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Selection of Italian cardoon genotypes as industrial crop for biomass and polyphenol production

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ABSTRACT

Eight cardoon genotypes, of which seven Italian cultivated and one wild cardoon, belonging to the ENEA-Tuscia University joint collection, were assessed over two growing seasons with the aim of evaluating biomass production for pharmaceutical uses. Nine morphological descriptors (plant height and diameter, number of lateral shoots, main flower stem diameter, first fully developed leaf length and width, number of leaf lobes, plant fresh and dry weight) were selected to describe plant vigor and dry matter accumulation. Biochemical characterization of aboveground biomass was also undertaken, using accelerated solvent extraction (ASE) and HPLC analyses. Significant differences among genotypes were found for many of the agro-morphological traits evaluated. In particular, results identified aerial biomass yield, which ranged between 1095 and 2495 g plant^{-1}, as a useful trait in discriminating among genotypes. Significant differences among genotypes were also found for biomass polyphenol content. The most representative compounds detected were caffeoylquinic acids (3-*O*-caffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid) and flavonoid derivatives (cynaroside). The 3-*O*-caffeoylquinic acid content in biomass extracts ranged from 0.01 to 2.65 g kg^{-1} DM, while the 1,5-*O*-dicaffeoylquinic acid varied from 3.08 to 4.07 g kg^{-1} DM. With regard to cynaroside, the cultivated cardoon "AFN" showed the highest value (4.20 g kg^{-1} DM). Taking into account that the analyzed germplasm has never been selected for biomass or polyphenol compound production, our data highlight the potential use of cardoon genotypes for biomass and pharmaceutical purposes.

1. Introduction

Cynara cardunculus L. is an herbaceous species of the *Asteraceae* family, which comprises three botanical varieties: *C. cardunculus* L. var. *scolymus* (L.) Hegi (globe artichoke), *C. cardunculus* L. var. *altilis* DC (cultivated cardoon) and *C. cardunculus* L. var. *sylvestris* Lam. (wild cardoon) (Foury, 1989; Rottenberg and Zohary, 1996). Since ancient times, both globe artichoke and cultivated cardoon have been grown as vegetables for human consumption and are still cultivated today, for the immature inflorescences and the enlarged bleached petiole and fleshy leaves, respectively, mainly in the Mediterranean countries (i.e. Spain, Greece, Italy, France). Wild cardoon, the ancestor both of cultivated cardoon and globe artichoke (Rottenberg and Zohary, 2005), is widespread in the Mediterranean area as well as in many countries of Central and South America. It is a non-domesticated perennial plant, and is a weed in many agricultural areas. The wild cardoon is not cultivated as a commercial crop (Cravero et al., 2012) and its *capitula* are sold in local markets and used in traditional recipes only in some areas of Sicily (Ierna and Mauromicale, 2010).

Both cultivated and wild cardoons are well adapted to the Mediterranean environments, where they are seed-propagated as annual crops. The main plant growth stages in Mediterranean areas are: new shoot development in September; leaf rosette development in November–January; flower stem elongation in April–May; full bloom in June; fruit maturation in July; and, fully dry aerial biomass by August (Raccuia and Melilli, 2007). The cultivated cardoon has a high growth rate with significant dry matter accumulation (Cravero et al., 2012). For this reason, there is an increasing interest in this species principally due to: its (i) high aboveground biomass with values of 29.3 t ha^{-1} for the Bianco Avorio cardoon

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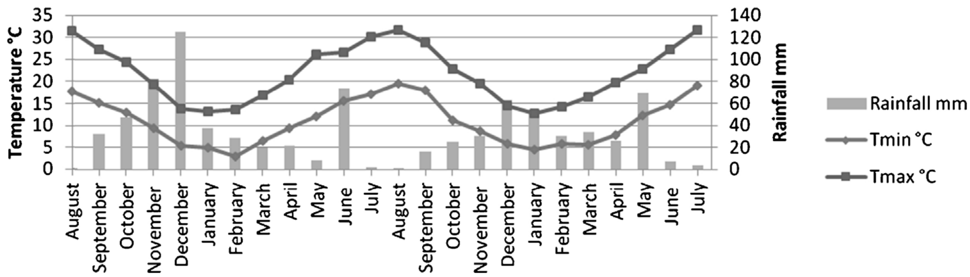

Fig. 1. Meteorological parameters recorded during the experimental years (2008/2009 and 2009/2010) at the experimental field station of Tarquinia (Viterbo, Italy).

genotype (Ierna et al., 2012); (ii) high dry matter content ranging between 29.50% and 33.80% (Cravero et al., 2012); (iii) high calorific value, which reached values of 3652 kcal kg^{-1} (Fernández et al., 2006); (iv) low crop energy input (i.e. irrigation, fertilization); and, (v) high degree of adaptability to semi-arid cultivation areas.

