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

CCDN News

Cassava Cyanide Diseases & Neurolathyrism Network

(ISSN 1838-8817 (Print): ISSN 1838-8825 (Online)

**Issue Number 19, June 2012**

## Contents





## Editorial

In June 2001 I started the Cassava Cyanide Diseases Network (CCDN) as a free, worldwide network of people interested in the elimination of cyanide poisoning, konzo and tropical ataxic neuropathy (TAN) resulting from consumption of cassava. In June 2003 the CCDN Newsletter, called CCDN News was commenced, with publication every six months. In all of this I was supported and helped by Dr Julie Cliff of Eduardo Mondlane University, Maputo, Mozambique.

In 2009, as a result of a very successful Workshop on "Konzo and Neurolathyrism," organised by Professor Fernand Lambein in Ghent, Belgium, the CCDN was expanded to include neurolathyrism, a disease closely related to konzo. The name of the Network was changed to the Cassava Cyanide Diseases and Neurolathyrism Network (CCDNN). The CCDNN has grown steadily since its inception and now has in excess of 350 members from 60 countries. Thankfully the Newsletter has kept its original name of CCDN News.

The time has come for me to relinquish my role as Coordinator of the CCDNN and as Editor of CCDN News. I am really pleased that Professor Fernand Lambein of Ghent University, who is well known for his many contributions to the understanding of both konzo and neurolathyrism, has kindly agreed to take on these roles. I wish him every success in this work and hope and pray that the CCDNN may continue to be of service to all those doing research in this very interesting field and to the untold thousands of people who eat cassava and grass pea.

In the current issue there are three articles that present new and interesting concepts and results. The first is a paper by Kawaguchi et al, which shows the importance of stress on the incidence of neurolathyrism. The second development is the introduction of cassava into areas in Ethiopia where grass pea has a long tradition as a reserve food source in times of drought. This is the first time that the geographical areas where cassava and grass pea are grown have overlapped. The toxins that are linked to konzo and to neurolathyrism are cyanogens in cassava and ODAP in grass pea respectively, and each of these toxins doubles in amount during a drought. Growing cassava and grass pea in the same area in periods of drought and famine seems to be heading for DISASTER. Now for some good news. The third article reports the prevention of konzo in three villages in Boko Health Zone, Bandundu Province, DRC, and follows on from earlier success in prevention of konzo in Kay Kalenge village in an adjacent health zone of DRC.

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It is a great honour but not an easy task to get into the footsteps of a really great and highly respected man (http://www.anu.edu.au/BoZo/staffandstudents/staffprofiles/bradbury.php). I hope the CCDNN community will continue their support and continue contributing to the Newsletter. The CCDNNewsletter has considerably helped to develop awareness of the medical consequences of inappropriate consumption of cassava by rural poor in neglected communities in Africa, The similar clinical presentation of neurolathyrism and konzo and the similar socio-economic condition of its victims seems to indicate some similarity in the etiology and in the prevention of both diseases. It was fortunate that the aim of the CCDNNetwork could be broadened to include neurolathyrism as topic for research and communication. This cross-fertilization of ideas should be important to make progress in the prevention of these two very unfair but often neglected diseases of the poor. Prevention of konzo and neurolathyrism is intimately linked to knowledge of the plants cassava and grass pea. An upcoming conference on *Lathyrus sativus* at the National Institute of Nutrition in Hyderabad, India, will hopefully give adequate attention to the risks of overconsumption of grass pea and the prevention of neurolathyrism.
**http://www.ninindia.org/confnin_web_site.pdf**

Fernand Lambein, Institute for Plant Biotechnology Outreach, Ghent University, Belgium

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## The effect of social as well as physiological stress as a risk factor of neurolathyrism outbreaks.

Neurolathyrism (NL) is a motor neuron disease characterized by spastic paraparesis of the hind legs described already by the ancient Greek philosopher Hypocrates. An excitatory amino acid L-β-*N*-oxalyl-α,β-diaminopropionic acid (L-β-ODAP) present in grass pea (*Lathyrus sativus* L.) has been proposed as the cause of this disease. During periods of food insufficiency grass pea was consumed as the sole staple food in drought prone areas of India, Bangladesh and Ethiopia, where poor subsistence farmers consider it a life-saving crop during drought and famine.

