PROGETTO/PROJECT

"**C**onoscenze **I**ntegrate per la **S**ostenibilità e l'**I**nnovazione del *made in Italy* **A**groalimentare"

Integrated Knowledge for Sustainability and Innovation in Agriculture

(CISIA)

SCHEDA PROPOSTA ATTIVITÀ/PROPOSAL FORM

GENERALITIES

ISTITUTO CNR Istituto di Biologia e Biotecnologia Agrarie (IBBA) - CNR.

Istituti CNR / Altre Istituzioni Pubbliche/ Associazioni di Categoria/ Privati
Istituto di Metodologie Chimiche - CNR (IMC). Via Salaria km 29,300. Monterotondo-Scalo. RM.
Azienda Enza Zaden (EZ). Strada Statale Aurelia, KM. 96.400. 01016. Tarquinia (VT)
Azienda La Noria, ISPA - CNR (LN). Contrada Scannacinque - Zona Industriale CP 93, 70042. Mola di Bari, Bari

Scientific Responsible: Donato Giannino; 06/90672529; giannino@ibba.cnr.it

REALIZATION OBJECTIVE

- ☐ OR1 "Risorse genetiche"
- **X** OR2 "Diagnostica Avanzata"/Enhanced Diagnostics
- ☐ OR3 "Innovazioni per migliorare la sostenibilità della produzione agroalimentare"
- ☐ OR4 "Innovazione di prodotto alimentare e rapporti alimenti e salute"

PROPOSAL TITLE
Identity, traceability and value increase of endive, escarole (prickly lettuce) and "puntarelle" (*Chicorium* spp.) from Lazio and Puglia shires through "olistic" technologies focussed on agro-traits of nutritional and economic relevance.
Acronym: **VISP-LP**

GENERAL OBJECTIVE (*max 2000 characters*)

Primary objective: production of knowledge and technological application regarding metabolites and molecules of liguliflorous Asteracee (*Chicorium* spp.) to both assess identity and traceability of local varieties from Lazio and Puglia and enhance the nutritive value for consumers' health and producers' profit.

In response to local SME demands, **VISP-LP** intends to develop tools and services to create a complex and validated system of variety-specific markers based on the aromatic and anti-feeding compound contents, the expression variability and polymorphism of key genes that regulate the metabolic pathways of these compounds. **VISP-LP** will produce important information on organoleptic qualities and nutritional values (including anti-oxidant properties and effects on human health) of typical vegetables addressed to markets of fresh consumption and of minimally processed foods.

VISP-LP focuses on: *C. intybus* – "puntarelle di Galatina/Molfetta", "cicoria all'acqua/Otrantina", which are included in the National lists of traditional products (DLg 16/10/2010, supp. n.145, 5/7/2010); *C. endivia* var. *crispum* (endive) and *C. endivia* var. *latifolium* (escarole) as both marketed and/or on-trial cultivars from Lazio and Puglia shires.

Sub-objectives:

1. Assessment of the content and variability of a) a wide range of metabolites for cultivar typing and b) target molecules (sesquiterpenes, inulin, citochinin, brassinosteroids, phytins metal micronutrient and heavy metals) for the enhancement of cultivar nutritional value.
2. Assessment of differential transcriptomics of genes involved in target metabolic pathways in distinct cultivars
3. Assessment of allelic diversity of genes involved in target metabolic pathways in distinct species/cultivars (targeted genotyping)
4. Identification of integrated and functional markers based on gene sequence polymorphism, gene expression profile and metabolite content.
5. Marker validation for typicality, traceability and potential identification of traits.

DESCRIPTION OF ACTIONS, PARTNERS AND ROLES, TIME DIAGRAMS, MID-TERM OBJECTIVES AND MILESTONES.

Structured in 6 actions for 3 years, in attachment the Gannt diagram and milestones.

1. PLANT MATERIAL MANAGEMENT

1.1. Cultivation techniques and Production (EZ/LN)

1st year: cultivation of 2 cultivars (cvs) of endive and escarole (with distinct and divergent traits) at EZ, and 2 cvs of puntarelle at LN (open field and/or greenhouse at standardized conditions). 2nd year: cvs from the 1st year and 3 new cvs (either registered or on-trial) derived from the cvs of the 1st year. 3rd year: swapped cultivation, that is: selected Apulian cvs in Lazio and Lazio cvs in Puglia (2 cvs puntarelle in VT, 1 cv escarole and 1 cv of endive at BA)

1.2. Sampling, stocking and distribution (IBBA/LN)

Sampling will be mainly performed at head harvesting, synchronised for the different cvs. Leaves will be frost (liquid nitrogen), lyophilized and stocked at -80°C at IBBA and LN, who will guarantee the distribution. 1st year: sampling at harvesting; 2nd year: sampling at precocious stages and at harvesting of the same cv as the previous year and of new selected varieties; 3rd year: sampling of cvs (selected on the basis of efficient markers) cultivated in swapped shires.

