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Caffeoylquinic acids and flavones profile in *Cynara cardunculus* L. seedlings under controlled conditions as affected by light and water-supply treatments

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ABSTRACT

Cynara cardunculus L. is an Asteraceae member widely diffused in the Mediterranean Basin, rich in phenolic acids (caffeoylquinic acids and derivatives), flavones (luteolin, apigenin and their conjugates), anthocyanins, inulin and vitamin C. Thanks to their numerous biological activities, these compounds are in high demand for food and no-food applications. To match such request, in this research we evaluated the effect of three water-supply (100%, 75% and 50% of plant requirement) and light (24 h, 12 h and 0 h) treatments on the polyphenols profile of *C. cardunculus* seedlings, with the aim of developing a production system under controlled conditions. Overall, the 100% of plant water requirement increased the amount of caffeoylquinic acids (+28%), luteolines (+27%) and total measured polyphenols (+26%) respect to water-stressed plants (75% and 50% of plant requirement), with cultivated cardoon showing a higher concentration than the globe artichoke. Concerning the light treatment, the trend 0 < 12< 24 h was found for all phytochemical compounds. In particular, 24 h of light strongly induced the biosynthesis of caffeoylquinic acids (+119%), luteolines (+273%) and total measured polyphenols (+129%) compared to 0 h of light. In both experiments, the most abundant compounds were 5-*O*-caffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid. Regardless of experiment, the genetic background showed a significant role, since the responses were genotype-dependent. From these results clearly emerged the possibility of producing polyphenols-enriched *C. cardunculus* seedlings in controlled conditions.

1. Introduction

Cynara cardunculus L. is an herbaceous perennial C_3 plant widely diffused in the semi-arid zones of the Mediterranean Basin, especially in Southern Europe. It is a complex species belonging to the *Cynara* genus from the Asteraceae family and comprising three cross-pollinated and cross-compatible botanical varieties: the globe artichoke [var. *scolymus* (L.) Fiori], the cultivated cardoon [var. *altilis* (DC.)] and the wild cardoon [var. *sylvestris* (Lamk) Fiori]. Altogether, the three types are recognized as a multipurpose crop with several food-alternative uses such as the inclusion in the animal feed as a forage, the production of biofuels (direct combustion, biodiesel, biomethane, bioethanol), paper-pulp, lightwood panel, and the use as ornamental and phytoremediation plant (Lanteri et al., 2012; Gominho et al., 2018; Capozzi et al., 2020; Pandino and Mauromicale, 2020). Moreover, the wide-range of biological activities possessed by *C. cardunculus* extracts have recently caught the attention of both the scientific community and companies (Silva et al., 2022). These biological activities comprise antimicrobial (Scavo et al., 2019a), antioxidant, anticancer (Shallan et al., 2020), anti-inflammatory (Salekzamani et al., 2019) and phytotoxic (Scavo et al., 2020a, 2019b) effects, as well as a number of non-classical medical applications (Zayed et al., 2020). These biological properties are related to the high content of bioactive compounds accumulated in several plant parts: seeds, leaves, stems, bracts, receptacles and heads (Pandino et al., 2012; Zayed et al., 2020). As reported in literature, they are mainly polyphenols (phenolic acids, caffeoylquinic acids, flavones, coumarins and anthocyanins) and terpenoids (sesquiterpene lactones, mono- and triterpenes), along with inulin and vitamin C (Pandino et al., 2012; Rial et al., 2014; Petropoulos et al., 2018; Scavo et al., 2020b; Silva et al., 2022). Among polyphenols, caffeoylquinic acids (e.g. chlorogenic acid, cynarin, dicaffeoylquinic acids, etc.), flavones (apigenin and luteolin) and their glycosides (e.g., apigenin 7-*O*-glucoside, apigenin malonylglucoside, luteolin 7-*O*-glucoronide, etc.) play a key role in the antioxidant, nutraceutical and pharmaceutical effects of

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Table 1
Calibration data for used standards including regression equations, correlation coefficients, precision, LOD[a] and LOQ[b] for the proposed method.

Standards	Regression equation	Correlation coefficient (r^2)	Precision (%CV)	LOD (mg L^{-1})	LOQ (mg L^{-1})
Chlorogenic acid	$y = 22.75x - 85.95$	0.9934	0.91	0.29	0.77
Cynarin	$y = 46.72x - 95.70$	0.9993	0.55	0.30	1.13
Luteolin-7-*O*-glc[c]	$y = 40.49x - 25.97$	0.9999	0.68	0.57	1.80
Luteolin	$y = 14.05x + 13.24$	0.9933	0.85	0.35	1.77
Apigenin-7-*O*-glc	$y = 38.94x - 82.74$	0.9932	0.99	0.22	0.61
Apigenin	$y = 41.78x - 4.20$	1	0.88	0.87	2.05

[a] = limit of detection.
[b] = limit of detection.
[c] = glucoside.

C. cardunculus extracts (Pandino et al., 2013, 2015).

Their synthesis and amount are closely influenced by genotype, plant part, harvest time, crop management (e.g. irrigation, fertilization, etc.) and environmental factors such as solar radiation, air temperature and rainfall (Lombardo et al., 2009, 2018; Pandino et al., 2017a). Overall, these factors do not produce standardized features in the raw material. This has prompted industries, as well as scientists, to consider the production of pharmaceutical plant compounds under controlled conditions (Mulabagal and Tsay, 2004; Pandino et al., 2017b). However, because of the growing public interest in replacing the synthetic antioxidants in foods with natural ones, the exploitation of a cheap and valuable source of health-promoting compounds could represent an important challenge for the near future (Zayed et al., 2020). For example, Toscano et al. (2020), studying the possibility of producing cultivated cardoon seedlings with a high content of bioactive molecules, reported that salt stress enhanced the total phenols content and the antioxidant activity in the obtained sprouts. Lobiuc et al. (2017) indicated that modulating blue and red LED ratios improved the growth, chlorophyll a, anthocyanin and phenolic synthesis (rosmarinic and gallic acid), as well as the free radical scavenging activity of *Ocimum basilicum* microgreens. Management of abiotic elicitors which trigger secondary metabolic pathway of *in vitro* cultures or plants under controlled conditions has been used for several horticultural and aromatic plant species (Toscano et al., 2019; Chandran et al., 2020).

