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First approach to introduce wild species of interest as vegetable crops: study of germination process, crop density and post-harvest conservation

M.Sc. Thesis

Elaborated by: Bc. Jindřich Sojka
Supervisor: Ing. Zbyněk Polesný, Ph.D.

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ABSTRACT

Two common weedy species *Silene vulgaris* and *Sinapis arvensis* traditionally used as wild food plants for culinary purposes throughout Mediterranean basin were studied. The species were subjected to study of morphological characteristics, growth response under different cultivation and harvest regimes, and postharvest processing methods with the aim to contribute to species domestication. Thousand seed weight and number of seeds per 1g was 1.15 g / 693 seeds and 1.01 g / 956 seeds in *Silene vulgaris* and *Sinapis arvensis*, respectively. A portion of germinated seeds of *Silene vulgaris* decreased from 66.0% to 20.0% with decreasing temperature characteristics. In *Sinapis arvensis* better germination ability was observed, also greater tolerance to lower temperatures was evidenced since the share of germinated seeds reached 93.5% and 72.5% in lower temperature characteristics. The highest yield in *Sinapis arvensis* of 7246.23 kg per ha was reached at the densest intra-row spacing of 15 cm. *Silene vulgaris* reached the highest yield of 1189.93 kg per ha at the sparsest intra-row spacing of 60 cm. Both species resprout repeatedly after being harvested. In postharvest analyses, no significant difference was observed in dry matter content among different spacings in any of the species. Decay and quality deterioration was the lowest in harvested material stored under ambient (non-modified) atmosphere. Modified atmosphere storage provided material of lower quality in terms of organoleptic characteristics. Both crop species introducing is assumed to be successful as for plants' low growth requirements as for the content of health beneficial compounds.

Key words:

Ethnobotany, traditional, vegetable, crop, domestication, *Silene vulgaris*, *Sinapis arvensis*, post-harvest, IV gamma

ABSTRAKT

Rostlinné plevelné druhy *Silene vulgaris* a *Sinapis arvensis*, které jsou tradičně používány ve Středomořské kuchyni, byly podrobeny studii, jenž má za cíl přispět svými výsledky k domestikaci obou druhů. Byla zkoumána morfologie semen obou druhů a byl zhodnocen vliv teplotních podmínek na jejich klíčivost. Dále byl zkoumán vliv hustoty výsevku na výnos a následné zpracování a uskladnění sklizeného materiálu. Hmotnost tisíce semen a počet semen na 1 g byla 1,15 g / 693 semen u *Silene vulgaris* a 1,01 g / 956 semen u *Sinapis arvensis*. Klíčivost byla u *Silene vulgaris* 66% a při nižších teplotách klesla na 20,0%. U *Sinapis arvensis* byla klíčivost 93,5%, při nižších teplotách 72,5%. Nejvyššího výnosu 7246,23 kg/ha bylo u *Sinapis arvensis* dosaženo při sponu 15 cm. U *Silene vulgaris* byl nejvyšší výnos 1189,93 kg/ha dosažen při sponu 60 cm. Oba rostlinné druhy obrážely při opakované sklizni. Mezi různými spony nebyl pozorován rozdíl v obsahu sušiny sklizeného materiálu. Uskladnění sklizeného materiálu bez ochranné atmosféry přineslo oproti kontrolovaným atmosférám nejlepší výsledky. Na základě nízkých pěstebních nároků a díky obsahu zdraví prospěných látek se předpokládá, že introdukce obou divokých druhů zeleniny bude úspěšná.

Klíčová slova:

Etnobotanika, tradiční, zelenina, plodina, domestikace, *Silene vulgaris*, *Sinapis arvensis*, posklizňová úprava, ochranná atmosféra

DECLARATION

I, Jindřich Sojka, declare that this thesis, submitted in partial fulfilment of requirements for the MSc. degree at the Institute of Tropics and Subtropics of the Czech University of Live Sciences, is wholly my own work unless otherwise referenced or acknowledged.

April 19 2012

Jindřich Sojka

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CONTENTS

PREFACE

The human diet changes markedly within the time and domestication of the wild growing species contributes to this amendments. Some wild plants are consumed especially during the times when conventional food resources are scarce and nowadays many of these plants species are very often recognised as famine plants. On the other hand, in traditions and popular wisdom knowledge of wild plants which are used also for preparation of traditional dishes is conserved. It is a great work of ethnobotanists who collect and record this knowledge. This precious information together with often unexpected situations and other factors contribute well to a diversification of the human's diet.

A contemporary tendency of "returning back to the roots" may be observed as well in national cuisines. This culinary fashion favours domestication of the species that were used and consumed even as a staple food. A modern science frequently reveals that traditionally used plants are rich in some bioactive compound. It is then where ethnobotany and ethnopharmacology meet.

This study presents first approach to domestication of two wild plant species, *Silene vulgaris* (Moench) Garcke and *Sinapis arvensis* L., which are traditionally used as leafy vegetables in various parts in southern Europe and northern Africa. The studied species are interesting for their low growth requirements as well as for their dietetic properties. This study intends to evaluate the species from the agronomical point of view with the aim to contribute to species domestication.

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SEM	Scanning Electron Microscope
UV-B	Ultraviolet B, medium wave (λ = 315-280 nm)
UV-C	Ultraviolet C, short wave (λ = 280 – 100 nm)

1 INTRODUCTION

1.1 Wild edible plants

Wild plant species have been always collected by the humankind for various purposes, such as for agricultural, food, medicinal, ritual and religious, and other purposes. Plants and their knowledge are somewhat a part of a culture; a culturally important plant can be defined as a species desired, preferred, or with an affective evaluation by most members of this culture (Tardío & Pardo-De-Santayana, 2008). Ethnobotanical studies may help to specify which plants are consumed by each ethnic group in actual cultural and geographical context (Nebel *et al*., 2006; Rivera *et al.*, 2007).

The knowledge of the traditionally utilised plants is of high importance and therefore ethnobotanists keen to collect this human wisdom (Lentini, 2002; Ogle *et al*., 2003; Pieroni *et al*, 2002; Scherrer *et al*., 2005; Tardío *et al*., 2005). This traditional knowledge, nowadays, survives mainly in the memory of the elderly and is in danger of disappearing (Tardío *et al*., 2006). The development of the current modern society results in erosion of this knowledge (Bonet & Vallès, 2002). The main keepers of traditional knowledge in the domain of local food plants are women, while men play an important role in collecting plants and fungi growing in sites distant from the village (Nebel *et al*., 2006).

The species of interest are mostly perennials and often are discovered during the hand-weeding of the cereal crops, as for example *Chondrilla juncea* L., a species that is traditionally used in province of Madrid in Spain as a leafy vegetable. (Tardío *et al*., 2005; Wujisguleng & Khasbagen, 2010). In natural habitats, the plants are commonly collected in the mountains, in the forests and thickets, in open areas and riparian environments such as lagoons and springs (Rivera *et al.*, 2007). Traditionally used species occur as well in the secondary habitats and areas influenced by humans, e.g. reforested sites or even regularly disturbed (sub)urban areas (Cristaudo *et al.*, 2009; Marsden-Jones & Turrill, 1957; Tardío *et al*., 2005).

The wild plant species form an important phytogenetic heritage of precious agricultural values, principally for indentifying new species suitable for further cultivation, as a genetic resource for breeding programmes, nonetheless for studying the crops' evolution (Branca, 2002). For many people the wild species form a fundamental resource of alimentation, especially due to the absence of other resources that also referred to small range of vegetable species offered on the market (Arcidiacono, 2002a).

The staple food is currently based on agricultural and livestock production. Until now, many wild plants were used as supplementary foods, and hence as an important source of vitamins and minerals. Currently, with the great development of marketing techniques, it is easy to find fruits and vegetables (i.e. source of vitamins and other dietetically essential elements) in markets throughout the year (Tardío *et al.*, 2005). There seems to be a strong pattern of avoidance of using the green parts of wild plants as either vegetables or for flavouring, particularly strong tasting ones, during periods when food is not scarce (Łuczaj, 2008).

The wild plants are collected regularly by the local inhabitants. The plant is usually collected in a well define growth stage to which very often refers also the vernacular name (Branca, 1992). Wild vegetables are usually harvested for leaves, apical parts and shoots, green fruits and underground parts (Wujisguleng & Khasbagen, 2010). Wild sources of food, in general, remain particularly important for the poor and landless, and are important more than ever during times of famine or conflict when normal food supply mechanisms are interrupted (Tardío *et al.*, 2006).

1.2 Wild plants used as leafy vegetables

The number of wild plant species which are traditionally used as leafy vegetables in different parts of Mediterranean region is rather impossible to estimate. Various ethnobotanical studies have been performed throughout the region (Arcidiacono, 2002b; Branca, 2002; Lentini, 2002; Di Tonno & Lamusta, 2002; Tardío *et al.*, 2006; Nebel *et al.*, 2006). Table 1 presents the list of the most represented botanical families among the species of interest. Although, some of the botanical families listed are represented by one or few species only, e.g. *Boraginaceae* or *Malvaceae*, yet such species are considered important (or even essential) in traditional cuisine. The collected plants are mostly herbs traditionally consumed as green vegetables (Arcidiacono, 2002b; Lentini, 2002; Nebel *et al.*, 2006; Tardío *et al.*, 2005; Tardío *et al.*, 2006).

The traditionally utilised species growing spontaneously in their natural habitats are very often considered weeds in conventional agriculture (Pieroni *et al.*, 2002; Rivera *et al.*, 2007; Scherrer *et al.*, 2005). In Poland, *Rumex* sp. L. and *Chenopodium album* L. used to make part of a common diet (Łuczaj, 2008). Nowadays, those species are commonly considered invasive weeds (Ficko *et al.*, 2010).

Table 1. Most representative botanical families of wild plant species traditionally used as vegetable in Mediterranean region (ordered alphabetically)

Family	References
Apiaceae	Arcidiacono, 2002b; Branca, 2002; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Hadjichambis *et al*., 2008; Lentini & Venza, 2007; Nebel *et al.*, 2006; Sánchez-Mata *et al.*, 2012; Tardío *et al.*, 2006
Asteraceae	Arcidiacono, 2002b; Branca, 2002; Giusti & Pieroni, 2009; Hadjichambis *et al*., 2008; Lentini, 2002; Lentini & Venza, 2007; Mustafa *et al.*, 2011; Nebel *et al.*, 2006; Sánchez-Mata *et al.*, 2012; Tardío *et al.*, 2006
Boraginaceae	Arcidiacono, 2002b; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Lentini, 2002; Lentini & Venza, 2007; Nebel *et al.*, 2006; Tardío *et al.*, 2006
Brassicaceae	Arcidiacono, 2002b; Branca, 2002; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Lentini, 2002; Lentini & Venza, 2007; Nebel *et al.*, 2006; Tardío *et al.*, 2006
Caryophyllaceae	Arcidiacono, 2002b; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Lentini & Venza, 2007; Nebel *et al.*, 2006; Sánchez-Mata *et al.*, 2012; Tardío *et al.*, 2006
Chenopodiaceae	Arcidiacono, 2002b; Branca, 2002; Giusti & Pieroni, 2009; Lentini, 2002; Lentini & Venza, 2007; Sánchez-Mata *et al.*, 2012; Tardío *et al.*, 2006
Fabaceae	Arcidiacono, 2002b; Di Tonno & Lamusta, 2002; Lentini & Venza, 2007; Nebel *et al.*, 2006; Tardío *et al.*, 2006
Lamiaceae	Arcidiacono, 2002b; Branca, 2002; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Hadjichambis *et al*., 2008; Lentini & Venza, 2007; Mustafa *et al.*, 2011; Nebel *et al.*, 2006
Liliaceae	Branca, 2002; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Hadjichambis *et al*., 2008; Lentini, 2002; Lentini & Venza, 2007; Mustafa *et al.*, 2011; Nebel *et al.*, 2006; Tardío *et al.*, 2006
Malvaceae	Arcidiacono, 2002b; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Lentini & Venza, 2007; Tardío *et al.*, 2006
Poaceae	Arcidiacono, 2002b; Lentini & Venza, 2007
Polygonaceae	Arcidiacono, 2002b; Branca, 2002; Giusti & Pieroni, 2009; Lentini & Venza, 2007; Nebel *et al.*, 2006; Sánchez-Mata *et al.*, 2012; Tardío *et al.*, 2006
Portulacaceae	Arcidiacono, 2002b; Lentini & Venza, 2007; Nebel *et al.*, 2006; Tardío *et al.*, 2006
Rosaceae	Arcidiacono, 2002b; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Hadjichambis *et al*., 2008; Mustafa *et al.*, 2011; Nebel *et al.*, 2006; Tardío *et al.*, 2006
Urticaceae	Arcidiacono, 2002b; Di Tonno & Lamusta, 2002; Giusti & Pieroni, 2009; Lentini & Venza, 2007; Tardío *et al.*, 2006

Collection and use of the concrete product is pretty seasonal. Generally, the harvest of the wild species takes place all year round. However, the majority of the species is harvested during winter and spring, or at the beginning of summer, depending on the region and its climatic conditions. The wild species are very valuable as vegetable substitutes in early spring and are available several weeks before the garden varieties (Branca, 2002; Nebel *et al*., 2006; Rivera *et al.*, 2007; Tardío *et al*., 2006).

