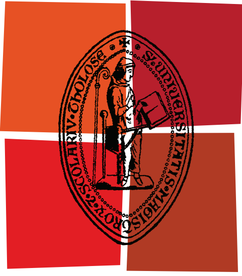

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A mio marito, gioia infinita dei miei giorni.

Ai miei genitori, punto fermo del mio essere.

Alla vita che porto dentro con tutto l'amore che posso.

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SUMMARY

Globe artichoke and cardoon, belonging to the *Asteraceae* (*Compositae*) family, are herbaceous perennial plants native to the Mediterranean area, which are traditionally grown as vegetables for the heads and the fleshy petiole leaves, respectively.

Italy is the richest reserve of globe artichoke (*Cynara cardunculus* var. *scolymus* L.) autochthonous germplasm, which is vegetatively propagated and well adapted to the different pedoclimatic conditions of the Country. On the basis of head harvest time, these traditionally grown genetic resources are classified as early or late distinct clonal varietal groups. Other distinct varietal groups are also identified, according to the morphological traits of the head, into four main typologies such as Violetto di Sicilia, Spinoso Sardo, Catanese and Romanesco.

In Central Italian environments, the Romanesco type, characterized by a spherical or sub-spherical and non-spiny green-violet heads, is widespread. In the last years, the development of *in vitro* technologies allowed the propagation of Romanesco globe artichoke type and its rapid expansion. As a result, the Romanesco clone C3 has been *in vitro* micropropagated and widely distributed to the farmers. This clone has replaced many Romanesco landraces traditionally grown in the Latium Region and has led either to a significant erosion of local genetic resources or a loss of diversity. Moreover, introduction of new seed-propagated F_1 hybrids such as Madrigal, Concerto, Opal, Tema, and Romolo, well suited to market demands, represents a further factor increasing the risk of genetic erosion for autochthonous germplasm. As regards Italian cultivated cardoon (*Cynara cardunculus* var. *altilis* DC) germplasm, there are few studies on its genetic characterization and identification and there is a lack of information about the genetic variability existing within and among accessions. For the wild cardoon (*Cynara cardunculus* var. *sylvestris* Lam.), no specialized crop is present and it represents mainly a weed in Italian environments.

The great variability existing in *Cynara* spp. has not been described, the nomenclature of Italian germplasm is not always very clear since there are many cases of homonyms. In addition to this risk of genetic erosion, in the last years, Italian globe artichoke sector is facing a crisis due principally to the appearance on the market of foreign products and to the high labor cost required for crop cultivation and harvesting. In order to overcome this crisis several possible uses of *Cynara* spp. were considered such as i) seeds for oil, ii) roots for inulin, iii) biomass for energy, iv) fiber for pulp and paper and as potential reinforcement in polymer composites v) green forage for ruminant feeding, vi) proteins as natural rennet for traditional cheese making, and vii) entire plant for metal-accumulation. These new possible applications

of the crop are linked principally to the European Union research support on new agricultural by-products (industrial raw materials) and led to an increasing interest also in aboveground globe artichoke biomass.

Considering these preliminary remarks, a strategy for valorizing Italian germplasm using biomass and biocompound production has been carried out during the three years of PhD program. In particular, nine Italian spring landraces (Ascolano, Campagnano, Castellammare, Jesino, Montelupone A, Montelupone B, Bianco di Pertosa, Pisa and Tondo Rosso di Paestum), ten ‗Romanesco' clones (S2, S3, S5, S11, S17, S18, S22,S23, Grato 1 and Campagnano), seven cultivated cardoon accessions (AFB, AFFG, AFGR, AFGI, AFM, AFM2, AFN) and AFS wild cardoon belonging to the joint germplasm collection of Tuscia University and ENEA have been considered in our PhD study.

Open field trials were conducted at the Experimental Field Station of ARSIAL (Latium Regional Agency for the Development and the Innovation of Agriculture) in Cerveteri, Rome and in Tarquinia, Viterbo while greenhouse experiments were carried out at the Experimental Station of Tuscia University in Viterbo (Italy).

The first objective of PhD work consisted in (i) characterizing agro-morphologically Italian germplasm using UPOV descriptors, (ii) assessing the genetic variability existing within and among landraces/clones and (iii) identifying and preserving genetic resources for the development of future plant breeding programs. As a result of this characterization, three genotypes have been selected and registered under the names of Michelangelo, Donatello and Raffaello. In order to analyze Italian *Cynara spp*. germplasm also from a biomass point of view, different traits explaining plant vigor and dry matter production have been considered. The aerial biomass yield resulted very high underlining the possibility of using this crop as raw industrial material. In particular, some genotypes, such as Ascolano, Campagnano, Pisa, Bianco di Pertosa for globe artichoke and AFFG and AFM for cardoon showed the highest biomass yield.

A focal point of PhD program was to set up biocompound extraction methods and analysis techniques to optimize polyphenol recovery from biomass of *Cynara* spp at a laboratory scale. In particular, conventional (i.e. Soxhlet and maceration) and modern extraction techniques (such as Microwave-Assisted Extraction MAE, and Accelerated Solvent Extraction ASE) have been compared and, using a full two-level factorial experimental design, ASE was found as the best extraction technique which allows to reduce extraction time and solvent consumption, increase nutraceutical yield and improvement of extract quality. Moreover, the

kinetics of biomass and bio-compound production has been evaluated and the optimal physiological stage to collect plant material grown in open field has been identified.
Biochemical characterization has been performed using the methods set up and collecting plant material in the optimal physiological stage identified in order to distinguish which genotypes were more suitable for bio-compound production purpose. As a result of morphological and biochemical characterization, genotypes were well distinguished from each other and were identified those which are the most suitable for food use, for biomass production and/or for dual-production (food and non-food).
The last focal point of PhD program was the development of an alternative technique for biomass and biocompound production in greenhouse grown conditions. The adaptation of globe artichoke and cardoon genotypes to floating system has been evaluated and the biochemical and molecular responses of the plant to the salinity stress have been also investigated.
Results obtained in the three PhD years, highlighted the possibility of using successfully some *Cynara* spp. genotypes for biomass and bio-compound production, in particular in open field condition. Also the real prospect of using some globe artichoke genotypes for food and non-food dual-production (biomass for biocompound extraction and heads for human food) has been underlined.

RÉSUMÉ

L'artichaut et le cardon, appartenant à la famille des *Asteraceae* (*Compositae*), sont des plantes pérennes herbacées natives du bassin méditerranéen, et qui sont traditionnellement cultivées comme plantes maraîchères, respectivement pour leurs têtes et leurs cardes (pétiole charnu des feuilles).

L'Italie est le pays possédant la plus importante collection de germoplasmes autochtones d'artichaut (*Cynara cardunculus* var. *scolymus* L.), la plante étant bien adaptée aux différentes conditions pédoclimatiques du pays. Sur la base de la période de récolte des têtes, ces ressources génétiques sont classées en groupes variétaux précoces ou tardifs. D'autres groupes variétaux distincts sont également identifiés selon les caractères morphologiques de leur tête en quatre principales typologies telles que Violetto di Sicilia, Spinoso Sardo, Catanese et Romanesco. Dans le centre de l'Italie, le type Romanesco, caractérisé par des têtes sphériques ou quasi-sphériques, d'un vert-violet et non épineuses, est étendu. Ces dernières années, le développement des techniques *in vitro* a permis la multiplication de l'artichaut Romanesco et sa rapide expansion. Le clone Romanesco C3 a ainsi été multiplié *in vitro* et grandement distribué aux agriculteurs. Il a remplacé de nombreuses races locales de Romanesco traditionnellement cultivées dans la région de Latium, contribuant de la sorte à une érosion significative des ressources génétiques locales et a une perte de la diversité. De plus, l'introduction d'hybrides F1 multipliés par graines, tels que Madrigal, Concerto, Opal, Tema, and Romolo, répondant bien à la demande du marché, représentent un facteur supplémentaire d'accroissement du risque d'érosion génétique pour les germoplasmes autochtones. Concernant le germoplasme de cardon cultivé (*Cynara cardunculus* var. *altilis* DC), seules quelques études sont disponibles sur son identification et sa caractérisation génétique, et les informations sur la variabilité génétique existant entre les accessions sont manquantes. Le cardon sauvage, quant à lui, n'est pas du tout cultivé; il est davantage considéré comme une adventice dans le paysage italien.

La grande variabilité existant entre les espèces de *Cynara* n'est pas correctement décrite, la nomenclature du germosplasme italien n'est pas toujours très claire et il existe plusieurs cas d'homonymes. Ajouté à ce risque d'érosion génétique, le secteur de l'artichaut italien a du faire face ces dernières années à une crise causée principalement par l'apparition sur le marché de produits étrangers et par les coûts élevés de mise en culture et de récolte. Afin de surmonter cette crise, de nombreuses valorisationss du *Cynara* ont été envisagées, à partir i) des graines pour l'obtention d'huile, ii) des racines pour l'inuline, iii) de la biomasse pour l'énergie, iv) des fibres pour la pulpe et le papier et comme renfort potentiel des composites

en polymères, v) du fourrage pour l'alimentation des ruminants, vi) de protéines comme présure naturelle pour la fabrication traditionnelle de fromages, et vii) la plante entière pour ses propriétés phytorémédiatrices. Ces potentielles applications pour la culture ont pu voir le jour grâce au support de l'Union européenne pour la recherche sur les co-poduits issus de l'agriculture, et ont mené à un intérêt croissant pour la biomasse entière d'artichaut.

Considérant ces remarques préliminaires, une stratégie, qui permettrait de valoriser le germoplasme italien par la production concomitante de biomasse et de biocomposés, a été mise en place durant ces trois ans de doctorat. Neuf races locales de printemps (Ascolano, Campagnano, Castellammare, Jesino, Montelupone A, Montelupone B, Bianco di Pertosa, Pisa et Tondo Rosso di Paestum), dix clones ‗Romanesco' (S2, S3, S5, S11, S17, S18, S22,S23, Grato 1 et Campagnano), sept accessions de cardon cultivé (AFB, AFFG, AFGR, AFGI, AFM, AFM2, AFN) et le cardon sauvage AFS, co-détention de l'Université de la Tuscia et de l'ENEA, ont été considérés dans cette étude.

Les essais en champ ont été conduits à la station expérimentale de l'ARSIAL (Agence Régionale de Latium pour le Développement et l'Innovation de l'Agriculture) à Cerveteri, Rome, et à Tarquinia, Viterbe, alors que les expérimentations sous serre ont été menées à la Station Expérimentale de l'Université de la Tuscia, à Viterbe (Italie).

Le premier objectif de ce travail de doctorat consistait en i) la caractérisation agro-morphologique du germoplasme italien par le biais de descripteurs UPOV, ii) l'évaluation de la variabilité génétique à l'intérieur et entre les races/clones et iii) l'identification et la préservation des ressources génétiques pour le développement de futurs programmes d'amélioration des plantes. Suite à cette caractérisation, trois génotypes ont été sélectionnés et enregistrés sous les noms de Michelangelo, Donatello and Raffaello. Afin d'analyser également le germoplasme italien de *Cynara* d'un point de vue de la biomasse, différents traits expliquant la vigueur de la plante et la production de matière sèche ont été considérés. Le rendement en biomasse aérienne s'est révélé très élevé, soulignant la possibilité d'utiliser cette culture comme matière première industrielle. Quelques génotypes en particulier, comme Ascolano, Campagnano, Pisa, Bianco di Pertosa pour l'artichaut et AFFG et AFM pour le cardon ont donné les meilleurs rendements en biomasse.

Un point particulier du programme de thèse était de mettre au point les méthodes d'extraction de biocomposés et les techniques d'analyse afin d'optimiser le rendement en polyphénols à partir de la biomasse de *Cynara* à l'échelle laboratoire. Des techniques d'extraction conventionnelles (soxhlet et macération) et plus modernes (extraction assistée par micro-ondes et extraction au solvant accélérée) ont été comparées, et grâce à un plan d'expérience

factoriel complet à deux niveaux, l'ASE a été reconnue comme étant la meilleure technique, qui permet de réduire la durée d'extraction et la consommation de solvant, d'accroître le rendement en molécules d'intérêt, et d'améliorer la qualité de l'extrait. De plus, les cinétiques de production de biomasse et de biocomposés ont été évaluées et le stade physiologique optimal pour collecter le matériel végétal en champ a été identifié.

La caractérisation biochimique a été réalisée grâce aux méthodes mises au point et en collectant le matériel végétal au stade physiologique optimal identifié afin de distinguer les génotypes les plus appropriés pour la production de biocomposés. De façon préliminaire, les cinétiques d'accumulation de biomolécules dans la plante ont été étudiées afin de détecter le stade de développement auquel l'accumulation de polyphénols est maximale. Suite aux caractérisations morphologiques et biochimiques, les génotypes ont pu être correctement distingués les uns des autres et les plus appropriés pour un usage alimentaire, pour la production de biomasse et/ou pour une double valorisation (alimentaire et non alimentaire) ont été identifiés.

Le dernier point du programme de thèse était centré sur le développement d'une technique alternative de production de biomasse et de biocomposés en conditions de croissance sous serre. L'adaptation de l'artichaut et du cardon à la culture hydroponique a été évaluée, et les réponses biochimiques et moléculaires de la plante au stress salin ont été également recherchées.

Les résultats obtenus durant ces trois années de thèse mettent en exergue la possibilité d'utiliser avec succès certains génotypes de *Cynara* pour la production de biomasse et de biocomposés, en particulier en condition de culture en plein champ. La perspective réelle d'utiliser certains génotypes d'artichaut pour une double valorisation alimentaire et non-alimentaire (la biomasse pour l'extraction de biocomposés et les têtes pour l'alimentation humaine) a ainsi été soulignée.

RIASSUNTO

Il carciofo e il cardo, appartenenti alla famiglia delle *Asteraceae* (*Compositae*), sono delle piante erbacee, perenni, originarie del basino del Mediterraneo e tradizionalmente coltivate a scopo alimentare rispettivamente per i capolini e le foglie carnose.

L‘Italia rappresenta una delle più ricche riserve di germoplasma autoctono di carciofo (*Cynara cardunculus* var. *scolymus* L.), il quale è propagato vegetativamente e ben adattato alle differenti condizioni pedoclimatiche del nostro Paese. Sulla base dell‘epoca di maturazione commerciale dei capolini, i genotipi di carciofo vengono classificati in tipologie precoci e autunnali. Un altro criterio di classificazione, basato sulle caratteristiche morfologiche dei capolini, suddivide i suddetti genotipi in quattro gruppi varietali: ‗Violetto di Sicilia‘, dello ‗Spinoso Sardo‘, del ‗Catanese‘ e del ‗Romanesco‘.

La tipologia ‗Romanesco‘, caratterizzata da capolini inermi di forma rotonda o ellittica e di colore verde-viola, è molto diffusa negli ambienti di coltivazione cinaricola dell‘Italia centrale. Negli ultimi anni, la messa a punto della tecnica di propagazione *in vitro* per il carciofo*,* ha portato ad una rapida diffusione di cloni della tipologia ‗Romanesco‘. In particolare, il clone C3 propagato *in vitro* ha conosciuto una grande diffusione nelle aree di coltivazione cinaricola, sostituendo molte varietà afferenti alla tipologia ‗Romanesco‘ tradizionalmente coltivate nel Lazio e determinando rischi di erosione genetica per queste risorse genetiche. Inoltre, l‘introduzione sul mercato di nuovi ibridi F_1 come Madrigal, Concerto, Opal, Tema, and Romolo, meglio rispondenti alle richieste dei consumatori, rappresenta un ulteriore fattore di rischio per la perdita del germoplasma autoctono.

Per quanto riguarda il cardo coltivato (*Cynara cardunculus* var. *altilis* DC), esistono pochi studi sulla caratterizzazione genetica e sull‘identificazione del germoplasma autoctono e mancano dati riguardanti la variabilità genetica esistente tra ed entro accessioni diverse. Per il cardo selvatico (*Cynara cardunculus* var. *sylvestris* Lam.), non sono presenti coltivazioni specializzate ed esso rappresenta un‘infestante negli ambienti di coltivazione cinaricola italiani.

L‘ampia variabilità esistente nel germoplasma di *Cynara* spp. non è stata descritta; la nomenclatura delle risorse genetiche non è spesso chiara e spesso si trovano casi di omonimia. Oltre a questo rischio di erosione genetica, negli ultimi anni assistiamo anche ad una forte crisi del settore cinaricolo dovuta alla comparsa sul mercato di prodotti stranieri e all‘alto costo di produzione e di raccolta previsti dalla coltura. Negli ultimi decenni, per far fronte a questa crisi, sono stati considerati diversi possibili usi per la specie quali, ad esempio, i) la produzione di olio dai semi; ii) l‘estrazione di inulina dalle radici; iii) la produzione di

energia dalla biomassa; iv) l'estrazione della fibra per l'industria cartaria; v) la produzione di foraggio da destinare all'alimentazione animale; vi) la produzione di formaggi come caglio vegetale mediante impiego di estratti fiorali; vii) l'accumulo di metalli pesanti nella pianta.

Lo studio di questi nuovi possibili utilizzi è stato reso possibile grazie ai finanziamenti della Comunità Europea che ha incentivato e supportato la ricerca nel settore delle agrorisorse e ha portato anche ad un forte interesse nell'uso della biomassa di carciofo.

Nel corso dei tre anni di dottorato è stata portata avanti una strategia di ricerca volta alla valorizzazione del germoplasma italiano di carciofo e cardo basata sull'uso della biomassa per l'estrazione di biomolecole. In particolare, sono stati considerati nove genotipi autoctoni primaverili (Ascolano, Campagnano, Castellammare, Jesino, Montelupone A, Montelupone B, Bianco di Pertosa, Pisa and Tondo Rosso di Paestum), dieci cloni della tipologia ‗Romanesco' (S2, S3, S5, S11, S17, S18, S22, S23, Grato 1 and Campagnano), sette genotipi di cardo coltivato (AFB, AFFG, AFGR, AFGI, AFM, AFM2, AFN) e un genotipo di cardo selvatico (AFS), tutti appartenenti alla collezione di germoplasma dell'Università della Tuscia e dell'ENEA. Le prove sperimentali di campo sono state svolte presso l'Azienda sperimentale ARSIAL (Agenzia Regionale per lo Sviluppo e l'Innovazione in Agricoltura) di Cerveteri (Roma) e di Tarquinia (Viterbo), mentre le prove di serra sono state condotte nella Azienda Sperimentale dell'Università della Tuscia, a Viterbo (Italia).

Il primo obiettivo del dottorato ha riguardato i) la caratterizzazione agro-morfologica del germoplasma italiano per mezzo dei descrittori UPOV, ii) la valutazione della variabilità genetica esistente tra ed entro i genotipi, e iii) l'identificazione e la conservazione delle risorse genetiche in collezione per lo sviluppo futuro di programmi di miglioramento genetico.

La caratterizzazione del germoplasma ha portato alla selezione di tre genotipi validi per la produzione edule che sono stati validati e proposti per l'iscrizione al Registro Nazionale delle Varietà con i nomi di Michelangelo, Donatello e Raffaello. Al fine di caratterizzare il germoplasma anche da un punto di vista della biomassa, sono stati anche considerati diversi caratteri morfologici atti a descrivere il vigore della pianta e a quantificare la produzione di sostanza secca. La produzione di biomassa è risultata davvero molto interessante nel nostro lavoro e si è sottolineato il grande potenziale di utilizzo della coltura nel settore industriale. Alcuni genotipi come Ascolano, Campagnano, Pisa e Bianco di Pertosa, per il carciofo, e AFFG e AFM, per il cardo, sono risultati davvero interessanti dal punto di vista della produzione di biomassa.

Un altro importante obiettivo del dottorato è stato quello di mettere a punto un metodo per l'estrazione di polifenoli al fine di ottimizzare le rese. Sono state messe a confronto tecniche

convenzionali (ad esempio metodo Soxhlet e macerazione) ed altre più innovative (ad esempio Microwave Assisted Extraction - MAE e Accelerated Solvent Extraction - ASE). La tecnica ASE ha fornito i migliori risultati, consentendo anche di ridurre i tempi d'estrazione e il consumo di solvente, fornendo al tempo stesso alte rese e elevata qualità degli estratti.
E' stato anche condotto lo studio della cinetica di accumulo della biomassa e delle biomolecole in campo al fine di individuare il momento migliore per la raccolta del materiale in campo ed ottimizzare le rese. Una volta messo a punto il metodo d'estrazione e individuata l'epoca ottimale per il prelievo delle biomasse in campo, si è andati ad effettuate le estrazioni al fine di caratterizzare biochimicamente i genotipi in prova e di selezionere quelli più rispondenti a tale attitudine.
La caratterizzazione morfologica e biochimica hanno permesso di ben identificare i genotipi e di selezionare quelli più adatti per la produzione di capolini (‗food production'), di biomassa o per la duplice attitudine (food and non-food).
Nel corso del dottorato si è anche valutato un sistema alternativo per la produzione di biomassa e di biomolecole in serra. E' stata valutata l'adattabilità del carciofo e del cardo al sistema di coltivazione fuori suolo (*floating system*), soprattutto per evidenziare risposta biochimica e molecolare delle piante sottoposte allo stress salino in termini di aumento nella produzione di sostanze fenoliche.
I risultati ottenuti nel corso del dottorato hanno messo in luce la reale possibilità di usare alcuni genotipi di carciofo e di cardo per la produzione edule, altri per quella di biomassa e di biomolecole, soprattutto in condizioni di pieno campo. Si è inoltre delineata una reale prospettiva per l'impiego di alcuni genotipi di carciofo in una duplice destinazione commerciale (biomassa per l'estrazione di biomolecole e capolini per la destinazione alimentare).

Introduction

1.1 *CYNARA* SPP. ORIGIN AND DOMESTICATION

The origin of the genus *Cynara* dates back to Würm‘s glaciation era, when an unknown wild cardoon was spread from the Southern area of the Mediterranean basin to the Sahara region. At the end of glaciation era, the species had even invaded the Eastern and Western part of the Mediterranean basin. In fact, some wild cardoons still today exist in these areas; for example, this is the case of *C. syriaca*, *C. cyrenaica*, *C. cornigera* in the Eastern part of Mediterranean region and *C. baetica*, *C. algarbienis*, *C. humilis* in the western one (Pignone and Sonnante, 2009). Genetic differences between the types coming from these two areas are evident.

The wild progenitor of globe artichoke is at present widespread both in Eastern and Western part of Mediterranean basin but presents genetic differences between the types coming from the different two areas.

Cynara cardunculus spp. was known by Greeks and Romans, as reported by Columella in *De Rustica* and by Plinio in *Naturalis Historia*. In 371–287 BC, Theophrastus referred that cultivated artichoke cultivation was already present in Sicily but not in Greece (Montelucci, 1962) while, in 1890, De Candolle supposed that cultivated artichoke was unknown in classical times. On the contrary, in 1967, Marzi affirmed that globe artichoke cultivation was widespread at the beginning of the Christian era and, in 1989, on the basis of Plinius and Columella literary works, Foury deduced that globe artichoke cultivation started around in the 1st century AD.

Reliable information on globe artichoke spreading dates back to XV century AD, when it has been introduced by Filippo Strozzi from Sicily to Campania and Tuscany regions (Bianco *et al.*, 1990). These reports support the hypothesis that globe artichoke was originally domesticated in Sicily and then spread by Arabs to the other regions (Pignone and Sonnante, 2004). At the begininnig of 16th century, cultivation of globe artichoke was widespread to all countries of Mediterranean area and to Central Europe and United States (Oliaro, 1967).

The name of the genus *Cynara* could come from the Greek word ‗kinara‘, which means spiny plants while ‗scolymus‘ is a Greek word indicating a pointed-shape (Bianco *et al.*, 1990). Italian (carciofo), Spanish (alcachofa), and Portuguese (alcachofra) current names used for globe artichoke come from the Arabic word ‗qarshuff‘ while, in English, French, German and in other languages of North Europe, the current name originates from the Latin composed word ‗articoculum‘, which means artus=spiny and cocolum=sphere (Bianco *et al.*, 1990; Pignone and Sonnante, 2009). The origin of the name suggests that Arabs played a crucial role on the crop spreading in Mediterrenean area, while Italy was an important connection to

the spread of globe artichoke into Central and Northern Europe. In *Naturalis historia,* Plinio referred on the cultivation of cardoon and defined the plant as precious.

1.2 Taxonomy and nomenclature

Globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.), Fiori], cultivated cardoon (*C. carduncul*us var. *altilis* DC.= *C. cardunculus* subsp. *cardunculus*) and wild cardoon [*C.cardunculus* var. *sylvestris* (Lamk) Fiori], all belonging to the *Asteraceae* family (*Asterales* order), subfamily *Tubuliflorae*, *Cynara* genus, are angiosperm dicotyledonous diploid plants with 2n = 2x = 34 chromosomes (Bianco *et al.*, 1990; Pignone and Sonnante, 2009).

Fig. I.1 Globe artichoke, cultivated and wild cardoon heads

In 1753, Linneo named globe artichoke as *Cynara scolymus* L. but, being its taxonomic position quite questionable for the lack of knowledge of the wild form, in 1982, Pignatti considered this species as a subspecies of *C. cardunculus* L. and classified globe artichoke as *C. cardunculus* L. subsp. *scolymus* (L.) Hayek. In 1848, on the basis of morphological profile, De Candolle for the first time proposed that globe artichoke would derive from the species *C. cardunculus* L. var. *sylvestris* (Lamk) Fiori and, in 1981, Basnizki demonstrated that there was a fully cross-compatibility between these two *C. cardunculus* forms with the possibility to obtain fertile inter-varietal hybrids (Basnizki and Zohary, 1994). The primary wild gene pool (GP1) of the cultivated globe artichoke is represented by that of wild cardoon, which is also considered as the wild progenitor of the cultivated cardoon (Bianco *et al.*, 1990; Basnizki and Zohary, 1994).

The genus *Cynara* consists of two groups, the first one of C. *cardunculus* comprises globe artichoke as well as wild and cultivated cardoon while the second one, more heterogeneous, includes seven wild species characterized by large spiny leaves and heads such as *C. syriaca* Boiss, *C. auranitica*, *C. cornigera*, *C. algarbiensis* Cosson, *C. baetica*, *C. humilis* L. and *C.*

cyrenaica (Wiklund, 1992; Rottenberg and Zohary, 1996; Pignone and Sonnante, 2009). This second group is considered the secondary wild gene pool (GP2) of the artichoke crop (Rottenberg and Zohary, 1996a). *C. syriaca,* coming from the South Syria and Israel, is a wild plant characterized by long floral stem and large flower heads. *C. baetica*, coming from the Western part of the Mediterranean area, is characterized by medium size plants with black coloured bract margin and white coloured leaf underside; it consists of two subspecies: subsp. *baetica* and *maroccana*.

C. humilis, widespread in the South-Western Iberian Peninsula to the North region of Marocco and Algeria, is characterized by small-size plants, winged achenes and revolute margin leaves. *C. cyrenaica*, is widespread in Lybia, while *C. cornigera,* characterized by small-size plants with short floral stem and white coloured flower heads, come from the Eastern part of the Mediterranean region. *C. auranitica,* native to the South-East of Turkey and to the South-West of Syria, is often misclassified as *C. syriaca*. *C. algarbiensis,* native to the South-West of Iberian Peninsula, is characterized by small plants.

Fig. I.2 From the left to the right: heads from C. cyrenaica, C. baetica and C. syriaca

1.3 BOTANICAL DESCRIPTION

Globe artichoke and cardoon are herbaceous perennial plants. If seed propagated, they are characterized by a main tap-root and several secondary roots while, plants vegetatively propagated by offshoots or underground dormant buds present fleshy adventitious roots that, during the growing season, lose the nutrient assimilation function to take that of reserve organ. At the beginning of the growth, a leaf rosette spread from plant caule (compressed stem) and, at the base of the floral stem, axillary buds producing lateral offshoots are originated. Each offshoot produces adventitious roots which, at the end of the first growth year, become rhizomes (Bianco *et al.,* 1990; Bianco and Calabrese, 2009).

After the winter, an apical bud appears on the caule apex and, after an induction period which depends on temperature and photoperiod, a floral stem develops and can reach above 1.20 m in height for globe artichoke and above 3.00 m for cardoon. The floral stem is erect,

cylindrical-shaped and grayish-green coloured. Other buds develop on branching floral stems, which depart from leaf axis of the main stem. The main floral stem and other stems of different order (primary, secondary, tertiary and high-order) terminate at the apex with a flower bud. Alternate leaves, green-grayish coloured and more or less incised, are present on the main and other order stems. Leaf shape and size depend on the genotype and on growth stage. Globe artichoke and cultivated cardoon are characterized by leaves without spines while, on the contrary, wild cardoon present many or few spines on the leaves (*Figure I.3*) depending on genotype.

Fig. I.3 Wild cardoon plant

The flower head, is composed by a petiole and a fleshy receptacle where multiple rows of bracts are inserted in the external part while, in the internal one, several florets are included (*Figure I.4*) (800-1200 for globe artichoke and 300-400 for cardoon). The flower head bracts are different in colour, ranging from green to purple, and in shape (elliptical or elongated, more or less incised); generally, they are larger and more fibrous outside but smaller and tenderer inside (Bianco and Calabrese, 2009). In general, globe artichoke and cultivated cardoon have not spines on the bracts while wild cardoon is characterized by spiny bract apex.

Fig. I.4 Globe artichoke plant

The head florets are hermaphrodites, blue-violet-white coloured, with fused petals at the base which form a tubular corolla. The flowering is centripetal and proterandric. Self-pollination is avoided by the proterandry of the flowers (stigmatic surfaces mature two or three days after pollen shedding).

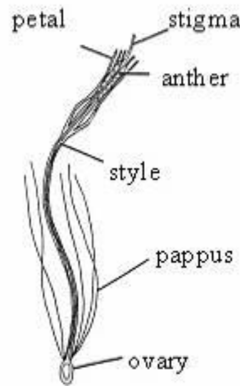


Fig. I.5 Head florets

The fruits are achenes of elliptical shape, grey/ brown/ black coloured with a weight ranging from 30-70 mg for globe artichoke and 25-45 mg for cardoon which mature 60 days after fecondation (Bianco and Calabrese, 2009). Achenes have a prominent pappus that contributes to the wind dispersion.

Fig. I.6 Globe artichoke achenes

1.4 Biology and ecology

The development cycle and the main plant growth stages of the species shown in Mediterannean environments have been widely described as follows: plant sprounting in September-October, winter leaf rosette in November, stem elongation in April-May, full blossom in June, ripe fruits in July, fully dry aerial biomass in August (Raccuia and Melilli, 2007, 2010) (*Figure I.7*).

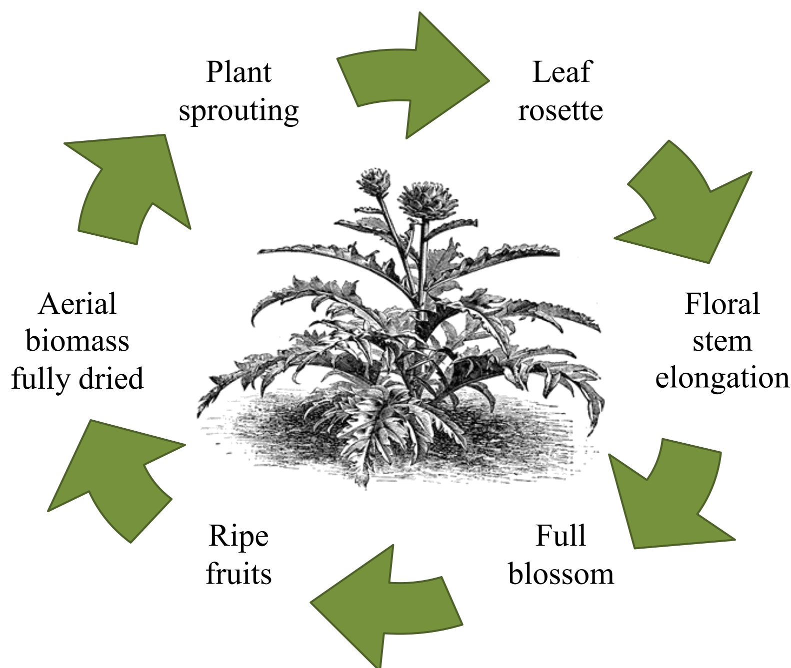


Fig. I.7 Main globe artichoke and cardoon plant growth stages

The optimal seed germination is generally reached at temperatures of 15-20ºC (Bianco *et al.*, 1990). In the Mediterranean environment, the aerial part of plants both seed propagated and vegetatively propagated by offshoots or ovoli at the end of the first growing season, dries up in May-June period leaving in the soil the rhizome as a reserve organ. Normally, in specialized cultivation, the ‗cut-back' operation, which consists in cutting back the aerial part of the plant slightly below the soil surface to induce offshoot spread, is applied to start a new cropping cycle. After the first autumn rains (September) or in specialized cultivations, after forced irrigation in August, offshoots emerge from the rhizomatous stem under the surface of the soil. The differentiation phase of the offshoots is not contemporaneous and, therefore, shoots of different ages are present on the same plant. The number of offshoots depends on the age of the plant and ranges from 1-12 or more. Each offshoot forms a rosette of large leaves and, at the center, grow up the flower-bearing stalks. Edible heads are produced both at the tips of these elongated stalks and their branches.

The minimal biological temperature ranges between 7-9ºC, while the lethal one is lesser than -10ºC (Bianco *et al.*, 1990). Plants are also tolerant to high temperatures (> 30°C) that, however, tend to decrease the quality of edible heads. Temperatures of 20-22ºC in the day and 12-14ºC in the night represent the optimal values to obtain good quality and high yields. The species is an obligated long-day plants with a critical photoperiod of 10.5 hours. Both globe

artichoke and cardoon can be grown on a wide range but well-drained soils with a pH between 6.4 and 7 as optimum. The species is quite tolerant to salinity and intolerant to prolonged waterlogging.

The seasonal water requirements of globe artichoke correspond to 4,000-5,000 m^3 per hectare and per year, with the highest crop water requirement during head formation stage (Bianco *et al.*, 1990). The species has not high nutrient demands and the removal values are estimated at 286-44-368-178-157 and 28 kg ha^{-1} of N, P_2O_5, K_2O, Ca, Na and Mg, respectively for globe artichoke (Bianco *et al.*, 1990) and at 300-100-400 kg ha^{-1} of N, P_2O_5, K_2O, respectively for cardoon (Bianco and Calabrese, 2009).

1.5 Geographic distribution and production

The globe artichoke harvested area in the world is about 125,000 hectares, of which about 75% are located in the Mediterranean area and, in particular, in Italy (50,321 ha), Spain (13,200 ha), Egypt (8,909 ha), France (8,690 ha) and Morocco (3,710 ha). In the last years, globe artichoke cultivation has been also extended to China (9,500 ha), to USA (2,910 ha), as well as to some countries of South America such as Peru (6,848 ha), Chile (4,651 ha), Argentina (3,700 ha) (FAOSTAT, 2010) (*Table I.1*).

Table I.1 Globe artichoke harvested area (Ha) and production (tonnes) in the World

Country	Harvested area	Head production
Italy	50,321	480,112
Spain	13,200	166,700
China	9,500	59,900
Egypt	8,909	215,534
France	8,690	42,153
Peru	6,848	127,503
Chile	4,651	35,000
Morocco	3,710	45,460
Argentina	3,700	84,800
United States of America	2,910	40,820
Algeria	2,700	39,200
Tunisia	2,400	19,000
Turkey	2,400	29,070
Greece	2,100	20,400
Islamic Republic of Iran	870	15,800
Syrian Arab Republic	510	6,100
Israel	330	2,638
Uzbekistan	220	2,200
Malta	145	1,566
Mexico	133	1,626
Cyprus	121	2,760
Lebanon	60	670
Réunion	-	250
Romania	50	500
Zimbabwe	20	270
Kazakhstan	10	100
Kenya	-	20
Switzerland	3	1
Zambia	750	-

FAOSTAT, 2010

As regard globe artichoke production, Mediterranean countries such as Italy (480,112 t), Egypt (215,534 t), and Spain (166,700 t) are the world leaders accounting almost 60% of the overall world production (*Table I.1*). In the last years, Peru (127,503 t), Argentina (84,800 t), China (59,900 t) as well as Morocco (45,460 t), Algeria (39,200 t), USA (40,820 t), Chile (35,000 t) and Turkey (29,070 t) provided high productions (FAOSTAT, 2010). In particular, production remained stationary or decreased in some countries where globe artichoke is a typical cultivation while, in some emerging countries, showed a very positive increasing

trend. China and some countries of South America (i.e. Peru, Argentina and Chile) confirm this remark.

Italy represents the world leader for globe artichoke production and its cultivation area is exceeded only both by tomato and potato. The majority of the globe artichoke cultivation is located in the central and southern areas of Italy; in particular, Apulia (16,525 ha), Sicily (14,765 ha) and Sardinia (13,267 ha) represent the most important production areas (*Figure I.8*). Generally, in south Italy, globe artichoke is grown for autumn-winter (e.g. ‗Violetto di Sicilia', ‗Spinoso Sardo', ‗Brindisino'), and early spring harvesting while, in central Italy, it is cultivated only for spring production (e.g. ‗Campagnano', ‗Castellammare', ‗C3', ‗Terom', ‗Violetto di Toscana').

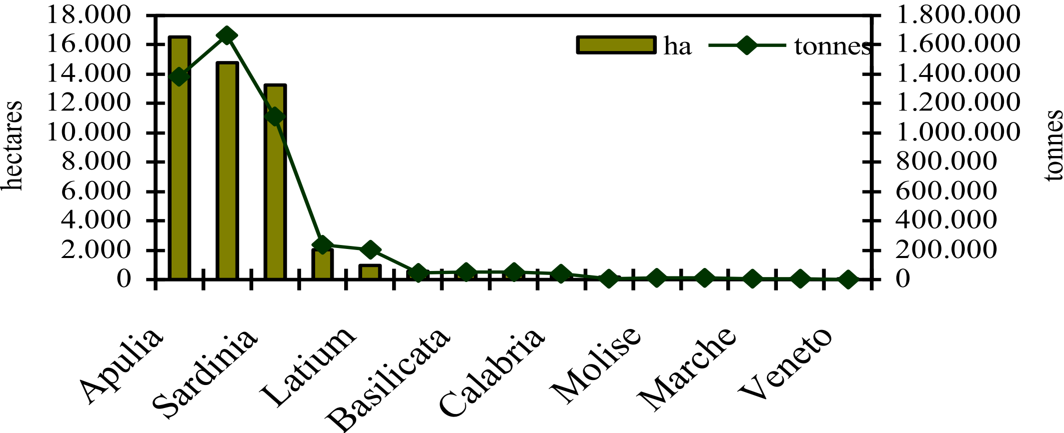


Fig. I.8 Italian globe artichoke harvested area (Ha) and head production (tonnes) (ISTAT, 2011)

Cardoon ditribution is found principally in the Mediterrenean area, its origin center. In the last years, the cardoon growing areas decreased significantly and there is a lack of information about the distribution and the production of the crop. In Italy, the cultivated cardoon cultivation decreased from 3,500 ha in 1960 to about 800 ha in 1980, and only in the last years the increased interest for biomass has led to a new interest also for cardoon. Today, the land area devoted to this crop (about 2,000-3,000 ha, even if official statistics are an understimated) is mainly localized in Spain, Italy, France and Greece (Ierna and Mauromicale, 2010).

1.6 GENETIC RESOURCES

Italy represents the richest globe artichoke primary cultivated ‗*gene pool*‘ with many distinct varietal groups well adapted to different local environments and consumers (Mauromicale and Ierna, 2000; Lanteri *et al.,* 2001, 2004a, Saccardo, 2009). The total number of globe artichoke genetically distinct genotypes cannot be easily determined and, even if 100-120 genotypes are at present estimated, only 11-12 can be considered more important for their commercial interest (Basnizki and Zohary, 1994; Mauro *et al.,* 2009). The measurement of genotype divergence on the basis of capitulum morphology by multivariate analysis allowed to identify four main following groups (*Figure I.9*):

1. ‗Spinoso‘, containing genotypes with long sharp spines on the bracts and leaves;
2. ‗Violetto‘, with medium-sized and violet-coloured heads;
3. ‗Romanesco‘, with spherical or sub-spherical heads;
4. ‗Catanese‘, with relatively small and elongated heads.

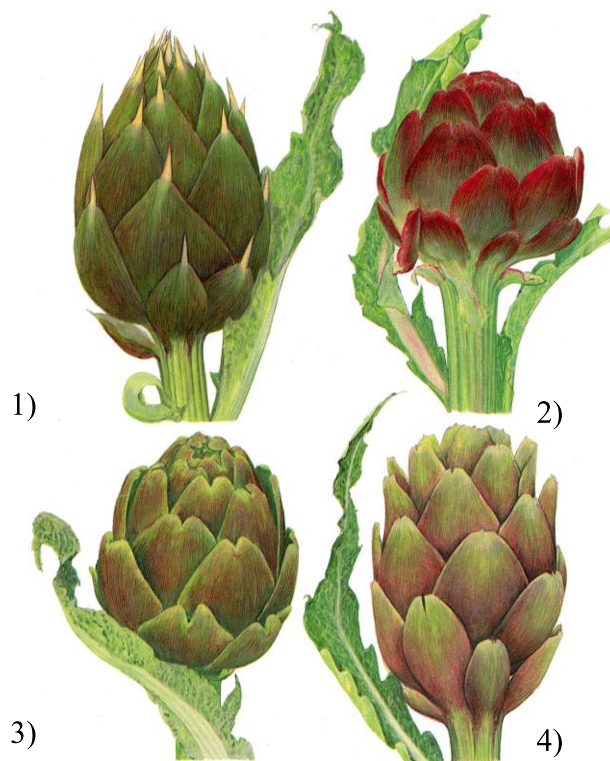


Fig. I.9 Four groups of globe artichoke classified on the basis of head morphological traits: 1) 'Spinoso', 2) 'Violetto', 3) 'Romanesco',4) 'Catanese'

On the basis of head appearance and harvest time, globe artichoke germplasm has been also classified as early and late flowering types (Ierna and Mauromicale, 2000; Acquadro *et al.*, 2010). The early flowering type, also named ‗reflowering‘ type, includes Violetto di Sicilia,

Spinoso Sardo, Spinoso di Palermo, Spinoso ligure, Masedu, Brindisino, Catanese, Violet de Provence, Tudela, Locale di Mola and produces heads continuously from the autumn to the spring. In Italy, this typology is grown mainly in the Southern part of the Country and in the islands (i.e. Sicily and Sardinia). The late flowering type, which is grown mainly in the central regions of Italy, produces during spring and early summer and includes Romanesco and Violetto di Toscana genotypes (Ierna and Mauromicale, 2000; Saccardo, 2009). Apart from these major classification groups, many traditional landraces cannot be classified according to these varietal group traits. In Italy, this occurrence could be related to the wide genetic variability existing and for which, therefore, our Country represents a largest reservoir of artichoke germplasm (Pignone and Sonnante, 2004; Sonnante *et al.,* 2007). Recently, morphological analysis of 104 artichoke accessions based on eight quantitative traits allowed the identification, using cluster analysis, of five main varietal groupscharacterized by similar morphological characters and presumably similar genomes (Elia and Miccolis, 1996). However, up to date different nursery companies produce some micropropagated cultivar and among these Apollo, Explorer 1 and 2, Life, Albani, Tema, Sms 09, Saturn A, Temasar, Albert, as well as different selections of clone C3 (i.e. C3 S4, C3 S2, C3 P2, C3 pepe, C3 rosso b, C3 zar 08 and 09, C3 vz, C3 niscemi, C3 battipa) are widespread on the Italian market.

Other genotypes, including the Spanish cv. Tudela (Dellacecca *et al.*, 1976; Porceddu *et al.*, 1976), belong to an intermediate group while French germplasm has been classified into two main group: 1) the ‗Brittany‘ typology characterized by large green coloured heads (ex.: Camus de Bretagne, Caribou, Camerys) and 2) the ‗Midi‘ typology, which includes genotypes coming from South France (ex.: Violet de Provence and Violet de Hyères) characterized by purple heads (Saccardo, 2009).

As regards cultivated cardoon, different genotypes are available on the market and they are mainly distinct from each other on the basis of morphological leaf traits such as spines presence/absence on the leaves, leaf colour (i.e. green, white, self-bleaching) and leaf rib size. In general, some genotypes are named with the name of cultivation area and, for this reason, it is difficult to determine the total number of genetically distinct genotypes for this species. In Italy, the most cultivated accessions registered to the Italian Register of the Varieties are as follows: ‗Bianco avorio‘, also named ‗Bianco avorio d‘Asti‘ characterized by medium-large fleshy leaf ribs, spine absence on the leaves and incised leaf margin; ‗Gigante di Romagna‘ with medium incised leaf margin, light green leaf colour, large leaf rib and very large plant size (plant height can exceed 160 cm); ‗Bianco gigante inerme a foglia intera‘ characterized

by wide spineless leaf, entire leaf margin and very wide and fleshy leaf rib (Raccuia and Melilli, 2009), ‗Cento foglie' also called ‗Pieno inerme' or ‗Gobbo di Nizza Monferrato' with spineless and very numerous leaves (SIAN, 2011 MiPAF).

1.7 Propagation

1.7.1 Agamic

Traditionally, globe artichoke genotypes are vegetatively propagated by offshoots, ovoli, stumps or micropropagated plants. For cardoon, agamic propagation has not applied and seed propagation is usually used.

The most widely system of agamic propagation used by farmers is *via* offshoots (Calabrese, 2009). Offshoots (*Figure I.10*) come from underground buds present on the plant rhizome and originate a new plant capable of rooting. Shoots of a good quality for propagation must have a well developed root system and 4-5 leaves with entire blade; indeed, this seems related to an early and satisfactory head production (Bianco *et al.*, 1990). This propagation method is widely used in Italy and the percentage of rooting depends on the quality of source material. Usually, shoots are collected from autumn to spring (Bianco *et al.*, 1990). Head production in the autumnal transplanting of rooted shoots starts generally in April while transplanting in summer (from the end of June to August), with the use of rooted offshoots grown in nurseries, provides earlier production (Calabrese, 2009). Generally, this technique is not very specialized and presents a high percentage of plant losses. Usually, artichoke planting is renewed every 2-3 years to obtain high productive performance.

Fig. I.10 Globe artichoke offshoots

Another agamic propagation system used for globe artichoke is *via* ‗ovoli'. Ovoli are dormant cylindrical shaped branches on the rhizomes which present one apical and several lateral buds (Bianco *et al.*, 1990). They can reach 13-14 cm in height and 1.0-3.5 cm in diameter. The

number of ovoli produced per plant can exceed 20 with the highest value for the genotypes belonging to ‗Catanese‘ and ‗Spinoso Sardo‘ groups grown in the South Italy including Sicily and Sardinia islands. Both the elimination of the apical bud and tamping operation increase the ovoli number per plant and their size. Ovoli are the underground part of offshoots, which are collected during summer (from the end of July to August) from resting mother plants with dried aerial part. After collection, ovoli are placed in straw layers for the pre-sprouting maintaining warm-humid conditions and, after one week, they are ready to be transplanted in open field (Bianco *et al.*, 1990).

A not specialized agamic propagation system used in some traditional artichoke cultivation areas involves the use of the stumps also named in Italian language ‗ceppaie‘. The stumps are collected at the end of production cycle and then they are divided into small portions called ‗zampe‘ in Italian language. Each portion of the stumps present roots and has a several number of buds which originate new plants. In general, ‗zampe‘ are left in the field during the winter and transplanted in April. This technique gives heterogeneous plants characterized by late head production (Bianco *et al.*, 1990).

The propagation systems aforementioned are not very specialized and present many phytosanitary problems. Moreover, the vegetative propagation using these traditional techniques present several disadvantages such as low multiplication rate, high percentage of plant losses, high labor costs and late plantation. Since the end of 1970s, some studies have been carried out to develop *in vitro* propagation technique for globe artichoke and to overcome the problems linked to the vegetative propagation using shoots, ovoli and stumps (*Figure I.11*).

Fig. I.11 Micropropagation

In particular, De Leo and Greco in 1976 and then in the 1980s, Ancora *et al.* (1981, 1986), Pécaut *et al.* (1983), developed the first *in vitro* propagation techniques for globe artichoke

obtaining satisfactory results. The explants used for globe artichoke micropropagation were root segments (De Leo and Greco, 1976), seed (Benoit and Ducreux, 1981) and shoot apices (Ancora *et al.*, 1981). In general, *in vitro* propagation has solved the main phytopathological problems caused by *fungi* and *bacteria* and allowed the reduction of labor cost and to the simplification of cultural management that is fundamental in specialized crop. The production of virus-free plant using micropropagation seems to be linked to the primary meristematic explant size which must not exceed 0.8 mm in size (Bianco *et al.*, 1990). In Italy, Ancora *et al.* (1981, 1986) and Morone-Fortunato *et al.* (2005, 2006) developed *in vitro* propagation technique for ‗Romanesco‘ globe artichoke and early typologies, respectively. At the Tuscia University, a vegetative propagation technique which allows to obtain disease-free and uniform plants has been developed (Temperini *et al.*, 2000; Cardarelli *et al.*, 2005a, 2005b). This technique consists in using pathogen-free micropropagated plants, grown in pots under greenhouse conditions, as ‗mother-plants‘ to obtain offshoots for the transplantations in open field. In order to promote offshoot production, mother-plants are periodically subjected to chemical treatment with growth regulator [6-benzylamino purine (BA)] and to cutting-back operation. The offshoots so obtained are cold stored after harvesting (3 harvesting per year) and, in the spring, are grown in multi-pack trays under high humidity conditions for rooting and transplantation in summer.

1.7.2 GAMIC

In the last years, propagation of globe artichoke by seeds (achenes) has been considered to overcome the aforementioned problems linked to agamic propagation. The development of seed propagated cultivars represents an important goal in globe artichoke breeding program (Bernal *et al.*, 2005) and in the modernization of its cultivation (Lo Bianco *et al.*, 2011). Seed-propagated cultivars are becoming popular in some countries such as Israel, USA and Spain but few of these have a commercially acceptable uniformity (Lanteri *et al.*, 2001, 2006). The main advantages of this system (Basnizki and Zohary, 1994; Zaniboni, 2009) consist in:

- the possibility to introduce globe artichoke cultivation in new areas;
- the reduction of labor and agricultural management costs;
- the major flexibility of the crop cultivation cycle and the possibility to include globe artichoke in annual crop rotations;
- the production of virus-free plants and the prevention of transfer and spread soil-borne pathogens;

- the development of long vertical tap roots, which utilize more efficiently the soil moisture and fertilizer content (Foti *et al.,* 2005);
- the possibility to mechanize sowing and harvesting agronomical operations.

Gamic propagation can be performed using open pollinated genotypes, inbreed lines, F_1 hybrids and synthetic varieties. The majority of new seed propagated cultivars were obtained in Israel and United States. New cultivars were obtained by crossing traditional clones and selecting new plants among segregant progenies. In Italy, an example of such new clone is Terom, derived from open pollination of ‗Violetto di Toscana' (Tesi, 1981; Basnizki and Zohary, 1994). As regards the inbreed lines, Talpiot was obtained after five self pollinations by Basnizki and Zohary (1987). Other inbred and open pollinated seed propagated cultivars are Imperial Star, Emerald, Green Globe and Colorado. F_1 hybrids such as HU #044, HU 137, and HU #223 were developed since the 1980s by Basnizki and Zohary, (1994). Other hybrids such as Romano, Napoleone, Romolo, Orlando, Madrigal, Simphony, Harmony, Concerto, Opal, Tempo, Nun 4021 F1 and Nun 4051 F1 (Zaniboni, 2009), have many advantages in overcoming inbreeding problems and showing positive effects of heterosis.

1.8 Agronomic aspects

1.8.1 Planting

As aforementioned, globe artichoke can be propagated by offshoots, ovoli, stumps or seeds while gamic propagation is preferred for cardoon. Usually, for the vegetatively propagated genotypes and in some cases also for the seed-propagated ones, transplanting in open field is done after a soil plowing and leveling. Generally, transplantation is made manually in a not specialized cultivation system while it is mechanical, from spring to summer depending on genotypes and on environmental conditions, in a specialized one. For seed-propagated genotypes, direct-seeding is widely used, expecially in well specialized cropping systems even if, in many cases, transplantation is preferred due to the non-uniformity of seed germination. Generally, the plant density used is 7,000-10,000 plants per hectare (using a intra- and inter-row distances of 0.8-1.0 m and 1-1.4 m, respectively) (Calabrese, 2009).

1.8.2 Fertilization

During a growing season, globe artichoke nutrient uptakes are estimated to be 286-44-368-178-157 and 28 kg ha^{-1} of N, P_2O_5, K_2O, Ca, Na and Mg (Bianco *et al.*, 1990). For the early genotype ‗Locale di Mola', the nutrient uptakes for the head production of 0.1 tonnes were estimated to be 1.9-0.3-2.4 of N, P_2O_5, K_2O, respectively (Magnifico *et al.*, 1984). For the

spring ‗Romanesco' genotype Grato1, Graifenberg *et al.* (2004) estimated nutrient uptake values of 1.6-0.4-2.7 of N, P_2O_5, K_2O, respectively. The application rates suggested by Tesi (1994) for Italian globe artichokes are 100-150 kg ha^{-1} of N, 150-200 kg ha^{-1} of P_2O_5 and 150 kg ha^{-1} of K_2O, with a further split application of urea (150-200 kg ha^{-1}) in winter. Bianco *et al.* (1990) suggested application rates of 200 kg ha^{-1} of N, 150 kg ha^{-1} of P_2O_5 and 50-150 kg ha^{-1} of K_2O. The N fertilization should be done before planting in the first year and, then, in concomitance with the first soil tillage. Nutrient uptake for cultivated cardoon are estimated by Raccuia and Melilli (2009) to be 250-300 kg ha^{-1} of N, 50-100 kg ha^{-1} of P_2O_5 and 350-400 kg ha^{-1} of K_2O for a leaf rib production of 25 tonnes ha^{-1}.

1.8.3 IRRIGATION

Traditionally, the irrigation volume for globe artichoke is around 4-5,000 m^3 ha^{-1} (Bianco *et al.*, 1990). In Italian specialized cultivation system, irrigation starts during summer (in July for the early genotypes and in August for the late ones) to anticipate head production. This operation is fundamental to assit the growth of the crop and to obtain good yield performance. For this reason, some authors (Tarantino and Caliandro, 1979; Linsalata, 1979) calculated the evapotraspiration of the crop during the growing season. In particular, the crop water consumption was estimated to be 3.0 mm per day from August to October, 1.5-2.0 mm per day from November to February and 2.0-4.0 mm per day from March to May for early genotypes (Tarantino, 1984). Also Bianco *et al.* (1990), estimated the crop water consumption ranging from 3.0-4.0 mm during summer to 1.5-2.0 mm during autumn.

The irrigation system most frequently used for globe artichoke is sprinkler or overhead irrigation with high-pressure sprinklers or guns. In the last years, the localized irrigation system has widespread also in globe artichoke cultivation (Tarantino, 1984; Bianco *et al.*, 1990) and, in particular, the micro-irrigation system which allows the use of fertirrigation (Mansour *et al.,* 2005). Considering that globe artichoke is moderately tolerant to salinity, water irrigation quality is important and Electrical Conductivity (EC) should not exceed 2.7 dS m^{-1} (Graifenberg *et al.*, 1993).

1.8.4 WEED CONTROL

In traditional cultivation systems, globe artichoke and cardoon as perennial crops are submitted to a wide spectrum of weeds which represent a problem for the nutrient and water removals; in addition, weeds provide a habitat for many insect pests and other pathogens. The most relevant weeds are *Portulaca oleracea* L., *Digitaria Sanguinalis* (L.) Beauv.,

Amaranthus spp. and *Sonchus* spp. (Bianco *et al.*, 1990). Several systems for weed control are available. They can be listed as follows:

- mechanical, involving the use of mechanical weeding or hoeing; in not specialized crop, the hand hoeing is sometimes used (Bianco *et al.*, 1990; Grafeinberg *et al.,* 2006);
- chemical, with the use of some herbicides such as trifularin, diphenamid, linuron and pendimethalin (Bianco *et al.*, 1990)
- physical, including the use of pyroweeding (Raffaelli *et al.*, 2004).

1.8.5 Parasites and pathogens

Several parasites and pathogens have been widely described in *Cynara cardunculus* spp. by Ciccarone (1967). Among the parasites, many species of aphids (ex.: *Aphis fabae*, *Brachycaudus cardui*, *Dysaphis cynarae*) and *Lepidoptera* (ex.: *Depressaria erinaceella*, *Gortyna xanthenes*, *Agrotis ipsilon*, *Spodoptera littoralis*, *Helicoverpa armigera*, *Cossus cossus*, *Agonopterix subpropinquella*) attack the crop and can cause serious damages to the production. Also some species of *Coleoptera* (ex.: *Ceratapion carduorum*, *Cleonis pigra*, *Larinus cynarae*) and some animal parasites such as mouses (ex.: *Microtus savi*) and snails (ex.: *Cantareus apertus*, *Milax gagates*) represent a problem for the crop (Delrio, 2009; De Lillo, 2009). Among pathogens capable of attacking the aerial part of the plant, *Leveillula taurica* f.sp. *cynarae, Bremia lactucae*, and *Botrytis cinerea* can provoke the most important damages. In particular, *Leveillula taurica* f.sp. *cynarae* can attack seriously the aerial part of the plant causing withering and dying back the leaves, so reducing head production. Among soil borne root pathogens, *Verticillium dahliae, Sclerotina sclerotiorum* and *Erwinia carotovora* subsp. *carotovora* must be considered (Marras, 2001, Marras and Cirulli, 2009). In particular, *Verticillium dahliae* which causes stunted plant growth, die-back of the leaves; in serious attacks, also the death of the entire plant has been frequently observed in warm growing areas such as South Italy (Cirulli *et al.*, 2000), Tunisia (Jabnoun-Khiareddine *et al.*, 2008), Spain (Armengol *et al.*, 2005), California (Bhat and Subbarao, 1999), Greece (Tjamos and Paplomatas, 1988). The strategy for the *Verticillium* wilt control is based mainly on the use of disease-free propagation material as well as planting in non-infested soils (Amenduini *et al.*, 2006). In organic cultivation systems, solarization and microbial antagonists were evaluated in controlling soilborne pathogens but the results are often contrasting (Cirulli *et al.*, 1996; Colella *et al.,* 2001). Selection of tolerant or resistant genotypes result to be the

most efficient strategy for *Verticillium* wilt control (Cirulli *et al.*, 2000; Ciancolini *et al.*, 2010).

