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Biomass, Seed and Energy Yield of *Cynara cardunculus* L. as Affected by Environment and Season

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Abstract: *Cynara cardunculus* is a perennial plant that adapts well to Mediterranean climate conditions. The possibility of cultivating *C. cardunculus* with low or zero input and in low productivity or marginal lands makes it one of the most promising crops for bioenergy production in the Mediterranean areas. The objective of the research was to study the effects of two marginal and contrasting southern Italian environments (plain, 42 m a.s.l. and hilly area, 419 m a.s.l.) during a three-year period on biomass, seed, energy yield, and oil composition of two genotypes of *C. cardunculus* (cultivated cardoon and wild cardoon). When compared to the plain, plants that were grown in the hills gave higher biomass yield (10.9 vs. 9.7 t DM ha^{-1} year^{-1}), higher seed yield (0.46 vs. 0.44 t DM ha^{-1} year^{-1}) and, consequently, higher total energy yield (190 vs. 172 GJ ha^{-1} year^{-1}), attributable to higher average annual rainfall (680 vs. 565 mm year^{-1}). The season, although only evaluated for three years (short cycle), showed a different effect based on the genotype, highlighting a greater over-time production constancy of wild cardoon (though less yielding) as compared to cultivated cardoon. Oil yield and composition were only slightly affected by environment and genotype. Overall, the results of this research suggest using *C. cardunculus* in marginal hilly areas, where, in addition to the production of bioenergy, it may represent a good chance to fight erosion and improve soil fertility, without competing with food crops.

Keywords: *Cynara cardunculus* L.; marginal areas; plain; hills; cultivated cardoon; wild cardoon

1. Introduction

Cynara cardunculus L. is a member of the Asteraceae family, including the globe artichoke [*C. cardunculus* L. var. *scolymus* (L.) Fiori], the cultivated cardoon [*C. cardunculus* L. var. *altilis* DC.], and their ancestor, the wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori] [1–3]. In addition to the traditional use for food [4,5], in recent years cultivated and wild cardoon have been considered for different industrial applications. These include for the production of cellulose, pulp, and paper, use in animal feeding, use of florets in the dairy industry, extraction of inulin and phytochemicals for pharmacological and cosmetic use [2,6,7], as well as use for its antimicrobial and bioherbicide action [8,9]. However, it is as a bioenergy crop that *C. cardunculus* is most promising in southern areas of Europe in relation to good adaptation to Mediterranean climate conditions of low rainfall and hot dry summers. This is attributable to the positive balance between the phases of the growth and development cycle under Mediterranean climatic trends, the capacity of photosynthesizing during winter time, as well as the capacity of nutrient uptake from deep soil layers [10]. In Mediterranean environments, *C. cardunculus* is able to provide high biomass, seed, and energy yields under low external management energy supplies thanks to its excellent adaptation [11–15]. Energy can be produced from the biomass via combustion and oil that accumulated in the seeds is suitable as solid biofuel and as a feedstock for biodiesel [16]; alternatively, biomass can be utilized for bioethanol [17] or biomethane production via fermentation [18]. The economic analysis of cardoon as compared to other herbaceous annual crops, demonstrated the low cultivation costs, the higher total revenues, and its suitability for inclusion in arable cropping systems in marginal lands [19,20]. The use of marginal lands for the cultivation of a bioenergy crop would ease the conflict that, in Europe, bioenergy crops have with food production [20–22]. Moreover, the use of marginal areas for energy crops may contribute to the EU policy objective to reduce CO_2 emissions by 40% in 2030 and, in particular, to achieve the contribution of 32% with renewable sources to the total energy consumption in that year contained in the new Directive of Renewable Energies 2021–2030 that promotes the use of renewable energy sources [23].

The possibility of cultivating *C. cardunculus* as a bioenergy crop with low or zero input and in low productivity or marginal lands has prompted great interest and research activities in the last thirty years [24]. The studies conducted mainly in Italy, Greece, Portugal, and Spain showed great variability in dry biomass yields [12–14,25–32], and seed yields and oil contents [11,25,33] in relation to the pedo-climatic conditions, cropping techniques and genotypes. Furthermore, data concerning the evaluation of the suitability of *C. cardunculus* to different environments at a local level in terms of biomass production are very limited in the literature. It would also be important to evaluate the adaptability of different genotypes and their productivity over time in different environments in order to provide more precise and timely information to farmers who want to invest in this bioenergy crop. The objective of this research was to study the effects of two marginal contrasting southern Italian environments during a three-year period on biomass, seed, energy yield, and oil composition of two genotypes of *C. cardunculus* (cultivated cardoon and wild cardoon).

2. Materials and Methods

2.1. Location, Climate and Soil

The field experiments were conducted during three growing seasons (from 2013–2014 to 2015–2016, hereafter referred as S1, S2, and S3) at two locations Ispica and Modica (hereafter referred as 'plain' and 'hills'), whose geographical coordinates and soil characteristics are listed in Table 1.

Table 1. Geographical coordinates, soil characteristics and climate characteristics (long-term 1977/2006) of the two locations [34].

	Location	
	Ispica (Plain)	**Modica (Hills)**
Geographical coordinates		
Latitude and longitude	36°47′ N 14°54′ E	36°53′ N 14°51′ E
Altitude (m a.s.l.)	42	419
Soil characteristics		
Clay (>0.002 mm) (%)	19	38
Silt (0.02–0.002 mm) (%)	10	26
Sand (2–0.02 mm) (%)	71	36
Total N (g kg^{-1})	0.8	1.4
Organic matter (g kg^{-1})	12.2	14.0
P_2O_5 available (mg kg^{-1})	57	53
K_2O exchangeable (mg kg^{-1})	302	351
pH	7.7	7.4
Climate characteristics		
Annual total rainfall (mm)	481	613
Annual rainy days (N)	53	63
Average maximum temperatures (°C)	23.4	24.2
Average minimum temperatures (°C)	14.8	13.9

The two locations, although not very far from each other, represent two different marginal environments. The plain location has a history of arable cropping and fairly fertile soils, even if its productive potential is variable from around average–low values [35]; the soil is a moderately deep, calcic brown [36], with sandy loam texture, low N soil content, and organic matter. The hillside location is subjected to erosion and characterized by low-fertility soils; the soil is a moderately deep, typic xerochrepts [36] with loamy-clay soil texture, low N soil content, and organic matter.

