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Dry matter yield, feeding value, and antioxidant activity in Mediterranean chicory (*Cichorium intybus* L.) germplasm

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Abstract: Knowledge about the forage yield potential and bioactive compound contents of Mediterranean natural populations of chicory is still scarce, even though it could be very helpful in view of the exploitation of the feeding value of local chicory germplasm for ruminants. Dry matter yield, chemical composition, antioxidant capacity, total polyphenols, flavonoids, and condensed tannins content were determined in 7 natural populations of Mediterranean wild chicory and, as a comparison, in the chicory commercial variety Spadona. Original seed was collected in different pastureland areas of Sardinia (Italy) and plants were grown in North Sardinia. Statistically significant differences were found in the leaf dry matter yield, ranging from 1.3 to 3.8 t ha^{-1}, and were not found in protein and fiber contents. The local populations were comparable to the commercial variety; however, the antioxidant activities, detected by means of 2 in vitro assays, significantly differed between populations. Among bioactive compounds, total flavonoids ranged from 27 to 37 g catechin equivalent kg^{-1} dry weight and statistically significant differences were also found in total polyphenol content and nontannic polyphenols; condensed tannins were not detected. Considering the importance of some bioactive compounds for the enhancement of animal nutrition and welfare, the exploitation of variability in bioactive compound content and antioxidant capacity of the chicory natural populations can be important for a more complete valorization of its Mediterranean germplasm.

Key words: Chicory, dry matter yield, forage quality, natural populations, Trolox equivalent antioxidant capacity

1. Introduction

Chicory (*Cichorium intybus* L.) is a typical Mediterranean plant, belonging to the family Asteraceae, indigenous to Europe, West Asia, Egypt, North America, and Italy. There are many varieties of chicory having different commercial uses, with some misunderstanding about the botanical classification of such varieties. Vegetable commercial varieties (e.g., Bruxelles chicory, Treviso red chicory, endive) all belonging to the species *intybus* have remarkable economic importance. Spontaneous chicory plants are also still used for human consumption, cooked or uncooked, in some Mediterranean regions. In Sardinia, gastronomic uses and medicinal properties of chicory have been well known for a long time (Manca Dell'Arca, 1780); 2 other authors have referred to chicory ethnobotany (Moris, 1840; Atzei, 2003). Lancioni et al. (2007) reported on the alimentary and therapeutic uses of chicory in the folk traditions of central Sardinia.

Moreover, chicory is a spontaneous component of pasturelands, crops, and uncultivated land. Seeds of chicory plants, growing as weeds in forage legume crops, after seed harvesting and cleaning have been occasionally used as a component of forage seed mixtures grazed in Mediterranean basin areas and should be considered for their forage potential (Molle et al., 2008). Chicory is palatable for ruminants, low in fiber, and high in nonstructural carbohydrates (Athanasiadou et al., 2007). In a recent paper, Di Grigoli et al. (2012) remarked on the potential of chicory to provide lasting good-quality herbage and the positive effect of this species on dairy performance in grazing ewes. In fact, in such an experiment, the crude protein (CP) content of the chicory was higher than that of an oat-berseem (*Avena sativa* L. and *Trifolium alexandrinum* L.) mixture and similar to that of sulla (*Hedysarum coronarium* L.). Moreover, the chicory forage had less dry matter (DM) and fewer structural carbohydrates, as it can remain green longer than other forage species. Because of such characteristics, chicory has been highly appreciated by farmers (Barry, 1998; Sitzia et al., 2006).

In spite of its natural distribution in many countries of Europe, forage chicory is a relatively new crop in many countries. In fact, much of the breeding for improved forage characteristics has been done in New Zealand,

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where the first variety, Puna, was released in 1985 (Moloney and Milne, 1993; Hume et al., 1995). Other forage chicory varieties, such as Lacerta, Forage Feast, Oasis, and Puna II, have been developed in France, the United States, Australia, and China (Rumball et al., 2003; Wang and Cui, 2011). This confirms the increased interest in forage chicory at a worldwide level. Currently, forage chicory is also being considered as a species with potential for reducing methane emissions from ruminants (Prusty et al., 2012, 2013; Sun et al., 2012). In addition, it has been tested in Turkey as a possible biomonitor of heavy metals in sites with different degrees of pollution (Aksoy, 2008).

