

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



CCDNN

Cassava Cyanide Diseases & Neurolethyrism Network

News

Issue Number 14, December 2009

Contents

Ghent Workshop	1
Committees	1
Program	2
Foreword	3
Keynote Lecture	3
Epidemiological and nutritional aspects abstracts	4
Toxicological aspects and other risk factors abstracts	8
Neurobiological aspects abstracts	13
Breeding and biotechnological approach for crop improvement abstracts	17
Recommendations	22
Author and E-mail index	24

As a result of the relationship between konzo and neurolethyrism and the recent Workshop held in Ghent, Belgium, we have decided to expand the Cassava Cyanide Diseases Network to include neurolethyrism. The name of the expanded Network is the Cassava Cyanide Diseases & Neurolethyrism Network (CCDNN). The CCDNN is a free worldwide network which is working towards the elimination of cyanide poisoning, konzo, tropical ataxic neuropathy (TAN) and neurolethyrism.

The Newsletter of the CCDNN is now called the Cassava Cyanide Diseases & Neurolethyrism News (CCDN News). This issue is No 14 in the series that commenced in June 2003.

In this issue we are privileged to publish the abstracts of the papers given at the recent Workshop on konzo and neurolethyrism as well as the recommendations from the Cassava/konzo – Grass pea/neurolethyrism Group.

We plan to expand the CCDNN, to include those interested in neurolethyrism, and also CCDN News to make it a venue for the publication of short articles and letters (1-3 pages A4 double spaced) written in English.

WORKSHOP ON TOXICO-NUTRITIONAL NEURODEGENERATIONS KONZO AND LATHYRISM

September 21-22, 2009
Ghent, Belgium

COMMITTEES

Conveners

J.Howard Bradbury (Australian National University, Canberra, Australia)
Fernand Lambein (Ghent University, Belgium)

Steering Committee

Marc Van Montagu (Institute of Plant Biotechnology for Developing Countries, IPBO, Ghent University, Belgium), J.Howard Bradbury (Australian National University, Canberra, Australia), Luc Leybaert (Faculty of Medicine and Health Sciences, Ghent University, Belgium), Patrick Van der Stuyft (Institute for Tropical Medicine, Antwerp, Belgium), Geert Angenon (Department of Plant Genetics, VUB, Brussels, Belgium), Godelieve Gheysen (Faculty of Bioscience Engineering, Ghent University, Belgium)

Scientific Committee

J.Howard Bradbury (Australian National University, Canberra, Australia), Fernand Lambein (IPBO, Ghent University, Belgium), Jean-Pierre Banea-Mayambu (University of Kinshasa, DR Congo), Yu-Haey Kuo (IPBO, Ghent University, Belgium), Peter Nunn (Portsmouth

CCDN Coordinator:

Dr. J. Howard Bradbury
Botany and Zoology, Research School of Biology,
Australian National University
Canberra ACT 0200, Australia
Phone: +61-2-6125 0775
E-mail: howard.bradbury@anu.edu.au

Coordinating Group:

J.P.Banea, Julie Cliff, Arnaldo Cumbana, Ian Denton, Fernand Lambein, N.L.V.Mlingi, Humberto Muquingue, Bala Nambisan, Dulce Nhassico, S.L.N. Rao

Country Contacts:

Cameroon: E.E. Agbor;
D.R. Congo: D.D. Ngudi;
Indonesia: A. Hidayat;
Mozambique: Anabela Zacarias;
Nigeria: M.N. Adindu and P.N. Okafor;

Website:

www.anu.edu.au/BoZo/CCDN

University, U.K.), S. L. N. Rao (Osmania University, Hyderabad, India), Dulce de Oliveira (IPBO, Ghent University, Belgium and Brazil), Delphin Diasolua Ngudi (IPBO, Ghent University, Belgium), Marijke Van Moorhem (Faculty of Medicine and Health Sciences, Ghent University, Belgium).

Local Organizing Committee
Fernand Lambein, Yu-Haey Kuo, Sylvia Burssens, Delphin Diasolua Ngudi, Marijke Van Moorhem, Katerine Kotsirkof and Véronique Kervyn de Meerendré.

PROGRAM

Monday September 21

Opening

Registration + mounting posters

Opening speech by professor em. Dr. Marc Van Montagu (IPBO, Ghent University, Belgium)

General Introduction by Prof. Dr. Fernand Lambein (IPBO, Ghent University, Belgium)

Keynote speaker

S.L.N. Rao (Hyderabad, India):
"A look at the brighter facets of ODAP, Homoarginine and the Grass Pea"

Coffee break and Poster session

Session 1: Epidemiological and nutritional aspects

Chair: Prof. H. Rosling
Co-chair: Prof. P. Nunn

Darna Dufour (University of Colorado, USA):
"Methods for the collection of dietary data in konzo and lathyrism prone populations"

Julie Cliff (Universidade Eduardo Mondlane, Maputo, Mozambique):
"Konzo and continuing cyanide intoxication from cassava in Mozambique"

Nicholas Mlingi (Tanzania Food and Nutrition Centre, Dar es Salaam, Tanzania):
"Re-occurrence of konzo disease in cassava consuming districts in Southern Tanzania"

Lunch break

Tylleskar Thorkild (Centre for International Health, Bergen, Norway):

"Dietary intake and sulphur nutrition in a konzo-affected population in DR Congo"

Jean-Pierre Banea-Mayambu (Programme National de Nutrition, Kinshasa, DR Congo):

"Bitter cassava consumption and konzo in DRC"

Presentation of selected posters

Coffee break and Poster session

Session 2: Toxicological aspects and other risk factors

Chair: Prof. S. L. N. Rao
Co-chair: Dr. L. Chiwona Karltun

Howard Bradbury (Australian National University, Canberra, Australia): (presented by Julie Cliff)
"Uptake of simple wetting method in Africa to reduce cyanide poisoning and konzo from cassava"

Haileyesus Getahun (Ethiopia):
"Risk and protective factors for neuropathy in Ethiopia"

Leon Brimer (University of Copenhagen, Denmark):
"Chemical food safety of cassava products in geographical regions new to cassava production and processing. Examples from Africa"

Presentation of selected posters

Coffee break and Poster session

Panel discussion open to the public and the press:

"Neglected diseases: Who or what to blame?"

Moderators: Prof. M. van Montagu and Prof. H. Rosling

Conference dinner

Tuesday September 22

Session 3: Neurobiological aspects

Chair: Prof. L. Leybaert
Co-chair: Dr. H. Getahun

Peter Nunn (University of Portsmouth, U.K.):
"Dietary and plasma sulphur-containing amino acids as factors in neurological diseases"

Kuniko Kusama-Eguchi (Nihon University, Japan):

"An improved neuropathy rat model: impaired VEGF systems have a critical role in the degeneration of motor neurons"

Desire Tshala-Katumbay (Oregon Health and Science University, USA):

"On the mechanisms and biomarkers of konzo"

Coffee break and Poster presentation

Marijke Van Moorhem (Ghent University, Belgium):

"Unraveling the mechanism of beta-ODAP induced excitotoxicity and oxidative stress, relevance for neuropathy prevention"

Dirk Enneking (Waite Agricultural Research Institute, Adelaide, Australia):

"A suitable bioassay is needed to model lathyrism in mono-gastric animals"

Jordi Llorens (Universitat de Barcelona, Spain):

"A new unifying chemical hypothesis on lathyrism, konzo and tropical ataxic neuropathy"

Presentation of selected posters

Lunch break

Session 4: Breeding and biotechnological approach for crop improvement

Chair: Prof. G. Gheysen
Co-chair: Prof. M. Tate

Geletu Bejiga (ICARDA, Aleppo, Syria) (presented by S. K. Agrawal):
"Integrated management of grass pea to reduce risk of lathyrism"

Bala Nambisan (Central Tuber Crops Research Institute, Trivandrum, India):

"Elimination of anti-nutritional factors for quality improvement of cassava"

Chongying Wang (Lanzhou University, China):

"The factors influencing β -ODAP content and their possible mechanisms in grass pea (*Lathyrus sativus* L.)"

Presentation of selected posters

Coffee break and Poster presentation

Round table discussion:

Priorities for future research for the prevention of konzo and lathyrism. Policy recommendations.

Moderator: Ashutosh Sarker (ICARDA)

Closing remarks + refreshments

Foreword

It is intriguing that two diseases with very similar clinical symptoms seem to be caused by the consumption of totally different plant foodstuffs. The occurrence of konzo is associated with consumption of the carbohydrate rich roots of *Manihot esculenta* (cassava) of the plant family Euphorbiaceae. The occurrence of lathyrism is associated with consumption of the protein rich seeds of the legume *Lathyrus sativus* (grass pea, Fabaceae). Identification of cyanogenic glucosides in cassava roots as toxin of the respiratory chain and a neuro-active amino acid in the seeds of grass pea does not bring the aetiology of these diseases much closer.

Epidemiology of the two diseases is more similar as both occur mainly among very poor rural people with little or no formal education, living as subsistence farmers on drought prone and marginal lands. Drought or other causes of food insufficiency leads them to consumption of either bitter cassava roots, and cassava flour or grass pea seeds as an almost exclusive staple food for extended periods. During droughts, both the cyanogens in cassava roots and the neuro-active amino acid in grass pea seed can double in concentration. Consumers who buy cassava roots or grass pea seeds at local markets are rarely affected. Apparently the food supply at the markets has more variation than what subsistence farmers harvest during periods of food insufficiency. The greater incidence of konzo among women of child bearing age, and of lathyrism among young men is not yet adequately explained. A cheap and reproducible animal model for konzo and lathyrism could bring the answers to such persistent problems much closer.

Droughts can trigger epidemics of konzo as well as lathyrism. Recent epidemics of konzo in Mozambique and

Tanzania, epidemics of lathyrism in Bangladesh (1980's), India (1980's) and Ethiopia (1980's and 1990's) have all occurred due to drought. Political and military instability is another factor that has facilitated the occurrence of konzo in D.R. Congo, Mozambique and Central African Republic, as well as cases of lathyrism during the second world war in Europe. Both drought and war reduce the amount of post harvest processing below what is required to ensure safe consumption. Both drought and war also reduce the number of different food items available in the diet.

As of now, there is no geographic overlap of cassava production and grass pea cultivation. Demographic pressure and global warming may be responsible for the increasing consumption of cassava in much of Africa and for the increasing area of grass pea cultivation in Ethiopia. While the exact aetiology of konzo and lathyrism is not completely understood, the result of consumption of either bitter cassava or grass pea in an unbalanced diet is so similar that a potential combination of the two foodstuffs could be predicted as a "worst case scenario". With regard to konzo there are three approaches to reduce its incidence. These are broadening of the diet, development by plant breeding and transgenic technology of new cultivars of low cyanogen content and increased removal of cyanogens through better processing. The same or similar approaches can be considered for lathyrism. Transgenic technology can be applied for the reduction of cyanogens in cassava and the reduction of the neurotoxin in grass pea, and perhaps of equal importance, to improve the content of essential amino acids and micronutrients.

The workshop is intended as an interface between researchers on konzo and on lathyrism to facilitate the identification of common ground. The workshop is also intended as an interface between agronomists and bio-medical researchers. Agronomists are developing the crop in order to satisfy the interests of farmers and the health of consumers, while bio-medical researchers study the molecular events in the aetiology of these diseases at a cellular or sub-cellular level or in animal models. Both groups are increasingly using

biotechnological techniques but normally do not communicate with each other in person or through the literature. However, they need each other's information and opinion to come to an integrated approach to solve the problem and to prevent these diseases.

We hope that during this workshop the community of cassava/grass pea researchers will widen their view and deepen their understanding of these neglected diseases, konzo and lathyrism. Both diseases, once established, are irreversible and there is no cure, no medicine and hence no interest from the pharmaceutical industry, that normally is a major sponsor of medical research. Perhaps this is one reason for their status as neglected diseases. It is expected that the round table discussion will come up with proposals to give guidance to the younger generation of researchers and stakeholders on how to prevent these unfair diseases of the poor, while at the same time improving the socio-economic conditions of farmers on the ever increasing areas of marginal land, boosted by global warming.

We wish all participants a fruitful meeting with productive discussions and increased networking.

