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Terracrepolo (*Reichardia picroides* (L.) Roth.): Wild food or new horticultural crop?

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ABSTRACT

The extreme adaptability of *Reichardia picroides* to stressful environments motivated experiments aimed to investigate the genotype-environment interactions on the nutraceutical parameters of this ancient food. The concentrations of antocyanins, flavonol glycosides, carotenoids and total phenols and the antioxidant capacity were significantly higher in the inland "Agnano" ecotype than in the coastal "Calafuria" ecotype. As expected, the cultivation of *R. picroides* generally led to a decrease in the compositional parameters except the content of carotenoids. A sodium chloride solution was sprayed onto the cultivated plants to simulate the stress caused by marine aerosols. However, the hypothesis that salt stress could act as an elicitor for nutraceutical substances was not validated, particularly in the Calafuria ecotype that evolved close to the sea shore. The nutraceutical performances of the wild ecotypes could be retained in cultivation through a chronic stress, which could allow the activation of the physiological response.

1. Introduction

The growing need for nutraceutical foods (Ozen et al., 2012) has elicited an increasing interest for ethnobotanical studies (Tardío et al., 2006), which have been addressed mostly to edible wild herbs (Pieroni, 2000; Guarrera and Savo, 2016). Indeed, important health benefits of the plant kingdom are mainly provided by the wild species, for their richness in secondary metabolites such as polyphenols (Hättenschwiler and Vitousek, 2000). Overall, secondary metabolites are the result of evolutionary processes in natural ecosystems, especially related to self-defence from both biotic and abiotic adversity (Jwa et al., 2006), and have a crucial role as a source of nutraceuticals. Paradoxically, the rediscovery of ancient local foods represents a promising healthy innovation of the daily Mediterranean diet (Heinrich et al., 2005).

Terracrepolo (*Reichardia picroides* (L.) Roth.), belonging to the Asteraceae botanic family, is a steno-Mediterranean herb of high ethnobotanic interest as medicinal food, since it was traditionally used as a depurative (Pieroni, 2000) or tonic (Loi et al., 2004) agent. In Sardinia it was even used as a popular treatment against heart diseases such as angina pectoris (Atzei et al., 1991). This species, utilized row or cooked (Nebel et al., 2006) was found to be a valuable source of antioxidants (Vanzani et al., 2011), probably due to its richness in phenolics (Recio et al., 1992). Terracrepolo is spread throughout the climatic area of olive grove (Pignatti, 1982), and grows in dry, rocky and calcareous soils in open space. It is also very common on buildings in the urban environment (Benvenuti, 2004), even on ancient monuments such as the Colosseum (Caneva et al., 2002). Moreover, its multiple stress tolerance allows it to be commonly present among the sand-dune vegetation in the saline environment of the Mediterranean coast (Sýkora et al., 2003).

The annual regrowth dynamics of this perennial species occurs through: i) the sprouting of basal buds (life cycle of hemicryptophyte) and/or ii) autumnal and/or spring seed germination (Benvenuti and Pardossi, 2016). Dispersal is carried out by anemocory, due to a white plumose pappus able to be moved by the wind (Andersen, 1993).

On account of this attitude to spatial dispersal, this species is a good example of "pioneer" flora belonging to the *Reichardia* botanic *Genus* (Parraga-Aguado et al., 2013), typically able to colonize biologically inhospitable areas and allow a floristic transition to other successive, more exigent species. The survival of this invasive species in new environments is also favoured by a genetic variability able to select the desired characters in the various colonized habitats (Lee, 2002). Plant species are often characterized by both phenotypic plasticity and large genetic variation. Indeed, the successful occupation of many ecological niches depends on the occurrence of many genotypes (Joshi et al., 2001) specialized to co-evolve in particular environmental conditions (Van Tienderen, 1990), and this could be the case also for some ecotypes of *R. picroides* (number of chromosomes n = 7; Siljak-Yakovlev, 1981). However, although it is clear that the abiotic stresses are elicitors of secondary metabolites (Zhao et al., 2005) necessary for plant

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survival (Namdeo, 2007), such as flavonoids (Treutter, 2006), anthocyanins (Chalker-Scott, 1999), or total phenolics (Michalak, 2006), and carotenoids (Young, 1991), it is not known whether this metabolic over-expression could be genetically retained even in different ecotypes that do not have to endure the same stress conditions. On the other hand, it is not even known which is the most effective environmental stress for the elicitation of secondary metabolites in *R. picroides*, since this species can colonize diversified environments (inland or immediately near the sea). In addition to the typical poor fertility, calcareous matrix and drought, some ecotypes adapted to grow near the sea may withstand salt stress (Mittler, 2002), due to the periodic deposition of marine aerosol on the coastal vegetation (O'Dowd and De Leeuw, 2007).

Recently, it has been reported that the chemical composition of soybean seeds can be affected by genotype, environment and their interaction (Shaw et al., 2016). Information about the genotype-environment interaction (Lila, 2006) could assume a crucial role in the agronomic perspective of cultivating *R. picroides* as a new "nutraceutical crop". Anyway, it is not clear whether and to what extent cultivation could imply changes in the nutraceutical performances typical of the plants from the native environment.