1.2.1 Wild leafy vegetables as a part of a diet

Besides the nutritive value that the edible species possess, use of the wild edible plants can broaden and diversify the cultural and dietary habits (Branca, 1992; Tilahum & Mirutse, 2010). Additionally, wild edible plants are suggested to be regarded as a potential food resource that may decrease hunger and provide sources of healthy food that would be acceptable for humans (Redzic, 2006).

The use of wild species is supported by a revealing interest and rediscovery of traditional dishes; moreover, new crop varieties or any amendment in products' assortment seems to be appreciated by the customer (Branca, 1992). A usage of plants belonging to the wild flora is common today as a supplement for healthy diet, even in the most developed regions of the world (Redzic, 2006). Consumer's current expectation in the supply of all types of fresh fruit and vegetables is to have these available throughout the year (Thompson, 2010).

The wild edible plants used as vegetables are commonly eaten raw as a snack or dessert. Nebel *et al*. (2006) reported that in Southern Italy *Chrysanthemum segetum* L., *Portulaca oleracea* L. or *Reichardia picroides* (L.) Roth. are traditionally used to make salads, often seasoned with olive oil and/or vinegar. Traditional preparation also includes steaming, boiling, frying in olive oil or baking, moreover the plants are usually eaten in one or two manners only. Accordingly, the basal leaves of *Arctium minus* Bernh. or young shoots and tender stems and leaves of *Chondrilla juncea* L. are stewed (Tardío *et al.*, 2005) or tender sprouts of *Ruscus aculeatus* L. or *Humulus lupulus* L. are used only scrambled with eggs. Conversely e.g. *Foeniculum vulgare* Mill. ssp. *piperitum* is traditionally eaten raw in salads or as a snack, or is used fried or boiled in soups or stews, or can be processed to make spirits and liquors (Rivera *et al.* 2007). The greens are further used in traditional soups with other vegetables, or in other main dishes, such as pasta traditionally prepared in Cantabria, Italy with leaf stalks of *Scolymus hispanicus* (Nebel *et al*., 2006; Rivera *et al.*, 2007; Tardío *et al.*, 2005).

Other important use of wild and sometimes also cultivated plant species is flavouring of dishes. In Sicily instead of expensive imported spices so called *fògghie mmiscitàti* or *viddùri mmischìgghi*, which consisted of collected wild *Cichorium* sp., *Sonchus* sp., or *Reichardia picroides* (L.) Roth, were traditionally added to simple quotidian dishes to flavour these, as well as to dishes prepared for special occasions (Di Tonno & Lamusta, 2002). People frequently ascribe a particular taste to singular plant species; the plants are then collected so that the tastes of the prepared dishes are balanced. The taste of wild greens is often connected with a beneficial property of the species or is even considered healthy (Branca, 1992; Cornara *et al*., 2009; Nebel *et al*., 2006).

1.2.2 Health beneficial value of wild leafy vegetables

Many plants develop underground organs which are a useful source of carbohydrates, proteins, and fats. Young shoots and leaves are rich in vitamin C and carotene and therefore play an important role in human nutrition especially in spring when conventional vitamin sources are scarce. Beside vitamins, keratin, and minerals, fruits of many wild plants also contain large quantities of sugar and pectin (Redzic, 2006).

The study of wild species is particularly interesting from both ethnobotanical and ethnopharmacological points of view since these species may have a great potential as a source of unusual colours and flavours, bioactive compounds and as sources of dietary supplements or functional foods (Rigat *et al*., 2009; Sánchez-Mata *et al*., 2012; Tilahum & Mirutse, 2010).

Active non-nutrients (chemical compounds, such as phenolic compounds, flavonoids, flavanols or anthocyanins) together with macro- and micronutrients in human diet are very important. These biologically active compounds are present in some wild edible plants used as vegetables and are highly appreciated for their antioxidant activity (Pereira *et al*., 2011). A recently growing interest in research into the role of plant-derived antioxidants in food and human health has been evidenced (Miguel, 2010). The antioxidant activity was observed as well in ascorbic acid and other organic acids that are commonly present in vegetable species (Sánchez-Mata *et al.*, 2012).

The results of the study conducted on the wild species traditionally utilised as vegetables, *Borago officinalis* L., *Montia fontana* L., *Rumex acetosella* L. *Rorippa nasturtium-aquaticum* (L.) Hayek, and *Rumex induratus* Boiss. & Reut., have revealed that they are nutritionally balanced. Furthermore, based on the antioxidant potential of their extracts, they might find applications in the prevention of free radical related diseases (Pereira *et al*., 2011).

Anthocyanins, are water-soluble compounds that can be found in colourful fruits, flowers and leaves. They are responsible for the brilliant colours of the plant – red, blue or purple (Zheng *et al*., 2011). Anthocyanins are potent antioxidants; they scavenge the free radicals and inhibit the oxidative damaging mechanisms which is involved in degenerative or pathological process such cancer or atherosclerosis (Shie *et al*., 2009).

Vegetables are excellent sources of natural antioxidants, and consumption of fresh plants in the diet may thus contribute to the daily antioxidant intake (Pereira *et al*., 2011). The presence of anthocyanins in highly pigmented vegetable species was recorded by Li *et al.* (2010). The anthocyanins were identified and the contribution of biochemical structure on the antioxidant bioactivity was assayed. For their beneficial properties the plant species containing anthocyanin pigments are suggested to be natural colorants or beneficial foods (Zheng *et al*., 2011).

1.2.3. Multiple use of wild edible plants

A continuum between medicinal and food plants is often found and many plants are used for more than one purpose (Scherrer *et al.*, 2005). These multipurpose foods are often called functional foods (Annunziata & Vecchio, 2011).

Some species are commonly used not only as vegetables, but also for therapeutic purposes. The medicinal use of *Borago officinalis* L. or *Olea europaea* L. was evidenced (Scherrer *et al*., 2005); also *Olea europaea* L. is an oil yielding crop. According to Rigat *et al*. (2009) *Foeniculum vulgare* Mill., *Taraxacum officinale* F.H.Wigg. and *Urtica dioica* L. are traditionally used for nutritive and therapeutic purposes. Similar results have been recorded by Tilahum & Mirutse (2010) in Ethiopia where some edible plant species, e.g. *Saba comorensis* (Bojer) Pichon or *Moringa stenopetala* (Baker f.) Cufod. are used as a food as well as a remedy. Vietnamese women that consume traditionally used wild vegetables, so called *Rau Dai*, rationalise the consumption of these and emphasise the need to balance the overall food intake accordingly for optimal health. In some species, leaves and young shoots are used as vegetables; whilst roots, seeds or the whole plant are used for a specific medicinal purpose, such as *Piper sarmentosum* Roxb. or *Sauropus androgynus* Merr. (Ogle *et al*., 2003).

Some plants used for alimentation in Ethiopia are used for several other purposes, namely *Grewia bicolor* Juss. is also used for decoration; *Celtis africana* Burm.f. and *Grewia kakothamnos* K.Schum. whose wood is used for making tools for fishing or hunting; *Ximenia americana* L. is utilised in processing goat skin and to make traditional clothing; *Ficus sycomorus* L. is used as timber for making bee hives or canoes (Tilahum & Mirutse, 2010).

1.3 Domestication of wild food plants

In this study wild food plants refer to those non-domesticated. Wild food plants can be cultivated; however the cultivation itself does not attribute to the plants the domesticated status. Not all cultivated plants are domesticated. For most species the transition from cultivation to domestication never happens (Cruz-Garcia & Price, 2011).

The cultural requests of the wild growing plants are less intensive mainly due to their often very vigorous growth. Therefore the production inputs can be greatly reduced, moreover, the unexploited marginal areas can be utilised and their value can increase (Branca, 1992). Tilahum & Mirutse (2010) highlighted the health potential of traditionally used wild edible (and still neglected) plants and suggested that direct cultivation of these may improve their beneficial properties.

Very often the most potentially “edible” plants are not actually consumed in localities where they are abundant; they are used only as fodder or in scarcity of other food sources. The plants are collected for almost exclusive subsistence use, only a few are collected for commercial purposes (Rivera *et al.*, 2007; Tardío *et al.*, 2006). On the other hand, Scherrer *et al*. (2005) reported that some greens collected in the wild have been currently replaced by their cultivated varieties.

A study on cultivation of species of alimentary interest that grow spontaneously in Sicily, *Beta vulgaris* ssp. *maritima* Arcang, *Borago officinalis* L., *Brassica fruticulosa* Cyr., *Cichorium intybus* L., *Foeniculum vulgare* Mill ssp. *vulgare* var. *dulce*, was conducted by Branca (2002). The following factors were analysed: seeding time, environment (field or greenhouse cultivation), shading, density and N-fertilization. The results revealed higher production rates in cultivations initiated rather delayed, i.e. sowing in winter times, respectively to the earlier ones, i.e. autumnal sowing. This was not verified in *Borago officinalis*. Plants pre-planted in greenhouse have provided significantly higher production. The doses of 100-200 kg of nitrogen per ha applied in cultivation of

Cichorium intybus resulted in rather modest production increase. However, in *Borago officinalis* the increase of the nitrogen doses applied revealed significantly higher yields; moreover, shading during the cultivation the fertilization effect and the yield decrease of approximately 50% was observed. Cultivation of *Brassica fruticulosa* at higher density did not show any decrease in production of biomass per plant, hence, with increasing density also an increasing tendency in the total yield was recorded.

1.4 Background for the studied species

This work intends to esteem the agronomical potential of two wild species that are traditionally used as vegetables, *Silene vulgaris* (Moench) Garcke and *Sinapis arvensis* L. belonging to family *Caryophyllaceae* and *Brassicaceae*, respectively.

The plant species are commonly known as weeds. However, the plants are collected and used for traditionally culinary purposes in different parts of southern Europe (Branca, 1992; Bonet & Vallès, 2002; Cornara *et al*., 2009; Nebel *et al.*, 2006; Pieroni *et al*., 2002; Rivera *et al*. 2007; Sánchez-Mata *et al*., 2012; Tardío *et al*., 2006).