Taking in mind these considerations, in the present research we focused on the management of water-supply and light treatments under controlled conditions as tool to produce *C. cardunculus* seedlings of seed-propagated globe artichokes and a cultivated cardoon enriched of polyphenol compounds.

2. Material and methods

2.1. Experimental design

This research was implemented to assay the effect of different water-supply and light treatments under controlled production systems able to obtain *C. cardunculus* seedlings and/or plantlets rich in polyphenols. In this view, it was performed a controlled production system, herein indicated as experiment 1, to evaluate the effect of three water-supply treatments on the leaf polyphenols profile of a globe artichoke and a cultivated cardoon. The other, refereed as experiment 2, assessed the influence of three light treatments on three seed propagated lines of globe artichoke. Both experimental designs were arranged in a completely randomized block design with three replicates.

2.2. Experiment 1-water-supply treatment

The experiment was carried out on the cultivated cardoon (‘Altilis 41’) and the new seed propagated line of globe artichoke (‘NP5’), both selected by researchers at the University of Catania within a breeding program on *C. cardunculus*. ‘Altilis 41’ is a cultivated cardoon commonly used for biomass and biomolecules production (Pandino et al., 2015); ‘NP5’ is a globe artichoke line with high achene production that was chosen by the need of having a homozygous seed-propagated progeny (Mauromicale et al., 2018; Mazzeo et al., 2020). Three seeds of both lines were placed into plastic vessels (Ø, 10 cm; height 8 cm) filled with a mixture of peat substrate (Profi- Substrate, Gramoflor, Germany)/sand (1:1). Each vessel was incubated into a growth chamber in alternating light (dark/light cycle 13/11 h) at 18±1 °C and daily moistened with deionized water (electrical conductivity $\leq$0.01 dS m^{-1}). Starting from “seeding”, all treatments were irrigated with 100 mL of deionized water, while from the first elliptic leaf visible phenological stage (code 11 according to the BBCH scale proposed by Archontoulis et al. (2010) to the five leaves visible (code 15), the following treatments were used: water-supply at 100% of plant requirement (W_{100}, control), water-supply at 75% of plant requirement (W_{75}), water-supply at 50% of plant requirement (W_{50}). Each replicate included four seedlings. Once reached the phenological stage 15 (Archontoulis et al., 2010), seedlings were removed from the vessels, washed and the following parameters were measured: leaf number $plant^{-1}$, fresh weight (g) and dry matter (%) of leaves. Part of leaves were lyophilized, ground and kept at −20 °C until HPLC analysis.

2.3. Experiment 2-light treatment

The effect of light was evaluated on three seed propagated lines of globe artichoke: ‘NP2’, ‘NP4’ and ‘NP5’. Genotypic and phenotypic characterization of these three lines are reported in Mauromicale et al. (2018). Ten seeds of each line were placed into 9 cm Petri dishes on two Whatman papers No. 2, imbibed with deionized water until the double paper layer was totally moistened. Further deionized water was added during the experiment when required. Petri dishes were sealed with parafilm to prevent evaporation and incubated at 18±1 °C with the following light treatments: 0 h (complete darkness), 12/12 h (dark/light cycle) and 24 h light. Only for complete darkness, Petri dishes were wrapped in sheets of aluminum foil, while transparent ones were used for the other two light treatments. Light derived from Osram cool white fluorescent lamps with an irradiance of 25 μmol m^{-2} s^{-1} and 400–750 nm. Seed incubation ended at the two elliptic leaves visible phenological stage (code 12) (Archontoulis et al., 2010). Before being subjected to light treatments, the seed quali-quantitative polyphenolic profile of each globe artichoke line (~2 g of seeds before explant for each line) was evaluated.

2.4. Reagents and solvents

Reagents and solvents were purchased from VWR (Leighton Buzzard, UK) and were of analytical or HPLC grade. Apigenin-7-*O*-glucoside, apigenin, luteolin-7-*O*-glucoside, luteolin, 5-*O*-caffeoylquinic acid (chlorogenic acid) and hesperetin were obtained from Extrasynthese (Lyon, France), cynarin (1,3-di-*O*-caffeoylquinic acid) was from Roth (Karlsrube, Germany). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used throughout this research.