In both locations (plain and hills), the climate is semiarid-Mediterranean, with mild wet winters and hot, rainless summers. However, the two locations differ for total mean rainfall (481 mm on the plain and 613 mm in the hills) and number of annual rainy days (53 and 63, respectively, in the plain and hills) in the 30-year period 1977/2006 [34]. Furthermore, when compared to the plain, the hilly location is characterized by higher monthly maximum temperatures on average of 1 °C and lower monthly minimum temperatures on average of 1 °C throughout the entire year (Table 1).

2.2. Experimental Design, Plant Material and Management Practices

In each location (plain and hills), a randomized block design with three replications was used. Each block included two genotypes with a plot size of 12 × 12 m, each containing 360 plants. The total area of the experiment was in each location 864 m^2 (six plots of 144 m^2). The two genotypes studied were: cultivated cardoon cultivar 'Bianco Avorio' and a wild cardoon landrace. 'Bianco avorio' (SAIS Sementi, Cesena, Italy) is a commercial cultivar cultivated for the fleshy leaf petiole and part of the central leaf vein used to prepare typical Italian dishes [13]. 'Bianco Avorio' was chosen due to its high biomass productivity and the adaptability to grow under low input in Mediterranean area [11,13,30]. Wild cardoon seeds were collected from native stands in the coastal plain area South of Ragusa (36°55′ N, 14°43′ E, 502 m a.s.l., South-Eastern Sicily). Four-week-old seedlings with three-four true leaves were transplanted in the field on 5 November on both the plain and in the hills, adopting a planting density of 2.5 plants m^{-2} with inter-row and intra-row spacing, respectively, of 1.00 and 0.40 m. In each location before transplanting, tillage consisted of ~35 cm depth ploughing, followed by harrowing. The same fertilizer regime was applied in each location, during seedbed preparation, the soil was fertilized with 60 kg ha^{-1} N (as urea), 100 kg ha^{-1} P_2O_5 (as triple superphosphate) and 80 kg ha^{-1} K_2O (as potassium sulphate). In late February, 60 kg ha^{-1} N (as urea) were applied. Soon after transplanting, an irrigation (30 m^3 ha^{-1}) and hand weeding were carried out, in order to improve crop establishment. In the following years (from second to third), no fertilization, irrigation, and weed control were carried out. Chemical products against pest and diseases were never necessary. In each location, the crop regrowth in the seasons after transplanting was naturally allowed by rains of mid-September to early October. The growth of the rosette leaves was rapid, competing very well against weeds, also thanks to its allelopathic action [37].

2.3. Data Collection

In each season of cultivation, the aboveground biomass was harvested when the fruits reached the ripened stage (at the end of July in both locations). As a member of the *Compositae* family, the fruit of *C. cardunculus* is a cypsela, i.e. an achene originating from an inferior ovary [2], but it is commonly known as 'seed' and it is thus called throughout the entire manuscript. The plants were harvested from the central area (4 × 4 m) of each plot, by cutting them at about 5 cm above the soil level. The harvested plants (about 40 for each plot) were immediately weighed in the field in order to determine their fresh weight. The number of plants per plot, height of plants and biomass components (stalks, leaves and heads) were also measured. The heads were threshed with a specific mini-thresher to separate seeds, which were then weighed. The moisture content of each biomass components (stalks, leaves, heads, and seeds) was measured in the laboratory by weighing ~200 g of plant material, and placing it in a thermoventilated oven (Binder, Milan, Italy) at 105 ± 1 °C until constant weight was reached. All of the biomass components (e.g., stems, leaves, heads and seeds) were milled in a MF10 IKA mill to 3.0 mm. In each growing season, the gross calorific value of harvested biomass was determined using a C200-System-IKA calorimeter (IKAWerke Staufen, im Breisgau, Germany), according to the standardized procedure ASTM E–711–87 (2004), and taking into account the biomass partitioning. The average values 16,517 kJ kg^{-1} DM for aboveground biomass and 22,650 kJ kg^{-1} DM for seed were utilized for the calculation of the energy yield.

The seed samples removed from ripe capitula harvested in the second growing season (on 30 July 2015) were sent to the Stazione Sperimentale per le Industrie degli Oli e dei Grassi (Milano, Italy) in roder to determine their oil yield and fatty acid composition. The moisture content of the seeds was determined before oil extraction by weighing 10 g of ground grain in pre-calibrated porcelain capsules and placing it in a thermoventilated oven (Binder, Milan, Italy) at 105 ± 1 °C, until a constant weight was reached. Seeds oil yield was determined by standard procedure according to the ISO 659:2009 norm; the fatty acids composition was determined by gas chromatography according to the procedure ISO 12966–4:2015; the free acidity as oleic acid was determined according to the procedure ISO 660:2009 [38].

2.4. Meteorological and Soil Measurements

The rainfall and air temperature were recorded in each location during the entire period of trials on a CR10 data logger (Campbell Scientific Inc., Loughborough, UK) connected to a meteorological station sited 100 m away from the experimental field.

