




Sainfoin (*Onobrychis viciifolia*) a legume with great ecological and agronomical potential under climate change

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Abstract

Climate change is a global challenge to ecosystem services, altering crop yields and food security worldwide. In the context of climate change, *Onobrychis viciifolia* Scop. (sainfoin) can offer a multitude of ecosystem services conferred by its multifaceted beneficial properties. We reviewed the morphological, biochemical and physiological responses to environmental stressors of *O. viciifolia*, summarized its ecological, agronomic, nutritional and biological interests, and we discussed its use under climate change. *Onobrychis viciifolia* is a hemicyrptophyte forage legume adapted to arid and semiarid regions by evolving a diverse array of protective mechanisms against abiotic stressors at morphological, biochemical and physiological levels. In the present scenario of climate change, *O. viciifolia* has desirable forage characteristics such as high nutritive value, high voluntary intake and palatability to grazing animals, leading to satisfying animal performance for milk, meat, honey and wool production. Recent studies suggest that *O. viciifolia* has several highly beneficial phytochemical properties including condensed tannins and polyphenol content, which have been demonstrated to have anthelmintic activities, enhance protein utilization, and prevent bloating. In addition, *O. viciifolia* also has the potential to reduce greenhouse gas emissions and sequester atmospheric carbon and nitrogen into the soil. Ethnobotanical investigations show that *O. viciifolia* possesses antimicrobial, antiseptic and vulnerary activities. This review could be helpful for understanding of *O. viciifolia* characteristics, interests and uses, thus promoting its reasonable cultivation under a changing climate.

Introduction

Global climate change has become a compelling environmental problem as it is hindering the yield performance of crops due to increasing environmental stresses including pest diseases and extreme climatic events (Jiang *et al.*, 2016; Zhang *et al.*, 2019a; Bakala *et al.*, 2021; Skendžić *et al.*, 2021). In addition, climate change may exacerbate soil erosion (Lal, 2012) and cause disturbances to ecosystem functions (van der Geest *et al.*, 2019). Ecosystems functions are the respective direct and indirect benefits arising from the ecological functioning of healthy productive ecological systems (Millennium Ecosystem Assessment, 2005; Beaumont *et al.*, 2007). Crop yields are projected to decline through the 21st century (Zinyengere *et al.*, 2013; Petersen, 2019) and there is a need to identify and characterized perennial forage legumes that would be able to stand high environmental stress levels while offering relatively high agronomic production (Seo and Mendelsohn, 2008; Komainda *et al.*, 2019). Moreover, ecological restoration of degraded lands is included in the array of adaptation and mitigation responses to climate change (Harris *et al.*, 2006; O'Mara, 2012; Simonson *et al.*, 2021).

The genus *Onobrychis* Miller (Fam. Fabaceae) comprises a few agronomically known forage legume species such as *O. transcaucasica* Grossh., *O. arenaria* (Kit.) DC. and *O. viciifolia* Scop. (sainfoin), the most commonly cultivated species of the genus (Lock, 2005; Maberley, 2008; Amirahmadi *et al.*, 2014). *Onobrychis viciifolia* has a long history of traditional cultivation in Europe, Asia and North America in the 19th and 20th centuries (Miller and Hoveland, 1995; Frame *et al.*, 1998). Indeed, its cultivation decline started in the middle of the 20th century due to the adoption of more intensive farming methods with the introduction of relatively low-cost nitrogen (N) fertilizers (Carbonero *et al.*, 2011). Consequently, *O. viciifolia* cultivation was gradually displaced by alfalfa (*Medicago sativa* L.) and clover species (*Trifolium* spp.) whose

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higher yields and easier establishment made them more desirable to farmers. *Onobrychis viciifolia* can be cultivated in multitude soils and climatic conditions (Tufenkci et al., 2006; Carbonero et al., 2011; Yin et al., 2020). Agronomically, *O. viciifolia* have positive characteristics such as a deep tap root that allows it to be very tolerant to drought (Irani et al., 2015a, 2015b; Malisch et al., 2016) and N fixation up to 168 kg N₂/ha via symbiosis with rhizobia (Malisch et al., 2017). Culture trials of *O. viciifolia* under harsh climatic conditions of dry areas in the Middle East and North Africa (MENA) region gave promising results (Le Houérou, 1969; Jafari et al., 2014; Sayar et al., 2022). In addition, *O. viciifolia* is also particularly valued for its content of condensed tannins, which have been shown to improve animal growth and health (Waghorn, 2008; Girard et al., 2016).

The Mediterranean Basin has been identified as one of the most climate-vulnerable regions and a climate change 'hotspot' (Salvia et al., 2021). In addition, the Mediterranean Basin is one of the areas with the most serious soil degradation and desertification rates in the world, reaching critical limits for its ability to provide ecosystem services and land productivity (Ferreira et al., 2022). In this sense, the large-scale grassland degradation in Mediterranean Basin aggravates the shortage of forage supply (Ferreira et al., 2022; Soares et al., 2022). Consequently, restoring degraded grasslands and increasing forage grass supply are urgent needs in this area, but the forage quality of *O. viciifolia* has not been deeply studied in the Mediterranean Basin. Additionally, *O. viciifolia* has obvious application advantages in soil and vegetation restoration that should be also analysed in detail in the Mediterranean Basin.

The study of *O. viciifolia* has regained interest in recent decades (Fig. 1) and four studies have reviewed its cultivation and agronomic potential (Carbonero et al., 2011; Bhattarai et al., 2016; Mora-Ortiz and Smith, 2018; Sheppard et al., 2019). However, no work has focussed on the role of *O. viciifolia* in the present scenario of climate change. In this work, we review the geographical distribution, main functional traits, stress tolerance and beneficial proprieties for humans of *O. viciifolia* under the ongoing climate change scenario. Our review is meant to provide information on the importance of

O. viciifolia for its possible utilization in cropping systems in a changing environment. Also, it is an attempt to recognize the lesser-explored aspects and knowledge gaps in the research on *O. viciifolia*.

Methods

Google Scholar, Web of Science, Springer and PubMed databases were used to search for published literature on *O. viciifolia*. The filtering was based on titles, abstracts and keywords including the words *Hedysarum onobrychis* L., *Onobrychis sativa* Lam., *Onobrychis viciaefolia* Scop. and *O. viciifolia* Scop. or sainfoin. Afterwards, the full text of all peer reviewed articles, books, book chapters and PhD thesis were reviewed. The deadline for the literature selected was up to July 2024. The Plant List (<http://www.theplantlist.org/tpl1.1/search?q=Onobrychis+viciifolia>), International Plant Name Index (<https://www.ipni.org/n/510168-1>), Kew Botanical Garden (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:510168-1>) and Global Biodiversity Information Facility (<https://www.gbif.org/fr/species/2972595>) were used for validating the scientific name as well as information on cultivars and the species synonyms.

Origin and distribution

Onobrychis viciifolia is derived from the natural hybridization between *O. arenaria* and *O. montana* (Falistocco, 1991), which are native to Central-Southern Europe, and temperate Southwest Asia and North Africa, respectively (Angevain and Prosperi, 1995; Jin et al., 2021). *Onobrychis viciifolia* is mostly tetraploid, through there are reports of diploid accessions (Carbonero et al., 2013). In addition, Abou-El-Enain (2002) reported the appearance of $2n = 22, 27, 28$ and 29 chromosomes that demonstrated the role of aneuploid alterations in the evolution of this species.

A few *Onobrychis* taxa, such as *O. viciifolia*, have been cultivated for hundreds of years as forage and ornamental crops in warm and temperate regions of Europe, Asia and North

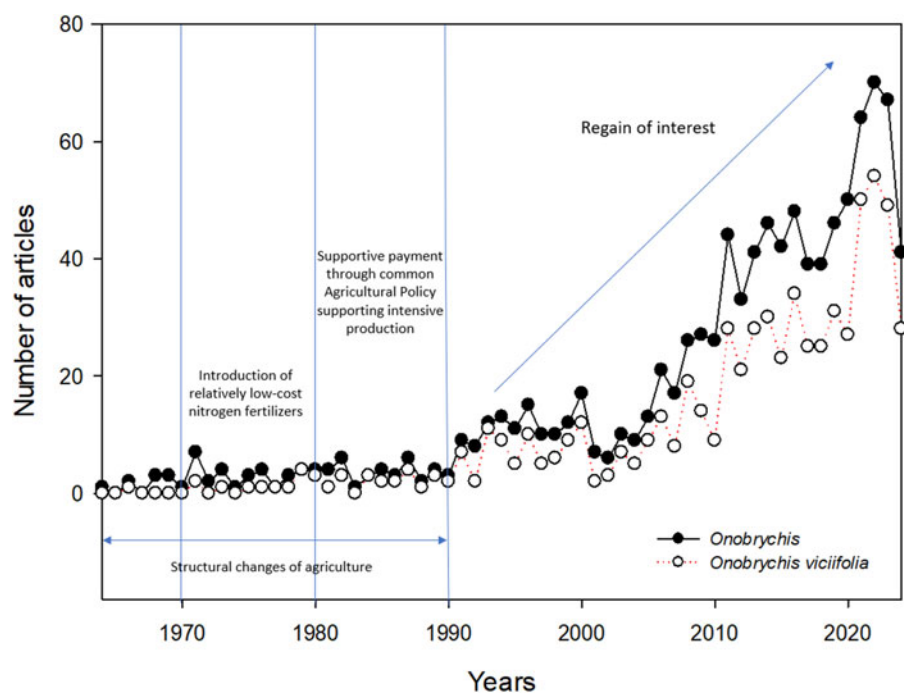


Figure 1. Evolution of annual number of publications on *Onobrychis* species and *O. viciifolia* published from 1963 to 2024 (Sources: Google Scholar, Web of Science, Springer and PubMed).

America (Lock and Simpson, 1991; Yakovlev *et al.*, 1996; Mabberley, 1997; Frame *et al.*, 1998; Frame, 2005; Carbonero *et al.*, 2011) (Fig. 2). Cultivated *Onobrychis* species were introduced to Central Europe from the Mediterranean Basin during the 16th century (Piper, 1924; Burton and Curley, 1968). *Onobrychis* cultivation was introduced to North America in 1786 (Bhattarai *et al.*, 2016), but was only grown occasionally until the 1960s, when improved varieties allowed wider cultivation. Today, *O. viciifolia* is cultivated mainly in Eastern Europe, Iran and around the Mediterranean Basin (Eken *et al.*, 2004; Avci *et al.*, 2014; Bolat, 2019). The Mediterranean Basin is one of the most affected regions by climate change (Cammarano *et al.*, 2019). One of the most relevant consequences of climate change is certainly water scarcity, as result of a reduction of surface runoff and groundwater levels (Noto *et al.*, 2023). *Onobrychis viciifolia* once represented an important forage legume in semi-arid environments of Italy, but its cultivation area has decreased from 160 to 9 thousand ha from 1983 to 2013 (ISTAT, 2013). It is recorded that more than 150 tonnes of seeds were sold every year in the late 1950s in the UK, enough for cropping 2500 ha (Hill, 1997). In the late 1970s, only approximately 150 ha were cropped. Today, *O. viciifolia* has become rare in the UK, and this is due, in part, to its poor response to the changing requirements and circumstance of British agriculture (Hutchinson, 1966). *Onobrychis viciifolia* could potentially be grown on 950 thousand ha in England and Wales, where the soil is sufficiently alkaline (Doyle *et al.*, 1984). On the other hand, Wang *et al.* (2018) mentioned *O. viciifolia* as a major fodder grass species cultivated in Gansu Province, Northwest China, in 2016.