In normal circumstances only sporadic cases of neurolathyrism occur, while during famine and in other extremely stressful conditions such as the well known Ethiopian famine of 1984-85, the Spanish civil war (1936-39) or at a German concentration camp in Vapniarca during World War II[1], there were outbreaks of NL with remarkably higher incidence of as much as 6% in Ethiopia and even 60% in Vapniarca[2]. On the other hand, neurolathyrism is very rare or non-existent among grass pea consumers in peaceful social situation with the availability of affordable alternative foods. Therefore we consider that social stress as well as unbalanced/poor nutrition is a risk factor of NL.

We have developed a reproducible model rat for neurolathyrism (NL) that showed hind-leg paraparesis after repeated L-β-ODAP treatment[3]. In our NL model, we exploited the stress-loading method i.e. the separation of pups from their mothers[4] and leave them for a 6 h fasting period in a group of littermates before parenteral injection of L-β-ODAP. This stress-loaded group produced higher incidence of paraparetic rats than the control group without-stress but with the same dose of L-β-ODAP. Considering the good reproducibility of the symptom incidence compared to the scarcity of symptoms in the no-stress group, maternal-separation and/or starvation-thirst stress actually played a role in the pathogenesis of 'rat neurolathyrism'.

We also observed the absence of paraparesis in pups after pretreatment with a moderate dose of dexamethasone (a synthetic glucocorticoid, 50 µg/kg; unpublished data). These facts suggest that stress might be an important risk factor for neuronal death in human neurolathyrism in addition to the neurotoxic effect of ingested high doses of L-β-ODAP.

The hypothalamo-pituitary-adrenal (HPA) axis is a major responder in mammals to stress[5]. To investigate the response of the HPA axis in relation to NL pathology, we measured corticosterone (CORT) a predominant peripheral steroid hormone of the rat HPA axis. As described previously, our rat NL model was prepared as follows: within 8 hours after birth, rat pups were injected subcutaneously with L-β-ODAP (100 mg/kg) or only saline as a control. From the second day, pups were separated from their mothers in another small polycarbonate cage for 6 hours (9:30-15:30) in a group of littermates (maternal separation)[4-6]. We also prepared the naive rats, which were not subjected to the maternal separation or to the L-β-ODAP administration. We measured serum CORT by enzyme immunoassay (EIA) of postnatal day 2 pups, the period of most frequent onset of hind-leg paraparesis. The serum CORT concentrations were increased 2.5-fold in maternal separation rats as compared with naive rats. Thus, maternal separation induced an increase of CORT as described by others even in such early period after birth like two day neonates[5]. But no statistically significant differences in CORT concentration were found among saline-treated controls, L-β-ODAP-treated pups with hind-leg paraparesis, and L-β-ODAP-treated rats without symptoms of paraparesis. Points that remain to be solved are the effect of sampling conditions on CORT level drifts.

The HPA activity is regulated by a glucocorticoid-negative feedback loop[6]. This feedback action is mediated by two different types of corticosteroid receptor, glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which differ distinctly in their distribution and pharmacological properties. GR mediates stress response of glucocorticoid. We measured GR mRNA using real-time RT-PCR. Our preliminary data showed a slight increase in the expression of GR mRNA in the spinal cord (~20%) and a higher increase in the whole brain (~80%) of the hind-leg paraparetic rat compared to the non-paraparetic L-β-ODAP-treated rat (unpublished). Considering the possible suppression of GR mRNA expression by dexamethasone, the observed GR induction might reflect the lowered endogenous CORT signal in the critical period, and either of these factors might have an accelerating role in motor neuron degeneration and/or uncontrolled inflammatory responses in the lower spinal cord as observed in rat models of other neurodegenerative diseases[7-8].

The lower reactivity of HPA axis with decreased CORT secretion may enhance the motor neuron sensitivity to the excitotoxicity induced by L-β-ODAP, although the study needs further confirmation.

**References**


[1] Kessler A. (1947) Lathyrism. Monatschrift Psychiatrie Neurol. 113, 41-54.

[2] Lambein F., Ngudi D.D., Kuo Y.H. (2001) Vapniarca revisited: Lessons from an inhuman human experience. Lathyrus lathyrism Newsletter 2, 5-7.

[3] Kusama-Eguchi K., Yamazaki Y., Ueda T., Suda A., Hirayama Y., Ikegami F., Watanabe K., May M., Lambein F., Kusama T. (2010) Hind-limb paraparesis in a rat model for neurolathyrism associated with apoptosis and an impaired vascular endothelial growth factor system in the spinal cord. J. Comparat. Neurol. 518, 928-942.

