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

CCDN News

Cassava Cyanide Diseases & Neurolathyrism Network

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EDITORIAL

Is grass pea (*Lathyrus sativus* L.) taking the stage in a world with changing climate?

The potential toxicity of grass pea consumption has been a matter of controversy since several decades. Poor farmers surviving on grass pea after a drought do not believe that this life-saving food can be toxic, but scientist exploring the toxic (or better: neuro-excitatory) properties can hardly recognise the beneficial properties of moderate consumption for human health. Similar controversy occurred at country level: Spain had many cases of neurolathyrism during the civil war and a ban on human consumption of grass pea was imposed in 1967, while the adjacent country Portugal that had no comparable civil war and also no cases of neurolathyrism has an annual *Lathyrus* festival in a grass pea growing locality: Alfalazere, calling itself the capital of 'Chicharo' or grass pea in Portuguese. The major grass pea growing country India has imposed a ban on the storage and sale of grass pea in 1961, but cases of neurolathyrism continued to occur among land labourers who were paid in kind with grass pea seed when they had little else to eat. This changed when rice became cheaper than grass pea and during the last several decades no new cases of neurolathyrism were reported. Also in Bangladesh, rice is cheaper than grass pea, and no new cases of neurolathyrism were recently reported. Although neurolathyrism cases were not reported from Nepal, a ban on the sale of grass pea was imposed in 1991 after the epidemic in Bangladesh and India in the 1980s. Also in Ethiopia, that was struck by an epidemic of neurolathyrism in 1998 after a drought-triggered famine, no recent cases are reported while the cultivation of grass pea is increasing. One of the conditions for high incidence of neurolathyrism, that grass pea seed is the cheapest food available, seems to have disappeared with the economic development of grass pea growing areas.
The Spanish authorities have commissioned a

comprehensive study on the potential toxicity of grass pea as used in a traditional dish and came to the conclusion that moderate consumption is not harmful when mixed with cereals or other foodstuffs rich in sulphur amino acids[1] (see the excerpt by de Oliveira in this issue). In other European countries where grass pea is cultivated, Italy, Greece and Poland, there is no ban on the sale of grass pea and a Greek company is distributor of grass pea under the name "Fava Feneou" to a number of retail shops in Europe. The seeds can be ordered 'on line'[2]. In India, similar studies as in Spain have been made by the Indian Council of Medical Research who came to propose the government that the 55 year old ban be lifted. This has been extensively discussed in the Indian media, but as of now the political decision was not made. A subtle change in focus, away from the toxic aspect, is seen in recent papers where ODAP is not mentioned as a toxin but as a 'neuroexcitatory amino acid'.[3,4] The increasing awareness of the global climate change and the resilience of grass pea in a changing climate make this crop more attractive for drought-prone regions and less fertile soils. The renewed interest in grass pea is also reflected in the financing of grass pea related projects and the use of new sophisticated technology including genome sequencing. A national Portuguese project is being implemented in ITQB (Instituto de Tecnologia Quimica e Biologica) in Lisbon and a major international project is being implemented by JIC (John Innes Centre, Norwich, U.K.) and ICARDA (International Centre for Agricultural Research in the Dry Areas); see Emmrich et al. in this issue.

As neurolathyrism and konzo are considered socio-economic diseases, the economic development of the country should have an impact on the incidence of both neurolathyrism and konzo. A socio-economic study may shed some light on this question. It is remarkable that while neurolathyrism is virtually a disease of the past, cases of konzo keep appearing and in D.R. Congo the media even mention a new epidemic of konzo in the Kwango province.[5]

Apparently the 'wetting method', to reduce cyanogen level in cassava preparations as developed by the late Howard Bradbury and associates, needs to be further propagated in rural areas of tropical Africa where people depend on cassava for their livelihood. The obvious need for better controlling the content of cyanogenic glycosides in food preparations based on cassava roots has prompted the development of a simple cyanide test as described by Zelder in this issue. The test uses the linamarin naturally present in fresh cassava to liberate free cyanide. The test is commercialised by a company Cyanoguard®. A separate test for thiocyanate is also available. We do hope this development may contribute to the prevention of konzo.