Responses to environmental stressors

Biotic stressors

Ongoing climate change poses considerable threats to sustainable food security, including increased number of generations of pests and plant pathogens resulting from a compressed life cycle due to a warmer climate combined to elevated CO₂ concentrations, increased risk of invasion by migratory pests and reduced effectiveness of biological control (Skendžić *et al.*, 2021). As climate change exacerbates the pest problem, there is a great need for future pest management strategies (Wei *et al.*, 2020). *Onobrychis viciifolia* is relatively resistant to most common pests and diseases in Western Canada and Northern Europe compared with other legumes such as *M. sativa* (Goplen *et al.*, 1991; Frame *et al.*, 1998). This has been attributed to the presence of a range of secondary metabolites, such as tannins and polyphenols, within the foliage of *O. viciifolia* (Malisch *et al.*, 2016). Even so, *O. viciifolia* can be damaged by fungal diseases related to certain *Fusarium*, *Stemphyllium* and *Sclerotinia* species (Mathre, 1968). In addition, an important number of insect and nematode species can damage *O. viciifolia* stands (Mathre, 1968; Wallace, 1968; Morrill *et al.*, 1998). But *O. viciifolia* is resistant to the alfalfa weevil (*Hypera postica* Gyll.), so it can be an alternative forage legume to *M. sativa* in areas where this pest causes severe damage (Morrill *et al.*, 1998; Böttger *et al.*, 2013).

Onobrychis viciifolia is relatively resistant and free from serious pest and disease problems compared with other legumes such as *M. sativa* (Goplen *et al.*, 1991). *Medicago sativa* suffers from several economically important insect pests such as *H. postica* and

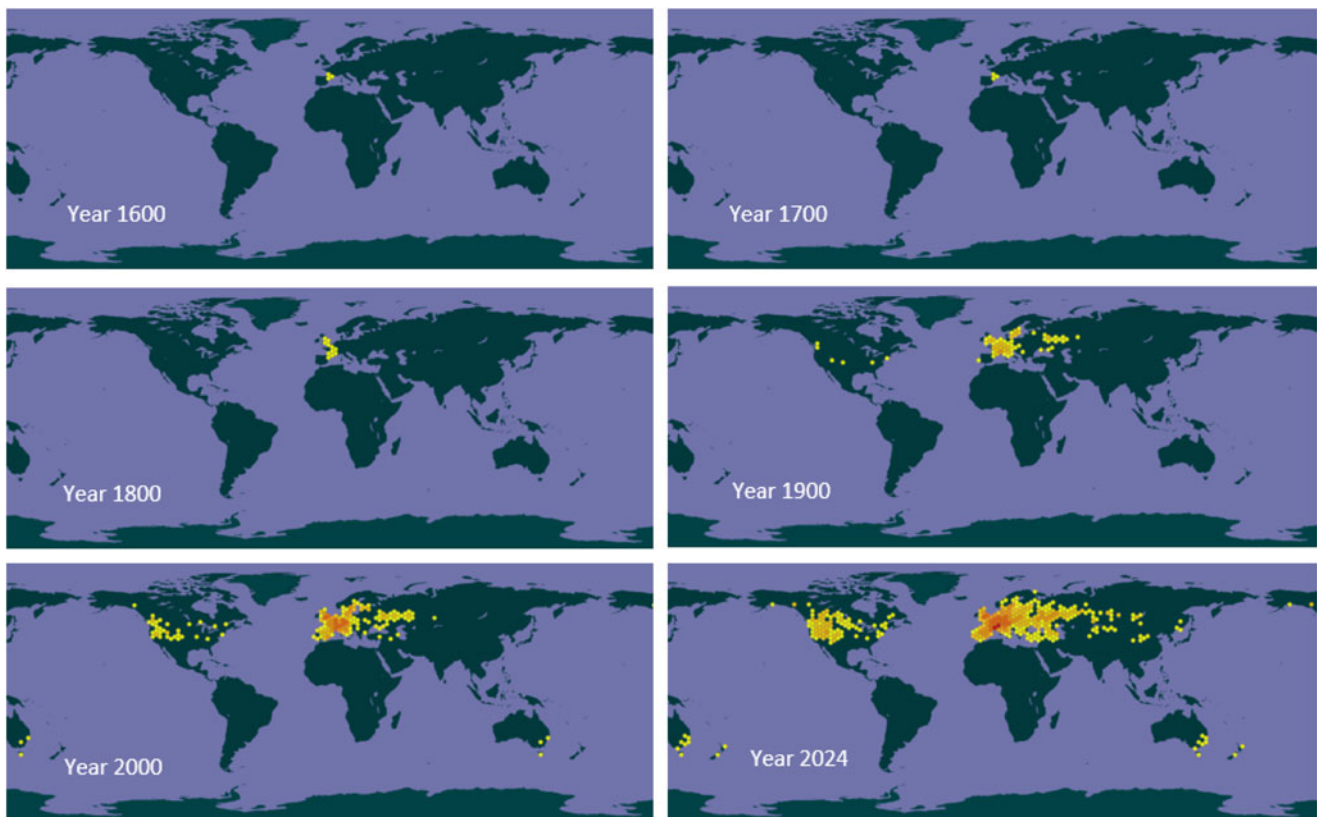


Figure 2. Centenary evolution of worldwide geographical distribution of *Onobrychis viciifolia* (88,635 occurrences) from 1600 to 2024. The colours from yellow to orange represent the density of occurrence for a given area.

Source: Global Biodiversity Information Facility, <https://www.gbif.org/>

Acyrtosiphon pisum, which do not affect *O. viciifolia*. This could encourage farmers to grow *O. viciifolia* as an alternative solution to *M. sativa* (Morrill et al., 1998). Two closely related species to *H. postica* (*Phytonomus farinosus* and *Hypera trilineata*) are mentioned as pests of *O. viciifolia* in some European countries (Wallace, 1968). Other pests such as *Empoasca fabae*, *Lygus elisus*, *L. hesperus* and *Adelphocoris lineolatus* appeared to have marginal effects on *O. viciifolia* and occurs only in localized areas (Morrill et al., 1998). Furthermore, only one pest (*Sitona scissifrons*), which feed on the roots of *O. viciifolia*, has been observed on accessions growing in United Kingdom, but there were no accessions that were severely affected (Carbonero et al., 2011). Adult *S. scissifrons* weevils become active in the field in June and eat the edges of the leaves of *O. viciifolia*, leaving characteristic notches along the leaves in Montana (USA). This damage could be disastrous at the seedling stage in the field (Wallace, 1968). Other members from this genus, including *S. lineata*, *S. calloso* and *S. crinite*, have been reported to cause minor damage in *O. viciifolia* in Europe (Wallace, 1968). However, *Contarinia onobrychidis* and *Eurytoma onobrychidis* represent a serious pest for *O. viciifolia* in some areas of Europe (Wallace, 1968). Other insects can also damage the seed production of *O. viciifolia* in Europe but marginally; these include *Perrisia onobrychidis*, *Apion pisi*, *Odontothrips intermedius*, *Otiorynchus ligustici* and *Melanotus erythropus*. Therefore, the inclusion of *O. viciifolia* as a rotation component could affect the presence of host-specific pests by disturbing their life cycle.

Abiotic stressors

Climate change is increasing the frequency and intensity of abiotic stress combinations that pose a serious threat to crop productivity (Zandalinas et al., 2022). *Onobrychis viciifolia* have evolved a diverse array of protective mechanisms against abiotic stressors at biochemical and physiological levels related to hormone homeostasis, transcriptional factors, photosynthesis, and the biosynthesis of antioxidants and osmotic adjustment-related substances (Yin et al., 2021). Consequently, *O. viciifolia* tolerates low nutrient conditions (Carbonero et al., 2011), high levels of active lime in the soil (De Falco et al., 2000a, 2000b), drought and alkalinity, and saline-alkaline stress (Fig. 3). In addition, *O. viciifolia* tolerates high concentrations of lead and copper (Beladi et al., 2011) and can grow in coal mined areas (Roy et al., 2021). Moreover, *O. viciifolia* can grow under seasonally cold and hot climatic conditions (Sengul, 2003; Tufenkci et al., 2006), and extreme climatic conditions at high altitudes (Yin et al., 2020, 2021). Rhizobia have the potential to be used in improving symbiotic N fixation on *O. viciifolia* under cold stress (Prévost et al., 2003). Climate change can affect the intensity and frequency of precipitation (Feng et al., 2019) and worsen ozone pollution over many populated regions, with larger impacts at higher concentrations (East et al., 2024). It has long been observed that the environmental gamma-ray dose rate increases noticeably during precipitation intervals (Mercier et al., 2009). *Onobrychis viciifolia* tolerates high levels of gamma radiation (Beyaz et al., 2016), but its growth is reduced after ozone exposure (Bungener et al., 1999).

Salt tolerance

Salt is one of the main abiotic stresses affecting crop yields around the world (Zörb et al., 2019). Seed germination is one of the most sensitive physiological phenomena to stress in the lifecycle of

plants (Al-Turki et al., 2022), and seeds of *O. viciifolia* withstand moderate saline environments (up to 400 mM). In this sense, seeds of *O. viciifolia* retain germinability under high salinity, displaying tolerance mechanisms such as physiological dormancy till the occurrence of favourable conditions (Li et al., 2021a, 2021b). Karamian and Ataei-Barazande (2013) reported decreasing germination rates in *O. viciifolia* with increasing salinity levels over 200 mM NaCl and indicated that germination was totally inhibited at 400 mM NaCl which is higher than for *Trifolium repens* L. (up to 180 mM) (Chu et al., 2022), *Trifolium pratense* L. (up to 240 mM) (Asci, 2011) and *M. sativa* (up to 257 mM) (Kadri et al., 2021). The germination of *O. viciifolia* is considered more tolerant to salinities c. 170 mM NaCl than other *Onobrychis* species (Uzun et al., 2017).

