Erosion of *Brassica incana* Genetic Resources: Causes and Effects

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Abstract: *Brassica incana* Ten., possessing a number of useful agronomic traits, represents a precious genetic resource to be used in plant breeding programs to broaden the genetic base in most *Brassica* crop species. *B. incana* that grows on limestone cliffs is at risk of genetic erosion for environmental constraints and human activities. We studied the pedological conditions of a Calabrian site where the *B. incana* grows, and we correlated the soil properties to the physiological and biochemical aspects of *B. incana* to identify the causes and effects of the genetic erosion of this species. Our results evidenced that physical soil conditions did not affect *B. incana* growth and nutraceutical properties; conversely, biological soil properties modified its properties. We identified leaf pigments and secondary metabolites that can be used routinely as early warning indicators of plant threat, to evaluate in a short term the dynamic behavior of plants leading to species extinction.

1. Introduction

The decline of biodiversity worldwide can lead to declines in overall levels of ecosystem functioning [1]. In the European context, there is a pressing need to assess the overall conservation status of species and habitats of community interest [2]. Rare species may be at greater risk of extinction because of their small geographic ranges, low abundances, and greater susceptibility to environmental changes [3], Further, incomplete information on their distributions, often gathered over long periods of time and with limited spatial accuracy, makes the assessment of these species particularly challenging [4], [5]. The genus *Brassica* is the most economically important genus within the Brassicaceae family being widely used in human diet as an important source of vegetables, condiments, and edible oils. The interest in Brassica vegetable crops has recently grown due to the breeding programs carried out in several countries - mainly in Asia, Europe and USA. Within *Brassica* species, an important loss of natural genetic diversity has been observed due to the introduction of highly performing F1 hybrids, characterized by a narrow genetic base that is contributing to the slow disappearing of landraces. In the last decades, an important loss of natural genetic diversity (loss of wild germoplasm) has also been caused by climatic changes, agricultural practices, deforestation and human activities. Calabria represents one of the main diversification center of the genus *Brassica*. The species most distributed in Calabria are *B. fructicolosa, incana, napus, rupestris, gravinae, nigra, montana and oleracea* [6]. These populations are wild relatives of kale crops [7] and therefore represent an important genetic resource. The populations are characterized by a considerable morphological variation [8] and show a strict genetic relationship [9]. Among these populations, *B. incana*, native to east and southeastern Europe (USDA, ARS, National Genetic Resources Program *2010, Marhold 2011),* possesses a number of useful agricultural traits. Additionally, from a nutraceutical point of view, it contains high amounts of glucosinolates and phenols, compounds with antioxidant, anticarcinogenic, cardiovascular and

biocide properties that confer to *B. incana,* phytotherapic properties, encouraging its use in medicine, and in organic farming. Additionally, seeds of *B. incana* are a source of mustard powder as *B. juncea* and *B. nigra*, and could represent a benefit for the local economy. Many International Institutions, involved in the nature protection, listed this species in the "IUCN Red List of Threatened Species" that deserve attention and conservation priority. As a conservation tool, red list data are recommended to be used at various scales, including site level evaluations and national resource management and legislation [10], [11]. At the local level, the presence of species recognized as threatened by an authoritative system can be accurate pointers for prioritizing key habitats and their conservation [12], [13]. *B. incana* is assessed as Data Deficient as there are currently insufficient information available to evaluate this species, thus researches are needed to determine the current demographic status of this species. Based on this background, the goal of this work was to correlate the soil properties of the Calabrian sites where the *B. incana* grows to the physiological and biochemical aspects of *B. incana* to identify the causes and effects of the genetic erosion of this species.

2. Materials and methods

The soils were collected under *B. incana* vegetation in Stilo, Calabria, South Italy, (Lat. 38° 30' 29,2'' N, Long. 16° 22' 45,8'' E, altitude of 600 m above sea level, with a mean annual air temperature of 15.6 ℃ and a mean annual rainfall of 905 mm) from 2 different layers (0-10; 10-20 cm) separated on the basis of morphological differences which could be perceived by the naked eye. Soil samples (1 kg) were taken from each layer and analyzed separately. The samples were brought to the laboratory on the same day. Prior to the soil analysis, except for dehydrogenase (DH) activity and fluorescein diacetate hydrolysis (FDA), all the soil samples were air-dried, sieved (<2mm), and visible roots were removed.