2.METABOLITE TYPING (IBBA/IMC/LN)

Inter-species/intra-cultivar fingerprinting will be assessed on the basis of nutritive/metabolite variability so a to generate typicality clusters. The variability of compound contents (bottom-up approach) paves the way to addressed studies of gene transcriptomic and diversity. The levels of "wide range" compounds, namely hydro and lipo – soluble fractions (H/L-S) and a special focus on **nitrates**(NO_3^-) will be measured together with a set of novel ***target molecules***: **sesquiterpenes** (S), **inulin** (I), **citochinin** (CK), **brassinosteroids** (BR), **phytin** (F) **metal micro- and macro-nutrients** (M). These compounds have been targeted for they both regulate important traits in *Cichorium* spp. and have been poorly investigated in local varieties. Briefly, S regulate the bitter taste; I has dietary properties. CK control leaf shape and senescence; high levels can increase leaf vegetable shelf-life. BR contribute to plant development and stress defence, have anti-cancer and anti-cholesterol properties. F is a major phosphorous reserve with metal-chelating and anti-feeding power but also anti-oxidant/cancer properties. Some M are essential for life, others can turn highly harmful when accumulated. The **anti-oxidant capacity** is a widely reckoned parameter for food quality.

2.1 Extraction, assignment, and quantification of metabolites (IBBA/IMC/LN).

1st year; cultivar-specific optimisation of: extraction methods, extract treatments, instrumental parameters. The table below includes: compounds, partners' roles and standard technologies, which are coupled to others with high resolution power (Ion-Trap3D, MALDI-FT-ICR).

2.2 Production of metabolite variability profiles in distinct cultivars and statistical analysis (IBBA/IMC/LN).

2nd and 3rd year: measurements will be performed on biological and technical replicates followed by ANOVA, PCA and LDA processing aimed to the cultivar typing.

2.3 Production of anti-oxidant profiles in distinct cultivars and health benefit appraisal (IBBA).

The anti-oxidant capacity of leaf hydro- and lipo- soluble compounds will be assayed by the ORAC method. Using animal model systems, the effects of leaf extracts (at variable time/concentrations) will be tested on: a) anti-oxidant enzymes (biochemical and immuno-blot assays) and b) gene expression response (qRT-PCR) of key transcription factors.

Compound	Technology	Partner
NO_3^-	Ion Chromatography	LN
I/L-S	NMR	IMC
I	NMR	IMC
S	NMR	IMC
CK	HPLC	IBBA-IMC
BR	GC/MS	IBBA-IMC
F	HPLC	IBBA-IMC
M	Atomic absorption	IBBA
CO	Spectrofluor.	IBBA

3. GENE EXPRESSION TYPING (IBBA)

3.1 Identification of expressed gene sequences of target pathways

1st year: identification of genes (synthesis/catabolism/transduction/transcription regulation) involved in the pathways of target compounds (S, I, BR, CK, F, M) by retrieving sequences from EST databases of *Cichorium* e *Lactuca* spp. Oligo will be designed to isolate target genes from the study cultivars and to unequivocally identify alleles after RNA-sequencing. The gene pool can be enriched of related markers such as genes specifically/sensibly responding to target metabolite variations.

3.2 Transcriptome sequencing (RNA-seq)

1st year: production of 100 bp pair-end sequences from cNDA libraries by RNA-seq technology (Illumina HiSeq2000). The libraries will derive from distinct tissues at different developmental stages to widen the trasnscriptome complexity and enhance the transcriptome assembly. Assembly ansd automatic annotation of transcripts by comparative analyses with public databases. As a start, one cultivar of *C. endivia* and one cv of *C. intybus* will be RNA-sequenced based on the fact that they are recurrent parent lines in breeding programmes. The re-sequencing of a set of cultivars will be performed (50 bp pair-end sequences). The NA-sequencing is in outsourcing, while all the computational processes will be carried out by IBBA.

