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INVITED REVIEW

Lathyrus diversity: available resources with relevance to crop improvement – *L. sativus* and *L. cicera* as case studies

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• *Background* The *Lathyrus* genus includes 160 species, some of which have economic importance as food, fodder and ornamental crops (mainly *L. sativus*, *L. cicera* and *L. odoratus*, respectively) and are cultivated in >1·5 Mha worldwide. However, in spite of their well-recognized robustness and potential as a source of calories and protein for populations in drought-prone and marginal areas, cultivation is in decline and there is a high risk of genetic erosion.
• *Scope* In this review, current and past taxonomic treatments of the *Lathyrus* genus are assessed and its current status is examined together with future prospects for germplasm conservation, characterization and utilization. A particular emphasis is placed on the importance of diversity analysis for breeding of *L. sativus* and *L. cicera*.
• *Conclusions* Efforts for improvement of *L. sativus* and *L. cicera* should concentrate on the development of publicly available joint core collections, and on high-resolution genotyping. This will be critical for permitting decentralized phenotyping. Such a co-ordinated international effort should result in more efficient and faster breeding approaches, which are particularly needed for these neglected, underutilized *Lathyrus* species.

Key words: Diversity, genetic resources, *Lathyrus sativus*, *L. cicera*, Fabaceae, legumes, plant breeding, protein crops.

INTRODUCTION

Predictions are that population and income growth will double the global demand for food by 2050, effectively increasing competition for crops as sources of bioenergy and fibre and for other industrial purposes (http://www.fao.org). Compounding the pressure for increased agricultural output are looming threats of water scarcity, constraints on soil fertility, and climate change. The highly resilient *Lathyrus* species (Fabaceae) can play an important role in these immense agricultural challenges. More sustainable management of renewable soil and water resources, in concert with more efficient utilization of genetic diversity, will be key to achieving the necessary productivity gains (Cobb *et al.*, 2013). Genetic diversity provides the basis for all plant improvement.

In this review, we begin by briefly assessing current and past taxonomic treatments of the *Lathyrus* genus. We then discuss a survey of interesting variable characters used in characterization of its germplasm collections and examine new approaches for diversity analysis, with a particular emphasis on the importance of diversity analysis for *L. sativus* and *L. cicera* breeding.

AGRONOMIC POTENTIAL OF *LATHYRUS* SPECIES

The *Lathyrus* genus, which includes some 160 species (Allkin *et al.*, 1986), is distributed throughout temperate regions of the northern hemisphere and extends into tropical East Africa and South America. Its main centre of diversity is in the Mediterranean and Irano-Turanian regions, with smaller centres in North and South America (Kupicha, 1983).

Members of the *Lathyrus* genus include food and fodder crops, ornamentals, soil nitrifiers, dune stabilizers, important agricultural weeds, and model organisms for genetic and ecological research (Kenicer *et al.*, 2005). Most members of *Lathyrus* are mesophytes of open woodlands, forest margins and roadside verges, but littoral, alpine and more drought-tolerant species are also represented (Kenicer *et al.*, 2005). Both annual and perennial species of *Lathyrus* occur, many of which have a climbing habit using simple or branched tendrils. Among the cultivated *Lathyrus* species, *L. sativus* (grass pea) is the most important as a food crop and has been central for animal feed or fodder since ancient times. Grass pea cultivation originated around 6000 BC and might have been the first crop domesticated in Europe (Kislev, 1989). Although its cultivation is in regression, it is still grown in 1·5 Mha, mainly in South Asia and Sub-Saharan Africa (Kumar *et al.*, 2011; Girma and Korbu, 2012; Hillocks and Maruthi, 2012). Grass pea is considered the most promising underutilized source of calories and protein for populations in drought-prone and marginal areas of Asia and Africa (Hillocks and Maruthi, 2012), with the potential for introduction in Australia (Hanbury *et al.*, 2000), North America (Rao and Northup, 2011; Calderón *et al.*, 2012; Gusmao *et al.*, 2012) and China (Yang and Zhang, 2005).

However, grass pea suffers from a reputation of being toxic, as its overconsumption under certain circumstances has caused neurolathyrism, a neurotoxic disease (Lambein and Kuo, 2009). The disabling effects of prolonged dependence on grass

pea due to its content of the neurotoxin β-*N*-oxalyl-L-α,β-diaminopropionic acid (ODAP) led to the decision that the crop should be abandoned as human food, and seed sales were banned in some countries (Enneking, 2011). However, given the increasing need for resilient food crops, improvement of grass pea is still considered a priority by national and international research centres. Major efforts in grass pea breeding in the last 50 years have been aimed at reducing the ODAP content, resulting in a number of cultivars with low ODAP being released (Kumar *et al.*, 2011). There is also agreement today that ODAP content in itself does not seem to be a problem because grass pea is harmless to humans and animals when consumed as part of a balanced diet (Getahun *et al.*, 2002, 2003, 2005; Lambein and Kuo, 2009) and because seeds can be partly detoxified by various processing methods such as fermentation, or pre-soaking in alkaline solutions and cooking (Kuo *et al.*, 2000; Kumar *et al.*, 2011). There is even the hypothesis that nitriles are the causative agents of neurolathyrism rather than ODAP (Llorens *et al.*, 2011). Additionally, we should not neglect any potential pharmacological benefits of ODAP (Lan *et al.*, 2013).

Other economically important species grown commercially are the forage crop chickling vetch (*L. cicera*) and the ornamental sweet pea (*L. odoratus*). *Lathyrus cicera* has been cultivated since ancient times, and was domesticated in Southern France and the Iberian Peninsula soon after the introduction of agriculture into the area (Kislev, 1989). It is used as animal feed (White *et al.*, 2002). *Lathyrus odoratus* originates from Southern Italy and has become an economically important ornamental plant grown for its cut flowers and for garden decoration. Other species such as *L. belinensis*, *L. chloranthus*, *L. vernus*, *L. tingitanus*, *L. grandiflorus*, *L. latifolius*, *L. rotundifolius* or even *L. sativus* also have potential ornamental use (Parsons, 2009). Other species are important for human consumption only in certain countries, such as *L. clymenum* or *L. ochrus* in areas of Greece, Cyprus, Italy or Turkey (Sarpaki and Jones, 1990; Jones, 1992).

Lathyrus species such as *L. sativus* also have potential as sources of variation for closely related important legumes such as pea (*Pisum sativum*) and, although they are cross-incompatible, there is potential for somatic hybridization (Durieu and Ochatt, 2000). Schaefer *et al.* (2012) also point out that a group of often overlooked Mediterranean *Lathyrus* species, *L. gloeosperma*, *L. neurolobus* and *L. nissola*, might be particularly appealing for pea breeding because of this group's close relationship to the *Pisum* genus. Their beneficial traits include drought tolerance and a perennial life form.