J. Howard Bradbury and
Fernand Lambein
September, 2009

Keynote Lecture

A LOOK AT THE BRIGHTER FACETS OF ODAP, HOMOARGININE AND THE GRASS PEA

S.L.N. RAO

Professor of Biochemistry (Retd), Osmania University, Hyderabad, 500007 (India)

It is now fairly clear that neurolathyrism is the result only of an excessive consumption of *L. sativus* for prolonged periods. However, vast populations are traditionally consuming the pulse over many years as a part of their diet without any apparent ill effects, with the result that the ban on its cultivation and sale are being relaxed (in India). It is appropriate at this juncture to examine in the context of some recent

developments, the likely benefits that might result from the use of *L. sativus* as a part of a regular cereal based diet.

Humans alone are endowed with some yet uncharacterised but unique mechanism to detoxify/metabolise orally ingested ODAP and this may well be the reason for the low incidence of neurolathyrism. Humans excrete less than 1% of ingested ODAP compared to animals. Metabolic oxidation of ODAP may be a likely pathway in humans and thus a natural detoxifying mechanism.

L-homoarginine, the other unusual amino acid in *L. sativus* is a substrate for nitric oxide synthase (NOS) and also inhibits liver arginase and is itself a poor substrate. Homoarginine thus stays in circulation much longer than arginine and may contribute to a sustained nitric oxide (NO) generation in addition to providing essential lysine. Though NO is both a good and bad messenger, endogenous overproduction of NO is a remote possibility in humans and thus, *L. sativus* in limited quantities as part of a normal diet may be beneficial to the general vasculature and overall performance. Hard physical labour is in fact a hallmark of *Lathyrus* consumers. *L. sativus* may be an effective natural alternative in dietary management and control of cardiovascular health and performance instead of the numerous arginine and citrulline formulations and allied products in the market. Homoarginine also inhibits the synaptosomal uptake of glycine and may even benefit long term potentiating (LTP) at the synapse.

Hypoxia-inducible factor (HIF-1 α) is an inducible transcription factor and a master regulator of cellular oxygen homeostasis. Very recent findings show that L-ODAP stimulates the stabilization and nuclear translocation of HIF-1 α in SK-N-MC human neuroblastoma and MCF7 cells under normoxic conditions and also in chick brain in a time dependent manner as shown by transient transfection and western blot analysis. The nuclear translocation of HIF-1 α is inhibited by staurosporine indicating the involvement of protein kinase C (PKC). Since HIF-1 α is a global regulator of hypoxic gene expression and a transcription factor for more than a dozen target genes

and its activation however, could be both pro-survival and pro-apoptotic. HIF-1 is increasingly studied for its perceived therapeutic potential as it promotes angiogenesis by upregulating vascular endothelial growth factor (VEGF) and enhancement of this gene within ischemic patients could promote vessel proliferation very much needed for oxygenation. PKC activation by ODAP could be a target for amelioration of hypoxia faced at high altitudes and also of cognitive impairment in Alzheimer's disease.

L. sativus is an efficient nitrogen fixer and is also a very heavily nodulated legume. ODAP is reportedly a derepressor of nitrogenase in certain nitrogen fixing bacteria and selectively enhances GSII activity and can derepress nitrogen fixation in free living Rhizobia. The role of ODAP in plant nitrogen fixation needs to be explored further.

Epidemiological and nutritional aspects

METHODS FOR THE COLLECTION OF DIETARY DATA IN KONZO AND LATHYRISM PRONE POPULATIONS

D. L. Dufour

Department of Anthropology, University of Colorado, Boulder, Colorado (USA)

OBJECTIVES: Evaluate the usefulness of different methods for the collection of dietary intake information.

BACKGROUND: A number of methods have been developed to collect information on food intake. Interview based methods like food frequency questionnaires (FFQs) and 24-hour recalls are easy to administer and can provide a general picture of the diet. However, both are subject to recall bias and provide only crude estimates of the quantities of foods ingested. In addition, FFQs require validation in each cultural setting. Food records (estimated or weighed) are more time consuming to administer, but avoid recall bias and provide accurate estimates of food quantities. If the goal is to assess

the dietary intake of specific nutrients and non-nutrients (toxins), food records are the method of choice. Sample size needs to be adequate to adjust for day-to-day and season variability in the intake of different foods. Adequate data on the composition of foods in their ready-to-eat form are also needed. This is especially important in the cases like that of cassava where cyanide content is a function of the value in the crop as harvested and the processing applied.

RESULTS: Accurate estimates of the dietary intake of toxins like cyanide and beta-ODAP as well as substances required for metabolic detoxification require the use of food records, adequate sample sizes and food composition data.

KEYWORDS: dietary intake methods, cassava, cyanide.

KONZO AND CONTINUING CYANIDE INTOXICATION FROM CASSAVA IN MOZAMBIQUE

J. Cliff¹, H. Muquinque², D. Nhassico², H. Nzwalo³, J.H. Bradbury⁴

¹ Department of Community Health, Faculty of Medicine, Universidade Eduardo Mondlane, Maputo (Mozambique)

² Department of Physiological Sciences, Faculty of Medicine, Universidade Eduardo Mondlane, Maputo (Mozambique)

³ Hospital Central, Faro (Portugal)

⁴ School of Botany and Zoology, Australian National University, Canberra (Australia)

BACKGROUND: In Mozambique, konzo was first reported in the northern provinces of Nampula and Cabo Delgado in 1981, when a large epidemic (over 1100 reported cases) occurred during drought. In 1992-93, a further large epidemic (over 600 cases) was reported in districts further south in Nampula Province, associated with war. More recently, in 2005, over 100 cases were reported in Zambezia province in central Mozambique, in a drought-associated epidemic. Smaller epidemics have occurred along the years, and sporadic cases continue to be reported in epidemic areas. Konzo is now persistent.

RESULTS: Epidemics have always occurred at times of agricultural crisis, during the cassava harvest, when the population has been dependent on a diet of insufficiently processed bitter cassava. Sporadic

cases occur in the poorest families, with konzo now passing down through generations. Konzo mostly affects women of child-bearing age and children over two years of age. The clinical picture is monotonous, with a presentation of irreversible, non-progressive spastic paraparesis of acute onset and varying severity. When measured, blood or urinary thiocyanate concentrations have always been high in konzo-affected patients or populations, and sulphur amino acids concentrations have been low. In non-epidemic years, concentrations of urinary thiocyanate remain high in patients and the population at the time of the cassava harvest.

CONCLUSIONS: Programs to prevent konzo have focused on distributing less toxic varieties of cassava and disseminating new processing methods, such as grating and the flour wetting method. There have been also attempts to rehabilitate konzo sufferers through physiotherapy using local, basic resources. Attention should also be given to the wider question of agricultural development and food security in the regions of Africa where dependence on bitter cassava results in chronic cyanide intoxication and persistent and emerging konzo.

KEYWORDS: konzo, cyanide, cassava, Mozambique.

RE-OCCURRENCE OF KONZO IN CASSAVA CONSUMING DISTRICTS IN SOUTHERN TANZANIA

N. L. V. Mlingi¹, S. Nkya¹, S. R. Tatala¹, H. Ngude², J. H. Bradbury³

1 Tanzania Food and Nutrition Centre (TFNC), P.O. Box 977, Dar es Salaam (Tanzania)

2 Tanzania Red Cross Society, P.O. Box 1133, Dar es Salaam (Tanzania)

3 School of Botany and Zoology, Australian National University, Canberra, ACT 0200 (Australia)

BACKGROUND: The first outbreak of konzo in Tanzania was in the north in 1984-1985 where 39 cases were diagnosed in three villages of Tarime district. The second outbreak occurred in southern Tanzania with three cases in Masasi district in 1988. In all cases there was an association between cassava consumption and konzo outbreak.

The outbreaks were due to food shortage in rural areas after a prolonged drought. A similar situation occurred in Mkuka village, Mbinga district, southern Tanzania in 2001-2002 where 24 cases were diagnosed in equal numbers of children and women of child bearing age.

Another outbreak occurred during the 2002-2003 drought, where 214 cases were reported in Mtwara region in southern Tanzania. These were spread over 13 villages in Mtwara rural district and five villages in Newala district. The cases in Mbinga, Mtwara and Newala districts were followed up in 2008 for rehabilitation by screening 497 subjects of whom 136 subjects needed assistance in walking. Sixty were given crutches and two given wheelchairs.

RESULTS and CONCLUSIONS: Observations and recommendations made by the screening team included the following:

1. The konzo problem is made worse by some villagers through ignorance in eating improperly processed cassava. Also food insecurity due to lack of rains leads to use of bitter cassava.
2. The wetting method is the simplest way to prepare cassava flour before cooking, it was well accepted by women activists and should be disseminated country wide to all who use cassava flour.
3. There is a need for cyanide test kits from Australia for health officers in konzo-prone districts to do regular monitoring of cyanide in cassava flour during regular village visits.
4. More workshops are needed on the wetting method and improved processing of cassava roots and testing using cyanide kits.
5. More collaboration between TFNC, Tanzania Red Cross Society and AusAID.

KEYWORDS: Konzo, cassava consumption, crutches, Tanzania, wetting method.

DIETARY INTAKE AND SULPHUR NUTRITION IN A KONZO-AFFECTED POPULATION IN D. R. CONGO

T. Tylleskar

BITTER CASSAVA CONSUMPTION AND KONZO IN KAHEMBA TERRITORY, BANDUNDU PROVINCE, DRC

J. P. Banea-Mayambu¹, D. Nahimana Gitebo¹, A. Mulumba Nkuadi²

1 Programme National de Nutrition, Ministère de la santé Publique, Kinshasa (DRC)

2 Institut Supérieur des Techniques médicales, Kinshasa (DRC)

OBJECTIVE: Association between consumption of insufficiently processed bitter cassava roots and konzo occurrences in Kahemba, Bandundu province, DRC.

BACKGROUND: Bitter cassava is the main staple food and also the most important cash crop in Bandundu province in DRC. Earlier studies in poor rural areas of Bandundu have associated monotonous and prolonged consumption of insufficiently processed bitter cassava roots with konzo, a paralytic disease affecting mostly women and children. In 2008, several konzo occurrences were reported in the territory of Kahemba in Bandundu province and health authorities in Kinshasa are willing to conduct an intervention project focusing on safe cassava processing and consumption. To confirm the association of consumption of bitter, insufficiently processed cassava with konzo, a rapid nutrition survey was conducted in one of the affected villages in Kahemba territory in February 2008.

RESULTS: Interviews with medical officers revealed 1047 cases of konzo from 2004 to 2009. In the village studied, of 840 inhabitants 42 cases of konzo were identified, of which 31 appeared before 2008, 8 in 2008 and 3 in 2009. The aetiology of konzo was not well known by the affected population. *Zeku*, a local bitter variety of cassava, was the main staple food. Roots were processed by soaking during two days followed by sundrying for 1-3 days. Food was monotonous and dominated by the consumption of the *zeku* paste with cassava leaves; while meat and legumes were rare.

CONCLUSIONS: Consumption of insufficiently processed cassava has been associated with konzo in several remote areas in Bandundu. This may be the same case for konzo in Kahemba. Like elsewhere,

a nutrition education program integrated in a health functional system can be of a durable effectiveness in the prevention of konzo in Kahemba.

KEYWORDS: bitter cassava, konzo.

SPASTIC PARAPARESIS IN BURHINYI: EVIDENCE FOR THE FIRST OUTBREAK OF KONZO IN EASTERN DEMOCRATIC REPUBLIC OF CONGO (DRC)

J. N. Chabwine^{1,5,6,7}, O. C. Masheka^{1,3,4}, B. Maheshe^{1,3}, B. Rutega², K. Mutendela³, M. J. Bonnet⁴, S. Balegamire¹, J. M. Lola², S. Z. Balol'ebwami¹, O. Shangalume², J. M. Balegamire^{5,6}, B. Nemery⁵

1 Faculty of Medicine, Université Catholique de Bukavu (UCB) (DR Congo)

2 Faculty of Agronomy, UCB (DR Congo)

3 Hôpital Provincial de Référence de Bukavu (DR Congo)

4 Bureau Diocésain des Oeuvres Médicales, Bukavu (DR Congo)

5 Katholieke Universiteit Leuven, Occupational, Environmental and Insurance Medicine (Belgium)

6 NGO Africa Tomorrow

7 University Hospital of Geneva (Switzerland)

BACKGROUND: Like other eastern provinces of the DRC, South-Kivu has been the site of war and violent conflicts for more than a decade. The appearance of paralytic disease in Burinhyi, a populous area in South-Kivu, led to the hypothesis that this represented an outbreak of konzo, an irreversible upper motor neuron disease caused by the consumption of insufficiently processed cassava containing cyanogenic glycosides in nutritionally compromised people.

