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

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EDITORIAL

HAPPY NEW YEAR 2022!

A challenging year is over and a new one brings with it promises and hope for better times.

Politics in Ethiopia, Afghanistan and Congo are causing food shortages and misery, forcing people to rely for food on whatever is available to them, including too much grasspea and poorly processed bitter cassava, respectively.

In this issue the distribution of grasspea in Afghanistan is described and attention is drawn to the potential problems associated with its excessive consumption during nutritional stress.

We also received two articles from China where research on grasspea is gathering momentum.

One is a tribute to the work and life of Fernand Lambein and the importance of communication in our multicultural and highly inderdisciplinary field.

The other reviews the considerable research effort on grasspea in China. It is nice to also be able see many of the people who are involved in this endeavour.

We also learn about Konzo in Mozambique, where political will and better funding are needed to achieve practical results.

The same can be said for the Republic of Congo, from where we received a report on a survey of cyanide in processed cassava. It is noteworthy how a simple tool such as a chemical colour test can provide useful results for action.

We hope that the donor and political community also reads our news in order to bring hope and effective prevention of neurolathyrism and Konzo to those who most need effective help.

D. Enneking

ARTICLES

Grasspea (Lathyrus sativus L.) in Afghanistan – famine food and the need for risk management

"The fight against lathyrism is … a fight against poverty and ignorance" (1).

The current food shortage in Afghanistan and the preceding drought conditions are likely to have led to increased production of grasspea (*Lathyrus sativus*) and consequently to its use as a famine food. The excessive and exclusive consumption of grasspea causes the crippling disease neurolathyrism which has been previously reported from this country (1,2,3). To manage this potential problem through provision of additional food sources containing protective sulfur amino acids and to inform the local population about safe use practices, it is important to know where in Afghanistan the species is cultivated and traded (4).

The distribution of *L. sativus* is shown in Figure 1. The circles indicate areas where the species has been observed and may still be grown today. In the absence of other food sources *L. sativus* will be used as a staple food in these regions. Excessive consumption of the seed coupled with malnutrition leads to the development of a permanent paralysis of the lower limbs (neurolathyrism) and thus to long term disability of often young people.

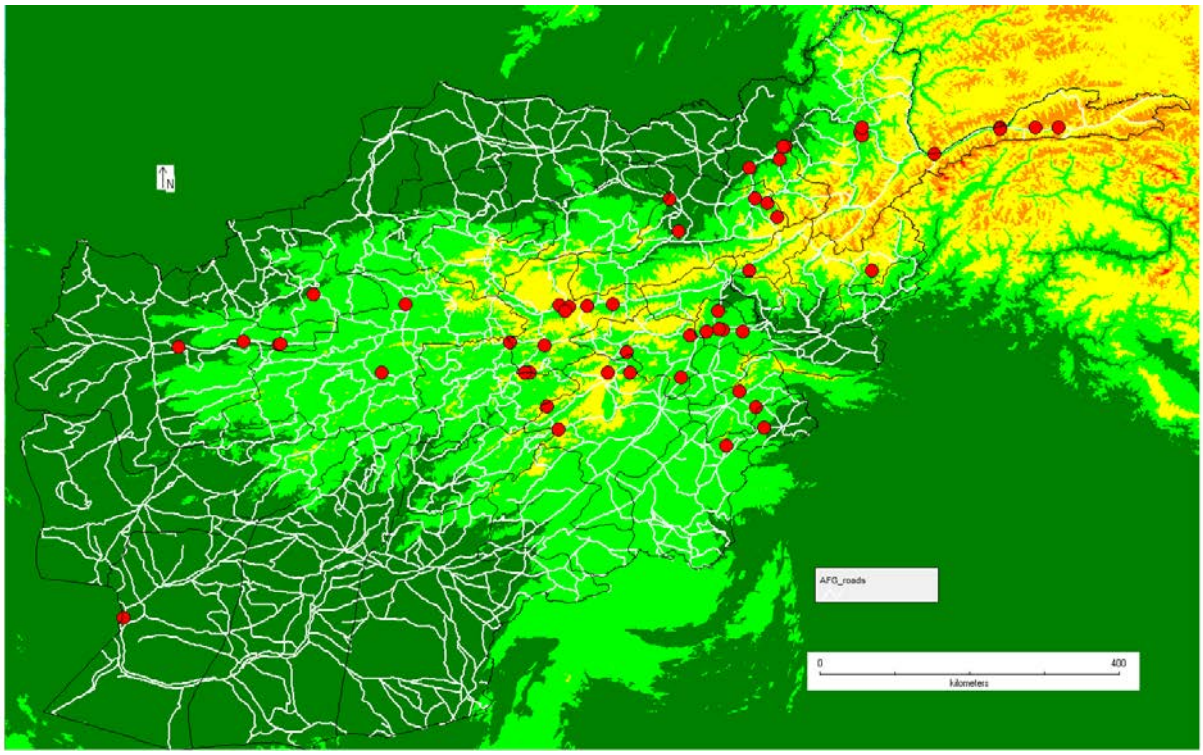

Fig 1. Distribution of *Lathyrus sativus* in Afghanistan (circles) (source: Global Biodiversity Information Facility, GBIF (5)). Roads are indicated by white lines. Elevation is shown by shading.