Soil analysis was conducted in each location before the start of the experiment (early September) by collecting three soil samples per each *Cynara* cultivated plot, with a 4 cm (i.d.) core auger to a depth of 30 cm, fractured into aggregates by hand pressure, air-dried, and sieved (<2 mm). The three samples were taken from the middle of each plot, ~100 cm away from each other. The soil analyses were carried out according to procedures that were approved by the Italian Society of Soil Science [39].

2.5. Statistical Analysis

Levene's test was used to test for homoscedasticity, following which the data were subjected to a three-way analysis of variance (ANOVA), based on a factorial combination of two locations × two genotypes × three seasons. The means were separated on the basis of the Least Significant Difference (LSD) test, when the F-test was significant. The percentage data were arcsine transformed before ANOVA (untransformed data are reported and discussed). All of the calculations and analyses were performed using the appropriate options within CoStat1 version 6.003 (CoHort Software, Monterey, CA, USA). Collected data were submitted to multiple correlation analyses in order to define the relationship among variables.

2.6. Temperature and Rainfall

The monthly maximum and minimum temperatures at the plain location during 2014 and 2016 were similar; instead, lower monthly maximum and minimum temperatures were recorded in January and February in 2015 as compared to 2014 and 2016 (Figure 1). In addition, in June, July, and August in 2015, higher monthly maximum and minimum temperatures were recorded when compared to 2014 and 2016. In the hills, the monthly maximum and minimum temperatures during 2014 and 2015 were similar, whereas, throughout March, April, and May, higher monthly maximum and minimum temperatures were found in 2016 as compared to 2014 and 2015. There was considerable variability in rainfall from year to year at the two locations. On the plain, the annual rainfall in 2013–2014 (679 mm) and 2014–2015 (669 mm) was almost double when compared to 2015–2016 (348 mm) and higher than the long-term average (481 mm). In the hills, the annual rainfall in 2013–2014 (620 mm) was in line with the long-term average (613 mm); on the contrary, in 2014–2015 (950 mm) and in 2015–2016 (470 mm), it was higher and lower than 2013–2014 and the long-term average, respectively (Figure 1).

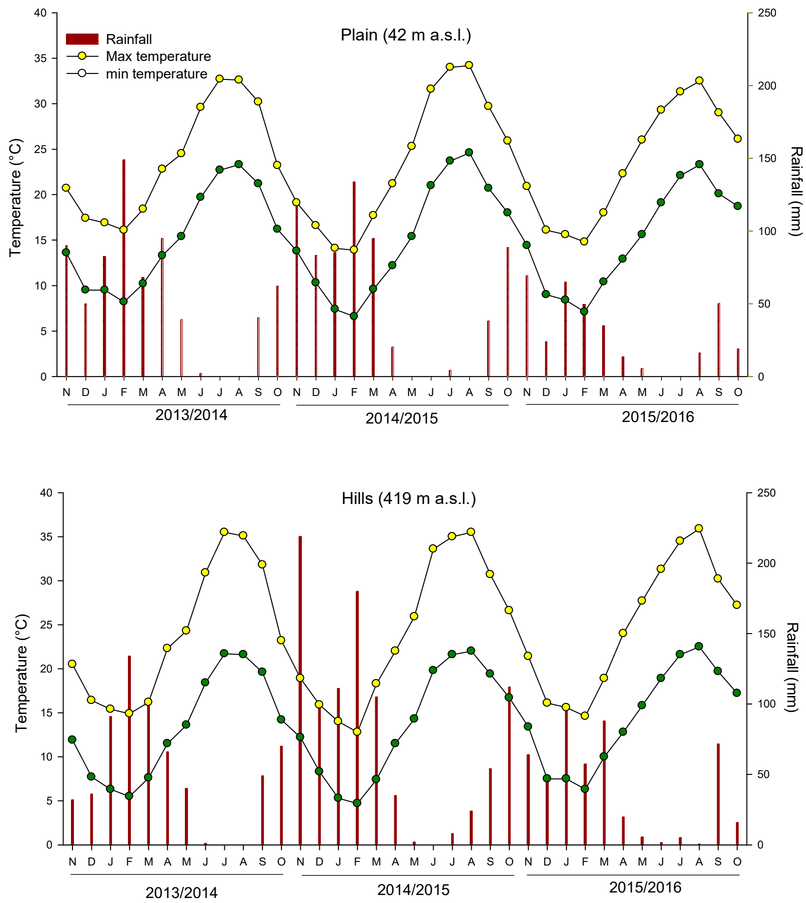

Figure 1. Maximum and minimum monthly air temperatures and total monthly rainfall recorded through the three seasons in the two environments.

3. Results

All of the data were subjected to ANOVA, whose results are reported in Table 2.

Table 2. F values resulting from analysis of variance for all studied variables.

Variable	Location (L)	Genotype (G)	Season (S)	(L) × (G)	(L) × (S)	(G) × (S)	(L) × (G) × (S)
Degree of freedom	1	1	2	1	2	2	2
Plants survival (%)	NS	9 **	19 ***	NS	NS	NS	NS
Plant height (cm)	21 ***	997 ***	112 ***	NS	NS	25 ***	NS
Biomass yield (t DM ha^{-1})	14 **	697 ***	69 ***	NS	NS	37 ***	NS
Biomass DM content (%)	15 **	NS	7 *		8 **	NS	NS
Leaves incidence (%)	NS	70 ***	NS	NS	NS	5	NS
Stalks incidence (%)	NS	347 ***	5 *	NS	NS	5	NS
Heads incidence (%)	NS	513 ***	4 *	NS	NS	4	NS
Seed yield (t DM ha^{-1})	5 *	1343 ***	NS	NS	NS	20 ***	NS
N heads plant^{-1}	NS	NS	10 ***	NS	NS	10 ***	NS
Seed weight head^{-1}	NS	103 ***	3 ***	NS	NS	4 ***	NS
Biomass E yield (GJ ha^{-1})	14 **	698 ***	69 ***	NS	NS	37 ***	NS
Seed E yield (GJ ha^{-1})	5 *	1343 ***	NS	NS	NS	20 ***	NS
Total E yield (GJ ha^{-1})	14 **	787 ***	68 ***	NS	NS	36 ***	NS

DM = Dry Matter; E = Energy; *, **, *** indicate significant at $p \leq 0.05$, 0.01, 0.001, respectively; NS = Not Significant.

