ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2018) **16**(5); 403–418
doi:10.1017/S1479262118000230

Onobrychis viciifolia; a comprehensive literature review of its history, etymology, taxonomy, genetics, agronomy and botany

M. Mora-Ortiz[1,2] and L. M. J. Smith[1]*

[1]*National Institute of Agricultural Botany, Huntingdon Road, Cambridge, CB3 0LE, UK and* [2]*Department of Twin Research, King's College London, St Thomas' Hospital Campus, 3rd Floor South Wing Block D, Westminster Bridge Road, London SE1 7EH, UK*

Received 26 July 2018; Accepted 26 July 2018 – First published online 1 October 2018

Abstract

Onobrychis viciifolia (sainfoin) is a forage legume crop with many positive agronomic, environmental, nutritional and nutraceutical attributes. Farmers also benefit from its drought tolerance in areas of low rainfall and light free draining soil, mainly due to its deep taproot. It is resistant to most common pest and diseases and is a valuable resource for pollinators, specifically cultivated for honey production in some regions. It has many benefits for animals, being highly palatable and without danger of bloat, which can be life-threatening to livestock. Its decline in Northern Europe started during the Green Revolution and was impacted by changes towards more intensive farming. Unlike other forage legume crops such as red clover and lucerne, sainfoin does not respond well to inputs and is difficult to establish and maintain. Sainfoin could be classified as an 'orphan crop' with very little genetic improvement or agronomic studies in the past 60 years. In the past 5–10 years, however, there has been a resurgence in interest and this has given rise to a number of studies and initiation of systematic improvement of the crop, which is indispensable to its reintroduction into the farmed environment. Interest has been driven in part by considerable evidence to suggest that condensed tannins present in the legume foliage, together with other polyphenol compounds, have positive effects on animal nutrition together with anthelmintic properties. These compounds are also thought to play a role in environmental benefits. There remain many challenges to address in order to optimize the potential for cultivation of sainfoin and its use as a beneficial forage crop. This review makes particular reference to a recently completed project; 'Legume Plus', funded by the European Union and embracing a multi-disciplinary approach to both understand and improve the crop for farmers. The present review covers results from both this project and other studies during the past 5 years, also drawing on historic studies of etymology, taxonomy, genetics, agronomy and botany, aiming to be a useful resource for research and for practical plant breeders and agronomists.

Keywords: etymology, forage legume crop, *Onobrychis viciifolia*, sainfoin, tannins and polyphenol composition, taxonomy

Introduction

Onobrychis viciifolia, commonly called sainfoin in the UK, was widely cultivated in Europe, Asia and North America (Miller and Hoveland, 1995; Frame *et al.*, 1998) in the 19th and 20th centuries. Its decline started in the middle

*Corresponding author. E-mail: lydia.smith@niab.com

of the last century and as the Green Revolution gathered momentum in the UK, it was gradually replaced by alfalfa (*Medicago sativa*) and clover (*Trifolium* spp.) whose higher yields and easier establishment made them more desirable to farmers. This decline in sainfoin cultivation was also seen in other parts of Europe, notably France, Italy and Spain due to the adoption of more intensive farming methods and crop choice (Hayot Carbonero, 2011b; Demdoum, 2012b). This decline in Europe was, in part, due to the introduction of relatively low-cost nitrogen fertilizers in the early 1970s, which helped to enable the expansion of grassland for livestock. Furthermore, supportive payments through the Common Agricultural Policy added to an increase in more intensive production methods during the 1980s (Hayot Carbonero *et al*., 2011). The disappearance of livestock farms in hilly areas (Borreani *et al*., 2003) and the rise in animal feed imports from non-European Union (EU) countries further reduced the use and cultivation of sainfoin. This shift in cropping patterns has led to increasing dependence by Europe on imports of animal feed. It has been calculated that the EC received 2.3 Mt of nitrogen in the form of grain legumes from South America in 2004 (Galloway *et al*., 2008) and there are further collateral negative consequences of this change in protein sourcing. In parts of South American, for example, large areas of forests have been cleared in order to increase soya production. Europe is now concerned about its dependence on imported protein sources and negative impacts on national food security (Aigner, 2009; European-Commission, 2010; Weightman *et al*., 2011; Lüscher *et al*., 2014). The trend within the EU is now to reduce this resource-dependence through re-establishing the use of forage legumes and improving their agronomy. Leguminous forage crops such as Sainfoin, trefoil (*Lotus corniculatus)* and red clover (*Trifolium pratense*) not only enable more efficient, locally grown supply of nitrogen, they also reduce transit of inorganic nitrogen from the soil. It has been suggested that global changes in climate and weather patterns will increase the potential for economic returns from the cultivation of both forage legumes and grasses, which can optimize the capture of heat and light, allied to nutrient sequestration (Haynes, 1980; Clarke *et al*., 2000).

Recent studies show that sainfoin has anthelmintic properties, methane-control potential from ruminants and protein-protection capability from early degradation in the rumen when used as a forage crop in the diet of ruminant animals. These properties are attributed mainly to sainfoin's foliar tannin composition (Lorenz, 2011; Novobilský *et al*., 2011; Pellikaan *et al*., 2011; Theodoridou *et al*., 2011). Moreover, it is cited that sainfoin is a good nectar and pollen source for honey bees and many other pollinator species, including bumblebees, hoverflies and solitary bees. This characteristic, together with its drought tolerance, could play a vital role in the stability and sustainability of agro-ecosystems if sainfoin is included as a crop or as part of a pollinator mixture within the farmed environment (Kells, 2001; Hayot Carbonero *et al*., 2011) (Figure 1).

Unfortunately, the reintroduction of sainfoin remains a challenge and it is necessary to significantly improve our understanding of the crop and to improve both its agronomy and crop genetic resources. Of particular interest are improvements in weed control, establishment, seed dormancy and genetic characterization to facilitate targeted pre-breeding and breeding programmes in the future.

History of the crop, introduction to Europe from Asia and posterior expansion to America

The centre of origin of sainfoin is South Central Asia, where it was common as a component of mixed swards in Asia Minor, particularly on the Anatolian Plateau of Turkey and the districts of the Caucasus and the Caspian fringes, it was originally cultivated by Arabian cultures through whom it was introduced to Greece and Italy, although it was neither formally cultivated by the ancient Greeks as a crop nor by their descendants (de Candolle, 1883; Stebler *et al*., 1894), although a related wild species *Onobrychis caputgalli* was documented at this time (Stebler *et al*., 1894). Most authors agree that it was introduced into southern continental Europe in the late 14th century not reaching northern Europe and the UK until at least a century later. It was introduced into North America later (Burton and Curley, 1968; Frame *et al*., 1998) probably early in the 16th century, the exact date is difficult to establish due to inconsistencies and contradictions in the literature.

Different documents testify to the historic cultivation of sainfoin in the UK; mostly in England. Cultivation in England has been documented in the south and south-east, south of Wales; north to the Humber and west to the river Severn since 18th century; in the Vale of Glamorgan in the 19th and early 20th century (Davies, 1815; Rees, 1928) and in East Anglia (Bland, 1971). This distribution is linked to its preference for light free-draining, neutral to alkaline soil. In '*The English Improver Improved*' (Blith, 1652) and '*Horse Hoeing Husbandry*' there is evidence that many thousands of acres in England were used for sainfoin production due to its importance in animal nutrition and soil quality preservation. The cultivation methods are described in '*General View of the Agriculture of Oxforshire*' (Tull, 1733; Young, 1913) it is traditionally sown mixed with a companion species, usually a non-invasive grass such as *Festuca pratensis* or *Phleum pratense*. This strategy enabled farmers to suppress weed invasion, which is a significant challenge in establishment and cultivation.

The decline of sainfoin in Britain started in the 1920s, and increased significantly as long-term leys were ploughed up

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

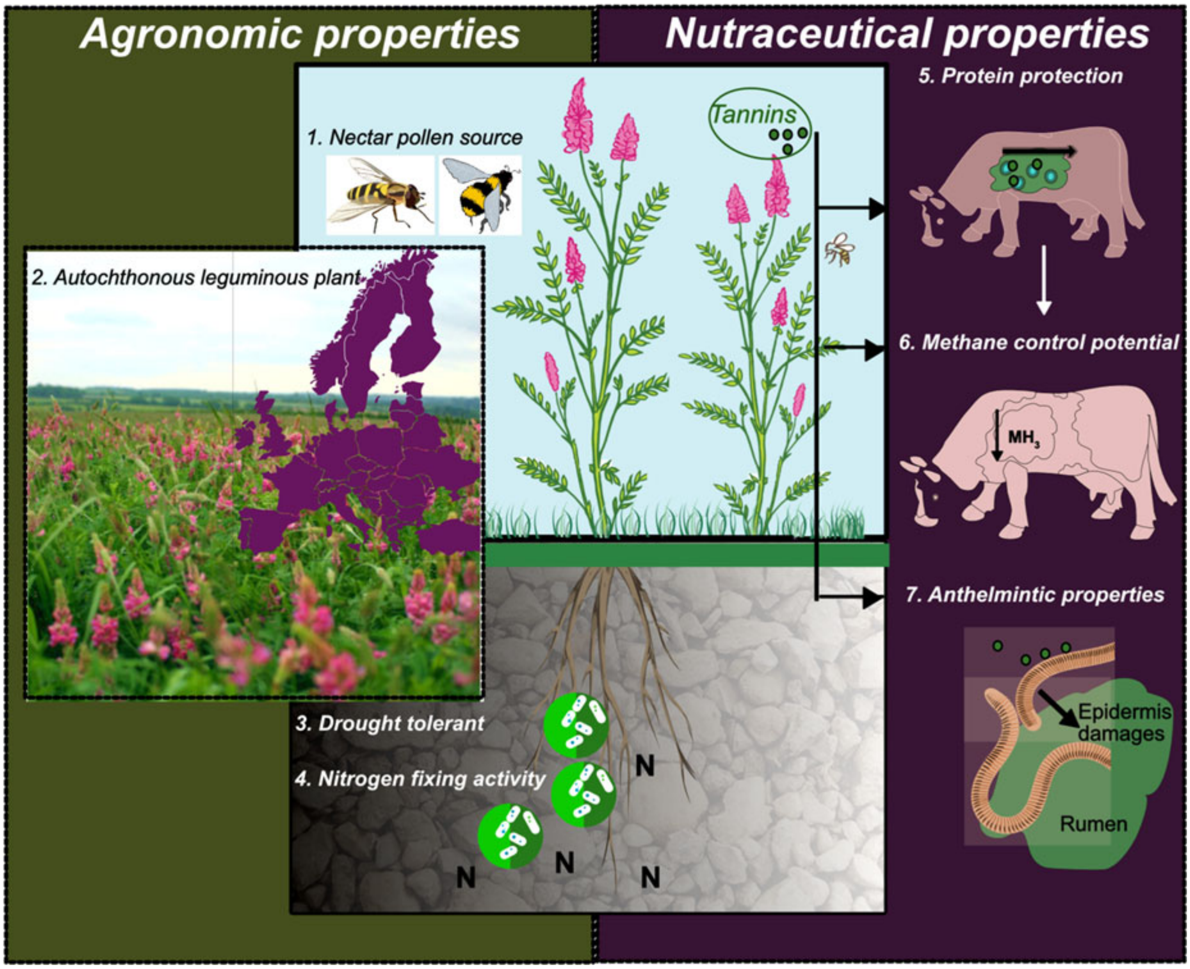

Fig. 1. (1) Sainfoin is a rich nectar and pollen source. The flowering is 'indeterminate' having with continuous flower development for several weeks. (2) UK Sainfoin field midway through peak flowering. Sainfoin is an autochthonous leguminous plant that could reduce the current level of nitrogen dependence from Europe. Photograph and figure donated by Cotswold Seeds Ltd. (3 and 4) Schematic representation of Sainfoin root and microbial associations. (5, 6 and 7) Schematic of impact on rumen microbiology and anthelmintic benefits.