Vavilov and Bukinich (6) provided a succinct description of Afghan grasspea cultivation, translated by google: "Tsina (*Lathyrus sativus* L.) is not widespread in Afghanistan, but individual spots of its culture are often found in mountainous regions, as if interspersed among other grain legumes. Significant crops can be seen only northeast of Herat (Karokh) and especially near Kabul, Charikar, Sheikhabad. Occasionally it is found in the villages of the central mountainous Afghanistan. We observed it in significant numbers in Badakhshan (Isketul), Ishkashim, where it reaches 3000 m. Along the Kandahar road near the Durani rabat, a sowing of a grasspea mixed with peas was found. Tsina (*L. sativus* L.) often comes across as a weed among other cereal legumes: peas, chickpeas, beans and lentils. It is curious, as it were, the insular settlement of this culture. At the entire distance from Herat through Maimene, Mazar and Sheriff to Kabul, we met her northeast of Herat and then at a distance of more than 1000 km near Bamiyan, moreover, under a different name. In the Herat region it is called "lyangash", in Badakhshan (Isketul, Ishkashim) - "molasses", near Bamiyan, Kabul, Charikar - "kalal"

(kalul, klol). Tsina is used as food for the population, and is also given in the form of grain to livestock (Charikar). At the bazaar in Kabul, it is regarded as lower than peas. The entire cultivated species is represented by *L. sativus* L. var. *coerulea* Al. (syn. var. *azurea* Korsh.) with blue flowers."

Recently grasspea cultivation has been observed in Ghor, the Hazarajat, highland Baghlan, Takhar, upper Panjshir and Badakhshan, with concerns about the poorer populations in Wakhan, where there is a tradition of subsisting on this crop, locally known as patak (7).

The present government and aid agencies need to pay attention to the potential threat of neurolathyrism and to provide contingency plans to deal with high risk conditions during the present famine.

Reduction of poverty, the provision of a balanced diet to consumers of grasspea, together with education about its safe use are likely the best solutions to this problem, while enhancing the utility of this life saving crop.

References

1. Arya, L. S., M. A. Qureshi, A. Jabor and M. Singh (1988). "Lathyrism in Afghanistan." Indian Journal of Pediatrics **55**(3): 440-442.
2. De La Vigne, R. A. A. (1953). "Lathyrisme en Afghanistan." Revue Médicale du Moyen-Orient **10**(3): 325-336
3. Simpson, R. A. (2002). "Mission to Afghanistan." Medical Journal of Australia **177**(11/12): 633-637.
4. Enneking, D. (2001) Famine in Afghanistan, threat of a new Lathyrism epidemic? https://tinyurl.com/5dr9xyf6
5. GBIF.org (24 November 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.u4fk8h
6. Vavilov, N. I. and D. D. Bukinich (1929). "Agricultural Afghanistan." Bulletin of Applied Botany, Genetics & Plant Breeding, Leningrad **Supplement 33** https://tinyurl.com/mjvk4xb9
7. Fitzherbert, A. (2014). An Introductory Guide to Sources of Traditional Fodder and Forage and Usage. Environmental Resilience in Pastoral Systems in Afghanistan. Kabul, United Nations Environment Programme

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The Link between Professor Fernand Lambein and Grass Pea Researches in China

Professor Fernand Lambein was a world class phytochemist. He devoted his life to research on grass pea (*Lathyrus sativus*) and to understand, in order to prevent, the diseases neurolathyrism and konzo. His achievements in the biochemical synthesis of β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) and its metabolism have significantly promoted the selection and breeding of low-toxicity varieties of grasspea worldwide. Moreover, he organized global workshops with outstanding organizational skills and dedication. Importantly, he actively helped developing countries to cultivate research talent in the field of *Lathyrus* and *Cassava*.

In China, a *Lathyrus* species was documented initially in "救荒本草" ("jiu huang ben cao" in Chinese or "several lifesaving crops" in English (1)) in 1406. In 1973, a neurolathyrism epidemic with a total of 2315 cases in eight cities of Gansu province (2) prompted the first Chinese research group on grass pea to be founded by Prof. Yaozu Chen of Lanzhou University (3). During the past 50 years, Prof. Lambein paid close attention to the study of grass pea in China. He always was in contact with several active grass pea researchers in China to discuss β-ODAP biosynthesis and metabolism, neurolathyrism epidemics and prevention, cultivation, genome sequence and other related topics of grass pea, which made the researches of grass pea in China greatly beneficial and helped their advance. Here, we would like to express our deepest gratitude for him and condolences to his wife, Dr. Yu-Haey Kuo and their family.

Investigating grass pea cultivation in China

In China, grass pea has been cultivated conventionally in mountainous areas of Gansu, Shaanxi, Shanxi, Sichuan province, etc. To learn more about its cultivation and utilization in China, Prof. Lambein and Dr. Kuo visited Northwest Agricultural University (now named as Northwest A&F University) in 1996. Prof. Lambein gave lectures to researchers and students on neurolathyrism, cultivation and re-utilization of grass pea, and other related topics. As a gift, he gave the proceedings of the international conference held in Ethiopia (1995) to Prof. Peihong Wang (1921-2015), entitled "Lathyrus and lathyrism: a decade of progress" (Eds. Redda Tekle Haimanot and Fernand Lambein). And then, they investigated the cultivation of grass pea in Wuqi County of Shaanxi province together with Prof. Peihong Wang (Figure 1).

Figure 1 *Prof. Fernand Lambein (fourth from the left) and Prof. Peihong Wang (first from the left) investigated the cultivation of grass pea in Wuqi County of Shaanxi province.*

Helping research teams from Lanzhou University

As early as the 1990s, Prof. Lambein paid attention to the work of the Lanzhou University research group and actively communicated with them, mailing new related-journals in time. Prof. Zhixiao Li still keeps several magazines sent by Prof. Lambein (Figure 2) and stored them away among his treasured things. In 2006, he wrote to Prof. Li to explain the purification methods of BIA (β -(3-isoxazolin-5-on-2-yl)-alanine), the precursor of ODAP, and sent the samples purified in his laboratory, which greatly promoted the functional investigation of the related synthetase. Prof. Lambein still contacted Prof. Li even after they had retired for many years.

