

Working together to eliminate cyanide poisoning, konzo, tropical ataxic neuropathy (TAN) and neuropathy



# CCDN News

Cassava Cyanide Diseases & Neurolathyrism Network

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## EDITORIAL

### HAPPY NEW YEAR 2023 🐰

Dear reader,

You might have been wondering if a new issue of the CCDN Newsletter would be published... here it is, ready for the year of the rabbit. It hasn't been easy to collect contributions, hence the delay since last June. We count on your support to be able to share the latest on *Lathyrus spp.* and *Manihot esculenta*. We welcome your reports on your local situation, your recent findings or research ideas and comments. Short reviews about related topics and information on conferences are also very welcome. The main goals of this newsletter are to keep this network connected, so people can contact, discuss and collaborate with others having similar interests; and to prevent the crippling diseases of Neurolathyrism and Konzo by exploring their apparently common causal mechanisms.

Climate change is leading to extreme weather conditions with droughts, floods and food shortages - so the research on these crops is topical today.

There are several interesting reports in this issue. Japanese researchers have a novel idea of using ODAP to screen active natural compounds as potential drug to treat motoneuron disease ALS. This work again draws attention to the utility of sulfur

amino acids, such as N-acetyl cysteine to protect cells against damage from ODAP. We would like to see studies assessing the strategic utility of this cheaply sourced compound to prevent neuropathy as a dietary supplement in emergency situations, as it is tempting to speculate that a few donkey loads of this compound could save a village in the Ethiopian highlands, the panhandle of Afghanistan or in war torn Africa from Neurolathyrism or Konzo in times of nutritional stress. The active group in Portugal led by Carlota Vaz Pato explored the resistance genes in *Lathyrus spp.* (*L. sativus* and *L. cicera*) against some fungal infections. This excellent work on resistances also gives potential benefits for the breeding of other legume crops. In Sichuan province of China, the beneficial effect of *Lathyrus spp.* as green manure by intercropping with rice or fruit trees was successfully demonstrated by the much higher yield. We hope these results will arouse some further interests and studies.

It is good to see work on agronomy and phytopathology validating often stated yield claims, while plant breeding is progressing rapidly with the development of modern low toxin genotypes of grasspea<sup>1</sup>. The key question, whether such cultivars continue to possess the yield capacity under adverse conditions can only be answered by further such studies and farmer acceptance, with Vaz Pato et al's work suggesting that the resistance traits are an important feature that can be maintained in future cultivars, while other work suggests that eliminating ODAP reduces the resilience of grasspea<sup>2</sup>. We also received some news of cassava research from Fiji and would like to see more reports about this crop.

We hope to be able to publish this newsletter twice a year again from 2023 and will have a special announcement in the next edition – keep an eye on your mailbox!

Best wishes for a peaceful and fruitful New Year 2023!

#### Editorial board

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## ARTICLES

### Exploring novel remedies of motoneuron disease using an assay based on the $\beta$ -ODAP pharmacology

Neuropathy is a motoneuron disease caused by the overconsumption of grass pea (*Lathyrus sativus*), a drought-resistant legume with a rich protein content (though relatively deficient in sulfur amino acids), which has been cultivated in Indian sub-continent and Ethiopia. It has been one of the staple foods in some area in the world since recorded history. One serious disadvantage of grass pea as a life-saving crop is its neurotoxic amino acid  $\beta$ -N-oxalyl-L- $\alpha$ , $\beta$ -diaminopropionic acid ( $\beta$ -ODAP, also called BOAA) which is implicated in neuropathy with an irreversible handicap at the legs of patients mainly with scissor gait, pushing their lives to more severity. The content is as much as about 1% of dry weight in the strains with higher content (1).