In recent years, *C. cardunculus* sp. have been assessed as biomass crops for energy (Foti et al., 1999; Fernández et al., 2006; Angelini et al., 2009; Mantineo et al., 2009; Ierna and Mauromicale, 2010; Ierna et al., 2012), for paper pulp production (Anthunes et al., 2000; Gominho et al., 2001, 2009; Fernández et al., 2006) and as green forage for ruminant feeding in winter (Fernández et al., 2005). Extraction of health-promoting biocompounds, mainly represented by mono- and dicaffeoylquinic acids and flavonoids, from cardoon biomass, has been also assessed (Fratianni et al., 2007; Lombardo et al., 2010; Bonasia et al., 2010; Pandino et al., 2010, 2011). Within the caffeic derivatives, chlorogenic acid (3-*O*-caffeoylquinic acid), which represents about 39% of the total caffeoylquinic acid content (Lattanzio et al., 2009), shows strong scavenging activity on superoxide anion radicals, inhibitory effect against oxidation of methyl linoleate (Takeoka and Dao, 2003; Gonthier et al., 2003) and inhibited lipid peroxidation (Nakajima et al., 2007). Also cynarin, presented marked antioxydative and protective effects in vivo studies (Gebhardt and Beck, 1996; Gebhardt, 1997). 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), which have been widely described in *Cynara* sp., are recognized in traditional or alternative medicine for their antimutagenic, anticarcinogenic, antioxidative, antiinflammatory, antiproliferative properties (Perez-Garcia et al., 2000; Romanova et al., 2001; Miadokova et al., 2008).

Although there is an abundance of biochemical and pharmacological studies, few papers are widely available on the genetic characterization and identification of Italian cardoon germplasm suitable for industrial and pharmaceutical purposes (Raccuia et al., 2004). In Italy, some cultivated cardoon genotypes have been released and registered into 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 these genotypes, often labeled according to the area of cultivation, are marketed by different seed companies and for culinary purposes only.

Based on the gaps identified, our work focuses on: (i) morphological characterization of seven Italian cultivated cardoons and one wild cardoon for biomass production; (ii) identification of useful morphological descriptors for discriminating genotypes under biomass and nutraceutical profiles; (iii) evaluation of the use of both biomass and immature flower heads as a source of nutraceuticals; and, (iv) selecting genotypes for potential biomass and biocompound production.

2. Materials and methods

2.1. Experimental field and plant material

Seven Italian cultivated cardoon genotypes (AFB, AFM, AFM2, AFGR, AFGI, AFN, AFFG) and a wild cardoon (AFS) genotype belonging to the Tuscia University and ENEA joint collection were considered in the study. 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″12N; 11°45′22″32E), Viterbo (Italy). The station is characterized by a temperate climate and an average annual rainfall of 900 mm. The values of weekly temperature and rainfall recorded during the two growing seasons are shown in Fig. 1. The station soil characteristics were as follows: sand, 62%; clay, 23%; silt, 15%; pH 7.2; organic matter, 0.97%; CE 0.25 mS. The textural class of the surface horizon (0–20 cm depth) fell within the sandy-loam USDA classification. The values of weekly temperature and rainfall recorded during the two growing seasons are shown in Fig. 1.

All genotypes were seed-propagated and transplanted in the field according to a randomized block experimental design with 4 replications. Each field plot (elementary unit area of 5.20 m^2) totally consisted of 9 plants (planting density of 7700 plants ha^{-1}, inter and intra-row distances of 1.30 and 1.00 m, respectively). The sowing date was 11th July 2008 and the transplanting date was 17th August 2008. Field experiments were conducted under low energy inputs. Manual weeding was carried out three times a year over the study period. Minimal fertilization (100 kg ha^{-1} of urea) was carried out before date of transplantation. Manual stalk removal was undertaken at the end of the first growing season. No crop diseases were detected. Overall, crop water requirement was satisfied by rainfed irrigation; however, in August of the first year, an additional 40 mm of watering was carried during transplantation.

2.2. Morphological characterization and biomass production

Plant growth and biomass descriptors were used for morphological characterization (Table 1). For qualitative evaluation of biomass production, one plant per each replication and per genotype was harvested at the stage of *capitula* production (on May, 29th 2009 and on June, 6th 2010, respectively) and immediately weighed and oven-dried at 103 °C, until a constant weight was reached, to determine the dry matter (DM) content of the whole plant. The flower stem diameter measurement was taken at the height of the first fully developed leaf. In total, nine morphological data were recorded, during each of the two growing seasons, prior to full bloom.

2.3. Phenolic compound extraction and analysis

2.3.1. Sample preparation

A representative sample (300 g FW) of biomass (leaves and flower stems) was collected from four plants per genotype (one