Two field surveys were conducted, in 2003 and 2005, using questionnaires and clinical examinations. Urinary thiocyanate (a marker of cyanide intake) was measured, as well as cyanide concentrations in processed cassava roots obtained from patients.

RESULTS: We identified 41 subjects (mean age 16 y) meeting the clinical criteria of konzo, yielding a prevalence of at least 6 per 10,000 people in 2005. Most patients showed evidence of chronic malnutrition. Urinary thiocyanate concentrations were elevated (mean 13 µg/L, range 2-69 µg/L) and several cassava samples contained

high cyanide levels (median 20 ppm, range 5-300 ppm). The shortened cassava processing did not efficiently reduce cyanide concentration. Interestingly, when prolonged for 24 more hours, the same cassava treatment allowed cyanide lowering below the WHO toxicity threshold.

CONCLUSIONS: The surveys and analyses confirm that the disease was indeed konzo, a condition never before reported in the area. The outbreak was triggered by food shortages and resulting shortcuts in the processing of the main staple food, cassava, because of the longstanding state of insecurity. Contributory factors include the introduction of varieties of bitter cassava and climate change, leading to soil erosion and prolonged drought. All these factors are still present in eastern Congo and there are indications that the outbreak of konzo is still ongoing and spreading. As for the prevention of cassava-derived cyanide intoxication, a brief extension of cassava processing time appears to be one possible solution.

KEYWORDS: konzo, cassava, malnutrition, war, Burhinyi.

AN OUTBREAK OF KONZO AMONG REFUGEES FROM CENTRAL AFRICAN REPUBLIC IN PROVINCE DE L'EST IN CAMEROON

I. Ciglenecki, R. Eyema, C. Kabanda, F. Taafu, V. Urbaniak

Medecins Sans Frontieres, 78 Rue de Lausanne, 1202 Geneva (Switzerland)

OBJECTIVES: Description of patients seen in MSF mobile clinics during an outbreak of konzo in Province de l'Est in Cameroon

BACKGROUND: Around 80'000 refugees from Central African Republic (CAR) had sought refuge in Cameroon since 2005, fleeing the insecurity caused by the conflict between rebel groups and national army, and regular attacks of armed bandits. MSF, an international non-governmental organization, was working in Province de l'Est from July 2007 on, providing nutritional assistance and medical care to the refugees. Mobile clinics were organised on a weekly basis at the refugee sites. Cases of konzo were

identified at the mobile clinics. Patients were classified as konzo cases if they had a history of sudden onset of spastic paralysis. Basic information about the history and clinical presentation was recorded on patient's cards. Patients were provided with nutritional supplements and selected cases were referred for physiotherapy in a rehabilitation center in a nearby town.

RESULTS and CONCLUSION: Between September 2007 and November 2008, 469 patients were diagnosed with konzo in the mobile clinics organized by MSF. Most of the patients developed the disease after 2007. New cases were arising throughout the year without a clear seasonal pattern. The majority (80%) were refugees from CAR. 82% of patients were aged between 5 and 30 years. Male to female ratio was 0.4. Number of male and female patients was similar in the age group below 15 years (male/female ratio 0.8), while in the age group above 15 years most of the patients were women (male/female ratio 0.2). Almost all patients complained about functional impairment of lower limbs, weight loss and muscle wasting. Over two thirds of patients reported vomiting and abdominal pain at the time of the onset of paralysis. 18% of patients also reported vision problems, and 8% reported hearing problems.

KEYWORDS: konzo, spastic paralysis, refugees.

KONZO AND DIETARY PATTERN IN CASSAVA-CONSUMING POPULATIONS OF POPOKABAKA, DEMOCRATIC REPUBLIC OF CONGO

D. Diasolua Ngudi^{1,2}, J. P. Banea-Mayambu¹, F. Lambein², P. Kolsteren³

1 Programme National de Nutrition, Kinshasa (D.R.Congo)

2 IPBO, Instituut Plantenbiotechnologie voor Ontwikkelingslanden, Ghent University, Ghent (Belgium)

3 Nutrition and Child Health Unit, Institute of Tropical Medicine, Antwerp (Belgium)

OBJECTIVES: Identification of konzo cases and description of associated household factors and

the dietary pattern in cassava consuming populations.

BACKGROUND: In sub-Saharan Africa, the paralytic disease konzo affects thousands of children and women of childbearing age. A study was conducted during the harvest period (February). The WHO criteria for konzo were applied for 3015 family members in 487 families to detect cases and to confirm the diagnosis. Food frequencies and 24h diet recall were registered from 487 randomly selected heads of household in Popokabaka rural health zone, DRC. Cassava flour ready to prepare luku, the cassava flour stiff porridge, for 135 persons distributed in 19 randomly selected households was weighed.

RESULTS: Konzo patients were detected in 33 (6.8 %) households. Female to male ratio of 3.3:1 was found. No reports on the life expectancy of konzo patients were found. High prevalence of konzo was associated with female gender ($P = 0.0024$) and marital status ($P = 0.030$). The diet was largely dominated by cassava. Luku was the main staple food consumed at least once during the day in 99.2 % of households. A median of 304 g (max 592g; min 120g) of cassava flour which should provide 1070 Kcal (max 2085 Kcal; min 422 Kcal) / day/ person was used to prepare luku. Saka - saka (pounded cassava leaves) (40 %), cowpeas (30 %), sesame (23.2 %), mbondi (*Salacia pynaertii*) (18.1 %), mushrooms (17.7 %) and mfumbwa (*Gnetum Africanum*) (11.3 %) were the main condiments consumed as side-dishes with luku.

CONCLUSIONS: The results obtained showed that major foods consumed are of poor quality in protein especially in sulphur containing amino acids. Appropriate information, communication and training in cassava processing and promotion of a better balanced diet may help in the prevention of this paralytic disease, konzo.

KEYWORDS: konzo, cassava (*Manihot esculenta*), dietary pattern, sulphur amino acids.

CHARACTERISTIC FEATURES OF NEUROLATHYRISM IN ETHIOPIA: THE BREAKEVEN POINT OF INTERVENTION IN THE SOCIO-CULTURAL PROCESSES

A. Fikre

Debre Zeit Agricultural Research Center, P. O. Box 32, Debre Zeit (Ethiopia)

OBJECTIVES: Studying the scenario of the disease over time and space in Ethiopia so that an intervention strategy can be designed.

BACKGROUND: Neurolathyrism in Ethiopia has long been stigmatised and hosted by the community at risk for mere cultural and social taboos. A survey of registering victims from all parts of the country with potential incidences was conducted in 2007 using skilled personnel trained for the purpose. Seven major administrative zones contributed more than 80% of cases and the disease incidence was included. The survey result is therefore based on the 1461 victims assessed and that is representative of the overall situation about the disease in the country. Description of physical symptoms of neurolathyrism patients was adapted as working guide, which of course was supported by studying the food history and experience to make decision.

RESULTS: Duration of disease was as high as 60 years with an average of 14 years. Regarding the severity of the disease more than half is in one stick stage (54.4%) followed by the mild symptomatic stage one (32.7%), two stick walker (7.3 %), crawling (1.7 %) and bedridden (0.07%). It was also found that nearly all patients (94.7%) do not change their residence, while the remainder left to look for better option. Using a simple economic status indicator some 88 % were identified as poor (no ox to plough the land). An interesting characterization of the aftermath is that most (66.4%) of the patients still live on agriculture while a significant amount (33.6 %) had changed their occupation to handicraft, religious school, begging and the like. These indicate the level of interference of the disease with socioeconomic and cultural life.

CONCLUSIONS: In summary, neurolathyrism is significant in Ethiopia and is still far from receiving the attention it deserves. It is important that neurolathyrism is a disease of the young that stays for life, hence any intervention has primarily to be focused on this age segment. Currently grass pea cultivation is expanding; information, education and communication (IEC) on its safe consumption should be spread as a minimal urgent preventive measure.

KEYWORDS: neurolathyrism, socio-cultural processes, grass pea.

IS LATHYRISM STILL ENDEMIC IN NORTHERN ETHIOPIA? – THE CASE OF LEGAMBO WOREDA (DISTRICT) IN THE SOUTH WOLLO ZONE, AMHARA NATIONAL REGIONAL STATE

R. Tekle-Haimanot¹, A. Feleke², F. Lambein³

1 Dept. of Internal Medicine, Faculty of Medicine, Addis Ababa University (Ethiopia)

2 Gondar College of Medicine and Health Sciences, University of Gondar (Ethiopia)

3 IPBO, Ghent University, Ledeganckstraat 35, 9000 Ghent (Belgium)

OBJECTIVE: This study is aimed at investigating whether lathyrism is still endemic in north Ethiopia based on the March 26, 2004 report that appeared in the Amharic daily *Addis Zemen*, which indicated the occurrence of an epidemic where 400 people were paralyzed in Legambo Woreda, South Wollo Zone of ANRS.

A house-to-house survey of 3,440 households was undertaken in Legambo Woreda, South Wollo Zone of Amhara National Regional State, using pre-tested questionnaires. The study subjects were identified as persons with walking difficulties due to weakness of the legs.

BACKGROUND: Lathyrism is a neurotoxic disorder caused by overconsumption of grass pea (*Lathyrus sativus*). It is endemic in Ethiopia, India and Bangladesh. The fact that grass-pea tends to replace the staples during food shortages, makes the disease particularly important in the Ethiopian context.

RESULTS: The study identified 424 cases of lathyrism which occurred over many years in the Woreda which has a population of 171,976, which gives a prevalence of 2.5/1000. Specifically, the survey revealed that there were 48 cases with onset occurring in 1997, 52 in 1998, 38 in 2000 and 37 in 2001. The study further revealed that there is ongoing endemicity of lathyrism in this typical highland of north Ethiopia.

CONCLUSION: It is important that lathyrism gets the attention of relevant governmental agencies that should ensure the existence of early warning systems to deal with food shortages promptly, so that the rural population does not resort to consuming large amounts of grass pea. Lathyrism has to also become a reportable disease within the Ministry of Health system in those areas of northern Ethiopia where grass pea is cultivated.

KEYWORDS: lathyrism, grass pea, northern Ethiopia, endemic.

GEOGRAPHICAL AND SEASONAL ASSOCIATION BETWEEN LINAMARIN AND CYANIDE EXPOSURE FROM CASSAVA AND THE UPPER MOTOR NEURON DISEASE KONZO IN DRC

D. Nahimana Gitebo¹, J.P. Banea-Mayambu¹, R. Nunga Matadi¹, T. Tylleskar², M. Gebre-Medhin³, H. Rosling⁴

¹ Programme National de Nutrition (PRONANUT) (D.R. Congo)

² Department of nutrition and Department of Pediatrics Stockholm, Uppsala University Hospital (Sweden)

³ Unit for International Child Health, Department of Pediatrics, Uppsala University Hospital (Sweden)

⁴ Division for International Health care Research, Karolinska Institutet, Stockholm (Sweden)

OBJECTIVES: Association between konzo and cyanide exposure from the consumption of cassava roots

BACKGROUND: High cyanide intake from consumption of insufficiently processed cassava has been advanced as a possible aetiology of the upper motor neuron disease konzo. However, similar neuronal damage has not been associated with cyanide exposure from any other source. With an ecological study design, we

compared 22 cases of konzo, 57 unaffected household members and 116 members from unaffected households, a total of 195 subjects in konzo-affected savanna villages, versus 103 subjects in adjacent non-affected forest villages in the Paykongila area in Bandundu Province, DRC.

RESULTS: In the dry season the mean value (\pm SEM) of urinary thiocyanate, the main cyanide metabolite, was higher in the three groups in konzo affected villages (563 ± 105 , 587 ± 44 and 629 ± 47 $\mu\text{mol/l}$) than in unaffected villages (241 ± 17 $\mu\text{mol/l}$). In affected villages in the dry season when konzo incidence was high, mean urinary thiocyanate was also higher than the levels found in the wet season when incidence was low. The wet season values (mean \pm SEM) were 344 ± 60 , 381 ± 35 and 351 ± 27 $\mu\text{mol/l}$, respectively. Urinary levels of inorganic sulfate were low in all groups, indicating low intake of the sulfur amino acids which provide a substrate for cyanide detoxification.

