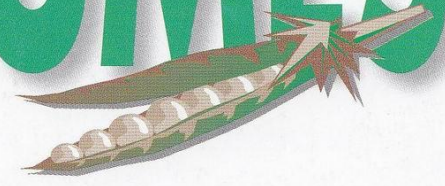


# GRAIN LEGUMES



AEP

The magazine of the European Association for Grain Legume Research  
Le magazine de l'Association Européenne de recherche sur les Protéagineux

ISSUE No. 54

October 2009

**Progress in *Lathyrus* genetic markers development**

**Grass pea as a multifunctional crop for sustainable production**

**Disease resistance in *Lathyrus* species**

**Iberian grass pea festivals**

**The word for *beauty* is... *Lathyrus*  
The *Lathyrus* research strikes back!**





# V<sup>th</sup> International Congress on Legume Genetics and Genomics

July 2-8, 2010

Asilomar Conference Grounds, Pacific Grove, California

Registration and Information Website  
[conferences.ucdavis.edu/ICLGG](http://conferences.ucdavis.edu/ICLGG)



We are pleased to announce the V<sup>th</sup> International Congress on Legume Genetics and Genomics (ICLGG) to be held at the Asilomar Conference Grounds in Pacific Grove, California on July 2-8, 2010. Asilomar is a picturesque venue on the coast of central California located near the historic city of Monterey.

The meeting will focus on fundamental discoveries that extend our understanding of the unique traits of legumes. A special feature of the ICLGG is its mission to bring together people working on fundamental aspects of legume biology in model species, using genetic and genomic tools, with those working on applied aspects and breeding of crop and pasture species.

Our goal is to create an intimate environment for collegial interaction, where all attendees are active participants. We are also striving to keep costs to manageable levels. Early registration is only \$400 and housing at Asilomar includes all meals. Participants can register and secure housing through the conference website.

Depending on our success with fund raising, we plan to offer travel awards to off set costs for young scientists, groups under-represented in science, and those in need. Please contact the organizing committee to request assistance. Sponsorship is very important to a successful meeting, so please let us know if you have suggestions in this regard.

#### **Deadlines**

April 1 – Register early to avoid late fees and to secure housing.

May 1 – Abstracts Due. Submission information on conference website.

May 1 – Last day to secure conference transportation and Asilomar housing.

[conferences.ucdavis.edu/iclgg](http://conferences.ucdavis.edu/iclgg)

## Tentative Program

Friday - Thursday

- |        |  |
|--------|--|
| July 2 | • Arrival<br>• Welcome & Keynote<br>• Reception  |
| July 3 | • Genomics<br>• Genome-Assisted Breeding<br>• Poster Session & Reception   |
| July 4 | • Pathogens, Pests, &<br>Resistance Mechanisms<br>• Abiotic Stress & Tolerance<br>Mechanisms<br>• Poster Session & Reception |
| July 5 | • Genes & Development<br>• Symbiosis<br>• Afternoon Free<br>• Poster Session & Reception                                     |
| July 6 | • Symbiosis<br>• Evolution & Diversity<br>• Gala Reception at the<br>Monterey Bay Aquarium                                   |
| July 7 | • New Faces & New Horizons<br>• Challenges<br>• Closing Remarks  |
| July 8 | • Departure  |

Contact: [ICLGG2010@gmail.com](mailto:ICLGG2010@gmail.com)

Organizing Committee:

Douglas Cook, Scott Jackson, Michael Udvardi

We are proud to present this special issue of the Grain Legumes magazine devoted to *Lathyrus*. In spite of the recent difficulties encountered by the AEP community, four special issues of GLM have been produced during 2009 thanks to the efforts of different voluntary teams. GLM51 was devoted to the outcomes of 2<sup>nd</sup> GL-TTP workshop hold at Novi Sad, Serbia. This issue was possible thanks to the constancy of our colleague Aleksandar Mikić who coordinated an editorial team composed by Noel Ellis, Tom Warkentin and Kevin PcPhee. This was followed by GLM52 devoted to "Pea", coordinated by Judith Burstein; then by GLM53 devoted to "Model Legumes" coordinated by Richard Thomson and Nicolas Rispaill; and finally this GLM54 devoted to "Lathyrus". We would like to warmly thank and acknowledge the additional people who contributed to them, Aleksandar Mikic and Dominique Millot for offset preparation, and the scientists who contributed by their reports to the various issues.

These issues were produced thanks to various specific sponsorships that are acknowledged in each issue. Printed issues will be freely distributed to AEP members only. One year after their production, pdf versions will be publicly available in the AEP website.

Future is always uncertain. However, even when AEP is facing a particular critical financial period, the network of scientists is alive, active and full of enthusiasm to continue with this dissemination endeavour. Any possible sponsorship will be most valuable and widely acknowledged.

**Carlota VAZ PATTO**

**Diego RUBIALES**

Managing Editors of GLM54

### Carte blanche

- 4 The *Lathyrus*/lathyrism controversy (F. Lambein)

### PhD Thesis

- 5 Is grass pea (*Lathyrus sativus*), a multi-tolerant plant species, able to cope with the presence of lead at root level? (J. Brunet)

### Special report: The Lathyrus research strikes back!

- 6 Ornamental *Lathyrus* species (R. Parsons)  
 8 Lathyrism (F. Lambein and Y.-H. Kuo)  
 10 Antioxidant activity in *Lathyrus* species (E. Pastor-Cavada and colleagues)  
 12 *Lathyrus* in vitro Biotechnology (S. Ochatt)  
 13 Flow Cytometry for *Lathyrus* breeding (S. Ochatt)  
 14 Agrobacterium-mediated transformation of grass pea (D. Barik)  
 15 *Lathyrus* spp. germplasm collection in the Iberian Peninsula (D. Rubiales and colleagues)  
 16 *Lathyrus* – diversity and phylogeny in the genus (G. Kenicer and colleagues)  
 19 Genetic resources at the CRF-INIA, Spain: collection, conservation, characterization and documentation in *Lathyrus* species (de la Rosa and Marcos)  
 22 Improving forage quality and availability in the southern Great Plains with grass pea (*Lathyrus sativus*) (S. Rao and B.K. Northup)  
 24 A brief history of quantitative analysis of ODAP (C.-J. Jiao and Z.-H. Li)  
 26 Grass pea (*Lathyrus sativus*): a novel plant species for phytoremediation systems? (A. Repellin and colleagues)  
 28 Drought resistance and management for water use efficiency on *Lathyrus sativus* (C.-J. Jiao and Z.-H. Li)  
 30 Development of cytogenetic stocks through induced mutagenesis in grass pea (*Lathyrus sativus*): Current status and future prospects in crop improvement (D. Talukdar)  
 32 Intercropping of grass pea (*Lathyrus sativus*) with other grain legumes for forage production (B. Čupina and colleagues)  
 33 A brief archaeological and linguistic review on *Lathyrus* in Europe (A. Mikić and colleagues)  
 34 Progress in genetic markers development for *Lathyrus* species (M.C. Vaz Patto and N.F. Almeida)  
 36 Disease resistance in *Lathyrus sativus* and *L. cicera* (D. Rubiales and colleagues)

### Around the world

- 38 Alvaiázere, Grass Pea Capital: A taste on patrimony (M.C. Vaz Patto)  
 40 From a survival food of the poor to a festivity main dish: "titos" (grass pea, *Lathyrus sativus*) in La Gamonal and in Padilla de Abajo (Burgos, Spain) (C. Caminero Saldaña and I. Grajal Martín)

### 42 Art

Carte blanche  
to...



Fernand Lambein\*

## The *Lathyrus*/lathyrism controversy

**A**neolithic crop with a modern future'; 'from royal offering in the ancient pyramids of Egypt to being listed as a toxic plant'; banned from the markets in much of India and Nepal, but promoted in Bangladesh and Ethiopia. How can a plant become so controversial and why? A simple answer is that grass pea (*Lathyrus sativus*) is the victim of its own agronomic success: surviving environmental calamities and producing the cheapest protein, helping millions to survive drought-triggered famines. The poorest of the poor then over-consume the only food available or affordable, mostly what they grow themselves on marginal land with no inputs but the seeds. Uninterrupted consumption as staple food during more than two months can cause symmetric spasticity of the legs (neurolathyrism) in up to 6 % of the consumers. This crippling with abrupt onset is irreversible. In 1964 a neuro-active amino acid  $\beta$ -N-oxalyl-L- $\alpha$ , $\beta$ -diaminopropionic acid ( $\beta$ -ODAP) was found in the seeds of this "toxic" plant and since then numerous breeders and toxicologists have focused on this small molecule. But breeders did not succeed in eliminating  $\beta$ -ODAP, and toxicologists could not determine a threshold of toxicity.

Only recently, epidemiology discovered a link with illiteracy, poverty and young age as risk factors for neurolathyrism, and fermentation of the seeds or consumption together with cereals, onions and ginger as protective factors. The low level of essential sulfur amino acids in grass pea seed is probably as important as  $\beta$ -ODAP as potential cause of neurolathyrism. Konzo (meaning: "tied legs"), a disease with the same symptoms as neurolathyrism, occurs among poor people in Africa surviving almost exclusively on cassava (*Manihot esculenta*) roots deficient in sulfur amino acids. At a recent workshop on konzo and neurolathyrism in Ghent (September 2009), participants concluded that "konzo and neurolathyrism are the clinical symptoms of abject poverty and malnutrition". In a well balanced diet grass pea can be a healthy and safe food, but legumes are not intended as staple food and neither is grass pea. The unique qualities of grass pea as resistant to biotic and abiotic stress, high nitrogen fixation and adaptable to high and low elevation and poor or contaminated soil (considered for phyto-remediation) should be better exploited by molecular breeders to arm the world against climatic changes and pollution, and *Lathyrus sativus* should no longer be treated as a pariah. ■

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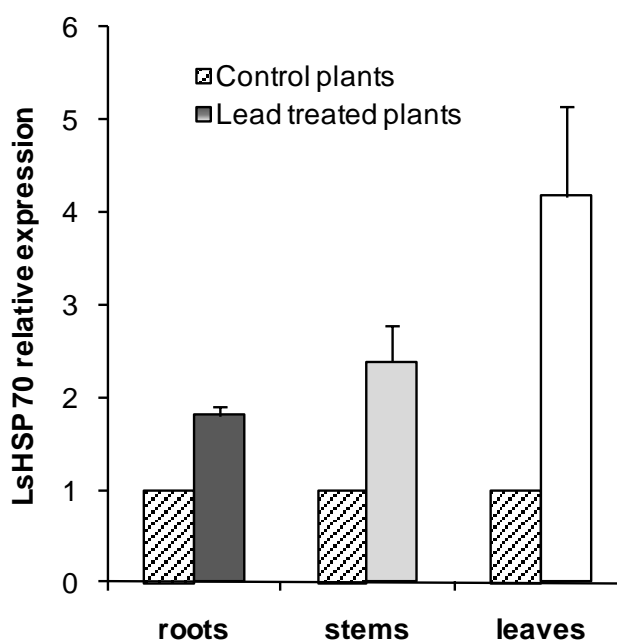
# Is grass pea (*Lathyrus sativus*), a multi-tolerant plant species, able to cope with the presence of lead at root level?

by Judicaëlle BRUNET\*

The tolerance of grass pea (*Lathyrus sativus* L.) to edaphic lead was assessed, using a hydroponic culture system including lead nitrate. Lead contents in roots, stems and leaves were determined and cell responses in these organs were studied through gene expression analysis, using 11 grass pea complementary DNA (cDNA) fragments newly isolated and sequenced here. The plants exposed to lead nitrate stopped growing while accumulating very important quantities of lead in their roots (up to 150 mg Pb g<sup>-1</sup> on a dry weight basis). Gene expression analysis showed that these tissues were under stress but were coping by stimulating the ascorbate-glutathione cycle, a scavenging pathway for lead-induced oxidant radicals. Despite the absence of detectable lead in the leaves, a number of genes were over-expressed in these tissues (Figure). This was a strong indication that lead-contaminated grass pea roots emitted a signal that was transported systemically to the rest of the plant where it stimulated controlled proteolysis and protection pathways against reactive oxygen species. This is the first evidence that such a mechanism is implemented in response to lead exposure and in grass pea plants.

Exposure of grass pea plants to EDTA-chelated lead in the liquid growth medium had a dramatic impact of the distribution of the element within the plants, with a significant accumulation of lead in leaf tissues (68 mg g<sup>-1</sup> on a dry weight basis). These results showed that grass pea plants could be induced to hyperaccumulate lead in aerial organs.

Furthermore, molecular analysis suggested that EDTA-Pb complexes were actively transported by a CNGC-like transporter. Together, these results suggest that the under-utilized grass pea species is able to cope with elevated amounts of lead and is therefore an interesting candidate for rhizofiltration of lead-contaminated liquid or semi-liquid wastes, or for phytoaccumulation of lead in the presence of a chelating agent. ■



**Relative expression of *LsHSP70*, a stress-induced chaperone (i.e. protecting) protein, gene in roots, stems and leaves of grass pea plants, grown (96 h) in modified Hoagland's medium supplemented with 0.5 mM Pb(NO<sub>3</sub>)<sub>2</sub> (Lead treated plants) or not (Control plants). Darker shades of grey in the "Lead treated plants" bars indicate higher accumulation of lead in roots (150 mg Pb g<sup>-1</sup> DW), stems (1.5 mg Pb g<sup>-1</sup> DW) and leaves (not detectable). A polyubiquitine gene was used as an internal control in quantitative RT-PCR analysis. Values are means ± (SD) (n = 3).**

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PhD thesis defended in December 2008 at the Université Paris Est – Créteil, France.

Available online at [BrunetPhDdissertationDec2008](http://BrunetPhDdissertationDec2008).

## Ornamental *Lathyrus* species

by Roger PARSONS\*

Around 50% of all the *Lathyrus* species have been grown and assessed for ornamental purposes in the UK. These are conserved in two National Collections ([www.nccpg.org.uk](http://www.nccpg.org.uk)). One is held by Sylvia Norton near Cambridge and the other by myself near Chichester. The Cambridge collection places more emphasis on conserving perennial species and securing stocks of known provenance collected in the wild. The Chichester collection places more emphasis on conserving annual species and securing stocks of superior horticultural value. A seedbank at the Chichester Collection was started in 1998 and stores around 900 sweet pea (*L. odoratus*) cultivars and around 100 accessions of other species.

Both collections would like to acquire new accessions of species not currently in cultivation. Potentially decorative species appear to exist that have never been introduced into cultivation, particularly in Sections *Lathyrostylis* and *Notolathyrus*.

### Importance of sweet peas

By far the most economically important of the species is sweet pea, grown throughout Europe and many other parts of the world for its cut flowers and for garden decoration. Sweet pea was sent from Sicily to England and the Netherlands in 1699 and has been in continuous cultivation since. It is generally seen as a tall growing species, up to 2.4 m high, with tendrils enabling the plant to support itself.

Many accounts of the history of sweet pea are inaccurate so the National Sweet Pea Society has published a researched and referenced account in 2000 on the occasion of the society's centenary (5). The species has a high mutation rate such that by 1793, five flower colours existed. Serious hybridisation begun in the late 1800s and received further impetus with the advent of the Spencer flower form in 1901. Most existing cultivars have it but the older plain type remains popular for garden decoration and its strong scent. Some cultivars have been developed with a dwarf habit, growing to 30 cm high, and these are available with plain or Spencer flower type. The tendrils on sweet peas arise from the reduction of leaflets and some cultivars are found with non-tendrils, or multijugate, leaves.

### Cut flower production

There are specialist societies dedicated to growing this species in the UK and the Netherlands. These are the National Sweet Pea Society ([www.sweetpeas.org.uk](http://www.sweetpeas.org.uk)) and the Nederlandse Lathyrus Vereniging ([www.lathyrus-vereniging.nl](http://www.lathyrus-vereniging.nl)). Sweet peas are not as extensively grown as they were 100 years ago but remain popular for their scent and variety of flower colours. The commercial cut flower market for Sweet Peas has declined because of their relatively short vase life but remains reasonably buoyant in Japan.

The summer flowering cultivars, that are most popular in northern Europe and temperate parts of North America, require around 12 hours daylight for flower initiation. Commercial cut flower growers generally prefer early-flowering cultivars which need a shorter daylength. Early-

flowering cultivars are also more popular with gardeners in warmer climates. Japanese growers use cold treatment of seeds to induce early flowering in summer cultivars (2). Sweet Peas could be induced to flower throughout the year but this is not considered economically viable. The main cut flower season in the UK is February to June, and in Japan is November to April.

All Sweet Peas have an annual life cycle. The main location of seed production has for 100 years been the Lompoc area of California, USA. Lompoc produces a hard seed coat, requiring mechanical abrasion of seeds or soaking in water prior to sowing. Seed produced in more temperate climate can be sowed naturally so that the Gisborne area of New Zealand has now become an important secondary centre of seed production.



*L. odoratus* 'Henry Eckford' shows typical flower form

\*Roger Parsons Sweet Peas, Chichester, UK ([roger@rpsweetpeas.co.uk](mailto:roger@rpsweetpeas.co.uk))

## Other annual species

Other species with an annual life cycle are often promoted for garden decoration. They lack the scent of the Sweet Pea and often have a short flowering season but their advantages include a very wide range of flower colours and being easy to grow. They are very suitable for meadow or prairie-style planting. The more decorative species include:

*L. belinensis*: grows up to 1 m high and carries red / yellow bicoloured flowers that shine in the sunlight. Has been incorrectly sold as a perennial under the cultivar name 'Goldmine'.

*L. chloranthus*: a tall growing species (to 2 m) that benefits from autumn sowing. It has attractive foliage and yellow-green flowers, sometimes splashed with red. The cultivar 'Lemonade' is the most popular form because of its pure yellow-green flowers.