3.1. Plant Survival and Height

Regardless of locations, plant survival was very high and it obviously declined with the seasons (Table 3). Higher plant survival values over the period S1–S3 were recorded in wild cardoon (99.3%) than in cultivated cardoon (98.2%) (Table 3).

Table 3. Plant survival, height, biomass yield, biomass dry matter content, and incidence of various components (leaves, stalks, and heads) on total biomass in *Cynara cardunculus* L. as affected by the main factors.

	Plant Survival (%)	Plant Height (cm)	Biomass Yield (t DM ha^{-1})	Biomass DM Content (%)	Leaves [(1)] Incid. (%)	Stalks [(1)] Incid. (%)	Heads [(1)] Incid. (%)
Location							
Plain	98.8 a	127.3 b	9.7 b	91.6 a	35.5 a	35.9 a	28.6 a
Hills	98.8 a	134.0 a	10.9 a	86.6 b	36.0 a	36.5 a	27.5 a
Genotype							
Cultiv. cardoon	98.2 b	153.8 a	14.2 a	89.0 a	38.5 a	42.6 a	18.7 b
Wild cardoon	99.3 a	107.5 b	6.4 b	89.3 a	32.9 b	29.8 b	37.4 a
Season							
S1	100 a	118.7 c	7.9 b	89.6 ab	35.4 ab	34.9 b	29.7 a
S2	98.9 b	128.1 b	11.6 a	91.9 a	36.8 a	36.1 ab	27.2 b
S3	97.4 c	145.2 a	11.6 a	85.8 b	35.0 b	37.7 a	27.3 b

S1 = season 1, S2 = season 2, S3 = season 3. Different letters within main factors indicate significance at Fisher's protected least significant difference (LSD) test ($p \leq 0.05$; [(1)] incidence values of leaves, stalks and heads on total biomass were calculated on the basis of dry weight.

Plants that were grown at the hilly location showed greater height than those grown on the plain (134.0 vs. 127.3 cm). Cultivated cardoon showed taller plants as compared to wild cardoon (153.8 vs. 107.5 cm) (Table 3). The plant height increased significantly through the seasons in both genotypes, but, in wild cardoon, it showed a greater increasing pattern from S1 to S3 (34%), when compared to cultivated cardoon (from S1 to S3, 15%, respectively) (Figure 2).

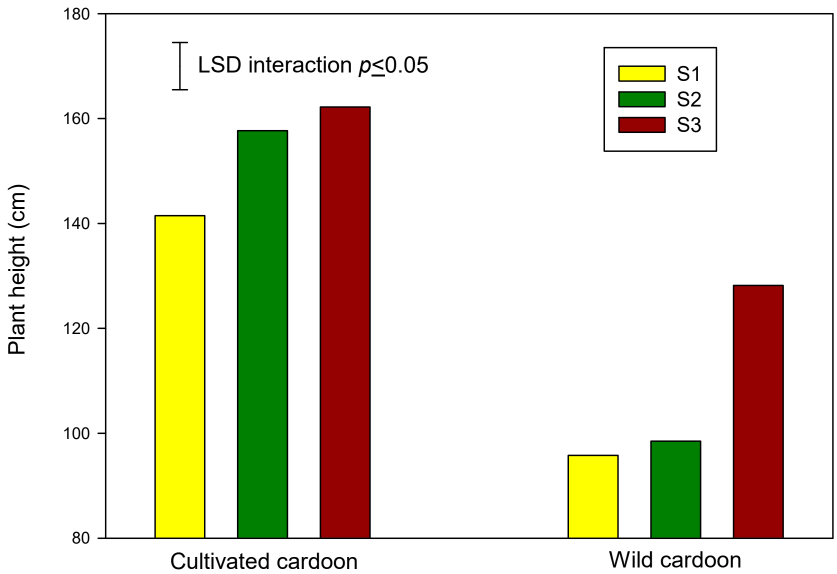

Figure 2. Plant height as affected by interaction 'genotype × season'. S1 = season 1, S2 = season 2, S3 = season 3.

3.2. *Aboveground Dry Biomass Yield and Its Partitioning*

Averaged over the period S1–S3 and genotypes, the biomass yield was significantly higher in the hills than on the plain (10.9 vs. 9.7 t DM ha^{-1} year^{-1}). Cultivated cardoon was characterized by a biomass yield almost more than double than that of wild cardoon (14.2 vs. 6.4 t DM ha^{-1} year^{-1}) (Table 3).

However, as revealed by the significant 'genotype × season' interaction, in cultivated cardoon aboveground dry biomass yield significantly increased by 65 % from S1 to S2 (from 10.0 to 16.5 t DM ha^{-1} year^{-1}) and then remained constant in the third season studied (16.2 t DM ha^{-1} year^{-1}); in wild cardoon, biomass yield increased poorly from S1 to S3 (from 5.7 to 6.9 t DM ha^{-1} year^{-1}) with no significant differences on statistical analysis (Table 4). Biomass dry matter content at harvest was significantly higher in plants from the plain than the hills (91.6 vs. 86.6 %) (Table 4); in addition, as revealed by the significant 'location × season' interaction, biomass dry matter content in plants from the plain significantly increased from S1 to S2 (from 87.3 to 92.5%) and it then dropped to 80.1% in S3, whereas in plants from the hills it remained constant (around 91%) throughout the seasons (Figure 3). With reference to biomass partitioning, cultivated cardoon showed a significantly higher stalks and leaves incidence as compared to wild cardoon (42.6 vs. 29.8 and 38.5 vs. 32.9%, respectively), while the latter exceeded cultivated cardoon for heads incidence (37.4 vs. 18.7%) (Table 3). Regardless of location and genotype, the plants showed a different shift in biomass partitioning over seasons, with an increase from S1 to S3 of stalks incidence (from 34.9 to 37.7%), and a decrease in heads incidence over the same period (from 29.7 to 27.2%); leaves incidence showed the highest value in S2, the lowest in S3 (Table 3).

