (Huelva, Spain)

What scenario is the *queen* of the Mediterranean Basin facing?

God save the queen! How and why the dominant evergreen species of the Mediterranean Basin is declining?

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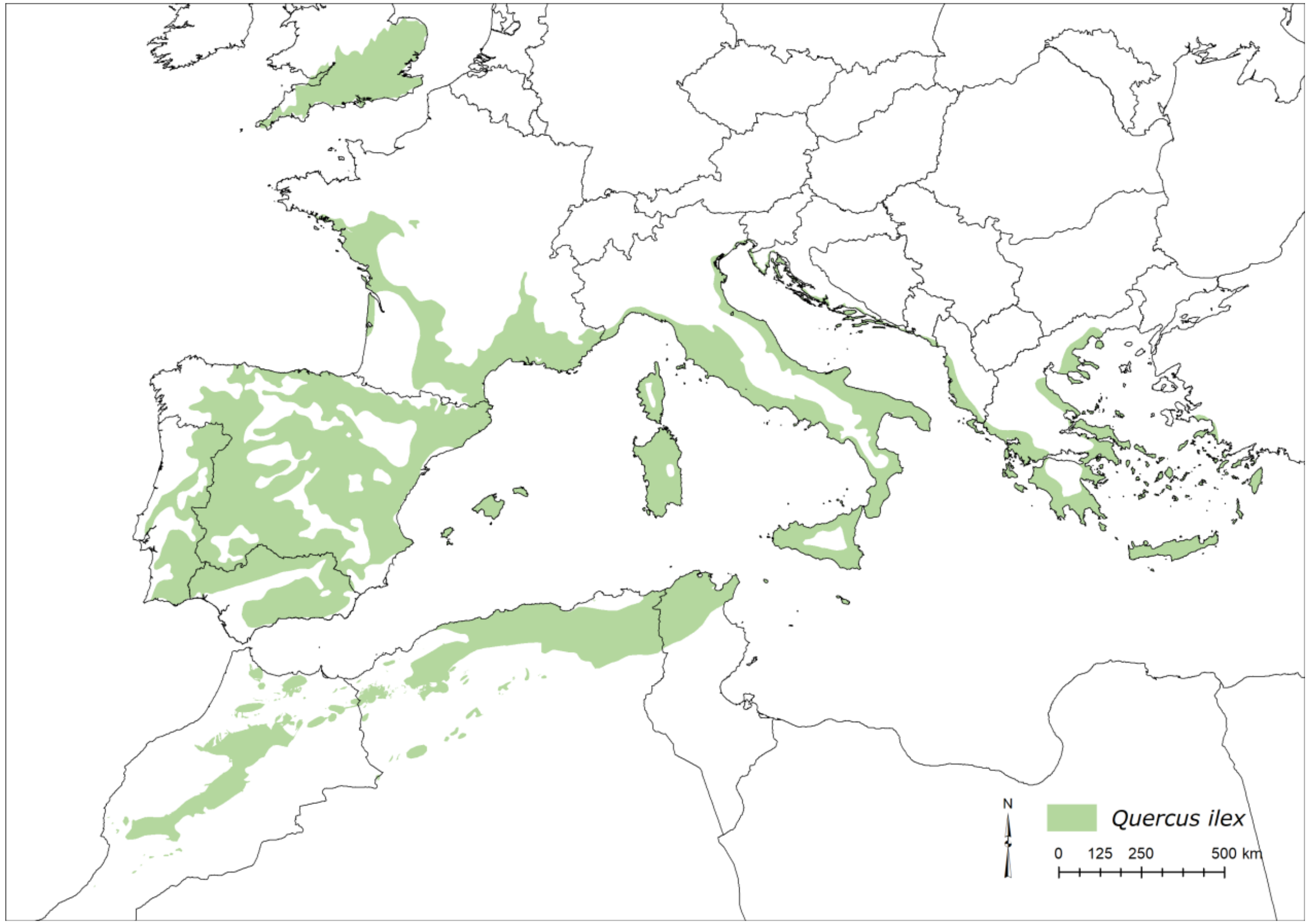


Distribution of holm oak (*Quercus ilex* L.) within the Mediterranean Basin

(Martin-Luther-University Halle-Wittenberg, 2006)

What scenario is the *queen* of the Mediterranean Basin facing?

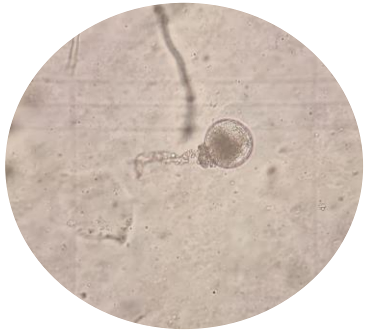
The long-term survival of holm oak is threatened

ABIOTIC FACTORS

Extreme and long drought episodes

BIOTIC FACTORS

Phytophthora cinnamomi



Chlamydospores of *P. cinnamomi*

+++ Climate Change Scenario

Photographs provided by F. Moreno Romero
Individuals located in Hornachuelos, Córdoba

Dieback episodes

What scenario is the *queen* of the Mediterranean Basin facing?

Disease spiral with main interacting factors associated with holm oak decline

Constança de Sampaio e Paiva Camilo-Alves *et al.* 2013

Decline syndrome

Loss of vigour by trees

(i) Shoot death and leaf detachment
(ii) Production of epicormic shoots
(iii) Fine root loss
(iv) Root rot induced by *P. cinnamomi*
(v) Decreased growth and increased mortality

Molecular research and breeding programs as a novel management strategy

The holm oak has **peculiar biological characteristics, that makes its research very challenging:**

Long life-cycle | Allogamy | Non-domesticated

Recalcitrance | High genetic diversity

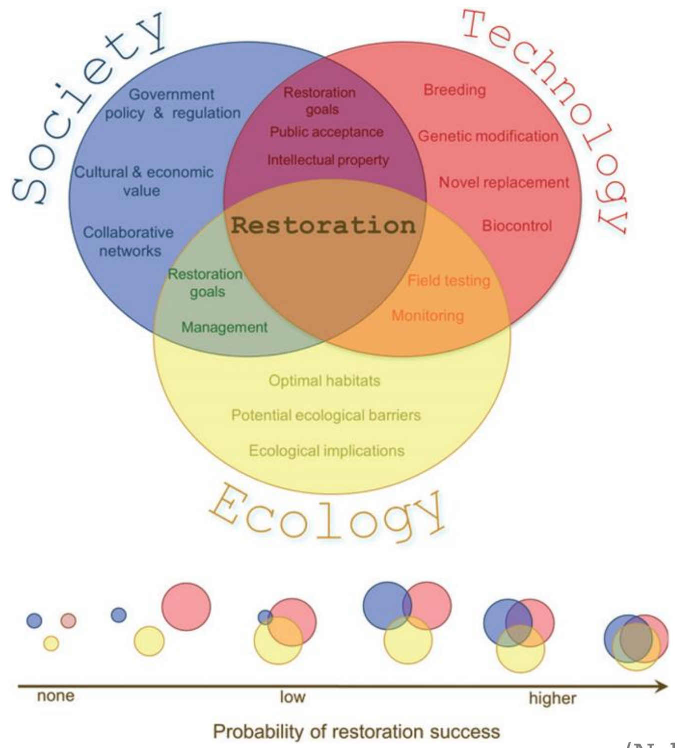
BIOTECHNOLOGY

Modern and future forestry based on biotechnology

Shihui Niu[1] | Jihua Ding[2] | Changzheng Xu[3] | Jing Wang[4]

Tree breeding, a necessary complement to genetic engineering

C. Dana Nelson[1]



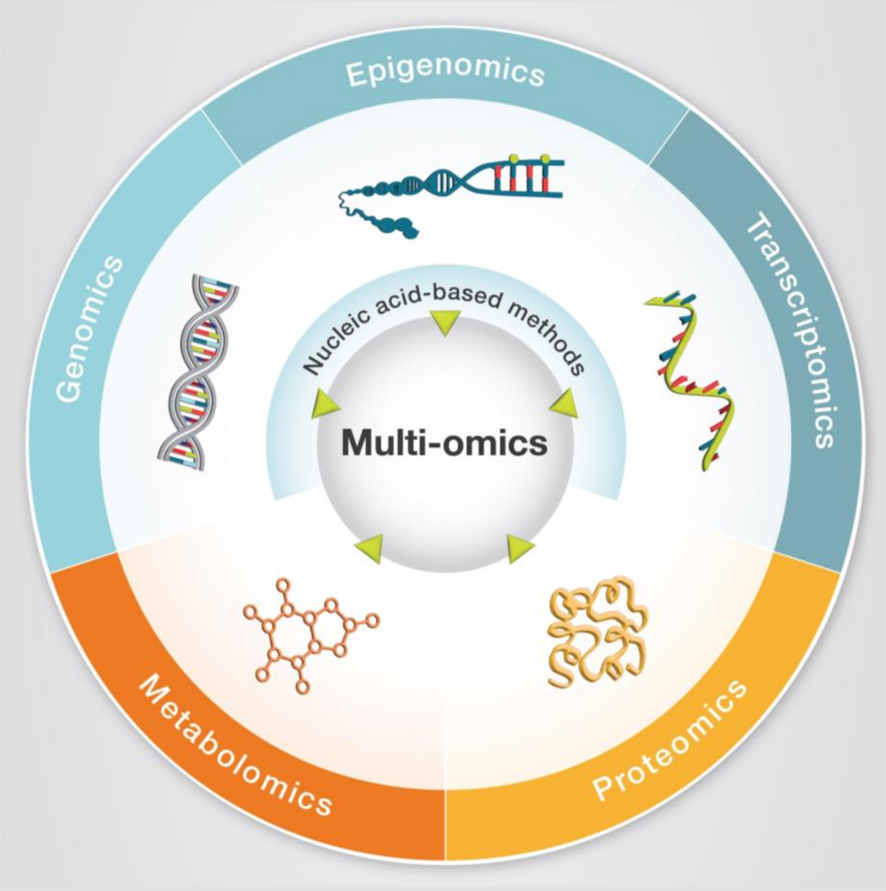
(Nelson C. 2023)

Molecular research can be useful in order to:

- Catalogue inter- and intra-**population variability**
- Develop **breeding programs** based on the **selection of elite genotypes** with **phenotypes of interest** (growth, resilience or productivity)

Genotype selection is based on **morphological, physiological, and molecular markers**, including nucleic acids (DNA and RNA), proteins and metabolites

Unravelling the **SPECIFICITY** of the holm oak's response to the decline phenomenon



Review

Multiomics Molecular Research into the Recalcitrant and Orphan *Quercus ilex* Tree Species: Why, What for, and How

