Chapter 8
Grass Pea

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1 Introduction

Grass pea (*Lathyrus sativus* L.) is a multipurpose robust grain legume crop. It can grow in both drought- and flooding-prone environments and poor soils due to its hardy and penetrating root systems (Campbell 1997; Vaz Patto et al. 2006b). It has a high nutritional value (protein content ranging from 25 to 30 %), being important both for human food and animal feed. In what concerns human consumption, it can be consumed uncooked as a green snack, cooked in a stew, milled into flour or by roasting the seed (Peña-Chocarro and Peña 1999). In addition to its uses as food and feed, symbiosis with rhizobia allows an efficient nitrogen fixation in the soil, lowering the inputs needed in crop rotation and making them suitable to be used as green manure in sustainable farming systems (Hanbury et al. 2000). As an example of its versatility, grass pea is easily introduced in intercropping systems, rotations or used along with paddy rice in relay cropping systems (Abd El Moneim et al. 2001; Campbell et al. 1994; Hillocks and Maruthi 2012).

There is great potential for the expansion in the utilization of grass pea in dry areas or zones which are becoming more drought prone, with increased salinity or increased tendency to suffer from biotic stresses. However, the crop is unpopular

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A. M. De Ron (ed.), *Grain Legumes,* Handbook of Plant Breeding 10,
DOI 10.1007/978-1-4939-2797-5_8

with governments and donors because the plant contains small amounts of a toxin, β-*N*-ozalyl-L-α, β-diaminopropanoic acid (ODAP). Although this toxin can cause a neuronal disorder, known as "lathyrism", the condition develops in humans with a 6 % chance only when grass pea is consumed in large quantities, unaccompanied by other foodstuffs in an unbalanced diet and during a long period of time (Lambein et al. 2009). Also, seeds can be partly detoxified by various processing methods (Kumar et al. 2011; Kuo et al. 2000).

Even though this robust crop is rightly considered as a model crop for sustainable agriculture and despite the lathyrism stigma, the development of new breeding technologies and the growing interest in its use in Mediterranean-type environments, all over the world, will provide a bright future to this crop (Vaz Patto et al. 2006b; Vaz Patto and Rubiales 2014).

2 Origin and Systematics

Grass pea belongs to genus *Lathyrus,* within the Fabaceae family (syn. Leguminosae), subfamily Faboideae (syn. Papilionoideae), tribe Fabeae (syn. Vicieae), along with genera *Pisum, Vicia, Lens* and *Vavilovia* (Kenicer et al. 2005; Schaefer et al. 2012; Smýkal et al. 2011; Wojciechowski et al. 2004).

Natural distribution of grass pea has been completely obscured by human cultivation. Its use for food, feed and forage makes it difficult to distinguish between wild and domesticated populations, toughening the task to precisely locate its centre of origin (Kumar et al. 2013). The most probable grass pea centre of origin is believed to have been in the eastern Mediterranean or Fertile Crescent, around 6000 before present (BP). This has been supported by archaeobotanical and recent phylogenetic reports (Kislev 1989; Schaefer et al. 2012), refuting the hypothesis by Smartt (1984) that the centre of origin was located in Southwest or Central Asia. Domestication of grass pea seems to have occurred alongside with other pulses, being normally found with early domesticates of pea (*Pisum sativum* L.), lentils (*Lens culinaris* Medik.) and bitter vetch (*Vicia ervilia* (L.) Willd.; Erskine et al. 1994).

Hopf (1986) hypothesized that *L. sativus* is a derivative from *Lathyrus cicera,* its genetically nearest wild species. In addition, in what concerns domestication in Southern Europe (France and Iberian peninsula), evidences of cultivation of *L. cicera* were found, dating from 4000 or 3000 BP, suggesting that expansion of *L. sativus* farming may have also led to the domestication of the local *L. cicera* (Campbell 1997).