[4] Daniels W.M.U., Fairbairn L.R., van Tilburg G., McEvoy C.R.E., Zigmond M.J., Russell V.A., Stein D.J. (2009) Maternal separation alters nerve growth factor and corticosterone levels but not DNA methylation status of the exon $1_7$ glucocorticoid receptor promoter region. Metab. Brain Dis. 24, 615-627

[5] Stanton M.E., Gutierrez Y.R., Levine S. (1988) Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. Behavioral Neuroscience 102, 692-700.

[6] Poltsky P.M., Meanny M.J. (1993) Early postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, media eminence CRF content and stress-induced release in adult rats. Mol. Brain Res. 18, 195-200.

[7] Hendricks S., Ojuka E., Kellaway L.A., Mabandla M.V., Russell V.A. (2012) Effect of maternal separation on mitochondrial function and role of exercise in a rat model of Parkinson’s disease. Metab. Brain Dis. DOI 10.10007/s11011-012-9305-y

[8] Smith L.K., Jadavji N.M., Colwell K.L., Perehudoff S.K., Metz G.A. (2008) Stress accelerates neural degeneration and exaggerates motor symptoms in a rat model of Parkinson’s disease. Eur. J. Neurosc. 27, 2133-2146.



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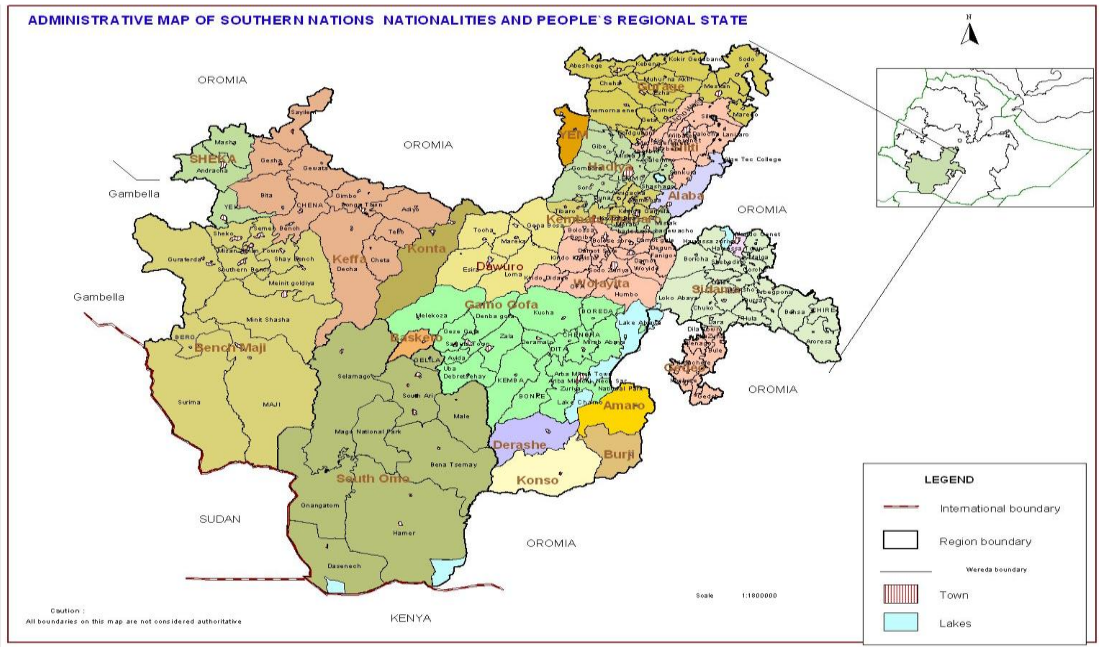
## Minimizing risks of cyanide in cassava based diets in Ethiopia

Cassava is the fourth most important food source worldwide after wheat, rice and maize[1]**.** It is valued for its outstanding ecological adaptation, low labor requirements, ease of cultivation and high productivity[2]. Cassava (*Manihot esculenta*) was believed to be introduced in Ethiopia around 1960‘s by missionaries and/or merchants from neighboring countries[3,4]. But the consumption was not practiced until 1984 during a period of severe famine in the country. The plant has been cultivated in the southern and southwestern regions for decades as an alternative food security crop[5]. Processing methods, storage experience and modes of consumption are not yet customized unlike other cassava producing and consuming African countries. Cassava is one of the underutilized root crops in the country. The crop has been cultivated and consumed in south western areas of Ethiopia mainly to tackle seasonal food shortage. This area is one of the more populous and food deficient areas in the country. Currently, some cassava varieties are being promoted in food insecure northern areas of Ethiopia, where a legume based (mainly grass pea) diet is consumed[3]. The purpose of this paper is to evaluate methods of minimizing the risks of cyanide and to improve the nutritional value of cassava based Ethiopian dishes.