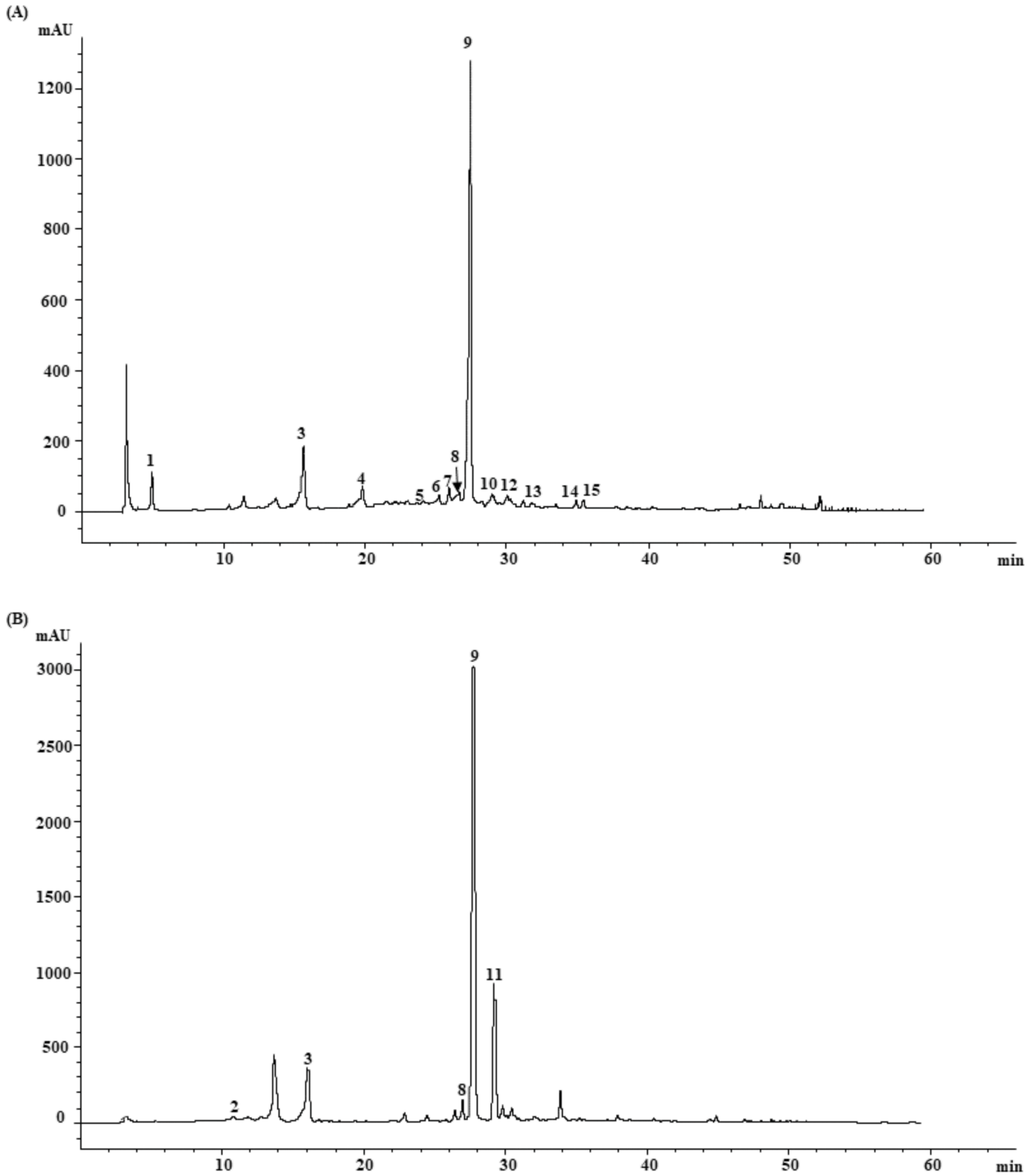

Fig. 1. HPLC/DAD chromatograms of an extract of *C. cardunculus* seedling (A) and seed (B) at 310 nm. 1-*O*-caffeoylquinic acid (**1**); 3-*O*-caffeoylquinic acid (**2**); 5-*O*-caffeoylquinic acid (**3**); 1,3-*O*-dicaffeoylquinic acid (**4**); luteolin-7-*O*-rutinoside (**5**); luteolin-7-*O*-glucoside (**6**); luteolin-7-*O*-glucuronide (**7**); 3,5-*O*-dicaffeoylquinic acid (**8**); 1,5-*O*-dicaffeoylquinic acid (**9**); monosuccinildicaffeoylquinic acid (**10**); dicaffeoylquinic acid derivative (**11**); luteolin malonylglucoside (**12**); monosuccinildicaffeoylquinic acid (**13**); apigenin malonylglucoside (**14**); luteolin (**15**).

2.5. Extraction procedure and HPLC analysis

The extracts were prepared by shaking the lyophilized samples (100 ±0.5 mg) and methanol/water (1 mL; 70:30 v/v) for 1 h at 25 °C, containing 1 mM butylated hydroxytoluene to preserve compounds during extraction, and hesperetin, as internal standard. The extract was then centrifuged and the clear upper layer was collected to a microfuge tube. The described extraction procedure was repeated twice, and the supernatants were combined in order to improve the recovery compounds yield and kept at −20 °C until analysis. This extraction procedure was performed for three independent samples.

The HPLC conditions for caffeoylquinic acid and flavone profile was carried out as described by Pandino et al. (2017b). Each extract was analysed using a series 1200 HPLC instrument (Agilent Technologies, Palo Alto, CA) equipped with ChemStation software (B.03.01) and a diode array detection system. Separations were achieved on a Zorbax Eclipse XDB-C18 (4.6 × 150 mm; 5.0 µm particle size), operated at 30 °C, with a 0.2 µm stainless steel inline filter. The mobile phase was 1% formic acid in water (solvent A) and in acetonitrile (solvent B) at a flow rate of 0.5 mL min^{-1}. The gradient started with 5% B to reach 10% B at 10 min, 40% B at 30 min, 90% B at 50 min, 90% B at 58 min. Chromatograms were recorded at 280, 310 (caffeoylquinic acids and apigenins), and 350 (luteolines) nm from diode array and data collected between 200 and 600 nm. Quantification was performed by a calibration curve using the available standards. Mono- and dicaffeoylquinic acids were calculated using 5-*O*-caffeoylquinic acid and 1,3-*O*-dicaffeoylquinic acid as references, respectively. Apigenin and luteolin conjugates were quantified as apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside,

Table 2
Number, fresh weight and dry matter content of leaves in cultivated cardoon ('Altilis 41′) and seed-propagated line of globe artichoke ('NP5') in relation to water-supply treatment and genotype.