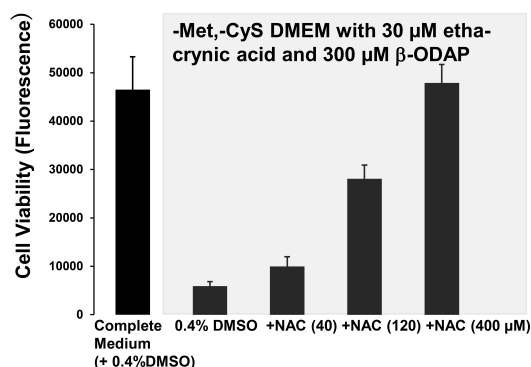
$\beta$ -ODAP was first reported in 1964 by Dr. Rao et al. (2).  $\beta$ -ODAP resembles L-glutamic acid, a major excitatory neurotransmitter in the cerebrospinal tract (the motor conducting system) of humans and other animals. Dr. Nunn's group first clarified that  $\beta$ -ODAP is an agonist of non-NMDA glutamatergic receptors (3,4), and thus the excess pulse consumption causes 'excitotoxicity' and results in the crippling disease neuropathy. Our group added some results which confirmed their data using the cloned glutamate receptors expressed in oocytes.  $\beta$ -ODAP behaved as an AMPA-type glutamatergic agonist especially activating at the  $\text{Ca}^{2+}$ -permeable subtype (5). Dr. Spencer's group clarified that  $\beta$ -ODAP has inhibitory actions on the glutamate transporters, which results in a higher level of L-glutamate at the excitatory synapse and increases the resultant neuronal death due to excitotoxicity (6). We also confirmed the finding using cloned glutamate transporters (7). These reports in the pharmacological mechanism of  $\beta$ -ODAP are important part of neuropathy study, and have relations to other neuronal diseases.

Another important factor that is implicated in the incidence of neuropathy is the monotonous food intake associated with a deficiency of sulfur amino acids, which was evident in famine, war, and detention in concentration camps. The epidemiological and experimental evidence shows that insufficient intake of these amino acids, i.e., not compensate for the amino acid imbalance, predisposes subjects to increased neuropathy (8). The fact indirectly indicates that anti-oxidative system in our body especially glutathione is protective against the development of neuropathy. An earlier *in vivo* report has already shown that guinea pigs, which depend on dietary vitamin C, developed neuropathy at a rate of 26 individuals out of 35 treated animals showing the importance of oxidative condition to accelerate this motoneuron-specific disease and providing vitamin C antagonized the insult (9).

On this line, we showed the neurotoxic action of  $\beta$ -ODAP was significantly increased *in vitro* at the

sulfur amino acid-deficient condition in a motoneuron-like cell NSC-34<sub>d</sub> (a differentiated form). The concentrations of cytoplasmic and mitochondrial glutathione of this cell have dramatically decreased in this condition, the state reminiscent of the monotonous grass pea diet. Interestingly,  $\beta$ -ODAP further decreased mitochondrial glutathione (10). The neurotoxicity of  $\beta$ -ODAP was reversed by exogenous GSHEE, a reduced glutathione analogue, or dithiothreitol, a reducing agent often used *in vitro* (the latter is our unpublished result). These data suggested that motoneuron death by  $\beta$ -ODAP is strongly related to the decrease in mitochondrial glutathione level and cellular redox condition shifted to the highly oxidative state. *N*-acetylcysteine (NAC) was the most effective agent in this system by probably replenishing glutathione (10). On the contrary, some antioxidants such as melatonin and vitamin E (Trolox) were not neuroprotective against  $\beta$ -ODAP in the sulfur amino acid deficiency. Further study is needed to clarify the signal transduction of the mechanism of motoneuron toxicity of  $\beta$ -ODAP.

Using the research outcomes of neurodegeneration as stated above, we recently developed an assay system to discover some novel compounds as Amyotrophic Lateral Sclerosis (ALS) drugs from the fungal kingdom. ALS is a devastating motoneuron disease with pathology of partial resemblance to neurodegeneration, although the former is progressive, and the latter is not. Our assay condition is composed of the medium deprived of L-methionine and L-cystine and added ethacrynic acid to decrease mitochondrial glutathione further to find metabolites to rescue motor neurons. The condition dramatically reduces intracellular glutathione of NSC-34<sub>d</sub> (10). The addition 100 to 300  $\mu$ M  $\beta$ -ODAP in this medium killed the cell by about 60-80% from the original viability with the complete medium containing sulfur amino acids (Fig. 1).