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ARTICLES

Grass pea (*Lathyrus sativus* L.) improvement in Nepal

Introduction

Grain legumes play an important role for food and nutritional security in the country. Grass pea (*Lathyrus sativus* L.) also known as Kheshari, Latara or Matara in local languages is adapted to both drought and excess soil moisture conditions.[1] In fact it is an important climate change resilient crop which plays a significant role and has great impacts on the livelihood improvement, food security and economic development in the grass pea growing countries Nepal, India, Bangladesh, Pakistan and Ethiopia. It has a very hardy and penetrating root system and therefore can be grown on a wide range of soil types, including very poor soil and heavy clays. This hardiness, together with its ability to fix atmospheric nitrogen, makes the crop one that seems designed to grow under adverse conditions.[2] Compared with other legumes, grass pea is resistant to many pests including storage insects.[3] Grass pea is endowed with many properties that combine to make it an attractive food crop in drought-stricken, rain-fed areas where soil quality is poor and extreme environmental conditions prevail.[3] Despite its tolerance to drought it is not affected by excessive rainfall and can be grown on land subject to flooding.[2,4;5]

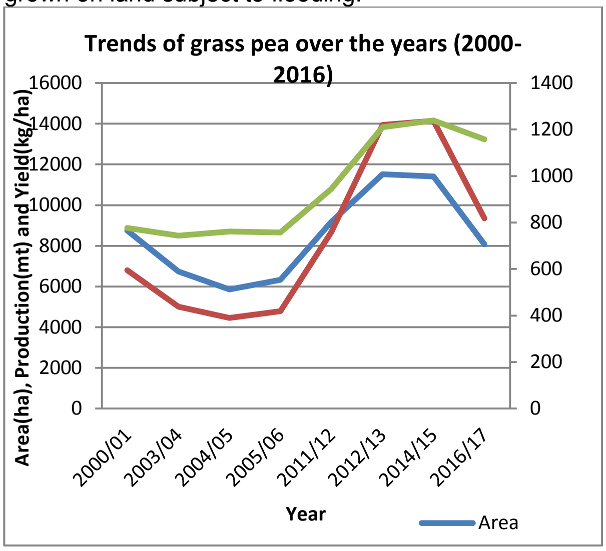
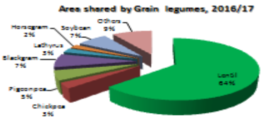
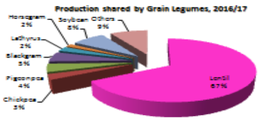


Fig .1 Area, production and yield trends of grass pea over the years

Area and Production of Grain Legumes in Nepal, 2016





Crops	Area (ha)	Production (mt)	Productivity (kg/ha)
Lentil	**206969**	**254308**	**1229**
Chickpea	9933	10969	1104
Pigeonpea	17091	16497	965
Blackgram	23429	19497	832
Lathyrus	8075	9354	1158
Horsegram	6351	5690	896
Soybean	23563	29061	1233
Others	30644	32817	1071
Total	326055	378193	1160

Source: Statistical Information of Nepalese Agriculture, 2016

Grass pea scenario

Currently the area of grass pea is 8075 ha only and the production is 9354 mt, representing 3% resp. 2% of total legumes in the country.[6] The major grass pea growing districts in Nepal were Kapilbastu, Bardiya, Sarlahi, Parsa, Morang, Rautahat, Rupandehi, Bara, Banke and Dang. However, the area has substantially reduced by -8.28% as compared to the year 2000/01but the production trend increased by 27.34% and the yield by 32.92% respectively over the same period (Fig 1). One of the reason for the decreasing area of grass pea was because the dietary intake of large quantities over a longer period is believed to cause the neurological disorder neurolathyrism paralysis of the lower limbs) due to the presence of a neurotoxin, β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP, also known as BOAA) and reported to be present in all parts of the plant.[7] During the 1980s in Bangladesh had a great drought-triggered famine and many farm families depended on grass pea for food security and this resulted in a well-documented epidemic of neurolathyrism. This event was widely disseminated in the markets of South Asian countries. Consequently, the government of Nepal of that time had banned its cultivation and consumption in Nepal. ODAP was first found in *L. sativus* by Bell in 1962 when he found ninhydrin reacting compounds in many *Lathyrus* species.[8] The biosynthesis of β-ODAP from its precursor β-(isoxazolin-5-on-2-yl)-alanine (BIA) was demonstrated in young pods by Kuo et al.[9] In general, ODAP content in our local varieties ranges from 0.6-0.8 %. Occasional consumption is not harmful to humans and does not appear to have any negative effect to sheep. A breeding and selection program using recurrent mutagenic treatment was started to produce high-yielding, low-toxin lines of Lathyrus.[10]