CONCLUSION: These findings support an aetiological role for cyanide in konzo. However, urinary linamarin, the cyanogenic glucoside and source of cyanide in cassava, was more closely associated with the occurrence of konzo. The mean value (\pm SEM) of urinary linamarin in the konzo cases was 632 ± 105 $\mu\text{mol/l}$, and in their households members 657 ± 52 $\mu\text{mol/l}$, which was significantly higher than in members of reference households in the same village (351 ± 28 $\mu\text{mol/l}$) and unaffected villages (147 ± 18 $\mu\text{mol/l}$). This suggests that a specific neurotoxic effect of linamarin, rather than associated general cyanide exposure resulting from glucoside breakdown in the gut, may be the cause of konzo.

KEYWORDS: konzo, cassava, cyanogens, cyanide, thiocyanate, linamarin.

Toxicological aspects and other risk factors

UPTAKE OF SIMPLE WETTING METHOD IN AFRICA TO REDUCE CYANIDE POISONING AND KONZO FROM CASSAVA

J. H. Bradbury¹, J. Cliff², I. C. Denton¹

¹ Botany and Zoology, Research School of Biology, Australian National University, Canberra, ACT 0200 (Australia)

² Faculdade de Medicina, Universidade Eduardo Mondlane, CP 257, Maputo (Mozambique)

OBJECTIVES: The reduction of cyanide poisoning and konzo from cassava in tropical Africa.

BACKGROUND: Cassava, the staple food of tropical Africa, contains cyanogenic glucosides (mainly linamarin) which are hydrolysed by an enzyme linamarase to produce cyanohydrins which hydrolyse to toxic cyanide. Cyanide ingestion from bitter cassava can cause cyanide poisoning with symptoms of headache, dizziness, stomach ache, diarrhoea and vomiting, sometimes leading to death. It can also produce konzo, an irreversible paralysis of the legs mainly in children and young women. There are many thousands of konzo cases in DRC, Mozambique, Tanzania, Cameroon, Central African Republic and probably Angola.

RESULTS: In 2005 we developed a simple wetting method that reduces the total cyanide content of cassava flour 3-6 fold. It involves wetting the flour, spreading it in a thin layer in the shade for 5 hours and using it the same day to make traditional thick porridge (ugali). The method was field tested in Mozambique and readily accepted by rural women. It requires no additional equipment or water and the ugali produced tasted better than that from untreated flour. Coloured, laminated posters describing the method are available for free in 3 European and 5 African languages, see web site address. Recent research has shown that an equally effective treatment method is to expose wet flour in the sun for 2 hours. Projects for rehabilitation and prevention of konzo occurred in Mozambique in 2007 and in 2008-9 in Tanzania involving Dr N. Mlingi, funded by AusAID. The Ministry of

Health in Mozambique are now using our posters in Macua. In Uvira DRC, collaborating with APAA Congo, the wetting method has been taught in many villages and 500 posters distributed in Kifuliru.

CONCLUSION: We hope that the wetting method will be used extensively and will reduce cyanide poisoning and konzo in Africa.

KEYWORDS: Konzo, cyanide poisoning, cyanogenic glucosides, cassava, wetting method.

RISK AND PROTECTIVE FACTORS FOR NEUROLATHYRISM IN ETHIOPIA

H. Getahun

Rue Liotard 56, Geneva (Switzerland)

OBJECTIVE: Describe risk and protective factors associated with neurolathyrism in Ethiopia.

BACKGROUND: Neurolathyrism is a neglected problem of public health importance in Ethiopia, which often occurs during times of food shortages in rural communities living in the most inaccessible parts of the country. We conducted community based epidemiological studies during and in the aftermath of the third major neurolathyrism epidemic that occurred in Ethiopia and identified the risk factors associated with it.

RESULTS: We have found that the risk factors associated with neurolathyrism are quite complex and of diverse nature ranging from individual factors to household and socio-political factors. The individual risk factors include male gender, pubertal age, blood group O and having a history of other illnesses during the epidemic. Household risk factors include illiteracy of the head of household and ownership of grass pea farmland. Other cooking and eating habits within the family were also found to increase the household risk. This includes exclusive cooking of grass pea food using the traditional handmade clay utensils, which are known to leach iron. Consumption of the green unripe form of the seed also increases the risk of neurolathyrism. Other socio-political factors that contributed for increased risk of neurolathyrism include politically motivated land redistribution and

resettlement schemes. Protective factors of neurolathyrism include consumption of the gravy or fermented form of the grass pea food, and the combination of grass pea with cereals during preparation of foods. Addition of spices and condiments with antioxidant quality into particularly the gravy form of the food was also protective.

CONCLUSION: To reduce the burden of neurolathyrism, consumption of grass pea in any non-mixed forms should be discouraged. Enhancing the content of antioxidants and/or sulphur amino acids in grass pea seeds through plant genetics along with reducing the content of the neurotoxin are essential.

KEYWORDS: neurolathyrism, grass pea seeds, neurotoxin, antioxidants, sulphur amino acids.

CHEMICAL FOOD SAFETY OF CASSAVA PRODUCTS IN GEOGRAPHICAL REGIONS NEW TO CASSAVA PRODUCTION AND PROCESSING. EXAMPLES FROM AFRICA

L. Brimer¹, D. B. Nyirenda², L. Chiwona-Karlton³, M. Chitundu⁴

1 Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen (Denmark)

2 Department of Animal Science, University of Zambia, Lusaka (Zambia)

3 Department of Urban and Rural Development, Swedish University of Agricultural Sciences, Uppsala (Sweden)

4 Programme Against Malnutrition (PAM) (Zambia)

OBJECTIVES: Study the influence of knowledge concerning cultivar characteristics and processing technologies on the chemical food safety of cassava products.

BACKGROUND: Within recent years a number of risk assessments and proposals for maximum limits of residual cyanide and cyanogenic compounds in food products were published. The EU (EFSA) and Food Standards Australia New Zealand have been active, but also a number of national standards agencies in Africa took initiatives. The risk assessments pointed to the possibility, that effects harmful to the developing central nervous system (CNS) may be seen at lower systemic exposure to cyanide than

previously anticipated. Also during the recent years growing and processing of cassava has spread in regions of Southern Africa. Through a North to South Public-Private Partnership Pilot Development Project funded by SIDA the potential of cassava transformation (commercialization) in Southern Africa was investigated. The value chain approach research included processing and chemical food safety of products; as varying between regions and seen in the light of recent risk assessments.

RESULTS and CONCLUSIONS: Cassava tubers, chips, mixed biscuits (soy, groundnut, sweet potato) and flour procured from households and local markets in three regions of Zambia (high, medium and low cassava consuming) and products from the Northern, Central and Southern regions of Malawi, respectively, were analyzed for total cyanogenic potential (CNp). Fresh tubers were in the interval of 40 to 210 mg HCN equivalents/ kg f.w. In Zambia processed products from Luapula Province in the North all showed low CNp; i.e. 10 mg HCN eq./k.g. a.d.w. (air dried weight) or less. Processed samples obtained around the town of Mongu, Western Province, in general were much higher in CNp. Thus cassava chips from Mongu were analyzed to have CNp's in the interval of 50 to 290 mg HCN eq./k.g. a.d.w. These, and similar results from Malawi will be discussed.

KEYWORDS: cassava, cyanogenic glucosides, food safety, processing technologies.

STUDY ON CLIMATIC, EDAPHIC AND ALTITUDINAL FACTORS AFFECTING YIELD AND TOXICITY OF GRASS PEA GROWN IN ETHIOPIA

A. Fikre¹, F. Lambein², M. Eshete¹, Y.-H. Kuo², S. Ahmed³

1 Ethiopian Institute for Agricultural Research, P.O. Box 32, Debre Zeit (Ethiopia)

2 Institute of Plant Biotechnology for Developing Countries (IPBO), Ghent University (Belgium)

3 International Center for Agricultural Research in Dry Areas, Aleppo (Syria)

OBJECTIVE: Identification of important environmental factors

associated with the seed yield and β -ODAP content of grass pea.

BACKGROUND: *Lathyrus sativus* L. (grass pea) is a drought tolerant legume grown in Ethiopia as a multi-purpose crop for food, feed, soil fertility restoration and income generation. The seeds, rich in protein, contain variable amounts of a neuro-excitatory amino acid β -ODAP that is held responsible for a crippling disease neurolethyrism after prolonged over-consumption. Despite all the undesired qualities about the crop, its rank among principal highland legume crops is growing fast and will surpass all in a short while, both in area covered and production volume because it is very hardy, and has low production cost and higher yield. Variance, path coefficient and correlation analyses were implemented to identify the interrelations and estimate the level of effects of climatic, edaphic/geographic factors on yield and β -ODAP level using ten grass pea genotypes grown for two seasons (2005 - 2006) at five eco-divergent locations.

RESULTS: Analysis of variance indicated that crop yield, yield related parameters and β -ODAP levels revealed considerable variability among locations, years and genotypes. Path coefficient analysis indicated that days to maturity had the most positive direct effect on yield while the dominant variables with high total effect ($r > 0.75$) on β -ODAP under field conditions were K^+ , sunshine hours (ssh) and days to maturity, suggesting for due consideration in the crop β -ODAP management. From linear correlation analysis, we found that K^+ , long reported to be high in Ethiopian soil, and ssh positively; while pH, days to maturity and yield negatively have the strongest correlations (>0.70) with β -ODAP levels among 35 factors considered in this study for Ethiopia.

CONCLUSIONS: Optimization of production conditions for grass pea could give a double advantage simultaneously of higher yield and lower toxicity. The strongest correlation of sunshine hours with β -ODAP level during the phase of crop maturity (ssh III) in this study may suggest that β -ODAP biosynthesis and its response to environmental stress could be highest during post-anthesis.

KEYWORDS: Lathyrus, neurolethyrism, β -ODAP, climatic, edaphic.

EFFECT OF AGRO-ECOLOGICAL FACTORS ON THE ACCUMULATION OF NEUROTOXIN AND OTHER AMINO ACIDS IN *Lathyrus sativus*

M. R. Haque¹, Y.-H. Kuo², F. Lambein²

¹ Department of Biochemistry, Patuakhali Science and Technology University, Dumki, Patuakhali (Bangladesh)

² IPBO, Ghent University, K. L. Ledeganckstraat 35, B-9000, Ghent (Belgium)

OBJECTIVE: To evaluate the effect of some environmental factors including salinity, water stress and trace elements on the biosynthesis of β -ODAP and other amino acids in *Lathyrus sativus* seeds and callus tissues.

BACKGROUND: *L. sativus* plants were grown in the fields of the coastal belt of Bangladesh, in artificially salinised soils, in sand culture and in hydroponic nutrient solutions to investigate the effect of salinity on ODAP and on other free amino acids. Experiments with habituated callus tissue by feeding radioactive BIA ([β -(isoxazolin-5-one-2-yl)-alanine], the biosynthetic precursor of ODAP, were also done to study the effect at cellular levels.

RESULTS: Low to medium salinity reduced ODAP and other excitatory amino acids (aspartic acid and glutamic acid) in *L. sativus*, while very high salinity enhanced those amino acids. Homoarginine, arginine and cysteine were increased several fold by increased salinity. In callus tissues the conversion of BIA into ODAP decreases proportionately with the increase in salinity of the medium.

Water stress (-0.14 to -0.9 Mpa) resulted in enhanced syntheses of ODAP and other excitatory amino acids. The trends of homoarginine, arginine and cysteine accumulation under water stress were similar to those occurring under salinity stress. Conversion of BIA into ODAP in callus tissues increased linearly with the increase in osmotic potential. In *L. sativus* seed, optimum (as in Hoagland nutrient solution) and double doses of Cu, Mo, B, Mn, I

and Co reduced the content of toxin but excess of almost all the trace elements enhanced aspartate and glutamate contents. Deficit or excess of almost all the trace elements resulted in total failure of seed formation except boron, manganese and cobalt. Cobalt reduced ODAP content significantly and in all cases arginine and homoarginine were enhanced, except with Mn. In seeds ODAP, asparagine and homoarginine contents were enhanced significantly by Al^{+3} . In callus tissues double doses of Cu and Mo showed a reducing effect on the synthesis of ODAP from BIA but both deficit and excess of all other trace elements enhanced ODAP content. Aluminium increased the synthesis of ODAP in callus tissues at an extremely high rate.