**Fig 1. An assay to measure the protective activity from the excitotoxicity in NSC-34, a motoneuron-like cells.** Differentiated NSC-34 cell (kindly supplied from Prof. N. Cashman (12,13)) was plated in a 96-well plate, and a medium replaced from the growth medium ('Complete Medium') to an excitotoxicity medium (gray), i.e. DMEM deprived of methionine (-Met) and cystine (-CyS) with added 30  $\mu$ M ethacrynic acid and 300  $\mu$ M  $\beta$ -ODAP. *N*-acetylcysteine (NAC), a neuroprotectant, at the concentrations in the parentheses was added. After 16 hr, cells' viability was measured using alamarBlue™ reagent, and the fluorescence was measured at  $\lambda_{e\_x}$  at 544 nm and  $\lambda_{e\_m}$  at 590 nm.

NAC was the most active substance in this assay named ODAP-EA. Some neuroprotective agents, including polyphenols such as phenethyl caffeate and resveratrol, recovered the viability in this system, although the level was lower than NAC (up to 40 % of NAC). A screening has been performed for 400 fungal extracts using this assay, and a novel compound with potency to rescue cell death were found from the culture media of an Ascomycetes fungi. It showed the positive *in vivo* therapeutic action on an ALS model mice SOD1-G93A. Our assay system using  $\beta$ -ODAP under an oxidative stress to screen drug candidates has made a success in finding ALS remedy-candidates or lead compounds (11).

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### Can an underutilized legume offer solutions for crop protection? – The continuum of *Lathyrus sativus* responses to rust disease

Grass pea (*Lathyrus sativus* L.), a cool-season  
legume crop, is considered an important source of  
protein and calories, particularly relevant in drought-  
prone and marginal areas<sup>1,2</sup>. In addition to its high  
tolerance of drought stress, grass pea germplasm is  
also highlighted as an interesting source of  
resistance to several diseases and pests<sup>2</sup>. However,  
the impact of disease epidemics on the yield of  
*Lathyrus* spp. under field conditions has not yet been  
critically studied.

One example of a biotic threat particularly relevant  
worldwide on a wide-range of legume crops is rust  
disease, caused by air-borne biotrophic  
pathogens<sup>3,4</sup>. Previous studies showed that rust in  
grass pea is mainly attributed to *Uromyces pisi*, the  
causal agent of pea rust<sup>5</sup>. A more detailed  
characterization of the genetic architecture of  
resistance to rust in grass pea, which would be of  
interest in efforts toward precision breeding, is still  
lacking. This may be in part attributed to the still  
limited genomic resources available in this species,  
hampering a more efficient exploitation of previously  
identified resistant grass pea accessions as sources  
of favorable alleles in breeding. The few efforts taken  
to revert this unfavorable situation resulted in the  
development of a high-throughput transcriptome  
assembly of contrasting grass pea accessions<sup>6</sup> and

the closely related *Lathyrus cicera* L. accessions<sup>7</sup>  
against *U. pisi* infection.

Although management practices can be  
implemented to control disease outbursts (using  
early-maturing cultivars to promote escape from  
the disease, or adopting cereal-legume intercropping  
systems), disease control still relies heavily on the  
application of chemical fungicides<sup>8</sup>. However, this  
scenario often risks human health, food security, and  
ecosystems sustainability while increasing the  
economic costs of farming practices. For this reason,  
identifying new sources of resistance and developing  
new resistant cultivars, together with disease  
management practices, are widely recognized as the  
most efficient strategies to promote the durability and  
stability of disease control<sup>4</sup>.

Efforts to develop resistant cultivars rely on exploiting  
the natural variation in germplasm collections for the  
identification of new sources of resistance. In order to  
assess the variability in disease responses to  
*Uromyces pisi* and identify interesting sources of  
resistance, we characterize the response of a  
worldwide collection of grass pea accessions to this  
rust disease. A continuous variation in the disease  
response to the mentioned pathogen was observed  
among the grass pea accessions<sup>9</sup>. This variation  
encompassed a wide range of responses from  
partially resistant to highly susceptible accessions.  
The most frequently observed phenotype was a  
compatible interaction, with reduced severity, but not  
associated with a hypersensitive reaction, fitting the  
partial resistance definition<sup>10</sup>.