Future Prospects and Research Strategy

Even though grass pea cultivation has been banned in Nepal, the farmers of southern parts of Nepal are still growing plenty of local landraces for livestock forages, green vegetables and pulse purposes. In general, young leaves are consumed as a delicious green vegetable. Farmers are also dried it for using as off-season vegetable.[11] Grass pea fodder is a valuable livestock feed. Basically in lean season, this crop might be an alternative source of fodder and grasses to small ruminants. Fresh biomass yields of 5-6 t/ ha in addition to 1.8 t/ha of seed yields have been reported for local varieties.[12] Green pods and seeds are eaten as snacks directly or whole pods are cooked and eaten as vegetable. Grass pea flour is used to prepare pancake (Pakoda) which is widely used as the breakfast in rural areas. Being of important economic value, Grain Legumes Research, Khajura, NARC had sent the local land races to Centre for Legumes in Mediterranean Agriculture (CLIMA), Australia with the aim to develop low ODAP lines by using recurrent mutagenic treatment. Right now the available grass pea genetic materials have low ODAP lines (< 0.2%) which are considered safe for human consumption as a pulse or other products. Grain Legumes Research Program (GLRP), Khajura had received some low ODAP grasspea lines under the phase-out ACIAR project CS1/1964 "Lentil and lathyrus in the cropping system of Nepal with the aim to evaluate an adaptive trial in Nepalese condition". Because of its adaptability both in waterlogged and drought conditions, there are opportunities for area expansion if low ODAP lines are made available. The Nepal Government has given priority in "National Flagship", which focuses on poverty alleviation by increasing income generating opportunities. In these circumstances, the adaptability of lathyrus even in mid hills (1000 - 1500 m) has golden opportunities for its cultivation and use as food, feed and fodder.[13] Now, GLRP is also receiving low ODAP grasspea lines through shuttle breeding program with ICARDA, Syria in the form of international screening nurseries. Many of them were found suitable and adaptive for the mid-western terai. Some of them have fodder value and some are dual purposes. Some of the lines like CLIMA Pink, Ratanetc are selected as low ODAP grain concentration which is completely safe for human consumption. These lines are being promoted in farmer's field of Kapilbastu, Banke, Sarlahi and Kailali districts through an IFAD-ICARDA Project "Enhancing food and nutritional security and improved livelihoods through intensification of rice-fallow system with pulse crop in South Asia (Bangladesh, India and Nepal)". Since its hardy nature, ability to adapt in the climate change context, pre-breeding activities, multi-environment evaluation and participatory trials are being tested across the environment and being selected under control condition to produce the seed for future use. The main objectives of the breeding improvement are to evaluate, select and conserve the high yielding, disease stress tolerance and most adaptive lines in different domains of Nepal.

Germplasm source status

A total of 46 different low-ODAP lines including few local landraces are being evaluated at the station. Every year GLRP is receiving 24-46 exotic germplasm for adaptive research and promote in the yield advance trials. Besides about 441 local landraces are safely conserved at the National Agriculture Genetic Resources Centre (Gene Bank), Khumaltar, Nepal for future use.

Breeding improvement activities

1. Collection, evaluation and characterization of grass pea accessions
2. International Grass pea Screening Nurseries
3. International Grass pea Yield Trial (IGYT) –Low ODAP
4. Observation nursery
5. Coordinated varietal trial
6. Participatory trial

Research strategy

- Collect local materials and obtain segregating materials from International Agricultural Research Centers (IARCs) for evaluation, selection and recommendation of grass pea varieties.
- Select the best adaptive grass pea lines through International Elite Nurseries (Segregating materials and stress tolerance accessions)
- Initiate breeding program on grass pea for Stress tolerance (diseases, pests, drought and cold)
- Categorized breeding plan to develop short, medium, late maturity, drought, cold, tolerance grass pea varieties
- Develop low-toxin (ODAP) grass pea for improved nutrition and health

- Plan for adequate quality source seed production within the NARC stations and through farm cooperatives/seed growers and supply source seed
- Strengthen seed delivery systems (Availability of Breeder seed, Foundation seed, quality improved seed,
- Improved methodologies and tools for genetic improvement (pre-breeding, advanced biometry, crop information system, etc.).