Table 4. Biomass yield, seed yield, number of heads per plant, and average seed weight per head, as affected by interaction 'genotype × season'.

		Biomass Yield Yield	**Seed Yield**	**Heads**	**Seed Weight**
Genotype	**Season**	**(t DM ha^{-1})**	**(t DM ha^{-1})**	**(N plant^{-1})**	**(g head^{-1})**
Cultiv. cardoon	S1	10.0	0.65	6.7	3.97
	S2	16.5	0.71	5.7	5.26
	S3	16.2	0.59	4.7	5.33
Wild cardoon	S1	5.7	0.22	5.5	1.66
	S2	6.6	0.22	5.2	1.69
	S3	6.9	0.27	5.5	2.05
LSD interaction $p \leq 0.05$		1.7	0.06	1.1	0.62

S1 = season 1, S2 = season 2, S3 = season 3.

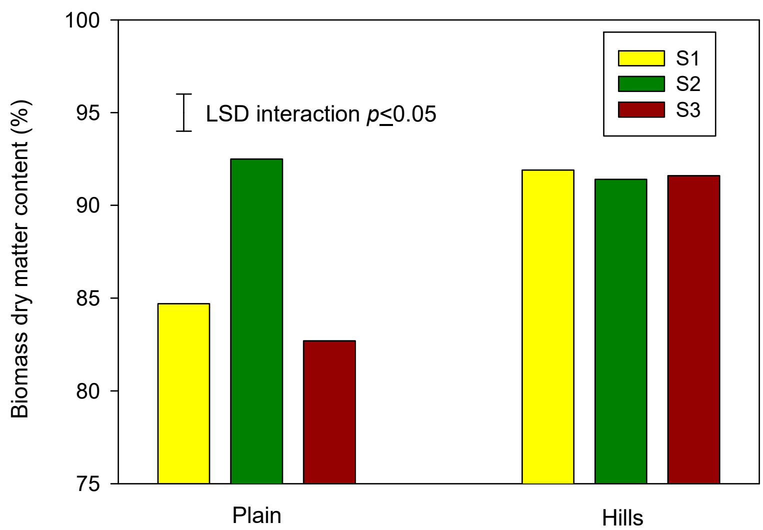

Figure 3. Biomass dry matter content as affected by interaction 'location × season'. S1 = season 1, S2 = season 2, S3 = season 3.

3.3. Seed Yield and Components

Averaged over the period S1–S3 and genotypes, the seed yield was higher from the hills when compared to the plain (0.46 vs. 0.44 t DM ha^{-1} year^{-1}). Averaged over the locations, cultivated cardoon yielded seed 174% more than wild cardoon (0.65 vs. 0.24 t DM ha^{-1} year^{-1}) (Table 5), attributable not to the number of heads per plant (5.7 vs. 5.4), but to the higher seed average weight per head (4.8 vs. 1.8 g head^{-1}) (Table 4). The seed yield trend during the seasons was substantially different between the two genotypes. In fact, in cultivated cardoon, the seed yield was almost constant from S1 to S2 (around 0.68 t ha^{-1}) and it then dropped down to 0.59 t ha^{-1} in S3, mainly due to the reduction in the number of heads from S1 to S3 (from 6.7 to 4.7 N plant^{-1}), while the average weight of seeds per head instead slightly increased from S1 to S3 (from 3.97 to 5.33 g head^{-1}); in wild cardoon, on the contrary, some stability over the years of the seed yield was observed without significant differences (Table 4).

Table 5. Seed yield, number of heads per plant, average seed weight per head, and energy yields in *Cynara cardunculus* L., as affected by the main factors.

	Seed Yield (t DM ha^{-1})	Heads (N plant^{-1})	Seed Weight (g head^{-1})	Biomass E [(1)] Yield (GJ ha^{-1})	Seed E [(1)] Yield (GJ ha^{-1})	Total E [(1)] Yield (GJ ha^{-1})
Location						
Plain	0.44 b	5.4 a	3.2 a	162 b	10 a	172 b
Hills	0.46 a	5.6 a	3.4 a	180 a	10 a	190 a
Genotype						
Cultiv. cardoon	0.65 a	5.7 a	4.8 a	236 a	15 a	251 a
Wild cardoon	0.24 b	5.4 a	1.8 b	106 b	5 b	111 b
Season						
S1	0.44 ab	6.1 a	2.8 b	130 b	10 a	140 b
S2	0.46 a	5.4 b	3.5 a	191 a	10 a	201 a
S3	0.44 b	5.1 b	3.7 a	191 a	10 a	201 a

S1= season 1, S2 = season 2, S3 = season 3. Different letters within main factors indicate significance at Fisher's protected least significant difference (LSD) test ($p \leq 0.05$); [(1)] E = Energy.