*L. sativus*: grows to 1m high. Three colours are commonly encountered in cultivation. The most popular is var. *azureus*, with bright blue flowers. Var. *albus* has white flowers and cultivar 'Blue Stripe' is white with a blue blotch. Other flower colours, such as pink and purple, are described from Asia (1) and it would be good to introduce these into cultivation.

*L. tingitanus*: grows to 2m high and has attractive glaucous foliage. Typically has purple flowers but var. *roseus* is also seen with pink flowers. A white variant is reputed to exist and would be a welcome introduction into cultivation.

## Perennial species

Perennial species are sometimes preferred by modern gardeners as being less work than annuals but they have no scent so remain less popular than *L. odoratus*. Cultivars exist in some species but these rarely come true from seed. There is no reason why they cannot be fixed to breed true, as occurs with new Sweet Pea cultivars. The popular species *L. latifolius* and *L. vernus* may be likened to *L. odoratus* in 1793 i.e. a small number of colours have arisen through mutation but the potential exists to really widen the range and improve the quality through hybridisation. The more ornamental ones include:

*L. grandiflorus*, has the largest flowers of the genus. It very rarely produces seed, but reproduces by stolons, and is normally only sold as plants.

*L. latifolius* is a widely grown climber that makes up to 3m. It is well known and grown throughout Europe and the more temperate parts of North America. Easily reproduced from seed and easy to grow. Flower colours include magenta, mauve, pink and white. Known in the UK as the Everlasting Pea.

*L. rotundifolius*, is similar to *L. latifolius* but has more refined foliage. The flowers are smaller but are red, a colour not found in the common Everlasting Pea.

*L. vernus* is popular in Europe for its spring flowers and easy cultivation. It makes up to 40cm and has a self-supporting, upright habit. Typically with mauve blue flowers, there is a pink / white bicoloured form that is particularly welcome at a time of the year when the garden is dominated by mauve and yellow flowers. Forms with narrow leaflets, some very fine, are preferred.

The South American species are very decorative and many are scented. Some are tall growing and a little prone to frost damage. Others are short, compact plants. None are easy to maintain in UK gardens but *L. nervosus*, is quite well known.

## *L. odoratus* 'Eclipse' shows Spencer flower form



Those wishing to grow better Sweet Peas have a choice of guides (3, 6). The best guide for gardeners on other species is by Sylvia Norton (4). I can supply seeds of many other *Lathyrus* species for study or for those who want to try this delightful genus in their own gardens; see [www.rpsweetpeas.co.uk](http://www.rpsweetpeas.co.uk). ■

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# Lathyrism

by Fernand LAMBEIN\* and Yu-Haey KUO\*

**G**rass pea is perhaps the most successful legume in terms of nitrogen fixation and productivity on marginal lands and under adverse environments. It was once a royal offering to the Pharaoh in ancient Egypt. How did it become the food for the poor with a stigma of toxicity?

Because of its unusual drought tolerance, it became the life-saving survival food for the poor after environmental calamities, especially during drought triggered famine, when it becomes the only surviving crop and is used as staple food. However, over consumption can have dramatic side effects: uninterrupted and almost exclusive consumption of grass pea during two to three months can cause upper motor neurone degeneration which leads to an irreversible spasticity of the legs in up to 6% of the consumers.

The discovery of a neuro-active compound in seeds of grass pea, identified as  $\beta$ -N-oxalyl-L- $\alpha,\beta$ -diaminopropionic acid ( $\beta$ -ODAP) in 1964, has triggered intensive studies on the precise action of this  $\beta$ -ODAP in animals and, recently, in cultured neuronal cells. Also plant breeders have performed many and often long-term experiments to develop grass pea varieties with low  $\beta$ -ODAP. These studies did not result in any  $\beta$ -ODAP-free varieties of grass pea. A possible explanation for this may be that enzymes of primary metabolism are involved in the biosynthesis of  $\beta$ -ODAP (5) and their elimination would be lethal for the plant. Also the environmental effects on the level of  $\beta$ -ODAP in the seeds has been ignored or underestimated: drought, zinc deficiency, heavy metals and oversupply of iron and manganese in the soil can considerably increase the level of  $\beta$ -ODAP in the seeds.

The double stigma as being the food of the very poor and as the cause of the irreversibly crippling neurolathyrism has made grass pea into a neglected crop with a reputation of being toxic. However, this toxic reputation has to be taken *cum granum salum*, as lathyrism is never lethal and even does not affect longevity in contrast to kitchen salt that can be lethal as well as shorten one's life.

## Clinical aspects of neurolathyrism

Most frequently, a latency period of 2-3 months is observed with continuous daily intake of at least 400 g *L. sativus* seeds (3). The most common presentation of neurolathyrism is a sudden onset in which the affected individual complains of heavy, weakened legs after falling down or awakening from sleep, often following physical exhaustion. Spasticity of the calf muscles results in toe-walking and unsteady scissor gait. The clinical symptoms vary from mild walking difficulties to severe incapacity and unable to stand.

## History

During the 18<sup>th</sup>, 19<sup>th</sup> and 20<sup>th</sup> centuries, outbreaks of neurolathyrism occurred throughout Europe, Northern Africa, the Middle East, Afghanistan, Russia and India. The most remarkable account comes from the description of the epidemic in the German prisoners of war camp in Vapniarca, a town in Ukraine occupied by Germany during the Second World War. In this camp, the highest incidence of neurolathyrism on record developed between December 1942 and the end of February 1943. Dr. A. Kessler, who was among the prisoners, noted many details on the diet and neurological symptoms. The prisoners received daily rations of 400 g boiled grass pea and 200 g barley bread, and 60 % of the inmates developed various stages of neurolathyrism.

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From Kessler's report it has been deduced that a threshold level of  $\beta$ -ODAP intake may be close to 1 g per day per adult person under the conditions of the forced labour camp (6). Malnutrition and stress may have been responsible for this high incidence. Recent experiments with rat pups showed a 5-fold higher incidence of neurolathyritic symptoms when the pups were kept away from the mother during a few hours per day (4).

### Risk factors for neurolathyrism

The variability in the human susceptibility for neurolathyrism is such that no threshold of toxicity for grass pea intake or for  $\beta$ -ODAP content in the seed or  $\beta$ -ODAP intake could be scientifically established. Besides over-consumption of grass pea seed, major risk factors are famine and chronic food shortage (2), but also young age and male sex, heavy physical labour (3), a diarrhoeal episode, a history of acute illness, zinc deficiency (7), illiteracy, large family size and exclusive use of clay utensils (2). In addition, the type of *L. sativus* seed preparation is a critical factor: consumption of grass pea as roasted, boiled and raw unripe seeds has been associated with an increased risk for neurolathyrism.

### Protective factors for neurolathyrism

A reduced risk of paralysis was associated with soaking *L. sativus* seeds in water before preparation, fermentation, mixing the seeds with gravy that contains condiments with antioxidant activity or mixing with cereals rich in sulphur amino acids is shown to be protective (2).

### Conclusions

It can be questioned, whether this focusing on  $\beta$ -ODAP might have blinded researchers for other potential factors leading to neurolathyrism. For example, manganese is abundantly present in Ethiopian soils and may potentiate the neurotoxicity of  $\beta$ -ODAP by altering the blood-brain barrier permeability (8). Among commercial legumes, grass pea contains the lowest level of the sulphur amino acids methionine and cysteine and this can also contribute to neurodegeneration by its effect on oxidative stress. Nunn and co-workers (9) found a significant decrease of methionine in the serum of volunteers after ingesting a single meal of grass pea. This amino acid is a crucial precursor for glutathione which is responsible for the protection of cells (including motor neurons) against oxidative stress. In young chicks on a grass pea diet, neurological symptoms are prevented by addition of methionine to the diet (1).

Apparently, neurolathyrism only occurs when grass pea is the cheapest food available to the poor. Banning the sale of grass pea can increase the risk for neurolathyrism for the poor farmers who have no choice but producing this life-insurance crop during droughts, and then can not sell for a fair price. Unfortunately the environmental conditions virtually forcing poor farmers to produce grass pea may very well increase if the global warming continues. It's time to properly re-evaluate this crop by modern molecular tools for its superior agronomic characters, to remove it from the list of "toxic plants" and to rescue it from its status of "neglected crop". Giving it its proper economic value should prevent over-consumption by the poor and can be the simplest way to prevent neurolathyrism. ■

(1) Fikre, A. *et al.* (2009). Food and Chemical Toxicology (on-line: 10.1016/j.fct.2009.08.020)

(2) Getahun, H. *et al.* (2003). *Lancet* **362(9398)**, 1808-1810.

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(6) Lambein, F. (2001). *Lathyrus Lathyrism Newsletter* **2**, 5-7

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(8) Mishra, G. *et al.* (2009). *Toxicol Mechanisms Methods* **19(4)**, 318-326.

(9) Nunn, P.B. *et al.* (1994). *Archives of Physiology and Biochemistry* **102(6)**, B89.

## Antioxidant activity in *Lathyrus* species

By Elena PASTOR-CAVADA\*, Rocio JUAN\*\*, Julio E. PASTOR\*\*, Julio GIRÓN-CALLE\*, Manuel ALAIZ\* and Javier VIOQUE\*

**L** *athyrus sativus* represents an ancient crop that was extensively cultivated in the past for human and animal consumption. Other economically important *Lathyrus* species are *L. cicera*, and *L. tingitanus*, which are cultivated for their grain, and *L. ochrus*, *L. latifolius*, and *L. sylvestris*, which are cultivated as forage species. Most of the *Lathyrus* species are cultivated because their seeds are rich in protein of good nutritional value. Other quantitatively important components in the seeds are starch and fibre.

### Polyphenols: from antinutritional to antioxidant components

Polyphenols are a large group of secondary metabolites with functional value, and include more than 8000 different chemicals divided in several subclasses including flavones, isoflavones and anthocyanines. They have in common a hydroxy-substituted benzene ring in their structure, and have a variety of functions, including structural functions, protection against UV radiation, protection against oxidants, defensive functions, and signalling.

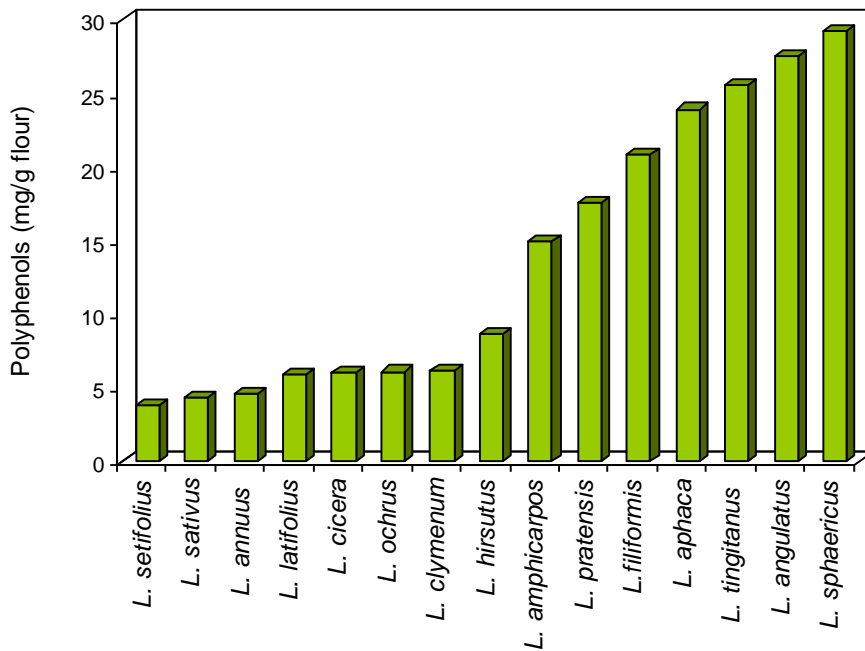
Traditionally, polyphenols have been considered antinutritional compounds because of their chelating properties that decrease mineral absorption and because of their bitter taste. In recent years however, polyphenols have been the object of increasing research due to their antioxidant and disease preventing properties. In human nutrition, polyphenols have been associated with the health promoting effects of consuming fruits and vegetables, and these positive effects have been related mainly with their antioxidant activity. In general, there is a correlation between intake of foods rich in polyphenols and prevention of major diseases. Increasing evidence shows the antiproliferative and proapoptotic properties of polyphenols, and the use of polyphenols in the treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's disease has been suggested.

### Polyphenols in *Lathyrus*

The polyphenol content, antioxidant activity, and functionality have only been studied in some of the better known species of *Lathyrus*. The polyphenol content in *Lathyrus* seeds is between 0.1 and 5 % (w/w). In 100 lines of *L. sativus* polyphenols were found to range from 39 to 999 mg/kg (1), being the concentration in tannins and polyphenols associated with the colour of the seed coat. In 1993 Ranabahu and co authors (3) studied the flavonoids (a class of polyphenols abundant in legumes) in the leaves of 36 species of *Lathyrus*, although the antioxidant properties of these chemicals were not determined. The comparison of flavonoid patterns observed in studied *Lathyrus* with those of *Cicer*, *Lens*, *Pisum* and *Vicia* indicated that each genus had a different flavonoid profile. Shahidi and co authors (4) studied and fractionated polyphenols from the non cultivated *L. maritimus* and determined their antioxidant activity. Polyphenol fraction with highest antioxidative activity showed the presence of (+) catechin and (-) epicatechin as the main phenolic compounds present.

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**Polyphenols contents in Andalusian *Lathyrus* species**

Recently we have reported the antioxidant activity of the polyphenols extracted from the seeds of 15 *Lathyrus* species from Andalusia, Spain (2). The polyphenol content ranged from 3.8 mg/g in *L. setifolius* to 29.2 mg/g in *L. sphaericus* (Figure). Among the cultivated species, *L. annuus* and *L. sativus* yielded the polyphenols with the highest specific antioxidant activity, while the flour with the highest antioxidant activity was found in *L. aphaca*. Comparing with some of the better known pulses, the antioxidant activity of the polyphenolic extracts from *L. annuus* and *L. aphaca* was higher than the antioxidant activity of the polyphenols extracted from chickpea, lupin and soybean. In addition, the polyphenols extracted from *Lathyrus* showed higher antioxidant activity than catechin, an abundant polyphenol found in tea beverages.

### **Lathyrus as promising functional foods**

There is an increasing interest in considering foods not only as a source of nutrients needed for maintenance and growing of the human body, but also as a source of functional components that can help in the prevention of certain diseases. Nowadays, many plant derived foods are recognized to be rich in non-nutritional compounds with health promoting properties. Consumption of polyphenols, which are especially abundant in fruits, red wine, tea, and legumes, reduces the risk of suffering cardiovascular diseases and cancer. The beneficial effects of soybean polyphenols have been extensively studied (5). However, the seeds from other legumes such as *Lathyrus* sp. may represent also a good source of these beneficial compounds. In fact, the antioxidant activity of the polyphenolic extracts from a number of *Lathyrus* species is higher than the antioxidant activity in extracts from chickpea, lupin and soybean seeds.

Some of the *Lathyrus* species that have been studied are marginal or neglected crops. They may represent an interesting source of antioxidant polyphenols that may facilitate the extension of these crops and their preservation. The *Lathyrus* genus is a potential new source of natural antioxidants in addition to containing high quality proteins for human and animal nutrition.

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## *Lathyrus in vitro* biotechnology

by Sergio OCHATT\*

**G**rass pea (*Lathyrus sativus*) is a cheap source of dietary protein for subsistence farmers in low-income food deficit countries, where it is vastly grown (6), but the sometimes-high seed content of ODAP (neurolethyrism toxin) limits grass pea consumption. Cultivars with reduced toxin levels would ensure a safer use of this adaptable, yet largely under-utilised crop (2,4). As *Pisum*, *Lens* and *Vicia*, *Lathyrus* are members of the *Viciae* tribe and may share genes, defence mechanisms and physiological processes with more widely cultivated crops (8, 9), becoming a useful genetic resource for resistance breeding (2, 4, 6, 9). However, grass peas have a long generation cycle, are cross-incompatible (5) with most other seed legumes and have received insufficient research input, so that little is known on their floral biology, cytogenetics and breeding. *In vitro* biotechnology may produce new grass pea genotypes that remain rustic but are better adapted for human consumption (6, 8, 9) and, also, introduce interesting traits from *Lathyrus* into the cross-incompatible major legume crops, as pea, by somatic hybridisation (3).

Few teams studied *Lathyrus in vitro*, the main bottleneck being plant regeneration from cultured tissues (6). Rooted plants were rarely obtained and, often, shoots grew on the callus surface probably from meristems pre-existing in initial explants. Grass pea *in vitro* regenerants are often subjected to hyperhydricity (Figure), and recently the genetic background of this problem was unravelled (5).

Many groups attempted interspecific hybridization with *Lathyrus* but most failed (2, 8). Incompatibility is even worse at the intergeneric level. Success rates may be increased by embryo rescue after fertilization (2, 6, 8), but when viable F1 hybrids were produced, they showed low fertility.

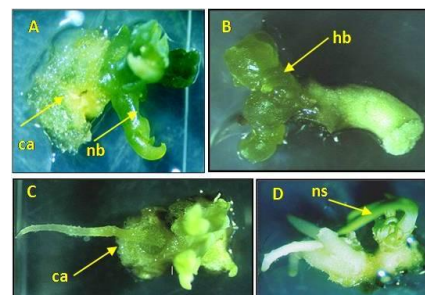
Breeding involving alien genetic transfer is only feasible via the readily crossable species *L. cicera* and *L. amphicarpos* (8), while other crosses will have to be assisted by

biotechnology tools (6) such as protoplast technology, i.e. the isolation and culture of protoplasts and their fusion with those of other species (and even genera) to recover somatic hybrid plants. The isolation and fusion of leaf protoplasts of grass pea with those of pea was reported, 20% heterokaryons were produced and divided to give somatic hybrid calluses, but have not regenerated plants yet (3).