1.4.1 *Silene vulgaris* (Moench) Garcke

1.4.1.1 Botany and ecology

A bladder campion belongs to family *Caryophyllaceae*. It is a perennial herb, wildly distributed throughout the Northern hemisphere. It is native to Europe; a phylogenetic investigation of chloroplast DNA supported a history of range expansion in Europe from Mediterranean glacial refugia (Keller, 2008).

The species is deep-rooted. The plants appear to be largely indifferent to the chemical nature of the soil, at least to its acidity and its lime content, however well-drained and aerated soils are certainly preferred (Marsden-Jones & Turrill, 1957). It naturally grows on bedrocks, meadows, fields or steep rough pastures. In secondary habitats and disturbed areas can be found along the roads or brick walls and similar.

The species is hemicryptopfytic. It is predominantly erected or, when bigger, erected-procumbent to prostrate. It is multi-stemmed, grows in clusters and reaches up to 60 cm when fully matured. Stems are smooth and thin, branched, nodules bear the leaves. Leaves are opposite and sessile, glaucous, hairless and fleshy. They vary in a form from lanceolate or spatulate to oblong or linear; the size of the leaves varies considerably from 3.8 cm to 6.6 cm as well. This characteristic is known as homoblasty.

The inflorescence is a dichasium. Flowers are peduncled, zygomorphic. Calyx is gamosepalous, pale-green or pinkish with darker green or purplish veins, and is noticeably bloated or even swollen, hence the species' vernacular names. Corolla is composed of five free petals, white and deeply lobbed. Stamens and styles are outstanding, often of livid violet colour. Flowers are protandrous (Marsden-Jones & Turrill, 1957).

The species is well known for a genetic feature called gynodioecy, a dimorphic breeding system in which hermaphroditic individuals and male-sterile (hereafter female) individuals co-exist in populations (Miller & Stanton-Gedde, 2007). Female flowers are always of smaller in comparison to the hermaphrodite ones; independently on as if the plant is female or hermaphrodite (Marsden-Jones & Turrill, 1957).

The fruit is an ovoid capsule. The mean fruit weight is 0.05 g, the mean length and width are 13 mm and 8 mm respectively. The capsule contains on average 50 oblong to kidney-shaped rather flattened seeds. The thousand seeds weight is 0.33 g (± 0.01) (Branca, 1992). Hilum is considerable in relation to the seed size and is visible by eye. For a taxonomical approach, it meets difficulties to distinguish different species of the genera. The tegumental cells lay in lines rising from the hilum in a fan-like pattern, giving to the seed surface a particular ornamentation. The SEM (Scanning Electron Microscope) analysis of seed-coat was used for scrutinising the surface of the seeds since it had been observed that the seed-coat ornamentation differed among the species. Such method revealed diverse forms of the lobbed exosperm cells accordingly to which the species can be distinguished (Valsecchi, 1995; Villa, 1995; Ladero *et al.*, 1999).

1.4.1.2 Nutritive and health beneficial properties

The production of anthocyanin pigments has been observed in vegetative parts as well as in calyx, petals and stamens. It is determined by the genotype – present genes regulate the production of the pigment and its intensity and distribution in the plant, besides, the inhibitor genes are present. However, environmental factors, such as light, temperature, water supply or location, can modify the phenotypic expression (Marsden-Jones & Turrill, 1957).

The study of UV-B (ultraviolet radiation) pigments reported that the leaves from plants exposed to such radiation contain flavonoids (Van de Staaij, 1995), as stated, particularly vitexine and/or iso-vitexine glycosides, flavonol glycosides and isoflavones. A short (ten minutes) UV-B and UV-C irradiation of the *in vitro* callus culture results in amendments in the pectin silenan which is contained in the cell walls. This polysaccharide possesses an antioxidant activity. The exposure of the callus to the ultraviolet irradiation increases the content of phenolic compound and the antioxidant activity of the pectin is more pronounced (Günter *et al*., 2009).

Two samples of young and tender leaves with different origin were analysed for the content of total vitamin C (ascorbic acid and dehydroascorbic acid). The results of the study reported the content of 26.32 mg (± 3.05) 27.66 mg (± 1.78) per 100 g. The plant is hence suggested to be selected for its high nutritional interest; moreover, the fact that the plant can be eaten raw is advantageous for the vitamin C preservation (Sánchez-Mata *et al.*, 2012).

A lipophilic extract (containing non-polar compounds) was assayed for antiviral activity and was reported to be very potent against *Herpes simplex* virus type-1 and *Parainfluenza*-3 virus (Orhan *et al*. 2009).

In the seed oil of *Silene vulgaris* seventeen fatty acids were detected. The ratio of unsaturated fatty acids to the saturated was found to be 0.3. The content of essential linoleic acid (18:2ω-6) in the oil is 38.4%. A notable antibacterial activity against the Gram-negative bacterium *K. pneumoniae* and a significant antifungal activity against *Candida albicans* were recorded (Kucukboyaci *et al.*, 2010).

The data reported in Table 2 show the detected nutritional values of the final product of the plants collected at different locations in central Spain (Alarcón *et al.*, 2006). Within the research, high amounts of polyunsaturated fatty acids (linoleic and α-linolenic acids) were determined. The mean content of erucic acid of 3.3% was reported. For domestication purposes populations with low contents of erucic acid should be selected.

Table 2. Nutritional values of the fresh harvested leaves and young shoots

Property	Weight (g) per 100 g of fresh material
Moisture	87.6 g
Ash	0.3 g
Lipids	0.7 g
Fibre	2.8 g
Protein	3.3 g
Available carbohydrates	3.4 g

(Adapted from Alarcón *et al*., 2006)

1.4.1.3 Ethnobotany

In Northern Italy, in Val d'Aosta region, the apical shoots called *coietti* are collected and consumed raw or stewed and flavoured more or less like spinach. The shoots are also used for preparation of soups, *minestrone* or *risotto* (Laghetti & Perrino, 1994). In Eastern Riviera, Liguria, where the plant is locally called *grisuéli* or *puntétti* the leaves are also utilised for *ravioli* or *gattafin* stuffing (Cornara *et al*., 2009).

In Southern Italy the plant is locally called *culicidd* in Apulia, *cannatedda* or *strigoli* (other dialectal names: *bubbolini*, *schioppettine*, *schioppetti*, *verzuli*, *erba del cucco* or *ebba priracatura*, *crepaterra* or *mazzettone minute*), in Sicily, *cavuráci* or *cavuleddhu* in Calabria. The tender young leaves and apical shoots are fried in olive oil and garlic and chilli, or with eggs for omelette preparation or are added to meat balls (Arcidiacono, S. 2002b.; Bonet & Vallès, 2002; Cornara *et al*., 2009; Laghetti & Perrino, 1994; Lentini & Venza, 2007; Nebel, *et al*., 2007). They are also used in mixture with other vegetables for soups preparation (Lentini & Venza, 2007).

In many parts of Spain, tender leaves and young stems of the first species, known as *collejas* (also *coletas*, *colissos* or *conillets*), are fried in olive oil and eaten or together with scrambled eggs, or in omelettes. Also the leaves are eaten with rice. They are also prepared as a garnish for *cocido*, or as a garnish for *potaje*, a typical Spanish dish often consumed during Lent (Rivera *et al.*, 2007; Sánchez-Mata *et al.*, 2012; Tardío, *et al*., 2005; Tardío *et al*., 2006).

The young leaves and upper parts are also eaten raw in salads, seasoned with olive oil (Alacrón *et al*., 2006; Rigat *et al*., 2009; Sánchez-Mata *et al.*, 2012; Tardío *et al*., 2006). These are also prepared as a simple side dish (Cornara *et al*., 2009).

In Morocco, the roots of *Silene vulgaris* are also well known for their detergent properties. The registered content of saponins in locally harvested roots is about 7% per root dry weight (Bouguet-Bonnet *et al*., 2002).

1.4.1.4 Growth and development

The species' biological cycle in the zones of its probable origin, in the Mediterranean (Keller, 2008), is initiated during autumn. The species is reproduced generatively via seeds. The plantlet grows modestly during the autumn and winter; new sprouts appear and initiate their growth once the temperature increases. During April and May first apical buds develop and consequently the anthesis takes place. The harvest of the product is set during the winter or early spring months, right before the first buds appear (Branca, 1992).

The flowering period is usually in midsummer, from June to July. The anthesis period is significantly determined by temperature; whilst at lower altitudes it can be observed so far at the end of May, at higher altitudes the blossoming does not initiate before June-July (Branca, 1992). Fruit is fully matured approximately a month after pollination. Self-pollination does not seem to play a very important role in plants reproduction. The fruit persist until the midsummer whereupon the seeds are spread, mainly by the wind (Marsden-Jones & Turrill, 1957). The plant then passes to vegetative quiescence which is interrupted at the beginning of the period of autumn rains, which stimulates the proliferation of new sprouts.

Arreola *et al.* (2006) made a research on effects of different water amounts applied during the nursery cultivation and after the plantlets transplantation. A root development was examined during the nursery period; as well, growth of aerial parts and roots was studied after transplanting. Moderately- and highly-stressed seedlings demonstrated greater roots to aerial parts fresh weight ratio. Moderately-stressed seedlings (irrigated at 50% of the water-holding capacity) were provided with the best developed root system. Highly-stressed seedlings resulted way too over-hardened and were not used for transplantation. After the transplantation the moderately-stressed derived plantlets showed greater root growth activity and higher tolerance to drought stress.

Mardsen-Jones & Turrill (1957) conducted a transplantation experiment. The study was conducted on different soils: sand, calcareous sand, clay and chalky clay and ordinary Potterne Upper Greensand soil. Seeds were seeded directly to the ground. The study revealed highest susceptibility to *Marssonia* disease on sand, the lowest one was recorded on the calcareous sand. It was observed that damages to flowers, fruits and seeds were mainly caused by insects *Hadena cucubali* (Fabricius, 1775), *Hadena bicruris* (Hufnagel, 1766), *Eupithecia venosata* (Fabricius, 1787), or *Anuraphis subterranea* (Walker, 1852) causing root-attack, and fungus *Marssonina* causing stem and leaf attack (damage may cause plant sterility), and *Ustilago silenesinflatae* (smut disease).

1.4.2 *Sinapis arvensis* L.

1.4.2.1 Botany and ecology

It is native to most of the temperate regions of the Old world, also occurs in Asia, North Africa (Fogg, 1950);by accident, it has been introduced to North and South America, South Africa and Australia and New Zealand (Mulligan & Bailey, 1975).

The plant easily grows over 1.5 m height. Plant size is subjected to density and decreases with increasing plant density. Moreover, water and nutrients competition limits the plant's growth (Soltani *et al*., 2011). The root system is much extended. The stem is coarsely hairy and branched. The leaves are petiolate, approximately 80 mm wide and 200 mm long, and are deeply lobbed, the edges are toothed. The leaves that grow close to the inflorescence are rather lanceolate and smaller, but still broad, often purplish in the axials.

Flowers are bisexual, sepals are purplish and petals yellow. Flowering takes place from March to June. Fruit is a pedunculed capsule, more often in *Brassicaceae* family called siliqua. The reported mean length and width are 27 mm and 2 mm, respectively. Each fruit contains approximately 11 seeds (Branca, 1992). Seeds are globose, smooth and brown to blackish. The weight of thousand seeds ranges from 1.05 to 1.89 g (Soltani *et al*., 2011), Branca (1992) evidenced a weight of 1.24 g (± 0.05).

Sinapis arvensis is an annual plant that reproduces generatively. The laboratory experiment, which had revealed that the seeds become dormant nearly immediately after being developed, suggests that the species reproduces via at least one year old seeds (Branca, 1992). This characteristic is important especially in modelling the dynamics of the weeds seedbank (Pekrun *et al*., 2005). The species name *arvensis* in latin means *of the* [*cultivated*] *field*, and indeed, it is generally considered a common and persistent weed, mainly in oil-seed crops or cereals (Branca, 1992; Deshpande & Hall, 2000; Meiss *et al*., 2010; Soltani *et al*., 2011).