Twenty-four viruses, belonging to 13 genera, have been up now isolated from globe artichoke (Martelli and Gallitelli, 2008). The majority of these viruses have been found in many countries of Europe and Mediterranean area and they are transmitted mainly by aphids, nematodes and infected propagated materials. The most detrimental viruses for globe artichoke are the AILV (*Artichoke Italian latent virus*), which is widespread in Apulia region, the ArLV (*Artichoke latent virus*) that is widely often distributed in mixed infection with AILV in globe artichoke cultivation areas, the TSWV (*Tomato spotted wilt virus*) and the AMCV (Artichoke mottled crinkle virus) (Acquadro *et al.*, 2010). In particular, TSWV and AMCV severely affect plant growth and provoke deformities on flower heads along with serious economic losses. The use of virus-free planting material coming from meristem-tip culture represents an important tool for the virus infection control and allows a significant increase in productivity (Gallitelli and Barba, 2003). Instead, seed propagation does not guarantee to obtain virus-free plants. In fact, some globe artichoke infecting viruses such as AILV and ArLV are transmitted both *via* seed and pollen (Bottalico *et al.*, 2002; Acquadro *et al.*, 2010).

1.8.6 Agricultural management of the plants

1.8.6.1 Stalk removal

This operation is very common for globe artichoke vegetatively propagated genotypes and consists in cutting off the top of the plants 4 cm below the soil level at the end of the growing season when the aerial part of the plants are completely dried (in June in Mediterranean basin) (Bianco *et al.*, 1990). Stalk removal allows the elimination of the buds present on the superficial part of the rhizome. These buds are already differentiated and, if they are left on the rhizome, will give rise to many offshoots after the summer dormancy, which should be removed to obtain satisfactory and early production (Bianco *et al.*, 1990).

1.8.6.2 Offshoot removal

This operation, very common in globe artichoke and cardoon cultivation, consists in the elimination of lateral offshoots. The number of offshoots produced per plant depends on the genotypes as well as on the agricultural management. In particular, early genotypes produce more offshoots than the late ones. Generally, in ‗Romanesco' genotypes, 1 central offshoot is left on the plant, while, in reflowering genotypes, 3-4 offshoots are left. Also the number of

‗offshoot removal‘ operation, ranging from 1 to 3, depends on genotypes, plant age and agricultural practices. In fact, plant density as well as propagation method influence the number of the offshoot removal. This is a hand-made operation, which requires many hours of labor and represents approximately 30% of the labor costs (Bianco *et al.*, 1990).

1.8.6.3 Bleaching

This agricultural practice is used in cardoon cultivation and consists in tying and wrapping autumnal leaf rosette for 3-6 weeks in burlap, paper or black film to enhance the sweet flavour and the tenderness of the bleached leaves. The bleaching operation usually starts in September-November and lasts until winter (December), when the bleached leaf rosette are cut off and used as vegetables for traditional recipes (Fernández *et al.*, 2006; Raccuia and Mellilli, 2009).

1.9 Edible product harvesting and commercialization

In cardoon crop, the fleshy bleached leaf petioles are traditionally harvested at the end of the bleaching operation, when heads still have not been developed (Cravero *et al.*, 2012). Generally, harvesting of the bleached leaf rosette is a hand-made operation and consists in cutting off the aerial part of the plant. In specialized system, the mechanical harvesting consists in cutting off the leaf rosette at the collar height. The commercial product is represented by the bleached fleshy leaf and in Mediterranean environments average yields of 0.4 tonnes ha^{-1} are obtained. Commercialization of the fresh edible product is done immediately after harvesting (Raccuia and Melilli, 2009).

Globe artichoke is traditionally grown for its immature inflorescences (heads), which are used as vegetable in many typical dishes of Mediterranean countries. The flower heads are destined to the fresh market, or are industrially processed and commercialized as frozen, cooked, canned product or preserved in oil. The EU marketing standards for the head globe artichoke commercilization classify heads into three categories (extra, first and second commercial category) on the basis of the morphological traits and the quality standards (i.e. caliber, homogeneity, etc). In general, the flower heads must not have any defects or damages and must be sold entire. The package must follow hygienic standards and labeling with information on the cultivation origin must be shown (Piazza and Caccioni, 2009).

1.10 USES

1.10.1 TRADITIONAL USES

Since ancient time, globe artichoke has been used for its immature inflorescences (heads), which can be sold entire for the fresh consumption or can be industrially processed. Also cardoon has been traditionally cultivated for centuries as vegetable for human food. The species is commonly known as ‗Spanish cardoon'. In particular, the fleshy leaf petioles of young plants, at the vegetative phenological stage, are used as human food in Mediterranean countries (Fernández *et al.*, 2006). Since ancient times, *Cynara* flowers have been traditionally used as natural rennet for the production of typical sheep cheese in some area of Portugal and Spain (Verissimo *et al.*, 1995). In particular, these milk-clotting properties are related to the enzyme' composition and proteolytic specificity of proteinases cardosin A and cardosin B (Vieira and Barbosa, 1972). In fact, these enzymes were found similar, in terms of specificity and activity, to chymosin and pepsin (Veríssimo *et al.*, 1995; Ordiales *et al.*, 2012).

1.10.2 INDUSTRIAL USES

In the last years, several alternative uses have been proposed for *C. cardunculus* spp. In particular, the use of the seeds for oil and biodiesel production has been widely investigated (Foti *et al.*, 1999; Maccarone *et al.*, 1999; Curt *et al.*, 2002; Fernández *et al.*, 2006; Raccuia and Melilli, 2007; Ierna *et al.*, 2012). The seed yield, seed oil content and also the fatty acid profile show the great potential of this crop for a such purpose even if all are affected by genotype and environmental conditions. Some authors found a great variability in seed yield depending on globe artichoke or cardoon genotype (Foti *et al.*, 1999; Raccuia and Melilli, 2007; Raccuia *et al.*, 2011; Ierna *et al.*, 2012). For example, Foti *et al.,* (1999) reported seed yield rangin from 1.93 to 2.60 tonnes ha^{-1} in cultivated cardoon genotypes and from 0.25 to 0.85 tonnes ha^{-1} in globe artichoke genotypes. However, some authors, such as Raccuia and Melilli (2007), selected some cardoon genotypes of interest for seed and oil production (i.e E438, which produce 6 and 1.6 tonnes ha^{-1} of seed and oil, respectively). The *Cynara* seed content oil results to be 24% (Sengo *et al.*, 2010) and its composition is similar to common sunflower oil (Benjelloun-Mlayah *et al.*, 1996; Curt *et al.*, 2002; Fernández *et al.*, 2006), with average percentages of 11, 4, 25 and 60% of palmitic, stearic, oleic and linoleic fatty acids, respectively (Fernández *et al.*, 2006). Raccuia *et al.,* (2011) reported percentages of 44.5, 42.6, 9.8 and 3.1% of linoleic, oleic, palmitic and stearic fatty acids in cardoon oil, respectively. However, the unsatured acids (oleic and linoleic) are predominanted over the satured ones (stearic and palmitic acids). The *Cynara* seed oil can easily extracted by cold

pressing at 20-25°C, which allows the composition conservation and the use in food applications. In fact, as reported by Maccarone *et al.* (1999), the high content of oleic and linoleic acids and the low amount of free acids peroxides and saturated as well as the optimal α-tocopherol content, which offers a guarantee of stability against oxidation, ensure a good alimentary quality. In particular, the genotypes characterized by high oleic acid content are very suitable for edible purposes. However, *Cynara* oil application for biodiesel production has also been recommended and the quality of the final product show high quality characteristic, similar to those of diesel no. 2, according to DIN EN 14214 standard specifications (European Standard requirements for biodiesel) (Encinar *et al.,* 1999; Fernández *et al.*, 2006; Sengo *et al.*, 2010; Raccuia *et al.*, 2011) (*Table I.2*).

Table I.2 European standard requirements for biodiesel and Cynara biodiesel properties

Fuel property	EN 14214	Ethyl esters	Methyl esters
Density 15ºC ($g\ cm^{-3}$)	0.86-0.90	0.88	0.89
Viscosity 40º ($mm^2 s^{-1}$)	3.5–5	4.48	5.10
Flash point (ºC)	> 101	184	182
Cloud point (ºC)	--	-5	-4
Cold filter plugging point (CFPP) (ºC)	≤ -10	-10	-10
Cetane number	> 51	66	59
Carbon residue (% m/m) (10% distillation residue)	< 0.3	0.28	0.36
Iodine index	<120(140)	109	117
Phosphorus ($mg\ kg^{-1}$)	< 10	<5	<5
Sulphur (% m/m)	< 0.02	<0.02	<0.02

Source: Fernández and Curt, (2004)

C. cardunculus spp. has been also considered as biomass energy crop (Foti *et al.,* 1999; Fernández *et al.,* 2006; Angelini *et al.,* 2009; Mantineo *et al.,* 2009; Ierna and Mauromicale, 2010; Ierna *et al.,* 2012). In particular, application as a solid biofuel has been investigated by several authors in the framework of European projects (Foti *et al.,* 1999; Piscioneri *et al.,* 2000; Curt *et al.,* 2002; Angelini *et al.,* 2009) mainly for heating production or power generation. The heating value of *Cynara* aboveground lignocellulosic biomass was found very interesting [Higher/Lower Heating Value 4083/3795 kcal kg^{-1} DM according to Fernández *et al.* (2006); 15 MJ kg^{-1} according to Angelini *et al.* (2009)]. Different conversion technologies

such as combustion, pyrolysis and gasification have been investigated for biomass exploitation (Encinar *et al.,* 2001; Gonzàles *et al.,* 2004; Damartzis Th. *et al.,* 2011). The main advantages of the use of *Cynara* spp. biomass as energy crop are linked to the high biomass productivity [15 tonnes ha^{-1} in cardoon genotypes according to Angelini *et al.* (2009); from 7.5 tonnes ha^{-1} to 20.2 tonnes ha^{-1} in cultivated and wild cardoon genotypes according to Raccuia and Melilli (2007); 12.36 tonnes ha^{-1} in globe artichoke genotypes according to Ierna and Mauromicale (2010); from 7.4 tonnes ha^{-1} for ‗307‘ globe artichoke genotype to 29.3 tonnes ha^{-1} for Bianco Avorio cardoon genotypes according to Ierna *et al.* (2012)], to the low moisture content and the high calorific value, to the low crop energy input (i.e. irrigation, fertilization) and also to the high degree of adaptability to semi-arid cultivation areas. It is important taking into account that the biomass and the energy yield, as reported by Ierna *et al.* (2012), is strongly affected by genotype, agricultural management and environmental conditions.

Also the potential of *Cynara spp.* as biomass crop for paper pulp production has been widely studied by several authors (Anthunes *et al.,* 2000; Gominho *et al.,* 2001; Fernández *et al.,* 2006; Gominho *et al.,* 2009). In particular, Gominho *et al.* (2001) evaluated anatomical and chemical composition of plant stalks and found interesting strength properties especially in relation to tensile strength (fibres were found 1.3 mm long, 18.8 μm wide and had a 4.8 μm wall thickness). The stalk pulping yield resulted to be over 46% with low residual lignin content (Gominho and Pereira, 2006). Also the use of hairs and pappi from *capitula* as new raw material for paper production has been investigated (Gominho *et al.,* 2009).

The possibility of using *Cynara* spp. biomass as green forage for ruminants feeding in wintertime has been proposed by Fernández *et al.* (2005) with yield of 6.5 tonnes DM ha^{-1}. Also the use of the seed as feed for ruminants has been evaluated by Cajarville *et al.* (2000). The crude protein (CP), ether extract (EE) and neutral detergent fibre (NDF) contents of cardoon seed were found as 225, 250, and 338 g kg^{-1} DM, respectively. The results obtained in literature showed a real possibility of using the crop for ruminant feeding.

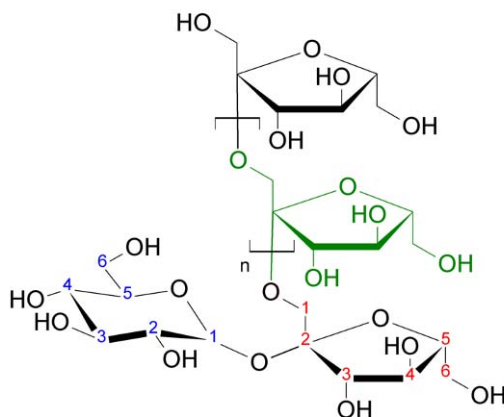


Fig. I.12 Structural formula of inulin (CAS number 9005-80-5)

In addition, the use of the crop as ornamental species has been also proposed by some authors (Lanteri *et al.,* 2012; Ciancolini *et al.,* 2012a).

Interest for this crop has been growing in recent years also for the recovery of inulin mainly from roots (Raccuia and Melilli, 2004, 2010). Indeed, in *Asteraceae* family, the reserve of carbohydrates is represented by inulin (*Figure I.12*) which is a linear polysaccharide consisting of β-(2─>1) linked fructofuranosyl units with a terminal glucose residual (Raccuia and Melilli, 2004, 2010). Inulin with a terminal glucose unit is also called as fructooligosaccharide. The general formula may be depicted as GFn or Fn, G=glucose and F=fructose, and n for the total unit number (Ronkart *et al.,* 2007). Inulin is stored in storage organs (i.e. rhizome and taproots) and it is brokendown during the summer dormancy stage before the plant growth.

Inulin also supports flowering demands and the seed maturation. The accumulation of this polysaccharide is enhanced by conditions increasing photosynthesis (i.e. long photoperiod) (Raccuia and Melilli, 2010). The inulin of *Cynara* spp. was found characterized by a high degree of polymerization (DP). For example, Ronkart *et al.* (2007) determined in ‗Petit Violet' globe artichoke inulin with DP over 80. Hellwege *et al.* (2000) found in globe artichoke inulin extracts chain length up to 200 DP. The inulin composition and yield depend on genotype, harvesting date, environmental conditions and extraction processes. The growing interest in *Cynara* spp. crop for inulin recovery is principally due to the high raw material (roots) and inulin yields. As regards the yield, Raccuia and Melilli (2004) found average roots yield for globe artichoke and cardoon genotypes of 9.8 tonnes ha^{-1} DM with average inulin yield of 3 tonnes ha^{-1}.

In non-food industrial application, inulin is used as a source of carbohydrates for ethanol production. In particular, inulin with high chain length is recommended for this application.

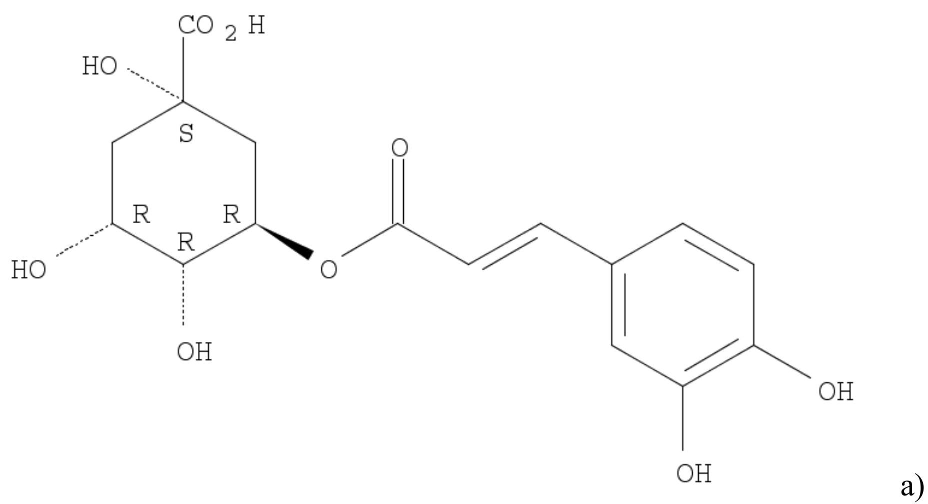
Inulin has also been recognized as a beneficial food ingredient. Indeed, food industry has increased in the last decades the use of this polysaccharide as nutritional ingredient, and in particular as fat replacer and prebiotic agent. Inulin is also used in yoghurt and ice cream preparations. Indeed, it forms a gel, when emulsified with water that is similar as texture to fat but with much lower calories (Raccuia and Melilli, 2004). Health-promoting properties of inulin, which is a non-digestible oligosaccharide (NDO), are widely investigated and its beneficial effects could be summarized as follows (Lattanzio *et al.,* 2009):

- prebiotic effects, inulin consumption enhance the activity of bifidobacteria and lactic acid bacteria in the human large intestine;
- positive effects on bowel habit being a NDO;
- increase of Ca absorption;
- positive interaction in the lipid metabolism;
- anticarcenogenic properties, in particular for the colon cancer.

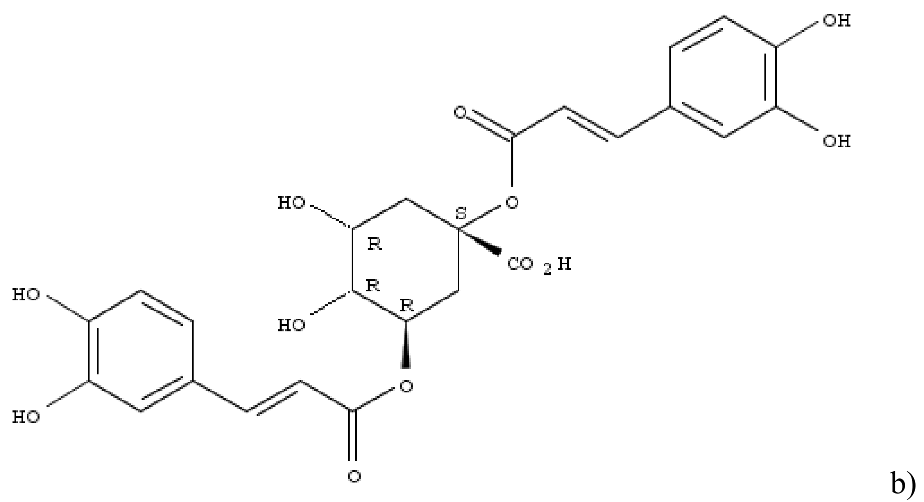
1.10.3 Pharmaceutical uses and major phenolic compounds

Since ancient time, *Cynara* spp. has been used in traditional medicine for its recognized therapeutic effects (i.e. hepatoprotective, anticarcinogenic, antioxidative, antibacterial, urinative, anticholesterol, glycaemia reduction) (Gebhardt, 1997; Kraft, 1997; Clifford, 2000; Saénz Rodriguez *et al.,* 2002; Coinu *et al.,* 2007; Rondanelli *et al.,* 2011; Fantini *et al.,* 2011). In the last years, interest in natural antioxidants for food or pharmaceutical supplements applications is increasing against the use of synthetic antioxidants (i.e. BHA and BHT), which is limited due to their suspected carcinogenicity (Llorach *et al.,* 2002; Falleh *et al.,* 2008). Today, *Cynara* spp. dry extracts are already commercialized as drugs mainly for liver and choleretic disease treatment (Lattanzio *et al.,* 2009). Several studies, both *in vivo* and *in vitro,* have been carried out to demonstrate the health-promoting effects of *Cynara* spp. extracts. In particular, leaf extracts are reported to decrease blood cholesterol levels with a reduction of its synthesis in liver and a decrease in fat accumulation in other tissues (Kraft, 1997). Also hepatoprotective and choleretic properties have been demonstrated for globe artichoke extracts (Adzet *et al.,* 1987). These health-promoting properties are mainly linked to the high content of polyphenolic compounds, which includes mono- and dicaffeoylquinic acids and flavonoids (Wang *et al.,* 2003; Fratianni *et al.,* 2007; Lattanzio *et al.,* 2009; Lombardo *et al.,* 2010; Menin *et al.,* 2010; Pandino *et al.,* 2010, 2011a, 2011b, 2011c, 2012). In particular, within the caffeic derivatives, chlorogenic acid (3-*O*-caffeoylquinic acid) (*Figure I.13*), which is an ester formed between transcinnamic and quinic acids, is the most abundant component

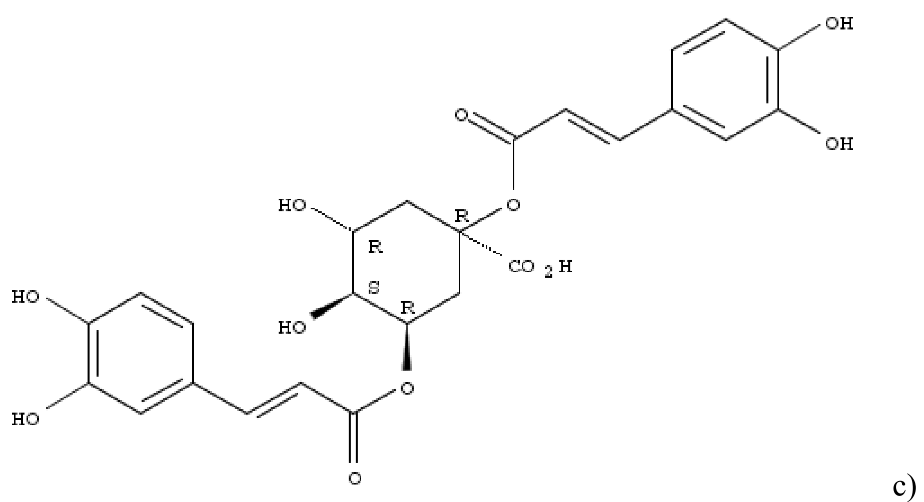
and represents about the 39% of the total caffeoylquinic acid content (Lattanzio *et al.,* 2009). Other major caffeoylquinic acid derivatives found in *Cynara* spp. leaf and head extracts are represented by 1,5-*O*-dicaffeoylquinic acid (21% of total caffeoylquinic acids), 3,4-*O*-dicaffeoylquinic acid (11% of total caffeoylquinic acids) and cynarin or 1,3-*O*-dicaffeoylquinic acid (1.5% of total caffeoylquinic acids) (Lattanzio *et al.,* 1994, 2009) (IUPAC nomenclature, 1976) (*Figure I.13*).



a)

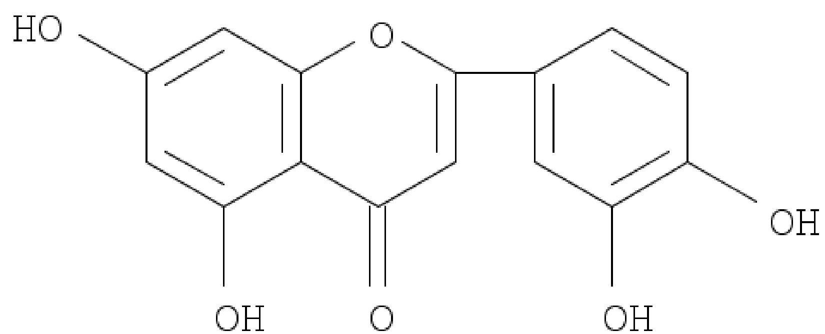


b)

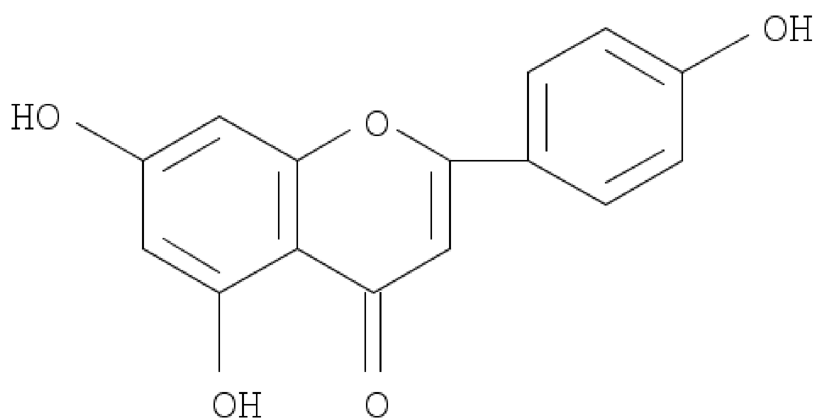


c)

Fig. I.13 Structural formula of (a) chlorogenic acid (3-O-Caffeoylquinic acid) (CAS number 327-97-9), (b) 1,5-O-dicaffeoylquinic acid (CAS number 19870-46-3), and (c) cynarin (1,3-O-dicaffeoylquinic acid) (CAS number 30964-13-7)



a)



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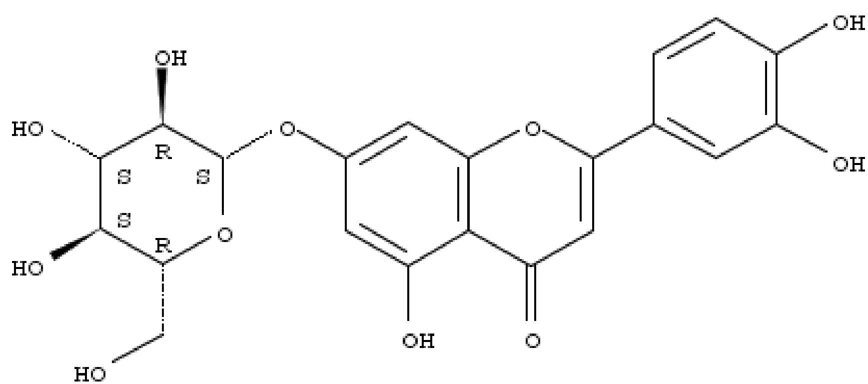


c)

Fig. I.14 Structural formula of (a) luteolin (CAS number 491-70-3), (b) apigenin (CAS number 520-36-5), and (c) cynaroside (CAS number 5373-11-5)

However, it is important to consider that, in plant tissue, phenolic content is strongly related to physiological stages, genotype, plant parts, environmental conditions, agricultural management and post-harvest conditions (Lattanzio and Morone, 1979; Fratianni *et al.,* 2007; Lattanzio *et al.,* 2009; Lombardo *et al.,* 2010; Negro *et al.,* 2011; Pandino *et al.,* 2011a, 2011b, 2011c, 2012). Indeed, several authors revealed a great variability in the phenolic content of *Cynara* spp. leaf and head extracts depending on the aforementioned factors (*Table I.3*). Also the flavonoids apigenin and luteolin and their glycosides (luteolin-7-*O*-glucoside or cynaroside, luteolin-7-*O*-rutinoside or scolymoside and apigenin-7-*O*-glucoside) have been widely described in *Cynara* spp (*Figure I.14*). However, these flavonoids are minor constituents of the total phenolic content (<10%) (Lattanzio *et al.,* 2009). Chlorogenic acid, dicaffeoylquinic acids and flavonoids derive from the phenylpropanoid pathway (*Figure I.15*).

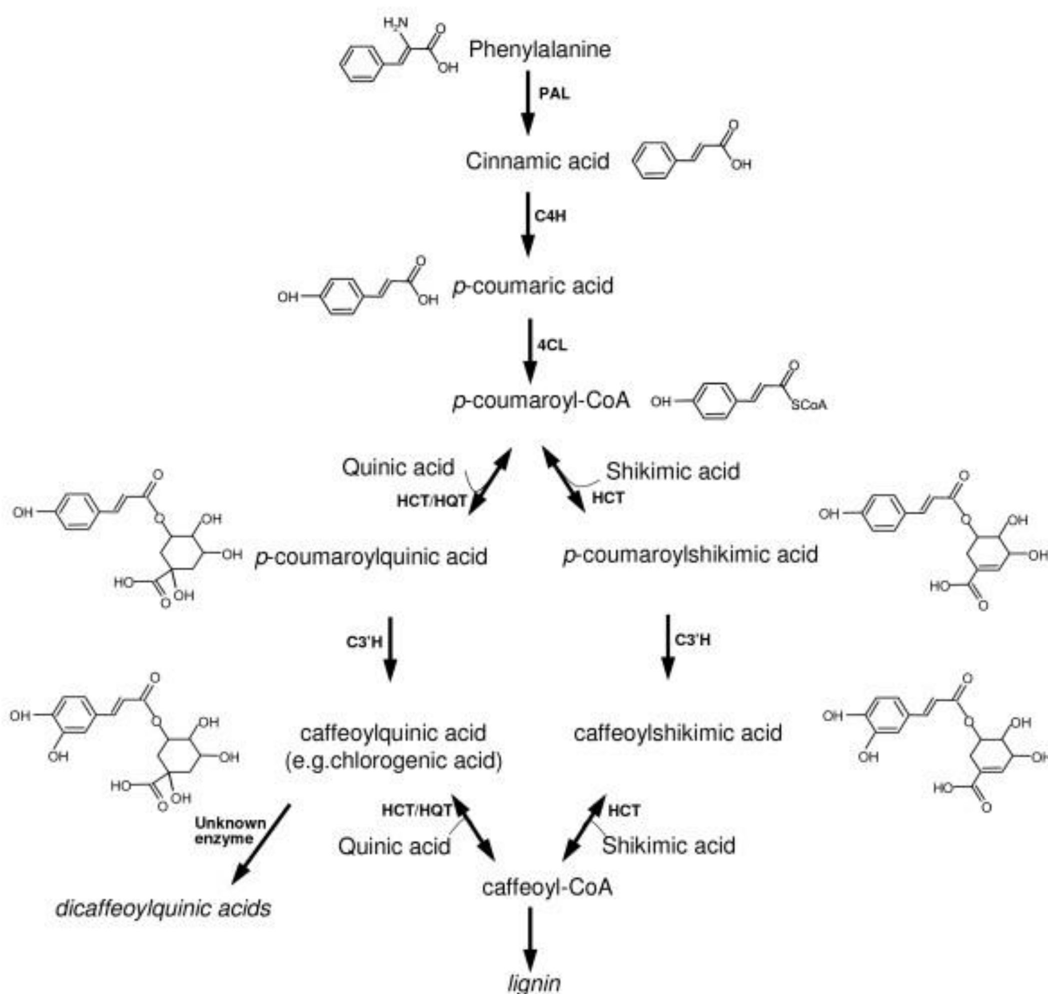


Fig. I.15 Phenylpropanoid pathway in plants

Phenylalanine represents the precursor that starts this pathway and leads to the biosynthesis of secondary metabolites such as flavonoids (i.e. luteolin and apigenin), phenolic acids (i.e. chlorogenic acid and dicaffeoylquinic acids) and lignin (Dixon and Paiva, 1995; Dixon *et al.,* 2002; Moglia *et al.,* 2008).

The function of these secondary metabolites seems to be involved in plant defence against abiotic (i.e. UV irradiation, wounding) and biotic (pathogen attacks) stresses (Moglia *et al.,* 2010). In fact, the enzymatic steps involved in this pathway are under the control of corresponding genes which are induced by signal molecules involved in local and systemic defence signaling (Dixon *et al.,* 2002).

Table I.3 Variability in phenolic content of leaf and head extracts depending on genotype and plant parts

Source	Species	Plant parts	Total caffeoylquinic acids	Total measured polyphenols
Negro *et al.*, 2012	Globe artichoke	Heads	Heads: range 11,390-28,787 mg kg^{-1} FM	Heads: range 24,621-66,658 mg kg^{-1} FM
Pandino *et al.*, 2012	Globe artichoke	Heads	Outer bracts: range 35-1756 mg kg^{-1} DM Inner bracts: range 412-3644 mg kg^{-1} DM	Outer bracts: range 1696-4895 mg kg^{-1} DM Inner bracts: range 2872-7566 mg kg^{-1} DM
Pandino *et al.*, 2011b	Globe artichoke	Heads	Outer bracts: Range nd-443 mg kg^{-1} DM Inner bracts: Range nd-2771 mg kg^{-1} DM	Outer bracts: Range 991-2684 mg kg^{-1} DM Inner bracts: Range 1774-9385 mg kg^{-1} DM
Pandino *et al.*, 2011c	Globe artichoke Cardoon	Leaves and floral stems	Globe artichoke Leaves: range nd-2.4 g kg^{-1} DM Floral stem: range 2.9-19.6 g kg^{-1} DM Cardoon Leaves: range nd-1.0 g kg^{-1} DM Floral stem: range 3.7-17.7 g kg^{-1} DM	Globe artichoke Leaves: range 5.3-13.1 g kg^{-1} DM Floral stem: range 3.7-20.6 g kg^{-1} DM Cardoon Leaves: range 5.4-9.3 g kg^{-1} DM Floral stem: range 4.2-18.6 g kg^{-1} DM
Melilli *et al.*, 2011	Globe artichoke	Heads		Range 1863-11,972 mg g kg^{-1} DM
Pandino *et al.*, 2010	Globe artichoke and cardoon	Heads		Globe artichoke: Range 4357-7574 mg kg^{-1} DM Wild cardoon: Range 4207-7573 mg kg^{-1} DM Cultivated cardoon: 9107 mg kg^{-1} DM
Bonasia *et al.*, 2010	Globe artichoke	Heads		Range 2.6-6.5 g GAE kg^{-1} FW
Wang *et al.*, 2003	Globe artichoke	Heads and leaves		Mature Heads: range 1.60-2.23% DM Young Heads: range 2.58-3.10% DM Leaves: range 6.81-9.81% DM

1.11 FOCUS ON PHENOLIC EXTRACTION TECHNIQUES

Several techniques for the extraction of biocompound from plant material have been developed with the aim of using plant extracts in food, pharmaceutical and cosmetic applications. Traditional techniques such as Soxhlet and maceration have been widely used in the last decades, but in general these conventional techniques are very time-consuming and require large amount of solvent. In the last years more environmentally friendly techniques have been investigated and used for nutraceutical extraction from plants, so allowing to reduce extraction time and solvent consumption as well as to increase nutraceutical yield (Wang and Weller, 2006).

Soxhlet is a conventional technique that provides the use of a Soxhlet apparatus invented in 1879 by Franz von Soxhlet (*Figure I.16*) and equipped with a condenser. This technique is very simple and cheap (Luque de Castro and Garcia-Ayuso, 1998). During extraction, solid plant material is placed inside a thimble-holder made from thick filter paper, which is putin the extraction chamber of the Soxhlet extractor. The solvent is placed in a boiling flask which is located in the lower side of the extractor. The solvent in the boiling flask is heated and the vapor goes up into a distillation arm and floods into the extraction chamber after condensation where the solid matrix is placed. Nutraceutical compounds are dissolved in the warm solvent and when the Soxhlet chamber is almost full, a siphon aspirates the solution from the extraction chamber and unloads it back into the

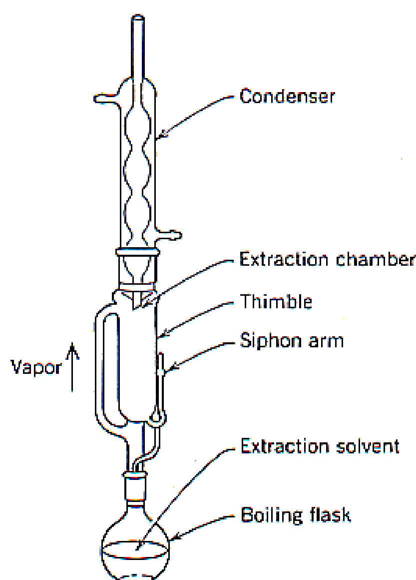


Fig. I.16 Soxhlet apparatus

distillation flask. The cycle is repeated until complete extraction is reached. Soxhlet technique requires long extraction time and high solvent consumption (Zheng *et al.,* 2009; Wang *et al.,* 2010). In addition, the use of large amount of solvent requires evaporation/concentration procedures after extraction (Wang and Weller, 2006). This method has been also used in *C. cardunculus* L. for oil extraction from the achenes (Raccuia *et al.,* 2011).

Maceration is a solid-liquid extraction process usually performed at low temperature and at ambient pressure. Plant material is placed in direct contact with the solvent during extraction. Long extraction time and large amount of solvent are required for a sufficient extraction yield of nutraceuticals. In addition, after extraction, the mixture must be strained, the damp solid material is pressed, and the extracts are clarified by filtration, this requiring long labor time. Maceration has been widely used for nutraceutical recovery in several species such as in *Corylus avellana* (Contini *et al.,* 2008), in Vitis *vinifera* (Spigno *et al.,* 2007) and also in *C. cardunculus* spp. (*Table I.4*).

Table I.4 Nutraceutical recovery from C. cardunculus spp. plant material using maceration

Source	**Species**	**Plant parts**	**Solvent used**	**Extraction Conditions**
Wang et *al.,* 2003	Globe artichoke	Heads and leaves	Methanol 70%	Not described
Fratianni *et al.,* 2007	Globe artichoke	Heads and leaves	Acetone:Ethanol:Methanol (70:15:15)	1h at 4°C twice
Lombardo *et al.*, 2010	Globe artichoke	Floral stem and heads	Methanol 60%	1h at room temperature
Pandino *et al.,* 2010	Globe artichoke, cultivated and wild cardoon	Heads	Methanol 70%	1h at room temperature
Pandino *et al.*, 2011c	Cultivated and wild cardoon	Floral stem and leaves	Methanol 70%	1h at room temperature
Negro *et al.,* 2011	Globe artichoke	Leaves and heads	Acetone:Ethanol:Methanol (70:15:15)	2h at 4°C twice
Lombardo *et al.,* 2012	Globe artichoke	Floral stem and heads	Methanol 70%	Procedure reported by Pandino *et al,* 2010

Microwave Assisted Extraction (MAE) is based on the use of microwaves which are electromagnetic radiations with a frequency from 0.3 to 300 GHz (Wang and Waller, 2006; Mandal *et al.,* 2007). Microwaves penetrate plant material during the extraction and interact with polar molecules. The principle of heating using microwaves is based upon its direct impact with polar materials/solvents and is governed by ionic conduction and dipole rotation phenomena (Mandal *et al.,* 2007). Rotation of molecules leads to rapid heating of the solvent and the sample (Wang *et al.,* 2010). There are two types of MAE systems: closed extraction vessels (Eskilsson and Björklund, 2000), which perform extraction under controlled pressure and temperature, and focused microwave ovens where only an extraction chamber containing plant material is irradiated with microwave (Luque-Garcia and Luque de Castro, 2004) (*Figure I.17*).

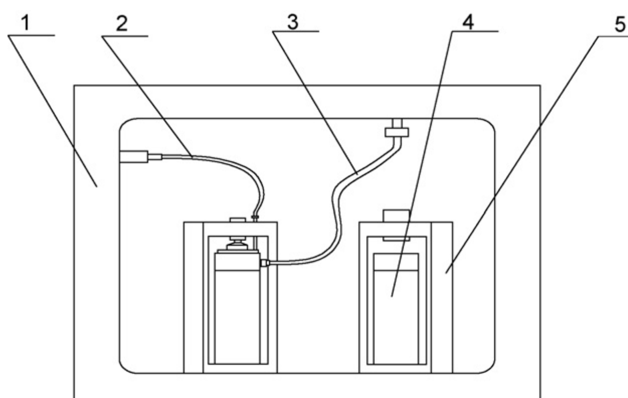


Fig. I.17 MAE apparatus (1) cavity inner wall; (2) temperature sensor; (3) pressure sensor; (4) Teflon vial; (5) container fixing-frame. Source: Zheng et al., (2009)

The effect of microwave is strongly dependent on moisture sample content, dielectric solvent and solid material susceptibility (Wang and Waller, 2006). The MAE technique allows i) to obtain high yields due to the cell distruption caused by the internal superheating, ii) to reduce extraction time and solvent consumption (Mandal *et al.,* 2007). The limit of this method is represented by the low extraction efficiency for non-polar solvent and target compounds. MAE has been successfully used for the recovery of nutraceuticals in several species such as *Nicotiana tabacum* (Zhou and Liu, 2006), *Erythroxylum coca* (Brachet *et al.,* 2002), *Capsicum annum* (Williams *et al.,* 2004), *Camellia sinensis* (Pan *et al.,* 2003).

Accelerated Solvent Extraction (ASE) is a solid-liquid extraction procedure which provides the use of high temperature (ranging from 50 to 200°C) and pressure (ranging from 10 to 15 MPa). This method is a form of pressurized solvent extraction (PSE). The

high pressure used during extraction keeps the solvent in the liquid state and allows the passage of the liquid solvent into the solid matrix. In ASE solid sample is placed into the extraction cells and the extracts are recovered in collection vial at the end of the extraction procedure (Zuloaga *et al.,* 1998).

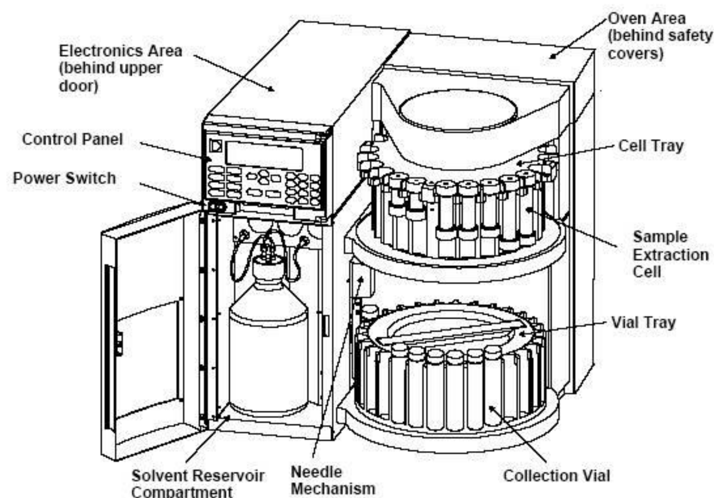


Fig. I.18 ASE apparatus

This technique allows biocompound yield increase, reduction in extraction time and solvent consumption and it is totally automatised. The main disadvantage is that thermolabile compounds could be degradeted during ASE extraction for high temperature used (Wang and Waller, 2006). In the last years, ASE has been used for the extraction of high-temperature stable organic pollutants from environmental matrices (Ezzell *et al.,* 1995; Fisher, 1997; Hubert *et al.,* 2000). Some applications are carried out also for nutraceutical extraction from plants, for example of *Erythroxylum coca* (Brachet *et al.,* 2001), *Sylibum marianum* (Benthin *et al.,* 1999), *Maclura pomifera* (De Costa *et al.,* 1999), *Thymus vulgaris* (Benthin *et al.,* 1999).

Experimental part

Chapter 1

MORPHOLOGICAL ANALYSIS AND GERMPLASM SELECTION FOR EDIBLE USE

GLOBE ARTICHOKE

1.1 INTRODUCTION

Globe artichoke as aforementioned is an herbaceous perennial plant native to the Mediterranean basin (Bianco, 2005), where about 90% of world production is made. Several uses such as (i) human food (typical component of the Mediterranean diet), (ii) lignocellulosic biomass for energy (Angelini *et al.,* 2009; Ierna and Mauromicale, 2010; Gominho *et al.,* 2011) and paper pulp (Gominho *et al.,* 2001, 2009), (iii) seed oil for biodiesel fuel production (Raccuia and Melilli, 2004), (iv) roots for inulin (Raccuia and Melilli, 2004, 2010), (v) leaves and heads for pharmaceutical compounds (Fratianni *et al.,* 2007; Lattanzio *et al.,* 2009; Bonasia *et al.,* 2010; Lombardo *et al.,* 2010; Pandino *et al.,* 2010, 2011a, 2011b, 2011c, 2012), are considered for the species.

Italy is the richest reserve of globe artichoke autochthonous germplasm, i.e. the ‗primary cultivated gene pool' (Pignone and Sonnante, 2004), which is vegetatively propagated and well adapted to the different pedoclimatic conditions of the Country. On the basis of head harvest time, these traditionally grown genetic resources are classified as early or late distinct clonal varietal groups. Early varieties are typically autumn-winter crops of the southern regions and continue to produce until around spring, while late varieties are represented by spring genotypes (e.g. Romanesco) that produce from February to April (Crinò *et al.,* 2008). Distinct varietal groups, each well adapted to different local environments, are generally identified also on the morphological traits of the head, into four typologies such as Violetto di Sicilia, Spinoso Sardo, Catanese and Romanesco (Porceddu *et al.,* 1976). In particular, the ‗Romanesco' type accounts for about 9% of Italy's production (ISTAT, 2007) playing an economic importance for Italy, its major world producer.

The germplasm nomenclature is not always clear and there are many cases of homonyms. Genotypes are often labelled with the name of their place of cultivation (Portis *et al.,* 2005), without any study on their genetic identity and characterization or even on their discrimination with other genotypes grown in nearby areas. As a result, it is necessary to carefully distinguish the many traditionally grown landraces that, only when recognized each other, can be properly identified and validated.

Real prospects of germplasm recovery are possible by use of rational management of traditional material and by intra-landrace selection of clones that well meet production (uniformity, aptitude to mechanical harvest) and market (precocity) requirements. For

globe artichoke, the European Directive no. 184/1/2004 imposes several well-defined quality standards to be fulfilled in order to release new varieties. In Italy, little has been up now done in this sector except for some clonal selections carried out to obtain the Romanesco C3 clone from the traditional populations of Castellammare and Terom from Violetto di Toscana (Soressi, 2003). In particular, the Romanesco clone C3, which matures earlier than local landraces Castellammare, has been *in vitro* micropropagated and widely distributed to the farmers. This clone replaced many Romanesco landraces traditionally grown in the Latium region, this leads to a significant erosion of local genetic resources and a loss of diversity.

Moreover, introduction of new seed-propagated F_1 hybrids such as Madrigal, Concerto, Opal, Tema, (Zaniboni, 2009; Bonasia *et al.,* 2010) and Romolo, as well as the use of Italian micropropagated clones well suited to market demands, represent factors increasing the risk of genetic erosion for autochthonous germplasm.

Genetic variability among and within germplasm accessions has been assessed using morphological and molecular analyses (Lahoz *et al.,* 2011), the latter exhibiting no variation due to environment, plant part and stage. Molecular markers such as AFLP (Lanteri *et al.,* 2004a; Pagnotta *et al.,* 2004; Raccuia *et al.,* 2004a; Portis *et al.,* 2005; Mauro *et al.,* 2009; Acquadro *et al.,* 2010), RAPD (Lanteri *et al.,* 2001; Sonnante *et al.,* 2002), ISSR (Crinò *et al.,* 2008; Lo Bianco *et al.,* 2011), and SSR (Acquadro *et al.,* 2005; Sonnante *et al.,* 2008; Mauro *et al.,* 2009) have been developed and are already applied to globe artichoke. ISSR, RAPD and AFLP markers have been used for assessments of genetic diversity in the different globe artichoke types i.e. ‗Romanesco‘ (Pagnotta *et al.,* 2004; Crinò *et al.,* 2008), ‗Spinoso Sardo‘ (Lanteri *et al.,* 2001), ‗Violetto di Sicilia‘ (Portis *et al.,* 2005; Raccuia *et al.,* 2004a) and ‗Spinoso di Palermo‘ (Portis *et al.,* 2005), where AFLP and ISSR have been used to generate DNA fingerprints from single plants (Lanteri *et al.,* 2004b). Over the last few years, a set of globe artichoke SSR assays (Lanteri *et al.,* 2006; Portis *et al.,* 2009), as well as EST-SSRs (Sonnante *et al.,* 2011a), have been also used to construct genetic linkage maps.

Nine spring landraces from different central Italian areas, cultivated for centuries by local farmers as example of man-made *in-situ* selection (Portis *et al.,* 2005), and ten ‗Romanesco‘ clones have been considered in the work. Their genetic variability and identity have never been analyzed. Their value lies in preserving traditional genetic resources and using them to provide genotypes/genes useful for the production of new materials suitable for market requirements. The present PhD study aims at (i)

characterizing these genotypes in terms of agro-morphological profile; (ii) investigating the genetic variability existing within and among genotypes; (iii) identifying genetic resources for the development of future plant breeding programs; iv) selecting genotype of interest under agronomic profile to obtain new varieties. As result, three ‗Romanesco' clones characterized by different dates of maturity, size of head, production weight, and receptacle thickness have been presented for release at the Italian National Variety Register in collaboration with ARSIAL (Latium Regional Agency for the Development and the Innovation of Agriculture) and accepted.

1st year: Morphological characterization of germplasm using UPOV descriptors
Germplasm description and identification
2nd year: Morphological characterization of germplasm using UPOV descriptors
Germplasm description, identification and validation
Genotype selection
3th year: Registration requirement to the Italian National Variety Register

Fig. 1.1 Work activity diagram of the PhD program for globe artichoke germplasm characterization

1.2 ITALIAN SPRING LANDRACES

1.2.1 MATERIALS AND METHODS

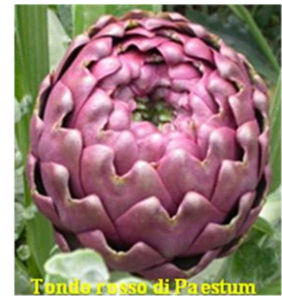
1.2.1.1 GENETIC RESOURCES AND OPEN FIELD EXPERIMENTS

Nine landraces of Italian spring globe artichoke [Campagnano, Castellammare, Bianco di Pertosa, Tondo Rosso di Paestum, Ascolano, Jesino, Montelupone A, Montelupone B (Romanesco type) and Pisa (Violetto type)], traditionally cultivated in Central Italy were considered in our study. The cultivation areas are described in Figure 1.2, while general information on landrace characteristics was obtained from various farmers involved in traditional cultivation (Noorani *et al.,* 2011).

Fig. 1.2 Growing areas of Italian analyzed landraces (modified from Noorani et al., 2011)

1. **Ascolano** comes from the area of Ascoli Piceno (42°51′N 13°34′E). This landrace grows best in the deep alluvial soils of valleys but is also found in the loamy soils of hillsides. The flower head is conical shaped and green coloured.
2. **Jesino** is found near Jesi (43°31′N 13°14′E), province of Ancona, in the valley of the Jesi river at around 200 m asl. The flower head is compact, more ellipsoid than round and medium in size.
3. **Montelupone A** and 4. **Montelupone B** come from the area around Montelupone, which is characterized by hills of up to 400 m asl, and occur inland and along the coast. The original landraces were divided by plant precocity forming the two subgroups. Montelupone A is the earliest of the two varieties, more frost-tolerant with less indented and greener leaves; it has head flowers larger than Montelupone B.

Fig. 1.3 Central flower heads of the Italian genotypes studied

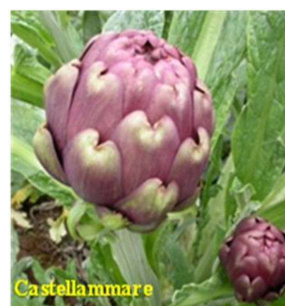


5. **Tondo Rosso di Paestum** was registered in 2004 within the EU as PGI (Protected Geographical Indication) for its origin certification and identification. It originates from the plain of the Sele river (40°25′N 15°0′E), at 0-6 m asl. It is characterized by round, dark red/purple, compact, medium-sized flower head and spine-free leaves.

6. **Bianco di Pertosa** is found in the valleys of Campania region in South Italy, near the National Park of Cilento, at 300-400 m asl (40°33′N 15°27′E), where the climate is mild. It is known locally as the ―white" artichoke of Pertosa because the flower head has a pale green colour. It is medium-large in size and indented in the middle; the plant is spine-free.

7. **Pisa** is also known as Violetto di Toscana. The plants used for this study were obtained from the areas around Pisa (43°43′N 10°24′E). The flower head is pale purple and darkens into a deep purple as it matures. It is ovoid in shape and the weight ranges from 120-200 g.
8. **Castellammare** and 9. **Campagnano** were widely grown in the coastal areas of Latium. The flower heads of both landraces are round shaped and green-purple coloured. Castellammare heads are harvested earlier than Campagnano and have a greater head. Also the Campagnano's plants are bigger than those of Castellammare.

Castellammare and Campagnano belong to the globe artichoke germplasm joint collection of Tuscia University and ENEA (Italy), while the other seven landraces, from Italian Regions Marche, Campania



and Tuscany, were kindly provided by Dr. N. Ficcadenti, Prof. V. Magnifico, and Prof. A. Graifenberg, respectively. All landraces were vegetatively propagated by rooted offshoots.

The trials were conducted for 2 years at the Experimental Field Station of ARSIAL (Latium Regional Agency for the Development and the Innovation of Agriculture) in Cerveteri, Rome - Italy (41° 59‘ N 12° 01‘ E). The station has temperate climate, clay soil and an average annual rainfall of 900 mm. Plant material was arranged in a randomized block experimental design with three replications of 20 plants each. Globe artichoke plants with 4-5 leaves were transplanted on 17 August 2007. Planting density was 0.77 plants m^2, with inter and intra-row distances of 1.3 and 1.0 m, respectively. The field experiments were conducted under low chemical inputs.

1.2.1.2 Morphological characterization using UPOV descriptors

Forty-seven morphological and agro-physiological data were recorded on 12 plants per landrace, for two years and during almost two months per year, from the harvest time of the central flower head to the commercial maturity and harvest time of the tertiary heads. Morphological characterization was carried out using standard UPOV (International Union for the Protection of New Varieties of Plants) (CPVO-TP/184/1 Final) descriptors for globe artichoke (*Table 1.1*) (Pagnotta *et al.,* 2009, 2012) and a well-defined group of complementary ‗Romanesco‘ type descriptors validated in a previous work (Crinò *et al.,* 2008). Leaf attitude was estimated by 1-5 (1=erect; 3=semi-erect; 5=horizontal attitude) visual rating. For the traits such as length of leaf spines, leaf incision and mucron on outer bracts of the central flower head, a 1-9 visual rating (1=presence and 9=absence) was used. Intensity of leaf green and grey colour as well as inner bract density, depth of outer bract incision, and hue of secondary colour of outer bract in the central flower head were assessed according to a 3-7 scoring scale. For the traits leaf blistering and hairiness, anthocyanin coloration at the base both of petiole and the central flower head inner bracts, and size of outer bract spine, a 1-9 scoring scale was used, according to UPOV descriptors. The ratio length/diameter of the flower head was calculated as *capitulum* shape index. Earliness was established counting the number of days from a reference date, which was chosen arbitrarily as October 1st, to the first flower head harvesting.

Fig. 1.4 Intensity of lobbing on the basis of UPOV descriptors (a=weak; b=medium; c=strong)

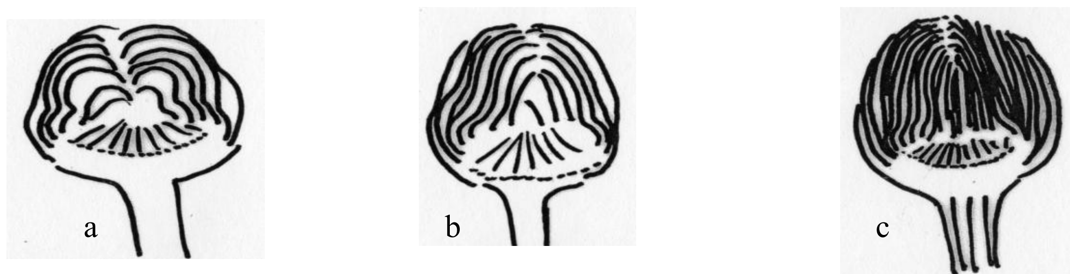


Fig. 1.5 Density of inners bracts of the central flower head on the basis of UPOV descriptors (a=sparse; b=medium; c=dense)

1.2.1.3 STATISTICAL ANALYSES

For the morphological traits, data were analyzed by ANOVA, using the GLM (Generalized Linear Model) procedure, Principal Component Analysis (PCA), Cluster analyses and Correlation analyses of SPSS software version 15.0. Mean separations were performed by the Duncan test. Significance was accepted at $p \leq 0.05$ level.

Table 1.1 Morphological traits measured, their codes and the level of significance from the ANOVA analysis in the three sources of variation

Code descriptor	Descriptor	Landraces	Year	Landrace *Year
Pheight	Plant height (including central flower head)	***	***	***
NLShoots	Number of lateral shoots on main floral stem	**	ns	ns
MSHeight	Main floral stem height (excluding central flower head)	***	***	***
DistHL	Distance between central flower head and youngest well developed leaf	***	***	ns
MSDiam	Main floral stem diameter	**	**	**
Leafatt	Leaf attitude	***	ns	ns
Spines	Spines	***	ns	ns
Llength	Leaf length	***	**	***
Linc	Leaf incision	ns	ns	ns
Nlob	Number of leaf lobes	***	**	***
ShTip	Shape of leaf tip	***	**	***
Nseclob	Number of secondary leaf lobes	ns	ns	ns
Shapeseclob	Shape of tip of leaf secondary lobes	***	ns	***
IntGreen	Intensity of leaf green colour	**	ns	***
HueGreen	Hue of leaf green colour	***	ns	ns
GreyHue	Intensity of leaf grey hue	***	* **	***
Hair	Leaf hairness	***	***	***
Blistering	Leaf blistering	ns	ns	ns
Anthocpet	Anthocyanin coloration at base of petiole	ns	ns	ns
HeadLength	Length of the central flower head	***	ns	*
HeadDiameter	Diameter of the central flower head	***	ns	ns
Length/DiamHead	Length/Diameter ratio of central flower head	***	ns	ns
TipShape	Shape of central flower head tip	***	ns	ns
AnthoHeadBract	Anthocyanin coloration of central flower head inner bracts	***	***	***
DensityBracts	Density of inner bracts	***	***	***
LengthBract	Length of outer bract base	***	ns	**
WidthBract	Width of outer bract base	***	**	***
ThickBract	Thickness of outer bract base	***	ns	**
ShapeBract	Main shape of outer bract	***	ns	ns
ApexBract	Shape of bract apex	ns	ns	ns
DepthBract	Depth of incision of outer bract	***	ns	***
ColBract	Colour of outer bract	***	***	ns
HueColBract	Hue of secondary colour of outer bract	***	***	ns
SizeSpine	Size of outer bract spine	***	ns	ns
Mucron	Mucron	***	***	***
LengthHead1	Length of first flower head on lateral shoot	***	ns	***
DiamHead1	Diameter of first flower head on lateral shoot	***	**	***

Table 1.1 Morphological traits measured, their codes and the level of significance from the ANOVA analysis in the three sources of variation (continuation)

ShapeHead1	Shape of first flower head on lateral shoot	***	**	***
RecepDiam	Receptacle diameter	***	ns	***
RecepThick	Receptacle thickness	***	***	***
RecepShape	Receptacle shape	***	***	**
HeadWeight	Central flower head weight	***	ns	***
TimeHead	Central flower head harvest time	***	*	**
TotalHeadNumber	Total number of heads	***	***	ns
TotalHeadWeight	Total weight of heads	***	***	***

*Ns= not significant, * p=0.05; 0.01 <p<0.05; p=0.01 (Duncan test)*

1.2.2 Results

Table 1.1 shows the results of ANOVA analysis with the statistical significant differences among landraces, between years and for landrace per year interaction related to each morphological trait. For the majority of qualitative traits, no significant landrace per year interaction was found. The landrace per year interaction was significant for most of the quantitative traits (i.e. number of lateral shoots of the main floral stem, distance between central flower head and the youngest first fully developed leaf, number of secondary leaf lobes, diameter of the central flower head, total number heads); however, showing that environmental conditions and cultural practices has a different influence on the landrace morphological profile. As regard the year, significant differences were found for the majority of the quantitative traits (i.e. plant height, main floral stem height, distance between the central flower head and the first fully developed leaf, main floral stem diameter, leaf length, number of leaf lobes, width of outer bract base, diameter of first flower head, receptacle thickness, central flower head harvest time, total head number and weight) showing a strong environmental influence. No significant differences among landraces were found for the traits: leaf incision, number of secondary leaf lobes, leaf blistering, anthocyanin coloration at the base of petiole and shape of outer bract apex. In both growing seasons, Bianco di Pertosa showed the greatest plant height while Tondo Rosso di Paestum, in the 1st year, and Bianco di Pertosa, in the 2nd one, had the highest number of lateral shoots (*Table 1.2*). In the 1st year, Castellammare and Tondo Rosso di Paestum and, in the 2nd one, Castellammare had the biggest main floral stem diameter. In both growing season, Bianco di Pertosa and in the 2nd year also Campagnano, were characterized by the maximum distance between the central flower head and the first fully developed leaf which is suitable for

mechanical harvesting. In both growing seasons, Castellammare and Campagnano had the longest leaves (*Table 1.2*). Among the landraces studied, only Pisa had leaves with spines.

