BioAltilis

Tuning of a sustainable cropping model for the production of bioactive compounds in *Cynara cardunculus* var. *altilis*

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Barchi Lorenzo –Università di Torino - AI

Lombardo Sara – Università di Catania - AI

KICK-OFF MEETING
9 January 2024

THE TEAM

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Principal investigator
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GIULIA CONVERSA
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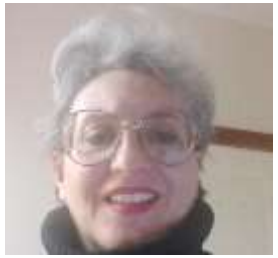

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Cynara cardunculus L.

subsp. *scolymus* (L) Hayek	subsp. *cardunculus* (L) Hayek (= var *altilis* DC	subsp. *cardunculus* (L) Hayek (= var *sylvestris* Fiori
Artichoke	Cultivated cardoon	Wild cardoon

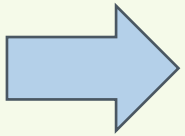
C. cardunculus taxa

PHENOLICS

- mono- and di-caffeoylquinic acids
- luteolin
- apigenin

SESQUITERPENE LACTONES

- cynaropicrin
- grosheimin



CYTOTOXICITY AGAINST SEVERAL TYPES OF CANCER CELLS

ANTI-INFLAMMATORY

ANTIOXIDANT

ANTI-PHOTOAGING ACTIVITIES

BioAltilis

GENERAL OBJECTIVE

Extraction of natural bioactive compounds (NBC) from *Cynara cardunculus* L. var. *altilis* DC (selection 'Altilis 41')

- setting up an eco-friendly cropping system
- genetic mechanisms involved in the quantitative and qualitative production of NBCs

SPECIFIC OBJECTIVES

OBJECTIVE 0

Coordination and management

OBJECTIVE 1

Development of an eco-sustainable and standardized agronomic cropping model

OBJECTIVE 2

Evaluation of agronomic effects on phytochemical composition of leaf biomass

OBJECTIVE 3

Genome sequencing of the cultivated cardoon genotype

OBJECTIVE 4

Transcriptomic assessment after AMF symbiosis establishment in CCGs for DEGs identification

PROJECT TASKS

Task 0
Project coordination and Risk Assessment
(UNIFG)

Task 1
Evaluation of the new eco-sustainable cropping model
(UNICT, UNIFG]

Task 2
Biochemical evaluation of plant material
(UNICT, UNIFG)

Task 3
Plant material for genetic analyses
(UNIFG)

Task 4
Chromosome scale assemblies
(UNITO)

Task 5
Transcriptomics and bioinformatics
(UNITO)

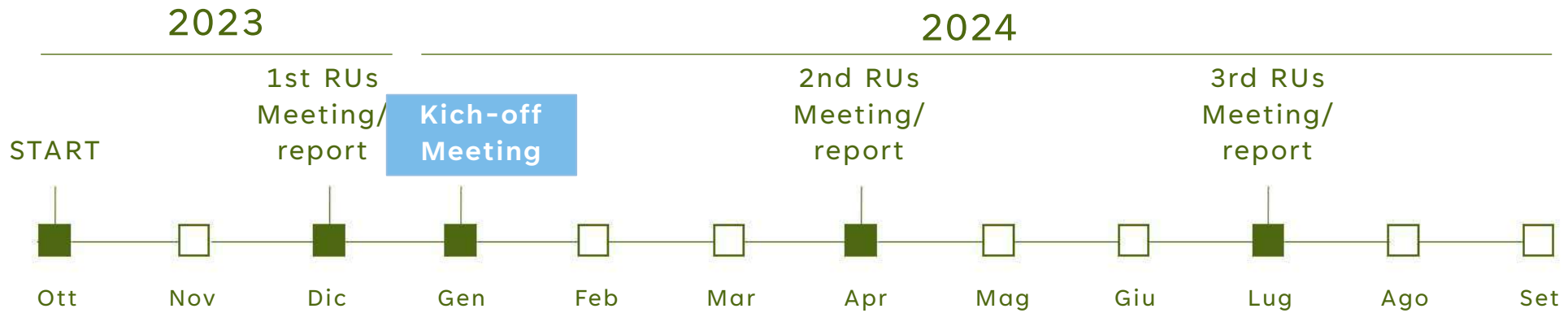
Task 6
Dissemination
(UNIFG, UNICT, UNITO)