Zinc had a highly significant role in reducing ODAP synthesis while Fe^{2+} enhanced ODAP level. ODAP was found to form stable chelates with Zn^{2+} . The ODAP: $^{65}Zn^{2+}$ chelate was taken up and readily transported to the shoot top of Zn^{2+} -deficient *L. sativus* plants much quicker than the chelate of Zn^{2+} with any other organic ligands (BIA, glutamate, aspartate, etc.) tested. Increase in the phosphorus/zinc ratio, rather than the phosphorus or zinc content of the plant and seed, correlated significantly with the increase in ODAP accumulation. Slightly acidic to near-neutral pH of the medium resulted in the highest Zn^{2+} and the lowest P/Zn ratio of *L. sativus* plant and seed, where seed also had the lowest ODAP values.

CONCLUSIONS: β -ODAP content of the grass pea seeds is variable and depends on the environmental conditions (salinity, water supply and presence of trace elements) where grass pea grows. Ample evidence suggests that ODAP might have a role as the biological carrier of Zn^{2+} in *L. sativus* plants.

Keywords: neurotoxin ODAP, *Lathyrus sativus*, callus, BIA, trace elements.

β -ODAP ACCUMULATION COULD BE RELATED TO LOW LEVELS OF H₂O₂ IN LATHYRUS SATIVUS L. (GRASS PEA) LEAVES

C.J. Jiao, Y.C. Xiong, C.Li, L.M. Ke,
J.L. Jiang, X.Y. Wang, F.M. Li, Z.X.
Li, C.Y. Wang*

Key Laboratory of Arid Agroecology under the
Ministry of Education / Institute of Cell
Biology, School of Life Sciences, Lanzhou
University, Lanzhou (China)

OBJECTIVES: To find the relationship between levels of β -ODAP and H₂O₂ in grass pea.

BACKGROUND: Toxin β -ODAP (β -N-oxalyl-L- α , β -diaminopropionic acid), a causative agent of lathyrism, exists in both seeds and vegetative tissues of grass pea. Previous studies have shown that drought stress can increase β -ODAP levels and also lead to the generation of H₂O₂ in the plant. It is important to find out the relationship between the levels of β -ODAP and H₂O₂ during water deficit and get an insight into the mechanism of β -ODAP accumulation. Therefore, their contents in leaves were investigated under different growth conditions.

RESULTS: Under the field conditions with normal watering, higher contents of β -ODAP (0.963 mg/g FW for young and 1.233 mg/g FW for mature leaves) and lower levels of H₂O₂ (8 μ mol/g FW for young and 0.846 μ mol/g FW for mature leaves) occurred in 15-day-old seedlings. However, very little β -ODAP (0.096 mg/g FW for young and 0.052 mg/g FW for mature leaves) and markedly high contents of H₂O₂ (25.152 μ mol/g FW for young and 50.426 μ mol/g FW for mature leaves) was detected in 60-day-old plants. Under the drought/osmotic stress induced by polyethylene glycol (PEG), young leaves from 12-day-old seedlings contained higher levels of β -ODAP and lower H₂O₂, but the mature leaves showed lower levels of β -ODAP and higher H₂O₂, compared with the control. Furthermore, the detached leaves pretreated with exogenous H₂O₂ exhibited less β -ODAP, but higher levels of β -ODAP were detected when pretreated with exogenous pyridine, an inhibitor of H₂O₂-generating NADPH oxidase, compared to the control.

CONCLUSIONS: The present results indicate that β -ODAP accumulation could be related to low

levels of endogenous H₂O₂ in the leaves under either field conditions or osmotic stress.

KEYWORDS: *Lathyrus sativus* L., β -ODAP, H₂O₂, accumulation, PEG.

IMPROVEMENT OF THE TECHNIQUES OF DETOXIFICATION OF THE LOCAL CASSAVA FOOD IN DEMOCRATIC REPUBLIC OF CONGO

D. L. M. C. Yandju¹, O. N. G.
Kabena¹, Y. D. Masika²

¹ Laboratory of Applied Microbiology and Nutrition, Department of Biology, Faculty of Science, University of Kinshasa (DRC)

² Department of Biology, ISP Gombe, Kinshasa (DRC)

OBJECTIVES: We have made a study on the evaluation of techniques of treatment of cassava and propose technological improvements in order to maximize the elimination of cyanides in various cassava based foods in DRC.

BACKGROUND: For more than half of the Congolese population, cassava constitutes the principal source of energy. To reduce the risks of exposure to cyanides and to increase the shelf life of cassava, the autochthones developed several techniques of preparation and transformation of the cassava with varied meals. By taking account of the strong dependence of the populations with respect to cassava and the persistence of the pockets of konzo in DRC, fermentation following steeping with water made up the only technical recommendation for elimination of cyanides in the manioc. And yet, there exist many recipes containing the non fermented cassava which enter the basic diet of much of the Congolese population.

RESULTS: Fourteen identified recipes of manioc were analyzed and improved during their cooking. Five practical techniques allowed to reduce the cyanides contained in the fresh manioc by 65-90%. Fermentation, steeping in water and exposing to the free air constitutes the best method of detoxification. However, a combination of steeping in water and continued evaporation during cooking of manioc made it possible to appreciably reduce the

risks of exposure to cyanides in the recipes containing the unfermented cassava.

CONCLUSIONS: In order to fight effectively against the extension of the epidemic of konzo in the new pockets with high prevalence, an investigation of prevalence and consumption should be planned in order to establish the status of the disease in the zones where the basic food largely consists of fresh or slightly fermented manioc in the context of current poverty.

KEYWORDS: Cassava food, cyanides, konzo, detoxified food.

ARE EXISTING TRADITIONAL PROCESSING METHODS PRODUCING SAFE FOOD PRODUCTS?

D. B. Nyirenda¹, L. Brimer², L.
Chiwona-Karlton³

¹Department of Animal Science, University of Zambia, Lusaka (Zambia); ²Dept of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen (Denmark); ³Dept of Urban and Rural Development, Swedish University of Agricultural Sciences, Uppsala (Sweden)

OBJECTIVES: A North to South Public Private Partnership Pilot Development Research Project funded by SIDA was carried out to investigate the potential of cassava transformation (commercialization) in Southern Africa.

BACKGROUND: The value chain approach research included cassava processing and food safety. Samples of cassava tubers, cassava chips and flour procured from households and local markets in three areas of Zambia (high, medium and low cassava consuming) were analysed for cyanogenic glucosides equivalents content.

RESULTS: The results revealed great variability in cyanogenic equivalents in the various processed cassava chips and flour samples. Some samples contained low cyanide content (0 to less than 10 mg HCN eq/kg) which is within the recommended WHO level of 10mg HCN eq/kg maximum for human consumption. Other samples contained up to 294 mg HCN eq/kg after processing.

The results showed that traditional cassava processing and consuming

areas like Kaoma and Luapula produced products containing 10 or less mg HCN eq/kg cyanide content, indicating that the processing technology meets the WHO standard. The results for gari flour from the farmers who had been trained in this new Nigerian technology showed no cyanide at all and the product is of good quality and was liked by consumers. The fermented cassava chips and flour procured from the households and markets in the town and neighbourhood of Mongu showed varied and high levels of cyanogenic equivalents (49 to 293 mg HCN eq/kg). Fresh roots showed high cyanogenic equivalents content that also varied within local (from Monze, Southern province of Zambia) and hybrid varieties (from Chongwe, in Lusaka province). The hybrid varieties ranged from 91 to 212 mg HCN eq/kg and local varieties from 37 to 185 mg HCN eq/kg.

CONCLUSIONS: These results indicate that the sampled local and hybrid cassava varieties in Zambia do contain high level of cyanogenic glucosides and that the traditional fermentation processing technology is adequate to remove the cyanide toxicity. However, the differences in the cyanide levels of processed products would indicate a very serious flaw in processing by those living near or selling products to cities, either resulting from making short cuts in post harvest processing or that the environment may affect the level of cyanide in the tubers. This alarming result would indicate exposure to cyanide toxicity and increased risk for konzo. This will need further investigation to improve the processing technology such that a product from any source done by the same processing technology should produce the same good quality (no cyanide) results. This also emphasises the importance of developing quality and safety standards by which to assess and regulate cassava products on the market.

KEYWORDS: cassava, cyanogenic glucosides, food safety, processing technologies.

LATHYRISM IN NORTH SHEWA ZONE OF ETHIOPIA: ASSESSMENT OF SOCIO ECONOMIC FACTORS AFFECTING GRASS PEA CONSUMPTION AND FARMERS PERCEPTION ON ITS EFFECT AND PREVENTION METHODS

A. Girma, B. Tefera, L. Dadi

Debre Brehan Agricultural Research Center, P.O.BOX 112, Debre Brehan (Ethiopia)

OBJECTIVE: Study of grass pea production, processing, consumption and farmers' indigenous knowledge about its toxicity and related attitude towards its consumption.

BACKGROUND: Lathyrism in Ethiopia is caused by food dependency on grass pea (*Lathyrus Sativus* L., Guaya in amharic), an environmentally tolerant but potentially neurotoxic legume that resists the prevailing droughts.

RESULTS: Both primary and secondary data were used. Primary data were collected from 100 farmers from two sub-districts using two-stage random sampling from purposively selected districts of north Shoa, Ethiopia. In the study area, a large proportion of the farmers are growing grass pea since it withstands harsh environment and it is a good source of protein and energy, where different food types /dishes are prepared from it. Socio economic (poverty; lack of money to buy other food legumes) and technical (i.e. pests, water logging, frost hazards, etc) problems influence consumption of grass pea. Most of respondents have the idea that some chemical contained in grass pea causes the health problem. This poses a problem on its production, processing, preparation and consumption. Different processing and preparation methods are used to prepare grass pea into different food forms. The major processing methods include washing and soaking. The farmers are applying these methods mainly because they assume that the amount of chemical that causes lathyrism, ODAP (β -N-oxalyl- α,β -diaminopropionic acid), is reduced through continuous washing and soaking. Mixing grass pea porridge with milk/butter, grass pea consumed as 'kita', blending/mixing

it with other crops, applying different processing/detoxification methods are among the other ways that farmers in this area use to minimize the effect of the toxin.

CONCLUSION: Since grass pea is consumed with a fear of lathyrism, future research should concentrate either on developing a grass pea variety with a safe and/or low level of ODAP content or improving the traditional/indigenous processing methods such as soaking method and time, washing and washing frequency, heating and time of heating.

KEYWORDS: grass pea, lathyrism, farmers' indigenous knowledge, Ethiopia.

PERCEPTIONS OF KONZO IN AFFECTED POPULATIONS IN KAHEMBA TERRITORY D. R. CONGO

A. Mulumba Nkuadi¹, J.P. Banea-Mayambu², Malela Pingudi³

¹ School of Nutrition and Dietetics, ISTM, University of Kinshasa (DRC)

² Programme National de Nutrition, Ministère de la Santé, Kinshasa (DRC)

³ Zone de santé de Kahemba, Bandundu province (DRC)

OBJECTIVES: Identification of perceptions of the local populations on konzo for better targeting of appropriate interventions.

BACKGROUND: Konzo is spastic paralysis reported in several remote areas in sub-Saharan Africa, particularly in the province of Bandundu, DRC. Although the precise aetiology is not yet highlighted in Bandundu, implementation in Bandundu in 2000 of the programs of nutritional education accepted by the local population, and targeted on sufficient soaking of more than 3 days and sufficient drying of more than 2 days, with the diversification of food, resulted in a decrease of konzo occurrence in the centre of Bandundu. From 111 cases reported in 1998, only one case was so far reported in the centre of Bandundu. In order to prepare a similar durable program of nutritional education for the Territory of Kahemba in the South of Bandundu an investigation into the perception of konzo was

held in the konzo affected villages of the territory of Kahemba.

RESULTS: Among 420 inhabitants in the 3 villages, we identified, using the WHO criteria, 22 konzo cases, of which 8 were from the year 2007. Most of the inhabitants in affected villages were the displaced populations from Angola in 2004 or 2005. In affected villages 36% of inhabitants think that konzo results from a bad fate of the ancestors, 25% attribute konzo to their drinking water and 25% to the alteration of cassava in the soil. Only 14% attribute konzo to cyanide exposure from the consumption of shortly soaked cassava roots.