3.4. Energy Yield

The crop grown in the hilly location provided greater energy from biomass than the plain (180 vs. 162 GJ ha^{-1} year^{-1}), but equal energy from seeds (10 GJ ha^{-1} year^{-1}) and, consequently, greater total energy (190 vs. 172 GJ ha^{-1} year^{-1}) (Table 5). As regards the genotypes tested, the cultivated cardoon more than doubled the biomass energy yield of wild cardoon over the S1–S3 cropping period (236 vs. 106 GJ ha^{-1} year^{-1}) and tripled the energy of the seeds (15 vs. 5 GJ ha^{-1} year^{-1}), thus giving a total energy greater than 225% (251 vs. 111 GJ ha^{-1} year^{-1}) (Table 5). Moreover, when comparing their energy yield pattern, the genotypes showed distinct trends passing from season to season, as revealed by the ANOVA. Indeed, cultivated cardoon biomass energy yield significantly increased from S1 to S2 (165 to 273 GJ ha^{-1} year^{-1}), in order to remain constant in S3 and seed energy yield increased from S1 to S2 and then dropped in S3 (Table 6). Differently, wild cardoon showed a slight and not significantly increase from S1 to S3 of biomass energy yield (from 95 to 114 GJ ha^{-1} year^{-1}) and seed energy yield (from 5 to 6 GJ ha^{-1} year^{-1}). Overall, total energy yield in cultivated cardoon increased significantly from S1 to S2, to remain constant at S3, whereas, in wild cardoon, it increased slightly, showing no significant differences between years (Table 6).

Table 6. Biomass energy yield, seed energy yield and total energy yield, as affected by interaction. 'genotype × season'.

Genotype	Season	Biomass E [(1)] Yield (GJ ha^{-1})	Seed E [(1)] Yield (GJ ha^{-1})	Total E [(1)] Yield (GJ ha^{-1})
Cultivated cardoon	S1	165	15	180
	S2	273	16	289
	S3	269	13	282
Wild cardoon	S1	95	5	100
	S2	109	5	114
	S3	114	6	120
LSD interaction $p \leq 0.05$		30	2	30

S1= season 1, S2 = season 2, S3 = season 3. [(1)] E = Energy.

3.5. Oil Yield and Fatty Acids Composition

Seed moisture, oil yield, acidity, and fatty acid composition were influenced by the location and genotype (Table 7). The seed moisture was significantly higher in the hills when compared to the plain (8.1 vs. 5.9%), whereas oil yield and acidity were substantially similar between the two locations. With regard to fatty acid composition oil of plants grown in the hills, these recorded higher concentrations of palmitoleic acid (0.18 vs. 0.15 g 100 g^{-1} DW), hepatdecanic acid (0.05 vs. 0.04 g 100 g^{-1} DW), and linolenic acid (0.06 vs. 0.04 g 100 g^{-1} DW) compared to the plain. Regardless of locations, cultivated cardoon showed a higher oil yield as compared to wild (25 vs. 23 g 100 g^{-1} DW) and with higher acidity (1.3 vs. 0.4%); in addition, the oil of cultivated cardoon was found to have higher palmitoleic acid (0.21 vs. 0.12 g 100 g^{-1} DW), heptadecanoic acid (0.06 vs. 0.03 g 100 g^{-1} DW), as well as higher linolenic acid (0.06 vs. 0.04 g 100 g^{-1} DW) than wild cardoon (Table 7).

Table 7. Seeds moisture, oil yield, acidity, and fatty acid composition in *Cynara cardunculus* L., as affected by location and genotype (mean ± standard deviation, *n* = 6).

		Location		Genotype	
Variable	**Unit**	**Plain**	**Hills**	**Cultiv. Cardoon**	**Wild Cardoon**
Seeds moisture	%	5.9 ± 0.3	8.1 ± 1.0	7.2 ± 0.2	6.8 ± 0.1
Oil yield	g 100 g^{-1} DW	23.9 ± 2.0	24.1 ± 1.6	25.0 ± 1.3	23.0 ± 1.5
Acidity	% oleic acid	1.0 ± 0.2	0.7 ± 0.1	1.3 ± 0.1	0.4 ± 0.03
Myristic, C14:0	g 100 g^{-1} DW	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.02
Palmitic, C16:0	"	11.1 ± 1.4	10.8 ± 1.1	11.0 ± 1.3	10.9 ± 1.2
Palmitoleic, C16:1	"	0.15 ± 0.01	0.18 ± 0.02	0.21 ± 0.01	0.12 ± 0.01
Heptadecanoic, C17:0	"	0.04 ± 0.003	0.05 ± 0.004	0.06 ± 0.005	0.03 ± 0.004
Stearic, C18:0	"	3.45 ± 0.2	3.25 ± 0.3	3.30 ± 0.3	3.40 ± 0.2
Oleic, C18:1	"	28.5 ± 1.6	27.6 ± 1.9	28.4 ± 2.3	27.7 ± 1.8
Linoleic, C18:2	"	55.3 ± 2.7	54.3 ± 2.8	54.8 ± 2.7	54.8 ± 2.5
Linolenic, C18:3	"	0.04 ± 0.003	0.06 ± 0.004	0.06 ± 0.005	0.04 ± 0.003
Arachidic, C20:0	"	0.40 ± 0.05	0.37 ± 0.03	0.39 ± 0.04	0.38 ± 0.05
Behenic, C22:0	"	0.14 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
Lignoceric, C24:0	"	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.02	0.20 ± 0.01

3.6. Correlation Among Variables

The total energy yield was positively correlated with the aboveground dry biomass and seed yield (0.99 *** and 0.87 ***, respectively), with plant height (0.90 ***) and percentage incidence of stalk (0.91 ***), whereas it was negatively correlated with the percentage incidence of heads (−0.93 ***). Seed yield showed a positive correlation with aboveground dry biomass (0.86 ***), incidence of stalk (0.90 ***), incidence of leaves (0.84 ***), and negatively with incidence of heads (−0.95 ***). Both biomass and seed yield were correlated also with plant height (0.89 *** and 0.88 ***, respectively) and with seed weight per head (0.94 *** and 0.92 ***, respectively) (Table 8).