PHYLOGENY AND PHYLOGEOGRAPHY

The *Lathyrus* genus belongs to the tribe Fabeae (syn. Vicieae) along with *Vicia*, *Lens*, *Pisum* and *Vavilovia* (reviewed in Smýkal *et al.*, 2010). Recently, Schaefer *et al.* (2012) concluded that the Fabeae tribe evolved in the Eastern Mediterranean in the middle Miocene, and it spread from there across Eurasia, into Tropical Africa, and at least seven times across the Atlantic and Pacific to the Americas. Long-distance dispersal events seem to be the most probable causes for these Atlantic crossings, with Schaefer *et al.* (2012) rejecting the hypothesis of ancient steppingstone dispersal via the Atlantic islands. These same authors, using phylogenetic data, suggested that the genus *Lathyrus* is not monophyletic and that a more natural classification of Fabeae should also include *Pisum* and *Vavilovia*. This regrouping is also supported by the currently available whole-plastid genomes of *L. sativus* and *P. sativum* (Magee *et al.*, 2010). Furthermore, the genera *Pisum* and *Lathyrus* share the phytoalexin pisatin, which is not found in *Vicia* or *Lens* (Robeson and Harborne, 1980).

Most *Lathyrus* species are diploid ($2n = 14$), with a few natural autopolyploids or allopolyploids, or contain both diploid and autopolyploid forms. Many species show similar chromosome morphology although their nuclear DNA content may vary from 6·9 to 29·2 pg/2C (10·6 and 13·4 pg/2C for *L. sativus* and *L. cicera*, respectively) (Ali *et al.*, 2000, and references therein).

After several historically important treatments of their infrageneric classification, Kupicha (1983) recognized 13 sections within the genus *Lathyrus* based on morphological studies (Fig. 1). This same author hypothesized the origin of *Lathyrus* in the Old World at high altitudes, during the Cretaceous or early Tertiary periods, as an inhabitant of the Boreal–Tertiary woodland flora. This primitive ancestral stock must have migrated in Europe to the Mediterranean region and to the North American continent via Greenland or from Asia to Alaska. Later, by the end of the Tertiary period, primitive *Lathyrus* ancestors migrated from North to South America. Therefore, similarities between South American and Mediterranean/Irano-Turanian *Lathyrus* sp. would be, according to this author, due to parallel evolution.

Later on, Asmussen and Liston (1998) performed the first phylogenetic study using molecular data on both Eurasian and New World *Lathyrus* species. These authors used chloroplast DNA (cpDNA) characters [cpDNA-restriction fragment length polymorphism (RFLP)] to test the monophyly and relationships of Kupicha's *Lathyrus* sections, suggesting that some of these sections should be combined in order to form monophyletic groups (Fig. 1). Agreement with Kupicha's classification was otherwise very good (Kenicer *et al.*, 2009). For instance, these cpDNA-RFLP parsimony analyses supported the North American origin of the South American *Lathyrus* species, earlier suggested by Kupicha (1983).

A later study based on amplified fragment length polymorphism (AFLP) (Badr *et al.*, 2002) confirmed the monophyly of the section *Lathyrus*, but only for the species sampled and, unfortunately, in this study the sections *Orobon* and *Orobastrum* were not included. Recent molecular studies using sequence data for the internal transcribed spacer (ITS) region and from cpDNA (Kenicer *et al.*, 2005, 2009) support Kupicha's morphological-based classifications and resolved clades that were left unresolved by previous studies (i.e. *Lathyrus*) (Fig. 1). Nevertheless these analyses also questioned the monophyly of some other clades. For instance, further DNA data from other species are required before any firm systematic decision can be made within the *Clymenum* and the *Linearicarpus* sections *sensu* Kupicha. Kenicer *et al.* (2005) also suggested that *Lathyrus*, contrary to what was stated by Kupicha (1983), originated in the eastern Mediterranean region during the mid to late Miocene rather than dispersing into this area from northern Eurasian Eocene or Oligocene lineages. However, Kupicha's proposal that North American taxa derived from a primitive ancestral stock (from Eurasia) is well supported, with the Bering

***Lathyrus* sectional treatments**			**Distribution (Kenicer *et al.* 2005, 2009)**
Kupicha (1983)	**Asmussen & Liston (1998)**	**Kenicer *et al.* (2009)**	
Notolathyrus [23]	*Orobus*	*Notolathyrus*	S. America (e.g. *L. nervosus*)
Orobus [54]		*Orobus*	N. America (e.g. *L. vestitus*) Holarctic (e.g. *L. japonicus*) E. Asia (e.g. *L. davidii*) C./W. Eurasia (e.g. *L. vernus*)
Pratensis [6]	*Pratensis*	*Pratensis*	C./W. Eurasia (e.g. *L. pratensis* or *L. aphaca*)
Aphaca [2]	*Aphaca*	*Aphaca*	
Lathyrostylis [20]	*Lathyrostylis*	*Lathyrostylis*	Mediterranean/W. Eurasia (e.g. *L. sativus*, *L. cicera*, *L. odoratus* or *L. digitatus*)
Neurolobus [1]	*Neurolobus*	*Neurolobus*	
Orobon [1]	*Lathyrus*		
Lathyrus [33]	*Circercula*	*Lathyrus*	
Orobastrum [1]	*Orobastrum*		
Linearicarpus [7]	*L. sphaericus*	*L. sphaericus*	
	L. angulatus	*L. angulatus*	
Viciopsis [1]			
Nissolia [1]	*Nissolia*	*Nissolia*	
Clymenum [3]	*Clymenum*	*Clymenum*	
	L. gloeospermus	*L. gloeospermus*	

FIG. 1. Sectional treatments and world distribution of *Lathyrus*. Numbers of species are given in square brackets. Shaded areas represent groups not studied. Adapted from Kenicer *et al.* (2005, 2009).

land bridge identified as the main route by which taxa have been exchanged between the two continents (Kenicer *et al.*, 2005). Furthermore, these authors suggested that the relationship between the South American clade and the Eurasian species was not due to parallel evolution, but rather was the result of a long-distance dispersal from Eurasia.

Several other traits were surveyed for potential use in defining closely related *Lathyrus* species, especially among the cultivated species. Patterns of protein electrophoresis (El-Shanshoury, 1997; Przybylska *et al.*, 1999; Emre, 2009) reflected the profound interspecific hybridization barriers in the genus *Lathyrus*, although *L. sativus* and *L. cicera* seem to be closer phylogenetically (Sáenz de Miera *et al.*, 2008; Emre, 2009). However, compliance with the Kupicha classification was not complete. Different levels of diversity have been detected in the different species, reflecting their different perenniality and breeding systems (Ben Brahim *et al.*, 2002). More recently, analyses of the differential composition of essential amino acids and seed oil fatty acids have proven useful to discriminate among the most closely related *Lathyrus* species (Pastor-Cavada *et al.*, 2009*a*, 2011).