According to Clark et al. (1990), a proportion of 70% leaves and 30% stems should be desirable in forage chicory because of the higher digestibility of leaves compared to stems. Therefore, frequent grazing has been proposed to produce a high proportion of leaves, up to 78% of the total aboveground biomass (Li et al., 1997).

Ozturk et al. (2006) studied the evolution of the nutritive value of wild chicory forage, harvested at different maturity stages in Turkey, and demonstrated that chicory should be harvested at the vegetative stage to obtain higher quality forage. Sulas et al. (2007) studied wild chicory plants from Sardinia and found that local populations of forage chicory can represent a valuable opportunity for semiarid environments under rainfed conditions. Moreover, wild chicory was shown to be a moderately salt-tolerant species (Sergio et al., 2012), which allows the use of brackish waters for its irrigation.

In the last 10 years, forage chicory has been studied for its secondary compounds, such as polyphenols, which can reduce intestinal infections in grazing animals (Marley et al., 2003). Polyphenols are among the most significant compounds related to the antioxidant properties of plant materials. Flavonoids have also been shown to act as scavengers of various oxidizing species [i.e. superoxide anions (O_2^-), hydroxyl radicals, and peroxy radicals], and they may also act as quenchers of single oxygen (Harborne and Williams, 2000).

Mulinacci et al. (2001) investigated the content of phenolic compounds, mainly cinnamic acids and flavonoids in *C. intybus* L. var. *silvestre*. Di Venere et al. (2004, 2009) also studied phenolic composition and antioxidant activity in wild chicory; they found the presence of caffeic acid derivatives and flavonoids (quercetin and kaempferol glycosides) and a very high antioxidant activity compared to other wild edible species. However, information about the dry matter yield potential and bioactive compounds contents of natural populations of chicory is still scarce, even if these details could be very helpful in establishing relationships such as that between forage polyphenol contents and their feeding values for ruminants. Within a general activity aimed at the in loco exploitation of Sardinian forage germplasm, the dry matter yield, chemical composition, bioactive compounds content (total polyphenols, flavonoids, condensed tannins), and antioxidant capacity were examined in the leaves of Mediterranean natural populations of chicory.

2. Materials and methods

2.1. Origin, management, and samplings of plant materials

Cichorium intybus seeds utilized in this investigation were previously collected from local natural populations in pastureland areas from 3 up to 962 m a.s.l. (Table 1) of Sardinia, Italy, where the species is usually grazed by ruminants and also used for human consumption (Atzei, 2003); the *C. intybus* Italian commercial variety Spadona was also included as a control.

The experiment was carried out in 2005 and 2006 in North Sardinia (41°N, 8°E, 81 m a.s.l.). The soil at the site is a flat sandy clay loam overlaid on limestone (Xerochrepts),

Table 1. List of collection and surveying sites of forage chicory germplasm in Sardinia (Italy).

Populations	FAO Soil Classification*	Altitude (m a.s.l.)	Latitude	Longitude
CIT01	Petric Calcisols	3	40°35′	8°18′
CIT02	Lithic Leptosols	275	40°28′	9°07′
CIT03	Eutric Cambisols	962	40°21′	8°55′
CIT04	Hypocalcic Calcisols	40	40°40′	8°21′
CIT05	Vertic Cambisol	158	39°41′	8°54′
CIT06	Rock outcrop	374	40°55′	8°38′
CIT07	Calcaric Cambisols	85	40°36′	8°29′

*According to FAO et al. (2006) soil classification manual.

with low organic C (12 g kg^{-1}) and N (0.8 g kg^{-1}), pH 7.5, low P_2O_5 content, and adequate K_2O content. The climate is typically Mediterranean, with a long-term average annual rainfall of 554 mm and a mean annual air temperature of 16.2 °C.

In November 2005, plots (2.5 m^2 each) of 7 Sardinian natural populations of chicory and the Spadona commercial variety were manually transplanted, with 25 cm between rows and 20 cm apart within rows, under a randomized block design with 3 replicates. Fertilization of 40 kg ha^{-1} of N and 100 kg ha^{-1} of P_2O_5 was applied at sowing. In spring 2006, when plants were at the vegetative stage, 3 plants per plot were cut at 5 cm above ground for production and quality determinations. Leaf length was measured in 4 leaves per plant.