Research achievements

- Low ODAP (<0.04%) containing lines CLIMA Pink, Ratan, 19B, 20A and Bari 2 were promising in terms of grain yield.
- Grass pea genotypes Sel-1959, Sel-290, Sel-2119, Sel-2177, Sel-387, Sel-299, Sel-449 and Sel-1942 showed good forage value.
- In observation nursery, 2007 : Genotypes showed variables among the tested genotypes in days to maturity level ranged 69-97, days to maturity level ranged 142-150 and the grain yield ranged from 400-1600 kg/ha. The genotypes 38.2(1600 kg/ha), 36.3(1525 kg/ha), 26.3(1500 kg/ha) were the best yielder than the check Siraha local (1325 kg/ha) and standard check Sarlahi local was found early in maturity; whereas the exotic lines was late maturity.
- In observation nursery 2008-2009 : Mean performances of yield and yield attributes of low ODAP Grass pea over the years (2008-2009) indicated that varied in days to maturity (138-147), plant height(37-82 cm), pods per plant(12-28) and grain yield(1650-2920 kg/ha). Grass pea genotype 38.2 (2920 kg/ha) produced the highest yield followed by 45.3(2870 kg/ha), 2.3(2820 kg/ha) and LHH-0066(2685 kg/ha) than the local check Siraha local (2450 kg/ha).
- In Regional Elite Varietal Trial: Grass pea Low ODAP Lines LHH-0012(2715 kg/ha), 26.2(2501 kg/ha), DL7-1(2483 kg/ha) and 38.1(2415 kg/ha) were the better performer over the years (2008-2009) in REVT.
- In Relay Lathyrus -2010 : Genotypes Prateek, Pink, white and siraha local were the best performer (yield ranges 1050-1600 kg/ha)
- In International Grass pea Yield Trial (IGYT) –Low ODAP, 2015 : Six grass pea cultivars namely IF-1310, IF-1332, IF-1344, IF-1347 IF-1393 and IF-1348 were selected out of the 25 lines based on the vigour and adapted to the climate for promoting the yield advance trial in future
- In International Grass pea Yield Trial (Low-ODAP), 2016: In international grass pea yield trial (Low-ODAP) at GLRP Khajura, accession #170 (2850 kg ha^{-1}) produced the highest yield followed by GP-62 (2813 kg ha^{-1}) than the local check (2763 kg ha^{-1}).
- In Coordinated varietal Trial, 2017: Genotypes GP67, GP97, 190 and local check were found better performer in terms of grain yield.

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The Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) clears Spanish gachas. The risk of neurolathyrism is marginal.

(Excerpt from the Spanish official document[1])
Grass pea (*Lathyrus sativus* L.) is used in different regions of the world both for food and feed. The dried seeds are consumed either as such or in the form of flour. It serves a variety of purposes including food, feed and fodder, owing in part to its nutritive qualities. Grass pea is considered to be a promising crop for adaptation under climate instability due to global warming. The legume is tolerance to drought, water-logging and salinity, and highly resistant to insect-pests and diseases. Despite its popularity the development of grass pea into an important food legume has been hindered by the presence of the neurotoxic amino acid - beta-N-oxalyl-L-alpha,beta-diaminopropionic acid in all parts of the plant. Widely known as β-ODAP, the neurotoxin is the main causative factor of neurolathyrism, a neurological disorder that causes a gradual paralysis of lower limbs in adults who

consume large quantities of it for several consecutive months.

Human consumption of grass pea is prohibited in Spain since 1967, despite the rooted use of its flour for the preparation of some traditional culinary dishes, such as *gachas* (porridge). *Gachas* is a traditional dish cooked frequently in Castilla-La Mancha. In its preparation, the flour of grass pea is mixed with water and oil and heated until obtaining a dough to which other ingredients can be added. In this dish, since it is the grass pea flour that is used, there is no possibility to previously wash the seeds or to eliminate the washing water, factors that facilitate the reduction of the content in β-ODAP.
In a 2009 report, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) highlighted the importance of informing consumers on the maximum rations of grass pea flour meal and that an excessive consumption could provoke neurolathyrism. It was also advised to carry out quantitative studies to recommend the appropriate thresholds.

Notwithstanding the prohibition for food use, grass pea flour is available in the Spanish market for use as animal feed. In 2012 the National Food Center (NCA) carried out the analysis of 32 different lots of flours and 2 lots of seeds in processing establishments and retail stores in Castilla-La Mancha. The β-ODAP content ranged between 0.255 and 1.039%, reaching this percentage in only one of the samples.

Recently, the Scientific Committee of AESAN reviewed the issue, considering new scientific information available since the 2009 report. The document was approved by the Food Security and Nutrition Section of the Scientific Committee in its plenary session of May 23, 2018. The Scientific Committee of the AECOSAN has concluded, in accordance with the up to date information, that the health risk of the sporadic consumption of *gachas* by the general population may be considered negligible (excluding people with metabolic difficulties to detoxify the β-ODAP). It is considered sporadic intake when the daily portion does not exceed the limit of 25 g of grass pea flour/day with a content of β-ODAP not higher than 1%, and in the context of a varied diet that includes sulfur amino acids present in foods of animal and plant origins such as meat, fish, eggs and dairy products, whole grains and nuts.

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Corrin-based Chemosensors for Rapid Detection and Quantification of Endogenous Cyanide in Cassava

Summary: This article summarizes the author's group activities in the development of corrin-based chemosensors for detecting cyanide in cassava (*Manihot esculenta Crantz*). The new reagent represents a powerful, safe and reliable alternative to traditional methods such as the picrate reagent. A test-kit for the detection of cyanide in foodstuff is now available to anyone and quantification with a smartphone instead of a bulky and expensive spectrophotometer is principally possible.