In 2008 Ascolano and in 2009 Tondo Rosso di Paestum had the highest number of lobes on the leaves. Differences in leaf colour were significant among landraces; the differences are related to different intensity and tonality of green and grey colour. As regard the intensity of green colour, in 2008, Ascolano and Tondo Rosso di Paestum and, in 2009, Ascolano, Castellammare and Tondo Rosso di Paestum showed the most pale green-coloured leaves. As regard the central flower head, length/diameter ratio of the heads was calculated as representative shape index. In 2008 Pisa and in 2009 Pisa, Jesino, Montelupone A and Montelupone B were the landraces with the most elongated heads. On the contrary, Castellammare, Tondo Rosso di Paestum, and Bianco di Pertosa showed elliptical heads in both growing seasons. In both years, Jesino, Pisa and Montelupone B had the most acute head tip. In 2008, Jesino, Montelupone A and Montelupone B and, in 2009, Bianco di Pertosa, Montelupone A and Montelupone B had the greatest value for the width of outer bract. In both growing seasons, Bianco di Pertosa showed the most green coloured heads (absent hue of secondary colour) while Tondo Rosso di Paestum was the landrace with completely purple heads (absent hue of secondary colour). Other landraces showed different green striped with violet heads.

Regarding yield, Tondo Rosso di Paestum in the 1st year and Pisa in 2nd year showed the highest values of total head weight per plant (*Table 1.3*). In 2008, Ascolano, Castellammare, Bianco di Pertosa and Tondo Rosso di Paestum showed the highest weight of central flower head while, in 2009, only Castellammare provided the highest value. For earliness, in both growing seasons, Castellammare was the earliest landrace while, in 2008, Bianco di Pertosa, Jesino and Montelupone B and, in 2009, Montelupone B were the latest landraces (*Table 1.3*).

Table 1.2 Differences in some quantitative and qualitative traits of the plant of the nine landraces studied

Landraces	**Year**	**Pheight (cm)**	**NLShoots (no.)**	**MSDiam (cm)**	**MSHeight (cm)**	**DistHL (cm)**	**Llength (cm)**	**Nlob (no.)**	**Shtip (no.)**	**ShapeSeclob (no)**	**HueGreen (no.)**	**GreyHue (no.)**	**Hair (no.)**	**IntGreen (no.)**	**Spines (no.)**
Ascolano	1	63.38bc	3.33ab	2.76ab	58.03b	27.17ce	95.06cd	7.67a	3.00a	3.00ns	2.00b	3.00d	1.00b	5.00b	No
Campagnano	1	72.35b	3.17ab	2.75ab	63.75b	33.50ac	110.31ab	7.00ab	2.00b	3.00ns	1.50c	3.00d	1.00b	7.00a	No
Castellammare	1	63.85bc	2.67b	3.08a	55.07bc	24.33de	114.81a	5.83bd	1.50bc	3.00ns	1.00d	3.00d	1.00b	6.67a	No
Jesino	1	56.35cd	2.83b	2.41bc	48.15c	22.00e	86.32d	5.33cd	1.00c	3.00ns	3.00a	5.00b	2.33a	7.00a	No
MonteluponeA	1	52.23e	3.50ab	2.28c	47.10c	25.50de	83.69d	5.00d	1.00c	2.83ns	3.00a	3.00d	1.00b	7.00a	No
MonteluponeB	1	66.36b	3.20ab	2.70ab	61.02b	20.80e	88.32d	5.00d	1.00c	3.00ns	3.00a	6.20a	1.40b	7.00a	No
B. Pertosa	1	106.40a	3.33ab	2.57bc	102.90a	38.33a	95.53cd	5.83bd	2.00b	3.00ns	3.00a	3.00d	1.00b	7.00a	No
Pisa	1	66.97b	3.20ab	2.59bc	58.66b	30.00bd	102.45bc	6.80ac	1.00c	2.80ns	3.00a	4.20c	1.00b	7.00a	Yes
TR Paestum	1	66.73b	4.17a	2.94a	63.93b	35.33ab	95.03cd	7.33ab	2.00b	3.00ns	3.00a	3.00d	1.00b	5.00b	No
Ascolano	2	75.72c	3.17bc	2.54c	65.16d	22.67c	94.06b	7.50cd	3.00a	3.00a	2.00b	3.00e	1.00b	5.00b	No
Campagnano	2	98.02b	3.33ab	2.54c	88.96b	31.83a	120.98a	8.50ab	2.67a	3.00a	2.00b	3.00e	1.00b	7.00a	No
Castellammare	2	66.68d	2.17d	2.98a	57.80e	24.83c	113.65a	8.33ac	1.83b	3.00a	1.00c	3.00e	1.00b	5.33b	No
Jesino	2	63.24d	2.83bc	2.09d	53.58e	14.33d	65.53c	6.17e	2.00b	3.00a	3.00a	5.00c	3.00a	7.00a	No
MonteluponeA	2	59.23d	2.67c	2.43c	53.39e	25.17c	95.86b	8.17bc	1.00c	3.00a	3.00a	3.00e	1.00b	7.00a	No
MonteluponeB	2	66.58cd	3.00bc	2.13d	59.83de	10.67d	50.64d	6.50e	2.00b	3.00a	3.00a	6.00b	3.00a	7.00a	No
B. Pertosa	2	102.90a	3.67a	2.46c	98.70a	31.33a	99.03b	7.00de	2.00b	3.00a	3.00a	7.00a	3.00a	7.00a	No
Pisa	2	90.83b	3.33ab	2.77b	81.71c	26.33bc	102.63b	6.67de	1.00c	2.00b	3.00a	7.00a	3.00a	7.00a	Yes
TR Paestum	2	63.56d	3.00bc	2.55c	59.49de	30.20ab	90.22b	9.20a	2.80a	3.00a	3.00a	4.60d	1.00b	5.00b	No

Different letters indicate statistical significant differences among genotypes at $p \leq 0.05$ (ANOVA analysis, Duncan test), 1= 2008/2009 and 2=2009/2010

Table 1.3 Differences in some quantitative and qualitative traits of the head and yield of the nine landraces studied

Landraces	Year	Length/Diam Head	HeadDiameter (cm)	HeadLength (cm)	TipShape (no.)	AnthoHead Bract (no.)	DensityBracts (no.)	WidthBract (mm)	ThickBract (mm)	LengthBract (mm)	DepthBract (no.)	ColBract (no.)	HueColBract (no.)
Ascolano	1	0.96c	9.30bc	8.97b	3.00b	5.00a	6.00ab	2.78cd	0.63ab	1.10a	7.00a	3.33bc	3.33b
Campagnano	1	0.88d	8.92c	7.92c	3.00b	4.67a	6.20a	2.50de	0.43c	0.87b	6.33ab	3.67bc	3.00b
Castellammare	1	0.79e	10.20a	8.10c	3.00b	3.67a	5.80ab	2.30e	0.52bc	1.23a	5.00bd	2.80cd	3.00b
Jesino	1	1.09b	9.48ac	10.35a	1.00d	5.00a	4.20c	3.63a	0.75a	0.98b	4.33cd	3.00bc	4.67a
Montelupone A	1	1.03b	8.98c	9.23b	2.00c	1.00b	4.00c	3.28ab	0.55bc	0.87b	4.67bd	2.00d	3.33b
Montelupone B	1	1.05b	8.90c	9.38b	1.00d	1.00b	5.80ab	3.58a	0.70a	1.10a	6.00ac	3.80b	3.40b
Bianco Pertosa	1	0.76e	9.95ab	7.60cd	3.00b	1.00b	7.00a	3.00bc	0.63ab	1.28a	5.00bd	1.00e	3.00b
Pisa	1	1.19a	7.80d	9.34b	1.00d	5.40a	4.60bc	2.36de	0.58b	0.98b	4.00d	3.80b	3.40b
TR Paestum	1	0.74e	9.20bc	6.90d	4.00a	4.67a	7.00a	2.62ce	0.55bc	1.07a	5.00bd	5.00a	3.00b
Ascolano	2	0.93b	9.48bc	9.17bc	3.00b	3.00c	6.00ab	2.44b	0.56bc	1.15bc	5.33bc	2.17c	5.67a
Campagnano	2	0.95b	8.80cd	8.37d	3.00b	3.67b	6.33ab	2.30bc	0.57bc	1.07c	4.67c	2.67b	5.00a
Castellammare	2	0.76c	10.75a	8.20d	3.00b	2.67c	6.00ab	2.03c	0.54c	1.08c	6.33ab	2.67b	5.00a
Jesino	2	1.10a	8.26d	9.08c	1.00d	1.00d	5.33b	2.32bc	0.64a	0.88d	4.33cd	3.00b	4.67ab
Montelupone A	2	1.03ab	9.71bc	9.95a	2.00c	1.00d	7.00a	3.39a	0.59bc	1.16bc	4.33cd	2.00c	5.00a
Montelupone B	2	1.07a	8.04d	8.67cd	1.00d	1.00d	6.67ab	3.32a	0.61ab	1.10c	6.00ab	2.83b	4.67ab
Bianco Pertosa	2	0.80c	10.39ab	8.29d	3.00b	1.00d	7.00a	3.24a	0.57bc	1.62a	7.00a	1.00d	3.00c
Pisa	2	1.28a	8.71cd	9.79ab	1.00d	3.00c	6.67ab	2.24bc	0.53cd	1.13bc	3.00e	3.00b	5.00a
TR Paestum	2	0.80c	8.85cd	7.10e	4.00a	5.40a	6.20ab	2.59b	0.48d	1.25b	3.40de	4.40a	3.80bc

Different letters indicate significant differences among genotypes at $p \leq 0.05$ (ANOVA analysis, Duncan test), 1= 2008/2009 and 2=2009/2010

Table 1.3 Differences in some quantitative and qualitative traits of the head and yield of the nine landraces studied (continuation)

Landraces	**Year**	**SizeSpine (no.)**	**Mucron (no.)**	**RecepDiam (mm)**	**RecepThick (mm)**	**RecepShape (no.)**	**HeadWeight (kg)**	**TimeHead (no.)**	**TotalHead Number (no.)**	**TotalHead Weight (kg)**
Ascolano	1	1.00b	1.00c	4.80ab	0.54b	2.00cd	0.39a	181.00bc	11.33bc	1.88b
Campagnano	1	1.00b	1.00c	4.28bc	0.52b	1.83de	0.32ab	178.17c	14.0ab	1.97b
Castellammare	1	1.00b	1.00c	5.08a	0.62a	1.67e	0.36a	166.33d	13.67ab	1.94b
Jesino	1	1.00b	3.67bc	4.85ab	0.52b	2.50b	0.37a	200.83a	5.67d	0.99c
Montelupone A	1	1.00b	2.33bc	5.08a	0.52b	2.17c	0.33ab	183.00b	8.00cd	1.23bc
Montelupone B	1	1.00b	2.60bc	4.32bc	0.48b	3.00a	0.32ab	199.75a	8.50cd	0.92c
Bianco Pertosa	1	1.00b	1.00c	4.35ac	0.50b	3.00a	0.36a	199.00a	10.83bc	1.49b
Pisa	1	3.00a	7.40a	3.22d	0.50b	3.00a	0.28b	183.60b	13.40ab	1.94b
TR Paestum	1	3.00a	5.00ab	4.05c	0.50b	2.00cd	0.35a	180.17bc	16.33a	2.43a
Ascolano	2	1.00c	1.00b	4.38b	0.66de	2.67ab	0.34ac	189.67ed	9.17bc	1.59b
Campagnano	2	1.00c	1.00b	4.02b	0.63e	2.33bc	0.30ce	188.33e	10.67bc	1.84b
Castellammare	2	1.00c	7.67a	5.03a	1.20a	1.83d	0.41a	174.50g	11.83ab	1.91ab
Jesino	2	1.00c	9.00a	3.92b	0.83bc	2.83a	0.27de	204.67b	4.00d	0.98d
Montelupone A	2	1.00c	7.67a	5.46a	0.78cd	2.83a	0.38ab	184.67f	8.67c	1.56b
Montelupone B	2	1.00c	9.00a	4.02b	0.71ce	3.00a	0.24e	208.00a	4.00d	0.96d
Bianco Pertosa	2	1.00c	9.00a	5.36a	0.91b	3.00a	0.39ab	201.00c	10.33bc	1.64b
Pisa	2	4.33a	1.00b	3.20c	0.73ce	3.00a	0.33bc	191.07d	13.83a	2.20a
TR Paestum	2	3.00b	1.00b	3.19c	0.62e	2.00cd	0.27de	188.60e	8.60c	1.16c

Different letters indicate significant differences among genotypes at $p \leq 0.05$ (ANOVA analysis, Duncan test), 1= 2008/2009 and 2=2009/2010

Based on these morphological traits, a similarity dendrogram was constructed using an agglomerative hierarchical cluster analysis (*Figure 1.6*). On the basis of the similarity dendrogram, our landraces could be classified into three major clusters. Cluster 1 consisted of two landraces, Jesino and Montelupone B. Cluster 2 consisted of Ascolano, Bianco di Pertosa and Montelupone A. Castellammare, Campagnano, Pisa, and Tondo Rosso di Paestum were grouped in cluster 3.

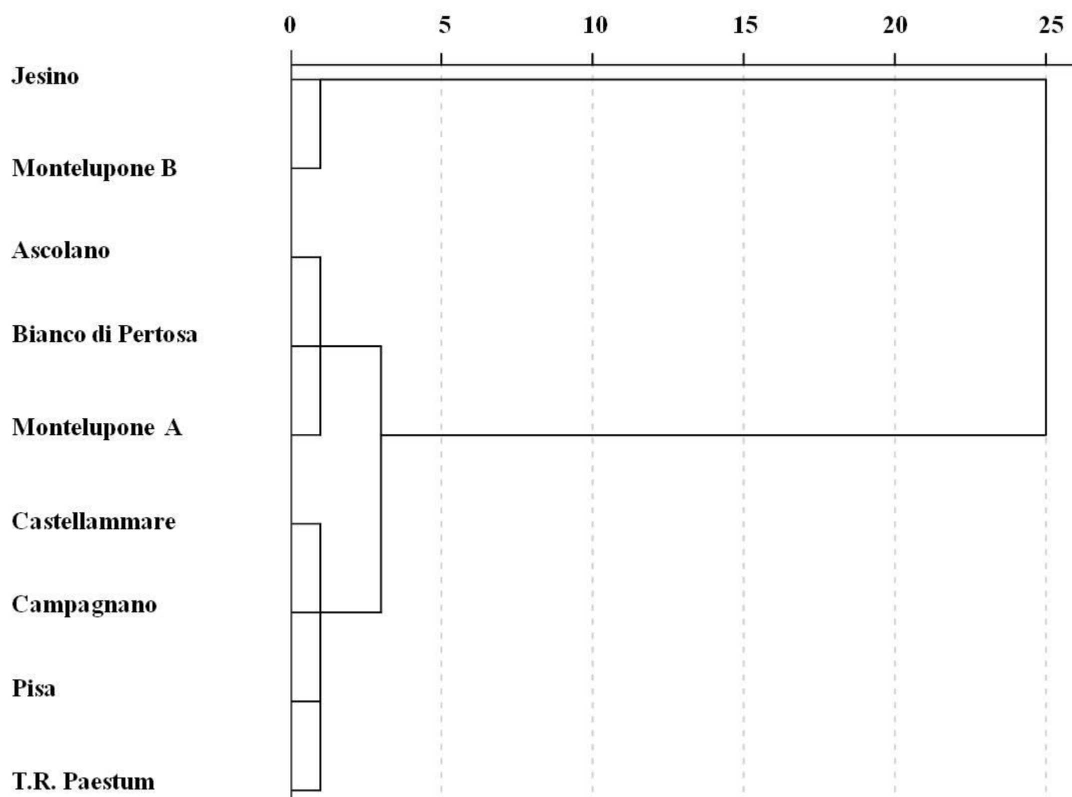


Fig. 1.6 Similarity dendrogram based on agglomerative hierarchical cluster analysis

An among-landraces pairwise similarity matrix was generated with the quantitative and qualitative morphological traits for the two growing seasons and a PCA was then applied. These first three functions explained 51.08% of the variance and each landrace was plotted against these three functions (*Table 1.4*). The first PC factor (20.76% of variance) included contributions from the following primary traits: NLShoots, Spines, IntGreen, Huegreen, Greyhue, Hair, Length/DiamHead, ShapeBract, LengthHead, RecepShape, and TimeHead (see abbreviations on *Table 1*). The second factor (16.30% of the variance) involved Pheight, MSHeight, MSDiam, Llength, RecepThick, TotalHeadNumber and TotalHeadWeight. The third factor (14.01% of the variance) included HeadDiameter, WidthBract, ApexBract, RecepDiam and DepthBract.

Table 1.4 Variance explained for each principal component

Component	Total Eigenvalues	Variance explained (%)	Cumulative variance (%)
1	8.099	20.766	20.766
2	6.359	16.305	37.071
3	5.463	14.008	51.079
4	4.200	10.770	61.849
5	2.396	6.142	67.991
6	1.773	4.546	72.537
7	1.691	4.336	76.873
8	1.429	3.665	80.538
9	1.073	2.752	83.290
10	0.832	2.134	85.425

The distribution of the landraces against the first three discriminant functions (*Figure 1.7*) showed that three groups could be identified, with the landraces from Latium and Campania regions on the right and those from Marche region on the left while Pisa landrace from Tuscany region is on the upper right.

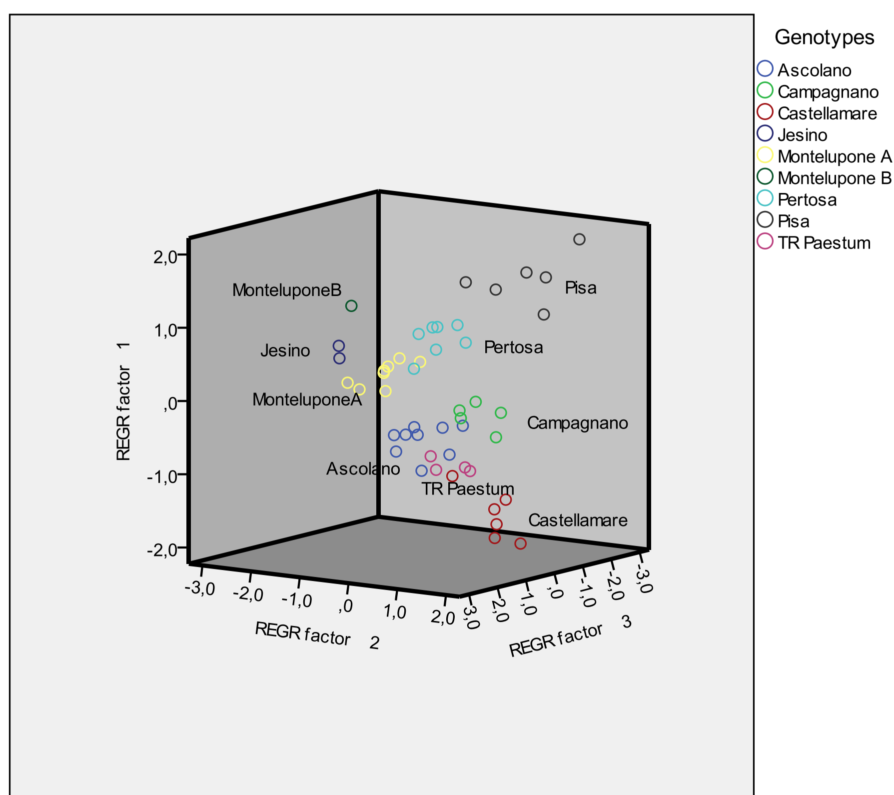


Fig 1.7 Distribution of the analysed landraces against the three discriminant functions

1.2.3 Discussion

In the present work, spring type germplasm has been characterized morphologically and their morphological profile has been given. It was found that significant intra-landrace differences exist, due mainly to their multiclonal composition derived from selection adopted by farmers during vegetative propagation (Lanteri *et al.,* 2001; Raccuia *et al.,* 2004a; Lanteri and Portis, 2008; Mauro *et al.,* 2009). Despite this important source of genetic variation, significant differences also exist among the nine landraces and most of these may be well identified, confirming the importance of preserving this germplasm against the risk of genetic erosion. In particular, some traits allowed a clear identification of certain landraces. For example, Pisa is characterized by the presence of spines on the leaves, purple elongated heads and high yields, while Bianco di Pertosa is characterized by green-coloured, elliptical and spineless heads. Traits such as number of lateral shoots and primary flower heads, spine presence on the leaves, intensity and hue of leaf colour, leaf hairiness, shape of central flower head and bract, and earliness explained the majority of total variance in the PCA and can be used for landrace classification. In agreement with previous observations (Crinò *et al.,* 2008), the contributions from length of first order heads, plant height, diameter of main floral stem, total head number and weight are also important. The distribution of our nine traditional landraces against the first three discriminant functions based on morphological traits (*Figure 1.8*) showed that they could be grouped into three clusters, each corresponding to a distinct geographical location: (i) Latium and Campania (in the bottom right), (ii) Marche (on the left), and (iii) Tuscany (in the upper right) regions.

It is interesting to note that the landrace per year interaction was significant for most of the quantitative traits, which are differently affected by the environmental conditions and cultural practices. This makes difficult a clear landraces identification on the basis of morphological traits only (Cravero *et al.,* 2010; Lahoz *et al.,* 2011). In fact, morphological characterization may represent only an initial step for the description and classification of many species plants (Lahoz *et al.,* 2011). The complementarity of both morphological and molecular analyses can contribute to the accurate identification and classification of landraces in the management of plant genetic resources for *in-situ* and *ex-situ* conservation as well as for use in plant breeding programs (Crinò *et al.,* 2008; Lo Bianco *et al.,* 2011). In fact, although the Montelupone A and Montelupone B landraces are phenotypically different and placed in two different clusters, their molecular profiles revealed a common genetic origin (Ciancolini *et al.,* 2012). The

division of Montelupone landraces into the two sub-groups A and B was based on morphological traits such as earliness, head characteristics and yield as well as plant aspect, but not on their overall genetic structure. Jesino and Montelupone B, already placed in the same cluster by the morphological analysis, maintain the same grouping with Montelupone A when molecular analysis is applied; this indicates that the three landraces could have been selected from the same ancestral origin (Ciancolini *et al.,* 2012). On the contrary, Castellammare, Campagnano, and Tondo Rosso di Paestum, belonging to the ‗Romanesco' typology but coming from different geographic locations and showing some different phenotypic characteristics (earliness, plant and head size), have been grouped in the same cluster when morphological descriptors are used. Molecular analysis discriminates rather well these three landraces. Despite, although of the same regional origin as Jesino, Montelupone A, and Montelupone B, also the genotype Ascolano has been well separated from the other three landraces on the basis of both molecular and morphological profiles. Instead, cluster analyses for morphological and molecular data showed different classifications for Pisa and Bianco di Pertosa.

Information obtained so far on genetic characterization is an important tool to well identify germplasm for the certification of typical products as added value for autochthonous landraces. Selection of clones within local landraces is a fundamental pre-requisite for the release of new varieties or for the development of homogeneous lines useful as parents of hybrid combinations in plant breeding programs. The need to preserve traditional globe artichoke germplasm is important for its safeguarding against the risk of genetic erosion. This opens new perspectives for the genetic improvement of globe artichoke, which has not up now widely studied.

1.3 ITALIAN ‗ROMANESCO' CLONES

1.3.1 MATERIALS AND METHODS

1.3.1.1 GENETIC RESOURCES AND OPEN FIELD EXPERIMENTS

Ten Italian genotypes of spring globe artichoke (S2, S3, S5, S11, S17, S18, S22, S23, Campagnano, Grato1) [‗Romanesco' type], cultivated in Latium Region, were recovered from the farmers and considered in the present work. The cultivation areas are described in Figure 1.8.

All trials were conducted for two growing seasons, at the Experimental Field Station of ARSIAL (Latium Regional Agency for the Development and the Innovation of

Agriculture) in Cerveteri, Rome - Italy (41° 59‘ N 12° 01‘ E). The ten vegetatively micropropagated ‗Romanesco‘ genotypes were morphologically characterized. Plant material was arranged in a randomized block experimental design with four replications of 20 plants per genotype. Globe artichoke plants with 4-5 leaves were transplanted on 17 August 2007. Planting density was 0.77 plants m^2, with inter and intra-row distances of 1.30 and 1.0 m, respectively.

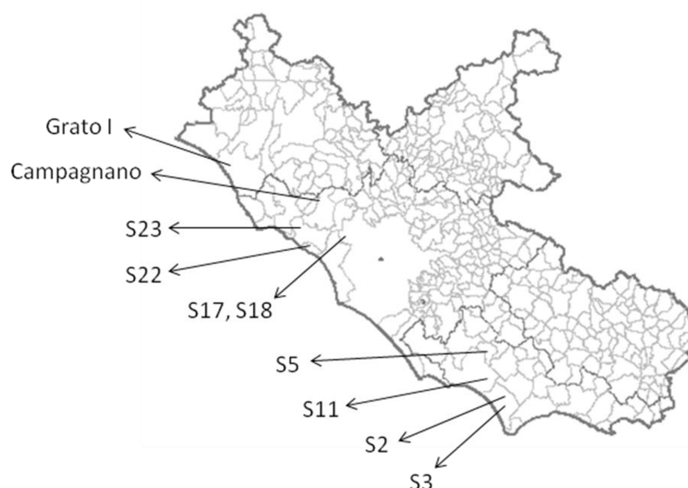


Fig. 1.8 Cultivation areas of the 'Romanesco' landraces morphologically characterized

1.3.1.2 MORPHOLOGICAL CHARACTERIZATION USING UPOV DESCRIPTORS

Forty-seven morphological and agro-physiological data were recorded, for two years at the harvest time, on 12 plants per genotype. The morphological characterization was done using standard UPOV (International Union for the Protection of New Varieties of Plants) (CPVO-TP/184/1 Final 2001) descriptors for globe artichoke (*Table 1.5*) (Pagnotta *et al.,* 2009, 2012) and a well-defined group of complementary ‗Romanesco‘ type descriptors validated in a previous work (Crinò *et al.,* 2008). The morphological characterization was performed as aforementioned (session 1.2.1.1.1).

1.3.1.3 STATISTICAL ANALYSES

Statistical analyses were performed as above described in Section 1.2.1.3.

1.3.2 RESULTS

Forty-seven agro-morphological traits were measured in present work. The statistical significance of the genotype, the year and their interaction is shown in the Table 1.5. For the majority of quantitative and qualitative traits measured significant differences

among genotypes were found except for the traits: shape of leaf secondary lobes, intensity of leaf green colour, intensity of leaf grey hue, leaf hairiness and blistering, anthocyanin coloration at the base of petiole, size of head spine, and depth of head outer bract. For most of quantitative and qualitative traits, also significant genotype per year interactions were found, except for the traits: distance between the central flower head and the first fully developed leaf, main floral stem diameter, leaf incision, number of leaf lobes, shape of head tip, density of inner head bracts, width and thickness of outer bract base, central flower head receptacle diameter and weight. As regard the growing season, significant differences were found for all traits evaluated except for the traits: leaf incision, shape of leaf tip, thickness of outer bract base, apex of head outer bract, depth of emargination of outer bract, length of the central flower head and receptacle diameter.

Table 1.5 Statistical significance of the genotype, the year and their interaction in the morphological characterization of the 10 'Romanesco' genotypes

Code descriptor	Descriptor	Genotype	Year	Genotype* Year
Pheight	Plant height (including central flower head)	*	***	***
NLShoots	Number of lateral shoots on the main floral stem	*	**	***
MSHeight	Main floral stem height (excluding central flower head)	*	***	***
DistHL	Distance between central flowerhead and youngest well developed leaf	*	***	ns
MSDiam	Main floral stem diameter	*	***	ns
Leafatt	Leaf attitude	*	***	***
Spines	Spines	*	***	***
Llength	Leaf length	*	***	***
Linc	Leaf incision	*	ns	ns
Nlob	Number of leaf lobes	*	***	ns
ShTip	Shape of leaf tip	*	ns	ns
Nseclob	Number of leaf secondary lobes	*	**	***
Shapeseclob	Shape of tip of leaf secondary lobes	ns	ns	ns
IntGreen	Intensity of leaf green colour	ns	***	***
HueGreen	Hue of leaf green colour	*	***	***
GreyHue	Intensity of leaf grey hue	ns	**	***
Hair	Leaf hairness	ns	ns	ns
Blistering	Leaf blistering	ns	***	***
Anthocpet	Anthocyanin coloration at base of petiole	ns	**	*
HeadLength	Length of the central flower head	*	**	**

Table 1.5 Statistical significance of the genotype, the year and their interaction in the morphological characterization of the 10 'Romanesco' genotypes (continuation)

Length/DiamHead	Length/Diameter ratio of central flower head	*	***	**
TipShape	Shape of tip of the central flower head	*	ns	ns
AnthoHeadBract	Anthocyanin coloration of central flower head inner bracts	*	**	***
DensityBracts	Density of inner bracts	*	*	ns
LengthBract	Length of outer bract base	*	***	***
WidthBract	Width of outer bract base	*	***	ns
ThickBract	Thickness of outer bract base	*	ns	ns
ShapeBract	Main shape of outer bract	*	***	***
ApexBract	Shape of bract apex	*	ns	**
DepthBract	Depth of emargination of outer bract	ns	ns	**
ColBract	Colour of outer bract	*	***	***
HueColBract	Hue of secondary colour of outer bract	*	**	**
SizeSpine	Size of spine	ns	ns	ns
Mucron	Mucron	*	***	***
LengthHead1	Length of first flower head on lateral shoot	*	ns	***
DiamHead1	Diameter of first flower head on lateral shoot	*	**	***
RecepDiam	Receptacle diameter	*	ns	ns
RecepThick	Receptacle thickness	*	***	***
RecepShape	Receptacle shape	*	***	**
HeadWeight	Central flower head weight	*	*	ns
TimeHead	Time appearance central flower head	***	***	***
TotalHeadNumber	Total number of heads	*	**	***

*Ns= not significant, * p=0.05; 0.01 <p<0.05; p=0.01 (Duncan test)*

As reported in Table 1.6, in 2008, the genotypes S5, S11, S18, Campagnano and Grato 1 and, in 2009, S18 and Campagnano showed the highest value for plant height. In the first year, S18 and Grato 1 and, in 2009, Campagnano provided the highest number of lateral shoots. In both growing season, there were no genotypes characterized by spine presence on the leaves and heads. In 2008, the genotypes S3, S11, S18, Campagnano and Grato 1 and, in 2009, S18 and Campagnano showed the longest distance between the youngest leaf on the main floral stem and the central flower head. In 2008, S17 and, in 2009, S18 had the biggest main floral stem diameter. In 2008, the genotype S2 and, in 2009, S5 and S11 showed the shortest leaves. As regard the leaf colour, in 2008, the genotype S2 and, in 2009, S22 and S23 showed the most pale green colour. In both growing season, all genotypes were characterized by no hairy leaves and blistering.

As regard the central flower head, in 2008, Grato 1 showed the biggest value of the head length while, in 2009, S11 and Grato 1 had the highest value for this trait. For the central head diameter, in 2008, the genotype S22 and, in 2009, S2, Grato 1 and S22 provided the best value for this trait. As regard the head shape, in both years, Campagnano showed the most elongated heads while S2, S17, S18, S22, and S23, in 2008, and the genotype S22, in 2009, provided the most elliptical heads. In 2008, S11 and Grato 1 and in 2009, S5 and Campagnano showed the strongest anthocyanin coloration of head inner bracts. As regard the head colour, in 2008, the genotypes S11 and Grato 1 and, in 2009, only Grato 1 showed the most purple striped heads while S17, S22 and S23, in 2008, and, S5 and S17, in 2009, were characterized by the most green-coloured heads. The genotypes S23 in the first growing season and S17, S22 and S23 in the second one presented mucron upon the head bract. In both growing season, S17 showed the earliest time of central flower head appearance while S2, in 2008, and S2, S5, S11, in 2009, showed the latest production. As regard the total head number, Campagnano, Grato 1, S22 and S23, in 2008, and S22 and S23, in 2009, showed the highest value. In 2008, Grato 1 and S23 and, in 2009, Grato 1, S22 and S23 provided the highest heads yield, expressed as kg of head per plant.

Table 1.6 Differences in some morphological traits of the ten 'Romanesco' genotypes analyzed for two years

Genotypes	Year	Pheight (cm)	NLShoots (no.)	MSHeight (cm)	DistHL (cm)	MSDiam (cm)	Llength (cm)	Nlob (no.)	Shtip (no.)	IntGreen (no.)	HueGreen (no.)	GreyHue (no.)	Hair (no.)	Blistering (no.)	Spines (no.)
2	1	59.50c	2.58cd	52.12d	23.12c	2.86c	92.75c	5.42c	2.42a	5.33b	1.33c	3.00ns	1.00 ns	1.00ns	1.00ns
3	1	66.33b	2.42d	59.77c	32.92a	2.72cd	104.17b	6.08bc	1.50c	6.17ab	1.50bc	3.33ns	1.00 ns	1.00ns	1.00ns
5	1	80.92a	2.42d	72.59a	31.75ab	2.61d	107.42ab	5.75bc	1.83bc	6.00ab	1.75ab	3.17ns	1.00 ns	1.00ns	1.00ns
11	1	77.83a	2.50cd	69.91ab	34.00a	2.84cd	109.42ab	6.50ab	1.33c	6.17ab	1.50bc	3.00ns	1.00 ns	1.00ns	1.00ns
17	1	54.92c	3.25ab	47.39d	29.08ac	3.13a	111.83ab	6.42ab	2.50a	7.00a	1.00d	3.00ns	1.00 ns	1.00ns	1.00ns
18	1	75.12a	3.50a	68.30ab	35.42a	2.91bc	114.58ab	5.83bc	2.17ab	6.33ab	1.92a	3.00ns	1.00 ns	1.00ns	1.00ns
Campagnano	1	79.83a	3.25ab	72.50a	36.25a	2.73cd	112.33ab	7.33a	2.25ab	6.67a	1.75ab	3.17ns	1.00 ns	1.00ns	1.00ns
Grato1	1	74.33a	3.58a	65.67b	35.40a	2.78cd	107.38ab	6.33bc	2.67a	6.50a	1.33c	3.00ns	1.00 ns	1.00ns	1.00ns
22	1	57.37c	2.67bd	48.97d	25.05bc	3.11ab	117.13a	6.00bc	1.50c	6.50a	1.50bc	3.00ns	1.00 ns	1.00ns	1.00ns
23	1	54.37c	3.08ac	46.82d	24.29c	2.93ac	111.37ab	6.58ab	1.83bc	6.33ab	1.33c	3.00ns	1.00 ns	1.00ns	1.00ns
2	2	77.71c	2.92ac	69.65c	23.58bc	2.75bc	95.21de	6.83bc	2.25b	5.50ce	2.42ab	3.00ns	1.00 ns	1.00ns	1.00ns
3	2	76.46cd	2.58bd	67.75c	26.29b	2.59cd	94.21de	7.33ac	1.50c	5.67cd	2.75a	3.00ns	1.00 ns	1.00ns	1.00ns
5	2	89.25b	2.83ac	80.54b	23.17bc	2.47d	89.42e	6.67bc	1.25cd	5.83bc	2.75a	3.50ns	1.00 ns	1.00ns	1.00ns
11	2	81.83c	2.50cd	72.79c	24.92bc	2.67bd	92.25e	7.33ac	1.00d	5.50ce	2.75a	4.00ns	1.00 ns	1.00ns	1.00ns
17	2	53.21f	2.75ac	46.16e	21.33cd	2.73bc	120.98a	6.25c	2.08b	5.17de	1.83cd	3.33ns	1.00 ns	1.00ns	1.00ns
18	2	97.58a	2.83ac	89.38a	34.58a	2.98a	113.42ab	7.58ab	2.67a	6.00bc	1.83cd	3.00ns	1.00 ns	1.00ns	1.00ns
Campagnano	2	101.50a	3.25a	93.03a	32.92a	2.50d	120.50a	8.42a	2.83a	6.67a	2.33b	3.00ns	1.00 ns	1.00ns	1.00ns
Grato1	2	69.75d	3.08ab	60.89d	23.46bc	2.50d	93.42de	6.42bc	3.00a	6.33ab	2.50ab	4.00ns	1.00 ns	1.00ns	1.00ns
22	2	62.33e	2.25d	54.55d	21.08cd	2.83ab	112.71ab	7.42ac	1.58c	5.00e	1.50d	3.00ns	1.00 ns	1.00ns	1.00ns
23	2	53.96f	2.42cd	46.21e	18.29d	2.77ac	106.92bc	6.83bc	1.25cd	5.00e	1.92c	3.17ns	1.00 ns	1.00ns	1.00ns

Different letters indicate statistical significant differences among genotypes at $p \leq 0.05$ (ANOVA analysis, Duncan test), 1=2008/2009 and 2=2009/2010

Table 1.7 Differences in some morphological traits of the flower head and yield of the ten 'Romanesco' genotypes analyzed for two years

Genotypes	Year	HeadLength (cm)	HeadDiameter (cm)	Length/Diam Head (no.)	TipShape (no.)	AnthoHeadBract (no.)	DensityBract (no.)	LengthBract (mm)	WidthBract (mm)	ThickBract (mm)	DepthBract (mm)
2	1	7.37b	10.11ab	0.73d	3.50ab	4.00bd	4.67c	0.74b	3.05a	0.59a	6.67a
3	1	7.93ab	9.38bc	0.85c	2.50cd	4.25bd	5.00bc	0.93b	2.61bc	0.55ab	6.50a
5	1	8.32ab	9.55b	0.87b	2.50cd	5.00ab	5.00bc	0.91b	2.87ab	0.58a	6.50a
11	1	7.92ab	9.44bc	0.84c	1.33e	6.00a	5.33bc	1.05ab	2.68bc	0.58a	5.33b
17	1	7.52ab	10.03ab	0.74d	3.58a	4.33bd	4.50c	0.75b	2.52cd	0.54ab	4.50b
18	1	7.52ab	9.32bc	0.81d	2.82bd	5.00ab	5.89ab	0.87b	2.71bc	0.51ab	5.33b
Campagnano	1	7.94ab	8.77c	0.90a	2.92ad	4.50bc	6.50a	0.86b	2.47cd	0.48b	5.00b
Grato1	1	8.66a	9.97b	0.87b	2.25d	6.17a	4.83c	0.90b	2.64bc	0.49b	4.33b
22	1	8.41ab	10.73a	0.78d	2.83bd	3.50cd	5.50bc	1.26a	2.27d	0.52ab	5.00b
23	1	7.56ab	9.85b	0.76d	3.17ac	3.17d	5.17bc	0.94b	2.27d	0.53ab	4.50b
2	2	8.06cd	9.76a	0.83cd	3.58a	2.50ac	4.17c	1.13ab	2.43a	0.57a	4.83cd
3	2	8.75ab	9.38ab	0.93ab	2.58c	2.50ac	6.00a	1.19a	2.39a	0.53a	4.83cd
5	2	8.71ab	9.39ab	0.93ab	2.42cd	3.17a	6.33a	1.27a	2.60a	0.59a	5.17bd
11	2	9.04a	9.57ab	0.95ab	2.00d	2.33ac	6.50a	1.15ab	2.31ab	0.56a	5.00cd
17	2	7.04e	8.23c	0.87bd	3.17ab	2.50ac	4.83bc	0.95bc	1.72c	0.42bc	5.33bc
18	2	8.20bd	9.34ab	0.88bc	3.08b	3.00ab	6.50a	1.14ab	2.44a	0.55a	4.33d
Campagnano	2	8.47ac	8.81bc	0.96a	3.08b	3.50a	6.33a	1.13ab	2.31ab	0.57a	4.83cd
Grato1	2	8.86a	9.86a	0.90ac	2.42cd	2.33ac	6.00a	0.97bc	2.02bc	0.39c	5.83ab
22	2	7.78d	9.92a	0.80d	3.33ab	1.83bc	5.83a	0.91c	1.79c	0.48ac	6.17a
23	2	7.75d	9.37ab	0.83cd	3.17ab	1.67c	5.50ab	0.92c	1.77c	0.52ab	5.83ab

Different letters indicate statistical significant differences among genotypes at p≤0.05 (ANOVA analysis, Duncan test), 1=2008/2008 and 2=2009/2010

Table 1.7 Differences in some morphological traits of the flower head and yield of the ten 'Romanesco' genotypes analyzed for two years (continuation)

Genotypes	Year	ColourBract (no.)	HueColBract (no.)	Mucron (no.)	RecepDiam (mm)	RecepThick (mm)	RecepShape (no.)	HeadWeight (kg)	TimeHead (no.)	TotalHeads (no.)	Wheads (kg)
2	1	3.25de	3.67ns	1.00b	5.02a	0.63ab	1.75be	0.36ac	190.00a	8.67e	1.32d
3	1	3.50de	3.17ns	1.00b	4.36cd	0.49b	2.00ac	0.33bc	183.17b	12.75bd	1.56cd
5	1	3.08e	3.25ns	1.00b	4.82ac	0.51b	2.18a	0.36ac	183.67b	10.92ce	1.49cd
11	1	4.58a	3.00ns	1.00b	4.40bd	0.52b	2.08ab	0.37ac	182.91b	13.67ac	1.69bd
17	1	2.25f	3.67ns	1.67b	5.24a	0.72a	1.08g	0.38ab	154.83f	11.00ce	2.06ab
18	1	3.92bc	3.36ns	1.00b	4.93ab	0.52b	1.17fg	0.36ac	176.82c	10.67de	1.93bc
Campagnano	1	3.75bd	3.00ns	1.00b	4.01d	0.52b	1.83ad	0.32b	177.92c	13.00ad	1.72bd
Grato1	1	4.17ab	3.17ns	1.00b	4.76ac	0.53b	1.67ce	0.41a	169.17d	15.75a	2.35a
22	1	2.42f	3.00ns	1.00b	5.33a	0.72a	1.50df	0.37ac	163.58e	14.58ab	1.88bc
23	1	2.25f	3.50ns	3.00a	5.02a	0.62ab	1.42fg	0.34bc	160.45e	15.25ab	2.37a
2	2	2.75bc	5.50a	1.00b	4.75ab	0.75c	2.17cd	0.35a	193.08a	7.50d	1.27cd
3	2	2.67bc	5.00ab	1.00b	4.03cd	0.65c	2.58b	0.32ab	189.92ab	8.58cd	1.12d
5	2	2.25c	5.00ab	1.00b	4.46ac	0.71c	3.00a	0.32ab	193.58a	8.08cd	1.16d
11	2	2.75bc	4.50bc	1.00b	4.37ac	0.76c	3.00a	0.34ab	194.08a	8.08cd	1.10d
17	2	2.25c	4.00c	4.33a	4.11bd	1.08b	1.17g	0.29b	142.58d	10.17bc	1.58bc
18	2	3.08b	5.00ab	1.00b	4.77a	0.77c	2.00de	0.35a	188.58ab	7.75d	1.45cd
Campagnano	2	2.67bc	5.00ab	1.00b	4.02cd	0.69c	2.42bc	0.31ab	189.17ab	9.75bd	1.58bc
Grato1	2	3.67a	4.50bc	2.33ab	3.86d	1.02b	2.25cd	0.35a	181.00b	9.25cd	1.99a
22	2	2.75bc	5.00ab	5.67a	4.44ac	1.16b	1.67f	0.35a	168.58c	12.50a	1.94a
23	2	2.83b	4.83b	5.67a	4.57ac	1.36a	1.75ef	0.32ab	163.00c	11.58ab	1.85ab

Different letters indicate statistical significant differences among genotypes at p≤0.05 (ANOVA analysis, Duncan test), 1=2008/2008 and 2=2009/2010

On the basis of morphological data, a cluster analysis was applied and a similarity dendrogram was generated (*Figure 1.9*). The dendrogram obtained has divided the 10 genotypes into three main clusters: the first cluster grouped the genotypes S2, S3, S5 and S11, the second one the genotypes S17, Campagnano, S18, and in the last one, the genotypes S22, S23 and Grato 1 were included. The three identified clusters were congruent with the agro-morphological traits observed. In particular, the genotypes were grouped mainly on the basis of the date of appearance of the central flower head.

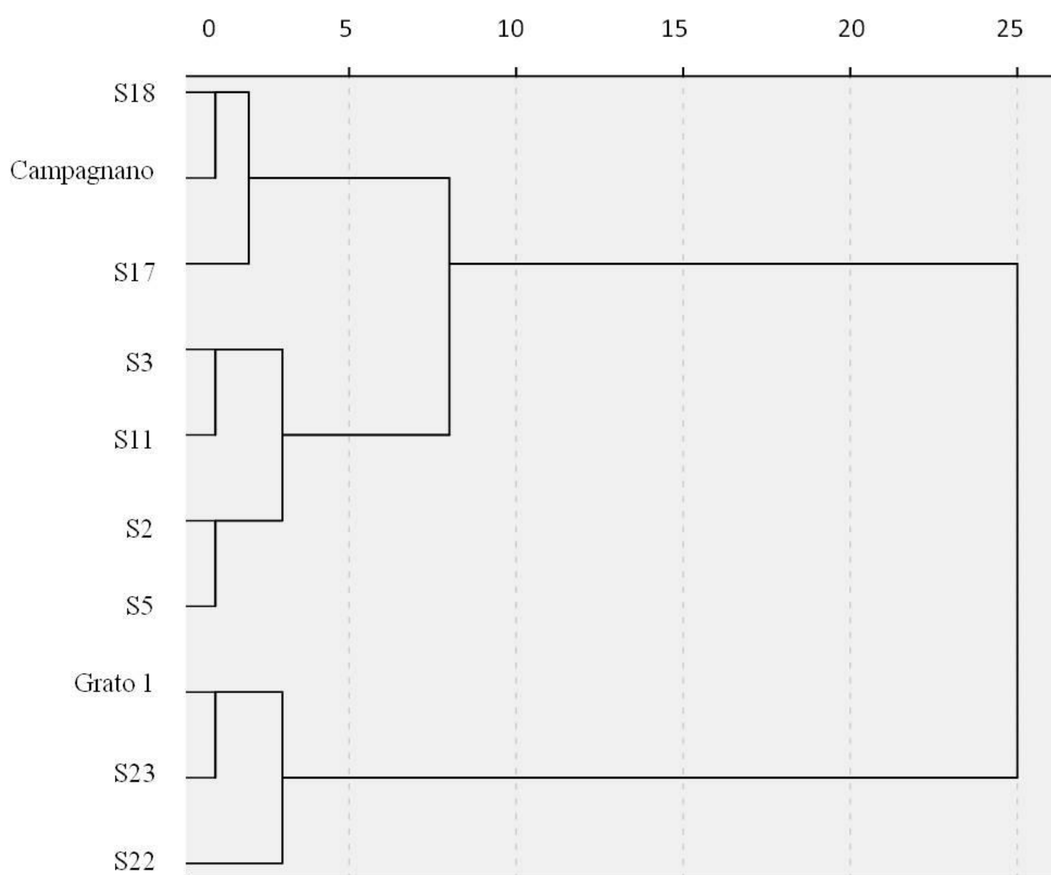


Fig. 1.9 Similarity dendrogram of the ten 'Romanesco' genotypes based on agglomerative hierarchical cluster analysis of the 47 morphological traits considered

An among genotypes pair wise similarity matrix was created with the total agro-morphological traits detected and a principal component analysis was applied. The percentage of total variance explained by the first four components is 44.03% of the whole variation. The first PC factor (15.84% of variance) included contributions from the following primary traits: plant height, leaf attitude, length of the first fully developed leaf, leaf incision, intensity of leaf green colour, shape of head bract, depth of bract incision, hue of bract colour and total head number. The second factor explained

the 13.29% of the variance and involved: number of lateral shoots, head bract colour, shape and thickness of receptacle and total heads weight. The third factor (8.48% of variance) included: main floral stem height, number of secondary lobes, hue of green colour, central flower head length, shape of head tip, anthocyanin coloration, density of head bracts and central flower height. The distribution of the ten genotypes against the first three discimant functions (*Figure 1.10*) showed that two main clusters could be individuated with the genotypes characterized by the earliest head production on the bottom side and the latest ones on the upper side.

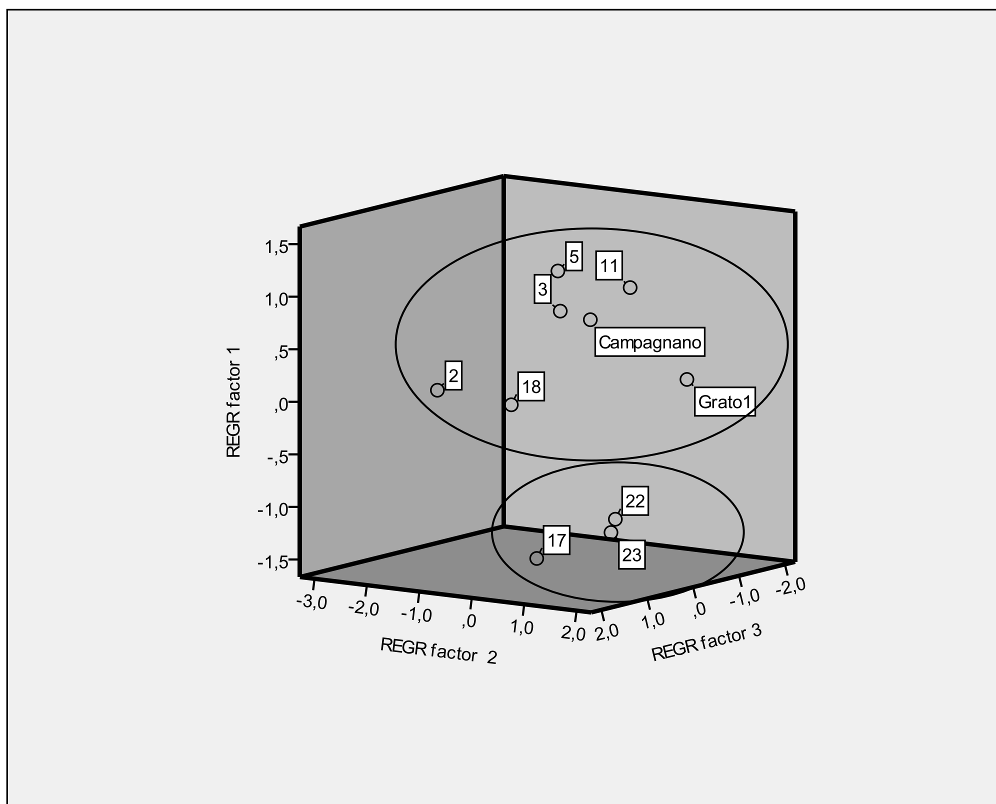


Fig. 1.10 Distribution of the analyzed genotypes against the three discriminant functions

1.3.3 Discussion

In Italy, plant breeding programs of globe artichoke have been up now limited to few studies, focused mainly on intra-clonal selection and/or hybridization of parental lines and aimed to obtain genotype/hybrid fitting the market demand (Lanteri *et al.,* 2004b; Raccuia *et al.,* 2004; Portis *et al.,* 2005; Sonnante *et al.,* 2008; Mauro *et al.,* 2009; Lanteri *et al.,* 2001). The ‗Romanesco' clones C3 and Terom are examples of clonal selections from the traditional populations Castellammare and Violettodi Toscana (Soressi, 2003), respectively; new seed-propagated F_1 hybrids such as Madrigal, Concerto, Opal, Tema, (Zaniboni, 2009; Bonasia *et al.,* 2010) and recently Romolo have been introduced in Italian market. However, the cultivation of these genetic materials has led to an increasing risk of genetic erosion for autochthonous germplasm. In fact, in the last years in the Central Italian regions, the traditional heterogeneous ‗Romanesco' germplasm was mostly substituted by the uniform micropropagated clone C3. The interest for this clone was mainly due to the market demand for early and uniform ‗Romanesco' materials, and to the adoption of *in vitro* propagation, which allows a great multiplication rate. To reduce the genetic erosion caused by the cultivation of micropropagated C3 plants, the present study has been addressed to identify the levels of diversity present within the ‗Romanesco' globe artichoke germplasm cultivated in the Latium Region, to conserve and utilize in plant breeding programs the traditional genetic resources and to create *in-situ* and *ex-situ* collections. The variability within the farmers' fields is an important factor in the design of a rational strategy of *in-situ* germplasm conservation.

In the present work, the morphological profiles of ‗Romanesco' germplasm did not show any significant intra-genotype difference, this revealing their clonal composition derived from vegetative micro-propagation. Despite the genotypes here studied belong to the same ‗Romanesco' typology, significant differences among genotypes were found and most clones can be recognized from each other and identified, confirming the importance of preserving this germplasm against the risk of genetic erosion. In particular, some genotypes can clearly identified; for example, S17 was the earliest clone characterized by small plant size, elliptical green-coloured striped with violet heads along with good and early yields.

Traits such as plant height, leaf attitude, length of the first fully developed leaf, leaf incision, intensity of leaf green colour, shape of head bract, depth of bract incision, hue of bract colour and total head number explained the majority of total variance in the

PCA and can be used for genotype classification. In line with previous observations (Crinò *et al.,* 2008), contributions from length of first order heads, plant height, diameter of main floral stem, total head number and weight are also important. The distribution of genotypes against the first three discriminant functions based on morphological traits (*Figure 1.10*) showed that they could be grouped into two main blocks, each corresponding to an early and late head production. Three of these genotypes, characterized by interesting agronomic traits (i.e. earliness, yield, and head morphological traits), have been selected within Romanesco germplasm here analyzed. Request for the release of these genotypes as varieties has been submitted to the National Variety Register in collaboration with ARSIAL (Latium Regional Agency for the Development and the Innovation of Agriculture) and recently approved. The 3 selected genotypes, S17, S23, and S22, will be registered under the names of Michelangelo, Donatello and Raffaello, characterized by differences referred to the following traits: maturity dates ranging from beginning to end of March; size of head (diameter from 8 to 10 cm); production weight (from 1.7 to 2.3 kg per plant); receptacle thickness (from 0.5 to 0.9 cm). In particular, Donatello is characterized by late maturity (end of March), tall and large plants, absence of spines, medium-sized heads and a short period of production (within about 23 days); Michelangelo and Raffaello are characterized by early maturity, small plants, good production in terms of total weight but differently distributed between primary and secondary heads, thick receptacles, uniformity in the head shape.

The present study highlights the need of implementing clonal selection among traditional germplasm to conserve this genetic resource and to provide uniform and selected material to farmers.

CARDOON

1.4 INTRODUCTION

Since ancient time, cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC) has been used as vegetable for human food and also today the species is still cultivated, mainly in the Mediterranean countries (Spain, Greece, Italy, France), over few thousand hectares for its bleached petiole and fleshy leaves. Wild cardoon (*Cynara cardunculus* L. var. *sylvestris* Lam.), considered the ancestor both of cultivated cardoon and globe artichoke, is widespread mainly as a weed, in the Mediterranean area but also in many countries of

Central and South America, and it was for centuries used as natural rennet for cheese making in the Iberian area.

Both cultivated and wild cardoons are well-adapted to the Mediterranean enviroments and climates, where they are seed propagated as annual crops. In the last years, the species has known a growing interest linked principally to its high aboveground biomass, which can be used either as solid biofuel (Piscioneri *et al.,* 2000), as green forage (Fernández *et al.,* 2006), and also as a source of bioactive compounds (Fratianni *et al.,* 2007; Lombardo *et al.,* 2010; Bonasia *et al.,* 2010; Pandino *et al.*, 2010, 2011a, 2011b). Until now, many studies on the possible applications of *Cynara cardunculus* plants have been done but there are few works on the genetic characterization and identification of Italian cardoon germplasm (Raccuia *et al.,* 2004). In particular, there is a lack of information about the genetic variability existing within and among cultivated and wild cardoon genotypes (Lahoz *et al.,* 2011). In Italy, some cultivated cardoon genotypes are registered in the Italian Register of the Varieties (i.e. Bianco avorio a foglia Frastagliata, Bianco avorio d'Asti, Bianco gigante inerme a foglia intera, Cento foglie, Gigante di Romagna, Gobbo di Nizza Monferrato, Pieno inerme, Spadone) but many genotypes, often labelled with the name of the cultivation place, are marketed by different seed companies without a clear genetic identification. On the contrary, wild cardoon genotypes are not registered in the Italian Register of the Varieties and this species does not exist as a specialized crop. Also wild cardoon genotypes are labelled with the distribution area name. The great variability existing in Italian authoctonous *Cynara cardunculus* spp. germplasm has not been described and there is a risk of genetic erosion. The present study aims at i) characterizing some Italian cultivated and wild cardoon genotypes, ii) identifying some morphological descriptors useful to well discriminate among genotypes, and iii) selecting genotypes of interest for future plant breeding programs.