Table 8. Pearson's correlation coefficients and significance of correlations among all of the variables recorded in the three season experiment and in the two environments.

	Plant Height	Biomass Yield	Biomass DM	Leaves Incid.	Stalks Incid.	Heads Incid.	N Heads plant^{-1}	Seed Weight head^{-1}	Seed Yield	Biomass E Yield	Seed E Yield	Total E Yield
Plant height	-											
Biomass yield	0.89 ***	-										
Biomass DM	NS	NS	-									
Leaves incid.	0.75 ***	0.77 ***	NS	-								
Stalks incid.	0.89 ***	0.91 ***	NS	0.70 ***	-							
Heads incid.	−0.91 ***	−0.93 ***	NS	−0.86 ***	−0.97 ***	-						
N heads plant^{-1}	NS	NS	NS	NS	NS	NS	-					
Seed weight head^{-1}	0.91 ***	0.94 ***	NS	0.76 ***	0.93 ***	−0.94 ***	NS	-				
Seed yield	0.88 ***	0.86 ***	NS	0.84 ***	0.90 ***	−0.95 ***	NS	0.92 ***	-			
Biomass E yield	0.90 ***	0.99 ***	NS	0.77 ***	0.91 ***	−0.93 ***	NS	0.94 ***	0.86 ***	-		
Seed E yield	0.88 ***	0.86 ***	NS	0.84 ***	0.90 ***	−0.95 ***	NS	0.92 ***	0.99 ***	0.86 ***	-	
Total E yield	0.90 ***	0.99 ***	NS	0.78 ***	0.91 ***	−0.93 ***	NS	0.95 ***	0.87 ***	0.99 ***	0.87 ***	-

DM = Dry Matter; E = Energy, *** indicate significant at $p < 0.001$, respectively; NS = Not Significant.

4. Discussion

The biomass yields that were obtained in both environments in our research are substantially in agreement with other authors operating in rainfed conditions in Italy [12,26,28,32]. On the contrary, our results were lower than those that were reported in a previous work [14], in which, differently from the current experiment, the *C. cardunculus* crop was grown on a high fertility soil and managed with supplements in terms of both irrigation and fertilization. The seed yields obtained in this research (about 0.45 t DM ha^{-1} year^{-1}) are also consistent with the results that were obtained under rainfed conditions in Spain [10] and Portugal [13], and lower than those reported in the same environment [14,25,30] with the aid of fertilization and irrigation. Biomass and seed yields in this research are not so high, but they are still respectable when considering that they have been obtained in marginal areas with soils with low-medium productivity and managed under zero/minimal inputs in rainfed conditions.

The two environments that were studied in this research, different for geographical, pedological, and climatic characteristics, influenced the productive response of the plants in both cultivated cardoon and wild cardoon. Indeed, regardless of seasons, the plants grown in the hilly area provided greater aerial biomass (10.9 vs. 9.7 t DM ha^{-1} year^{-1}) when compared to the ones grown on the plain. The hills compared to the plain recorded higher maximum temperatures and lower minimum temperatures and, above all, higher annual rainfall (680 vs. 565 mm year^{-1}) throughout the three seasons. Weather conditions, in particular rainfall, can affect biomass production, as was clearly shown in a nine-year experiment in Spain [40], in which biomass productivity of cultivated cardoon, in rainfed conditions, was extremely variable from one year to the next according to rainfall. The yields of whole biomass were reported in the range of 10–20 Mg DM ha^{-1} year^{-1} for crops receiving around 500 mm annual rainfall, and a mean value of 14 Mg ha^{-1} year^{-1} for the drylands of Central Spain in years of severe drought; much lower yields e.g. 3.4 Mg ha^{-1} year^{-1} were reported for Madrid (Spain) in a year with only 280 mm [10]. In a recent research that was conducted in a Mediterranean cropland [41] evaluating the productivity levels of different genotypes of cultivated cardoon ('Altilis', 'Gigante' and 'Trinaseed') in two different environments (plain vs hills), no differences in lignocellulosic biomass yield (on average 19 t DM ha^{-1} year^{-1}) were found during three years between the environments, which were different for minimum and maximum temperatures, but very similar for rainfall (900 mm year^{-1}). In addition, the loam-clay texture soil in the hills, as compared to the sandy loam of the plain, has certainly allowed for greater water retention and water conservation in the soil and, therefore, better conditions for plant growth and development. This soil characteristic (texture) is of greater importance in rainfed conditions.

The differences found in the biomass dry matter content between locations (lower in the hills than the plain) are also attributable to the different weather conditions of the two environments. In our research, the higher seed yields found may also be attributed to the higher rainfall in the hills when compared to the plain (0.46 vs. 0.44 t DM ha^{-1} year^{-1}, respectively).