Cassava is consumed mainly in boiled form and for making bread. It is a staple in diets of Konso people (Konso district, SNNPRS), especially for “Cheqa” (non-distilled local beverage which can be made in alcoholic or non-alcoholic form) preparation. In other areas it is consumed seasonally for example, April-June in Kindo Koisha district , Feburary-May in Amaro districtand May-June in Sahula district (all located in South Nations Nationalities People Regional State, SNNPRS, at an elevation of 600-2100 m above sea level). People in Konso district complain of the burning effect and increased alcoholic strength of the local beverage made of cassava. The problem could be attributed to ethanol. To reduce this burning effect of the local drink, people used to boil or make it warm before drinking. In all the study districts, problems such as nausea, vomiting, distress in abdomen, blotting, weakness, headache and dizziness are common symptoms occurring mainly in children and seldom in adults when boiled cassava is consumed frequently[3, 4]. Reports in other countries also show similar trends[6]. The problem is serious when raw or boiled bitter cassava is frequently consumed during periods of hunger and the diet is not complemented with protein rich foods especially foods with higher level of sulfur containing amino acids[7]. Moreover, the toxin inhibits iodine uptake by the thyroid gland thereby aggravating iodine deficiency. The symptom seen agrees with studies reported from cassava consuming populations in Ethiopia and elsewhere in the world[3]. The summary from focus group discussions show that death of cattle is common in January just after the dry season when rain begins




and the first new buds of cassava appear. This may be due to the higher cyanogen level in the young fresh leaves[8].

As a response to problems that occurred after consuming boiled cassava and from a few studies conducted concerning health impact of cassava consumption, people have information on its cyanogenic effects. Most people in the south west of Ethiopia consume cassava only as a means to tackle seasonal food shortage[4]. Among the study group physically visible neurological problems such as konzo, occurring from consuming poorly processed cassava during extended periods as seen in some countries are not observed[6, 7]. Fatality due to intoxication is not reported. According to key informants from Amaro district, communities traditionally prevent the problems associated with cassava consumption by using cow milk. Milk or milk mixed with sheep or goat blood was reported to prevent cyanide fatality from cassava consumption in similar areas[9].

Cassava is one of the very few tropical crops where cyanide content has not restricted its use as an important food for human consumption. This is because a large variety of processing techniques have been developed in different parts of the world resulting in a variety of products. The degree of reduction of cyanide in the final product varies greatly with the type of processing techniques used[6,7]. As the plant has a long history of cultivation and consumption in other parts of the world, different processing methods had developed to neutralize or to remove the toxin. But in Ethiopia the crop became popular very recently. The population of the south western part of Ethiopia has the dietary habit of consuming tubers and roots, unlike other parts of the country where cereals and legumes are the main staple foods. Although not given a priority, cassava is being produced and becoming a staple food for people in the study areas. Most people consume cassava in boiled form. Therefore the purpose of this study is to evaluate the effectiveness of washing, boiling, drying and fermentation in reducing cyanide level and improving nutritional value of cassava foods.

In this study the effect of peeling, taking out the center, washing, sun drying, grinding and fermenting on cyanide content were tested. Peeling, removing the center and washing 2-times reduced the cyanide content by about 38%. Peeling and boiling reduces over 60 % of the cyanide provided that the water used for boiling is disposed of. Combined use of peeling, washing and boiling reduced the cyanide to less than 15% of the original content. Peeling, chopping, sun drying on aluminium pan and grinding (electrical) removed almost 90% of the cyanide content in the original sample. Fermentation of cassava flour for 24-hr according to traditional customs for making 'Injera" (fermented pancake traditionally made from teff, *Eragrostis teff*, and fermented with some left-over dough from the previous fermentation) and "Dabbo" (a circular leavened bread) is found to be the best method in totally removing the cyanide content of cassava below detection limit as proved by both qualitative and quantitative test described by Bradbury[10]. The removal efficiency of the processing methods described here agrees with the results reported in by other authors[11].