Another biotechnology breeding tool is haplo-diploidisation. Haploids have the same chromosome complement as gametes and, after chromosome doubling, double haploids (DHs) become homozygous in all loci and can be used as parental breeding genotypes or *per se* (7). Legumes are unresponsive to DH research and, until recently, no successful reports existed. Microspores of several species, including 10 genotypes of *L. sativus*, *L. cicera*, *L. oculus* and *L. chymenum* were cultured (7), and those of 4 landraces of *L. sativus* gave microcalluses and then calluses. Ultimately a few plants confirmed as haploid by flow cytometry, were recovered for *L. sativus* genotypes LB and L3 (7).

Aimed at fastening breeding of legumes, including grass pea, generations were shortened *in vitro* (5) by transferring shoots germinated or regenerated *in vitro* to a medium where they elongated, flowered and set seed. Use of this with three *L. sativus* genotypes over 10 successive generations shortened each cycle from 150-180 days in the field to 100-120 days *in vitro*. This method allows a constant supply of flower buds (e.g. for haplo-diploidisation), and a better exploitation of breeding where seed supply is short (e.g. for SSD) or where ethical or political issues make field-testing difficult (e.g. with GMOs).

Gene transfer using *Agrobacterium tumefaciens* or biolistic techniques has also been tried, but only one reliable procedure for grass pea was developed (1), by co-cultivation with disarmed strains carrying *neomycin phosphotransferase II* and  $\beta$ -*glucuronidase* as reporter genes. Transient GUS expression occurred and transgene integration was confirmed by Southern hybridization of genomic DNA of the kanamycin-resistant



**Regeneration *in vitro* from explants and callus of *Lathyrus sativus* L. A. A callus (ca) with regeneration of normal buds (nb); B. Regeneration of hyperhydric buds (hb) from an explant cultured on a medium with low auxin and high cytokinin, or with cytokinin alone and no auxin in the medium; C. Regeneration of normal buds (nb) and a root (r) from callus (ca); D. Regeneration of roots (r) and normal shoots (ns) from an explant.**

GUS-expressive shoots to a *gus* intron probe. Transformed shoots were rooted, acclimatized and established in field, with germ-line transformation proven by progeny analysis (for more details see Barik D.P. contribution, this issue). Gene transfer to reduce ODAP level in the grain protein may be at hand-reach even if grass pea regeneration protocols are genotype specific (6), and it may be needed to develop generally applicable protocols or to adapt them after transformation.

The international research input efforts of these last few years should contribute to slowly but surely taking the *Lathyrus* species away from the list of neglected crops. It is now the time also for farmers to do the same, so that grass peas may also leave the list of under-utilised crops of the planet.

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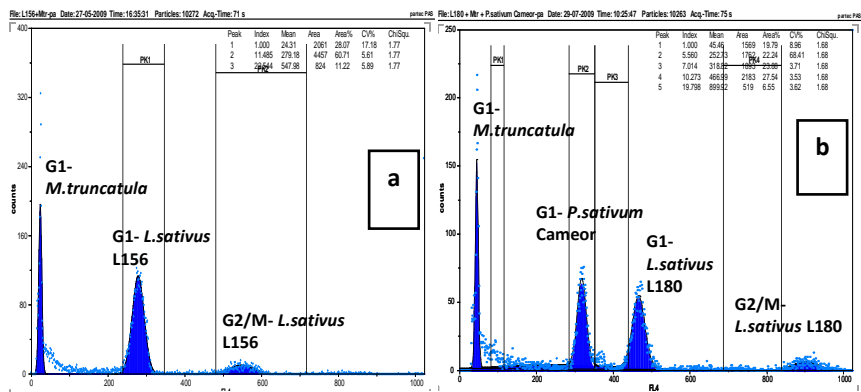
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# Flow cytometry for *Lathyrus* breeding

by Sergio OCHATT\*

**F**low cytometry may be a tool to palliate the lack of cytogenetic data for nuclear DNA content in *Lathyrus* (1, 2). The most frequent use of flow cytometry to date has been for determination of ploidy level of tissues and plants following *in vitro* manipulations or treatment with mitogenic substances. However, with the advent of new molecular tools, flow cytometry in plants has left its infancy (1, 2). The range of variation in genome size in the plant kingdom spans nearly 2000-fold (2), and this area has been controversial because of significant differences in nuclear DNA content of a same species analysed over a several years interval, perhaps due to the more refined practices used in more recent studies (1, 2). For the analysis of biotechnology-derived material, tissues (calluses or leaves from regenerated plants) are compared with leaf controls from seed-derived plants of the respective species and landraces. Nuclei are mechanically isolated by chopping tissues in a two-step method (nuclei extraction buffer plus staining buffer) or directly in a one-step procedure (Partec GmbH, Germany), and the suspension is filtered through a fine mesh prior to reading (2). A range of stains of differing specificity, which all bind stoichiometrically to the DNA bases can be used to determine relative nuclear DNA content compared to an internal (or, eventually, external) standard of known DNA content (Figure). The flow cytometer transforms the epifluorescence signal into numerical data, plotted with the epifluorescence intensity in the abscissa and the count of objects emitting at each intensity in the ordinate. Typically, flow cytometry profiles show two peaks where the first one

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**Flow cytometry profiles of *L. sativus* genotypes. a. L156 analysed with *Medicago truncatula* as internal standard. The relative nuclear DNA content of grass pea L156 is cca 11.5-fold that of barrel medic, with a genome size of 5391.7 MPb (calculated based on 1 pg DNA = 978 Mpb); 3). b. L180 analyzed with both *M. truncatula* and *Pisum sativum* cv. Cameor as internal standards. Note that DNA content of L180 differs from that of L156, useful for taxonomy**

corresponds to nuclei in the G0/G1 phase of cell cycle (2C DNA) and the second to nuclei in the G2/M phase (4C DNA) (1, 2).

Recently flow cytometry was used to unravel the mechanism(s) underlying hyperhydricity, and hyperhydric grass pea (*L. sativus*) tissues showed abnormal profiles with supernumerary peaks (4), indicating DNA content disorders (2). We have studied *in vitro* several genotypes of *L. sativus*, *L. cicera*, *L. chymenum*, *L. oculus* and *L. odoratus*, which we also characterized by flow cytometry. We analysed the cell cycle and calculated the relative DNA content per nucleus (measured DNA content being more a magnitude than an accurate content; Figure) and the AT/GC ratio. Leaf tissues of *Medicago truncatula* and pea were used as internal standard (i.e. run together with the grass pea nuclei). To assess AT/GC ratio, we used DAPI (which forms a fluorescent complex by attaching in the minor groove of A-T rich sequences of DNA) and propidium iodide (non-base pair specific but that exhibits GC-preference) or chromomycine A3 (specific for G-C; [1, 2]).

Although no callus phase intervened in our regeneration strategy and true-to-typeness should be commonplace among

regenerated plants, we undertook further flow cytometry analyses. All plants were fertile, true-to-type and euploid, except for one tetraploid *L. sativus* L156 regenerant. We also analysed the mother plants of all genotypes, and data fitted well with those available, with other DNA measurement methods used), but were not exactly the same, particularly because samples had been contrasted with an internal standard and, interestingly, this had never been done before. This stresses how different ecotypes, genotypes and even clones in a given species may differ in relative DNA content per nucleus, and is particularly true when dealing with *Lathyrus*, where little information exists on the cytogenetics of the species and even less on its many landraces (3). ■

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# Agrobacterium-mediated transformation of grass pea

by Durga P. BARIK\*, Uma B. MOHAPATRA\* and Pradeep K. CHAND\*

**L**athyrus sativus (grass pea) is the cheapest source of dietary lysine-rich protein for people of Low Income Food Deficit Countries (LIFDCs). This is well-adapted to adverse agricultural condition such as flooding, drought, salinity, low soil fertility and pathogen-infested soil (5). Unfortunately, efforts to exploit the potential of this grain legume are limited because of the presence of a neurotoxic amino acid,  $\beta$ -N-oxalyl-L- $\alpha,\beta$ -diaminopropionic acid (ODAP/BOAA) in the grain protein, which causes neuropathy in humans on prolonged consumption. As the coding sequence for ODAP degrading gene (ca. 3.3 kb) is already characterized and cloned from a soil microbe, *Pseudomonas stutzeri* (3), the remaining requirement was to develop a reproducible procedure for genetic transformation of grass pea using *Agrobacterium*. Therefore, the present study was aimed at examining some of the key factors influencing *Agrobacterium*-mediated genetic transformation of grass pea. This resulted in a complete protocol for obtaining transgenic grass pea plants expressing a reporter (*gus-int*) gene as well as a selectable marker (*npt II*) gene (1).

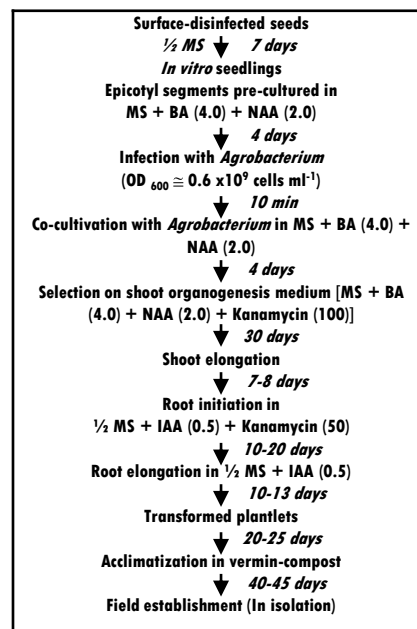
## Grass pea transformation procedure

Several key factors influence *Agrobacterium*-mediated genetic transformation of grass pea such as explant type showing adventitious shoot organogenesis, bacterial strains, growth phase and cell density, explant wounding method, infection time, co-cultivation period and pH of the co-cultivation medium. A single bacterial cell colony was inoculated in liquid YMB

medium (20 ml) containing 100  $\mu\text{g ml}^{-1}$  kanamycin and grown in dark (16-18 h, 28 °C, 180 rpm). The final cell density was adjusted to  $10^9$  cells  $\text{ml}^{-1}$  with liquid YMB. Epicotyl explants were submerged in the bacterial suspension (10 min), blot dried on sterile paper and finally transferred to agar-solidified regeneration medium without kanamycin following different durations of co-cultivation. The explants were then transferred to 0.8 % agar-solidified shoot regeneration medium containing MS salts augmented with 4.0  $\text{mg l}^{-1}$  BA and 2.0  $\text{mg l}^{-1}$  NAA and cefotaxime (500  $\mu\text{g ml}^{-1}$ ). Following a 2-week culture period, explants were sub-cultured on fresh medium as above with cefotaxime as well as the phytotoxic antibiotic kanamycin (100  $\mu\text{g ml}^{-1}$ ). Kanamycin-resistant shoots were sub-cultured at 15-day interval. The histochemical assay of GUS activity was carried out as described by Jefferson and coauthors (2) and southern blot analysis was done according to Sambrook et al. (4).

## Protocol optimization

A reproducible protocol (Figure) was developed for genetic transformation of grass pea using epicotyl segment co-cultivation with *Agrobacterium*. Two disarmed *Agrobacterium tumefaciens* strains, EHA 105 and LBA 4404, both carrying the binary plasmid p35SGUSINT with the *nptII* gene and *gus-int* gene, were examined as vector systems of which the latter had a higher transforming ability. Several key factors which influenced transformation were optimized. The highest transformation was achieved using hand-pricked explants for infection with an *Agrobacterium* culture corresponding to  $\text{OD}_{600} \approx 0.6$  and diluted to a cell density of  $10^9$  cells  $\text{ml}^{-1}$  for 10 min followed by co-cultivation for 4 days in a



medium with pH 5.6. Putative transformed explants capable of forming shoots were selected on regeneration medium stressed with kanamycin (100  $\mu\text{g ml}^{-1}$ ). Up to 36 % transient expression was evident based on the GUS histochemical assay. Southern hybridization of genomic DNA of the kanamycin resistant GUS-expressive shoots to a *gus*-intron probe substantiated the integration of the transgene. Transformed shoots were rooted on half-strength MS containing 0.5  $\text{mg l}^{-1}$  IAA, acclimated in vermi-compost and established in the experimental field. Germ-line transformation was evident through progeny analysis. Among  $T_1$  seedlings of most transgenic plant lines, kanamycin resistance to sensitivity segregated in a ratio close to 3 : 1.

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# *Lathyrus* spp. germplasm collection in the Iberian Peninsula

by Diego RUBIALES\*, Monica, FERNÁNDEZ-APARICIO\*, A. MORAL\*, Maria Carlota VAZ PATTO\*\*, S. S. NEVES\*\*, A. PUJADAS-SALVÁ\*\*\*

**L**athyrus species have a great potential in crop rotation, improving soil physical conditions; reducing the amount of disease and weed populations, with the overall reduction of production costs. However, in the last decades, the cultivation of *Lathyrus* species has shown a regressive pattern in many areas. The Iberian Peninsula is rich in *Lathyrus* species, 32 in total (2), whose specimens are well studied and preserved in herbaria. *L. sativus* and *L. cicera* are well preserved in the collections of CRF-INIA (3). However, germplasm from other *Lathyrus* species has seldom been collected and conserved in germplasm banks, and thus is little accessible for breeding purposes.

In an initial germplasm collection survey in the Iberian Peninsula, during the spring of 2005 and 2006, seeds from 117 populations of 13 *Lathyrus* species were collected and preserved. Several field trips were carried out across Spain and Portugal. Herbarium specimens (vouchers) were also collected for each population and were later deposited at the Herbarium of the Botanical Garden of Cordoba.

**Table 1. Accessions collected in the Iberian Peninsula during 2005 and 2006**

<i>Lathyrus</i> species	Number of accessions collected and preserved
<i>L. angulatus</i> L.	18
<i>L. annuus</i> L.	6
<i>L. aphaca</i> L.	2
<i>L. cicera</i> L.	16
<i>L. clymenum</i> L.	34
<i>L. hirsutus</i> L.	1
<i>L. latifolius</i> L.	8
<i>L. nissolia</i> L.	1
<i>L. odoratus</i> L.	3
<i>L. pratensis</i> L.	2
<i>L. setifolius</i> L.	3
<i>L. sphaericus</i> Retz.	5
<i>L. tingitanus</i> L.	18



Comparison of *L. cicera* vs *L. sativus* seeds (above)  
 Lathyrus collecting fact (left)

Botanical identification of the species was performed according to *Flora Iberica* (2) and *Flora Europaea* (1). Mature pods were collected from as many plants as possible from each population, with an average of 20-30 pods. These seeds are now preserved at the gene bank of the Institute for Sustainable Agriculture, CSIC, Córdoba, Spain. ■

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# Lathyrus – diversity and phylogeny in the genus

by Gregory KENICER\*, Esther María NIETO-BLÁSQUEZ\*\*, Aleksandar MIKIĆ\*\* and Petr SMÝKAL\*\*\*

The Eastern Mediterranean and Caucasus is undoubtedly the cradle of diversity for tribe Fabaeae, both in the wild and in cultivation. Also known as tribe Viciae, this group contains many of our major grain legumes including *Lens* (the lentils – 4 species.), *Pisum* (peas – 2/3 species), *Vicia* (the vetches and faba bean ~ 140 species) and the monotypic genus *Vavilovia*. The fifth genus in the tribe is *Lathyrus*, (chickling vetches and sweet peas), which is the most diverse with around 160 species. *Lathyrus* contains fodder crops such as *L. ochrus*, and *L. chymenum*; some of horticulture's most popular ornamentals, such as *L. odoratus* (the sweet pea) and *L. latifolius* (everlasting pea), and some of humanity's earliest crops in *L. cicera* and *L. sativus*. Both of these last species are known from some of the earliest Neolithic sites from the Fertile Crescent to Greece, and *L. sativus* is widely investigated today as it offers great potential as an arid lands crop.

The genus is almost exclusively temperate and shows an interesting 'antitropical' distribution. The greatest diversity of species is found around the Mediterranean and Irano-Turanian floristic region, and this area includes almost all of the annuals in the group. More mesophytic-temperate areas of Eurasia include a limited range of around 25 perennial species. North America also has around 25 species, mainly in the western coastal region and Rocky mountain chain. South America's 26 species are most diverse in a wide range of habitats through northern Argentina, although the

group ranges from the Paramó vegetation of the Venezuelan high Andes to the Straits of Magellan in the utter south.

## Key morphological characters

Originally a Linnaean genus, *Lathyrus* included mainly the plants that used tendrils to climb. Linnaeus placed erect species without tendrils in a separate genus, *Orobus*. These two genera were later merged, although some of the members of *Orobus* were placed in *Vicia*, and a more subtle suite of characters were identified that distinguished the general in the tribe *Lathyrus* is now characterised by the following characters:

- Styles adaxially pubescent (shared with *Pisum*, *Lens*, *Vavilovia* and only a few members of *Vicia*)
- Staminal tube tip truncate in most species (squared off – oblique in other members of the tribe)
- Supervolute leaflet vernation (the leaflets are rolled into each other in bud)
- Stems in some species are winged
- Leaflet veins in some species are parallel, and in reticulate-veined species, they do not loop back from the margins of the leaflet (as they do in *Vicia*)

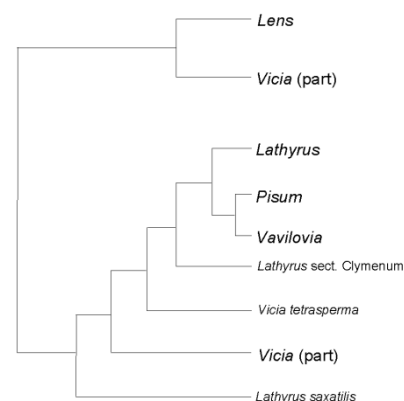
Current molecular phylogenetic evidence from DNA suggests the tribe is paraphyletic, although a complete picture is still a long way off (Figure 1).

## Sectional classifications

As *Lathyrus* is such a large and diverse genus, it has traditionally been broken into sections. The superb morphology-based work of Francis Kupicha (8) split the group into thirteen sections. Kupicha was the first researcher to consider the entire genus throughout its range and her work remains the definitive sectional classification (Table), with most of her sections standing up to DNA evidence (1, 6, 7). Kupicha's work refined and added to the studies of Simola (9), Czefranova (4), and Bässler (2).