Cassava (*Manihot esculenta Crantz*) is a staple food for many people in Africa and South America. It contains cyanogenic glycosides that enzymatically convert to toxic cyanide (CN^-) after cell damage. Although this conversion protects the crop against undesired animal predation, it represents a potential threat to humans upon consumption of cassava products. Food processing is therefore of utmost importance to avoid any acute and chronic cyanide intoxications such as *konzo*. For ensuring the quality of these food-processing procedures, detection and quantification of cyanide is strongly recommended. A convenient straightforward analytical method would additionally allow controlling the quality and safety of cassava products on rural markets or supermarkets. Today, the most established procedure for detecting cyanide in cassava is probably based on the semi-quantitative alkaline picrate method. In this easy-to-use test, yellow coloured picrate is converted by cyanide into reddish-brown isopurpuric acid. Howard Bradbury pioneered this area and developed a kit that was available to anyone.[1] Despite advantages of the picrate method and its wide dissemination, the test exhibits certain disadvantages. The reaction is very slow (~16 hours), the chemical needs special handling and storage, and the response is sometimes imprecise.

My group introduced corrin-based chemosensors for the rapid, sensitive and selective detection of cyanide.[2] These vitamin-B_{12}-derivatives bind cyanide with high affinity to the cobalt (Co) center of the complex as schematically depicted in Figure 1. This reaction is accompanied by a change of colour from yellow to violet, proceeds in less than 2 seconds and is compatible with biological matrices.

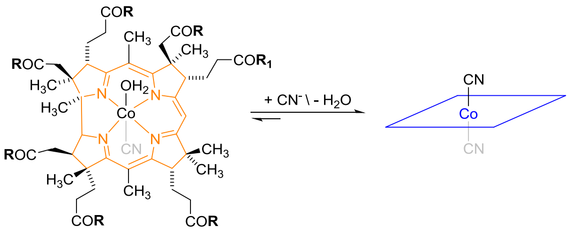

Figure 1: Corrin-based chemosensors before (left) and after reaction with CN^- (right). Detection of cyanide is associated with a change of colour from orange to violet.[2]

Figure 2 shows a photograph of the corrin-based chemosensor before (i) and directly after applications to a crude aqueous cassava suspension (ii), the surface of a freshly cut cassava slice (iii) and a crude cassava slurry (iv). As mentioned earlier, the presence of cyanide was indicated by a change of colour of the chemosensor from orange to violet.

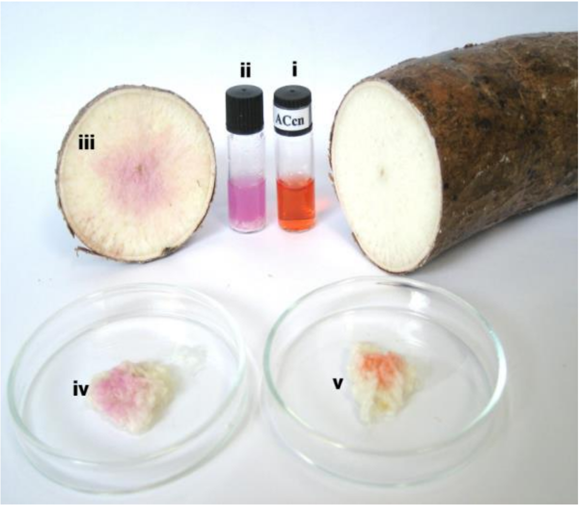

Figure 2: Corrin-based chemosensor (i) upon coordination of endogenous cyanide from (ii) a manioc extract, (iii) a freshly sliced cassava and (iv) a manioc pulp. After several washings cyanide is not anymore detected in the pulp (v).[3]

Having washed the cyanide containing cassava slurry repeatedly with water (v), cyanide was successfully removed as indicated by the orange colour of the sensor. The robustness of the test was further improved by immobilizing the orange coloured sensor on hydrophobic surfaces. Only if cyanide-containing solutions are passed through the kits, the detection zone changes its colour from orange to violet. Such kits with the name CyanoKit (Figure 3) are now commercially available from CyanoGuard, a chemical company located in Switzerland.

