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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