Particle size analysis was detected following Bouyoucos [14] method; pH was measured in distilled water and in 1 M KCl using a 1:2.5 (soil:water) suspension; organic carbon (OC) was determined by dichromate oxidation, following the method of Walkley and Black[15]; soil total nitrogen (TN) was measured by the method of Kjeldahl [16]; electric soil conductivity (EC) was measured in distilled water using a 1:2.5 (soil:water) suspension by using a conductometer. Phenols were extracted with distilled water, 1:10 (w/v) [17]. Total water-soluble phenols (WSP) were measured by using the Folin–Ciocalteau reagent, following the Box method [18]. Tannic acid was used as a standard and the concentration of water-soluble phenols was expressed as tannic acid equivalents (mg TAE/g dw) [19]. FDA hydrolysis reaction was determined according to the methods of Adam and Duncan [20]. Dehydrogenase (DH) activity was determined by the method of von Mersi and Schinner [21].

Leaves of *B. incana* were homogenized in a chilled mortar with distilled water at a ratio of 1:4 (leaves/water; w/v) and centrifuged at 14000 g for 30 min. All steps were performed at 4 ℃. The supernatants were filtered through two layers of muslin cloth and were used to determine the total antioxidant capacity by the spectrophotometric method of Prieto et al. [22]. Chlorophyll a and b content, expressed in μg/g fresh weight, was determined following the method of [23]. Total phenolic content was determined with the Folin–Ciocalteu reagent according to a modified procedure described by Singleton and Rossi [24]. Carotenoid content was determined according Lichtenthaler [25].

All variables analyzed were first tested for data normality by using Shapiro- Wilk's test. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test at a 95% confidence level. All data collected were statistically analyzed using SYSTAT 8.0 software.

3. Results

Tables 1 summarizes physical, chemical and biochemical properties of the soils collected under *B. incana* vegetation.

Table 1. Physical chemical and biochemical properties of soil under B. incana vegetation: electric soil conductivity (EC, dS /m); organic carbon (OC %); organic matter (OM %); total nitrogen (TN %); carbon/nitrogen ratio (C/N); water-soluble phenols (WSP, mg TAE/g dw); Dehydrogenase (DH, μg TFF g-1h-1); Fluorescein diacetate (FDA μg fluorescein g-1h-1). Different letters in the same column indicate significant differences $p \leq 0.05$.

Depth (cm)	Sand %	Loam %	Cla %	pH (H_2O)	pH (KCl)	EC	OC	OM	TN	C/N	WSP	DH	FDA
0-10	85	10	5	7.94[b]	6.86[b]	< 1	0.350[a]	0.602[a]	0.045[a]	7.8[b]	120.51[a]	17.39[a]	85[a]
10-20	86	9	5	8.12[a]	7.31[a]	< 1	0.130[b]	0.224[b]	0.015[b]	8.6[a]	105.63[a]	9.78[b]	64[b]

pH was lower at the soil surface than at the depth. pH (H_2O) was slightly alkaline ranging from 7.94 to 8.12. pH (KCl) ranged from 6.86 to 7.31, and it was consistently less than that measured in H_2O. Soil had a sandy texture, an EC lower than 1 dS/m, and a low content of organic matter and total nitrogen. The C/N ratio increased with increasing soil depth. DH and FDA hydrolysis activities were higher in the surface layers. The greatest amount of WSP was detected in the surface layer. The plants measured, in mean, 40 cm (data not shown). The amount of total chlorophyll and chlorophyll *a* was low as well the Chlorophyll *a* and *b* ratio and carotenoids.