DIVERSITY IN THE *LATHYRUS* GENUS

Breeding efforts in any cultivated plant species rely on the identification and characterization of the germplasm resources of the crop and the study of its evolution (Yunus and Jackson, 1991). Detailed knowledge of its closest relatives and geographic origin (Schaefer *et al.*, 2012) are important steps in this breeding process.

Gene pools

The gene pool concept originally proposed by Harlan and de Wet (1971), based on crossability and ease of gene transfer, was intended to provide a better classification of crop plants and their wild relatives. Exploitation of germplasm resources for the improvement of *L. sativus* currently concentrates on landrace material (Yunus and Jackson, 1991) by conventional means. The potential for a high level of improvement exists within this material since high variability has been found in the primary gene pool within *L. sativus* accessions, as will be discussed

below. However, there is the potential for exploitation of related species.

Yunus and Jackson (1991) were the first to identify the gene pools of *L. sativus*, with *L. amphicarpos* and *L. cicera* placed in a restricted secondary gene pool and the other *Lathyrus* species in an extended tertiary gene pool. More recently, Heywood *et al.* (2007) extended this *L. sativus* secondary gene pool to include *L. chrysanthus*, *L. gorgoni*, *L. marmoratus* and *L. pseudocicera*, with which *L. sativus* can cross and produce ovules, and possible more remotely *L. amphicarpos*, *L. blepharicarpus*, *L. chloranthus*, *L. cicera*, *L. hierosolymitanus* and *L. hirsutus*, with which *L. sativus* can cross and with which pods are formed (Table 1). The remaining species of the genus can be considered members of the tertiary gene pool.

There is also a lot of interest in exploitation of secondary gene pool resources in *L. odoratus* to obtain new pigmentations and scents. *Lathyrus odoratus* has been successfully crossed with *L. hirsutus*, *L. chlorantus* (Khawaja, 1988) and *L. belinensis* (Hammet *et al.*, 1994).

TABLE 1. Lathyrus sativus *gene pools (Heywood* et al.*, 2007)*

Primary gene pool	Secondary gene pool	Tertiary gene pool
Wild and cultivated *L. sativus* races	*L. cicera* *L. amphicarpus* *L. chrysanthus* *L. gorgoni* *L. marmoratus* *L. pseudocicera* *L. blepharicarpus* *L. choranthus* *L. hierosolymitanus* *L. hirsutus*	Other *Lathyrus* sp.

Germplasm collections

The most economically important *Lathyrus* species grown commercially are found in the section *Lathyrus*, and include *L. sativus*, *L. cicera* and *L. odoratus.* Although there are relatively few efforts being made throughout the world for the genetic improvement of these species compared with other crops, some important programmes exist that aim to improve its yield, quality and adaptability. All these breeding efforts require access to suitable genetic resources. Due to its importance as a survival food for some of the world's poorest people, yet recognizing the dangers that can be caused by excessive consumption, *L. sativus* was listed among the crops included in the multilateral system of access and benefit sharing under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2009). Some significant collections of cultivated and wild *Lathyrus* species have already been assembled and are maintained *ex situ* in a number of different institutes throughout the world. The largest collections are maintained by the Conservatoire Botanique National des Pyrénées et de Midi-Pyrénées (BP 70315) in France (4477 accessions) and by ICARDA in Syria (3239 accessions), both comprising about 50 % *L. sativus*. Details of other important *ex situ Lathyrus* collections are listed in Table 2. Co-ordinated international efforts to collect and conserve *Lathyrus* crop species have been initiated during the last decades, with the establishment of a '*Lathyrus* Genetic Resources Network' (Mathur *et al.*, 1998), and more recently with the development of a grass pea *ex situ* conservation

TABLE 2. *Main* Lathyrus *germplasm collections*

Institute	Location	No. of accessions	W/C*	Ls/Lc†	Contact
International Center for Agricultural Research in Dry Areas (ICARDA)	Syria	3239	45/54	53/6	www.icarda.cgiar.org/
Conservatoire Botanique National des Pyrénées et de Midi-Pyrénées (CBNPMP)	France	4477	NA	53/18	contact@cbnpmp.fr www.cbnpmp.fr
National Bureau of Plant Genetic Resources (NBPGR)	India	2619	3/85	98/0·04	www.nbpgr.ernet.in/
Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI)	Bangladesh	1841	0/100	100 %	www.bari.gov.bd/
Instituto Nacional de Investigación Agraria (INIA)	Chile	1424	NA	NA	www.inia.cl/
Ustymivka Experimental Station of Plant Production	Ukraine	1215	NA	NA	sluds@kot.poltava.ua
N.I. Vavilov All-Russian Scientifc Research Institute of Plant Industry	Russian Federation	1207	43/30	74/23	www.vir.nw.ru
Australian Grains Genebank	Australia	1020	28/39	60/30	www2.dpi.qld.gov.au/extra/asp/AusPGRIS/
Plant Gene Resources of Canada (PGRC)	Canada	840	10/90	93/0	pgrc3.agr.gc.ca/
Germplasm Resource Information Network (GRIN) United States Department of Agriculture (USDA)	USA	505	NA	36/5	www.ars-grin.gov/npgs/
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)	Germany	515	40/NA	45/47	www.ipk-gatersleben.de/en/
Centro de Recursos Fitogenéticos (CRF) Instituto nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)	Spain	429	NA	20/60	wwwx.inia.es/crf

*W/C: % wild/% cultivated material.
†Ls/Lc: % *Lathyrus sativus*/% *Lathyrus cicera* accessions.
NA, information not available.

strategy as part of the Global Crop Diversity Trust (Crop Trust, 2007). Both efforts focused on *L. sativus*, *L. cicera* and *L. ochrus*. Relatively large collections of *L. cicera* and *L. odoratus* exist (>800 accessions) in a number of countries due to its agricultural use, with many fewer accessions of other *Lathyrus* species (de la Rosa and Marcos, 2009; Rubiales *et al.*, 2009; Gurung and Pang, 2011; Parsons and Mikic, 2011; Shehadeh *et al.*, 2013).

As we will describe below, several phenotypic and genotypic germplasm characterization studies have taken place using these collections. These studies are enhancing the use of germplasm collections in crop improvement via plant breeding while also aiding the management of collections themselves through an improved understanding of the relationships between accessions and underlying patterns of diversity (Davenport *et al.*, 2004). However, the large sizes of many of these collections, either individually or collectively, complicate the characterization, evaluation and maintenance of the conserved germplasm, hindering their successful use (Odong *et al.*, 2013).