The nutritional value of cassava blend with cereals and legumes was assessed using physicochemical and organoleptic test[3]. Fermentation is the best method in reducing the toxin to below detection limit. Moreover, blending of cassava with cereals and beans seem to increase the nutritional quality and value of cassava based foods apart from diluting the cyanogenic effect. Blending with cereals is very important to improve the level of sulfur containing amino acids needed for the metabolisation of cyanide into thiocyanate[12].

Recommendations- Solar drying and fermentation were found to be the best methods in removing cyanide content and detoxifying cassava based foods. Moreover, blending with cereals, legumes, vegetables and animal products in preparation of cassava based foods might play a great role in detoxification as well as increasing nutritional quality of cassava based foods. The investigation reported here shall be considered and implemented in the study areas as well as in areas where plantation of cassava is currently being promoted throughout the country. Since cassava is being promoted to northern dry-land and food insecure areas of the country, cassava blend of staple diets of these areas should be attempted in order to reduce the cyanide content, increase nutritional quality without affecting consumer acceptance. Legumes such as Grass pea and Chickpea might be used to increase the protein content of cassava based diets. But caution must be taken when consumed with grass pea which is very low in sulfur containing amino acids and has been associated with Neurolathyrism. Concerned governmental and non-governmental bodies shall collaborate in promotion of safer processing methods and nutrition education regarding cassava plantation, harvest, processing, detoxification and consumption based on accepted and registered manual to promote cassava utilization and make use of the underutilized benefits of cassava.

**References**


[1] Cassava Cyanide Diseases Neurolathyrism Network (CCDN) news (2011), 18, 1838-8825 (Online).

[2] CGIAR, Consultative Group on International Agricultural Research (2001). CGIAR Areas of Research: Cassava (*Manihot esculenta*).

[3] Abuye C., Berhane Y., Ersumo T. (2008) The role of changing diet and altitude on Goitre prevalence in five Regional States in Ethiopia. East Afr. J. Public Health 5(3), 163-168)

[4] Aweke K, Beka T. Asrat W., Adamu B., Birhanu W. and Aynalem L. (2012) Detoxification and Consumption of Cassava Based Foods in South West Ethiopia. Pakistan J. Nutrition 11 (3): 237-242.

[5] Taye, M. (2000) Some Quality Changes During Storage of Cassava Root, *The Journal of Food Technology in Africa* 5 (2), 64-65.

[6] Nhassico D., Muquingue H., Cliff J., Cumbana A., Bradbury,, J.H. (2008) Review Rising African cassava production, diseases due to high cyanide intake and control measures, *J Sci Food Agric* 88:2043–2049.

[7] Cardoso A.P., Ernesto M., Nicala, D., Mirione E., Chavane L., N'zwalo H., Chikumba S., Cliff J., Mabota,, A. P., Haque M. R., Bradbury, J. H. (2004) Combination of cassava flour cyanide and urinary thiocyanate measurements of school children in Mozambique, *International Journal of Food Sciences and Nutrition*, 55 (3), 183-190.

[8] Metre T.K. (2006) Cassava Consumption in South Kivu Province of DR Congo: For Survival or Death? In: Cassava Cyanide Diseases Network, CCDNNews 7.

[9] Abuye C., Kelbessa U., Wolde-Gebriel S. (1998) Health effects of cassava consumption in south Ethiopia, East Afr. Med. J. 75(3):166-70.

[10] Bradbury J.H., Egan S.V., Lynch M.J. (1991) Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. *J Sci Food Agri*. 55. 277-290.

[11] Cardoso, A.R., E. Mirione, M. Ernesto, F. Massaza, J. Cliff, M.R. Haque and H.J. Bradbury, (2005). Processing of cassava roots to remove cyanogens. J. Food Comp. Anal., 18: 451-460.

[12] Getahun H., Lambein F., Vanhoorne M., Van der Stuyft P. (2003) Food-aid cereals to reduce neurolathyrism related to grass-pea preparations during famine Lancet 362: 1808–10.