3.3 Transcriptional profiles by RNa-seq and statistic elaboration.

The expression analysis will be performed by RNA-seq by using 50 bp sequences, with the option of either 15billion (6samples/lane) or 25 billion (4 samples/lane) of reads per sample. The reads will be mapped on the transcript-sequences of reference, the values of Reads Per Kilobase of exon model per Million mapped reads (RPKM) will be determined and reported in tables of relative expression (vs internal controls). The transcription profile validation will be performed by qRT-PCR on representative genes, which will be further investigated for structural polymorphism analyses. 2nd year: expression profiles will be produced using the model cv at harvesting of year 1 and year 2; and the novel cultivar at harvesting; 3rd year: the profiles will be produced on selected cvs grown in swapped areas.

4. GENETIC DIVERSITY TYPING (IBBA)

4.1 Polymorphism identification of genes involved in target pathways

1st and 2nd year: single nucleotide polymorphysms (SNP) will be identified from the RNa-seq data, therefore the transcript reads will be mapped and the variants will be named. SNP analyses will be enhanced by computational analyses from public db of EST of *C. intybus* vs *C. endivia*, and among alleles within the same species (Quality SNP software). The analysis is limited to EST due to the absence of a fully sequenced genome. The sequences of the varieities of the project will widen the chances to draw oligos for genotype fingerprinting strategies.

4.2 Genotyping by allele polymorphism

2nd and 3rd year: methods to identify/confirm gene SNP will be developed by the high melting resolution technology (HMR-SNP) using low cost fluorescent dyes. 3rd year: testing of markers on cv derived from the cross of parents examined in the 1st and 2nd year.

5. DATA MANAGEMENT AND IDENTIFICATION OF FUNCTIONAL MARKERS (IBBA, IMC, EZ, LN)

5.1 Database production and management

The IBBA web site will host a db of phenotypical/metabolic/transcriptional/genomic data for the distinct cvs, including links of useful tools (see also section of result dissemination)

5.2 Data collection and stock (IBBA/IMC/LN/EZ)

1st -3rd year: LN/EZ will produce phenotypic (quali-quantitative) data of the cv from *Cichorium* spp. As for specific traits measurement methods can be developed to improve the association with the metabolic pathways and genetic determinants. IBBA/IMC/LN will produce raw and processed "olistic" data according to agreed procedures.

5.3 Identification of functional markers (IBBA/IMC/LN/EZ)

Based on collected profiles for a given trait within a given species/cv, correlation analyses of the phenotype variability *vs* the variability of metabolite levels/gene transcription and the allelic diversity will be performed through statistical matrices (co-variance/correlation/regression etc.)

6. VALIDATION OF MARKERS FOR TARGET METABOLIC PATHWAYS (IBBA/IMC/LN)

6.1 Re-test of markers selected on 1st year cultivars at distinct stages.

For gene trasncripts qRT-PCR methodologies will be used for they are faster and less costly than RNA-seq.

6.2 Assay of markers on novel cultivars grown in the native areas

A set of varieties of escarole/endive/puntarelle will be tested for the selected markers to validate the marker efficiency/behaviour.

6.3. Assay of markers on novel cultivars grown in swapped areas

The selected markers will be assayed on a set of test-varieties in swapped growth experiments (endive and escarole from Lazio will be grown in Puglia and apulian puntarelle will be grown in Lazio)

EXPECTED RESULTS (*max 4000 characters*)

- Typing, traceability and valorisation of escarole, endive and puntarelle varieties (*Chicorium* spp.) from Lazio and Puglia through:

-assessment of morphological, development and growth parameters (fine phenotyping) of study cv
-identification of metabolite clusters within the hydro and lipo-soluble fractions, including nitrates, aimed to inter-specific/intra-varietal distinction
-Optimisation of extraction protocols and analysis methods for the target metabolites (sesquiterpenes, inulin, citokinins, brassinosteroids, phytin, metals)
-production of profiles of target metabolite variability and anti-oxidant capacity in the study cultivars
-identification of diagnostic metabolic markers for typicality, traceability and nutritional value
-identification of gene sequences regarding synthesis, catabolism, transduction and transcriptional regulation of target metabolites from EST databases of *Cichorium* spp. And related species (*Lactuca* spp)
-production of custom expression chips (30000 oligomeric probes) for the transcriptional monitor of genes involved in the target metabolic pathways and of genes of potential interest
-production of gene expression variation for the cultivar characterization
-identification of allelic polymorphisms of target metabolic pathways in distinct cultivars
-production of an integrated database including phenotypic, metabolic, transcriptomic data and allelic polymorphisms of the study cultivars
-identification of functional markers through the integration and analyses of collected data and marker validation on novel cultivars, on cultivars grwn in distinct areas and at precocious developmental stages.
- characterisation of metabolites and genes that regulate: the bitterness grade, the post-harvest/shelf-life, dietetic, anti-oxidant, anti-tumour, anti-cholesterol and anti-feeding properties of cultivated *Cichorium* spp.