Saline-alkaline conditions resulted in oxidative stress and the accumulation of proline in seedlings of *O. viciifolia* (Wu et al., 2021). Along with organic osmolytes, *O. viciifolia* under salinity also increased the production of reactive oxygen species (ROS) scavengers such as catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase (Beyaz, 2019). In fact, *O. viciifolia* can grow without much yield and quality loss in salt-affected areas (c. 109 mM NaCl), where it can provide enough high-quality forage production for livestock without altering its macro-mineral content (Temel et al., 2016a, 2016b) (Fig. 3a). Nonetheless, Wu et al. (2017a, 2017b) reported reduced growth and chlorophyll and water contents, diminished root potassium concentration, and increased malondialdehyde (MDA) concentration and relative membrane permeability in *O. viciifolia* shoots under salinity (100 mM NaCl). This study also reported that, when supplied exogenously, proline and silicon improved salt stress tolerance in *O. viciifolia* by mitigating sodium toxicity (Fig. 3a). Under salinity, *O. viciifolia* maintain better cellular function and overall physiological homeostasis evidenced by less drastic imbalance in intracellular Na^+/K^+ ratio than *M. sativa* (Li et al., 2010; Beyaz 2019) which is one of the key determinants of plant salt tolerance under climate change (El Sabagh et al., 2021).

Drought tolerance

Drought is the largest contributor to world-wide crop losses (Lesk et al., 2016; Santos-Medellín et al., 2021). In this general context that is exacerbated by climate change (Cook et al., 2018), *O. viciifolia* produces indehiscent fruits (pods) that break down slowly, promoting seed survivorship during drought periods (Majidi and Barati, 2011; Avci and Kaya, 2013). In this sense, organic, hydrogel and mineral seed coating improved germination speed of *O. viciifolia* with and without drought (Mehrabi and Chaichi, 2012). Contradictorily, Kintl et al. (2021) showed that *O. viciifolia* did not respond positively to the seed coating under drought. Uncoated seeds exhibited a greater drought resistance than the coated seeds which showed a sharp, significant decline of germination capacity and a great increase in the dead seed percentage due to the death of a fraction from the hard seeds.

Nasirzadeh et al. (2005) reported that *O. viciifolia* can be considered as a semi-resistant species to drought. Adult plants of *O. viciifolia* tolerate exposure to the combined effects of drought and ozone (Bungener et al., 1999). The response mechanisms of *O. viciifolia* to drought include osmotic adjustment (Dehabadi, 1997; Irani et al., 2015a, 2015b; Beyaz, 2019; Beyaz and Yildiz, 2021), ROS scavenging, reduced transpiration (Roy et al., 2021), increasing stomatal resistance and water use efficiency (Dehabadi et al., 1993a, 1993b; Dehabadi, 1997; Huang

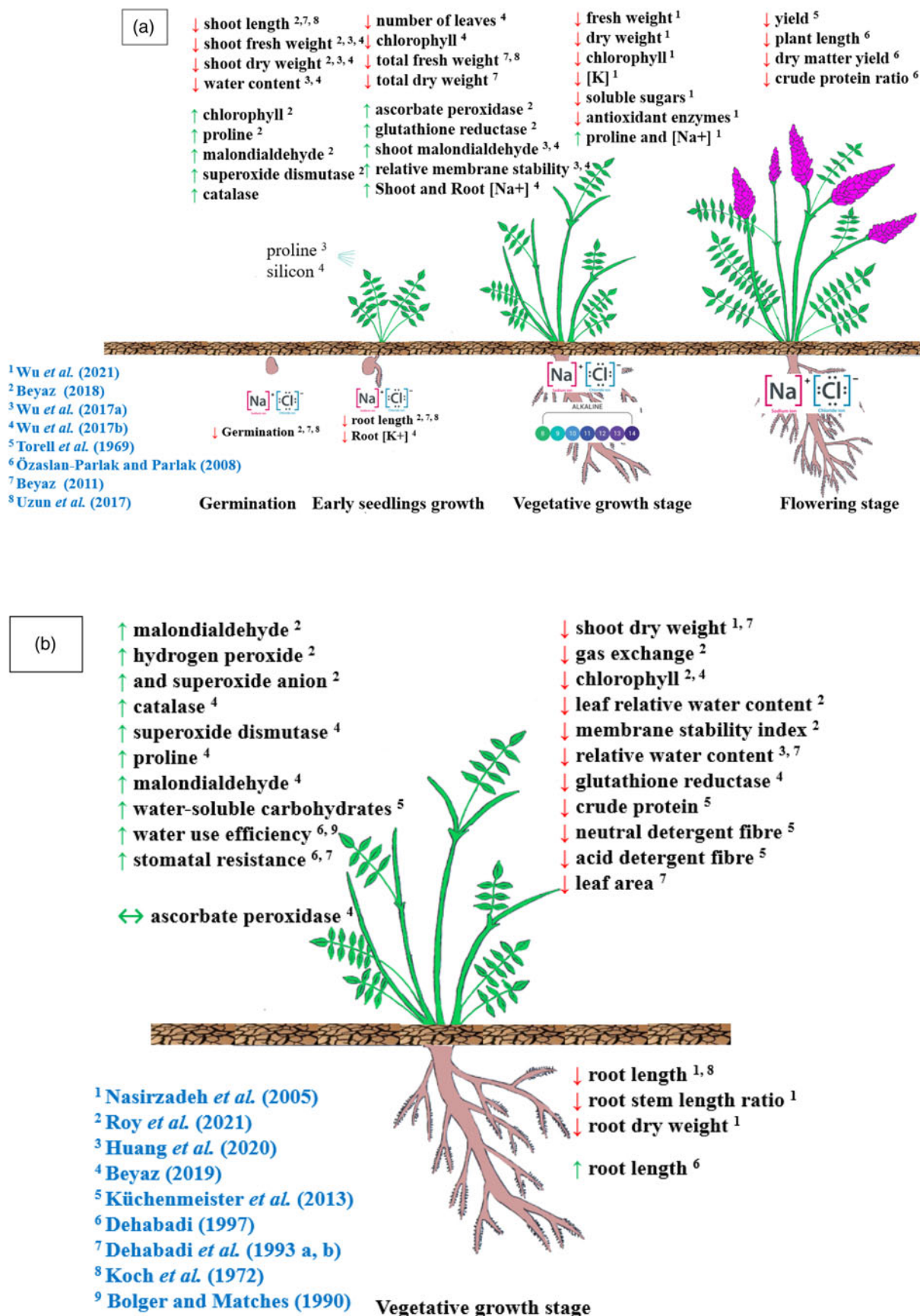


Figure 3. Summary of the main tolerance mechanisms of *Onobrychis viciifolia* to (a) salinity and (b) drought stress.

et al., 2020), and increasing carotenoid contents with decreasing chlorophyll contents (Irani *et al.*, 2015a, 2015b). In addition, *O. viciifolia* tolerance to drought involves also morphological adaptations such as a deep root system (Koch *et al.*, 1972; Dehabadi, 1997; March-Salas *et al.*, 2021) and reduced leaf area (Dehabadi *et al.*, 1993b). Besides all its responses to drought, *O. viciifolia* shows reduced plant heights which resulted in significant yield losses when exposed to severe drought (Irani *et al.*, 2015a, 2015b). In this sense, Bolger and Matches (1990) found a higher yield potential for *O. viciifolia* in spring than in summer, possibly indicating higher water use efficiency. To tide over periodic drought stress, Malisch *et al.* (2016) highlighted the importance of harvesting at optimal stages to have good forage performance for *O. viciifolia*.

Onobrychis viciifolia have greater ability to resist drought and adapt to dry habitats compared to several forage species. Considering the field capacity as the upper limit of soil water availability, *O. viciifolia* had the widest range of adaptability to soil water compared to other crop species such as *Astragalus adsurgens*, *Elymus nutans* and *Lolium multiflorum* (Huang *et al.*, 2020). Moreover, *O. viciifolia* exhibited a lower average lower limit of relative soil water content compared to *A. adsurgens*, *E. nutans*, and *L. multiflorum*. In addition, *O. viciifolia* closed stomata more rapidly with the decrease of relative soil water content, suggesting that the species had better drought resistance and allowed plants to keep water in plant tissues (Huang *et al.*, 2020). The intrinsic water use efficiency (WUE_i) represents an important indicator of the adaptability for higher plants to climate change (Weiwei *et al.*, 2018). WUE_i increased more rapidly in *O. viciifolia* than the other three forage under moderate water deficit (Huang *et al.*, 2020).

High temperatures tolerance

Onobrychis viciifolia is an alternative forage for semi-arid regions, where *M. sativa* and *Trifolium* sp. cannot be sown. Although there is little published data, there is considerable observational evidence that *O. viciifolia* is tolerant to relatively high temperatures. Maximum air temperatures of above 32°C did not affect *O. viciifolia* in small plots in northern Greece and southern Spain (Carbonero *et al.*, 2011). However, *O. viciifolia* exhibits poor growth following periods of high ambient temperatures and few plants survived at 35°C due to severe leaf loss causing plant death because high metabolic rates cannot be supported by existing leaf area or taproot carbohydrates even under well-watered conditions (Kallenbach *et al.*, 1996). High temperatures at the beginning of summer interrupt regrowth under traditional and intensive cutting regimes in southern Italy (De Falco *et al.*, 2000a, 2000b). *Onobrychis viciifolia* had a higher rate of biomass accumulation compared to *M. sativa*. However, *M. sativa* tended to grow faster during the warmer months (July–September) in Texas (USA).

Interests and uses

Agronomic characteristics and value

Changing climatic conditions have reduced plant productivity and generated food security issues. In this context of food security, legumes exhibit promising benefits making them an exceptional food to meet nutritional needs (Akram *et al.*, 2018). *Onobrychis viciifolia* is considered as an excellent component of a rotation in cropping systems by enhancing productivity and improving

soil physicochemical properties including soil texture, fertility, water retention and organic matter content (Decourtye *et al.*, 2007; Malisch *et al.*, 2017; Sariyildiz and Savaci, 2020).

Agronomic advantages

Global climate change is predicted to impact on soil fertility through the physical, chemical, and biological properties of soil due to rise in temperature, alternation in precipitation patterns, increase in greenhouse gases concentration in the atmosphere, etc. (Mondal, 2021; Bibi and Rahman, 2023). In addition, climate change could lead to the loss of soil function for fertility maintenance and greater dependence on mineral fertilizers (Pareek, 2017). For centuries, *O. viciifolia* was widely grown across Europe before commercial fertilizers were used. The species does not need fertile soil to thrive if its requirements for lime and humidity are satisfied. *Onobrychis viciifolia* can thrive in less fertile soils than *M. sativa* and *T. repens* and can also grow well in more fertile soils. *Medicago sativa* and *T. repens* will, however, produce better yields in fertile and irrigated lands, but *O. viciifolia* provides better outcomes growing in low fertility soils compared with *M. sativa* (Benaiges, 1971; Demdoun, 2012).

Mature plants of *O. viciifolia* have over 2-m-long taproots, partly responsible for its drought tolerance. The root is quite branched, especially at the bottom and multiples thin lateral roots constitute the bulk of the root system (Carbonero *et al.*, 2011; Mora-Ortiz and Smith, 2018). The *O. viciifolia* root systems rivals *M. sativa* for its ability to access deep subterranean waters (Mora-Ortiz and Smith, 2018). In the Mediterranean Basin, *O. viciifolia* prefers altitudes above 600 m, but it performs well when cultivated in a range between 100 and 2500 m (García Salmerón *et al.*, 1966; Demdoun, 2012).

