Functional genomics of drought response in *Pinus pinaster*

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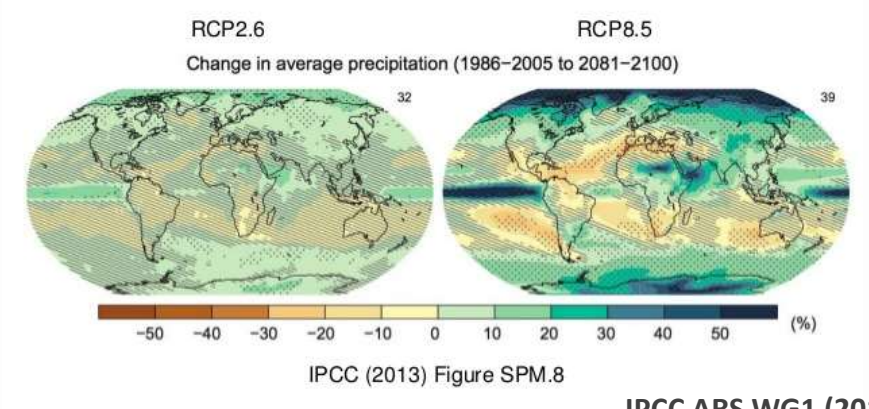
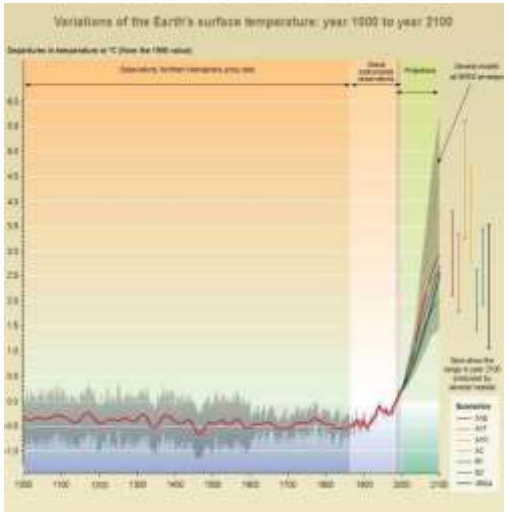
Climate change impacts on the forests



IPCC (2013) Figure SPM.8

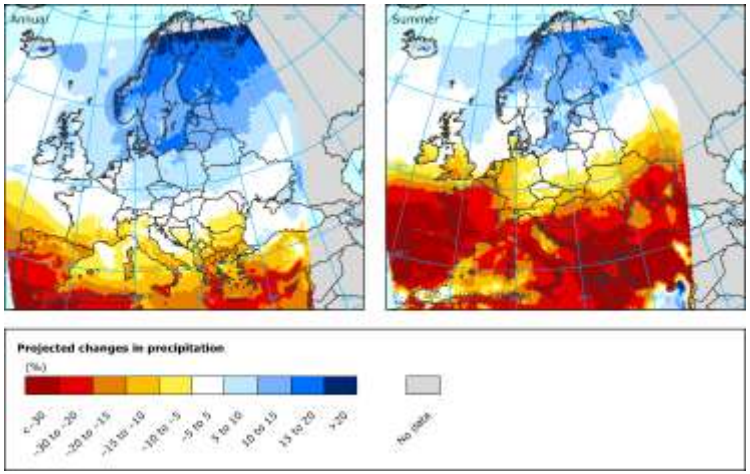
IPCC ARS WG1 (2013)



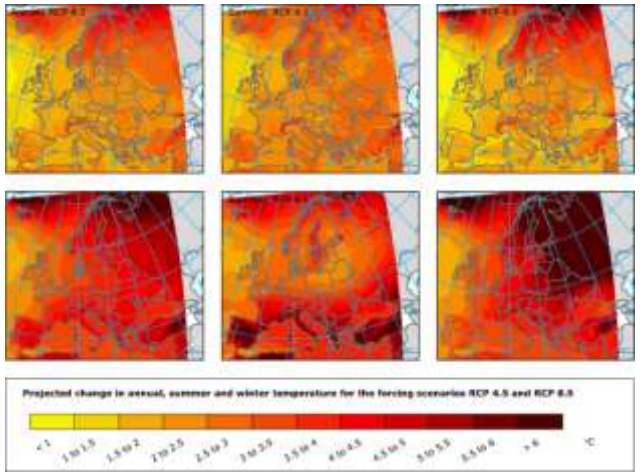
Climate Change in the Mediterranean area

increased T°
more heterogeneous rainfall
marked differences between seasons
increased occurrence of storms

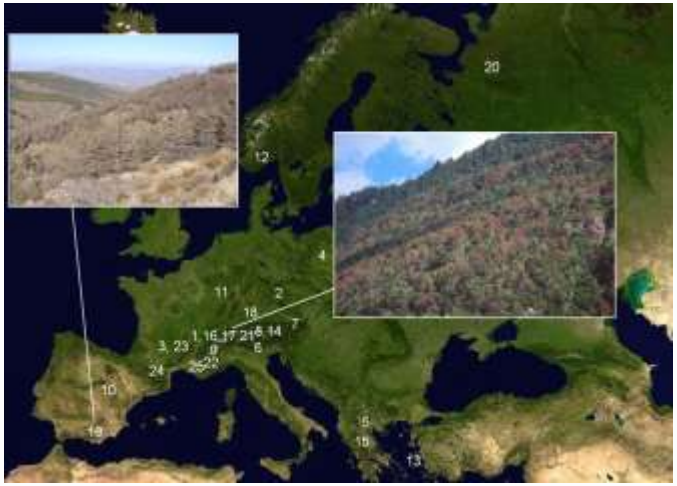
Change in annual and summer **precipitation** for 2071–2100 vs 1961–1990 (%) *eea.eu_2012*