Within the economically important legume crops and model species, *P. sativum* is reported as the closest relation to grass pea, followed by lentil, faba bean (*Vicia faba* L.), barrel medic (*Medicago truncatula* Gaertn.), chickpea (*Cicer arietinum* L.) and *Lotus corniculatus* L. (Asmussen and Liston 1998; Ellison et al. 2006; Wojciechowski et al. 2004).

The infrageneric classification of *Lathyrus* genus has been revised several times, the one reported by Kupicha (1983) being the most accepted one. In this treatment, the genus is organized in 13 clades (Orobus, Lathyrostylis, Lathyrus, Orobon, Pratensis, Aphaca, Clymenum, Orobastrum, Viciopsis, Linearicarpus, Nissolia, Neurolobus and Notolathyrus). This morphological-based classification has been recently supported by molecular phylogenetic studies using sequence data from the internal transcribed spacer (ITS) region and from cpDNA (Kenicer et al. 2005, 2009). Schaefer et al. (2012), using nuclear and chloroplast phylogenetic data, further suggested that the genus *Lathyrus* is not monophyletic and recommended that a more natural classification would be to transfer *Pisum* and *Vavilovia* to a then monophyletic *Lathyrus* genus.

3 Varietal Groups

Great morphological variation is reported in grass pea, especially in vegetative characters such as leaf length, while, for instance, its floral characters are much less variable, showing a clear grouping in flower colour (Fig. 8.1; Jackson and Yunus 1984), as well as its seed and yield traits (Hanbury et al. 1999). Several studies divided grass pea accessions broadly into two groups: those from the Indian subcontinent and those from the Mediterranean region. Jackson and Yunus (1984) reported that all blue-flowered accessions came from Southwest and South Asia, while the white and mixed-coloured accessions had a more western distribution, from the Canary Isles to the western republics of the Soviet Union. These authors also pointed out that white-flowered accessions only had white seeds with no secondary markings on the seed coat. In accordance with this, Hanbury et al. (1999) reported that Mediterranean accessions were characterized by larger and whiter seeds, selected for human consumption, with higher yield potential than the Indian accessions. Grass pea small-seeded accessions are considered more primitive types

Fig. 8.1 *Blue*-flowered *Lathyrus sativus* genotype

and normally associated with hardened seeds like what happens in other Old World grain legumes such as pea, chickpea or lentil (Chowdhury and Slinkard 2000).

A particular case is the germplasm selected for forage, in the Mediterranean region, with landraces with broad leaves and pods, but low seed yield (Chowdhury and Slinkard 2000; Kumar et al. 2013).

4 Genetic Resources and Utilization

Conservation of *Lathyrus* genetic resources has recently attracted more attention because of the potential role of this species under the climate change scenario (Kumar et al. 2013).

Grass pea is mentioned in two conservation programmes for major food legumes. One is the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA; FAO 2009), which aims at guaranteeing food security through conservation of biodiversity, fair exchange and sustainable use of plant genetic resources. This is being accomplished by establishing a global system to provide farmers, plant breeders and scientists access to plant genetic materials, ensuring that recipients share benefits with the countries where they have been originated and by recognizing the contribution of farmers to the diversity of crops used as food.

The other, a more specific programme developed by the Global Crop Diversity Trust (CGDT) in collaboration with the International Center for Agriculture Research in the Dry Areas (ICARDA), aims for a long-term conservation strategy of *L. sativus*, *L. cicera* and *L. ochrus* (GCDT 2009). This programme is detailing the current status of national collections and identifying gaps in collections of these three species from areas of diversity. Their strategy recommends that documentation on collections should be upgraded and that more work should be carried out on characterizing and evaluating collections for key traits, making this data widely available (Gurung and Pang 2011).