Agronomic disadvantages

Onobrychis viciifolia is a forage legume of renewed interest worldwide, with equally weighted advantages and disadvantages that prevent many farmers from considering this crop a viable alternative to other forage legumes. In this sense, a wide distribution of *O. viciifolia* in productive grassland systems is hampered by the limited availability of high-performing cultivars adapted to different environmental conditions (Subedi, 2018; Sheppard *et al.*, 2019). In fact, *O. viciifolia* weaknesses are related to its lower yield when compared to other forage legumes, its poor competitive ability against weeds especially during the establishment year, a limited persistence, susceptibility to waterlogging and frost (Sheehy and Popple, 1981; Liu *et al.*, 2010), low tolerance to frequent cutting (Malisch *et al.*, 2017), and susceptibility to diseases such as powdery mildew or *Phytophthora* root rot (Sears *et al.*, 1975; Carbonero *et al.*, 2011). *Onobrychis viciifolia* prefers well-drained soils and does not grow well in heavy soils or under flood irrigation (García Salmerón *et al.*, 1966; Demdoun, 2012; Anderson, 2016). *Onobrychis viciifolia* does not perform properly in acidic soils. Poor *O. viciifolia* establishment was obtained on soils at pH lower than 6 (Bland, 1971; Carbonero *et al.*, 2011). In Spain, *O. viciifolia* is traditionally cultivated on neutral or slightly alkaline brown-earth soils.

Onobrychis viciifolia died out primarily during winter due to aerial interspecific competition, resulting in reduced root reserves (Liu *et al.*, 2010). Persistence of *O. viciifolia* appears to be dependent on minimal pressure from competing plants, harvest or grazing, and good growth conditions from midsummer into fall, allowing for adequate root reserves for survival. It is possible that judicious use of glyphosate in late season might lessen

competition in that critical period as *O. viciifolia* is more glyphosate-tolerant than *M. sativa* (Peel *et al.*, 2013).

Crop residue

Due to its deep, extensive and nodulated roots system, *O. viciifolia* uses water reserves in deep soil layers, increases carbon sequestration, reduces moisture and nutrient loss through leaching and runoff, prevents soil erosion, and improves the physicochemical and microbiological properties of the soil, including reductions on nitrification rates (Sergeeva, 1955; Komainda *et al.*, 2019; Clemensen *et al.*, 2022). The irrigated cultivation of *O. viciifolia* accumulate in the soil up to 16.2 t/ha/year of residues, which represents approximately four times the quantity left by *M. sativa* (4.2 t/ha/year) (Sergeeva, 1955). This could be explained by its high root biomass compared to other cultivated legumes (Bolat, 2019; Rossi *et al.*, 2020). In fact, *O. viciifolia* can be used for soil organic matter improvement (Porqueddu *et al.*, 2000; Wu *et al.*, 2018). In view of these studies, cultivating *O. viciifolia* would help increasing the role of agricultural soils as carbon sinks to mitigate climate change.

In addition to supplies biologically fixed N₂, *O. viciifolia* displays a range of adaptations for the acquisition and retention of other important resources. *O. viciifolia* has excellent resource use efficiency in low input environments (Carbonero *et al.*, 2011). By virtue of their large active root systems and mycorrhization, *O. viciifolia* can efficiently accelerate the absorption of water and nutrients (Kong *et al.*, 2014). *Onobrychis viciifolia* has the ability to acquire phosphorous via specialized root structures, through arbuscular mycorrhizal associations under controlled environment (Kong *et al.*, 2014). The large rooting systems of *O. viciifolia* function to increase soil organic C by reducing erosion, reducing microbial respiration (via lack of tillage). As a result, nutrients are retained in the cropping system for use by subsequent crops (Carbonero *et al.*, 2011).

Fossil fuel burning in the energy sector is a major contributor to greenhouse gas emissions. Biofuels, considered as a substitute for fossil fuels, have become top priority due to its eco-friendly nature (Prasad *et al.*, 2020). In addition to the biodiesel production from the legume (Ndukwu and Onyeoziri, 2022), all the biomass of *O. viciifolia* can serve to produce second-generation biofuel due to its high N content (Slepetys *et al.*, 2012). Moreover, the use of *O. viciifolia* showed potential for methane production in biogas plants (Hunady *et al.*, 2021).

Onobrychis viciifolia contains phenolic compounds (tannins) that can influence soil nutrient dynamics by inhibiting microbial activity, which could slow N mineralization rates and minimize N losses in field (Clemensen *et al.*, 2020, 2022; Slebodnik *et al.*, 2019). Tannins, at low concentrations in the soil, may increase some enzyme activity (Adamczyk *et al.*, 2017), inactivate other soil enzymes, in part due to their antibiotic properties (Benoit and Starkey, 1968; Field and Lettinga, 1992; Joannis *et al.*, 2007; Triebwasser *et al.*, 2012), and to the formation of tannin–protein complexes (Adamczyk *et al.*, 2019). Dehydrogenase enzymatic activity was reported to be higher in *O. viciifolia* than *M. sativa* sown plots (Clemensen *et al.*, 2020, 2022).

Atmospheric di-nitrogen fixation

Crop production is dependent on inorganic N and other fertilizers inputs to resupply nutrients lost as harvested grain and forage, via soil erosion/runoff, and by other natural or anthropogenic causes (Schlautman *et al.*, 2018; Bibi and Rahman, 2023). Nitrogen-fertilizers are one of the most monetary and environmentally expensive inputs in agricultural settings,

which are currently more expensive than ever before (Herrera *et al.*, 2016; Adjesiwor and Islam, 2016). The mobility of applied inorganic N fertilizers results in less than 50% fertilizer N-recovery efficiency by the first crop with substantial amounts of the remaining N leaving the cropping system as N₂O and NO₃ (Crews and Peoples, 2004; Ladha *et al.*, 2005). *Onobrychis* species are soil enriching via the fixation of atmospheric N. The symbiosis between legumes and root-nodule bacteria supplies biologically fixed N to natural and agroecosystems around the globe (Crews, 1993). This form of N is directly incorporated into the growing plant, overcoming problems of low fertilizer N-recovery efficiency. *Onobrychis viciifolia* is able to provide relief from reliance on synthetic N while supplying high forage quality in low-input agricultural ecosystems (Carbonero *et al.*, 2011). While N₂ fixation in legumes is considered to have higher energy and carbon requirements than N assimilation by plants using reduction of NO₃ for growth, the energy is supplied via solar radiation rather than through fossil fuels; thus, the resulting CO₂ respired by the nodules originates through photosynthesis and is not a net contributor to atmospheric CO₂ concentrations (Crews and Peoples, 2004; Jensen *et al.*, 2012).

Onobrychis species form symbioses with bacteria belonging to the genera *Mesorhizobium*, *Rhizobium* and *Bradyrhizobium* (Baimiev *et al.*, 2007). In field conditions, Nitrogen deficiency symptoms were reported in *O. viciifolia* despite plants being abundantly nodulated, which indicates that the strain of N-fixing bacteria present was inefficient or short lived (Burton and Curley, 1968; Schreiner *et al.*, 1969), but these symptoms disappeared with time in plants nodulated by effective strains (Prévost *et al.*, 1987a). Small young nodules are generally the most effective in *O. viciifolia* and are scarce in early stages of growth in the field (Burton and Curley, 1968). In this sense, it seems likely that *O. viciifolia* is dependent on some mineral N at early growth stages and later growth stages benefit significantly from an effective symbiosis (Carbonero *et al.*, 2011). However, the N-fixing system of *O. viciifolia* is very sensitive to low levels of nitrate under glasshouse conditions (Hume and Withers, 1985). *Onobrychis viciifolia* can be cross inoculated by *Rhizobium* species isolated from different leguminous species (Burton and Curley, 1968; Prévost *et al.*, 1987a). Furthermore, inoculating *O. viciifolia* with rhizobia isolated from three arctic legume species improved biological nitrogen fixation during cold phases of the growing season (Prévost *et al.*, 1987a, 1987b). Based on acetylene reduction rates, the general effect of adding strains of *Rhizobium* to other strains of *Rhizobium* in symbiosis with *O. viciifolia* was additive (Hill, 1980). Kong *et al.* (2014) demonstrated that the inoculation of arbuscular mycorrhizal fungi can improve mycorrhizal infection rate and plant growth, accelerating the absorption of water and nutrients by the roots compared with uninoculated plants. Kon (1980) established that *O. viciifolia*, when infected with the appropriate *Rhizobium* spp. and arbuscular fungi, produced more and larger nodules and, consequently, a greater nodule dry weight and exhibited greater N₂ fixation than plants infected with only the rhizobia in a glasshouse conditions. Over two-year experiments, *O. viciifolia* fixed 106 kg N ha/year in rain-fed Mediterranean conditions, which was below the usual range of 130–160 kg N ha/year and quite far from the potential value of 270 kg N ha/year from non-Mediterranean areas (Provorov and Tikhonovich, 2003; Re *et al.*, 2014) (Table 1). Issah *et al.* (2020) quantified biological N₂ fixation using ¹⁵N isotope dilution and estimated resource partitioning in *O. viciifolia* under controlled conditions. The percentage of N derived from atmosphere was

Table 1. Effective bacterial and mycorrhiza strains for *Onobrychis viciifolia* inoculation

	Origin	Variety	References
Bacteria			
Arctic N ₃₁	<i>Astragalus alpinus</i>	Melrose	Prévost <i>et al.</i> (1987b)
Arctic N ₁₁ , N ₂₈	<i>Oxytropis maydelliana</i>		
Temperate SM-2	<i>Onobrychis viciifolia</i> cv Melrose		
Temperate 116M15	Commercial inoculant (Nitragin Co., Milwaukee, WI)		
116A27, 116A14, 116A8, 124Z1, 124B1	Commercial inoculant (Nitragin Co., Milwaukee, WI)	Remont	Hill (1980)
NZP 5301	<i>Onobrychis viciifolia</i> cv Fakir	Fakir	Kon (1980)
<i>Rhizobium ciceri</i>	<i>Onobrychis spinacrisibi</i>	–	Yousef and Abdul-Karim (2012)
Arbuscular mycorrhiza			
<i>Gigaspora magarita</i>	–	–	Kon (1980)
<i>Glomus fasciculata</i>			
<i>Glomus tenuis</i>			

81% corresponding to estimated N contributions of 65 kg N ha/year. In view of previous works, cultivating *O. viciifolia* would help mitigating climate change through the reduction of N fertilizers and related greenhouse gasses emissions. As well, the cultivation of *O. viciifolia* would support farmers on their efforts to cope with climate change and to face increasing costs of N fertilizers.