Wild mustard is well adaptable to a wide range of climatic and soil conditions (Branca, 1992). It is a quantitative long-day species and is apparently sensitive to photoperiodism prior to anthesis. Nevertheless, it is able to complete its life cycle in both long and short photoperiod (Huang, *et al*., 2001). Stress factors, such as low light, applied during the plant development cause significant asymmetry in growth of vegetative parts and seed weight. However the fertility does not seem to be affected (Roy & Stanton, 1999).

The species survives more effortlessly under lower temperatures. The minimal temperature for growth was registered at 5°C. At the day temperatures higher than 23°C the formation of new leaves and shoots is drastically reduced (Huang, *et al*., 2001).

1.4.2.2 Nutritive and health beneficial properties

A study on free radical scavenging capacity of the essential oils extracted from fruits of *Sinapis alba* L., a close relative to *Sinapis arvensis*, reported no efficiency in inhibition of *in vitro* peroxynitrite-induced tyrosine nitration (Chericoni *et al.*, 2005). Other type of antioxidation assay is then suggested to be applied.

On the other hand, in the leaves of *Sinapis arvensis* 3-sophoroside-7-glucosides of kaempferol, quercetin and isorhamnetin were found (Durkeet & Harborne, 1973). Glycosides are known for being potent antioxidants and thus beneficial for human health. The presence of glycosides in the seeds oil was reported and a proper use of the seed oil was suggested by Warwick (2000). Seeds contain respectively 24.0%. 38.8% and 11.9% of protein, oil and fibre (Tkachuk &Mellish, 1977).

Several studies reported anthocyanins presence in other species of *Brassicaceae* family, e.g. in cauliflower and cabbage (Lo Scalzo *et al.*, 2008) or in broccoli sprouts (Moreno, *et al*., 2010). In the epidermis of wild mustard leaves the presence of light-dependent anthocyanin and flavonols was recorded (Beggs *et al.*, 1987). Within the coloured flavonoids, anthocyanins are the most important group of plant pigments. These are considered as multifunctional components of food due to their antioxidant activity and other beneficial biological properties (Moreno, *et al*., 2010; Tkachuk &Mellish, 1977).

It could be assumed that plant are attractive to bees and can be hence suggested as a suitable melliferous plant. In accordance with the reported analyses of various samples, share of pollen of *Sinapis arvensis* of 4.7-9.5% in a summer honey and of 4.8-12.0% in a beebread, both collected in Lithuania, were registered (Čeksterytė *et al.*, 2006). Another analyses of honey samples from Canada or Morocco confirmed the content of pollen up to 6% (Feller-Demalsy *et al*., 1987; Terrab *et al*., 2003).

1.4.2.3 Ethnobotany

The part of interest is either entire juvenile plant or, when bigger, tender leaves or shoots from leaf axils. The species is sometimes cultivated as a garden crop (Pieroni *et al*., 2002). The plants are generally harvested from January to May (Branca, 1992). They are eaten like vegetables, boiled with oil and lemon. In Sicily, the plant is traditionally considered as *cauru* food (hot food) and an excessive use can cause stomach pains and diarrhoea (Lentini & Venza, 2007).

The species can be easily confused with an almost equally looking species *Raphanus raphanistrum* L. which is also used for alimentary purposes (Branca, 1992). In Poland, both species are not distinguished by local people and are given the same name *pszonak*, *psconak* or *hodrych* (Łuczaj, 2010).

By the local inhabitants of Lucania, Southern Italy, wild mustard is commonly called *sënap* and is traditionally used boiled or fried. It is utilised together with cooked dried stockfish or anchovies to prepare a special Christmas Eve dish called *sënap e bakala* (Pieroni *et al*., 2002.) In Calabria it is known as *rapa* and is used to make an omelette with egg, flour and *pecorino* (goat cheese). In Sicily, it is called *alassani* in province of Agrigento and *qualeddu* in province of Trapani (Lentini & Venza, 2007) or simply *sinàpa* (Lentini, 2002); in province of Catania the plant is commonly called *Senape selvatica*, in dialect *sinapi* (Arcidiacono, 2002b).

The use of *Sinapis arvensis* in Cyprus, where the species is called *sinapiss to lefko*, is prevalently as a condiment for a typical dish called *moungrac chenaf el khadal lift* (Arnol-Apostolides, 1991). Furthermore, its use as a remedy for muscular-skeletal problems has been reported (Gonzalez-Tejero *et al*., 2008). In Tunisia the species is commonly used as a laxative or for seasoning a local dish *chenaf el khadad lift* (Lemordant *et al*., 1977).

In central Anatolia (Turkey) the aboveground parts of the plant are traditionally consumed roasted with eggs (Dogan *et al*., 2004). The plant is commonly called *haldar* in Turkey. It is recommended to drink one teacup of the infusion of flowering branches two times a day for 2-3 weeks to cure diabetes (Polat & Satil, 2012). A relative species *Sinapis alba* L. is also a traditionally used wild plant species. The leaves and tender shoots are prepared in a similar way. In Spain it is called *jaramago amarillo* (Tardío *et al*., 2006). In Western part of Granada province, the inflorescence of this species is used against liver diseases, in accordance with a "theory of the colours of flowers", according to which white flowers are beneficial to the heart, purple ones for the lungs, and yellow ones for the liver (Benítez *et al*., 2010).

1.4.2.4 Growth and development

A study conducted by Soltani *et al*. (2011) observed growth of the plants and seed production under different cultural condition. The experimental field was arranged in a fan-like form, thus inter-row spacing varied from 0 to 150 cm. The intra-row spacing was 5-10 cm. Two sowing periods were settled: in mid-November and mid-December. The biomass production per plant ranged from 5.5 g to 103.7 g, on average 32.8 g per plant. The number of seeds ranged from 264 to 10336, the minimum was performed at the highest densities in the second planting date. This apparent relation between number of produced seeds and plant size (weight) was described and verified by a correlation coefficient $R^2 = 0.95$. However, Lutman (2002) evidenced that seed number regressed against plant dry matter resulted $R^2 = 0.75$, with small differences between years in the individual regression lines.

1.5 Postharvest processing of leafy vegetables

Minimally processed vegetable is any fresh vegetable that has been physically altered from its original form, but remains at a fresh state (Gomez-Lopez *et al*., 2008). Ultimately, the consumption of fresh-cut or minimally-processed fruit and vegetables has reported a large increase (Abadias *et al*., 2008), mainly for the product convenience (Ragaert *et al*., 2004). Modified-atmosphere-packed vegetables also maintain their antioxidant capacity and scavenging capacity and they show no significant losses in such properties with respect to fresh vegetables (Murcia *et al*., 2009; Lavelli *et al*., 2009).

Controlled atmosphere storage and modified atmosphere packaging can greatly prolong an availability of packed fruit and vegetables by extending their postharvest marketable life and retaining the quality of the product. Packaging can reduce losses and preserve quality in fruit and vegetables, yet it adds an extra cost to a product which a customer has to pay (Thompson, 2010). The marketing period of vegetables (excluding fruiting vegetables) is usually limited to approximately 15-20 days (Watada & Qi, 1999).

The main benefits of the modified atmosphere packaging are respiration reduction and decay hindering. A successful preservation of the product is achieved once an equilibrium modified atmosphere is established (generally at elevated concentrations of carbon dioxide and reduced levels of oxygen) and a packaging film with correct gas permeability is chosen (Jacxsens *et al*., 2001; Kader *et al*., 1989).

For fresh vegetable products, atmosphere modification in the package headspace relies on the natural interplay between two processes, the respiration of the product and the transfer of gases through packaging, which leads to an atmosphere enriched in CO_2 and poor in O_2 (Torrieri *et al.*, 2010). Oxygen concentrations below 8% reduce the production of ethylene, a key component of the ripening and maturation process. Respiring products, like raw or processed vegetables and ready-made salads, generate equilibrium gas conditions inside the package that are typically very low in O_2 (2-3%) and moderately high in CO_2 (5-20%). Modified-atmosphere packaging of produce, however, should always incorporate packaging materials that will not lead to an anoxic package environment (Amanatidou *et al*., 1999; Farber *et al.*, 2003). Rather anaerobic conditions, indeed, favour the fermentation process. Fermentation by-products alcohols and aldehydes cause tissue necrosis (Thompson, 2010).

A development of anaerobic conditions and undesirable fermentation may occur also at higher temperatures. Higher temperatures, conversely to the lower ones, induce respiration increase. Temperature seems to regulate the respiration rate more than the gases concentration (Torrieri *et al*., 2010).

The quality and persistence of the product is also affected by other factors. Maturity and age of the harvested vegetable influence its persistence: mature tissues are generally more senescent, chlorophyll integrity in younger leaves seem to be greater. Sanitation is essential to minimise the external microbial contamination. Use of sanitizers is recommended, however their application is limited to the surface, sanitizers will not eliminate the microorganisms inside the tissue. Additionally, the native microorganisms should not be removed completely since they are beneficial for further control of pathogens growth. (Watada & Qi, 1999).

Minimally processed vegetables are susceptible to degradation caused by microbiological and physiological activities (Lavelli *et al*., 2009). Fresh-cut processing causes increase in rates of physiological activity and leakage of nutrients, which also favours growth of microorganisms. Discoloration (darkening or lightening) is a common problem with cutting surfaces (Ragaert *et al*., 2007; Watada & Qi, 1999).

Texture degradation is observed as a sensory characteristic when a relatively large part of the textural structure becomes degraded. Conversely, odour or visual defects can originate from some small areas of the tissue (Ragaert *et al*., 2007).

Microbial growth is reported to be the main degradation process that limits the shelf life of minimally processed vegetables (Lavelli *et al*., 2009). Microbial loads tended to be high for leafy vegetables, carrots and sprouts; moreover, the load has been reported lower on edible parts of unprocessed vegetables rather than in fresh-cut vegetables (Abadias *et al*., 2008).

High levels of O_2 can inhibit the growth of both anaerobic and aerobic microorganisms since the optimal O_2 level for growth (21% for aerobes, 0-2% for anaerobes) is surpassed (Farber *et al.*, 2003). Nevertheless, Amanatidou *et al*. (1999) reported that high concentrations of O_2 (90%) stimulate the growth of pathogens *Listeria monocytogenes* and *Salmonella typhimurium*, adding a CO_2 10% share even contributed to further growth increase. Conversely, the growth of *Enterobacter agglomerans* and *Pseudomonas fluorescens* and almost duplicate in high O_2 concentrations (90%) but was reduced for almost a half once an extra portion of CO_2 (10%) was added. Furthermore, the yeast strain of *Candida sake* reduced its growth under 20% CO_2 and almost blocked it under combined 80% O_2 and 20% CO_2 atmosphere. Nitrogen is used to displace the atmospheric oxygen. It also retards the growth of aerobic spoilage organisms. Finally, it is applied as a filler to maintain package conformity (Parry, 1993). Carbon dioxide is the only mainly utilised gas that has significant and direct antimicrobial activity, its inhibitory action has unequal effects on microorganisms: while aerobic bacteria such as the pseudomonades are inhibited by moderate to high levels of CO_2 (10-20%), lactic acid bacteria can be stimulated though (Amanatidou *et al*., 1999).

The above presented wild species *Silene vulgaris* and *Sinapis arvensis* are traditionally used as vegetables in diverse parts of Mediterranean region (Lentini & Venza, 2007; Nebel *et al.*, 2006; Sánchez-Mata *et al.*, 2012; Tardío *et al.*, 2006). Until now the number of studies which could contribute to their domestication is very limited (Arreola *et al.* 2006; Branca, 1992; Huang, *et al*., 2001; Mardsen-Jones & Turrill, 1957). The morphological analysis and investigation on cultivation, harvesting and postharvest handling is essential for successful introduction of new crops. Consequently, this thesis should contribute to the species domestication.