Based on the above considerations, the aim of this study was: i) to quantify some important nutraceutical parameters (anthocyanins, chlorophylls, carotenoids, flavonol glycosides, total phenols, antioxidant capacity) of two different ecotypes of *R. picroides*, ii) to verify whether the cultivated progeny retains the same nutraceutical performances as the mother plants, iii) to artificially elicit the synthesis of secondary metabolites by a simulated marine aerosol.

2. Materials and methods

2.1. Plant material and sampling

2.1.1. Germplasm collection

Wild plants of *R. picroides* belonging to different ecotypes were collected in Tuscany (central Italy), in the inland (Agnano) and close to the coast (Calafuria). Table 1 reports some details on the two different areas, while Fig. 1 shows the ecotypes from Agnano and Calafuria, respectively. Seed collection was carried out in September 2015, by removing the whole inflorescences from the senescent tissues in the laboratory. The seeds were cleaned, dried in dry room, and kept in glass containers at 20 °C.

2.1.2. Greenhouse cultivation

The plants were cultivated during winter-spring 2015 in a greenhouse at the Department of Agriculture, Food and Environment of the University of Pisa, Italy (43°70′ N 10°43′ E). The seeds were sown in alveolar polystyrene containers (50 holes) commonly used in horticultural nurseries. Each hole (3 cm diameter, 5 cm depth) was filled with a peat-perlite substrate (1:1 v/v) and hosted one seed, which was placed on the surface and covered with an additional substrate layer (1 mm). Irrigation was carried out daily by water nebulization (about 3 mm day^{-1}).

After 3 weeks from emergence, 30 seedlings per each ecotype were transplanted in plastic pots (10 cm height, 9 cm diameter) filled with the same substrate enriched with 3 g l^{-1} of controlled-release fertilizer (Osmocote® plus organics vegetable tomato & herb plant food & soil improver, Scotts, Australia; 13-10-10 N, P_2O_5 and K_2O, respectively). Daily irrigation was carried out through a 2 l m^{-2} over-head (boom) at each application. The growing conditions were: 20 °C average temperature, 70–80% humidity, approximately 12/12 h photoperiod, 300 µmol m^{-2} s^{-1} light intensity.

2.1.3. Sampling of cultivated plants

For each ecotype, plant sampling was carried out 4 weeks after transplantation (6 weeks from seedling emergence), at the vegetative phenological stage, when the plants had produced a basal rosette of leaves. Completely developed young leaves were collected for the laboratory analyses during the first light hours (8.00–9.00 a.m.). Four samples (1 g) were prepared by pooling the leaf tissues of seven distinct plants. The samples were immediately wrapped in aluminium foil, placed in refrigerator bags and stored at −80 °C. They were analyzed within 3–4 weeks from collection. An aliquot of the fresh material was kept one week in ventilated oven at 60 °C for dry weight determination.

2.1.4. Sampling of wild-grown plants

Wild plants were sampled at the same time as the cultivated ones, in the same environments where seeds had been collected the previous year (Calafuria rocky coast, and the drystone walls of Agnano). For each ecotype, leaf samples from plants in the same phenological stage as the cultivated ones were prepared as described in the previous subsection and kept in refrigerated bags (0 °C) during the short way to the laboratory (about 30 min), where they were immediately freezed at −80 °C, or oven-dried at 60 °C. The samples were analyzed together with those from the cultivated plants.

2.1.5. Salt stress

The experiment aimed at evaluating the effect of a saline aerosol was performed twice, using a completely randomized experimental design with four replicates, each composed of the leaves of seven greenhouse cultivated plants. For each of the two ecotypes, the pot plants were grown under the above described conditions (unstressed) or were subjected to a simulated marine aerosol treatment (salt stressed). A 3.5 g l^{-1} sodium chloride solution was sprayed onto the latter plants (14 ml m^{-2}) by means of a microairbrush after 3 weeks from transplanting. To ensure that the desired amount of solution was entirely conveyed onto a known surface, a plastic shield was pierced on the airbrush at the base of the nozzle insertion. The resulting salt dose of 0.049 g m^{-2} could resemble a deposition left by marine aerosol after wind events (Franzén, 1990). Leaves sampling was carried out as described in the previous subsection, after 10 days of salt spraying.

2.2. Reagents and apparatus

HPLC grade methanol was purchased from Sigma–Aldrich (Milano, Italy). Reagent grade chemicals were purchased from the same manufacturer or from Carlo Erba Reagents (Cornaredo, Milano, Italy). All the determinations were performed by spectrophotometric assays, measuring the absorbance of the solutions with a Lambda35 UV–vis double beam spectrophotometer (Perkin Elmer, Waltham, Massachusetts, USA).

Table 1
Geographical and environmental information on the two different localities of *Rheicardia Picroides* germplasm collection.