Histological observations revealed that differences  
among partially resistant and susceptible grass pea  
accessions against *U. pisi* were evident only at later  
stages of infection (48 HAI, Hours After Infection,  
onwards). Rust exclusion mechanisms in partially  
resistant accessions limited pathogens' intercellular  
growth and the number of haustorium per infection  
unit (D.C. Martins, pers. comm.).

Furthermore, the genetic architecture of grass pea  
partial resistance against rust was clarified as an  
initial step for a more efficient precision resistance  
breeding in grass pea. To achieve this, a genome-  
wide association study (GWAS) was conducted,  
combining the collected phenotypic data (disease  
response) with a previously generated genotypic  
dataset<sup>11</sup> from a high-throughput single nucleotide  
polymorphism (SNP) screening of the same  
collection. Seven SNPs were detected as  
significantly associated with disease response of  
grass pea to *U. pisi*<sup>9</sup>. Each SNP-trait association  
explained only a portion of the phenotypic variation  
measured. The described oligogenic nature of the  
disease response and the partially resistant  
phenotype previously described suggest the  
presence of a quantitative nature of resistance to rust  
in grass pea germplasm. Among the genomic regions  
highlighted through GWAS, a number of candidate  
resistance genes with biological relevance in the  
context of plant-pathogen interaction were  
highlighted, through comparative mapping with the  
pea reference genome. Examples are the genes  
encoding for a NB-ARC domain, leucine reach-

repeat (LRR), and the basic leucine zipper (bZIP) TGA transcription factor family<sup>9</sup>. However, detailed functional characterization is still needed to better describe the underlying molecular mechanisms. The results presented here highlight the usefulness of exploiting the natural variation in grass pea germplasm to reveal genomic regions controlling resistance to *U. pisi*. The identified favorable SNP alleles constitute an important molecular tool to assist precision resistance breeding initiatives in grass pea.

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## ***Lathyrus sativus* – an unexplored source of resistance to *Fusarium* wilt disease**

It is known that the yield of most legume species worldwide is limited and unstable due to environmental challenges and susceptibility to pests and diseases<sup>1</sup>. Grass pea (*Lathyrus sativus*) is an underused but resilient grain legume with potential to be a source of important traits to tackle climate change, including resistance to several important fungal diseases<sup>2,3</sup>. The development of resistant cultivars supported by precision breeding techniques, such as molecular markers associated with the genetic factors controlling resistance, allows a more efficient, faster, and affordable selection approach compared with conventional plant breeding programs<sup>1,4</sup>. However, the information available on the genetic basis of disease response in grass pea is still scarce. To clarify the genetic control of grass pea resistance to fungal diseases, studies have been performed in the last decade under the scope of the research projects LEGATO (FP7 EU funding grant agreement 613551) and QuaLaty (Portuguese FCT national funding, PTDC/AGR-TEC/0992/2014).

One of the diseases affecting legume production is *Fusarium* wilt, a vascular disease responsible for important yield losses<sup>5</sup>. In pea and grass pea, it is mainly caused by the soilborne hemibiotrophic pathogen *Fusarium oxysporum* Schltdl. f. sp. *pisii* (*Fop*)<sup>6,7</sup>. Vascular wilt symptoms include stunting, progressive wilting of leaves and stems, and defoliation, ultimately resulting in plant death<sup>8</sup>. As a soilborne pathogen able to survive in the soil for many years in the absence of a host, *Fop* eradication is hard and can only be controlled with complementing disease management measures. Among all, the use of resistant cultivars is widely known as the most efficient, cost-effective, and eco-friendly measure to prevent the extensive losses promoted by this pathogen<sup>1,4,9</sup>.

To identify new resistance sources to *Fop* and the associated resistance mechanisms, we, the Vaz Patto team at the PLANTX Lab, ITQB NOVA, Portugal, characterized the response of a worldwide collection of grass pea accessions (with diverse seed colors and sizes) to one of the most frequent *Fop* isolates worldwide (*Fop* race 2), under controlled conditions<sup>7</sup>. A quantitative resistance response to *Fop* race 2, ranging from highly to partially resistant and susceptible accessions, was detected, with resistance being the most frequent phenotype. This agreed with the observations made on pea with the same isolate<sup>10,11</sup>. We found that Ethiopian grass pea accessions - mainly characterized by small dark seeds - were in general more susceptible than accessions from America, Europe, Northern Africa, or Asia, which showed instead a considerable range of responses, including resistance and susceptibility<sup>7</sup>.