CONCLUSION: Adherence to sufficient soaking and drying has been an efficient means to prevent konzo in Central Bandundu. In Kahemba, populations need to be well informed on konzo in order to get their participation in any intervention that can be planned in that area, such as nutrition education.

KEYWORDS: konzo, cassava, nutrition education, Kahemba.

DETOXIFICATION OF *LATHYRUS SATIVUS*: REDUCTION OF TOXIC PRINCIPLE 3-N-OXALYL-L- 2,3-DIAMINOPROPIONIC ACID (ODAP) BY STEAMING PROCESS: A HOUSEHOLD APPROACH

A. K. Geda¹, R. L. Pandey²

1 Department of Medicinal & Aromatic Plant Sciences, Indira Gandhi Krishi Vishwavidyalaya, Raipur 492006 (India)

2 Department of Plant Breeding & Genetics, Indira Gandhi Krishi Vishwavidyalaya, Raipur (India)

BACKGROUND: In the present study, detoxification of *Lathyrus* seeds by a steaming process is an economical and household approach to minimize the neurotoxin component i.e. 3-N-oxalyl-L-2,3-diaminopropionic acid (ODAP), without affecting the quality of protein, sugar and carbohydrates.

RESULTS: The results indicate that steaming of seeds reduces 50 to 62 percent of ODAP content. The seeds are pre-soaked in edible non-toxic chemicals at different concentrations and intervals of time. The results also reveal that increase

in duration of steaming decreases the ODAP content with a few changes in nutritional quality of products, which have also been studied. The maximum ODAP reduction was 61.46% under the treatment of tartaric acid (5%, 5 hrs) with loss of 4.22% protein, 25.5% of carbohydrate and 5.55% of sugar.

CONCLUSION: The result of steaming process reveals that increase in duration of processing decreases the neurotoxin with a minimum loss in quality of products.

KEYWORDS: *Lathyrus sativus*, neurotoxin (ODAP), detoxification, steaming.

Neurobiological aspects

BRAIN GLUTATHIONE AS A TARGET FOR AETIOLOGICAL FACTORS IN NEUROLATHYRISM AND KONZO

P. B. Nunn¹, J. R. A. Lyddiard²

1 School of Pharmacy and Biomedical Sciences, University of Portsmouth (UK).

2 School of Biomedical Sciences, King's College London (UK)

OBJECTIVE: To review the roles of plasma sulphur amino acids in neurological diseases of dietary origin.

BACKGROUND: The chronic neurological diseases neurolathyrism and konzo are associated with the nutritional dependence of human populations on a single plant food. In each case the disease expresses itself as a primary disorder of upper motor neurones, leading to clinical signs and symptoms that are characteristic of sporadic amyotrophic lateral sclerosis (motor neurone disease). The plant food associated with neurolathyrism is the seed of *Lathyrus sativus*. This seed contains the neurotoxic amino acid β -N-oxalyl- α , β -diaminopropionic acid (β -ODAP). The plant food associated with konzo is cassava which, when improperly processed, contains significant concentrations of a cyanogenic glycoside. Mechanistic studies have shown that β -ODAP has numerous effects within the central nervous system that potentially may result in neuronal death. However, a

monotonous diet of *Lathyrus sativus* is likely to generate nutritional deficiencies and it is proposed that one of these, plasma methionine deficiency, may predispose neurones to the neurotoxic effects of β -ODAP. In the case of konzo cyanide released from cyanogenic glycosides, reacts with plasma cystine. Subjects suffering from konzo have low plasma cystine concentrations, probably resulting from the reaction of cystine with cyanide to form 2-imino-4-thiazolidine carboxylic acid. Since both plasma methionine and cystine are used for glutathione synthesis it seems likely that one common feature that leads to motor neurone death in neurolathyrism and konzo is the depletion of neural glutathione. It is known that depletion *in vitro* of glutathione in neural cell cultures increases the sensitivity of neurones to glutamate excitotoxicity.

CONCLUSIONS: It is proposed that a major mechanism that precipitates the dietary-related upper motor neurone diseases neurolathyrism and konzo is the depletion of glutathione within the central nervous system. The role of ancillary components, β -ODAP and 2-imino-4-thiazolidine carboxylic acid, will be discussed.

KEYWORDS: neurolathyrism, konzo, cystine, methionine, glutathione.

AN IMPROVED NEUROLATHYRISM RAT MODEL: IMPAIRED VEGF SYSTEMS HAVE A CRITICAL ROLE IN THE DEGENERATION OF MOTOR NEURONS

K. Kusama-Eguchi¹, Y. Yamazaki¹,
F. Ikegami², F. Lambein³, T.
Kusama¹

1 College of Pharmacy, Nihon University, Funabashi, Chiba (Japan)

2 Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Chiba (Japan)

3 Institute of Plant Biotechnology for Developing Countries (IPBO), Ghent University (Belgium)

OBJECTIVE: Elucidating the pathology of neurolathyrism using a rat model.

BACKGROUND: Neurolathyrism is a motor neuron disease characterized by lower limb

paraparesis and associated with a plant excitotoxin β -N-oxalyl-L- α , β -diaminopropionic acid (L- β -ODAP), an agonist of AMPA/kainate-type glutamatergic receptors. We have reported a rat model of neuropathy to study the aetiology of human neuropathy.

RESULTS: As the incidence of the model rat was quite low, another condition with separation-starvation resulted in the 4.6 fold higher incidence of the paraparesis in newborn pups. In the adult (up to 36 week) paraparetic rats, the numbers as well as the sizes of motor neurons (MN) were measured thoroughly from cervical to sacral spinal cord resulting in the prominent decreases only in the lumbar and sacral cord. In addition to these insults, the surviving motor neurons revealed pathological insults typical of physical and ischemic spinal cord injury. In the early period of treatment, we found extensive but transient hemorrhage to occur in their spinal cord parenchyma located at the ventral side associated with numerous TUNEL-positive cells. In parallel, vascular endothelial growth factor receptor (VEGFR)-2 (Flk-1) levels were significantly lower in the lumbo-sacral spinal cord of the paraparesis rats compared to their controls suggesting a failure to protect neurons through VEGF receptors against the neurotoxicity of L- β -ODAP.

CONCLUSION: The early insult on the vasculature was observed with simultaneous motor neuron apoptosis during the early, critical period of this rat neuropathy model.

ACKNOWLEDGMENT: This work was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan to promote multi-disciplinary research projects.

KEYWORDS: rat model, motor neuron, VEGF, apoptosis, stressor.

ON THE MECHANISMS AND BIOMARKERS OF KONZO

D. Tshala-Katumbay

Center for Research on Occupational and Environmental Toxicology & Department of Neurology, Oregon Health & Science University, Portland OR (USA), & Department of Neurology, Kinshasa University, Democratic Republic of Congo (DRC)

OBJECTIVE: To elucidate biomarkers and mechanisms of konzo.

BACKGROUND: Konzo is an upper motor neuron disease that affects populations with heavy reliance on insufficiently processed cyanogenic cassava as staple food. Disease outbreaks have been reported in Sub-Saharan countries notably in the Democratic Republic of Congo and Mozambique but also in Central African Republic, Tanzania, and possibly Angola and Uganda. The disease mainly affects children and women of childbearing age for reasons that are not understood. The mechanisms by which the disease preferentially strikes the central motor system have yet to be elucidated.

RESULTS: Epidemiological studies conducted across konzo-affected areas consistently show a relationship between the onset of disease outbreaks, poor nutrition, and increased consumption of insufficiently processed cyanogenic (bitter) cassava as demonstrated by very high levels of the principal cyanide metabolite thiocyanate (SCN, up to thousands μ mol/L in serum/urine) in subjects affected by the disease. Clinical and electrophysiological studies suggest a pre-synaptic failure in the upper motor system. The culpable agent responsible for cassava-associated motor system disease remains unknown. Laboratory studies indicate that oxidative metabolism of cyanide is favored under circumstances of sulfur amino acid (SAA) deficiency leading to an increased production of cyanate (OCN), a protein-carbamoylating agent. Therefore, culpable agents in the pathogenesis of konzo include CN (improbable), SCN (AMPA receptor chaotrope, conceivable), and/or OCN (a protein-carbamoylating agent, likely). Ongoing modeling studies in laboratory animals seek biomarkers and molecular mechanisms associated with linamarin intoxication under conditions of SAA deficiency.

KEYWORDS: konzo, sulfur amino acid deficiency, linamarin, biomarkers, cassava.

UNRAVELING THE MECHANISM OF β -ODAP INDUCED EXCITOTOXICITY AND OXIDATIVE STRESS, RELEVANCE FOR NEUROPATHY PREVENTION

M. Van Moorhem¹, F. Lambein², L. Leybaert¹

¹ Department of Physiology and Pathophysiology, Ghent University, Ghent (Belgium). ² Institute of Plant Biotechnology for Developing Countries (IPBO), Ghent University, Ghent (Belgium)

OBJECTIVES: β -N-oxalyl- α , β -diaminopropionic acid (β -ODAP) – present in *L. sativus* seeds – is a glutamate analogue at α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate (KA) receptors in motor neurons and therefore potentially acts as an excitotoxic substance. Additionally, *L. sativus* seeds are deficient in the sulphur-containing amino acids methionine (Met) and cysteine (Cys). In the present work we aimed at investigating the effect of β -ODAP on the cellular Ca^{2+} homeostasis and the role of oxidative stress to define prevention of neuropathy.

BACKGROUND: Chronic consumption of *L. sativus* seeds can result in the neurodegenerative disease neuropathy. β -ODAP is proposed to be the causative agent, especially as an excitotoxic substance. A key factor in excitotoxic neuronal cell death is a disturbance of the intracellular Ca^{2+} homeostasis. Epidemiological data suggest that balancing the diet with Met and Cys containing foods significantly lowers the risk for neuropathy.

RESULTS: To study Ca^{2+} homeostasis, the photoprotein aequorin was used in N2a neuroblastoma cells to investigate Ca^{2+} dynamics specifically in the cytosol, ER or mitochondria. Treatment of N2a cells with β -ODAP (24 h) enhanced bradykinin (BK) - induced Ca^{2+} transients and endoplasmic Ca^{2+} uptake. Moreover, BK-triggered mitochondrial Ca^{2+} uptake was stimulated by 35 %. Additionally, the mitochondrial membrane potential (Ψ_m) was more negative after β -ODAP treatment. Oxidative stress was studied by evaluating survival of primary motor neurons after treatment with β -ODAP, including antioxidants in the

treatment medium. Both Met and Cys protected the neurons from β -ODAP induced toxicity.

CONCLUSION: The emerging working hypothesis is that the β -ODAP-triggered hyperpolarisation of Ψ_m with increased mitochondrial Ca^{2+} uptake is an early event in the excitotoxicity of this neurotoxin. Oxidative stress is also involved in β -ODAP induced motor neuron death, indicating that antioxidant supplements in the diet may prevent people from developing neuropathy.

KEYWORDS: excitotoxicity, calcium homeostasis, mitochondria, β -ODAP, oxidative stress.

A SUITABLE BIOASSAY IS NEEDED TO MODEL LATHYRISM IN MONO-GASTRIC ANIMALS

D. Enneking

School of Food and Agriculture, University of Adelaide, Waite Agricultural Research Institute, Waite Rd. Glen Osmond, 5064 South Australia (Australia)

OBJECTIVES: To discuss the need for a bioassay to model neuropathy in combination with contributing factors.

BACKGROUND: It is well known that neuropathy is a consequence of eating too much grass pea. Malnutrition and oxidative stress appear to be prerequisite factors leading to the onset of neurodegeneration. There is no animal model that combines predisposing factors with grass pea consumption in order to replicate the symptoms of neuropathy observed in humans.

RESULTS: There are numerous older reports about animals occasionally being intoxicated by grass pea containing feeds. Generally, grass pea appears to be a useful and nourishing ingredient for animal diets. Why is grass pea only toxic in very specific circumstances? Is a reduction in dietary ODAP intake sufficient to prevent neurodegeneration? What are the thresholds for safe consumption of grass pea? How do other dietary, environmental and genetic factors contribute to the onset of neurodegenerative symptoms? Which nutrients have protective functions?

CONCLUSION: To maximize grass pea benefits, an experimental animal model is needed to dissect the range of risk and protective factors that influence the possible outcomes of excessive grass pea consumption.