Site of germplasm collection	Tuscany province	Geographical coordinates	Environment	Substrate type	Altitude	Distance from the sea
Agnano	Pisa	43°73'N 10°48' E	Drystone wall in open spaces of Mediterranean chaparral	Calcareous soil	75 m a.s.l.	18.000 m
Calafuria	Livorno	43°47'N 10°33' E	Coastal rocky and arid environment	Calcareous rocks	10 m a.s.l.	10 m

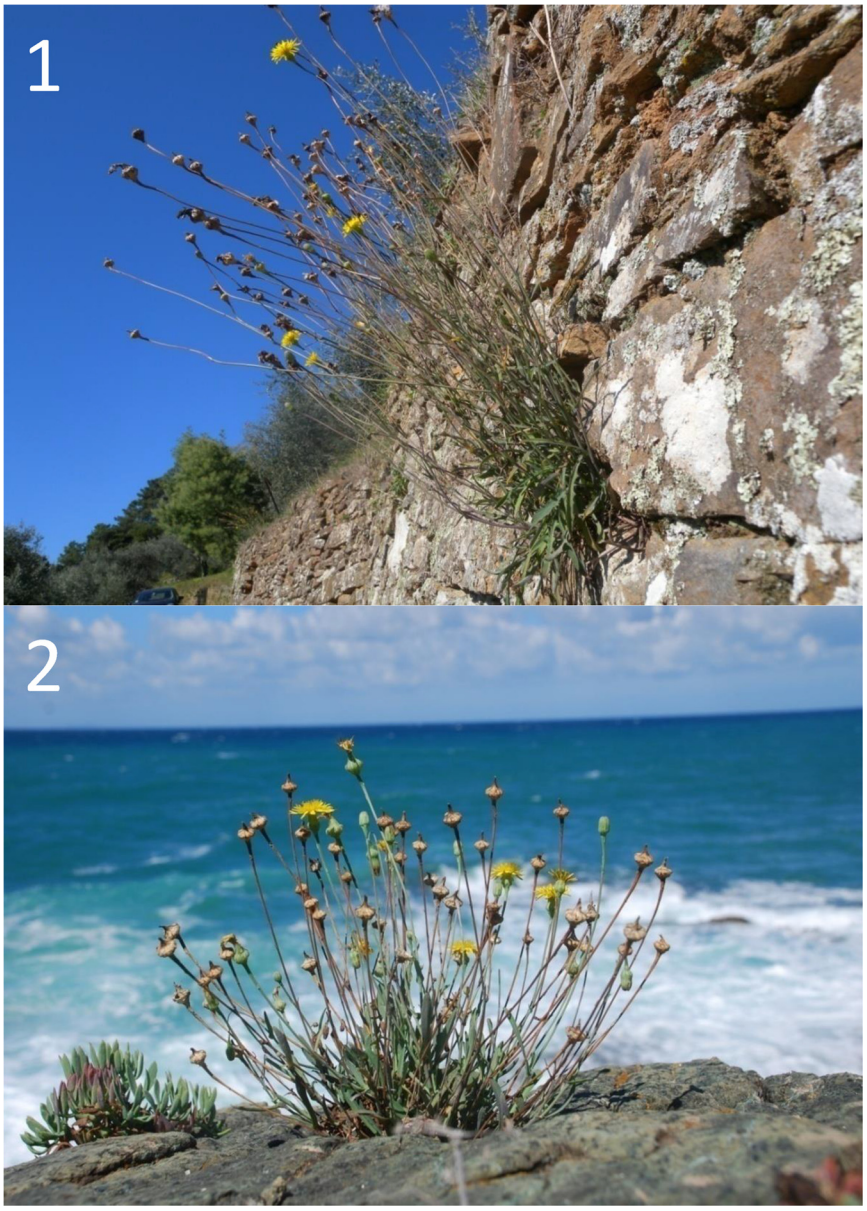

Fig. 1. The two different *Rheicardia picroides* ecotypes in their respective environments: 1) drystone wall of Agnano (Pisa) and 2) coastal rocks of Calafuria (Livorno).

2.3. Plant analyses

2.3.1. Extraction

Acidified 80% methanol (containing 1% hydrochloric acid) was used for the extraction of total anthocyanins and flavonolglicosides; pure methanol was used for all the other determinations. The extraction protocol reported by Maggini et al. (2013) was used with modifications. The leaf samples (1 g) were soaked with 5 ml extraction solvent, ground with mortar and pestle, and transferred in 10-ml test tubes. The tubes were sonicated 15 min in ice bath four times, stored overnight at −20 °C and centrifuged 5 min at 2700*g*. After separation of the supernatant, the extraction was repeated on the pellet with 5 ml fresh extraction solvent. The two supernatant aliquots were pooled and used for the subsequent analyses within a few days. All the parameters were expressed on a fresh weight (FW) basis.

2.3.2. Chlorophylls and carotenoids

For the determination of chlorophylls and carotenoids, the methanol extracts were diluted 1:10 with methanol. The absorbance of the diluted extracts was read at 665.2, 652.4 and 470 nm, and the concentrations of the pigments (μg g^{-1} FW) were calculated according to Lichtentahler and Buschmann (2001).