We also studied fungal colonization dynamics in grass pea through confocal laser scanning microscopy (CLSM), using *Fop* race 2 expressing green fluorescent protein (GFP)<sup>7</sup>. The utilization of *Fop*-GFP transformants allowed a detailed visualization of fungal progression, enabling

the clarification of the resistance mechanisms in highly and partially resistant accessions. For instance, in highly resistant accessions, we observed the arrest of pathogen progression occurring early, with the fungus being unable to penetrate the root. On the other hand, in partially resistant accessions, the pathogen progression was stopped by a mechanism active only within the root, with no detection of fungal mycelium from the hypocotyl onwards. Moreover, GFP fluorescence also exposes the plant responses to fungal infection in the partially resistant accessions such as an accumulation of filling substances in the hypocotyl vascular tissue and vessel wall thickening caused by lignin deposition. In contrast, with susceptible accessions, 10 days after inoculation (dai), the fungus had already reached the upper part of the plant, and the entire plant was colonized by 21 dai<sup>7</sup>. The highly and partially resistant accessions identified can be exploited in grass pea breeding for *Fusarium* wilt resistance, and due to the phylogenetic relatedness to pea, potentially contribute also to pea improvement.

Moreover, to unveil the genetic basis of resistance, a genome-wide association study (GWAS) was performed in the previously phenotyped grass pea collection<sup>12</sup>. Seventeen single-nucleotide polymorphisms (SNPs) were identified as significantly associated with grass pea response to *Fop* race 2, suggesting an oligogenic control of resistance. In particular, one of these detected trait-associated SNPs (SNP5205), located on the grass pea linkage group IV might share the same chromosomal location with Fnw4.1, a pea *Fop* race 2 resistance gene, located on pea chromosome 4 since grass pea linkage group IV is considered highly syntenic<sup>13</sup> with pea chromosome 4. The favorable SNP alleles and putative candidate genes identified through GWAS constitute important tools to assist precision breeding for *Fusarium* wilt resistance in grass pea.

Asian and Ethiopian accessions, characterized by darker and smaller seeds, presented predominantly a lower frequency of the identified beneficial SNP alleles. Thus, it might be possible to increase the resistance levels of Ethiopian and Asian accessions by the introgression of the identified resistant alleles in these grass pea accessions. European and especially Northern African accessions revealed to be promising sources of resistance alleles, with high frequencies of the favorable allele in almost all the associated SNPs.

All the previously described grass pea advances might also be valuable for pea breeding if crossability barriers are overcome.

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## Advances on *Lathyrus cicera* resistance to rust and powdery mildew diseases

*Lathyrus cicera* L. (red chickling pea) together with *L. sativus* (grass pea) are the *Lathyrus* species most cultivated for food and feed<sup>1,2</sup>. *L. cicera* has resistance to biotic stresses, such as rust and powdery mildew diseases<sup>3-6</sup>. This makes *L. cicera* an attractive choice for sustainable feed and food production, mainly in more marginal environments<sup>4</sup>. Because of pathogen sharing, *L. cicera* could also act as a promising alternative source of disease or pest resistance for related species, such as grass pea and pea<sup>3,4,7</sup>.

Sources of resistance to rust (*Uromyces pisi*) and powdery mildews (*Erysiphe pisi* and *E. trifolii*) have been identified in *L. cicera*. In an Iberian collection of *L. cicera* accessions, microscopic and macroscopic variable levels of resistance were identified against *U. pisi* and *E. pisi*<sup>5,6</sup>. A segregating recombinant inbred line (RIL) population ('BGE023542' × 'BGE008277') was developed from the cross of the most contrasting *L. cicera* accessions against both pathogens. This RIL population was used for the development and refinement of the first *L. cicera* genetic linkage map<sup>8,9</sup>. This genetic linkage map contains transcriptome based Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSR) markers, retrieved from the RIL parental lines leaf transcriptome response to *U. pisi* infection<sup>8</sup>. In that transcriptomic study, upregulated genes in response to rust infection were identified and predicted to be involved in hormone metabolism, cell wall degradation, secondary metabolism, radical oxygen species (ROS) production, signalling and regulation of transcription of defence<sup>8</sup>. More recently, genotype-by-sequencing based markers were added to the *L. cicera* RIL ('BGE023542' × 'BGE008277') genotypic dataset allowing the construction of a high-density genetic linkage map: 1468 polymorphic loci mapped on seven major and two minor linkage groups, covering a total of 712.4 cM<sup>9</sup>.