Change in annual, summer and winter **temperature** for 2071–2100 vs 1961–1990 (%) *eea.eu_2012*

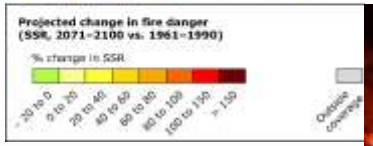


Climatic change predictions suggest more frequent and severe drought events in the Mediterranean region



Allen et al Forest Ecol Manag 2010

Fire danger *eea.eu_2012*

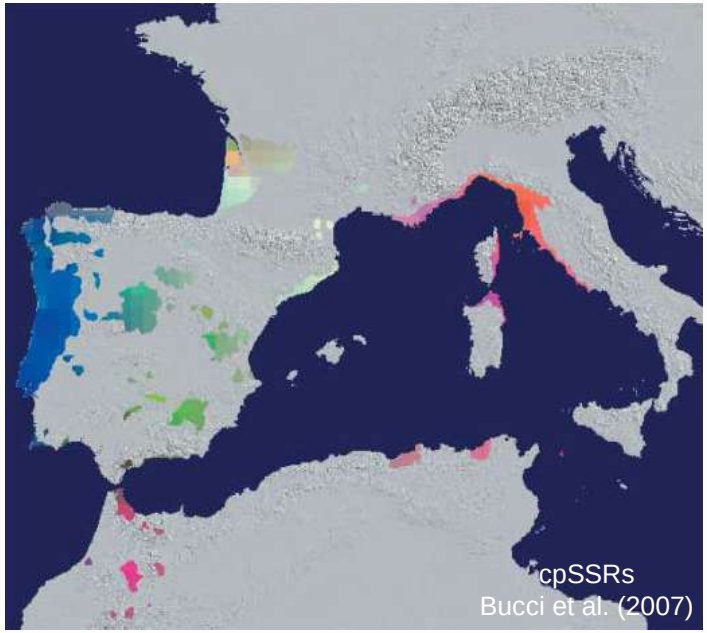


↑ tree mortality

Global warming ⟷ **Long lived species**

Same time scale

Pinus pinaster



Fragmented & reduced natural distribution

Present in different ecosystems / High level of local adaptation

High level of population differentiation for osmotic adjustment, carbon isotopic discrimination & growth under drought.

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Environmental services

Resin

Wood

Biomass & bioenergy

Reforestation

Dissecting maritime pine drought response

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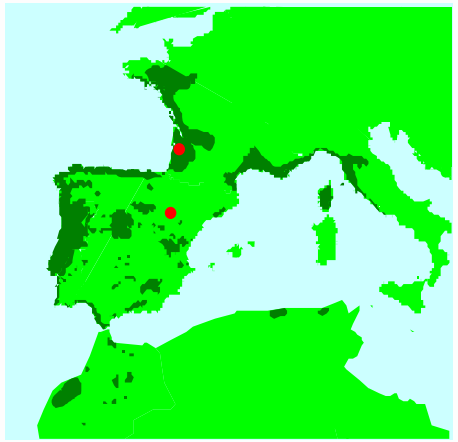
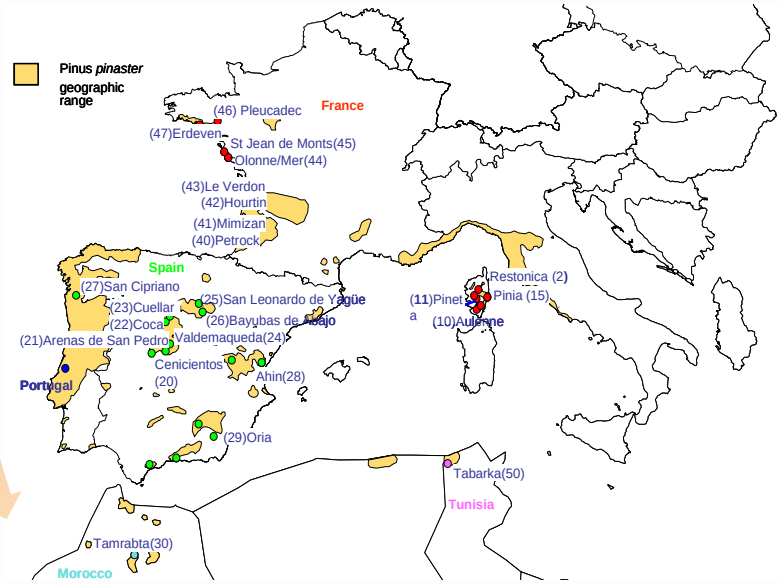
Provenance / progeny trials

Evaluation: field trials + controlled conditions





22 populations from the rainfall range
x 20 to 30 families x 15 progeny / family

348 mm
2 mmdm

1343 mm
18 mmdm

Spain

France

Traits

*Isotope discrimination (related to WUE)

*Growth (height, diameter, annual increments)

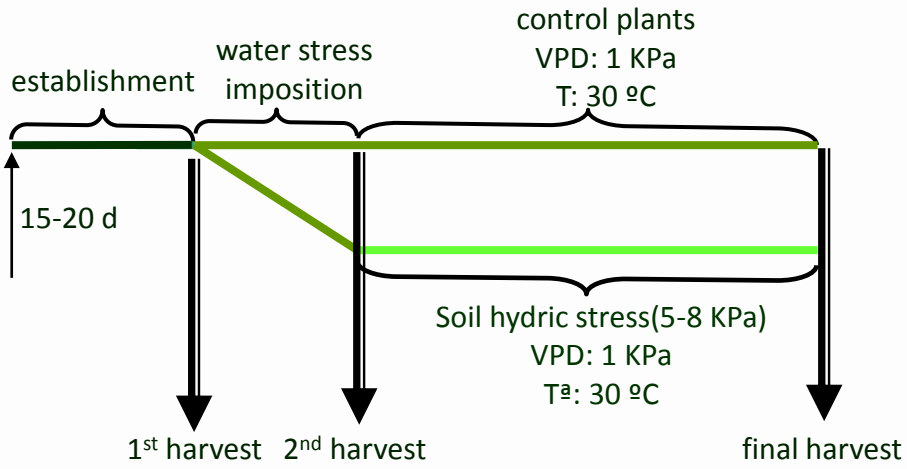
*Biomass (total and aerial)