Weed and pest control

Climate change is opening new geographic windows for disease outbreaks, insect herbivory and weed infestations in crops worldwide (Bajwa *et al.*, 2020; Gullino *et al.*, 2022). The inclusion of *O. viciifolia* can reduce number and cover of weeds in cereal fields (Cirujeda *et al.*, 2019). Mummey and Ramsey (2017) concluded that *O. viciifolia* may be a useful bridge species for improving soil conditions while allowing for weed control during restoration of invasive crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) stands, improving conditions for native species establishment in dry rangeland in North America. In addition, *O. viciifolia* can offer a multitude of ecosystem services by supporting biodiversity and hosting important enemies of crop pests in agricultural landscapes. For example, González *et al.* (2022) found almost 147 morphospecies of hymenopteran parasitoids in *O. viciifolia* patches providing a low-cost strategy for biological pest control in *Brassica napus* L. fields. According to these studies, cultivating *O. viciifolia* would improve biological control of pests just when their impacts are growing due to climate change.

Honey production

The change in climatic conditions is bound to have an impact on the physiology, distribution and survival of bees (Le Conte and Navajas, 2008; Smoliński *et al.*, 2021). Under this scenario, conservation of honeybees remains a challenging task (Wood *et al.*, 2020). As *O. viciifolia* is self-sterile, it relies on flower visitors for cross-pollination and requires multiple visits to maximize pollination (Bogoyavlenskii, 1955, 1974; Kropacova and Haslbachova, 1969). Bee pollination of *O. viciifolia* gave an increase of seed productivity by more than 30% (Pankov, 2013). Consequently, its flowers are a rich source of pollen and nectar, attracting ten times more bees than *Trifolium repens* L. and *M. sativa* (Rosov, 1952; McGregor, 1976; Kells, 2001; Devenci and Kuvanci, 2012). *Onobrychis viciifolia*

is visited by managed and indigenous pollinator insect species, including *Apis*, *Bombus* and *Osmia* in southern Alberta (Canada), Eastern Washington (USA), the British Isles (UK), Europe and Japan (Richards and Edwards, 1988; Horne, 1995; Clement *et al.*, 2006; Howes, 2007; USDA SARE, 2007; Westphal *et al.*, 2008; Taki *et al.*, 2009). Rozen *et al.* (2010) noted that *O. viciifolia* is the only pollen source for *Osmia avosetta* Warncke bees in Turkey. In addition, different studies have described a very diverse pollinator community, dominated by *Bombus* and different bee species, foraging on *O. viciifolia* in Italy, Canada, USA, Europe and Turkey (Ricciardelli d'Albore and Roscioni, 1990; Kells, 2001; Clement *et al.*, 2006; Decourtye *et al.*, 2010; Manino *et al.*, 2010; Özbek, 2011; Pearce *et al.*, 2012; Richards, 2019). Richards and Edwards (1988) found that bumblebees visited *O. viciifolia* flowers at a much greater rate than western honeybees in Canada. As previously stated, *O. viciifolia* is a good nectar and pollen source for many pollinator species and, additionally, it shows a long-lasting flowering period to offer bee pasture for western honeybees (*Apis mellifera* L.) and wild bees (Szalai, 2001). In addition, weeds in *O. viciifolia* crops are mainly represented by melliferous species in Russia (Pankov, 2014). When grown as a forage crop, *O. viciifolia* is mown late so flowering has normally finished, maximizing its value as a bee forage crop (Ayers, 1993). With a reduced number of flowers per plant under drought, *O. viciifolia* maintained similar per-flower nectar production (Phillips *et al.*, 2018). *Onobrychis viciifolia* can yield up to 400 kg/ha of honey (Howes, 2007) with a distinctive taste, smell, texture and colour (Vereshchagin *et al.*, 2015), which constitute an additional revenue (Pankov, 2012). Recently, it has been demonstrated that the caffeine present in the nectar of *O. viciifolia* reduces the infection of bumblebees (*Bombus terrestris*) by the microsporidian parasite *Nosema bombi* Fantham and Porter (Folly *et al.*, 2021). Growing *O. viciifolia* may promote a diversification of agronomic production with honeybee related products, which is key to stand the impacts of climate change (Zhang *et al.*, 2022).

Erosion control

Global warming is expected to lead to a more vigorous hydrological cycle, including more total rainfall and more

frequent torrential rainfall events, which may lead to higher soil erosion in many locations worldwide (Eekhout and de Vente, 2022; Dash and Maity, 2023). Cover of *O. viciifolia* and *Rosa canina* L. provided year-round soil protection, improving the soil structure, increasing its water holding capacity and its nutrient retention in Northwest Turkey (Sariyildiz and Savaci, 2020). *O. viciifolia* enhanced soil productivity by increasing soil organic carbon, soil total N and soil organic carbon stock capacity (Sariyildiz and Savaci, 2020). Yu *et al.* (2015) highlighted the superiority of *O. viciifolia* in water retention compared to different tree and herbaceous species in China. The structural stability of the soil under *O. viciifolia* continued for a long period and the infiltration rate was high, preventing an increase of runoff with time. Reduced-tillage systems coupled with *O. viciifolia* cropping should be considered as an efficient management practice and should be improved to withstand extreme rainfall conditions. When used as ridge-furrow rainwater harvesting with mulch, *O. viciifolia* offers farmers means to address drought, water loss and soil erosion in arable lands in arid and semiarid regions (Zhang *et al.*, 2019b). Yüksek and Yüksek (2015) reported that plant cover of *O. viciifolia* was the main factor reducing surface runoff. Hairy leaves of *O. viciifolia* accumulate high sediment loads per leaf area, presumably, because hairs create a buffer zone of reduced water flow velocity enhancing sedimentation (Kretz *et al.*, 2020). *O. viciifolia* is used for erosion control in northwest China and Turkey (Turk and Celik, 2006; Xu *et al.*, 2006). Yakupoglu *et al.* (2021) showed that *O. viciifolia* cultivation exhibited the lowest soil losses when compared to fallow and wheat (*Triticum aestivum* L.) in Southern Turkey. *O. viciifolia* is an efficient cover crop in the conservation of soil and water, for example, in Mediterranean vineyards (Ben-Salem *et al.*, 2018). According to these studies, cultivating *O. viciifolia* would reduce carbon emissions from soils since lowering erosion also reduces soil carbon dioxide emissions (Mandal *et al.*, 2020; Ran *et al.*, 2021).

Ecological restoration

Ecological restoration is a major nature-based solution towards meeting a wide range of global development goals by improving food and water security, protecting biodiversity and promoting adaptation and mitigation of climate change (Liu *et al.*, 2020; Simonson *et al.*, 2021). The improvement of semi-arid and arid degraded lands is one of the most important uses of *O. viciifolia*, since leguminous crops naturally increase soil productivity due to their association with N fixing microbes (Singh *et al.*, 2019). In this sense, Roy *et al.* (2021) suggested that *O. viciifolia* is suitable when designing an appropriate strategy for achieving a successful revegetation of coal mined areas. Moreover, Ibragimov *et al.* (2019) highlighted the importance of the cultivation of multi-component mixtures, including *O. viciifolia*, to face desertification in Southeast Russia.

Recently, there has been growing concern over the potential impacts of global climate change on the sensitivity of weeds to herbicides. Reduced glyphosate sensitivity is projected in response to climate change (Matzrafi *et al.*, 2019). Crop tolerance to glyphosate can reduce competition from weeds, leading to potentially higher yields and increased efficiency in weed control (Raza *et al.*, 2023). *Onobrychis viciifolia* is relatively tolerant to glyphosate application. Based on the dose estimated to cause 50% mortality (LD₅₀), *O. viciifolia* seedlings are over six times, and mature plants are over 20 times, more tolerant to glyphosate than *M. sativa*. Based on the dose required to produce a 50%

reduction in biomass yield (GR₅₀), glyphosate has a 10-fold greater negative impact on *M. sativa* biomass yield than it does on *O. viciifolia*. In addition, the GR₅₀ in *O. viciifolia* seedling was over two-fold and six-fold higher than *M. sativa* at the first and second harvests, respectively (Peel *et al.*, 2013). However, no study has addressed the impact of glyphosate application on seed production. Hard seed levels vary considerably in *O. viciifolia* (up to 90%), and it appears to vary considerably with the cultivars (Bhattarai and Biligetu, 2018). Hard seed pod character may increase weediness of glyphosate tolerant *O. viciifolia* as reported for *M. sativa* (USDA, 2005) suggesting that *O. viciifolia* might pose weediness in ecological sensitive areas where it is not native and/or disperse into wild populations and persist in seed banks.

Glyphosate-resistant crops have become a significant part of cropping systems in North America while it remains used extensively in the mediterranean basin probably due to lawsuits and concerns about gene flow and impacts on organic agriculture (Putnam and Orloff, 2013). *Medicago sativa* is a relatively recent crop to have glyphosate-resistant technology introduced. While weed control in sainfoin still a complex issue, *O. viciifolia* has not yet benefited from this technology. Given the low vigour of sainfoin, glyphosate resistant *O. viciifolia* offers new weed control options for sainfoin establishment for an improved long-term crop vigour and yield (McCordick *et al.*, 2008). Adopted this technology could encourage many growers to adopt sainfoin as an alternative forage for apparent improvements in crop safety, quality, profitability and herbicide application simplicity.

Nutritional interest and animal performance

Livestock production is a major and highly diverse component of agriculture that is being exposed to changes in climate impacting on forage and feed crop production (Moore and Ghahramani, 2013; Rojas-Downing *et al.*, 2017; Godde *et al.*, 2021). *O. viciifolia* has aroused renewed interest in its use in livestock diets, as it has important nutritional properties such as high palatability and great nutritional value leading to very satisfactory animal performance (Gayrard *et al.*, 2021). Scharenberg *et al.* (2007a) found that the palatability of dried and ensiled *O. viciifolia* was 20–24% higher than that of grasses and 10–29% higher than for *Trifolium pratense* L. and *M. sativa* (Waghorn *et al.*, 1990). *O. viciifolia* hay was preferred to *L. corniculatus* hay by sheep (Scharenberg *et al.*, 2007b) and nonlactating cows (Scharenberg *et al.*, 2009; Lagrange *et al.*, 2020). Contrary to forages like alfalfa, sainfoin is a non-bloating legume due to the presence of condensed tannins that attenuate the formation of biofilms in the rumen (Wang *et al.*, 2006). In addition, condensed tannins attenuate the excessive accumulation of ammonia in the rumen through reductions in proteolysis (Lagrange *et al.*, 2020). Finally, sainfoin has been shown to contain high concentrations of non-structural carbohydrates that provide carbon skeletons for an efficient synthesis of microbial protein (Lagrange *et al.*, 2021; Villalba *et al.*, 2021). Collectively, these nutritional benefits contribute to explain the high preference for this forage displayed by ruminants. Mammalian herbivores base their dietary preferences on the association between the orosensorial properties of forages and their post-ingestive consequences (Provenza, 1995) and animals form strong preferences for forages that supply carbohydrates and nitrogen in amounts and proportions that satisfy their daily requirements (Villalba and Provenza, 2000). In contrast, herbivores avoid feeds that enhance rumen distension

such as those that induce bloat (Villalba *et al.*, 2009), as well as those forages that yield high concentrations of rumen ammonia (Villalba and Provenza, 1997).