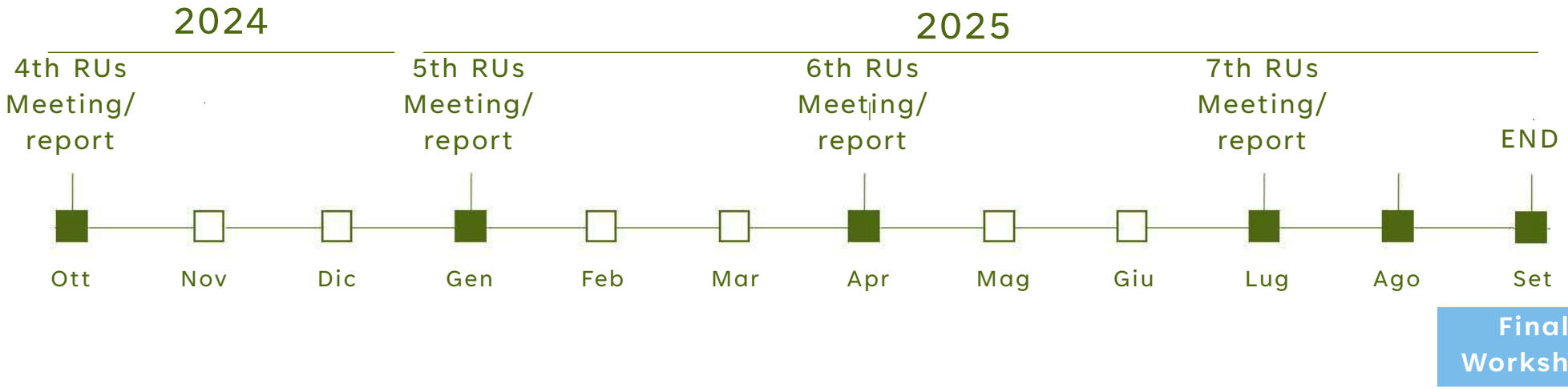
OBJECTIVE 0

Coordination, management and dissemination

- Kick-off meeting
- 4-month reports/meetings
- Meeting with RUs, end of the 1st year
- Workshop, end of the project

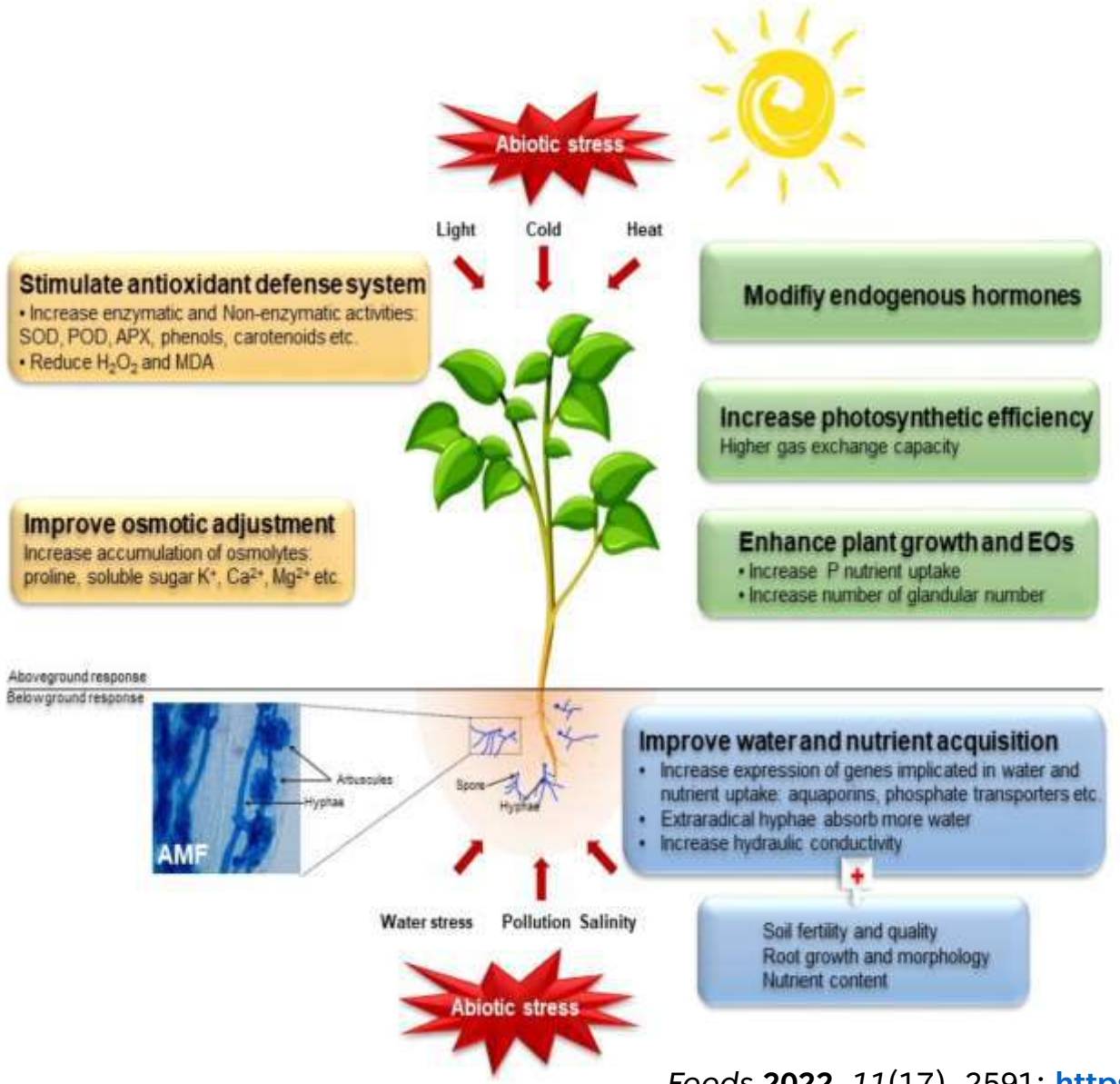
2-YEAR
COORDINATION, MANAGEMENT, DISSEMINATION PLAN