1.5 Italian Accessions

1.5.1 Materials and methods

1.5.1.1 Genetic resources and open field experiments

Seven Italian genotypes of cultivated cardoon (AFB, AFM, AFM2, AFGR, AFGI, AFFG, AFN) and AFS as wild cardoon, belonging to the Tuscia University-ENEA joint collection, were considered in our study (*Figure 1.11*).

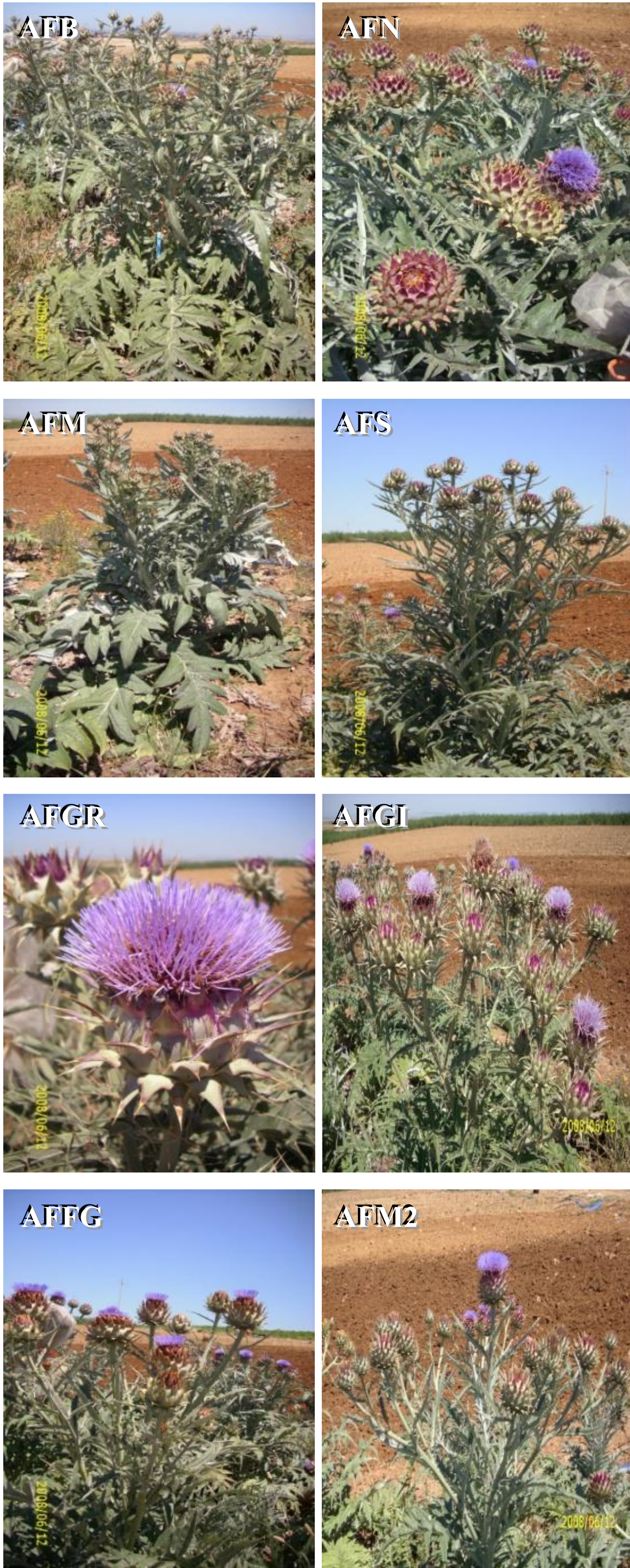


Fig. 1.11 Cardoon Italian accessions analyzed in the present study

All trials were conducted for 2 years, during the 2008-2009 and 2009-2010 growing seasons, in Tarquinia, Italy (42°14‘57‘‘12 N; 11°45‘22‘‘32 E). Plant material, obtained by seed propagation, was arranged in a randomized block experimental design with 4 replications of 5 plants each. The planting density was 0.77 plants m^2, with inter and intra-row distances of 1.30 and 1.0 m, respectively.

1.5.1.2 MORPHOLOGICAL CHARACTERIZATION

Twenty-five morphological and agro-physiological data were recorded on 4 plants per genotypes at the central flower heads appearance for two years (at May 5th 2009 and at May 20th 2010). The morphological characterization was performed using some standard UPOV (International Union for the Protection of New Varieties of Plants) descriptors for globe artichoke (CPVO-TP/184/1 Final 2001) (*Table 1.8*) (Pagnotta *et al.,* 2009, 2012) and some complementary other descriptors validated in a previous work (Crinò *et al.,* 2008). In particular, the morphological descriptors which could be well-adapted to cardoon plants have been chosen to characterize cardoon plants. Leaf attitude was estimated by a visual rating 1-5 (1=erect; 3=semi-erect; 5=horizontal attitude). The presence of leaf and head spines, leaf hairiness and incisions was evaluated using a visual rating (1=presence and 9=absence), according to UPOV descriptors. Intensity of leaf green and grey colour were estimated by a 3-7 scoring scale (3=pale; 7=strong). The colour of central flower head outer bract was determined using a visual 3-7 rating scale (3= green; 7=purple). As head shape index, the ratio length/diameter of the flower head was estimated.

1.5.1.3 STATISTYCAL ANALYSES

For the morphological traits, statistical analyses were performed as aforementioned in section 1.2.1.3.

1.5.2 RESULTS

Significant differences were found among genotypes analyzed on the basis of morphological characterization for most of the quantitative and qualitative traits, except for the traits such as distance between central flower head and the first fully developed leaf on the main floral stem, main floral stem diameter, leaf attitude, leaf length, leaf incision, number of leaf secondary lobes, intensity and hue of leaf green colour, central flower head tip shape, shape of head outer bract and shape of bract apex (*Table 1.8*). As regards the years, no significant differences were found for the NLShoots, MSDiam,

Spines, Linc, Hair, HeadDiameter, TipShape, ShapeBract, ApexBract, ColoBract, HueColBract showing that environmental conditions have not a influence on the qualitative traits. For most of the parameters considered, no significant genotype per year interactions have not been found, this means that the growing season has not influence on the morphological profile of each genotype.

Table 1.8 Statistical significance of the genotype, the year and their interaction in the morphological characterization of the cardoon germplasm

Code descriptor	Descriptor	Genotype	Year	Genotype*year
Pheight	Plant height (including central flower head)	**	***	ns
NLShoots	Number of lateral shoots on the main floral stem	**	ns	ns
MSHeight	Main floral stem height (excluding central flower head)	**	***	ns
DistHL	Distance between central flower head and youngest well developed leaf	ns	**	**
MSDiam	Main floral stem diameter	ns	ns	ns
Leafatt	Leaf attitude	ns	***	**
Spines	Spines	**	ns	ns
Llength	Leaf length	ns	***	ns
Linc	Leaf incision	ns	ns	ns
Nlob	Number of leaf lobes	**	***	ns
Nseclob	Number of leaf secondary lobes	ns	***	ns
IntGreen	Intensity of leaf green colour	ns	**	ns
HueGreen	Hue of leaf green colour	ns	***	ns
GreyHue	Intensity of leaf grey hue	***	**	ns
Hair	Leaf hairness	***	ns	ns
HeadLength	Length of the central flower head	***	***	**
HeadDiameter	Diameter of the central flower head	**	ns	**
Length/DiamHead	Length/Diameter ratio of central flower head	**	**	ns
TipShape	Shape of tip of the central flower head	ns	ns	ns
ShapeBract	Main shape of outer bract	ns	ns	ns
ApexBract	Shape of bract apex	ns	ns	ns
ColBract	Colour of outer bract	***	ns	**
HueColBract	Hue of secondary colour of outer bract	***	ns	***
SizeSpine	Size of spine	**	***	ns
TotalHeadNumber	Total number of heads	**	***	**

*Ns= not significant, * p=0.05; 0.01 <p<0.05; p=0.01 (Duncan test)*

Table 1.9 Differences in some morphological traits of cardoon genotypes analyzed for two years

Genotypes	Year	Pheight (cm)	NLShoots (no.)	MSHeight (cm)	DistHL (cm)	MSDiam (cm)	Spines (no.)	Llength (cm)	Nlob (no.)	Nseclob (no.)	IntGreen (no.)
AFB	1	190.37ab	5.63b	133.88ab	54.38ns	2.22ns	1.00b	43.88ns	7.50ab	3.00a	6.00ns
AFFG	1	187.62ac	4.88b	126.88bc	59.00ns	2.30ns	1.00b	53.38ns	6.25b	3.00a	5.50ns
AFGR	1	149.86d	4.57b	101.00c	48.71ns	2.21ns	1.00b	44.71ns	6.71b	3.29a	5.29ns
AFGI	1	157.86d	4.29b	102.57c	56.00ns	2.05ns	1.00b	49.00ns	6.71b	1.29b	6.43ns
AFM	1	195.62a	7.25a	155.13a	50.75ns	2.11ns	2.00b	45.75ns	6.50b	2.75a	7.00ns
AFM2	1	168.12bd	4.13b	117.63bc	53.00ns	2.15ns	1.00b	51.50ns	6.50b	2.75a	6.50ns
AFN	1	165.00cd	5.63b	119.00bc	54.38ns	2.06ns	3.00b	44.88ns	6.50b	3.50a	5.50ns
AFS	1	147.14d	5.14b	111.00c	43.14ns	1.68ns	5.57a	44.86ns	8.14a	2.43a	6.43ns
AFB	2	157.50ab	5.25ab	104.50ab	53.00ns	2.22ns	1.00b	48.25ns	8.00ns	3.00bc	5.50ns
AFFG	2	163.75ab	5.00ab	90.00bc	73.75ns	2.30ns	1.00b	63.50ns	7.25ns	3.50ab	6.50ns
AFGR	2	124.75bc	4.00b	71.50c	53.25ns	2.21ns	1.00b	51.50ns	7.75ns	4.00ab	5.00ns
AFGI	2	144.00ac	4.00b	79.75bc	64.25ns	2.05ns	1.00b	57.00ns	7.50ns	1.50c	7.00ns
AFM	2	165.75a	6.50a	122.00a	43.75ns	2.11ns	1.00b	55.75ns	7.50ns	4.00ab	7.00ns
AFM2	2	142.50ac	4.25b	85.75bc	56.75ns	2.15ns	1.00b	66.25ns	7.75ns	3.50ab	7.00ns
AFN	2	129.00bc	5.25ab	75.75bc	53.25ns	2.06ns	3.00ab	51.50ns	7.75ns	5.00a	6.00ns
AFS	2	114.25c	4.75ab	70.50c	43.75ns	1.68ns	5.00a	51.50ns	9.00ns	3.00bc	6.50ns

Different letters indicate statistical significant differences among genotypes at $p \leq 0.05$ (ANOVA analysis, Duncan test), 1=2008/2009 and 2=2009/2010

Table 1.9 Differences in some morphological traits of cardoon genotypes analyzed for two years (continuation)

Genotypes	Year	HueGreen (no.)	GreyHue (no.)	Hair (no.)	HeadLength (cm)	HeadDiameter (cm)	Length/Diam Head (no.)	ColBract (no.)	HueColBract (no.)	SizeSpine (no.)	TotalHead Number (no.)
AFB	1	3.25ns	4.50bc	3.00bc	4.85c	6.34ac	0.86ab	2.00a	4.50cd	2.75bc	16.88b
AFFG	1	2.63ns	3.75ce	3.00bc	5.05bc	5.66c	0.93ab	1.38ce	5.50ab	2.00bc	17.63b
AFGR	1	2.29ns	5.00b	4.14ab	5.66b	6.57ac	0.87ab	1.00e	4.43cd	1.57bc	14.29b
AFGI	1	2.71ns	6.43a	5.00a	4.68c	5.73bc	0.82b	1.86ab	5.00bc	1.29c	13.43b
AFM	1	2.25ns	3.00e	1.50de	5.31bc	6.22ac	0.93ab	1.63ac	4.50cd	2.75bc	22.88a
AFM2	1	2.38ns	3.75ce	2.50cd	6.42a	7.39a	0.92ab	1.13de	6.00a	2.00bc	14.88b
AFN	1	2.50ns	4.25bd	3.25bc	5.59b	7.08a	0.84ab	1.50bd	4.00d	3.00b	17.88b
AFS	1	2.14ns	3.29de	1.29e	6.65a	6.86ab	1.03a	1.00e	4.14d	5.00a	16.14b
AFB	2	3.00ns	5.00b	3.00bc	4.75d	5.57b	0.71b	2.00ab	5.00a	1.50b	12.25c
AFFG	2	3.00ns	4.50bc	3.50b	5.32bd	5.75b	0.88ab	1.50bc	5.00a	1.00b	16.00a
AFGR	2	3.00ns	5.00b	3.00bc	6.40ab	7.55a	0.91a	1.00c	5.00a	1.00b	12.00c
AFGI	2	3.00ns	7.00a	5.00a	4.87d	5.97b	0.82ab	2.25a	3.50b	1.00b	12.50c
AFM	2	3.00ns	3.00d	1.00d	5.20cd	5.57b	0.79ab	1.50bc	5.00a	1.00b	14.75ab
AFM2	2	2.25ns	3.50cd	2.00cd	6.35ac	6.90ab	0.83ab	1.25c	5.00a	1.00b	12.75c
AFN	2	3.00ns	4.50bc	3.00bc	6.22ac	7.47a	0.77ab	1.50bc	5.00a	2.00b	13.75b
AFS	2	2.50ns	3.50cd	1.50d	6.92a	6.77ab	0.92a	1.00c	5.00a	5.00a	14.25ab

Different letters indicate statistical significant differences among genotypes at p≤0.05 (ANOVA analysis, Duncan test), 1=2008/2009 and 2=2009/2010

Table 1.9 shows differences in some morphological traits of the cardoon genotypes analyzed. Among the genotypes studied, AFM provided the highest plants and showed the highest value of lateral shoots number in both growing seasons. AFS was characterized, obviously in both growing seasons, by the presence of spines on the leaves. As regards the leaf colour, there were no significant differences among genotypes for the traits intensity and hue of green colour while, in both growing seasons, AFGI genotype showed the most hairy grey-coloured leaves. In 2008-2009, AFM2 and AFS, and only AFS in 2009-2010 showed the most elongated central flower head while, as regards the central flower head diameter, AFM2 and AFN in 2008-2009, and AFGR and AFN in 2009-2010 were characterized by the highest value. The ratio length/diameter of the central flower head was calculated as shape index. On the basis of this index in both years, AFS was the genotype with the most elongated heads while AFGI, in 2008-2009, and AFB, in 2009-2010, had the most elliptical heads. In both growing seasons, AFM were the genotypes with the highest lateral shoots and total heads number. AFGI was characterized by grey and hairy leaves. No significant differences among genotypes were found for the traits: plant diameter, hue of leaves green colour, floral stem diameter, attitude, leaf incision and width.

Based on the morphological traits, a similarity dendrogram (*Figure 1.12*) was constructed using an agglomerative hierarchical cluster analysis. On the basis of the similarity dendrogram, our genotypes could be classified into five clusters. Cluster 1 consisted of two genotypes: AFGR and AFGI. Cluster 2 consisted of the wild cardoon AFS, that is well separated from the rest of the cultivated cardoon. In cluster 3, two genotypes such as AFN and AFM2 were grouped together while, in cluster 4, AFM and AFB were found. Finally, cluster 5 consisted of the AFFG genotype.

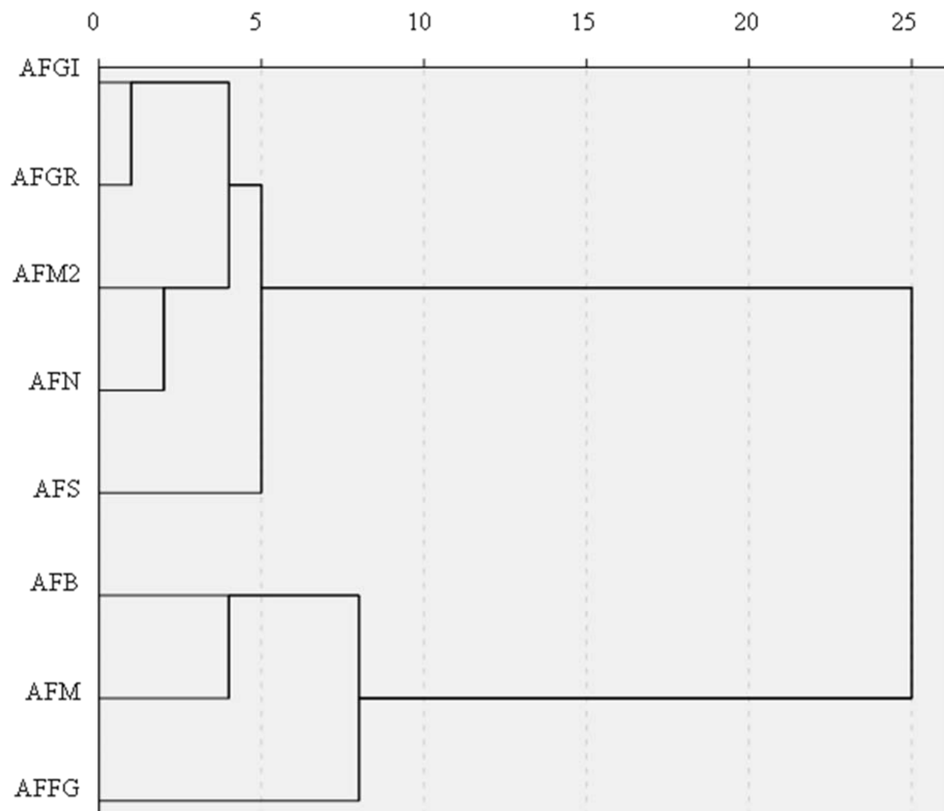


Fig. 1.12 Similarity dendrogram of the cardoon genotypes generated using an agglomerative hierarchical cluster analysis

An among genotypes pairwise similarity matrix was generated on the basis of agro-morphological traits considered and a principal component analysis was applied. The percentage of total variance explained by the first three components is 46.42%. The first PC factor (22.45% of variance) included contributions from the traits: plant and main floral stem height, flower head bract apex and total flower head number. The second factor (12.92% of the variance) involved the intensity of leaf grey colour and the leaf hairness. The third factor (11.05% of variance) included the number of lateral shoots and central flower head diameter.

The distribution of the genotypes here considered against the first three disciminant functions is reported in Figure 1.13. On the basis of this analysis, the cultivated cardoon genotypes analyzed resulted to be grouped in the upper side while AFS wild cardoon was located in the bottom side. Correlation analysis was also performed using SPSS 15. On the basis of morphological traits no positive correlations were found except for the intensity of leaf grey colour and leaf hairness (Pearson's correlation coefficient $r=0.699$, $p \leq 0.05$)

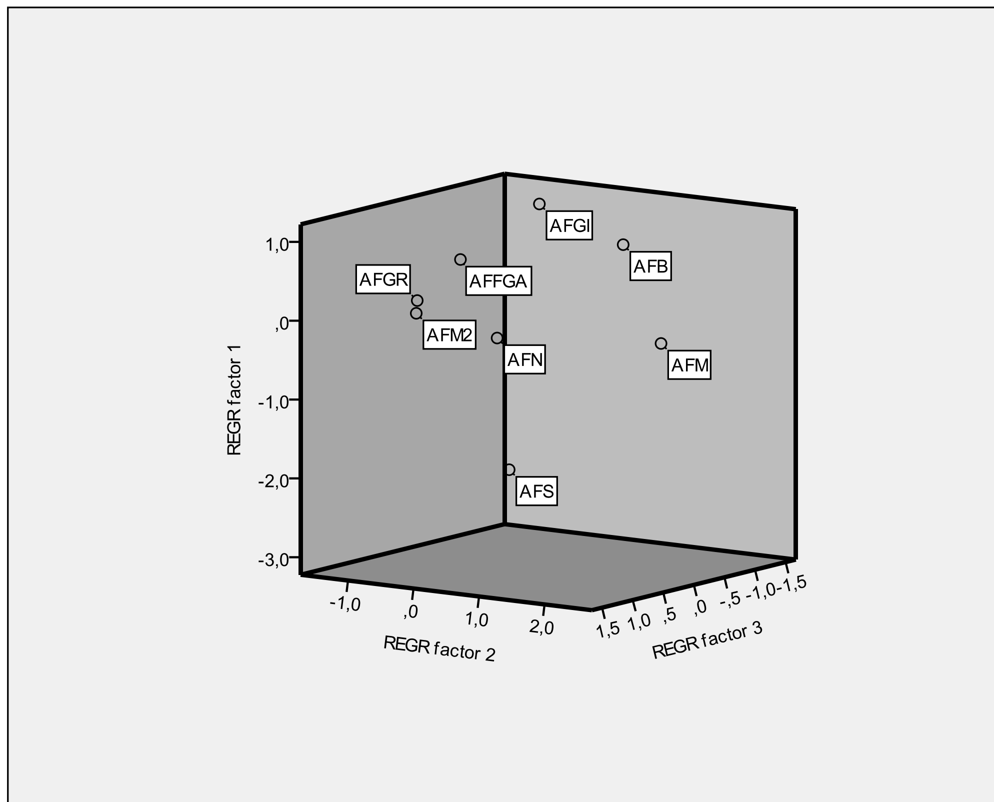


Fig. 1.13 Distribution of the analyzed genotypes against the three discriminant functions

1.5.3 Discussion

Few studies on *Cynara* spp. germplasm characterization and identification have been up now conducted (Lahoz *et al.,* 2011). In particular, Italian cardoon germplasm has never been analyzed using agro-morphological descriptors with the aim of selecting genotypes more stable and useful as parents of hybrid combinations in plant breeding programs. In addition, it is important to consider that standard UPOV descriptors are not yet available for cardoon crop. In PhD work, some Italian cardoon genotypes have been characterized for the first time on the basis of their morphological profile, adapting some of standard UPOV descriptors used for globe artichoke. Among the fourty-seven standard UPOV descriptors for globe artichoke, only twenty-five have been selected and adapted to cardoon plants. Some traits regarding flower head receptacle or the primary and secondary head characteristics have not been considered in this work because of the different cardoon plant structure. On the basis of morphological characterization, results from ANOVA analysis showed significant differences between cultivated and wild genotypes, but also among the seven cultivated cardoon genotypes analyzed. In particular, most of the genotypes here evaluated could be clearly distinguished from each other and represents an important genetic resource to be preserved.

Some traits such as plant height, number of lateral shoots and heads, spine presence on the leaves and heads, flower head length, diameter, shape and colour, resulted very useful for the identification of some genotypes. For example, wild cardoon AFS could be well identified from other genotypes for the presence of spines on the leaves and on the head bract apex, for small-sized plants and elongated heads, while cultivated cardoon AFM is characterized by vigourous plants and by a high number of lateral shoots and flower heads. It is also interesting to notice that in PCA traits such as plant height, leaf grey colour and hairiness, number of lateral shoots and heads and central flower head diameter explain the majority of total variance. In line with Lahoz *et al.* (2011), contributions from plant height and leaf colour are important traits to discriminate among cardoon genotypes. On the contrary, traits such as distance between central flower head and the youngest fully developed leaf, main floral stem diameter, leaf attitude, leaf length and incision, number of secondary lobes, intensity and hue of leaf green colour, shape both of central flower head tip and bract apex resulted not useful in characterizing cardoon plants and did not show any significant difference among genotypes. Moreover, significant differences were found between the two growing seasons for most of the quantitative and qualitative traits and the genotype per year interaction was significant for some of the traits considered, showing that environmental conditions have an unpredicatable influence on the phenotypic expression of the genetic material. Despite this strong influence of environmental conditions on phenotypic expression, morphological approach represents an initial important step for the description of the plants (Crinò *et al.,* 2008; Lo Bianco *et al.,* 2011; Lahoz *et al.,* 2011). Certainly, the combination of morphological and molecular approaches could provide more complete informations and could allow to better characterizing germplasm.

Data obtained from morphological characterization allowed to group genotypes into five main clusters providing useful informations on genetic variability existing among genotypes and suggesting a possible strategy for germplasm utilization in plant breeding programs. In the distribution of the eight genotypes against the first three discriminant functions based on morphological traits, two groups could be identified: the first one on the upper side grouping cultivated cardoon genotypes and the second one the lower side with AFS wild cardoon. This shows that descriptors here considered could allow only a discrimination between the two *C. cardunculus* botanical varieties (var. *altilis* and

sylvestris), which are characterized by well-distinct characteritics. Further descriptors, more suitable for cardoon species, have to be applied.

The information obtained in the present work highlighted the need of collecting authoctonous cardoon germplasm and preserving this material against the risk of genetic erosion. The morphological characterizion of this germplasm provide challenging information for plant breeders which could select parental line of interest for globe artichoke and cardoon breeding programs.

Chapter 2

DEVELOPMENT OF POLYPHENOL EXTRACTION TECHNIQUE FROM *CYNARA* SPP. BIOMASS

2.1 Introduction

In the last years, there is an increasing interest in active biocompounds, especially phenolic compounds, from natural sources. This interest in natural compounds is mainly linked to the increasing demand of natural antioxidants due both to health concerns on the present use of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), and to consumers‘ preference (Llorach *et al.,* 2002; Ghafoor *et al.,* 2009).

In particular, the possibility of recovering phenolic compounds from different fresh or dried plants, or part of plants, has been widely investigated. The extraction of bioactive compounds from vegetable materials is part of phytopharmaceutical and food technology and herbal preparations, which include herbal extracts, and was introduced in the pharmacopoeias of numerous countries (Vinatoru, 2001). Indeed, extraction methods of phenolic compounds from plant material have become an important research issue. In fact, extraction represents the first fundamental step for recovering, isolating and using these biocompounds (Ghafoor *et al.,* 2009). Biocompound extraction is usually performed through a solvent-extraction procedure and different parameters such as solid/liquid ratio of the mixtures, extraction time and temperature are also optimized (Spigno *et al.,* 2007; Ghafoor *et al.,* 2009). Until now, conventional extraction techniques employed to extract active biocompound are Soxhlet and maceration which are very time-consuming and require large quantities of solvents (Wang and Weller, 2006; Michel *et al.,* 2011). In addition, in these traditional techniques high amounts of toxic and expensive solvent, such as methanol and acetone, are used (Monrad *et al.,* 2010; Michel *et al.,* 2011). In order to use bioactive plant extracts in pharmaceutical and cosmetic formulations, the development of environmental friendly and efficient extraction techniques is required. In the last years, novel sustainable extraction techniques such as Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE) and Accelerated Solvent Extraction (ASE), which could allow to reduce extraction time and solvent consumption, increase nutraceutical yield and improve plant extract quality (Zheng *et al.,* 2009) have been investigated. However, few or no applications of these modern techniques have been considered in *Cynara cardunculus* L. spp. to optimize phenolic compound extraction from plant material. In literature, conventional methods have been widely used for the extraction of phenolic compounds from globe artichoke and cardoon plants (Llorach *et al.,* 2002; Wang *et al.,* 2003; Fratianni *et al.,* 2007; Pandino *et al.,*

2011). For example, Fratianni's method (Fratianni *et al.,* 2007) consists in a first extraction using 5 volumes of acetone: ethanol : methanol (70:15:15) for 1h at 4°C and then a second extraction performed on the residue obtained from the first step using 5 volumes of ethylacetate for 1h at 4°C. On the basis of these preliminary remarks, in the present study conventional (Soxhlet and maceration) and novel extraction methods (ASE and MAE) for the recovery of phenolic compounds from *C. cardunculus* spp. using recognized as renewable ethanol solvent have been compared. In particular, the possibility of using the more efficient and environmentally friendly novel techniques which decrease energy cost and increased extraction speed in *C. cardunculus* spp. has been investigated. Moreover, the effect of independent variables of novel techniques on polyphenol yields using a full two-level factorial experimental design has been studied and the most efficient technique and the maximal levels of independent variables for polyphenol extraction from globe artichoke and cardoon leaves have been determined.

2.2 Materials and methods

2.2.1 Plant material, solvents and reagents

Cardoon leaves of the accession ―Plein blanc amelioré" were collected in Salvagnac, south west France, in November 2009. Plant material was oven-dried at 40°C until the moisture level was constant (48h). Dried leaves were ground to a fine powder using an electrical grinder Freitsh Pulverisette. Ethanol solvent and reagent grade formic acid (96%) were purchased from Scharlau, (Barcelona, Spain). De-ionized water was home-made using a Milli-Q water purifying system purchased from Millipore Co. (Bedford, MA, USA). Standard molecules (luteolin CAS number *491-70-3* and apigenin CAS number *520-36-5*) were obtained from Sigma Chemical Co. (St. Louise, MO, USA).

2.2.2 Extraction techniques

Based on laboratory experience, we chose 80% ethanol as extraction solvent instead of methanol or acetone for all extraction methods because it is more suitable for nutraceutical or cosmetic applications.

2.2.2.1 Soxhlet extraction

An aliquot (5g) of oven-dried cardoon ―Plein blanc amelioré" leaves were placed in a paper filter like a drop and then were put in Soxhlet apparatus. Extraction was done

using 100 mL of 80% ethanol for 5h. The average temperature during the experiment was the boiling point of the azeotrope (78.1°C). Each trial was performed in duplicate.

2.2.2.2 Maceration

The extraction was performed mixing 1 g of oven-dried cardoon ―Plein blanc amelioré" leaves to an aliquot of ethanol 80% solvent in a shaking-water bath for 1h at 4°C. The supernatant was collected and filtered under-vacuum and the residue was redissolved in the solvent for the second extraction for 1h at 4°C (*Table 2.1*). The second supernatant was collected and filtered under-vacuum and added to the first until the further analysis. Each experiment was performed in duplicate.

Table 2.1 Independent variables and their levels used for polyphenol extraction by maceration of Cynara spp. leaves

Independent variables	**Levels**	
Temperature (°C)	4	
Solvent volume (mL)	10	20
Time (min)	60 twice	

2.2.2.3 Microwave Assisted Extraction

MAE experiments were performed using a Mars HP 500 Plus Microwave Extraction System. The Mars instrument has an internal temperature control system using a temperature probe. An aliquot of ethanol solution 80% was added to 1g of oven-dried cardoon ―Plein blanc amelioré" leaves into the Mars vessel fixing the extraction system. All vessels were closed to avoid volatilization losses during the extraction process and were placed in the sample tray. The extractions were performed using 1/10, 1/15 or 1/20 mL solid/liquid ratio, at 50, 75, or 100°C for 2, 8.5 or 15 min.

2.2.2.4 Accelerated Solvent Extraction

ASE was performed using a Dionex ASE 200 Extraction System. An aliquot (1 g) of cardoon leaves (Cardoon ―Plein blanc amelioré") was packed into the extraction cell and an aliquot of washed sea sand was used to fill the cell. Extraction trials were carried out with ethanol 80% at 50, 100 or 150°C, with two static cycles for 1, 7.5 or 15 min at 50, 100 or 150 bars of pressure.

After each extraction the mixtures were filtered on a hydrophilic system (0.45µm) under vacuum and then the extracts were diluted until 50 mL using ethanol 80%. All samples were stored at -20°C until the further HPLC analysis.

2.2.3 Determination of moisture content

In order to evaluate moisture content, an amount (10 g three times replicated) of homogenized plant material was oven-dried at 103°C until the moisture level was constant (24h) to determine the dry matter (DM) content.

2.2.4 Colorimetric analysis of total phenolics

Total phenolics were estimated using the Folin-Ciocalteu method based on the reduction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products. One mL of diluted extract sample was added to 5 mL of the Folin-Ciocalteu reagent. Then, 10 mL of 20% Na_2CO_3 were added to the mixture. The solution was then adjusted with distilled water to a final volume of 100 mL and mixed. Total phenolic content was spectrophotometrically estimated at 760 nm. The quantification was based on the standard curve generated using gallic acid. The standard preparation was made adding 5 mL of Folin-Ciocalteu reagent to 0-1-2-3-4 mL of gallic acid. Total phenolic content of plant parts was expressed as mg of gallic acid equivalents (GAE) per g of DM through the calibration curve.

2.2.5 HPLC analysis

Each extract was analyzed using Dionex HPLC chromatography equipped with a UV detector HP 1100. HPLC separation was performed on a Pursuit C18 column at room temperature. The mobile phase was 0.1% formic acid in water (solvent A) and in methanol (solvent B) at a flow rate of 1 mL/min. The mobile phase was increased to 65% in a linear gradient in 25 min and then returned to 30% in 5 min. The injection volume was 20 µL. Chromatograms were recorded at 230, 286 and 350 nm. The standards used in the experiments were weighed and dissolved in methanol. The calibration curves were generated with concentration ranging from 1 to 200 mg/L of luteolin and apigenin.

2.2.6 Statistical analyses

The Microsoft Excel software (Microsoft Corporation, Redmond, USA) was used to analyze the responses for the factorial design, to calculate the regression coefficients and to obtain the two-dimensional response surface analyses. The NemrodW software (LPRAI, Marseille, France) allowed us to analyse regression coefficients. The ANOVA analysis of the regression models, the F-test and the determination of R^2 of the polynomial model was performed using SPSS version 15.0.

2.2.7 Experimental design for Microwave Assisted Extraction and Accelerated Solvent Extraction

Optimization of MAE and ASE methods has been performed using a two-level full factorial design (-1, 0, +1). For each method, this design has been used in order to evaluate the most significant parameters on the polyphenol, apigenin and luteolin contents. The variables chosen were extraction temperature, solid:liquid ratio and extraction time for MAE (*Table 2.2*) and extraction temperature, extraction pressure and extraction time for ASE (*Table 2.3*).

Table 2.2 Independent variables and their levels employed in the factorial experimental design for the optimization of polyphenols MAE of Cynara spp. leaves

		Coded Levels		
Independent variables	**Coded variables**	-1	0	1
Extraction Temperature (°C)	Xi	50	75	100
Solid:liquid ratio	Xj	1:10	1:15	1:20
Extraction Time (min)	Xk	2	8.5	15

Table 2.3 Independent variables and their levels employed in the factorial experimental design for the optimization of polyphenols ASE of Cynara spp. leaves

		Coded Levels		
Independent variables	**Coded variables**	-1	0	1
Extraction Temperature (°C)	Xi	50	100	150
Extraction Pressure (bars)	Xj	50	100	150
Extraction Time (min)	Xk	1	7.5	15

For both MAE and ASE extraction techniques, a matrix of 8 factorial points was defined with two replicates at the center of the design leading to 10 sets of experiments. Total polyphenol, luteolin and apigenin contents obtained by MAE and ASE were used as response values (Y) (*Tables 2.4* and *2.5*). These responses recorded for the 10 experiments led to mathematical models. The equations allowed us to predict the values of the responses all over the experimental field. They consisted of first-order polynomials:

$$Y = a_0 + a_iX_i + a_jX_j + a_kX_k + a_{ij}X_iX_j + a_{ik}X_iX_k + a_{jk}X_jX_k$$

where Y represents the response, X_i, X_j et X_k the independent variables, a_0, ai, aj, ak, aij, aik and ajk the regression coefficients mathematically estimated from the experimental data and calculated by the formula :

$$\hat{a} = (X'X)^{-1}X'Y$$

a_0 is the response value at the middle of the experimental domain.

a_i, a_j and a_k are the effects of the independent factors

a_{ij}, a_{ik} and a_{jk} are the first order interaction between factors

The quality of the fit of polynomial model was expressed by the R^2 coefficient and its statistical significance was checked using F-test.

2.3 Results

2.3.1 Optimization of Microwave Assisted Extraction (MAE) and Accelerated Solvent Extraction (ASE) for Phenolic Compounds

In order to evaluate the influence of the independent factors on phenolic compound extraction yield and to optimize MAE and ASE extraction methods of polyphenols from *Cynara* spp. leaves, a two-level full factorial experimental design was applied on this study. A set of 10 experiments for each method was performed, as shown in Tables 2.4 and 2.5, and results obtained from colorimetric Folin-Ciocalteu and HPLC analyses of the extracts were considered as response values of the experimental design and analyzed using regression analysis.

Table 2.4 Experimental design conditions and responses for Cynara spp. leaves MAE

Experiments	Coded levels			Responses		
	Temperature	Time	Solid/liquid ratio	Luteolin (µg/gDM)	Apigenin (µg/gDM)	Total polyphenols (mg GAE/gDM)
1	-1	-1	-1	38.00	74.16	2.44
2	-1	-1	1	33.01	70.40	3.65
3	-1	1	-1	45.96	69.38	3.78
4	-1	1	1	55.41	88.77	3.68
5	1	1	-1	94.44	88.32	5.49
6	1	1	1	124.63	87.19	8.40
7	1	-1	-1	93.04	89.87	4.67
8	1	-1	1	98.99	90.49	5.53
9	0	0	0	66.03	77.14	4.54
10	0	0	0	63.68	81.80	4.56

DM: dry matter; GAE: gallic acid equivalent

Table 2.5 Experimental design conditions and responses for Cynara spp. leaves ASE

Experiments	Coded levels			Responses		
	Temperature	Pressure	Time	Luteolin (µg/gDM)	Apigenin (µg/gDM)	Total polyphenols (mg GAE/gDM)
1	-1	-1	-1	62.18	100.06	9.84
2	-1	-1	1	85.56	111.21	5.77
3	-1	1	-1	58.23	92.51	12.89
4	-1	1	1	83.41	105.27	7.92
5	1	1	-1	192.34	110.74	19.24
6	1	1	1	223.24	111.40	24.76
7	1	-1	-1	184.52	108.68	14.40
8	1	-1	1	249.46	120.60	31.86
9	0	0	0	114.37	87.79	15.63
10	0	0	0	117.68	83.79	15.62

DM: dry matter; GAE: gallic acid equivalent

The regression coefficients calculated on the basis of luteolin, apigenin and polyphenols contents for both MAE and ASE extraction techniques are shown in Figures 2.1, 2.2 and 2.3 for MAE and in Figures 2.4, 2.5 and 2.6 for ASE. In MAE the highest value of regression coefficient was obtained for the temperature variable while the solid:liquid ratio and extraction time had a slight effect on polyphenol recovery. Also in ASE, temperature regression coefficient presented the highest values for luteolin, apigenin and total polyphenol responses. On the contrary, pressure independent variable showed the lowest one.

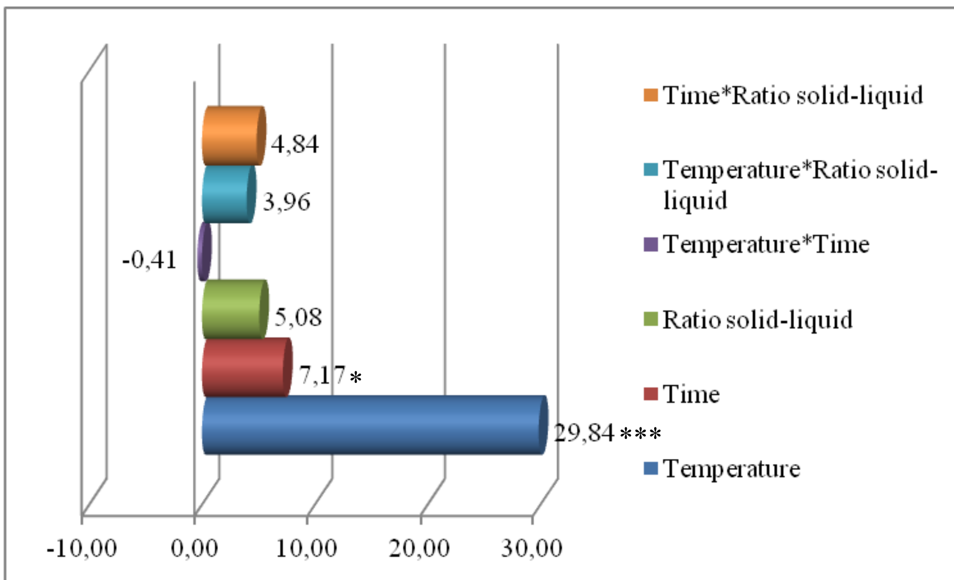


*Fig. 2.1 Pareto charts of factors and their first order interactions for Luteolin content response in MAE determined by HPLC analysis. *Denotes significant at 5% level, *** Denotes significant at 0.1% level*

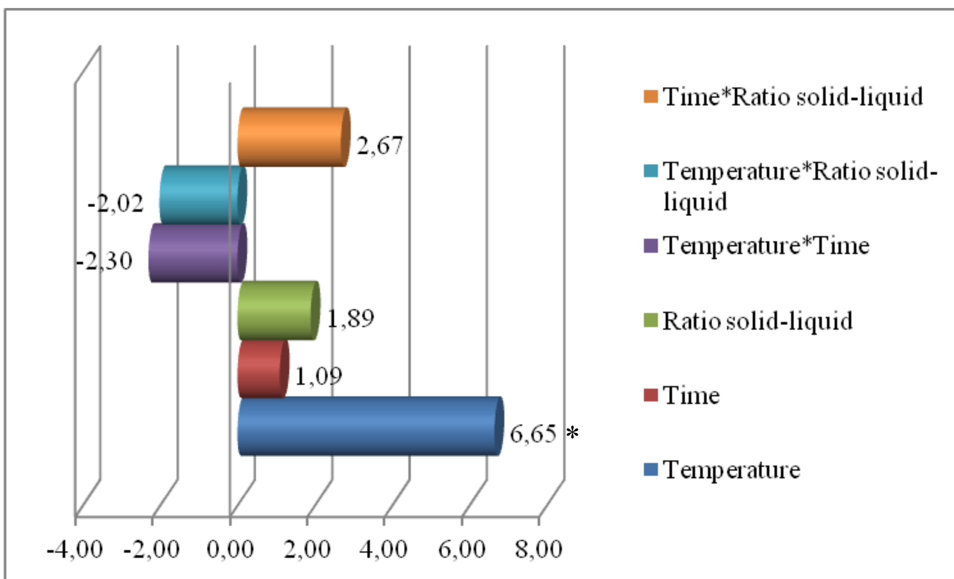


*Fig. 2.2 Pareto charts of factors and their first order interactions for Apigenin content response in MAE determined by HPLC analysis. *Denotes significant at 5% level*

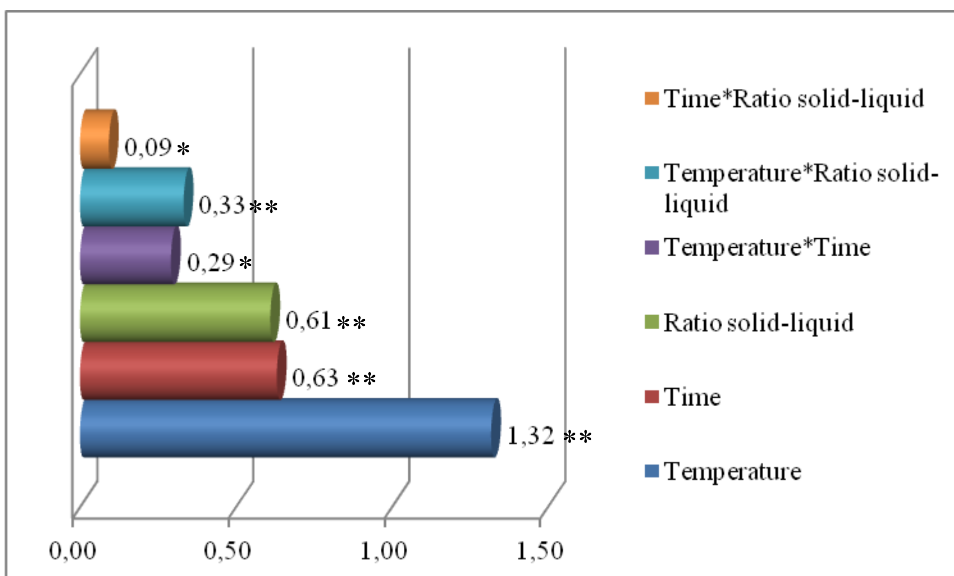


*Fig. 2.3 Pareto charts of factors and their first order interactions for total polyphenols content response in MAE determined by Folin-Ciocalteu colorimetric method. *Denotes significant at 5% level, **Denotes significant at 1% level*

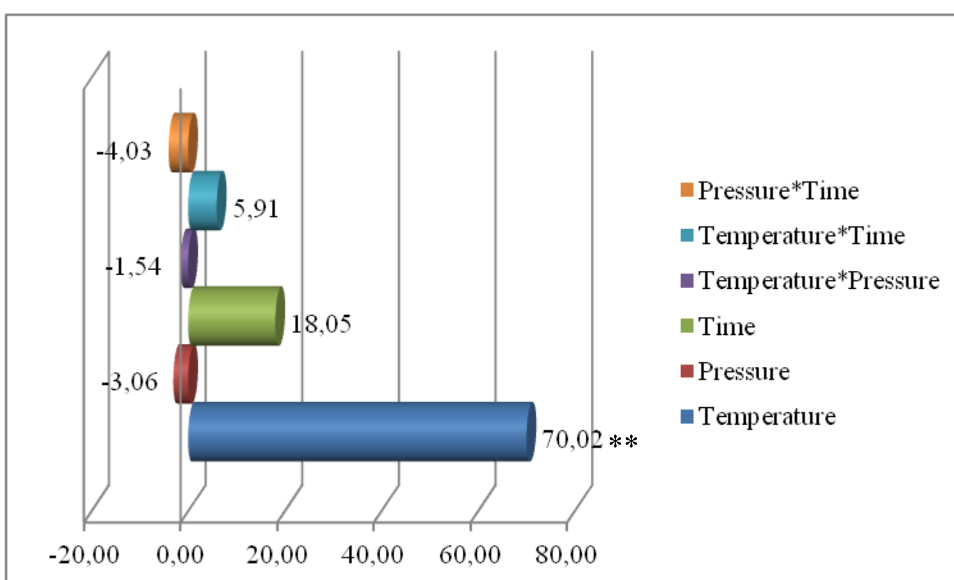


*Fig. 2.4 Pareto charts of factors and their first order interactions for Luteolin content response in ASE determined by HPLC analysis. **Denotes significant at 1% level*

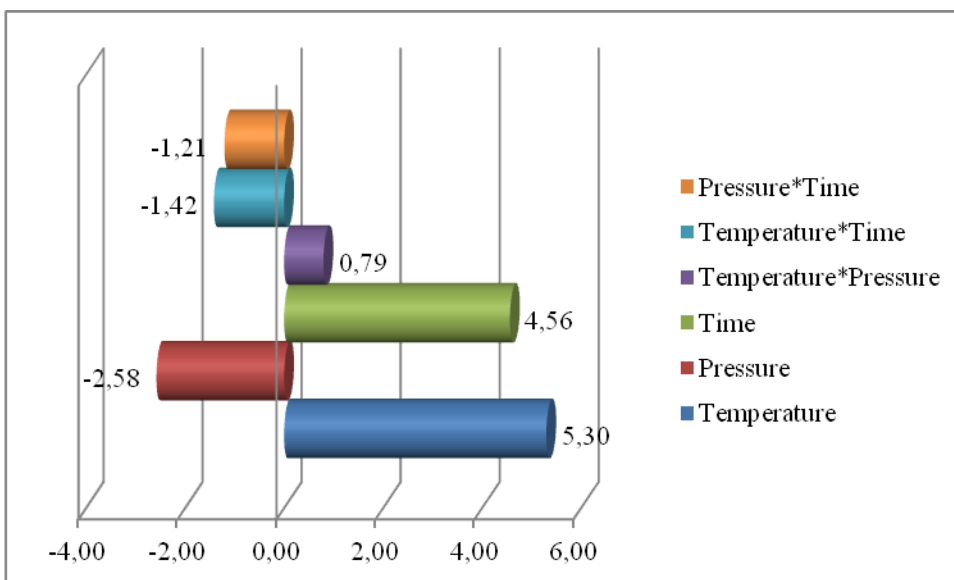


Fig. 2.5 Pareto charts of factors and their first order interactions for Apigenin content response in ASE determined by HPLC analysis

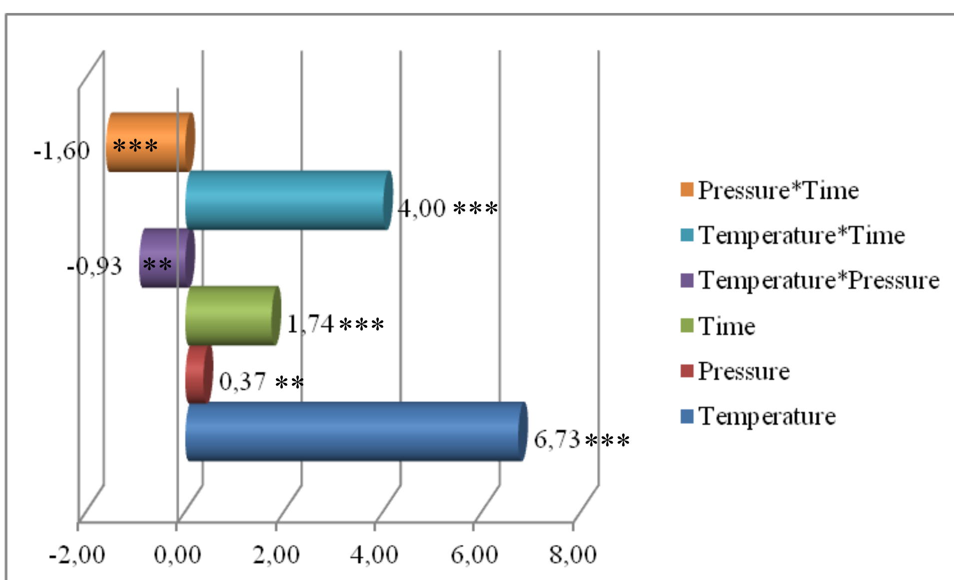


*Fig. 2.6 Pareto charts of factors and their first order interactions for total polyphenols content response in ASE determined by Folin-Ciocalteu colorimetric method. **Denotes significant at 1% level, *** Denotes significant at 0.1% level*

These regression coefficients were used to construct the relative first-order polynomial equations given in annex 1.

In order to determine the validity of the model for each extraction method the experimental and the predicted yields of total polyphenols, luteolin and apigenin were compared. Both for ASE and MAE, Pearson's correlation coefficients were estimated to evaluate the correlation degree between observed and predicted values. In MAE,

Pearson‘s coefficient values resulted 0.999, 0.993, 0.981 for total polyphenol, luteolin and apigenin yield, respectively. In ASE the value of Pearson‘s coefficients for total polyphenols, luteolin and apigenin were 0.999, 0.987 and 0.627, respectively. The ANOVA of the regression models showed a high significance per $p \leq 0.05$, except for the model designed for apigenin in ASE which resulted no significant ($p=0.052$).
Predicted contour levels were obtained using the first-order polynomial equations calculated for each method and response by keeping constant the independent variable, which resulted to have the lowest regression coefficient. In particular, for MAE the solid:liquid ratio independent variable has been fixed at 1:15 while for ASE the pressure independent variable has been fixed at 100 bars. In Figures 2.7, 2.8 and 2.9, the effects of extraction time and temperature on total phenolic content (expressed as mg GAE per g DM), luteolin and apigenin content (expressed as µg per g DM) in MAE are reported. Total polyphenols and luteolin content was strongly related to extraction temperature and also positively related to the extraction time (*Figures 2.7* and *2.8*, respectively). Apigenin content resulted strongly related to the extraction temperature while were slightly affected by extraction time (*Figure 2.9*).

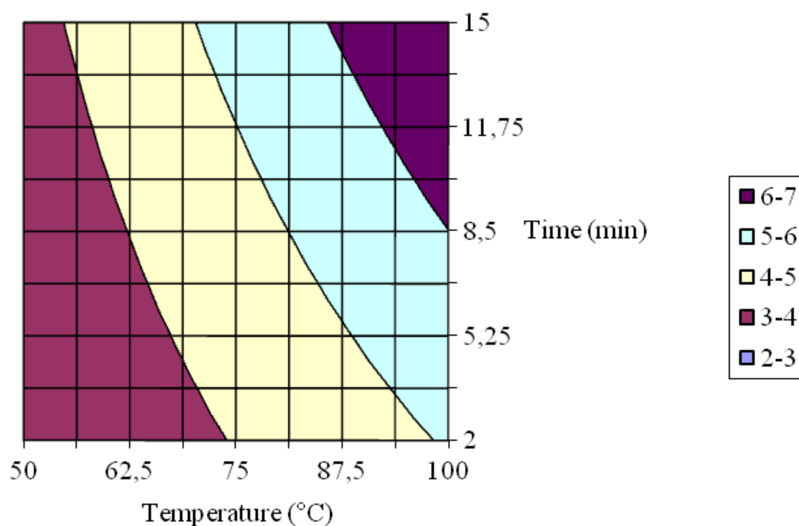


Fig. 2.7 Predicted contour levels for the total polyphenols content (mg GAE/g DM) as a function of temperature and time in MAE

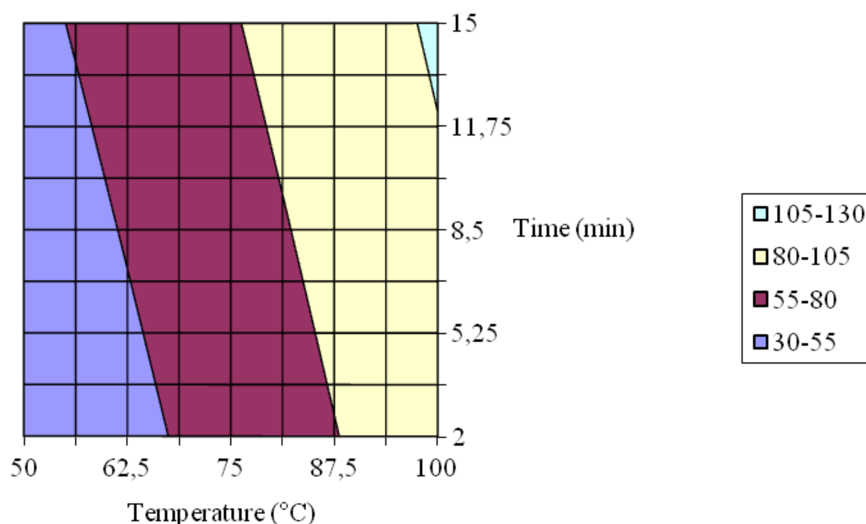


Fig.2.8 Predicted contour levels for the Luteolin content (μg/g DM) as a function of temperature and time in MAE

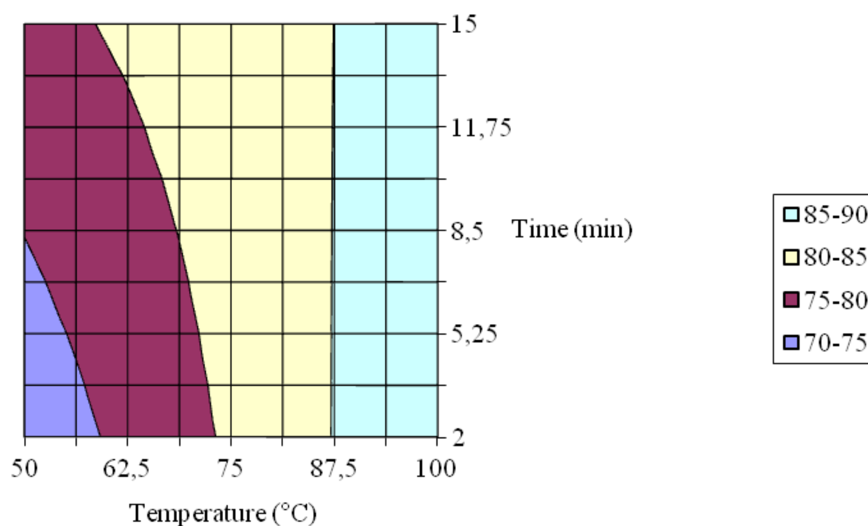


Fig. 2.9 Predicted contour levels for the Apigenin content (μg/g DM) as a function of temperature and time in MAE

In Figures 2.10, 2.11 and 2.12, the effects of extraction time and temperature on total polyphenols (expressed as mg GAE per g DM), luteolin and apigenin content (expressed as μg per g DM) in ASE are shown. The results showed that total polyphenols, luteolin and apigenin contents in ASE were strongly related to extraction temperature. Indeed, at higher extraction temperature the biocompound contents increased significantly. In addition, at 150°C the molecule content increases with an increase in time showing an influence of this variable on the response.