Variable	Leaf (n plant^{-1})	Fresh weight (g)	Dry matter (%)
Genotype			
Altilis 41	3.9 ± 0.5 a	2.7 ± 0.3 a	11.8 ± 0.1 b
NP5	2.5 ± 0.2 b	1.8 ± 0.1 b	12.4 ± 0.2 a
Water-supply treatment			
W_{100}	3.6 ± 0.5 a	2.8 ± 0.4 a	12.1 ± 0.1 a
W_{75}	2.8 ± 0.3 a	2.2 ± 0.2 b	12.0 ± 0.1 a
W_{50}	3.2 ± 0.3 a	1.8 ± 0.2 b	12.2 ± 0.1 a

W_{100}: water-supply at 100% of plant requirement (control); W_{75}: water-supply at 75% of plant requirement; W_{50}: water-supply at 50% of plant requirement. Values are means ± standard deviation. Values within each column and variable followed by different letters are significantly difference at $P \leq 0.05$ (Student-Newman-Keuls test).

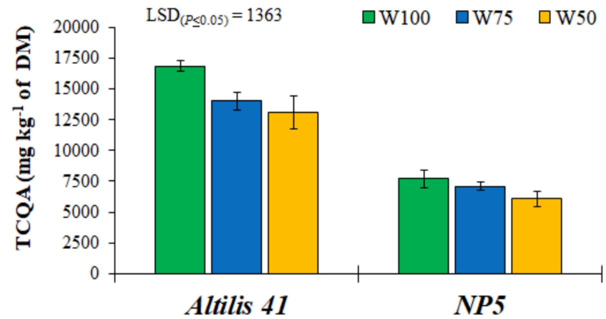

Fig. 2. 'Genotype × water-supply treatment' interaction of TCQA (total caffeoylquinic acids) in cultivated cardoon ('Altilis 41′) and seed-propagated line of globe artichoke ('NP5') seedling. Values are means ± standard deviation. The LSD value was calculated with the Student-Newman-Keuls test at $P \leq 0.05$. W_{100}: water-supply at 100% of plant requirement (control); W_{75}: water-supply at 75% of plant requirement; W_{50}: water-supply at 50% of plant requirement. DM: dry matter.

Table 3
Total caffeoylquinic acids, luteolin, apigenin and their derivatives, and measured polyphenols (mg kg^{-1} of DM$^{(1)}$) in cultivated cardoon ('Altilis 41′) and seed-propagated line of globe artichoke ('NP5') seedling in relation to genotype and water-supply treatment.

Variable	Total caffeoylquinic acids	Total luteolines	Total apigenines	Total measured polyphenols
Genotype				
Altilis 41	14,674 ± 1959 a	2314 ± 167 a	233±12	17,221 ± 1907 a
NP5	6943 ± 827 b	1279 ± 285 b	nd$^{(2)}$	8222 ± 1087 b
Water-supply treatment				
W_{100}	12,285 ± 741 a	2018 ± 170 a	–	14,304 ± 200 a
W_{75}	10,551 ± 539 b	1777 ± 104 b	186±10 a	12,515 ± 171 b
W_{50}	9588 ± 141 c	1594 ± 31 c	164±6.1 a	11,347 ± 169 c

W_{100}: water-supply at 100% of plant requirement (control); W_{75}: water-supply at 75% of plant requirement; W_{50}: water-supply at 50% of plant requirement; $^{(1)}$DM = dry matter. $^{(2)}$nd = not detected. Values are means ± standard deviation. Different letters within each column and variable indicate significant differences at $P \leq 0.05$ (Student-Newman-Keuls test).

respectively (Table 1). The 5-*O*-caffeoylquinic acid, 1,3-*O*-dicaffeoylquinic acid, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, apigenin and luteolin were located on the chromatogram by comparison with a commercial standard. When commercial standards were not available, peak identities were assigned by their UV spectrum and sequence of elution/retention time relative to hesperetin (as internal standard) using method validated in our laboratory.

All samples were assayed in triplicate. All data are presented as mean ± standard deviation and results were expressed as mg kg^{-1} of dry matter (DM).

2.6. Statistical analysis

Data were statistically analysed by applying analyses of variance (ANOVAs) with the computer software CoStat® version 6.003 (CoHort Software, Monterey, CA, USA). In detail, general linear models (GLMs) were used to test the significance of 'genotype', 'water-supply treatment' and their interactions for experiment 1, 'genotype', 'light treatment' and their interactions for experiment 2, on the quali-quantitative composition of polyphenols in *C. cardunculus* seedlings. One-way ANOVAs for single compound or chemical group were also applied on seeds and seedlings. The assumptions of homoscedasticity and normality were checked with the Bartlett's and the Shapiro-Wilk's tests, respectively. Post-hoc comparisons of means were performed by the Student-Newman-Keuls test at $P \leq 0.05$.

3. Results

3.1. Identification of caffeoylquinic acids and flavones in C. cardunculus seeds and seedlings extracts

In the *C. cardunculus* seeds and seedlings extracts were identified and calibrated, by HPLC/DAD, 15 phenolic compounds between caffeoylquinic acids and flavones (Fig. 1). In detail, the following caffeoylquinic acids were identified: 1-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 1,3-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, two monosuccinil-dicaffeoylquinic acids and a no-identified dicaffeoylquinic acid derivative. The caffeoylquinic acids are presented according to the recommended IUPAC numbering system. As flavones, were indentified: luteolin 7-*O*-rutinoside, luteolin 7-*O*-glucoside, luteolin 7-*O*-glucuronide, luteolin 7-*O*-malonilglucoside, luteolin, apigenin 7-*O*-rutinoside, apigenin 7-*O*-glucoside, apigenin 7-*O*-glucuronide, apigenin 7-*O*-malonilglucoside, apigenin.