- Enhancement of the integration between CNR activities and the production of agro-enterprises in Lazio and Puglia
- Knowledge implement on the biology, physiology and genetics of crop species widely grown in Italy
- Increase of scientific competitiveness and technological innovation capacity in the integrated private-public system (applied agro-sciences)
- Creation of an open database including validated functional markers, useful for sector workers dealing with *Cichorium* spp. and related crop vegetables
- High scientific education for young researchers in the agro-food sector and widening of employment perspectives in the productive chain
- In the short term: value increase speed up of endive/escarole seeds; in a longer term: value increase of local putarelle and sustain of more widely diffused products
- Support for the marketing of vegetables with high quality for healthy and nutritional properties

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ACTIONS FOR THE TECHNOLOGICAL TRANSFER AND SPREAD OF RESULTS AND FOR THE LINKS WITH THE PRODUCTION SYSTEM

(*max 4000 characters*)

TECHNOLOGICAL TRANSFER

The trascriptome sequence of *C. intybus* e *C. endivia* and the release of userfriendly databases can be exploited by the enterprises involved for the development of other markers related to other traits of interest. Analogously, the set of SNP markers and the genotyping method (HMR-SNP), of free use for EZ and LN, will be developed to provide low cost services and extended to those farms that do not have adequate facilities. The markers will be used to type, trace and qualify non-marketed, local and wild varieties of *Cichorium* spp. The identification of functional markers for quality traits will lead to effective tools for the precocious selection of genotypes with desired traits within breeding populations. The project will allow transferring the knowledge acquired on the metabolic pathways affecting target traits to other related species (e.g.: lettuce, artichoke, radicchio). The "olistic" trait-linked markers will also facilitate the accurate description of novel cultivars in the national/international patenting.

DISSEMNINATION OF RESULTS

A website will be created including the access to the project description and up-dates, annual reports with open results, scientific articles released by the partners, technical sheets addressed to different sector categories (e.g.: breeders, consumers, dealers, associations etc.). The results will be disseminated through local institutions such as associations of horticultural valorisation from Viterbo (and Latina) and Bari, trans-regional groups (UIAPOA) and the support of technical secretary of agro-national organizations (Confagricoltura, Coldiretti). Side farms/SME involved in the agribusiness will be also invited to evaluate and promote the use of *Chicorium* spp. agro-waste (use of roots for inulin and bio-alcohol). VISP-LP will also benefit from the support and experience of the EZ marketing group in the issuing of brochures, presentations to diverse market segments, direct visits to farms and publicity on magazines.

SOCIAL AND ECONOMIC IMPACTS (referring to shires and provinces listed in the law 191/2009 art. 2 com. 44) (*max 4000 characters*)