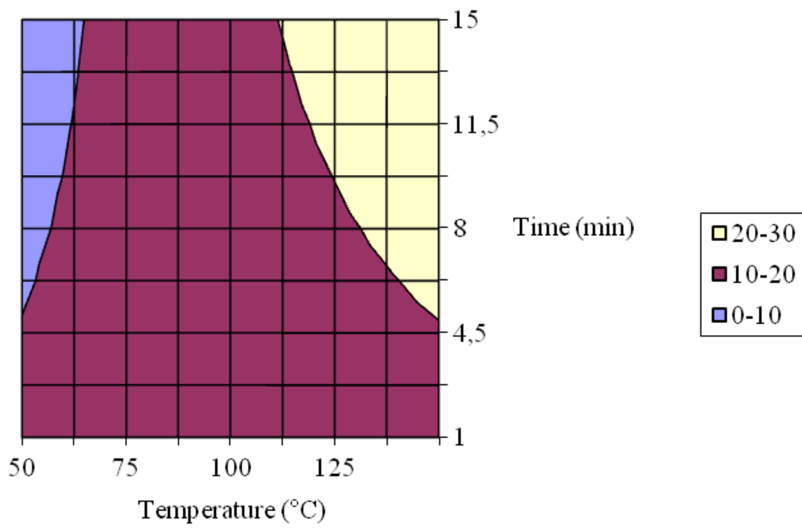


Fig. 2.10 Predicted contour levels for the total polyphenols content (mg GAE/g DM) as a function of temperature and time in ASE

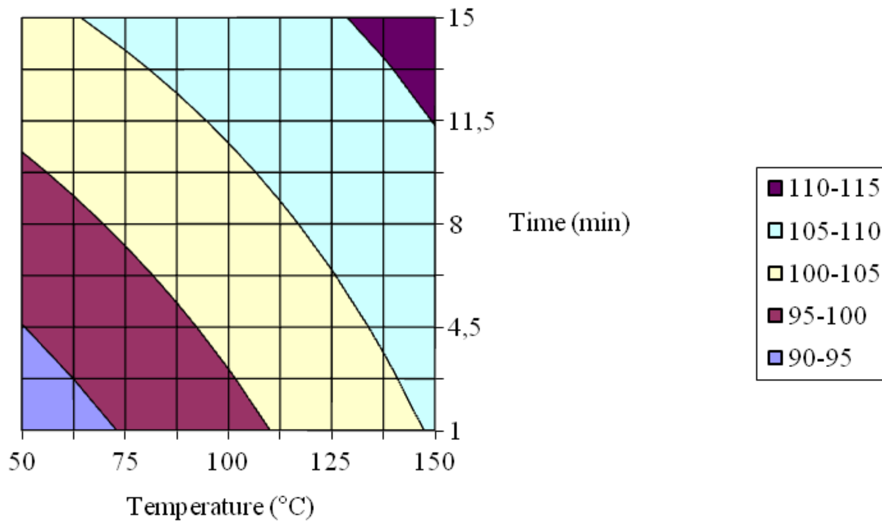


Fig. 2.11 Predicted contour levels for the Apigenin content (µg /g DM) as a function of temperature and time in ASE

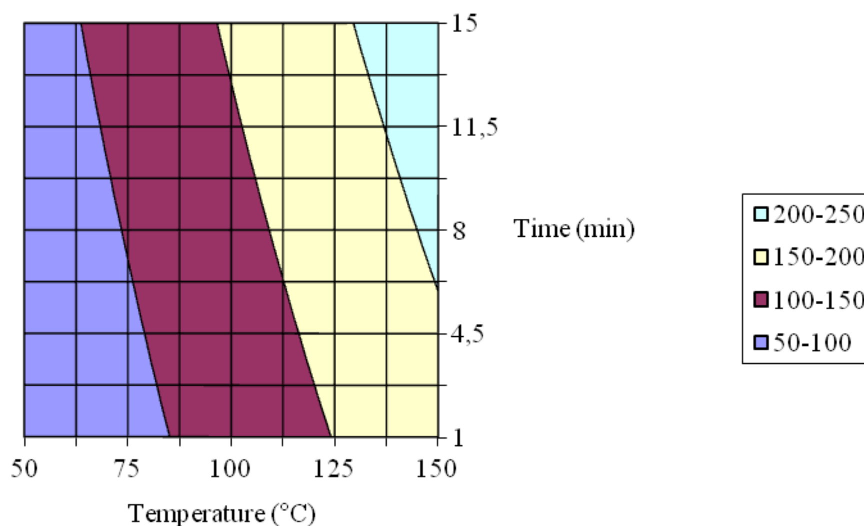


Fig. 2.12 Predicted contour levels for the Luteolin content (μg/g DM) as a function of temperature and time in ASE

In order to evaluate the effect of the extraction cycle number (1st and 2nd cycle) on the biocompound recovery from the same plant matrix in ASE, the apigenin, luteolin (expressed as mg per L of leaf extract) and total polyphenols concentration (expressed as mg GAE per mL of leaf extract) for two extraction cycles has been determined.
Results obtained indicated that already the first ASE cycle allowed to solubilise the majority of the biocompounds of interest (*Table 2.6*). In particular, strongest conditions of extraction (i.e. temperature at 150°C) allowed to obtain the best phenolic extraction performance at the first extraction cycle.

Table 2.6 Effect of ASE extraction cycle number on the Apigenin, Luteolin and total phenolic concentration (expressed as mg/L and mg GAE/mL, respectively)

Sample Name	Extraction conditions	Apigenin mg/L	Luteolin mg/L	Total polyphenols mg GAE/mL
	50°C			
extract ASE 1	50°C 50bars 1 min cycle 1	1.60	0.90	0.11
extract ASE 2	50°C 50bars 1 min cycle 2	0.18	0.21	0.05
extract ASE 3	50°C 50bars 15 min cycle 1	1.82	1.30	0.07
extract ASE 4	50°C 50bars 15 min cycle 2	0.16	0.23	0.04
extract ASE 5	50°C 150 bars 1 min cycle 1	1.58	0.93	0.18
extract ASE 6	50°C 150 bars 1 min cycle 2	0.07	0.11	0.05
extract ASE 7	50°C 150 bars 15 min cycle 1	1.82	1.37	0.08
extract ASE 8	50°C 150 bars 15 min cycle 2	0.06	0.12	0.06
	150°C			
extract ASE 9	150°C 150 bars 1 min cycle 1	1.97	3.35	0.24
extract ASE 10	150°C 150 bars 1 min cycle 2	0.00	0.07	0.09
extract ASE 11	150°C 150 bars 15 min cycle 1	1.98	3.85	0.49
extract ASE 12	150°C 150 bars 15 min cycle 2	0.00	0.12	0.12
extract ASE 13	150°C 50 bars 1min cycle 1	1.93	3.22	0.18
extract ASE 14	150°C 50 bars 1min cycle 2	0.00	0.07	0.04
extract ASE 15	150°C 50 bars 15min cycle 1	2.15	4.31	0.36
extract ASE 16	150°C 50 bars 15min cycle 2	0.00	0.13	0.05

GAE: gallic acid equivalent

2.3.2 Comparison between novel and traditional extraction methods

Extraction by ASE and MAE under the optimized conditions consists in the use of high extraction temperatures (150°C and 100°C for ASE and MAE, respectively) which enhances biocompound diffusivity and increase the recovery of the phenolic compounds but may also degrade thermolabile molecules. In order to evaluate temperature effect on phenolic profile and to evaluate the efficiency of these novel techniques, ASE and MAE phenolic contents were compared to those obtained using other conventional extraction methods (i.e. Soxhlet and maceration).

2.3.2.1 Colorimetric analysis of total phenolics

The total phenolic content of leaf extracts obtained using the aforementioned extraction methods was determined by Folin-Ciocalteu analysis. The results of this colorimetric method, expressed as mg GAE per g DM, are shown in Table 2.7.

In maceration method, the total phenolic content of leaf extracts obtained using 20 or10 mL of 80% ethanol for 60 minutes at 4°C ranged from 4.26 to 5.97 mg GAE g^{-1} of dry

matter (DM), respectively. The extraction performed by Soxhlet method using 79°C 5 h and 100 mL of 80% ethanol provided 1.90 mg GAE per g of dry leaf matter (DM).

Table 2.7 Luteolin, apigenin (expressed as µg/g DM) and total polyphenol (expressed as mg GAE/g DM) content of cardoon leaf extracts obtained by different extraction techniques

Methods	**Conditions**	**Luteolin content (µg/g DM)**	**Apigenin content (µg/g DM)**	**Total Polyphenol content (mg GAE/g DM)**
Maceration	4°C 60 min 10 mL	45.21±2.19	88.64±1.69	5.97±0.16
Maceration	4°C 60 min 20 mL	44.31±2.61	91.32±3.55	4.26±0.14
Soxhlet	79°C 300 min 100 mL	127.85±2.50	116.18±10.35	1.90±0.03

Analytical results are the means ± SD (n=2); DM: dry matter; GAE: gallic acid equivalent

Conventional extraction methods (maceration and Soxhlet) have been compared with optimized ASE and MAE techniques for total polyphenols recovery (*Figure 2.13*).
Results obtained from Folin-Ciocalteu colorimetric analysis showed that ASE provided the highest yield of total polyphenols (31.86 mg GAE g^{-1} DM, respectively) compared to those obtained using Soxhlet and maceration conventional methods (1.90 and 5.97 mg GAE g^{-1} DM, respectively), and MAE at 100°C, 15 minutes and 1:20 ratio solid:liquid (8.40 mg GAE g^{-1} of DM) (*Figure 2.13*).

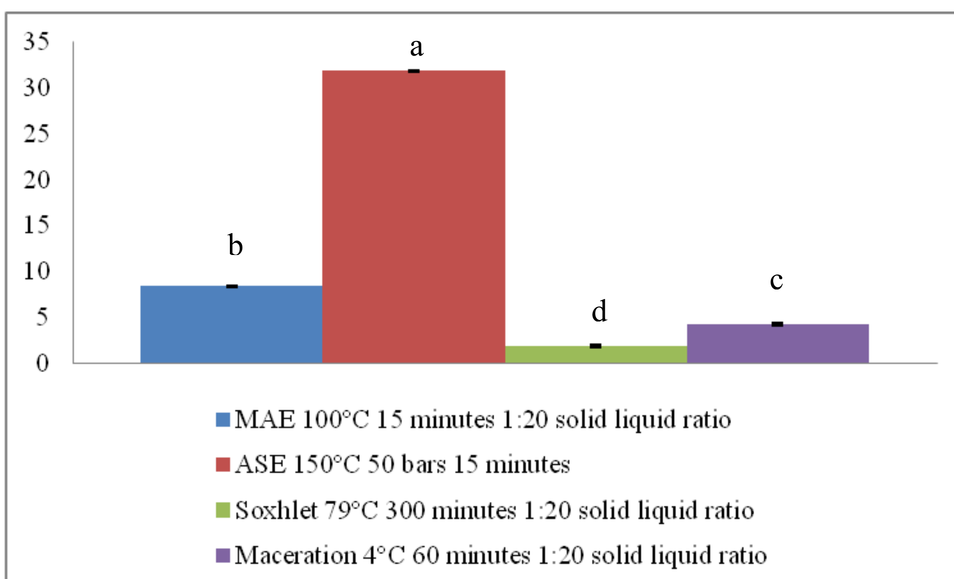


Fig. 2.13 Means ± SD of total polyphenols obtained using four extraction techniques (MAE, ASE, Soxhlet and maceration) from leaf extracts of Cynara cardunculus. Different letters indicate statistical significant differences among extraction methods at $p \leq 0.05$ (Duncan test)

2.3.2.2 HPLC ANALYSIS

Results obtained from HPLC analysis are shown in Table 2.7. Luteolin content of leaf extracts obtained using maceration, with 10 or 20 mL as solvent volume, was 45.21 and 44.31 µg g^{-1} of DM, respectively. Apigenin content was 88.64 µg g^{-1} of DM for the extract obtained by maceration with 10 mL of solvent volume and 91.32 µg g^{-1} of DM with 20 mL. As regards Soxhlet extraction, the content of luteolin and apigenin resulted in 127.85 and 116.18 µg g^{-1} of DM, respectively (*Table 2.7*).

Luteolin and apigenin content of leaf extracts obtained using the traditional extraction methods, maceration and Soxhlet, have been compared with optimized ASE and MAE techniques (*Figures 2.14* and *2.15*).

Optimized ASE method provided the highest yield of luteolin compared to those obtained using Soxhlet, maceration and optimized MAE method (*Figure 2.14*).

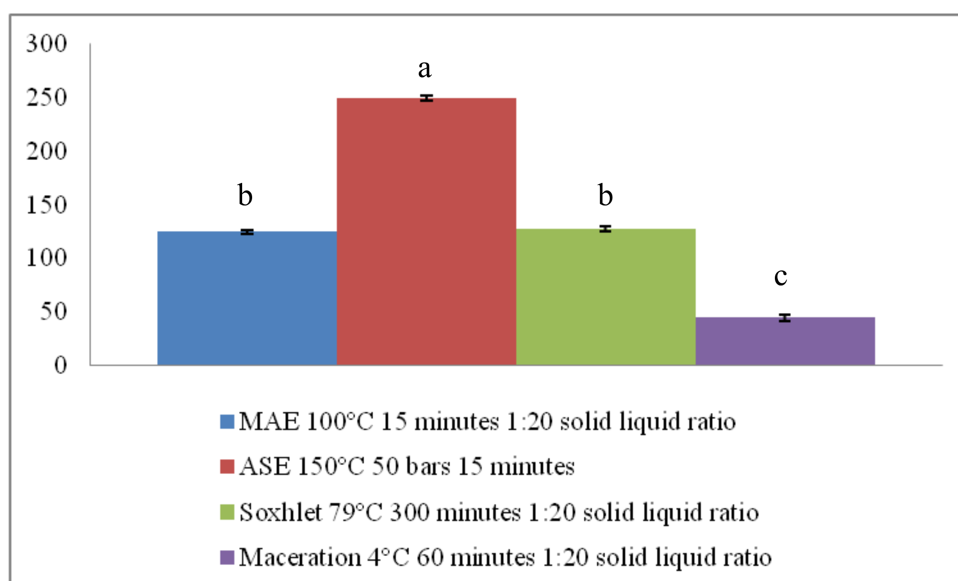


Fig. 2.14 Means ± SD of Luteolin obtained using four extraction techniques (MAE, ASE, Soxhlet and maceration) from leaf extracts of Cynara cardunculus. Different letters indicate statistical significant differences among extraction methods at p≤0.05(Duncan test)

As regards apigenin, optimized ASE and Soxhlet provided the highest similar yields respect to those obtained using both maceration and optimized MAE methods (*Figure 2.15*).

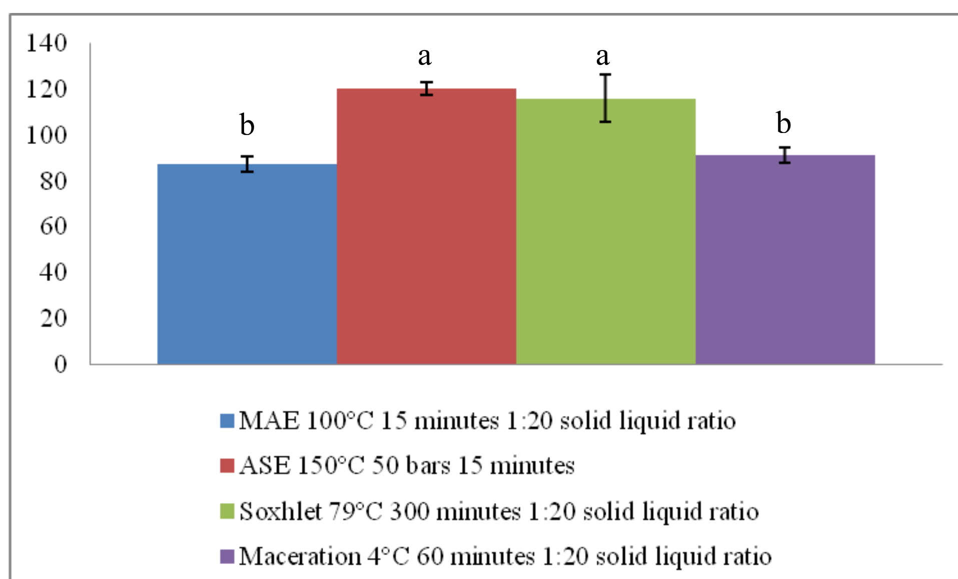


Fig. 2.15 Means ± SD of Apigenin obtained using four extraction techniques (MAE, ASE, Soxhlet and maceration) from leaf extracts of Cynara cardunculus. Different letters indicate statistical significant differences among extraction methods at p≤0.05(Duncan test)

Figures 2.16 and 2.17 depict the characteristic chromatographic fingerprints obtained from the same amount of leaves material extracted either at elevated temperature (*Figure 2.16*) or at 4°C (*Figure 2.17*). The profile similarity between technique using low and high temperature indicates that the heating does not induce evident break down of phenolic compounds.

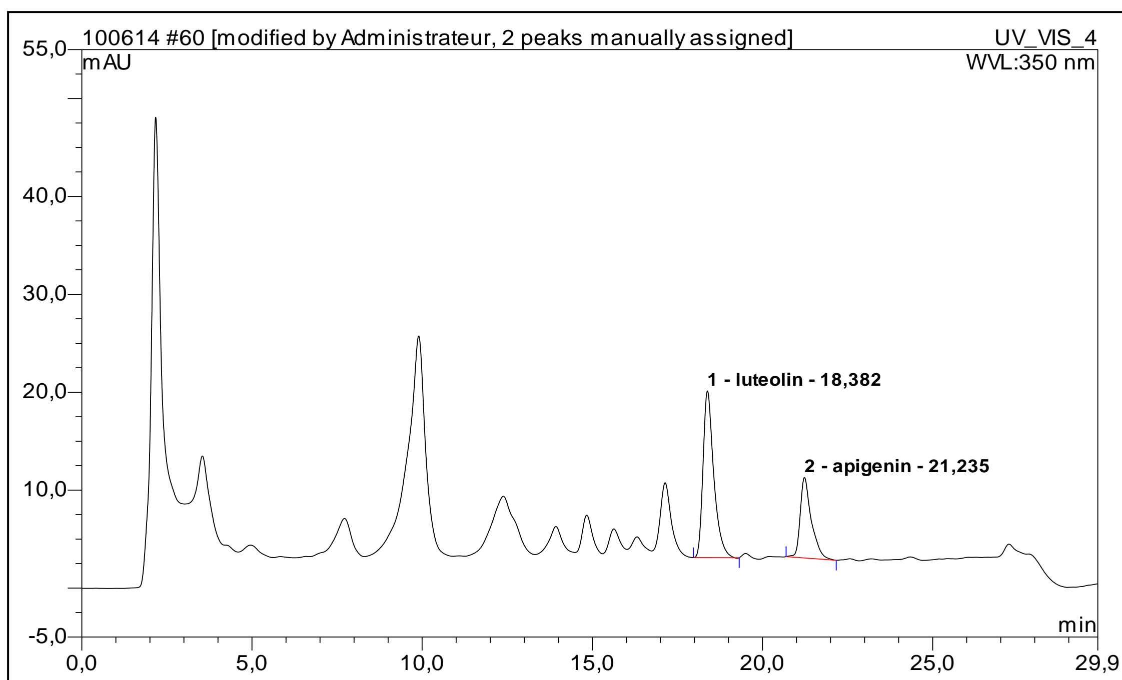


Fig. 2.16 Chromatogram recorded at 350 nm of leaf extract (1g DM) obtained using ASE at 150°C, 150 bars and 15 minutes. HPLC analysis operating conditions: mobile phase 0.1% formic acid in water (solvent A) and in methanol (solvent B), flow rate 1 mL/min. The mobile phase was increased to 65% in a linear gradient in 25 min and then returned to 30% in 5 min. Injection volume 20 µL

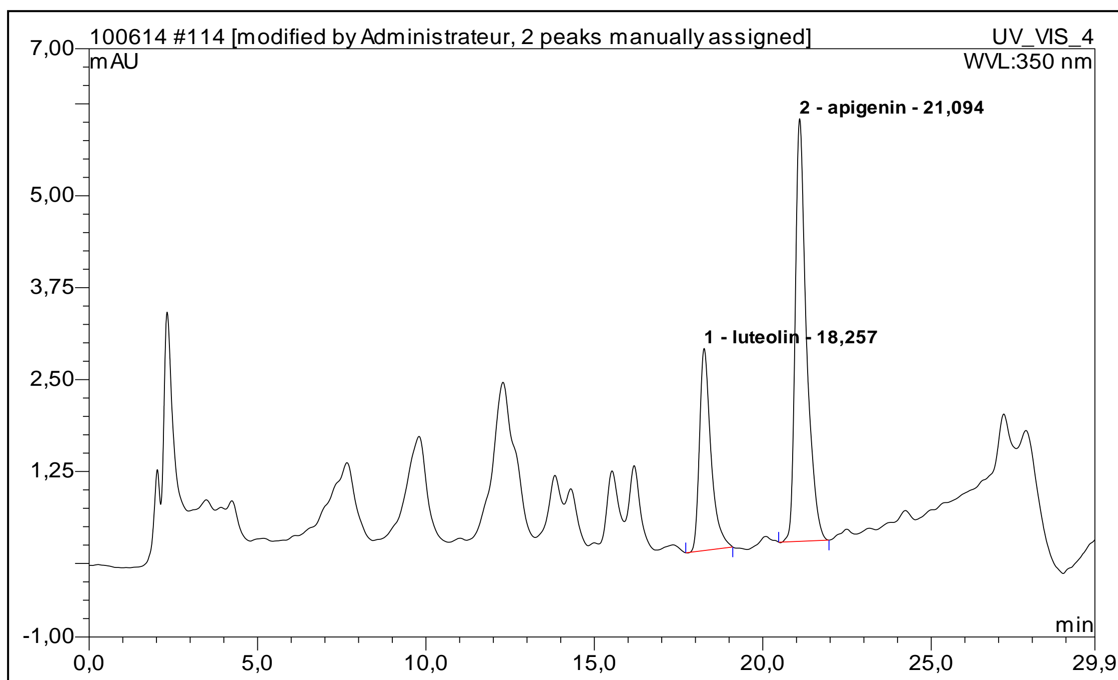


Fig. 2.17 Chromatogram recorded at 350 nm of leaf extract (1g DM) obtained using maceration at 4°C, 1:10 solid:liquid ratio and 60 minutes. HPLC analysis operating conditions: mobile phase 0.1% formic acid in water (solvent A) and in methanol (solvent B), flow rate 1 mL/min. The mobile phase was increased to 65% in a linear gradient in 25 min and then returned to 30% in 5 min. Injection volume 20 µL

2.4 Discussion

Optimization of MAE and ASE extraction methods was achieved by a two-level full factorial design (-1, 0, +1) consisting in a 10 sets of experiments (8 factorial points with two replicates at the center of the design) (*Tables 2.4* and *2.5*). This experimental design was used to evaluate the influence of independent variables and their interactions on phenolic compounds recovery. Total polyphenols, luteolin and apigenin contents, molecules previously found in *C. cardunculus* spp. extracts by other authors (Wang *et al.,* 2003; Schütz *et al.,* 2004; Fratianni *et al.,* 2007; Melilli *et al.,* 2011; Pandino *et al.,* 2010, 2011a, 2011b, 2011c, 2012), have been chosen as responses of the experimental design. Results obtained from colorimetric and HPLC analyses highlighted the strong influence of independent factors on phenolic extraction yield (*Tables 2.4* and *2.5*). Regression analysis of these results allowed to estimate the regression coefficients for both MAE and ASE extraction techniques (*Figures 2.1, 2.2, 2.3* for MAE and *Figures 2.4, 2.5, 2.6* for ASE). In agreement with other findings (Kwon *et al.,* 2003; Zheng *et al.,* 2009; Michel *et al.,* 2011), a positive relationship between extraction temperature and phenolic compounds yield was found in MAE (*Figures 2.1, 2.2* and *2.3*). For this technique a slight influence of the solid:liquid ratio and extraction time was assessed.

However, it is important to consider that the effect of independent factors on polyphenol yield may also depend on the different level chosen for independent variables, plant materials and solvent used.

In ASE, regression coefficients for extraction temperature variable presented the highest values for luteolin, apigenin and total polyphenols (Sharma *et al.,* 2007; Monrad *et al.,* 2010) while pressure variable showed the lowest ones (*Figures 2.4, 2.5* and *2.6*). In ASE, as reported by other authors (Alonso-Salces *et al.,* 2001; Sharma *et al.,* 2007), pressure independent variable has only the function of keeping solvent in liquid state to ensure that it remains in intimate contact with the sample during the extraction, and to force diffusivity of the solvent into solid matrix. In our experiment, pressure of 50 bars was sufficient to assure these functions, and increasing the pressure to 150 bars had only little influence on biocompound recovery. Regression coefficients estimated for each method were used to determine the relative first-order polynomial equations (Section 2.3.1). On the basis of these equations, Pearson‘s correlation coefficients were calculated for each method and a good correlation between the observed and predicted values of the test was found confirming the validity of the experimental design. Considering the first-order polynomial equation, predicted contour level were obtained for each method and response by keeping constant the independent variable characterized by the lowest regression coefficient (i.e. solid:liquid ratio for MAE and extraction pressure for ASE) (*Figures 2.7, 2.8, 2.9* for MAE and *Figures 2.10, 2.11, 2.12* for ASE). In the predicted contour level obtained as aforementioned, the effects of independent variables, temperature and time for both MAE and ASE, on the phenolic compound recovery are shown and confirmed the general trends previously observed on the basis of regression coefficients.

The developed experimental models allowed to set optimal extraction conditions at 150°C, 15 minutes and 100 bars for ASE and at 100°C, 15 minutes and 1:15 solid:liquid ratio for MAE. ASE at the optimal conditions showed the best performance respect to MAE. For ASE, the effect of the extraction cycle number (1st and 2nd cycle) on biocompound recovery was also evaluated (*Table 2.6*) and the results obtained indicated that one ASE cycle allowed to solubilise the majority of the biocompounds of interest in agreement with Çam and Hışıl (2010).

In order to evaluate the efficiency of ASE and MAE, these novel techniques were compared to Soxhlet and maceration traditional methods for the phenolic compound yield. Results obtained from Folin-Ciocalteu and HPLC analyses showed that ASE and

MAE provided highest yield of phenolic compounds compared to those obtained from maceration and Soxhlet. Certainly, the highest temperature used in MAE and ASE in a short time positively influenced polyphenol recovery and no evident biocompound degradation phenomena have been observed. Indeed, extraction at elevated temperatures increases solubility, diffusion rate, and mass transfer, coupled with the ability of the solvent to disrupt the analyte-matrix interactions (Sticher, 2008). Moreover, secondary metabolites are localized in superficial tissues. As reported by several authors (Santiago *et al.,* 2000; Sakihama *et al.,* 2002; Agati *et al.,* 2009), phenolic molecules including flavonoids, both in glycosidic and non-glycosidic forms, are indeed confined mainly to hydrophilic regions, such as vacuoles and apoplasts, of epidermal cells of plant organs such as leaves and stems. Because of this, in ASE, cell disruption due to the forced diffusivity of the solvent into solid matrix could improve nutraceutical recovery compared to traditional extraction techniques (Soxhlet and maceration) (Wang and Weller, 2006).

Considering the satisfactory results obtained in ASE, using one extraction cycle at the optimum conditions, respect to MAE and other conventional techniques and taking into account the possibility of reducing extraction time and solvent consumption (Kaufmann and Christen, 2002), this technique has been chosen for the further extractions and was found an attractive method for polyphenols recovery from *Cynara* spp. leaves at a laboratory scale.

Chapter 3

BIOMASS AND NUTRACEUTICAL KINETICS EVALUATION IN GLOBE ARTICHOKE AND CARDOON PLANTS GROWN IN OPEN FIELD

3.1 INTRODUCTION

In recent years, the interest for the nutraceutical compounds present in whole *Cynara carduncului s*pp. plants has increased and several studies on the biochemical profile determination of globe artichoke and cardoon plants has been carried out. In particular, as aforementioned, beneficial properties of this species is linked to the special chemical composition which includes high levels of phenolic compounds. The major phenolic components found in this plant are caffeic acid derivatives with mono- and dicaffeoylquinic acids which represent the majority of these compounds (Lattanzio *et al.,* 2009). Plants phenolic content undergoes important variations during the growing season (Lattanzio and Morone, 1979). Indeed, these secondary metabolites not represent a final product of a metabolic process, but are constantly evolving and are subject to changes based on phonological and environmental conditions. The biosynthesis of phenolic compounds seems to be linked to their protective role against biotic and abiotic stresses such as oxidative damages caused by free radicals and is involved as substrates in oxidative browning reactions (Lombardo *et al.,* 2010). In literature, few works about the kinetics of polyphenol accumulation in *Cynara* plant grown in open field are available (Lattanzio and Morone, 1979; Lombardo *et al.,* 2010; Negro *et al.,* 2011; Pandino *et al.,* 2012). In particular, Lattanzio and Morone (1979) studied the variation of the orthodiphenol fraction composition in leaves at different physiological stages. Lombardo *et al.* (2010) evaluated the phenolic compound profile variation in relation to the harvest time in ‗Romanesco' clone C3 head parts and floral stem (winter and spring). All authors found that the climatic conditions (such as temperature and light) during the growing season and the physiological stage of the plants strongly influenced the phenolic profile. For this reason it is important to well know the maximum polyphenol accumulation stage in *Cynara* plant, in relation to the specific cultivation environment, to improve biocompound yield. Indeed, for a large-scale production of biomass for biocompound extraction, the knowledge of the maximum biocompound accumulation stage in the plant could lead to a rational management of genetic resources and to an exploitation of plant material by pharmaceutical industry. On the basis of these preliminary remarks, before starting the biochemical characterization of globe artichoke and cardoon genotypes belonging to the Tuscia University and Enea joint collection, the evaluation of the phenolic compound kinetics in globe artichoke and cardoon reference genotypes grown in the coastal area of central Italy has been carried out.

The aims of the present work were:

i) evaluating the biomass and polyphenol accumulation kinetics in plants grown in open field;

ii) comparing biomass and polyphenol production in cardoon and globe artichoke genotypes;

iii) individuate the plant physiological stage of maximum polyphenol accumulation.

3.2 MATERIALS AND METHODS

3.2.1 FIELD EXPERIMENTS

Field experiments were conducted during the 2009-2010 and 2010-2011 growing seasons at the experimental station of Arsial (Latium Regional Agency for the Development and the Innovation of Agriculture), Cerveteri (Latium, Italy). Three genotypes, belonging to the ENEA-Tuscia University germplasm joint collection, were considered in our study: Cavi and Campagnano globe artichoke genotypes and AFB cultivated cardoon genotype. Both Cavi and AFB genotypes were propagated by seed. For AFB cardoon genotype seeds from two subsequent self-pollinations and for Cavi seeds from inbred line have been used. The sowing date was July, 14th 2009. Campagnano genotype was vegetatively propagated by rooted offshoots. Four-week-old plants of Cavi and AFB genotypes with 3-4 leaves and Campagnano rooted offshoots were transplanted and arranged in a split-plot experimental design with three replications and five main theses (physiological stages). Each plot consisted of 5 plants adopting inter- and intrarow distances of 1.10 and 1.00 m, respectively. The transplanting date was August, 17th 2009.

Field experiments were conducted under low energy inputs for crop management (irrigation once in each year in August using 60 mm of water, organic fertilization 100 kg N ha^{-1}).

3.2.2 AGRO-MORPHOLOGICAL CHARACTERIZATION

During both years, in order to evaluate the morpho-physiological development of *Cynara* spp. plants, eight agromorphological traits were evaluated (i.e. plant height, floral stem diameter, plant diameter, total number of leaves, plant fresh and dry weigth, plant root fresh and dry weight) on three plants for each genotype at different physiological stages (i.e. young plant with < 10 leaves= October, winter leaf rosette=

December, floral stem elongation= February-March, flower head appearance= April, full blossom= May).

3.2.3 Biochemical characterization

During both growing seasons, biomass (leaves and floral stems) was collected from two plants for each genotype at different stages of development (October-February/March-April in 2009-2010 growing season and October-December-February/March-April-May in 2010-2011 growing season) to determine the kinetics of fresh, dry matter and phenolic compound accumulation in the plants. Also primary flower heads were harvested at the commercial maturity stage and analyzed. In the first growing season, only plant material from Cavi and AFB genotypes was biochemically analyzed. In the second growing season, samples from the three genotypes (Cavi, Campagnano, and AFB) were considered in the biochemical analysis. All samples (300 g FW) were collected and freeze-dried, milled to a fine powder (diameter 1 mm) and stored at -20°C until the analyses were carried out at the Institut National Polytechnique de Toulouse - Laboratoire de Chimie Agroindustrielle, in France.

3.2.4 Determination of moisture content

In order to evaluate moisture content, an amount (10 g three times replicated) of homogenized plant material was oven-dried at 103°C until the moisture level was constant (24h) to determine the dry matter (DM) content.

3.2.5 Solvents and reagents

Ethanol solvent and reagent grade formic acid (96%) were purchased from Scharlau, (Barcelona, Spain). Deionized water was home-made using a Milli-Q water purifying system purchased from Millipore Co. (Bedford, MA, USA). Luteolin-7-*O*-glucoside (cynaroside) *(CAS number 5373-11-5)*, silybin *(CAS number 22888-70-6)*, 1,3-*O*-dicaffeoylquinic acid (cynarin) *(CAS number 30964-13-7)*, and 1,5-*O*-dicaffeoylquinic acid *(CAS number 19870-46-3)* were purchased from Extrasynthese (Lyon, France) while apigenin *(CAS number 520-36-5)*, luteolin *(CAS number 491-70-3)*, taxifolin *(CAS number 480-18-2)* and 3-*O*-caffeoylquinic acid (chlorogenic acid) *(CAS number 327-97-9)* were obtained from Sigma Chemical Co. (St. Louise, MO, USA).

3.2.6 Extraction procedure

The extraction was performed using Accelerated Solvent Extraction (ASE). The instrument Accelerated Solvent Extractor ASE100 Dionex Corporation (Sunnyvale, CA, USA) was used for pressurized solvent extraction. An amount (1g) of powdered dried sample was packed into the extraction cell and an amount of washed sea sand was used to fill the cell. Ethanol 80% was used as solvent. The ASE was performed at 150°C, 100 bars for 15 min with one static cycle using extraction condition optimized in the aforementioned full-factorial experimental design (see chapter 2). After each extraction, the vessels were allowed to cool at room temperature and then each extract was filtered under vacuum and diluted up to 25 mL using ethanol 80%. All samples were stored at -20°C until further analysis. All extractions were performed in duplicate.

3.2.7 HPLC analysis

Polyphenol analysis was carried out using a Dionex HPLC chromatography equipped with an UV detector HP 1100. HPLC separation was performed on a Pursuit C18 AQ18 (250 x 4.6 mm, 5 μm) column at room temperature. The mobile phase was 0.1% formic acid in water (solvent A) and in acetonitrile (solvent B) at a flow rate of 1 mL/min. The gradient program started with 6% solvent B to reach 30% solvent B at 30 min until 35 min and then decreased to 6% solvent B at 37 min until 40 min. The injection volume was 20 μL. Calibration curves for each available standard was generated with concentrations ranging from 1 mg/L to 200 mg/L. Identification of single compounds was done using retention time and UV spectrum, while quantification was performed by standard calibration curves. For each standard, the limits of detection and quantification (LOD and LOQ) were determined at their maximum UV-vis absorbance wavelength (*Table 3.1*). Chromatograms were recorded at 230 nm (taxifolin and silybin), 325 nm (caffeic acid, 3-*O*-caffeoylquinic acid, 1,3-*O*-dicaffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid) and 350 nm (luteolin, apigenin, cynaroside) (*Figure 3.1*). All samples were analyzed in duplicate.

Table 3.1 LOD and LOQ of standards used in HPLC analysis

Standards	LOD ($mg \cdot l^{-1}$)	LOQ ($mg \cdot l^{-1}$)
3-*O*-caffeoylquinic acid (chlorogenic acid)	0.050	0.167
1,3-*O*-dicaffeoylquinic acid (cynarin)	0.050	0.166
1,5-*O*-dicaffeoylquinic acid	0.056	0.184
luteolin 7-*O*-glucoside (cynaroside)	0.026	0.086
luteolin	0.054	0.180
apigenin	0.050	0.167
taxifolin	1.010	3.360
silybin	1.020	3.400
caffeic acid	0.050	0.167

3.2.8 Colorimetric analysis of total phenolics

Total phenolics were estimated using the Folin-Ciocalteu method based on the reduction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products. One mL of diluted extract sample was added to 6 mL of distilled water. Then, 1 mL of 20% Na_2CO_3 and 1 mL of the Folin-Ciocalteu reagent were added to the mixture. The solution was then mixed and incubated for 20 min at 70°C. Total phenolic content was spectrophotometrically estimated at 760 nm. The quantification was based on the standard curve generated using gallic acid. The standard preparation was made adding 6 mL of distilled water to 1 mL of 0-5-10-50-100-150 mg/L gallic acid solutions. Then, 1 mL of 20% Na_2CO_3 and 1 mL of the Folin-Ciocalteu reagent were added to the mixture. Total phenolic content of plant parts was expressed as mg of gallic acid equivalents per g of dry weight through the calibration curve.

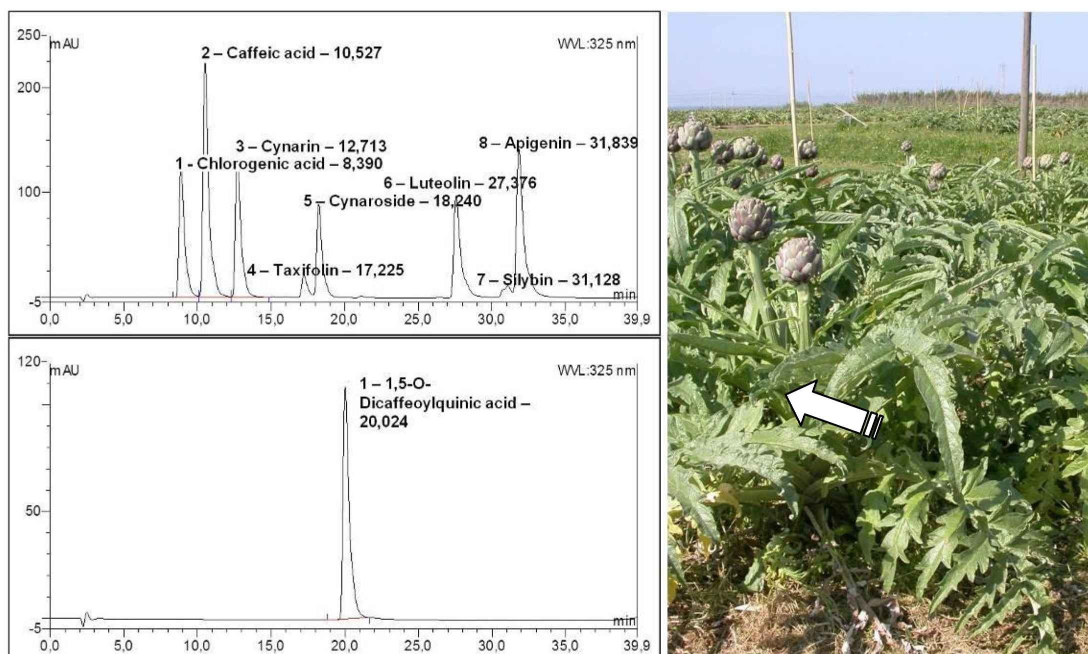


Fig. 3.1 HPLC chromatograms at 325 nm of standards used for biochemical characterization of globe artichoke and cardoon genotypes

3.2.9 Statistical analyses

All data were analyzed by ANOVA with the Generalized Linear Model (GLM) procedure using SPSS software version 15.0. Mean separations were performed by Duncan test. Significance was accepted per $p \leq 0.05$ level.

3.3 Results

3.3.1 Agro-morphological characterization and plant growth evaluation

2009-2010 growing season

In the first growing season, no significant differences among genotypes were found for all traits evaluated in the first three sampling times (October, December and February) except for plant fresh and dry weight in December (*Figures 3.2* and *3.3*, respectively). Indeed, in this second sampling time, cardoon plants (AFB) showed a higher plant fresh and dry weight (5.20 and 0.65 kg per plant, respectively) compared to globe artichoke plants (Cavi and Campagnano). For plant fresh and dry weight, there were no significant differences in all sampling times between Cavi and Campagnano genotypes which showed a similar growth trend. On the contrary, significant differences for plant fresh and dry weight resulted for AFB genotypes compared with the other genotypes in December and March sampling times. Significant differences were found for the traits plant height and leaf number starting from March in particular between cardoon (AFB) and globe artichoke genotypes (Cavi and Campagnano) analyzed (*Figures 3.4* and *3.5*,

respectively). Indeed, the highest growth rate starts after the winter rest period from the floral stem elongation to the plant full blossom stage. In March and April, AFB cardoon presented the significant highest value of plant height and leaf number (in April 225.33 cm and 63.67 no., respectively) compared with both Cavi and Campagnano globe artichoke genotypes which showed no significant differences between each other for these traits. As regards floral stem diameter, no significant differences were found among genotypes studied which presented an average value of 2.89 cm. For plant diameter, significant differences were found among genotypes in December and April while in February only AFB cardoon showed a significant highest value for this trait (211.67 cm) comparing with Cavi and Campagnano (*Figure 3.6*). As regards root fresh and dry weight, no significant differences were found among genotypes considered in the first four sampling times while only at the end of the growing season (April) AFB cardoon provided the highest value for these traits (3.39 and 1.29 kg per plant) compared with the globe artichoke genotypes (*Figures 3.7* and *3.8*). Generally, at the begin of the growing season, from August to February, the plant growth rate was very slow while starting from March a rapid growth has been found for all genotypes. In particular, for each trait evaluated AFB cardoon genotype provided the best values compared with globe artichoke genotypes. Even though, Campagnano and Cavi were propagated differently (offshoots *vs* seed), no significant differences were found for most of the considered traits and a similar agro-physiological behavior has been observed. Moreover, AFB cardoon presented the highest genetic variability (represented by standard deviation) which may depend on the high degree of heterozygosity.

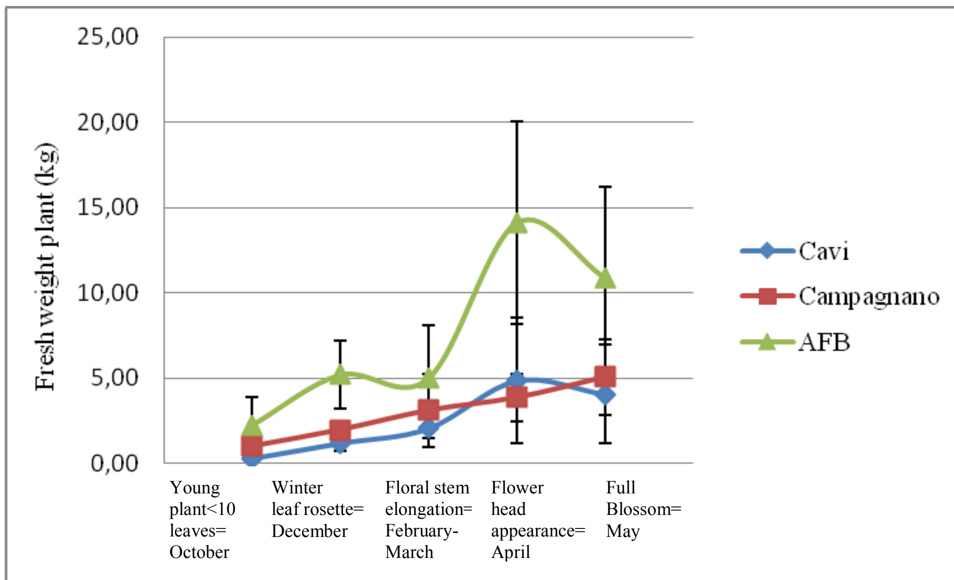


Fig. 3.2 Kinetics of the fresh weight of the plant for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

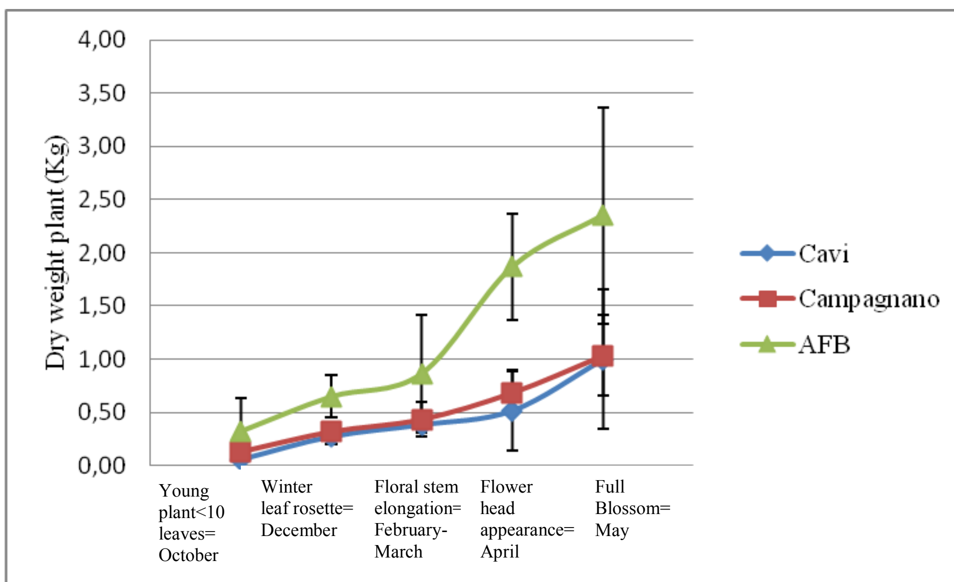


Fig. 3.3 Kinetics of the dry weight of the plant for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

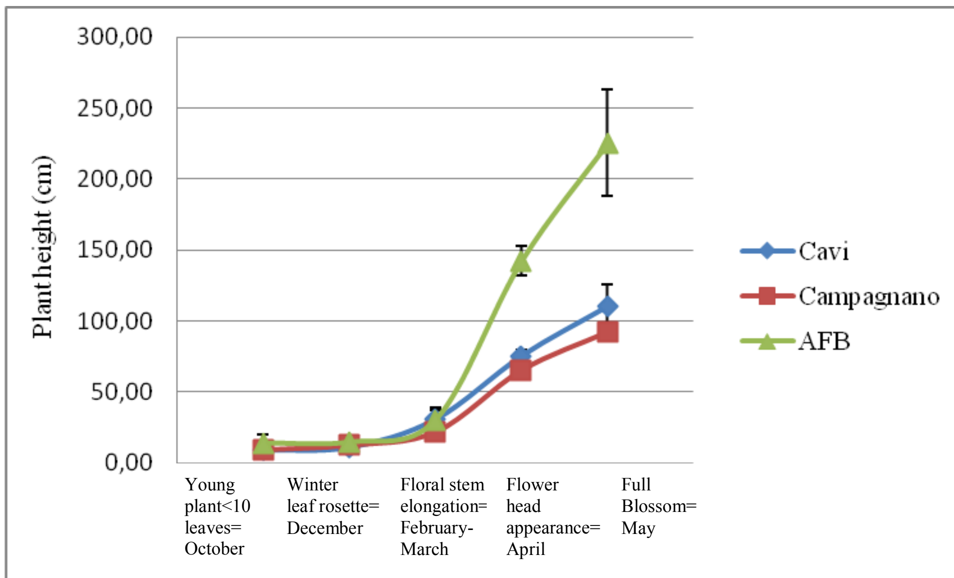


Fig. 3.4 Kinetics of the plant height for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

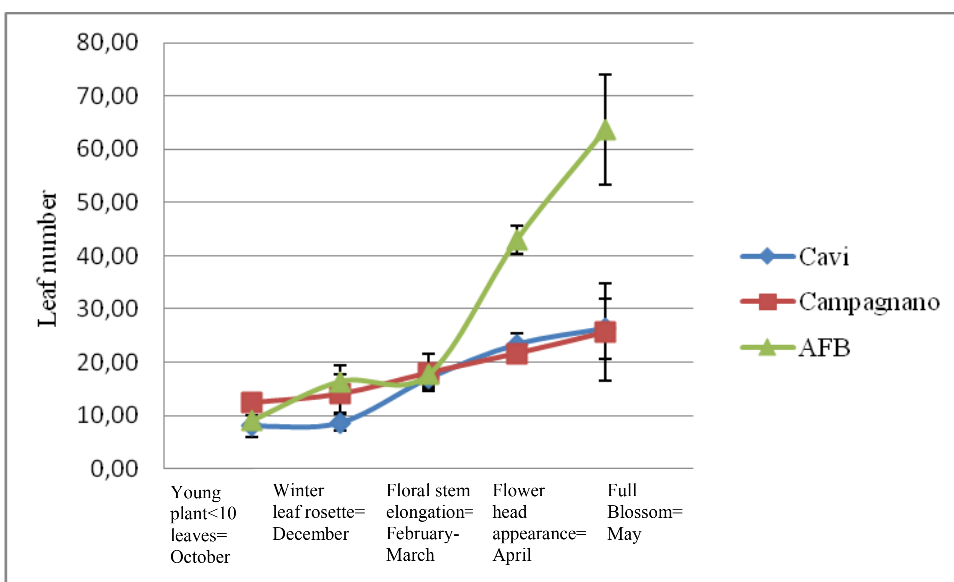


Fig. 3.5 Kinetics of the plant leaf number for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

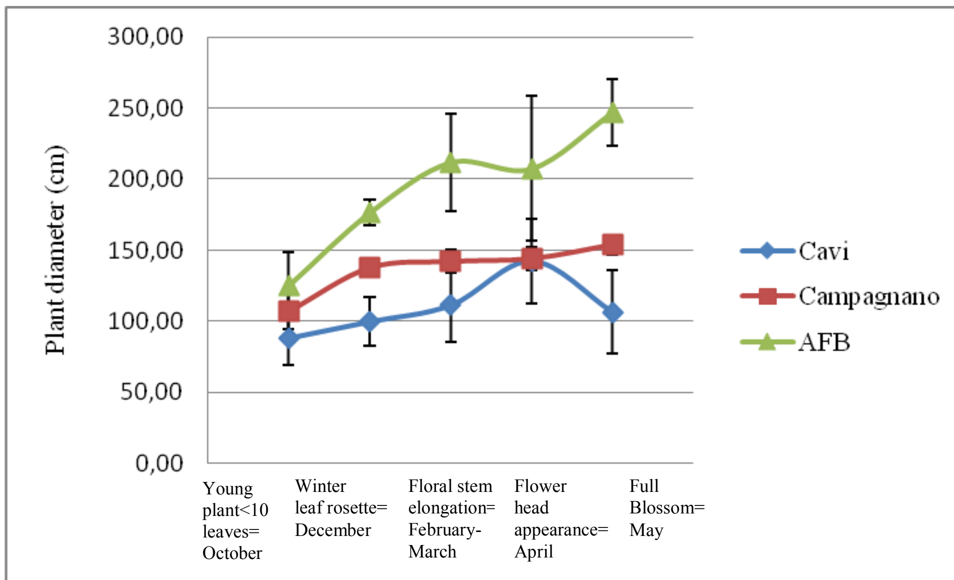


Fig. 3.6 Kinetics of the plant diameter for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

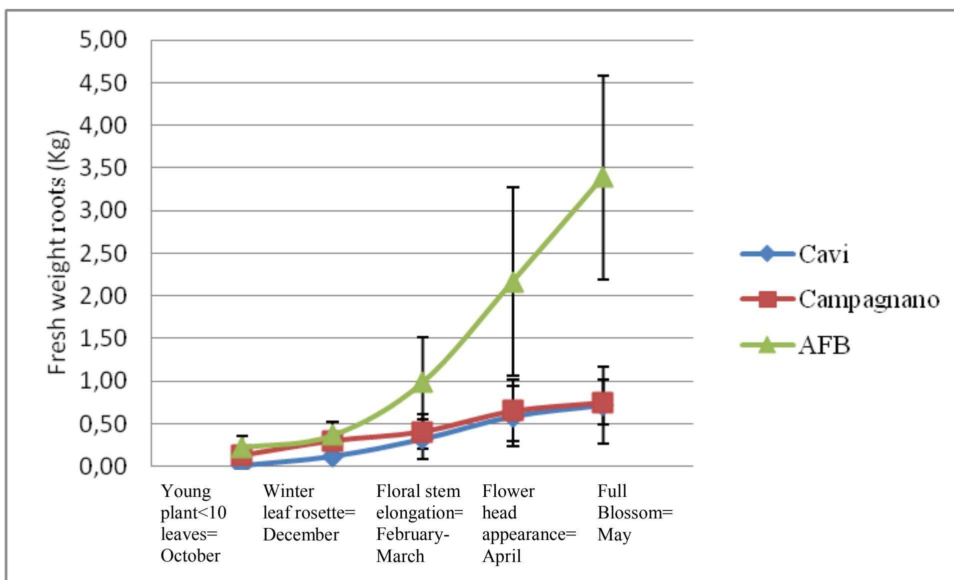


Fig. 3.7 Kinetics of the fresh weight of the roots for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

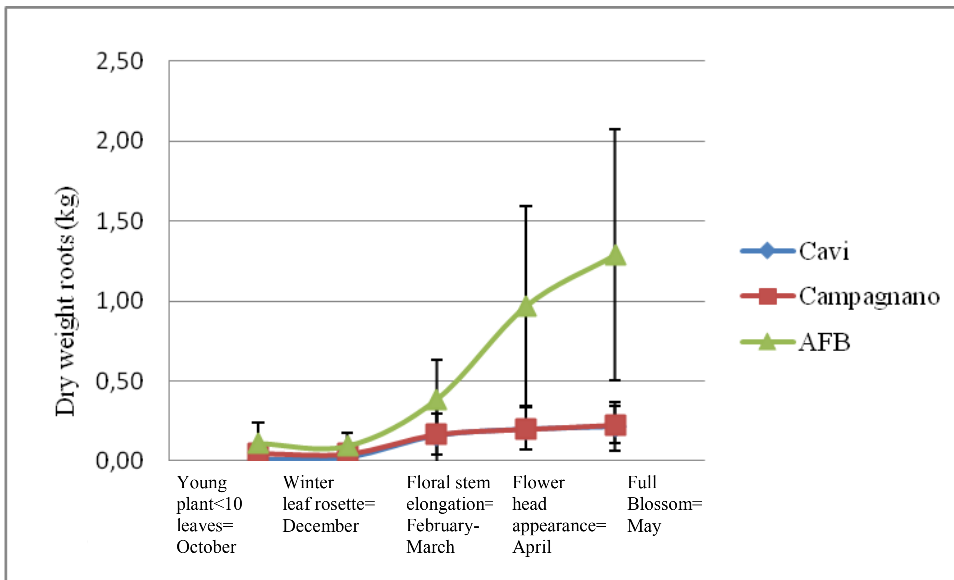


Fig. 3.8 Kinetics of the dry weight of the roots for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

2010-2011 GROWING SEASON

In the second growing season, no significant differences among genotypes were found for plant height in the first two sampling times (October-December) while in March AFB resulted significantly different from Campagnano. In April and May, AFB was significantly different from Cavi and Campagnano and showed the highest values for this trait (193.67 and 201.00 cm, respectively against 122.33 and 119.33 cm for Cavi and 122.67 and 144.33 cm for Campagnano) (*Figure 3.9*). As regards total leaf number, for Cavi and Campagnano there were no significant differences for all sampling times except in May when Campagnano showed 42.67 leaves and Cavi 35.00. For this trait, AFB genotype resulted to have significant higher leaf number than Cavi in all sampling times and than Campagnano only in December, March and April (*Figure 3.10*). The plant diameter was not significantly different among genotypes during the first three sampling times while in April and in May, AFB showed the highest value of (298.33 and 333.00 cm, respectively) compared with the other genotypes (*Figure 3.11*). In all sampling times, AFB showed the highest value of plant fresh weight except in October and in March. At the end of the growing season it was characterized by a total fresh weight of 22.07 kg per plant while Cavi by a total fresh weight of 9.93 kg per plant and Campagnano of 6.37 kg per plant (*Figure 3.12*). It is interesting to notice that AFB resulted also the genotype with the highest accumulation of dry matter and showed the highest value of plant dry weight in all sampling times except in October and March (*Figure 3.13*). As regards floral stem diameter, significant differences were found

among genotypes, in particular, in this second year, AFB presented the highest value for this trait (5.70 cm) compared with Cavi and Campagnano (3.42 and 3.55 cm, respectively). The fresh and dry weight of plant roots were evaluated only at the end of the second growing season. Significant differences were found among genotypes for these traits and AFB and Campagnano provided the highest value of root fresh and dry weight (FW 11.4 and DM 1.6 kg, FW 10.6 and DM 1.4 kg, respectively) compared with Cavi genotype (FW 1.2 and DM 0.22 kg). During the first months of the second experimental year, the plant growth rate was very low while starting from March a rapid growth has been found for all genotypes. AFB confirmed to have the highest values for all traits considered compared with the globe artichoke genotypes. Campagnano and Cavi genotypes showed a similar agro-physiological behavior, but it should remember that Cavi was the earliest genotype which terminated its physiological growth already at the begin of April while Campagnano ended its growth cycle at the end of April. Both Cavi and Campagnano genotypes have been entered the senescence period after the reproductive stage in May; while, AFB resulted the latest genotype with a good growth cycle up to May. Indeed, plant fresh weight for Cavi and Campagnano decreased from April to May while in AFB increased until May.