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## Control of konzo in DRC by reducing cyanide intake from bitter cassava

Konzo is an irreversible paralysis of the legs that occurs mainly in children and young women after childbirth, due to consumption of large amounts of cyanogens from bitter cassava[1,2]. The cyanogens are cyanogenic glucosides made by the plant to deter predators, and cyanohydrins produced by their decomposition catalysed by an enzyme linamarase, also made by the plant. On ingestion these cyanogens are mainly converted to poisonous cyanide in the body which can cause konzo and cyanide poisoning that may lead to death.

We have developed a wetting method to reduce greatly the total cyanide content of cassava flour, that simply involves leaving wet flour in a thin layer for 2 hours in the sun or 5 hours in the shade, to allow the enzyme present to break down the cyanogenic glucoside to hydrogen cyanide gas that escapes into the air[3-5]. This method was taught to the women in Kay Kalenge village in Popokabaka Health Zone, Bandundu Province, DRC, who accepted it gladly. There are 36 cases of konzo in Kay Kalenge. It reduced the total cyanide content of cassava flour and prevented the occurrence of any new konzo cases. Control of the paralytic disease konzo has thus been achieved for the first time, by a simple preventative strategy[6].

The same strategy has now been repeated with 3 villages (Imboso Mwanga, Ikusama and Ikialala) in Boko Health Zone, which is adjacent to Popokabaka Health Zone in Bandundu Province. These villages have a total population of 1315 and 61 konzo cases, with an average prevalence of 4.6%, compared with 2.7% in Kay Kalenge. The villages are located 10-20 km from a main road and two of them have been inaccessible by road since 2008. In July 2011 women leaders in the village were trained in the detoxification (wetting) method and preparation of the thick porridge (fufu) and they in turn trained 10-15 other women in the village. The families were provided with a knife, plastic basin and a mat needed to spread out the wet flour. The women accepted the method spontaneously and the fufu produced from the detoxified flour was found to be more delicious than that from untreated flour, which has a bitter flavour from the linamarin present.

On subsequent visits of the full team in November 2011 and February 2012 it was found that not all the women were using the method, particularly in Imboso Mwanga where 25% were not using the method, compared with about 6.5% in the other two villages. Total cyanide analyses of cassava flour[7] and urinary thiocyanate analyses of school children[8] (urinary thiocyanate measures cyanogen intake of the child over previous days) have all shown considerable reductions since the intervention in July, but the reductions for Imboso Mwanga are less than those for the other villages. This shows conclusively that (as expected) the effectiveness of the preventative strategy depends on the continued use by all the women of the detoxification method.

The good news is that there have been no new cases of konzo in the Boko villages since the intervention started in July 2011. There has been a reduction in the mean total cyanide content of cassava flour from 50 to 18 ppm and in the mean urinary thiocyanate content from 930 to 225 µmole/L urine. Current levels are still higher than those in Kay Kalenge village[6,] partly due to the fact that initial conditions were much worse in the Boko villages than in Kay Kalenge and also because the uptake of the detoxification method by the women is not as complete as at Kay Kalenge. It is hoped to improve the uptake in the final three months of the Boko village project, that has been funded by the Australian Agency for International Development (AusAID).

Konzo is known to occur in Bandundu, Kasai Occidental, Kasai Oriental and South Kivu Provinces of DRC, but the number of cases of the disease in DRC is unknown. A survey in 2009 by ACF (Action Against Hunger) in most of the health zones of Kwango District in Bandundu Province reported 2218 konzo cases[9], and we are currently conducting a konzo survey in Kwilu District in Bandundu Province. The simple prevention strategy used in Kay Kalenge[6] and in the Boko villages, will be used in further villages in DRC in 2012-3 and also in Nampula Province of Mozambique, supported by AusAID.

**References**


[1] Cliff J., Martensson J., Lundquist P., Rosling H. Sorbo B. (1985) Association of high cyanide and low sulphur intake in cassava induced spastic paraparesis. Lancet 11, 1211-1213.

[2] Howlett W.P., Brubaker G.R., Mlingi N. Rosling H. (1990) Konzo, an epidemic upper motor neuron disease studied in Tanzania. Brain 113, 223–235.

[3] Bradbury J.H. (2006) Simple wetting method to reduce cyanogen content of cassava flour, J Food Comp Anal, 19, 388-393.

[4] Cumbana A., Mirione E., Cliff J. Bradbury J.H. (2007) Reduction of cyanide content of cassava flour in Mozambique by the wetting method. Food Chem 101, 894-897.