during the 1939–1945 war period (Bland, 1971). This loss of popularity was partly associated with a decrease in cultivation of long-term pasture for sheep production in favour of the cultivation of higher yielding ryegrasses (Edmunds, personal communication cited in Hayot Carbonero (2011b)). The decline was also attributable to the replacement of horses by machinery (Newman, 1997). It was recorded that about 150 tonnes of sainfoin seed were sold every year in the late 1950s, this was sufficient for circa 2400 hectares (Hill, 1997). Seed sales dropped to an amount sufficient for 150 hectares in the 1970s (Sheehy and Popple, 1981) and just 50 hectares in the 1980s (Aldrich, 1984), but UK cultivation has increased in the past decade to more than 1970s levels and is still rising. Sainfoin is still an important crop in parts of Asia, Turkey and Iran. Currently, sainfoin cultivation also persists in parts of North America, Italy and Spain (Koivisto, 2001).

Etymology and common denomination of sainfoin

The name *Onobrychis viciifolia* is probably derived from the Greek, *ónos* (ὄνος, 'donkey') and *brýkein* ('to eat avariciously') (Carniol, 1771). However, Jaques (1894–1897) and (Demdoum, 2012b) suggest that the real origin of *–brychis* is *brýcho* ('bray') is due to the happy sound that is made by donkeys when they eat it.

The crop has many common names, the most popular is probably sainfoin, but it has been known as St. Foin, which comes from the old French *sain foin* ('healthy hay'). It has also been called Medicinal Plant and Luzerne. Other names and derivations originate from the French and Spanish name *esparceto*. Some examples of this include *esparceta* or *pipirigallo* in Spanish, *esparsette* in Danish, *esparcette* in Dutch, *sparceta* in Polish, or Эспарцет (espartset) in

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

Russian. It has been called 'holy grass' due to its beneficial properties, 'French grass' or 'Cock's head' in English, or 'crête de coq' in French (Zolla, 1904). The Spanish *pipirigallo,* also originates from this source meaning 'cockscomb', all of which latter names refer to the morphology of the spiny husk of the seed (Moliner, 1982). Foin de Bourgogne, Fenasse, Bourgogne or Herbe éternelle (Stebler *et al*., 1894) are other names found in the literature, referring to its use and perennial growth.

Taxonomy of sainfoin

The crop known as 'sainfoin' has been located in many different genera during its history, for example, *Hedysarum* and *Sartoria* (Badoux, 1965). Old drawings were published by Johann Georg Sturm in 1796, illustrating an example of *Onobrychis viciifolia* called Esparsette, but classified as *H. onobrychis*. Some authors have named it *Onobrychis foliis viciae* due to its similarity to those species in the genus *Vicia* and due to the resemblance between foliage morphology of *Onobrychis sativa* (Stebler *et al*., 1894). Sainfoin has also been called *Dendrobrychis* and *Xanthobrychis* in the past (Table 1).

Onobrychis viciifoila is now the accepted name for the crop known as sainfoin and most authors now accept that this genus has 126 species closely–following the classification originally developed by (Širjaev, 1925). This inlcudes a large representation from Turkey, where 57 species are described of which, 27 are endemic (Aktokly, 1995). *Onobrychis* is in the Fabaceae family, previously called Leguminoseae. In this widely accepted classification (Table 1) the genus is organized into two sub-genuses, Eunobrychis and Sisyrosemae, with four sections each.

Classification by other authors has used various contradictory systems based on different components of morphology (Emre *et al*., 2007). ILDIS included between 140 and 150 species (ILDIS, 2005) in contrast, only 23 were included in the encyclopaedia of *European Flora* (Wallace, 1969). Based mainly on the seed morphology, 170 species were estimated by Yildiz (1999) but this was shown to be flawed due to the high variability observed in the seeds depending upon environmental conditions (Yildiz *et al*., 1999; Hayot Carbonero, 2011b). Guner *et al*. (2000) building on earlier work (Badoux, 1965) estimated that the 54 species can be divided into five sections.

Of the species within the genus *Onobrychis*, only five have been shown to have useful agricultural attributes: *Onobrychis sativa* Lam. (*O. Viciifolia* Scop.), *Onobrychis sativa* var. *Persica* (Širjaev pro var.), *Onobrychis arenaria* (Kit.) Ser., *Onobrychis transcaucasica* Gross. and *Onobrychis montana* D.C. (Badoux, 1965).

Badoux (1965) named these five species as *O. viciifolia* Scop. *sensu lato*, and *O. viciifolia* Scop. sensu stricto

Table 1. *Onobrychis viciifolia*, a summary of the classification (Širjaev, 1925)

Genus Onobrychis	
Subgenus Euonobrychis = Onobrychis	Subgenus Sisyrosemae
Dendrobrychis	Anthyllium
Lophobrychis	Afghanicae
Hemicyclobrychis	Heliobrychis
Eubrychis (=Onobrychis)	Hymenobrychis

O. sativa, in its three forms and varities: *f. communis*, *f. bifera* and var. *persica* (cited in Demdoum (2012b) and Lewke Bandara *et al*. (2013)). Hayot Carbonero *et al*. (2011) re-defined the classification of divisions according to flowering date and morphological traits, indicating that there are two coherent clusters dividing *O. viciifolia* originally from Western Europe and Eastern Europe, Asia and USA (Hayot Carbonero, 2011b). The taxonomic classification of *O. viciifolia* still remains a matter of debate (Lewke Bandara *et al*., 2013).

Polyploidy and karyotype of *Onobrychis viciifolia*

Sainfoin has been characterized in terms of the chromosome number and morphology. An analysis of the ploidy of world seed collections of sainfoin yielded three possible ploidy levels ($2x$, $4x$ and $8x$) and 2 basic chromosome numbers ($x = 7$ and $x = 8$) (Abou-El-Enain, 2002). Sainfoin can be diploid ($2n = 2x = 14$) or tetraploid ($2n = 4x = 28$) (Figure 2), the basic number of chromosomes being 7. Whereas diploid accessions of sainfoin are infrequent and not very well described in the literature, tetraploid makes up the majority of sainfoin accessions held in most collections (Sacristan, 1966; Frame *et al*., 1998; Tamas, 2006; Hayot Carbonero *et al*., 2013). Polyploidy level has been linked with domestication and improvement of the crop, such that productive tetraploid plants have been selected (Hayot Carbonero, 2011b). These tetraploid accessions can be either autopolyploids or allopolyploids, and it is uncertain whether the inheritance is tetrasomic or disomic (Corti, 1930; Sacristan, 1966; Vicente and Arús, 1996; Abou-El-Enain, 2002).

Most of the cytological research on *Onobrychis* has been focused on ploidy characterization (Karshibaev, 1992; Slavicvk *et al*., 1993), while karyological studies are less common (Surayya and Syed Irtifaq, 1991; Karshibaev, 1992; Mesicek and Sojak, 1992). The karyotype analyses of *O. viciifolia* show an average size of 3.39 μm (Tamas, 2006) and more recent analysis indicates that arm ratios range from 1.41 to 2.22 μm (Somay Akcelik *et al*., 2012). The analysis of the karyotype was also used to define the

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

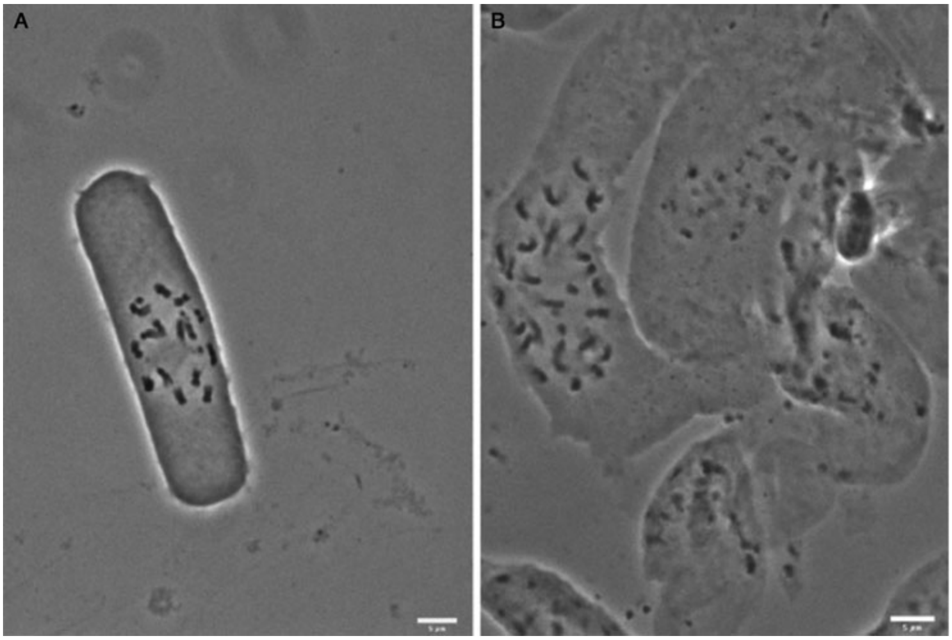

Fig. 2. Meristematic root tips of *O. viciifolia* stained with feulgen solution showing (a) diploid nucleus with $2n = 2x = 14$ chromosomes (accession 1257), and (b) tetraploid nuclei with $2n = 4x = 28$ chromosomes (accession 1292). Scale bars are 5 µm (reproduced by permission of (Hayot Carbonero, 2011b)).

taxonomy of different species within the *Onobrychis* genus and to determine its evolution. Through the karyotype analysis of different *Onobrychis* species, it has been suggested that the two populations of *Onobrychis transcaucasica* should be placed in one group, while *Onobrychis altissima* and *O. viciifolia* should be placed in a second group (Massoud *et al.*, 2010). During one of the last karyotype analyses developed in sainfoin, the 2C value was determined to be approximately 2.5 pg (Hayot Carbonero *et al.*, 2013). This cytological data is potentially useful alongside genetic profile to support crop genetic improvement in the future.