3.2. Experiment 1-effect of water-supply treatment

3.2.1. Number, fresh weight and dry matter of leaves

The effect of the water-supply treatment in globe artichoke seedling was significant only for leaf fresh weight, which progressively increased from W_{50} to W_{100} (1.8 to 2.8 g) (Table 2). Number plant^{-1}, fresh weight

Table 4
Caffeoylquinic acids and flavones (mg kg^{-1} of DM[(1)]) in cultivated cardoon ('Altilis 41') and seed-propagated line of globe artichoke ('NP5') seedling in relation to genotype and water-supply treatment.

Compound	Genotype		Water-supply treatment		
	Altilis 41	*NP5*	W_{100}	W_{75}	W_{50}
Caffeoylquinic acids					
1-*O*-caffeoylquinic acid	95 ± 1.0	nd[(2)]	trace	0.09 a	0.05 b
5-*O*-caffeoylquinic acid	10,102 ± 861 a	6139 ± 58 b	8806 ± 217 a	8133 ± 148 ab	7423 ± 174 b
1,3-*O*-dicaffeoylquinic acid	445 ± 7.2	nd	261 ± 1.8 a	211 ± 1.4 b	196 ± 2.3 b
3,5-*O*-dicaffeoylquinic acid	133 ± 3.2	nd	61 ± 0.4 a	70 ± 1.1 a	68 ± 0.8 a
1,5-*O*-dicaffeoylquinic acid	3079 ± 78 a	803 ± 1.9 b	2666 ± 55 a	1659 ± 36 b	1499 ± 30 b
monosuccinildicaffeoylquinic acid	671 ± 12	nd	404 ± 2.5 a	316 ± 1.4 b	287 ± 2.0 b
monosuccinildicaffeoylquinic acid	148 ± 0.9	nd	88 ± 1.0 a	73 ± 0.6 b	62 ± 1.0 c
Flavones					
Luteolin 7-*O*-rutinoside	nd	686 ± 15	431 a	305 b	293 b
Luteolin 7-*O*-glucoside	1112 ± 12 a	353 b	875 ± 14 a	673 ± 9.9 b	649 ± 10 b
Luteolin 7-*O*-glucuronide	284 ± 1.6	nd	151 ± 2.2 b	189 ± 1.6 a	86 ± 0.4 c
Luteolin malonylglucoside	600 ± 5.6 a	117 ± 1.9 b	396 ± 3.1 a	363 ± 1.9 a	316 ± 3.3 b
Luteolin	318 ± 1.9 a	123 ± 4.1 b	165 ± 2.1 b	248 ± 4.0 a	249 ± 1.6 a
Apigenin 7-*O*-rutinoside	nd	nd	nd	nd	nd
Apigenin 7-*O*-glucoside	nd	nd	nd	nd	nd
Apigenin 7-*O*-glucuronide	ndr	nd	nd	nd	nd
Apigenin malonylglucoside	233 ± 12	nd	nd	186 ± 10 a	164 ± 6.1 a
Apigenin	nd	nd	nd	nd	nd

W_{100}: water-supply at 100% of plant requirement (control); W_{75}: water-supply at 75% of plant requirement; W_{50}: water-supply at 50% of plant requirement.
(1) DM = dry matter.
(2) nd = not detected. Each value represents the mean of $n = 3 \pm$ standard deviation. Different letters within each compound and factor under study indicate statistical differences at $P \leq 0.05$ (Student-Newman-Keuls test).

and dry matter of leaves were significantly affected by genotype (Table 2). In details, 'Altilis 41' showed a higher number of leaves plant^{-1} and leaf fresh weight than 'NP5' (3.9 *vs.* 2.5 and 2.7 *vs.* 1.8 g), while dry matter was higher in 'NP5' than 'Altilis 41' (12.4 *vs.* 11.8%).

3.2.2. Polyphenol profile

The total measured polyphenols, referred as the sum of detected polyphenols, leaves of globe artichoke seedling was significantly affected by both water-supply treatment and genotype (Table 3).

Concerning the effect of the water-supply treatment, the trend $W_{100} > W_{75} > W_{50}$ was observed for all chemical groups. Except for apigenin and its conjugates, both water-stressed seedling (W_{75} and W_{50}) showed an average apigenin content of 175 mg kg^{-1} of DM, while any apigenin and its conjugates were detected in W_{100} (Table 3). On the contrary, W_{100} achieved +28% of total caffeoylquinic acids (TCQA), +27% of total luteolines (TLut) and +25% of total measured polyphenols (TMP) content than W_{50} (Table 3).

Table 5
Polyphenol profile (mg kg^{-1} of DM[(1)]) of three lines of globe artichoke seeds, before explant, in relation to genotype.

Compound	Genotype		
	NP2	*NP4*	*NP5*
1-*O*-caffeoylquinic acid	nd[(2)]	nd	nd
3-*O*-caffeoylquinic acid	166 ± 24 a	trace	189 ± 9 a
5-*O*-caffeoylquinic acid	1147 ± 132 ab	1047 ± 91 b	1305 ± 42 a
3,5-*O*-dicaffeoylquinic acid	317 ± 25 b	577 ± 59 a	255 ± 15 b
1,5-*O*-dicaffeoylquinic acid	5942 ± 323 b	6262 ± 168 b	7419 ± 161 a
dicaffeoylquinic acid derivative	1680 ± 147 b	2508 ± 208 a	1238 ± 162 c
Total caffeoylquinic acid	**9252 b**	**10394 a**	**10406 a**
Luteolin 7-*O*-rutinoside	nd	nd	nd
Luteolin 7-*O*-glucoside	nd	nd	nd
Luteolin	nd	nd	nd
Total luteolin	–	–	–
Total measured polyphenols	**9252 b**	**10394 a**	**10406 a**

(1) DM = dry matter.
(2) nd = not detected. Each value represents the mean of $n = 3 \pm$ standard deviation of the mean. Different letters within each compound indicate statistical differences at $P \leq 0.05$ (Student-Newman-Keuls test).