2.2. Forage quality analysis

Forage samples were dried in an oven at 65 °C for 48 h, then ground through a 1-mm screen to be analyzed for quality traits. Total N was determined using the Kjeldahl method and crude protein was calculated by multiplying the N content by 6.25. Neutral and acid detergent fiber (NDF and ADF) and acid detergent lignin (ADL) were determined using the procedure of Goering and Van Soest (1970). Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), and net energy for lactation (NE_l) were estimated according to the following equations adapted from Horrocks and Vallentine (1999):

$TDN = (-1.291 \times ADF) + 101.35$,

$DMI = 120 / \%NDF$ dry matter basis,

$DDM = 88.9 - (0.779 \times \%ADF$, dry matter basis),

$RFV = \%DDM \times \%DMI \times 0.775$,

$NE_l = (1.044 - (0.0119 \times \%ADF) \times 2.205$.

2.3. Bioactive compounds and antioxidant capacity

Plant samples were kept on ice during harvesting, freeze-dried, and ground to a fine powder for the chemical analysis. Ground leaf samples (50 mg) were treated with 2.5 mL of acetone/water (7:3) mixture and shaken for 60 min. The samples were then centrifuged for 10 min at 3900 rpm and the supernatant was stored at 4 °C until use for the following determinations. Total phenolic content (TotP) of extracts was determined using the Folin–Ciocalteu reagent according to Singleton and Rossi (1965), with some modifications by Piluzza and Bullitta (2010). Results were expressed as g gallic acid equivalent kg^{-1} dry weight of plant material (g GAE kg^{-1} DW). Nontannic phenolics (NTP) were determined after precipitation of tannin components with polyvinylpolypyrrolidone according to the FAO/IAEA (2000). Results were expressed as g GAE kg^{-1} DW. The butanol assay of Porter et al. (1986) was adapted (Piluzza and Bullitta, 2010) for quantification of extractable condensed tannins content from our samples. Total flavonoids (TotF) were quantified by colorimetric assay with the $AlCl_3$ method (Kim et al., 2003). Catechin was used as a standard and the flavonoid content was expressed as g catechin equivalent kg^{-1} dry weight of plant material (g CE kg^{-1} DW). Antioxidant capacity was determined by means of ABTS [(2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)] and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays (Surveswaran et al., 2007) with some modifications (Piluzza and Bullitta, 2011). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analog of vitamin E, was used as the reference standard. The results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), as mmol Trolox equivalent 100 g^{-1} dry weight of plant material (mmol TEAC 100 g^{-1} DW).

2.4. Statistical analysis

Statistical significance was performed by 2-way analysis of variance (ANOVA). Differences between means were assessed with the least significant difference (LSD) test for means separation. The significance level was fixed at $P \leq 0.05$ for all the statistical analyses. Coefficients of determination (R^2) were calculated using Microsoft Excel 2000.

3. Results

Considering that a high proportion of leaves is desirable in chicory (Clark et al., 1990) and that phytomass partitioning demonstrated that stems' contribution to total plant dry matter was very limited compared to leaves at spring cutting harvest, the data referred to are for leaves only. Leaf dry matter content was on average 11.5% and it ranged from 10.1% (Spadona) to 13.7 % (CIT01). Leaf length (Figure 1), leaf DM yield, and CP yield (Table 2) significantly varied between populations. The commercial variety Spadona exhibited the longest leaves, with an average of 56 cm, and statistically significant variations

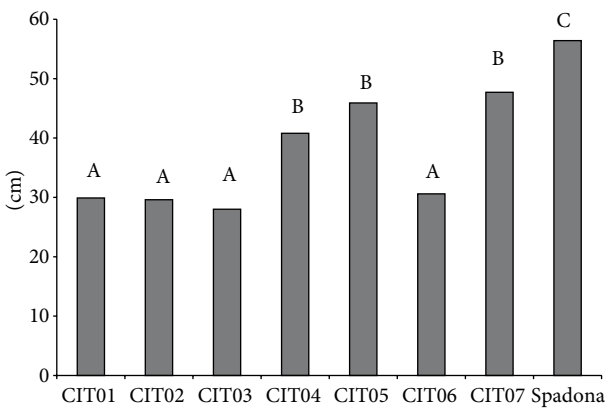

Figure 1. Average leaf length (cm) of the natural populations of forage chicory and the commercial variety Spadona. Values marked by different letters are significantly different.