Figure 3: CyanoKit for cyanide detection in cassava (left) and a handhold analysed (right) (Reproduced by permission of CyanoGuard AG, Wädenswil, Switzerland)

With these kits, semi-qualitative and quantitative detection is possible. For a semi-qualitative analysis, the colour of the test-kit is compared to a colour chart and allows a simple yes/no evaluation. Quantitative measurements are based on a smartphone device. The method is schematically depicted in figure 3 and consists of five steps (i-v). First (i); an aqueous sample is prepared from the foodstuff using standard food processing protocols. Afterwards, the sample is passed through the kit containing the immobilized chemosensor (ii). Only in the presence of cyanide a change of colour from orange to violet is observed. The kit is then inserted into a device accessory (iii) photographed and analysed using a smartphone app (iv), Using common smartphone technologies; it is also possible to transmit the results immediately to a server (v). Consultancies with a remotely located expert in almost real-time are principally possible and can strongly improve food-processing procedures.

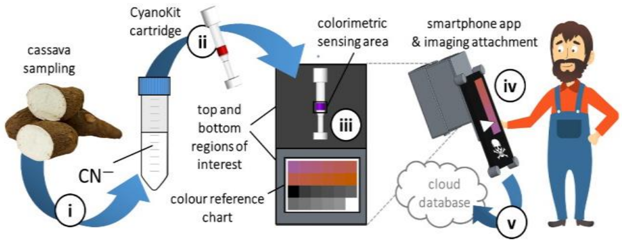

Figure 3: Smartphone based detection of cyanide in foodstuff. For the details of the individual steps see text (Reproduced by permission of W. Karlen, ETH Zürich, Switzerland).[4]

The smartphone method was tested in food quality control. Analysis of three different cassava samples and four other food samples demonstrated that the smartphone-based quantification is comparable to that with a high-end UV-spectrophotometer. Following the idea of Howard Bradbury, I envision that the smartphone-based test will not only be a useful solution for commercial applications, but will also be available in remote locations where it is urgently required but unfortunately not affordable. To make this dream come true, we are looking for philanthropists that are sponsoring such program.

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New tools for grass pea improvement from the UPGRADE project

The development of grass pea as a major crop has been impeded by a number of issues. Firstly, the perceived risk of toxicity has discouraged farmers from cultivating it except in the most stressful environments where other pulse crops would likely fail. Because of this, its pattern of cultivation is as a minor crop in marginal environments. This, in turn, has kept pioneer scientists and breeders from focussing attention on improving it, which again in turn, has led to the paucity of improved varieties. All these factors together have cemented grass pea's status as an orphan crop, despite its excellent resilience to multiple environmental stress factors and good yield potential.

A growing body of evidence highlights the crucial role of sulphur-containing amino acids in the aetiology of neurolathyrism [1, 2], but still, the presence of β-L-ODAP in grass pea seeds remains the primary factor in the perception of grass pea as a dangerous food, limiting the adoption of the crop in areas where its environmental resilience could be beneficial. In Ethiopia, which has a long history of grass pea consumption and no regulations against its use and cultivation, grass pea grain retails at a 40-60% lower price [pers. comm. Shiv Kumar Agrawal, ICARDA] than other pulses despite similar taste and nutritional profiles.

To truly shift this perception, improved grass pea varieties would need to be safe to consume even under the extreme nutritional conditions that would otherwise give rise to neurolathyrism.

Achieving this, through a better basic understanding of β-L-ODAP biosynthesis and its interaction with the environment, as well as building tools to enable the rapid improvement of grass pea through breeding are the two goals of a new project, entitled 'Unlocking the Potential of Grass pea for Resilient Agriculture in Dry Environments' (UPGRADE). Launched in July 2018, UPGRADE is funded by the UK government through the Global Challenges Research fund (GCRF), a collaboration between the Department for International Development and the Biotechnology and Biological Sciences Research Council. The project is a collaboration bringing together the fundamental plant science expertise of the John Innes Centre (JIC), UK, and the Queensland University of Technology, Australia, with the experience in grass pea improvement of ICARDA and the connections for technology deployment in Sub-Saharan Africa of the Ethiopian Institute for Agricultural Research and the Biosciences eastern and central Africa Hub at the International Livestock Research Institute in Kenya.
As part of UPGRADE, we will introgress novel low-ODAP traits generated through EMS-mutagenesis [3] into elite material adapted to Ethiopia. These mutants will broaden the gene pool for low-ODAP traits and may result in a further reduction of β-L-ODAP contents when combined with the already deployed low-ODAP traits developed from the P-24 lineage, such as Mahateora, Ratan, Wasie and Prateek [4].

In addition, the low-ODAP mutants grown along multiple high-ODAP siblings allow us to assess the physiological impacts of the low-ODAP trait. By comparing the performance of high- and low-ODAP plants to simulated stress conditions in controlled environments (including drought, salinity, waterlogging, powdery mildew, heat, and soil zinc deficiency) and in the field (using multiple locations in Ethiopia), we will measure any agriculturally relevant effects that would compromise the usefulness of low-ODAP varieties to farmers, and generate evidence to strengthen or dismiss existing hypotheses for the physiological function of β-L-ODAP in grass pea. Conversely, by measuring β-L-ODAP-concentrations in seeds and green tissues of plants produced in stress and control environments, we will quantify genotype-by-environment effects on β-L-ODAP production in low-ODAP lines to better predict potentially hazardous β-L-ODAP-concentrations produced in farmers' fields – or prove the safety of future low-ODAP varieties.