Genetic characterization and phylogenetic analysis

The understanding of sainfoin molecular genetics is limited to a few recent studies. These studies focused on DNA extraction methods, marker discovery, phylogenetic analysis of sainfoin accessions, gene discovery (focused on polyphenol metabolism) and most recently, *de-novo* transcriptome investigation for gene identification and putative marker discovery (Hayot Carbonero, 2011b; Demdoum *et al.*, 2012; Hayot-Carbonero *et al.*, 2012; Thill *et al.*, 2012; Lewke Bandara *et al.*, 2013; Kempf *et al.*, 2016; Mora-Ortiz *et al.*, 2016).

Polyphenols and high levels of many types of high molecular weight tannins are present in sainfoin foliage. These interfere with successful DNA and RNA extraction, producing problems in isolating high-quality samples. Very low yield, contamination by (i) phenols, (ii) proteins and (iii) RNA, or (iv) degradation of the samples, are some of the most common issues described in the literature (Hayot Carbonero, 2011b; Mora-Ortiz, 2015). Several extraction methods have been tested in order to extract high-quality DNA. These methods included standard procedures such as those described by (Hormaza, 1999) and (Fulton *et al.*, 1995). Other commercial options such as the kits from Qiagen (DNeasy Plant Mini Kit) and GE Healthcare (Nucleon PhytoPure Genomic DNA) have also been tried. The most successful method reported was Nucleon PhytoPure and the modified (Doyle and Doyle, 1987) 'DNA extraction protocol' described by Hormaza (1999) (Hayot Carbonero, 2011b, Demdoum *et al.*, 2012, Hayot-Carbonero *et al.*, 2012). Similar methodological difficulties were encountered when attempting to obtain high-quality RNA extracts. Many methods have been evaluated including (i) TRIzol® (Invitrogen, USA) RNA isolation method, (ii) RNeasy® (Qiagen, Germany) and (iii) CTAB Total RNA Extraction Protocol. The Plant/Fungi Total RNA Purification Kit from NORGEN from Biotek Corporation was identified as a suitable method to extract high-quality RNA for Next Generation Sequencing (Mora-Ortiz, 2015; Mora-Ortiz *et al.*, 2016).

In view of the lack of markers available for sainfoin, initial marker development studies were based on the transferability of genetic markers from related species like

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

Medicago truncatula and *Glycine max* (L.), nuclear internal transcribed spacer region and the *trnH-psbA*, *trnT-trnL* intergenic spacers of the chloroplast genome, and nuclear (ITS) and chloroplast (*matK*) markers (Hayot Carbonero, 2011b; Demdoum *et al.*, 2012; Lewke Bandara *et al.*, 2013). EST-SSR from *Medicago truncatula* were found to be 81% amplifiable in sainfoin, and 52% were polymorphic. The amplification size found in sainfoin was 79–865 bp, and between 79 and 240 bp in *Medicago truncatula* (Demdoum *et al.*, 2012). In a recent study, *de-novo* transcriptome interrogation was completed, which allowed the identification of 3786 potential SSRs and 77,000 putative SNPs markers (Mora-Ortiz *et al.*, 2016).

Phylogenetic studies have indicated that there is a significant division between western European varieties of sainfoin *versus* eastern European and Asiatic. This division is similar to that identified using morphological and agronomic traits (Hayot Carbonero, 2011b; Hayot-Carbonero *et al.*, 2012). Furthermore, in a phylogenetic study (Lewke Bandara *et al.*, 2013) using nuclear (ITS) and chloroplast (matK) markers, *Onobrychis* species were resolved as paraphyletic, with species of the genera *Eversmannia* Bunge and *Hedysarum* L. nested within it. In this study, uncertainty in defining species delimitation in the *Onobrychis* genus has been attributed to recent speciation, hybridization, and introgression events, particularly between cultivated species and their wild relatives (Lewke Bandara *et al.*, 2013) (Figure 3). More recent phylogenetic studies interrogating sainfoin transcriptome have shown that *O. viciifolia* is more closely linked to red clover and *Medicago truncatula*, than other legumes like *Lotus japonicas*, bean and soybean, which are more distant relatives (Mora-Ortiz *et al.*, 2016).

Similar findings were observed using SSRs markers in different subsequent studies. When the polymorphism information content (PIC)[1] (Botstein *et al.*, 1980) was studied for 25 different accessions from *Onobrychis* species in Demdoum (2012b), it was shown that PIC ranged from 0.45 to 0.85. *Onobrychis viciifolia* lines clustered with a high genetic similarity. This was much lower compared with *O. argentea* and *O. pyrenaica*. This closely clustered with a PIC of 0.95, which was unexpected by the authors because, in terms of morphology, *O. argenta* is more similar to *O. viciifolia* than to *O. pyrenaica* (Demdoum *et al.*, 2012). This investigation also showed that British accessions are phylogenetically associated with Western European accessions such as UK varieties known as 'Cotswold-Common' and 'Sombourne', which are phylogenetically more distant to those from the cluster formed by Eastern and West continental European accessions (Demdoum *et al.*, 2012). This study was, however, based on EST-SSR markers obtained from *Medicago truncatula*. A later study based on *O. viciifolia* putative SSR markers showed similar results; confirming clusters according to geographical origin and separating species into two major groups from Southern and Eastern Europe, and Switzerland and UK, respectively. In this case, PIC reached lower values, ranked between 0.14 and 0.36 (Kempf *et al.*, 2016) (Figure 4). These results agree with the phylogenetic study based on the morphology developed by Hayot Carbonero (2011b); Hayot Carbonero *et al.* (2011).

These latest advances in molecular genetics, have provided a large number of molecular markers for further breeding programmes and a better understanding of sainfoin phylogeny. Breeding and pre-breeding programmes now have tools for focussed development in the future. The selection of new highly productive varieties is demanded by farmers and the seed industry (Mora-Ortiz and Smith, 2016). Breeding programmes will need to include recently identified attributes in selection processes to ensure that multi-beneficial properties attributed to its condensed tannins content are maximized. There are 12 cDNAs encoding genes implicated in sainfoin flavonoid biosynthesis pathway, which have been cloned and sequenced (Thill *et al.*, 2012). These sequences, together with a collection of genes encoding enzymes in this pathway from KEGG, allowed the identification of 63 transcripts involved in the tannin biosynthesis pathway and their associated transcriptional levels (Mora-Ortiz *et al.*, 2016). During this study, the gene ontology (GO) analysis of the transcriptome also enabled the annotation of 18,000 transcripts with at least one GO term (Mora-Ortiz *et al.*, 2016).

Botanical description of sainfoin

Plant morphology

Sainfoin has epigeal germination, which means that during germination, the hypocotyl extends and the cotyledons emerge from the soil during early growth, as opposed to hypogeal germination. In hypogeal germination the epicotyl extends and the cotyledons stay below the soil surface until germination whereby the cotyledons are pushed above ground after germination. Crop plant vigour during this early stage depends on the stored substrate in the seed during the first 7 d of growth. Following initial germination, photosynthesis in the cotyledon leaves then plays an important role in the development of normal first-leaves and their expansion (Cooper and Fransen, 1974).

The plant habit is mainly erect or sub-erect; however, some accessions have a more prostrate or rosette habit and most accessions will die back to a short prostrate

1 The PIC is calculated using the equation $\mathrm{PIC} = 1 - \sum_{j=1}^{n} P_{ij}^2$ (Botstein *et al.*, 1980), where P_{ij} is the frequency of the '*i*th' allele for marker '*i*' in the '*j*th' population and summation extends over *n* alleles

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

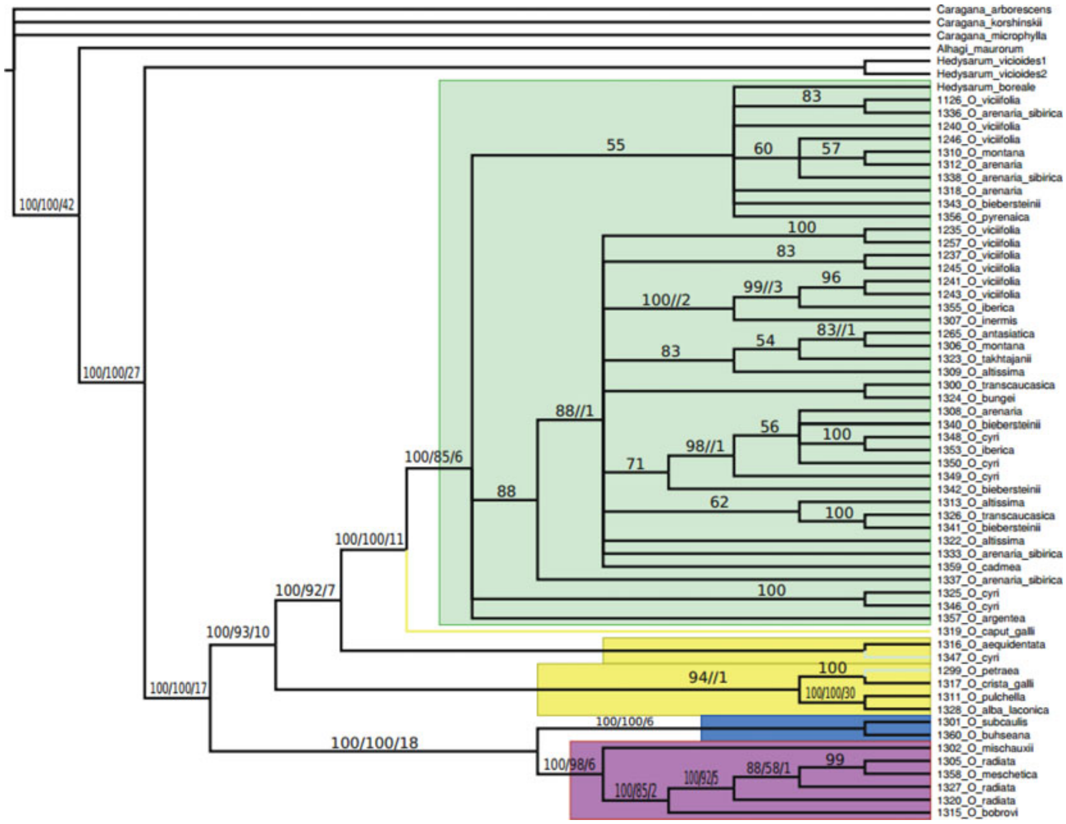

Fig. 3. Phylogenetic study developed using nuclear (its) and chloroplast (matk) markers. The section in green represents *Onobrychis* subgenus *Onobrychis* section *Onobrychis*. The lower section from accession 1319 to 1328 represents *O.* subgenus *Lophobrychis*. The bottom section from 1302 to 1315 represents, *O.* subgenus *Sisyrosema* section *Hymenobrychis* and from 1301 to 1360, *O.* subgenus *Sisyrosema* section *Heliobrychis*. Robustness of the analysis is explained above branches: the first number corresponds to the Bayesian support, the second to the bootstrap (maximum parsimony) support, and the third to the decay values (Lewke Bandara *et al.*, 2013). Reproduced with permission from the authors.

plant over the winter months. The morphological plasticity of sainfoin adds to difficulties in characterizing varieties, malleability contributes to its ability to cold stress during winter and early spring (Frame *et al.*, 1998; Seker *et al.*, 2003; Drobná, 2010; Hayot Carbonero, 2011b).