Figure 2 *Several Lathyrus Newsletters treasured by Prof. Li and sent by Prof. Lambein.*

Prof. Lambein very enthusiasticly helped Prof. Chongying Wang and her doctoral student Chenjin Jiao to attend the international academic workshop hosted by IPBO in Ghent, Belgium, on September 21-22, 2009. Prof. Wang was invited to be the guest speaker at the conference and Dr. Jiao's poster was selected for a short oral presentation in the plenary session (Figure 3). Due to Prof. Lambein's efforts to get full financial support for their Ghent travel and accommodation, this conference provided the opportunity for them to meet the senior researchers in the field such as Prof. S.L.N. Rao and Peter Nunn. When they arrived in Ghent, Prof. Lambein and Dr. Kuo went to the hotel to visit them and invited them to dinner together. After the conference, he gave them the proceedings of the international conference held in Ethiopia (1995) and some grass pea seeds preserved in his laboratory. His enthusiasm made their trip to Ghent much more memorable.

Figure 3 From Left to right: Prof. Fernand Lambein, Dr. Chengjin Jiao and Dr. Yu-Haey Kuo presenting the workshop in Ghent in 2009, photographed by Prof. SLN Rao.

Concern about the safety of grass pea sprouts eaten in China

Around the mid of February 2013, Prof. Chongying Wang received an email from Prof. Lambein, in which he wrote: "Apparently in some places in China the seedlings of grass pea were consumed. As you know, the seedlings contain high concentrations of isoxazolinones, including the precursor of ODAP and also 2-cyanoethyl-isoxazolin-5-on, which can be hydrolysed or photolysed into beta-aminopropionitrile or BAPN, and this happens to be the compound causing osteo-lathyrism. Perhaps those people could be warned that young people should not eat too much or too often of these grass pea seedlings. It may be a mind-opener to the readers to have a contribution on grass pea research going on in China, or a short overview of the production and ways of consumption of grass pea in China". Prof. Wang was very pleased to accept the invitation by Prof. Lambein and promised to have a deep investigation on the consumption of grass pea around Lanzhou and to write a review paper, including the planting, consumption and current studies of grass pea in China, also together with the epidemic of neurolathyrism in Gansu

province during the 1970s. Thus, we can see Prof. Lambein's love to all people in the world and his persistence to prevent neurolathyrism.

Hereafter, they kept a communication between February to July in 2013. When the review entitled "The cultivation, consumption and research progress of Lathyrus in China" sent to Prof. Lambein and published in CCDN News (2), Prof. Lambein expressed his appreciation for this contribution, as it provided important factual information and access to Chinese literature which is out of reach for most people. Prof. Lambein always genuinely treated people politely and encouraged them with his enthusiasm. He will be best remembered, by all of us, for his contribution to the prevention of konzo and neurolathyrism and the utilization of grass pea while protecting human health.

Inspiring Chinese researchers during the conference in Morocco

At the 7th International Food Legumes Research Conference held on 6-8 May, 2018 in Marrakesh, Morocco, as a grass pea researcher and a witness, Prof. Xuxiao Zong felt very happy for Prof. Lambein's achievement of "Honorary Academy Award", and enjoyed very much his award-winning speech on his grass pea researches and utilization prospects.

During coffee break after the prize-awarding ceremony, Prof. Zong talked with Prof. Lambein about the β-ODAP content of grass pea, its biological activity and potential usage in traditional Chinese medicine. Prof. Lambein told Prof. Zong that he had read Zong's published grass pea research papers. He said that β-ODAP is neuroexcitatory and good for health, but not a poisonous component (4). He entrusted Prof. Zong to bring his opinions to grass pea researchers in China for future researches and utilization of β-ODAP. It is a pity that this meeting was their last (Figure 4).

With the great concern and support from Prof. Lambein, research and utilization of grass pea in China has developed significantly, especially in regulation of ODAP biosynthesis, cropping grass pea as green manure, etc (Xu et al., 2021). Particularly, the timing of the latest paper on grass pea co-authored by Dr. Tao Yang and Prof. Zong is coincided with the day of Prof. Fernand Lambein's funeral (November 6, 2021).

Prof. Lambein reached beyond himself as an outstanding phytochemist, he spend his professional life helping to make grass pea a safe and nutritious food, and soliciting sponsorship to facilitate researchers from Africa and developing countries to participate in associated and synergistic academic activities. Furthermore, he considered to modulate our focus of research on both grass pea and cassava, to make it no longer be blindfolded by ODAP or cyanogenic glycosides, because lathyrism and konzo, the neglected human diseases, are caused by overconsumption of grass pea and improperly processed cassava, respectively, in an unbalanced diet (4).

Figure 4 Prof. Fernand Lambein (middle), Dr. Jinguo Hu (research director of USDA-ARS, right) and Prof. Xuxiao Zong (left) presenting the conference (photo by Dr Yu-Haey Kuo).

We praise him, for his devoted research career, for his brilliant life, for all he has done. We wish him in peace in heaven! He will live in our hearts forever!