KEYWORDS: neuropathy, malnutrition, bioassay, risk factors, protective factors.

A NEW UNIFYING CHEMICAL HYPOTHESIS ON LATHYRISM, KONZO AND TROPICAL ATAXIC NEUROPATHY

J. Llorens

Departament de Ciències Fisiològiques II, Universitat de Barcelona, Hospitalet de Llobregat (Spain)

OBJECTIVE: This communication will review data supporting an alternative unifying hypothesis on the causative agents for these diseases, namely, that they are nitriles, compounds containing cyano groups.

BACKGROUND: Konzo and neuropathy are two similar diseases associated with consumption of two different toxic plants. In addition to konzo, cassava consumption has also been associated with a third disease, tropical ataxic neuropathy (TAN). Widely accepted but unproven hypotheses are that β -ODAP is the causative agent for neuropathy, and that cyanide or cyanide metabolites are the causative agents for konzo and TAN. The diseases would be caused by different but similar nitriles through direct neurotoxic actions requiring no cyanide release. Cassava nitriles include linamarin, lotaustralin, acetone cyanohydrin, and 2-hydroxy-2-methylbutyronitrile. *Lathyrus* seeds contain at least 2-cyanoethyl-isoxazolin-5-one. Other nitriles can be generated during food processing, which includes fermentation and/or cooking.

RESULTS: Available data indicate that a number of small structurally related nitriles cause a rich variety of neurotoxic effects. In experimental animals, 3,3'-iminodipropionitrile (IDPN) and allylnitrile are toxic to sensory systems whereas hexadienenitrile cause selective neuronal degeneration in discrete brain nuclei. These effects are

differentially caused by the isomers of crotonitrile: *cis*-crotonitrile causes sensory toxicity whereas *trans*-crotonitrile causes the neuronal effect. IDPN also causes a neurofilamentous axonopathy affecting both the central and the peripheral nervous systems. In addition, dimethylaminopropionitrile is known to cause autonomic (genitourinary) neurotoxicity in both humans and rodents. Some of these actions appear to depend on metabolic bioactivation of the parental nitriles by enzymes that display notable sex-, age- and species-dependent differences in expression levels. Recently, neuronal degeneration has been found in rats exposed to acetone cyanohydrin.

CONCLUSION: The neurotoxic properties of nitriles make them ideal candidate causative agents for konzo, neuropathy and tropical ataxic neuropathy. This hypothesis has still little direct support, but further investigation seems warranted.

KEYWORDS: Konzo, neuropathy, nitriles, cyanogenic glycosides, 2-cyanoethyl-isoxazolin-5-one.

VISUAL EVOKED POTENTIALS IN KONZO

L. K. Nyamabo¹, J. C. Mwanza², D. Tshala-Katumbay³.

¹ Université de Kinshasa, Faculté de Médecine, Département de Neurologie (D.R. Congo)

² Université de Kinshasa, Faculté de Médecine, Service d'Ophthalmologie (D.R. Congo)

³ Oregon Health & Science University (USA)

OBJECTIVES: To assess whether or not visual evoked potentials (VEPs) are abnormal in konzo, a para/tetraparesis of sudden onset, and to correlate the findings to the clinical picture of the disorder.

BACKGROUND: VEPs were recorded in 23 patients (9 men and 14 women, mean age: 23 ± 10 years) suffering from konzo, and 38 healthy subjects (20 men and 18 women, mean age: 27 ± 15 years). The mean P100 latencies and peak-to-peak N75-P100 amplitudes of each eye were measured and compared in the two groups. The mean interocular P100 latency and amplitude differences were calculated and also compared.

RESULTS: VEPs were abnormal in 11/23 patients (48%) consisting of P100 prolongation (7 subjects), absence of P100 wave (2 subjects) or an atypical waveform (2 subjects). The mean P100 latency value of the konzo group was significantly increased as compared with the mean (+2.5 SD) of the reference values from healthy subjects ($p < 0.05$). There was a statistically significant decrease of amplitude in konzo patients compared to normal subjects ($p < 0.05$) with, however, only 2 patients outside the 95% confidence limits. Six patients (27%) had abnormal VEPs despite normal visual acuity. These abnormalities were symmetric and no relation could be found between the duration or the severity of the disease and the VEP perturbation.

CONCLUSION: The main features of these abnormalities are delayed P100 latency and decreased amplitude. These findings indicate involvement of visual pathways and seem to suggest the presence of axonal loss in the prechiasmatal visual pathways in konzo. This study provides evidence that the neurodamage in konzo extends to the visual pathways.

KEYWORDS: konzo, upper motor neuron, visual evoked potentials.

MECHANISM OF OXIDATIVE STRESS BY L- β - ODAP TO MOTOR NEURONS IN RELATION TO NEUROLATHYRISM ROLE OF METHIONINE DEFICIENCY

N. Yoshino¹, A. Minoura¹, F. Ikegami², K. Watanabe¹, T. Kusama¹, K. Kusama-Eguchi¹

¹ College of Pharmacy, Nihon University, Funabashi, Chiba 274-8555 (Japan)

² Chiba University, Kashiwa, Chiba (Japan)

OBJECTIVES: To clarify whether oxidative stress has a chief role in the pathogenesis of neurolathyrism.

BACKGROUND: Neurolathyrism has been found at higher incidence in periods of famine or other food insufficient episodes when other food or total food supply is extremely limited. The amino acid composition of *Lathyrus sativus* is deficient in methionine and cysteine (sulfur-containing amino acids; SAA), which are the precursors of glutathione.

Using rat primary neurons and NSC34 cell line, the role of SAA on cell viability was evaluated.

RESULTS: When both SAAs were deprived from the medium, the toxicity of L- β -ODAP was greatly increased examined by the mitochondrial activity. The increased toxicity was affected by agents that affect intracellular glutathione and other unknown factors controlling ROS level. In fact, ethacrynic acid (EA) that lowers mitochondrial glutathione level was the most toxic agent when added with β -ODAP in a condition of SAA deprivation. N-acetylcysteine was the only effective compound to protect from β -ODAP toxicity among several antioxidants suggesting that glutathione level was more critical in these cells. Our ongoing project to screen the extracts of natural products or fungal culture to save the neurons has picked up nearly 20 candidates. In this project, the toxicities of β -ODAP as well as of transfected gene G93A from ALS patients (another complex motor neuron disease) were the targets. If some effective agents were found to protect against β -ODAP toxicity, they would be worth analysing as powerful drugs to cure motor neuron diseases including neurolathyrism and ALS.

CONCLUSION: β -ODAP had a deleterious effect on the redox status of neurons through the decrease of mitochondrial glutathione.

KEYWORDS: motor neuron, NSC34, glutathione, antioxidants.

NEURO- AND GLIOTOXICITY OF THE EXCITATORY AMINO ACID BETA-ODAP INVOLVES IMPAIRED FREE RADICAL DEFENCE

M. Van Moorhem¹, E. Decrock¹, M. De Bock¹, N. Wang¹, E. De Vuyst¹, G. Callewaert³, L. Van Den Bosch², F. Lambein⁴, L. Leybaert¹

¹ Department of Basic Medical Sciences, Ghent University, Ghent (Belgium)

² Department of Neurosciences, University of Leuven, Leuven (Belgium)

³ Subfaculty of Medicine Campus Kortrijk, University of Leuven, Kortrijk (Belgium)

⁴ Institute of Plant Biotechnology for Developing Countries (IPBO), Ghent University, Ghent (Belgium)

OBJECTIVES: The aim of the present work was to determine the role of oxidative stress on β -ODAP-triggered cell death in motor neurons and glial cells.

BACKGROUND: The excitatory amino acid β -N-oxalyl- α , β -diaminopropionic acid (β -ODAP) is present in grass pea and is related to the neurodegenerative disease neurolathyrism. β -ODAP acts as an agonist of AMPA/KA receptors and metabotropic glutamate receptors, it inhibits Na⁺-dependent glutamate uptake, glutamate-dependent cystine uptake and complex-I of the mitochondrial electron transfer chain and it alters mitochondrial Ca²⁺ handling. These effects take place in neurons as well as in astrocytes and result in the accumulation of glutamate into the extracellular space, the generation of free radicals and a compromised defence against free radicals. The latter probably play an important role in the pathogenesis as the grass pea that triggers this disease is deficient for methionine and cysteine, two precursors of glutathione that confers antioxidant protection.

RESULTS: We studied neurotoxicity by exposing primary rat spinal motor neurons co-cultured with astrocytes to β -ODAP or glutamate over 24 h. β -ODAP and glutamate triggered significant cell death but β -ODAP was more potent when applied at the same concentration. Inclusion of methionine or cysteine in the treatment medium protected the neurons against cell death. Gliotoxicity was evaluated in a simple model system of C6 glioma cells after applying β -ODAP or glutamate for 24 h and quantifying cell survival/death. Inclusion of cysteine or ascorbic acid in the medium decreased the toxicity of β -ODAP and glutamate.

CONCLUSIONS: Our results suggest that oxidative stress is involved in β -ODAP neuro- and gliotoxicity. Future work will be directed to determine the contribution of astrocytes in neurotoxicity, i.e. whether gliotoxic effects increase the sensitivity of the neurons for β -ODAP and glutamate. Evidence from other diseases like ALS suggests that this is indeed the case.

KEYWORDS: β -ODAP, neurotoxicity, gliotoxicity, oxidative stress.

Breeding and biotechnological approach for crop improvement

INTEGRATED MANAGEMENT OF GRASS PEA TO REDUCE RISK OF LATHYRISM

G. Bejjga

Biodiversity and Integrated Gene Management Program
International Center for Agricultural Research in the Dry Areas (Syria)

OBJECTIVE: To reduce risk of lathyrism through integrated management of grass pea production.

BACKGROUND: Grass pea (*Lathyrus sativus*) has been grown for seed and fodder production in many countries. It is tolerant to drought, water logging, cool conditions and moderate alkalinity. It thrives well on poor marginal lands and gives relatively better yield without much management and inputs. The area of grass pea production has been increasing over years in countries like Ethiopia due to frequent occurrence of drought and its ability to survive. It is also an important pulse crop in Bangladesh, India and Nepal. However, it contains a neurotoxin, 3-(N-oxalyl)-L-2,3-diaminopropionic acid (β -ODAP) or β -oxalylamino-L-alanine (BOAA) that causes lathyrism disease (an irreversible paralysis of the lower part of the legs). Breeding efforts have been made to develop varieties with zero or low β -ODAP content to reduce the risk of this disease. Less emphasis has been given to genetic and / or management practices that can reduce β -ODAP content.

RESULTS: Efforts have been made to identify varieties with zero or low β -ODAP content, but, there is no report of grass pea varieties with zero β -ODAP content. Many lines have as high as 0.8% β -ODAP while many lines with less than 0.1% have been identified. Several ICARDA lines like SEL-190, SEL-288, SEL-289, SEL-290, SEL-299, SEL-387, SEL-390, SEL-449, SEL-111 and SEL-222 have less than 0.1% β -ODAP content. Of these SEL-222 (Wasie) was released for production in Ethiopia. A sowing date

experiment conducted at Debre Zeit and Sheno, Ethiopia showed that early sowing will greatly reduce (up to 28%) β -ODAP content of the seed. An application of zinc sulfates at the rate of 15 to 20kg/ha reduced high β -ODAP content of the seed to safe level (<0.2%). Early planting of grass pea coupled with an application of 20 kg/ha of zinc sulfate significantly reduced (up to 42.5%) β -ODAP content of the seeds. Therefore, the use of low β -ODAP varieties coupled with an application of zinc sulfate and early sowing reduces the β -ODAP content of the seed and can minimise the risk of Lathyrism disease. Results of grass pea seed priming with zinc sulfate solution grown at ICARDA in 2009, the effect of soaking and modifying cooking methods are recommended.

KEYWORDS: grass pea, β -ODAP, lathyrism, sowing date, zinc sulfate.

ELIMINATION OF ANTINUTRITIONAL FACTORS FOR QUALITY IMPROVEMENT OF CASSAVA

B. Nambisan, S. Sundaresan

Division of Crop Utilization, Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, - 695017 (India)

OBJECTIVES: Identification of factors influencing the cyanoglucoside content in cassava and elimination of toxicity by processing.