2 OBJECTIVES

The principal aim of the thesis is to determine the appropriate cultivation and postharvest handling practices for further domestication of two wild leafy vegetable species (*Silene vulgaris* and *Sinapis arvensis*), which are traditionally collected and consumed in Mediterranean region.

The obtained data from morphological analysis of seeds and germs and data from harvest and postharvest handling will be applied to determine the optimal cultivation and harvest process, to reveal the suitable storage manner and so to extend the marketable life of the harvested product, leading to new crop development.

3 MATERIALS AND METHODS

The study took place at the Faculty of Agricultural Sciences of the University of Catania, Italy. The investigation comprised laboratory experiments as well as field trials. Laboratory experiments have been conducted at the Laboratory of Department of Agricultural and Food Science, Faculty of Agriculture, University of Catania, while the field trials have been established at the faculty experimental field area at the suburb of Catania city.

Sicily and its fauna and flora are determined by the particular climatic conditions, typically Mediterranean. The annual rainfall usually does not exceed 1200 mm; the precipitations are not distributed regularly and the major part falls in 50-100 days in autumn and winter. The annual mean temperatures vary from 11°C in areas of higher altitudes to 19°C in coastal zones. The soils are highly heterogeneous (Di Martino, 2002).

For the research the seeds of *Silene vulgaris* and *Sinapis arvensis* were used The research approach combined the study of the germination of the seeds and the husbandry of the plantlets, their subsequent transplantation on the experimental field and cultivation, the harvest of the parts of the agricultural interest of these plants and the analysis of the harvested material.

3.1 Plant material

The seeds were obtained from the collection of Department of Agricultural and Food Science, Faculty of Agriculture, University of Catania. The seeds in the collection were stored at the temperature of 5°C and kept in dry conditions using silica gel.

3.2 Morphological analysis

To comply with the standard domestication procedures the following morphological characteristics were analysed: seed length, width and perimeter and size of developed radical, hypocotyl and cotyledons of germinated seeds. The germination of the seeds was analysed in order to determine the ratio of germinated seeds under different conditions, as described in chapter 3.2.2.

3.2.1 Seed analysis

For each species, the weight of 1000 seeds was measured and the number of seeds per 1g was counted. The weight of 250 seeds was determined using the electronic balance

Adventure® SL. The value was multiplied by 4 to give the mass of 1000 seeds (Coskuner & Karaba, 2007).

The morphological characteristics of ten randomly selected seeds were determined. Since the dimensions of the seeds were far too small to allow the manual measurement as an alternative the analysis of a digital photograph was established. The picture of the seeds on the millimetre paper was taken and subsequently the seed length, width and perimeter were measured using Leica IM500 Image Manager Software.

3.2.2 Germination test

The germination tests were done in four repetitions. Each repetition comprised 50 randomly selected seeds that were put into a Petri dish with a moistened filter paper inside the bottom part to provide a hydrating medium. No pre-treatment was applied on the seeds for the germination analysis. The seeds were selected randomly. However, those ones damaged or attacked by a fungal disease were not selected for the analysis. After equally spread on a filtration paper 100 ml of distilled water was poured. As soon as the water was consumed another portion of distilled water was added to maintain the sufficient moisture.

The germination test was done twice, the temperature characteristics differed (Yaniv *et al.*, 1995). Two tests were established to simulate different conditions under which the seeds germinate. The difference in germination ratio was observed in the tests and the results were compared. The seeds were put in the fridge without light access. The temperature was automatically controlled by an electronic sensor.

In the first test, the initial temperature of 15°C was set and from the tenth day on it was decreased to 10°C. During the second test the initial temperature of 5°C was set for first three days and was subsequently increased and maintained at 10°C until the end of the test.

The test proceeded until all germinated seeds were provided with fully developed radical, hypocotyl and cotyledons, and the remained seeds did not seem to show any germination activity. Therefore for an additional couple of days the test was held to prevent its precocious ending, whence it was considered as finished.

The Petri dishes were observed daily to control the number of germinated seeds and the temperature stability. The number of developed germs was counted at each observation. After being counted, the germs were put apart to reduce the water competition with the other still not germinated seeds. The particular attention was paid to the beginning of the germination (time of the first germs appeared) and to the very last day when the last germs appeared.

The duration of the test was measured in days and the results were compared for both test variations. The germinated seeds of each repetition were counted, and relevant germination ratio was calculated using the following formula:

$$\%(germ.) = \frac{100 \cdot n_0}{n} \text{ (Mo \textit{et al}., 2011),}$$

where n_0 is the total number of the seeds (50 seeds) and n is the number of the germinated seeds. Subsequently, the total germination rate was counted as a mean of all four repetitions.

3.2.3 Germ analysis

Several seeds were let to germinate for twelve days to provide material for the analysis of the dimensions of developed radical, hypocotyl and cotyledons. From the newly germinated seeds ten randomly selected germs were measured. The germs of *Silene vulgaris* were obtained from the seeds grown at the initial temperature of 5°C, maintained for seven days, that was subsequently increased to 25°C for next five days. In case of *Sinapis arvensis* two different temperature characteristics were established, in order to reveal the influence of temperature on the development of the vegetative organs. The obtained material was then compared and the potential impact of different temperatures was discussed. First sample provided the germs developed after eleven days at the stable temperature of 5°C; the second one provided germs grown at the initial temperature of 5°C kept for five days, thereafter the temperature was upgraded to 20°C for another days.

3.3 Field trials

3.3.1 Planting material

The seeds of both species were sown in the multi-cell seed trays composed of 104 cells each. In total, five trays were sown with each species. The trays were placed in the greenhouse to avoid unfavourable outdoor conditions (excess of rainfall and low temperatures), which commonly occur in the month of November when the sowing took place. The seed trays were filled with a substrate and 2-3 seeds per cell were sown. The trays sown were regularly irrigated to maintain the substrate moistened.

The seedlings were thinned to facilitate development of one seedling per cell. The seedlings were at the growth stage of first to third real leaf (Fig. 1 and Fig. 2), approximately twenty days after sowing. Before transplanting into the field the seedlings of *Sinapis arvensis* and those of *Silene vulgaris* were left in the greenhouse for additional five and seven weeks, respectively.

3.3.2 Experimental design

The plantlets were transplanted into an experimental field plot 20 m long and 8 m wide, longitudinally of east-west orientation. The plot was arranged in 2 equal sections, one for each species. Sections were arranged in seedbeds on the basis of a settled pattern (Fig. 3). Every flowerbed was 3,3m long and 1m wide (Fig. 4 and 5).

Inter-row distance was 100 cm. Three different spacings between plants were set, A = 15 cm, B = 30 cm and C = 60 cm. Each spacing was applied in three and four repetitions on *Silene vulgaris* and *Sinapis arvensis*, respectively. In *Silene vulgaris* each repetition was formed by two seedbeds planted with seedlings along the micro irrigation pipe in a zigzag pattern (Fig.7). Additionally, for both species two marginal seedbeds of the plants destined to only-seed-production were established. The plantlets were planted at the equal density between the plantlets of 30cm.

On the experimental plots the drip irrigation system was constructed. The irrigating frequency was not regular since the climate conditions were not stable, therefore only in times of necessity the irrigation system was turned on. The field was fertilized with mineral fertilizer N-P-K 11-22-16 that contains 11% of ammonia, 22% of phosphorus pentoxide and 16% of potassium dioxide.

3.3.3 Transplanting

The seedlings of *Sinapis arvensis* were transplanted in a growth stage of fourth or fifth real leaf (Fig. 7); the radical apparatus was well developed (growing forth of the seed trays). The original scheme needed to be re-arranged due to the damage caused by pest, hence planting was done in four repetitions for every density, one flowerbed per repetition. In comparison with those of *Sinapis arvensis*, the seedlings of *Silene vulgaris* have shown slower growth. Therefore the transplanting took place seven weeks after sowing. The planting was done in three repetitions, two flowerbeds per repetition. In order to protect the plants in the field against further birds’ attack a textile net was put onto the ground (Fig. 6).

3.3.4 Harvesting

The cultivated plants of *Silene vulgaris* and *Sinapis arvensis* were double and triple harvested, respectively. The first harvest started approximately ten weeks after planting. Different spacing variants and their repetitions were harvested separately. The whole aerial plant part was harvested. The stems were cut approximately 5 cm above the ground level to allow the plants to re-sprout. Subsequently, the final product (i.e. young shoots and small tender leaves) of both species was separated from the harvested material and further analysed.

Prior to harvesting, number of plants per plots for spacing variants and their repetitions was counted and directly registered to field note book. For each spacing and repetition 10 plants were harvested. The yield was then proportionally calculated accordingly to the total number of plants in a proper spacing and repetition. Following values were calculated: yield per plant, yield per square meter and the ratio of the net harvest to the total harvested material.

After harvest, the total fresh biomass was weighed. The harvested material contained also non-utilizable parts, such as stem pieces or large matured leaves. Therefore a subsequent cleaning was applied to obtain only the parts of culinary interest that are traditionally used (Branca, 1992; Cornara *et al*., 2009; Nebel *et al.*, 2006; Pieroni *et al*., 2002; Rivera *et al*. 2007; Sánchez-Mata *et al*., 2012; Tardío *et al*., 2006). The cleaned material was weighed again and net harvest was determined. The clean final product was kept separated in plastic bags labelled with sample coding.

The harvest was set only when the plants had re-sprouted successfully and had grown enough to provide sufficient amount of biomass. The yields of all harvests were compared among the distinct densities; either decreasing or increasing tendency in biomass production was observed.

Approximately fifteen weeks after, mainly for the weather change, the vegetative growth ceased. After the last harvest was established the culture was left to flower and to produce the fruit.

3.4 Post-harvest analysis

3.4.1 Dry matter content

To determine a nutritional value of the plants the moisture content in the final product was assessed. For the analysis of the proportion of the dry matter a previously harvested and cleaned plant material was used. Only the final product was analysed. Other plant parts (stems, large leaves, flowers) were discarded. For each spacing and repetition a sample of approximately 100 g was prepared and put in a small aluminium dish. The dishes were placed in an electrical dryer and left to dry for four days at 70°C. Subsequently, the dried matter, considered absolutely water-free, was weighed again. Dry to fresh mass ratio and the water content were counted (Garnier *et al.*, 2001; Shipley & Vu, 2002) and the values for different spacing variants were compared.

3.4.2 Product conservation under modified atmospheres

The material from the first harvest was used for the analysis. The harvested material was analysed at The Institute for Mediterranean Agriculture and Forest Systems, National Research Council in Catania.

Approximately 4.5 kg of the fresh and cleaned harvested material of *Silene vulgaris* material was used. This was split in 18 plastic bags; each bag contained approximately 250 g of the fresh matter. For the conservation of the product two atmospheres were applied: ambient atmosphere and the mixture of nitrogen/carbon dioxide (70/30%). For each atmosphere 9 bags were used, 3 bags per repetition. The material was stored at the stable temperature of 4°C.

As for *Sinapis arvensis*, approximately 10.5 kg of the fresh and clean harvested material was used for the analysis. The material was split in 27 plastic bags; each bag was filled with approximately 400 g of the material. The following atmospheres were applied for the material conservation: ambient atmosphere, pure carbon dioxide atmosphere, and the mixture of nitrogen/carbon dioxide (70/30%). For each atmosphere 9 bags were used, 3 bags per repetition. The temperature was maintained constantly at 4°C.