References:

1. *Jiuhuang Bencao* (Famine relief herbal) https://en.wikipedia.org/wiki/Jiuhuang_Bencao last accessed 19.11.2021
2. Sun ZX, Hou HZ, Zhou LN, Cheng W, Wang CY. The cultivation, consumption and research progress of *Lathyrus* in China. CCDN NEWS, 2013, 21:4-8
3. Xu QL, Jiang JL, Jiao CJ, Zhang DW, Turner NC, Kumar S, Xiong YC. Process and progress of researches on *Lathyrus sativus* L. over the past 160 years. Acta Bot. Boreal.-Occident. Sin., 2021, 41(9):1583-1604
4. Lambein F, Travella S, Kuo YH, Montagu MV, Heijde M. Grass pea (*Lathyrus sativus* L): Orphan crop, nutraceutical or just plain food? Planta, 2019, 250(3): 821-838.

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Konzo management, disability, and inclusion in Mozambique – Memba and Erati Districts

It is exactly 40 years since the first epidemic of konzo was reported in Mozambique in 1981 during a severe drought in northern Nampula Province (1). Further epidemics have since been reported, and konzo has become endemic in areas of Nampula and neighbouring Zambezia Province (2). Pockets of food insecurity afflict the endemic areas, forcing the population to subsist on unprocessed bitter cassava, especially during drought.

A recent study conducted by the non-governmental organization, NLR Mozambique, in partnership with governmental institutions and public universities, looked at the needs of persons affected by konzo, with a focus on disease management, disability, and inclusion (DMDI) services. The first part of the study consisted of structured interviews of persons with konzo in Memba and Erati Districts of Nampula Province. The second, qualitative, part looked at existing policies, strategies and practices related to konzo at all levels of the health system, using semi-structured interviews. At national level, the interviews were conducted with policy makers, and at provincial level with managers, technical health personnel and implementers from the Provincial Directorate of Health. At district and community level, health and social services technicians, persons affected by konzo, and community leaders were interviewed. Two other disabling neglected tropical diseases were studied, but results are only presented for konzo.

The study of persons with konzo included 133 participants, of whom 47 were female (35%). The mean age was 45 years (SD 14). The most common disability was moderate (47%), with a majority walking with the support of assistive devices or a stick. Most persons affected by konzo were not married (62%); this high percentage may be related to the early onset of disabilities in persons with konzo (mostly during childhood). Of persons affected by konzo, 65% experienced mental distress. Those with severe konzo (26%) experienced high levels of mental distress, and often reported headaches. Persons affected by konzo were restricted in their participation in activities (65%), especially in work and visits to public places. Restriction of participation was much higher for those with moderate and severe disabilities. Their physical impairment was clearly of great impact. Sixty-nine percent of persons affected by konzo reported anticipated and experienced stigma because of their disability. Persons affected by konzo preferred to keep others from knowing about their disability, and they often thought less of themselves.

In the qualitative study, we found that the National Directorate of Public Health in the Ministry of Health did not refer to konzo in their national policies and regulations. No surveillance system was in place for konzo, and hence outbreaks were not monitored. Attempts to mount a surveillance system through the creation of a multisectoral taskforce had not progressed.

The national health system has not had funds to provide physical rehabilitation services for persons with konzo. Small and sporadic donations have come from NGOs and private donors, but they have been insufficient for the needs. District health professionals do, however, treat konzo patients, as any other case of severe spastic paralysis. Most konzo cases live far from district capitals.

In practice, peripheral health workers receive people with konzo and address them as best as they can, without formal training on how to treat konzo and manage complications. Training provided by the government health service for these workers on physical therapy and rehabilitation is poor, and konzo-specific training is not included. The organisation of konzo-specific training depends on the availability of funds from donor organisations. Many health workers have gained some knowledge of konzo, but only through peer learning. The retention of capacity to treat konzo in the health system is also challenged because health technicians and nurses often change their place of work and/or move up in the system.

Barriers in access to health and social services were extensively discussed. The main barrier was the distance from the remote communities where konzo occurs to health units. This distance impeded patients from reaching health units, but also restricted health workers from visiting communities. Persons affected by konzo often had to walk long distances to reach a health unit. Most of the health units are in areas only reachable via small side roads or sandy and/or rugged terrain, which makes it difficult to use mobility aids such as wheelchairs or crutches. Patients reported that it was difficult and painful to obtain the required access to diagnosis and treatment, and thus many gave up on efforts to reach health units.

Available health services for persons affected by konzo at local level depended on the expertise and perseverance of individual health workers. In areas supported by donor organisations these services were better organized, with active case finding activities, and some morbidity management through self-help groups. These activities, however, lacked continuity because NGO funds were limited and time bound. The government health service has not had the resources to continue support of activities initiated by donor organisations.

Health units in the district capitals have physiotherapy and rehabilitation technicians who can provide basic physical therapy and rehabilitation activities. However, they are barely seen by persons with chronic disabilities, including those affected by konzo. The provision of services for the chronically disabled is mostly carried out by mobile teams going into communities. The chronically disabled are therefore dependent on the infrequent visits of such teams. The provision of assistive devices is coordinated at provincial level through social services. The government health system does not provide assistive devices for persons affected by konzo; provision is dependent on funding from a private donor. In a new district hospital built recently in Memba, no room was allocated for the physical therapy and rehabilitation department, impeding the provision of adequate services.

The tasks of the social services system should include the provision of information about health, where and how to access services, support for access to financial aid, food, psychosocial support, and provision of assistive devices. Most people with disabilities, especially those affected by konzo, do not have access to these services. Persons with disabilities need an official statement from a physiotherapy and rehabilitation technician that they are eligible to receive them. This requires accessing the health services, but health workers are generally unaware of their role in this and rarely refer persons with disabilities to the social services. Persons working for the social services do not have a referral system to the clinical services and those in the clinical services do not have a referral mechanism to the social services.