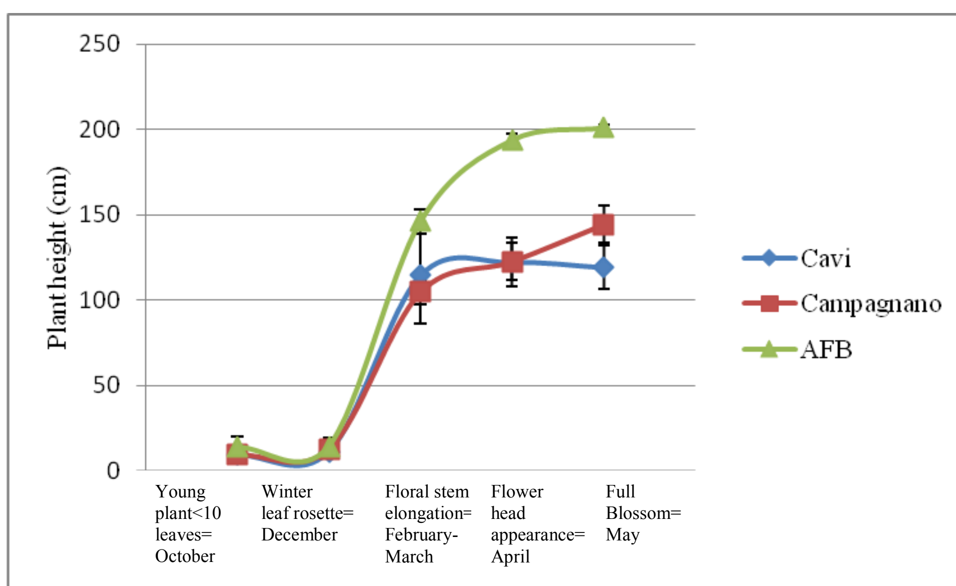


Fig. 3.9 Kinetics of the plant height for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

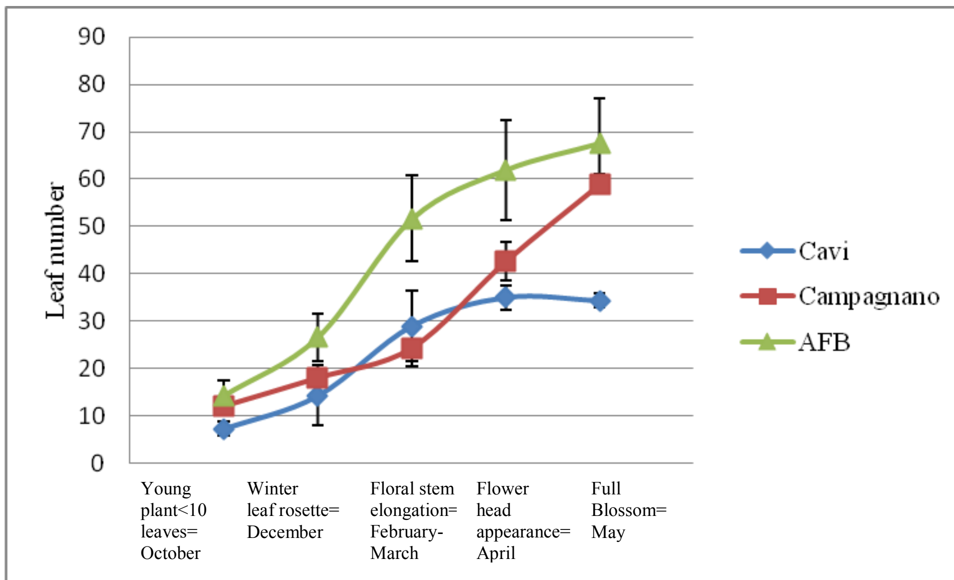


Fig. 3.10 Kinetics of the plant leaf number for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

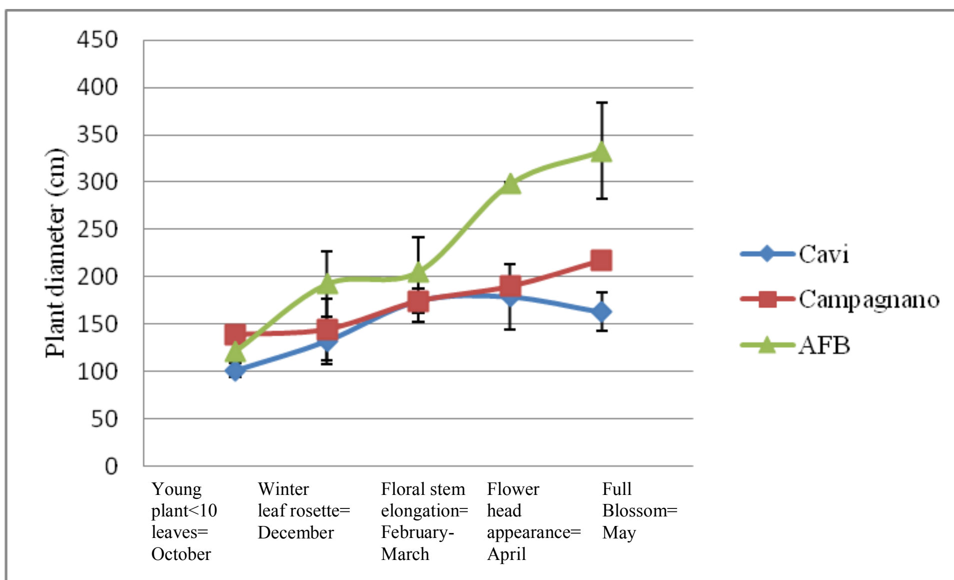


Fig. 3.11 Kinetics of the plant diameter for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

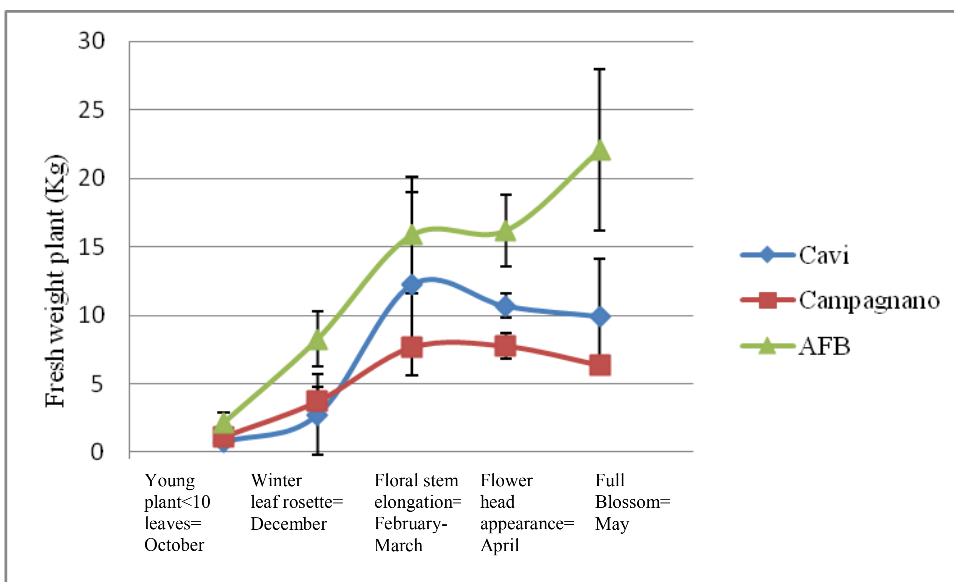


Fig. 3.12 Kinetics of the fresh weight of the plant for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

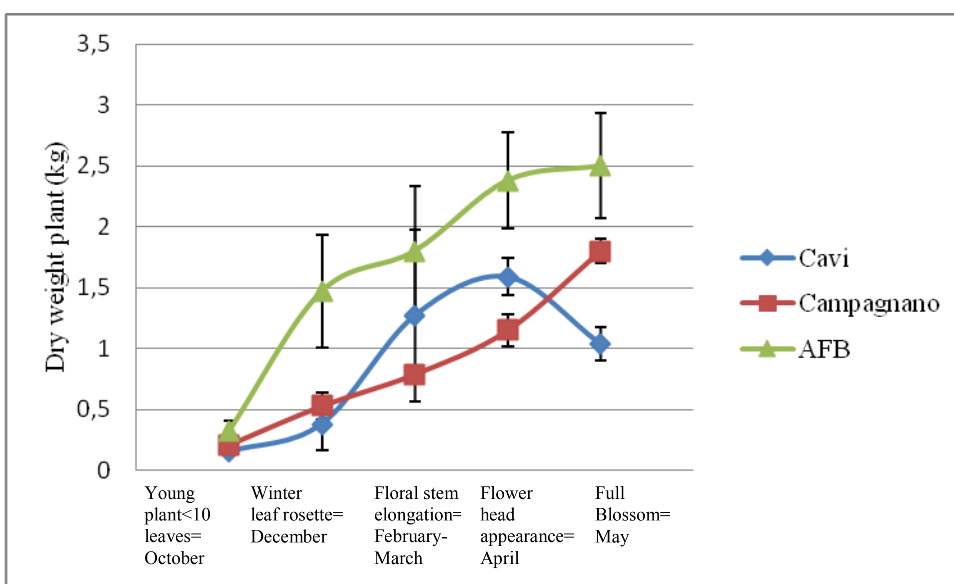


Fig. 3.13 Kinetics of the dry weight of the plant for the genotypes analyzed. Values are the means of measurements on 3 plants ± SD

3.3.2 BIOCHEMICAL CHARACTERIZATION AND PHENOLIC COMPOUND KINETICS EVALUATION

2009-2010 GROWING SEASON

In the present study biomass (leaves and floral stems) phenolic content of two genotypes (AFB and Cavi) at different developmental stages has been investigated in order to evaluate phenolic compound accumulation kinetics in plants grown in open field and individuate the plant physiological stage of maximum polyphenol accumulation.

Polyphenols such as chlorogenic acid, 1,5-*O*-dicaffeoylquinic acid, luteolin, cynaroside and apigenin were identified using HPLC analysis while the content of total polyphenols was determine by Folin-Ciocalteu method. The biomass biocompound content of the genotypes analyzed, expressed as mg per g of DM, is shown in Table 3.2. Taxyfolin, silybin and cynarin have not been detected in all samples collected in the two growing seasons and analyzed using HPLC. As regards chlorogenic acid, in AFB biomass the highest content was found in April while in Cavi biomass in October (*Table 3.2*). The highest 1,5-*O*-dicaffeoylquinic acid content was found in October and April in AFB biomass while in Cavi this biocompound was detected only in October. For Cavi genotype, the maximum cynaroside content resulted in April while in AFB genotype this compound has not been detected. Both luteolin and apigenin have not been detected in Cavi samples for all sampling times. In AFB biomass, luteolin has not been detected in April while there were not significant differences for the biocompound content between October and February sampling times. As regards apigenin, in AFB biomass this compound has not been detected in April samples while no significant differences for the apigenin content were found for the other sampling times (October and February).
Results obtained using Folin-Ciocalteu method showed that there were no significant differences in AFB biomass polyphenol content among different sampling times while in Cavi biomass the highest content was found in October. In general, AFB cardoon genotype provided the highest contents of 1,5-*O*-dicaffeoylquinic acid, luteolin, apigenin and total polyphenols while Cavi showed the best contents of chlorogenic acid and cynaroside (*Table 3.2*).

Table 3.2 Polyphenol content of AFB and Cavi genotype biomass extracts at different physiological stages (2009-2010 growing season)

Genotypes	**Physiological stage**	**chlorogenic acid mg/g DM**	**1,5–*O*-dicaffeoylquinic acid mg/g DM**	**cynaroside mg/g DM**	**luteolin mg/g DM**	**apigenin mg/g DM**	**Total polyphenols mg GAE/ g DM**
AFB	October = young plant	0.29±0.11	17.10±3.28	nd	1.76±0.12	0.19±0.04	31.30±18.89
AFB	February/ March = floral stem elongation	0.32±0.12	0.49±0.69	nd	1.12±0.55	0.14±0.04	35.13±4.30
AFB	April = head appearance	2.20±0.18	19.40±2.86	nd	nd	nd	39.81±4.24
Cavi	October = young plant	6.73±0.62	5.05±1.88	4.48±0.72	nd	nd	35.48±0.51
Cavi	February/ March = floral stem elongation	0.50±0.11	nd	1.75±0.31	nd	nd	12.59±1.99
Cavi	April = head appearance	0.71±0.18	nd	6.18±0.68	nd	nd	30.60±2.06

In order to evaluate the polyphenol yield obtained from globe artichoke and cardoon biomass, the biocompound accumulation in plant grown in open field has been estimated taking into account the phenolic content (expressed in mg per g DM) and the plant dry weight (expressed in g per plant) at different developmental stage. In Figure 3.14 is reported the total polyphenols kinetics in AFB and Cavi biomass during the first growing season. Results obtained using Folin-Ciocalteu method showed a highest total polyphenol accumulation in spring than in autumn-winter. This could be linked to the different composition of solar radiation during the year.

AFB cultivated cardoon provided the highest yield of total polyphenols, expressed as g GAE per plant, at all development stages compared to Cavi. In April, polyphenol biomass yield obtained from AFB and Cavi was 93.55 ± 9.56 g per plant and 30.57 ± 2.06 g per plant, respectively.

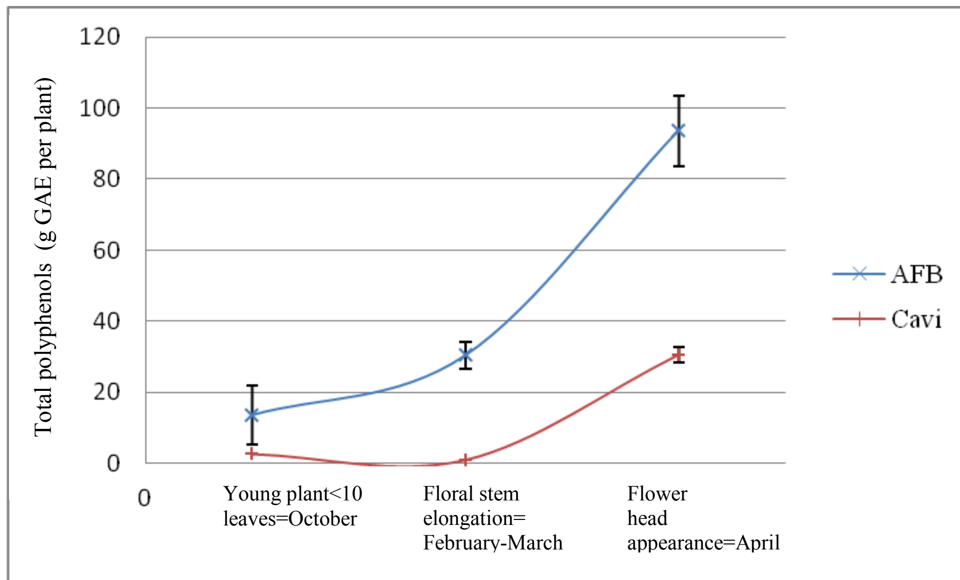


Fig. 3.14 Kinetics of total polyphenols (expressed as g GAE per plant) of AFB and Cavi genotype biomass during the 2009-2010 growing season

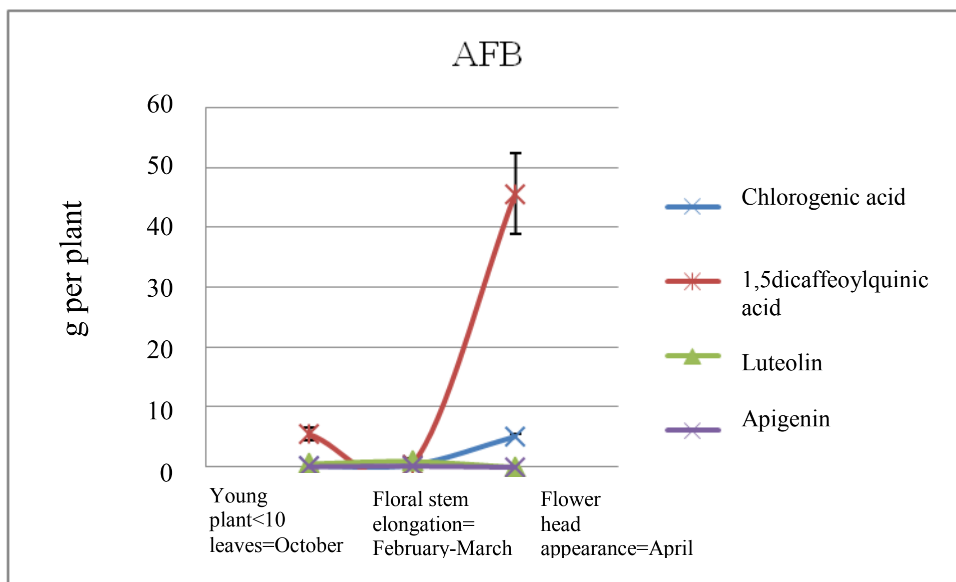


Fig. 3.15 Kinetics of chlorogenic acid, 1,5-O-dicaffeoylquinic acid, luteolin and apigenin (expressed as g per plant) of AFB cardoon genotype biomass during the 2009-2010 growing season

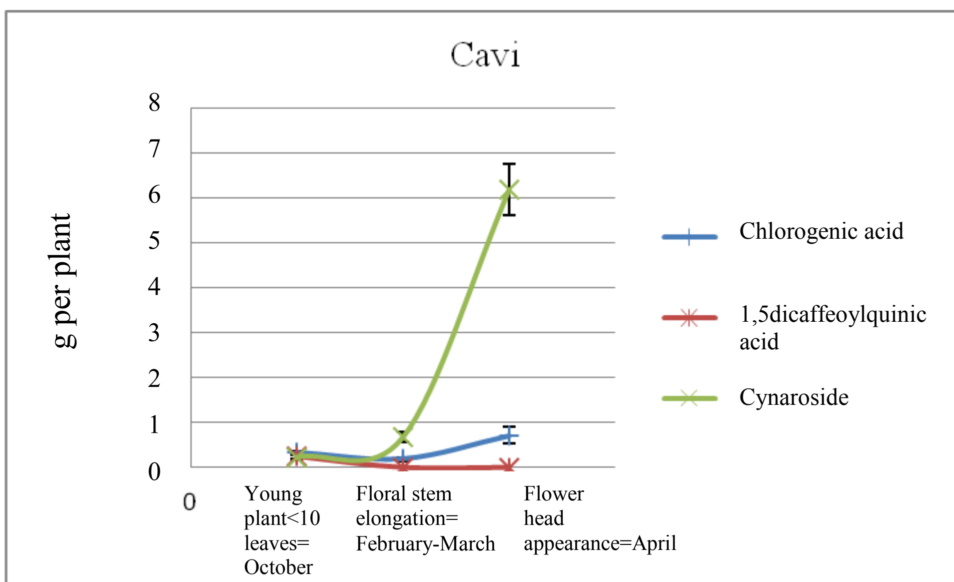


Fig. 3.16 Kinetics of chlorogenic acid, 1,5-O-dicaffeoylquinic acid, luteolin and apigenin (expressed as g per plant) of Cavi globe artichoke genotype biomass during the 2009-2010 growing season

Also concerning individual phenolic compound yield investigated, remarkable differences between genotypes (AFB and Cavi) and sampling times (October, February and April) were found (*Figures 3.15* and *3.16*). As for total polyphenols, the highest biocompound yields were obtained in April when the plant dry weight was highest except for apigenin and luteolin in AFB and for 1,5-*O*-dicaffeoylquinic acid in Cavi. It is interesting to note that AFB provided highest yield of 1,5-*O*-dicaffeoylquinic acid, chlorogenic acid, luteolin and apigenin while Cavi showed the highest cynaroside yield. As previously observed in agro-morphological characterization, AFB cardoon presented the highest genetic variability (represented by standard deviation) which may depend on the high degree of heterozygosity due to the use of seed coming from only two self-pollinations.

2010-2011 GROWING SEASON

The biomass (leaves and floral stems) phenolic content has been evaluated for three genotypes (AFB, Cavi and Campagnano) at different developmental stages during the second growing season.

The biomass phenolic content of the genotypes considered, expressed as mg per g of DM, is reported in Table 3.3.

As regards AFB biomass, the highest 1,5-*O*-dicaffeoylquinic acid and chlorogenic acid content was found in April while in Cavi biomass in October (*Table 3.3*). The highest 1,5-*O*-dicaffeoylquinic acid content was found in October and April in AFB biomass

while cynaroside content resulted highest both in March and April. Apigenin has not been detected in AFB samples for all sampling times considered while luteolin has been found only in October. In general, biocompound content increases from October to December, then decreases from December to March and increases again from March to April (*Table 3.3*).

For Cavi biomass, both apigenin and luteolin have not been detected in all samples. As regards 1,5-*O*-dicaffeoylquinic acid, the highest content was found in October while for chlorogenic acid and cynaroside in April. In Cavi, biocompound content decreases from October to December and increases from December to April (*Table 3.4*).

In Campagnano biomass, luteolin and apigenin have not been detected in all sampling times. The highest content of 1,5-*O*-dicaffeoylquinic and chlorogenic acid was found in October while cynaroside content resulted highest in December and in April. From April to May, there is a rapid decrease in chlorogenic acid and cynaroside content and an unchanged content of 1,5-*O*-dicaffeoylquinic acid (*Table 3.5*).

In general, AFB cardoon genotype provided the highest contents of 1,5-*O*-dicaffeoylquinic acid, luteolin and cynaroside while Campagnano showed the best content of chlorogenic acid.

Table 3.3 Polyphenol content of AFB genotype biomass extracts at different physiological stages (2010-2011 growing season)

AFB	1,5-*O*-dicaffeoylquinic acid mg/g DM	chlorogenic acid mg/g DM	apigenin mg/g DM	cynaroside mg/g DM	luteolin mg/g DM
October = young plant	15.44±2.90	12.63±3.16	nd	3.69±0.69	0.06±0.15
December = winter leaf rosette	12.69±3.34	24.65±2.86	nd	6.48±1.45	nd
February/March = floral stem elongation	7.64±3.38	18.88±1.34	nd	12.25±0.52	nd
April = head appearance	22.02±3.59	32.47±1.61	nd	11.24±1.15	nd
May = full blossom	1.53±0.11	2.34±0.19	nd	2.56±0.14	nd

Table 3.4 Polyphenol content of Cavi genotype biomass extracts at different physiological stages (2010-2011 growing season)

Cavi	1,5-*O*-dicaffeoylquinic acid mg/g DM	chlorogenic acid mg/g DM	apigenin mg/g DM	cynaroside mg/g DM	luteolin mg/g DM
October = young plant	2.63±0.99	12.36±3.82	nd	4.08±1.48	nd
December = winter leaf rosette	0.91±0.21	10.59±0.55	nd	6.92±0.19	nd
February/March = floral stem elongation	1.37±0.15	18.03±5.29	nd	7.22±1.39	nd
April = head appearance	1.42±0.29	26.49±0.67	nd	10.75±0.49	nd

Table 3.5 Polyphenol content of Campagnano genotype biomass extracts at different physiological stages (2010-2011 growing season)

Campagnano	1,5-*O*-dicaffeoylquinic acid mg/g DM	chlorogenic acid mg/g DM	apigenin mg/g DM	cynaroside mg/g DM	luteolin mg/g DM
October = young plant	5.94±1.38	34.56±0.84	nd	5.77±0.80	nd
December = winter leaf rosette	1.66±0.26	23.82±4.09	nd	10.66±1.21	nd
April = head appearance	2.17±0.72	24.00±3.30	nd	10.19±0.51	nd
May = full blossom	2.23±0.39	14.27±0.87	nd	4.12±1.88	nd

DM : Dry matter

Also in the second growing season, the biocompound yield obtained from globe artichoke and cardoon biomass has been evaluated taking into account the phenolic content (expressed in mg per g DM) and the plant dry weight (expressed in g per plant) at different developmental stage.

In Figures 3.17, 3.18 and 3.19 is shown the biocompound kinetics in AFB, Cavi and Campagnano biomass during the second growing season, respectively.

In AFB cultivated cardoon the 1,5-*O*-dicaffeoylquinic acid and chlorogenic acid yields followed the same trend. Indeed, these molecule contents showed an increase from October to December, then a slight decrease from December to March, from March to April there was a strong increase and from April to May there was a rapid decrease. As regards cynaroside yield, a positive trend from October to April was found while from April to May there was a strong decrease. On the basis of these observations, April resulted for AFB the best sampling time to obtain the highest biocompound yield. Indeed 1,5-*O*-dicaffeoylquinic acid, chlorogenic acid and cynaroside yields obtained in April from AFB plants were 534.78 ± 87.19, 788.56 ± 39.21 and 272.89 ± 27.88 g per plant, respectively. In AFB plants, luteolin has been detected only in October with a yield of 0.05 ± 0.02 g per plant (*Figure 3.17*).
As regards phenolic kinetics in Cavi biomass, it is interesting to observe that there was a positive trend from October to April for all biocompound detected. In April, the 1,5-*O*-dicaffeoylquinic acid, chlorogenic acid and cynaroside yield resulted 2.25 ± 0.47, 42.03 ± 1.07 and 17.06 ± 0.77 g per plant, respectively (*Figure 3.18*).
In Campagnano biomass there was a positive chlorogenic acid and cynaroside yield trend from October to April. Also, for 1,5-*O*-dicaffeoylquinic acid there was a slight positive yield trend from October to April. On the contrary, there was a strong decrease for all biocompound detected yield from April to May (*Figure 3.19*).
Results obtained in our experiments showed that AFB cardoon provided in both growing seasons the highest biocompound yield respect to globe artichoke genotypes analyzed. In both growing seasons, April resulted the best biomass harvesting time in open field experiments to obtain the best biocompound yield.

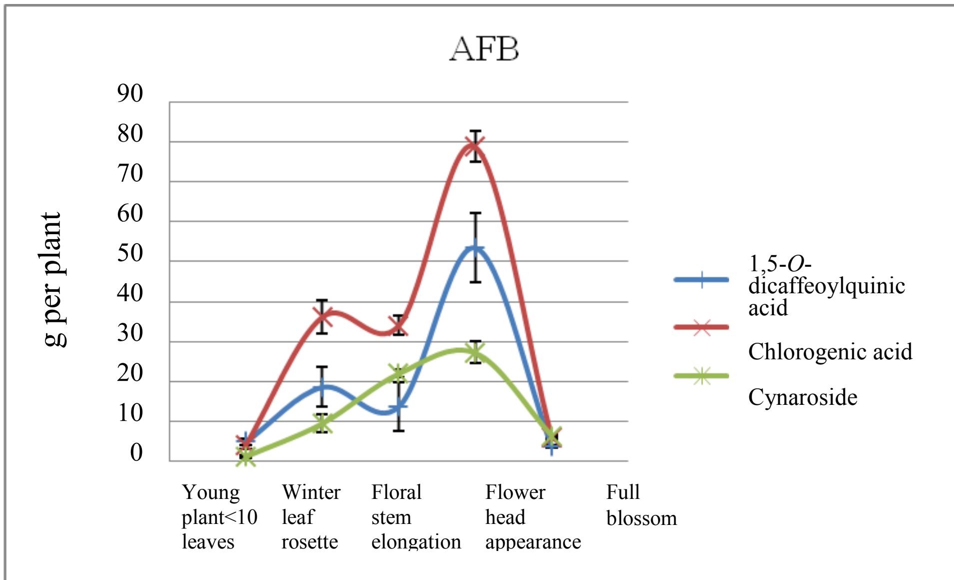


Fig. 3.17 Kinetics of 1,5-O-dicaffeoylquinic acid, chlorogenic acid and cynaroside (expressed as g per plant) of AFB cardoon genotype biomass during the 2010-2011 growing season

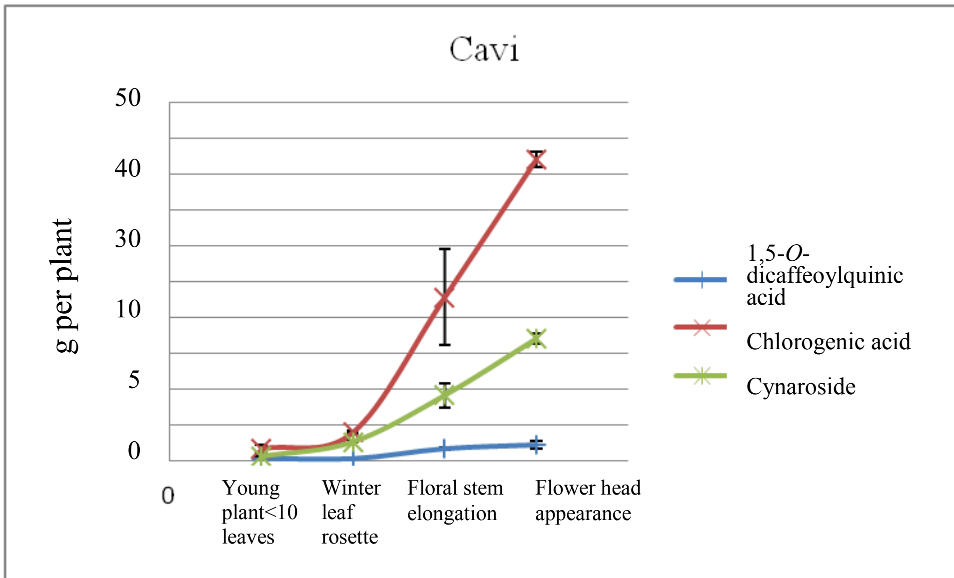


Fig. 3.18 Kinetics of 1,5-O-dicaffeoylquinic acid, chlorogenic acid and cynaroside (expressed as g per plant) of Cavi globe artichoke genotype biomass during the 2010-2011 growing season

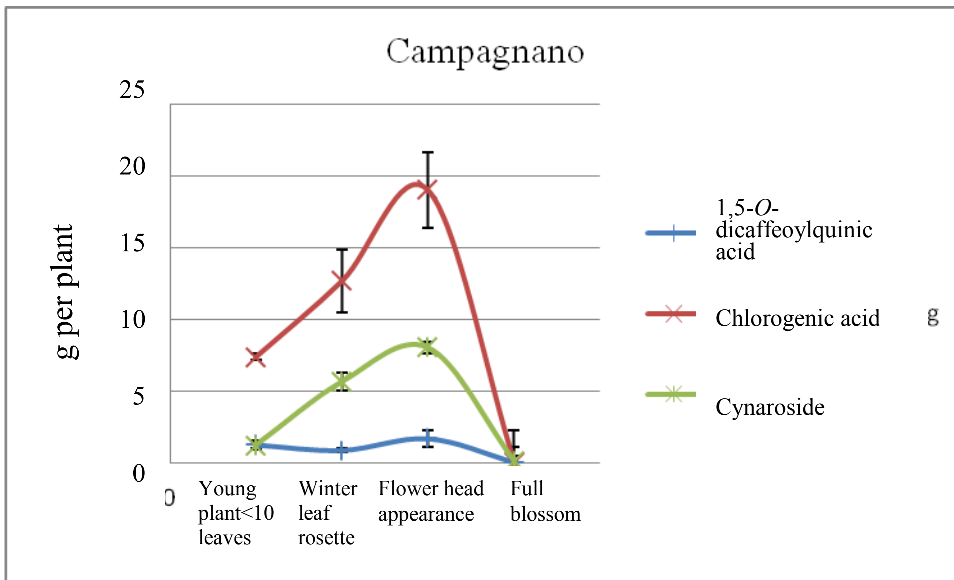


Fig. 3.19 Kinetics of 1,5-O-dicaffeoylquinic acid, chlorogenic acid and cynaroside (expressed as g per plant) of Campagnano globe artichoke genotype biomass during the 2010-2011 growing season

3.4 DISCUSSION

In the last years, European policies of energy, agriculture and environment have led to a growing interest for biomass crops and also for *Cynara cardunculus* (Fernández *et al.,* 2007). Several works on the use of *Cynara* spp. biomass principally for energy and pharmaceutical purposes and other applications have been conducted (Foti *et al.,* 1999; Falleh *et al.,* 2008; Pandino *et al.,* 2010, 2011c). The development cycle and the main plant growth stages of the species in Mediterannean environments have been widely described as follows: plant sprouting in September-October, winter leaf rosette in November, stem elongation in April-May, full blossom in June, ripe fruits in July, and fully dry aerial biomass in August (Raccuia and Melilli, 2007, 2010). Also the *Cynara* spp. aboveground and root biomass yields have been considered by other authors (Foti *et al.,* 1999; Raccuia and Melilli, 2007; Mantineo *et al.,* 2009; Gominho *et al.,* 2011; Lahoz *et al.,* 2011) but the complementarity of agromorphological and biochemical data are lacking in literature. In particular, the kinetics of plant agromorphological and biochemical traits during the growing season has been considered in the present study in order to provide useful informations to extraction industry on the stage of the maximum biomass and biocompound accumulation in open field.

The agro-morphological traits considered resulted very useful to clearly characterize our genotypes. In particular, significant differences among genotypes have been found for all traits considered starting from March. In agreement with Raccuia and Melilli (2010),

in the first part of the growth cycle (autumn-winter) the dynamics of biomass accumulation and plant development were similar in all genotypes considered while significant differences were found after the winter rest period. In spring, the influence of genotype on agromorphological profile and biomass accumulation was very clear (Raccuia and Melilli, 2010). In general, in both growing seasons AFB cultivated cardoon has been found very interesting for all agro-morphological traits evaluated respect to globe artichoke genotypes (Campagnano and Cavi). This is in line with Foti *et al.* (1999) which found some cultivated cardoon genotypes more interesting in the aboveground biomass yield respect to globe artichoke genotypes. The aboveground dry biomass, expressed as kg per plant provided useful information to describe plant vigor in agreement with previous works (Fernández *et al.*, 2006; Raccuia and Melilli, 2007, 2010; Angelini *et al.,* 2009; Ierna and Mauromicale, 2010; Gominho *et al.,* 2011). In line with other authors (Raccuia and Melilli, 2004, 2010; Gominho *et al.,* 2011), the biomass yield obtained from *Cynara* spp. genotypes in the two growing seasons resulted very high. In particular, the highest biomass yield was obtained in April in the first growing season and in April for Cavi and May for AFB and Campagnano in the second growing season. In particular, in the first growing season the dry weight per plant obtained in April was 1.00 ± 0.66 kg DM for Cavi, 1.03 ± 0.37 kg DM for Campagnano and 2.35 ± 1.01 kg DM for AFB; while in the second growing season resulted 1.04 ± 0.14 kg DM in April for Cavi, and in May 1.8 ± 0.10 kg DM for Campagnano and 2.5 ± 0.43 kg DM for AFB.

In both growing seasons, the biochemical characterization showed significant differences among genotypes under this profile. Among the biocompounds analysed, the caffeoylquinic acids, i.e. chlorogenic acid and 1,5-*O*-dicaffeoylquinic acid, and the luteolin derivative, i.e. cynaroside, were found predominants as reported by several authors (Lattanzio *et al.,* 2009; Lombardo *et al.,* 2010; Pandino *et al.,* 2010, 2011c). In particular in AFB biomass extracts, chlorogenic acid (ranged from 0.27 to 2.20 mg g^{-1} DM in 2009/2010 and from 2.34 to 32.47 mg g^{-1} DM in 2010/2011) and 1,5-*O*-dicaffeoylquinic acid (ranged from 0.49 to 19.40 mg g^{-1} DM in 2009/2010 and from 1.53 to 22.02 mg g^{-1} DM in 2010/2011) are the most abundant compounds. Also in Cavi and Campagnano biomass extracts, the choloregenic acid (range in Cavi extracts from 0.49 to 6.73 mg g^{-1} DM 2009/2010 and from 10.59 to 26.49 mg g^{-1} DM in 2010/2011 while for Campagnano range from 14.27 to 34.56 mg g^{-1} DM in 2010/2011) and 1,5-*O*-dicaffeoylquinic acid (in Cavi extracts in October 5.05 mg g^{-1} DM 2009/2010 and from

0.91 to 2.63 mg g^{-1} DM in 2010/2011 while for Campagnano range from 1.66 to 5.94 mg g^{-1} DM in 2010/2011) are predominants as well as cynaroside (in Cavi extracts range from 1.75 to 6.18 mg g^{-1} DM in 2009/2010 and from 4.08 to 10.75 mg g^{-1} DM in 2010/2011 while for Campagnano in 2010/2011 range from 4.12 to 10.66 mg g^{-1} DM). On the contrary, the 1,3-*O*-dicaffeoylquinic acid (cynarin) has not been detected in all samples analysed and this could be related to the isomerization of this compound to 3,5- or 1,5-*O*-dicaffeoylquinic acid as reported by Schütz *et al.* (2004).

The flavonoids apigenin and luteolin have not been detected in all extracts of the two growing seasons except in October and February 2009-2010 in AFB biomass samples. This is in line with previous studies which have not detected luteolin and apigenin in some globe atichoke and cardoon genotypes (Lombardo *et al*., 2010; Pandino *et al.,* 2011c). Indeed, phenolic profile is strongly affected by genotype variability as well as by environmental conditions (Pandino *et al.,* 2010). It is interesting to notice that the phenolic content was generally higher in the second experimental year than the biocompound content obtained in the first growing season and this may depend on the different climatic conditions as also observed by Lombardo *et al.* (2010).

The variation of phenolic profile in relation to harvest time has been also evaluated in AFB cardoon and Cavi globe artichoke genotypes for two years while for Campagnano only in the second growing season starting from October to April in 2009/2010 and from October to May in 2010/2011. In line with previous findings (Lombardo *et al.,* 2010), the phenolic content resulted highest in spring (April) respect to winter (December) sampling time for all genotypes considered. As regards autumn sampling time (October), in this period the content of some compounds was found higher than spring. In particular, in the first growing season the chlorogenic acid and 1,5-*O*-dicaffeoylquinic acid content of Cavi and in the second year the content of 1,5-*O*-dicaffeoylquinic acid of Cavi and Campagnano was found higher in October than in April.

Generally, considering a large-scale biomass production and taking into account the phenolic content (expressed in mg g^{-1} DM) and the plant dry weight (expressed in g per plant) at different developmental stage, the highest yield for the majority of biocompounds analyzed was found in April, at the flower head appearance. For this reason this physiological stage has been chosen in our experiments as the optimal time for biomass harvesting in open field conditions.

As regards root biomass accumulation, in line with Raccuia and Melilli (2010), no significant differences among genotypes were found in the first four sampling times (from October to March) of the first year; while only at the end of the growing season (in April) AFB provided the highest yield (FW 3.39 and DM 1.29 kg per plant) respect to Cavi genotype. The root biomass (expressed as kg of fresh and dry weight per plant) has been evaluated only at the end of the second growing season and was found significant different among genotypes and in particular AFB and Campagnano provided the highest value (FW 11.4 and DM 1.6 kg, FW 10.6 and DM 1.4 kg, respectively) compared to Cavi (FW 1.2 and DM 0.22 kg). In agreement with previous observations (Raccuia and Melilli, 2010), the root biomass yield was significantly higher in the second growing season than in the first one. Indeed, in the first year root development was very slow and most of the photoassimilates were used to leaf aerial apparatus growth while starting from the end of the first growing season and during the second year there was a predominated root growth and a high translocation of sucrose to underground storage organs where it is converted into inulin (Raccuia and Melilli, 2010).

Chapter 4

MORPHOLOGICAL ANALYSIS AND GERMPLASM SELECTION FOR BIOMASS AND BIOCOMPOUND PRODUCTION

4.1 INTRODUCTION

Since ancient time, *Cynara* spp. has been used in traditional medicine for its recognized therapeutic effects (i.e. hepatoprotective, anticarcinogenic, antioxidative, antibacterial, urinative, anticholesterol, glycaemia reduction) (Kraft *et al.,* 1997; Saénz Rodriguez *et al.,* 2002; Coinu *et al.,* 2007; Rondanelli *et al.,* 2011; Fantini *et al.,* 2011) linked principally to the high content of polyphenolic compounds, which include mono- and dicaffeoylquinic acids and flavonoids (Fratianni *et al.,* 2007; Lattanzio *et al.,* 2009; Lombardo *et al.,* 2010; Menin *et al.,* 2010; Pandino *et al.,* 2010, 2011a, 2011b, 2011c, 2012; Negro *et al.,* 2012). In particular, within the caffeic acid derivatives, chlorogenic acid (3-*O*-caffeoylquinic acid) is the most abundant component (Lattanzio *et al.,* 2009). Also the flavonoids apigenin and luteolin and their glycosides have been widely described in *Cynara* spp. All these compounds are strong antioxidants and protect low density lipoproteins from oxidative damages (Lattanzio *et al.,* 2009). In this regard, some studies have been done to analyze biochemically *Cynara* spp. germplasm suitable principally for fresh consumption or/and industrial processing of the heads (Fratianni *et al.,* 2007; Bonasia *et al.*, 2010; Lombardo *et al.,* 2010; Pandino *et al.*, 2010, 2011a, 2011b). In the last years, other possible applications of *Cynara* spp. alternative to the traditional ones were envisaged. Different types of products can be harvested and utilized to obtain i) oil from seeds (Foti *et al.*, 1999; Curt *et al.*, 2002; Raccuia and Melilli, 2007, 2011); ii) inulin from roots (Raccuia and Melilli, 2004, 2010); iii) energy from biomass (Raccuia and Melilli, 2007; Angelini *et al.*, 2009; Ierna and Mauromicale, 2010; Gominho *et al.*, 2011); iv) fiber for pulp and paper industry (Antunes *et al.*, 2000; Gominho *et al.*, 2001, 2009) and as potential reinforcement in polymer composites (Fiore *et al.*, 2011); v) green forage for ruminant feeding (Fernández *et al.*, 2006); vi) natural rennet for traditional cheese making (Fernández *et al.*, 2006; Galán *et al.*, 2008; Aquilanti *et al.*, 2011). Globe artichoke can also be used as crop for metal-accumulation (Hernández Allica *et al.*, 2008). These new possible uses of the crop are linked principally to the European Union research support on new agricultural byproducts (industrial raw materials) and have led to an increasing interest also in aboveground globe artichoke biomass. This interest is due mainly to the great adaptation of the crop to Mediterranean climate, characterized by low annual rainfalls and hot dry summer (Fernández *et al.*, 2006), to the relatively low crop energy input and to the large biomass productivity (Angelini *et al.*, 2009). Until now, several studies on globe artichoke as

energy crop have been done (Raccuia and Melilli, 2007; Angelini *et al.*, 2009; Ierna and Mauromicale, 2010; Gominho *et al.*, 2011), but there are only a few suggestions on the use of its biomass as raw industrial material to recover phenolic active compounds. In particular, not many data are available in the literature on the extraction from globe artichoke and cardoon biomass of active biocompounds which are of interest to the pharmaceutical industry.

Therefore in the present work, a sustainable production of *Cynara* spp. biomass and biocompound of interest for pharmaceutical industry have been evaluated and the possibility of using a such biomass, without upsetting traditional agricultural practices, has been also considered to allow possible increase of farmers‘ income. Taking into account these preliminary remarks, the objectives of the present PhD experiment aimed at:

i) evaluating biomass production of some Italian globe artichoke and cardoon genotypes;
ii) characterizing globe artichoke and cardoon aboveground biomass under biochemical profile;
iii) investigating the possibility for globe artichoke to obtain food and non-food production (heads for human consumption and biomass for pharmaceutical active compound extraction);
iv) selecting Italian globe artichoke and cardoon genotypes more suitable for this double purpose.

4.2 Globe artichoke: evaluation of biomass and biocompound production

4.2.1 Materials and methods

4.2.1.1 Experimental field and plant material

Seventeen Italian spring globe artichoke genotypes were considered (*Table 4.1*). Field trials were conducted for two years, during the 2008-2009 and 2009-2010 growing seasons, at the experimental station of ARSIAL (Latium Regional Agency for the Development and the Innovation of Agriculture) in Cerveteri, Rome - Italy (41° 59‘ N 12° 01‘ E). The station is characterized by a temperate climate, clay soil and average annual rainfall of 900 mm. All genotypes were vegetatively propagated by offshoots and were assessed in a completely randomized block experimental design with three replications. Each field plot consisted of 20 plants (planting density of 7,692 plants ha^{-1}, inter and intra-row distances of 1.30 and 1.00 m, respectively). The transplanting date

was 17th August 2007. Field experiments were conducted under low energy inputs (manual weeding, basic fertilization using N 100 kg ha^{-1}, P_2O_5 90 kg ha^{-1}, K_2O 125 kg ha^{-1}) for crop management.

Table 4.1 Globe artichoke spring genotypes evaluated

Genotypes	**Type**	**Donor Institute**	**Origin**
S2	Romanesco	Enea (RM)- Tuscia University (VT)	Latium, Italy
S3	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
S5	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
S11	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
S17	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
S18	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
Castellammare	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
S23	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
Campagnano	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
Grato 1	Romanesco	Enea (RM) - Tuscia University (VT)	Latium, Italy
Ascolano	Romanesco	CRA-ORA Monsampolo del Tronto (AP)	Marche, Italy
Jesino	Romanesco	CRA-ORA Monsampolo del Tronto (AP)	Marche, Italy
MonteluponeB	Romanesco	CRA-ORA Monsampolo del Tronto (AP)	Marche, Italy
MonteluponeA	Romanesco	CRA-ORA Monsampolo del Tronto (AP)	Marche, Italy
B. Pertosa	Romanesco	CRA-ORT Pontecagnano (SA)	Campania,Italy
TR. Paestum	Romanesco	CRA-ORT Pontecagnano (SA)	Campania,Italy
Pisa	Violetto	Pisa University (PI)	Tuscany, Italy

4.2.1.2 Morphological characterization

Morphological characterization was performed using thirteen UPOV descriptors established for globe artichoke (i.e. plant height, number of lateral shoots, floral stem diameter, first fully developed leaf length, leaf lobe number, central head weight and time of appearance, central head length along with diameter and shape index, primary head number along with weight and time of appearance) and eight complementary descriptors (*Table 4.2*) describing different components of the biomass production. A visual 0-3 rating as biomass index (BI; 0=not vigorous plant; 3=very vigorous plant) has been used to express the plant vigor. In order to evaluate quantitatively the biomass production, three plants per genotype were harvested after the primary head removal, and immediately weighed and oven-dried at 103°C, until a constant weight was reached to determine the dry matter content. In total, 21 morphological and agro-physiological

data were recorded, during the two growing seasons, on nine plants per genotype at the central and primary head harvest times.

Table 4.2 Morphological traits used for biomass characterization of globe artichoke spring genotypes and level of significance from the ANOVA analysis

Code	Descriptors	Genotypes	Year	Genotype*Year
PH	Plant height	*	*	*
LSh	Number of lateral shoots	ns	ns	*
FSD	Main floral stem diameter	*	*	*
Llength	First fully developed leaf length	*	*	*
Lwidth	First fully developed leaf width	*	ns	*
NSL	Main floral stem leaf number	*	*	*
LSL	Lateral shoot leaf number	*	ns	*
DL	Dry leaf number	*	ns	*
PD	Plant diameter	*	*	*
BI	Biomass index	*	*	*
Llob	Number of leaf lobes	*	*	*
PFW	Plant fresh weight	*	*	*
PDM	Plant dry weight	*	*	*

*ns = not significant; * significant differences per $p \leq 0.05$*

4.2.1.3 Phenolic compounds extraction and analysis

4.2.1.3.1 Sample preparation

A representative sample (300 g FW) of biomass (leaves and floral stems) was collected from nine plants per genotype at the primary head harvesting date in 2009/2010 growing season. Nine primary heads per genotype were also harvested at the same

sampling time. All samples were immediately weighed, freeze-dried and ground to 1 mm diameter fine powder. For each genotype, an amount (10 g three times replicated) of homogenized plant material (heads and biomass, separately) was oven-dried at 103°C until a constant weight was reached to determine the dry matter content.

4.2.1.3.2 Extraction procedure and analysis

Extraction and analysis of samples were performed as aforementioned in chapter 3. All extractions and analysis were performed in duplicate.

4.2.1.4 Statistical analyses

All data were analyzed by ANOVA with the Generalized Linear Model (GLM) procedure, Principal Component Analysis (PCA), Correlation and Cluster analyses using SPSS software version 15.0. Mean separations were performed by Duncan test. Significance was accepted per $p \leq 0.05$ level.

4.2.2 Results

4.2.2.1 Biometric characteristics

4.2.2.1.1 Biomass production

Thirteen agro-morphological traits were utilized to characterize biomass production of the seventeen genotypes. The ANOVA statistical significant differences related to each morphological trait among genotypes, between years and for genotype per year interaction have been reported in Table 4.2. Significant genotype per year interactions have been found for all traits, whereby the profile of morphological expression of each genotype varies between the years. The most relevant biometric parameters per each genotype are shown in Table 4.3. Significant differences among genotypes have been found for all traits evaluated, except for the number of lateral shoots in the 2008-2009 growing season. In the same growing season, Bianco di Pertosa showed the greatest plant height while, in 2009-2010, Bianco di Pertosa, S5, S18, Campagnano and Pisa revealed the highest value for this trait. Only in the second experimental year, Tondo Rosso di Paestum and Bianco di Pertosa had the highest number of lateral shoots, while no significant differences have been detected among genotypes in the first year. In 2008-2009, Ascolano, S18, Grato 1, S5 and Campagnano had the widest first fully developed leaf of the main floral stem; the last three genotypes confirmed their behavior also in the subsequent year but, in addition, S2, S11 and Castellammare revealed

interesting values for the same trait. In both growing seasons, Montelupone B had the highest number of leaves, while Pisa has been added to this genotype in the second year. In 2008-2009, Campagnano and S18 and, in 2009-2010, Campagnano, S18, Ascolano, Bianco di Pertosa and Pisa showed the highest plant diameter. A biomass index (BI) was determined by the visual 0-3 rating scale and was used to express plant vigor. The genotypes S18, Campagnano, Bianco di Pertosa and Pisa, in 2008-2009, and Bianco di Pertosa and Pisa, in 2009-2010, showed the highest values for this trait. For dry weight of the whole plant (leaves and floral stem), only Ascolano confirmed the highest value in both years; S18, Campagnano, Pisa and Bianco di Pertosa provided interesting values only in the first year. In general, it is interesting to notice that S5, S18, Ascolano, Bianco di Pertosa, Campagnano and Pisa were the most interesting genotypes in term of biomass yield among the other parameters studied in both growing seasons.

Table 4.3 Biomass characterization of the globe artichoke spring genotypes in 2008-2009 (2009) and 2009-2010 (2010) growing seasons

Descriptors	**PH (cm)**		**LSh (no.)**		**Llength (cm)**		**Lwidth (cm)**		**NSL (no.)**		**PD (cm)**		**BI (no.)**		**PDM (kg)**	
Genotypes	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
S11	69.1 bc	76.2 cd	2.5 ns	2.7 ef	99.1 ab	91.8 bc	37.8 cd	47.8 ab	10.9 fg	12.7 bc	167.3 cd	165.1 b	1.7 gh	2.3 b	1.3 b	1.4 de
S17	71.7 bc	74.0 cd	3.1 ns	2.8 de	108.3 a	78.8 d	39.7 cd	32.0 ef	11.0 ef	8.1 hi	178.4 bc	152.7 de	1.8 fg	0.6 h	0.5 d	0.5 g
S18	79.0 b	89.3 ab	2.7 ns	2.9 de	104.4 ab	102.5 ab	46.9 ab	35.4 de	13.1 cd	13.0 bc	201.8 ab	173.1 ab	2.8 ab	2.2 b	1.9 a	1.4 de
S2	74.1 bc	77.3 cd	3.0 ns	2.5 fg	94.1 ab	99.1 ab	43.8 bc	45.5 ab	11.8 de	13.5 bc	178.5 bc	167.4 b	1.7 gh	2.0 bc	1.3 b	1.4 de
Campagnano	77.5 bc	90.3 ab	2.7 ns	3.0 cd	105.3 ab	110.7 a	53.8 a	48.0 ab	12.5 de	12.4 bc	215.1 a	174.9 ab	3.0 a	2.3 b	1.8 a	1.8 b
Grato 1	75.9 bc	82.5 bc	2.7 ns	3.4 bc	98.9 ab	88.0 cd	50.2 ab	36.5 cd	11.1 ef	9.4 fg	188.1 bc	159.1 bc	2.4 bc	0.9 gh	0.7 c	0.8 f
Castellammare	77.3 bc	77.8 cd	2.9 ns	3.2 bc	87.7 c	104.1 ab	42.0 bc	52.8 a	9.4 h	8.5 hi	189.0 bc	169.8 b	2.5 bc	1.1 fg	0.9 bc	1.1 ef
S23	54.9 h	68.5 de	2.7 ns	3.5 bc	98.8 ab	88.2 cd	43.7 bc	35.1 de	10.2 gh	7.2 i	181.0 bc	166.8 b	2.2 de	0.7 h	0.8 bc	0.7 f
S3	63.9 de	77.8 cd	2.8 ns	2.9 de	94.9 ab	94.7 bc	40.1 cd	40.7 bc	11.4 de	11.4 de	172.1 cd	161.7 bc	1.8 fg	1.4 ef	1.0 bc	1.2 ef
S5	60.9 ef	89.5 ab	3.7 ns	2.2 g	92.2 bc	104.9 ab	46.2 ab	49.4 ab	11.9 de	13.2 bc	179.5 bc	169.5 b	2.3 cd	2.0 bc	1.1 bc	1.5 cd
Ascolano	67.0 cd	74.3 cd	3.3 ns	3.2 bc	96.3 ab	95.3 bc	50.3 ab	54.3 a	12.5 de	12.2 bc	184.5 bc	176.7 ab	1.8 fg	1.9 bc	1.9 a	2.5 a
Jesino	58.5 fg	62.7 e	2.8 ns	2.8 de	89.2 c	65.5 e	31.7 ef	31.0 fg	12.8 cd	11.7 cd	161.0 e	161.8 bc	1.7 gh	1.6 de	0.9 bc	0.8 f
MonteluponeA	56.3 gh	63.3 e	3.5 ns	2.7 ef	86.7 d	98.8 ab	41.0 bc	41.3 bc	14.3 bc	12.8 bc	162.7 de	159.5 bc	2.0 ef	2.0 bc	1.3 b	1.6 bc
MonteluponeB	70.4 bc	68.5 de	3.2 ns	3.0 cd	91.0 bc	51.7 f	28.8 f	27.7 g	17.2 a	18.0 a	155.0 f	143.7 e	2.0 ef	1.7 cd	1.0 bc	1.0 ef
Bianco Pertosa	110.5 a	107.0 a	3.3 ns	3.7 ab	98.5 ab	102.0 ab	40.0 cd	43.3 bc	12.7 cd	10.8 ef	184.8 bc	184.3 a	2.7 ab	2.7 a	1.8 a	1.6 bc
Pisa	68.0 bc	91.5 ab	3.2 ns	3.3 bc	102.0 ab	103.2 ab	32.2 de	37.0 cd	15.7 ab	14.0 b	185.8 bc	180.3 ab	2.8 ab	2.7 a	2.0 a	1.7 bc
TR. Paestum	66.6 cd	70.8 de	3.0 ns	4.2 a	94.4 ab	98.0 ab	30.2 f	36.3 cd	9.2 i	9.0 gh	155.2 f	153.7 cd	1.4 h	1.2 fg	1.0 bc	1.2 ef

Values are the means of measurements on 9 plants. Means followed by different letters for each parameter are significantly different per $p \leq 0.05$ (Duncan test)

A similarity dendrogram, based on these morphological traits and describing plant biomass, was constructed using an agglomerative hierarchical cluster analysis (*Figure 4.1*). On the basis of the similarity dendrogram, the genotypes could be classified into five major clusters. Cluster 1 consisted of six genotypes: S3, S11, S17, S23, Montelupone A and Tondo Rosso di Paestum. Cluster 2 consisted of S2, S5, Grato 1, Castellammare and Ascolano. In cluster 3, S18, Pisa and Campagnano were grouped, while cluster 4 consisted of Jesino and Montelupone B. Finally, only Bianco di Pertosa was grouped in cluster 5.

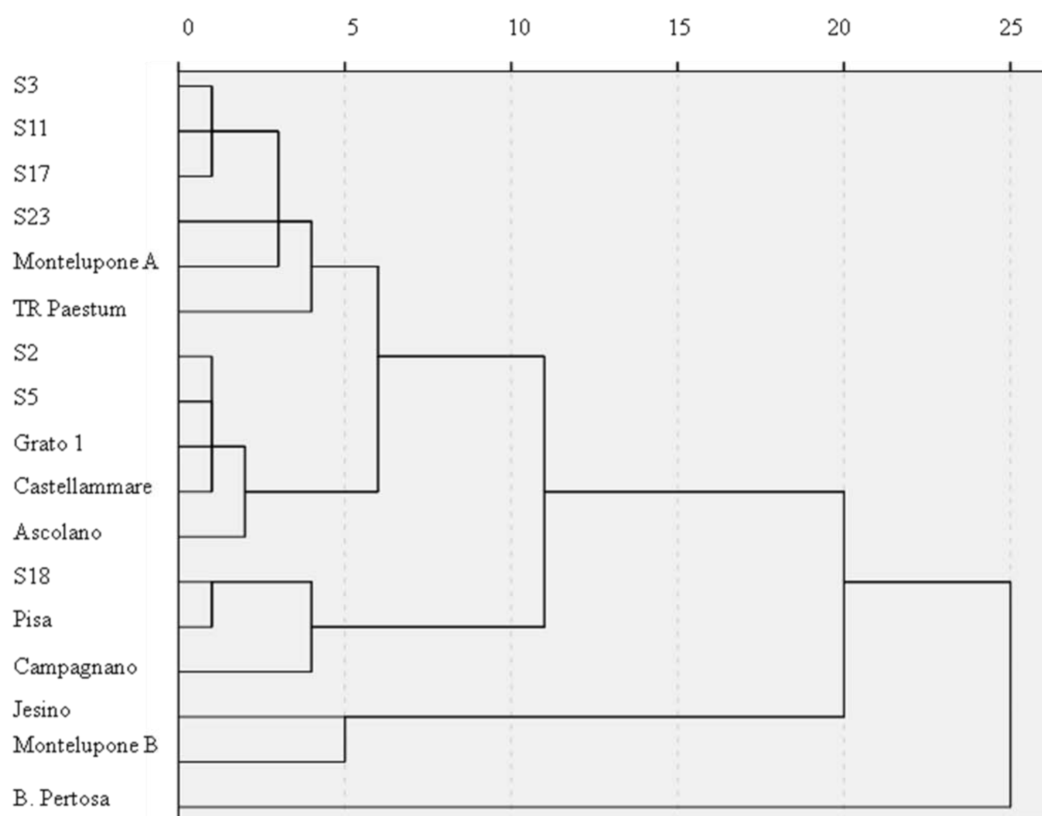


Fig. 4.1 Similarity dendrogram constructed on the morphological biomass characterization of the seventeen genotypes analyzed using agglomerative hierarchical cluster analysis

An among-genotype pairwise similarity matrix was generated with the quantitative and qualitative morphological traits explaining biomass production for the two growing seasons and a PCA was then applied. The first PC factor (39.75% of variance explained) included contributions from the following traits: plant height, floral stem diameter, first fully developed leaf length and width, number of shoot leaves, plant diameter, biomass index and plant dry weight. The second factor (24.35% of the variance explained) involved the main floral stem and dry leaf number. The third factor

(15.97% of variance explained) considered the number of lateral shoots. These first three functions all together explained 80.01% of the variance and each genotype was plotted against these three functions (*Figure 4.2*). PCA showed that four groups could be identified: one on the upper right side with the genotypes S18, Campagnano, Pisa, Bianco di Pertosa and Ascolano, one on lower right side with the genotypes Jesino and Montelupone B from Marche region, one on the lower left side including Tondo Rosso di Paestum, S17 and S23 genotypes and the last one, on the center of the graphic, with all other genotypes.

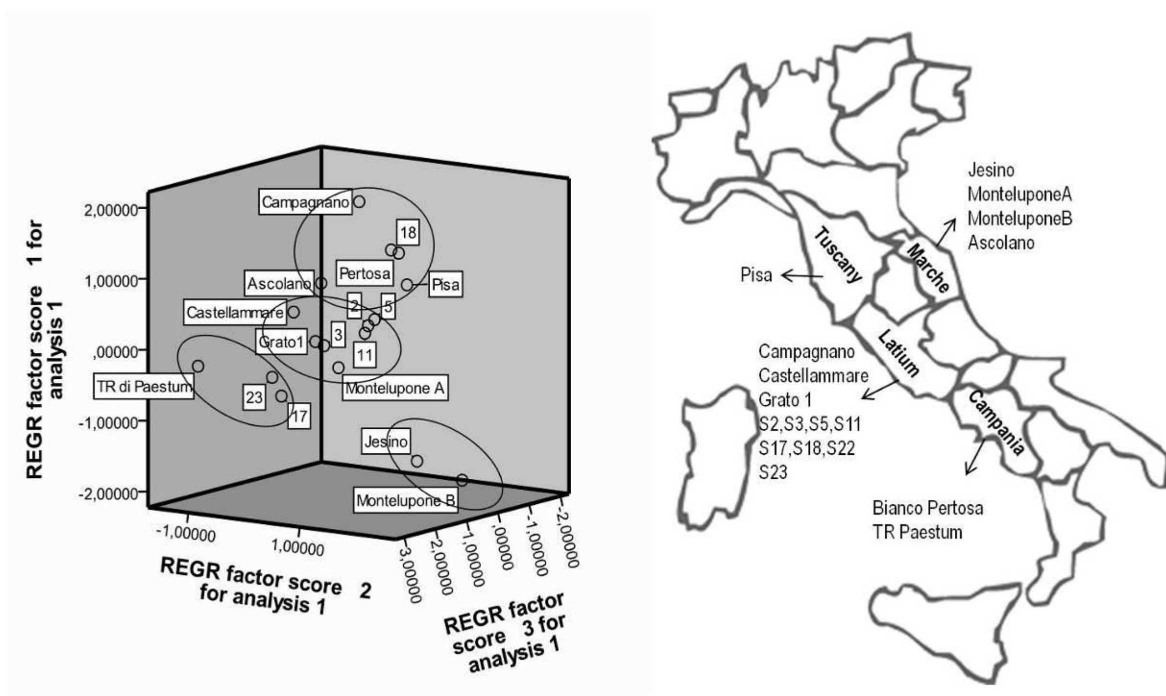


Fig. 4.2 Distribution against the three discriminant functions (on the left side) and Italian growing areas (on the right side) of the seventeen globe artichoke spring genotypes analyzed for biomass production

A correlation matrix among the morphological traits analyzed was generated (*Table 4.4*). Plant dry weight was strongly correlated to the biomass index, while biomass index was positively related to the plant dry weight obviously along with plant height and diameter, and to dry leaf number.