*Ontogeny scores

*Survival

Controlled conditions

10 populations from the rainfall range x 5 mother trees x 5 progenies x 5 ramets x 2 water regimes
[long and moderate drought event versus watered controls]



Physiology

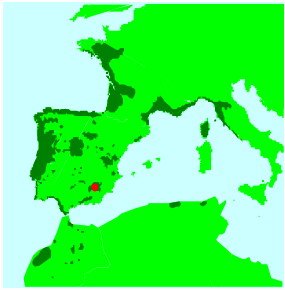
- Isotopic discrimination ($^{\delta}C$ 13 –WUE_i)
- Gas exchange

Growth & morphology

- Growth (height, diameter, annual increments) & biomass
- Changes in allometric patterns

Interpopulation and intrapopulation variation in biomass allocation & growth in seedlings submitted to contrasting watering regimes

Provenance / progeny trials → Evaluation: field trials + controlled conditions → Selection: population → **Oria** inducible response



Provenance: Sierra de Oria

altitude ~1400 m

annual rainfall = 351.5 mm

seasonal drought: May-September

Tm = 14.4ºC

Provenance / progeny trials → Evaluation: field trials + controlled conditions → Selection: population & genotype

Oria

Oria 6

Seedlings

cDNA libraries (UPM-INIA)

Megagametophytes
callus lines (UV)
→ **genome sequencing**

Controlled crosses

♀ elite clone **Galicia 1056**
+ wood production
+ stem straightness
- drought response
- cold response

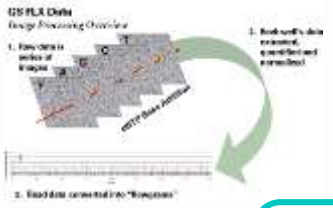
×

♂ **Oria 6**
- wood production
-/+ short stem
+ drought response
+ cold response

cDNA libraries (UMA)

SNP identification (UMA)

Segregating progeny → cDNA libraries (INIA-UAH) & ncRNAs (INIA-UAH-IBET)
→ Physiological and metabolic analysis
→ Reference genetic map → Trait dissection (QTL analysis)
→ Consensus map



Roche GS-FLX Titanium

Assembly & annotation

cDNA libraries from Gal1056 x O6 F1 progenies

NGS

cDNAs

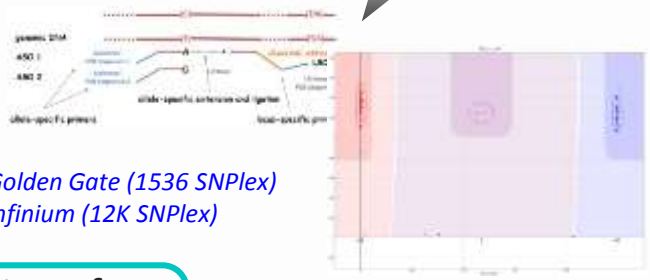
Unigene

Gene expression

Microarrays

SNPs identification

Genotyping (SNPs, ESTP-illing, SSRs, SAMPLs)



Golden Gate (1536 SNPlex)
Infinium (12K SNPlex)

P. pinaster consensus map

Construction of SNP-based genetic maps

Galicia x Oria

QTL analysis

Functional characterization

Identification of genomic regions underlying adaptive traits

Candidate genes

Comparative analysis

Validation RT-PCR

transformation

Genetic variability

Epigenetic variability

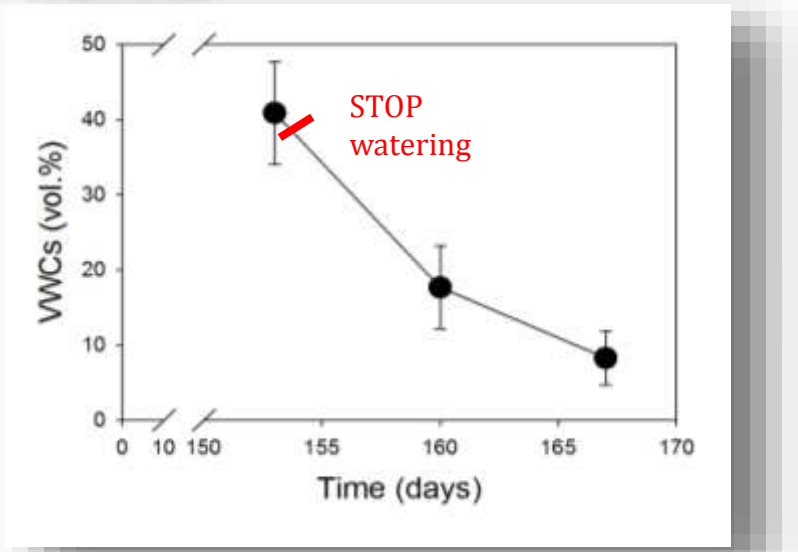
Stress interaction

G1056 × O6

controlled cross → vegetative propagation → clonal bank → destructive assays / non-destructive field assay

162 full-sibs

Characterization of G1056 × O6 family response to drought: *short and intense drought*



Traits

Photosynthesis (A_n)

Stomatal conductance (g_{sw})