The existence of stigma towards persons affected by konzo was widely acknowledged. This stigma is mainly caused by lack of knowledge and fear of transmission, but religious and cultural beliefs also play a role. Persons affected by konzo are regularly left by their spouses and abandoned by their families, causing them to live in isolation. This leads to a worsening of their disability and has a negative impact on their mental and social wellbeing.

At community level, people are confronted with the shortcomings of the government health and social services systems. Persons with disabilities and those affected by konzo are often dependent on the generosity of their neighbours for food, water, and transportation. Community leaders may play a role in mobilising people to access health services.

The study recommended improvements at all levels, but especially at district level, to effectively provide DMDI services for persons affected by konzo. Chief among the recommendations is the official recognition of the disease by the Ministry of Health and immediate inclusion in the national surveillance system.

References

1. Ministry of Health, Mozambique (1984) Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. Bulletin of the World Health Organization, 62:477-84.

2. Cliff J, Muquingue H, Nhassico D, Nzwalo H, Bradbury JH (2011) Konzo and continuing cyanide intoxication from cassava in Mozambique. Food Chem Toxicol 49:631-5.

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History, progress and perspectives in the research & development of grass pea in China

Lathyrus palustris and L. maritimus were recorded in China as early as 1406, at the time of the Ming Dynasty, in an ancient Chinese book entitled "救荒本草" ("jiu huang ben cao" in Chinese) in which several lifesaving crops were documented (1). Grass pea (*Lathyrus sativus* L.) used to be widely planted in the Chinese provinces of Gansu, Shanxi, Shaanxi, Neimeng, Ningxia, Sichuan, Yunnan. Its current planting, however, is mainly restricted to the mountainous areas of Gansu province (Figure 1) for two major reasons. One was the neurolathyrism epidemic which took place in 1970s, the other is the increasing availability of other food with the improvement of living conditions in China.

Figure 1. Cultivated grass pea in mountainous areas of Dingxi district in Gansu province, northwest China (From left to right: Dr. Hao Zhu, Song Wang, Professor Junmin Jin, Ning Ma, Youcai Xiong, Chengjin Jiao, Meichun Wang and Quanle Xu, 2021).

In the event of the neurolathyrism outbreak, a total of 2315 cases were recorded across eight cities including Gangu, Qinan, Longxi, Huining, Tongwei, Wushan, Yuzhong and Tianshui of Gansu province in 1970s (2). To address this issue, the first research group on grass pea biology in China was established, which was led by Prof. Yaozu Chen from Lanzhou University with the aim to clarify the pathogenic mechanism and find the solution (3). Over the past five decades since then, more than 40 academic institutions subsequently joined in this program. Here, a brief history and recent advances are summarized to report on grass pea research in China.

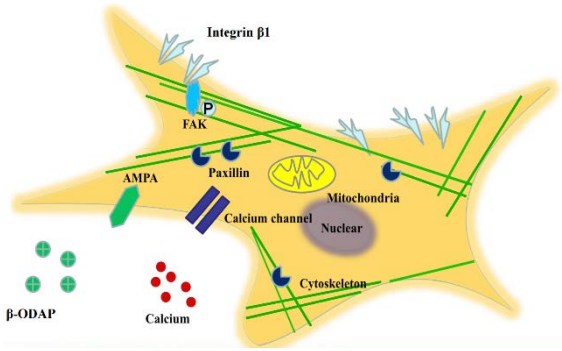

Figure 2. The mechanism of neural decay induced by β-ODAP acting on AMPA receptors, causing an influx of calcium into the cell, ultimately leading to cell disintegration (5)

(1) Neurolathyrism investigation and low-toxic cultivar screening

As a result of joint efforts early in the 1970s, it was clearly established that the neurolathyrism epidemic was closely associated with the over-consumption of grass pea seeds. This was followed by a study on the toxin content and detoxification of *L. sativus* (4) . Recently, through a study of gene expression in human glioma cell line M059K, the research group led by Prof. Youcai Xiong, found that β-ODAP can activate the integrin β1 and focal adhesion pathways (Figure 2), which appear to be the main mechanism leading to neural cell damage, and thus identifying the possible causal mechanism of neurolathyrism (5).

On the other hand, the methodology of β-ODAP determination has also been updated. To improve the accuracy of β-ODAP content detection, a HPLC method incorporating different derivatization reagents, including 2,4-dinitrofluorobenzene (DNFB) was tested and successfully explored by Prof. Zhixiao Li and his colleagues (6). As a result of this work, five cultivars with lower ODAP content, but better agronomic performance, were screened from the germplasm resource of sixty-five cultivars collected from various regions in China. They have been identified as safe fodder to be used locally (7).

Figure 3. Professor Zhixiao Li (left) and Yafu Wang (right) in a grass pea field

(2) Physiology and biochemistry of β-ODAP activities

β-ODAP is not just an excitatory amino acid, but it is also well-known as dencichine for its ability to stop bleeding, a feature of several Chinese traditional medicines like *Panax notoginseng* etc.. To investigate the physiological and biochemical activities of β-ODAP, Prof. Yafu Wang, Chongying Wang, and Youcai Xiong et al., carried out multidisciplinary research on the relationship between drought resistance of grass pea and β-ODAP accumulation. The mechanism is still unknown and needs further investigation.