Several ex situ and a few in situ conservation examples exist for grass pea germplasm. The largest *Lathyrus* ex situ collections are maintained at the Conservatoire Botanique National des Pyrénées et de Midi-Pyrénées in France (4.477 accessions; previously at Pau University), by the ICARDA comprising 3.239 accessions and by the National Bureau of Plant Genetic Resources (NBPGR) in India (2.619 accessions). Smaller, but still relevant, collections are maintained by other banks such as the Germplasm Resource Information Network (GRIN) from the US Department of Agriculture (USDA) in the USA, the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Germany and the Centro de Recursos Fitogenéticos (CRF) from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) in Spain. Backups from 2.134 grass pea accessions, from 44 countries, are deposited at the Svalbard Global Seed Vault (http://www.nordgen.org/sgsv/, accessed June 2014). In what concerns in situ conservation, five genetic reserves for *Lathyrus* diversity conservation have been proposed in Syria and Turkey (Heywood et al. 2007). These authors also stressed the importance of increasing public awareness for the significance of crop wild relatives in agricultural development and the need for their simultaneous conservation.

This conserved germplasm represents a valuable reservoir of diversity, providing access to sources of a wide range of interesting agromorphological traits such as earliness, plant architectural traits, disease and pest tolerance, as well as low ODAP content. Characterization of this diversity through phenotyping and genotyping studies will unveil novel alleles that can be used to improve this crop. Diversity characterization in *Lathyrus* germplasm has focused, for example, on ODAP content (Fikre et al. 2008; Grela et al. 2012; Kumar et al. 2011), phenology and yield (Grela et al 2012; Mera 2010), parasitic weed resistance (Fernández-Aparicio et al. 2012), disease resistance (Gurung et al. 2002; Vaz Patto et al. 2006a; Vaz Patto and Rubiales 2009) or quality traits (Granati et al. 2003). Some of these characterization studies have represented the first step of selection programmes.

5 Major Breeding Achievements

Conventional grass pea-breeding programmes have been established in several countries, including Australia (Hanbury et al. 1995), Bangladesh (Malek 1998), Canada (Campbell and Briggs 1987), China (Yang and Zhang 2005), Chile (Mera et al. 2003), Ethiopia (Tadesse and Bekele 2003), India (Lal et al. 1986; Pandey et al. 1996), Nepal (Yadav 1996) and Syria (Abd El-Moneim et al. 2000). Some of these breeding programmes are still active, but most are small in comparison to other legume crops (Vaz Patto et al. 2011).

Due to the occurrence of lathyrism in humans, major breeding programmes are essentially aimed for low ODAP content, besides productivity and adaptability. This has resulted at present in several *L. sativus* or *L. cicera* breeding lines or released varieties with reduced ODAP content (from 0.5 to 1.5 %, down to 0.01 % or less; Kumar et al. 2011). For instance, low ODAP cultivars have been released in several countries, such as ‘Wasie’ in Ethiopia, ‘Ali-Bar’ in Kazakhstan and ‘Gurbuz 1’ in Turkey (ICARDA 2006, 2007). Similarly, low ODAP, high-yielding cultivars have been released in India such as ‘Pusa 24’, ‘Prateek’, ‘Ratan’ and ‘Mahateora’ (ICAR 2009). In Bangladesh, examples of the low ODAP and high-yielding varieties are ‘BARI Khesari 1’, ‘BARI Khesari 2’ and ‘BARI Khesari 3’ (Malek 1998) or the ‘BINA Khesari 1’ (Kumar et al. 2011). In Canada, high yield and low ODAP (0.03 %) ‘LS8246’ was released for feed and fodder (Campbell and Briggs 1987), and in addition, a high N-fixation variety, ‘AC Greenfix’ was released specially as green manure (Krause and Krause 2003). In Chile, ‘Luanco-INIA’, a large-seeded, high-yielding grass pea variety was released, used locally as feed and for export, especially for some European markets where larger seed size is desirable for human consumption (Mera et al. 2003). Finally, in Australia, the variety ‘Ceora’ was bred to be used as forage, hay or as a green manure crop (Siddique et al. 2006). Also in Australia, a *L. cicera* cultivar, ‘Chalus’, was selected for high yields and low ODAP levels (Hanbury and Siddique 2000).

6 Specific Goals in Current Breeding

Low ODAP content is still one important goal of many of the current grass pea-breeding programmes. Nevertheless, other traits have always been associated with this.