In addition to the above-mentioned *ex situ* collections, *in situ* preservation is recommended. *In situ* genetic reserve conservation may be defined as 'the location, designation, management and monitoring of genetic diversity in natural wild populations within defined areas designated for active, long-term conservation' (Maxted *et al.*, 1997). However, there has been very limited effort to conserve *Lathyrus* diversity *in situ*, and native populations are susceptible to genetic erosion or even extinction (Maxted and Bennett, 2001). Gap analysis studies of *Lathyrus* species to guide future collecting missions and *in situ* preservation efforts have been proposed (Shehadeh *et al.*, 2013). A multigenepool approach has been used by Maxted *et al.* (2012) for several legume genera including *Lathyrus*. This involved the collation of 61 081 unique geo-referenced *Lathyrus* records collected between 1884 and 2008.

Besides these co-ordinated conservation efforts, there is an urgent need to establish a global phenotyping and genotyping network for comprehensive and efficient characterization of *Lathyrus* germplasm for an array of target traits particularly for biotic and abiotic stress tolerance and nutritional and technological quality. *Lathyrus* descriptors (IPGRI, 2000) have been established as a result of the effort to define a set of common morphological traits, in order to have common tools focusing on the phenotyping of the genus. Those descriptors were mainly based on diversity observed for *L. sativus*, *L. cicera* and *L. ochrus*; however, they are also recommended for use for other *Lathyrus* species. This is expected to aid in effective identification and use of novel alleles for *Lathyrus* crop improvement.

Core collections

To unlock the genetic potential of these large collections, a general proposal is to construct smaller core collections to increase the efficiency of characterization and utilization, while preserving as much as possible the genetic diversity of the entire collection (Frankel, 1984; Brown, 1989). Frankel (1984) defined a core collection as a limited set of accessions representing, with minimum repetitiveness, the genetic diversity of a crop species and its wild relatives. These sub-sets have been reported for most legumes and have proven useful in identifying new sources of variation (Upadhyaya *et al.*, 2011).

In this way, and for the time being, an initial representative core collection for grass pea could be developed using passport data, but also employing the existing characterization and evaluation data normally more available on *L. sativus*, *L. cicera* and *L. amphicarpus*. In a second stage, as in the approach proposed by Upadhyaya *et al.* (2011), the core collection would be evaluated for various detailed morpho-agronomic, genotypic and quality traits to select a sub-set of 10 % of accessions to form a mini-core collection. At both stages, standard clustering procedures would be used to separate groups of similar accessions combined with various statistical tests to identify the best representatives (Upadhyaya *et al.*, 2011). On the other hand, accessions not included in core/mini-core collections would still be maintained as reserve collections for more in-depth study for specific traits and gene variants. Depending on the future progress of *Lathyrus* genetic engineering technology, other *Lathyrus* species besides those comprising its primary and secondary gene pool could also be considered as sources of novel genes for breeding.

However, insufficient efforts have been made in *Lathyrus* so far apart from the attempts of Shehadeh (2011) who compared several core sub-set selection strategies based on eco-geographical information. These authors also proposed the selection of alternative best-bet sets for particular traits (here named specific or thematic collections), through the Focused identification of Germplasm Strategy (FIGS). The FIGS approach is a trait-based and user-driven approach to select potentially useful germplasm for crop improvement. It was conceived to provide indirect evaluation of germplasm for specific traits, using, as a surrogate, the environment based on the hypothesis that the germplasm is likely to reflect the selection pressures of the environment in which it was originally sampled (Mackay *et al.*, 2005). This is especially appealing for improvement of adaptive traits such as abiotic stress (heat and drought) resistance, which can be more directly correlated with the climatic data (maximum temperature and aridity index) from the collecting sites (Endresen *et al.*, 2011).

Germplasm characterization

In order to achieve effective conservation and enhance the use of the germplasm collections, there is a need for detailed characterization of the existing diversity. Information regarding different levels of diversity in *Lathyrus* germplasm would help to identify sources of broadening improved breeding pools and in seeking genes and alleles that have not been tapped in modern breeding.

Diversity analysis through morphological phenotyping. By studying the morphological variation of a collection of *Lathyrus* accessions covering the known worldwide geographical distribution, Jackson and Yunus (1984) showed that *L. sativus* is differentiated into several distinct forms, primarily on the basis of flower colour, seed size, and size of leaves. In this way, they identified a clear distinction between the blue-flowered forms from South-west Asia, Ethiopia and the Indian sub-continent, and the white-and blue-flowered forms with white seeds that have a more western distribution (from the Canary Islands to the western ex-republics of the Soviet Union). This array of variation is undoubtedly the result of geographical separation as well as selection by man.

This grouping, of white-seeded with large seeds, originating mainly from Europe and North Africa, and coloured-seeded with relatively small seeds, originating mainly from Asia and Ethiopia, was also supported by Przybylska *et al.* (1998, 2000), based on quality analysis, and by Hanbury *et al.* (1999), based on agronomic testing. Those lines of Mediterranean/European origin were consistently higher yielding, with much larger seeds and later phenology (Hanbury *et al.*, 1999). They also had lower ODAP content (Abd El Moneim *et al.*, 2001). Preference for larger seeds in this area is common to other grain legumes such as lentil (*Lens culinaris*), chickpea (*Cicer arietinum*) and faba bean (*Vicia faba*), and is a product of human selection (Chowdhury and Slinkard, 2000). Similar studies on field evaluation of grass pea landraces, but in a more restricted germplasm study, where high variability in morphological and agronomical traits was detected, were also performed with Chilean (Tay *et al.*, 2000), Ethiopian (Tadesse and Bekele, 2003*a*, *b*), Italian (Tavoletti *et al.*, 2005), Indian (Kumari, 2001), Spanish (De la Rosa and Martín, 2001) and Slovak germplasm (Benková and Záková, 2001). In the majority of these studies, diversity among and within populations has been detected for several of the characterized traits (Table 3), indicative of high breeding potential in these materials.

Diversity analysis through biochemical and molecular markers. Biochemical and molecular markers can be used to better document the organization of genetic diversity between possible parental materials of new breeding programmes. The high agronomical and morphological diversity within *L. sativus* germplasm is also found at the biochemical and molecular level. Considerable genetic diversity, as revealed by isozymes and molecular markers, exists in *L. sativus* throughout the world (Table 4). These markers are normally very efficient in distinguishing among different *L. sativus* genotypes. However, it was not always possible to associate genetic diversity with morphological or geographical diversity (Yunus *et al.*, 1991; Tadesse and Bekele, 2001; Belaid *et al.*, 2006; Vaz Patto *et al.*, 2011). The lack of correlation between genetic diversity and the region of origin supports the idea that the natural distribution of *L. sativus* has been completely obscured by cultivation.