(3) Biosynthesis of β-ODAP and germplasm utilization

In recent decades, Prof. Chengjin Jiao and Quanle Xu have focused their research efforts on the regulation of β-ODAP biosynthesis. They investigated firstly the accumulation pattern of β-ODAP during the complete lifespan of grass pea and the effect of nutrient elements on β-ODAP content (8). Lately, the biosynthesis pathway of β-ODAP in grass pea was found to be clearly linked with primary metabolism via metabolomic and transcripomic analysis (9; 10). Recently, they confirmed that β-cyanoalanine synthase regulates the accumulation of β-ODAP via interaction with serine acetyltransferase to form a cysteine regulatory complex (11). These results provided new insights into the molecular regulation of β-ODAP content in *L. sativus*.

As a key event of the grass pea program, a monograph entitled "*Grass Pea Biology*" was edited by Prof. Youcai Xiong, Chengjin Jiao and Gengmei Xing and published in 2013. Moreover, Prof. Xuxiao Zong developed "*Descriptors and data standard for grass pea* (*Lathyrus* spp.)" to promote germplasm utilization and suggested to remodel the ancient insurance crop for climate resilience. In fact, grass pea and red pea (*Lathyrus cicera* L) have been widely used as green manure in the orchard management of orange and other fruit trees, with a cultivated area of more than 140,000 hectares for soil quality improvement in Sichuan province under the supervision of Prof. Wenbin Han (Figure 4).

Figure 4. Intercropping with red pea (*Lathyrus cicera*) to improve fruit quality of orange orchards in Xichong district, Sichuan province, China (Credit: Photo Prof. Wenbin Han, 2019).

At the beginning of this year, Professor Quanle Xu and other colleagues successfully organized a national academic conference with the title of "2021 Symposium on *Lathyrus sativus* Biology". More than 60 people participated in this event. As a key outcome of this conference, a consortium named by "The alliance for grass pea industrial technology innovation and strategic development" was established (Figure 5). The Alliance consisted of 21 academic and industrial institutions in China, with the major mission of enhancing grass pea research and development. This organization is able to promote the fundamental research for the industrial utilization of this species. As a support, a review on grass pea in Acta Botanica Boreali-Occidentalia Sinica was just published (3) accompanied by several studies (12,13,14). All the efforts by Chinese colleagues ultimately support the development of grass pea cultivars with reduced or zero seed β-ODAP content and lead to better utilization of the available germplasm resources. The outcome is likely to contribute to improved food security under global climate change, due to grasspea's resilience to environmental constraints.

Figure 5. The group photo of 2021 Symposium on *Lathyrus sativus* Biology that was held in Yangling, Shaanxi province of China

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References:

1. Chapman, G. P. and Wang, Y.-Z. (2002) A Summary of Famine Foods Listed in the Chlu Huang Pen Tsao (1406 A.D.) The Plant Life of China - Diversity and Distribution. Heidelberg, New York, Springer**:** 193-202. https://link.springer.com/content/pdf/bbm%3A978-3-662-04838-2%2F1.pdf last accessed 25.11.2021
2. Sun Z. X., Hou H. Z., Zhou L. N. , et al. (2013) The cultivation, consumption and research progress of *Lathyrus* in China. CCDN News 21:4-8.
3. Xu Q. L., Jiang J. L., Jiao C. J., Zhang D, Turner, N.C., Kumar, S , Xiong Y. (2021) Process and progress of researches on *Lathyrus sativus* L. over the past 160 years. Acta Botanica Boreali-Occidentalia Sinica 41(9):1583-1604.
4. Department of Chemistry, Lanzhou University (1975) Analysis on the toxin and studies on the removal of the toxin in *Lathyrus sativus*. Journal of Lanzhou University (Natural Sciences) 11(2), 45-65.
5. Tan R.Y., Xing G.Y., Zhou G. M., et al. (2017) Plant toxin β-ODAP activates integrin β1 and focal adhesion: a critical pathway to cause neurolathyrism. Scientific Reports 7:40677
6. Jiao C. J., Li Z. X. (2009) A brief history of quantitative analysis of ODAP. Grain Legumes 54: 24-25.
7. Chen Y. Z., Li Z. X., Lu F. H., et al. (1992) Studies on the screening of low toxic species of *Lathyrus*, analysis of toxin and toxicology. *Journal of Lanzhou University* (*Natural Sciences*) 28(8): 93-98.
8. Jiao C. J., Xu Q. L., Wang C. Y., Li F. M., Li Z. X., Wang Y. F. (2006) Accumulation pattern of toxin β -ODAP during lifespan and effect of nutrient elements on β-ODAP content in *Lathyrus sativus* seedlings. The Journal of Agricultural Science 144 (4), 369–375.
9. Liu F. J., Jiao C. J., Bi C. X., et al. (2017) Metabolomics approach to understand mechanisms of β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) biosynthesis in grass pea (*Lathyrus sativus* L.). Journal of Agricultural and Food Chemistry 65(47): 10206-10213
10. Xu Q. L., Liu F. J., Qu R. H., et al. (2018) Transcriptomic profiling of *Lathyrus sativus* L. metabolism of β-ODAP, a neuroexcitatory amino acid associated with neurodegenerative lower limb paralysis. Plant Molecular Biology Reporter, 36(5-6):832-843
11. Song Y. Y., Wang L., Liu F. J., et al. (2021) β-cyanoalanine synthase regulates the accumulation of β-ODAP via interaction with serine acetyltransferase in *Lathyrus sativus*. Journal of Agricultural and Food Chemistry, 69(6):1953-1962