The analysis comprised observing of the freshness of the material in plastic bags filled with different atmospheres. The analysis was done in three repetitions for each atmosphere. The initial freshness of the material was analyzed 2 hours after the establishment of the analysis, and subsequently on the day 3 and day 7 from the initiation of the test (Cliffe-Byrnes *et al.*, 2003; Olarte *et al.*, 2009). The bags were analyzed one by one; the concentrations of oxygen, carbon dioxide and the remaining atmospheric gases (especially nitrogen) were determined using PBI Dansensor CheckPoint portable analyser. The weight of the material was determined as well. The stored product was subjected to sensory evaluation to complete the vitality analysis. The colour and odour of the material was assessed by a specially trained committee, using the evaluation scale described in Table 3.

In order to maintain the relevance of the data, the control samples were analyzed. For the blind test empty plastic bags containing a piece of a tissue paper were used. The bags were filled with the same gases and stored at the same conditions. The measurement of the gas concentration was done at once with the analysis of the investigated samples.

Table 3. The scale for sensory evaluation of the conserved material.

Sensoric properties	1	2	3	4	5
Appearance	Fresh. Not withered	Good appearance. Slightly withered.	Acceptable appearance. Material lightly crushed. No tracks of decay	Not good appearance. Material squashed; “mucilaginous” aspect. Cut surface wet and/or slightly brownish	Bad appearance. Severe damages to the material. Partly decayed. Cut surface brown
Odour	Fresh. No bad odour	Weak odour. Not disturbing	Acceptable. Strengthened odour	Strong odour. Persistent	Strong odour. Disturbing

The scale used for the evaluation of the sensoric properties: 1 = the best, 5 = the worst

3.5 Data analysis

A distribution normality of the gathered data was tested by the Shapiro-Wilk analysis. It was verified for both recorded values of dimensions of the germs of *Sinapis arvensis*. The equality of both variations allowed the performance of two-sample *t*-test, which was done for each character separately, i.e. for radical, hypocotyl and cotyledons dimensions. Furthermore, a multivariate test allowed the comparison of the results considering all characters at once.

Yield per plant, yield per square meter and final product ratio within single harvests were compared among all the spacings. Normality of the data distribution was not verified hence the Kruskal-Wallis one-way analysis of variance was done. Calculation of confidence interval of the registered data revealed the significant difference among all the spacings.

Regarding the post-harvest moisture content determination, the normality of the distribution of recorded data was not verified. Therefore the Kruskal-Wallis one-way analysis of variance was performed.

4 RESULTS

4.1 Morphological analysis

The characteristics relevant for further domestication were determined. For sowing objectives ten randomly selected seeds of *Silene vulgaris* and *Sinapis arvensis* were measured, thousand seeds weight and number of seeds per 1 g were counted. Furthermore, the germs dimensions were measured and germination tests were conducted, the germination ratio under different conditions was determined.

4.1.1 Seed analysis

The recorded thousand seed weight and number of seeds per 1g was 1.15 g / 693 seeds and 1.01 g / 956 seeds in *Silene vulgaris* and *Sinapis arvensis*, respectively.

4.1.2 Germs analysis

From the analysis of the germs of *Sinapis arvensis* two sets of dimensions were obtained from two different conditions which were settled to determine the impact of temperature on germs development. It is apparent from the results that under higher temperatures during germination the hypocotyl and cotyledons grow bigger. The second sample was also showing more lushness and a better development. However, a multivariate analysis of the gathered data did not verify a significant difference in complex development between two samples.

Table 4. Mean values of the dimensions (mm) of seeds and the developed organs of germs

Morphological measures	*Silene vulgaris*	*Sinapis arvensis*	
Seeds			
Perimeter	4.8		4.4
Length	1.6		1.4
Width	1.4		1.3
Germs		*(5°C)*	*(5°C → 20°C)*
Radical	38.1	13.1a	16.0a
Hypocotyl	7.1	25.1a	31.4b
Cotyledons	6.1	2.3a	2.8b

Mean values were calculated from 10 samples measured. Values in line signed with the same letter do not differ significantly.

4.1.3 Germination test

Generally, the seeds of *Silene vulgaris* seemed to require longer imbibitions period. The second germination test took 10 days more in *Silene vulgaris* than in *Sinapis arvensis*. In both germination tests the ratio of germinated seeds was significantly higher in *Sinapis arvensis.* Additionally, germination of the seeds of *Sinapis arvensis* did not seem to be as much subjected to temperature as germination of the seeds of *Silene vulgaris*.

4.1.3.1 First germination test

The first germination test was performed under temperature characteristic of 15°C and 10°C. The mean ratio of germinated seeds of *Silene vulgaris* reached 66.0%. For the seeds of *Sinapis arvensis* a mean germination ratio of 93.5% was recorded.

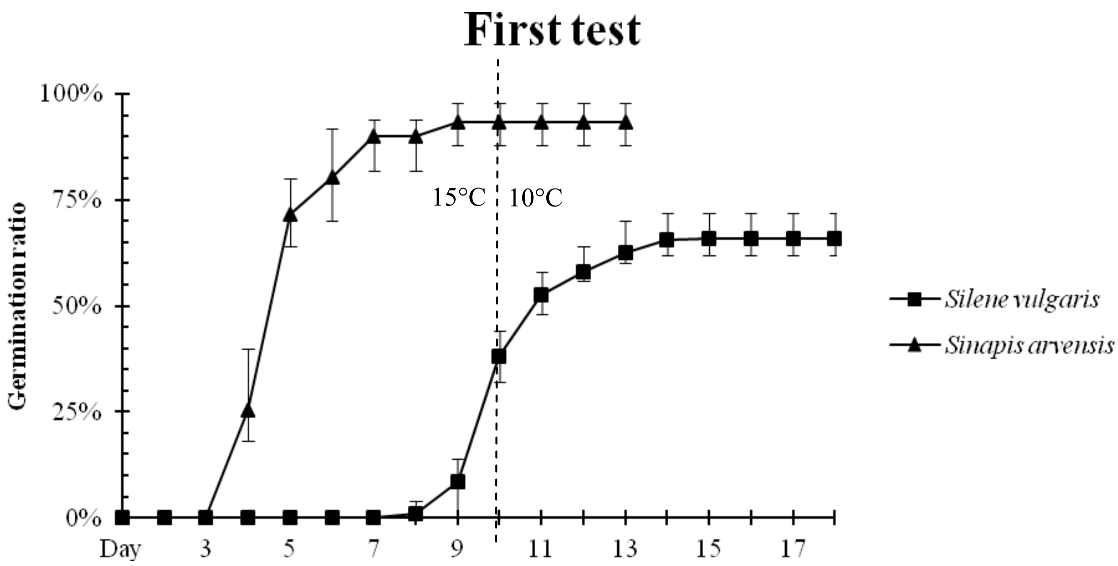

Fig. 1. Germination characteristic of *Silene vulgaris* and *Sinapis arvensis*. Test I.

4.1.3.2 Second germination test

The second germination test was performed under the temperatures of 5°C and 10°C. The test revealed a significantly lower ability to germinate in seeds of *Silene vulgaris*. The mean ratio did not exceed 20.0%. The mean ratio of the germinated seeds of *Sinapis arvensis* in the second test reached 72.5%.

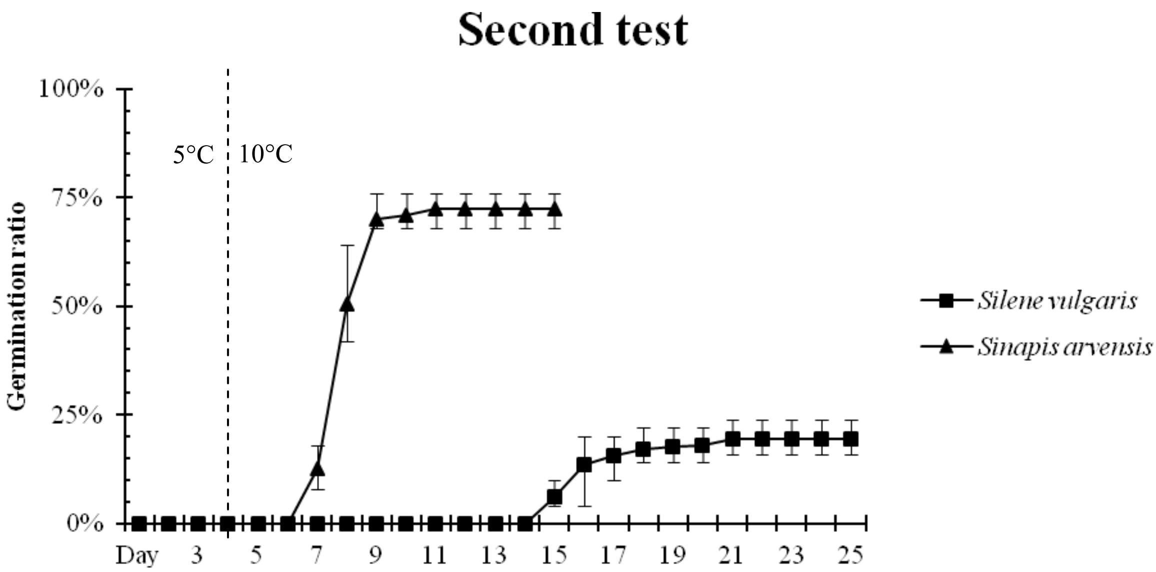

Fig. 2. Germination characteristic of *Silene vulgaris* and *Sinapis arvensis*. Test II.

4.2 Harvest

The parts of interest of the plants, i.e. young tender leaves, shoots and apical parts were harvested. Both species provide an attitude to re-sprout once the upper parts of the plant is cut. The yield per plant, yield per square meter and the ratio of final product to total harvested material were counted.

4.2.1 *Sinapis arvensis* L.

The first harvest showed the highest yield of the final product in comparison with the consequential ones. Although the plant re-sprouted after the first harvest again, the second and third harvests provided lower amount of material. The second harvest took place 18 days after the first one. The plants have shown faster growth in the sense of development of generative organs; the buds were close to anthesis. The third harvest was set two weeks after the second one. Very poor material was provided, some plants were already flowering, stems were highly lignified, the growth of leaves was almost ceased; all the aerial parts of the plants were harvested.

During the field trials several plants died. Before the first harvest took place the mean number of plants decreased: for spacing A, B and C from 44 to 40, from 22 to 17 and from 11 to 10, respectively. Subsequently, before the third harvest took place, the numbers further decreased from 40 to 35 and from 10 to 9 plants per spacing A and C, respectively; the number of plants in spacing B did not change further.

Table 5. Yield of young, tender leaves and shoots per plant and per unit area (m^2) and share of utilizable product on total biomass

Harvest	Spacing (cm)	Number of plants		Yield per plant (g)		Yield per m^2 (g)		Share of young leaves and shoots on total aerial biomass (%)	
		SV	SA	SV	SA	SV	SA	SV	SA
1st harvest									
	15	87	40	7.03 (± 1.67)a	61.25 (± 32.89)a	93.05 (± 22.43)a	724.62 (± 373.48)a	64.74 (± 2.97)a	35.53 (± 7.33)a
	30	44	17	9.29 (± 0.94)b	60.00 (± 27.74)a	61.54 (± 6.74)b	281.06 (± 104.42)b	67.08 (± 3.81)a	44.99 (± 8.48)b
	60	22	10	17.56 (± 1.58)c	57.56 (± 8.50)a	58.53 (± 5.26)b	175.64 (± 33.53)c	71.84 (± 1.29)b	40.85 (± 3.36)b
test result				$p < 0.0001$	$p = 0.8065$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
2nd harvest									
	15	87	40	8.13 (± 0.37)a	9.88 (± 3.94)a	107.17 (± 4.94)a	117.82 (± 45.90)a	76.91 (± 1.72)a	29.91 (± 10.25)a
	30	44	17	7.18 (± 0.49)b	9.43 (± 4.19)a	108.73 (± 7.47)b	50.79 (± 30.13)b	65.39 (± 0.91)b	28.54 (± 4.08)a
	60	22	10	7.85 (± 1.25)b	9.45 (± 2.47)a	118.99 (± 18.89)b	29.09 (± 9.23)c	70.67 (± 4.57)c	38.73 (± 8.80)b
test result				$p < 0.0001$	$p = 0.9936$	$p = 0.0117$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
3rd harvest									
	15	-	35	-	15.84 (± 3.74)a	-	171.68 (± 52.39)a	-	14.20 (± 2.44)a
	30	-	17	-	12.68 (± 3.44)b	-	61.19 (± 8.79)b	-	12.67 (± 1.76)b
	60	-	9	-	13.02 (± 4.76)a	-	36.93 (± 15.70)c	-	14.07 (± 4.97)a
test result					$p = 0.0007$		$p < 0.0001$		$p = 0.0321$

SV = *Silene vulgaris*, SA = *Sinapis arvensis*. Values in a column (within a single harvest) signed with the same letter do not differ significantly. Results of the statistical analysis (*test result*) are described as *p-value* for each test performed. 1st harvest set 71 and 69 days after transplanting of *Sinapis arvensis* and *Silene vulgaris*, respectively. 2nd harvest set 18 and 31 days after the 1st harvest of *Sinapis arvensis* and *Silene vulgaris*, respectively. 3rd harvest of *Sinapis arvensis* set 14 days after the 2nd harvest.

