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

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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 breeding of L. sativus and L. cicera.

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 neurolatyrism 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. ciceria) and the ornamental sweet pea (L. odoratus). Lathyrus ciceria 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 Fabaeae (syn. Vicieae) along with Vicia, Lens, Pisum and Vavilovia (reviewed in Smykal et al., 2010). Recently, Schaefer et al. (2012) concluded that the Fabaeae 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 Fabaeae should also include Pisum and Vavilovia. This regrouping is also supported by the currently available plastid genomes of L. sativus and P. sativum (Magee et al., 2010). Furthermore, the genera Pisum and Lathyrus share the phytotoxin pisatin, which is not found in Vicia or Lens (Robeson and Harborne, 1980).

Most Lathyrus species are diploid (2n = 14), with a few natural autoploids or allopolyploids, or contain both diploid and autoploidy 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. ciceria, 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/Iranian-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) (Bade 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
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., 2009a, 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. amphicarpus* 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. amphicarpus*, *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 pigmentation and scents. *Lathyrus odoratus* has been successfully crossed with *L. hirsutus*, *L. chloranthus* (Khawaja, 1988) and *L. belinensis* (Hammet et al., 1994).

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

<table>
<thead>
<tr>
<th>Primary gene pool</th>
<th>Secondary gene pool</th>
<th>Tertiary gene pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild and cultivated <em>L. sativus</em> races</td>
<td><em>L. cicera</em></td>
<td>Other <em>Lathyrus</em> sp.</td>
</tr>
<tr>
<td></td>
<td><em>L. amphicarpus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. chrysanthus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. gorgoni</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. marmoratus</em></td>
<td></td>
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<tr>
<td></td>
<td><em>L. pseudocicera</em></td>
<td></td>
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<tr>
<td></td>
<td><em>L. blepharicarpus</em></td>
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<td></td>
<td><em>L. chloranthus</em></td>
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<td></td>
<td><em>L. cicera</em></td>
<td></td>
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<tr>
<td></td>
<td><em>L. hierosolymitanus</em></td>
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<td></td>
<td><em>L. hirsutus</em></td>
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**Table 2. Main Lathyrus germplasm collections**

<table>
<thead>
<tr>
<th>Institute</th>
<th>Location</th>
<th>No. of accessions</th>
<th>W/C</th>
<th>Ls/Lc</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>International Center for Agricultural Research in Dry Areas (ICARDA)</td>
<td>Syria</td>
<td>3239</td>
<td>45/54</td>
<td>53/6</td>
<td><a href="http://www.icarda.cgiar.org/">www.icarda.cgiar.org/</a></td>
</tr>
<tr>
<td>Conservatoire Botanique National des Pyrénées et de Midi-Pyrénées (CRNPMP)</td>
<td>France</td>
<td>4477</td>
<td>NA</td>
<td>53/18</td>
<td><a href="mailto:contact@cbnpmp.fr">contact@cbnpmp.fr</a></td>
</tr>
<tr>
<td>National Bureau of Plant Genetic Resources (NBPGR)</td>
<td>India</td>
<td>2619</td>
<td>3/85</td>
<td>98/0.04</td>
<td><a href="http://www.nbpgr.ernet.in/">www.nbpgr.ernet.in/</a></td>
</tr>
<tr>
<td>Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI)</td>
<td>Bangladesh</td>
<td>1841</td>
<td>0/100</td>
<td>100%</td>
<td><a href="http://www.bari.gov.bd/">www.bari.gov.bd/</a></td>
</tr>
<tr>
<td>Instituto Nacional de Investigación Agraria (INIA)</td>
<td>Chile</td>
<td>1424</td>
<td>NA</td>
<td>NA</td>
<td><a href="http://www.inia.cl/">www.inia.cl/</a></td>
</tr>
<tr>
<td>Ustymivka Experimental Station of Plant Production</td>
<td>Ukraine</td>
<td>1215</td>
<td>NA</td>
<td>NA</td>
<td><a href="mailto:sluds@kot.poltava.ua">sluds@kot.poltava.ua</a></td>
</tr>
<tr>
<td>N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry</td>
<td>Russian Federation</td>
<td>1207</td>
<td>43/30</td>
<td>74/23</td>
<td><a href="http://www.vir.nw.ru">www.vir.nw.ru</a></td>
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<tr>
<td>Plant Gene Resources of Canada (PGRC)</td>
<td>Canada</td>
<td>840</td>
<td>10/90</td>
<td>93/0</td>
<td>pgrc3.agr.gc.ca/</td>
</tr>
<tr>
<td>Germplasm Resource Information Network (GRIN) United States Department of Agriculture (USDA)</td>
<td>USA</td>
<td>505</td>
<td>NA</td>
<td>36/5</td>
<td><a href="http://www.ars-grin.gov/npgs/">www.ars-grin.gov/npgs/</a></td>
</tr>
<tr>
<td>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)</td>
<td>Germany</td>
<td>515</td>
<td>40/30</td>
<td>45/47</td>
<td><a href="http://www.ipk-gatersleben.de/en/">www.ipk-gatersleben.de/en/</a></td>
</tr>
<tr>
<td>Centro de Recursos Fitogenéticos (CRF) Instituto nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)</td>
<td>Spain</td>
<td>429</td>
<td>NA</td>
<td>20/60</td>
<td>wwwx.inia.es/crf</td>
</tr>
</tbody>
</table>