12. Jia H., Li C., Song Y., Liu F., Jiao C., Xu Q. (2021) Prokaryotic Expression and Protein Polymerization Analysis of β-cyanoalanine Synthase in *Lathyrus sativus*. Acta Botanica Boreali-Occidentalia Sinica 41(9):1605-1610

13. Chen H., Zeng P., Xu Q. (2021) Cloning and Expression Analysis of LsSULTR Gene in *Lathyrus sativus*. Acta Botanica Boreali-Occidentalia Sinica 41(11):1954-1961

14. Zhou Z., Liang Q., Quan Z., Qi Y., Mo K., Han W. (2021) Effects of Turning Green Manure *Lathyrus sativus* and Reducing Nitrogen Application on Growth, Nutrient Absorption and Yield of Rice. Acta Botanica Boreali-Occidentalia Sinica 41(11):1962-1970

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Report on konzo study in Uvira territory, South Kivu Province, DR Congo

The villages of Kiliba, Biriba and Kalungwe were visited by the APAA (1) Congo team from 10 September. To 10 October 2021

The objectives were:

- to know if there is the possibility for the existence of Konzo cases in the three villages
- to measure the concentration of cyanide (ppm) found in locally consumed cassava food (roots and leaves)
- to propose prevention and rehabilitation of Konzo in villages visited
- to set up a village committee to monitor and prevent Konzo and other diseases due to consuming bitter cassava

The Villages of Kiliba, Biriba and Kalungwe are located in east of DR Congo, in Uvira Territory where the Bafuliiru and Bavira tribes are the majority. For many decades this community is cultivating cassava for several reasons: annual harvesting, adaptation to climate change, tolerance to poor soils and seed availability. These favourable attributes allow all people to grow it, even the very poor. Most cassva cultivators are women (75% of labor) who can be helped by their children or occasionally by their husbands (who are mainly involved in small business, pastoralism or administration).

Cassava still is the crop which is more resistant to climate change and diseases than other crops

Cassava is still, until now, the principal crop in these villages, due to its drought tolerance and its utility to the extreme poor. A farmer whose family income is under 1$ per day, can eat cassava leaves and Ugali because other food like fish or meat are very expensive.

Due to armed conflict, the population of these villages is not stable, with people escaping to safer villages or into the bush, obliging them to eat fresh bitter cassava and leaves, thus increasing the chances for them to suffer from Konzo and other diseases.

With the complete kit on determination of total cyanide in cassava products and leaves and laminated posters from CCDN Australia, we organized the survey and research on Konzo in the three villages of Kiliba, Biriba and Kalungwe, Uvira territory, South Kivu Province, DR Congo.

Results from the Survey

How is cassava (roots and leaves) prepared?

- Kivunde (food's local name)

It consists of soaking the peeled roots in water for about 3-4 days, followed by sun drying, grinding by wooden pestle and mortar, and sieving to obtain the flour to prepare Ugali

- Kapalanga

It consists of peeled roots exposed to the sun for 3-4 days, then dried, ground by wooden pestle and mortar and sieved to obtain the flour to prepare Ugali

- Nyange

It consists of peeled roots exposed to the sun for 3-4 hours before putting in a sack for fermentation, then it is dried, ground in a wooden pestle and mortar and and sieved to obtain the flour to prepare Ugali

- Mitewe 1

Cassava tubers are cut into small pieces and cooked in water before eating.

- Mitewe 2

Cassava tubers are cut into small pieces, cooked in water and then put in [fresh?] water 2-3 days before eating.

- Sombe 1 (cassava leaves)

It consists of cut tender leaves from cassava, boiled for a few minutes (blanched) before drying in a pot or a pan over a fire. Then the leaves are squeezed to remove liquid before pounding, grinding in wooden pestle and mortar before further cooking.

- Sombe 2 (cassava leaves)

It consists of cut tender leaves from cassava, blanched before drying in a pot or a pan to remove liquid before pounding, grinding in wooden pestle and mortar before cooking.

a. How often do you eat food made using bitter cassava?

Cassava roots and leaves are the main food in these villages. About of 95% of persons eat cassava food, and the very poor can eat it every day because they said cassava is tolerant to climate change and seeds are available. Other seeds such as as maize or banana are very expensive and do not tolerate poor soil or water scarcity.

Generally, farmers prefer to grow bitter cassava as a way to avoid beggars, vandals and thieves who can damage or reduce cassava production.

Women are preparing cassava roots

b. Do you understand that bitter cassava contains poison that can make you sick and give you Konzo?

Some of the community members have understood superficially that bitter cassava is dangerous, but due by its adaptation and socio-economic importance in our community, we are obliged to grow it. They don't know specifically what diseases; their causes and symptoms are due to bitter cassava.

Women are preparing cassava leaves for daily food

c. Does your family get sick (dizziness, vomiting, stomachache, diarrhea…)?

Recently, 2 weeks ago, 3 children from a family when they came home from school ate bitter cassava. Suddenly they vomited and had stomach pains. They were brought to the hospital for care. It should be emphasized that due to extreme poverty and Covid 19, many children do not have access to lunch before they go to school (and in the school no food is available). During the break they can forage for cassava in the bush, or by begging the farmers coming from the fields for fresh bitter cassava with contains high cyanide poison.

Many cases of dizziness, vomiting, stomachache and diarrhoea after eating bitter cassava are reported.

d. In your village are there any people who have Konzo (there were able to walk normally but suddenly get sick, and they were unable to walk unaided or at all)?