Table 2. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents in leaves of Mediterranean natural populations of forage chicory.

Populations	DM (kg ha^{-1})	CP (kg ha^{-1})	CP (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)	ADL (g kg^{-1} DM)
CIT01	1963.5	363.4	173.9	372.7	303.4	188.1
CIT02	1729.3	334.5	200.7	396.1	332.9	175.2
CIT03	1372.9	255.8	185.2	406.5	336.8	205.8
CIT04	2889.3	515.8	177.0	379.8	309.0	157.9
CIT05	2176.4	400.2	182.6	364.9	290.6	166.2
CIT06	2113.3	407.5	193.0	395.2	343.2	188.2
CIT07	3808.4	689.2	182.5	406.2	336.5	186.3
Spadona*	3945.3	646.3	162.1	366.0	300.9	178.5
$LSD_{0.05}$	809.1	196.7	ns	ns	ns	ns

*: Commercial variety.

for the length of leaves were found among the wild populations. CIT07 leaves were about twice longer than those of CIT03. Leaf DM ranged from about 1.3 (CIT03) to 4 t ha^{-1} of DM (Spadona); leaf DM yield of Spadona and CIT07 significantly differed from the remaining ones. Among Sardinian chicory populations, CIT07 exhibited significantly higher leaf lengths and leaf DM yields.

Chemical composition of chicory leaves did not significantly differ between populations. CP concentration ranged from 162 (Spadona) to 200 (CIT02) g kg^{-1} DM, whereas, considering the CP production per hectare, leaf CP yield varied from 255.8 (CT03) to 689.3 (CT07) kg ha^{-1}, showing wide differences between populations due to the combined effect of both DM and CP content, the latter considered one of the most important criteria for forage quality evaluation. Other important characteristics for forage quality are NDF and ADF; NDF content ranged from 366 (Spadona) to 406.5 (CIT03) g kg^{-1}; ADF content ranged from 290.6 (CIT05) to 336.8 (CIT03) g kg^{-1} DM, whereas ADL content ranged from 157.9 (CIT04) to 205.8 (CIT03) g kg^{-1} DM. TDN, DDM, DMI, RFV, and NE_l (Table 3) did not significantly differ between populations.

Table 3. Total digestible nutrients (TDN), digestible dry matter (DDM), digestible dry matter intake (DMI), relative feed value (RFV), and net energy for lactation (NE_l) in leaves of Mediterranean natural populations of forage chicory.

Populations	TDN (g kg^{-1} DM)	DDM (g kg^{-1} DM)	DMI (g kg^{-1} of body weight)	RFV (%)	NE_l (Mcal kg^{-1})
CIT01	621.8	652.6	32.4	165.1	1.505
CIT02	583.7	629.7	30.3	148.5	1.428
CIT03	578.7	626.6	29.5	143.7	1.418
CIT04	614.5	648.2	31.6	159.0	1.491
CIT05	638.2	662.6	33.1	170.6	1.539
CIT06	570.4	621.6	30.3	146.3	1.401
CIT07	578.9	626.8	29.7	145.1	1.418
Spadona*	624.9	654.5	32.8	166.4	1.512
$LSD_{0.05}$	ns	ns	ns	ns	ns

*: Commercial variety.

TDN ranged from 570.4 (CIT06) to 638.2 (CIT05) g kg^{-1} DM and variations were related to the ADF concentration. DDM ranged from 621.6 (CIT06) to 662.6 (CIT05). The DMI range was 29.5–33.1 and it was negatively correlated with NDF. RFV exceeded a value of 151, which is indicative of prime forage (Horrocks and Vallentine, 1999), in CIT01, CIT04, CIT05, and Spadona; a similar trend was recorded for NE_l.