In spring, many hollow stems grow from basal buds and form a branched crown. They are defined as sub-woody until the height of about 70 cm and can be hairless to slightly hairy, and hardly ramified. Height varies between 100 and 20 cm and normally has between 16 and 18 stems per plant, with a variable thickness of 3–9 mm (Frame *et al.*, 1998; Valdes, 2000; Hayot Carbonero, 2011b). Foliage is green, rarely with some red pigmentation. Significant variability has been observed in the green colour of the different accessions and occasionally the leaves have hairs in the middle nerve (Canals *et al.*, 2009; Hayot Carbonero, 2011b). Stems have pinnate leaves in a variable number between 6 and 14 and the leaves are compound normally with between 10 and 28 leaflets per leaf. The individual leaflets are oblong, oblong-elliptic and elliptic on average 10.3 mm long and 6 mm wide. The leaves are classified as impapirinnante, pinaticomposed and oppositepinnate (Allaby, 1987; Font Quer, 1987; Lancha and Sempere, 1988; Polunin, 1991; Valdes, 2000; Canals *et al.*, 2009; Hayot Carbonero, 2011b).

Root morphology and development

Sainfoin has over a 2-meter-long taproot in mature plants, partly responsible for its drought tolerance. The root is quite branched, especially at the bottom and multiples of thin lateral roots constitute the bulk of the root system (Hayot Carbonero, 2011b; Kempf, 2016; Mora-Ortiz and Smith, 2016). Roots have often been measured at more than 2 m and in dry conditions over 3 m. The Sainfoin root systems rival Lucerne for its ability to access water in the lower soil horizons (personal communication, Beat Boller, ETH, 2011)

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

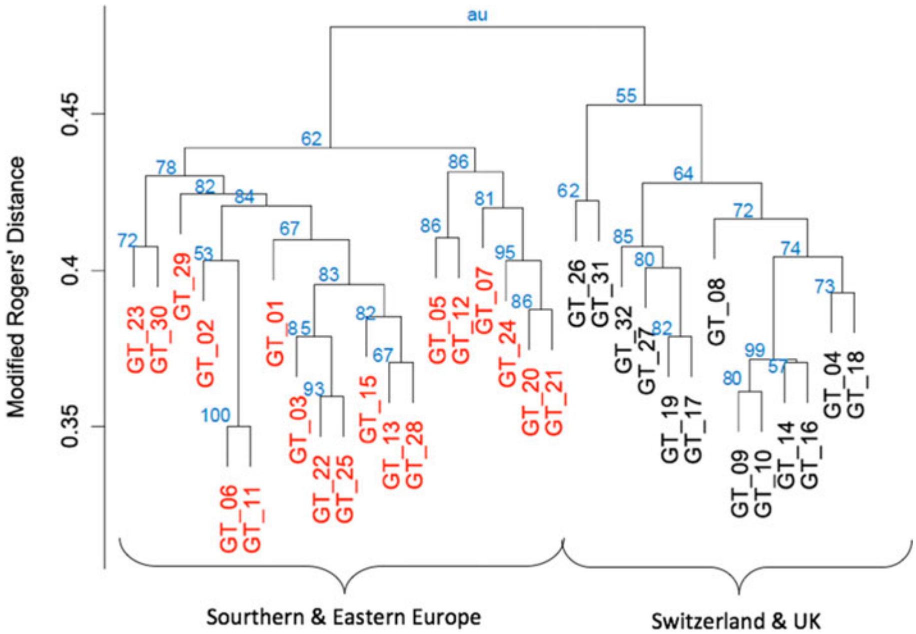

Fig. 4. Cluster dendrogram of individuals based on the modified Rogers' distance. Values at branches are AU *P*-values (blue). Different colours of genotype labels give the affiliation to the two groups determined by *k*-means partitioning. The accessions that were tested separated into two main clusters. One was associated with varieties from southern & eastern Europe and a second one included accessions from the UK and Switzerland. Figure is adapted from Kempf *et al*. (2016).

Flower development and morphology

Inflorescences, developed on auxiliary tillers have broad finely pointed stipules. The inflorescences are dense with 10–80 flowers and peduncle between 12 and 20 cm. The calyx is loosely tomentose or sub-glabrous. The corolla of the flowers is pinkish. This base colour has high diversity from white to purple with darker linear patterns of greater intensity than the primary colour. The corolla is 1.5–2 times larger than the calyx. The keel is curved in an obtuse angle and 1.5 times longer than the calyx. The flowers have bracteoles 0.6–1.5 mm (Valdes, 2000; Canals *et al*., 2009; Hayot Carbonero, 2011b).

Sainfoin is an indeterminate species and generally flowers for more than 5 weeks, from green flower buds to set seed in UK field conditions. During this period, nine phonological stages have been defined: (1) green bud, (2) red bud, (3) keel out, (4) open flower, (5) wilted flower, (6) calyx, (7) swollen calyx, (8) green seed and (9) dry seed. Fruit set depends upon the variety, but it can be correlated with the suitability and adaptability of its environment. The flowers open gradually over 24-h especially between night and sunrise (McGregor, 1976; Demdoum, 2012b). Authors disagree on the time at which the flower becomes receptive to fertilization. This may occur before the stage of keel out or not until the flower is fully open (Galloni *et al*., 2007; Demdoum, 2012b). This may be a strategy to avoid self-pollination (Knuth, 1906). The pollen is more viable at the stage of keel out (Pavlova and Manova, 2000; Demdoum, 2012b). Sainfoin was thought to be an obligate allogamous species due to its flower morphology; however, recent studies (Demdoum, 2012b) suggest that although the species requires pollination by insects, it can tolerate a low level of selfing. The lack of success in selfing could be due to a combination of the morphology of the flower and the level of activity of pollinating insects to cover the asynchronous maturation of the stigmas (Demdoum, 2012b). It has also been observed that a physical jolt is required by visiting pollinators in order to break the stigma cuticle, which improves pollination by 4.1% (Thomson, 1938).

The seeds are kidney-shaped with a brown pod whose size varies between 2.5 and 7 mm long, 2–3.5 wide and 1.5–2 mm thick (Valdes, 2000; Hayot Carbonero, 2011b; Hayot Carbonero *et al*., 2011). The pod-spinyness is variable and has been used as a taxonomic character (Thomson, 1951). The hull is downy due to the presence of short hairs. The contour of the seed is described as orbicular and from 0.1 to 0.3 mm in length. The embryo is large and rich in starch reserves, protein and lipid. The maturity of the fruit at the time of harvesting is the major factor that determines the final colour of the seed. Seed are either sold with their hull intact (those that have not been processed and retain the pod coat, are known as 'un-milled seeds' among farmers) or as de-hulled seeds (which have been processed to remove the pod coat, also known as 'milled seeds' among farmers). Seed weight is between 24 g/1000 seed for the former and 15 g/1000 for the latter.

The dispersion of the fruit is by animals, which is improved by the presence of the spines on the seed hull (Valdes, 2000; Hayot Carbonero, 2011b; Hayot Carbonero *et al.*, 2011). Under certain conditions, dehulling or 'milling' the seed assists in early synchronous germination (Hayot Carbonero, 2011b).

Agronomic characterization

Varieties

Sainfoin has been traditionally divided into two types, 'giant or two-cuts sainfoin' and 'common or single-cut sainfoin'. They have different characteristics and morphology (Table 2). In general, the common type has more stems per plant, while the giant type has longer stems, more internodes per stem and more leaflets per leaf. The giant type is normally recommended for fertile lands, while the common type is more suited to a higher altitude (Badoux, 1965; Michelena, 1983; Prosperi *et al.*, 1994; Delgado *et al.*, 2008; Demdoum, 2012b).

Most of the varieties that are now commercialized were developed in the 1970s and are an intermediate type between the giant and common sainfoin. The most popular varieties available in Europe are Ambra, Vala and Zeus from Italy; Perly from Switzerland, Emyr from Hungary; Fakir from France; Višňovský from Czech Republic; and Cotswold-Common and Cholderton-Hampshire-Common from the UK. In Canada, varieties such as Melrose and Nova are popular; Eski, Remont, Remunex and Shoshone are from USA and G35 in New Zealand (Hayot Carbonero, 2011b; Demdoum, 2012b).

Climate, habitat and soil

Sainfoin is adapted to a range of climatic and abiotic conditions existing in Asia, Europe, North America, New Zealand and Australia. (García Salmerón *et al.*, 1966). It prefers the Mediterranean sub-humid climates with some Central-European trend, being compatible with Mediterranean semi-arid climates, moderately warm and dry, and with the climates of High Mountain. In the Mediterranean basin, it prefers altitudes above 600 m but is cultivated in a range between 100 and 2500 m (García Salmerón *et al.*, 1966; Demdoum, 2012b). In a survey of 40 Spanish farmers producing sainfoin seeds, 90% of their farms were located in altitudes between 600 and 1474 m, where the climate was semi-arid and the soil was limestone (Delgado *et al.*, 2002; Demdoum, 2012b).

Sainfoin grows well in very slightly acid, neutral and alkaline soils with a pH above 6.5; sainfoin is intolerant to acid soil, especially of subject to high rainfall, and can grow in both, dry-lands and irrigated areas (Bland, 1971; Frame *et al.*, 1998). In the absence of irrigation, annual rainfall should be at least 330 mm (Miller and Hoveland, 1995). Sainfoin is not tolerant to waterlogging and prefers well-drained areas (Sheldrick *et al.*, 1987). In the UK, sainfoin has generally been linked to calcareous chalky or limestone soils (Frame *et al.*, 1998). The poor establishment was obtained on clay soil at pH 6 with failures on the alluvial sand at/or below 5 (Bland, 1971; Hayot Carbonero *et al.*, 2011). In Spain, it is traditionally linked to neutral or slightly alkaline brown-earth soils. It is incompatible with poor draining soils such as podzols, greysolic acid brown, grey forest and oxisols and combinations of any of the latter (García Salmerón *et al.*, 1966; Demdoum, 2012b). Sainfoin does not need fertile soil to thrive as long as the requirement for lime and humidity are satisfied. Sainfoin can thrive in less fertile soils than alfalfa and clovers, but can also grow well in more fertile soils. Alfalfa and clover will, however, produce better yields in fertile and irrigated lands, but sainfoin provides better outcomes when the soil is of low fertility compared with alfalfa (Benaiges, 1971; Demdoum, 2012b).