Lazio and Puglia play a key role in the market of endive, escarole and puntarelle. As for endive and escarole (ISTAT 2010), the apulian production was 57.200t, 25% of national yield (n.y.) and 34% of "Mezzogiorno"(M.), whilst 7.300t was yielded in Lazio that was 3,5% of n.y. and 22% of Central Italy (C.I.). As for *C. intybus* (radicchio and chicory), the apulian production was ca. 32.300t (13% of n.y., 45% of M.). The **puntarelle from Galatina, Molfetta and Otranto** are listed as apulian traditional and typical products. In the Lazio shire, ca .100t was harvested (4% of n.y., 31% of C. I.), including the typical **puntarelle from Gaeta**. These vegetables are consumed as fresh products (called "I gamma" in France and Italy, acronym: I-G), but there is a strong interest in their use as minimally processed foods (fresh products chopped, washed, combined into multi-product packs labelled and bar-coded) since Italy is European leader in this sector (English synonym: fresh-cut; French-Italian synonyms: IV gamma, IV-G sectors). This sector has been remarkably activating both network of suppliers and related activities in the industries of seed production, food machinery and packaging. Several factors affect the quality of fresh-cut horticultural products such as the cultivar, cultural techniques, post-harvest conditions, process of transformation (% of waste), transport and selling conditions. **VISP-LP** will exert impact in the I-G and IV-G sectors, which often intersect and overlap, through the qualification of natural resources and traceability. Upstream the production chain, **VISP-LP** will support the direct valorisation of cv and sustainable methodologies for the rapid identification of target traits (moreover genotype bar-coding can be used to reveal parentage and genetic relations among *Chicorium* spp.). Downstream the production system, **VISP-LP** will produce data and tools responding to the standards required by the institutional and market laws. These request precise and rigorous info in the food labelling for the protection, benefit and conscious shopping of consumers. The profiles of metabolite, gene transcription and allelic variability identified through VISP-LP will function to effectively identify the origin of cultivars, and possibly the cultivation areas, in the interest of both producers and consumers, in support of the consolidation of I-G and IV-G compartments. With regard to agro-waste recycling sectors (pre-GDO), **VISP-LP** will disseminate results regarding sesquiterpenes and inulin which are expected to hook SME/farms from the herbal medicine and fuel areas. **VISP-LP** will produce results with direct fallouts (antioxidant properties on animal systems) or potential impact (anti cancer properties) with the medical industry. In the seed industry, research has generated added value to products which has lead to a multiplier economic effect on related industries and a direct increase of employment. As for the **escarole case** (source EZ), the improved cultivars generated elite seeds for healthy production and trading. One direct effect was the shift from direct seed sowing to transplanting, which impacted on related activities such as expansion of nursery farms, transplant machinery firms and increase of employment of specialized work units. The research on seed allows the penetration in new market segments, the spreading into diversified areas and a continuous/constant product supply. In ca. 10 years the seed price increased of 4.5 folds (from 2 to 9€/1000seeds); for a fixed cultivated area and product (ca. 10.000ha; 500billion seeds), the profit raised from 2 to 4.5 billion Euros, igniting new research lines, employment of crop-specialists, widening of cultivar supply in the I-G e IV-G sectors, quantified into a 1.5 fold increase of work units and in an intensive use of a research lab devoted to endive traits. A similar reasoning can go for the Apulian puntarelle. Due to the low cost of local varieties, the valorisation of improved open pollinated cvs can be esteemed as 9 fold raise in ca. 10 years (e.g.. from 1 to 9 euros/1000 seeds), generating similar effects as those described for the endive case. The presented analyses just dealt with the seed sector, which bears the costs for research; the distribution and trade sectors benefit of research valorisation up to 100 fold of profit increase.

ATTACHMENT-PERSONNEL LIST

IBBA-CNR-Director: Dr. Roberto Bollini (30/5/2013). Dr. Aldo Ceriotti (1/6/2013)

1. Donato Giannino, Researcher. Project Responsible. Expertise: mol. genetics and biotech of crop species (nitrates).
2. Giovanna Frugis, Senior Researcher. Expertise: plant molecular biology and biotechnology (Cytokinins).
3. Maria Adelaide Iannelli, Researcher. Expertise: plant molecular biology and biochemistry (Metals)
4. Chiara Nicolodi, Researcher. In vitro culture and crop phenotyping.
5. Giulio Testone, Researcher. Expertise: plant molecular biology and biotechnology (Inulin and Sesquiterpene).
6. Giovanni Mele, Senior Researcher. Expertise: plant molecular biology and biotechnology (Brassinosteroids).
7. Vincenzo Longo, Researcher. Expertise: plant physiology and biochemistry (Antioxidant capacity).
8. Francesca Sparvoli, Senior Researcher. Expertise: plant molecular biology and biotechnology (Phytin).
9. Sergio Mapelli, Technician. HPLC of crops and food.
10. Elisabetta Di Giacomo. Post-doct. Expertise: plant molecular biology and biotechnology, allelic variation (Inulin).

IMC-CNR. Director: Dr. Giancarlo Angelini (Operative Unit responsible)

1. Zeineb Aturki, Researcher. Expertise: Chromatography and Mass Spectrometry of crop species and food
2. Donatella Capitani, Senior Researcher. Expertise: NMR methodologies of crop species and food
3. Anatoly Sobolev, Researcher. Expertise: NMR methodologies and statistical analyses
4. Ornella Ursini, Senior Researcher. Chromatography and Mass Spectrometry of crop species and food (GC-MS, MS/MS/MS and high resolution MS FT-ICR)

"LA NORIA", ISPA – CNR – BA (http://noria.ba.cnr.it/azienda.phtml)

1. Maria Gonnella, Researcher of ISPA-CNR, Operative Unit Responsible. Expertise: nutrition and quality of leafy crops.
2. Francesco Serio, Researcher of ISPA-CNR. Expertise: Ionic Chromatography, management of indoor/glasshouse horticulture.
3. Pietro Santamaria, Researcher of Dept. Plant Production Sciences, University of Bari. Expertise: hydroponic cultures and agro-techniques to control the nitrate content in leafy crops.