Statistically significant differences between populations were found for antioxidant capacity, TotP, NTP (Table 4), and TotF content (Figure 2). ABTS assay exhibited a variation of antioxidant capacities among populations, from 13.63 (CIT04) to 17.51 (CIT01) mmol TEAC 100 g^{-1} DW. However, all Sardinian populations, except CIT04, did not significantly differ from Spadona for antioxidant capacity determined by means of the ABTS method. Statistically significant differences were found for the total antioxidant capacity determined through the DPPH assay, ranging from 15.63 (CIT04) to 23.86 (CIT05) mmol TEAC 100 g^{-1} DW and, except for CIT04 and CIT05, natural populations did not significantly differ from Spadona. TotP content (Table 4) showed statistically significant variation ranging from 33.76 (CIT07) to 43.89 (CIT05) g GAE kg^{-1} DW; except for CIT04 and CIT07, TotP content of natural populations did not significantly differ from that of Spadona. The NTP content (Table 4) varied significantly from 20.27 to 25.85 g GAE kg^{-1} DW in CIT07 and CIT01, respectively. TotF (Figure 2) showed a statistically significant variation and ranged from 27.13 (CIT04) to 36.94 (CIT05) g CE kg^{-1} DW. Significant correlations were found between the CP (CP kg ha^{-1}) and DM yield (DM kg ha^{-1}) (Figure 3) and leaf length and CP (Figure 4a) and DM yield (Figure 4b). Significant correlations were also found between the TotF and total antioxidant capacity assayed by means of ABTS (Figure 5a) and DPPH (Figure 5b) methods. ABTS and total phenolics and nontannic phenolics showed a significant correlation of $R^2 = 0.5651$ and $R^2 = 0.7511$, respectively, whereas statistical significant correlations were not found between antioxidant capacity and DM yield.

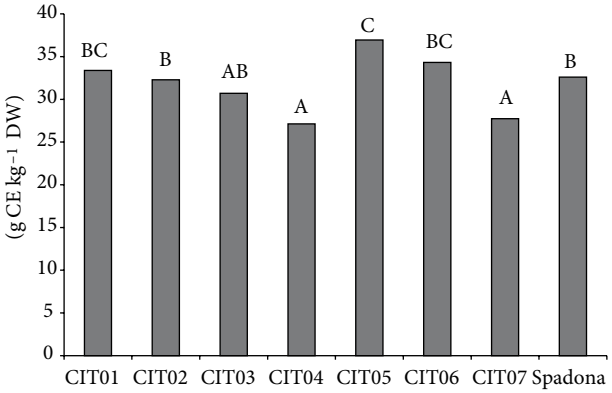

Figure 2. Total flavonoid content of the natural populations of forage chicory and the commercial variety Spadona. Values marked by different letters are significantly different.

Table 4. Trolox equivalent antioxidant capacity (TEAC) determined by means of ABTS and DPPH methods, total phenolic (TotP), and nontannic phenolic (NTP) content in leaves of the Mediterranean natural populations of forage chicory.

Populations	TEAC (mmol 100 g^{-1} DW)		TotP (g GAE kg^{-1} DW)**	NTP (g GAE kg^{-1} DW)**
	ABTS	DPPH		
CIT01	17.51	20.66	41.92	25.85
CIT02	16.03	20.14	39.86	24.65
CIT03	15.75	19.58	38.60	23.49
CIT04	13.63	15.63	34.87	20.60
CIT05	17.49	23.86	43.89	24.01
CIT06	16.23	21.84	40.63	25.17
CIT07	14.10	18.12	33.76	20.27
Spadona*	15.93	20.18	40.60	22.06
$LSD_{0.05}$	2.1	3.3	5.4	2.78

*: Commercial variety. **: Grams of gallic acid equivalent per kilogram of dry weight.