Ana María Maldonado-Alconada [1], María Ángeles Castillejo [1], María-Dolores Rey [1,*], Mónica Labella-Ortega [1], Marta Tienda-Parrilla [1], Tamara Hernández-Lao [1], Irene Honrubia-Gómez [1], Javier Ramírez-García [1], Víctor M. Guerrero-Sanchez [1,2], Cristina López-Hidalgo [1,3], Luis Valledor [3], Rafael M. Navarro-Cerrillo [4] and Jesús V. Jorrin-Novo [1,*]

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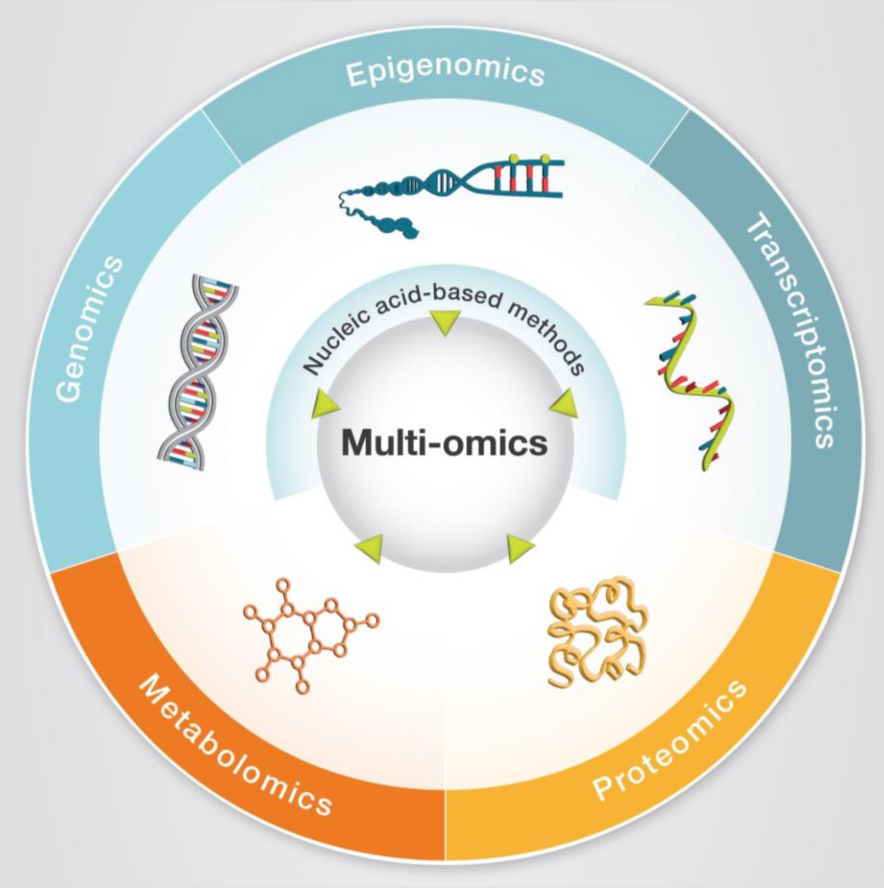

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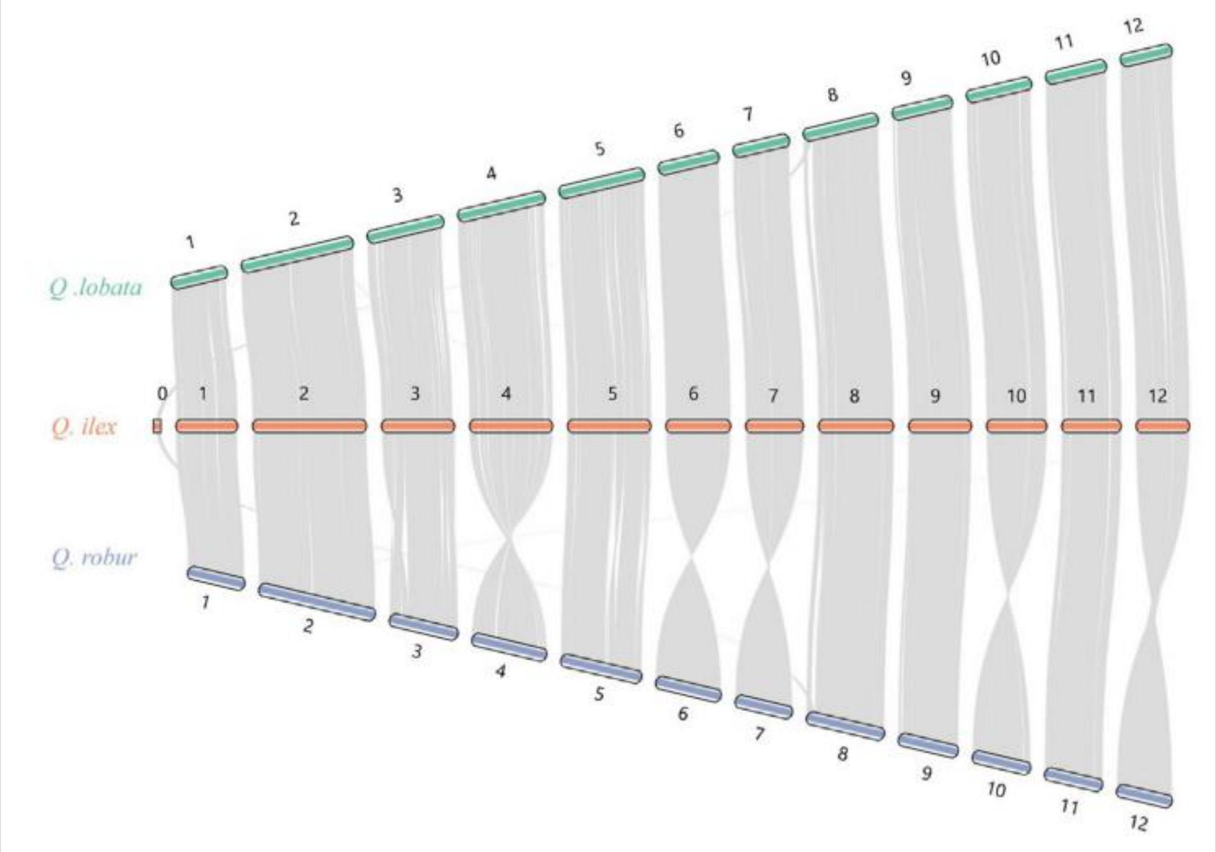
A first draft genome of Holm oak (*Quercus ilex* L.), the most representative species of the Mediterranean forest and the Spanish agrosilvopastoral ecosystem "*dehesa*"

María-Dolores Rey, Mónica Labella-Ortega, Víctor M. Guerrero-Sánchez, Rômulo Carleial, María Ángeles Castillejo, Antonio Rodríguez-Franco, Richard G. Buggs, Valentino Ruggieri, Jesús V. Jorrín-Novo

doi: https://doi.org/10.1101/2022.10.09.511480

DNA



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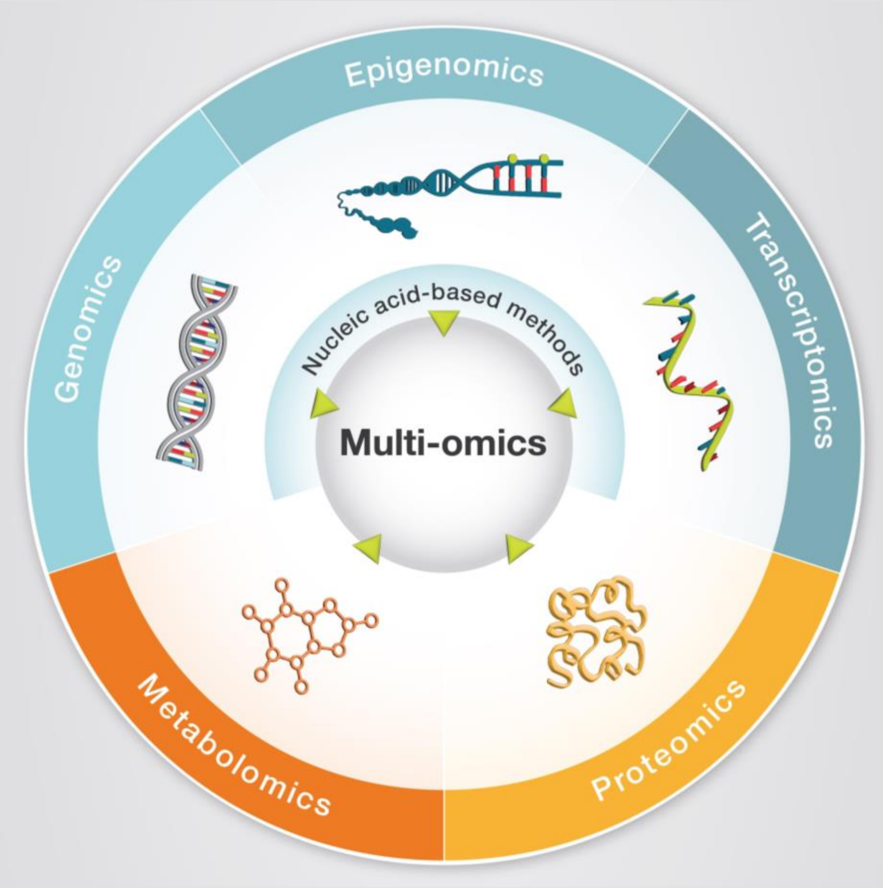

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María
Jesús

doi: http

DNA

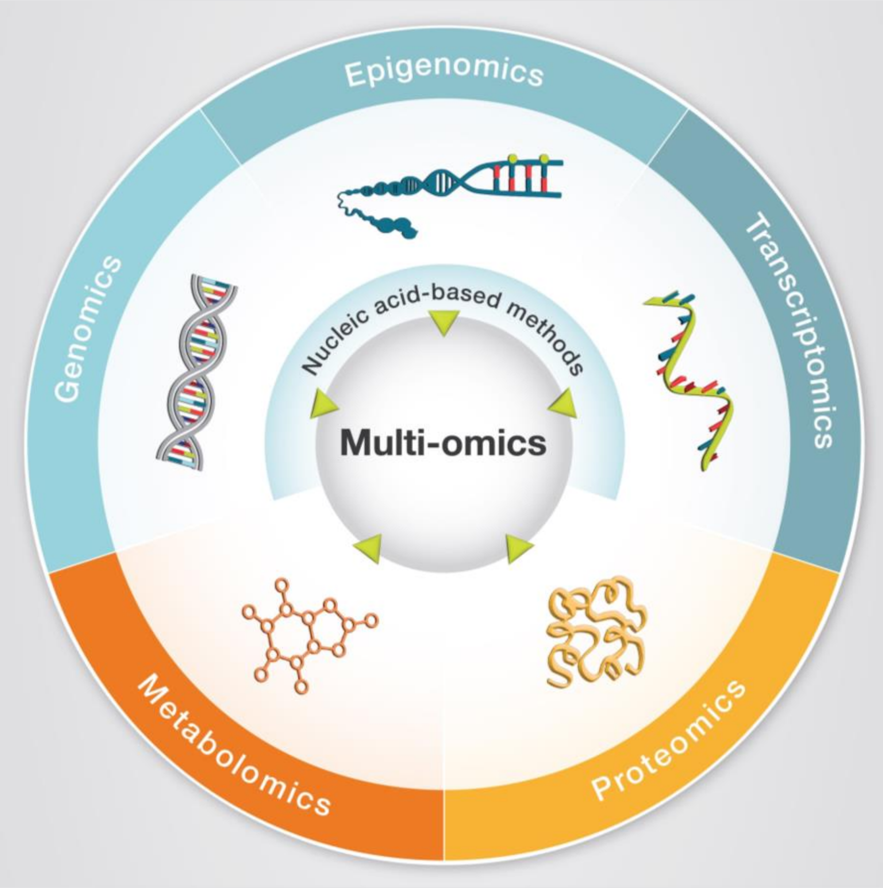
Unraveling DNA methylation dynamics during developmental stages in *Quercus ilex* subsp. *ballota* [Desf.] Samp.