Long periods of hot temperature can negatively affect sainfoin and therefore, reduce yields and this is particularly important following defoliation when the ability of the plant to cope with high metabolic rates is decreased (Kallenbanch *et al.*, 1996). Although sainfoin is considered

Table 2. Morphological and agronomic differences between giant and common sainfoin also called 'two-cuts' and 'single-cut', respectively

Attribute	Giant sainfoin	Common sainfoin
Synonym	Two-cuts	Single-cut
Origin	*Onobrychis sativa* var. *bífera* Hort	*Onobrychis sativa* var. *communis*
Geographical origin	Middle East	Central Europe
Growth habit	Erect habit during the year of sowing	Slightly prostrate during the year of sowing
Re-growth	Re-flowers after being cut – and can be cut more than once per year	Slow and vegetative – tolerates only one cut per year. Unlikely to flower during the establishment year
Survival	Up to 3 years	Between 7 and 10 years

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

to be intolerant to high temperatures, there is some evidence to show it can grow at temperatures above 32°C in Spain and Greece when irrigation is well managed (Hayot Carbonero *et al.*, 2011). There are few studies on its ability to tolerate frost, and this relates to variety choice. Most accessions can withstand winter frost, but not frost in combination with prolonged snow cover. Young sainfoin seedlings were found to be better able to withstand such conditions than seedlings from other legumes, such as *M. sativa* and several *Trifolium* species, with the exception of *Trifolium hybridum* (Benaiges, 1971; Meyer and Badaruddin, 2001).

Sowing

In the warm Mediterranean basin, sainfoin is normally drilled either in early autumn or at the beginning of spring. Conversely, in colder areas like the UK, it is recommended to drill sainfoin between April and July after the soil temperature has become warm and humid enough to facilitate a quick germination and subsequent growth (Jensen and Sharp, 1968; Goplen *et al.*, 1991). Sainfoin has an extended optimum temperature range for germination, but it is normally advised to drill it between 10 and 20°C and never below 5°C (Jensen and Sharp, 1968; Smoliak *et al.*, 1972). Early sowing can improve the development of the plants thanks to the early development of the vegetative plant and roots, and yield in the first year.

The seeds can be drilled either de-hulled or hulled (Thomson, 1951). There is a controversy among authors as to the preferred option (Wiesner *et al.*, 1968; Chen 1992). Use of de-hulled seed could provide staggered germination and thus cushion potential weather disturbances (Wiesner *et al.*, 1968; Chen, 1992; Demdoum, 2012b). The use of large fully mature seeds increases establishment success giving stronger plants, with more nodules and higher rates of nitrogen fixation (Cash and Ditterline, 1996).

In order to establish a population of 70–150 plants/m^2 in the first year, authors recommend seed rates of 40–50 kg/ha of de-hulled seed (or 80–120 kg/ha hulled) (Sheldrick *et al.*, 1995; Frame *et al.*, 1998) at a depth of 1 and 2 cm in Canada (Hill, 1997). Conversely, in China, a depth of 4–5 cm was recommended (Chen, 1992). These variations in planting depth are attributed to differences in the soil texture and moisture at the different sites (Hayot Carbonero, 2011b). The recommended row spacing is between 50 and 60 cm (Goplen *et al.*, 1991; Stevovic *et al.*, 2010).

Inoculation and nitrogen fixation indications in sainfoin

Sainfoin, along with other leguminous species, will establish symbiotic relationships with gram-negative bacteria from the family *Rhizobiaceae* and with arbuscular mycorrhizal fungi. The symbiosis with Rhizobiaceae is sited in specialized root nodules, which in sainfoin can exhibit a range of morphologies; spherical, to branched and coralloid are formed (Figure 5). In these nodules, differentiated bacteria use nitrogenase enzyme complex to reduce atmospheric nitrogen to ammonia. Sainfoin benefits from this ammonia to synthesize amino acids and proteins (Baimiev *et al.*, 2007). Both, mycorrhizal fungi and the *Rhizobia*, associated with sainfoin plants benefit from food in the form of carbohydrates produced from photosynthesis in the host plant. The inoculation of sainfoin with *Rhizobium* sp. can be developed using strains isolated from related legumes such as *Hedysarum, Coronilla* or *Dalea* or from healthy nodules on sainfoin (Burton and Curley, 1968) Isolation of *Rhizobia* from more cold tolerant legumes such as *Astragalus alpinus, Oxytropis madelliana* and *Oxytropis arctobia*, led to an improvement in nitrogen fixation during cold conditions (Prevost *et al.*, 1987). Several authors have noted that the level of nitrogen fixation in sainfoin nodules is inadequate in some situations and nitrogen deficiency symptoms can be seen, even when the crop has been inoculated (Burton and Curley, 1968; Sims *et al.*, 1968; Sheehy and Popple, 1981). The higher requirement of energy from sainfoin has been attributed to the smaller leaf area index compared with alfalfa. This would reduce the use of the light energy and carbon fixation, which is indirectly related to the level of nitrogen fixation. That would explain the high nodular activity of sainfoin and weight of their nodules compared with other legumes (Sheehy and Popple, 1981; Hayot Carbonero, 2011b; Demdoum, 2012b) (Figure 5).

The nitrogen fixation rate of sainfoin has been compared with that of alfalfa. In sainfoin, it was estimated at between 130 and 160 kg N/ha and for alfalfa between 140 and 160 kg N/ha. This resulted in a yield improvement of 17 and 25%, respectively (Provorov and Tikhonovich, 2003). In an experiment with the nitrogen-free growing medium, the sainfoin variety Melrose was tested against 47 rhizobia strains. The results ranked from 8 to 140 mg total nitrogen/pot showing that efficient symbiosis depends on finding an efficient rhizobial symbiont (Prevost *et al.*, 1987). A combined application of phosphorus, nitrogen and *Rhizobium* inoculum was found by some authors to produce the best improvement in sainfoin yields over untreated controls (Tufenkci *et al.*, 2006).

Arbuscular mycorrhizal (AM) symbiosis

AM symbiosis is a close association between plant roots and fungi; at least 80% of the vascular flowering plants worldwide are able to form this type of symbiosis, and it is one of the most widespread symbioses found in plants. The fungus partner(s) supply sainfoin with phosphate and

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

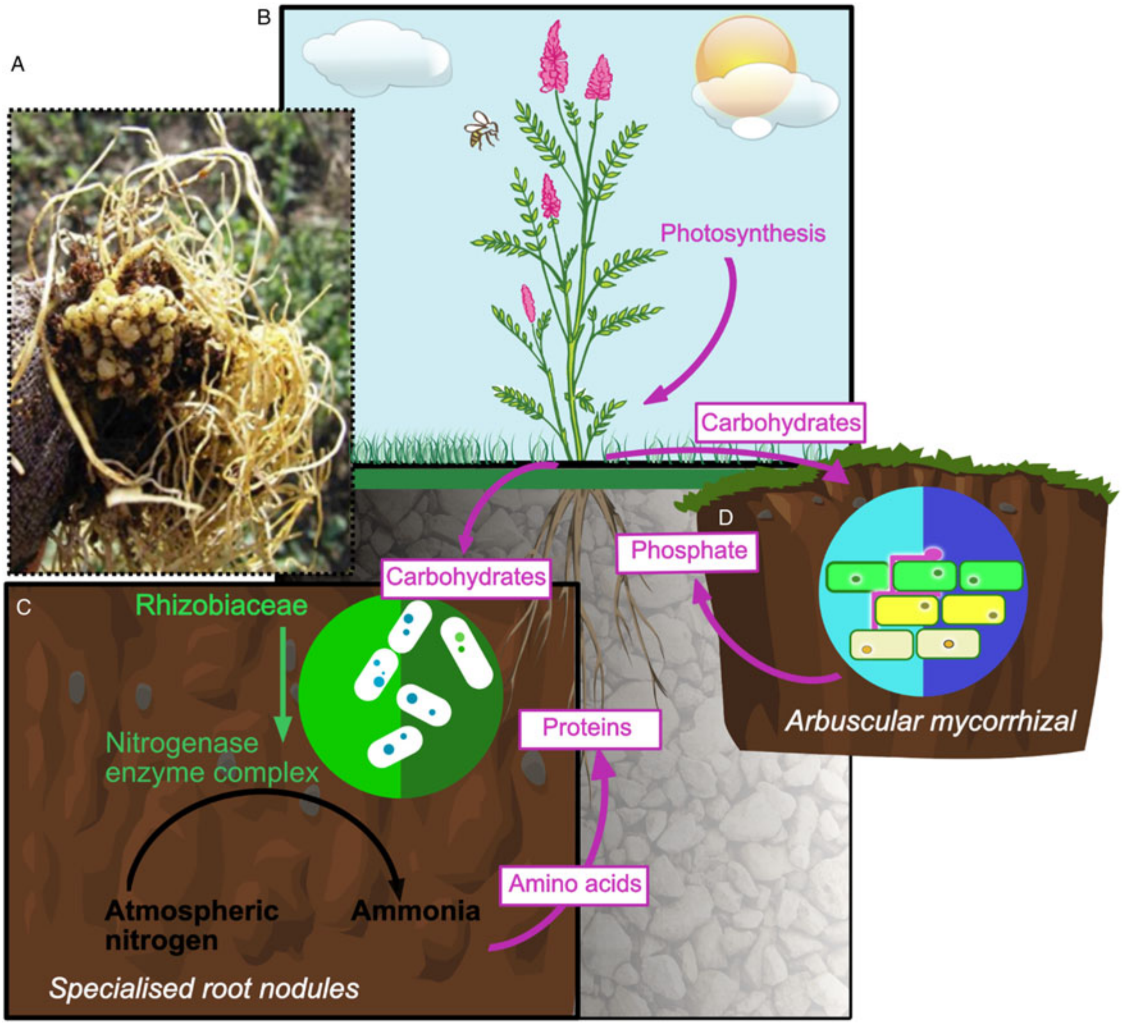

Fig. 5. (a) Nodules found in sainfoin. The size of sainfoin nodules is unusually large compared with other legumes. Research on these symbioses is limited. (b) Products of photosynthesis, are transferred to both rhizobium and arbuscular mycorrhizal partners. (c) Rhizobium fixes nitrogen that sainfoin will use for protein synthesis and (d) Mycorrhizas enhance phosphate uptake.

other nutrients from the soil, improves water use efficiency and promotes plant resistance to pathological infections. The plant provides the fungus with carbon compounds (Harrison, 1998). Pilot studies of mycorrhizal inoculation have indicated that sainfoin can benefit from improved access to symbionts under field conditions, especially in the presence of Rhizobia inoculants (NIAB, L. M. J. Smith, unpublished data).

Fertilization

Fertilization requirements in sainfoin can be highly variable. It is traditionally believed that sainfoin does not require fertilizers. Studies have, however, shown that use of low levels of inorganic N fertilizer applications stimulated nitrogen fixation in sainfoin (Sims *et al*., 1968). But high dosages inhibited nodulation and fixation rates (Badoux, 1965; Koter, 1965; Meyer, 1975; Sheehy and McNeill, 1988; Hartwig and Nösberger, 1996). These contradictory responses to nitrogen could be partly attributed to differences in the original nitrogen content of the soil, which is not always defined (Hayot Carbonero, 2011b; Demdoum, 2012b).