Causative variants controlling partial resistance to *U. pisi* were recently identified in *L. cicera*, by combining phenotypic- and expression-quantitative trait loci analysis (pQTLs and eQTLs, respectively)<sup>10</sup>. *L. cicera* RILs' *U. pisi* disease severity (DS) was recorded in three independent screenings at seedling stage (growth chamber) and in one season of exploratory screening at adult plant stage (semi-controlled field conditions). Different pQTLs were identified under growth chamber and semi-controlled field conditions, indicating a distinct genetic basis depending on the plant developmental stage and/or the environment. One cis-eQTL and one trans-eQTL were also identified controlling the expression variation of one gene related to rust resistance – a member of glycosyl hydrolase family 17. Integrating phenotyping, gene expression and linkage mapping allowed prioritising four candidate genes relevant for disease-resistance precision breeding involved in adaptation to biotic stress, cellular, and organelle homeostasis, and proteins directly involved in plant defence<sup>9,10</sup>.

Regarding to powdery mildew, three pQTLs for partial resistance against *E. pisi* were mapped in the *L. cicera* ('BGE023542' × 'BGE008277') high-density genetic linkage map, suggesting that partial resistance to this pathogen has an oligogenic genetic basis. On the other hand, a single pQTL was detected for the *L. cicera* response against *E. trifolii* infection<sup>9</sup>. Different candidate genes for partial resistance to each powdery mildew pathogen were identified, revealing different genetic bases involved in the response to *E. pisi* and *E. trifolii*. Partial resistance to *E. pisi* may involve the secretion of antifungal compounds and signalling integration during infection, whereas specific pathogen recognition may have a central role in response against *E. trifolii*<sup>9</sup>. Besides these candidate genes for response against powdery mildews, the *Mildew Locus O 1* (*MLO1*) gene was isolated and characterized in *L. cicera* (*LciMLO1*). Phylogenetic analyses revealed that *LciMLO1* belongs to Clade V, where all dicot MLO proteins associated with powdery mildew response are included, suggesting a role of *LciMLO1* in powdery mildew response<sup>11</sup>.

Comparative mapping and synteny analyses of *L. cicera* genetic linkage maps and QTLs mentioned above have been performed with *L. sativus* genetic linkage map and *Pisum sativum*, *Lens culinaris* and *Medicago truncatula* reference genomes<sup>8,10,11</sup>. These studies revealed: 1) ortholog genes underlying the QTL regions, which are potentially involved in resistance against rust and powdery mildew diseases<sup>9,10</sup>; 2) important aspects of the conservation of the *MLO1* locus position<sup>11</sup> and 3) overall chromosomal rearrangements occurring during legume evolution<sup>9,11</sup>.

All the recent advances here reported have extended the previously available genetic and genomic tools in *Lathyrus* species, providing clues about different rust and powdery mildews resistance mechanisms useful for future resistance breeding of *L. cicera* and related species.

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## Application of *Lathyrus* spp. as green manure in Sichuan province of China

*Lathyrus* spp., annual legume crops with a strong nitrogen fixation capacity reach up to 40~210 kg/hm<sup>2</sup>, are also useful as green manure crops. In Sichuan province of China, an important contribution to the agriculture system was made when *L. sativus* and *L. cicera* were used as green manure, especially in grain production. It was reported that the cumulative release of C, N and P is accelerated, and the content of organic carbon and inorganic nitrogen in soil can be effectively increased by turning over *L. cicera*<sup>[1,2]</sup>. Here, we summarize briefly the developmental history of production and application status of *Lathyrus* spp. in Sichuan.