ENZA ZADEN – VT (http://www.enzazaden.it/)

1. Giuseppe Arnesi. General Manager, Operative Unit Responsible. Expertise: plant selection and breeding, indoor/glasshouse horticulture.
2. Alessandro Schiappa. Breeder specialist (fennel, radicchio, scarole, endive), indoor/outdoor orticulture
3. Tiziano Biancari. Enzazaden agronomic specialist consultant.

ATTACHMENT 1- GANNT DIAGRAM

ACTIVITY	YEAR		
	I	II	III
1. Plant Material Management	1.1, 1.2	1.1, 1.2	1.1, 1.2
2. Metabolite Typing	2.1	2.2, 2.3	2.2, 2.3
3. Gene Expression Typing	3.1, 3.2	3.2, 3.3	3.3
4. Genetic Diversity Typing	4.1	4.2, 4.3	4.2, 4.3
5. Data Management & Functional Marker Identification	5.1	5.2, 5.3	5.2, 5.3
6. Marker Validation	-	6.1, 6.2	6.3

Legend.

1.1. Cultivation techniques and Production (EZ/LN)

1.2. Sampling, stocking and distribution (IBBA/ LN)

2.1 Extraction, assignment and quantification of metabolites (IBBA/IMC/LN)

2.2 Production of metabolite variability profiles in distinct cultivars and statistical analysis (IBBA/IMC/LN)

2.3 Production of anti-oxidant profiles in distinct cultivars (IBBA)

3.1 Identification of expressed gene sequences of target pathways (IBBA)

3.2 Transcriptome sequencing by RNa-seq (IBBA)

3.3 Transcriptional profiles by RNA-seq/ qRT-PCR and statistic elaboration (IBBA)

4.1 Polymorphism identification of genes involved in target pathways (IBBA)

4.2 Genotyping by allele polymorphism (IBBA)

5.1 Database production and management (IBBA)

5.2 Data collection and stock (IBBA/IMC/LN/EZ)

5.3 Identification of functional markers (IBBA/IMC/LN/EZ)

6.1 Re-test of markers selected on cv of the 1st year at distinct stages (IBBA/IMC/LN).

6.2 Assay of markers on novel cv grown in the native areas (IBBA/IMC/LN)

6.3. Assay of markers on novel cv grown in swapped areas (IBBA/IMC/LN)

MILESTONES (checkpoints tied to deliverables)

End of year 1. Production of sequenced transcriptome

End of year 2. Production of metabolic, transcriptional and genetic diversity profiles in *Chicorium* spp.

End of year 3. Production of markers effective for traceability and valorisation of local and new cultivars

ATTACHMENT- PLANT MATERIAL

ENDIVE

Chicorium endivia var. *crispum* (small leaves, narrow lamina, highly fringed margin); common names: curly endive, curly leaved endive.

Model study (summer-fall cycle)

E02.7162 (reference for RNA-seq)

Myrna (http://www.enzazaden.it/Products/leafvegets/endive/openfield/myrna.aspx)

ESCAROLE

C. endivia. var. *latifolium* (medium leaves, expanded lamina, lobed margin); common names: escarole, smooth endive, broad-leaved endive

Model study (summer-fall cycle)

Flester (http://www.enzazaden.de/products/leafvegets/endive/openfield/flester.aspx)

Confiance (http://www.enzazaden.de/products/leafvegets/endive/openfield/confiance.aspx#)

Novel cultivars for marker validation (summer-fall cycle) used in the second year of project

E02C.2314 (Myrna x Curly-leaved F_3), endive type

E02S.0338 (Confiance x Broad-leaved F_1), escarole type

A32861 (Curly-leaved F_5 x E02.7162), endive type

PUNTARELLE

C. intybus L. "Catalogna" group

Model study ecotypes (summer-fall cycle)

Galatina (reference for RNA-seq), a.k.a. "Puntarelle" (http://www.freshplaza.it/news_detail.asp?id=42637)

Molfetta, a.k.a. "Puntarelle" (http://www.freshplaza.it/news_detail.asp?id=42913)