Unpublished data

Labella-Ortega M*[1], Martín-Fernández MC[2], Valledor L[3], Castiglione S[4], Castillejo MA[1], Jorrín-Novo JV[1], Rey MD*[1]

Epigenetic modifications

Unravelling the **SPECIFICITY** of the holm oak's response to the decline phenomenon



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Labo

Nov

Epigenetic modifications

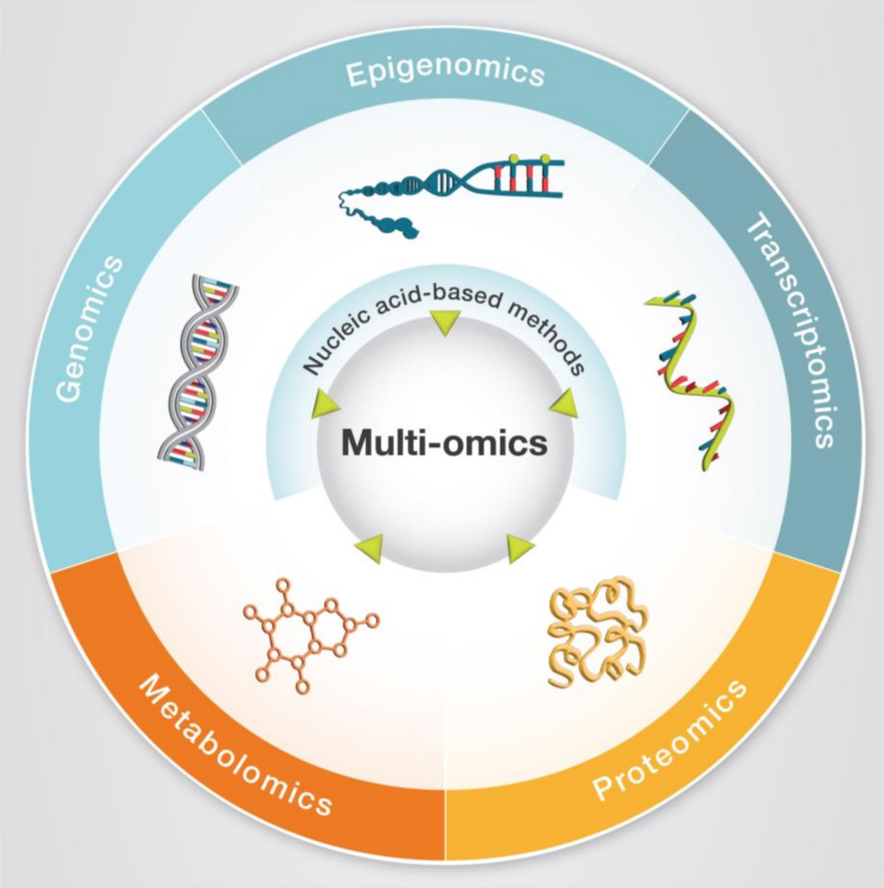
Changes in the transcript and protein profiles of *Quercus ilex* seedlings in response to drought stress

Víctor Manuel Guerrero-Sánchez [1], María Ángeles Castillejo, Cristina López-Hidalgo [2], Ana María Maldonado Alconada, Jesús Valentín Jorrín-Novo, María-Dolores Rey

Agroforestry and Plant Biochemistry, Proteomics and Systems Biology, Department of Biochemistry and Molecular Biology, University of Cordoba, UCO-CeiA3, 14014 Cordoba, Spain

RNA

Unravelling the **SPECIFICITY** of the holm oak's response to the decline phenomenon



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Labe

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Víctor Manu ... dalgo [2],
Ana María M ... Rey
Agroforestry and Plan ... Córdoba, UCO-CeiA3, 14014 Córdoba, Spain

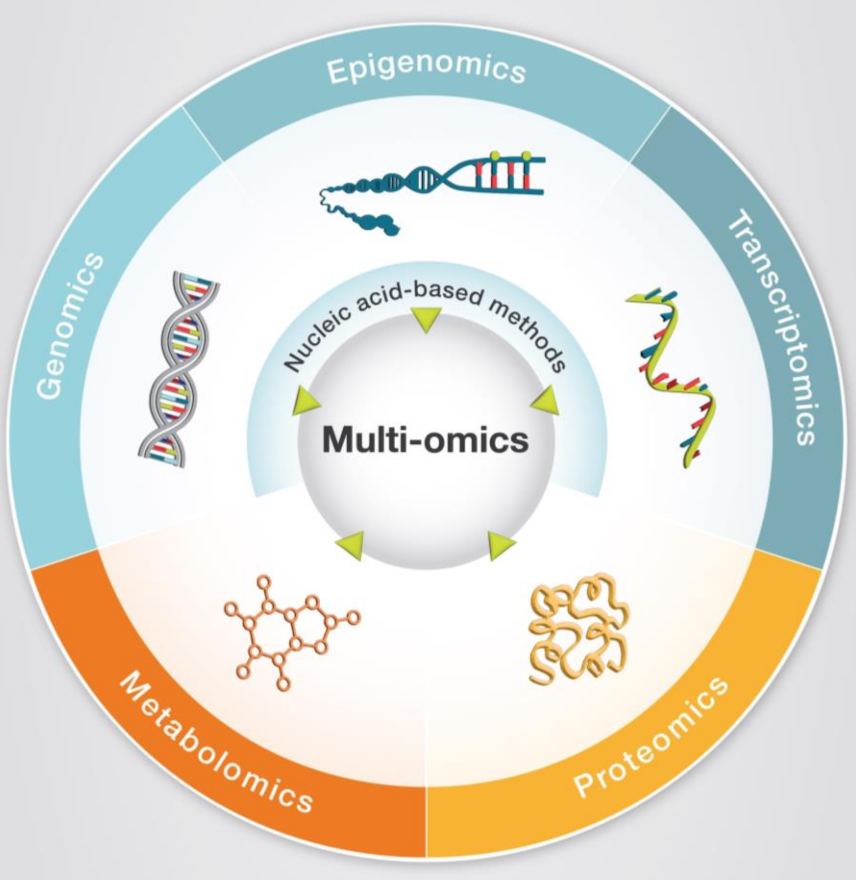
Proteins

Effect and Response of *Quercus ilex* subsp. *ballota* [Desf.] Samp. Seedlings From Three Contrasting Andalusian Populations to Individual and Combined *Phytophthora cinnamomi* and Drought Stresses

Bonoso San-Eufrasio [1], María Ángeles Castillejo [1], Mónica Labella-Ortega [1], Francisco J. Ruiz-Gómez [2], Rafael M. Navarro-Cerrillo [2], Marta Tienda-Parrilla [1], Jesús V. Jorrín-Novo [1] and María-Dolores Rey [1*]

[1] Agroforestry and Plant Biochemistry, Proteomics and Systems Biology, Department of Biochemistry and Molecular Biology, University of Córdoba, Córdoba, Spain, [2] Evaluation and Restoration of Agronomic and Forest Systems ERSAF, Department of Forest Engineering, University of Córdoba, Córdoba, Spain

Unravelling the **SPECIFICITY** of the holm oak's response to the decline phenomenon



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Proteins

Article

Untargeted MS-Based Metabolomics Analysis of the Responses to Drought Stress in *Quercus ilex* L. Leaf Seedlings and the Identification of Putative Compounds Related to Tolerance

Marta Tienda-Parrilla [1,†], Cristina López-Hidalgo [1,2,*,†], Victor M. Guerrero-Sanchez [1,3], Álvaro Infantes-González [1], Rocío Valderrama-Fernández [4], María-Ángeles Castillejo [1], Jesús V. Jorrín-Novo [1] and María-Dolores Rey [1,*]

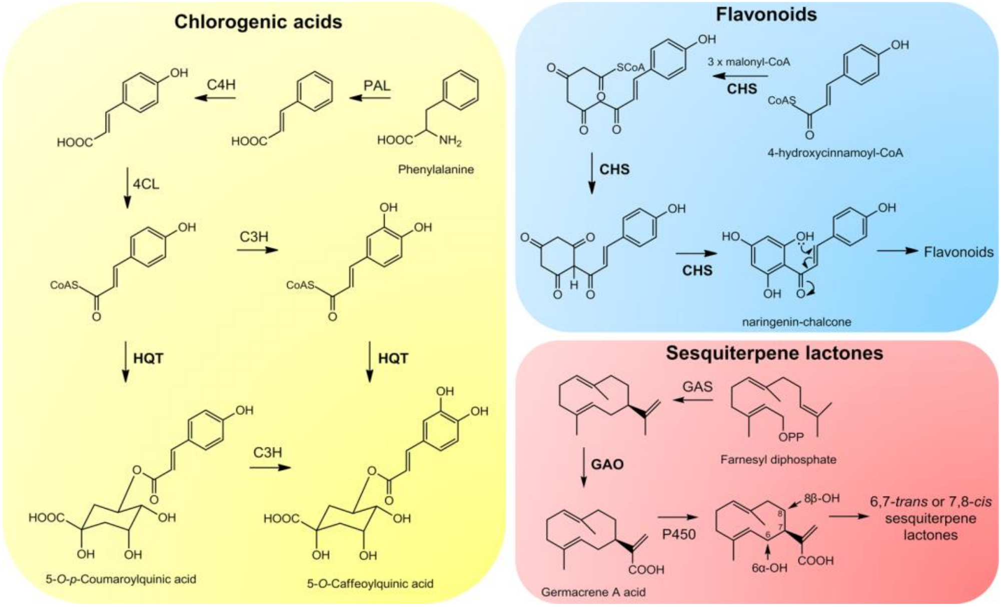
Metabolites ↔ **Phenotype**

Unravelling the **SPECIFICITY** of the holm oak's response to the decline phenomenon