SYMBIOSIS WITH ARBUSCULAR MYCORRHIZAL FUNGI (AMF)

The soil inoculation with AMF is crucial to define a cropping model tuned to eco-sustainable agricultural practices



symbiosis with AMF may improve nutrients and water use efficiency as well as influencing the quantity and quality of NBCs

selected non-native AMF has been proved effective in improving growth both in wild cardoon and globe artichoke as well as NBCs content in globe artichoke

AMF colonization causes a significant cell reorganization which modulates plant gene expression by influencing metabolic processes

Foods **2022**, *11*(17), 2591; https://doi.org/10.3390/foods11172591

Table 1
Phenolic content (g kg^{-1} of DM) of *C. cardunculus* leaves in relation to genotype.

Compound	Genotype								
	Altilis	Blanc Hyèrois	Nobre	Sylvestris Creta	Sylvestris Kamaryna	Tempo F_1	Tondo di Paestum	Tema 2000	Violetto di Sicilia
1-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	Trace	Trace	nd
3-Caffeoylquinic acid	Trace	nd	nd	nd	nd	nd	nd	Trace	Trace
5-Caffeoylquinic acid	0.3	0.9	0.8	0.2	nd	nd	0.7	1.4 ± 0.2	2.3 ± 0.2
3,5-Dicaffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,5-Dicaffeoylquinic acid	nd	0.08	0.09	nd	Trace	nd	nd	0.1	0.1
Monosuccinyldicaffeoylquinic acid	Trace	nd	nd	nd	nd	nd	nd	Trace	nd
Monosuccinyldicaffeoylquinic acid	nd	Trace	nd	nd	nd	nd	Trace	Trace	Trace
Total caffeoylquinic acid	**0.3**	**1.0**	**0.9**	**0.2**	**–**	**–**	**0.7**	**1.5**	**2.4**
Luteolin rutinoside	nd	3.1	2.5 ± 0.1	0.1	0.2	1.0 ± 0.1	1.8 ± 0.1	6.5 ± 0.1	4.3 ± 0.4
Luteolin glucoside	0.1	2.4	nd	1.2 ± 0.1	0.8	3.6 ± 0.2	1.3 ± 0.1	2.4	4.2 ± 0.4
Luteolin glucuronide	2.4 ± 0.3	nd	1.9 ± 0.1	nd	1.9 ± 0.1	nd	nd	nd	nd
Luteolin malonylglucoside	nd	1.6	0.6	nd	nd	2.2 ± 0.1	0.6	2.2	1.9 ± 0.1
Luteolin	0.9 ± 0.1	0.2	0.2	0.1	0.3	0.3	0.3	0.2	0.3
Total luteolin	**3.4**	**7.3**	**5.2**	**1.4**	**3.2**	**7.1**	**4.0**	**11.3**	**10.7**
Apigenin rutinoside	0.5	nd	0.7	0.3	0.2	nd	0.3	nd	nd
Apigenin glucoside	0.1	nd	nd	0.1	Trace	nd	0.08	nd	Trace
Apigenin glucuronide	3.3 ± 0.3	nd	nd	1.6	3.3 ± 0.1	nd	nd	nd	nd
Apigenin malonylglucoside	0.4	Trace	Trace	0.3	Trace	Trace	Trace	Trace	Trace
Apigenin	1.3 ± 0.2	nd	Trace	1.5	1.3	nd	0.2	nd	nd
Total apigenin	**5.6**	**–**	**0.7**	**3.8**	**4.8**	**–**	**0.6**	**–**	**–**
Total measured polyphenols	**9.3**	**8.3**	**6.8**	**5.4**	**8.0**	**7.1**	**5.3**	**12.8**	**13.1**

nd = not detected.