Chowdhury and Slinkard (2000), using a wide *L. sativus* germplasm collection, managed, however, to associate different levels of genetic diversity, measured by isozymes, with the different geographical origins. These authors identified the Near East and North Africa regions as those with the most isozyme variability, suggesting that the centre of diversity for *L. sativus* was this general area.

Also using a worldwide collection of *L. sativus* accessions from several different geographical origins, and the seed proteins, albumins (Przybylska *et al.*, 1998) and globulins (Przybylska *et al.*, 2000), it was possible to separate two groups of *L. sativus* accessions: white-seeded with large seeds, originating mainly from Europe and North Africa, and coloured-seeded with relatively small seeds, originating mainly from Asia and Ethiopia. Nevertheless, in a restricted study using only Southern Italian *L. sativus* germplasm, seed storage proteins were revealed to be unsuitable for detecting any variability among the studied landraces (Lioi *et al.*, 2011). This may be an indication of the high level of genetic affinity among these landraces collected from a restricted geographical region.

PCR-based molecular markers, such as randomly amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers, have also proven to be efficient in distinguishing between different *L. sativus* accessions and in assessing the within-species genetic variability (Chtourou-Ghorbel *et al.*, 2001; Belaid *et al.*, 2006; Barik *et al.*, 2007). The presence of considerable intra-population variation among the *L. sativus* accessions, revealed in many of these diversity studies (Chowdhury and Slinkard, 1997; Gutierrez-Marcos *et al.*, 2006), was greater than would have been expected given the predominantly autogamous breeding system of *L. sativus*. In fact, although *L. sativus* appears to be autogamous, outcrossing rates as high as 36 % have been recorded (Rahman *et al.*, 1995; Chowdhury and Slinkard, 1997; Gutierrez-Marcos *et al.*, 2006), which have implications in breeding and germplam maintenance.

Tavoletti and Iommarini (2007) evaluated the genetic diversity of a collection of *L. sativus* populations collected in central Italy using AFLPs. Two main clusters were found: one included large-seeded populations from farms and the second included small-seeded populations, cultivated in market-oriented farms. AFLP markers have also been used more recently in a Southern Italian collection of *L. sativus* and, even though the detected polymorphism was low, these populations were completely discriminated using 12 AFLP primer combinations (Lioi *et al.*, 2011). The genetic diversity of a collection of Iberian *L. sativus* germplasm was also studied using AFLPs as

TABLE 3. *Examples of* Lathyrus *diversity studies in morphological traits*

Traits	Germplasm	Reference
Flower colour, seed and leaf size	*L. sativus*, wild sp. (worldwide)	Jackson and Yunus (1984)
Plant vigour, time to flowering, to end of flowering and to podding, physiological maturity, seed weight and yield	*L. sativus*, *L. cicera* (worldwide)	Hanbury *et al.* (1999)
Seed size, shape and colour, days to flowering	*L. sativus* (Chile)	Tay *et al.* (2000)
Time to maturity, plant height, first pod height of setting, plant dry weight, pods/plant, seeds/plant, seed weight and yield, lodging resistance	*L. sativus* (Slovakia)	Benková and Záková (2001)
Time to flowering, podding and maturity, pods/plant, seeds/pod, seed weight and yield	*L. sativus* (India)	Kumari (2001)
Phenological, plant, inflorescence and fruit *Lathyrus* IPGRI descriptors	*L. sativus*, *L. cicera*, ten other *Lathyrus* sp. (Spain)	de la Rosa and Martín (2001)
Time to flowering and maturity, pods/plant, plant height, seed weight, harvest index, leaflet and seed size, flower and seed colour	*L. sativus* (Ethiopia)	Tadesse and Bekele (2003*a*, *b*)
Stem height, leaflet length and width, pod height, pod length, seeds/pod, seed weight and yield	*L. sativus* (Italy)	Tavoletti *et al.* (2005)

TABLE 4. *Examples of* Lathyrus *diversity studies in biochemical and molecular markers*

Marker type	Germplasm	Reference
ODAP	*L. sativus*, *L. cicera* (worldwide)	Hanbury *et al.* (2000)
	L. sativus (worldwide)	Abd El Moneim *et al.* (2001)
	L. sativus, *L. cicera* (worldwide)	Granati *et al.* (2003)
	L. sativus (Ethiopia)	Tadesse and Bekele (2003*a*)
	L. cicera (Iberian Peninsula)	Sánchez-Vioque *et al.* (2009)
	L. sativus (worldwide)	Kumar *et al.* (2011)
	L. sativus (central and southern Italy)	Piergiovanni *et al.* (2011)
	L. sativus, *L. cicera* (European)	Grela *et al.* (2010, 2012)
Isozymes	*L. sativus* (worldwide)	Yunus *et al.* (1991)
	L. sativus (worldwide)	Chowdhury and Slinkard (1997, 2000)
	L. sativus (Ethiopia)	Tadesse and Bekele (2001)
	L. sativus (worldwide)	Gutierrez-Marcos *et al.* (2006)
Seed storage proteins	*L. sativus*, *L. amphicarpos*, *L. blepharicarpus*, *L. cicera*, *L. gorgoni*, *L. marmoratus*, *L. pseudocicera*, *L. stenophyllus* (worldwide)	Przybylska *et al.* (1998, 2000)
	L. sativus (southern Italy)	Lioi *et al.* (2011)
RAPD	*L. sativus*, *L. cicera*, *L. latifolius*, *L. ochrus* (worldwide)	Chtourou-Ghorbel *et al.* (2001)
	L. sativus (worldwide)	Barik *et al.* (2007)
ISSR	*L. sativus*, *L. cicera*, *L. ochrus* (worldwide)	Belaid *et al.* (2006)
AFLP	*L. sativus* (central Italy)	Tavoletti and Iommarini (2007)
	L. sativus (southern Italy)	Lioi *et al.* (2011)
	L. sativus (Iberian Peninsula)	Vaz Patto *et al.* (2011)
L. sativus- and *Lotus japonicus*-derived EST-SSR	*L. sativus* (southern Italy)	Lioi *et al.* (2011)
M. truncatula- and *L. sativus*-derived EST-SSR	*L. sativus* (Ethiopia)	Shiferaw *et al.* (2012)
Pisum sativum-and *Medicago truncatula*-derived ITAP and *P. sativum*-derived gSSR and EST-SSR	*L. sativus*, *L. cicera* (Iberian Peninsula)	Almeida *et al.* (2014)

a first step towards the selection of appropriate parental lines for the establishment of a disease-resistant cross-breeding scheme (Vaz Patto *et al.*, 2011).