Metabolites ⟷ **Phenotype**

OBJECTIVE

Study the **effect and response to stresses** associated to the decline syndrome and climate change in Holm oak: **drought** and ***Phytophthora cinnamomi***, and to **identify metabolites as molecular markers** associated with **resilience**



MATERIAL AND METHODS

Plant material and experimental design

Metabolite extraction and LC-MS/MS analysis

Data acquisition and processing, and statistical analysis

- **Nine-month-old seedlings** (from three holm oak individuals)
- **30-day experiment** (two sampling times corresponding to a drop in leaf fluorescence of 30 and 50%)
- **Experimental conditions:** control (well watered and not inoculated) and combined stresses (drought and *P. cinnamomi* inoculation*)*

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Declined Area

Control

30-day experiment

Combined stresses

"*Quercus* breeding and conservation program in Spain" (Pérez, 2020 Foresta 78:56-61)

Damage symptoms and seedling mortality
Relative Leaf Water Content (RLWC)
Leaf fluorescence (Fv/Fm)

MATERIAL AND METHODS

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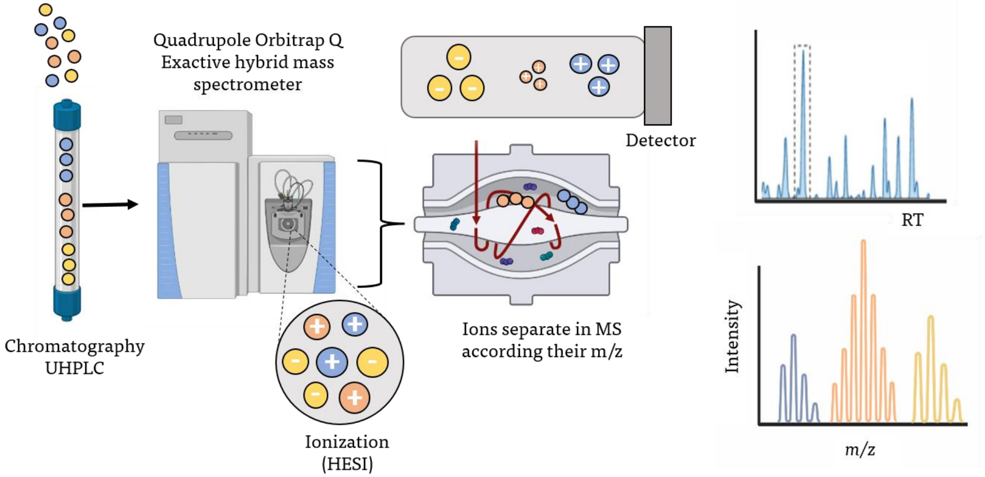
Metabolite extraction and LC-MS/MS analysis

Leaves metabolites extraction: 80:20 (ethanol: water)

Metabolite Identification and Quantification (UHPLC-QToF)

Data acquisition and processing, and statistical analysis



Leaf extract metabolite

Untargeted metabolomic analysis

MATERIAL AND METHODS

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Metabolite extraction and LC-MS/MS analysis

Leaves metabolites extraction:
80:20 (ethanol: water)

Metabolite Identification and Quantification
(UHPLC-QToF)

Data acquisition and processing, and statistical analysis

Multivariate Analysis (PCA)

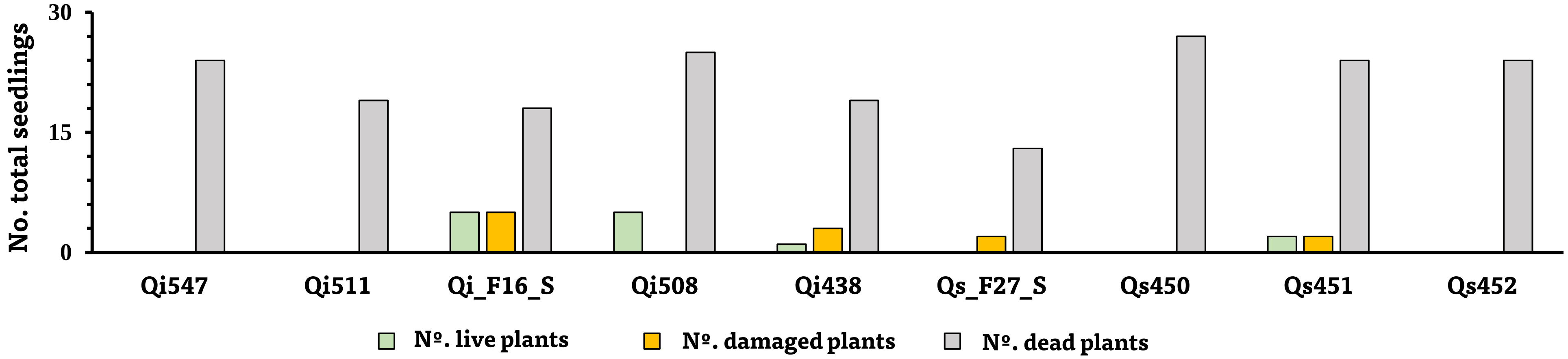
Univariate Analysis (Kruskal-Wallis)

Fold-Changes

Venn Diagram

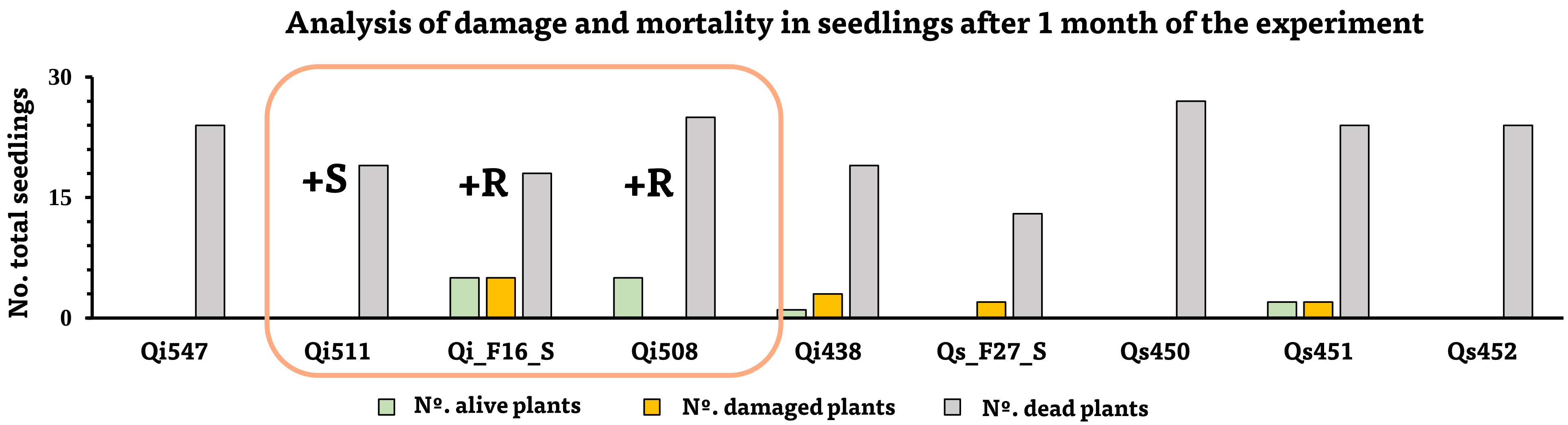
Physiological response to combined (abiotic and biotic) stresses