Another part of the project is concerned with investigating potential uses of grass pea in fodder production in Sub-Saharan Africa, via intercropping with the resilient and highly productive forage grass *Brachiaria spp.* A limitation of *Brachiaria* deployment has been the observed decline in yield of these perennial grasses over multiple seasons. Intercropping with a legume capable of fixing nitrogen and mobilising phosphate [5] may mitigate this problem if it can persist in competition with the forage grass even in harsh environments.

A first annotated draft genome of grass pea has recently been produced by researchers at the JIC and is under preparation for release on bioRxiv. Pre-publication access can be granted to interested researchers on request. The relatively large size of the grass pea genome (8.12 Gbp) and abundance of repetitive regions made it difficult to assemble. The current draft assembly, based on a combination of Illumina paired-end sequencing and 2kb, 5kb, 8kb and 14kb mate-pair libraries, remains highly fragmented, comprising 669,893 scaffolds with an N50 of 59,728. More sequencing with long read technologies (Oxford Nanopore or Pacific Biosciences), optical mapping (BioNano) or other scaffolding technology (e.g. 10X, HiC) would be needed to further improve this assembly, by spanning long, repetitive regions.

This genome draft will allow us to establish gene editing as a tool for grass pea research. As a first step, we will develop a robust transformation platform, building on the expertise of the Mundree lab at the Queensland University of Technology in transforming the tropical legume species *Cicer arietinum* [6] and *Cajanus cajan*. Using this transformation platform we will attempt CRISPR/Cas9-mediated knockouts/knockdowns of genes associated with β-L-ODAP biosynthesis and agronomically important mutations known from other legume species. Due to the high licensing costs of CRISPR/Cas9 for commercial applications and complex regulatory environments hindering the deployment of genetically modified or gene edited crops in most countries of Sub-Saharan Africa, we intend this platform to be used primarily to identify useful traits in grass pea that could be achieved through mutagenesis or selection from existing germplasm.

To this end, a TILLING platform, also under development at JIC, will be instrumental. These reverse genetics technology will allow breeders and research scientists to identify mutations in genes of interest rapidly and cheaply, adding to the pool of available diversity. Applying the advances made in the breeding of other legumes to grass pea through knowledge of the genetic basis of certain traits, such as reduced trypsin inhibitor content [7], altered shade-avoidance responses [8], early flowering [9], powdery mildew resistance [10] and enhanced branching [11], could greatly accelerate the improvement of this crop, allowing varietal development to 'catch up' with more established pulses. Another highly useful platform, currently beyond the scope of this project, would be an EcoTILLING platform that would leverage the global diversity of grass pea present in the ICARDA collection of *Lathyrus sativus* accessions.

To accelerate the introgression of novel traits into new varieties, JIC has developed a speed-breeding protocol for grass pea, using 22h photoperiod in a glasshouse with LED lights tuned to be as close to sunlight as possible. Under these conditions plants start flowering 31 days post-sowing, providing a much shorter generation time [12]. However, better genetic maps and markers for agronomic traits are needed to enable rapid marker-assisted breeding in grass pea and deliver urgently needed, safe and high-yielding varieties to the field.

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ABSTRACTS

Konzo Cases in the Eastern Region of Cameroon, May 2077
(7th AFENET Scientific Conference Maputo, 12-16 November 2018).

Background: Konzo, caused by cyanogenic glycoside intake from a monotonous diet of bitter cassava, is a neurological disease characterized by abrupt, irreversible and symmetric spastic paralysis. In Africa, more than 4000 cases have been reported mainly in malnourished children and women of childbearing age. In April 2017, the East Region of Cameroon reported a cluster of cases with spastic paraplegia. We investigated to identify the causes and emolument control measures.

Method: We conducted a case-control study in the affected village in the East Region. A case was any person presenting with spastic lower limb weakness occurring between January and May 2O17 .We reviewed hospital registers and actively searched for cases in the community. Each case was matched to two controls by sex and place of residence. We used a structured questionnaire to collect data on disease onset, clinical presentation and eating habits from consenting persons. We assessed the nutritional status using mid-upper arm circumference, weight/height (z-score) in children and body mass index in adults. Biological samples were taken from cases and controls to exclude other possible causes (infectious, toxic, and vitamin and protein deficiency).