[5] Bradbury J.H., Denton I.C. (2010) Rapid wetting method to reduce cyanogen content of cassava flour. Food Chem., 121, 591-594.

[6] Banea M., Nahimana G., Mandombi C., Bradbury J.H., Denton I.C., Kuwa N. (2012) Control of konzo in DRC using the wetting method on cassava flour. Food Chem. Toxicol. 50, 1517-1523.

[7] Bradbury M.G., Egan S.V., Bradbury J.H. (1999) Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. J. Sci. Food Agric. 79, 593–601.

[8] Haque M.R. Bradbury J.H. (1999) Simple method for determination of thiocyanate in urine. Clin. Chem. 45, 1459-1464.

[9] Kasongo E., Calo M. (2011) A cross-sectoral approach to addressing konzo in DRC. Field Exchange, Issue 41, P 2-4.



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## *Lathyrus* research in Portugal

*Lathyrus* spp. in Portugal are nowadays underutilized crops. The main species grown are *L. sativus* (grass pea) for food and *L. cicera* (chickling vetch) for feed.

Important in the past for the poorer population and overlooked by the wealthy, grass pea has been gaining a new niche as a traditional delicacy, with a special place on the menu of trendy restaurants (normally stewed with different kinds of meat and vegetables) in the southern part of the country and serving as main theme for a couple of annual gastronomic festivals in the central region of Portugal. There are no records of neurolathyrism outbreaks during historical times in Portugal. This contrasting situation with our neighbour Spain, is probably because Portugal has never faced such extreme hardship times and famine as the one felt during the civil war in Spain, and due to that grass pea has never been used as a staple food for the Portuguese. The only known available cultivated grass pea germplasm are traditional landraces selected by the farmers. Farmers produce it mainly for their own consumption, selling only the surplus. Therefore grass pea can only be found in Portugal in some local markets in low quantities. Chickling vetch on the other hand has a high potential to conquer the big animal farmers from the Central-south of Portugal, as an alternative crop for the more marginal lands. The National Institute of Agronomic Research (Instituto Nacional de Investigação Agrária - INIA) has registered two *L. cicera* cultivars “grão de gramicha” and “grão de comenda”[1], to be used as animal feed and fodder crop, known to perform well in these regions.

*Lathyrus* spp. have considerable potential on neutral to alkaline soils. Not accidentally, in the country areas were *Lathyrus* spp. are known to be grown, the pH values of the soils range from 6.6 to 8.5 (Figure 1).

In Portugal, as in the rest of Europe there has been increasing emphasis on local production of legumes. Initially, this was mainly meant for animal feed, to avoid the hazardous use of animal based cattle feed (linked to “mad cow disease”) and the importation of soya meal, but lately also an increased diversity of protein sources for human consumption is sought due to the growing concern about healthy food habits. Chickling vetch and grass pea fulfill these feed and food purposes. Their cultivation is also justified by the need to recover marginal lands (as through green manuring). Due to the above, breeding efforts are needed to supply the Portuguese farmers with highly productive, locally adapted, sustainable varieties.

With the aim to provide biotechnological support to breeding programmes we develop research on several *Lathyrus* species, presently with support from the national FCT funding (PTDC/AGR

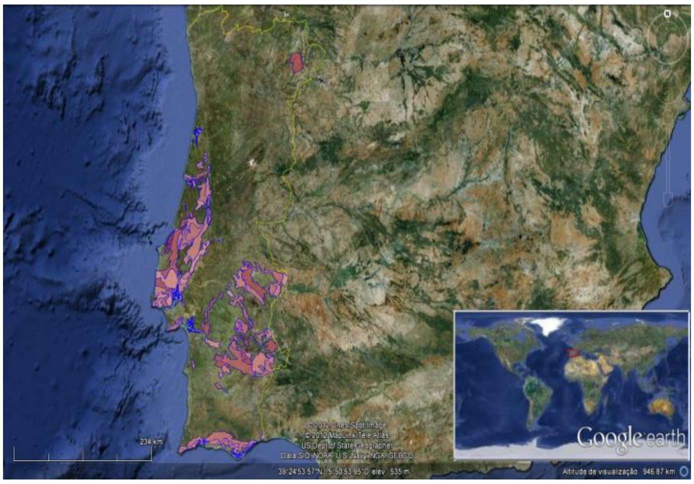


**Figure 1 – Neutral to alkaline soil areas in Portugal identifying the potential *Lathyrus* spp. production areas. Google earth plugin with soil pH values acquired at http://googleearthpt.blogspot.pt/2009_04_01_archive.html (accessed 31-05-2012) based on the report by Câmara-Freitas[11].**