## Phylogeny

DNA-based phylogenies have been generated from RFLP data (1), and from sequence data of the ITS (nuclear) and the *trnL-F*, *trnS-G* and *matK* regions of the chloroplast. Only sect. *Linearicarpus sensu* Kupicha is poorly resolved in this analysis and some members appear to be closely allied to sections *Lathyrus* and *Lathyrostylis*. Agreement with Kupicha's classification is otherwise very good. For this reason Kupicha's work remains the standard classification pending a complete revision of the genus.



**Figure 1: Hypothesised generic relationships in tribe Fabaeae, based on DNA sequence data – Kenicer, Nieto-Blásquez and Smýkal, unpublished data. *Lathyrus* in the traditional sense (8) is evidently paraphyletic.**

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\*\*\*Agritec Plant Research Ltd., Šumperk, Czech Republic

Grenier & Godron (1848)	Boissier (1872)	Czefranov (1971)	Kupicha (1983)	Asmussen & Liston (1998)	Kenicer (2007)
			<i>Notolathyrus</i>	<i>Orobus</i>	<i>Notolathyrus</i>
<b>Orobus</b>	<i>Orobus</i> *	<i>Lathyrus</i>	<i>Orobus</i>		<i>Orobus</i>
	<i>Orobastrum</i>	<i>Orobus</i>	<i>Lathyrystylis</i>	<i>Lathyrystylis</i>	<i>Lathyrystylis</i>
		<i>Pratensis</i>	<i>Pratensis</i>	<i>Pratensis</i>	<i>Pratensis</i>
		<i>Eurytrichon</i>			
<b>Aphaca</b>	<i>Aphaca</i>	<i>Aphaca</i>	<i>Aphaca</i>	<i>Aphaca</i>	<i>Aphaca</i>
<b>Orobus</b>	<i>Orobastrum</i>	<i>Neurolobus</i>	<i>Neurolobus</i>	<i>Neurolobus</i>	<i>Neurolobus</i> *
		<i>Orobon</i>	<i>Orobon</i>	<i>Lathyrus</i>	
<b>Eulathyrus</b>	<i>Eulathyrus</i>	<i>Lathyrus</i>	<i>Lathyrus</i>		<i>Lathyrus</i>
<b>Cicerula</b>	<i>Cicerula</i>	<i>Cicerula</i>		<i>Cicerula</i>	
<b>Orobus</b>	<i>Orobastrum</i>	<i>Orobastrum</i>	<i>Orobastrum</i>	<i>Orobastrum</i>	
			<i>Linearicarpus</i>	<i>L. sphaericus</i>	<i>L. sphaericus</i>
				<i>L. angulatus</i>	<i>L. angulatus</i>
			<i>Viciopsis</i>		
<b>Nissolia</b>	<i>Nissolia</i>	<i>Nissolia</i>	<i>Nissolia</i>	<i>Nissolia</i>	<i>Nissolia</i> *
<b>Clymenum</b>	<i>Clymenum</i>	<i>Clymenum</i>	<i>Clymenum</i>	<i>Clymenum</i>	<i>Clymenum</i> *
				<i>L. gloeospermus</i>	<i>L. gloeospermus</i>

\*Boissier's (3) *Orobus* was treated as a genus

**Orobon** [1] *L. roseus* – from the Black Sea coast is an erect almost shrubby species without tendrils. DNA suggests it is closely allied to section *Lathyrus*.

**Orobus** [50] a very diverse group of perennial, mainly mesophytic plants from Eurasia and North America (includes all the N. American species).

**Pratensis** [6] a mainly Mediterranean group characterised by sagittate (arrow-head-shaped) stipules. *Lathyrus pratensis* is very widespread. Throughout temperate Eurasia and appears as a weed in all other continents (except Antarctica).

**Viciopsis** [1] *L.* is probably a *Vicia*. It has very small flowers, and although the styles are only pubescent on the inner face, this seems to be an independent origin.

There is a huge range of diversity in *Lathyrus* – it is one of the top 25 most species-rich genera in the leguminosae. Many produce high yields of edible seeds and vegetative tissues. Because they are common and relatively easy to grow and identify, they have been the subject of many research projects as model organisms. As such they offer enormous potential for development as crops. ■

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These analyses suggest that the annual Mediterranean species are from the earliest evolved lineages, and that *Pisum* may be a part of this annual Mediterranean complex. There is also an interesting geographical split in the genus. Those species centred around the Mediterranean (particularly sects. *Clymenum*, *Lathyrus*, *Lathyrystylis*, *Linearicarpus*, *Neurolobus* and *Nissolia*) form one group distinct from the Eurasian-American remainder of the genus (mainly species of sects *Orobus* and *Notolathyrus*). These two major groups are roughly equal in species numbers yet the latter covers a far larger geographical area than the Mediterranean groups. Within this group, it appears that the mainly South American sect. *Notolathyrus* probably arose from Eurasian ancestors.

Section summary (species numbers in square brackets). See Figure 2 for estimated phylogenetic relationships:

**Aphaca** [2] small Mediterranean annuals with leaves reduced to a sagittate (arrow-head-shaped) stipule and simple tendril. Apparently closely allied to sect. *Pratensis*.

**Clymenum** [4] medium-sized, *Pisum*-like Mediterranean plants with leaves reduced to phyllodes when young, later developing leaflets. DNA evidence from this research group (7) suggests that some species may lie outside *Lathyrus*, *Pisum* and *Vavilovia* as a distinct clade.

**Neurolobus** [1] *L. neurolobus* is a small perennial species, endemic to the Mediterranean island of Crete.

**Nissolia** [1] *L. nissolia* is a pan-European species that is remarkable for its convergence with grasses. When not in flower or fruit, it is incredibly similar to the meadow grasses among which it grows.

**Lathyrus** [34] This Eurasian and African section contains some of the most significant crops (e.g. *L. sativus* and *L. cicera*) and ornamentals (*L. odoratus* and *L. latifolius*) as well as the type species (*L. sylvestris*). Most members have only one pair of leaflets and climb using tendrils. They range in size from 20cm to 3m in height and contain a range of perennials and annuals.

**Lathyrystylis** [20] Europe and eastern Mediterranean, often in more temperate areas. Small, erect perennials lacking tendrils. Extremely similar to some members of sect. *Orobus*, but DNA evidence (6, 7) suggests no close relationship.

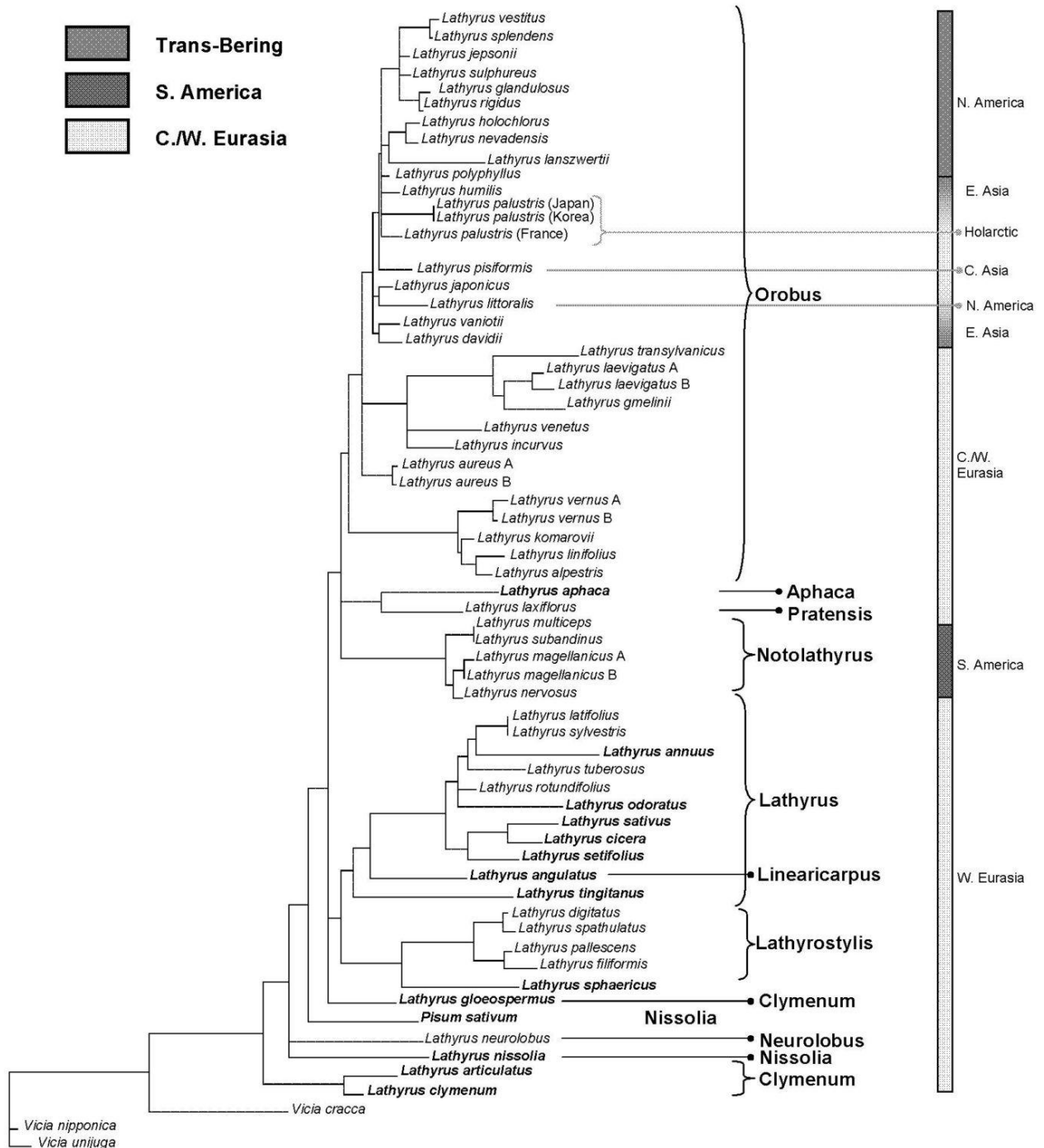
**Linearicarpus** [7] a group of small, difficult annuals, often with simple tendrils, few flowers per raceme and broad styles—this section does not hold up well to DNA based phylogenetic analysis. Further data from other species is required.

**Notolathyrus** [26] South American, with only one representative in the southeastern states of the USA. Very morphologically diverse, but apparently monophyletic. Subject of a complete revision in Kenicer (7).

**Orobastrum** [1] *L. setifolius* a delicate little annual from the Mediterranean. Considered (1) to be part of sect. *Lathyrus*, based on RFLP data.



SPECIAL REPORT  
**THE LATHYRUS RESEARCH  
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**Figure 2: Hypothesised relationships in the genus *Lathyrus*, showing sectional classifications according to Kupicha (8). Geographical distributions of the species are indicated by coloured bars, and annual species are indicated by bold font (modified from 7)**



Examples of Spanish *Lathyrus* accessions: a : *L. clymenum* ; b : *L. annuus*; c: *L. cicera* ; d: *L. ochrus*; e: *L. tingitanus*; f: *L. sativus*  
(see the article on the following two pages)

# Genetic resources at the CRF-INIA, Spain: collection, conservation, characterization and documentation in *Lathyrus* species

by Lucía DE LA ROSA\* and Teresa MARCOS\*

The genus *Lathyrus* comprises over 160 species and subspecies, spread throughout the temperate region in the Old World and the New World, with a well defined centre of diversification for the Old World in Asia Minor and the Mediterranean Basin (8).

Thirty two *Lathyrus* species have been reported in Spain (6). There is archaeological evidence of the presence of *Lathyrus* in Spain from the Neolithic Era. Written data can be found in “Twelve Agricultural Books” by Columella in the I century A.D (5). Until the second half of the XX century, several species of the genus were cultivated as food (*L. sativus* L.), forage (*L. hirsutus*, L., *L. tingitanus*, L., *L. sylvestris*, L., *L. cicera* L. and *L. aphaca* L.) or as ornamental plants (*L. odoratus* L. and *L. latifolius* L.). However, during the last decades, the area cultivated with all these species has been reduced dramatically. For example, the acreage of *L. sativus* in Spain has decreased from 55,000 ha in 1950 to 15 ha only in 2007.

Neither the European Common List nor the Spanish Commercial and Protected Varieties Catalogue include any *Lathyrus* variety, which is an indication of the scarce breeding effort made in this genus, in spite of its great potential value for Mediterranean environments and dry areas.

## CRF-INIA collection

The National Plant Genetic Resources Centre-National Institute for Agricultural and Food Technology Research (CRF-INIA) holds an important collection of grain legumes (2). About 400 accessions belonging to several species of the genus *Lathyrus* are maintained as active collection and most of them are also duplicated in the base collection. Three hundred and seventy seven accessions have been collected in Spain. The most represented species are chickling vetch (*L. cicera*), named in Spain as “titarro”, “tito negro”, “cicérula” or “galgana”, with 177 accessions, and grass pea (*L. sativus*) named as “almorta”, “mucla”, “tito”, “guija”, “arveja” or “chícharo” with 152 accessions. The rest of the Spanish accessions belong to ten different species (Table 1, Fig. 1). The non-Spanish samples come from Portugal (five), Morocco (four), Italy (two), and Peru (one). All the accessions are identified by a

set of passport descriptors according to the FAO/IPGRI Multicrop standards ([www.ecpgr.org](http://www.ecpgr.org)). This information is available at INIA web site ([www.inia.es/webcrf](http://www.inia.es/webcrf)) and also in the European Plant Genetic Resources Database EURISCO (<http://eurisco.ecpgr.org>).

*Lathyrus* seeds are orthodox in their behaviour in conservation. Seeds are desiccated to and internal moisture below 7% and stored in open able glass jars at -4° C as active collection and in hermetically closed tins at -18° as base collection.

The initial seed viability is determined by germination trials and monitored every ten years. Accessions showing a low level of germination or with scarce amount of seed are included in the regeneration process. A small plot of each accession is assigned on the field at CRF during the autumn where they are manually managed until harvest. *L. sativus* is managed as an allogamous species, and for this reason isolation measures between accessions, such as isolation cages with bumblebee colonies in each of them in order to increase the fertilization rate, are implemented.

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Species	Number of accessions
<i>L. amphicarpos</i> L.	1
<i>L. annuus</i> L.	5
<i>L. aphaca</i> L.	1
<i>L. cicera</i> L.	<b>177</b>
<i>L. clymenum</i> L.	10
<i>L. hirsutus</i> L.	2
<i>L. ochrus</i> (L.) DC.	7
<i>L. sativus</i> L.	<b>152</b>
<i>L. setifolius</i> L.	1
<i>L. sphaericus</i> Retz.	1
<i>L. tingitanus</i> L.	9
<i>L. tuberosus</i> L.	1
	<b>367</b>

**Number of Spanish accessions, by species, of *Lathyrus* held at the CRF-INIA active collection**

Part of the collection was characterized with agro /morphological descriptors (1), before the publication of the IBPGR (now Bioversity International) *Lathyrus* descriptor list. The agro /morphological descriptors used at that time were chosen after preliminary studies and based on Franco – Jubete (3). Information on 60 accessions of grass pea and 160 accessions of *L. cicera* are available at CRF web site. This includes the results of the study of 4 phenological descriptors, 7 plant descriptors, 5 inflorescence and fruit descriptors and 5 seed descriptors.

These collections have also been studied in other institutions. Thus, the work carried out at IAS-CSIC in Córdoba, where part of the collection has been evaluated for resistance to powdery mildew (*Erysiphe pisi*) (7), could be cited.

Knowledge about the traditional uses of *Lathyrus*, together with the information of the collection site, has been recorded during the collecting expeditions organized by the CRF staff. In the case of grass pea, the most common use is for human nutrition, as flour, but the use of the whole fresh seed in salads

or the whole grain boiled together with vegetables as Lent food has also been reported. Additionally in some places of collection, farmers have told us that grass pea is “good for people with sugar problems”; this property has been confirmed by scientific studies which have demonstrated that the inositol phosphoglycan (IPG) isolate from grass pea seed has insulin-mimetic activity (4). This activity could explain the use of this species to lessen diabetic symptoms. As animal feed, grass pea has been used as forage and dry grain, either whole or broken. In the case of chickling vetch, the most common use is as forage but in the past, the dry grains, alone or in a mixture with grass pea, have been frequently used for animal feeding. In addition with the utilization as food, there are other interesting uses for *Lathyrus* species like the prevention of soil erosion or the ornamental use as in the case of *L. clymenum*. The use of toasted seeds of *L. ochrus* as coffee substitute and the human consumption of tubers produced by *L. tuberosus* are, likewise, good examples of the diversity of uses that *Lathyrus* can provide. ■

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# Improving forage quality and availability in the southern Great Plains with grass pea (*Lathyrus sativus*)

by Srinivas C. RAO\* and Brian K. NORTHUP\*

The rising cost of inorganic commercial fertilizer has renewed interest in introducing legumes into paddocks of tame grass. Legumes fix atmospheric nitrogen (N), which will be available to the legume and some will be available to the following non-legume crop, reducing the need for N fertilizers (1, 3). Inter-seeding legumes into paddocks of tame grasses can improve forage quality by supplying high N biomass. Legumes generally have greater relative feed value than warm-season grasses (2).

Grass pea (*Lathyrus sativus*) is a cool-season annual pulse known for its tolerance to dry conditions and adaptability to difficult environments. Proper agronomic practices are necessary to maximize the yield of grass pea.

## Planting Date

We examined the influence of planting dates (March 15, April 01 and April 15) on yield and nutritive values of grass pea cv. AC Greenfix in the southern Great Plains. Planting date effects on biomass yield was minimal, though nitrogen accumulation varied among planting dates. At peak production, N accumulation was 135, 153, and 125 kg N ha<sup>-1</sup>, respectively for 15 March, 01 April, and 15 April planting dates.