The yield per square meter was the highest in the first harvest for all three spacings. Absolutely highest yield per square meter of 7246.23 kg per ha was reached in the first harvest in the A spacing, while the lowest yield of 290.87 kg per ha was reached in the second harvest in the C spacing. The highest yield per plant, 61.25 g per plant, was recorded in A spacing in the first harvest, while the lowest yield of 9.43 g per plant was reached in the B spacing in the second harvest. In regards to yield per plant, an increasing tendency was recorded between the second and third harvest, nevertheless, the ratio of the final product was the lowest in the third harvests. The ratio of the final product has shown similar decreasing tendency within subsequent harvests. The highest percentage of the final product, nearly 45% of all harvested material, was recorded in the B spacing in the first harvest; the lowest percentage of approximately 12.5% was registered in the B spacing in the last harvest.

The mean values of yield per plant, yield per square meter and final product ratio are presented in Table 5. Both yield per plant and yield per square meter express the weight of the clean final product, indeed the utilizable part of total harvested material.

4.2.2 *Silene vulgaris* (Moench) Garcke

The plants were harvested two times. The second harvest took place 35 days after the first one. The plants re-sprouted after the first harvest, the buds were close to anthesis, the cluster seemed denser and the plants even more ramified. The mean number of plants changed from 88 to 87 plants in the A spacing; no changes were observed in the B and C spacing. This is very probably thanks to the protective net that had been put onto the field.

Although the yield per plant has shown an opposite tendency the yield per square meter was higher in the second harvest in all three spacings. The ratio of the final product to the total harvested material subsequently increased only in the A spacing after the first harvest.

The highest yield per square meter, 1189.93 kg per ha, was recorded in the C spacing in the second harvest, the lowest yield of 585.33 kg per ha was recorded in the same spacing in the first harvest. The highest yield per plant of 17.56 g was reached in the C spacing in the first harvest, the lowest one, 7.03 g per plant, was recorded also in the first harvest, but in the A spacing. The highest ratio, almost 77%, was reached in the A spacing in the first harvest, while the lowest percentage of the final product on the total harvested material was recorded in the A spacing in the third harvest.

The mean values of yield per plant, yield per square meter and final product ratio are presented in Table 5. Both yield per plant and yield per square meter express the weight of the clean final product, indeed the utilizable part of total harvested material.

4.3 Dry mass weight

The portion of the dry mass was evaluated. The harvested and cleaned material was dried and the weights difference was recorded; the percentage content of water was counted. The mean values have shown that regarding the nutritional value of the plants, *Silene vulgaris* is more valuable than *Sinapis arvensis* in terms of the water content. There was no significant difference in the water content among the spacings in any of the species.

Table 6. Mean values of dry matter content (g per 100 g of fresh material) of harvested and cleaned young leaves and tender shoots.

Spacing	*Silene vulgaris*	*Sinapis arvensis*
15 cm	11.22 g	13.95 g
30 cm	10.97 g	13.81 g
60 cm	11.30 g	13.81 g

Mean values per spacing were calculated from three repetitions in *Silene vulgaris* and four repetitions in *Sinapis arvensis*.

4.4 Product conservation under modified atmospheres

The cleaned harvested parts of plants that are used in traditional cuisine – young and tender leaves and shoots – were put in plastic bags and filled with modified atmospheres. Different gases showed different impact on vitality of the material. The quality of the product, in regards to its appearance and odour, was assessed.

Generally, the tomentose leaves of *Silene vulgaris* seemed to be more persistent and did not wither quickly in respect to *Sinapis arvensis*. Also bad odours were less pronounced. The product weight did not decrease a lot, after a week the weight difference ranged from 2 to 5 g. On the contrary, softer leaves of *Sinapis arvensis* shrivelled more quickly; as early as by the third day the leaves demonstrated the tracks of decomposition and especially on more crushed ones even some mucilaginous compounds were present. Typical bad odours developed also rather precociously and were pretty pronounced and persistent. The weight of the stored product was reduced by 5 to 7 g after a week.

4.4.1 Ambient atmosphere

The atmosphere used to fill the plastic bag was not modified at all; the product was stored in an ambient air. This way of storage resulted to be the most efficient for storage of the final product both species.

The leaves and young shoots of *Silene vulgaris* showed the greatest persistence. Even after a week the leaves proved very few crushing features. No signs of decay have been evidenced. After a week, a weak odour was present – however, it was not disturbing in any way.

The stored leaves of *Sinapis arvensis* were slightly crushed after 3 days, after a week no further damage was observed. Bigger and relatively matured leaves were covered with a thin layer of mucilage. A typical bad odour was present. Nevertheless, in the ambient atmosphere it developed the least pronounced and disturbing in respect to other two modified atmospheres.

4.4.2 Conservation under 30% carbon dioxide atmosphere

The absence of oxygen revealed worse results especially regarding the odours. Bad smell was present in both species and developed persistent and disturbing very soon in *Silene vulgaris*. In other species the bad smell occurred as well. However, it was not as much disturbing and did not develop sooner than after a week.

The aspect of the material resulted definitely worse in *Sinapis arvensis*. The product quality deteriorated much after a week. The leaves looked squashed and especially on the cut surface first tracks of decomposition were apparent. In *Silene vulgaris*, no great damage on the leaves was evidenced. However, in comparison to the ambient atmosphere the product quality resulted less stable.

4.4.3 Conservation under 100% carbon dioxide atmosphere

The presence of carbon dioxide only did not show high efficacy in preventing severe damages to the leaves of *Sinapis arvensis*. The cut surface did not show any signs of decay nor browning. However, the product appearance was not acceptable in terms of marketable quality, the leaves were heavily squashed and their structure and texture was pretty damaged. Additionally, within a week a very strong and disturbing odour developed.

The leaves and shoots of *Silene vulgaris* were not stored in this atmosphere and no investigation was conducted in this case.

5 DISCUSION

The two studied species, *Silene vulgaris* and *Sinapis arvensis* are both wildly growing and are considered weeds (Benoit *et al*, 2006; Branca, 1992; Deshpande & Hall, 2000; Meiss *et al*., 2010; Pomar & Hidalgo, 1998; Wei *et al*., 2005). The species naturally occur in various, also secondary habitats and both feature somewhat vigorous growth. None of the species has special cultural requirements. Indeed one can assume that any melioration of cultural conditions may only have positive effects on growth and development of the plants, in respects to natural environment.

The traditional use of the species as vegetables has been registered in various parts of Mediterranean basin – in Italy, Spain, Morocco, Tunisia, Turkey and Cyprus (Branca, 1992; Bonet & Vallès, 2002; Cornara *et al*., 2009; Nebel *et al*., 2006; Pieroni *et al*., 2002; Rivera *et al*. 2007; Sánchez-Mata *et al*., 2012; Tardío *et al*., 2006). The traditional use of *Silene vulgaris* was evidenced also in Poland. However, its traditional use has not been evidenced since 1960's or its traditional use has been forgotten (Łuczaj, 2010).

A rather strong tradition of the species utilisation may indicate probably successful domestication. However, very little has been published about agronomical research dealing with the cultivation or even proper domestication of the species (Branca, 1992; Arreola *et al.* 2006; Mardsen-Jones & Turrill 1957). Hence, further studies concerning mainly the agronomic experiments are suggested. Despite a little information available about cultivation, the species domestication however should not be turned down a priori. The species are easy to grow and possess dietetically interesting properties that could be even meliorated be controlled cultivation.

5.1 Laboratory analyses

The seeds' dimension match closely to those reported by Branca (1992), but in general, the analysed seeds were slightly longer and wider. Furthermore, especially the weight of thousand seeds was almost quadruple in *Silene vulgaris* in respect to that reported previously. Such difference could only be explained by the origin and age of the seeds, however there is no information available in the mentioned study. In *Sinapis arvensis* smaller differences were found in respects to formerly recorded values of the weight of thousand seeds (Branca, 1992; Soltani *et al*. 2011). The determined weight resulted approximately 0.2 g lower than that evidenced by Branca (1992) and 0.1-0,88 g lower than that evidenced by

Soltani *et al.* (2011). The results somewhat confirm that seed weight decreases under stress conditions , as observed by Roy & Stanton (1999).

The seeds of *Silene vulgaris* have shown a very poor germination rate especially under low temperatures. Branca (1992) reported that germination has already been superior to 65 % at 10°C. On the other hand, a reported germination ratio of 27.5 % can be considered as verification of the hypothesis that temperatures below 10°C reduce seed germination potential. The results also confirmed that the lower the temperature, the longer the time needed for initiation of the germination.

On the other hand, no such phenomenon was observed during testing the germination of *Sinapis arvensis* seeds. This can be explained by unknown origin and age of the seeds. It can be assumed that the seeds were at least six months old – since it was proposed by Branca (1992) that the seeds probably turn into dormancy immediately after maturation and in older seeds better germination is obtained. The seeds, of 6-7 month of age, sown directly to soil germinated properly under mean temperatures of 12.7-13.5°C (Soltani *et al.*, 2011). It was reported by Huang *et al.* (2001) that the plant is capable to complete its reproductive development and to produce mature seeds at day/night temperatures ranging from 12/2°C to 35/25°C. The optimum day temperature for germination and plant development seems to be between 10°C and 15°C (Branca, 1992; Huang *et al*., 2001).

5.2 Harvest

A more thorough investigation of this issue requires adequate quota of trials and repetitions. No previous study on biomass production has been performed. Therefore no comparative study is available. Nevertheless, some recommendations seem to be noticeable.

According to Soltani *et al*. (2011) in *Sinapis arvensis*, a total biomass production per plant decreases with increasing density. However, this was not verified, since the narrowest spacing, 15 cm between the plants, showed the best results in both yield per plant and yield per area unit and the yields were repeatedly the highest in the second and the third harvest. Also, the recorded minimum, maximum and mean values of a plant size (g per plant) are all higher than those reported by Soltani *et al*. (2011). The production of aerial plant parts seems to be influenced by number of harvests rather than by intra-row spacing. With repetitive harvests the mass of total harvested material decreased significantly. Furthermore, the share of the young and tender leaves and shoots, which are traditionally utilised as vegetable (Arcidiacono, 2002b; Branca, 1992; Lentini & Venza, 2007; Pieroni *et al*., 2002), fell even under 15% of the total harvested biomass. Since no investigation on seed production has been conducted, no correlation could have been counted for number of seeds against weight of plant. According to the published studies (Lutman, 2002; Soltani *et al*., 2011), the relation is of high statistical importance.