*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 multi-genepool 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). Preference for larger seeds in this area is common to other 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, 2003a, 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; Barik et al., 2007). 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 (Chitourou-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 germplasm 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>
<thead>
<tr>
<th>Traits</th>
<th>Germplasm</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower colour, seed and leaf size</td>
<td>L. sativus, wild sp. (worldwide)</td>
<td>Jackson and Yunus (1984)</td>
</tr>
<tr>
<td>Plant vigour, time to flowering, to end of flowering and to podding, physiological maturity, seed weight and yield</td>
<td>L. sativus, L. cicera (worldwide)</td>
<td>Hanbury et al. (1999)</td>
</tr>
<tr>
<td>Seed size, shape and colour, days to flowering</td>
<td>L. sativus (Chicago)</td>
<td>Tay et al. (2000)</td>
</tr>
<tr>
<td>Time to maturity, plant height, first pod height of setting, plant dry weight, pods/plant, seeds/plant, seed weight and yield, lodging resistance</td>
<td>L. sativus (Slovakia)</td>
<td>Benková and Záková (2001)</td>
</tr>
<tr>
<td>Time to flowering, podding and maturity, pods/plant, seeds/pod, seed weight and yield</td>
<td>L. sativus (India)</td>
<td>Kumari (2001)</td>
</tr>
<tr>
<td>Phenological, plant, inflorescence and fruit Lathyrus IPGRI descriptors</td>
<td>L. sativus, L. cicera, ten other Lathyrus sp. (Spain)</td>
<td>de la Rosa and Martín (2001)</td>
</tr>
<tr>
<td>Time to flowering and maturity, pods/plant, plant height, seed weight, harvest index, leaflet and seed size, flower and seed colour</td>
<td>L. sativus (Ethiopia)</td>
<td>Tadesse and Bekele (2003a, b)</td>
</tr>
<tr>
<td>Stem height, leaflet length and width, pod height, pod length, seeds/pod, seed weight and yield</td>
<td>L. sativus (Italy)</td>
<td>Tavoletti et al. (2005)</td>
</tr>
</tbody>
</table>
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, 2003a; 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 <0.1 %) (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 (Kao et al., 2003), where, under the name Dencichine, it is known for its haemostatic property to promote ginseng root (Kuo et al., 2003), where, under the name Dencichine, it is known for its haemostatic property to promote ginseng root (Kuo et al., 2003). Another potential beneficial application of *L. sativus* seeds is to ameliorate diabetic symptoms, as they possess glycosylphosphatidylinositol with insulin-mimetic activity (Pańeda 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., 2006a, 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 (Fondèvila 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. vicieae-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* (Siliero et al., 2005). Other relevant reports include resistance to *Mycosphaerella pinodes* (Robertson and Abd El-Moneim, 1996; Gurung et al., 2002), *Fusarium oxysporum* (Benková et al., 2009).
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., 2006b); 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, Viciea 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. cicer 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., 2006b).

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 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 < 0.05) overexpressed or 900 (P < 0.05) 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, overexpression, deletion or silencing due to the non-existence of proof-of-function studies 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 < 0.05) overexpressed or 900 (P < 0.05) 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.

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).

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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