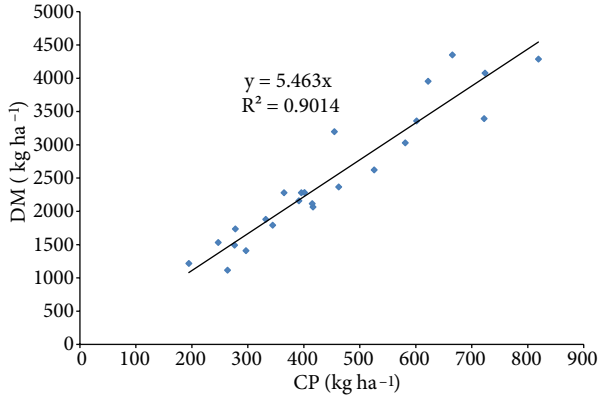

Figure 3. Linear regression between chicory crude protein (CP kg ha^{-1}) and dry matter (DM kg ha^{-1}).

4. Discussion

Notwithstanding the wide variations recorded, our results highlight the remarkable potential of some of the Mediterranean wild populations of chicory under study as a forage pasture species, because of their production of relevant amounts of leaves. In fact, Spadona and CIT07 produced a similar amount of dry matter that was 2 times higher than that of CIT03 and, compared to the remaining populations, showed higher plant vigor in winter, reaching an early complete ground cover rate of the plots (data not shown). With regard to protein and fiber content, Sardinian populations did not significantly differ from the commercial variety Spadona. According to Molle et al. (2008), the chicory commercial cultivar Spadona (positively evaluated for forage purposes), when ingested by sheep (who are able to select the more proteinaceous and less fibrous part of plants), can exhibit an improved nutritional quality compared to other forages available. The statistically nonsignificant variations recorded between Sardinian populations in terms of protein and fiber content suggest that substantial differences can arise from the different production potential of populations in terms of forage yield per hectare. In Sardinia, Nieddu et al. (2000) and Sulas (2004) suggested the use of chicory as a fodder crop; Sitzia et al. (2006) used cultivar Spadona and sulla as forage crops in a dairy sheep system, and they concluded that both chicory and sulla, cultivated under Mediterranean conditions, can serve as high-quality pasture for sheep. According to Sulas et al. (2009), the same

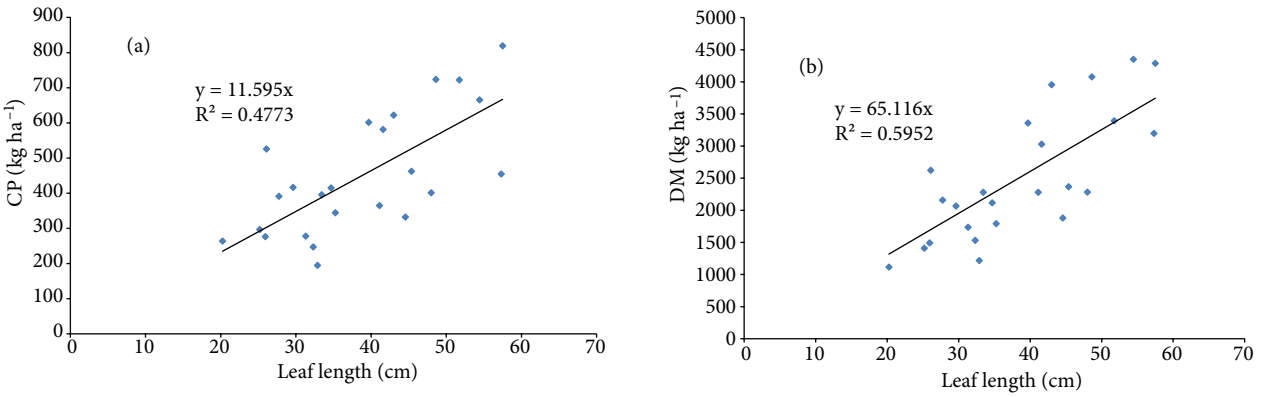

Figure 4. Linear regression between chicory leaf length and CP (a) and DM yield (b).