(Food Chem 2011, 126, 417-422)

OBJECTIVE 1

DEVELOPMENT OF AN ECO-SUSTAINABLE AGRONOMIC CROPPING MODEL FOR HIGH LEAF BIOMASS AND NBCs

BioAltilis

- Up to **162%** more biomass than wild cardoon and **260% than globe** artichoke (Ind Crops Prod 1999, 10, 219-228)
- **Requirement of limited inputs,** as a result of the positive balance between the phases of growth and development under Mediterranean climate, the capacity of photosynthesizing during wintertime and the capability of nutrient uptake from deep soil layers
- **Releases of allelochemicals** into the soil system, thus reducing the crop need for chemical weed control

'Altilis 41' genotype

Known and promising NBCs profile
High level of biomass production
High adaptability to Mediterranean environment

Table 1
Phenolic content (g kg^{-1} of DM) of *C. cardunculus* leaves in relation to genotype.

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Apigenin malonylglucoside	0.4	Trace	Trace	0.3	Trace	Trace	Trace	Trace	Trace
Apigenin	1.3 ± 0.2	nd	Trace	1.5	1.3	nd	0.2	nd	nd
Total apigenin	**5.6**	**–**	**0.7**	**3.8**	**4.8**	**–**	**0.6**	**–**	**–**
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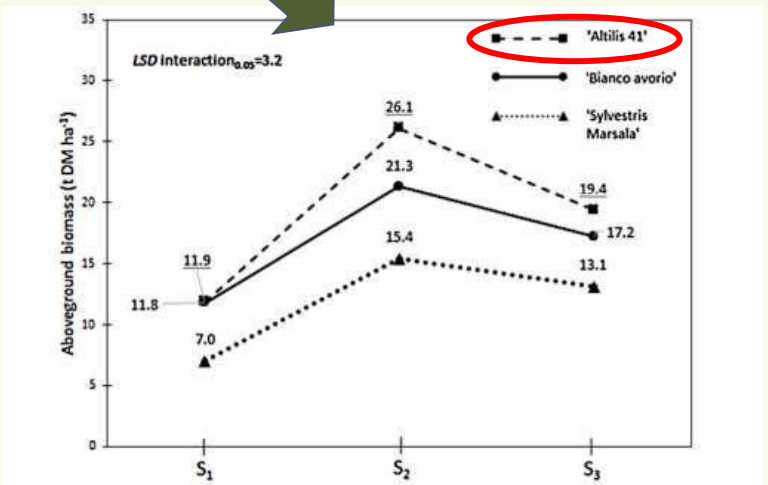


Fig. 2. Effect of interaction "Genotype x Season" on cardoon aboveground biomass yield.

(Ind Crops Prod 2017, 103, 233-239)

A potential extraction of about **6, 107 and 65 kg ha^{-1}** of **caffeoylquinic acids, apigenin and luteolin derivatives**

OBJECTIVE 1

The eco-sustainable approach of the cropping model:

- ❑ exploitation of the **AMF bioresource**
- ❑ **high-density pattern** of the crop
- ❑ **envisaged shift of the cycle** compared with a traditional cultivated cardoon crop

AMF BIORESOURCE

A significant effect of AMF inoculation in the improvement of crop's Water Use Efficiency (WUE) and Nutrient Use Efficiency (NUE), under the reasonable assumption that plants may benefit from symbiosis in terms of ameliorated water status and nutrients uptakes.

HIGH-DENSITY PATTERN OF THE CROP

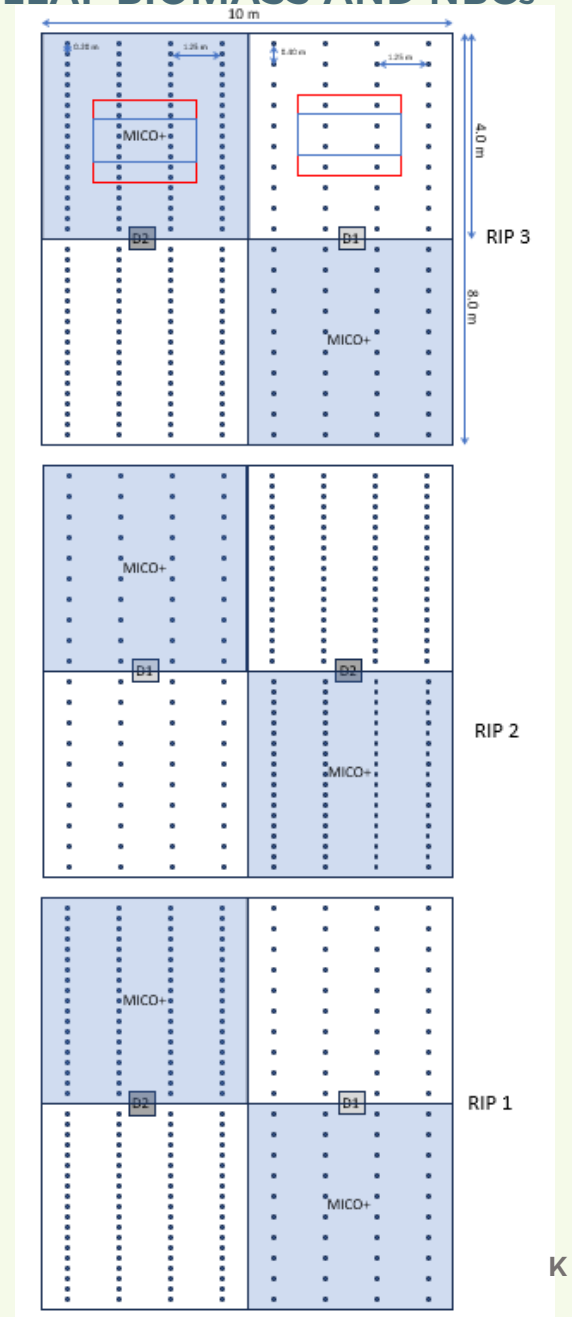
The adopted high planting density will allow the crop to better exploit the light and soil resources and to be more competitive with weeds, resulting in a higher biomass production