### 1. The introduction and breeding of *L. cicera* in Sichuan

In 1977, Yinghui Li (the Regional Agricultural Science Institute of Nanchong City) introduced *L. cicera* firstly to Sichuan from the Nanjing Botanical Garden. She compared adaptability between different varieties and investigated the cultivation techniques, which greatly promoted the production and utilization of *L. cicera* in the hilly areas of Sichuan

province. However, the large-scale transfer of rural labor force and excessive application of chemical fertilizer resulted in the rapid shrinking of the planting area, lack of germplasm resources, and the serious degradation and mixing of *Lathyrus* varieties. Until 2008, special scientific projects of green manure were restarted in China, and the research group of Nanchong Academy of Agricultural Sciences recollected several *Lathyrus* varieties. Through identification and evaluation, regular and radiation breeding approaches, they found a novel variety of *L. cicera* that was suitable as a green manure with a higher thousand grain weight (62-70g), the content of nitrogen (0.53%), phosphorus (0.22%), potassium (0.42%), crude protein (22.7%), crude fat (3.4%), the biological yield (17.2%) compared with wild type<sup>[3]</sup>.

### 2. Technology for high yield of *Lathyrus*

After years of research, the team led by Professor Wen-bin Han, developed technologies for high yield of *Lathyrus*<sup>[4]</sup>. One focusses on seed yield of up to 1500~2000 kg/hm<sup>2</sup>. It includes timely and early sowing, appropriate sparse sowing, increased phosphorus to promote factors related to high yield, etc. The other focus is on fresh biomass production up to 30000-40000 kg/hm<sup>2</sup>. It includes a combination of no-tillage broadcast sowing with shallow rotation early sowing, and increased application of nitrogen and phosphate fertilizer appropriate to improve biological yield.

### 3. Integration of *Lathyrus* planting technologies

Based on the climatic characteristics and tillage system of Sichuan, the team carried out researches on the cultivation technologies of *Lathyrus* in Nanchong, Guangan, Suining and Yibin<sup>[5-7]</sup> by integrating the crop rotation model of *Lathyrus* - paddy field, the intercropping model of *Lathyrus*-orchard and the crop technology model of *Lathyrus* in dry land. The results suggested that: (1) paddy rotation of *Lathyrus* can effectively promote the growth and nutrient absorption and accumulation of rice. Under the condition of replacing 30-40% nitrogen fertilizer, rice still shows stable yield, quality is improved and soil fertility is effectively improved<sup>[6,7]</sup>. (2) Under intercropping conditions of *Lathyrus*-orchard, it has an obvious inhibition effect on nearly 20 species of weeds in more than 10 families, such as *Gramineae*, *Asteraceae*, *Scrophulariaceae*, *Ranunculaceae* and *Caryophyllaceae*. Soil organic matter significantly increased, soluble solids increased by 1.9%, Vc content increased by 1.1mg/100g, titratable acid decreased by 0.13%<sup>[8,9]</sup>. (3) Under the broad-band (2 m) intercropping pattern of "wheat-bean // reserved belt and corn", the yield of wheat increased by 9.2% and maize increased by 6.3% in the same season, and the comprehensive efficiency by 9.1%, and the annual benefit by 1398 RMB/hm<sup>2</sup><sup>[10]</sup>.

### 4. Promotion and application of *Lathyrus*

#### 4.1 Application in orchard

Fruit is one of the "10+3" dominant industries of modern agriculture in Sichuan province.



Intercropping green manure in orchards is an important measure of modern orchard management and a soil management tool to realize sustainable development of the fruit industry. Using *L. sativus* and *L. cicera* as green manure has been promoted and applied in large areas of peach and plum orchards in Nanchong, Guang'an, Suining, Meishan and other citrus producing areas, with a cumulative promotion area of more than 200,000 hectares.



Figure 1. Intercropping with *L. cicera* to improve fruit quality of orange orchards in Jialing district, Sichuan province, China (Credit: Photo Prof. Wenbin Han, 2019)

#### 4.2 Application in rice field

As a major grain province, Sichuan province has a large paddy field area, most of which are idle winter fields, resulting in a great waste of land resources. Planting and turning over *Lathyrus* as a winter catch crop in paddy fields can effectively improve soil fertility, reduce the amount of chemical fertilizer application, improve rice yield and quality to a certain extent. This technology has become one of the important measures for high standard farmland construction of paddy fallow land. *Lathyrus* have been popularly used in rice field in Northeast, central and South Sichuan, with a total area of 15,000 hectares.