The season, also as a consequence of the crop age, had an important effect in both environments. Indeed, the rather modest dry biomass yield at S1 (7.8 t DM ha^{-1}) increased in S2 (11.6 t DM ha^{-1}) to remain constant in S3 (11.7 t DM ha^{-1}). Like all perennial biomass crops, cardoon has low yields in the first year, which can be considered to be a crop stabilization stage (about 60% of maximum yield), and it reaches the maximum values at the fifth or sixth year of cultivation [12,42]. Although the research was carried out for only three seasons, there was a progressive increase in the average height of the plant (from about 119 cm in S1 to 145 cm in S3), a significant increase in the incidence of stalks on the total biomass (from 34.9% in S1 to 37.7% in S3) and a decreasing incidence of the heads (from 29.7% in S1 to 27.3% in S3). The highest incidence of stalks on biomass yield, as well as the decreasing incidence of the heads over time, substantially agree with a previous experiment in a similar environment [14,43], reflecting a change in the overall plant organography and subsequent modifications in intra-plant competition relationships. Beyond the mean annual biomass yield, a very different biomass and seed yield pattern was recorded between genotypes in this study across seasons. Indeed, cultivated cardoon showed oscillations over the three cropping season periods in biomass yield and seed yield according to other authors [12,32]. Differently, wild cardoon, although less yielding, showed a more conservative profile in biomass and seeds yield over seasons, as observed in the same environment [32]. The stability of biomass and seed production over time is an advantage, as it allows for a more reliable quantification of the land for the cultivation of energy plants and the possibility of ensuring a programmable biomass production. Therefore, stabilizing the biomass production over time should be one of the objectives of the breeding programs in *C. cardunculus* as an energy crop. Averaged over seasons and environments, cultivated cardoon yielded 122% more biomass than wild cardoon (14.2 vs. 6.4 t DM ha^{-1} $year^{-1}$), in agreement with the results of various researches that were conducted both in conditions of good soil fertility and supplying fertilization and irrigation [14,30], but also in marginal and low fertile environments, such as that of our research [32]. In addition, cultivated cardoon yielded seed 174% more than wild cardoon (0.65 vs. 0.24 t DM ha^{-1} $year^{-1}$). It is known that high growth generally results in a greater number of heads, and in heads of larger size that contain a higher proportion of seeds (% *w*/*w*) [44]. This plasticity of *Cynara* explains the wide range of values that were reported in the literature for seed yields, from 0.2 to 4.3 t ha^{-1} $year^{-1}$ [2]. Seed yield has been related to biomass yield, but what interestingly emerged in this research is that both biomass and seed yield were positively correlated with plant height, with incidence of stalks and leaves and with seed weight per head and negatively with incidence of heads. These indications can be important for breeding, as they indicate that, to increase the yield capacity of genotypes for producing both biomass and seed yield, it is necessary to increase the formation and development of stalks in the plants and seed weight per head, but also, with respect to wild cardoon alone, in order to improve plant height. Breeding for the aforementioned characteristics should be facilitated by marker-assisted selection for quantitative traits [45], since the genome of the artichoke has recently been decoded [15,46]. With regard to energy yield, it substantially reflects the quantity of biomass produced per hectare, since the difference in heating values between the botanical varieties is small [2]. Consequently, the crop grown in the hills provided higher biomass energy yield as compared to the plain (180 vs. 162 GJ ha^{-1} $year^{-1}$), but equal seeds energy yield (10 GJ ha^{-1} $year^{-1}$) and, consequently, a greater total energy yield (190 vs. 172 GJ ha^{-1} $year^{-1}$). These results suggest the advisability of moving the *C. cardunculus* as energy crop to inland marginal hilly or submontane areas, generally characterized by higher average annual rainfall, and loamy-clay soils compared to the plain in the Mediterranean environment. In these marginal hilly areas *C. cardunculus* could also represent a good chance to fight erosion and to improve soil fertility, as shown in a multi-year research [32]. Between the two genotypes tested, the cultivated cardoon more than doubled the biomass energy yield of wild cardoon (236 vs. 106 GJ ha^{-1} $year^{-1}$). However, wild cardoon could be more wind resistant compared to cultivated cardoon due to its lower height; this, together with greater survival and stability, means that wild rather than cultivated cardoon seems to be more appropriate for implanting a perennial crop in difficult and windy marginal areas.

With regard to oil yield, we found no difference between the two environments in accordance with other findings [47], whereas wild cardoon as compared to cultivated cardoon showed less oil yield (23 vs. 25 g 100 g^{-1} DW) and acidity (0.4 vs. 1.3% oleic acid) in agreement with the results of researches that were conducted in a similar environment [25,48]. The fatty acids profile was very similar to that of the sunflower (*Helianthus annuus* L.), a species of the same family as the Compositae [11]. The main components (linoleic, oleic, and palmitic acids) showed no differences between environments and genotypes, whereas differences were found in minor fatty acids between environments with plants grown in the hills as compared to the plain, showing higher concentration of palmitoleic (0.175 vs. 0.15 g 100 g^{-1} DW), hepatdecanic (0.05 vs. 0.04 g 100 g^{-1} DW), and linolenic (0.06 vs. 0.045 g 100 g^{-1} DW) acids, and between genotypes with wild cardoon showing lower palmitolenic (0.12 vs. 0.20 g 100 g^{-1} DW), heptadodecanic (0.03 vs. 0.05 g 100 g^{-1} DW), and linolenic (0.04 vs. 0.06 g 100 g^{-1} DW) acids than cultivated cardoon. In the context of bio-energy applications, the suitability of biomass and seed oil of *C. cardunculus* for biodiesel [49,50], biomethane, and bioethanol production [17,18] has already been studied. For the two-fold application of the crop—lignocellulosic biomass for energy and oil seeds for biodiesel production—a strategy was suggested [2], i.e. to harvest the whole biomass, mechanically separate the different biomass fractions at the facility; in this way, each biomass fraction could be directed to different applications, following the biorefinery concept.

5. Conclusions

Overall, the results of this research show that cultivated and wild cardoon as energy crops are able, without competing with food crops, to enhance marginal areas, with soils with low-medium productivity and managed under zero/minimal inputs in rainfed conditions, where they may also represent a good chance to fight erosion and improve the soil fertility. The greater productivity in biomass and seed expressed by *C. cardunculus* in the marginal environment located at a higher altitude suggests the opportunity to move the crop to hilly and submontane areas, which are generally characterized by higher average annual rainfall. The season, although only evaluated for three years (short cycle), showed a different effect based on the genotype, highlighting a greater production constancy of wild cardoon over time (though less yielding) compared to cultivated cardoon; attention should be given to the stability together with agronomical performances in the breeding, recently facilitated by the decoding of the artichoke genome, in order to valorize the yield potential of marginal farmland in the Mediterranean area.

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