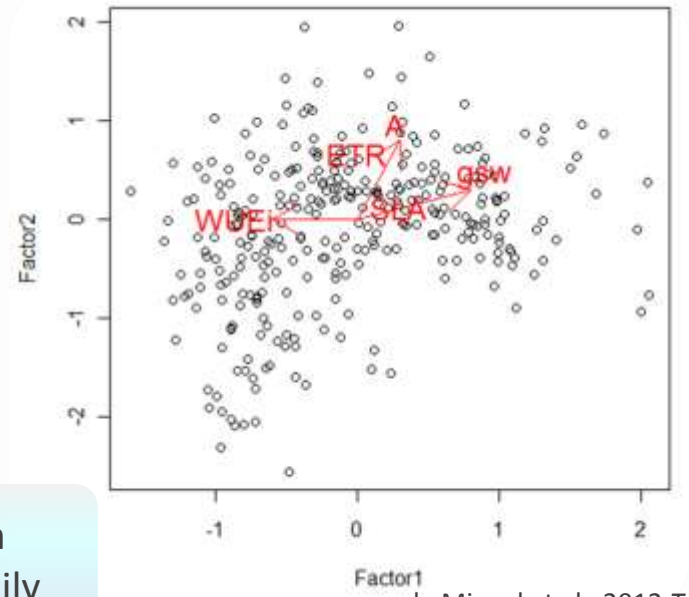
Chlorophyll fluorescence

$WUE_i = A_n / g_{sw}$

$^{\delta}C13$

SLA = leaf area / leaf biomass

Biomass

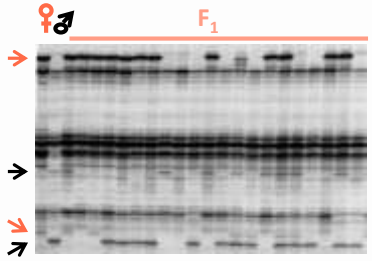


High variability in GxO F_1 full-sib family

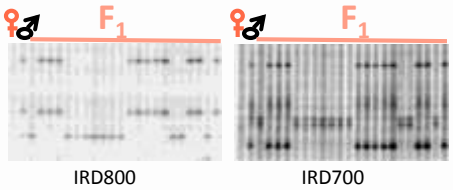
de Miguel et al., 2012 *Tree Physiol*

Gal1056 x O6 genetic maps

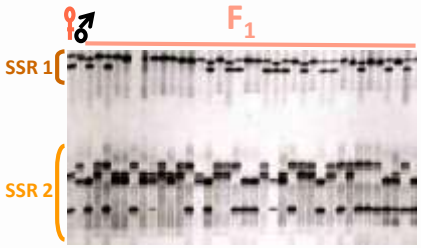
SAMPLs



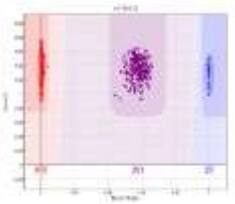
ESTPs (Tilling or SNPlex)