In a comparative study total N, P and K extracted from the soil by *Medicago sativa* and sainfoin were evaluated and expressed as fertilizer equivalents. It was concluded that sainfoin needs more P_2O_5 and NO_3 than alfalfa, and that alfalfa needs more K_2O and $CaCO_3$ than sainfoin (Sheehy *et al*., 1984). Meyer (1975) observed only small effects from applications of P_2O_5 and K_2O fertilizer while (Sheldrick *et al*., 1995) noted a better response.

https://doi.org/10.1017/S1479262118000230 Published online by Cambridge University Press

Seed production

Every sainfoin flower has the biological capacity to produce a seed, but on average only 55% of these will succeed and produce a viable seed (Goplen *et al.*, 1991). Sainfoin can produce between 5 and 40 tillers, each of which has between 3 and 5 inflorescences. Sainfoin inflorescences are composed of 5 and 80 flowers. Both the variety and the environment will have an impact on the final seed production (Carleton and Wiesner, 1968). Honey bees (*Apis mellifera*) and leafcutting bees (*Megachile rotundata*) are the recommended pollinators to assist in sainfoin seed production. Bee-assisted pollination in sainfoin is considered to be more successful than in alfalfa due to the longer morphology of the flower (Wallace, 1968).

Seed yield can be improved by the presence of at least two to three colonies of honey bees per hectare. Alternatively, it is possible to use leafcutting bees; in this case, it would be necessary to have at least 20,000 per hectare (Goplen *et al.*, 1991). To optimize seed yields, seeds are swathed after they have dried to a maximum of 40% water content and then allowed to dry further in the windrow before threshing. In this way, yields vary between 500 and 900 kg of clean seeds per hectare. Following these protocols, a maximum yield of 1100 kg/ha has been obtained in Canada (Thomson, 1951; Goplen *et al.*, 1991; Prosperi *et al.*, 1994). Some authors consider that it is better to leave the seeds with hulls intact if they are going to be stored, to maintain maximum viability for longer (Thomson, 1951).

There are several factors involved in seed production that can have a secondary impact on the final yields. The best yields have been obtained when the flowers are cross-pollinated. Seed size increases as the number of seeds per plant head decreases. Plant density also impacts on seed production; seed production per plant decreased when there was competition between densely planted individuals. In dry areas, such as Italy, seed production improves with irrigation (Carleton and Wiesner, 1968; Martinello and Ciola, 1994; Demdoum, 2012b).

Weed control

Weed invasion in recently drilled sainfoin fields in the UK often leads to the poor competitive establishment, especially with broad leaf weeds such as *Galium aparine*, *Senecio vulgaris*, *Chenopodium album*, *Lamium purpurum* and *Stellaria media* (Hayot Carbonero *et al.*, 2011). Sainfoin has 50% less leaf surface area than alfalfa and a diffuse canopy structure during the first 4 months of growth (Sheehy and Popple, 1981; Frame *et al.*, 1998). The main strategies used to control weed invasion in sainfoin fields are herbicide treatments and use companion crops. Different herbicide approaches have been tested, but research in this area is limited. Previous studies have shown that control of dandelion (*Taraxacum officinale*) in sainfoin using metribuzin [4-amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one] improved sainfoin yields by up to 28% (Moyer *et al.*, 1990). It has also been noted that, in the absence of herbicide treatments, weeds can represent up to 98% of the final yields in the first cut (Moyer, 1985). A range of herbicides aimed at pre and post-crop emergence was tested in a field scale screen including; bentazone [3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] and imazethapyr [5-ethyl-2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl] nicotinic acid] and weed control were improved by the inclusion of an adjuvant (tween 80 or ammonium sulphate). Imazethapyr performed best, which, unlike bentazone, did not reduce sainfoin biomass (Amiri *et al.*, 2013). The use of pre-emergence herbicides like pendimethalin, metazachlor and prosulfocarb significantly controlled weed numbers and increased yields by 8 and 30%, respectively in the UK. These findings highlight the importance of good weed control strategies during early establishment of the crop (Mora-Ortiz *et al.*, 2015a, b). The use of carbetamide [(R)-1-(ethylcarbamoyl) ethylcarbanilate] was tested for maintenance of a clean crop during the winter; and MCPA [a.i. 4-(4-Chloro-2-methyl-phenoxy) acetic acid] and MCPB [a.i. 4-(4-Chloro-2-methyl-phenoxy) butyric acid] for the control of broad leaf weeds during the spring (Sheldrick and Thomson, 1982; Frame *et al.*, 1998). In one study, yields were increased by 20% using hexazione [3-Cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-dion] and terbacil (3-tert-butyl-5-chloro-6-methyluracil) treatments (Malik and Waddington, 1988). In the USA, sainfoin natural tolerance to Glyphosate was the basis for weed control using post-emergence multiple applications of low dosage Glyphosate (N-(phosphonomethyl) glycine) (Lauriault *et al.*, 2009).

Weed control can also be addressed through the use of companion crops. Depending upon local environmental conditions, farmers have favoured the use of mixtures containing *Phleum pratense*, *Festuca pratensis* or have undersowed with spring barley (*Hordeum vulgare*), tetraploid perennial ryegrass (*Lolium perenne*), Russian wild rye (*Psathyrostachys juncea*) or crested wheatgrass (*Agropyron desertorum*). Co-cultivation with a second leguminous species, *Lotus corniculatus*, has also been considered (Dubbs, 1968; Bland, 1971; Cooper, 1972; Goplen *et al.*, 1991; Frame *et al.*, 1998; Liu *et al.*, 2009; Hayot Carbonero *et al.*, 2011). Chicory and oat have also been considered as potential alternatives due to their antiparasitic properties and nutritional profile respectively. Recent studies have shown that they can grow together with sainfoin during short periods, for example, in rotation systems, but further studies are necessary (Mora-Ortiz, 2015). The use of companion crops has been shown to reduce the proportion of weeds to crop by 65%, to increase the symbiotic N_2 fixation rate by up to 158 kg/ha and increase total yields by 31% compared with monocultures (Malisch *et al*., 2017). This approach can be combined with reduced herbicide use.

Finally, sainfoin crops have not suffered significant impact from most common pest and disease problems in Northern Europe compared with other legumes (Goplen *et al*., 1991; Frame *et al*., 1998). This has been attributed to the presence of a range of complex secondary metabolites within the foliage, including high molecular weight condensed tannins and polyphenols. Some minor damage through insect and nematode predation has been noted such as *Sitona scissifrons*, a weevil from the family Curculionidae (Morrill *et al*., 1998) and other members from this genus including *S. lineata, S. calloso* and *S. crinite* have been reported to damage sainfoin (Wallace, 1968). Similarly, sainfoin is rarely damaged by diseases, only certain *Fusarium spp* have been found to have an economic impact on the crop affecting survival over winter (Mathre, 1968). Farmers have occasionally noted the presence of other minor pathogens including, *Stemphyllium* sp. where infection led to black stems and characteristic pepper spots in leaves (Mathre, 1968).

General conclusions and perspectives

Sainfoin has significant potential for benefits to the farmer when included in a rotation, due to its environmental and nutraceutical attributes; however, its low productivity and difficulty relating to reliable establishment prevent many farmers from considering this crop a viable alternative to other forage legumes. Recently, advances in high-throughput sequencing have yielded markers that will enable further potential advances in targeted breeding programmes. Marker-Assisted Selection (MAS) programmes could ideally focus on improving the major disadvantages in comparison with other leguminous forage crops, such as slow establishment, poor competition with weeds and low yields; especially during the first establishment year. Phenotypic approaches like ‘phenomics’ or metabolomics strategies using NMR interrogation, could represent a significant step forward in the characterization of current varieties, and the combination of these techniques with MAS could promote the selection of new and more competitive varieties of sainfoin, that retain the many positive attributes it possesses. Weed control is another area where more research is necessary, the current knowledge of herbicide options and companion species choice have only been tested in a few geographical locations and some results are contradictory in the literature. In summary, advances in recent years have provided more opportunities for sainfoin to be considered as an alternative choice for farmers, particularly those interested in producing locally sourced protein and lower input, sustainable agricultural practices.

Acknowledgements

The authors thank the European Commission for funding this study (Marie Curie Initial Training Network, ‘LegumePlus’, PITN-GA-2011-289377). The authors wish to thank Javier Mora-Ortiz for support with graphics software and the authors who allowed them to replicate some of their pictures. Finally, thanks to Cotswold-Seeds LTD for the sainfoin drawing and picture.

References

Abou-El-Enain MM (2002) Chromosomal criteria and their phylogenetic implications in the genus *Onobrychis mill*. sect. *Lophobrychis* (leguminosae), with special reference to Egyptian species. *Botanical Journal of the Linnean Society* 139: 409–414.

Aigner I (2009) Our future grows in the country. Stuttgart: Federal Agriculture Minister. Available at http://www.bmelv.de/SharedDocs/Reden/EN/2009/07-02-German-Farmers-Day.html (accessed 15 March 2014).

Aktokly E (1995) *Turkiye'de yetisen Onobrychis Miller (Fabaceae) turlerinin revizyonu*. PhD, Inonu Universitesi Fen Bilimleri Enstitusu Biyoloji Anabilim Dali.

Aldrich DTA (1984) Lucerne, red clover and sainfoin-herbage production. In Forage legumes. Proceedings of a Symposium. Organised by the British Grassland Society, Maidenhead, pp. 126–131.

Allaby M (1987) *Diccionario del medio ambiente*. Madrid, España: Pirámide.

Amiri S, Karimmojeni H, Majidi MM and Boromand A (2013) Evaluation of post-emergence herbicides to control weeds of newly planted sainfoin (*Onobrychis sativa*). *Plant Knowledge Journal* 2: 4.

Badoux S (1965) *Etude des caractères morphologiques, physiologiques et agronomiques de populations d'esparcette (Onobrychis spp.)*. PhD, Recherche Agronomique Suisse.

Baimiev AK, Baimiev AK, Gubaidullin II, Kulikova OL and Chemeris AV (2007) Bacteria closely related to *Phyllobacterium trifolii* according to their 16S rRNA gene are discovered in the nodules of Hungarian sainfoin. *Russian Journal of Genetics* 43: 587–590.

Benaiges C (1971) *La esparceta*. Madrid, España: Ministerio de Agricultura.

Bland BF (1971) *Crop Production: Cereals and Legumes*. London, New York: Academic Press.

Blith W (1652) *The English improver improved, or the survey of husbandry surveyed discovering the improvableness of all lands*. London: J. Wright.

Borreani G, Peiretti PG and Tabacco E (2003) Evolution of yield and quality of sainfoin (*Onobrychis viciifolia* scop.) in the spring growth cycle. *Agronomie* 23: 193–201.

Botstein D, White RL, Skolnick M and Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32: 314–331.