Increased yield has been a selection criterion for most crop improvement programmes. However, some of the yield components that affect yield such as double podding or increased seeds per pod have received insufficient attention. Also the biomass yield of *L. sativus* has started to receive more attention during the past few years (Campbell 1997; Abd El Moneim et al. 2001; Vaz Patto et al. 2006b). This is a very important area due to the large potential of this crop for forage and straw in the North African and South Asian regions (Campbell 1997). Additionally, undesirable traits such as prostrate plant habit, indeterminate growth, late maturity and pod shattering (Rybinski 2003) are being handled by several breeding programmes.

The concentrated effort on reducing ODAP content resulted in many other areas of evaluation and crop improvement, such as resistance to biotic and abiotic stresses, being neglected. However, with the release of these low ODAP lines, the development of varieties with increased resistance to prevalent pests and diseases has gained new strength. This crop is usually grown by poor farmers and under poor management, where it is difficult to adopt chemical control for diseases and pests. Therefore, the development of varieties having resistance to prevalent biotic stresses is essential, and more efforts are required in this area of improvement of this very hardy pulse crop (Vaz Patto et al. 2006b).

7 Breeding Methods and Specific Techniques

Collection and evaluation of germplasm, local or introduced, is the cornerstone in any breeding programme. Subsequent hybridization and selection of the resulting progeny using different strategies will allow incorporating interesting traits into a more adapted background. This may include backcrossing, recurrent selection, single-seed descent and pedigree/bulk breeding methods. All of these methods can be applied on grass pea improvement.

Grass pea is predominantly a self-pollinated crop, although outcrossing up to 30 % has been reported (Ben Brahim et al. 2001; Chowdhury and Slinkard 1997; Rahman et al. 1995). Large size of flower, bright colour of petals, flower density and nectar production are reported to influence the outcrossing in *Lathyrus* species (Kiyoshi et al. 1985). Entomophilic pollination in grass pea is due especially to bees and bumblebees (Kumar et al. 2011). Due to this observed outcrossing level, in most grass pea-breeding programmes, crosses are done under controlled conditions, in greenhouse or under insect-proof coverings (Vaz Patto et al. 2011).

Conventional grass pea breeding focussed essentially on hybridization of selected accessions, with the screening and evaluation of the resulting progeny. In the particular case of breeding to reduce ODAP content, low ODAP accessions are crossed with high-yield material with good agronomic potential (Campbell 1997).

Intergeneric hybridization, although difficult, is possible with *L. amphicarpos* and *L. cicera* (Yunus and Jackson 1991). Crosses have also been made with other species such as *L. chrysanthus, L. gorgoni, L. marmoratus* and *L. pseudocicera* (Heywood et al. 2007), but only ovules were produced.

Also with the objective of reducing ODAP content, grass pea has been subjected to induced mutagenesis by physical and/or chemical mutagens. Other traits have been affected by mutagenesis such as plant habit, maturity, branching, stem shape, leaf size, stipule shape, flower colour and structure, pod size, seed size and colour and NaCl tolerance (Biswas 2007; Nerkar 1972, 1976; Rybinski 2003; Talukdar 2009a, b, 2011). In vitro culture was also employed, inducing somaclonal variation (Ochatt et al. 2002a; Roy et al. 1993; Zambre et al. 2002). Induced mutagenesis and somaclonal variation created new diversity, allowing the selection of lines with interesting traits.

Ochatt et al. (2002b) developed an in vitro system coupled with in vitro stages in order to shorten regeneration cycles, obtaining up to almost four cycles per year. However, this approach is only applicable when few seeds/plant are intended, as in single-seed descendant breeding schemes.

The advent of various molecular-marker techniques and the ability to transfer genes across different organisms, using transgene technology, have begun to have an impact on plant genome research and breeding. These techniques offer new approaches for improving important agronomic traits in *Lathyrus* species and breaking down transfer barriers to related legume species (Vaz Patto et al. 2006b). This would allow exploring the variability existing in other *Lathyrus* genepools and hopefully transfer the interesting grass pea traits to related legume species.