SSRs

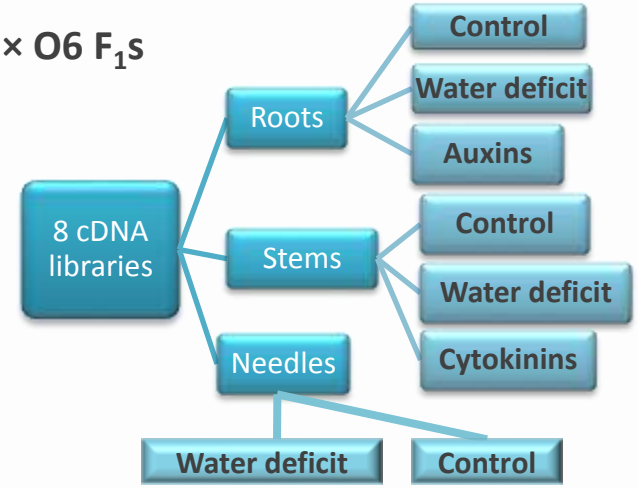


SNPs



Massive transcriptomics

Gal1056 × O6 F_1s



454 GS-FLX Titanium pyrosequencing

16 335 genes

1536 Golden Gate

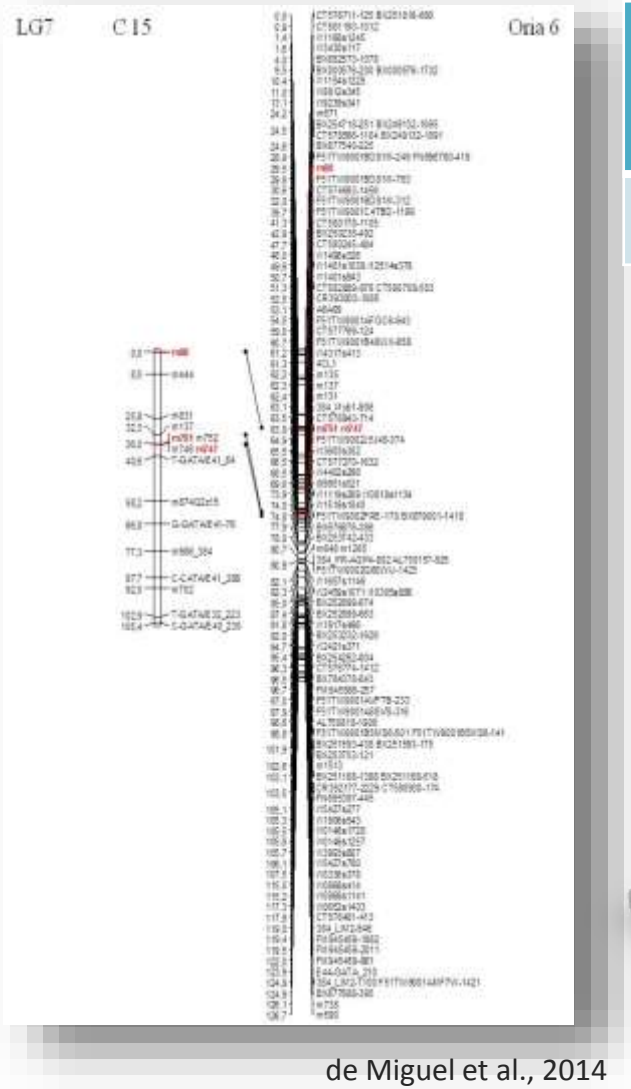
In vitro vs. *in silico* SNPs

Lepoittevin et al. 2010
Chancerel et al. 2011, 2013

1536 GoldenGate
384 VeraCode
12 K Infinium

Highly saturated maps

Gal1056 x O6 genetic maps



de Miguel et al., 2014

Segregating SNPs	1536 INIA	1536 INRA 384 INIA	12K INRA	Total available SNPs
Gal1056 × O6	574	159	1805	2538

Molecular markers in Gal1056 × O6 maps

1636 SNPs → **1204 Genes**

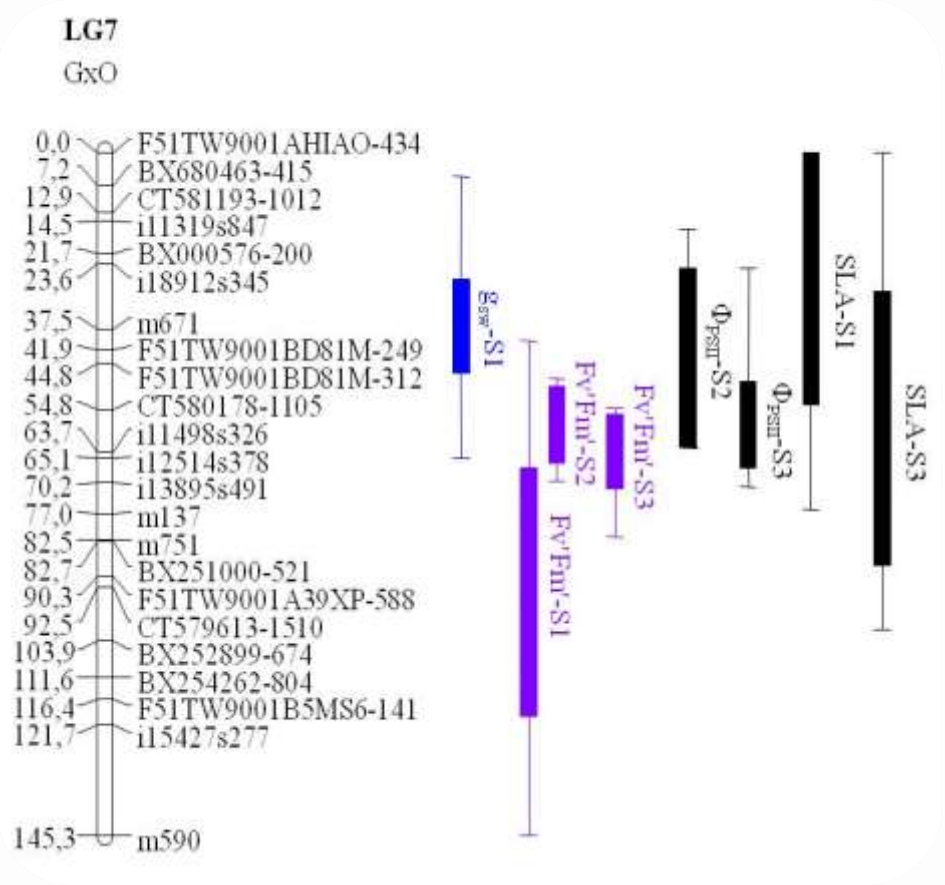
28 SAMPLs

5 SSRs

Comparative mapping
P. pinaster genetic maps:
INIA CocaxCoca
INRA F2 & G2

Chancerel et al., 2011 *BMC Genomics*, 2013 *BMC Biol*
de Miguel et al., 2012

Gal1056 x O6 response to drought: QTL analysis



28 significant QTLs
27 suggestive QTLs

Phenotypic explained variance 1 QTL
4.6% WUE_i – 20.9% Fv'Fm'

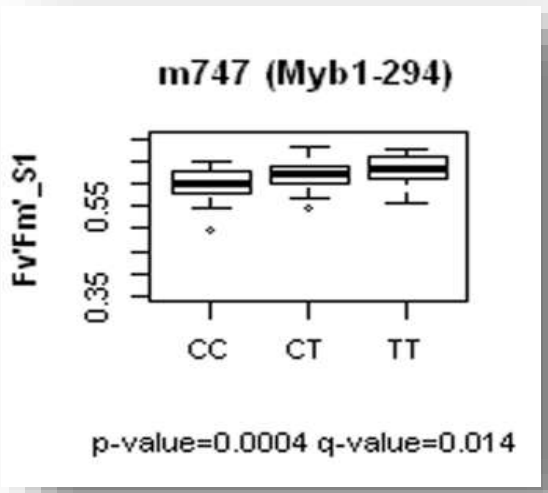
Total explained phenotypic variance
44% ∑Fv'Fm'