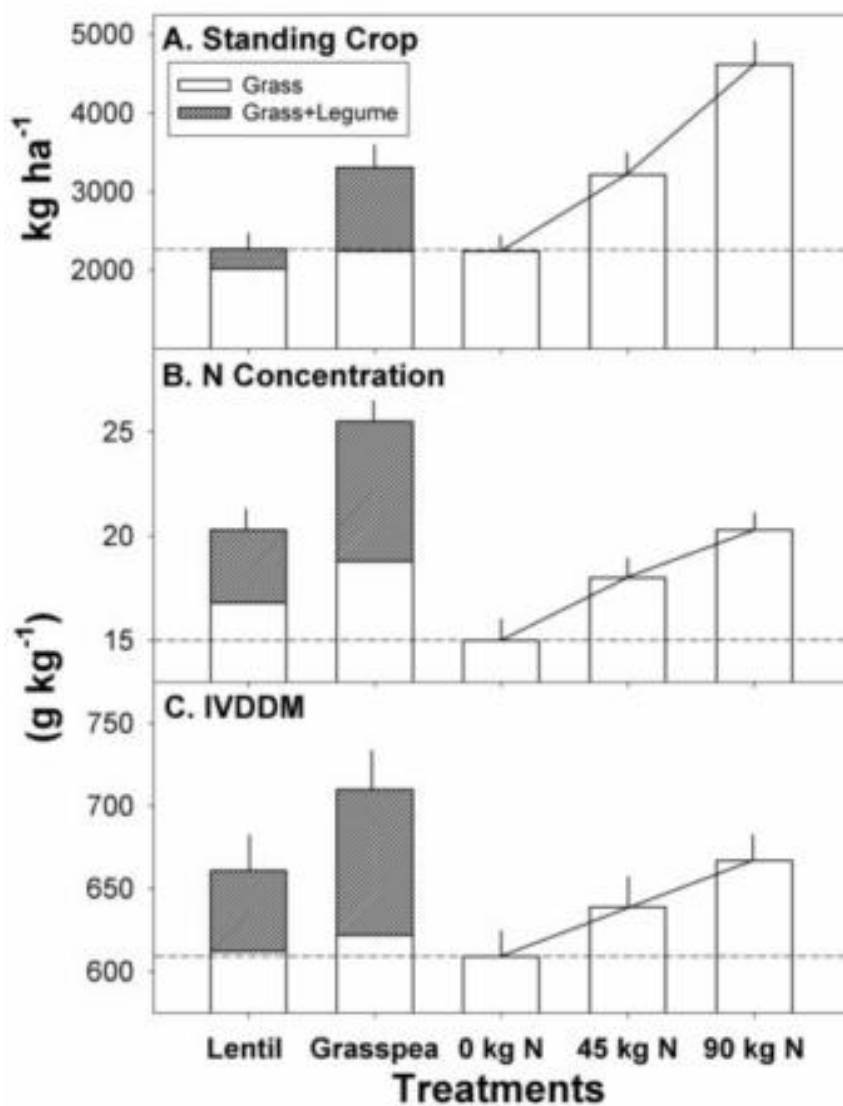
## Inter-seeding in warm-season pastures

Studies also tested the effect of inter-seeding cool-season pulses such as grass pea and lentils (*Lens culinaris*) into bermuda grass (*Cynodon dactylon*) – a warm-season perennial - paddocks to reduce N inputs. The objective was to compare forage yield and nutritive value of inter-seeded stands with pure stands of bermuda grass receiving three levels of N fertilizer (0, 45 and 90 kg N ha<sup>-1</sup>). Total end-of-season standing dry matter of bermuda grass and inter-seeded grass pea was 5550 ± 423 (SEM) kg ha<sup>-1</sup>, which was similar to biomass production of bermuda grass paddocks receiving 45 kg N ha<sup>-1</sup> (5305 ± 570 kg ha<sup>-1</sup> (Fig. 1). Seeding grass pea or lentil into bermuda grass resulted in large improvements in quality and total herbage. Seeding grass pea into bermuda grass paddocks allowed the quality of forage to exceed that of plant materials produced with 90 kg N ha<sup>-1</sup> of applied fertilizer, while inter-seeding lentils produced forage quality similar to 90 kg N ha<sup>-1</sup>.

Inter-seeding grass pea into bermuda grass paddocks can produce sufficient forage in the spring to allow the initiation of grazing 30 d earlier than the traditional way of grazing Bermuda grass in the southern Great Plains. Although additional studies are needed to optimize management for inter-seeded grass pea, this practice can improve the quality and alter the duration of available grazing for bermuda grass paddocks and reduce the input of chemical fertilizers.



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**Standing crop (A), N concentration (B), and digestibility (C) of bermuda grass forage with and without pulse crops**

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# A brief history of quantitative analysis of ODAP

by Cheng-Jin JIAO\*, Zhi-Xiao LI\*\*

A simple, rapid and accurate method that can be effectively used to quantify toxin ODAP content in grass pea (*Lathyrus sativus*) seeds or other tissues has always been needed for breeding programs since discovery of its neurotoxic activity. Furthermore, assaying the toxin is important in studying its metabolic fate in plant and animal tissues as well as its neurotoxic action in humans and other animals.

Because ODAP is a neuroexcitatory non-protein amino acid, the methods, which are mostly used for analysis of protein amino acid, are reasonably employed also to detect its presence. Relatively simple methods with the well known ninhydrin as color reagent (such as paper chromatography, paper electrophoresis and thin layer chromatography on silica gel) were first used to detect ODAP. However, these methods all have a deficiency with respect to resolution of ODAP, and generally are not applied to the analysis of low-toxin samples. In the 1970s and 1980s, when the technologies associated with high performance liquid chromatography (HPLC) were not yet available in many laboratories, the most commonly used method for determining ODAP was the spectrophotometric method developed by Rao (5), for this reason, the method is also called "Rao-method" proposed by Prof. Lambein. The method involved the alkaline hydrolysis of ODAP to yield  $\alpha$ ,  $\beta$ -diaminopropanoic acid, which in turn is complexed with o-phthalaldehyde in the presence of ethanethiol to form a colored

product that is quantified at 420 nm. However, the method does not differentiate between the two isomers of ODAP ( $\alpha$ -ODAP and  $\beta$ -ODAP).  $\alpha$ -ODAP is a nontoxic or much less toxic isomer, and easily formed from  $\beta$ -ODAP during heating. However, whenever new methods are developed for analysis of ODAP the standard for comparison is nearly always the "Rao-method".

The ion-exchange chromatography is a classical method used for separation and detection of amino acids. However, like many protein amino acid, ODAP itself does not exhibit a characteristic absorption spectrum in the ultraviolet or visible region. For detection, ODAP is generally derivatized to form a reaction product that will absorb light at some particular wavelength. It therefore, becomes a matter of choice whether the derivatization process precedes (known as pre-column derivatization) or follows (known as post-column derivatization) the column separation. In early years, the automatic amino acid analyzer, based on post-column derivation with ninhydrin was a popular tool for ODAP analysis. However, the arduous reaction conditions, i.e. 100°C at 15min, when generated in post-column flow systems caused significant loss of resolution and sensitivity.

The ion-exchange chromatography of amino acids represents one specific application of the general analytical technique of liquid chromatography and the high performance (or high pressure) liquid chromatography (HPLC) is simply the terminology applied to the technique as currently optimized. Therefore, modern HPLC equipment has been most frequently used by large number of researchers to quantify ODAP, and the trend in recent years has been towards pre-column derivatization in which the sample is reacted to form suitable derivatives. Various known derivatizing reagents have been used to develop large numbers of methods suitable for ODPA analysis (as shown in Table) (10).

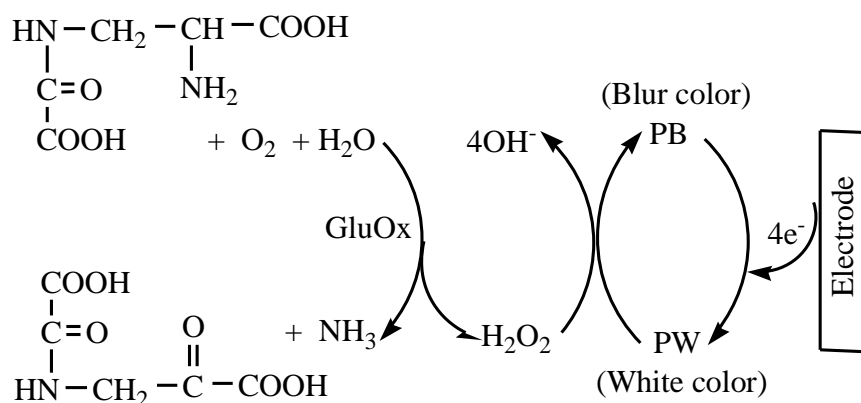
Pre-column derivatization with ortho-phthalaldehyde (OPA) prior to RP-HPLC (1, 6), has become an important analytical technique for the assay of the neurotoxin  $\beta$ -ODAP in low-toxin varieties of the grass pea seeds because of the relatively instantaneous derivatization procedure and high sensitivity. The derivatized  $\beta$ -ODAP could be detected either by UV at 340 nm or by fluorescence. Derivatization with fluorenyl methyl chloroformate (FMOC) followed by reversed-phase HPLC with fluorescence detection of total of ODAP (2) was a sensitive, reproducible method, but the two isomers of ODAP were not separated.

## Application of HPLC in the analysis of ODAP

Derivatizing reagent	Time of derivation (min)	Retention time (min)	Sensitivity
OPA	2	13.6	3.5 ppm
FMOC	30	8.4	15 pmol
PITC	20	~12	100 pmol
FDNB	30	2.3	60 pmol
AQC	10	3.85	1.8 ng
DNS-CI	25	10.5	2.3 ng
PNZ-CI	5	12.1	0.2 $\mu$ g/ml

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### $\beta$ -N-oxalyl- $\alpha,\beta$ -diaminopropionic acid ( $\beta$ -ODAP)



### $\beta$ -N-oxalylamino- $\alpha$ -keto-propionic acid

Phenyl isothiocyanate (PITC) used as a pre-column derivatizing reagent (3), can be successfully applied not only to the detection of  $\beta$ -ODAP, but also to analysis of several other toxic amino acids occurring in young seedlings of grass pea.

Our group have also published a few HPLC methods that successfully separate a- and b-isomers (9); these include pre-column derivatization with 1-fluoro-2,4-dinitrobenzene (FDNB), 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), 5-dimethylaminonaphthalene - 1 - sulfonylchloride (DNS-Cl) and para-nitrobenzyloxycarbonyl chloride (PNZ-Cl). It is noteworthy that the derivatized with FDNB is an important method because the derivatized  $\beta$ -ODAP is first eluted (7). Therefore, although the eluted program operated with a binary mixture system by changing the percentage of the organic phase, in practice, the method is also suitable for isocratic separations by using a constant solvent strength (such as 18% acetonitrile), which offers advantages over gradient conditions in baseline stability and reproducibility. By contrast, for almost other HPLC methods mentioned above, the derivatized  $\beta$ -ODAP (or ODAP) was not first eluted, the gradient elution program were necessary for adequate separation of  $\beta$ -ODAP from other amino acids, which increases the risk of instrumental breakdown and makes re-equilibration necessary for next sample detection.

$\beta$ -ODAP is also separated and detected by capillary zone electrophoresis (CZE) or flow injection assay. The former technique was originally designed to separate species based on their size to charge ratio in the interior of a small capillary filled with an electrolyte. Crude extracts of grass pea seeds without derivation were injected directly into the CZE system, making the analysis a simple fast procedure, although the detection limits were sometimes lower than those obtained from HPLC analysis.

Flow injection system application with an immobilized glutamate oxidase (GluOx) reactor led to a successful development for assaying  $\beta$ -ODAP, which included an immobilized horseradish peroxidase (HRP) reactor for detecting the generated hydrogen peroxide (4). The method was very fast compared with the usual colorimetric and the earlier-reported HPLC methods. Recently, the same group further optimized this system by replacement of HRP with prussian blue (PB) (as shown in Fig. 1) and addition of polyethyleneimine (PEI) to the immobilisation mixture. By further research, it is possible that the system can be developed into the integrated and portable  $\beta$ -ODAP biosensor suitable for a rapid and cost-efficient analysis of large numbers of samples even in field condition.

Interestingly, the gas chromatography-mass spectrometry (GC-MS) for ODAP detection was developed by many other researchers with roots of Ginseng (*Panax*

#### Proposed reaction scheme of the biosensor for $\beta$ -ODAP

*ginseng*) or some seeds of cycad because this non-protein amino acid was also found in those plants (8). Those methods, however, were time-consuming and inconvenient. Moreover, complex electronic instruments were required. Therefore, the methods are not suitable for handling large numbers of samples.

As mentioned above, none of these methods is always superior to other methods for analysis of toxin ODAP in grass pea. Finding the overall optimum method is therefore often a compromise between contradictory objectives. An efficient optimization method, therefore, should be a simple and practical method in terms of research objectives. Nevertheless, further development of new biosensor specific to  $\beta$ -ODAP may be proposed due to its simplicity, high sensitivity and potential ability on-site analysis. ■

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# Grass pea (*Lathyrus sativus*): a novel plant species for phytoremediation systems?

by Anne REPELLIN\*, Judicaëlle BRUNET\*, Yasmine ZUILY-FODIL\*

Contamination of soil and water with lead (Pb) has become a major issue in many countries, even though several strategies to reduce anthropogenic emissions have been implemented. Chemical and physical techniques to decontaminate environments soiled with lead exist. However, they are expensive and hardly applicable to large areas. The use of plants for the extraction of metallic elements like lead from contaminated media (a technology named phytoextraction) has recently emerged as a cost-effective alternative to chemical and physical cleaning techniques. Over 400 plant species have already been described as accumulators of a number of metallic trace elements [1]. As bioconcentrators, they confine metals in their biomass that can then be processed under controlled conditions. Lead, however, strongly differs from other metallic trace elements, because it is poorly mobile in both soils and plants [5]. As a result, the number of candidate plant species for phytoextraction of lead is extremely limited in comparison with the number of species that can accumulate nickel or cadmium.

In this context, we studied the capacity of grass pea (*Lathyrus sativus* L.) to tolerate and accumulate lead. Our choice was justified by the fact that this ubiquitous annual leguminous crop is amazingly tolerant to a wide variety of environmental stresses, such as drought, water-logging, poor soil conditions and various pests [6]. The main objective of this study was thus to determine the potentials of grass pea, as an accumulator of lead and its tolerance level to this pollutant.

## Approach

Young grass pea plants from a Bengalese local line were grown in liquid medium supplemented with lead nitrate (0.5 mM Pb(NO<sub>3</sub>)<sub>2</sub>), under fully controlled conditions. After four, eight and 14 days of exposure, roots, stems and leaves were collected separately and analysed for lead content, using a spectroscopic method (ICP-OES, Inductively Coupled Plasma Optical Emission Spectroscopy) with a Varian Vista MPX instrument.

## Results

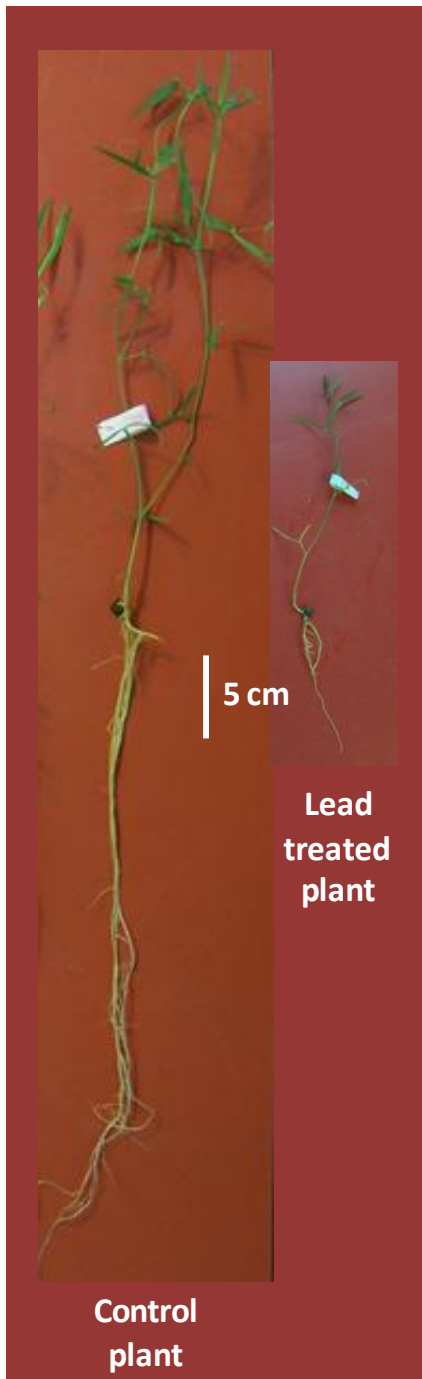
A major effect of lead on grass pea plants was a limitation on leaf and root elongation and leaf expansion (Fig. 1). This was not surprising since this is a widely acknowledged effect of this pollutant. It is noteworthy that growth arrest was complete in roots whereas stems retained a slight

capacity to elongate. This, with the fact that the cells of all tissues remained biologically responsive in terms of gene expression even after several days of exposure (J. Brunet, this issue) [2], suggested that the plants survived the treatments with lead.