Genetic transformation of grass pea was attempted with only one successful report obtaining stable transformed plants (Barik et al. 2005). Given that regeneration protocols for grass pea are often genotype specific, it may be necessary either to develop more generally applicable protocols or to adapt the protocol after transformation (Ochatt et al. 2013).

8 Integration of New Biotechnologies in Breeding Programmes

In order to be able to perform marker-assisted selection (MAS), it is necessary to identify molecular markers that are closely linked to the trait of interest. Once a trait is associated with a marker (or more), plants can be selected early on its growth stage, allowing a faster and more efficient breeding process.

Until now, only two linkage maps using molecular markers were developed for *L. sativus*. One developed by Chowdhury and Slinkard (1999) used 11 random amplified polymorphic DNA (RAPD) markers, 1 isozyme marker and 1 morphological trait (flower colour). The other linkage map was constructed by Skiba et al. (2004), using 47 RAPDs, 7 cross-amplified pea microsatellite simple sequence repeats (SSR) markers and 13 cleaved amplified polymorphic sequence (CAPS) markers and was used to study the genetic basis of resistance to Ascochyta blight. Nevertheless, these maps were not informative enough to allow bridging that mapping information between them, as reviewed by Vaz Patto et al. (2006b).

Compared to other grain legumes such as pea, faba bean or chickpea, genomic recourses for grass pea are still scarce. In July 2014, the National Center for Biotechnology Information (NCBI) database had made available the information of 178 EST sequences from a cDNA library of one *L. sativus* accession inoculated with *Mycosphaerella pinodes* (Skiba et al. 2005), 89 nucleotide sequences mainly from the Bowman–Birk (BBI) inhibitor coding sequences (41 accessions), chloroplast sequences (21 accessions) and 216 protein sequences (44 amino acid sequences from BBI inhibitors, 150 sequences from chloroplast proteins).

Specific molecular markers have been developed or adapted for grass pea in order to assist diversity studies and further develop linkage maps. Almeida et al. (2014a) studied the transferability of molecular markers from *M. truncatula, P. sativum, L. culinaris, Lupinus* spp. and *V. faba* to *Lathyrus* spp. and their application in mapping and diversity studies. Cross-genera amplification of molecular markers provided an alternative for the development of new molecular markers on understudied genus, allowing also performing comparative mapping between the sequence donor and the target species. This survey for similar genetic regions among closely related species will contribute to the potential future exchange of interesting traits among them.

Earlier molecular markers, specific or cross amplification studies in grass pea, included the work of Shiferaw et al. (2011) that successfully amplified nine expressed sequence tag-simple sequence repeats (EST-SSRs) developed from the EST sequences of Skiba et al. (2005) and 12 EST-SSRs from *M. truncatula,* which have been previously proven to be transferable to other legume species by Gutierrez et al. (2005).

Lioi et al. (2011) were able to genotype in a grass pea diversity study, ten SSRs developed from nucleotide sequences stored at public databases, being nine from *L. sativus* sequences and one from a *L. japonicus* sequence.

More recently, Yang et al. (2014) employed next-generation sequencing (NGS) to develop 144 specific grass pea SSRs, from which, 74 where polymorphic and therefore useful for diversity studies and genetic mapping.

The first grass pea expression analysis was performed by Skiba et al. (2005), identifying 29 potential defence-related genes differentially expressed in response to *M. pinodes* inoculation. These included genes associated with pathogen recognition, the phenylpropanoid pathway, hypersensitivity, pathogenesis-related and disease resistance response proteins.