Many cases of paralysis are reported now, while villagers are accusing each other of sorcery by lack of knowledge, we can estimate about 9% of inhabitants, but I think due by lack of knowledge on Konzo in the community, diagnoses made by nurses indicate that all paralyses are due to diabetes or Stroke (cerebrovascular accident) while they or a portion thereof, could be due to cyanide from cassava causing Konzo.

Research on konzo determination

The results from the determination of total cyanide in cassava roots and cassava leaves (Table 1) suggest that the cyanide concentration (ppm) depends on the processing and cooking method, since the values measured are quite similar for each method in the three villages, with Kivunde and Mutewe2, both

processes involving a leaching extraction, although not perfect, still the most effective to remove cyanide before food consumption.

We also observed that since 1990 until now, the temperature has increased and resulted in lower agricultural production (data not shown). Cassava is one crop that resisted and adapted during this time, but we discovered that all sweet cassava had become bitter, and only in the area with 22-25 °C we can find sweet cassava. All sweet cassava grown more than 35 °C had become bitter after one year. In 2015, some maize planted in Ruzizi Plain contained a bitter taste. We still don't understand that phenomenon. [Have you tested it for cyanide?]

Table 1. Cyanide concentration (ppm) found bitter cassava food in the surveyed villages

#	Villages	Ugali (cassava bread) ppm			Sombe (cassava leaves) ppm		Cassava Chop ppm	
		1	2	3	4	5	6	7
1	Biriba	10-20	45-50	20-30	18-20	30-45	15-25	12-17
2	Kiliba	12-19	40-50	20-25	14-20	32-44	18-29	10-15
3	Kalungwe	11-17	42-50	18-30	15-22	30-40	15-25	12-15
	Average	15	46	24	18	37	21	14

Legend:

1-kivunde, 2-kapalanga, 3-nyange, 4-sombe1, 5-sombe, 6-mutewe1, 7-mutewe

Our research was focused on bitter cassava of all variety of cassava found locally with their local name (kaliyai, nangunga, nakijuba, nakirisi, nabubembe and muzungu). (data not shown).

Generally fresh bitter cassava roots and leaves can contain more than 90ppm of cyanide. Such a high level is dangerous, while due to poverty people are obliged to eat these without processing, causing enormous problems as konzo and other diseases. Women and children are most affected.

C. **RECOMMENDATIONS AND SOLUTIONS**

- Sensitize and train local communities on how to remove cyanide from bitter cassava flour, tuber or leaves before consumption.
- Improve growing cassava and food systems to encompass the entire range of activities involved in the production, processing, marketing retail and consumption.
- Sensitize and train local communities on how to practice agricultural innovations (adaptation to climate change) to introduce new crops, pulses, fruits or vegetables tolerant to warming conditions.
- Find peaceful solutions to local resource and ethnic conflicts
- Provide nutritional supplements, food and walking sticks to people affected by Konzo and other diseases due by bitter cassava.
- Provide big bowls to very poor families to help to remove cyanide after soaking bitter cassava in the water 3-4 days

Aaron Kalala is testing cassava flour and leaves using Kit test from CCDN

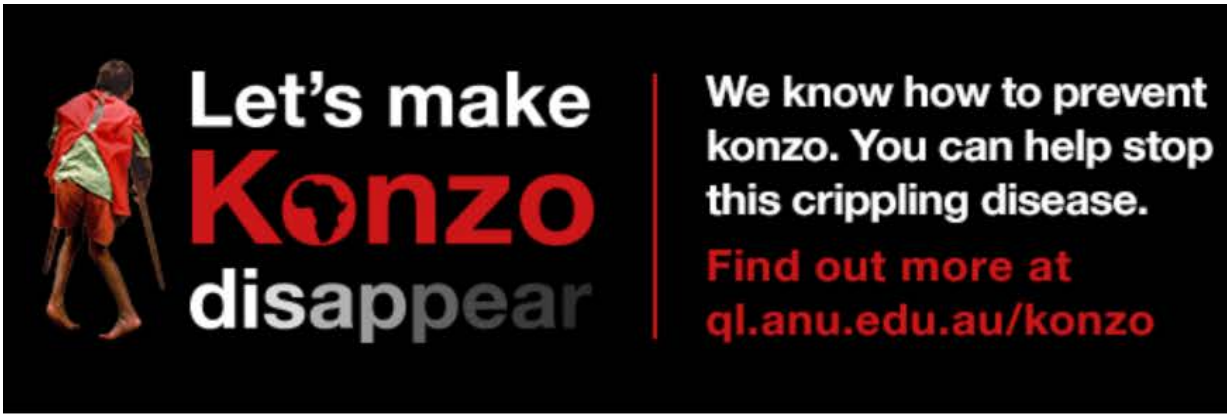
- Set up a hospital center where nurses and health workers will be trained to diagnose and care for Konzo cases.
- Communities and villagers, those affected directly by Konzo, the most vulnerable and those working in agriculture and food

systems are encouraged to actively engage and communicate with the other farmers to promote safe food and to prevent Konzo and other diseases due to consumption of bitter cassava.

References

1. APAA Congo Presentation https://tinyurl.com/4vwyz4hy [Google translation in english] of https://apaa-congo-ong.blogspot.com/2016/04/ [en français] last accessed 19-11-2021
2. Metre T.K. (2006) Cassava Consumption in South Kivu Province of DR Congo: For Survival or Death? In: Cassava Cyanide Diseases Network, CCDNNews 7: 2
3. Karumba, A.K., Denton, I.D., Bradbury, J.H., 2009. Cyanide poisoning and konzo from cassava in South Kivu Province, DRC. Cassava Cyanide Diseases Network, CCDNNews 13: 2-3.

Aaron Kalala Karumba



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