2.3.3. Anthocyanins and flavonol glicosides

The determinations of total anthocyanins and flavonol glicosides were accomplished following Hrazdina et al. (1982). For the evaluation of the content of total anthocyanins, the absorbance of the acidic extract was read at 530 nm, and the results were expressed as mg cyanidin-3-glucoside g^{-1} FW, using the value 38,000 M^{-1} cm^{-1} for the molar absorptivity. The total concentration of flavonol glycosides was determined on the same extracts after proper dilution by absorbance readings at 360 nm, using the molar absoptivity of quercetin-3-glucoside at the working wavelength (20,000 M^{-1} cm^{-1}), and expressing the results as mg quercetin-3-glucoside g^{-1} FW.

2.3.4. Total phenols

The determination of total phenols was carried out both by the Folin-Ciocalteu phenol reagent, and by absorbance readings at 320 nm, as reported by Kang and Saltveit (2002). For the former assay, 100 μl methanol extract, 2.0 ml distilled water and 300 μl Folin-Ciocalteu

phenol reagent were mixed in plastic test tubes. After four minutes, 7.5% sodium carbonate (1.6 ml) was added into the tubes and the solutions were kept 2 h at room temperature. The concentration of total phenols was determined by measuring the absorbance of the solutions at 765 nm, using standard gallic acid (0–500 mg L^{-1}) for calibration, and expressing the results as mg gallic acid g^{-1} FW. For the absorbance readings at 320 nm, the methanol extracts were diluted 1:100 with methanol. The results were expressed as absorbance units of the pure extract at 320 nm per gram leaf tissue, A(320 nm) g^{-1} FW.

2.3.5. Antioxidant capacity

The antioxidant capacity was determined by both the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) assays. The FRAP determination was carried out according to Benzie and Strain (1996). The FRAP reagent was freshly prepared immediately before the analyses and contained 2 mM ferric chloride and 1 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine). The following solutions were mixed in a spectrophotometric cuvette: 0.25 M acetate buffer pH 3.6 (2.0 ml); FRAP reagent (900µl); diluted 1:4 methanol extract (100 µl). A calibration curve was prepared with standard solutions containing ferrous ion (Fe(II); 0–1000 µM), obtained from ferrous ammonium sulfate. The absorbance was read at 593 nm and the results were expressed as µmol Fe(II) g^{-1} FW. The DPPH assay was performed following Dudonné et al. (2009) with slight modifications. 2.97 ml methanol DPPH solution (20 mg L^{-1}) and 30 µl methanol extract were mixed in a spectrophotometric cuvette. A blank solution was also prepared by replacing the plant extract with methanol. The cuvettes were kept 45 min in the dark at room temperature, and the absorbance was read at 515 nm. The percentage inhibition of the DPPH radical per gram tissue was calculated from the absorbance values of the blank (A_{blank}) and of the sample (A_{sample}) as follows:

$$\% \text{ Inhibition } g^{-1} \text{ FW} = 100 \cdot [(A_{blank}-A_{sample})/A_{blank}]/g \text{ FW}$$

2.4. Statistical analyses

The mean value and standard deviation of four samples of each type were evaluated in all the assays. For all the parameters under investigation, normal distribution and variance homogeneity of the data were verified by means of Kolmogorov-Smirnov and Levene tests, respectively. For the evaluation of the effect of cultivation, the data concerning wild or cultivated samples were subjected to pairwise comparisons by means of four distinct t-tests: Agnano wild versus Calafuria wild plants; Agnano cultivated versus Calafuria cultivated plants; Agnano wild versus Agnano cultivated plants; Calafuria wild versus Calafuria cultivated plants. For the Agnano ecotype, t-tests were also performed to compare wild and salt treated samples. For the evaluation of the effect of a saline aerosol on cultivated plants, the data were subjected to two-way ANOVA with the ecotype (Agnano or Calafuria) and the treatment (unstressed or salt stressed) as the sources of variation. The Bonferroni post-test was used for means comparisons. The linear regression analysis was applied to the results of distinct assays (FRAP versus DPPH or Folin-Ciocalteu versus absorbance at 320 nm). The Statgraphics Centurion Version 17 software (Statpoint Technologies, Warrenton, Virginia, USA) was used for the statistical analyses.

3. Results

Both wild and crop plants were collected under optimal turgidity conditions, and had 80–82% moisture content.

Fig. 2 shows that, although all the samples contained similar concentrations of total chlorophylls, the two wild grown ecotypes showed distinct levels of anthocyanins, carotenoids and flavonol glycosides, the plants from Agnano containing higher amounts than those from Calafuria. The concentrations of anthocyanins and flavonol glycosides were higher in the wild-collected samples than in the cultivated plants. In contrast, the concentration of carotenoids was significantly (Calafuria) or tendentially (Agnano) higher in the cultivated plants than in the wild-collected ones.