Molecular markers can also be developed using publicly available DNA sequencing data. Expressed sequence tags (ESTs) in public databases and cross-species transferable markers are considered to be a cost-effective means for developing sequence-based markers for less studied species (Ellwood *et al.*, 2008). Both approaches have been applied to *Lathyrus* sp. with variable achievements. Molecular markers developed for closely related legume species have been shown to be transferable to *L. sativus* and *L. cicera* (Almeida *et al.*, 2014). These included genomic and expressed sequence tag microsatellite (gSSR and EST-SSR) and intron-targeted amplified polymorphic (ITAP) markers, and were successfully used to discriminate within *L. cicera* and *L. sativus* accessions.

Shiferaw *et al.* (2012), using information on EST-SSRs derived from *Medicago truncatula* and also on publicly available (NCBI database) *L. sativus* EST sequences developed and validated polymorphic markers that were used successfully for exploring the genetic diversity of Ethiopian grass pea accessions. Lioi *et al.* (2011) developed SSR markers from publicly available (EMBL database) *L. sativus* and *Lotus japonicus* cDNA sequences and used them to study Southern Italian *L. sativus* accessions. In this case accessions were grouped into two clearly distinguishable clusters following a geographical pattern, but not consistent with the morphological data, AFLP- or SSR-based clustering. If we take into account the presence of polymorphism in the studies where this comparison could be performed, it can be concluded that more informative markers for genetic diversity studies were developed directly from *L. sativus* sequences than were transferable from *M. truncatula* or *Lotus japonicus*.

More recently, polymorphic EST-SSRs were developed from *L. sativus* sequence information available on a public database (NCBI database) (Sun *et al.*, 2012) and from an enriched grass pea genomic library (Lioi and Galasso, 2013), as additional resources for grass pea genetic studies, but they are not yet exploited in diversity analysis.

Diversity on quality traits. Several preliminary studies to establish quality breeding approaches in *Lathyrus* sp. resulted in characterization of the quality diversity of germplasm collections (e.g. Granati *et al.*, 2003). *Lathyrus* species are protein-rich legumes, the development of which into important food legumes has been hindered by the presence of ODAP, which, if consumed in large quantities for extended periods, can cause irreversible paralysis (Lambein and Kuo, 2009). The reduction in ODAP levels in *L. sativus* breeding has been the emphasis for a long time (Kumar *et al.*, 2011; Girma and Korbu, 2012; Hillocks and Maruthi, 2012). No *L. sativus* or *L. cicera* accession is ODAP free, although in several lines the ODAP content can be significantly low. This appears to be species related, since the average ODAP content of *L. cicera* is generally lower than that of *L. sativus* (Hanbury *et al.*, 2000; Abd El Moneim *et al.*, 2001; Kumar *et al.*, 2011). Variation of ODAP

content, in a range from 0·02 to 2·59 %, has been reported in *L. sativus* (Granati *et al.*, 2003; Tadesse and Bekele, 2003*a*; Grela *et al.*, 2010, 2012; Piergiovanni *et al.*, 2011) and from 0·09 to 0·49 % in *L. cicera* seeds (Granati *et al.*, 2003; Sánchez-Vioque *et al.*, 2009).

Selection for high yield and low ODAP can be practised simultaneously for *L. sativus* improvement. Most of the initial progress in the development of cultivars low in ODAP was by direct selection from landraces and lines with a worldwide origin, and several improved grass pea cultivars have been released as the result of various national and international breeding initiatives (Ali-Bar, Ceora, Gurbuz 1, Wasie, Prateek, Mahateora, Ratan, Bari Khesari 1 and 2, and Bina Khesari 1, all with an ODAP content $<0{\cdot}1$ %) (summarized by Abd El Moneim *et al.*, 2001; Kumar *et al.*, 2011). Similarly, improved cultivars with low ODAP have been released, such as Chalus (Hanbury and Siddique, 2000).

This strategy of prioritizing reduction of ODAP content in breeding programmes is under debate today. First, although a number of cultivars with low ODAP have been released, the long-term results of these efforts are frequently questioned because ODAP content is highly influenced by climatic and edaphic conditions, with strong genotype × environment effects (Fikre *et al.*, 2011; Jiao *et al.*, 2011; Girma and Korbu, 2012). Water stress can double the toxin content in the plant (Hanbury *et al.*, 1999) and there are indications that zinc fertilization can reduce the toxin accumulation (Lambein *et al.*, 1994), although the mechanism by which the ODAP content may be reduced by added zinc is not known (Abd El-Moneim *et al.*, 2010).

This long-term breeding priority did not take into consideration that ODAP in itself does not seem to be a problem when grass pea is consumed as part of a balanced diet, in which case grass pea is harmless to both humans and animals (Lambein and Kuo, 2009). Also, risks of overconsumption can be reduced by the fortification of grass pea with cereals rich in sulfur amino acids and condiments rich in antioxidants, such as onion, garlic and ginger (Getahun *et al.*, 2003, 2005). In addition to this, seeds can be partly detoxified by various food processing methods, as reviewed by Kumar *et al.* (2011). Therefore, it seems clear that the widespread school of thought held 50 years ago of the vital need to reduce the ODAP content in *Lathyrus* seeds by breeding does not exist today. Even with the possibility of its toxicity, we should not neglect the potential benefits of ODAP. For instance, there is the prospect of using ODAP as a haemostatic agent during surgery (Lan *et al.*, 2013). ODAP is not only produced by several *Lathyrus* sp. seeds, it is also present in the longevity-promoting ginseng root (Kuo *et al.*, 2003), where, under the name Dencichine, it is known for its haemostatic property to stop bleeding (Lan *et al.*, 2013).

In addition, there is the hypothesis that nitriles are the causative agents of neurolathyrism rather than ODAP (Llorens *et al.*, 2011). However, nitriles too, even though they are toxic, can have some benefits. For instance, β-aminopropionitrile (β-APN) inhibits the cross-linking of collagen and is the cause of osteo-lathyrism, but has a number of pharmacological applications. β-APN has the potential for the control of silicotic pulmonary fibrosis (Levene *et al.*, 1967); for the control of unwanted scar tissue in humans (Harrison *et al.*, 2006); and for diminishing the metastatic colonization potential of circulating breast cancer cells (Bondareva *et al.*, 2009). β-APN is a reagent used as an intermediate in the manufacture of β-alanine and pantothenic acid. Most reports on β-APN refer to *L. odoratus*. Genetic variation for content in other *Lathyrus* germplasm has not been explored.