Table 2. Content of antioxidants, chlorophylls, carotenoids and total phenols in leaves of *B. incana.*

Anthocyanins µg anthocyanin gr^{-1} FW	Chlorophyll *a* mg 100 gr^{-1}FW	Chlorophyll b mg 100 gr^{-1}FW	Chl	Chla/Chl b	Carotenoids mg gr^{-1} FW	Total Phenols µg TAET g^{-1} FW	Total Antioxidant capacity (µg tocopherol ml^{-1})
2.4	94.7	60.6	117.2	1.56	8.0	4342	462.0

Conversely a great amount of phenols was detected in leaves of *B. incana* the total antioxidant capacity was high too (Table 2).

4. Discussion

The loss of genetic variability represents not only the loss of wild germplasm but also the loss of evolved landraces resulting from the interaction of environmental selection with the genes present in both wild and cultivated populations [26]. The *Brassica* genus has not been an exception and, in particular, conservation of wild *Brassica* species has been a high priority [27]. During the 1970s, wild germplasm of *Brassica* was extensively collected and cytogenetic studies were started. Intensive efforts were made in the last decades to search and collect this material that, otherwise, would be irreversibly lost [28], [29]. After 1970s, the introduction of the concept of biodiversity was a strong support for many improvements in ex situ and in situ conservation strategies. *Brassica* wild relatives are valuable sources of genes for crop improvement [27]. They have been widely used since more than one century to increase resistance/tolerance to biotic and abiotic stresses in several cvs. Recently they have been used to increase nutritional value in edible parts like enhancing proteins or vitamins content. Conservation of species involves the selection of accessions to be conserved and the maintenance of these accessions for current and future users by regeneration. Decisions concerning both these aspects require specific knowledge of the environment, and soil characteristics where the species naturally grows and also physiological and biochemical knowledge of the species. Our results evidenced that *B. incana* was less high than the Sicilian *B. incana* [27] suggesting that is spread in sandy eroded soils with low amounts of organic matter not for choice but almost forced by climatic changes and human interventions that allowed other plants to take over confining this species to marginal places where other plants difficulty grow and/or animals hardly access to use this species as fodder. Thus, *B. incana* used these sites as shelters for own survival and conservation. Data of biochemical traits of this accessions supported this hypothesis. Plant secondary metabolites and pigment synthesis display an important role in the adaptation of plants to their environment [30], and generally low pigment

synthesis has been proven to be the consequence of hexogen stress, senescence or adaption to changing environments [31]. In this sense, plants to maintain a balanced physiological state in the respective tissues conferring protection against environmental stresses [32]-[34] use chlorophylls, carotenoids, anthocyanins, and phenols. Our data evidenced that *B. incana* had a lower content of anthocyanins, chlorophylls, carotenoids, and total phenols in respect to data reported in literature [35]. All these data evidence that the properties of the soil in situ is not optimal for *B. incana* growth. Soil under brassica with a low amount of organic matter, nitrogen, FDA and DH, enzymes related to soil quality, induced low production of pigments and antioxidant compounds, affecting health-properties in *B. incana.* A negative linear relationship between the amount of photosynthetic pigments, antioxidant compounds, organic matter and quality of soils and a positive linear relation between leaf phenols and amount of organic matter, and soil enzyme activities was observed. In short, these data demonstrate the low fertility of soil in terms of organic matter and the scarce soil quality in terms of enzymatic activity and soil ecosystem functioning are the cause of genetic erosion risk and scarce propagation of *B. incana* [36], [37].

5. Conclusion

Results from current study show that decrease in soil fertility (chemical and biological soil characteristics), caused by human interventions or environmental changes, can alter species distribution in a geographic area reducing plant its spread and causing erosion genetic risk. Additionally, soil biological properties can condition plant growth and metabolism, affecting the nutraceutical properties of a vegetal species. The results of this study support the idea of using early key leaf biochemical indicators (decrease in pigments and contemporary increase in total phenols) for investigating environmental constraints inducing species extinction, specifically in the case of brassicacae. Despite this progress, several areas of uncertainty remain to be investigated.

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