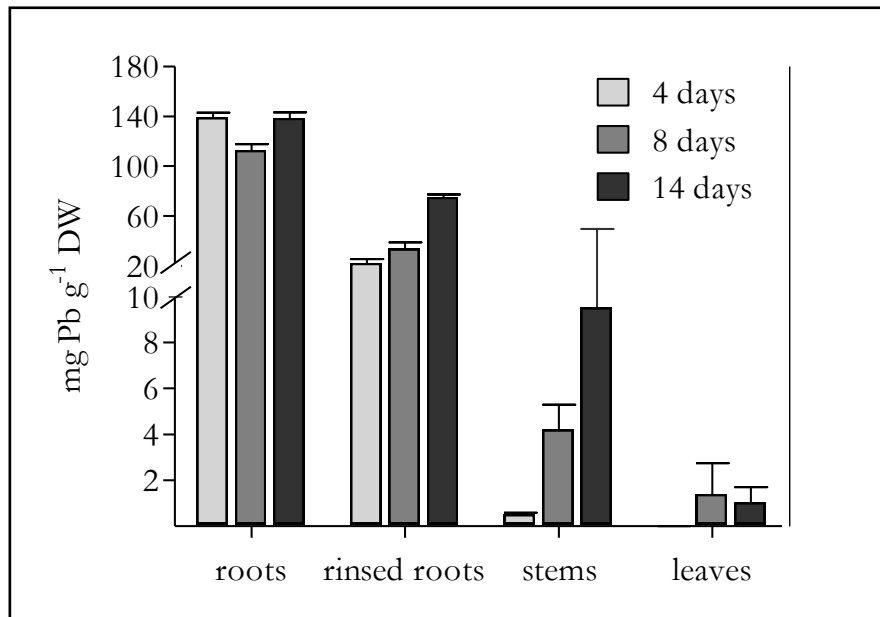
Distribution of the pollutant within the plants clearly showed that the roots were the main sinks for lead. These organs managed to accumulate an average of 130 mg of lead per unit of dry mass (Fig. 2). These values are in the range of those found in the roots of acknowledged efficient lead accumulators like *Brassica juncea* [4] and *Thlaspi rotundifolium* [3], in response to similar experimental conditions. Another interesting feature was that global fixation of lead developed rather rapidly (after four days) and did not evolve afterwards. On the other hand, the amounts of tightly bound, non-exchangeable lead, (i.e. lead that resisted desorption by rinsing of the roots with a strong chelator of Pb ions), increased with the duration of the treatment. The nature of lead binding to grass pea root tissues remains to be determined. Nonetheless, these results suggest the occurrence of intracellular lead processing, another indication of the resistance of grass pea root tissues to this pollutant.

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**Figure 1: The effect of lead nitrate (0.5 mM) on the elongation of grass pea plants grown in a liquid medium for 14 days. Control plants were not exposed to lead**



**Figure 2: Amounts of lead (mg g<sup>-1</sup> dry weight, DW) immobilized in the roots, stems and leaves of grass pea plants grown in a liquid medium supplemented with 0.5 mM lead nitrate for four days (light grey), eight days (middle grey) and 14 days (dark grey). Lead contents were also determined in roots after rinsing with 1 mM EDTA for 1h to determine the proportion of tightly-bound lead (rinsed roots).**

After four days of exposure, no lead was detected in the stems and leaves (Fig. 2). However, with time, small, yet significant amounts of lead were found in these organs, particularly in the stems. These were very encouraging results suggesting that grass pea plants are equipped with transport systems that can transfer lead to aerial organs. The impact of a chelating agent like ethylene diamine tetraacetic acid (EDTA), as a facilitator of lead translocation to the leaves in grass pea plants remains to be assessed.

### Conclusion

Despite a lack of elongation, grass pea plants exposed to high quantities of lead in a liquid growth medium retained essential biological activity. Furthermore, root tissues immobilized surprisingly large amounts of the pollutant, in a very short time. Combined with the fact that lead was tightly bound to the root tissues, these data clearly suggest

that grass pea is highly tolerant to the stress caused by the presence of lead. As a result, this multi-resistant plant species has interesting potentials, as an efficient lead phytoextracting species in rhizofiltration setups. Investigation is currently underway to determine if lead accumulation in leaf tissues can be increased in the presence of lead chelating agents.

The authors thank Dr. F. Lambein of Laboratory of Physiological Chemistry at the University of Ghent for kindly providing them with grass pea seeds.

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# Drought resistance and management for water use efficiency on *Lathyrus sativus*

by Cheng-Jin JIAO\* and Zhi-Xiao LI\*\*

**G** rass pea (*Lathyrus sativus*) is widely acknowledged as a drought tolerant crop. Under extreme conditions such as drought, grass pea is the only crop that remains available to the poor people in some rural or marginal areas of Asia and Africa. Nevertheless, in terms of drought resistance, a great variation exists among grass pea varieties. Thus, there is a growing recognition of the importance of understanding the drought resistance mechanisms for developing the proper strategies for improving drought tolerance.

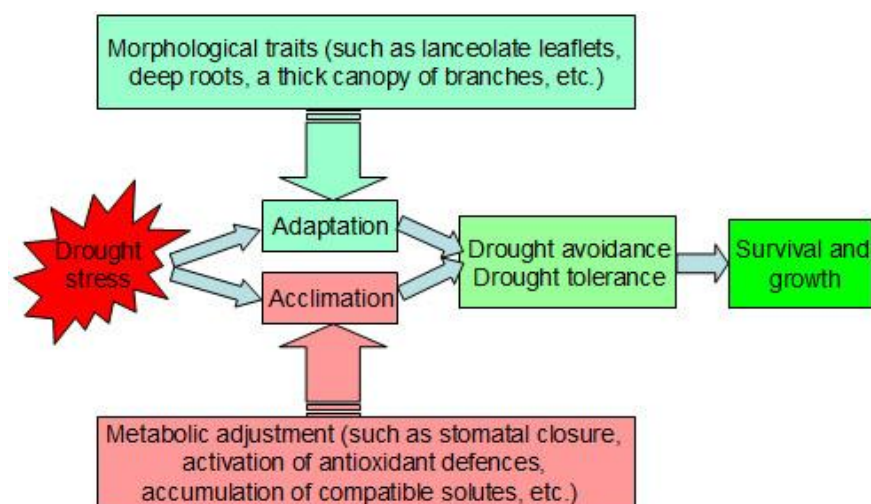
Plant drought resistance is a complex trait, which expression depends on the action and interaction of different morphological, physiological and biochemical characters, and involves changes at whole-plant, tissue, physiological and molecular levels. Drought resistance mechanisms can be grouped into three categories: escape, which enables the plant to complete its life cycle before the most intense period of drought, avoidance, which prevents exposure to water stress, and tolerance, which permits the plant to

withstand drought conditions (5). Some resistance mechanisms are constitutive and are active before exposure to drought. In other cases, plants exposed to drought alter their physiology, thereby acclimating themselves to a dry condition.

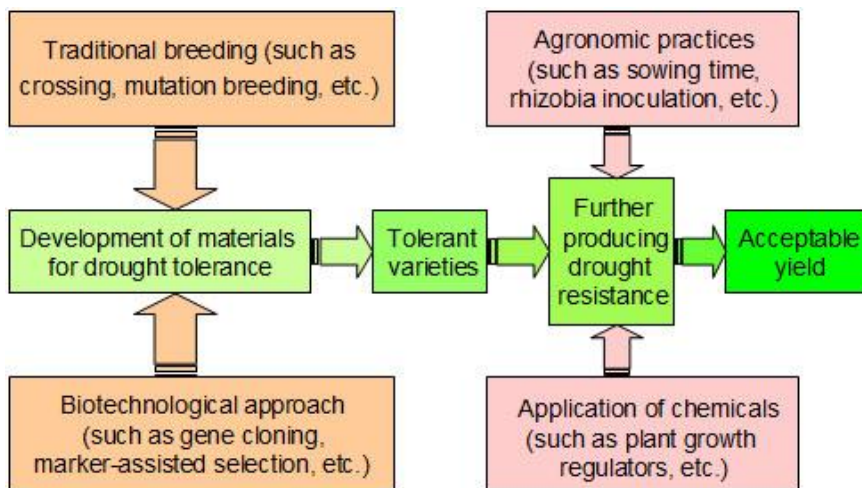
During the long-term evolution of grass pea and to cope with drought, this crop has developed a lot of constitutive drought tolerance traits (Figure 1), including linear-lanceolate leaflets, photosynthetic stems with winged margins, well-developed taproot system, etc. (1). It has long been established that plants bearing small leaves are typical of xeric environments (2). Grass pea characterized by the narrow linear leaves and the green stems with winged margins can not only photosynthesize efficiently but also

withstand drought by reducing the evaporation surface. Moreover, this type of leaf can easily roll so as to reduce transpiration surface under dry conditions, minimizing further water loss. Roots are the key plant organ for adaptation to drought. Grass pea plant with its deep and extensive root system can access water deep in the soil, even in the arid lands. In addition, in the early vegetative growth stage, these plants can easily connect together by the simple or much-branched tendrils to develop a thick canopy of branches, with which rapidly covers the field surface, declining soil moisture evaporation, and therefore, enhancing greatly water absorption from soil by the plants.

**Figure 1: Possible mechanisms of grass pea drought resistance**



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**Figure 2: Proposed grass pea water management**

Plants in general, when dry conditions are detected, decrease the levels of auxins, gibberellins and cytokinin, while increasing the synthesis of abscisic acid and ethylene. They subsequently trigger an array of biological processes to conserve water, including the rapid induction of stomatal closure to reduce transpiration water loss; the activation of antioxidant defenses to combat oxidative stress, which can cause an extensive membrane damage; the decrease in osmotic potential achieved by the decrease in cell volume as well as by the accumulation of solutes such as proline; the expression of a wide range of stress proteins to prevent macromolecule denaturation and cell dehydration, etc. In addition, various chemical signals involved in the complex protective responses at whole plant level, allow plant to alter its homeostasis, its steady-state physiology and to adapt to a drier external environment. In grass pea, relatively little attention was given in the past to these physiological responses to water deficit. Delayed senescence and maturity as forms of drought avoidance and/or tolerance were observed for grass pea by Thomson and co authors (7) and Leport and co authors (4). In these studies, although grass pea had lower yields and lower water use efficiency when compared to faba bean and pea, it presented a greater water extraction in the low to medium rainfall Mediterranean-type environments in southwestern Australia. Physiologically, Yang and

co authors (9) found that water deficiency in grass pea led to a decrease of stomatal aperture, but also to an increase in stomatal density and stomatal resistance, as well as an increase in leaf water use efficiency of the leaves. Sun and co authors (6) using grass pea seedlings in solution culture, reported that in the early water deficit induced by polyethylene glycol (PEG), stomatal conductance and transpiration decreased sharply, while the net photosynthesis rate and the water use efficiency fluctuated at a certain level. In a later stage (108 hours after treatment), proline had accumulated 14 times more than in the control.

Because of a wide range of variation in drought resistant characters exists among grass pea varieties, the drought stress effects can be first managed by breeding the most appropriate crop cultivars that tolerate water stress and at the same time yield well in the arid soil (Fig.2). The combination of the biotechnological approaches already existing in grass pea (8) and traditional breeding will allow a more rapid way to improve drought tolerance in this crop.

Higher water use efficiency can also be achieved through the adjustment of agronomic practices (sowing time, plant density and soil management), and the exogenous application of various growth regulating and other chemicals (2), and this should be also possible for grass pea. Foliar application of plant growth regulators such as gibberellic acid, or abscisic acid has

proven worthwhile and practicable for improving growth against water stress (2).

Additionally, it has already been proved in other legumes that drought-tolerant rhizobia can compete with naturalized rhizobia to form effective symbiosis under conditions of scarce moisture, improving nitrogen nutrition of the host legume (3). Inoculation of grass pea seeds with drought-tolerant rhizobia may be also an efficient and low-cost way to enhance its drought resistance.

Despite its reputation of being a drought-tolerant and hardy legume crop, absolutely drought-resistant grass pea varieties do not exist, as far as is known. To correctly assess grass pea drought tolerance, much study on phenological, physiological and molecular aspects will be essentially needed in fully utilizing its drought-tolerant potential. ■

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# Development of cytogenetic stocks through induced mutagenesis in grass pea (*Lathyrus sativus*): Current status and future prospects in crop improvement

by Dibyendu TALUKDAR\*

With rare combination of many desirable biological and agronomic characters, grass pea serves as a dependable cropper in many developing countries. Notwithstanding its rich seed protein content, low agronomic requirement and wide scale use as a food legume grass pea has not received much attention from geneticists. Development of high yielding and low seed neurotoxin containing lines are two prime objectives in grass pea breeding.

Low level of genetic variation due to predominant presence of self-pollination and inter-specific incompatibility hindered its improvement through conventional breeding. Induced mutagenesis has, therefore, been adopted as an important tool to create additional genetic variability providing wider scope on the isolation and characterization of novel genetic variants. In recent years, apart of valuable genetic mutants, a good number of cytogenetic variants including different types of trisomics, tetrasomics, double trisomics and translocations were identified (1, 3, 4) in induced mutant population of different diploid varieties of grass pea and are being used successfully in mapping of desirable mutations in grass pea.

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## Genetic mutant stocks

Genetic mutants are useful tools in basic and applied research to ascertain the developmental and regulatory systems of crop plants and their value is enhanced with the knowledge of the genome mutation location. A wide spectrum of viable diploid mutations, exhibiting contrasting modifications in different morphological traits, has been used as mutant stocks in grass pea (6). These mutants are true breeding, self-fertile, easily detectable and mainly recessive in nature. No chromosomal anomaly was observed in these mutants indicating nature of true gene mutation. The genetic basis of some of these mutations has been ascertained and a brief overview is given in Table.

## Aneuploid stocks

Different aneuploid cytotypes, including a complete set of seven different primary trisomics, seven tetrasomics and five double trisomics, were identified in induced mutant populations of different grass pea cultivars (1).

Trisomics with one extra chromosome ( $2n+1$ ;  $2n=15$ ) were phenotypically distinguishable from diploid plants ( $2n=14$ ) and from each other by the specific leaflet and stipule morphology even at early seedling stage (4). Trisomics were self-fertile and have been maintained at an average rate of 23% in selfed population.

Among higher aneuploids, seven different primary tetrasomics ( $2n+2$ ;  $2n=16$ ), with two extra homologous chromosomes

and higher pollen sterility (1), and five double trisomic plants ( $2n+1+1$ ;  $2n=16$ ), with two different non-homologous extra chromosomes, were isolated in advanced selfed generation of trisomics and gamma radiation induced  $M_2$  progeny of three grass pea cv., 'BioR-231', 'Biol-203' and 'P-27/3', respectively. Trisomic phenotypes were found exaggerated in tetrasomics. However, a combination of two different trisomic phenotypes was present in each double trisomic plant.

Presence of tertiary and interchange trisomics with one translocated chromosome as extra was traced in selfed and intercrossed progenies of reciprocal translocation (RT) by analyzing meiotic I chromosomal association of microsporocytes. Cytogenetic studies of this group of plants are in progress.

## Hybridization involving mutant lines and aneuploid stocks

The mutant lines showing true breeding nature for a number of contrasting morphological traits as compared to their parental cultivar were selected as parents in hybridization programs. From the grass pea cv. 'BioR-231' two dwarfs (2), one high yielding bold-seeded mutant (5) and one stipulate mutant, and from cultivar 'Hoogly Local', one dwarf and one tendril-less mutant line were selected for this purpose (2) (Table and Figure). The association of a number of desirable traits in the mutant lines seems to be either due to pleiotropic action or due to induction of mutations in a number of closely linked genes of the same genome (2).

Primary trisomics, double trisomics and tetrasomics produce modified segregation ratios (when crossed) which are used to assign genes on specific chromosome. Using primary trisomics *df1/df2*, *df3*, *lfi*, *ngn* and *cbl* genes have already been assigned on different chromosomes of grass pea (2). Diploid mutant lines also exhibited allozyme diversity in different isozyme loci and using aneuploids, linkage mapping between morphological and biochemical markers is being carried out. Detection of arm location and position of concerned gene/s with centromere may be deduced with the help of tertiary trisomics.



**A dwarf line with stipulate and tendril-less condition (2)**

### Recombinant inbred lines (RILs)

As many as 27 RILs (designated as RIL<sub>1</sub>-RIL<sub>27</sub>) descendent from different intercrosses involving parental varieties and induced mutant lines, have been randomly selected for different qualitative and quantitative characters.

RILs showing true breeding nature in successive selfed generations (F<sub>2</sub>-F<sub>10</sub>) were selected based on their qualitative traits (such as flower colour, floral architecture, plant habit, etc). Desirable characters combination, such as higher yield potential and low range of seed ODAP content, will result in the RILs superiority over parental varieties..

### Reciprocal translocation stocks

A Reciprocal Translocation (RT) contains a segment of one linkage group (LG) attached to another group and are therefore useful for the location of genes to LGs, for testing independence of LGs, and for map orientation. At present, 10RTs, also known as chromosomal interchanges were detected in gamma ray (300Gy, 350 Gy and 400 Gy) induced M<sub>2</sub> progeny of grass pea var. 'BioK-231', 'BioR-231', 'Nirmal' and 'LSD-3'. Multiple interchanges have been detected in four RT lines (3).The RT stocks involve both RT homozygotes showing 7II at meiosis I and marginal pollen sterility (2.5-3.0%) and heterozygote lines with one ring quadrivalent and partial pollen sterility

**List of induced morphological mutants indicating important features, mode of inheritance and gene/s identified in grass pea (\* Identification of other loci, involvement of pleiotropy or linkages still being studied)**

Name of the mutants	Important characters	Inheritance/loci identified*
1) Dwarf	dwarfism, erect, determinate, pod indehiscence, low ODAP	<i>df1/df2</i> and <i>df3</i>
2) Branching	sub-opposite/concentric, profuse branching, low ODAP, high yield	digenic mode
3) Leaflet	ovate-lanceolate, crinkle, circinnate	<i>lfts</i>
4) Stipule	ovate, linear-acicular or absent, blackish-purple, perpendicular, high ODAP, high biomass	<i>St<sup>m</sup></i> , <i>St<sup>c</sup>-St<sup>pp</sup></i> , <i>Par</i>
5) Tendril-less	no tendril, additional leaflet, double podding, high yield, low ODAP	monogenic, digenic
6) Internode	non-winged (afila), semi-dwarf, fasciated, high yielding	<i>wgn</i>
7) Flower colour	pale-violet, reddish-purple, pink, light blue, pinkish-purple, blue-patched white, white, low to high ODAP, early to late maturity	<i>B+</i> , <i>Pv</i> , digenic, etc.
8) Seed coat colour	black, green, yellow, light-brown, grey-white, yellowish white, white, monogenic, high yielding, low to moderate ODAP	<i>cbl1/cbl2</i>
9) Floral structure	malformed corolla, exposed stigma, extra sepals and petals	<i>mle</i> , <i>clf</i> , <i>crl</i>
10) Pedicel	distichous to multistichous, stiff, abnormal elongation, high grain, forage mono-,di-and yield, low ODAP	mono-,di-and polygenic
11) Seed size	bold, round, white, pod indehiscence, high yield, low ODAP	<i>st<sup>m</sup>-st<sup>c</sup></i>

(48.67-53.12%). RT homozygotes are important source of RT heterozygotes which produces tertiary and interchange trisomics in selfed and intercrossed populations.