In addition, expression analysis using RNA-sequencing was also employed in grass pea to tackle the molecular mechanisms underlying prehaustorial rust resistance (Almeida et al. 2014b). These authors identified several pathogenesis-related proteins as possibly involved in grass pea resistance to rust, that included some regulated by the well-studied mildew resistance locus O (MLO) gene. In this study, several potential rust effectors were also identified. These could be used as probes to identify target grass pea host proteins, as a first step in the development of effector-driven legume breeding, maximizing the durability of resistance against this quickly evolving pathogen (Vleeshouwers et al. 2011). Finally this RNA-sequencing study also identified several polymorphic single-nucleotide polymorphism (SNPs) and EST-SSRs between parental lines of existing grass pea segregating recombinant inbred lines (RILs), allowing its use for linkage mapping.

Expression analysis of the response to infection with *Ascochyta lathyri* in a resistant grass pea accession was performed using deepSuperSAGE. This approach has identified several differentially expressed genes (Almeida et al. 2015), opening the way to a powerful route of identification of candidate resistance genes and more detailed study of resistance gene networks in *L. sativus* (Vaz Patto and Rubiales 2014).

9 Future Prospects

The present paradigm change towards the study of crop species instead of focusing on model species will aid in the development of plant species that have been neglected. Lowering costs in high-throughput sequencing and the development of high-throughput phenotyping have encouraged the development of new molecular tools to boost the genetic characterization and utilization of the rich *Lathyrus* germplasm.

Grass pea research was tied to the persecution of an ODAP-free variety for several decades. This has hampered progress in this crop for the improvement of other traits. As an example and despite its importance, ODAP-related research should not block the understanding of the reasons behind the success of grass pea when dealing with biotic and abiotic stresses, and for which it is considered a survival crop. In an alternative to low ODAP varieties, an option might be improving quality traits that can lower ODAP's negative effects. These include increasing the content in homoarginine, cysteine or methionine. Although this is an old objective, it is still unachieved, due to the presence of technical barriers in the regeneration of transformed tissues (Girma and Korbu 2012) or the high influence of genotype × environment in those traits (Piergiovanni et al. 2011; Piergiovanni and Damascelli 2011).

For quicker progress on these and other quantitative traits improvement via MAS, it would also be useful to have a saturated linkage map, including cross-transferable markers to other related species such as pea, faba bean or the model *M. truncatula*, to apply in quantitative trait loci (QTL) mapping. In this way, comparative mapping would also be possible to other closely related legume species, assisting knowledge transfer among these species and facilitating candidate gene discovery for the detected potential QTL regions.

With the development of high-throughput and dense genotyping, 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 the analysis of 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 the collection genetic structure (Cobb et al. 2013). That is why Vaz Patto and Rubiales (2014) supported the idea of concentrating international evaluation efforts on to a grass pea core collection, representative of all the existing diversity, but of a manageable size. For adaptive traits, core and mini-core collections may not capture the needed diversity (Gepts 2006). As an alternative, the Focused Identification of Germplasm Strategy (FIGS) approach, which is a trait-based approach with high probability of identification of desired genetic material (Khazaei et al. 2013) is being applied at ICARDA to the *Lathyrus* germplasm collection to develop subset collections.

In terms of grass pea plant resources for functional genomics studies, various mapping populations including RILs, near isogenic lines (NILs) and targeting induced local lesions in genomes (TILLING) populations are critically needed for trait–marker association and gene inactivation/deletion studies (Kumar et al. 2013).

10 Seed Production

Several grass pea improved varieties have been originated from various breeding programmes as already described in the section "Major Breeding Achievements" section. As in any plant species with outcrossing frequency rate up to 30%, special efforts are needed for cultivar conservation. Strategies like an isolation distance or the use of a buffer crop between cultivars when producing seeds are essential to maintain genetic purity and phenological features of the developed cultivars.

Nevertheless, the most common seed available is from landraces or farmers' varieties, inherently heterogeneous. These farm-saved seeds are obtained and traded within an informal seed system where seeds are exchanged among farmers that mainly do not sell the product of the seed, but use it for self-consumption.

In conclusion, presently available genetic resources, established breeding achievements and recent biotechnological progress, associated with a growing international interest on grass pea cultivation, will definitely provide a bright future to this highly potential crop.

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