ENVISAGED SHIFT OF THE CYCLE

The repeated removal of all shoots will extend the vegetative stage of plants and will stimulate the growth of new shoots from the basal gems and the production of NBCs, due to the stress induced by the cut.

DEVELOPMENT OF AN ECO-SUSTAINABLE AGRONOMIC CROPPING MODEL FOR HIGH LEAF BIOMASS AND NBCs

OBJECTIVE 1



GROWING ENVIRONMENT

- Catania (planting date 19 Sept. 2023)
- Foggia (planting date 9 Sept. 2023)

PLANT DENSITY

- D1: 2 plants/m^2 (20*125 cm)
- D2: 4 plants/m^2 (40*125 cm)

SOIL MYCORRHIZAL INOCULATION (MYCO)

- MYCO+
- MYCO-

GROWING SEASON

- 1st year
- 2nd year

HARVESTING TIME

- Late autumn
- Early spring
- Late spring

Growing season (GS):

In the first season, the crop will be started by transplanting 4-week-old seedlings with 3-4 true leaves and two irrigation treatments will be carried out: (i) at transplanting, (ii) about 2 weeks after transplanting to favor crop establishment. During seedbed preparation, the soil will be fertilized considering both the crop NPK uptakes and its high degree of adaptability to grow under low input conditions, as well as on the basis of the initial soil's nutrients content.

At the end of the first annual cycle (end spring), the above-ground biomass will be completely cut down and the crop will enter a state of dormancy due to hot and dry summer conditions.

In the second GS, the crop regrowth in late summer will be possible by either rains or an irrigation treatment, and a further nitrogen fertilization will be provided to stimulate leaf biomass production.

Harvesting Time (HT):

with the plants still at a rosette stage and with a growth of 15-20 leaves all shoots will be repeatedly cut, since the scope of this crop model is the production of high leaf biomass, before the elongation of the floral stem.

OBJECTIVE 1

DEVELOPMENT OF AN ECO-SUSTAINABLE AGRONOMIC CROPPING MODEL FOR HIGH LEAF BIOMASS AND NBCs

AGRONOMIC and LAB EVALUATIONS

- Physical and chemical soil analysis
- Native AMF analysis (real-time PCR)
- Leaf number, total fresh weight and dry matter content
- Determination of the AMF root colonization indices

OBJECTIVE 2

EVALUATION OF NBCs - CHEMICAL ANALYSES

- Flavonoids*
- Phenolic acids*
- Sesquiterpene lactones*
- Free radical scavenging capacity (ABTS and FRAP methods)

*An HPLC system coupled in line with a triple quadrupole Mass Spectrometer (MS) will be used to identify and confirm the molecular identity of NBCs, thus defining the qualitative and quantitative profiles of flavonoids, phenolic acids and sesquiterpene lactones in the leaf biomass as affected by the treatments under study.