Development of a complete set of primary trisomics and tetrasomics along with RT lines will facilitate classical and molecular genetic linkage mapping in grass pea and can provide important information for comparative mapping in other closely related grain legumes. ■

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# Intercropping of grass pea (*Lathyrus sativus*) with other grain legumes for forage production

by Branko ČUPINA\*, Aleksandar MIKIĆ\*\*, Vojislav MIHAILOVIĆ\*\*, Đorđe KRSTIĆ\* and Branko ĐURIĆ\*\*\*

**G** rass pea and other vetchlings (*Lathyrus* spp.) are extensively present in the flora of Balkan countries. Grass pea was widely cultivated in the past for diverse purposes, such as grain, forage or green manure (3), but today it is almost forgotten and can be found rather sporadically and produced mainly as pulse crop only (1).

## Renewal of the research on *Lathyrus* in the Balkans

Within the Annual Forage Legumes Collection (AFLCNS) of the Institute of Field and Vegetable Crops (IFVCNS), Serbia, a small *Lathyrus* collection was founded, with about 120 accessions of diverse geographic origin and status, belonging to 18 species. Each year, expeditions in the region are undertaken and the wild populations are collected, *ex situ* conserved, characterized and evaluated.

The only breeding program on grass pea in the region is carried out at IFVCNS aimed at the development of multifunctional cultivars, it recently produced the first two registered Serbian grass pea cultivars ever, Studenica and Sitnica.

Supporting crop	Supported crop	Mixture ratio	Green forage yield (t ha <sup>-1</sup> )	LER (supporting crop)	LER (supported crop)	LER (total)
Faba bean	Grass pea	100 : 0 (faba bean monocrop)	25.6	1	0	1
		25 : 75	32.5	0.91	0.36	1.27
		50 : 50	39.5	0.82	0.62	1.44
		75 : 25	30.5	0.36	0.67	1.02
White lupin	Grass pea	100 : 0 (lupin monocrop)	24.8	1	0	1
		25 : 75	32.0	0.88	0.27	1.14
		50 : 50	28.5	0.56	0.48	1.04
		75 : 25	32.5	0.35	0.86	1.21
LSD <sub>0.05</sub>			5.2	0.19	0.21	0.14
LSD <sub>0.01</sub>			7.7	0.24	0.26	0.19



## Can an intercropping of grass pea with other legumes make sense?

During the last few years the first attempts have been made in intercropping grass pea and other annual legumes, with a main goal of increasing yields in both forage and grain production. A field trial was carried out by IFVC at Rimski Šančevi in 2008 to study various combinations of grass pea with faba bean (A) and white lupin (B), as supporting crops. In both combinations, there were pure stands of all three crops and the three mixing ratios, namely 75% : 25%, 50% : 50% and 25% : 75%. The results on forage yields had emphasis upon the determination of the Land Equivalent Ratio (LER), as an indicator of reliability of each combination (Table 1).

With the green forage yields at the same level with a long-term average for grass pea in the same conditions (2) and the LER > 1 in all mixtures (Table), the intercropping of grass pea with other annual legumes, based on the above stated principles, may be economically reliable and thus deserves attention. The study will be proceeded and enriched with the data on LER in grain production and impact of intercropping on forage and grain yield quality and stand morphology, especially yield components. ■

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# A brief historical review on *Lathyrus* in Europe

by Aleksandar MIKIĆ\*, Marija LJUŠTINA\*\*, Gregory KENICER\*\*\* and Petr SMÝKAL\*\*\*\*

Vetchlings (*Lathyrus* spp.) had been known to humans before they became cultivated. They were part of the everyday diet of the hunter-gatherers at the end of the last Ice Age in Europe, as witnessed by the archaeological remains from the site of Santa Maira in Spain, dating back to 12,000–9,000 years ago (1).

## Domestication and distribution

There is very little evidence of how, when and where *L. sativus* and *L. cicera* were domesticated, despite the fact that they often occur at high frequencies in finds from Near East during the 10th and 9th millennia BC. It is possible that these two species initially had been considered weeds in the lentil (*Lens culinaris*) crop and afterwards began to be cultivated separately (4).

It is quite certain that *L. sativus* and *L. cicera*, along with lentil, bitter vetch (*Vicia ervilia*) and pea (*Pisum sativum*), were among the most ancient crops that entered Europe from Asia Minor, following the end of the last Ice Age and its re-inhabitation. It could be assumed that *L. sativus* and *L. cicera* spread over Europe by two independent pathways. The first one was via the Iberian Peninsula, witnessed by early Neolithic sites such as Cova de les Cendres near Alicante (3). Another one was via Danube, with numerous findings in the Balkans, such as one in Kovačevo, southeast Bulgaria, from early 6th millennium BC. Afterwards, both crops quickly became widely present all over Europe until modern times.

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**Table 1. Words denoting vetchlings and pea in some modern European languages**

Family	Language	<i>Pisum</i>	<i>Lathyrus</i> spp.
Altaic	Turkish	<i>bezelye</i>	<i>mürdümük</i>
	Armenian	<i>oloř</i>	<i>tapoloř</i>
	Belarusian	<i>garoh</i>	<i>garošak</i>
	Breton	<i>piz</i>	<i>piz-moc'h</i>
	Bulgarian	<i>grah</i>	<i>sekirche</i>
	Croatian	<i>grašak</i>	<i>grahor, sastrica</i>
	Czech	<i>hrach</i>	<i>hrachor</i>
	Danish	<i>ært</i>	<i>fladbælg</i>
	Dutch	<i>erwt</i>	<i>lathyrus</i>
	English	<i>pea</i>	<i>vetchling</i>
	French	<i>pois</i>	<i>gesse</i>
	German	<i>erbse</i>	<i>platterbse</i>
	Greek	<i>bizéli</i>	<i>lathoúri, araká</i>
	Italian	<i>pisello</i>	<i>cicerchia</i>
	Lithuanian	<i>žirnis</i>	<i>peležirnis</i>
	Norwegian	<i>ert</i>	<i>erteknapper</i>
	Polish	<i>groch</i>	<i>grozek</i>
	Portuguese	<i>ervilha</i>	<i>chícharo, cizeirão</i>
	Romanian	<i>mazăre</i>	<i>orăstică</i>
	Russian	<i>goroh</i>	<i>čina, gorošek</i>
Serbian	<i>grašak</i>	<i>grahor, sastrica</i>	
Spanish	<i>guisante</i>	<i>guija, almorta, tifarro</i>	
Swedish	<i>ärt</i>	<i>vial</i>	
Kartvelian	Georgian	<i>mukhudo</i>	<i>matkvartzana</i>
	Estonian	<i>hernes</i>	<i>seahernes</i>
Uralic	Finnish	<i>herne</i>	<i>linnunherne</i>
	Hungarian	<i>lednek</i>	<i>borsó</i>

## Origin and diversity of words

The words denoting *Lathyrus* species in modern European languages are often derived from the words denoting pea and other grain legumes (Table 1). An example are Slavic languages where the words denoting pea and vetchlings were derived from, via the Proto-Slavic *\*gorxŭ* denoting the same, the Proto-Indo-European *\*ghArx-*, denoting a leguminous plant.

The Italic languages remain the richest in words denoting vetchlings, where an extreme linguistic abundance perfectly supports the archaeological evidence: *almorta*, *alverjón*, *arveja*, *arvejo cantudo*, *arvejote*, *bichas*, *cicércula*, *chícharo*, *diente de muerto*, *frijol de yerba*, *garbanzo de yerba*, *guija*, *muela*, *pedruelo*, *pinsol*, *pito* and

*tito*. Many of these terms have their ultimate origin in the Proto-Indo-European words *\*erǵʰ(b)-*, denoting a kernel of leguminous plant, and *\*kek-*, denoting pea. The well-known Latin word *lathyrus*, derived from the Greek *lathoúri*, is generally accepted as denoting something very strong, due to its supposed aphrodisiac properties (2). ■

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## Progress in genetic markers development for *Lathyrus* species

by Maria Carlota VAZ PATTO\* and Nuno Felipe ALMEIDA\*

Most investments in agricultural biotechnology have been centered on widely consumed crops that are traded internationally. Neither the public nor the private sector has invested significantly in genetic technologies in the more diverse minor or orphan crops that are often critical in the world's most disadvantaged regions (5). *Lathyrus* species are among these so called "orphan" species that tend to be locally or regionally important for food security, but receive little scientific focus or investment.

Opportunely, researchers working with these underused crops have realized that the developed powerful technologies in major crops and model species gave raise to important new opportunities/tools for improvement of orphan crops. Among these tools, DNA-based approaches have been applied in several areas of molecular breeding in underused species.

One of these areas is marker assisted breeding, aiming at enhancing the power of conventional genetic analysis. In this approach, molecular markers are used as tools to detect the extent and structure of genetic variation. Molecular tools are also used in the analysis of inheritance of key crop traits.

Several different types of molecular markers are available to perform these studies. Some, due to its broad applicability such as RFLPs, RAPDs or AFLPs, have

been readily exploited on underused crops such as several *Lathyrus* species for diversity analysis and genome mapping (reviewed by 7 and 8). Others, depending on the knowledge of the DNA sequence (SSR, SNP), are more cost demanding and were first developed in major crops or model species. Nevertheless their application in less studied species like the *Lathyrus* sp. can be attempted after some technical efforts, through cross amplification. This is possible due to the existence of synteny among related genomes. Evidence indicates that all plant genomes have a great deal in common in their gene content, biochemical pathways, and chromosomal organization. Different plant taxa have different versions of the same genes at a given position or locus in a genome, but the order of loci is conserved to varying degrees across even distantly related crops (5). In the case of *Lathyrus* sp., efforts are being made to increase transferability of pea and lentils genomic microsatellites (gSSRs) (1).

The increasing availability of express sequence tags (ESTs), short segments of sequenced gene transcripts that provide information on genetic expression, function and heritability, has allowed the identification of several candidate genes for complex traits. Tens or hundreds of thousands of ESTs are now available for major crop species, but in orphan species this number is quite meager. As an example, for *Lathyrus sativus*, databases show only 178 different EST (<http://www.ncbi.nlm.nih.gov/nuccore> retrieved on 26<sup>th</sup> November 2009). Nevertheless, the molecular markers generated by primers designed from these

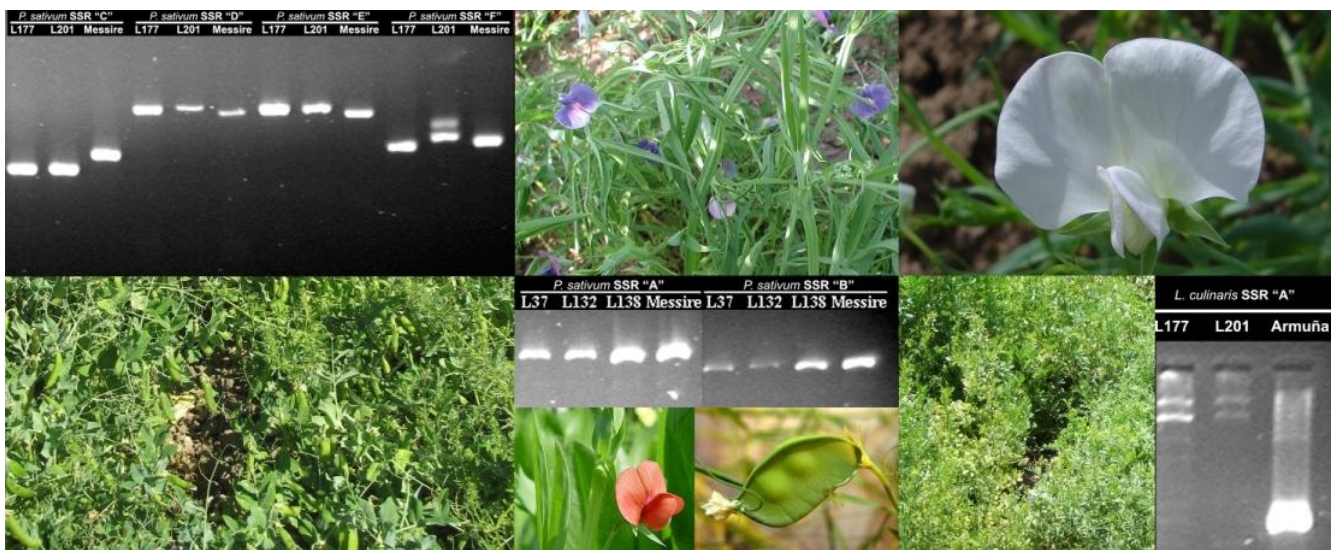
defense-related EST sequences, since they are derived from coding DNA (representing genes of known function), will have generally a higher degree of sequence conservation and may represent useful markers in closely related species such as *L. cicera*.

As stated before for gSSR, also in this particular type of markers (derived from EST sequences) it is possible to try cross species amplification. The recently initiated work of Almeida and Co authors, also explores the transferability of EST-SSR besides gSSR from the closely related pea and lentils (1). This study reports that, despite the low transferability, the cross-amplification of pea SSR in *Lathyrus* spp. is a good alternative to the development of specific grass pea microsatellites. This study also confirmed that the pea microsatellite motif was maintained, but in most cases with different repeat numbers. In relation to the lentil SSRs, cross amplification was less successful and some SSRs did not yield any amplification product and the others amplified a non-specific fragment.

The incorporation of these common markers in future linkage mapping studies of *Lathyrus* sp. will allow map alignment and facilitate comparative mapping and comparative genomics with several related major crops.

On the other hand, the potential defense-related ESTs, besides being useful as a source of molecular markers for the construction of high-density genetic linkage maps, provide also very interesting tools for expression analysis studies. Expression analysis studies were performed on *L. sativus* inoculated with *Mycosphaerella pinodes* using 29

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EST representing genes coding for enzymes and proteins involved in different levels of defense (6). The availability of this EST set allied with the microarray technology will provide a powerful way to identify further defense-related genes and pathways involved in *L. sativus* disease resistance, and also in biotic or abiotic resistance in other related pulses such as chickpea (3).

More recently, the advent of next generation sequencing technologies has boosted genome-wide quantitative gene expression profiling by SuperSAGE (4). This approach has been applied to the interaction *L. sativus* / *Ascochyta lathyri* within the frame of an ERA-PG European funded project (LEGRESIST) (2). This technique is also particularly adapted to organisms for which no extended DNA database is available (4) as is the case for *L. sativus*. With this approach it was possible to identify the differential expression on *L. sativus* of more than 3000 ( $P < 0.05$ ) or 900 ( $P < 0.005$ ) transcripts, respectively, between infected and control tissue (Peter Winter, pers. comm.).

In future, quantitative Real Time PCR (qRT-PCR) assays will be available from this European consortium that will make possible to perform eQTL (expression QTL) mapping in the *L. sativus* / *Ascochyta* interaction. The transcript abundance (also

referred as gene expression level) can be analyzed as any higher order trait in terms of QTL detection. Variation in gene expression can be mapped in segregating populations by expression profiling and marker-based fingerprinting of each individual of a segregating population.

This same European project is studying the allelic diversity of resistance genes on the level of Single Nucleotide Polymorphisms (SNPs) for genetic mapping of expressed resistance genes in major crop legumes. At this moment, the 3'-RACE and the 5'-RACE sequences of the Expressed Potential Resistance Genes (EPRG) are being screened for SNPs and small indels with the objective of delivering a SNP-array for rapid and cost efficient mapping in *L. sativus* (2) providing potential links to the structural genes themselves. EPRGs were isolated from cDNA using resistance gene specific primers.

*Lathyrus* researchers will then have available a combination of QTL and eQTL mapping data and mapped EST /EPRGs data as powerful tools for identifying candidate genes underlying quantitative traits and to study resistance gene networks on this underused crop. Reciprocal benefits exist for the major crops from incremental investments on these orphan crops, e.g. the

identification of genes conferring resistance to biotic or abiotic stresses as a set of interesting genes for transferring to major crops.

This European project is a clear example on that orphan crop improvement will have to consider fostering institutional arrangements that combine efforts across whole sets of major and underused crops. ■

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## Disease resistance in *Lathyrus sativus* and *L. cicera*

by Diego RUBIALES\*, Monica FERNÁNDEZ-APARICIO\*, Ana MORAL\*, Nuno Felipe ALMEIDA\*\* and Maria Carlota VAZ PATTO\*\*

**L** *athyrus sativus* and *L. cicera* are interesting alternatives for cropping systems diversification in marginal lands. There is a renewed interest in the improvement of these species. Achievements in resistance breeding will be summarized and discussed.

Powdery mildew (*Erysiphe pisi*) is among the major diseases on *Lathyrus*. Little is known about the availability of resistance against powdery mildew in the *Lathyrus* genus and its different responsible mechanisms. Resistance has only recently been identified and characterized in *L. sativus* and *L. cicera* germplasm (7, 8). Only Partial Resistance has been identified so far, resulting in reduction of disease severity in spite of a compatible reaction (high infection type), with no macroscopically visible hypersensitivity. The combination of different resistance genes and or mechanisms in new cultivars should provide longer durability of resistance than each resistance gene by itself.