Table 4.4 Correlation matrix among some morphological traits evaluated for biomass production of globe artichoke spring genotypes

	PH	LSh	Llength	Lwidth	NSL	PD	BI	PDM
PH	1.000							
LSh	.228	1.000						
Llength	.519	.042	1.000					
Lwidth	.282	-.202	.635	1.000				
NSL	.041	-.132	-.330	-.244	1.000			
PD	.632	-.086	.781	.681	-.090	1.000		
BI	.645	-.023	.465	.281	.549	.687	1.000	
PDM	.413	.166	.484	.455	.437	.588	.764	1.000

Pearson correlation test ($p \leq 0.05$)

4.2.2.1.2 Edible production

Ten agro-morphological traits were measured in order to evaluate production of the seventeen genotypes studied at the main and primary head harvesting times. The ANOVA analysis results for all genotypes evaluated during the two growing seasons (2008-2009 and 2009-2010) along with the average values of the two years for each trait have been reported in Table 4.5.

Significant differences among genotypes were found for all biomass traits analyzed. As regard the central and the primary head yield, Grato 1, Ascolano, Tondo Rosso di Paestum and Bianco di Pertosa showed the highest average value of total head weight per plant. The genotypes Grato 1, Ascolano, Tondo Rosso di Paestum, Bianco di Pertosa, Campagnano and Pisa showed the highest number of primary heads. Ascolano, Pisa, Jesino, Montelupone A and Montelupone B had the highest value for the central flower head length, while S2, Castellammare, Grato 1 and Bianco di Pertosa had the biggest central flower head diameter. The length/diameter ratio of the central flower head was used as a representative shape index. The genotypes Pisa and Jesino had the most elongated heads, while Tondo Rosso di Paestum, Bianco di Pertosa, S2, S17, S18, Castellammare and S23 showed elliptical heads.

Table 4.5 Yield and morphological characterization of globe artichoke spring genotype capitula

Genotypes	Primary head (no.)	Central flower head length (cm)	Central flower head diameter (cm)	Head shape (L/D ratio)	Central flower head weight (g)	Central flower head harvesting (day no.)	Primary head weight (g)	Primary head harvesting date (day no.)	Total head (no.)	Total head weight (g)
S11	2.44 f	8.48 cg	9.55 bf	0.89 df	357.50 ad	189.11 bc	222.75 ad	197.16 cd	3.44 f	900.4 dg
S17	2.77 cf	7.31 hi	9.07 eh	0.81 gh	330.33 be	148.00 g	231.29 ad	164.03 g	3.77 cf	982.17 bf
S18	2.94 cf	7.79 fi	9.17 dg	0.85 eh	340.22 ad	183.70 bc	250.45 ab	192.25 d	3.94 cf	1079.96 bd
S2	2.77 cf	7.74 fi	9.95 ac	0.77 h	359.72 ad	191.38 b	241.25 ac	199.88 bc	3.83 cf	1040.50 bd
Campagnano	3.27 ac	8.08 dh	8.75 gi	0.92 de	311.92 ce	183.44 bc	229.85 ad	193.66 d	4.27 ac	1063.47 bd
Grato 1	3.16 ad	8.83 bd	9.87 ad	0.89 df	371.22 ab	175.44 d	257.24 a	185.20 e	4.16 ad	1193.75 a
Castellammare	2.55 ef	8.54 cf	10.48 a	0.82 fh	367.33 ab	167.61 ef	217.86 bd	176.92 f	3.55 ef	806.31 fg
S23	2.66 df	7.66 gi	9.66 be	0.79 h	332.92 be	162.00 f	241.45 ac	175.82 f	3.66 df	902.72 dg
S3	2.61 ef	8.28 cg	9.30 cg	0.89 df	317.75 be	187.05 bc	230.62 ad	197.69 cd	3.61 ef	919.81 cg
S5	2.66 df	8.68 ce	9.58 bf	0.90 de	345.66 ad	188.22 bc	203.06 cd	195.55 cd	3.66 df	881.52 eg
Ascolano	3.25 ac	9.06 ac	9.39 cg	0.96 cd	387.00 a	170.41 de	223.33 ad	178.00 f	4.25 ac	1121.25 ab
Jesino	2.83 cf	9.71 a	8.87 fi	1.09 ab	364.63 ac	185.33 bc	195.75 d	192.25 d	3.83 cf	887.75 eg
Montelupone A	3.08 be	9.59 ab	9.34 cg	1.03 bc	310.00 de	183.25 c	222.00 ad	193.00 d	4.08 be	1005.25 be
Montelupone B	3.09 be	8.99 ac	8.43 hi	1.06 b	327.18 be	202.63 a	135.45 e	206.36 a	4.09 be	753.45 g
Bianco Pertosa	3.50 ab	7.94 eh	10.17 ab	0.78 h	349.58 ad	185.50 bc	212.41 bd	193.33 d	4.50 ab	1102.25 ab
Pisa	3.27 ac	9.58 ab	8.29 i	1.16 a	287.09 e	202.00 a	140.63 e	204.09 ab	4.27 ac	739.18 g
TR Paestum	3.63 a	6.99 i	9.04 eh	0.77 h	366.63 ab	200.54 a	223.36 ad	203.81 ab	4.63 a	1221.54 a

Values are the means of measurements on 9 plants. Means followed by different letters for each parameter are significantly different per $p \leq 0.05$ (Duncan test)

4.2.2.2 BIOCHEMICAL CHARACTERISTICS

The biomass and head polyphenol contents of all genotypes, determined by HPLC analysis, are reported in Table 4.6. The biocompounds caffeic acid, taxifolin, silybin and cynarin were no detected in all head and leaf samples analyzed. As regard the other biocompounds, statistical differences among genotypes were found for the biochemical composition of biomass (leaves and floral stems). In particular, S5 and S3 genotypes showed the highest content of 1,5-*O*-dicaffeoylquinic acid, while S5, S17, S23, Ascolano, Campagnano and Castellammare, resulted the genotypes with the highest concentration of luteolin-7-*O*-glucoside. The genotypes S3, S5, S17, S18, S23, Ascolano, Campagnano and Montelupone A showed the highest content of 3-*O*-caffeoylquinic acid.

Also the *capitula* of all genotypes were analyzed biochemically to determine their nutraceutical value and statistical differences among genotypes have been found (*Table 4.6*). Bianco di Pertosa was characterized by the highest content of 1,5-*O*-dicaffeoylquinic acid, while Montelupone A showed the highest head content of luteolin-7-*O*-glucoside and Montelupone B heads provided the highest value of 3-*O*-caffeoylquinic acid.

Table 4.6 Phenolic (g·kg^{-1} of DM) contents of globe artichoke spring genotype biomasses (leaves and floral stem) and heads

Genotypes	**Biomass**					**Heads**				
	1,5-*O*-dicaffeoylquinic acid (g·kg^{-1} DM)	Chlorogenic acid (g·kg^{-1} DM)	Cynaroside (g·kg^{-1} DM)	Total caffeoyquinic acids (g·kg^{-1} DM)	Total measured polyphenols (g·kg^{-1} DM)	1,5-*O*-dicaffeoylquinic acid (g·kg^{-1} DM)	Chlorogenic acid (g·kg^{-1} DM)	Cynaroside (g·kg^{-1} DM)	Total caffeoyquinic acids (g·kg^{-1} DM)	Total measured polyphenols (g·kg^{-1} DM)
Ascolano	1.46 bc	22.41 a	8.93 a	23.87 a	32.80 a	9.24 c	10.89 c	0.01 g	20.13 a	20.14 bc
Campagnano	1.52 bc	18.99 ab	8.27 a	20.51 ab	28.78 ab	4.17 g	3.62 h	0.04 df	7.79 g	7.83 g
Grato 1	0.91 d	10.66 c	4.30 cd	11.57 c	15.87 c	5.99 ef	6.52 ef	0.03 ef	12.51 ef	12.54 ef
Jesino	0.66 df	1.86 d	2.99 de	2.52 d	5.51 d	9.94 c	12.53 b	0.13 b	22.47 b	22.60 b
Montelupone A	1.10 cd	16.83 ac	5.57 bc	17.93 ac	23.50 ac	8.27 cd	9.18 d	0.16 a	17.45 cd	17.61 cd
Montelupone B	0.80 de	1.60 d	2.16 ef	2.40 d	4.56 d	12.76 b	14.47 a	0.07 cd	27.23 a	27.30 a
Bianco Pertosa	0.34 eg	0.35 e	0.53 f	0.69 e	1.22 e	18.11 a	10.67 c	0.08 c	28.78 a	28.86 a
Pisa	0.28 eg	0.38 e	1.08 ef	0.66 e	1.74 e	6.26 ef	8.02 de	0.07 cd	14.28 e	14.35 e
S11	nd	0.64 e	1.17 ef	0.64 e	1.81 e	5.96 ef	5.93 fg	0.04 df	11.89 ef	11.93 ef
S17	1.56 bc	20.94 a	6.92 ab	22.50 a	29.42 ab	3.49 g	6.63 ef	0.04 df	10.12 fg	10.16 fg
S18	1.04 cd	18.96 ab	5.10 bc	20.00 ac	25.10 ac	6.60 df	7.32 ef	0.03 ef	13.92 e	13.95 e
S2	0.18 g	2.66 d	2.18 ef	2.84 d	5.02 d	6.44 df	7.00 ef	0.04 df	13.44 ef	13.48 ef
Castellammare	0.67 df	12.37 bc	6.86 ab	13.04 bc	19.90 bc	4.93 fg	6.69 ef	0.04 df	11.62 ef	11.66 ef
S23	1.51 bc	20.61 ab	8.35 a	22.12 a	30.47 ab	3.53 g	4.42 h	0.06 cf	7.95 g	8.01 g
S3	1.96 ab	17.75 ac	6.14 bc	19.71 ac	25.85 ac	7.05 de	6.74 ef	0.03 ef	13.79 e	13.82 e
S5	2.10 a	21.18 a	8.96 a	23.28 a	32.24 a	7.38 de	7.30 ef	0.06 cf	14.68 de	14.74 de
TR Paestum	nd	0.16 e	0.72 f	0.16 e	0.88 e	7.47 de	5.05 gh	0.02 fg	12.52 ef	12.54ef

nd= not detected
Values are the means of measurements on 9 plants. Means followed by different letters for each parameter are significantly different per p ≤ 0.05 (Duncan test)

For a large-scale of biocompound production in open field, the polyphenol yield per hectare and per genotype was estimated, considering the biomass dry matter accumulated per plant and at the planting density used of 7,692 plant·ha^{-1}. Ascolano and Campagnano showed the highest yield of luteolin-7-*O*-glucoside (176.24 and 153.14 kg·ha^{-1}), 1,5-*O*-dicaffeoylquinic acid (28.89 and 28.25 kg·ha^{-1}) and 3-*O*-caffeoylquinic acid (442.23 and 351.70 kg·ha^{-1}), while S5 was very interesting for 1,5-*O*-dicaffeoylquinic acid producing 31.29 kg·ha^{-1} (*Table 4.7*).

Table 4.7 Biocompound yield from globe artichoke spring genotype aerial biomass (planting density 7,692 plants·ha^{-1})

Genotype	**Cynaroside kg·ha^{-1}**	**Chlorogenic acid kg·ha^{-1}**	**1,5-*O*-dicaffeoylquinic acid kg·ha^{-1}**	**Total measured polyphenols kg·ha^{-1}**
Ascolano	176.24 a	442.23 a	28.89 b	647.36 a
Campagnano	153.14 b	351.70 b	28.25 b	533.09 ab
Grato 1	33.32 ef	82.59 h	7.01 g	122.92 d
Jesino	22.00 fg	13.70 l	4.84 i	40.54 e
Montelupone A	74.71 d	225.70 e	14.80 d	315.21 c
Montelupone B	22.44 fg	16.59 l	8.34 e	47.37 e
Bianco Pertosa	9.79 i	6.57 l	6.20 gh	22.56 e
Pisa	22.58 fg	7.94 l	5.86 h	36.38 e
S11	16.74 gi	9.15 l	nd	25.89 e
S17	36.79 f	111.22 g	8.27 ef	156.28 d
S18	71.89 d	267.13 d	14.72 d	353.74 c
S2	33.35 ef	40.66 i	2.81 l	76.82 de
Castellammare	74.53 d	134.31 f	7.26 fg	216.10 cd
S23	57.48 de	141.88 f	10.42 e	209.78 cd
S3	76.83 d	222.04 e	24.51 c	323.38 c
S5	133.32 c	314.95 c	31.29 a	479.56 b
TR Paestum	7.68 i	1.71 l	nd	9.39 f

nd= not detected
Means followed by different letters for each parameter are significantly different per $p \leq 0.05$ (Duncan test)

In order to determine the connection among the polyphenolic compounds analyzed, a correlation analysis was performed. The 1,5-*O*-dicaffeoylquinic acid and 3-*O*-caffeoylquinic acid contents were positively related both in the heads (Pearson‘s correlation coefficient $r=0.826$, $p \leq 0.05$) and in the biomass (Pearson‘s correlation coefficient $r=0.915$, $p \leq 0.05$). On the contrary, there was no significant correlation among morphological and biochemical traits considered for genotype biomass characterization. As regard the heads, there was a slightly positive correlation between

the lateness of genotype production and the 1,5-*O*-dicaffeoylquinic acid content (Pearson's correlation coefficient r=0.646, p≤0.05).

4.2.3 DISCUSSION

Italian spring genotypes here studied have been previously classified by *capitula* morphology in ‗Romanesco' and ‗Violetto' groups (Crinò *et al.,* 2008; Ciancolini *et al.,* 2012). This germplasm was selected by local farmers taking into account both climatic adaptation of the genotype and culinary purposes. In Italian environmental conditions, the main plant growth stages of spring globe artichoke are: sprouting in September-October, leaf rosette during winter, stem elongation in April, full blossom in June, ripe fruits in July, and dry aerial biomass in July-August (Raccuia and Melilli 2004, 2007). In the agricultural management of these genotypes, the heads were traditionally harvested in March-April and the aboveground biomass was left in the field without any use and profit.

In the last years, the growing interest on renewable energy sources led to the evaluation of *Cynara* spp. for biomass purpose (Angelini *et al.,* 2009; Mantineo *et al.,* 2009; Ierna and Mauromicale, 2010; Gominho *et al.,* 2011). In addition to the use as energy source, a new possible application of biomass as raw industrial material to recover phenolic active compounds has been investigated (Fernández *et al.,* 2006; Falleh *et al.,* 2008; Pandino *et al.,* 2011c). In this regard, some globe artichoke and cardoon genotypes have been characterized for biomass production and/or biochemical profile (Raccuia and Melilli, 2010; Gominho *et al.,* 2011), but there is a lack of data about the germplasm most suitable for this purpose. In particular, several studies were focused on the polyphenolic composition of the head as a functional food and the possibility of using the outer bracts, the floral stem and other head waste products, obtained during the globe artichoke industrial processing, has been also evaluated for the recovery of active biomolecules (Llorach *et al.,* 2002; Lattanzio, 2009; Bonasia *et al.,* 2010; Lombardo *et al.,* 2010; Pandino *et al.,* 2010, 2011b). Taking into account the aforementioned remarks, in our work globe artichoke germplasm have been analyzed and characterized both morphologically and biochemically to select genotypes more appropriate for a large-scale biomass and pharmaceutical compound production.

In fact, the agro-morphological descriptors used allow a clear identification of the genotypes analyzed and significant differences among genotypes have been found for all the agro-morphological traits evaluated. In particular, the aboveground dry biomass

yield provided useful information for industrial uses. In line with previous works (Fernández *et al.,* 2006; Raccuia and Melilli, 2007, 2010; Angelini *et al.,* 2009; Ierna and Mauromicale, 2010), the aerial biomass yield, expressed in kg of dry matter (DM) produced per genotype, is considered an important trait to discriminate the most interesting genotypes under this profile. In our experiments, the field dry biomass yield was estimated 9.7 $t \cdot ha^{-1}$, as average value of all genotypes in the two growing seasons, in accordance with other works (Foti *et al.,* 1999; Raccuia and Melilli, 2004). Also the biomass index emerges as a very useful trait to describe plant size related to plant height and diameter, which both were considered in several studies on *Cynara* spp. biomass (Piscioneri *et al.,* 2000; Angelini *et al.,* 2009; Gominho *et al.,* 2011). However, it is interesting to notice that aerial biomass yield (expressed as kg·of DM·per plant) and many agro-morphological traits were also affected by the growing season (environment). In fact, the genotype per-year-interaction was significant for all traits evaluated, showing that environmental conditions have a different influence on the morphological profile expression of each genotype. Temperature and rainfall may represent the most important environmental factors which influence plant phenology, as reported by other authors (Raccuia and Melilli, 2010; Gominho *et al.,* 2011). In particular, in the second growing season (2009-2010), all genotypes showed the most vigorous plants and the highest dry matter accumulation. This is also in agreement with the results obtained on cardoon crop in previous works, which reported an increasing of biomass dry yield from the second year of cultivation (Angelini *et al.,* 2009; Raccuia and Melilli, 2010; Gominho *et al.,* 2011). In fact, in the first growing season, the *Cynara* spp. plants have a stabilization stage investing mostly in root system development while, from the second year of cultivation, there is a great aerial biomass production and leaf expansion (Angelini *et al.,* 2009; Gominho *et al.,* 2011).
In order to evaluate the possibility of using this crop for biomass production without upsetting traditional agricultural practices, food and non-food dual-production (biomass for biocompound extraction and heads for human food) could be proposed with the aim of increasing farmers' income. In particular, central and primary flower heads, both at the commercial stage, could be collected and sent to the food market before the aerial biomass harvesting. The head yield, referred to the central and primary flower head production and obtained in our work, was satisfactory and comparable with the productive performance of globe artichoke crop specialized in head production (Crinò *et*

al., 2008; *Ciancolini et al*., 2012). A proper economic analysis should be useful to evaluate the real possibilities of using biomass in a well-organized system.

In this work, polyphenol extraction from globe artichoke biomass was performed using Accelerated Solvent Extraction (ASE). ASE has recently become one of the most promising and sustainable extraction procedures allowing high polyphenol yield with low solvent use and time consumption (Zuloaga *et al.,* 1998; Garcia Salas *et al.,* 2010); this procedure has been used on several species for active biocompound extraction (Wijngaard and Brunton*,* 2009; Monrad *et al.,* 2010). As reported by several works (Wang *et al.,* 2003; Lattanzio *et al.,* 2009; Pandino *et al.,* 2010, 2011b, 2011c; Lombardo *et al.,* 2012), also in our experiments, globe artichoke biomass (leaf and floral stem) and head extracts showed a high content of caffeoylquinic acid and flavonoid derivatives. In particular, in aerial biomass extracts, the main caffeic acid derivatives detected are 3-*O*-caffeoylquinic acid (from 0.16 $g \cdot kg^{-1}$ DM in Tondo Rosso di Paestum to 22.41 $g \cdot kg^{-1}$ DM in Ascolano), and 1,5-*O*-dicaffeoylquinic acid (from 0.18 $g \cdot kg^{-1}$ DM in S2 to 2.10 $g \cdot kg^{-1}$ DM in S5), in agreement with Pandino *et al.* (2011c). In line with previous studies (Wang *et al.,* 2003), a higher content of 3-*O*-caffeoylquinic acid (from 3.62 $g \cdot kg^{-1}$ DM in Campagnano to 14.47 $g \cdot kg^{-1}$ DM in Montelupone B), and 1,5-*O*-dicaffeoylquinic acid (from 3.49 $g \cdot kg^{-1}$ DM in S17 to 18.11 $g \cdot kg^{-1}$ DM in Bianco di Pertosa) respect to other compounds analyzed, was found in head extracts. Limited to the flavonoids apigenin and luteolin, our results are in contrast with other works (Pandino *et al.,* 2010, 2011c); apigenin and its derivatives have not been detected by HPLC analysis in biomass and head extracts, and we only quantified luteolin-7-*O*-glucoside in aerial biomass (from 0.53 $g \cdot kg^{-1}$ DM in Bianco di Pertosa to 8.96 $g \cdot kg^{-1}$ DM in S5) and in heads (from 0.014 $g \cdot kg^{-1}$ DM Ascolano to 0.16 $g \cdot kg^{-1}$ DM in Montelupone A). This could be linked to genetic aspects, harvesting time and plant parts analyzed (Lombardo *et al.,* 2010, 2012), but also to environment, agricultural management and abiotic/biotic stress. In addition, differences in the biochemical profile could depend on the different extraction methods used. Indeed, it is important to consider that each compound shows different polarities and thermal stabilities and is characterized by a different optimal set of extraction conditions. For example, the relative abundance of caffeoylquinic acid derivatives, identified in globe artichoke extracts, is strongly related to the solvent, pH and temperature used for their extraction (Lattanzio *et al.,* 2009). In our case, 80% ethanol as extraction solvent fitted as more suitable for nutraceutical or cosmetic applications. Moreover, ASE with 80% ethanol at

high temperature provides higher yields of 3-*O*-caffeoylquinic acid, 1,5-*O*-dicaffeoylquinic acid and luteolin-7-*O*-glucoside than other extraction techniques currently used, such as maceration (Fratianni *et al.,* 2007; Lombardo *et al.,* 2010; Pandino *et al.,* 2011a, 2011b). Correlation analysis, performed on the morphological and biochemical data, showed that the head content of 1,5-*O*-dicaffeoylquinic acid is, although slightely, positively related to the lateness of the genotypes. This could be also linked to the favorable influence on polyphenolic profile of late spring climatic parameters (i.e. temperature and light). In this sense, Lombardo *et al.* (2010, 2012) showed that phenolic content increased in globe artichoke heads from winter to spring harvesting. In particular, results obtained by Lombardo *et al.* (2012) showed an increase in polyphenol content of 12% in Romanesco clone C3 passing from winter to spring harvest time.

The biocompound yield of biomass obtained per hectare, ranging from 9.39 kg·ha^{-1} in Tondo Rosso di Paestum to 647.36 kg·ha^{-1} in Ascolano, was very interesting, also considering that this material has never been selected for a biochemical purpose. In particular, significant differences have been shown among genotypes for all compounds analyzed, this highlighted the genetic variability existing in *Cynara* spp. germplasm, and enhances the possibility to run successfully selections. For this reason, the selection of genotypes of interest under both biochemical and edule profiles is an important step in opening new horizons in globe artichoke breeding programs. The present work showed that, in Central Italy environments, there could be a real opportunity for a dual-production of globe artichoke (food and non-food), as revealed by the analysis of our genotypes (i.e. Ascolano and Campagnano) which confirmed this perspective.

4.3 Cardoon: Italian authoctonous accessions

4.3.1 Materials and methods

4.3.1.1 Experimental field and plant material

Seven Italian cultivated cardoon genotypes and a wild cardoon genotype belonging to the Tuscia University and Enea joint collection were considered in the study (*Table 4.8*). Field trials were conducted for two years, during the 2008/2009 and 2009/2010 growing seasons, at the experimental station of Tarquinia, (42°14‘57‘‘12 N; 11°45‘22‘‘32 E) Viterbo- Italy. The station is characterized by a temperate climate, clay-sandy soil and average annual rainfall of 900 mm. All genotypes were propagated by seed and were assessed in a randomized block experimental design with 4 replications of 5 plants each (planting density of 7,692 plants ha^{-1}, inter and intra-row distances of 1.30 and 1.00 m, respectively). The planting date was 11th July 2007 and the transplanting date was 17th August 2007. Field experiments were conducted under low energy inputs (manual weeding, no fertilization for crop management).

Table 4.8 Cardoon genotypes evaluated

Genotypes	Type	Donor Institute	Origin
AFB	Cultivated cardoon	Enea (RM)- Tuscia University (VT)	Latium, Italy
AFM	Cultivated cardoon	Enea (RM) - Tuscia University (VT)	Latium, Italy
AFM2	Cultivated cardoon	Enea (RM) - Tuscia University (VT)	Latium, Italy
AFGR	Cultivated cardoon	Enea (RM) - Tuscia University (VT)	Latium, Italy
AFGI	Cultivated cardoon	Enea (RM) - Tuscia University (VT)	Latium, Italy
AFN	Cultivated cardoon	Enea (RM) - Tuscia University (VT)	Latium, Italy
AFFG	Cultivated cardoon	Enea (RM) - Tuscia University (VT)	Latium, Italy
AFS	Wild cardoon	Enea (RM) - Tuscia University (VT)	Latium, Italy

4.3.1.2 Morphological characterization

Morphological characterization was performed using some of the standard UPOV descriptors established for globe artichoke (i.e. plant height, number of lateral shoots, floral stem diameter, first fully developed leaf length, leaf lobe number) and a group of complementary other descriptors capable of explaining biomass production (*Table 4.9*). A visual 0-3 rating scale as biomass index (0=not vigorous plant; 3=very vigorous

plant) has been used to express the vigor of the plant. In order to evaluate quantitatively the biomass production, four plants per each genotype were harvested at the end of the growing season (July-August) and immediately weighed and oven-dried at 103°C, until a constant weight was reached to determine the dry matter (DM) content. In total, 10 morphological data were recorded, during the two growing seasons, from the central flower head appearance until the end of the growth cycle on four plants per genotype.

Table 4.9 Morphological traits used for biomass characterization of cardoon genotypes and their abbreviations

Code	Descriptors
PH	Plant height
LSh	Number of lateral shoots
FSD	Main floral stem diameter
Llength	First fully developed leaf length
Lwidth	First fully developed leaf width
PD	Plant diameter
BI	Biomass index
Llob	Number of leaf lobes
PFW	Plant fresh weight
PDM	Plant dry weight

4.3.1.3 Phenolic compound extraction and analysis

4.3.1.3.1 Sample preparation

A representative sample (300 g FW) of biomass (leaves and floral stems) was collected from four plants per genotype at the end of the growing season in 2009/2010. Four primary heads per genotype were also harvested at the same sampling time. All samples were immediately weighed, freeze-dried and ground to 1 mm diameter fine powder. For each genotype, an amount (10 g three times replicated) of homogenized plant material (heads and biomass, separately) was oven-dried at 103°C until a constant weight was reached to determine the dry matter content.

4.3.1.3.2 Extraction procedure and analysis

Extraction and analysis of samples were performed as aforementioned in chapter 3. All extractions and analysis were performed in duplicate.

4.3.1.4 Statistical analyses

All data were analyzed as described in section 4.2.1.4.

4.3.2 Results

4.3.2.1 Biometric characteristics

4.3.2.1.1 Biomass production

Ten agro-morphological traits were evaluated to characterize biomass production of the eight genotypes. The ANOVA results with the statistical significant differences related to each morphological trait among genotypes, between years and for genotype per year interaction have been reported in Table 4.10. No significant genotype per year interaction has been found for all traits, except for the biomass index. On the contrary, significant differences among genotypes have been found for all traits evaluated, except for plant diameter, leaf lobe number, first fully developed leaf length and width. Significant differences have been also shown between the growing seasons for all parameters except for the lateral shoot number, main floral stem and plant diameter, leaf lobe number, fresh and dry weight of the plants.

Table 4.10 Level of significance of the descriptors used for the morphological characterization from the ANOVA analysis

Code	Descriptors	Genotypes	Year	Genotype*Year
PH	Plant height	**	***	ns
LSh	Number of lateral shoots	**	ns	ns
FSD	Main floral stem diameter	*	ns	ns
Llength	First fully developed leaf length	ns	***	ns
Lwidth	First fully developed leaf width	ns	***	ns
PD	Plant diameter	ns	ns	ns
BI	Biomass index	***	**	**
Llob	Number of leaf lobes	ns	ns	ns
PFW	Plant fresh weight	***	ns	ns
PDM	Plant dry weight	***	ns	ns

*ns = not significant; * significant differences at $p \leq 0.05$*

In Table 4.11 some biometric parameters of the genotype plants are reported. As regard plant height, in 2008-2009 growing season, AFFG and AFM showed the highest values while, in 2009-2010, there were no significant differences among genotypes for this trait. In both years, AFM had the highest number of lateral shoots. In 2008-2009, AFFG and AFM2 were characterized by the longest first fully developed leaf of the main floral stem while in 2009-2010 no significant differences for this trait were found. In both growing seasons, AFFG showed the widest first fully developed leaf of the main floral stem. As regards plant diameter, no significant differences among genotypes were found. All genotypes except AFB, AFN and AFS in the first year, and AFS in the second year, were characterized by the best value of biomass index which has been used to determine plant vigor. In both growing seasons, AFFG had the highest plant dry weight, while AFM has been added to this genotype only in the first year. AFFG and AFM genotypes resulted very interesting in term of biomass yield among the other parameters studied in both growing seasons.

Table 4.11 Agro-morphological characterization of the cardoon genotypes under biomass profile (2008-2009 and 2009-2010 growing seasons)

Genotypes	Year	PH (cm)	LSh (no.)	Llength (cm)	Lwidth (cm)	PD (cm)	BI (no.)	PDM (g)
AFB	2008/2009	157.50ab	5.25ab	48.25b	24.00b	175.00ns	1.88ab	1650.00b
AFFG	2008/2009	163.75a	5.00ab	63.50a	41.75a	173.00ns	2.13a	2357.50a
AFGI	2008/2009	144.00ac	4.00b	57.00ab	28.25b	177.00ns	2.38a	1787.50b
AFGR	2008/2009	124.75bc	4.00b	51.50ab	27.25b	168.75ns	2.13a	1675.00b
AFM	2008/2009	166.75a	6.50a	55.75ab	31.50ab	177.50ns	2.50a	2415.00a
AFM2	2008/2009	142.50ac	4.25b	66.25a	31.25ab	185.00ns	2.25a	1675.00b
AFN	2008/2009	129.00bc	5.25ab	51.50ab	24.25b	150.25ns	1.38b	1387.50c
AFS	2008/2009	114.25c	4.75ab	51.50ab	32.00ab	150.25ns	1.25b	1097.50d
AFB	2009/2010	223.25ns	6.00ab	39.50ns	19.75ab	187.00ns	2.38a	1617.50cd
AFFG	2009/2010	211.50ns	4.75b	43.25ns	26.25a	183.25ns	2.50a	2495.00a
AFGI	2009/2010	183.33ns	4.67b	38.33ns	17.67b	165.00ns	2.33a	1750.00c
AFGR	2009/2010	183.33ns	5.33ab	35.67ns	15.67b	169.33ns	2.50a	1572.50de
AFM	2009/2010	225.50ns	8.00a	35.75ns	20.50ab	185.25ns	2.50a	2275.00b
AFM2	2009/2010	193.75ns	4.00b	36.75ns	17.25b	176.50ns	2.63a	1667.50cd
AFN	2009/2010	201.00ns	6.00ab	38.25ns	22.25ab	186.50ns	2.63a	1435.00e
AFS	2009/2010	191.00ns	5.67ab	36.00ns	21.67ab	174.00ns	1.17b	1095.00f

Values are the means of measurements on 4 plants. Means followed by different letters for each parameter are significantly different at $p \leq 0.05$ (Duncan test)

A similarity dendrogram, based on these morphological parameters, was generated using an agglomerative hierarchical cluster analysis (*Figure 4.3*). Four major clusters could be identified. Cluster 1 consisted of four genotypes: AFB, AFGR, AFM2 and AFGI. In cluster 2 AFN and in cluster 3 AFS were classified. In cluster 4, AFFG and AFM were grouped.

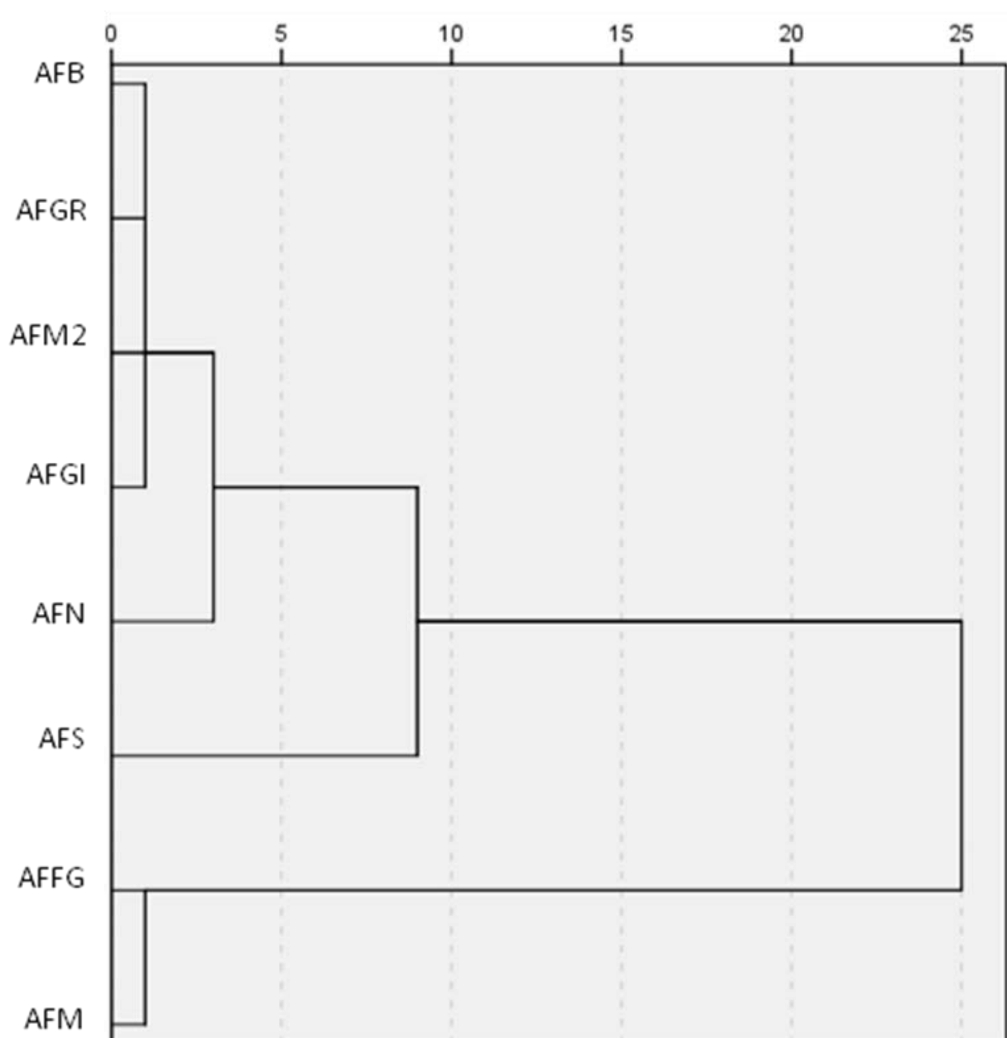


Fig. 4.3 Similarity dendrogram constructed on the morphological biomass characterization of the eight cardoon genotypes analyzed using agglomerative hierarchical cluster analysis

An among-genotype pairwise similarity matrix was created with the quantitative and qualitative morphological traits explaining biomass production for the two growing seasons and a PCA was then applied. The first PC factor (53.04% of variance explained) included contributions from the following primary traits: plant height, floral stem diameter, first fully developed leaf length, plant diameter, biomass index and plant dry weight. The second factor (19.67% of the variance explained) considered the lateral

shoot number. The third factor (16.26% of variance explained) involved the first fully developed leaf width. These first three functions explained 88.96% of the variance and each genotype was plotted against these three functions (*Figure 4.4*). PCA showed that three groups could be identified: one on the upper right side of the figure with the genotypes AFB and AFM2, one on the upper left side with the genotypes AFFG, AFGR, AFGI, AFN, AFM and the last one, on the lower side of the graphic, with AFS genotypes which was the only wild cardoon.

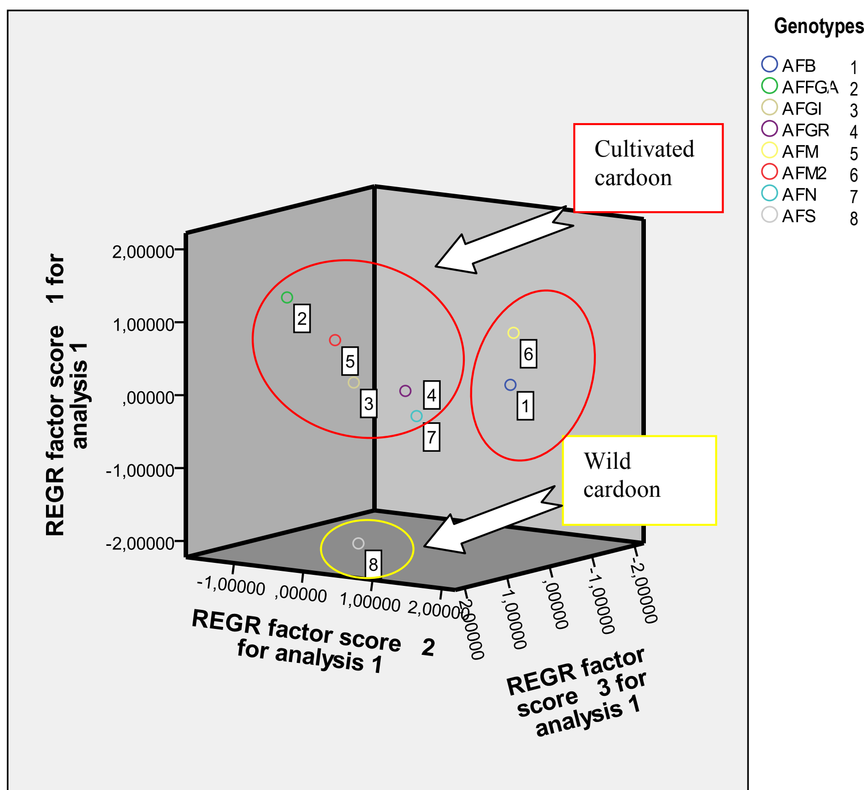


Fig. 4.4 Distribution on the basis of biomass production against the three discriminant functions of the cardoon genotypes analyzed

On the basis of all morphological traits evaluated a correlation matrix was generated using SPSS 15 (*Table 4.12*). As results, plant dry weight was positively correlated to the plant height and biomass index, while biomass index was positively related to the plant dry weight obviously along with the plant and floral stem diameter.

Table 4.12 Correlation matrix among morphological traits evaluated for biomass production of cardoon genotypes

	PH	LSh	PD	BI	FSD	Length	Lwidth	Llob	PDM
PH	1.00	.609	.843	.467	.547	.280	.359	-.214	.742
LSh	.609	1.00	.243	.003	-.066	-.401	.054	.093	.281
PD	.843	.243	1.00	.737	.712	.474	.142	-.392	.692
BI	.467	.003	.737	1.00	.827	.451	-.066	-.826	.710
FSD	.547	-.066	.712	.827	1.00	.426	.071	-.680	.666
Length	.280	-.401	.474	.451	.426	1.00	.665	-.571	.607
Lwidth	.359	.054	.142	-.066	.071	.665	1.00	-.180	.582
Llob	-.214	.093	-.392	-.826	-.680	-.571	-.180	1.00	-.658
PDM	.742	.281	.692	.710	.666	.607	.582	-.658	1.00

Pearson correlation test ($p \leq 0.05$)

4.3.2.2 Biochemical characteristics

In Table 4.13 biomass (floral stems and leaves) and head polyphenol contents of all genotypes, determined by HPLC analysis, are shown. Among the biocompounds analyzed, caffeic acid, taxifolin, silybin, luteolin and cynarin were no detected in all head and biomass samples. On the contrary, statistical differences among genotypes were found on biomass (leaves and floral stems) and head content of the other compound analyzed (i.e. chlorogenic acid, apigenin, and cynaroside). As regards the biomass, only in the genotypes AFGR and AFM the 1,5-*O*-dicaffeoylquinic acid was detected (4.07 and 3.08 g kg^{-1} DM, respectively). As regards cholorogenic acid, the genotype AFGR showed the highest content in biomass samples. In all genotypes, apigenin was not found except in AFGR and AFM2 biomass samples. The genotype AFN showed the highest concentration of luteolin-7-*O*-glucoside (4.20 g kg^{-1} DM).

Table 4.13 Phenolic (g·kg^{-1} of DM) contents of globe artichoke spring genotype biomasses (leaves and floral stem) and heads

Genotypes	Biomass						Heads					
	1,5 dicaffeoylquinic acid (g·kg^{-1} DM)	Chlorogenic acid (g·kg^{-1} DM)	Apigenin (g·kg^{-1} DM)	Cynaroside (g·kg^{-1} DM)	Total caffeoyquinic acids (g·kg^{-1} DM)	Total polyphenol measured (g·kg^{-1} DM)	1,5 dicaffeoylquinic acid g·kg^{-1} DM)	Chlorogenic acid (g·kg^{-1} DM)	Apigenin (g·kg^{-1} DM)	Cynaroside (g·kg^{-1} DM)	Total caffeoyquinic acids (g·kg^{-1} DM)	Total polyphenol measured (g·kg^{-1} DM)
AFB	nd	0.59d	nd	1.29e	0.59c	1.88c	3.33c	2.10b	0.03c	0.68ce	5.43b	6.14c
AFFG	nd	0.43e	nd	1.80d	0.43c	2.23b	4.20bc	2.08b	0.079b	0.95bc	6.28b	7.31b
AFGR	4.07a	2.65a	0.04a	0.63g	6.72a	7.39a	nd	0.33de	nd	0.59e	0.33e	0.92e
AFGI	nd	0.34f	nd	1.11ef	0.34c	1.45c	5.95a	3.65a	0.028c	0.62de	9.60a	19.82a
AFM2	nd	2.40b	0.02b	0.56g	2.40b	1.98b	nd	0.03e	nd	0.84bd	0.03e	0.87e
AFM	3.08b	0.01g	nd	2.08c	3.09b	2.08b	1.07e	0.84d	0.12a	0.86bd	1.91d	2.89d
AFN	nd	0.95c	nd	4.20a	0.95c	5.15a	2.33d	1.54c	0.03c	1.37a	3.87c	5.27c
AFS	nd	0.43e	nd	2.39b	0.43c	2.82b	4.45b	3.26a	0.075b	1.27a	7.71ab	9.05b

nd= not detected. Values are the means of measurements on 4 plants. Means followed by different letters for each parameter are significantly different at p≤ 0.05 (Duncan test)

Also for the flower heads, statistical differences among genotypes were found under biochemical profile (*Table 4.13*). The genotype AFGI was characterized by the highest content of 1,5-*O*-dicaffeoylquinic acid and chlorogenic acid, while only for this last compound AFS showed the highest head content. AFM genotype provided the highest value of apigenin (0.12 g kg^{-1} DM) while AFN and AFS showed the highest content of luteolin-7-*O*-glucoside (1.37 and 1.27 g kg^{-1} DM, respectively).

In order to evaluate a large-scale production of biocompounds from cardoon biomass, total polyphenol yield per hectare was estimated per each genotype taking into account the polyphenol concentration, expressed in g kg^{-1} DM, the biomass dry matter accumulated per plant and the planting density of 7,692 plants·ha^{-1} (*Table 4.14*).

AFGR genotype provided the highest yield of chlorogenic acid (35.48 kg ha^{-1}), 1.5-O-dicaffeoylquinic acid (54.47 kg ha^{-1}) and apigenin (0.52 kg ha^{-1}), while for cynaroside AFN showed the highest value (45.95 kg ha^{-1}) (*Table 4.16*).

Table 4.14 Biocompound yield from cardoon genotype aerial biomass (planting density 7,692 plants·ha^{-1})

Genotype	**cynaroside (kg ha^{-1})**	**chlorogenic acid (kg ha^{-1})**	**1,5-*O*-dicaffeoylquinic acid (kg ha^{-1})**	**apigenin (kg ha^{-1})**	**Total polyphenol measured (kg ha^{-1})**
AFB	16.82cd	7.74e	nd	nd	24.56f
AFFG	36.89b	8.69d	nd	nd	45.59d
AFGR	8.49e	35.48a	54.47a	0.52a	98.97a
AFGI	15.94cd	4.87f	nd	nd	20.82fg
AFM2	7.57e	32.39b	41.50b	0.32b	81.79b
AFM	39.47b	0.19h	nd	nd	39.66e
AFN	45.96a	10.35c	nd	nd	56.31c
AFS	20.21c	3.64g	nd	nd	23.89f

nd= not detected, Means followed by different letters for each parameter are significantly different at $p \leq 0.05$ (Duncan test)

A correlation analysis was performed in order to investigate the connection among the polyphenolic compounds analyzed. The 1,5-*O*-dicaffeoylquinic acid and 3-*O*-caffeoylquinic acid contents were positively related both in the heads (Pearson's correlation coefficient $r=0.963$, $p \leq 0.05$) and in the biomass (Pearson's correlation coefficient $r=0.958$, $p \leq 0.05$). The 1,5-*O*-dicaffeoylquinic acid content was also positively related to the apigenin content in biomass (Pearson's correlation coefficient

r=0.929, p≤0.05). On the contrary, no significant correlations were found among morphological and biochemical parameters evaluated for genotype biomass characterization.

4.3.3 DISCUSSION

In the last years, new possible uses for cardoon have been evaluated. Investigation of other possible applications of *Cynara cardunculus* crop started at the end of the last century and several industrial applications such as achenes for oil for human consumption (Maccarone *et al.,* 1999; Curt *et al.,* 2002) and biodiesel (Sengo *et al.,* 2010), roots for inulin extraction (Raccuia and Melilli, 2004, 2010), and aboveground biomass for energy production have been widely studied (Piscioneri *et al.,* 2000; Férnandez *et al.,* 2006; Raccuia and Melilli, 2007; Mantineo *et al.,* 2009; Ierna and Mauromicale, 2010).

The use of cardoon biomass for the extraction of health-promoting biocompound has been also considered in the last years (Falleh *et al.,* 2008; Pandino *et al.,* 2010, 2011c) but there is a lack of data useful for the pharmaceutical industry. In particular, Italian cardoon germplasm has never been analyzed taking into account both morphological and biochemical profile. Moreover, the selection of cardoon genotypes characterized by a high biomass and biocompound yields has never been carried out. Italian germplasm has been selected only by local farmers who have chosen genotypes taking into account mainly culinary purposes. Indeed, in the traditional agricultural management of this crop, the plants are grown from September to July, without high energy input, and the enlarged bleached petioles were harvested and used as ingredient of traditional recipes. However in Italy, cardoon is not widespread and there are no official data on its cultivation area. As regards wild cardoon, it is well adapted to the Italian environmental conditions; it is a non-domesticated perennial plant which represents a weed in many agricultural areas and is not cultivated as a commercial crop (Cravero *et al.,* 2012) and only in some areas of South Italy (i.e. Sicily) its *capitula* are sold in local markets (Ierna and Mauromicale, 2010). The eight genotypes here analyzed, using agro-morphological descriptors, have been clearly characterized and identified. In particular, no significant genotype per year interactions have been found for all traits, except for the biomass index, this showing the stability of environment influence on each genotype. Significant differences among genotypes have been found for many of the agro-morphological traits evaluated. In particular, the aboveground dry biomass obtained, expressed in kg

per plant provided useful information for industrial uses. In line with previous works (Fernández *et al.,* 2006; Raccuia and Melilli, 2007, 2010; Angelini *et al.,* 2009; Ierna and Mauromicale, 2010; Gominho *et al.,* 2011; Cravero *et al.,* 2012), the aerial biomass yield, expressed in kg of dry matter produced per genotype, is considered as an important trait to discriminate the most interesting genotypes under this profile. In line with previous works (Raccuia and Melilli, 2004, 2010; Gominho *et al.,* 2011), biomass yield obtained from all genotypes in the two growing seasons resulted very high (average value of 13.44 tonnes ha^{-1} of dry matter at planting density of 7,692 plants ha^{-1}). Also plant height, which has been considered in several studies on *Cynara* spp. biomass (Piscioneri *et al.,* 2000; Angelini *et al.,* 2009; Gominho *et al.,* 2011) resulted very useful to describe plant vigor and was positively correlated to plant dry weight. As regards plant diameter, this trait resulted not useful to discriminate among genotypes and no significant differences among genotypes were found. In line with other authors (Raccuia and Melilli, 2010) cultivated cardoon genotypes showed most vigorous plants than wild cardoon. Indeed, in both years AFS wild cardoon presented the lowest biomass index, plant dry weight and height than other genotypes of cultivated cardoon.
As regards biochemical characterization, performed by Accelerated Solvent Extraction (ASE) and HPLC analysis, significant differences among genotypes were found for polyphenol content. As reported by several works (Wang *et al.,* 2003; Lattanzio *et al.,* 2009; Pandino *et al.,* 2010, 2011c), also in our experiments, cardoon biomass (leaf and floral stem) and head extracts showed a high content of caffeoylquinic acid (3-*O*-caffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid) and flavonoid derivatives (cynaroside). In particular, in cardoon biomass extracts, the main caffeic acid derivatives detected have been 3-*O*-caffeoylquinic acid (from 0.01 $g \cdot kg^{-1}$ DM in AFM to 2.65 $g \cdot kg^{-1}$ DM in AFGR in biomass extract), and 1,5-*O*-dicaffeoylquinic acid (with 3.08 $g \cdot kg^{-1}$ DM in AFM and 4.07 $g \cdot kg^{-1}$ DM in AFGR in biomass extracts), in agreement with Pandino *et al.* (2011c) which found in leaf and floral stem extracts of different cardoon genotypes high content of these such compounds (average value of 0.55 and 0.85 $g \cdot kg^{-1}$ DM of 3-*O*-caffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid, respectively in leaf extracts). In line with previous studies (Wang *et al.,* 2003), also in cardoon *capitula* extracts the content of 3-*O*-caffeoylquinic acid (from 0.03 $g \cdot kg^{-1}$ DM in AFM2 to 3.65 $g \cdot kg^{-1}$ DM in AFGI), and 1,5-*O*-dicaffeoylquinic acid (from 1.07 $g \cdot kg^{-1}$ DM in AFM to 5.95 $g \cdot kg^{-1}$ DM in AFGI) was highest than other compounds analyzed. As regards luteolin, this compound has not been detected in HPLC analysis both in

biomass and head extracts in contrast with other works (Pandino *et al.,* 2010, 2011c) which found very interesting contents of this such compound in cardoon extracts (average value of 0.35 $g \cdot kg^{-1}$ DM in leaf extract). On the contrary, the luteolin-7-*O*-glucoside in aerial biomass (from 0.56 $g \cdot kg^{-1}$ DM in AFM2 to 4.20 $g \cdot kg^{-1}$ DM in AFN) and in heads (from 0.59 $g \cdot kg^{-1}$ DM in AFGR to 1.37 $g \cdot kg^{-1}$ DM in AFN), and the apigenin in biomass (with 0.04 $g \cdot kg^{-1}$ DM in AFGR and 0.02 $g \cdot kg^{-1}$ DM in AFM2) and in heads extracts (from 0.028 $g \cdot kg^{-1}$ DM in AFGI to 0.12 $g \cdot kg^{-1}$ DM in AFM) were detected and resulted the most representative flavonoids. However, it is important to consider that phenolic profile of the genotypes may depend on genetic factors, but also on the environmental conditions, agricultural practices and on the extraction methods (Pandino *et al.,* 2010). Correlation analysis, performed on the morphological and biochemical data, showed that both in head and biomass extracts the 1,5-*O*-dicaffeoylquinic acid is positively related to the 3-*O*-caffeoylquinic acid content, and only in biomass extracts, it is related to apigenin content. As regards the positive correlation between 5-*O*-caffeoylquinic and 1,5-*O*-dicaffeoylquinic acids, this could be linked to the phenol acids biosynthesis pathway in which chlorogenic acid is the precursor of dicaffeoylquinic acids (Moglia *et al.,* 2008).

Taking into account that the germplasm here analyzed has never been selected for a biochemical purpose, the biocompound yield obtained from cardoon biomass, ranging from 23.56 $kg \cdot ha^{-1}$ in AFB to 98.97 $kg \cdot ha^{-1}$ in AFGR, was very interesting. In particular, some genotypes (AFGR and AFM2) may be selected and used in plant breeding programs to improve biomass and biocompound yields. Our data highlight the great potential of cardoon as biomass crop for pharmaceutical uses and open challenging perspectives for this crop which is up now underutilized.

Chapter 5

BIOMASS AND BIOCOMPOUND PRODUCTION IN FLOATING SYSTEM UNDER SALT STRESS: MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR RESPONSES

5.1 INTRODUCTION

Globe artichoke and cardoon contain high levels of phenolic compounds, especially in their leaves, as reported by several authors (Fratianni *et al.,* 2007; Lattanzio *et al.,* 2009; Lombardo *et al.,* 2010; Pandino *et al.,* 2011c, Negro *et al.,* 2011, 2012). In particular, caffeic acid derivatives, as well luteolin glucosides, represent the majority of these compounds (Moglia *et al.*, 2008; Lattanzio *et al.,* 2009; Pandino *et al.,* 2011c, Negro *et al.,* 2012). Flavonoids and caffeic acid derivatives originate from the phenylpropanoid pathway. This biosynthesis pathway, starting from phenyalanine, is induced upon biotic and abiotic stresses, and is involved in defense mechanism operating in stress-afflicted cells (Dixon and Paiva, 1995; Moglia *et al.,* 2008). Indeed, phenylpropanoid compounds are induced in response to several agents such as: i) pathogen attack reported in *Nicotiana tabacum* by Chong *et al.* (2002), and in alfalfa by He and Dixon (2000); ii) wounding reported in *Lactuca sativa* by Loaiza-Velarde (1997), and in *Solanum tuberosum* by Reyes and Cisneros-Zevallos (2003); (iii) high visible light level exposure reported in *Lotus corniculatus* by Paolocci *et al.* (2005) and in globe artichoke by Moglia *et al.* (2008); iv) cold/heat reported in tomato and watermelon by Rivero *et al.* (2001); v) drought reported in *Ligustrum vulgare* by Tattini *et al.* (2004); vi) salinity reported in *Oriza sativa* by Walia *et al.* (2005); and vii) pollution reported in *Arabidopsis thaliana* by Sharma and Devis (2004). Although several works on phenylpropanoid pathway and on its induction due to different types of stresses are present in literature for several species, much less is known on the increase of phenolic compounds in *Cynara* spp. plants grown under stress conditions; only some studies have been recently conducted by Moglia *et al.* (2008) and Rezazadeh *et al*. (2012). In addition, in *C. cardunculus* spp., the phenylpropanoid pathway is still under study. Isolation and functional characterization of the gene sequences, involved in the synthesis of some compounds such as chlorogenic acid, is under investigation (Comino *et al.,* 2007, 2009; Moglia *et al.*, 2009; Sonnante *et al.*, 2011).

In order to evaluate accumulation of the major phenolic compounds and flavonoids in globe artichoke and cardoon plants in response to salinity stress condition, an experimental hydroponic system was developed in greenhouse at the experimental station of Tuscia University. Another focal point of the present PhD work consisted in setting up a biomass and biocompound production technique which may fulfill both the legal requirements for medicinal plants introduced by European Community and the commercial demands of the pharmaceutical extraction industry. Indeed, a regulatory

legislation has been recently introduced in European Union and in North America to discipline the safety and the quality specifications of herbal preparations (Maggini *et al.,* 2012). In addition, in the last years, several studies on medicinal plant cultivation under hydroponic (soilless) culture have been successfully carried out with the aim of facilitating biocompound extraction industry in the recovery of this raw material and in obtaining standardized and clean raw material (Dorais *et al.,* 2001). As known, medicinal plant demand was initially satisfied by the collection of wild plants in natural environment but the increasing interest for herbal products has promoted the field and greenhouse soilless cultivation of these species, as reported by Dell'Acqua *et al.* (2010) for *Echinacea angustifolia*.

Greenhouse hydroponic culture has many advantages respect to open field and in particular it allows: i) to standardize and control the production process; ii) to obtain year-round production and faster crop growth; iii) to produce high quality standard raw material; iv) to reduce pollutants and biotic (as fungal and bacterial) contaminations; v) to increase the efficiency in water use (Fallovo *et al.,* 2009; Gottardi *et al.,* 2012; Maggini *et al.,* 2012). Hydroponic cultivation includes several different techniques such as nutrient film technique (NFT) and floating raft system (FRS) where a nutrient solution ensures the nutrient supply to plants (Gottardi *et al.,* 2012). In particular, this last technique, which is usually used for short-cycle leafy vegetables (reported in *Lactuca sativa* L., *Cichorium intybus* L. and *Valerianella locusta* L. by Gonnella *et al.,* 2002; in *Spinacia oleracea* by Rodriguez-Hidalgo *et al.,* 2010; in *Lactuca sativa* L. by Scuderi *et al.,* 2011; in *Valerianella locusta* L. by Gottardi *et al.,* 2012), requires quite low investments and allows to cultivate plants for producing roots especially in closed-loop system. This technique has been already used for the caffeic acid derivatives production in *Echinacea angustifolia* DC. var. *angustifolia* by Maggini *et al.*, (2012). Considering these preliminary remarks, floating system has been chosen in our experiments also for the possibility of regulating secondary plant metabolism by efficient control of the nutrient solution (Briskin, 2000).