Onobrychis viciifolia can be grown as a monoculture or in mixtures with perennial grasses and *M. sativa* (Moyer, 1985; Goplen *et al.*, 1991; Frame *et al.*, 1998; Carbonero *et al.*, 2011). In recent years, the grazing of *O. viciifolia* as a monoculture, mixed with perennial grasses (*Festuca pratensis* Huuds. and *P. pratense*) or mixed with *M. sativa* has become a widespread practice in several regions of the world (Frame *et al.*, 1998; Carbonero *et al.*, 2011; Biliget *et al.*, 2021). *Onobrychis viciifolia*-grasses mixtures generally have higher yields and quality than grasses and *O. viciifolia* in monoculture. Liu *et al.* (2006) reported a forage yield of 9.1 t/ha of dry matter for a mixture of *O. viciifolia*-*F. pratensis* seeded in a ratio of 2:1 compared to the monoculture of *O. viciifolia* which gave 7.5 t/ha of dry matter. *Onobrychis viciifolia* can be used primarily as hay or grazed directly, but it can also be cut for silage (Bland, 1971; Sheldrick *et al.*, 1987; Waghorn *et al.*, 1998).

The daily gain reported in heifers was 0.96 and 0.91 kg with *O. viciifolia* and *M. sativa*-grass hay, respectively (Parker and Moss, 1981), and 0.80 and 0.67 kg for heifers on *O. viciifolia* and *M. sativa* pastures, respectively (Marten *et al.*, 1987). Thus, *O. viciifolia* has been reported as an excellent pasture plant (Bonciarelli and Coravelli, 1963; Ferret, 1975; Bencivenga and Negri, 1983). When *O. viciifolia* was grazed as a sole diet, cows, and lambs liveweight gain was similar to *T. repens* and about 20% greater than for *M. sativa* (Waghorn, 2008). Weight gains in beef cattle would be equivalent for *O. viciifolia* hay and that of *M. sativa* (Jensen *et al.*, 1968), and in the case of pasture, the weight gains were higher for *O. viciifolia* compared to *M. sativa* (Hanna and Smoliak, 1968), explained by the lower incidence of bloat and higher efficiency of nitrogen retention due to the presence of tannins in the former (Lagrange *et al.*, 2020). Despite lower concentrate supplementation for grazing rabbits, *O. viciifolia* grassland yielded the highest daily mean growth rates (29 g/day) when compared to natural (26 g/day) and to a pure stand of *Festuca arundinacea* Schreber. (19.2 g/day). Thus, it seems possible to reduce complete feed supplementation without reducing animal performance (Martin *et al.*, 2016; Legendre *et al.*, 2019). To achieve daily mean growth rates of 20 g/day, a grazing area of 0.4 m²/rabbit is sufficient when *O. viciifolia* is grazed while it should be increased to 0.65 m²/rabbit when grass and forbs are grazed (Legendre *et al.*, 2019).

Reducing greenhouse gas emissions from livestock

The livestock sector plays an important role in climate change as it accounts for 15% of human-induced greenhouse gases emissions (Hur *et al.*, 2024). Methane represents the second largest anthropogenic greenhouse-effect gas after carbon dioxide (CO₂) (IPCC, 2021). Livestock, enteric fermentations or effluents, contributes about 1/3 of the global methane emissions (Saunois *et al.*, 2020). From an environmental point of view, feeding ruminants with forage containing condensed tannins may offer potential benefits. Nitrogen balance studies performed on sheep fed with conserved *O. viciifolia* have shown a reduction in excretion of urinary N, whereas the opposite pattern was observed with *M. sativa* (Aufrere *et al.*, 2008; Theodoridou *et al.*, 2010). Urinary N can pollute water resources through excessive nitrate levels (Hoste and Niderkorn, 2019) and is quickly converted to nitrogen oxide, a greenhouse gas 298 times more potent than carbon dioxide (Vallero, 2019). Moreover, sheep fed *O. viciifolia* increased faecal N excretion, which contributes to organic matter

accumulation more than urinary N (Aufrere *et al.*, 2008; Theodoridou *et al.*, 2010), reducing N losses to the atmosphere. Condensed tannins contribute to shift the proportion of excreted N from urine to faeces (Stewart *et al.*, 2019; Lagrange *et al.*, 2020), given the capacity of tannins to bind proteins in the acidic rumen environment and release proteins at greater pH in the intestines for digestion and absorption (Mueller-Harvey *et al.*, 2019). Enteric methane emissions, another greenhouse gas, were reduced in ruminants consuming *O. viciifolia* (Hatew *et al.*, 2015, 2016; Petrič *et al.*, 2022). This reduction depends on the phenological stage and the chemical composition of the biomass. *In vitro*, methane production in *O. viciifolia* hays showed a tendency to increase with the advancement of phenological stage. The best period to cut *O. viciifolia* for hay making was between early and late flowering, when the forage offers high organic matter digestibility, low methane production and more efficient microbial fermentation (Guglielmelli *et al.*, 2011). At flowering stage, *in vitro* gas and methane productions were cultivar dependant (Kaplan, 2011; Ülger and Kaplan, 2016). Hatew *et al.* (2015) suggested that conserved *O. viciifolia* accessions collected worldwide exhibited substantial variation in terms of their effects on rumen *in vitro* methane production, revealing some promising accessions for future investigations. Additionally, the methane yield depended on the chemical composition of the biomass (Amaleviciute-Volunge *et al.*, 2020). The results obtained in metabolic cages indicated that the inclusion of *O. viciifolia* in silage reduces the digestibility of organic matter *in vivo* on castrated male sheep compared to pure timothy (*Phleum pratense* L.), and methane emissions were proportionately lower (Niderkorn *et al.*, 2016). A trial on dairy cows, including 50% *O. viciifolia* silage in a ration of grass silage, resulted in a reduction of 6.0% methane emissions per kg of dry matter ingested. Silage of *O. viciifolia* allowed improved milk in quantity and quality (Huyen *et al.*, 2016a, 2016b). The consumption of *O. viciifolia* pellets by gastrointestinal nematode-infected lambs decreased methane emission by affecting ruminal methanogens without undesirable changes in the ruminal microbiome or animals' health (Petrič *et al.*, 2022). Adding *O. viciifolia* into *M. sativa* prior to ensiling suppressed silage proteolysis and mitigated rumen CH₄ in a proportion-dependent manner, with a minor negative effect on dry matter digestibility (Rufino-Moya *et al.*, 2019). Therefore, co-ensiling of *M. sativa* with *O. viciifolia* could be used as a promising strategy not only to produce high-quality legume silage but also to reduce N excretion and mitigate rumen CH₄ (Wang *et al.*, 2022). Lower values of CH₄ per kg intake were recorded with *O. viciifolia* silage compared to pure *Phleum pratense* L. silage (Niderkorn *et al.*, 2019). Similar effects were observed *in vitro* when *O. viciifolia* was mixed with *Lolium perenne* L. or *Dactylis glomerata* L. (Niderkorn *et al.*, 2011, 2012) and *in vivo* when *O. viciifolia* was included in diets of dairy cows (Huyen *et al.*, 2016a). The significant reduction of total digestive tract neutral detergent fibre digestibility in the presence of *O. viciifolia* compared to pure *P. pratense* was likely the main driver for the reduction of CH₄ emissions. In addition, the acetate:propionate ratio in the rumen, which is strongly related to the availability of hydrogen as a substrate for methanogenic archaea to form CH₄, was lower for *P. pratense*-*O. viciifolia* and *Trifolium pratense*-*O. viciifolia* than for pure *P. pratense*, highlighting the potential of *O. viciifolia* silage to decrease CH₄ emissions via a modification of the fermentative microbial ecosystem in the rumen (Warner *et al.*, 2017). In addition, the reduction of NH₃ emissions and urea in milk were more pronounced than the

reduction in methane emissions (McMahon *et al.*, 1999; Guglielmelli *et al.*, 2011; Niderkorn *et al.*, 2011; Williams *et al.*, 2011; Theodoridou *et al.*, 2011; Chung *et al.*, 2013; Copani *et al.*, 2015).

Nutritive value of sainfoin

The nutritional value of *O. viciifolia* is determined by variety, growth stage and growing environment. Several studies have quantified the nutritional values, mainly the concentrations of crude protein, neutral detergent fibre and acidic detergent fibre, of *O. viciifolia* at different stages of growth. After 42 days of regrowth, the nutritive value of *O. viciifolia* was comparable to the first growth vegetative stage, with crude protein ranging between 148–186 g/kg, and neutral detergent fibre and acid detergent fibre concentration ranging 365–454 g/kg and 337–397 g/kg, respectively (Table 2).

Freshly harvested forage, silage and hay of *O. viciifolia* have similar quality and nutritive value (Table 3). The tannins in *O. viciifolia* silage can reduce the proteolysis that takes place in the silo (Wilkins and Jones, 2000). Incorporation of *O. viciifolia* into *M. sativa* forage improves fermentation in laboratory silos. Optimal ruminal fermentation with silage was obtained with a proportion of 60/40 for *M. sativa* and *O. viciifolia*, respectively (Wang *et al.*, 2007). The inclusion of *O. viciifolia* in grass silage has been shown to improve forage quality, fermentation as well as protein protection against microbial and enzymatic degradation (Lee *et al.*, 2008; Lorenz *et al.*, 2010; Copani *et al.*, 2014). *Onobrychis viciifolia* can be offered as dehydrated granules (Gaudin, 2017), but the necessary technological treatments, high temperature and high pressure, can cause a possible destruction of the tannins and a conversion of the tannins from the free majority form to the bound form attached to proteins (Terrill *et al.*, 1992, 2007; Minnee *et al.*, 2002; Lorenz, 2011).

Onobrychis viciifolia was found to increase by 17% the proportion of omega-3 polyunsaturated fatty acid and unsaturated fatty

acids in milk and cheese fat from lactating cattle (Girard *et al.*, 2015, 2016), due to condensed tannins modulating the activity of bacteria involved in the processes of biohydrogenation (Vasta *et al.*, 2010; Buccioni *et al.*, 2015). In addition, it has been reported that condensed tannins of *O. viciifolia* reduced protein degradation in the rumen leading to reductions in rumen ammonia concentrations and N losses in urine (Scharenberg *et al.*, 2007b). Condensed tannins also increased the plasma concentration of essential amino acids, indicating that the protein escaped from the rumen is digested in the intestine leading to faster animal growth rates and increased milk production (Waghorn *et al.*, 1990; Waghorn, 2008; Girard *et al.*, 2016).