Co-localizations
Fv'Fm', Φ_{PSII} –A_n
WUE_i - SLA

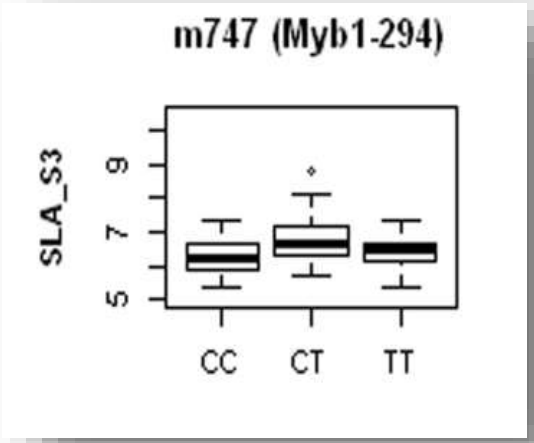
Fv'Fm' , Φ_{PSII} , SLA: S1, S2, S3

de Miguel et al., 2014

Gal1056 x O6 response to drought: candidate genes



14.4%



12.6%

74% mapped genes annotated

↓ QTL co-localization

58 Candidate genes

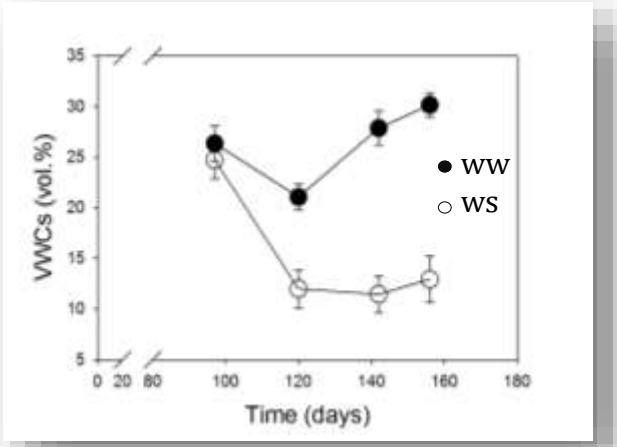
A_n, Fv'Fm', Φ_{PSII} : oxidative stress, ATPase family proteins, proteins of the light harvesting centres

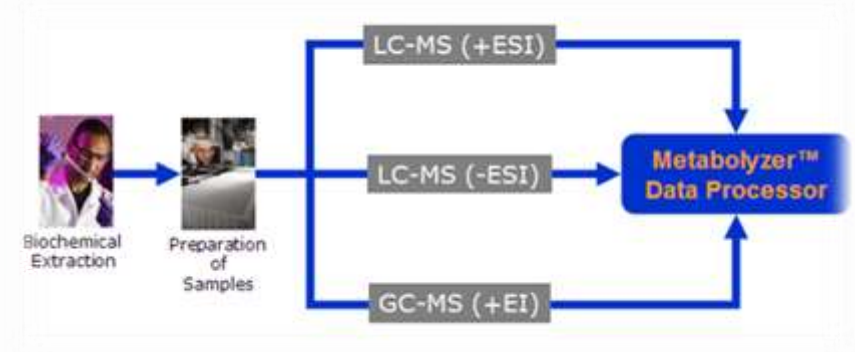
g_{sw} , WUE_i: stomatal regulation, ABA signaling pathways, cell wall composition

SLA: Auxin biosynthesis

$^{\delta}13C$: aquaporin

6 contrasting Gal1056 x O6 F1 genotypes subjected to a long and moderate drought event