Results: A total of eight cases were identified with a median age of I2.5 years (6 - 35) and 6 (67%) women. Disease onset was rapid, characterized by an internal deformation of the feet when walking (n=6): 2 needed walking sticks. Malnutrition and bitter cassava consumption was 75.O% (n=6), and 100% (n=B) in cases and 1,8.8% (n=3) and 87.5% (n=74) in controls.
Only malnutrition was significantly associated with the disease (OR=36.9, P=0.007). Biological assessments were normal.

Conclusion: Regular consumption of cassava, malnutrition, clinical picture and geographical context support Konzo as the likely cause of this outbreak. Malnutrition prevention coupled with education of the population on cassava preparation will help prevent new cases.

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Khesari dal (*Lathyrus sativus*), A nature`s gift to mankind: Use it or Abuse it

(National Conference on Advances in Research on Aging and Neurological Disorders 31st Annual meeting of Society for Neurochemistry (India) 2017 Banaras Hindu University, Varanasi, India, Sept 20 -22, 2017)

Accusing Khesari dal for human neurolathyrism was an epidemiologists blunder of the century since it did not take into account that it was only the sole consumption of the pulse as a staple that was the cause since whenever it is consumed as part of a diet there is never a problem as seen in the recent surveys. In fact no legume should be consumed as a staple. It is now a foregone conclusion that khesari dal is safe when used as part of a normal diet. This is very evident even from the most recent survey in the Gondia district of Maharashtra wherein there was no evidence of neurolathyrism.

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ODAP and Homoarginine are the two unusual amino acids in khesari dal discovered in the early sixties. Homoarginine is now known as a normal constituent in humans and is assigned special roles for a healthy cardiovasculature and also for a healthy bone especially in the elderly and also during pregnancy and child birth. ODAP is also present in the Chinese “ cure all “ herb Ginseng. Both Homoarginine and ODAP are now covered by several patents. The most recent patent for ODAP (Dencichine) covers its property to improve platelets count following chemotherapy in certain situations.

Khesari dal should also be able to raise dopamine levels in Alzheimers and Parkinson patients.

ODAP is now used in tooth pastes, surgical bandages and band Aid strips since it prevents Bleeding. Scientists at Osmania University, Hyderabad have recently shown that ODAP (Khesari dal) could be beneficial in overcoming hypoxia under certain conditions. Recently it has been shown that very low Homoarginine levels are associated with major cardiac events and in patients with acute chest pain the use of Homoarginine may be an effective therapeutic option. Some of these newer developments with ODAP and Homoarginine would be highlighted in this talk. In the coming days some of these newer findings and approaches would make khesari dal a high priced commodity as a most important functional food and may no longer be a poor man`s pulse.

Following extensive deliberations during the last 4 years the ICMR had authorised the FSSAI regulatory and statutory body in India to take appropriate steps and come out of archaic concepts to resurrect khesari dal as legal commodity throughout India and restore its deserved status. It remains to be seen how soon this is going to be implemented.

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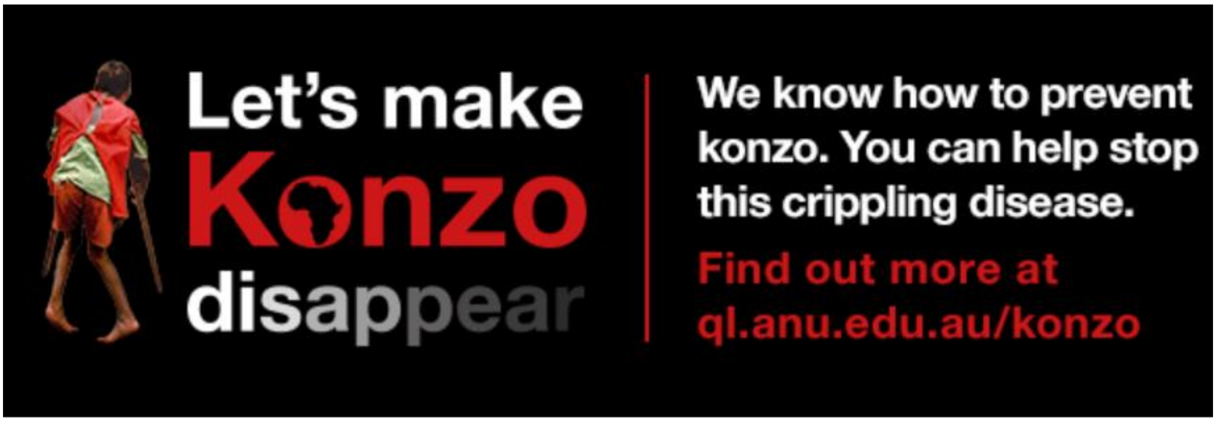

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