Table 6
Total caffeoylquinic acids, luteolin and its derivatives, and measured polyphenols (mg kg^{-1} of DM[(1)]) in three lines of globe artichoke seedlings in relation to genotype and light treatment.

Variable	Total caffeoylquinic acids	Total luteolines	Total measured polyphenols
Genotype			
NP2	3241 ± 421 b	87 ± 19 a	3328 ± 502 b
NP4	2786 ± 408 b	138 ± 32 a	2924 ± 510 b
NP5	5679 ± 1078 a	139 ± 31 a	5818 ± 1824 a
Light treatment			
0 h	2623 ± 436 b	nd[(2)]	2623 ± 336 b
12 h	3346 ± 469 b	92 ± 21 b	3438 ± 480 b
24 h	5736 ± 322 a	273 ± 37 a	6009 ± 741 a

(1) DM = dry matter. (2)nd = not detected. Values are means ± standard deviation. Different letters within each column and variable indicate significant differences at $P \leq 0.05$ (Student-Newman-Keuls test).

The TCQA content was also significantly affected ($P \leq 0.05$) by 'water-supply treatment × genotype' interaction (Fig. 2). 'Altilis 41' treated with W_{50} recorded a higher content than that of not water-stressed (W_{100}) 'NP5' (13,118 *vs.* 7697 mg kg^{-1} of DM). Within each genotype a different response to water-supply treatment was observed. For example, 'Altilis 41' treated with W_{100} showed a major TCQA level respect to those treated with W_{75} and W_{50}. On the contrary, in 'NP5' statistical differences were observed between the seedling subjected to W_{100} and W_{50} (Fig. 2).

With regard to single compounds, W_{100} determined significantly higher values than W_{75} and $W_{50,}$ excluding 3,5-*O*-dicaffeoylquinic acid, luteolin 7-*O*-glucuronide and luteolin (Table 4). 'Altilis 41' leaves showed also a wider qualitative profile of polyphenols than 'NP5' (12 *vs.* 6 detected compounds), as well as a higher level of all detected compounds. Regardless both water-supply treatment and genotype, 5-*O*-caffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid were the most abundant compounds.

With regard to the genotype, interestingly 'NP5' was devoid of apigenin and its conjugates, and 'Altilis 41' seedling showed a higher concentration of TCQA (+111%), TLut (+80%) and TMP (+109%) than clone 'NP5'. In addition, the apigenin and its conjugates were only recorded in 'Altilis 41' (Table 3).

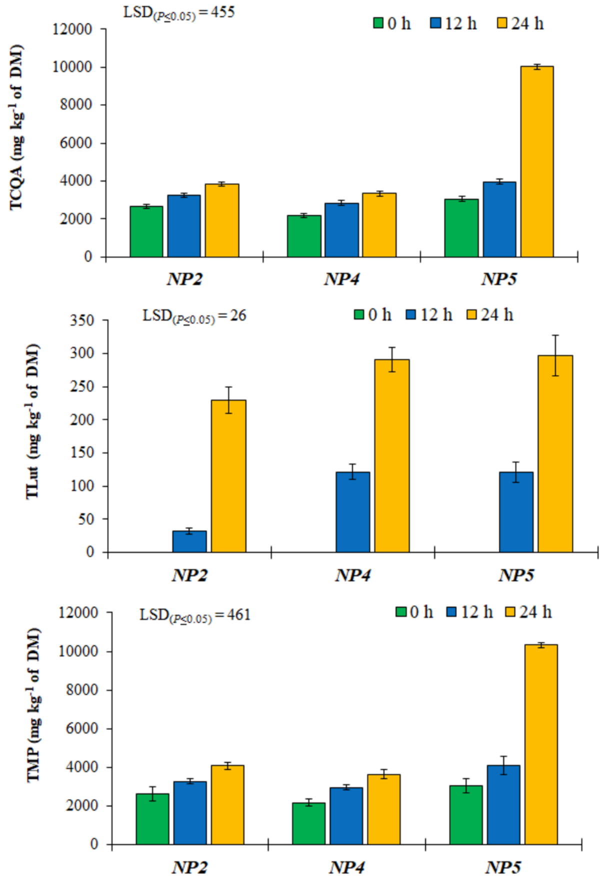

Fig. 3. 'Genotype × light treatment' interaction of TCQA (total caffeoylquinic acids), TLut (total luteolines) and TMP (total measured polyphenols) in three lines of globe artichoke seedlings. Values are means ± standard deviation. The LSD value was calculated with the Student-Newman-Keuls test at $P \leq 0.05$. (1)DM = dry matter.

3.3. Experiment 2-effect of light treatment

3.3.1. Polyphenol profile of seeds before explant

HPLC analysis revealed that the seeds, before explant, of three seed propagated lines of globe artichoke were characterised by only five caffeoylquinic acids (Table 5). Among these, 1,5-*O*-dicaffeoylquinic acid and 5-*O*-caffeoylquinic acid were the predominant compounds in all globe artichoke lines, with the highest levels recorded in 'NP5' (7419 and 1305 mg kg^{-1} of DM, respectively) than 'NP2' and 'NP4'. On the contrary, 'NP4' was characterised by a higher amount of the no-identified dicaffeoylquinic acid derivative (2508 mg kg^{-1} of DM) and 3,5-*O*-dicaffeoylquinic acid (577 mg kg^{-1} of DM) than both 'NP5' and 'NP2'. The latter showed the lowest amount of TCQA and, as consequence, of TMP (Table 5).

3.3.2. Polyphenol profile of seedlings

Light treatment and genotype affected the polyphenol profile of three lines globe artichoke seedlings (Table 6). Concerning the light treatment, the trend 24 > 12 > 0 h was observed for all chemical group, even if any statistical differences occurred between 12 and 0 h of light in terms of both TCQA and TMP content (Table 6). On the contrast, 24 h of light significantly enhanced the amount of TCQA (+70%), TLut (+89%) and TMP (+75%) than 12 h (Table 6). In addition, the seedling treated at 0 h of light did not accumulated luteolines.