On the other hand, *Silene vulgaris* showed the opposite behaviour. With harvest repeating the biomass production increased. Moreover, the differences among the spacings became less sharp in both yield per plant and yield per unit area, unlike in first harvest. Also, the portion of the utilisable plant parts (young leaves and tender shoots) on the total harvested material did not decrease much and it was even higher in the 15 cm spacing. High plants density might have reduced the competition with weeds.

Generally, cultivation at narrow spacing is recommended. Although larger spacing allows greater plants development and hence higher share of young leaves and shoots, eventually, along with decreasing yield per plant, the total yield per unit area might result lower than that of narrow spacing.

This may explain especially large standard deviations within single values, and also large differences in harvests among the spacings might be explained by little data obtained. The yields in the second and third harvest might have been significantly influenced by the period in which they were settled – in mid-April and on the beginning of May. The species naturally passes into flowering during this time, and the production of leaves decreases then (Branca, 1992). For both of the species regular weeding is recommended. During the study, weeds were controlled neither mechanically nor chemically. It is known that weeds compete with crops for water and nutrients, as well as for space, air, light, etc. *Silene vulgaris* withstands competition with other plants; nevertheless it can be crowded out by taller herbage (Marsden-Jones & Turrill, 1957). The competition for water seems to be the most important factor for that the field trials were performed in Catania, Sicily (37.5°N), where the temperatures are high and annual precipitations fall prevalently during autumn and winter and do not exceed 1200 mm (Di Martino, 2002). The probable water competition could have significantly reduced the total yield. Drip irrigation, naturally, should avoid such competition. However, the study was performed on rather coarse soils with low content in clay, which increases the water retention indeed (Nemes & Rawls, 2006). Hence, one should consider the leaching of water in deeper layers, where it becomes available for weeds as well.

5.3 Postharvest analysis

The samples of *Sinapis arvensis* showed a little higher dry matter content than those of *Silene vulgaris*. The dry matter share determination revealed no difference between the spacings in any of the species. The determined dry matter content of *Silene vulgaris* leaves and shoots is slightly lower than that reported by Alarcón *et al.* (2006), it can be thanks to different age of the analysed plant parts; although both samples were young leaves and shoots, it is likely to doubt whether the leaves were of similar age. It is also important to highlight that after being harvested on the field, the samples were transported to the laboratory in not utterly airtight plastic bags.

There were certainly present pathogens on the harvested leaves and young shoots as these were not washed or disinfected after being harvested. However, a development of spoilage pathogens (e.g. *Listeria monocytogenes* or *Salmonella typhimurium*) on the material was not assayed by laboratory analysis. Carbon dioxide possesses certain antimicrobial activity (Amanatidou *et al*., 1999). Yet, according to Torrieri *et al*. (2010), low temperature seems to have even greater impact on pathogens' growth regulation. Storage under non-modified atmosphere showed the best results, in both species, and therefore somewhat verifies that temperature regulation is a key factor for successful long-time storage of the material.

Microbial loads seem to be present more frequently on fresh-cut vegetables rather than on those unprocessed (Abadias *et al*., 2008). Even so, further microbiological analyses are strongly recommended as to confirm that the consumption of the harvested leaves and shoots is secure and presents no danger for the customers. Special attention should be paid to the presence of bad odours that develop rather quickly. Particular odour development might imply decay caused by a particular pathogen.

5.4 Species use perspective

From a dietetic point of view, the green parts of *Silene vulgaris* appear to be health beneficial. The presence of antioxidants, such as ascorbic acid, anthocyanins or flavonoids may suggest the species' classification to as functional food (Marsden-Jones & Turrill, 1957; Sánchez-Mata *et al.*, 2012; Van de Staaij, 1995). Nevertheless, the further analysis is highly recommended, especially the presence of relevant amount of anthocyanins should be verified. A noticeable content of essential linoleic acid (18:2ω-6) in the seed oil proposes also its use for alimentary purposes (Kucukboyaci *et al*., 2010). The leaves and shoots contain rather low portion of fibre (*Alarcón* et al.*, 2006)*. Even so, the edible parts of the plant do not contain any compound that would make their digestion more difficult (as e.g. oligosaccharides in legumes). The leaves are pretty fleshy and smooth. They can be easily consumed raw without any processing necessarily applied (Alacrón *et al*., 2006; Rigat *et al*., 2009; Sánchez-Mata *et al.*, 2012; Tardío *et al*., 2006). The result showed that medium-length dry periods (Arreola *et al*., 2006) or longer storage do not deteriorate the product quality significantly.

Some studies (Banásová *et al*., 2006; Martínez-Iñigo *et al*., 2009), conducted in different regions, have reported the species' tolerance to high concentrations of heavy metals. Plants are adapted to such environments by mechanisms of exclusion of the toxic heavy metals from its main metabolic pathways (Chaney *et al*., 1997). The toxic elements are prevalently accumulated in vacuoles. The accumulation was observed especially in the roots (Marques *et al.*, 2009). However, some studies reported the accumulation to the leaves as well (Xiao *et al.*, 2008; Nadgórska-Socha *et al.*, 2011). A proposal of the species use for phytoremediation of degraded or contaminated soils was evidenced (Chaney *et al*., 1997; Marques *et al.*, 2009). Nevertheless, as it has been approved that the species is capable to accumulate the noxious heavy metals, a direct consumption of the plants may reveal a potential danger. A probable occurrence of intoxicated plants in sites where these are of dietetic interest should be considered and further investigation is recommended. For cultivation purposes, also, the properties of the soil *ad hoc* should be determined.

Sinapis arvensis is rather vigorous plant with leaves producing trichomes, even young and tender ones. Before consumption the leaves are always boiled or processed in some way (Arnol-Apostolides, 1991; Branca, 1992; Dogan *et al*., 2004; Pieroni *et al*., 2002.). It was reported that overuse of the plant might cause digestion disorders (Lemordant *et al*., 1977; Lentini & Venza, 2007). Consequently, the species should be consumed carefully to avoid possible inconveniences.

Still, the plant is interesting also from a chemotaxonomical point of view. The content of anthocyanin was registered in other species from *Brassicaceae* family (Lo Scalzo *et al.*, 2008; Moreno, *et al*., 2010). Rather remotely published paper confirmed the presence of these pigments in the leaves (Beggs *et al*., 1987). A content of anthocyanins – which are potent antioxidants – should certainly be assayed by laboratory analysis. Additionally, a traditional remedial use of the plant confirms the species' medicinal properties (Benítez *et al*., 2010; Polat & Satil, 2012), which might be revealed and closely identified by further analyses.

Wildly growing plants, which *Silene vulgaris* and *Sinapis arvensis* belong to, are mostly associated with famine foods, particularly by elderly people who lived through times of poverty (Rigat et al. 2009). However, the neglected properties both discussed species make them interesting from a dietetic point of view. A pretty new phenomenon of “functional foods” labelling of some products contributes to higher consumption (Annunziata & Vecchio, 2011) and might also promote consumption of local plants species that are traditionally used as vegetables.

CONCLUSION

This study investigated main seed characteristics, different husbandry approaches and their impact on yield change, and storage of the harvested material. Results from the morphological analysis of the seeds and of their germination are convenient and utilisable mainly for seeding purposes and for determination of favourable temperature characteristics for seeds germination. The obtained data can be considered to verify that germination of seeds of *Silene vulgaris* is highly dependent on temperature. It is thus proposed to have the seedlings pre-cultivated in a greenhouse or a similar site where the favourable conditions can be easily established. Furthermore, it is proposed to study more the germination of seeds of *Sinapis arvensis* mainly in respects to their age, yet the influence of the seed age on their germination was not empirically approved. However results related to such phenomenon have been discussed.

For field trials more repetitions are recommended for further studies. The factors influencing yield, in both species, need to be observed and analysed, especially spacing and number of harvests. In terms of the sowing and harvest time, more work should be done as well. Harvest does not request any special techniques or particular equipment and can be set more than once since both species showed their ability to re-sprout after being harvested. This should be appreciated mainly from the economical point of view.

The part of the plants, which is traditionally harvested and used in local cuisine, are young and tender leaves and shoots. The evaluation of the final product storage revealed its relatively long shelf life. This is principally reached without necessity of application of modified atmosphere packaging. This indicates a simple postharvest handling and material storage. Even so, for commercial purposes a microbiological laboratory assay of the product is highly recommended.

Biologically active compounds have been identified in the aerial plant parts of the species studied. Consequently, a relevant content of dietetically interesting compounds along with a contemporarily increasing fascination by traditional dishes, which often require local wild edible species for their preparation, both contribute to a potential success of introduction of the species as new vegetable crops.

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APPENDIX A

Fig. A1. Seedlings of *Silene vulgaris* after thinning

Fig. A2. Seedlings of *Sinapis arvensis* before thinning

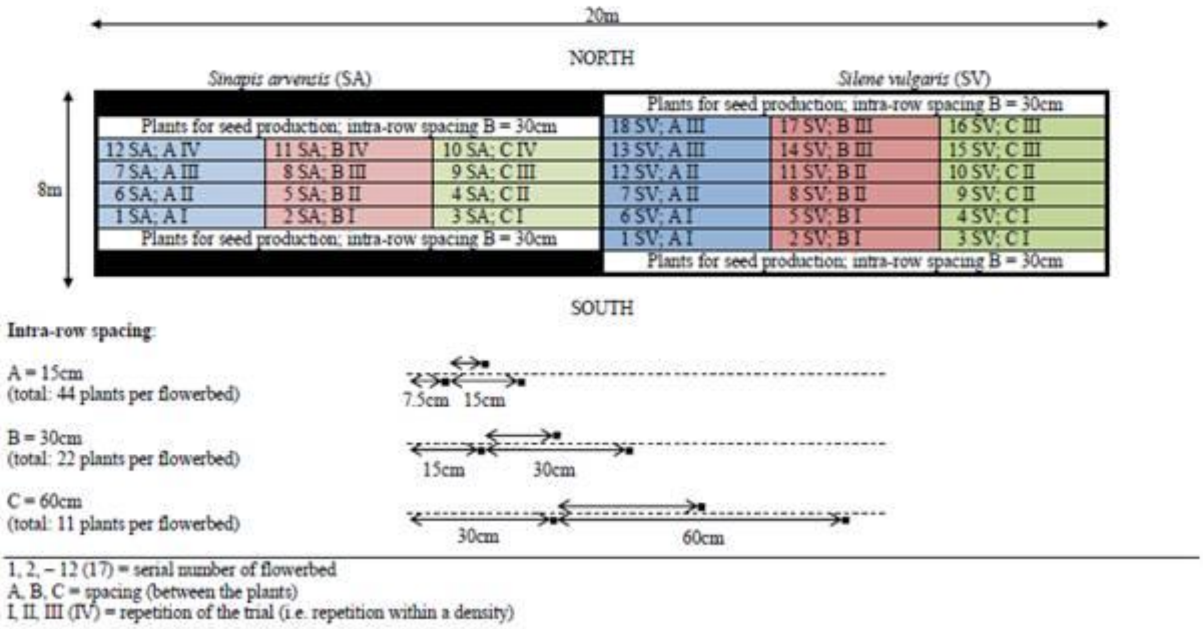

Fig. A3. Field arrangement

Fig. A4. Experimental field after transplanting the seedlings of *Sinapis arvensis*

Fig. A5. Two rows of one repetition of seedlings of *Silene vulgaris*. The 15 cm spacing

Fig. A6. Harvest of *Sinapis arvensis*

Fig. A7. Harvested young and tender leaves and shoots of *Sinapis arvensis*

Fig. A8. Shoots and young and tender leaves of *Silene vulgaris* before harvest

Fig. A9. Final product of *Silene vulgaris*