Nutritional benefits

Onobrychis viciifolia is particularly valued for its content of condensed tannins, which have been shown to improve animal health by reducing bloat (McMahon *et al.*, 1999; Sottie, 2014; Wang *et al.*, 2015), and by diminishing gastro-intestinal parasites (Hoste *et al.*, 2015; Desrues *et al.*, 2017). In fact, the intake of *O. viciifolia* fresh leaves (10–20% of the dry matter of *M. sativa*) reduces bloat in cattle by 27% (McMahon *et al.*, 1999) compared to those consuming 100% *M. sativa*. Consequently, *O. viciifolia* could be grazed, offered in as hay or silage *ad libitum*. When *O. viciifolia* is grown in a mixture with *M. sativa*, it has been shown to reduce bloat incidence in beef cattle relative to the grazing of pure *M. sativa* (Sottie, 2014; Malisch *et al.*, 2015). Condensed tannins of *O. viciifolia* hay has shown reduced egg per gram of faeces, and female fertility of parasitic nematodes when fed to livestock (Azuhwi *et al.*, 2013; Arroyo-Lopez *et al.*, 2014). Anti-parasitic properties of *O. viciifolia* tannins have been demonstrated by both *in vitro* (Barrau *et al.*, 2005) and *in vivo* studies (Molan *et al.*, 2000a, 2000b, 2002; Hoste *et al.*, 2015). Moreover, sheep increase their intake of *O. viciifolia* through time when parasitized (Gaudin *et al.*, 2019) and show

Table 2. Nutritional value of *Onobrychis viciifolia* at different stages of growth

Growth stage	Variety	Crude protein (g/kg DM)	Neutral detergent fibre (g/kg DM)	Acid detergent fibre (g/kg DM)	References
Vegetative	Anatolian	195–198	378–461	286–334	Bal <i>et al.</i> (2006)
	nd				Turk <i>et al.</i> (2011)
Flowering	Eski	116–143	372–457	368–392	Parker and Moss (1981)
	nd	121	478	433	Khalilvandi-Behroozyar <i>et al.</i> (2010)
	nd	114–177	433–476	343–433	Kaplan (2011)
	Anatolian	145	493	372	Bal <i>et al.</i> (2006)
	Nova	125–161	–	313–371	McMahon <i>et al.</i> (1999)
Grain filling	38 accessions	134–175	356–458	351–416	Bhattarai and Biligetu (2018)
	Anatolian	130	557	402	Bal <i>et al.</i> (2006)
Regrowth (42 Days)	nd	171	446	338	Turk <i>et al.</i> (2011)
	Moiry	148–186	365–454	337–397	Azuhwi <i>et al.</i> (2012)
	Sarzens				
	Premier				
	Visnovsky				
	Perly				

Table 3. Characteristics and nutritive value of *Onobrychis viciifolia* feeding forms

	Variety	Dry matter (g/kg)	Organic matter	Crude protein (g/kg DM)	Neutral detergent fibre (g/kg DM)	Acid detergent fibre (g/kg DM)	Condensed tannins (g/kg DM)	Metabolizable energy (%)	References
Fresh	nd	298	927	198	231	202	43.7	9.8	Scharenberg <i>et al.</i> (2007a)
Hay		968	897	220	239	210	46.2	9.7	
Silage		315	921	211	229	228	28.8	9.1	
Fresh	Perly	–	–	143	415	314	6.2	–	Theodoridou <i>et al.</i> (2010)
Frais		–	–	187	355	279	13.6	–	
Hay	nd	–	923	219	391	256	–	–	Guglielmelli <i>et al.</i> (2011)
Hay		–	933	207	433	346	–	–	
Hay		–	935	175	441	296	–	–	
Hay		–	942	122	514	409	–	–	
Hay	Zeus	–	924	157	346	249	9.8	–	Niderkorn <i>et al.</i> (2012)
Fresh	Reznos	–	–	194	392	258	–	–	Rufino-Moya <i>et al.</i> (2022)
Hay		–	–	169	470	340	–	–	
Silage		–	–	169	456	332	–	–	
Pellets	nd	918	–	121	460	357	–	–	Petrič <i>et al.</i> (2022)

high preferences for the legume despite the high availability of alternative legumes without tannins like cicer milkvetch (Villalba *et al.*, 2013).

Onobrychis viciifolia has an antiparasitic effect on the most important sheep nematodes (Ríos-De Álvarez *et al.*, 2008; Komáromyová *et al.*, 2021; Petrič *et al.*, 2022). Similar results were obtained with goats (Paolini *et al.*, 2005). While goats are more willing than sheep to consume tanniferous feeds, the potential for self-medication has been demonstrated in both species concerning *H. contortus* (Gaudin *et al.*, 2019). Regular feeding on *O. viciifolia* pastures by small ruminants could therefore be used to improve host resilience and thus reduce pasture contamination. The anthelmintic bioactivity of *O. viciifolia* is maintained in hay or silage (Ojeda-Robertos *et al.*, 2010). Berard *et al.* (2009) proved that *O. viciifolia* silage and hay reduced the excretion of *Escherichia coli* by cattle. The consumption of *O. viciifolia* by small ruminants has also been associated with effects on coccidia (Saratsis *et al.*, 2012) and on infestations by gastrointestinal nematodes (Hoste *et al.*, 2015). These findings contribute to the further development of sustainable grass–legume systems, as evidence for the successful cultivation of *O. viciifolia* as an alternative legume species (Malisch *et al.*, 2017).

Medicinal and pharmacological properties

Global environmental degradation and climate change threaten the foundation of human health and well-being (Pathak and McKinney, 2021; Rocha *et al.*, 2022). Several diseases are all likely to become more common (Faergeman, 2007). Ethnobotanical evidence supports the use of *O. viciifolia* in traditional medicine (Martini, 1981; Mbaveng *et al.*, 2014) (Table 4). Cornara *et al.* (2016) recently reviewed temperate forage legumes as a resource for nutraceuticals and pharmaceuticals. The nutraceutical activity

of *O. viciifolia* is due to the presence of a large phenolic complex, dominated by arbutin, rutin, catechin, kaempferol, quercetin, afzelin and condensed tannins (Marais *et al.*, 2000; Regos *et al.*, 2009). These phenolic metabolites are reported as antioxidants, detoxifying agents, reducing blood pressure and anti-cancer agents (Dai and Mumper, 2010).

Establishment characteristics

Climate change is predicted to cause significant changes in composition, establishment, growth and development of pastures and fodder crops (Sunil *et al.*, 2020; Martins-Noguerol *et al.*, 2023). Ease of stand establishment is considered an important trait in perennial forage utilization under climate change. *Onobrychis*

Table 4. Biological activity of *Onobrychis viciifolia*

Plant part	Biological activity	Target/	Reference
Stems and leaves	Anthelmintic properties Antimicrobial activity	<i>Teladorsagia circumcincta</i> , <i>Haemonchus contortus</i> and <i>Trichostrongylus colubriformis</i>	Paolini <i>et al.</i> (2004)
		<i>Escherichia coli</i> O157:H7	Barrau <i>et al.</i> (2005)
		<i>Escherichia coli</i>	Liu <i>et al.</i> (2013)
Dried and pulverized roots	Antiseptic and vulnerary	Placed directly on the wounds	Martini (1981)

viciifolia establishment depends on soil characteristics, seeding (size, colour of tegument, milled vs unmilled) and weed competition. *Onobrychis viciifolia* seeds germinate rapidly and are easy to establish in the Brown, Dark Brown and Black soil zones of western Canada, as well as stony clay loam soils in UK (Goplen *et al.*, 1991; Carbonero *et al.*, 2011).

The areal expansion of semiarid and subhumid zones under climate change will increase at the expense of the contraction of arid and humid zones (Li *et al.*, 2021a, 2021b). *Onobrychis viciifolia* performs well over multitude of climatic areas. In the warm Mediterranean Basin, *O. viciifolia* 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 *O. viciifolia* between April and July (Jensen *et al.*, 1968; Goplen *et al.*, 1991). Early sowing can improve the development of the plants due to the early development of roots (Nikola, 1998). Global warming due to climate change will expose plants and their seeds to novel climatic conditions and likely affect seed germination responses (Gremer *et al.*, 2020; Mondoni *et al.*, 2022). Species with a wide range of temperature for germination could have a better adaptability to climate change (Bandara *et al.*, 2019). Although there is limited information available on its seed germination, *O. viciifolia* has a wide range of optimum temperature for germination, but it is normally advised to drill it between 10–27°C and never below 5°C (Carleton *et al.*, 1968; Jensen *et al.*, 1968; Smoliak *et al.*, 1972). There are conflicting views on the relative germination of milled and unmilled *O. viciifolia* seeds. Wiesner *et al.* (1968) reported a higher germination percentage for milled seeds, but no significant difference in germination among the two types was observed by Chen (1992). Noorbakhshian *et al.* (2011) found improved germination and seedling vigour for *O. viciifolia* after removing the seed pods. Use of de-hulled seeds could provide staggered germination and thus cushion potential weather disturbances (Wiesner *et al.*, 1968; Chen, 1992; Demdoun, 2012). To enhance *O. viciifolia* germination and early establishment, several studies report the need for seed pod removal rather than seed scarification (Bhattarai and Biliget, 2018). In addition, seedlings emerging from seeds with intact pod may have a high probability of fungal infestation (*Alternaria* and *Fusarium* spp.). Slow and non-uniform seedling growth and high weed infestations may be other limiting factors for sowing seeds with pods (Noorbakhshian *et al.*, 2011).

Variation among *O. viciifolia* germplasm for seed size, seed weight and seed coat colour would enhance the species adoption by farmers under climate change. The germination percentage was higher for brown than green seeds as the former are physiologically mature (Thomson, 1938; Noorbakhshian *et al.*, 2011). Brown seeds colouration has important functions in plants including a role in camouflage and thermoregulation, and protection against UV-radiation and pathogens (Roulin, 2014). Germination is also affected by seed size, which varies among *O. viciifolia* cultivars. Bhattarai and Biliget (2018) found that final germination was increased by seed pod removal but not with seed size. In *O. viciifolia*, the medium seed size class (1000-seed weight of 21 g) and the large seed size class (1000-seed weight of 28 g) had a final germination of 90%–93% at temperature of 25°C, but the small seed class (1000-seed weight of 12 g) had a final germination lower than 10% due to high degree of physical dormancy (Bhattarai and Biliget, 2018). Cash and Ditterline (1996) reported that *O. viciifolia* seedlings emerged more rapidly from large seeds. The use of large fully mature seeds increases establishment success giving stronger

plants, with more nodules and high rates of N₂ fixation (Cash and Ditterline, 1996). The seeding density of *O. viciifolia* depends upon seed size, soil type, soil moisture, purpose and method of seeding. There is almost no data available to confirm the effect of *O. viciifolia* seeding density on stand establishment, forage yield and other agronomic performance. To establish a population of 70–150 adult plants/m² in the first year, authors recommend seed densities of 40–50 kg/ha of de-hulled seeds (or 80–120 kg/ha hulled seeds) (Sheldrick *et al.*, 1987; Frame *et al.*, 1998) at a depth of 1–2 cm in Canada (Hill, 1997). Conversely, in China, a depth of 4–5 cm was recommended (Chen, 1992). The recommended row spacing is between 50–60 cm for a better stand establishment (Goplen *et al.*, 1991; Stevovic *et al.*, 2010).