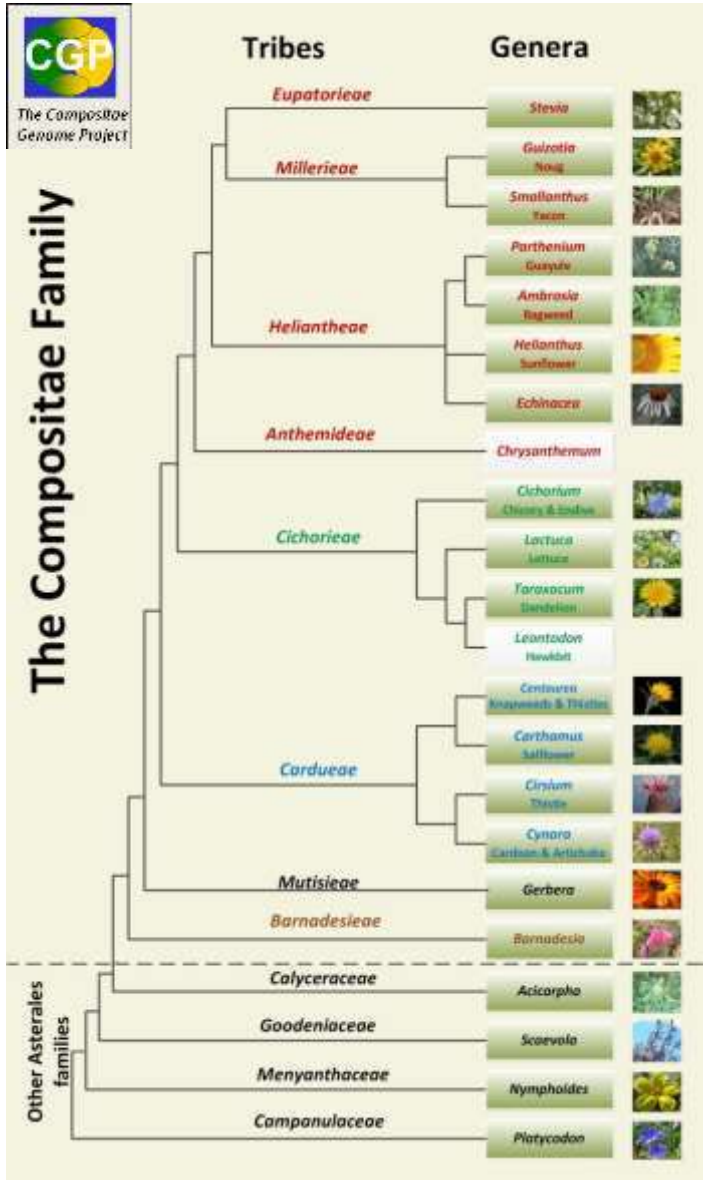
OBJECTIVE 3 & 4

Genomics and transcriptomics

Identify differentially expressed genes (DEGs) and molecular mechanisms involved in the response of the plant to root mycorrhizal colonization through:

- chromosome-scale de novo assembly of selection ‘Altilis 41’
- RNA sequencing performed in control and mycorrhized plants

Cynara cardunculus sequencing state of the art



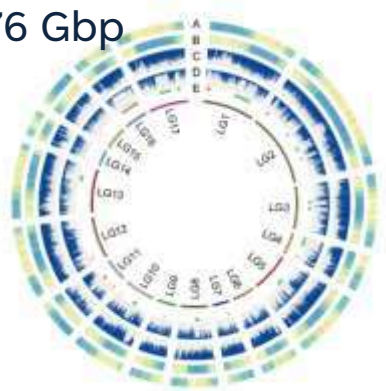
Sunflower – 3,600 Gbp

Chicory – endive - 1,360 Gbp

Lettuce - 2,500 Gbp

Globe artichoke: 1,076 Gbp

(January 2016)



Cynara cardunculus sequencing state of the art

Italy harbours the richest globe artichoke primary cultivated gene-pool

Classified on the basis of capitulum traits into:

‘SPINOSI’ ‘CATANESI’ ‘VIOLETTI’ ‘ROMANESCHI’ CULTIVATED CARDOON A41

Hiseq-2000 Illumina resequencing (35X/genotype)

Genomes’ sequence available and annotated

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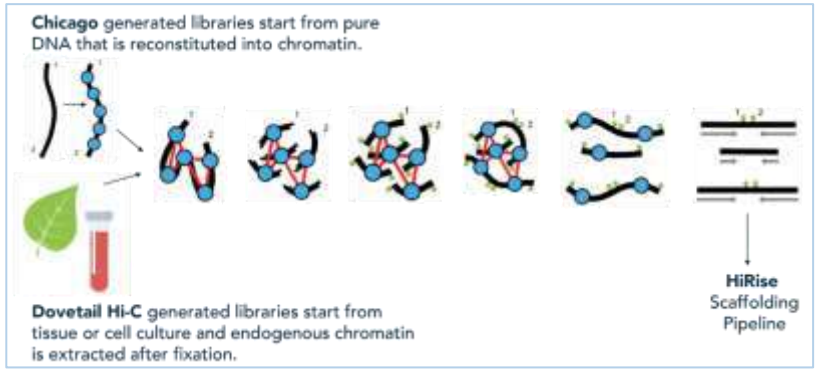
Article | Open access | Published: 17 July 2017

Genome reconstruction in *Cynara cardunculus* taxa gains access to chromosome-scale DNA variation

Alberto Acquadro, Lorenzo Barchi, Ezio Portis, Giulio Mangino, Danila Valentino, Giovanni Mauromicale & Sergio Lanteri