The antioxidant power of our samples was determined by means of both the FRAP and the DPPH assays (Fig. 3), and the results obtained with the two independent methods were linearly correlated (correlation coefficient $r^2 = 0.886$); however, the differences among the samples were more evident with the latter assay. The antioxidant capacity was higher for the wild grown plants from Agnano than for those from Calafuria, and was generally higher for the spontaneous plants than for the cultivated ones. The only exception to this trend was the Calafuria cultivated ecotype, whose antioxidant power in the FRAP assay was close to that of the corresponding wild grown plants.

Two independent methods were employed also for the determination of the concentration of total phenols (Fig. 3). In addition to the commonly used Folin-Ciocalteu assay, we measured the absorbance of the extracts at 320 nm according to Kang and Saltveit (2002). The linear correlation coefficient between the results of two assays was $r^2 = 0.989$. According to the former method, the concentration of total phenols in the wild plants was higher for the ecotype from Agnano, and with both methods the cultivated samples of this ecotype contained less phenolics compared to the plants at the spontaneous state. In contrast, the wild or cultivated plants from Calafuria contained similar concentrations of phenolics.

Table 2 reports the results of the two-way ANOVA concerning the salt stress experiment, which show that the effect of the ecotype was significant for all the parameters under investigation. Fig. 4 shows the variation of the individual constituents in both ecotypes consequent to the saline aerosol application to the cultivated plants. For each parameter, the difference between the values in stressed (S) and unstressed (U) plants is expressed as a percentage, according to the formula:

$$\% \text{ Difference from unstressed plants} = 100 * (S–U)/U$$

The saline aerosol caused a general decrease of the parameters in the Calafuria ecotype. On the other hand, the concentration of total phenols and the antioxidant capacity tended to increase in the Agnano ecotype.

4. Discussion

Despite the large diffusion of *R. picroides* all over Tuscany, a distinction could be made between the two ecotypes from the inland (Agnano) and the coast (Calafuria), based on their nutraceutical performances (Figs. 2 and 3). At the spontaneous state, the plants from Agnano showed overall higher contents of the metabolites of interest, which included important classes of plant pigments such as anthocyanins, carotenoids, chlorophylls, along with flavonol glycosides, whose concentrations were obtained by simple readings of the absorbance of the extracts. Both anthocyanins and flavonols belong to the widespread class of flavonoids. Specifically, flavonols are one of the largest subclasses of flavonoids, which in turn are the largest group of plant phenolics (Chang et al., 2002; Balasundram et al., 2006). The antioxidant capacity of our samples was determined both as the reducing ability towards the ferric ion (FRAP assay) and as the scavenging ability towards the DPPH free radical (DPPH assay). The use of independent methods is generally recommended because the antioxidant capacity of a complex mixture such as a plant extract is due to the contribution of its individual constituents. These are chemical compounds which often have very different structures and show distinct reactivity in dependence of the experimental conditions that they undergo. As a consequence, distinct assays could measure slightly different antioxidant properties. However, with our samples a good linear correlation was evidenced for the two methods.