Analysis of damage and mortality in seedlings after 1 month of the experiment



RESULTS

Physiological response to combined (abiotic and biotic) stresses



Sampling

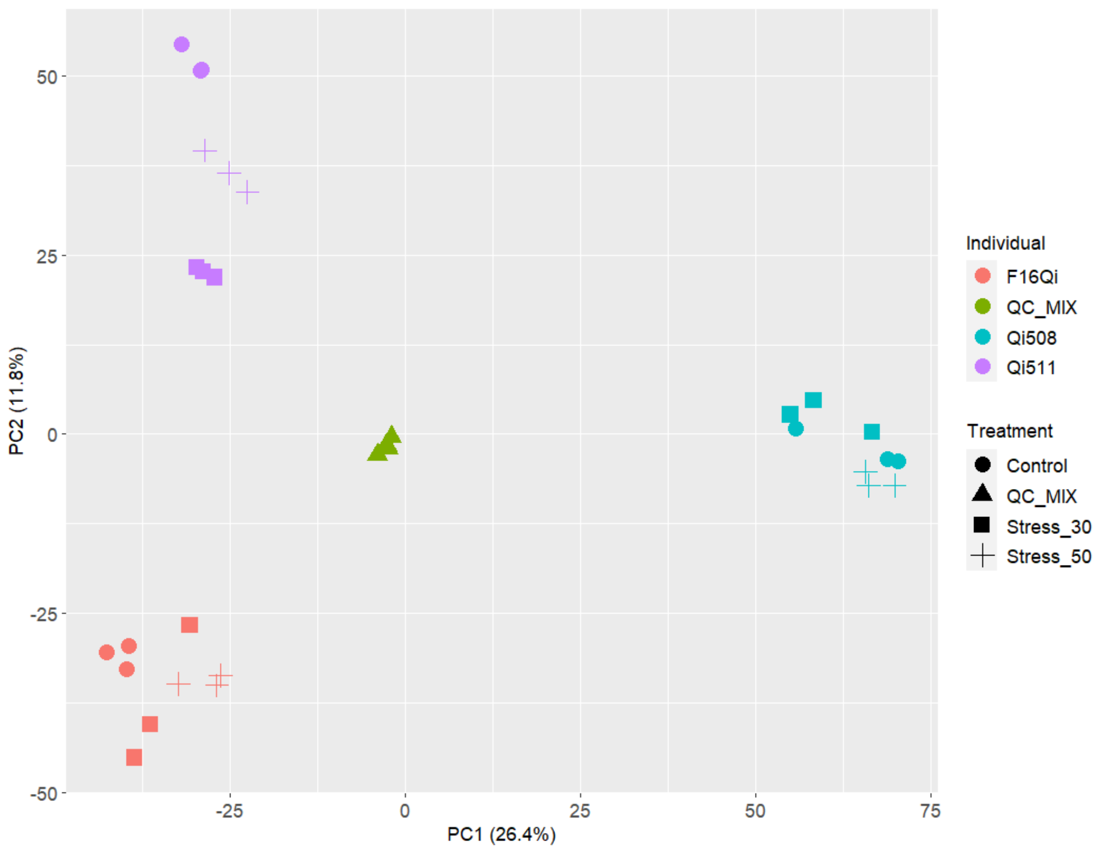
Fluorescence decay analysis in leaves →

↓ 30 % Early response

↓ 50 % Later response

Untargeted metabolome profiling in Q. *ilex* leaves

PCA Score plot

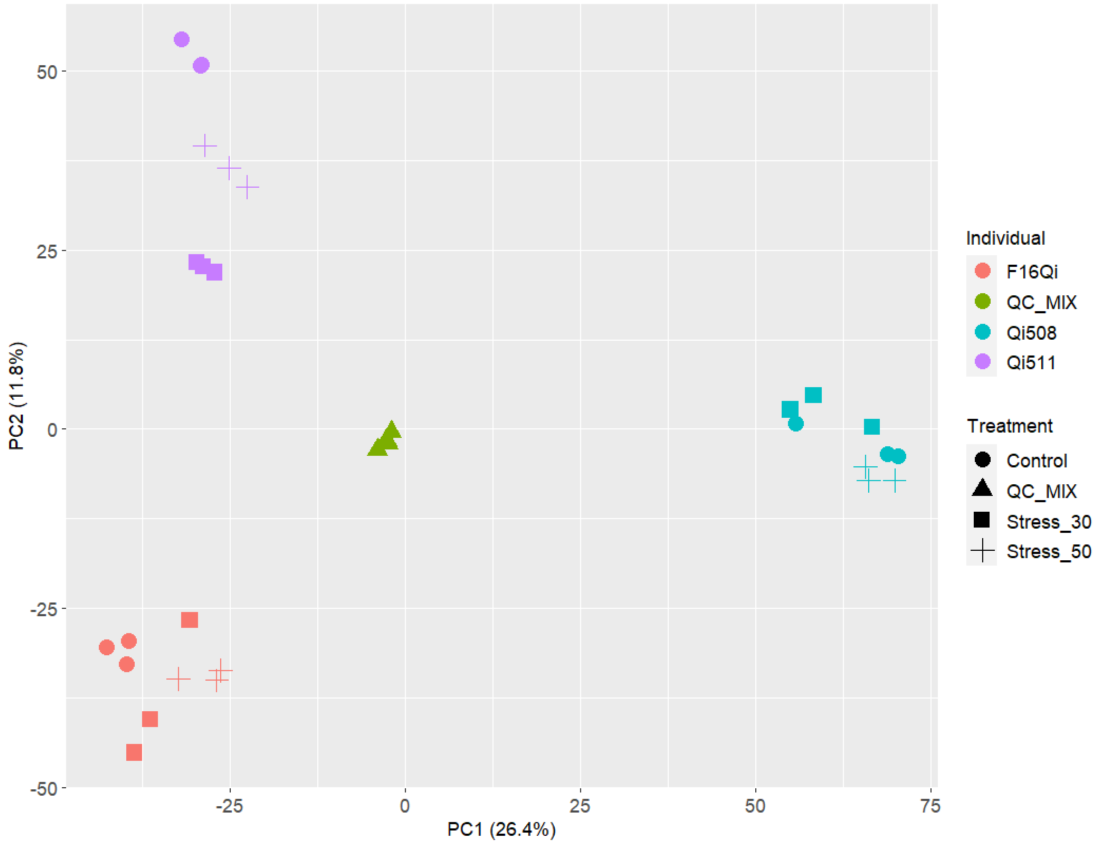


Dataset	Nº Features
Raw (both positive and negative ionizations modes)	17664
Selected features based on consistency filter	6996
Identified features *(Putative annotation without manual revision)	3100

RESULTS

Untargeted metabolome profiling in Q. *ilex* leaves

PCA Score plot



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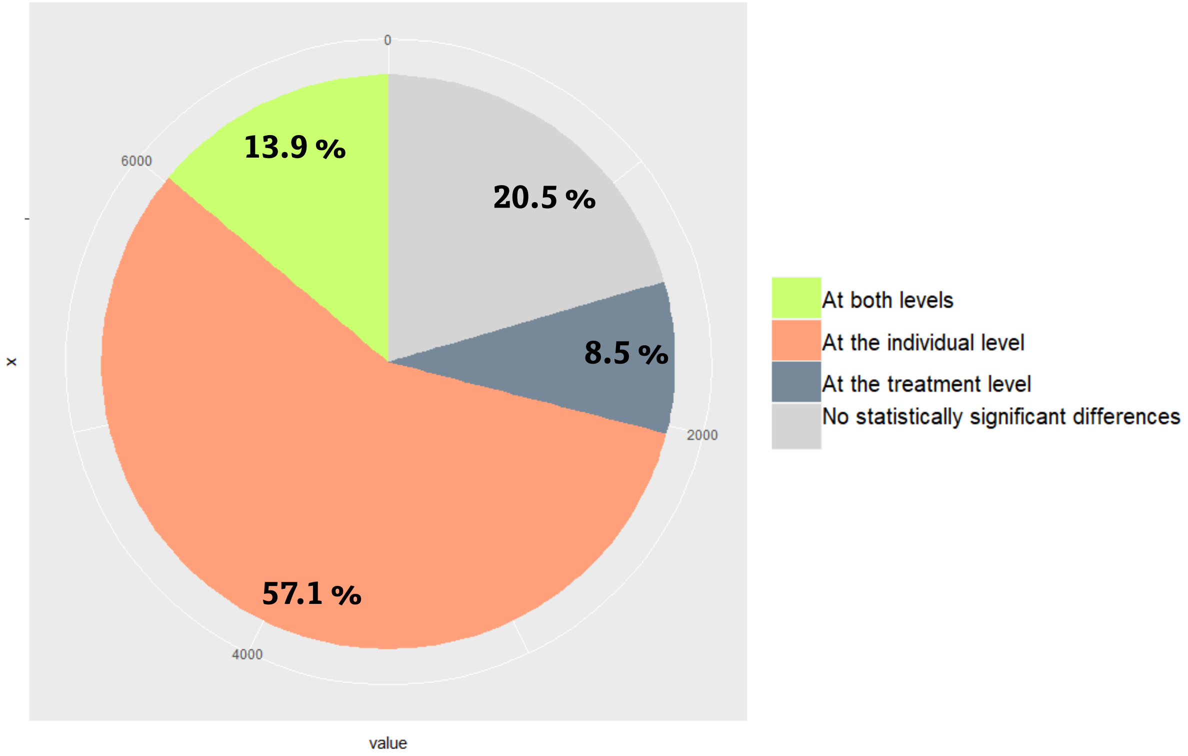
- Differences in the metabolome at the **individual level** are more pronounced than at the treatment level

RESULTS

Untargeted metabolome profiling in Q. *ilex* leaves



Dataset	Nº Features
Raw (both positive and negative ionizations modes)	17664
Selected features based on consistency filter	6996
Identified features *(Putative annotation without manual revision)	3100
Variable features (p-value < 0.05 in Kruskal-Wallis test)	1569 (treatment) 4967 (individual)

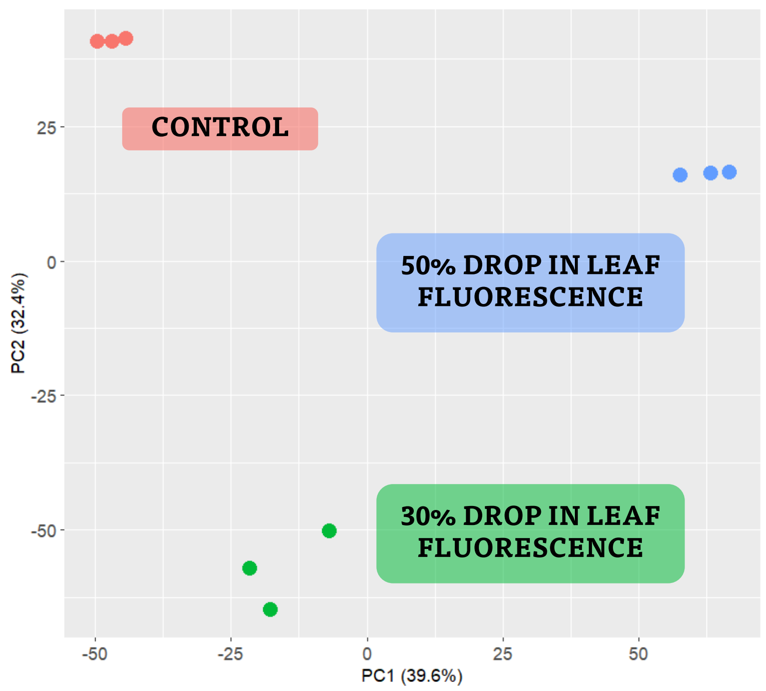
- Differences in the metabolome at the **individual level** are more pronounced than at the treatment level

RESULTS

Untargeted metabolome profiling in Q. *ilex* leaves

To avoid inter-individual variability…

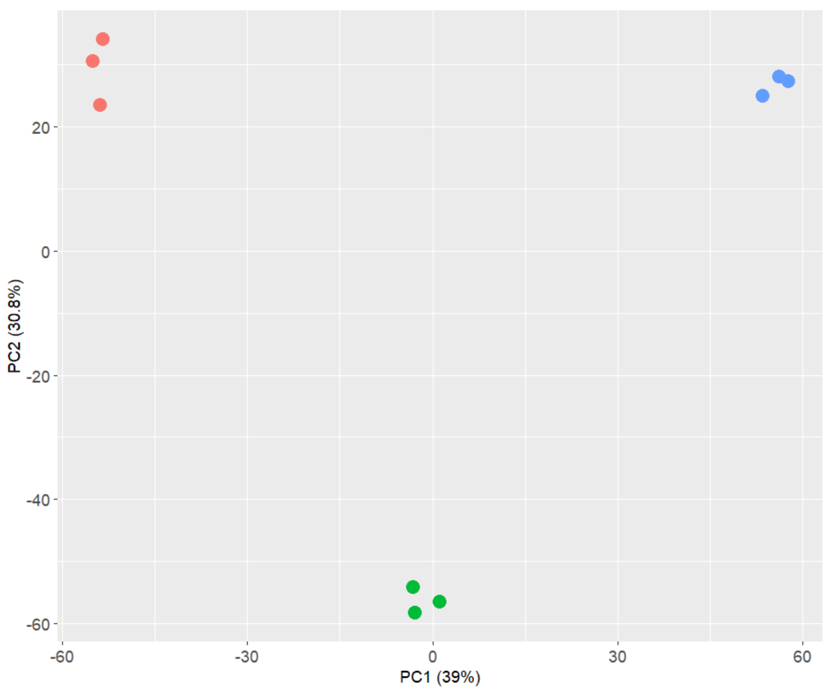
F16Qi **+R**



Dataset: 6085 features

(1133 identified)