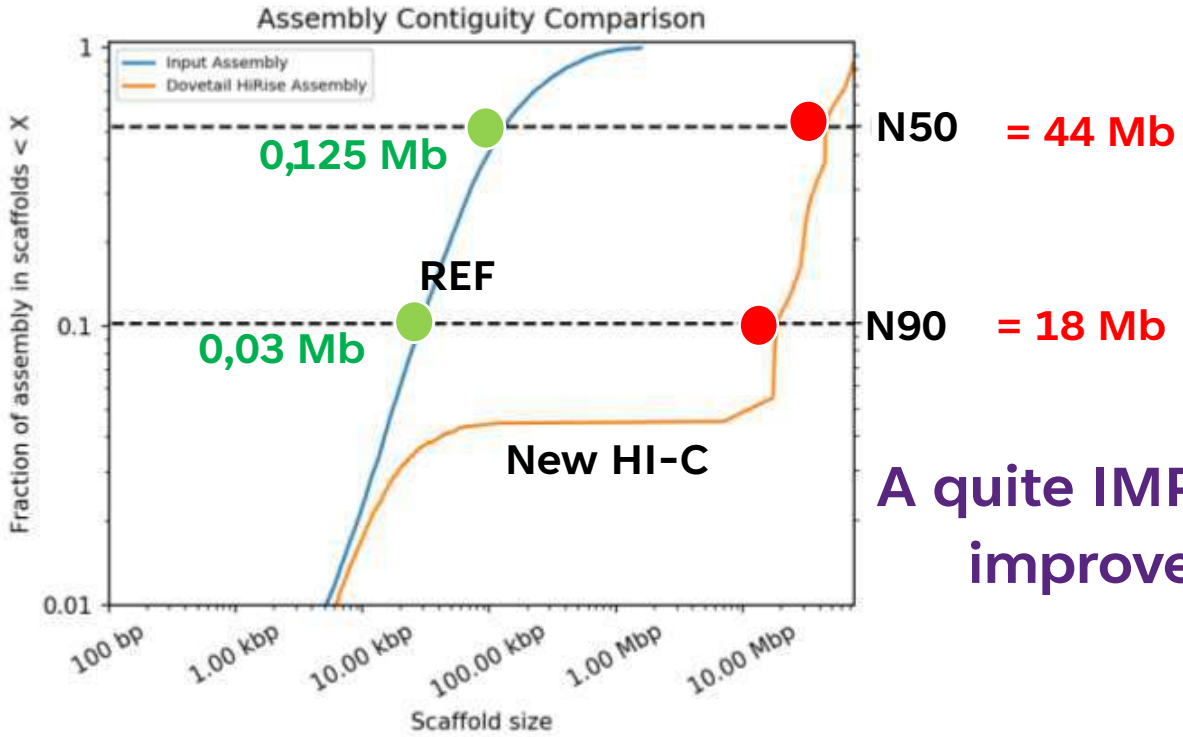
Scientific Reports 7, Article number: 5617 (2017) | Cite this article

Improving artichoke genome



HI-C libraries

(Chromosome conformation capture)



A quite IMPRESSIVE improvement

JOURNAL ARTICLE

"Mind the Gap": Hi-C Technology Boosts Contiguity of the Globe Artichoke Genome in Low-Recombination Regions

Alberto Acquadro, Ezio Portis, Danila Valentino, Lorenzo Barchi ✉, Sergio Lanteri

G3 Genes|Genomes|Genetics, Volume 10, Issue 10, 1 October 2020, Pages 3557–3564,
https://doi.org/10.1534/g3.120.401446
Published: 01 October 2020 **Article history** ▾

OBJECTIVE 3

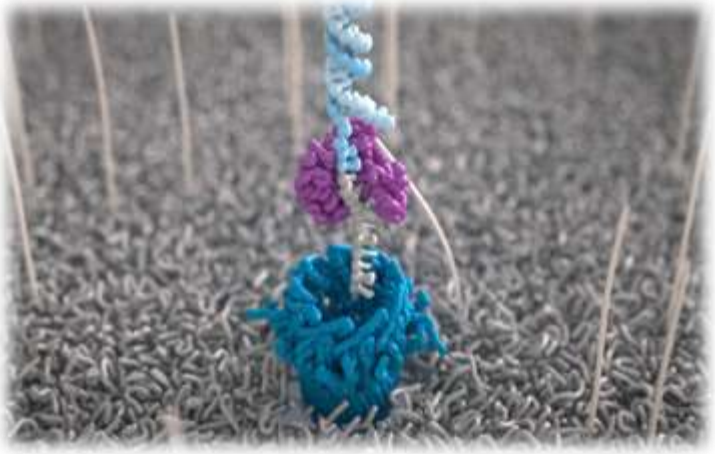
Genome sequencing of A41 genotype

- An improved sequence (chromosome-scale) of the cultivated cardoon ‘Altilis 41’ will be obtained.
- Due to the high level of heterozygosity characterising the species, the *de novo* assembly will be generated based on
  - long-read sequencing
  - Hi-C based scaffolding
- Genome will be structural and functional annotated