From the GLM emerged that the 'genotype x light treatment' interaction was highly significant ($P \leq 0.01$) for TCQA, TLut and TMP (Fig. 3). For all chemical compounds and all globe artichoke lines, the trend 0 *h* < 12 *h* < 24 h was found, with luteolines' synthesis which was inhibited by 0 h of light. 'NP5' treated with 24 h of light reported the highest values of both TCQA (10,024 mg kg^{-1} of DM) and TMP (10,321 mg kg^{-1} of DM) respect to both 'NP2' and 'NP4' (Fig. 3). If compared to 0 h of light, 24 h of light enahanced about 70% the TCQA and TMP in 'NP5'. Concerning the TLut content, any statistical differences were observed between 'NP4'0 and 'NP5' at both 12 and 24 h of light (Fig. 2). On the contrary, 'NP2' appeared more affected by light treatment, since its TLut content increased of 86% passing by 12 to 24 h of light, respect to 79% recorded in both 'NP4' and 'NP5' (Fig. 3).

Averaged over light treatment, 'NP5', compared to both 'NP2' and 'NP4', showed *a* + 75% and +104% of caffeoylquinic acids, as well as *a* + 75% and +99% of total measured polyphenols, respectively. No significant differences were observed among genotypes for luteolines.

With respect to the qualitative profile (Table 7), 3,5-*O*-dicaffeoylquinic acid was detected only in 'NP2' subjected to 24 h of light. On the contrary, the not identified dicaffeoylquinic acid derivative was recorded in all globe artichoke lines and light treatment, excluding in 'NP2' treated at 24 h of light. Similarly, at 12 h of light, both luteolin-7-O-rutinoside and luteolin-7-O-glucoside were only absent in 'NP2'. In all globe artichoke lines, 24 h of light allowed the widest polyphenol profile (8 out of 9 compounds), while 0 h the lowest one (3 out of 9 compounds). Overall, the most abundant detected compound in all globe artichoke lines and light treatment was 1,5-*O*-dicaffeoylquinic acid (Table 7). It is also worth to note the behavior of both not identified dicaffeoylquinic acid derivative and 5-*O*-caffeoylquinic acid at each light treatment and genotype. In particular, the amount of 5-*O*-caffeoylquinic acid increased from 0 to 24 h of light in each globe artichoke line, while opposite trend was observed for the not identified

Table 7
Polyphenol profile (mg kg^{-1} of DM(1)) of three lines of globe artichoke seedlings in relation to genotype and light treatment (0, 12 and 24 h).

Compound	NP2			NP4			NP5		
	0	*12*	*24*	*0*	*12*	*24*	*0*	*12*	*24*
1-*O*-caffeoylquinic acid	nd(2)	nd	trace	nd	nd	47 ± 8	nd	nd	122 ± 7
3-*O*-caffeoylquinic acid	trace	trace	141 ± 15	nd	31 ± 2 b	146 ± 15 a	trace	trace	279 ± 14
5-*O*-caffeoylquinic acid	208 ± 27 c	356 ± 52 b	672 ± 14 a	122±16 c	265 ± 12 b	633 ± 5 a	169 ± 11 c	304 ± 33 b	2357 ± 14 a
3,5-*O*-dicaffeoylquinic acid	nd	nd	205 ± 5	nd	nd	nd	nd	nd	nd
1,5-*O*-dicaffeoylquinic acid	2191 ± 315 b	2713 ± 134 a	2825 ± 12 a	1846 ± 172 b	2372 ± 212 a	2345 ± 5 a	2687 ± 339 c	3473 ± 426 b	7097 ± 14 a
dicaffeoylquinic acid	242 ± 33 a	169 ± 13 b	nd	210 ± 12 a	171 ± 10 b	169 ± 13 b	193 ± 26 a	186 ± 15 a	169 ± 14 a
Luteolin 7-*O*-rutinoside	nd	nd	66 ± 5	nd	35 ± 3 b	101 ± 6 a	nd	37 ± 5 b	94 ± 12 a
Luteolin 7-*O*-glucoside	nd	nd	120 ± 15	nd	44 ± 4 b	138 ± 9 a	nd	31 ± 4 b	123 ± 12 a
Luteolin	nd	33 ± 5 b	44 ± 4 a	nd	42 ± 5 a	52 ± 9 a	nd	53 ± 7 b	80 ± 9 a

(1) DM = dry matter.
(2) nd = not detected. Each value represents the mean of $n = 3 \pm$ standard deviation of the mean. Different letters within each compound and genotype indicate statistical differences at $P \leq 0.05$ (Student-Newman-Keuls test).

dicaffeoylquinic acid derivative (Table 7).

4. Discussion

In this work, we performed two experimental designes to assess the effect of water-supply and light treatment on the polyphenols profile of *C. cardunculus* seedlings of three seed-propagated lines of globe artichoke and a cultivated cardoon.