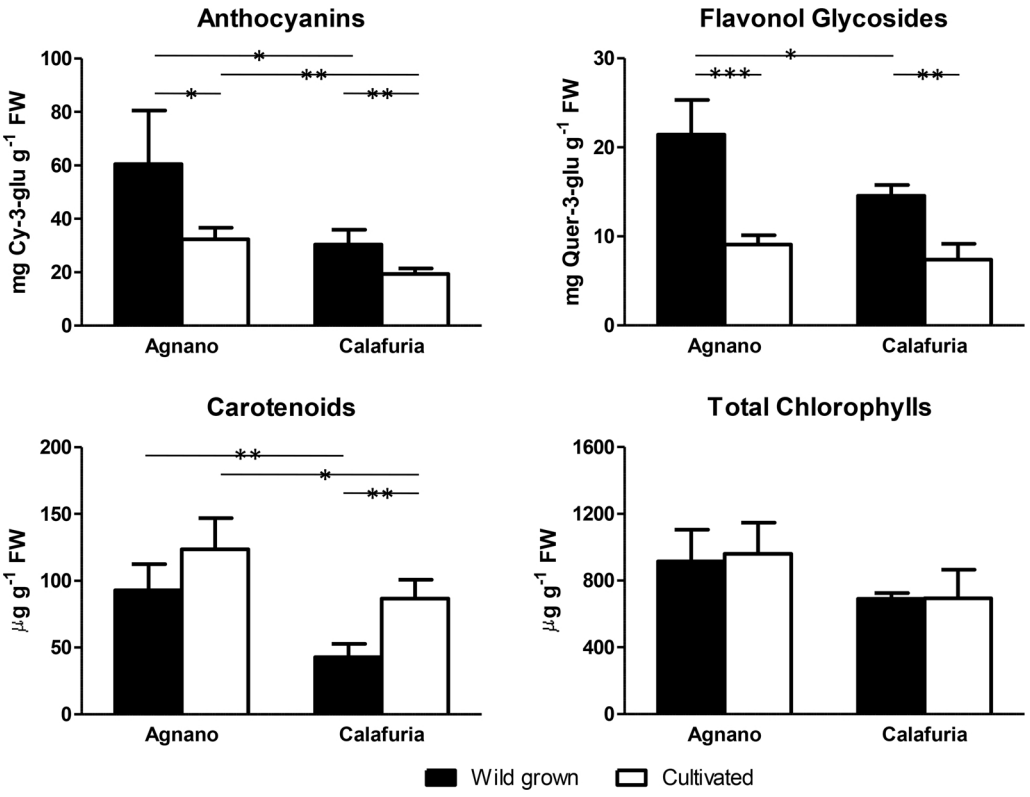

Fig. 2. The concentration of anthocyanins, flavonol glycosides, carotenoids and total chlorophylls in the leaves of *Reichardia picroides* from different ecotypes (Agnano or Calafuria) and growing environments (wild-collected or cultivated). Mean values and standard deviation of four samples. Data were subjected to pairwise means comparisons by *t*-test. Only significant differences at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) are indicated. according to.

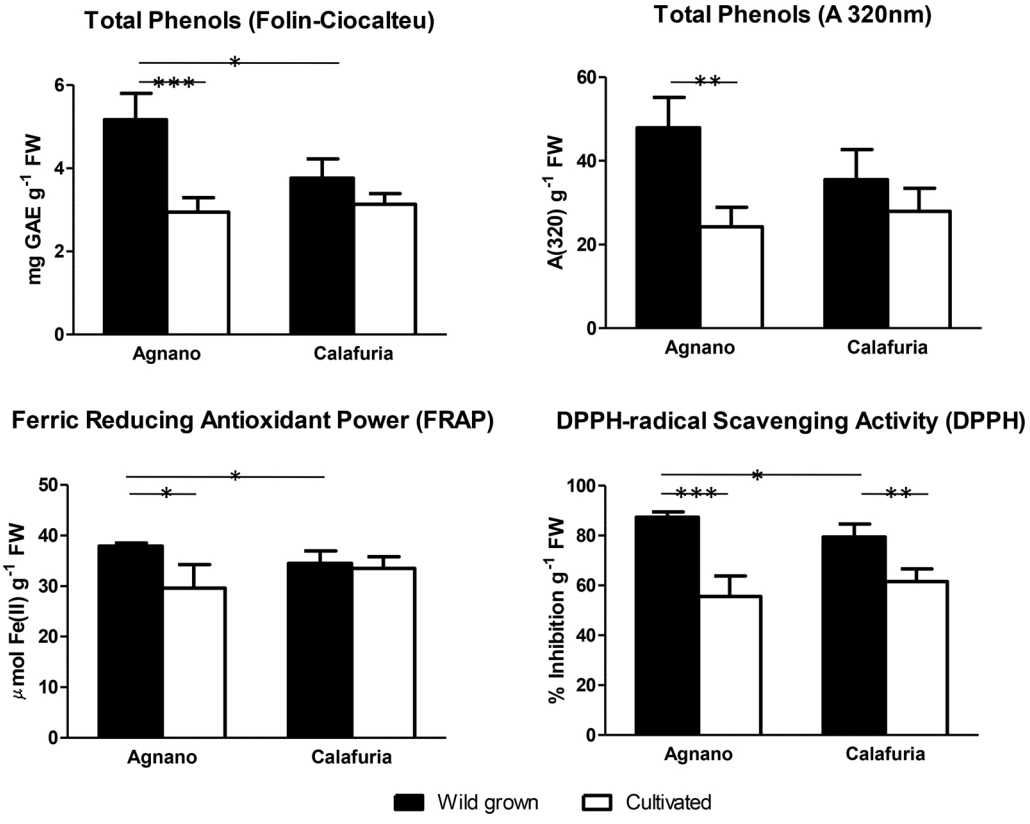

Fig. 3. The concentration of total phenols and the antioxidant capacity as determined by the FRAP or the DPPH assays, in the leaves of *Reichardia picroides* from different ecotypes (Agnano or Calafuria) and growing environments (wild-collected orcultivated). Mean values and standard deviation of four samples. Data were subjected to pairwise means comparisons by *t*-test. Only significant differences at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) are indicated.

In a similar way, a strong linear correlation was apparent also for the two independent assays for the determination of the concentration of total phenols (Folin-Ciocalteu assay or absorbance readings at 320 nm). This outcome was in total agreement with those obtained in lettuce with the same assays (Kang and Saltveit, 2002). Moreover, our results on the antioxidant power and the concentration of total phenols as obtained through the FRAP and the Folin-Ciocalteu assays, respectively, were in full agreement with those found with the same methods by Vanzani et al. (2011).

The reasons for the differences in the compositional parameters

Table 2
The statistical effects of treatment (unstressed or salt stressed), ecotype (Agnano or Calafuria) and their interaction on the contents of anthocyanins, flavonol glycosides, carotenoids, total chlorophylls, total phenols and antioxidant capacity (FRAP and DPPH) in the leaf tissues of cultivated *Reichardia picroides*, according to two way ANOVA. Four replicates were analyzed, each one consisting of seven plants. Asteriscs: significant at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***); ns: not significant.