5.2 Materials and methods

5.2.1 Greenhouse experiments

Greenhouse experiments were conducted, during both 2009-2010 and 2010-2011 growing seasons, at the experimental station of Tuscia University, in Viterbo (42°25'N; 12°6'E) (Italy). Two genotypes, belonging to the ENEA-Tuscia University germplasm joint collection, were considered in our study: (i) the globe artichoke line named Cavi and (ii) the cultivated cardoon accession named AFB. Both Cavi and AFB were seed-propagated and were chosen just for this feature, so to facilitate their cultivation under soilless culture. In order to avoid plant heterogeneity typically occurring in cardoon genotypes, seeds of AFB have been used after two subsequent selfing-pollinations, while being Cavi an inbred line its seed were used directly in the experimentation. The sowing dates were October 8th, 2009 and November 8th, 2010 in the two years. After emergence, which occurred in about two weeks, the trays were placed in plastic tanks with 65 L of nutrient solution. Thus, globe artichoke (Cavi) and cultivated cardoon (AFB) plants were grown, under soilless conditions, in a static floating system formed by expanded polystyrene boards (104 holes) floating on a nutrient solution contained in rectangular tanks. The standard nutrient solution composition is reported in Table 5.1. The solution was aerated by bubbling, which also provided its constant mixing. The fresh solution was supplied every thirty days and the pH, the Electrical Conductivity EC and the temperature were weekly measured (*Table 5.2*).

Table 5.1 Standard nutrient solution composition

Component	mg/L
$Ca(NO_3)_2$	812.90
KH_2PO_4	204.81
K_2SO_4	273.72
$Mg(NO_3)_2$	384.00
NH_4NO_3	81.00
$KHCO_3$	100.00
MIKROM	24.00

Table 5.2 Means of ph, electrical conductivity (EC) and temperature of nutrient solutions in the two growing seasons

	2009-2010 growing season		**2010-2011 growing season**		
	Salinity 0	Salinity 2	Salinity 0	Salinity 1	Salinity 2
Ph	6.97±0.14	7.03±0.11	6.12±0.65	5.66±0.12	5.65±0.17
EC (dS m^{-1})	1.69±0.05	3.05±0.09	1.78±0.05	2.80±0.05	3.92±0.08
Temperature (°C)	17.53		13.27		

In 2009-2010 growing season, plant material was arranged in a split-plot experimental design with two main-plots for genotype (AFB and Cavi), two sub-plots for salinity stress (salinity 0: standard solution and salinity 2: 2.9 g/L NaCl supplemented solution) and three replications. Also in 2010-2011 growing season, plant material was arranged in a split-plot experimental design using two main-plots (two genotypes: AFB and Cavi), three sub-plots (Salinity 0: standard solution, Salinity 1: 1.45 g/L NaCl supplemented solution, and Salinity 2: 2.9 g/L NaCl supplemented solution), and three replications. In order to evaluate the effect of salinity stress on polyphenolic content of leaf extracts, standard solution was supplemented with NaCl 2.9 g/L (Salinity 2) at the beginning of the experimental trials in 2009-2010 growing season (*Figure 5.1*) while; in the second year, standard solution was supplemented at one week before the sampling time with NaCl 1.45 (Salinity 1) and 2.9 g/L (Salinity 2), respectively (*Figure 5.2*).

2009-2010 growing season

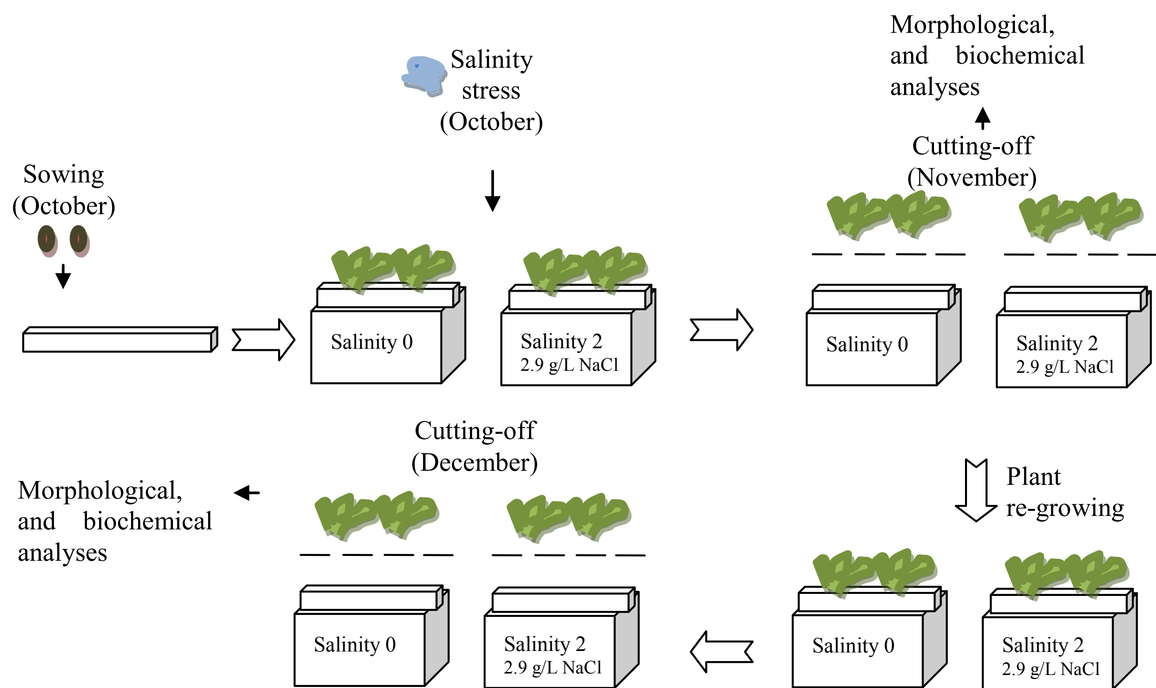


Fig. 5.1 Phases of the experimental trial conducted in floating system during the 2009-2010 growing season

2010-2011 growing season

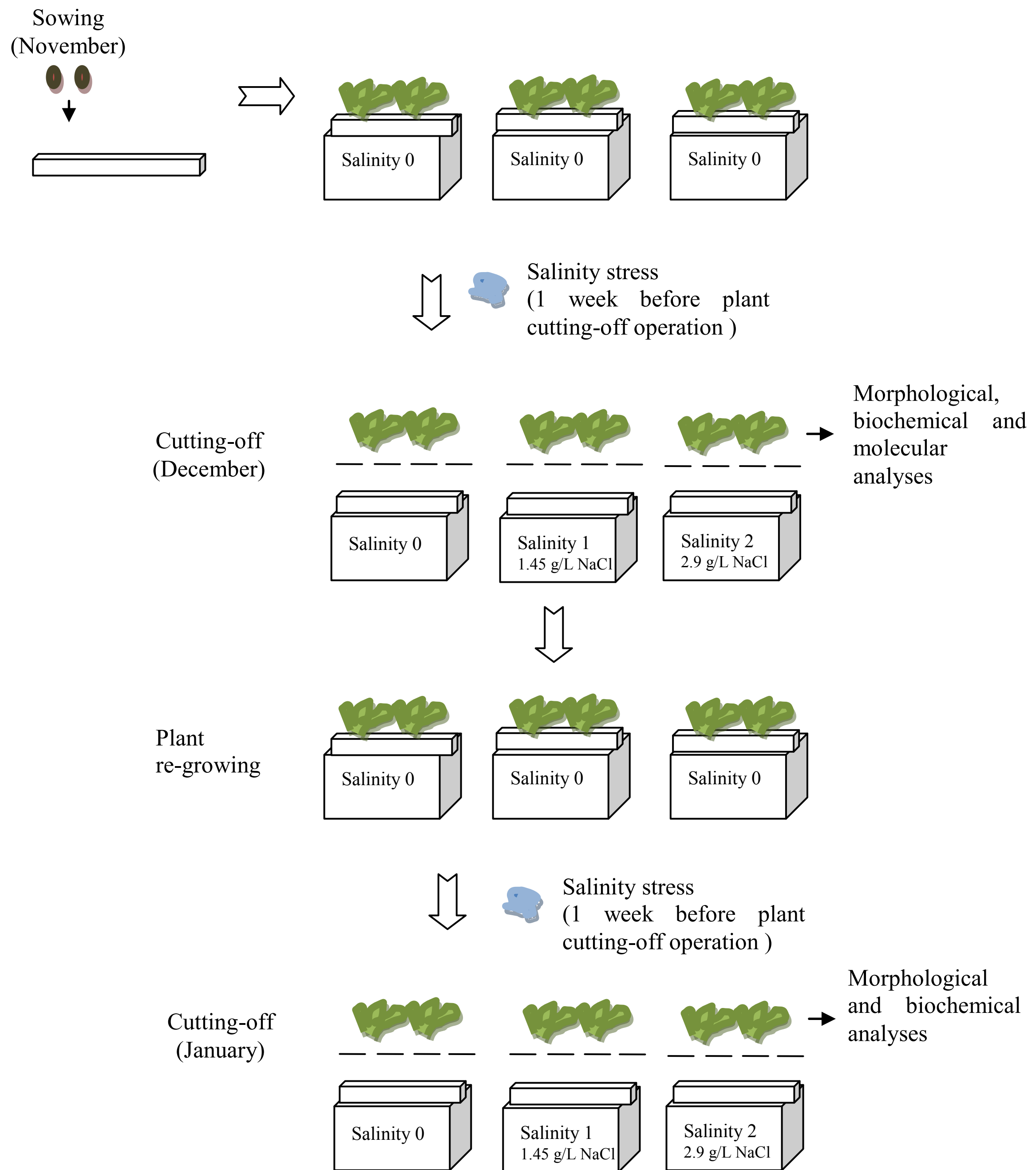


Fig. 5.2 Phases of the experimental trial conducted in floating system during the 2010-2011 growing season

Fig. 5.3 Globe artichoke Cavi and cultivated cardoon AFB grown in floating system; a) young plant at cotyledonary stage; b) experimental trial in 2009-2010 growing season; c) experimental trial in 2010-2011 growing season; d) Cavi roots grown in floating system; e) AFB plants of the Salinity 0 on the right and of Salinity 2 on the left (2009-2010)

5.2.2 AGRO-MORPHOLOGICAL CHARACTERIZATION

During both experimental growing seasons, morphological characterization was performed using 11 descriptors relating to biomass production in soilless culture (i.e. plant diameter, leaf number, leaf length and width, fresh and dry weight of aerial part of a single plant, fresh and dry weight of aerial biomass produced per boards, fresh and dry weight of roots produced per board, and total number of survived plants) (*Table 5.3*). In particular, for the morphological characterization, three plants per genotype and replication were evaluated using biometric traits while; for the evaluation of biomass production, 10 plants per each genotype and replication were collected at each sampling time (i.e. on November 20th, and on December 21th, 2009 in the first year; on December 20th, 2010 and on January 21th 2011 in the second year), immediately weighed and oven-dried at 103°C, until a constant weight was reached for the determination of dry matter (DM) content.

Table 5.3 Agro-morphological descriptors used and their abbreviations

Descriptor	**Code**
Leaf number	LN
Plant diameter	PD
Leaf length	Llength
Leaf width	Lwidth
Fresh weight of the aerial part of the plant	FWAP
Dry weight of the aerial part of the plant	DWAP
Fresh weight of the aerial biomass produced per board	FWB
Dry weight of the aerial biomass produced per board	DWB
Fresh weight of root produced per plant	FWRootP
Dry weight of root produced per plant	DWRootP
Fresh weight of the root produced per board	FWRootB
Dry weight of the root produced per board	DWRootB
Total number of survived plants	N survived plant

*ns = not significant; * significant differences at $p \leq 0.05$*

5.2.3 Phenolic compound extraction and analysis

5.2.3.1 Sample preparation

Twenty plants per genotype and replication were collected at each sampling time. All samples were immediately weighed, freeze-dried and ground to 1 mm diameter fine powder and stored at -20°C until the analyses were carried out at the Institut National Polytechnique de Toulouse- Laboratoire de Chimie Agroindustrielle, in France.

5.2.3.2 Extraction procedure

Biocompound extraction was performed using ASE system as described in section 4.3.1.3.2.

5.2.3.3 Solvents and Reagents

Solvents and reagents used were aforementioned in section 4.3.1.3.3.

5.2.3.4 HPLC Analysis

HPLC analysis was carried out as described in section 4.3.1.3.4.

5.2.4 Inulin extraction and analysis

5.2.4.1 Sample preparation

In 2010-2011 growing season, fresh roots of twenty plants per genotype and thesis were collected at the end of the experimental trial (January 21th, 2011), dried in a thermo-ventilated oven at 70°C until the constant weight was reached and then ground to a fine powder using an electrical grinder Freitsh Pulverisette.

5.2.4.2 Extraction procedure

Based on the laboratory experience, inulin extraction was performed using one gram of freeze-dried root sample for both Cavi and AFB. Briefly, each root sample was placed in a Becker containing 30 mL of deionized water;extraction was performed by maceration at 60°C for two hours. The extracts so far obtained were filtered under vacuum and the solid matrix was resuspended using 30 mL of deionized water for a second extraction performed as above described.

After the second extraction, the extract was filtered under vacuum and added to that obtained from the first extraction. The volume of the final extract so obtained was

reduced using rotavapor at 70°C until a final volume of 30 mL. Each extraction was performed in duplicate.

5.2.4.3 Solvents and Reagents

Sodium hydroxide and sodium acetate anhydrous were purchased from Scharlau (Barcelona, Spain). De-ionized water was home-made using a Milli-Q water purifying system purchased from Millipore Co. (Bedford, MA, USA). Inulin *(CAS number 9005-80-5)* standard molecule was obtained from Extrasynthese, (Lyon, France).

5.2.4.4 HPAEC-PAD analysis

Each extract was analyzed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (GP50 Gradient Pump, ED50 Electrochemical Detector and AS50 Autosampler), Dionex (Sunnyvale, CA, USA).
A Dionex PA-200 column was used for the analysis. The eluents were as follows: 100 mM Sodium Hydroxide as eluent A and 100 mM/ 1M Sodium Acetate as eluent B. All analyses were carried out at room temperature under the following elution conditions: eluent A at 7% and eluent B at 93% for 10 minutes followed by a linear gradient of 40 minutes, where eluent B reached 50 % for 10 minutes and then, in 10 minutes, the initial conditions have been reached with a flow-rate of 0.35 mL min^{-1}. The injection volume was 20 µL. Inulin standard used in the experiments was weighed and dissolved in deionized water. The calibration curves were generated with concentration ranging from 10 to 200 mg/L.

5.2.5 RNA extraction and molecular analysis

5.2.5.1 Sample preparation

In 2010-2011 growing season, young leaves of nine plants per each genotype (globe artichoke Cavi and cultivated cardoon AFB) and thesis (Salinity 0, 1 and 2) were collected in December, 10th 2010, before the salinity stress treatment (which was applied in December, 13th 2010), and five days later, in December, 17th 2010. All samples were immediately frozen in liquid nitrogen and stored at –80°C until the further analyses carried out at the Tuscia University, Department DAFNE, Italy.

5.2.5.2 RNA EXTRACTION AND MOLECULAR ANALYSIS

Globe artichoke and cardoon materials (young leaves) were ground to a fine powder in liquid nitrogen. RNA extraction was carried out using ‗Ambion PureLink RNA' kit (Invitrogen), while C-DNA was obtained using ‗ThermoScript RT-PCR System' kit (Invitrogen).

The extracted DNA were amplified with four ISSR and two SSR primer pairs. For the ISSR analysis, PCR reactions have been performed in a final volume of 10µl containing 10ng of DNA, 0.3µM primer, 100µM dNTP, 10mM Tris-HCl (pH 9.0) and 1U Taq polymerase. The amplification regime was 94°C/5min, followed by 39 cycles of 96°C/1min, 60°C/1min and 72°C/2min, and ending with an extension step of 72°C/10 min. ISSR primers where selected within those provided from the University British Columbia (called 810, 848, 841, 857); ISSR forward primers were labeled either with FAM or HEX. PCR amplicons were resolved in an ABI 3500 sequencer machine.

For the SSR analysis, the two primer pairs amplify one microsatellite *locus* isolated from *Cynara cardunculus* L. var. *scolymus* and named CLIB02 and the other found in database accessions with the name of CDAT01 (Acquadro *et al.*, 2003). The PCR reactions have been performed in a final volume of 10 µl containing 20 ng of DNA, 0.2 µM of forward primer and 2 µM of reverse primer, 100 µM dNTP, 10 mM Tris-HCl (pH 9.0) and 1U Taq polymerase. The amplification regime was 94°C/2 min, followed by 35 cycles of 94°C/30 sec, 55°C/30 sec and 72°C/1 min, and ending with an extension step of 72°C/10 min. The forward primers, of both markers typology, were labeled either with FAM or with HEX. PCR amplicons were resolved in an ABI 3500 sequencer machine and read utilizing Genemapper v 4.0.

The ISSR amplicon ranging from 50 to 500 bp were assumed to represent a single dominant *locus*; while, for SSR, fragments ranging from 200 to 250 bp were considered and assumed to represent a single co-dominant *locus*.

5.2.6 STATISTICAL ANALYSES

For morphological and biochemical characterization, data were analyzed by ANOVA, using the Generalized Linear Model of SPSS software version 15.0. Mean separations were performed by LSD analysis. Significance was accepted at $p \leq 0.05$ level.

5.3 Results

5.3.1 Morphological characterization of globe artichoke and cardoon plants grown in floating system and effect of salt stress on plant biometric traits

2009-2010 growing season

In the first growing season, no significant differences between the 0 and 2 salinity theses were found for each of both genotypes (AFB and Cavi) at each sampling time (November and December) and for all biometric traits referred to leaf and plant size (*Table 5.4*). No significant differences were also highlighted between the behaviors of AFB and Cavi, that showed, under their morphological profile, a similar growth in soilless floating system cultivation (LSD analysis, $p \leq 0.05$).

Table 5.4 Biometric traits considered for biomass characterization of AFB cultivated cardoon and Cavi globe artichoke genotypes grown in floating system

Genotypes	Thesis	Sampling Time	PD (cm)	LN (no.)	Llength (cm)	Lwidth (cm)
AFB	Salinity 0	November	50.3±4.69 ns	6.22±0.67 ns	43.00±2.45 ns	13.22±3.77 ns
AFB	Salinity 2	November	48.00±8.53 ns	6.00±0.87 ns	40.33±3.87 ns	11.78±1.39 ns
AFB	Salinity 0	December	58.00±7.45 ns	4.22±0.44 ns	52.33±10.84 ns	13.39±3.28 ns
AFB	Salinity 2	December	57.56±7.83 ns	4.33±0.50 ns	54.67±8.09 ns	12.11±1.54 ns
Cavi	Salinity 0	November	45.67±7.66 ns	5.56±0.73 ns	37.44±5.27 ns	10.44±1.51 ns
Cavi	Salinity 2	November	38.22±5.87 ns	5.44±0.53 ns	33.56±2.46 ns	11.78±1.99 ns
Cavi	Salinity 0	December	53.22±11.65 ns	3.78±0.44 ns	51.11±5.95 ns	10.72±1.82 ns
Cavi	Salinity 2	December	51.44±7.88 ns	4.44±0.53 ns	52.50±4.46 ns	11.89±2.57 ns

Values are the means of measurements on nine plants ± SD; ns= not significant per p≤0.05 (LSD analysis)

Also as regards FWAP, DWAP, FWRootP, DWRootP, FWRootB and DWRootB, no significant differences were found between the 0 and 2 salinity theses for both AFB and Cavi at each sampling times (November and December). Considering instead the fresh and the dry weight of biomass produced per board (FWB and DWB), significant differences were found between the two salinity theses of AFB at November sampling

time; as for Cavi at both sampling times, no significant differences were highlighted for AFB in December (*Table 5.5*). As regards the genotypes, no significant differences were evidenced for FWAP and DWAP for both salinity theses and sampling times (LSD analysis, $p \leq 0.05$). On the contrary, AFB genotype had significant higher values of FWRootP and DWRootP respect to Cavi for both salinity theses and sampling times. Considering biomass production, no significant differences related to FWB and FWRootB have been evidenced between genotypes; AFB-Salinity 0 was characterized by values significantly higher of the same traits respect to Cavi-Salinity 0 at both sampling times. According to LSD analysis ($p \leq 0.05$), significant differences between both Salinity 2 theses of AFB and Cavi were found for these last traits at both sampling times (*Table 5.5*). It is important to consider that, at the first sampling time, only the 55.12 and 58.67% of the total plants were survived for AFB and Cavi, respectively; at the second sampling time, only the 49.67 and 43.26 and % of plant survived for AFB and Cavi, respectively.

Table 5.5 Evaluation of aerial and root biomass production of AFB cultivated cardoon and Cavi globe artichoke genotypes grown in floating system

Genotype	AFB				Cavi			
Thesis	Salinity 0	Salinity 2	Salinity 0	Salinity 2	Salinity 0	Salinity 2	Salinity 0	Salinity 2
Sampling Time	November		December		November		December	
FWAP (g)	11.88±1.09ns	13.83±1.81ns	10.81±1.30ns	14.16±3.62ns	10.77±1.88ns	11.89±1.82ns	12.60±4.10ns	17.57±9.92ns
DWAP (g)	0.99±0.02ns	1.01±0.11 ns	1.37±0.09ns	1.42±0.51ns	0.69±0.11ns	0.76±0.14ns	0.70±0.22ns	1.06±0.67ns
FWB (g)	842.99±147.56a	608.33±56.63b	659.41±216.27ns	599.79±115.27ns	664.69±188.90ns	717.32±110.37ns	617.77±161.09ns	720.77±259.62ns
DWB (g)	69.96±4.97a	44.33±2.91b	84.00±27.91ns	60.33±16.21ns	42.91±10.58ns	46.09±8.98ns	34.66±8.63ns	43.58±19.3ns
FWRootP (g)			5.98±0.30ns	8.74±1.61ns			5.50±0.84ns	7.40±1.67 ns
DWRootP (g)			0.45±0.13ns	0.64±0.14ns			0.36±0.09ns	0.49±0.28ns
FWRootB (g)			365.33±187.75ns	370.10±43.03ns			269.94±58.34ns	303.78±124.69ns
DWRootB (g)			27.04±13.08ns	27.36±3.01ns			17.81±4.22ns	20.20±10.66ns
N survived plant (no.)	70.67±7.63a	43.98±5.86b	61.00±13.22a	42.33±2.08b	61.71±5.00ns	60.33±16.78ns	49.00±1.73ns	41.00±7.81ns

Values for FWAP, DWAP, FWRootP and DWRootP are the means of measurements on 3 plants ± SD, while values for FWB, DWB, FWRootB and DWRootB are the means of measurements of 30 plants

ns= not significant, different letters indicate statistical significant differences between theses for each genotype and sampling time per p≤0.05(LSD analysis)

2010-2011 GROWING SEASON

In the second growing season, no significant differences (p≤0.05) have been found for all biometric traits considered between both the salinity theses and between genotypes (AFB *vs* Cavi) at each sampling time (December and January) (*Table 5.6*). Considering these traits, no significant differences there were between AFB and Cavi, which showed a similar plant morphological profile (LSD analysis, p≤0.05) in all salinity theses.

Table 5.6 Biometric traits considered for biomass characterization of AFB cultivated cardoon and Cavi globe artichoke genotypes grown in floating system

Genotypes	**Thesis**	**Sampling Time**	**LN (no.)**	**PD (cm)**	**Llength (cm)**	**Lwidth (cm)**
AFB	Salinity 0	December	5.33±0.58 ns	52.33±2.88 ns	48.86±1.20 ns	11.42±1.02 ns
AFB	Salinity 1	December	5.00±0.00 ns	51.45±2.61 ns	48.28±0.88 ns	11.78±0.53 ns
AFB	Salinity 2	December	4.67±1.15 ns	54.10±2.52 ns	47.26±1.75 ns	13.29±0.72 ns
AFB	Salinity 0	January	4.67±0.58 ns	53.01±2.63 ns	47.96±0.87 ns	12.04±1.00 ns
AFB	Salinity 1	January	4.67±0.58 ns	50.41±0.74 ns	47.81±1.60 ns	12.02±0.61 ns
AFB	Salinity 2	January	4.67±0.58 ns	51.01±2.66 ns	48.28±0.60 ns	12.18±0.52 ns
Cavi	Salinity 0	December	5.33±1.15 ns	52.14±1.87 ns	47.42±1.97 ns	11.83±0.53 ns
Cavi	Salinity 1	December	4.67±0.58 ns	50.21±1.73 ns	47.44±1.72 ns	12.41±1.43 ns
Cavi	Salinity 2	December	5.00±0.00 ns	51.14±3.66 ns	47.92±0.96 ns	12.33±0.95 ns
Cavi	Salinity 0	January	5.00±0.00 ns	53.47±1.90 ns	47.80±0.45 ns	12.29±0.78 ns
Cavi	Salinity 1	January	5.33±0.58 ns	50.41±1.36 ns	47.22±1.69 ns	11.85±1.04 ns
Cavi	Salinity 2	January	5.33±0.58 ns	50.05±1.09 ns	48.29±0.94 ns	12.21±0.69 ns

Values are the means of measurements on nine plants ± SD; ns= not significant, different letters indicate statistical significant differences between theses for each genotype and sampling time per p≤0.05(LSD analysis)

The results of the aerial and root biomass production are shown in Table 5.7. Significant differences related to FWAP and FWB traits were found only between the 0 and 2 Salinity theses of Cavi at the December sampling time. Instead, no significant differences were highlighted for DWAP among theses for both genotypes and sampling times. As regards DWB, significant differences between Salinity theses 0 and 2 of Cavi at both sampling times (December and January) have been also evidenced. As regards root biomass production, FWRootP and FWRootB were not significantly affected by salinity theses for both genotypes and sampling times. On the contrary, DWRootP and

DWRootB were significantly different among theses for Cavi at January sampling time (*Table 5.7*).

As regards the genotypes, the cultivated cardoon AFB and the globe artichoke Cavi significantly differed for all traits referred to aerial and root biomass production in December while, at January sampling time, significant differences between genotypes have been maintained for all traits except for FWAP-Salinity 2, DWAP-Salinity 1, FWRootP-Salinity 0 and DWRootP-Salinity 1 (LSD analysis, $p \leq 0.05$). At the first sampling time of the 2nd growing season, the 79.00 and 56.83% of total plants survived for AFB and Cavi, respectively; this number was reduced to 66.36 and 39.93% for the same genotypes at the second sampling time.

Table 5.7 Evaluation of aerial and root biomass production of AFB cultivated cardoon and Cavi globe artichoke genotypes grown in floating system

Genotype	AFB						Cavi					
Thesis	Salinity 0	Salinity 1	Salinity 2	Salinity 0	Salinity 1	Salinity 2	Salinity 0	Salinity 1	Salinity 2	Salinity 0	Salinity 1	Salinity 2
Sampling Time	December			January			December			January		
FWAP (g)	19.03±1.52 ns	17.98±2.22 ns	14.69±3.90 ns	19.56±3.59 ns	16.92±1.70 ns	12.58±4.52	12.36±1.23 a	12.14±2.76 ab	7.80±2.12 b	12.79±3.83 ns	11.03±2.00 ns	8.49±3.36 ns
DWAP (g)	1.41±0.16 ns	1.31±0.43 ns	1.12±0.31 ns	1.70±0.35 ns	1.54±0.13 ns	1.19±0.41 ns	0.63±0.21 ns	0.75±0.09 ns	0.47±0.05 ns	1.26±0.59 ns	1.63±0.15 ns	0.88±0.35 ns
FWB (g)	1572.60±212.80 ns	1434.34±172.50 ns	1228.00±367.00 ns	1349.42±262.40 ns	990.04±234.40 ns	1001.14±221.60	898.28±246.60 a	688.34±215.20 ab	375.00±141.26 b	605.94±297.20 ns	403.34±94.18 ns	345.42±18.00 ns
DWB (g)	115.76±13.80 ns	102.82±21.20ns	93.34±29.52 ns	117.26±31.44 ns	90.10±19.84 ns	95.02±18.14 ns	46.02±5.08 a	42.66±9.50 ab	22.56±2.66b	59.80±14.26 a	43.06±7.84 ab	36.06±3.62 b
FWRootP (g)				2.23±1.17 ns	3.31±0.54 ns	2.74±1.00 ns				0.70±0.30 ns	0.85±0.33 ns	0.65±0.21 ns
DWRootP (g)				0.32±0.00 ns	0.32±0.04 ns	0.39±0.03 ns				0.04±0.03 b	0.19±0.04 a	0.03±0.01 c
FWRootB (g)				153.90±73.98 ns	193.72±39.40 ns	218.62±7.42 ns				33.42±13.78 ns	31.22±2.52 ns	26.68±2.52 ns
DWRootB (g)				22.12±3.68 ns	18.72±9.36 ns	31.03±6.92 ns				2.28±1.60 b	7.28±1.66 a	1.26±0.18 c
N survived plant (no.)	82.66±4.62 ns	80.66±7.57 ns	83.34±6.02 ns	68.98±4.16a	58.51±4.00b	79.58±6.81a	72.66±5.86 a	56.66±3.05 ab	48.00±2.64 b	47.37±8.54 ns	36.56±4.93 ns	40.66±6.11 ns

Values for FWAP, DWAP, FWRootP and DWRootP are the means of measurements on 3 plants ± SD, values for FWB, DWB, FWRootB and DWRootB are the means of measurements of 30 plants
ns= not significant, different letters indicate statistical significant differences between theses for each genotype and sampling time per $p \leq 0.05$(LSD analysis)

5.3.2 Biochemical characterization of globe artichoke and cardoon plants grown in floating system and evaluation of salt stress on biocompound content

5.3.2.1 Phenolic compounds

2009-2010 growing season

The phenolic content of AFB cultivated cardoon and Cavi globe artichoke biomasses (leaves), obtained from plants grown in floating system, is shown in Table 5.8. In particular, the influence of salinity stress (Salinity 2) on phenolic profile of each genotype was compared at each sampling times (November and December).

Among the biocompounds analyzed by HPLC, caffeic acid, taxifolin, silybin, apigenin and cynarin were not detected in all samples.

As regards chlorogenic acid content of biomass, significant differences between theses of both genotypes have been detected at November sampling time. In particular, both AFB and Cavi showed the highest values of chlorogenic acid in the thesis Salinity 2 respect to Salinity 0 (2.31 and 1.51 mg g^{-1} DM, respectively) (*Table 5.8*). In December, no significant differences in chlorogenic acid content of biomass were found between the same theses of AFB, while Cavi showed a significant higher value of this compound in Salinity 2 thesis (0.46 mg g^{-1} DM) respect to Salinity 0 (0.27 mg g^{-1} DM).

At both sampling times (November and December), both AFB and Cavi genotypes stressed by salinity (Salinity 2) were characterized by the highest values of 1,5-*O*-dicaffeoylquinic content, showing a positive influence of stress treatment on this compound.

For cynaroside, no significant differences between theses were found of both genotypes and sampling times, except for AFB at November sampling time (3.46 and 2.22 mg g^{-1} DM for Salinity 0 and Salinity 2, respectively).

At December sampling time, no significant differences were highlighted between theses for both genotypes for luteolin biomass content; while, at November, both AFB and Cavi showed the highest values of this compound in Salinity 2 (0.55 and 0.30 mg g^{-1} DM for AFB and Cavi, respectively) respect to Salinity 0.

As regards the genotypes, AFB cultivated cardoon was very interesting respect to Cavi globe artichoke for the phenolic production in floating system. In particular, AFB was characterized by the highest values of chlorogenic acid content in the Salinity 2 thesis of November respect to Cavi (2.31 mg g^{-1} DM). Also for 1,5-*O*-dicaffeoylquinic acid, AFB genotype provided the highest values for both theses and sampling times respect to

Cavi (1.81 mg g^{-1} DM in November Salinity 0, 7.57 mg g^{-1} DM in November Salinity 2 and 0.55 mg per g DM in December Salinity 2). As regards cynaroside and luteolin, AFB was characterized by the highest contents for both theses in December while, in November, only the Salinity 0 and 2 showed the highest values of cynaroside (3.46 mg g^{-1} DM) and luteolin (0.55 mg g^{-1} DM), respectively.

Table 5.8 Biocompound content (expressed as mg/g DM) of AFB cultivated cardoon and Cavi globe artichoke plants

Chlorogenic acid (mg/g DM)	***Thesis***	***November***	***December***
AFB	Salinity 0	0.27± 0.03b	0.26± 0.02ns
AFB	Salinity 2	2.31± 0.19a	0.32± 0.07ns
Cavi	Salinity 0	0.36±0.03b	0.27± 0.02b
Cavi	Salinity 2	1.51± 0.43a	0.46± 0.03a
1,5-O-dicaffeoylquinic acid (mg/g DM)	***Thesis***	***November***	***December***
AFB	Salinity 0	1.81±0.67b	nd
AFB	Salinity 2	7.57±1.33a	0.55±0.11a
Cavi	Salinity 0	nd	nd
Cavi	Salinity 2	2.08±0.56a	0.17±0.02a
Cynaroside (mg/g DM)	***Thesis***	***November***	***December***
AFB	Salinity 0	3.46±0.40a	0.50±0.20ns
AFB	Salinity 2	2.22±0.48b	0.48±0.10ns
Cavi	Salinity 0	1.71±0.21ns	0.15±0.11ns
Cavi	Salinity 2	2.62±0.89ns	0.17±0.01ns
Luteolin (mg/g DM)	***Thesis***	***November***	***December***
AFB	Salinity 0	0.13±0.00b	0.40±0.15ns
AFB	Salinity 2	0.55±0.04a	0.35±0.05ns
Cavi	Salinity 0	0.10±0.01b	0.21±0.02ns
Cavi	Salinity 2	0.30±0.02a	0.24±0.07ns

Different letters indicate statistical significant differences between theses for each genotype and sampling time per $p \leq 0.05$ (LSD analysis), ns=not significant, nd=not detected

2010-2011 GROWING SEASON

Biomass phenolic content of AFB cultivated cardoon and Cavi globe artichoke is reported in Table 5.9. Also in the second growing season, caffeic acid, taxifolin, silybin, apigenin and cynarin were not detected by HPLC analysis in all samples. The effect of stress treatments (Salinity 1 and 2) on phenolic profile was investigated for each genotype (AFB and Cavi) and at each sampling time (December and January).

The highest biomass chlorogenic acid contents were found in the Salinity 2 (standard solution supplemented with 2.9g/L of NaCl) for both genotypes and at each sampling time (6.22 and 9.74 mg g^{-1} DM for AFB in December and January, and 6.14 and 6.23 mg g^{-1} DM for Cavi in December and January, respectively). These results confirmed the positive influence of salinity stress on chlorogenic acid production, already observed during the first experimental year. As regards 1,5-*O*-dicaffeoylquinic acid, the Salinity 2 of both genotypes and at each sampling time was characterized by the highest biomass content of this compound except for AFB in January. No significant differences among theses were observed in biomass cynaroside content for both genotypes and sampling times except for Cavi genotype in January which presented the highest value of such compound in the Salinity 2 (1.77 mg g^{-1} DM). Luteolin has not been detected in any Cavi samples and only in AFB samples of theses Salinity 0 and 1 in December. As regards genotypes, AFB cultivated cardoon provided the highest phenolic compound production respect to Cavi also in this second experimental year (LSD analysis, $P \leq 0.05$). In particular, significant differences between genotypes were found for luteolin, 1,5-*O*-dicaffeoylquinic acid and cynaroside biomass content for all theses and sampling times. As regards chlorogenic acid, AFB showed the highest content of this compound respect to Cavi in Salinity 0 at January sampling time (4.28 mg g^{-1} DM).

Table 5.9 Biocompound content (expressed as mg/g DM) of AFB cultivated cardoon and Cavi globe artichoke genotype plants

Chlorogenic acid (mg/g DM)	***Thesis***	***December***	***January***
AFB	Salinity 0	2.50±0.25c	4.28±1.46b
AFB	Salinity 1	4.10±0.26b	9.88±5.06ab
AFB	Salinity 2	6.22±1.34a	9.74±3.73a
Cavi	Salinity 0	2.51±0.70b	0.02±0.00b
Cavi	Salinity 1	3.61±1.11b	4.74±1.19a
Cavi	Salinity 2	6.14±0.18a	6.23±2.14a
1,5-O-dicaffeoylquinic acid (mg/g DM)	***Thesis***	***December***	***January***
AFB	Salinity 0	1.60±0.27ab	3.43±1.15ns
AFB	Salinity 1	1.53±0.14b	2.22±0.98ns
AFB	Salinity 2	2.47±0.61a	3.40±1.05ns
Cavi	Salinity 0	0.33±0.12b	0.17±0.06b
Cavi	Salinity 1	0.62±0.31ab	0.73±0.16a
Cavi	Salinity 2	0.68±0.16a	0.63±0.20a
Cynaroside (mg/g DM)	***Thesis***	***December***	***January***
AFB	Salinity 0	1.28±0.36ns	1.87±0.29ns
AFB	Salinity 1	1.93±0.42ns	2.59±0.49ns
AFB	Salinity 2	1.93±0.30ns	2.54±0.85ns
Cavi	Salinity 0	0.78±0.27ns	nd
Cavi	Salinity 1	1.33±0.39ns	1.08±0.07b
Cavi	Salinity 2	1.21±0.13ns	1.77±0.20a
Luteolin (mg/g DM)	***Thesis***	***December***	***January***
AFB	Salinity 0	nd	0.67±0.33ns
AFB	Salinity 1	nd	0.78±0.11ns
AFB	Salinity 2	0.15±0.03a	0.78±0.32ns
Cavi	Salinity 0	nd	nd
Cavi	Salinity 1	nd	nd
Cavi	Salinity 2	nd	nd

Different letters indicate statistical significant differences among theses for each genotype and sampling time per $p\leq0.05$(LSD analysis), ns=not significant, nd=not detected

5.3.2.2 INULIN

Inulin content of the two genotypes varied from 14.12 to 110.29 mg g^{-1} DM and from 42.81 to 50.41 mg g^{-1} DM in AFB and Cavi roots, respectively (*Table 5.10*).

In AFB, significant differences on inulin content among salt levels were found. In particular, the Salinity 2 provided the highest value for this compound while the Salinity 0 the lowest one; its content in the Salinity 1 was significantly different respect to the Salinity 2 and 0. For Cavi genotype, no significant differences were observed in inulin content among the two salinity levels 1 and 2. Also for inulin, AFB cultivated cardoon allowed to obtain the highest inulin production respect to Cavi in the Salinity 1 and 2 (LSD analysis, p≤0.05).

Table 5.10 Inulin content (expressed as mg g^{-1} DM) of AFB cultivated cardoon and Cavi globe artichoke genotype root grown in floating system

Inulin (mg/g DM)	***Thesis***	***January***
AFB	Salinity 0	15.12±3.08c
AFB	Salinity 1	87.34±13.09b
AFB	Salinity 2	110.29±8.16a
Cavi	Salinity 0	45.88±14.37ns
Cavi	Salinity 1	50.41±17.33ns
Cavi	Salinity 2	42.81±17.80ns

Different letters indicate statistical significant differences among theses for each genotype and sampling time per p≤0.05(LSD analysis), ns=not significant

5.3.3 MOLECULAR RESPONSE TO STRESS TREATMENTS

A set of four ISSR and two SSR markers have been used in order to evaluate the effect of salinity stress (Salinity 1 and 2) on different genetic backgrounds (globe artichoke and cardoon plants). A quantitative analysis of the results on the expression experiments was undertaken. The number of amplicons per ISSR marker were 115, 289, 357 and 290 for 810, 841, 848 and 857, respectively; while for SSR markers the number of alleles were 2 for both CLIB02 and CDAT01. Unfortunately, the results obtained by ISSR revealed a huge level of polymorphism also between replications and, for this reason, the markers was considered, in this case, not reliable the evaluate differences between the two salinity stress treatments.

On the contrary, 2 marker associations were identified between CLIB02 and CDAT01 SSR markers and salinity stress treatments (Salinity 1 and 2). In particular there were no differences between the controls (Salinity 0) while changes are reveled for the other 2

salinity levels, with major changes at higher salt concentration. Indeed, the level of each transcript in AFB and Cavi plants under different salinity theses increased in response to salt stress compared to the control (Salinity 0) (*Figure 5.4*).

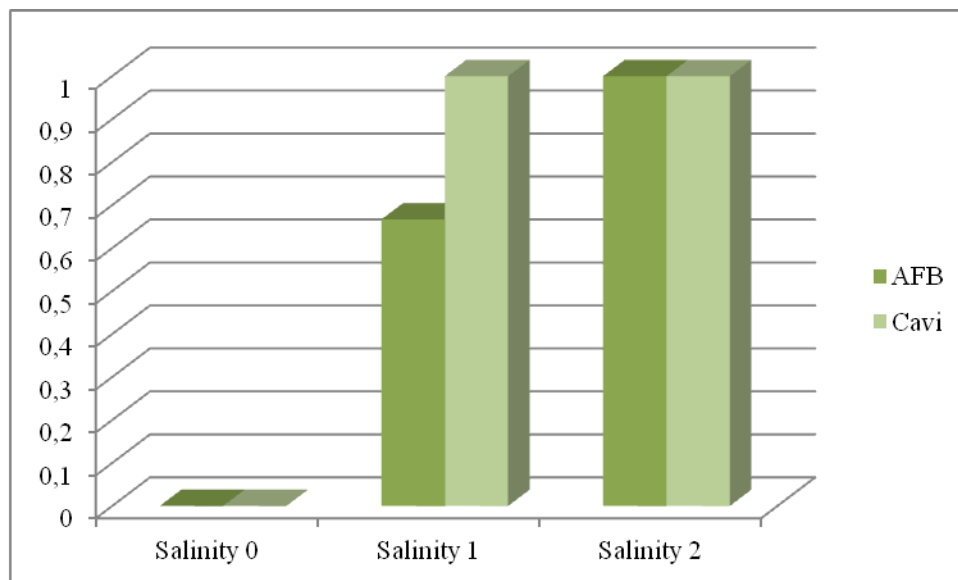


Fig. 5.4 Expression analysis (expressed as percentage) of CLIB02 and CDAT01 *markers in Cavi and AFB leaves under salt stress (Salinity 1 and 2) using PCR*

5.4 Discussion

In the present PhD work, cultivation of globe artichoke and cardoon plants in hydroponic system has been carried out with the aim of producing biomass (leaves and roots) for pharmaceutical compound extraction (phenolic compounds from leaves and inulin from roots). Both globe artichoke (Cavi) and cardoon (AFB) genotypes showed a quite good adaptability to floating system. In fact, some plants have collapsed within two months after sowing due to the disintegration of roots, as observed by Maggini *et al.* (2012) in *E. angustifolia*. This may depend on the physiological limit of adaptation of the species to the floating system, since a high number of collapsed plants was present also in the control (Salinity 0). Certainly, salt stress may have had some influence on the loss of plants. Indeed, the number of collapsed plants was generally higher in the salt solutions (Salinity 1 and 2) respect to the control (Salinity 0). As reported by Rajendran *et al.* 2009, plants have evolved three main mechanisms to tolerate salinity stress: i) osmotic tolerance, a mechanism not fully understood, which involves plant‘s ability to tolerate the drought aspect of salinity stress; ii) ionic tolerance based on the exclusion of Na^+ from leaves; and iii) ionic tolerance based on Na^+ compartmentalization in vacuoles or in other specific cell types where the damage to the

plant metabolism is minimized. However, high salinity levels cause serious damages to plants inducing Ca deficiency and detrmining root pressure and transpiration reduction (Francois *et al.,* 1991). In particular, sodium (Na^+) and chloride (Cl^-) are the two key ions responsible of osmotic and ion-specific damages that provoke crop growth reduction (Munns and Tester, 2008) and oxidative damages. The osmotic effect inhibits cell expansion and division as well as stomatal closure (Munns and Tester, 2008); ionic stress causes premature senescence, chlorosis and necrosis of older leaves due to sodium (Na^+) which interferes with protein synthesis and enzyme activity (Blaha *et al.*, 2000). Several authors reported plant size reduction and leaf restriction as principal response to salt stress in different species (as reported in *Zea mays* L. by Hichem *et al.,* 2009; in *Nigella sativa* L. by Bourgou *et al.,* 2012; and in *Capsicum annum* L. by Yiu *et al.,* 2012). In our experiments, moderate levels of salinity have been used and no effect on biometric characteristic of the survived plants of AFB and Cavi has been observed after salinity stress application in both growing seasons. Indeed, according to salt tolerance classification scheme of Maas and Hoffman (1977), who classified artichoke as moderately tolerant, no significant differences between salinity levels were found in both growing seasons and in both genotypes under morphological profile (i.e. leaf number, plant diameter, leaf length and width, fresh and dry weight of the plants). This may also depend on the stress duration but also on the species and genotypes. However, as observed by Francois (1995), globe artichoke vegetative growth was more tolerant to salt stress than bud production. Indeed, the author reported no vegetative growth reduction of globe artichoke plants respect to bud yield which had been reduced by 20% at EC of 7.8 dS m^{-1}. Also Graifenberg *et al.* (1993) reported that the globe artichoke plant fresh weight was less sensitive than yield to soil and water salinity and, only at EC up to 7.3 dS m^{-1}, a decrease of 50% in FM plant accumulation was observed. In particular, marginal leaf necrosis and brown areas were observed by the author at EC higher than 3.63 dS m^{-1}.

In our work, biomass (leaves) production was satisfactory (range of 599.79-842.99 g FM per board for AFB and range of 617.77-720.77 g FM per board for Cavi in 2009-2010; range of 990.04-1572.60 g FM per board for AFB and range of 345.42-898.28 g FM per board for Cavi in 2010-2011), taking into account that the present experiment represented the first experience of cultivation of globe artichoke and cardoon plants in floating system. In addition, biomass raw material obtained in floating system presented many advantages compared to that collected in open field, mainly for the absence of

microorganisms and soil contamination, as reported by other authors (Rodriguez-Hidalgo *et al.,* 2010; Maggini *et al.,* 2012).

In agreement with other authors (Incerti *et al.,* 2009), salt stress did not induce variations in the qualitative polyphenolic profile of the plants of both genotypes, confirming that the genetic component represents the primary factor while the quantitative phenolic composition can be affected by external factors (i.e. abiotic and biotic stress). Indeed, the biochemical characterization evidenced that the dominant phenolic acids and flavonoids were the same in both control (Salinity 0) and NaCl theses (Salinity 2 in 2009/2010 and Salinity 1 and 2 in 2010/2011). In particular, chlorogenic acid and 1,5-*O*-dicaffeoylquinic acid represented the main caffeic acid derivatives; luteolin and cynaroside were the dominant flavonoids. In both growing seasons, chlorogenic acid and 1,5-*O*-dicaffeoylquinic acid content (expressed as mg g^{-1} DM) in AFB and Cavi leaves were positively influenced by salt stress (*Tables 5.8* and *5.9*). The same positive effect was also evidenced for luteolin content (expressed as mg per g DM) of stressed plants that was enhanced by NaCl treatment in both AFB and Cavi in November of the first growing season and only in AFB in December of the second growing season (*Tables 5.8* and *5.9*). These results are in agreement with Rezazadeh *et al.* (2012) who found an increase in phenolic content of 20% in globe artichoke leaves of plants stressed using salt concentration of 6.9 dS m^{-1}. On the contrary, cynaroside content has not been enhanced by salinity stress and no significant differences among salt levels were evidenced for the majority of the samples analyzed. However, as reported by Rezazadeh *et al.* (2012), flavonoids had a significant reduction at higher salinity levels and this behavior has been also observed in phenolic content that decreased at too high level of salinity.

Our findings confirmed that, also in *Cynara* spp., polyphenols play an important role in protecting cell against the oxidative stress due to external factors (i.e. salinity). These observations are also in line with other authors who reported an increase of phenolic content due to salt stress treatments in other species (Incerti *et al.,* 2009; Maggini *et al.,* 2012; Bourgou *et al.,* 2012; Hichem *et al.,* 2012). As observed by several authors (Abdi and Ali, 1999; Chanwitheesuk *et al.,* 2005; Ksouri *et al.,* 2007), plants subjected to biotic/abiotic stresses produce Reactive Oxygen Species (ROS) which are inevitably produced when aerobic or photosynthetic metabolism is impaired by environmental stresses and cause cellular damages leading to the increase of secondary metabolites, such as polyphenols, for defending cell structures against oxidation.

As regards root production and evidenced by Maggini *et al.* (2012), floating system was very suitable method for plants cultivated for obtaining roots. Indeed, in this system, roots grow directly plunged in the nutrient solution and, for this reason, can be easily harvested. In the present experiment, no significant differences were found among salt levels under root morphological profile; root production was satisfactory (average values of 278.23 and 158.65 g FM per board for AFB and Cavi, respectively) and raw material was easily collected at the end of the trial in both growing seasons.
As regards inulin content of roots, no significant differences among salt levels were found in Cavi (average value of 32.43 mg g^{-1} DM). On the contrary, salt stress (Salinity 2) enhanced the inulin content (110.29 mg g^{-1} DM) of AFB respect to that obtained in the control (Salinity 0) (15.12 mg g^{-1} DM). Inulin, as known, represents a long-term reserve carbohydrates in underground storage organs and plays also other roles in the plants such as regulation of osmosis during flower opening and protection of plants against cold and drought stress through membrane stabilization (Valluru and Van den Ende, 2008). Some studies conducted in *Cichorium intybus* L. (De Roover *et al.*, 2000; Monti *et al.,* 2005) on the impact of water stress on the plants demonstrated that water shortage increased glucose, fructose, and sucrose concentrations in the roots and leaves of stressed plants, leading to increase fructan concentrations in the roots. Also Raccuia and Melilli (2007a), observed in *C. cardunculus* L. an increase of inulin content in the roots to prevent drought stress in winter time. In our experiments, induction of inulin synthesis in cardoon roots of salinity theses was evidenced and this may represent a defense response of the plant against osmotic stress to maintain a stable ion balance and to avoid ion injury.
Inulin content was lower than that found in roots of *Cynara* spp. after about 1 year of open field cultivation by other authors (Raccuia and Melilli, 2004); but the age of the plant must be considered. Indeed, Raccuia and Melilli (2010) reported a seasonal dynamics of inulin content, ranging from 0 to 367 mg g^{-1} DM, in the first year of cultivation of globe artichoke and cardoon genotypes. In particular, during the early phases of plant growth, inulin content was very low and entirely comparable with our findings.
Preliminary molecular studies carried out in the present work evidenced that salinity stress induce transcription changes of genes involved in the phenylpropanoid pathway, which probably contribute to confer tolerance to salt stress. However, the mechanism is still under studied (Comino *et al.,* 2007, 2009; Moglia *et al.*, 2009; Sonnante *et al.*,

2011) and only future researches should clarify the interactions existing between external factors and gene induction.

In conclusion, results so far obtained, indicate that floating system could be a suitable technique for biomass and biocompound production and future research programs should be developed to reduce the number of collapsed plants and increase plant growth rate.

General conclusion

GENERAL CONCLUSION

The present PhD work was carried out within the CYNARES (European Genetic Resources of *Cynara* spp.) international research project on the collection, characterization, conservation and valorization of *Cynara* spp. autochthonous germplasm from Italy, Spain and France. Few studies on genetic identity, characterization and discrimination of other genotypes grown in nearby areas have been performed in Italy, where homonyms and lack of clarity in the nomenclature of spring genotypes often occurs.

Since Italian traditional *Cynara* spp. germplasm is subjected to genetic erosion due to the increasing cultivation of micropropagated clones and new seed-propagated hybrids F_1, PhD program was developed with the main objectives of identifying, preserving and valorizing traditional genetic resources of Italian globe artichoke and cardoon belonging to the Tuscia University-ENEA joint germplasm collection. For the first time, these autochthonous genetic resources have been considered not only for food production but also for alternative non-food industrial applications. In particular, the use of biomass for biocompound extraction was the focused point of this research program.

In this context, nine spring local landraces from different Italian central areas, cultivated for centuries by local farmers as example of man-made *in-situ* selection and up now never enough studied, and ten ‗Romanesco‘ clones, have been characterized in terms of morphological profile; this allowed also investigations on the genetic variability existing within and among genotypes. Morphological characterization, performed using UPOV descriptors, allowed to identify the genetic resources and to select for food production three ‗Romanesco‘ clones (S17, S22 and S23), which were characterized by different earliness, head size and weight. These genotypes have been proposed and accepted for registration at the Italian National Variety Register within a collaboration among Tuscia University, ENEA and ARSIAL (Latium Regional Agency for the Development and the Innovation of Agriculture). In addition, other two genotypes from Campania region (Bianco di Pertosa and Castellammare) have been distinguished for their high productivity in terms of head number.

As regards cardoon germplasm, seven Italian genotypes of cultivated cardoon and one of wild cardoon, have been characterized in terms of morphological profile adopting some of the UPOV descriptors used for globe artichoke. The genetic variability existing within and among genotypes has been also investigated. Our findings showed that the descriptors considered could allow only discrimination between the two *C. cardunculus*

botanical varieties *altilis* and *sylvestris*. For this reason, further descriptors more suitable for cardoon species should be considered and applied.

In order to valorize *Cynara* spp. germplasm using biomass and biocompound production, optimization of Microwave Assisted Extraction (MAE) and Accelerated Solvent Extraction (ASE) methods was achieved using a two-level full factorial design; influence of independent variables along with their interactions on phenolic compounds recovery has been also evaluated. The optimal polyphenol extraction conditions were determined for both MAE and ASE methods (150°C, 15 minutes and 100 bars for ASE and at 100°C, 15 minutes and 1:15 solid:liquid ratio for MAE). ASE, at the optimal conditions, has been chosen as method for the further extractions because it shows the best performance in polyphenol extraction from *Cynara* spp. biomass. The modern extraction techniques (MAE and ASE), compared to the traditional ones (Soxhlet and maceration), revealed the best performances for the extraction of phenolic compounds from *C. cardunculus* biomass.

In order to find the optimal plant stage for the biomass production, plant development cycle of two genotypes (AFB cultivated cardoon and Cavi globe artichoke) was described using some agro-morphological traits. In correspondence with very specific phases of the plant growth, the kinetics of dry matter accumulation in the plant was determined. Harvesting of the main central flower head, corresponding to April in central Italian environmental conditions, was considered as the optimal stage for the highest biomass production under open field conditions.

More knowledge was achieved on the variation of the major phenolic compounds in AFB and Cavi plants during the two growing seasons considered. The highest phenolic content (expressed as mg per g DM) was that of autumn and spring sampling times compared to that obtained in winter sampling time. Considering the complementarities of the phenolic content and the dry weight of the plant at different developmental stage, the stage ‗flower head appearance' (April in our experiments) represents also the best compromise between high biomass production and the highest yield of phenolic compounds. For this reason, this physiological stage is advisable for pharmaceutical use.

Globe artichoke and cardoon genotypes were characterized under biomass production profile and some genotypes (S5, S18, Ascolano, Campagnano, Pisa, Bianco di Pertosa for globe artichoke and AFFG and AFM for cardoon), characterized by interesting dry matter accumulation, were selected for this purpose. Also biomass and heads of globe

artichoke and cardoon genotypes were biochemically characterized and the results obtained allowed the selection of genotypes for food (heads) (Montelupone B and Bianco di Pertosa) or non-food (biomass for pharmaceutical compound extraction) (Ascolano for globe artichoke and AFGR for cardoon) destination. In addition, the possibility of dual-production (food and non-food) was investigated for globe artichoke germplasm and some genotypes characterized by good head production, high biomass yield and interesting phenolic content were selected (Ascolano and Campagnano).

In order to find an experimental system capable of obtaining biomass (leaves and roots) and biocompound production in a reproducible way and with standardized production process, the hydroponic technique was set up under greenhouse conditions. Both globe artichoke and cardoon genotypes showed a moderate adaptability to soilless culture. On the basis of our findings, floating system could be considered profitably for the production of raw materials aimed at the extraction of polyphenols (from leaves) and inulin (from roots). However, further investigations are requested for the solution of problems still encountering with the globe artichoke and cardoon soilless culture.

More knowledge was also achieved on the phenylpropanoid pathway induction due to salinity stress in *Cynara* ssp. plants. In floating system, the leaf content of some phenolic compounds (chlorogenic acid, 1,5-*O*-dicaffeoylquinic acid and luteolin) was enhanced by salt stress treatment both in cardoon and globe artichoke genotypes. Preliminary molecular studies were carried out, these suggesting that salinity stress could induce transcription changes of genes involved in the phenylpropanoid pathway. Obviously, these findings must be verified.

Many investigations have been carried out during this PhD work, but some scientific aspects can further be developed:

- further selection of clones within traditional germplasm would be carried out for the release of new varieties or for the development of homogeneous lines useful as parents of hybrid combinations in plant breeding programs;
- other morphological descriptors would be applied to cardoon germplasm collection to well identify and characterizing plant material;
- further self-pollinations of cardoon genotypes should be done to reduce heterozygosis phenomena and to select the more stable genotypes for the release of new varieties;

- the evaluation of agricultural practices (irrigation, fertilization), as well as of plant density, should be carried out with the aim of optimizing biomass production in open field conditions;
- future research programs should be developed to optimize the production of *Cynara* spp. biomass in floating system.

Nevertheless, the results up now obtained open new horizons for possible alternative uses of globe artichoke and cardoon, species that at least in Italy, are currently focused mostly on the food use. Biomass is up now left in the field without any use and profit. A dual purpose-production could be considered as a real perspective to further increase farmer‘s income.

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ANNEX 1

The regression equations for MAE and ASE apigenin, luteolin and polyphenol contents (y) of leaf extracts resulted as follows:

MAE

$Y(luteolin) = 71.32 + 29.84\ Xi + 7.17\ Xj + 5.08\ Xk - 0.41\ XiXj + 3.96\ XiXk + 4.84\ XjXk$

$Y(apigenin) = 81.75 + 6.65\ Xi + 1.09\ Xj + 1.89\ Xk - 2.30\ XiXj - 2.02\ XiXk + 2.67\ XjXk$

$Y(total\ polyphenols) = 4.67 + 1.32\ Xi + 0.63\ Xj + 0.61\ Xk + 0.29\ XiXj + 0.33\ XiXk + 0.09\ XjXk$

ASE

$Y(luteolin) = 137.10 + 70.02\ Xi - 3.06\ Xj + 18.05\ Xk - 1.54\ XiXj + 5.91\ XiXk - 4.03\ XjXk$

$Y(apigenin) = 103.21 + 5.30\ Xi - 2.58\ Xj + 4.56\ Xk + 0.79\ XiXj - 1.42\ XiXk - 1.21\ XjXk$

$Y(total\ polyphenols) = 15.79 + 6.73\ Xi + 0.37\ Xj + 1.74\ Xk - 0.93\ XiXj + 4.00\ XiXk - 1.60\ XjXk$

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