4.1. Experiment 1 – water-supply treatment

Generally, plants acquire drought resistance by increasing their antioxidant metabolism and, in this sense, phenolic compounds are well-known antioxidant agents. A moderate or severe water stress is commonly associated to a greater L-phenylalanine ammonia lyase activity (PAL), enzyme responsible for the synthesis of phenolic acids (Tovar et al., 2002). Under controlled conditions, a moderate drought stress (50% of field capacity) was indicated as the optimum condition to highly enhance the total phenolic content and total flavonoid content in three *Achillea* species (Gharibi et al., 2016). In our research, we found a different trend to these findings, with an increased polyphenols production due to the total satisfaction of plant water requirement (W_{100}). In particular, it was stimulated the production of polyphenols, especially caffeoylquinic acids, with remarkable higher values detected in the cultivated cardoon than in the globe artichoke. Similarly, Pandino et al. (2015) indicated chlorogenic acid and 1,5-*O*-dicaffeoylquinic acid as the major *C. cardunculus* leaf phenolic compounds, with the globe artichoke containing more of both compounds than the cultivated cardoon. In the other hand, it is hard to compare our results with those of literature, since to our knowledge, data on the effect of water-supply treatment on *C. cardunculus* popyphenol profile is missing under controlled conditions. Nouraei et al. (2018), investigating the influence of three irrigation treatments on the polyphenolic compounds in globe artichoke leaves and heads under open-field conditions, indicated that a moderate drought stress reduced the DM content and significantly elevated the amount of phenolic acids such as chlorogenic acid and 1,5-dicaffeoylquinic acid, likely due to the lignification of the cell wall and the production of amino acids for osmotic adjustment. A similar response is reported by Sałata et al. (2022). Wu et al. (2017) found that a deficit irrigation increased the polyphenols content and the antioxidant activity in sorghum. It is likely that our results could be explained by the early phenological stage of seedlings on one side, and by the genetic background on the other side. Stomatal closure has been identified as an efficient way to reduce water loss in drying field conditions. This limits carbon uptake into leaves, affecting the photosynthesis during mild to moderate drought, and as consequence the primary metabolites (Lawlor et al., 2002). The polyphenol compounds are derived from primary metabolic processes in plants. Previous studies reported that sucrose affects biosynthesis of phenolic compounds. For example, sucrose deficiency reduced the lycopene accumulation in fruit pericarp of *Solanum lycopersicum* (Telef et al., 2006), while an addition of sucrose increased carotenoid content in cell cultures of *Daucus carot* (Yun et al., 1990). Therefore, our hypothesis was that the lower content of polyphenol content, observed in W_{75} and W_{50}, might be associated to the lower concentrations of primary metabolites in early phenological stage of seedlings that could slow down the synthesis of secondary metabolites. Moreover, the correlation between the plant water status and the polyphenols content was found to be cultivar-dependent in tomato, with some cultivars showing a positive relation and other cultivars a negative one (Gómez-Caravaca et al., 2014).

4.2. Experiment 2 – light treatment

Before evaluating the effect of light treatment on seedlings of globe artichoke lines, in this work, it was screened the polyphenol composition of their seeds. In all lines, the qualitative profile was characterized by only caffeoylquinic acids. These results were in line with previous studies. Petropoulos et al. (2019) detected only two caffeoylquinic acids (5-*O*-caffeoylquinic acid and 3,5-*O*-dicaffeoylquinic acid) in seed extracts obtained from globe artichokes, wild and cultivated cardoons. Mandim et al. (2022) reported six phenolic acids (mainly 3,5-*O*-dicaffeoylquinic acid) in cultivated cardoon seeds, with increasing levels at increasing seed maturity.

About the globe artichoke seedlings, it is worth pointing out how the polyphenol profile was light-dependent. At 0 h of light, total measured polyphenols was 100% represented by caffeoylquinic acids in all globe artichoke lines, while flavones were absent. Such percentage decreased to 96% at 12 h and 91% at 24 h in 'NP4', due to the presence of flavones. More in detail, apigenines were totally absent, while, luteolines significantly increased with the time of light. Similar trend in open-field conditions were observed by Pandino et al. (2013a). This result could be attribute to less effective free radical scavengers of apigenin derivatives (monohydroxyflavones) at dissipating absorbed UV energy than luteolin derivatives (dihydroxyflavones) (Smith and Markham, 1996), as also reported by Pandino et al. (2013b). According to our results, 24 h of light allowed to increase, respect to 12 h and 0 h of light, not only the number, but also the amount of polyphenol compounds. In a similar work, Moglia et al. (2008) found that the dicaffeoylquinic acid content was consistently increased at 24 h after UV-C radiation, and concluded that the production of these antioxidant compounds in chloroplasts under light exposure serves to protect young globe artichoke leaf tissue from light damage. In this sense, chlorogenic acid is a well-known compound involved in the protection from different biotic and abiotic stresses in several plant species. In a previous study, chlorogenic acid, dicaffeoylquinic acids and luteolin glucosides were indicated as the most active free radical scavengers in globe artichoke leaves (Wang et al., 2003). In this sense, the 5-*O* caffeoylquinic acid has been found to be significantly affected by UV radiation or daylight exposure in several Solanaceae members such as tobacco and potato (Izaguirre et al., 2007; Percival and Baird, 2000).

5. Conclusions

The results here observed clearly indicate that it is possible to produce *C. cardunculus* seedlings and/or plantlets rich in polyphenols under controlled conditions. This could allow to standardize the extraction of these compounds for industrial applications. It is well-documented that *C. cardunculus* species is an important constituent of healthy food, and hence its seedlings and/or plantlets could be a promising source of antioxidant phenolic compounds. In particular, our findings revealed also the role light and water-supply treatment on the polyphenol profile. According to our data, the higher polyphenol yield was reached in the seedlings treated with W_{100} (experiment 1) and 24 h of light (experiment 2). These findings were also genotypic-dependent, since each genotype had different response in relation to the applied treatment. Overall, the cultivated cardoon ('Altilis 41′) showed a higher concentration of polyphenols than seed-propagated line of globe artichoke in experiment 1, while 'NP5' had the better performing than the other globe artichoke lines in experiment 2. However, further studies are needed to standardize the method and assess other growing conditions and genotypes to improve and/or keep under control the profile of these compounds.

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CRediT authorship contribution statement

Gaetano Pandino: Methodology, Formal analysis, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Angelo Bonomo:** Data curation, Investigation. **Aurelio Scavo:**

Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Giovanni Mauromicale:** Conceptualization, Writing – review & editing, Supervision. **Sara Lombardo:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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