	Anthocyanins	Flavonol Glycosides	Carotenoids	Total Chlorophylls	Total Phenols (Folin-Ciocalteu)	Total Phenols (A 320 nm)	Ferric Reducing Antioxidant Power (FRAP)	DPPH-radical Scavenging Activity (DPPH)
Treatment	**	ns	ns	ns	ns	ns	ns	ns
Ecotype	***	**	**	*	**	*	*	**
Interaction	ns	ns	ns	ns	***	**	**	***

between the spontaneous plants from the two sites could be due to a different mechanisms of adaptation to environmental stress conditions. In particular, the reaction of the plants from Calafuria to their droughty, windy and saline environment might involve non-phenolic antioxidants such as vitamin C, proline or glutathione, or different classes of phenolics than those that have been examined in this work. Alternatively, in the coastal ecotype adaptation could be based mainly on antioxidant enzymes such as ascorbate peroxidase, catalase or superoxide dismutase, rather than on antioxidant molecules (Das and Roychoudhury, 2014; Demidchik, 2015).

Different response mechanisms to the natural environment could be explained by the strong adaptation attitude of *R. picroides*, which was able to develop ecotypes that can endure particular environmental stress conditions. In a recent paper, the genus *Reichardia* has been reported as an appropriate model to investigate on genome evolution (Siljak-Yakovlev et al., 2017).

In contrast with the spontaneous plants, significant differences between the two ecotypes were not apparent in cultivation, except for the contents of anthocyanins and carotenoids (Fig. 2).

As expected, the cultivated plants of both ecotypes, which had grown in a less stressful environment compared to those at the spontaneous state, contained lower concentrations of anthocyanins and phenol glycosides. The opposite trend that was observed for the content of carotenoids could be due to the much higher light intensity in the native environment than in the greenhouse, leading to a higher rate of carotenoid oxidation in the wild grown plants.

Anyway, although similar results were obtained in cultivation for both ecotypes, only the cultivated plants from Agnano contained a significantly lower concentration of total phenols and showed a significantly lower antioxidant capacity than the spontaneous ones (Fig. 3). These findings suggest that in this ecotype both anthocyanins and phenol glycosides could bring a relevant contribution to the pool of phenolics, and that phenolic antioxidants could play an essential role in determining the overall antioxidant activity. In contrast, in the Calafuria ecotype, cultivation did not have a strong overall effect on the content of total phenols or in the antioxidant power, since only the DPPH assay revealed a significant reduction of the radical scavenging activity.

Also the salt stress affected the two ecotypes in a different way, especially concerning the concentration of total phenols and the antioxidant capacity (Table 2 and Fig. 4), suggesting that the coastal grown plants and those from the inland could have developed distinct salt tolerance mechanisms.

In the Agnano ecotype, both the concentration of total phenols and the antioxidant capacity tended to increase in reaction to the saline aerosol. Although only a slight variation was observed, this could indicate a possible role of phenolics in the physiological response to salt stress. On the other hand, a similar trend was not observed for the other parameters, including anthocyanins and flavonol glycosides. This outcome suggests that different classes of phenolic substances could be involved in the mechanism of defence against salinity, such as phenolic acids or different subclasses of flavonoids. According to *t*-test comparisons, the salt treated plants of the inland ecotype contained lower levels of bioactive compounds than the corresponding wild samples, with the only exceptions of total chlorophylls and carotenoids, indicating that the application of a saline spray was not effective in restoring the nutraceutical properties of the spontaneous plants from Agnano.

In the ecotype from Calafuria, the concentrations of chlorophylls, flavonol glycosides and carotenoids tended to decrease with salt stress, and all the other parameters under examination were strongly lowered. This unexpected behaviour may be indicative of a salt tolerance mechanism not involving antioxidant compounds, or could be ascribed to a slow physiological response, not yet apparent after only ten days from the beginning of the salt treatment. Alternatively, a sudden salt stress during optimal plant growth could be ineffective in triggering a stress response in this ecotype, since the activation of the metabolic pathways of salt stress tolerance might require the adverse conditions to occur already during germination or in the early phenological phases.

By an overall comparison between the wild plants from the two sites, those from Agnano appeared more promising for ex situ cultivation, because they were naturally richer in important bioactive components and showed a higher antioxidant capacity, which tended to increase in cultivated plants with the application of salinity conditions. Anyway, even in this ecotype an acute stress caused by foliar treatment was not effective to stimulate a significant accumulation of antioxidant molecules, especially phenolics. In order to observe a marked effect on the compositional parameters, more severe conditions could be required, such as a chronic stress induced by root uptake.