Traits

Gas exchange

Chlorophyll Fluorescence

WUE_i

$^{\delta}C13$

SLA

Biomass

Osmotic potential

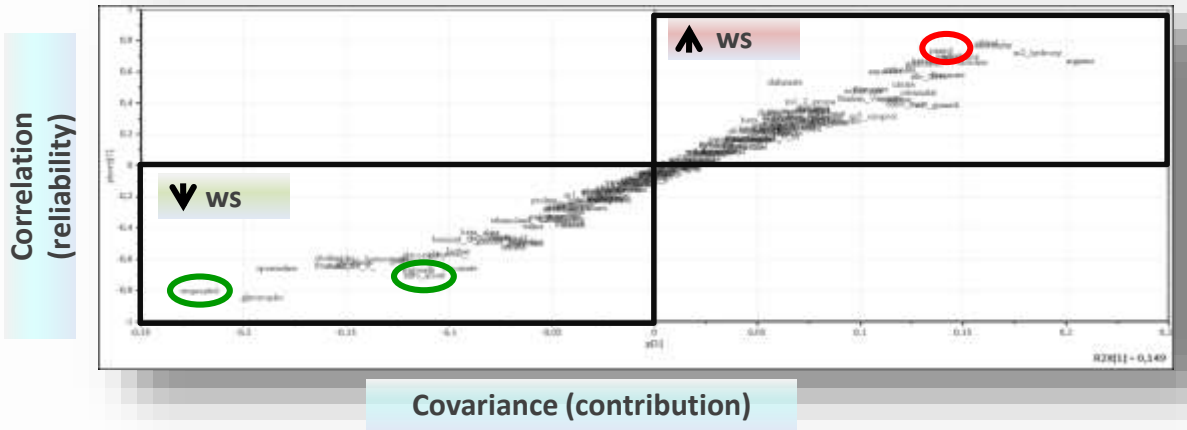
Metabolites

Root

Stem

Juvenile needle

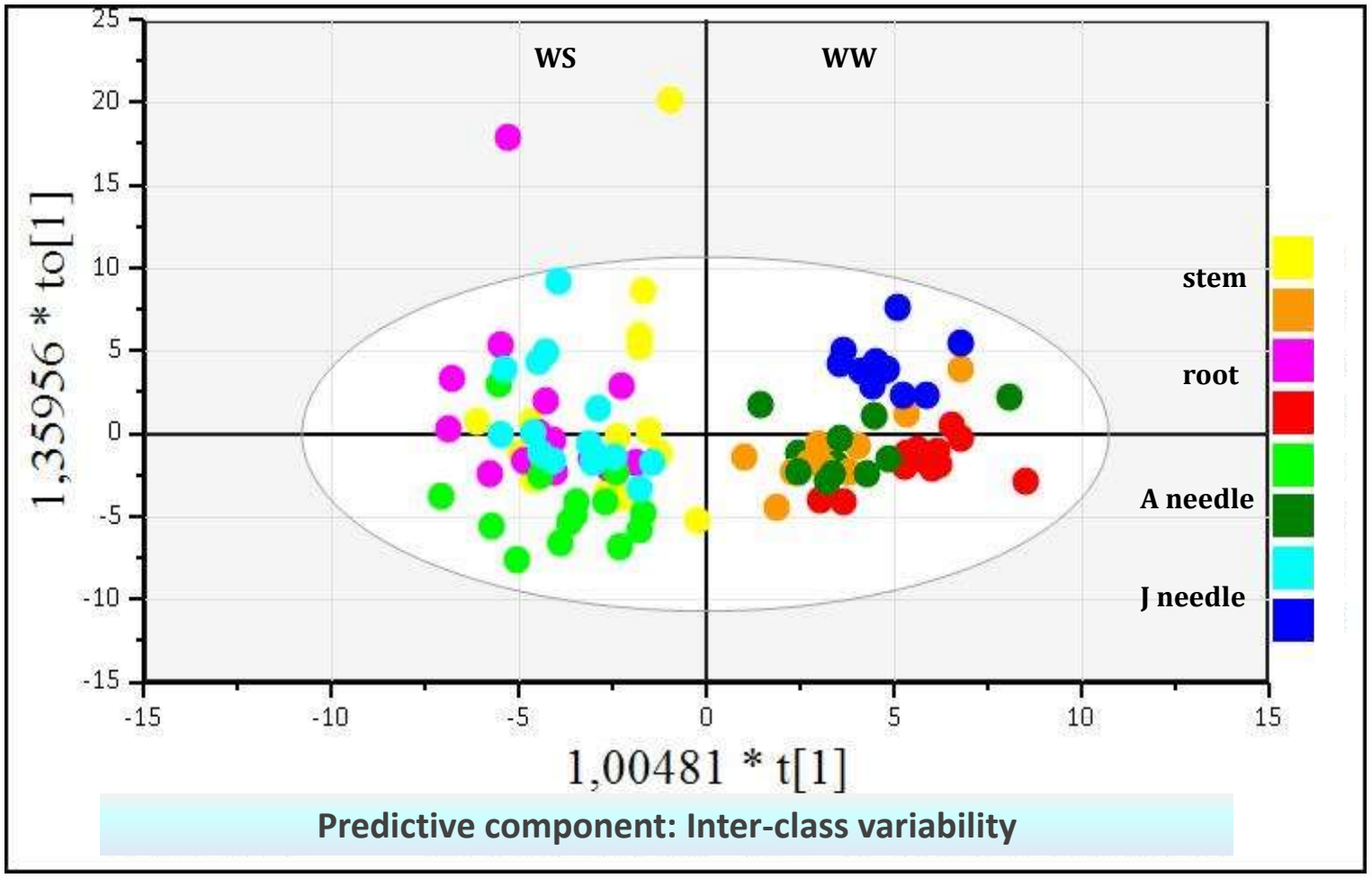
Adult needle



myo-inositol → sequoyitol → → pinitol

Organ-specific metabolic drought response

Intra-class variability

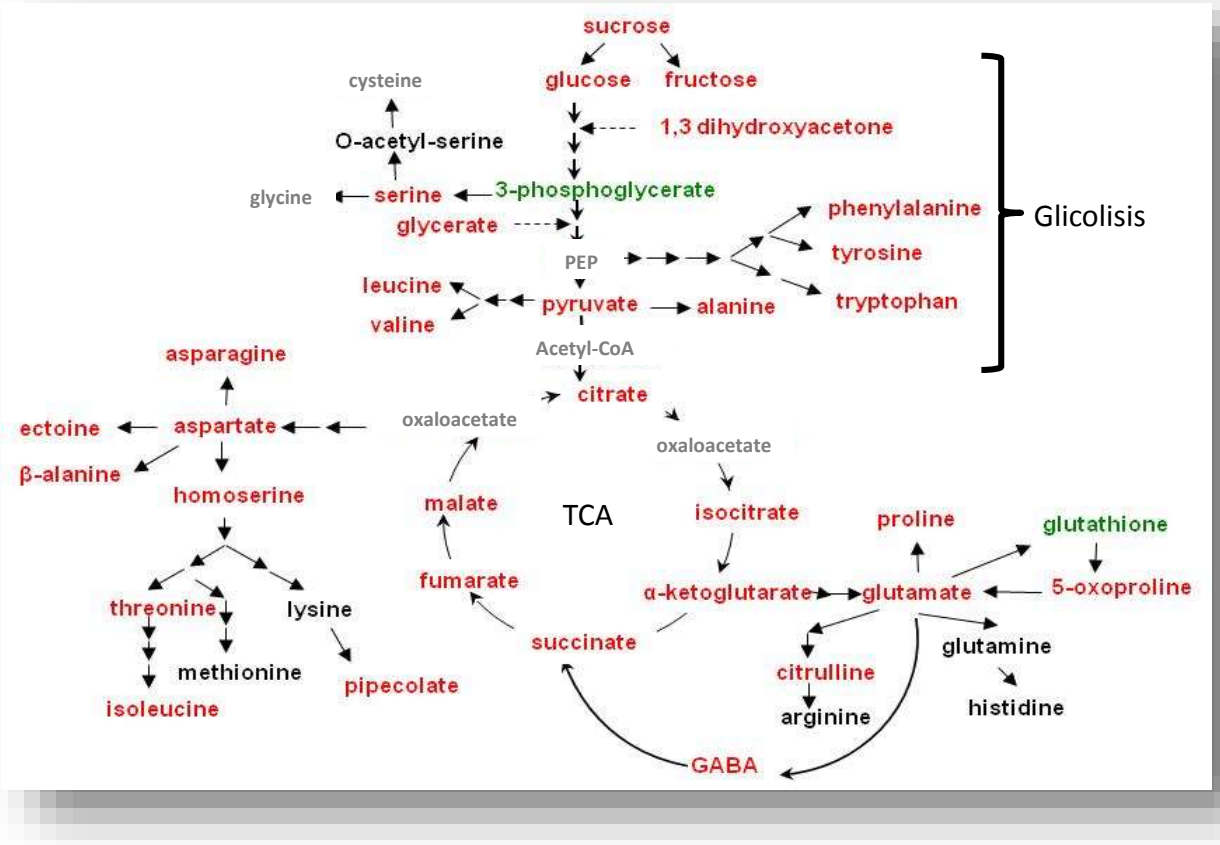


Predictive component: Inter-class variability

Variability in metabolic profiles in different plant organs in response to water stress

Metabolic drought response

Amino acid and energy metabolism



Roots clone 131

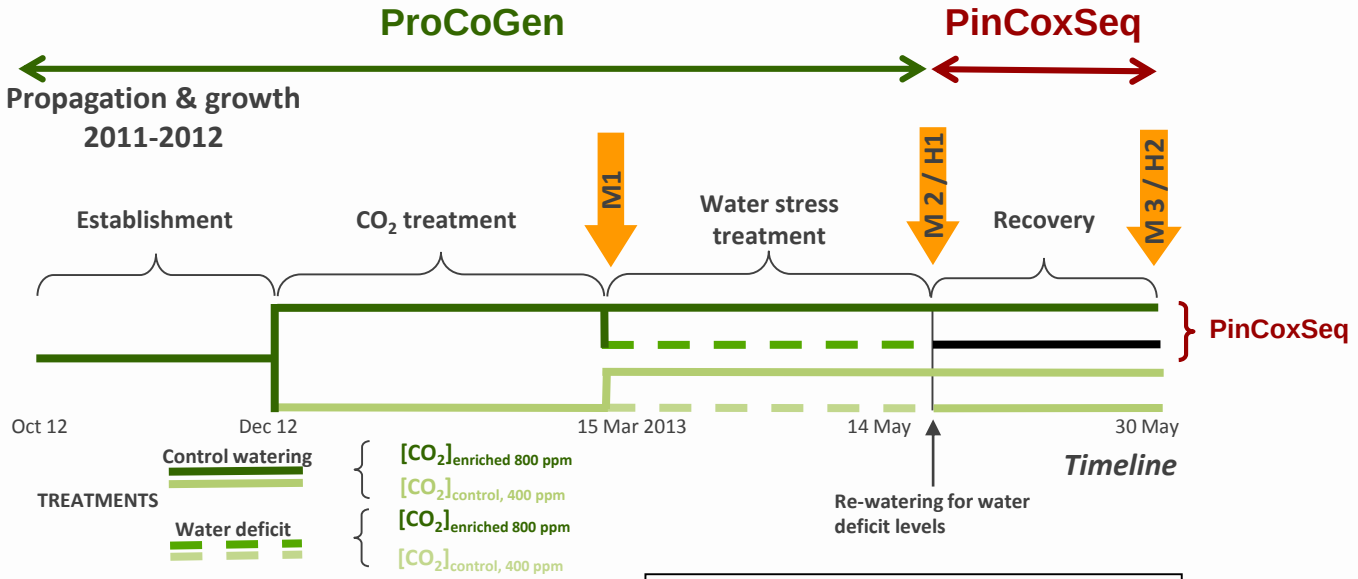
- Increase in ws
- Decrease in ws
- No change
- No detected

Ongoing: Expression analysis of CGs

Ongoing…

(col. UAH, UPM & IBET)

- ✓ Transcriptome dynamics associated to adaptive responses: drought, drought + recovery & drought x↑ CO_2 , drought x↑ CO_2 + recovery.
- ✓ Small non-coding RNAs discovery and profiling.
- ✓ Evolution of genome-wide DNA methylation patterns & characterization of the cytosine methylation levels of the most relevant CGs associated with drought, drought + recovery & drought x ↑CO_2 , drought x↑ CO_2 + recovery responses.