Rusts (*Uromyces* sp.) are important diseases of grass pea in some areas such as northwestern Ethiopia. In the *Lathyrus* genus infection by different rust species has been reported, although on *L. sativus* and *L. cicera*, data only refer to *U. pisi* and *U. viciae-fabae*. Recent studies have shown high specialization within *U. viciae-fabae*, with each morphologically distinct isolate exclusively infecting cultivars of the species from which it was collected (1). Results from cross

species inoculations show that *L. sativus* was only infected by *U. pisi* but not by other *Uromyces* species. A wide range of disease reaction severity has been found in *L. sativus* (10) and *L. cicera* (9) germplasm against these rust species. A compatible reaction (high infection type) was the rule with *U. pisi*, although in this case the level of resistance varied in terms of disease severity among accessions. *L. sativus* accessions were in general more resistant (lower DS) to *U. pisi* than accessions of *L. cicera* both under field and growth chamber conditions. However, low infection type was only found on some *L. cicera* accessions. Macroscopically visible hypersensitive resistance was detected not only in some *L. cicera* accessions to *U. pisi*, but also in *L. sativus* to *U. ciceris-arietini* and in *L. cicera* to *U. viciae-fabae*. At microscopic level, host cell death was also detected on *L. cicera* against *U. ciceris-arietini* and in *L. sativus* against *U. viciae-fabae* what resulted in reduced colony size. Relatively low infection in spite of high IT was detected in 17% of *L. cicera* and in almost all *L. sativus* accessions, indicating levels of partial resistance against *U. pisi*. However, different reactions have been recently observed to still unidentified rust species collected on *Lathyrus* spp. in the wild.

*Lathyrus* spp. are known to be resistant to *Mycosphaerella pinodes*, the causal agent of pea ascochyta blight. Resistance in *L. sativus* may be controlled by two independently segregating genes, operating in a complementary epistatic manner (6). However, species of *Lathyrus*, particularly *L. tingitanus* can be severely damaged by still unidentified *Ascochyta* species.

Research is being performed at present to determine the identity of these ascochyta, and to discern their host range and the availability of resistance within *L. sativus* and *L. cicera*.

Crenate broomrape (*Orobanche crenata*) is widely distributed in the Mediterranean region and West Asia (3) where it is a major constraint to the production of grain and forage legumes, including *L. sativus* and *L. cicera*. Several strategies have been developed for the control of broomrape, from cultural practices to chemical control (4), but none with unequivocal success. So far, a combination of sowing *Orobanche*-free crop seeds and delaying the sowing date or use of early maturing cultivars to escape *O. crenata* damage has been the only available strategy for controlling the broomrape infection in many cool-season legume crops. Resistance to *O. crenata* is scarce and of complex nature, but in spite of these difficulties, significant success has been achieved in some legume crops (3). Recent results show varying levels of infection under field conditions in *L. cicera* accessions. Avoidance due to early flowering plays a major role in the reduction of broomrape infection in *L. cicera* and *L. sativus* (2). Besides early maturing germplasm avoiding infection, valuable *L. cicera* germplasm was reported with additional interesting levels of genetic resistance that might be further exploited in breeding programs. In addition to escape due to precocity, vigor or root biomass, different mechanisms of resistance and avoidance to *O. crenata* are operative in *Lathyrus* sp. germplasm (5). ■

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Rust infecting *L. cicera*



Typical *Ascochyta* lesion on a *L. tingitanus* pod

**Partially resistat (left) vs susceptible *L. cicera* accessions against *Orobanche crenata***



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## Alvaiázere, Grass Pea Capital: A Taste on Patrimony

A case of successful recuperation of a local variety almost extinct, through an intelligent participatory exploitation of the grass pea gastronomic value

by Maria Carlota VAZ PATTO\*



The gastronomic festival of *chicharo* (grass pea) in Alvaiázere, a small village in the central region of Portugal, is now on its 8<sup>th</sup> annual edition, every first week of October. This initiative, started by two cousins (Carlos Furtado and Pedro Alves) with their family roots on the village of Alvaiázere, is intended for elevating local knowledge on this forgotten crop and their associated traditions. Moreover, it provides increased opportunities for local farmers to sell their products outside the conventional commercial systems.

This festival counts not only with grass pea based gastronomy (traditional and innovative recipes), but it gives also an opportunity to buy and sell all other different regional productions (cheese, honey, olive oil, wine, chorizos, figs, nuts, etc) and to attend several different cultural and musical activities. There is wide space to reinvent old traditions.

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### History

In this particular mountainous region of Portugal (Serra do Sicó), grass pea was highly cultivated in the past especially on hunger times, and because of that, it still reminds local old people of hard times that they normally want to forget. Consequently, besides low and inconstant productivity, due to the presence of several biotic and abiotic stresses, this cultural/sociological reluctance and this negative memory have also contributed for the present under use of the crop. Nevertheless, a few farmers of this region kept on producing grass pea due to tradition rather than for the profit they could get from it. These farmers were the ones who made possible the reintroduction and reborn of this crop in the Alvaiázere region. It is still hard to buy these seeds outside this festival in this part of the country but it is even harder to find them on big cities farther away.

Eight years ago, when this festival started, there were only about 10 farmers that kept on cultivating grass pea in the all municipality of Alvaiázere. Nowadays, and as a result of this gastronomic initiative, the demand for seeds of this crop increased, and many small farmers reintroduced its cultivation, that they had abandon more than 50 years ago. Maize is one the crops that is being replaced by this species, year after year, and farmers are including this legume on sustainable rotations with other crops with an obvious overall N fertilization improvement.

The support of the local authorities was crucial for the success and sustainability of this initiative. The local Municipality was

concerned about the human erosion that the region was suffering from and saw in this festival an opportunity to attract tourists and motivate the local population. In order to promote the market the it has developed this year a local brand package, with a proper barcode, for selling grass pea at this festival.

This initiative counts also with the support of more than 20 local restaurants in a joint effort to provide a great diversity of grass pea menus during the festival days.

From its first edition, the idea was that this event had to be participative, involving locals to increase their knowledge and proud for the region. This was the only way to make this initiative viable and economically sustainable. This type of initiative is everyday more valorised by urban people and their visits to the festival served, as a touchstone to motivate more the local population, usually doubting of the traditional agricultural systems and old crops.

This initiative survives on a reduced budget, but involves extensive publicity at national level, all over the year, through news papers articles, radio programs or direct contacts with environmental associations, schools and individual people. Organizers can be contacted through the e-address [alvachicharo@gmail.com](mailto:alvachicharo@gmail.com) and a webpage <http://festivaldochicharo.blogs.sapo.pt>. ■

Fig.1: *Chicharada*: a grass pea stew with different kinds of meat and vegetables.

Fig. 2: 2009 *Alvaiázere, Capital do Chicharo* festival poster.

Fig. 3-7: Different pictures from the Farmer's market at Alvaiázere with different grass pea products to sell (seeds, marmalade, liquors, etc).

Fig. 8: Carlos Furtado and Pedro Alves, the organizers of this festival with their uncle, an enduring grass pea farmer.





## From a survival food of the poor to a festivity main dish: “titos” (grass pea, *Lathyrus sativus*) in La Gamonal and in Padilla de Abajo (Burgos, Spain)

by Constantino CAMINERO SALDAÑA\* and Ignacio GRAJAL MARTÍN\*\*

**T**itos, almortas, muelas or guijas are some of the different names with which the grass pea (*Lathyrus sativus*) is known in different parts of Spain. Many years ago, this presently neglected species was an important crop in this country, mainly in some areas of Castilla La Mancha and Castilla y León regions. It was used with different purposes, from animal feed, as complement to cereals, to human consumption, both mashed, in soup, boiled in stew or directly consuming the green immature seeds. In fact, in these areas “correr los titos” (“running for grass peas”, means to slip into the neighbours grass pea fields and eat the immature green seeds while hoping for the owner not to catch you) was one of the most “healthy” and amazing custom for almost all the village children until the sixties decade. Today, in these same regions, grass pea is an almost neglected crop, just sown for human consumption in a few small plots.

Nowadays, probably the most known form of grass pea consumption in Spain is the “gachas” (a sort of purée made with grass pea flour) typical from some parts of Castilla La Mancha. Additionally, grass pea is also consumed nowadays in other parts of Spain but mainly in some religious festivities only. A couple of these examples can be found in La Gamonal, a suburb in the capital of Burgos province (Castilla y León) and in Padilla de Abajo, a small and lovely town from the same province.

In La Gamonal, where grass pea is known as “titos”, its consumption is related with the festivity of Saint Anton, the animals’ guardian, on January 17<sup>th</sup>. It is told that in the past during Saint Anton’s day cattle owners released their animals, leaving them to go freely into the mountains and forests. In the present times there are a lot of places where, in Saint Anton’s day, people attend a mass with their animals and pets, being all blessed by the priest at the church door. In La Gamonal, this celebration is organized by the “Cofradía de Nuestra Señora de Gamonal y San Antonio Abad” (a religious Brotherhood) and the animal blessing is accompanied by other well known and famous tradition: the distribution among visitors of tonnes of “titos” stew, prepared in enormous pots.

The tradition of distributing “titos” in La Gamonal is coming from the middle Ages, when “titos” were distributed as charity among the poor, needy and sick persons. From 1502, when the brotherhood was

born, this type of charity was included as one of the rules from the brotherhood codes. The brotherhood also nourished, in the same way, the pilgrims going to Santiago de Compostela.

Presently, this celebration begins with a mass in the Iglesia Real y Antigua de Gamonal (the Royal Ancient Church of Gamonal), and continues during the whole morning with the animals blessing and, finally, with the cooking, distribution and eating of “titos” (which, of course, are also previously blessed). Every year, the number of people present in this celebration is increasing, and, obviously, the number of kilos of cooked “titos” is increasing as well. As an example, last year, around two tonnes of grass pea were cooked and around 25,000 rations of “titos” stew were distributed among visitors.

For cooking, the main secret in the recipe, according with the members of the brotherhood, is to cook grass pea with a “lots of patience, love and pamper”. But also with some vegetables as onion, red pepper and leek, in a sauté done with garlic and chilli. Cooking takes around two and a half hours, and close to 7000 kilos of firewood and hundreds of litres of water are spent.

Grass pea seed used for this stew is sowed exclusively for this celebration in near by fields. Although this impressive consumption during this day in La Gamonal, grass pea it is not an everyday course neither there neither in the closest regions. It is normally just eaten during Saint Anton’s day.

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Other interesting, but less known, example comes from the small village of Padilla de Abajo in Saint Catalina's day (November 25th). In this place, Saint Catalina is celebrated also with a mass and with the "titos" cooking and distribution.

There is not known when exactly this tradition began, but for sure its origin comes at least from the beginning of last century. In that days, other religious Brotherhood (Cofradía de Santa Catalina de Padilla de Abajo) received a donation of some lands. These lands were used by the members of the brotherhood who, in exchange, sowed "titos" for giving to the community as charity for the poor and the needy people. During Saint Catalina's day "titos" were cooked and distributed freely to the poor.

Grass pea was a traditional crop in this zone until not so long ago. It was sown during spring, at the beginning of March and harvest in summer. Harvesting was done by hand by crews of numerous people, mainly women and children, called "peladoras" (peeler people). For harvesting, "peladoras" advanced in rows pulling out the plants and piling them in heaps called "morenas". Then, the "morenas" were picked up in carts. This harvesting and

grinding was done during the night, finishing the journey when the dew evaporated. If the night was very dry, simply they did not work.

Nowadays, grass pea is an almost neglected crop also in this area, but there are still some farmers who continue supplying the "titos" for the Santa Catalina's festivity. Similar to what happens in La Gamonal, it looks like grasspea is not normally eaten outside this celebration.

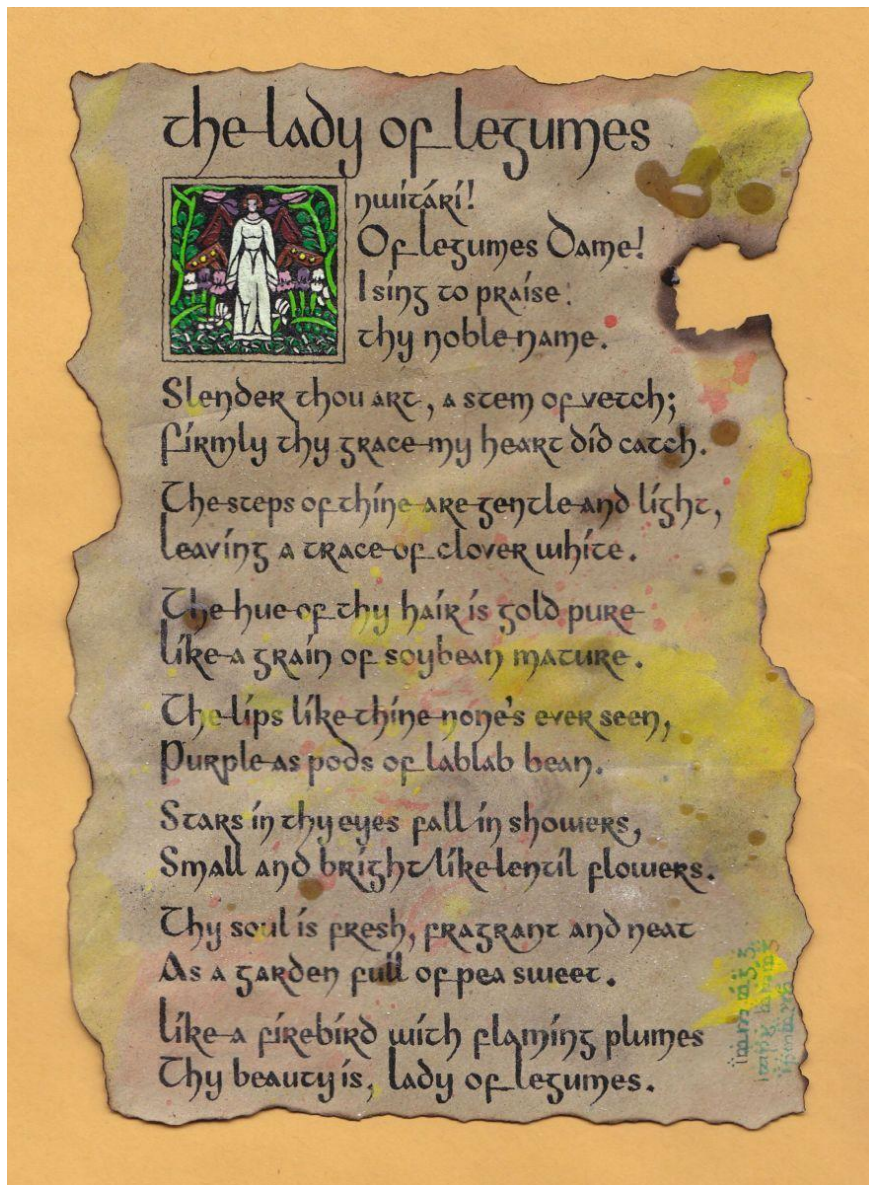
"Titos" are cooked by the members of the brotherhood with a recipe that have in common with the recipe from La Gamonal patience, love and pamper as the main ingredients. In the Padilla recipe other ingredients appear as lamb meat. After the mass, "titos" are eaten in community. The scale of consumption in Padilla de Abajo is quite different from the one in La Gamonal. For instance, last year 15 kg of "titos" were cooked, for around 120 eating people.

Although people in Padilla de Abajo are quite open and friendly, their "titos" tradition is a more family event compared with the so multitudinous festival in La Gamonal. But it is simply because Padilla de Abajo is in a more isolated place and it is less known. The main conclusion is that everybody can go and live this experience.

The cases here presented are just a couple of examples about traditions in which an already neglected grain legume continues playing a main role during festivities celebration. Sometimes, we forget that our traditional and neglected legumes are not just potentially recoverable crops for a sustainable and environmentally friendly cropping system, or species with fascinating genetics for developing conscientious scientific projects but they are also part of the childhood of our elders, part of the cultural wealth in different regions and, more importantly, they can work as a ticket to our own origins; a wonderful excuse, at least in Padilla de Abajo, for returning to the town once a year and have the pleasure of eating some "titos" surrounded by our loved ones. ■

## The Lady of Legumes

by anonymous legume (re)searcher, early 21<sup>st</sup> century



*Invitári! Of Legumes Dame!  
I sing to praise thy noble name.*

*Slender thou art, a stem of vetch;  
Firmly thy grace my heart did catch.*

*The steps of thine are gentle and light,  
Leaving a trace of clover white.*

*The hue of thy hair is gold pure  
Like a grain of soybean mature.*

*The lips like thine none's ever seen,  
Purple as pods of lablab bean.*

*Stars in thy eyes fall in showers,  
Small and bright like lentil flowers.*

*Thy soul is fresh, fragrant and neat  
As a garden full of pea sweet.*

*Like a firebird with flaming plumes  
Thy beauty is, Lady of Legumes.*

## Did Keats invent the name *sweet pea*?

by Tanja VUNJAK-KVAIĆ\*

*Many and many a verse I hope to write,  
Before the daisies, vermeil rimmed and white,  
Hide in deep herbage; and ere yet the bees  
Hum about globes of clover and sweet peas,  
I must be near the middle of my story.*

*Here are sweet peas, on tiptoe for a flight;  
With wings of gentle flush o'er delicate white,  
And taper fingers catching at all things,  
To bind them all about with tiny rings.  
Linger a while upon some bending planks  
That lean against a streamlet's rusby banks,  
And watch intently Nature's gentle doings:  
They will be found softer than ringdove's cooings.  
How silent comes the water round that bend!  
Not the minutes whisper does it send  
To the o'erhanging shallows: blades of grass  
Slowly across the chequered shadow pass.*

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# GRAIN LEGUMES



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*Lathyrus latifolius* (P. Smýkal)



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