#### 4.3 Application in dry land

*Lathyrus* plays an important role in improving soil physical and chemical properties, improving soil fertility, stabilizing and increasing yields of subsequent crops in dry farming. Intercropping with grain and oil crops in hilly areas of Sichuan basin and rotation with tobacco crops in mountainous areas around basin, with a total area of 7000 hectares.

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#### Growth, physiology and nutritional value of cassava in Fiji for adaptation solutions to changes in climate

Cassava (*Manihot esculenta* Crantz) is becoming increasingly popular in the Pacific Islands due to its ease of cultivation and ability to grow in poor soils and harsh conditions. However, it is not clear whether the varieties used in the Pacific Islands produce the high levels of toxic cyanide found in other countries or whether cultivation methods and changes in climate are affecting nutritional value. This study aimed to compare the growth and chemoecology of a bitter (Nadelei) and a sweet variety (Merelesita) of cassava planted and harvested in different seasons. The varieties were also sequenced genetically as part of a global consortium project. The field trial was conducted at the Koronivia Research Station, near Suva, Fiji over 18 months. The main trial was a split plot design with the two cassava varieties replicated four times. Vegetative phenology measurements were made weekly on a subset of plants from each plot for 72 weeks. A total of 200 plants from each variety were harvested destructively at 10 intervals over this period. To explore seasonal variation two additional

trials were conducted with plots established at the onset of the dry and wet season, six and twelve months into the main trial, and harvested in the same way. There were five main findings: (1) time of planting and harvesting were important in terms of yield and nutritional value; (2) Nadelei contained more cyanide in the leaves than the Merelesita variety but this difference was not observed in the tubers; (3) Nadelei yielded more than Merelesita but only when planted in the wet season; (4) cyanide concentration was higher in the leaves harvested during the dry season; and (5) genetic sequencing showed that the two varieties were distinct but the branched and unbranched forms of Nadelei were genetically the same.



Above: Cassava growing in the field trial at Koronivia, near Suva, Fiji.

Below: All parts of the cassava plants (stems, roots and leaves) from the field trial were harvested for biomass calculations.



Furthermore, an integral part of the study was also to use data generated to build the cassava Agricultural Production Systems Simulator (APSIM) model by collaborators at CSIRO, Australia.

#### Dr Poasa Nauluvula

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Street stall in Suva, Fiji with sweet potatoes, cassava, taro and coconuts. Cassava is an increasingly important staple food in the region. Photo: R. Gleadow

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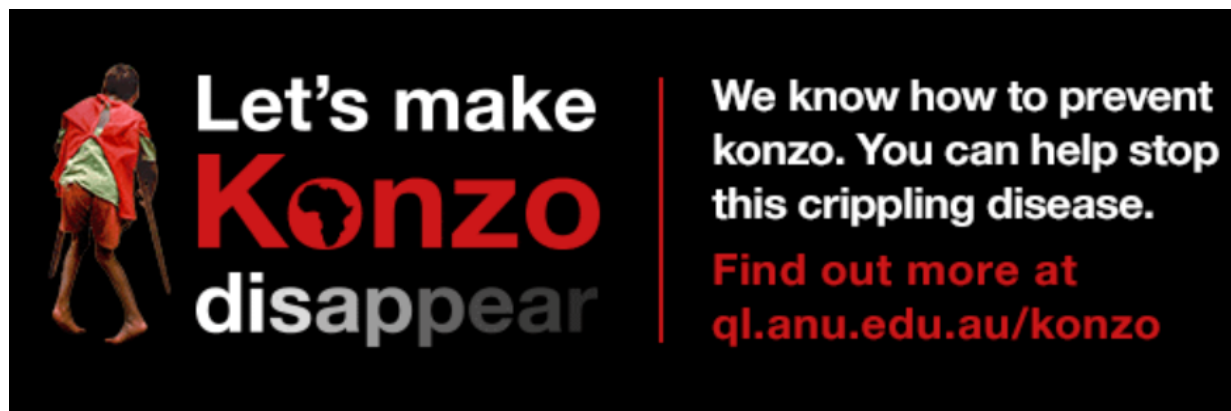
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