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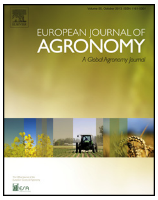

Germination ecology of nutraceutical herbs for agronomic perspectives

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ABSTRACT

The growing need for nutraceutical foods has elicited increasing interest in the herbs traditionally used for ethnobotanical purposes. Their richness in antioxidants inspired this research, which is aimed at cultivating them as crops. Sixteen wild species of crucial interest as food were studied in term of seed propagation. The frequent dormancy was reduced or removed by physical, or physiological seed treatments in laboratory experiments in Petri dishes. However, their direct field sowing, in spring and autumn, did not show the same performance in spite of the efficacy of the treatments. This specially occurs in the case of species with very small seeds suggesting a burial involvement of this soil-mediated inhibition. This inhibition was markedly lower after greenhouse sowing in a soft substrate (peat–pearlite) confirming the hypothesis that the soil acts (*via* hypoxia) as a crucial obstacle to germination. The study of the emergence reduction by a slight burial (1 cm), compared to unburied germination, allowed to evaluate, for each species, the burial-mediated inhibition. After the 1000 seed weight measurement a relation between these two parameters (burial inhibition and seed weight) was fitted. A polynomial regression confirmed the inverse relationship between seed weight and burial-mediated inhibition showing the remarkable burial-intolerance of very small seeds. Consequently the greenhouse sowing in substrate appears the most suitable agronomic strategy overall for food herbs characterized by little seeds. Critical issues are discussed regarding these weeds becoming real nutraceutical crops.

1. Introduction

The increasing focus on health and well-being has let to a rapid increase over the last few years in the study (Bernal et al., 2011), technology (Champagne and Fustier, 2007), marketing (Siro et al., 2008), and consumption (Ozen et al., 2012) of nutraceutical foods.

Vegetables have aroused great interest especially as a source of antioxidants (Vinson et al., 1998) and other functional properties (Stintzing and Carle, 2004) such as the discovery of nutraceutical crops, was considered an agronomic challenge of the twenty-first century (Raskin et al., 2002).

However, the greatest health benefits of the plant kingdom are mainly provided by wild species, above all as antioxidants (Vanzani et al., 2011), since these phytochemicals, which are the result of evolutionary processes in natural ecosystems, play an ecological role in self-defense from both biotic and abiotic adversity (Jwa et al., 2006). Many of these wild species were traditionally collected and used as food as highlighted by the results of ethnobotanical studies conducted in Europe (Paoletti et al., 1995; Trichopoulou and Vasilopoulou, 2000; Leonti et al., 2006; Redzic, 2006; Tardío et al., 2006), Mediterranean Africa (Addis et al., 2005; Kumar and Kumar, 2009), South America (Tanji and Nassif 1995; Ladio, 2001), Indonesia (Cruz-Garcia and Price, 2011), and Asia (Yang, 2008). Some of these species have a thousand-year-old history and have been found in various archaeological sites (Behre, 2008). In ancient times, although people were not aware of the chemical nature of plants, their health benefits were well-known thanks to ethnobotanical traditions handed down between generations. There is also evidence that some herbs were part of the Mediterranean diet (Trichopoulou et al., 2003).

Today the ancient philosophy of Hippocrates (460–370 BC) that stated "*Let food be your medicine and medicine be your food*" has been re-assessed.

Most of these pharmaceutical (Stepp, 2004) and mineral-rich (Tanji and Elgharous, 1998) food herbs are common weeds and are especially widespread in agro-ecosystems, that are rich in biodiversity due to eco-friendly cropping systems (McLaughlin and Mineau, 1995) and urban ecosystems (Benvenuti, 2004). It is surprising that we are now considering using these unwanted and neglected species as healthy food (Rapoport et al., 1995).

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Table 1
Botanical information (Pignatti,1982) and seed collection environments of the tested food species.

Species	Botanic family	Life form[a]	Chorology	Environment of germoplasm collection
Alliaria petiolata (M. Bieb.) Cavara & Grande	Brassicaceae	H	Euro-Asiat.	Woods borders
Borago officinalis L.	Boraginaceae	T	Euri-Medit.	Edges of ditches
Bunias erucago L.	Brassicaceae	T	Euri-Medit.	Agricultural fields
Campanula rapunculus L.	Campanulaceae	H	Euro-Asiat.	Woods borders
Hyoseris radiata L.	Asteraceae	H	Steno-Medit.	Arid grasslands
Hypochoeris radicata L.,	Asteraceae	H	Europ.-Cauc.	Arid grasslands
Picris echioides L.	Asteracee	T	Euri-Medit.	Agricultural fields
Plantago coronopus L.	Plantaginaceae	H	Euri-Medit.	Coastal hind dunes
Reichardia picroides (L.) Roth	Asteraceae	H	Steno-Medit.	Rocky meadows
Rumex acetosa L.	Polygonaceae	H	Circumbor.	Wet meadows
Sanguisorba minor Scop.	Rosaceae	H	Paleotemp.	Rocky meadows
Silene vulgaris (Moench) Garcke	Cariophyllaceae	H	Paleotemp.	Wood borders
Sonchus oleraceus L.	Asteraceae	T	Cosmopol.	Urban meadows
Taraxacum officinale Weber	Asteraceae	H	Cosmopol.	Urban meadows
Tordylium apulum L.	Apiaceae	T	Steno-Medit.	Rocky meadows
Urospermum daleschmpii (L.) Schmidt	Asteraceae	H	Steno-Medit.	Field margins

[a] T = Therophyte, H = Hemicryptophyte.