It is recognized that weed pressure associated with climate change is a significant threat to crop production, either through increased temperatures, rainfall shift and elevated CO₂ levels, but the current knowledge of these effects is very sparse (Ramesh *et al.*, 2017; Munda *et al.*, 2024). *Onobrychis viciifolia* is a non-aggressive crop during seedling establishment. Thus, weed control in the first year is important for good establishment and high forage production in subsequent years. In the first year of establishment, Moyer (1985) found weeds made up 98% of dry matter yield in *O. viciifolia* fields without any weed control measures Lethbridge (Canada). Koivisto and Lane (2001) suggested using a non-competitive grass as a companion crop to aid in weed control in the establishment year. Chemical weed control in pure stands of *O. viciifolia* resulted in higher seed yield than stands with *H. vulgare* as a companion crop in Europe (Dimitrova, 2010).

Recent advances

Climate change has a substantial bearing on crop productivity and food security, and hence there is a need to develop resilience to mitigate climate change induced impacts in crop plants (Acevedo *et al.*, 2020; FAO, 2020; Raj *et al.*, 2022). The challenge is to try to preserve the resilience of our ecosystems in the years to come, and to maintain food security by protecting important crop species and finding ways to increase their productivity. Despite *O. viciifolia* potential to tackle climate change adverse effects, the species is relatively understudied, and several aspects need to be addressed. In recent years, ‘HealthyHay’ (<http://legumeplus.eu/healthyhay-project>) and ‘LegumePlus’ (<http://legumeplus.eu>) projects conducted genetic analyses, agronomic, biological and chemical evaluations, nutritional analyses, environmental assessments and developed methodologies for screening for genetic improvement of 362 different *O. viciifolia* accessions across Europe. Nevertheless, there is no report of registration of new improved *O. viciifolia* cultivars from these large-scale research projects (Poudel *et al.*, 2023). In 2000, a new breeding initiative of *O. viciifolia* focusing on improvement of the compatibility of *O. viciifolia* in *M. sativa* stands to reduce *M. sativa* bloat led to the creation of two new Canadian cultivars, namely AAC-Mountainview and AAC-Glenview released in 2015 and 2018, respectively (Poudel *et al.*, 2023). AAC-Mountainview was derived from single-cycle selection under competition with *M. sativa*, whereas AAC-Glenview was selected for persistence in *M. sativa* stands, followed by improved grazing tolerance in a grazing trial. The variation in dry matter yield in both cultivars is low compared to the Romanian cultivar Splendid, but higher than the Kazakhstan cultivar Nova based on recent tests in Lethbridge

(South Owest Canada) in monocultures. Nevertheless, AAC-Mountainview and AAC-Glenview showed significant advantages over parents when seeded with *M. sativa*. These cultivars are successful, and demand for their seeds is increasing considerably (Poudel *et al.*, 2023).

Overall, climate change has exemplified the need for continued research into crop environmental stress tolerance (Jing *et al.*, 2024). Current updates and recent advances in the physiological, molecular and genetic perspectives of plant responses to environmental stresses may offer insights underlying these responses and how this pool of knowledge can be explored to develop plants for future climates (Kumar *et al.*, 2023). In recent years, there has been a growing focus on research endeavours aimed at enhancing the resilience of *O. viciifolia* to both abiotic and biotic stresses providing opportunity for climate change adaptation and mitigation. To address the challenge of establishing *O. viciifolia* in drought conditions, Irani *et al.* (2015a) identified *O. viciifolia* plants that exhibited resilience to drought and displayed high yields. These plants were characterized by elevated foliar proline contents, presenting a potential physiological marker for screening drought tolerance for climate change adaptation and mitigation. Moreover, enhancing the activity of two crucial antioxidant enzymes, glutathione reductase (GR) and ascorbate peroxidase (APX), can lead to successful breeding of *O. viciifolia* with improved drought tolerance (Beyaz, 2019). On the other hand, the growth of *O. viciifolia* is constrained by powdery mildew (*Erysiphe polygoni*), with several tolerant accessions having been previously identified (Jafari *et al.*, 2014; Alizadeh *et al.*, 2021). Li *et al.* (2021a, 2021b) investigated *O. viciifolia* seed response under saline conditions and offered valuable insights for advancing the establishment and cultivation of salt-tolerant *O. viciifolia* in saline lands in China. The challenges posed by environmental stresses may be influential in the reduced persistence of *O. viciifolia*. Therefore, forthcoming *O. viciifolia* breeding endeavours ought to prioritize the creation of cultivars resilient to both biotic and abiotic stressors.

Progress in enhancing the molecular characteristics of *O. viciifolia* has significantly trailed behind that of other forage legume species, primarily because of the limited genomic resources. While several genetic markers have been established for *O. viciifolia* (Kempf, 2016; Mora-Ortiz *et al.*, 2016; Shen *et al.*, 2019), and a complete chloroplast genome sequence has been recently disclosed (Jin *et al.*, 2021), there is currently no existing reference nuclear genome sequence for this species. Progress in molecular improvement has been advancing in recent years, with the accumulation of transcriptomic data from various tissues across a limited number of genotypes (Kempf, 2016; Mora-Ortiz *et al.*, 2016; Shen *et al.*, 2019; Yin *et al.*, 2020; Jin *et al.*, 2021; Qiao *et al.*, 2021). Additionally, there has been an evaluation of microRNAs (miRNAs) in *O. viciifolia* cultivated at different altitudes (Yin *et al.*, 2020). Considering the growing interest in *O. viciifolia*, further advancements should continue to unfold in this sphere in the years ahead, contributing to the facilitation of breeding activities. Alongside the existing limited supply of genetic data for tetraploid *O. viciifolia*, there exists a notable lack of accessible biotechnological tools. This shortfall has impeded both functional genetic investigations and progress in advanced molecular breeding initiatives. There has been some advancement in this domain with the introduction of a transient virus-induced gene silencing method in *O. viciifolia*. This method has proven successful in downregulating the expression of the phytoene desaturase gene, signifying recent progress in the field.

Future perspectives

In the present review, *O. viciifolia* appears as a plant with high application potential with agronomic, ecological and economic interest in the present scenario of climate change. Advances in recent years have provided more opportunities for *O. viciifolia* to be considered as an alternative for farmers, particularly those interested in producing locally sourced protein and sustainable agricultural practices (Sheppard *et al.*, 2019). Although the agronomical and ecological potential of *O. viciifolia* have recently received due attention, its general biological and physiological attributes have not been well-investigated. Evaluating these basic facets may extend its cultivation and pave the way for novel applications in the present context of climate change (Sakhraoui *et al.*, 2023). In this sense, research efforts on the ecology, stress tolerance and uses of *O. viciifolia* should be increased. Thus, use of both genetic manipulation and traditional breeding approaches will be required to develop salt-tolerant cultivars better able to cope with high salinities in marginal agriculture areas affected by salinization in the present climate change scenario.

In view of our review, there are mainly five deficiencies in the research on *O. viciifolia* and its relationship with climate change: (1) Most of the studies on *O. viciifolia* ecophysiology have been focused on seedlings, while research concerning adult plants is scarce. Plant physiological characteristics often show differences in different growth stages, so the seedling stage may not represent the physiological characteristics of the whole plant life cycle (Mganga *et al.*, 2019). Therefore, the research on physiological responses to environmental stressors related to climate change should be carried out for different growth stages of *O. viciifolia*, especially adult plants. (2) The studies on stress tolerance of *O. viciifolia* have been mainly focused on salt and drought tolerance, while the research on the responses to other environmental stressors, such as extremes of air temperatures, are scarce or non-existent. Therefore, more research is needed on the responses of *O. viciifolia* to different environmental stressors related to climate change, besides salinity and drought, and on the interactive effects of the combination of different environmental stresses. (3) The study of stress tolerance mechanism has been mainly focused on the observation of physiological changes, while the research on anatomical, morphological and genetic adaptation mechanisms is scarce. To further clarify the stress resistance of *O. viciifolia* to stressors related to climate change, anatomical, morphological and genetic studies should be conducted. Covering these knowledge gaps and their incorporation in hybridization and breeding programs can be useful in creating new cultivars of *O. viciifolia* better adapted to climate change. (4) *Onobrychis viciifolia* is relatively resistant to biotic stresses compared to other forage legume species and appears to rely on cultivar choices and thus represent a potential source for breeding (Carbonero *et al.*, 2011). This resistance will need to be confirmed by further rigorous field pathology studies to determine the susceptibility of selected lines, and the potential resistance mechanism should be studied to see if it is possible to transfer this into new cultivars. (5) The evaluations of ecological and economic benefits and impact of *O. viciifolia* production are mostly qualitative, lacking quantitative evaluations. This may limit farmers and the decision-making departments of public administrations to pay attention to its application values. Therefore, quantitative analysis should be carried out in the evaluation of ecological and economic benefits of *O. viciifolia*. (6) There is a lack of long-term observation experiments after sowing pastures with *O. viciifolia*

or using this legume in ecological restoration projects. This sort of long-term studies would be a great opportunity to improve our knowledge on the benefits of *O. viciifolia* as a biological tool to mitigate and adapt to climate change. (7) The genomes of *O. viciifolia* have not yet been fully sequenced, and a limited number of genetic markers are present, except for some transcriptomics data to apply in crop improvement studies and functional genomics (Kempf, 2016; Mora-Ortiz *et al.*, 2016; He *et al.*, 2024). In fact, recent breeding studies on *O. viciifolia* have led to improvements in this crop as bloat-free forage legume by enhancing its yield, biomass productivity, grazing tolerance and fatty acid composition (Subedi, 2018; Wijekoon *et al.*, 2021). However, only a few *O. viciifolia* cultivars are available and new cultivars with high dry matter yield and persistence under regional growing conditions are required (Bhattarai *et al.*, 2016; Sheppard *et al.*, 2019). Further breeding studies are necessary on the improvements in weed control, establishment, seed dormancy and genetic characterisation to enable effective pre-breeding programmes in different environments.

Conclusions

Onobrychis viciifolia is a forage legume of renewed interest worldwide, with equally weighted advantages and disadvantages that prevent many farmers from considering this crop a viable alternative to other forage legumes. However, advances in recent years have provided more opportunities for sainfoin to be considered as an alternative choice for farmers, particularly for its agronomical and ecological interests, nutritional benefits and nutraceutical properties. However, some knowledge gaps and application should be studied deeply to promote *O. viciifolia* use for climate change adaptation and mitigation. In this sense, research is required to select and breed potentially useful varieties combining nutritional, agronomic and environmental potential.

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