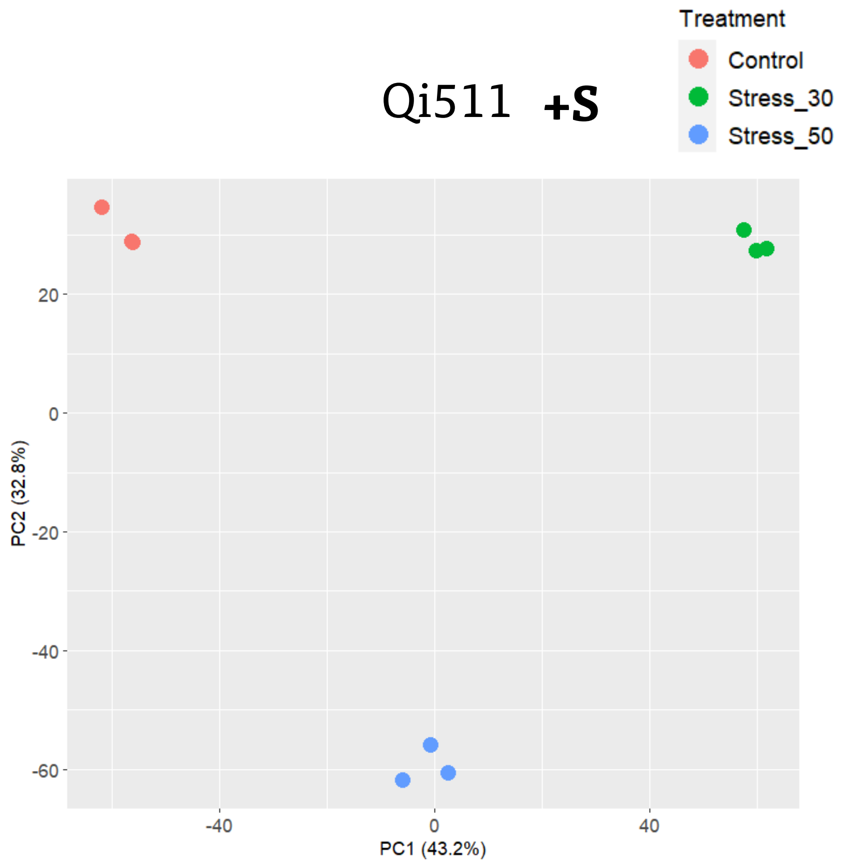
Qi508 **+R**



Dataset: 5812 features

(1144 identified)

Qi511 **+S**



Dataset: 6068 features

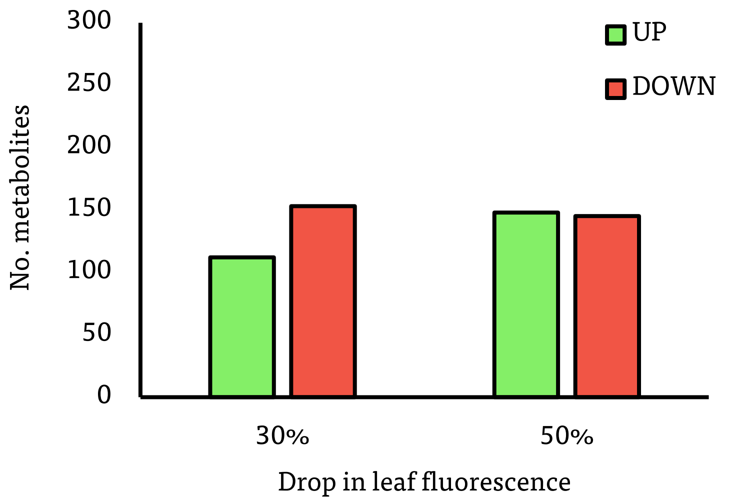
(1150 identified)

- PC1 (~40%) and PC2 (~30%) discriminate treatments and sampling times (early and late response VS Control) for each of the individuals

RESULTS

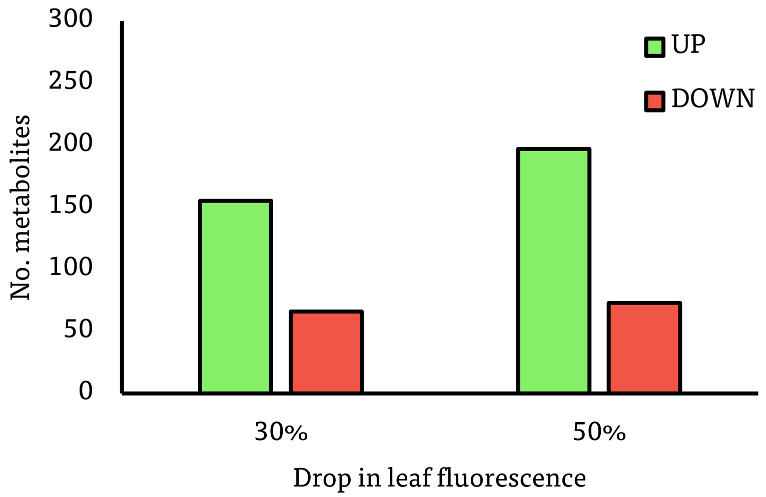
Search for resilience markers: Differential metabolites (DMs)

F16Qi +R



Early response		
Up (FC > 2)	**Qualitative response**	**63**
	Quantitative response	49
Down (FC < 0.5)	Qualitative response	70
	Quantitative response	83
Late response		
Up (FC > 2)	**Qualitative response**	**97**
	Quantitative response	51
Down (FC < 0.5)	Qualitative response	83
	Quantitative response	62

Qi508 +R



Early response		
Up (FC > 2)	**Qualitative response**	**80**
	Quantitative response	75
Down (FC < 0.5)	Qualitative response	44
	Quantitative response	22
Late response		
Up (FC > 2)	**Qualitative response**	**111**
	Quantitative response	86
Down (FC < 0.5)	Qualitative response	45
	Quantitative response	28

Qi511 +S

Early response		
Up (FC > 2)	**Qualitative response**	**103**
	Quantitative response	67
Down (FC < 0.5)	Qualitative response	96
	Quantitative response	162
Late response		
Up (FC > 2)	**Qualitative response**	**60**
	Quantitative response	41
Down (FC < 0.5)	Qualitative response	57
	Quantitative response	58

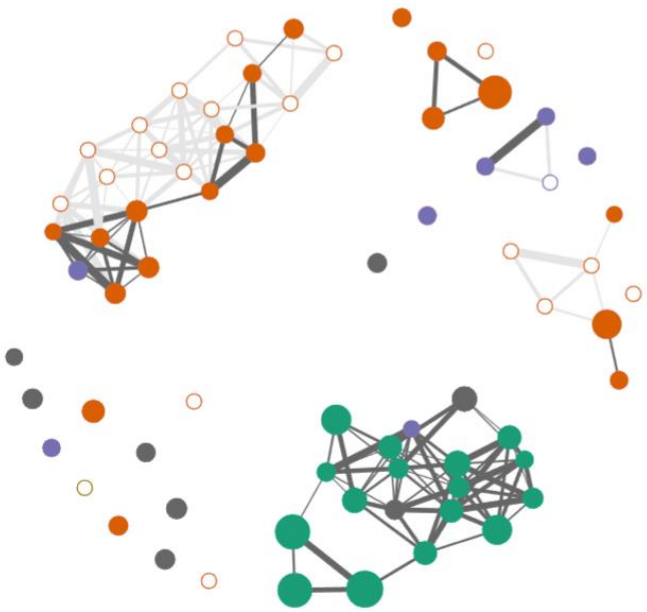
RESULTS

Exploring putative metabolites as molecular resilience markers


Methods

Quantifying chemodiversity considering biochemical and structural properties of compounds with the R package CHEMODIV

Hampus Petrén[1], Tobias G. Köllner[2] and Robert R. Junker[1,3]



Molecular Networking for metabolic diversity characterization and metabolic pathway enrichment analysis (chemodiv R package)

Up-accumulated stress response metabolites in resilient individuals

↓ *Chemodiv R package*

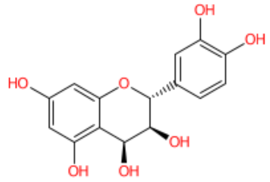
Pathway

- Alkaloids
- Amino acids and Peptides
- Carbohydrates
- Fatty acids
- Polyketides
- Shikimates and Phenylpropanoids
- Terpenoids
- NA

RESULTS

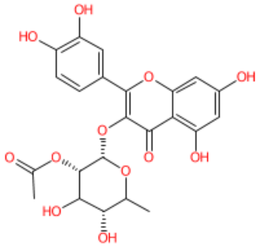
Exploring putative metabolites as molecular resilience markers

Phenolic Compounds



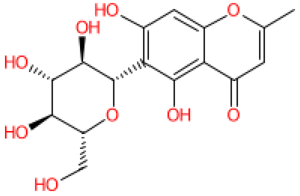
Catechin-4beta-ol

- Superclass: Phenylpropanoids and polyketides
  - Class: Flavonoids
    - Subclass: Flavans
      - Level 5: Flavan-3-ols
        - Level 6: Catechins



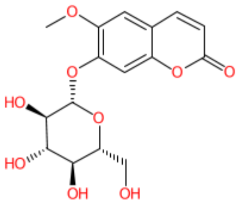
Quercetin 3- (2"-acetylrhamnoside)

- Superclass: Phenylpropanoids and polyketides
  - Class: Flavonoids
    - Subclass: Flavonoid glycosides



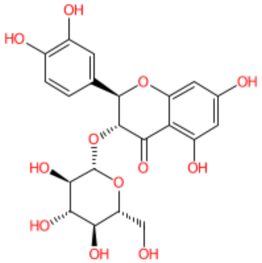
Biflorin

- Subclass: Carbohydrates and carbohydrate conjugates
  - Level 5: Glycosyl compounds
    - Level 6: Phenolic glycosides



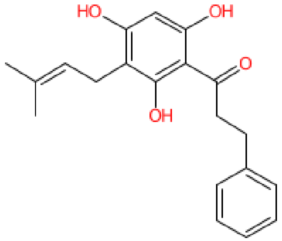
Scopolin

- Superclass: Phenylpropanoids and polyketides
  - Class: Coumarins and derivatives
    - Subclass: Coumarin glycosides



Taxifolin-3-glucoside

- Superclass: Phenylpropanoids and polyketides
  - Class: Flavonoids
    - Subclass: Flavonoid glycosides
      - Level 5: Flavonoid O-glycosides
        - Level 6: Flavonoid-3-O-glycosides



2',4',6'-Trihydroxy-3'-prenyldihydrochalcone

- Superclass: Phenylpropanoids and polyketides
  - Class: Linear 1,3-diarylpropanoids
    - Subclass: Chalcones and dihydrochalcones
      - Level 5: 2'-Hydroxy-dihydrochalcones

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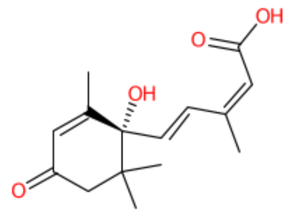
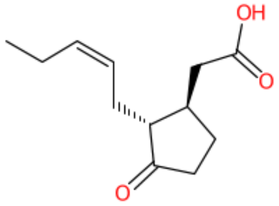
Other metabolites whose defensive function is well known in plants