5. Conclusions

Genotype, environment and their interaction may significantly affect the chemical composition of *R. Picroides*, as already found for common crops. Our results showed that only the chlorophyll content of the leaf tissues was not influenced by the ecotype or the growing environment. In contrast, the concentrations of antocyanins, flavonol glycosides, carotenoids and total phenols, along with the antioxidant capacity, were strongly dependent on both factors. This adaptable richness of health-friendly metabolites could arouse interest towards future collection and selection of *R. picroides* ecotypes evolved in different environments. Moreover, the nutraceutical performances of this "new vegetable" could be improved through appropriate cropping systems. In our experiments, the hypothesis that nutraceuticals may be elicited by a sudden salt stress on the leaf canopy was not validated. However, further work is in progress to test the chronic long-term effect of tolerable salt doses and investigate the influence of different types of abiotic stress on the phytochemical composition of this species.

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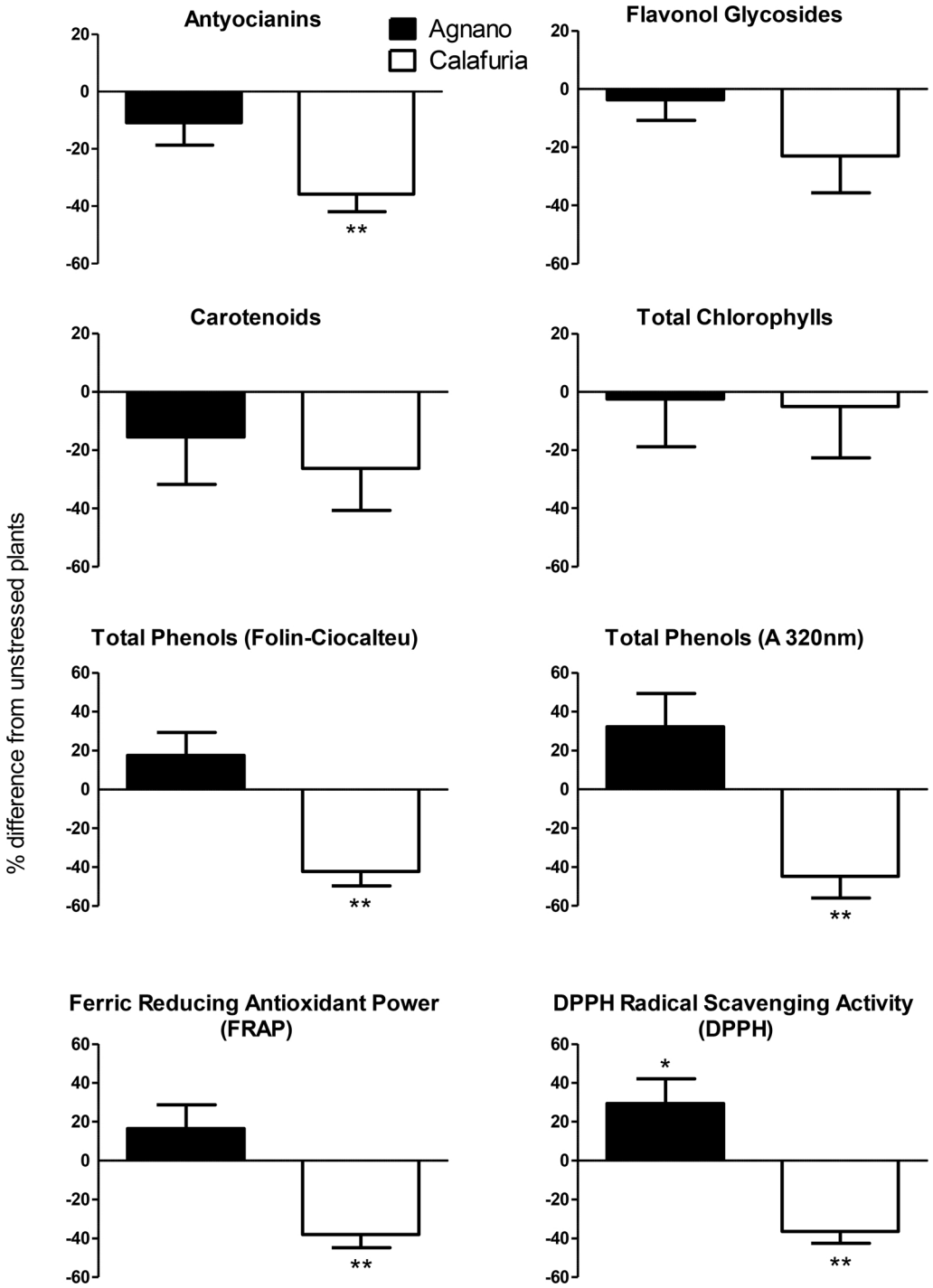

Fig. 4. The percentage variation of the contents of anthocyanins, flavonol glycosides, carotenoids, total chlorophylls, total phenols and antioxidant capacity (FRAP and DPPH) in the leaf tissues of cultivated *Reichardia picroides* of different ecotypes (Agnano or Calafuria), after 10 days spraying of sodium chloride solution (0.049 g m^{-2}). Mean values and standard deviation of four replicates. Only significant differences relative to the unstressed control are indicated (*: $P < 0.05$, **: $P < 0.01$), according to Bonferroni post-test following two way ANOVA.

practical application perspectives of the outcome of this work.

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