- ✓ Integration with ecophysiological, phenotypic, and metabolic data.



Control watering: well watered plants (substrate volumetric water content in the range 30-35 %)

Water deficit: plants submitted to a cycle of water depletion (substrate volumetric water content in the range 7-5 %)

M 1: Physiological measurements
M 2: Physiological measurements and harvest
M 3: Physiological measurements
H 1: Harvest
H 2: Harvest

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Forest Genomics & Ecophysiology

Genomics…

Ángeles Guevara
Nuria de María
José Antonio Cabezas
Mariló Vélez
Marina de Miguel
Quique Sáez-Laguna
Luis Díaz
Susana Ferrándiz

Ecophysiology…

Ismael Aranda
David Sánchez
José Antonio Mancha

Metabolomics

Maribri Fernández de Simón
Estrella Cadahía

EC: NoE EVOLTREE, FoResTTraC, NTB, **ProCoGen**
Plant-KBBE: SUSTANPINE
MICINN/MINECO: HIDROFOR, EPINEA,
ECOFISEPI, MAPINSEQ,
PINCOxSEQ

Universidad de Alcalá (transcriptomics epigenetics)

Carmen Díaz Sala
Dolores Abarca

Mixed Research Unit UPM / INIA-CIFOR

Carmen Collada
Álvaro Soto de Viana
Pedro Perdiguero

Maritime pine genome mapping

Christophe Plomion (UMR 1202 BioGEco)

ProCoGen partners!