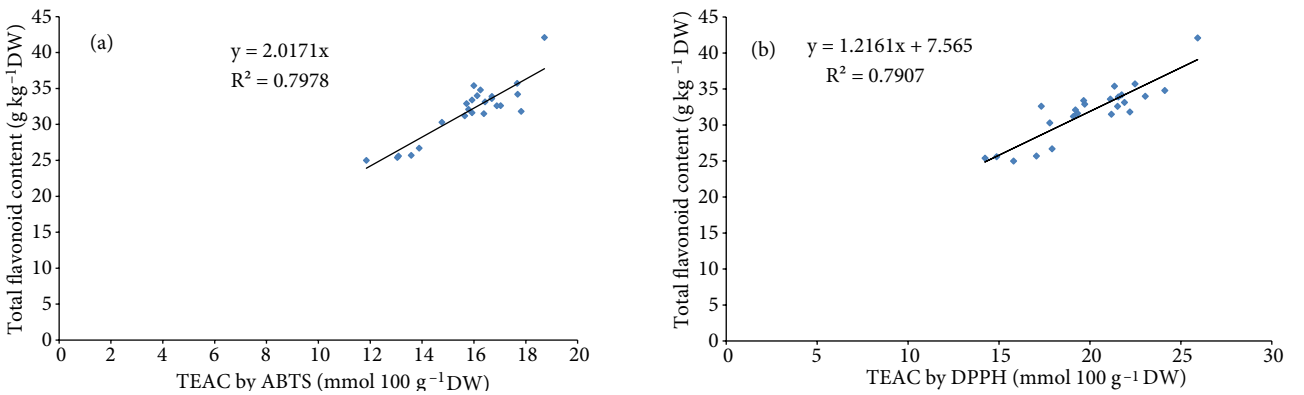

Figure 5. Linear regression between chicory total flavonoid content and antioxidant capacity determined by means of ABTS assay (a) and DPPH assay (b).

Spadona cultivar was able to produce up to 6 t ha^{-1} of DM per year when harvested at a later stage (early flowering) but with a relevant contribution of stems to the total DM yield. The recorded values for Spadona leaf chemical composition, except for ADL, are quite similar to those reported by Sitzia et al. (2006) and Di Grigoli et al. (2012) for the same chicory cultivar grown in different grazing experiments in Sardinia and Sicily (Italy), respectively. The overall mean values obtained in our quality evaluations were consistent with the values reported for forage chicory quality in Turkey by Ozturk et al. (2006).

Comparisons with other published data about polyphenols and the antioxidant capacity of the species are quite difficult, owing to variations in the methods, procedures, and standards used for analyses. Ivanova et al. (2005) studied the polyphenols and antioxidant capacity of Bulgarian medicinal plants, including chicory; the plant extracts were prepared as an infusion with boiling water and the standard used was quercetin. Kratchanova et al. (2010) found in Bulgarian chicory a polyphenol content of 1821 mg 100 g^{-1}, lower than our results. They prepared the plant extract with 80% acetone in 0.2% formic acid and the standard was gallic acid. Sulas et al. (2007) found a content of polyphenols of 30 g kg^{-1} DM in Sardinian chicory populations and the Puna variety. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. According to Pieroni et al. (2002), nondomesticated food vegetables play a central role as traditional functional foods; specifically, their antioxidant properties are regarded as quite noteworthy. Except for 2, all the other local populations showed total flavonoids content comparable or higher than Spadona and are therefore useful for both animal and human nutrition and health.

No CT was detected in the natural populations under study, although some authors referred to its presence in forage chicory (Terrill et al., 1992; Molan et al., 2002; Häring et al., 2007, 2008). According to Piluzza et al. (2013), there have been conflicting reports on the presence of CT in forage chicory; in fact, the presence of CT was not detected in other studies on chicory (Waghorn et al., 2002; Bullitta and Piluzza, 2005; Tava, 2005; Sulas et al., 2007).

Statistically significant variations were not recorded between Sardinian populations in terms of protein and fiber content. However, some Sardinian populations showed statistically significant differences in forage yield, bioactive compound contents, and antioxidant capacity, which can be important for animal feeding and also for human consumption. The results obtained show that some of the examined natural populations of chicory possess productive and qualitative traits comparable to Spadona, a commercial cultivar positively evaluated for forage purposes. The variability in bioactive compound contents and antioxidant capacity could be exploited for a more complete valorization of Mediterranean wild chicories and encourages investigations on Mediterranean germplasms of the species as sources of functional feed and food and natural antioxidants. Additional research needs to be accomplished in further investigation aimed at elucidating the chemical composition of phenolics.

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