OBJECTIVE 3

Genome sequencing of A41 genotype

- Third generation long read sequencing, based on nanopore



	SBS sequencing	Nanopore sequencing
Read length	2x150 bp	10–100 kb
Read accuracy	99.92% (Q31)	99.26% (Q21)
Run time	44 hours	72 hours
Yield	2,400–3,000 Gb	50–110 Gb
Variant calling – SNVs	✓	✓
Variant calling – indels	✓	X
Variant calling – SVs	X	✓
5mC methylation	X	✓
Phasing	X	✓

OBJECTIVE 4

RNA-seq after AMF symbiosis in A41 for DEGs identification

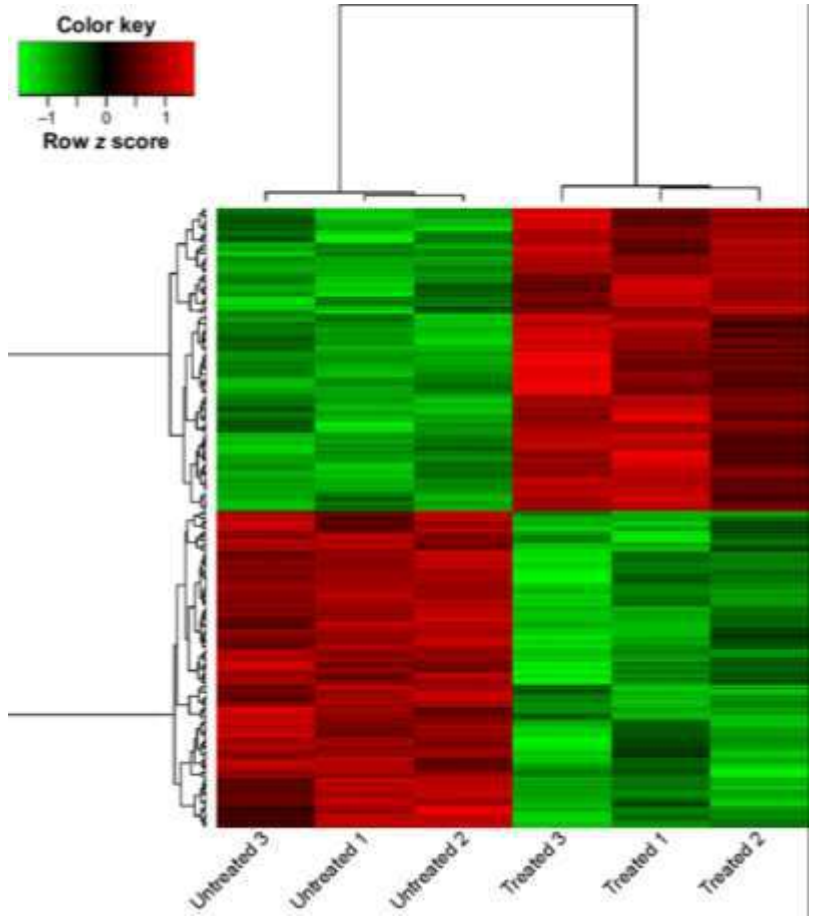
Rationale:

- By combining genomic information with RNA sequencing, it is possible to identify differentially expressed genes (DEGs) in response to a specific treatment
- In globe artichoke, AMF mycorrhization has proved to increase both biomass and total phenolic content in heads and leaves
- No information is currently available on the molecular mechanisms involved in the symbiosis in cultivated cardoon and on the effects of AMF on the quantity and quality of NBCs (flavonoids, phenolic acids, sesquiterpenes) production.

OBJECTIVE 4

RNA-seq after AMF symbiosis in A41 for DEGs identification

- Exploit the newly obtained chromosome-scale sequence of ‘Altilis 41’
- RNA sequencing will be performed in control and mycorrhized plants at different stages of development and grown in a controlled environment (greenhouse).
- Identification of differentially expressed genes (DEGs) to shed light on the molecular mechanisms involved in the response of the plant to root mycorrhizal colonization.



OBJECTIVE 4

RNA-seq after AMF symbiosis in A41 for DEGs identification

- Plants of the selected genotype ‘Altilis 41’ will be cloned by rooting off-shoots.
- AMF inoculation using the same commercial AMF inoculum used for field trials.
- Inoculated AMF plants (Myco+), together with controls (no-Myco) will be grown under controlled conditions.
- Three-time points will be assessed:
  - T0: after rooting and before AMF inoculation (leaves and root)
  - T1: 2 months post-AMF inoculation (leaves and root)
  - T2: 6 months post-AMF inoculation (leaves)