Burton JC and Curley RL (1968) *Nodulation and nitrogen fixation in sainfoin (Onobrychis sativa LAM.) as influenced by* *strains of rhizobia*., Sainfoin Symposium. Mont., Agric. Exp. Stn. Bull.

Canals RM, Peralta J and Zubiri E (2009) *Flora Pratense y Forrajera Cultivada de la Península Ibérica. Familia Leguminoseae, Onobrychis viciifolia Scop.: esparceta* [Online]. Available at http://www.unavarra.es/herbario/pratenses/htm/Onob_vici_p.htm (accessed 12 March 2013).

Carleton AE and Wiesner LE (1968) *Production of sainfoin seed. Sainfoin Symposium*. Montana: Montana State University.

Carniol FI (1771) *Onobrychis viciifolia Scop* Fl. Carniol. ed. 2, 2: 76 (1771) ["viciaefolia"].

Cash S and Ditterline RL (1996) Seed size effects on growth and N-2 fixation of juvenile sainfoin. *Field Crops Research* 46: 6.

Chen B (1992) *Sainfoin*. Lanzhou, China: Gansun Sci-tech Press.

Clarke PJ, Davison EA and Fulloon L (2000) Germination and dormancy of grassy woodland and forest species: effects of smoke, heat, darkness and cold. *Australian Journal of Botany* 48: 687–699.

Cooper CS (1972) Establishment, hay yield, and persistence of two sainfoin growth types seeded alone and with low-growing grasses and legumes. *Agronomy Journal* 64: 2.

Cooper C and Fransen S (1974) Contribution of cotyledons to growth of the sainfoin seedling. *Crop Sciences* 14: 3.

Corti SR (1930) Nuovi reperti sulla cariologica di alcune leguminosae. *Nuovo Giornale Botanico Italiano* 37: 679–680.

Davies WAM (1815) *General view of the agriculture and domestic economy of South Wales; containing the counties of Brecon, Caermarthen, Cardigan, Glamorgan, Pembroke, Radnor*, M'millan.

de Candolle A (1883) *Origine des plantes cultivees*. Paris, France: Cambridge University Press.

Delgado I, Andrés C, Sin E and Ochoa M (2002) Estado actual del cultivo de la esparceta (*Onobrychis viciifolia* Scop.). Encuesta realizada a agricultores productores de semilla. *Pastos* 12: 235–247.

Delgado I, Muñoz F, Demdoum S and Buil I (2008) La esparceta o pipirigallo. In: *Informaciones técnicas/Centro de Transferencia Agroalimentaria, Gobierno de Aragón*. Aragón, Spain: Centro de Transferencia Agroalimentaria, pp. 1–8.

Demdoum S (2012b) *Caracterización agronómica y composición química de una colección de variedades de Esparceta*. PhD Thesis, Universidad de Lleida.

Demdoum S, Munoz F, Delgado I, Valderrabano J and Wuensch A (2012) EST-SSR cross-amplification and genetic similarity in Onobrychis genus. *Genetic Resources and Crop Evolution* 59.

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 4.

Drobná J (2010) Morphological variation in natural population of *Lotus corniculatus* in association to geographical parameters of collecting sites. *Biologia* 65: 5.

Dubbs AL (1968) The performance of sainfoin and sainfoin-grass mixture on dryland in central Montana. Sainfoin symposium; 1968 December 12–13, Bozeman, MT: Montana Agricultural Experiment Station Bulletin 627: 22–25.

Emre I, Turgut-balik D, Sahin A and Kursat M (2007) Total electrophoretic band patterns of some *Onobrychis* species growing in Turkey. *American-Eurasian Journal of Agricultural and Environmental Sciences* 2: 123–126.

European-Commission (2010) *The CAP Towards 2020: Meeting the Food, Natural Resources and Territorial Challenges of the Future. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions*. Brussels, Belgium: European Commission.

Font Quer P (1987) *Diccionario de botanica*. Barcelona, SA: Editorial Labor.

Frame J, Charlton JFL and Laidlaw AS (1998) *Temperate Forage Legumes*. Wallingford: CAB International.

Fulton T, Chunwongse J and Tanksley S (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter* 13: 2.

Galloni M, Podda L, Vivarelli D and Cristofolini G (2007) Pollen presentation, pollen-ovule ratios, and other reproductive traits in Mediterranean legumes (fam. Fabaceae-subfam. Faboideae). *Plant Systematics and Evolution* 266: 17.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP and Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320: 889–892.

García Salmerón J, Montserrat P, Buendía F, Ruiz-del-Castillo A and Allue J (1966) *Studies of Botany, Ecology, Biology and Pascology of the Principal Existing Species in the Spontaneous Pasture-Grounds of Mountains of our Semiarid Regions*. Madrid, España: Instituto Forestal de Investigaciones y Experiencias.

Goplen BP, Richards KW and Moyer JR (1991) *Sainfoin for Western Canada*. Ottawa, Ontario: Agriculture Canada Publication, 1470E.

Guner A, Ozhatay N, Ekim T and Baser K (2000) *Flora of Turkey and East Aegean Islands*, vol. 11, pp. 98–99. Edited by Press, E.U.

Harrison MJ (1998) Development of the arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology* 1: 5.

Hartwig UA and Nösberger J (1996) Symbiotic nitrogen fixation: a physiological link between plant and environment. In: Younie D (ed.) *Legumes in Sustainable Farming Systems*. Occasional Symposium No. 30. England: British Grassland Society, pp. 36–43.

Haynes RJ (1980) Competitive aspects of the grass-legume association. In: Brady NC (ed.) *Advances in Agronomy*. Academic Press, pp. 227–261. https://doi.org/10.1016/S0065-2113(08)60168-6

Hayot Carbonero C (2011b) *Sainfoin (Onobrychis viciifolia), a forage legume with great potential for sustainable agriculture, an insight on its morphological, agronomical, cytological and genetic characterisation*. PhD, The University of Manchester.

Hayot Carbonero C, Mueller-Harvey I, Brown TA and Smith L (2011) Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. *Plant Genetic Resources* 9: 70–85.

Hayot-Carbonero CH, Carbonero F, Smith LMJ and Brown TA (2012) Phylogenetic characterisation of Onobrychis species with special focus on the forage crop *Onobrychis viciifolia* Scop. *Genetic Resources and Crop Evolution* 59: 1777–1788.

Hayot Carbonero C, Carbonero F, Smith LMJ and Brown TA (2013) Cytological characterisation of the underutilized forage crop Onobrychis viciifolia Scop. and other members of the Onobrychis genus. *Genetic Resources and Crop Evolution* 60: 1987–1996.

Hill R (1997) Sainfoin: the not quite forgotten Legume. In: Lane GPF and Wilkinson JM (eds) *Alternative Forages for Ruminants Papers, Conference*. Cirencester, UK, pp. 55–59.

Hormaza JI (1999) Early selection in cherry combining RAPDs with embryo culture. *Scientia Horticulturae*, 79, 5.

ILDIS (International Legume Database & Information Service) (2005) Website: https://ildis.org. Available at https://ildis.org/index.shtml.

Jensen E and Sharp M (1968) Agronomic evaluation of sainfoin in Nevada. *Sainfoin Symposium* Montana State University.

Kallenbanch R, Matches A and Mahan J (1996) Sainfoin regrowth declines as metabolic rate increases with temperatures. *Crop Sciences* 36: 6.

Karshibaev HK (1992) Chromosome numbers of some Fabaceae in Uzbekistan. *Soveshchanie Po Kariologii Ratenii* 27: 2.

Kells A (2001) Sainfoin: an alternative forage crop for bees. *Bee World* 82: 192–194.

Kempf K (2016) *Self-Fertilization and Marker-Trait Associations in Sainfoin (Onobrychis Viciifolia)* PhD, ETH Zürich.

Kempf K, Mora-Ortiz M, Smith LMJ, Kolliker R and Skot L (2016) Characterization of novel SSR markers in diverse sainfoin (*Onobrychis viciifolia*) germplasm. *BMC Genetics*, 17.

Knuth P (1906) *Handbook of Flower Pollination Based upon Hermann Müller's Work 'The Fertilisation of Flowers by Insects'*. Oxford: Clarendon Press.

Koivisto JM (2001) *Sainfoin, worth another look*. PhD, Royal Agricultural College.

Koter Z (1965) Nutrition of leguminous plants. 1. The effect of different forms of N on the growth and symbiotic N2-fixation by red clover and sainfoin plants. 2. The effect of increasing amount of N on the yield and n2-fixation by Sainfoin (*Onobrychis viciifolia*). *Pamielnik Pulawski* 20: 34.

Lancha J and Sempere T (1988) *Diccionario de ciencias naturales. Usos y etimologías*. Madrid: Siglo veintiuno de España editores.

Lauriault LM, Contreras F, VanLeeuwen DM and Kirksey RE (2009) Sainfoin has natural tolerance to Glyphosate. *Plant Management Network*.

Lewke Bandara N, Papini A, Mosti S, Brown T and Smith LMJ (2013) A phylogenetic analysis of genus Onobrychis and its relationships within the tribe Hedysareae (Fabaceae). *Turkish Journal of Botany* 37: 981–992.

Liu Z, Baines RN, Lane GPF and Davies WAM (2009) Survival of plants of common sainfoin (*Onobrychis viciifolia* Scop.) in competition with two companion grass species. *Grass and Forage Sciences* 65: 3.

Lorenz M (2011) *Sainfoin Tannins and Their Impact on Protein Degradation During Silage and Rumen Fermentation and Testing of Novel Techniques*. Uppsala: Swedish University of Agricultural Sciences.

Lüscher A, Mueller-Harvey I, Soussana JF, Rees RM and Peyraud JL (2014) Potential of legume-based grassland–livestock systems in Europe: a review. *Grass and Forage Science* 69: 206–228.

Malik N and Waddington J (1988) Weed control strategies for forage legumes. *Weed Technology* 3: 288–296.

Malisch CS, Suter D, Studer B and Luscher A (2017) Multifunctional benefits of sainfoin mixtures: Effects of partner species, sowing density and cutting regime. *Grass and Forage Science* 72: 794–805.

Martinello P and Ciola A (1994) The effect of agronomic factors on seed and forage production in perennial legumes sainfoin (*Onobrychis viciifolia* Scop.) and French honeysuckle (*Hedysarum coranarium* L.). *Grass and Forage Science* 49: 8.

Massoud R, Karamian R and Hadadi A (2010) Cytosystematics of three *Onobrychis* species (Fabaceae) in Iran. *Caryologia* 63: 12.

Mathre D (1968) Disease in sainfoin. *Sainfoin Symposium* 65–66.

McGregor SE (1976) Insect pollination of cultivated crop plants. In *Legumes and Some Relatives*. U.S.D.A. Agriculture Handbook No. 496. Chap. 4. Available at https://www.ars.usda.gov/ARSUserFiles/20220500/OnlinePollinationHandbook.pdf.