Another recent paradigm shift in the perception of the *L. sativus* research is its content of homoarginine, which is an alternative substrate for nitric oxide biosynthesis (Rao, 2011). Nitric oxide is well recognized for its role in cardiovascular physiology and general well-being, and thus a daily dietary intake of homoarginine through small quantities of *L. sativus* may have advantages and deserves to be exploited. The activation of protein kinase C (PKC) by ODAP adds a new dimension for investigating its therapeutic potentials in such areas as Alzheimer's disease, hypoxia and the long-term potentiation of neurons essential for memory (Rao, 2011). Genetic variation for homoarginine content in *L. sativus* germplasm has been identified (Piergiovanni and Damascelli, 2011). Also, *Lathyus* spp. have potential for use as functional foods as the antioxidant activity of their polyphenols is higher than that of other legumes such as chickpea, lupin (*Lupinus* sp.) and soybean (*Glycine max*) (Pastor-Cavada *et al.*, 2009*b*). Another potential beneficial application of *L. sativus* seeds it to ameliorate diabetic symptoms, as they possess glycosylphosphatidylinositol with insulin-mimetic activity (Pañeda *et al.*, 2001).

Diversity in stress resistance. Many more reports exist on the biotic stress resistance evaluation of *Lathyrus* germplasm collections than on abiotic stress evaluations. *Lathyrus sativus* and *L. cicera* accessions of Iberian origin have been screened for resistance against powdery mildew and rust fungi (Vaz Patto *et al.*, 2006*a*, *b*, 2007, 2009; Vaz Patto and Rubiales, 2009, 2014) and against the parasitic weed *Orobanche crenata* (Fernández-Aparicio *et al.*, 2009, 2012; Fernández-Aparicio and Rubiales, 2010), identifying a wide range of levels of resistance. Moderate levels of resistance to powdery mildew in *L. sativus* have also been reported in India and Syria (Campbell *et al.*, 1994; Robertson and Abd El-Moneim, 1996; Asthana and Dixit, 1998). Powdery mildew is among the major diseases affecting *L. sativus* (Campbell *et al.*, 1994) and *L. odoratus* crops (Cook and Fox, 1992), and rusts are important diseases of *L. sativus* in north-western Ethiopia (Campbell, 1997). However, insufficient information is often available on the identity of the fungus. Powdery mildew that infects *Lathyrus* is believed to be mainly *Erysiphe pisi*, but it might be that several other species are able to infect *Lathyrus* sp., as recently found in pea (Fondevilla *et al.*, 2013). The existence of specialized forms and races is still unclear, but a different ability to infect different plant species has been reported. Cook and Fox (1992) reported that a strain of *E. pisi* collected on *L. odoratus* was able to infect faba bean but not pea, whereas a different strain collected on *L. latifolius* was able to infect pea and faba bean. Similarly, rust in *Lathyrus* sp. is believed to be caused by both *Uromyces pisi* and *U. viciae-fabae* (Barilli *et al.*, 2011, 2012).

Resistance to, or escape from, the parasitic weed *O. crenata* has also been identified in *L. sativus* and *L. cicera* germplasm (Fernández-Aparicio *et al.*, 2009, 2012; Fernández-Aparicio and Rubiales, 2010). High levels of resistance to *O. crenata* have been reported in the species *L. ochrus* and *L. clymenum* (Sillero *et al.*, 2005). Other relevant reports include resistance to *Mycosphaerella pinodes* (Robertson and Abd El-Moneim, 1996; Gurung *et al.*, 2002), *Fusarium oxysporum* (Benková

TABLE 5. *Examples of* Lathyrus *diversity studies in biotic stress resistance*

Stress	Germplasm	Reference
Cercospora pisi-sativae	*L. sativus*	Mishra *et al.* (1986)
Meloidogyne hapla (root knot nematode)	*L. latifolius*, *L. sylvestris*, *L. hirsutus*	Rumbaugh and Griffin (1992)
Fusarium oxysporum	*L. sativus* (Slovakia)	Benková and Záková (2001)
Mycosphaerella pinodes	*L. sativus* (worldwide)	Gurung *et al.* (2002)
Erysiphe pisi (powdery mildew)	*L. sativus*, *L. cicera* (India, Syria, Iberian Peninsula)	Campbell *et al.* (1994); Robertson and Abd El-Moneim (1996); Asthana and Dixit, (1998); Vaz Patto *et al.* (2006*a*, 2007)
Uromyces pisi (rust)	*L. sativus*, *L. cicera* (Iberian Peninsula)	Vaz Patto and Rubiales (2009); Vaz Patto *et al.* (2009)
Orobanche crenata (broomrape)	*L. sativus*, *L. cicera*, eight other *Lathryrus* sp. (worldwide)	Sillero *et al.* (2005); Fernández-Aparicio *et al.* (2009, 2012); Fernández-Aparicio and Rubiales (2010)
Pseudomonas syringae	*L. cicera* (Iberian Peninsula)	Martín-Sanz *et al.* (2012)

and Záková, 2001) and *Cercospora pisi-sativae* (Mishra *et al.*, 1986) in *L. sativus* germplasm; to *Pseudomonas syringae* pv. *syringae* in *L. cicera* germplasm (Martín-Sanz *et al.*, 2012); and to the northern root-knot nematode (*Meloidogyne hapla*) in *L. latifolius*, *L. sylvestris* and *L. hirsutus* (Rumbaugh and Griffin, 1992). All these reports are summarized in Table 5.

In relation to *Lathyrus* abiotic stress resistance screening, the lack of methodologies to identify resistant genotypes has hampered the proper exploitation in breeding of *Lathyrus* sp. As a result, knowledge of the mechanisms underlying this resistance to environmental injuries is also missing. The effects of drought and salt stress on different *Lathyrus* sp. morphological and physiological traits have been studied with the objective of developing the missing efficient discrimination methods applicable to large germplasm screenings. Using a critical salt-induced stress treatment or the chlorophyll *a* fluorescence transient, several *L. sativus* salt- and drought-resistant genotypes, respectively, have been identified (Talukdar, 2011; Silvestre *et al.*, 2014).

PROSPECTS FOR *LATHYRUS* DIVERSITY ANALYSIS AND USE IN BREEDING

Conserved plant genetic resources are essential to meet the current and future needs of crop improvement programmes. However, progress in *Lathyrus* breeding has been slow due to the dispersal of the few available resources and evaluation efforts among several scattered germplasm collections, plus the modest molecular and biotechnological breeding tools currently in existence. More efficient and faster breeding approaches are needed on this neglected but promising, underutilized species.

Marker development for diversity analysis and for marker-assisted selection

Although there has been encouraging recent growth of available genomic information in the *Lathyrus* genus, these resources are still modest when compared with other legume crops such as pea. As mentioned before in this review, several neutral DNA marker systems have been applied successfully in *Lathyrus* diversity studies. However, this success has not been translated into gene discovery or development of trait-associated markers for marker-assisted selection (MAS) in *Lathyrus* breeding.