This transition from weed to crop is necessary not only to ensure their availability, irrespectively of their erratic spread in space and time, but overall, due to food safety in terms of potential contamination by herbicides or environmental pollutants. In other words it is only by cultivating these potential "new crops" that the growing demand for nutraceutical foods can be satisfied in the light of the increasing interest in healthy eating.

Unfortunately most of the wild species have dormant seeds, which typically involve poor and unsynchronized germination (Koornneef et al., 2002). In addition, herbaceous wild species are frequently characterized by small seeds with scarce energy reserves and consequently by vulnerable pre-emergence growth. Consequently they are typically strongly inhibited by soil burial due to the typical hypoxia-mediated mechanism (Benvenuti and Macchia, 1995) that occurs in the soil matrix.

Usually the seeds of many herbaceous species (wild and/or weeds) undergo towards an often dormant seed bank (Thompson et al., 1993) after their dispersal and natural burial (Benvenuti, 2004). However, this survival strategy, although it is useful in the natural environment it prevents synchronized germination. Consequently it is agronomically unacceptable for their hypothetical propagation and cultivation. Indeed a high, rapid and uniform emergence is one of the most important needs to use un-domesticated species for agronomic perspectives.

Despite the great interest for food herbs, very few experiments were focused on the propagation and cultivation of these species.

The aim was to study the ecology of seed dormancy, germination burial inhibition of some interesting food species and to verify their emergence in the following agronomic strategies: direct field sowing (in autumn or spring) and a greenhouse winter sowing in peat–perlite substrate.

In other words we tried to answer the question to know which species are suitable for a direct field sowing and which are suitable for a greenhouse sowing as typically occurs for many vegetables.

2. Materials and methods

2.1. Plant material and germoplasm collection

Some of the most interesting food herbs in terms of ethnobotanical use (Pieroni 2000, 2001) have been selected. During the summer of 2013 seeds of various wild species were collected from different environments (arid grasslands, agricultural fields, *etc.*) in Tuscany as shown in Table 1. Mother plants had grown in normal conditions without any symptoms of biotic or abiotic stress. The seeds were extracted from the respective fruits in the laboratory, cleaned, dried (max 12% humidity), and kept in glass containers (50% of air relative humidity) at 20 °C.

2.2. Seed treatments

Preliminary tests in Petri dishes showed a pronounced seed dormancy in some species and consequently a diversified treatment protocol (by previous experiences on wild species, Bretzel et al., 2009) was carried out in order to promote germination in spite of their relative physical, chemical or physiological dormancy. The treatments consisted of a physical type (seed coat removal), chemical (inhibitor oxidation using sodium hypochlorite), or physiological (chilling in moist conditions). In this last treatment, seeds were placed in Petri dishes (12 cm diameter) equipped with moistened filter paper (7 ml of distilled water each), and maintained (after sealing by using parafilm) at a low temperature (4 °C) in darkness for one month. Regarding the sodium hypochlorite treatment, seeds were soaked in a solution containing NaOCl diluted to 50% with distilled water for 30 min. Throughout the treatment, seeds were constantly stirred with a magnetic stirrer in order to achieve uniform seed contact with the solution. At the end of this hypochlorite treatment, seeds were washed under running water (about 2 l passed through a common sieve) to eliminate the residual solution adsorbed by the seed coats. A similar soaking and stirring procedure was adopted for the potassium nitrate treatment (KNO_3 solution $1\ g\ l^{-1}$) with the only exception that, in this case, the seed immersion was prolonged for two hours (to allow its full absorption). After each soaking or chilling treatment, seeds were air dried and stored at room temperature until use.

In the case of *Bunias erucago,* the dehiscent siliques (hereafter referred to as coats) were carefully removed using a scalpel.

The different above cited treatments were carried out on all species but were then illustrated and examined only the most effective ones for each species.

2.3. Germination tests

Seeds of all species were imbibed, both untreated and after their respective treatment, on a single sheet of moistened (7 ml of distilled water) filter paper (Whatman No. 1) and placed in 12 cm Petri dishes (50 seeds each). Their incubation was carried out in climatized cabinets at the alternate temperature of 10/20 °C light/dark respectively (photoperiod 12 h/12 h). Light (neon fluorescent lamps PHILIPS THL 20W/33) was regulated at the intensity of roughly $50\ \mu mol\ m^{-2}\ s^{-1}$. A spectroradiometer (model 1800, Licor Inc., Lincoln, NE, USA) was used to control the irradiance. The incubation