Mesicek J and Sojak J (1992) Chromosome numbers of Mongolian angiosperms. *Preslia* 64: 13.

Meyer D (1975). Yield, regrowth and persistence of sainfoin under fertilisation. *Agronomy Journal* 67: 2.

Meyer D and Badaruddin M (2001) Frost tolerance of ten seedling legume species at four growth stages. *Crop Sciences* 41: 4.

Michelena A (1983) *Estudio de la variabilidad de la esparceta cultivada (Onobrychis viciifolia Scop.) en España*. PhD thesis. *198pp*. Escuela Técnica Superior de Ingenieros Agrónomos de Lérida. Lérida. Spain.

Miller DA and Hoveland CS (1995) *Other Temperate Legumes*. Ames, Iowa, USA: Iowa State University Press.

Moliner M (1982) *Diccionario de uso del español*. Madrid, España: Gredos.

Mora-Ortiz M (2015) *Pre-breeding, functional genomics and agronomic improvement of the tanniniferous forage crop Onobrychis viciifolia*. PhD Philosophy Doctorate Thesis, University of Reading.

Mora-Ortiz M and Smith L (2016) *Sainfoin – Surprising Science Behind a Forgotten Forage*. Moreton-in-Marsh, UK: Cotswold Grass Seeds.

Mora-Ortiz M, Wood TA, Philpott H, Skøt L and Smith LMJ (2015a) Pre-emergence herbicide treatments to improve the early establishment of sainfoin (*Onobrychis viciifolia*). In: De Ron AM (ed.) *V Meeting AEL. Actas AEL 6*, 2nd Edn. Pontevedra, Spain: Eucarpia International Symposium on Protein Crops.

Mora-Ortiz M, Wood TA, Skøt L and Smith LMJ (2015b) Weed control in sainfoin; pre-emergence treatments for the development of new strategies for the establishment of *Onobrychis viciifolia*. In: De Ron AM (ed.) *Eucarpia International Symposium on Protein Crops. V Meeting AEL. Plant Proteins For the Future*. Pontevedra, Spain.

Mora-Ortiz M, Swain MT, Vickers MJ, Hegarty MJ, Kelly R, Smith LMJ and Skøt L (2016) *De-novo* transcriptome assembly for gene identification, analysis, annotation, and molecular marker discovery in *Onobrychis viciifolia*. *BMC Genomics* 17: 756.

Morrill WL, Ditterline RL and Cash SD (1998) Insect pests and associated root pathogens of sainfoin in western USA. *Field Crops Research* 59: 129–134.

Moyer JR (1985) Effect of weed control and a companion crop on alfalfa and sainfoin establishment, yields and nutrient composition. *Canadian Journal of Plant Science* 65: 9.

Moyer JR, Hironaka R, Kozub GC and Bergen P (1990) Effect of herbicide treatments on dandelion, alfalfa and sainfoin yields and quality. *Canadian Journal of Plant Science* 70: 8.

Newman G (1997) An overview of forage legumes. In: Lane GPF and Wilkinson JM (eds) *Alternative Forages for Ruminants*. Cirencester, UK, pp. 3–14.

Novobilský A, Mueller-Harvey I and Thamsborg SM (2011) Condensed tannins act against cattle nematodes. *Veterinary Parasitology* 182: 213–220.

Pavlova D and Manova V (2000) Pollen morphology of the genera *Onobrychis* and *Hedysarum* (hedysareae, Fabaceae) in Bulgaria. *Annales Botanici Fennici* 37: 10.

Pellikaan WF, Stringano E, Leenaars J, Bongers DJGM, Schuppen SvL-v, Plant J and Mueller-Harvey I (2011) Evaluating effects of tannins on extent and rate of in vitro gas and CH_4 production using an automated pressure evaluation system (APES). *Animal Feed Science and Technology* 166: 377–390.

Polunin O (1991) *Guía de campo de las flores de Europa*. Barcelona, Spain: Omega.

Prevost D, Bordeleau LM and Antoun H (1987) Symbiotic effectiveness of indigenous arctic rhizobia on temperate forage legume – Sainfoin (*Onobrychis viciifolia*). *Plant and Soil* 104: 6.

Prosperi J, Demarquet F, Angevain M and Mansat P (1994) Evaluation agronomique de variétés de pays de sainfoin (*Onobrychis sativa* L.) originaires du sud-est de la France. *Agronomie* 14: 13.

Provorov NA and Tikhonovich IA (2003) Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. *Genetic Resources and Crop Evolution* 50: 10.

Rees J (1928) A preliminary survey of the rough pastures of the Llyn peninsula. Thesis for the degree of M.Sc, University of Wales, Bangor.

Sacristan MD (1966) Estudios citotaxonomicos sobre al género Onobrychis (L). Adanson con referencia especial a la citogenetica de la esparceta (*O. viciifolia* Scop). *Anales de La Estación Experimental de Aula Dei* 8: 1–114.

Seker H, Rowe DE and Brink GE (2003) White clover morphology changes with stress treatments. *Crop Sciences* 43, 7.

Sheehy J and McNeill A (1988) Factors controlling legume nitrogen fixation and their measurement in the field. Physiological limitations and the genetic improvement of symbiotic nitrogen fixation. *Advances in Agricultural Biotechnology* 23: 9.

Sheehy JE and Popple SC (1981) Photosynthesis, water relations, temperature and canopy structure as factors influencing the growth of sainfoin (*Onobrychis viciifolia* Scop.) and lucerne (*Medicago sativa* L.). *Annals of Botany* 48: 113–128.

Sheehy JE, Minchin FR and McNeill A (1984) Physiological principles governing the growth and development of lucerne, sainfoin and red clover. *Forage Legumes* 13: 113–125.

Sheldrick RD and Thomson DJ (1982) *Management and Utilization of Sainfoin (Onobrychis Sativa). I. Institute ed Hurley, Berkshire*. Berkshire: Institute ed Hurley.

Sheldrick R, Thomson D and Newman G (1987) *Sainfoin. In: Legumes for Milk and Meat*. Marlow, UK: Chalcombe Publications.

Sheldrick R, Newman G and Roberts D (1995) *Legumes for Milk and Meat*. Canterbury, UK: Chalcome Publications.

Sims J, Muir M and Carleton A (1968) Evidence of ineffective rhizobia and its relation to the nitrogen nutrition of sainfoin (*Onobrychis viciaefolia*). *Sainfoin Symposium*. Montana: Montana Agriculture Experimental Station Bulletin.

Širjaev G (1925) *Onobrychis Generis Revisio Critica*. Brno: Faculté des Sciences de l'Université Masaryk ed.

Slavicvk B, Jarolivmovav V and Chrtek J (1993) Chromosome counts of some plants from Cyprus. *Candollea* 48: 8.

Smoliak S, Jonston A and Hanna M (1972) Germination and seedling growth of alfalfa, sainfoin and cicer milkvetch. *Canadian Journal of Plant Science* 52: 5.

Somay Akcelik E, Avci S, Uzun S and Sancak C (2012) Karyotype analysis in some Onobrychis (sainfoin) species in Turkey. *Archives of Biological Science*, *Belgrade* 64: 567–571.

Stebler FG, Schroeter C and Volkart A (1894) *Les Meilleures Plantes Fourragères*. Berne: K.J. Wyss.

Stevovic V, Stanisavljevic R, Djukic D and Djurovic D (2010) Effect of row spacing on seed and forage yield in sainfoin (*Onobrychis viciifolia* Scop.) cultivars. *Tubitak* 36: 10.

Surayya K and Syed Irtifaq A (1991) Chromosome numbers in subfamily papilionoideae (Leguminosae) from Pakistan. *Willdenowia* 20: 159–165.

Tamas E (2006) Citological aspects of the Onobrychis genus. *Bulletin USAM* 62: 154–158.

Theodoridou K, Aufrère J, Andueza D, Le Morvan A, Picard F, Stringano E, Pourrat J, Mueller-Harvey I and Baumont R (2011) Effect of plant development during first and second growth cycle on chemical composition, condensed tannins and nutritive value of three sainfoin (*Onobrychis viciifolia*) varieties and lucerne. *Grass and Forage Science* 66, 402–414.

Thill J, Regos I, Farag MA, Ahmad AF, Kusek J, Castro A, Schlangen K, Carbonero CH, Gadjev IZ and Smith LMJ (2012) Polyphenol metabolism provides a screening tool for beneficial effects of *Onobrychis viciifolia* (sainfoin). *Phytochemistry* 82: 67–80.

Thomson JR (1938) Cross and self-fertility in sainfoin. *Annals of Applied Biology* 25, 695–704. 25, 9.

Thomson JR (1951) Seed studies in sainfoin. *Journal of British Grassland Society* 6: 12.

Tufenkci S, Erman M and Sonmez F (2006) Effects of phosphorus and nitrogen applications and Rhizobium inoculation on the yield and nutrient uptake of sainfoin (*Onobrychis viciifolia* L.) under irrigated conditions in Turkey. *New Zealand Journal of Agricultural Research* 49: 4.

Tull J (1733) *The Horse-Hoeing Husbandry; or, an Essay on the Principles of Tillage and Vegetation and Tillage. Designed to Introduce a New Method of Culture; Whereby the Produce of Land will be Increased, and the Usual Expence Lessened. Together with Accurate Descriptions and Cuts of the Instruments Employed in it*. London, UK: A. Millar.

Valdes E (2000) *Ononbrychis*. *En: Flora Ibérica*. Madrid, Castroviejo. CSIC.

Vicente MC and Arús P (1996) Tetrasomic inheritance of Isozymes in Sainfoin (Onobrychis viciaefolia Scop.). *Journal of Heredity* 87: 54–62.

Wallace LE (1968) *Current and Potential Insect Problems of Sainfoin in America*. *Sainfoin Symposium*. Montana: Montana Agriculture Experimental Station Bulletin.

Wallace R (1969) *Flora Europaea. Vol. 2 by T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, D. A. Webb*. Madroño; a West American journal of botany.

Weightman RM, Cottrill BR, Wiltshire JJJ, Kindred DR and Sylvester-Bradley R (2011) Opportunities for avoidance of land-use change through substitution of soya bean meal and cereals in European livestock diets with bioethanol coproducts. *Global Change Biology Bioenergy* 3: 158–170.

Wiesner L, Carleton A and Cooper C (1968) Factors affecting sainfoin seed germination and emergence. *Sainfoin Symposium*. Montana Agriculture Experimental Station Bulletin. Bozeman, Montana: Montana State University.

Yildiz B, Ciplak B and Aktoklu E (1999) Fruit morphology of sections of the genus *Onobrychis mille*r (*Fabaceae*) and its phylogenetic implications. *Israel Journal of Plant Sciences* 47: 269–282.

Young A (1913) *General View of the Agriculture of Oxfordshire. Drawn up for the Consideration of the Board of Agriculture and Internal Improvement*. London, UK: M'Millan, B.

Zolla D (1904) *Dictionnaire-manuel-illustré d'Agriculture*. Paris, France: Librairie Armand Colin.