There is one report of an *L. sativus* molecular marker linkage map developed to identify genomic regions linked to agronomically important traits, ascochyta blight resistance (Skiba *et al.*, 2004). Also, there is no subsequent report on the use of the detected associated markers in breeding for resistance in *Lathyrus*. Moreover, this linkage map was not adequately saturated with markers, presenting numerous gaps and short linkage groups (Vaz Patto *et al.*, 2006*b*); due to the lack of anchor markers, it could not be aligned and compared with other legume species linkage maps. Earlier studies indicated that extensive genome conservation based on comparative genetic mapping was exhibited by members of the legume Papilionoideae subfamily (such as *Pisum*, *Lens*, *Vicia* or *Cicer*) (Zhu *et al.*, 2005). There is an urgent need to develop a more comprehensive genetic map for *Lathyrus*, with localization of useful genes and quantitative trait loci (QTLs) for MAS and with the possibility of alignment with other species in a comparative mapping approach.

The inclusion of cross-amplified anchor markers needs to be addressed to allow comparative mapping with other related legume species, opening the way for using *Lathyrus* as a source of interesting traits for other related species, and vice versa. Genomic and EST microsatellites were the most commonly attempted cross-species amplification marker systems in *Lathyrus*, but ITAP, RGA and DR genes have been used on these cross-amplification studies involving *Lathyrus* sp. (see above). Some of these marker systems, like microsatellites, have an additional advantage for linkage map development, since they are co-dominant markers. The incorporation of co-dominant markers will be very important for a correct estimation of genetic distances among markers in repulsion phase (Vaz Patto *et al.*, 2011).

As previously mentioned above, not only cross-amplifiable markers from other legume species are being used in *Lathyrus* genetic studies. *Lathyrus* EST are being made available in public databases, in particular for *L. sativus* and *L. odoratus*, and these are now being used to develop molecular markers associated with coding DNA. Very recently, cDNA libraries have also been developed for *L. cicera* and EST-SSR markers identified (Almeida *et al.*, 2011). This marker system generally has a high degree of sequence conservation and may potentially be more transferable among species, thus facilitating comparative genomic mapping (Vaz Patto *et al.*, 2006*b*).

With the development of high-throughput and dense genotyping, the assessment of the correlation between phenotype and genotype, needed for the development of MAS approaches, has shifted from focusing on two parental lines differing strongly

in phenotype to populations of unrelated individuals. Association mapping panels by sampling more genetic diversity can take advantage of many more generations of recombination and avoid the time-consuming generations of crosses (Morrell *et al.*, 2012). High-throughput genotyping associated with a core collection evaluation will facilitate trait dissection and gene discovery through association mapping as well as characterization of genetic structure (Cobb *et al.*, 2013). That is why it would be so important to concentrate the evaluation efforts on to a core sub-set collection representative of all the existing diversity, but of a manageable size.

Other biotechnological advances

In contrast to the development of, and now initiated use of different molecular markers with breeding objectives, other biotechnological advances conceived particularly for functional studies are not currently used on *Lathyrus* breeding.

Expression analysis studies were initially performed on *L. sativus* inoculated with *Mycosphaerella pinodes* using a limited number of 29 ESTs representing genes coding for enzymes and proteins involved in different levels of defence (Skiba *et al.*, 2005). Some of the *Lathyrus* EST libraries previously mentioned in this review are now being developed not only for identifying molecular markers useful for the construction of high-density genetic linkage maps, but also for allowing expression analysis studies in order to identify and assess the function of putative genes thought to be involved in plant disease resistance responses. This is the case of rust resistance in *L. sativus* and *L. cicera* (Almeida *et al.*, 2012). Next-generation sequencing technologies have also been recently applied to the *L. sativus*–*Ascochyta* sp., interaction through Super SAGE quantitative gene expression profiling (Almeida *et al.*, 2013). With this approach, it was possible to identify >3000 ($P < 0{\cdot}05$) overexpressed or 900 ($P < 0{\cdot}05$) underexpressed transcripts during the first 24 h after inoculation between infected and control tissue, opening the way to a powerful route of identification of candidate resistance genes and the study of resistance gene networks in *L. sativus*.

Gurung and Pang (2011) have recently prioritized the future construction of *Lathyrus* EST libraries from developing pods and seeds to achieve representation of reproductive tissues. In addition, these authors have called attention to the difficulties of proof-of-function studies of putative *Lathyrus* genes via overexpression, deletion or silencing due to the non-existence of a widely employed transformation system (only one event, reported by Barik *et al.*, 2005) or a broadly applicable virus-induced gene silencing (VIGS) reverse genetics approach, until now only reported for *L. odoratus* (Grønlung *et al.*, 2008). Likewise, the reduced size of the presently available mutant populations does not provide the opportunity to study the effects of gene deletions/silencing through targeting-induced local lesions in genome (TILLING) approaches (Gurung and Pang, 2011).

Mutation breeding has been employed on several occasions to create additional variability in a range of traits, from plant growth habit to ODAP or methionine and lysine content (reviewed in Vaz Patto *et al.*, 2011). Extensive variation was also detected in the course of tissue culture studies in several morphological traits, and has also been exploited in grass pea breeding (Kumar *et al.*, 2011).

Embryo rescue and protoplast fusion protocols meant to increase the range of species in successful interspecific crosses (Ochatt *et al.*, 2007) have unfortunately not been routinely used for grass pea improvement to date due to the difficult regeneration of hybrid plants. There are also survival problems with the recovered confirmed haploid plants that were reported in the haplo-diploidization method established from cultured isolated microspores in *L. sativus* (Ochatt *et al.*, 2009), hindering its application in breeding. An *in vitro* protocol for fast production of advanced progeny that drastically shortens generation cycles has been developed in *L. sativus* (Ochatt *et al.*, 2004). Over three generations per year can be obtained instead of the normal two, allowing a faster progress in *L. sativus* improvement. However this approach is only applicable when few seeds/plant are intended, as in single-seed descendant breeding schemes.

CONCLUSIONS

Today, due to the deluge of low-cost genomic information, phenotyping is quickly emerging as the major operational bottleneck and funding constraint of genetic analysis (Cobb *et al.*, 2013). Consequently, after overcoming the problem of availability of appropriate germplasm resources to address specific questions through the establishment of a core collection, further emphasis should be placed on overcoming the shortage of high-quality phenotypic information to associate with the high-throughput genotyping information.

We propose that future international efforts on *L. sativus* and *L. cicera* improvement should concentrate on the development of publicly available joint core collections, and on its high-resolution genotyping. This will be critical for permitting a decentralized phenotyping, where multiple researchers can interrogate the same genetic materials, phenotyping in environments and with technology and analytical expertise that are uniquely available to different research groups (Cobb *et al.*, 2013). Such co-ordinated international effort is sure to translate into more efficient and faster breeding approaches which are especially needed for such neglected but promising, underutilized species.

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