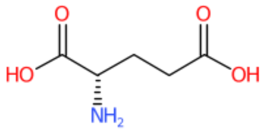
Abscisic acid (ABA)

- Superclass Lipids and lipid-like molecules
  - Class Prenol lipids
    - Subclass Sesquiterpenoids
      - Level 5 Abscisic acids and derivatives



Jasmonic acid (JA)

- Superclass Lipids and lipid-like molecules
  - Class Fatty Acyls
    - Subclass Lineolic acids and derivatives
      - Level 5 Jasmonic acids

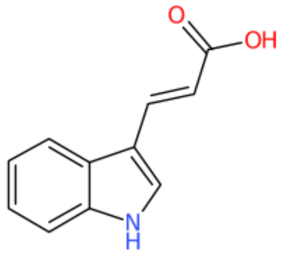


Glutamic acid

- Superclass Organic acids and derivatives
  - Class Carboxylic acids and derivatives
    - Subclass Amino acids, peptides, and analogues
      - Level 5 Amino acids and derivatives
        - Level 6 Alpha amino acids and derivatives
          - Level 7 Glutamic acid and derivatives

Azelaic acid

- Superclass Lipids and lipid-like molecules
  - Class Fatty Acyls
    - Subclass Fatty acids and conjugates
      - Level 5 Medium-chain fatty acids

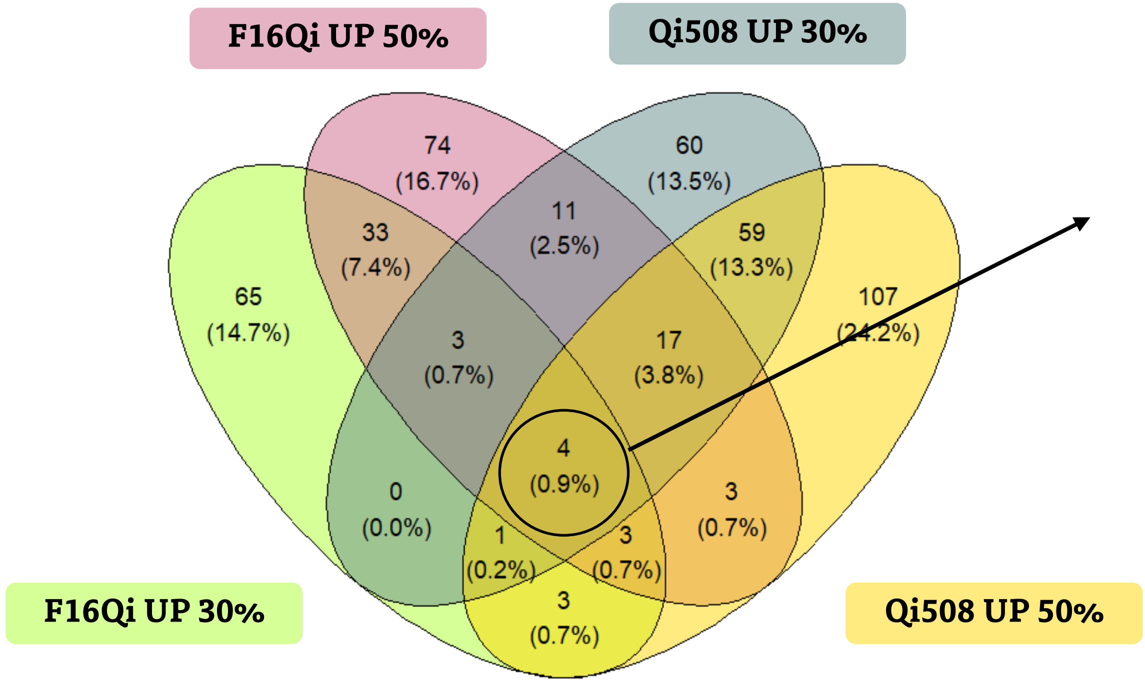


3-Indoleacrylic acid (IAA)

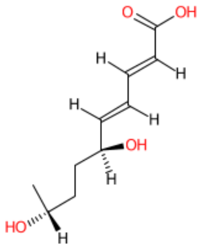
- Superclass Organoheterocyclic compounds
  - Class Indoles and derivatives
    - Subclass Indoles

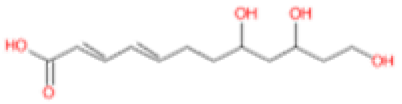
RESULTS

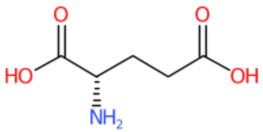
Exploring putative metabolites as molecular resilience markers

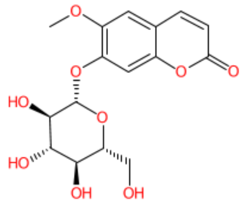


Compound	Formula	Chemical family
Murranoic acid A	C10 H16 O4	Lipids and lipid-like molecules
YF-0200R-B ((2E,4E)-8,10,12-Trihydroxy-2,4-dodecadienoic acid)	C12 H20 O5	Hydroxy acids and derivatives
Glutamic Acid	C5 H9 N O4	Amino acids and derivatives
Scopolin	C16 H20 O9	Coumarin glycosides









Metabolites from the Endophytic Fungus *Curvularia* sp. M12 Act as Motility Inhibitors against *Phytophthora capsici* Zoospores

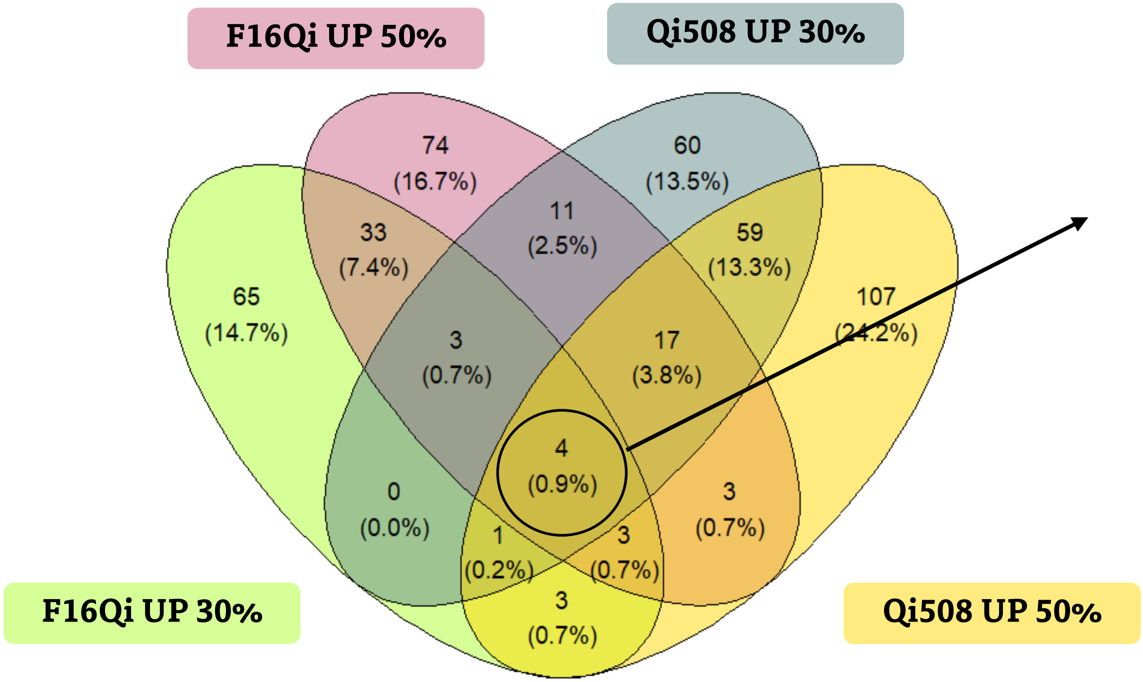
Muhammad Abdul Mojid Mondol,[†] Jannatul Farthouse,[‡] Mohammad Tofazzal Islam,[‡] Anja Schüffler,[§] and Hartmut Laatsch[*,†]

THE ROLE OF THE PHYLLOSPHERE MICROBIOME IN PLANT HEALTH AND FUNCTION

Bram W. G. Stone, Eric A. Weingarten and Colin R. Jackson
Department of Biology, University of Mississippi, University, MS, USA

RESULTS

Exploring putative metabolites as molecular resilience markers



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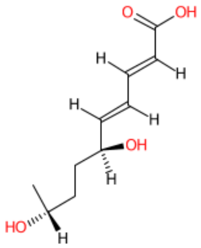
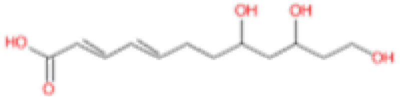
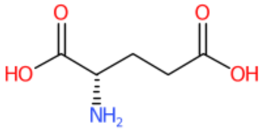
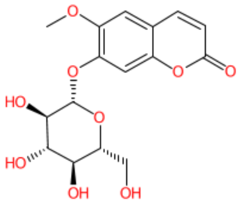








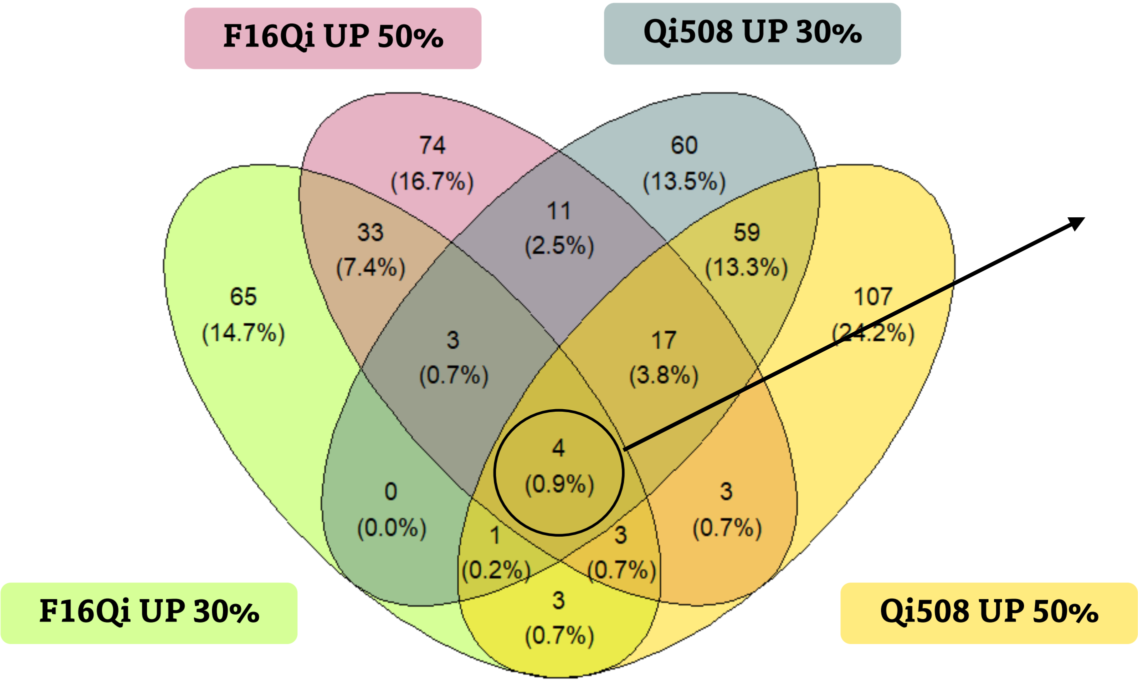
NOVEL ASPARTYL PROTEASE INHIBITORS, YF-0200R-A and B