GPL/103285). We are characterizing the physiological responses of different *Lathyrus* spp. to drought[2,3], developing new molecular tools for *L. sativus* and *L. cicera*[4] and adapting tools developed for the model legume specie *Medicago truncatula* and major crops like pea, lentil and faba bean to *Lathyrus* spp.[5]. Combined with the rust, powdery mildew and *Ascochyta* resistance evaluation that has been done in the past[6-10], these new biotechnological tools are allowing the identification of the underlying candidate resistance genes. These genes will speed up a more efficient resistance breeding. The transferred tools will also enable more fundamental comparative mapping and synteny studies between grass pea and chickling vetch and the other legume crops.

In the future we intend to support the development of a national *Lathyrus* grower's net to disseminate the accumulated scientific knowledge to the real end users, invest on the quality aspects that they value most, and assist on eventual participatory selection to increase quality and tackle their major constrains.

**References**


[1] Veloso M. (2008) State of Plant Genetic Resources for Food and Agriculture in Portugal - Second Portuguese National Report on Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture, Instituto Nacional de Recursos Biologicos, Oeiras.

[2] Silvestre S., Araújo S., Vaz Patto M.C., Marques da Silva J. (2011a) Comparison of Water Stress Resistance in *Lathyrus* spp., In: Proceedings of the Model Legume Congress, 15-19 May, Sainte-Maxime, France.

[3] Silvestre S., Araújo S., Vaz Patto M.C., Marques da Silva J. (2011b) Drought Effects in the Water Relations of 10 Genotypes of *Lathyrus* spp.. XII Congresso Hispano-Luso de Fisiologia Vegetal. Sociedad Española de Fisiología Vegetal (SEFV). 21-24 June, Universitat Jaume I de Castellón, Spain.

[4] Almeida N.F., Leitão S.T., Rotter B., Winter P., Rubiales D., Vaz Patto M.C. (2011) Development of molecular tools for *Lathyrus cicera* using normalized cDNA libraries, In: Proceedings of the 9th Plant Genomics European Meeting (Plant GEM), 4-7 May 2011, Istanbul, Turkey. pp. 26.

[5] Almeida N.F., Caminero C., Vaz Patto M.C. (2008) Cross-amplification of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) microsatellites in grass pea (*Lathyrus sativus* L.), In: Proceedings of the the 4th International Conference on Legume Genomics and Genetics, 7-12 December 2008, Puerto Vallarta, Mexico.

[6] Almeida N.F., Rispail N., Vaz Patto M.C., Rubiales D. (2010) Where do *Ascochyta* isolates infecting *Lathyrus* species stand on a grain legume-associated *Ascochyta* phylogenetic study?, In: Proceedings of the 5th International Food Legume Research Conference (IFLRC) & 7th European Conference on Grain Legumes (AEP), 26-30 April 2010, Antalya, Turkey.

[7] Vaz Patto M.C., Rubiales D. (2009) Identification and characterization of partial resistance to rust in a germplasm collection of *Lathyrus sativus* L. Plant Breeding 128:495-500.

[8] Vaz Patto M.C., Fernández-Aparicio M., Moral A., Rubiales D. (2006) Characterization of resistance to powdery mildew (*Erysiphe pisi*) in a germplasm collection of *Lathyrus sativus*. Plant Breeding 125:308-310.

[9] Vaz Patto M.C., Fernández-Aparicio M., Moral A., Rubiales D. (2007) Resistance reaction to powdery mildew (*Erysiphe pisi*) in a germplasm collection of *Lathyrus cicera* from Iberian origin. Genetic Resources and Crop Evolution 54:1517-1521.

[10] Vaz Patto M.C., Fernandez-Aparicio M., Moral A., Rubiales D. (2009) Pre and posthaustorial resistance to rusts in *Lathyrus cicera* L. Euphytica 165:27-34.

[11] Câmara-Freitas F. (1984) Acidez e Alcalinidade dos Solos / Notícia Explicativa (III.2), Portugal. Atlas do Ambiente, Comissão Nacional do Ambiente, Lisboa.



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