Tsutomu Sato, Koji Nagai, Mitsuyoshi Shibazaki and Kenji Abe

Drug Serendipity Research Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan

RESULTS

Exploring putative metabolites as molecular resilience markers



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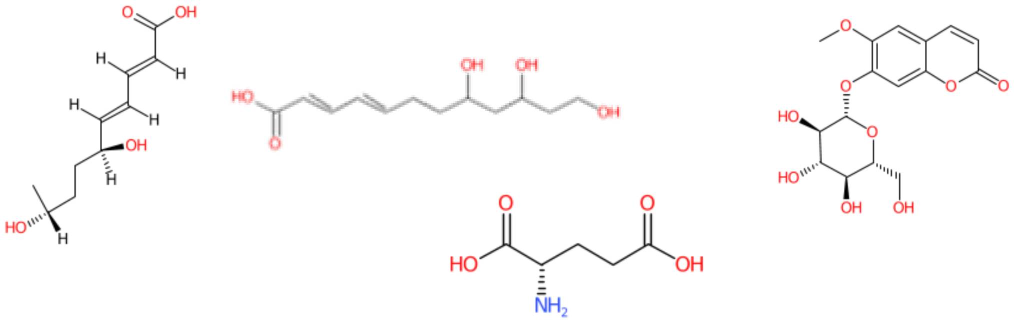


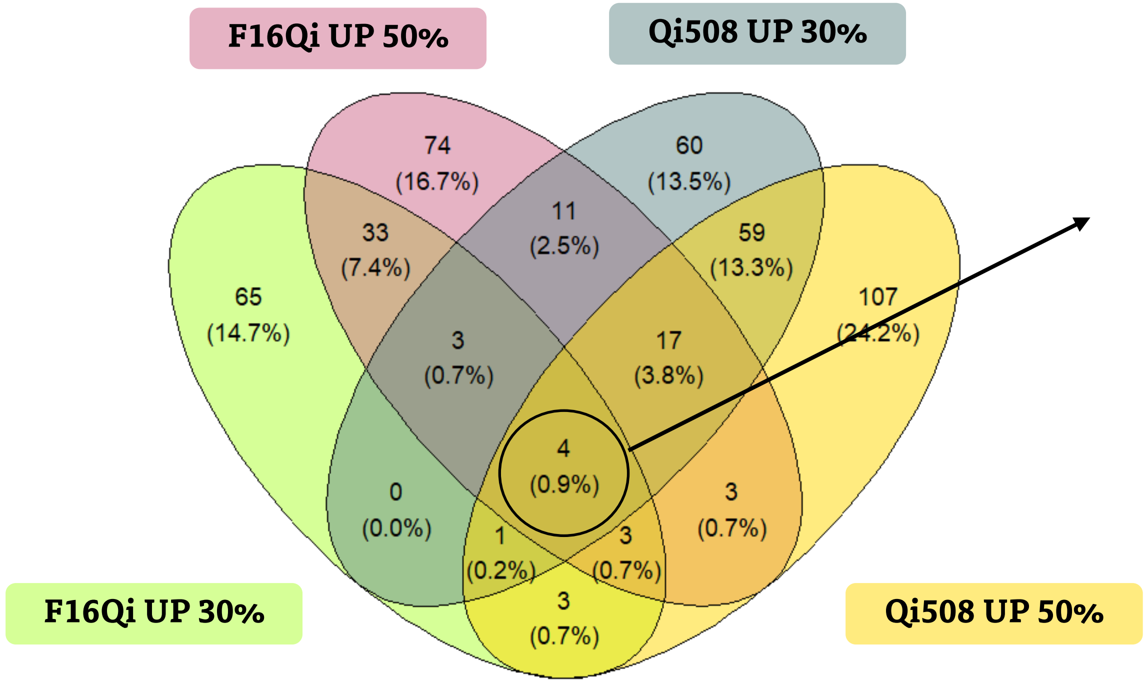
RESEARCH Open Access

Glutamic acid reshapes the plant microbiota to protect plants against pathogens

Check for updates

Da-Ran Kim[1], Chang-Wook Jeon[2], Gyeongjun Cho[2], Linda S. Thomashow[3], David M. Weller[3], Man-Jeong Paik[4], Yong Bok Lee[2] and Youn-Sig Kwak[1,2,5*]

Exploring putative metabolites as molecular resilience markers



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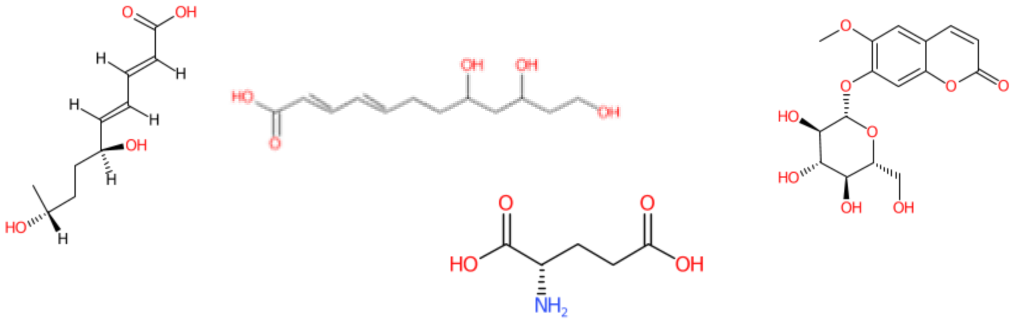

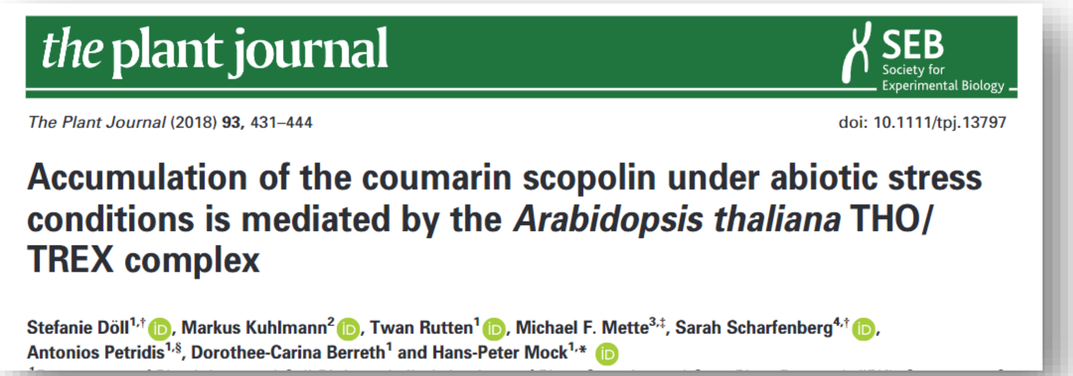
the plant journal
SEB Society for Experimental Biology

The Plant Journal (2018) **93**, 431–444 doi: 10.1111/tpj.13797

Accumulation of the coumarin scopolin under abiotic stress conditions is mediated by the *Arabidopsis thaliana* THO/TREX complex

Stefanie Döll[1,†], Markus Kuhlmann[2], Twan Rutten[1], Michael F. Mette[3,‡], Sarah Scharfenberg[4,†], Antonios Petridis[1,§], Dorothee-Carina Berreth[1] and Hans-Peter Mock[1,*]

CONCLUSIONS

- The leaf metabolome of *Quercus ilex* seedlings is highly variable between individuals, while it is possible to differentiate between the metabolomic profile of control and stressed seedlings in each individual.

- Metabolites up-accumulated in resilient individuals mainly belonged to secondary metabolism, highlighting coumarins, catechins, flavonoids and phenolic glycosides.

- Four metabolites were identified as putative molecular resilience markers, highlighting the microbial metabolites and the coumarin scopoline, as well as others whose defensive function is well known in plants such as glutamic acid, abscisic acid, jasmonic acid and azelaic acid

Work is now in progress to integrate metabolomic analysis with transcriptomics and proteomics in order to propose gene markers of resilience in Holm oak useful in breeding programs.

Non-targeted metabolomic analysis to dissect mechanisms of resilience to combined, drought and *Phytophthora cinnamomi*, stresses in Holm oak (*Quercus ilex*)

Tienda-Parrilla, M, López-Hidalgo C, Valderrama-Fernández R, Rey MD, Jorrín-Novo, JV

Department of Biochemistry and Molecular Biology

University of Cordoba, Spain

IUFRO FOREST ENVIRONMENT DIV 8 CONFERENCE 2023
October 24th – 27th
ÉVORA, PORTUGAL

Effect and Response of *Quercus ilex* subsp. *ballota* [Desf.] Samp. Seedlings From Three Contrasting Andalusian Populations to Individual and Combined *Phytophthora cinnamomi* and Drought Stresses

Bonoso San-Eufrasio[1], María Ángeles Castillejo[1], Mónica Labella-Ortega[1], Francisco J. Ruiz-Gómez[2], Rafael M. Navarro-Cerrillo[2], María Tienda-Parrilla[1], Jesús V. Jorrín-Novo[1] and María-Dolores Rey[1]*

UNIVERSIDAD DE CÓRDOBA

Tragsa GrupoTragsa

GOBIERNO DE ESPAÑA · MINISTERIO PARA LA TRANSICIÓN ECOLÓGICA Y EL RETO DEMOGRÁFICO

GOBIERNO DE ESPAÑA · MINISTERIO DE UNIVERSIDADES

Chlamydospores of *Phytophthora cinnamomi*



Molecular research as a novel management strategy

Conventional mitigation practices

Not sustainable over time
Sensitive to environmental alterations

Adaptive silviculture methods
(selective thinning)

Integrated pest management
(Recovery of transhumant-based seasonal grazing regimes)

Related to *P. cinnamomic* infection:

- Encouraging soil drainage
- Lime fertilization
- The use of biofumigant crops
- The elimination of alternative host herbaceous species
- The avoidance and soil movements
- **Chemical control** (K2HPO3 and aluminium tris-O-ethyl phosphonate)