BACKGROUND: Toxicity of cassava arises due to the presence of the cyanoglucosides linamarin and lotaustralin which are hydrolysed by an endogenous enzyme linamarase to acetonecyanohydrin (ACN) and cyanide (CN) which are toxic. Elimination /reduction of cyanoglucoside level to the minimum is necessary in order to make cassava safe for consumption. Major research efforts in this direction have focused on i) development of acyanogenic cassava varieties by breeding, ii) controlling its metabolism and iii) processing to remove cyanogens.

RESULTS and CONCLUSION: The cyanoglucoside (CNgl) content in cassava is genetically controlled and tubers of different varieties show

large variation in CN potential and can be classified as low (< 50ug/g), medium (50 – 100ug/g) and high CN (> 100ug CN equ/g) varieties. The difference in CN potential among tubers of different varieties is not reflected in the CN potential of leaves, stem or root cortex. Translocation of linamarin from leaves also influences the tuber CNgl content. However, the extent to which this contributes to the tuber CNgl level is not clear. Increased leaf senescence/leaf fall, especially during drought, enhances CNgl content in the tubers. Molecular techniques for reducing tuber CNgl have focused on development of transgenic plants with reduced expression of cyt P 450 in leaves, or increased expression of hydroxynitrilase in the tuber. Production of acyanogenic cassava is a long term process. For immediate solution, CNgl content can be reduced using several processing methods. Depending on the nature of the process, they are either hydrolysed, forming ACN and free CN which are lost on heating, or leached into the cooking medium. Traditional methods used for processing include boiling, drying, parboiling and drying, baking, steaming, frying and preparation of flour. These processes result in CN losses ranging from 25 – 98%. The cyanogen level in the final product is influenced both by the tuber CNgl and the method of processing. In order to obtain products with minimum safe level, it would be necessary to use different processing methods for low and high CNgl varieties. The development of new methods of processing especially for cassava containing more than 250ug CN equ/g remains a challenging problem.

KEYWORDS : cassava, cyanoglucosides reduction, minimum safe level.

THE FACTORS INFLUENCING β -ODAP CONTENT AND THEIR POSSIBLE MECHANISMS IN GRASS PEA (*Lathyrus sativus* L.)

C.J. Jiao, Y.C. Xiong, C.Li, L.M. Ke,
J.L. Jiang, X.Y. Wang, F.M. Li, Z.X.
Li, C.Y. Wang*

Key Laboratory of Arid Agroecology under the
Ministry of Education / Institute of Cell Biology,
School of Life Sciences, Lanzhou University,
Lanzhou (China)

OBJECTIVES: To review the factors affecting β -ODAP levels and their possible mechanisms and explore the possibility of decreasing β -ODAP levels in grass pea (*Lathyrus sativus* L.).

BACKGROUND: β -N-oxalyl-L- α , β -diaminopropionic acid (β -ODAP), a toxin causing lathyrism in humans, varies highly even in the same variety depending on different growing conditions. It is, therefore, necessary to study the factors affecting its accumulation and their possible mechanisms for the safe consumption of grass pea seeds.

RESULTS: 1) The levels of β -ODAP reached peaks at seedling and at seed ripening stage, although low levels of β -ODAP were found at other developmental stages; 2) β -ODAP was found in all tissues, but the young leaves and mature seeds contained the highest level of β -ODAP; 3) Under hydroponic conditions, β -ODAP sharply increased in ripening seeds when over-supplied with Fe^{2+} or reduced Zn^{2+} , NO_3^- , Mg^{2+} and K^+ concentrations. But in shoots of the young seedlings, β -ODAP obviously increased with deficiency of N, P, Ca, B, Zn or Mo. Field studies showed that β -ODAP levels in seeds were correlated positively with P, and negatively with Zn, N, Ca, K or Mg in soil; 4) β -ODAP content in seedlings obviously reduced when infected by *Rhizobium*; 5) Drought stress increased the levels of β -ODAP in the seeds. In soil, higher salinity reduced β -ODAP level of seed, while the presence of cadmium or aluminium increased β -ODAP level of seeds.

CONCLUSION: The factors possibly influencing β -ODAP level include development phase, tissue type, nutrition, biotic and abiotic stress. It is speculated that most of the factors influence β -ODAP levels possibly by interfering with its biosynthesis or

degradation, and that this interference may be related to the levels of reactive oxygen species (ROS) in tissues.

KEYWORDS: biotic and abiotic stress, β -ODAP levels, development phase and tissue type, nutrient, *Lathyrus sativus* L.

EXPLORING THE AFRICAN CASSAVA (*Manihot esculenta* Crantz) GERMPLASM FOR SOMATIC EMBRYOGENIC COMPETENCE

D. Ndofunso¹, L. Ndiku¹, K. Kejuni¹,
B. Otono¹, K. Nakweti¹, C. Mba², J.
Jamckowicz²

1 Commissariat Général à l'Énergie Atomique.
CGEA/CREN-K BP. 868 Kin XI Kinshasa
(République Démocratique du Congo)
2 Plant Breeding Unit, FAO/IAEA,
Biotechnology and Agricultural Laboratory.
Vienna. BP. 24440 (Austria)

BACKGROUND: A method for the induction of somatic embryogenesis in five Cassava genotypes from African countries is described. The explants used were leaf lobes isolated from *in vitro* grown plants.

RESULTS: Somatic embryogenesis was achieved in high frequencies by the addition in the induction medium of the auxins picloram and 2,4 dichlorophenoxyacetic acid over a wide range of concentrations in the dark. Pro-embryos obtained from *in vitro* tissue culture of the African cassava genotypes 98/2101, TMEI, Boma, Mpelo nlongi and 130572 were used to determine the effect of the concentration of the auxins.

CONCLUSIONS: The method could be used not only for the mass production of plants of the African cassava genotypes, but also to generate explants (green cotyledons of somatic embryos) as themselves excellent targets for induced mutation.

KEYWORDS : *Manihot esculenta*, cassava, somatic embryogenesis, picloram, 2,4-D.

THE POTENTIAL OF BIOTECHNOLOGY IN ETHIOPIA: PRESENT SITUATION AND EXPECTED DEVELOPMENT

K. G. Dereje

Era Agrilink PLC, P. O. Box 22927, Addis
Ababa (Ethiopia)

BACKGROUND: Ethiopia owns immense genetic wealth of flora and fauna. Vavilov, the Russian geneticist, in his report in 1951, designated Ethiopia as one of the eight crop diversity centers of the world. The country has contributed much for crop improvement globally. Some of the examples include Ryd2 and Ryd3 genes for BYDV resistance, mlo-11 and related genes for powdery mildew resistance, high lysine containing zera-zera sorghum varieties, drought tolerant traits in sorghum and grass pea. Due to incapacity in biotechnology research, however, the potential of the country has not been fully explored.

RESULTS: The Horn Biotechnology Forum's survey in 2006 to assess the national biotechnology capacity of Ethiopia revealed that 7 institutions with a total of 24 branches were involved in or under preparation to commence biotechnological activities. Out of these institutes/ centers, 10 have got modest to well-equipped molecular labs and the rest only had or planned tissue culture labs. These institutes hosted 105 scientists with different levels of acquaintance in biotechnology, and a total of 65 projects were undertaken in immunology, diagnostics, vaccine development, epidemiology, molecular markers, plant tissue culture, bio-fertilizer and embryo transfer. However, almost all advanced studies were conducted in collaboration with foreign laboratories. In agriculture, most activities across the country were in tissue culture. With the aim of commencing higher level plant, animal and microbial biotech research, the Ethiopian Institute of Agricultural Research has built a laboratory complex at Holetta Agricultural Research Center. However, this laboratory has not yet been functional due to logistic and technical reasons.

CONCLUSION: It is recommended that long term manpower

development plan, strong inter- and intra-institutional linkage/networking, flexible procurement procedure for consumables, fast custom release service, competitive incentive system, and constant electric power supply should be practiced. Moreover, researchers should be encouraged to develop joint projects (of any scale) with scientists worldwide.

KEYWORDS: BYDV, Ryd2, Ryd3, mlo-11, Zera-zera sorghum.

PRELIMINARY ATTEMPTS IN MOLECULAR BREEDING OF ETHIOPIAN GRASS PEA (*Lathyrus sativus* L.): PRODUCTIVE REGENERATION AND GENETIC TRANSFORMATION

D. Girma¹, A. Fikre², N. Terryn³,
Y.H. Kuo³, F. Lambein³

¹ Debre Brehan Agricultural Research Center, P.O.Box 112, Debre Brehan (Ethiopia)
² Debre Zeit Agricultural Research Center, P.O. Box 32, Debre Zeit (Ethiopia)
³ Institute of Plant Biotechnology for Developing Countries (IPBO), Ghent University (Belgium)

OBJECTIVES: Productive regeneration of grass pea (*Lathyrus sativus* L.) with an attempt for *Agrobacterium*-mediated genetic transformation.

BACKGROUND: Grass pea is an ancient crop adapted to marginal soils and producing protein rich seeds during droughts and even surviving floods. When grass pea is used as the sole staple during extended periods, the irreversible crippling lathyrism can occur. Reduction of a neuroactive amino acid in the seeds and increasing essential sulfur amino acids by breeding or genetic transformation may improve the nutritional quality of grass pea.

RESULTS: We report a productive regeneration and an *Agrobacterium*-mediated transient genetic transformation of several Ethiopian grass pea genotypes. For regeneration, a popular Ethiopian local cultivar from Debre Zeit area (DZ-local) was used. Buds without scale and shoot tips were used as source for callus generation. For genetic transformation three grass pea genotypes obtained from Wollo, Gondar, and Bahir Dar, Ethiopia

were used. Cotyledonary nodes from *in vitro* grown *Lathyrus sativus* seedlings were infiltrated with *Agrobacterium* suspension for 20 minutes under vacuum and aseptically cultured on MS medium containing two levels of BAP 1mg/l or 2mg/l. The bacterial strain used was C58C1Rif^R carrying the binary plasmid pTAG-arc5-M1,2 coding for extra methionine.

Effective callus development was obtained in the range 30 % to 55 % for zero-passage and a three passage treatment respectively. In agreement with other similar work in the literature apical or axillary buds produced more compact green tissues than explants from apical segments. In the transformation experiments, from each genotype after three weeks of co-cultivation on MS-medium six randomly selected explants with adventitious shoots were used for GUS test. Our reporter system revealed that two grass pea genotypes (Wollo and Gonder) have shown transient GUS expression, indicating the potential for genetic transformation.

CONCLUSION: Genetic transformation of grass pea via *Agrobacterium* technology should be further investigated as a way to improve the sulfur amino acid content for safer consumption.

KEYWORDS: Grass pea, regeneration, genetic transformation, *Agrobacterium*.

VIRUS INDUCED GENE SILENCING IN *LATHYRUS SATIVUS*

J. E. Johansen¹, G. D. Constantin¹,
O. S. Lund²

¹ Department of Genetics and Biotechnology, Faculty of Agricultural Sciences, Aarhus University, Frederiksberg (Denmark)
² Danish Seed Health Centre, Faculty of Life Sciences, University of Copenhagen, Frederiksberg (Denmark)

OBJECTIVES: To determine the function of candidate genes in *Lathyrus sativus*.

BACKGROUND: Plant defence against virus infection includes an RNAi based sequence specific degradation of virus RNA. The defence reaction is triggered by double stranded RNA and can be exploited to determine the function of candidate genes. This requires construction of an infectious clone of

a virus that can be manipulated by insertion of heterologous cDNA fragments. Upon inoculation with the virus carrying a gene fragment from the host plant, both the virus and host mRNA with sequence identity to the inserted fragment are degraded. Often the degradation of host mRNA is sufficiently efficient to induce a clear phenotype in the plant. This response is known as virus induced gene silencing (VIGS).

RESULTS: The RNA virus *Pea early browning virus* (PEBV) consists of two RNA molecules RNA1 and RNA2. Infectious cDNA clones have been constructed, which allow insertion of heterologous cDNA fragments into the cDNA of RNA2. The PEBV based virus vector has been used extensively as a tool to determine gene function in pea. Recently we found that PEBV also infects *Lathyrus sativus* and observed that PEBV can induce efficient silencing of a test gene.

CONCLUSION: We expect that PEBV can be used to determine the function of candidate genes in *L. sativus* and support the efforts towards making *L. sativus* a safe crop.

KEYWORDS: *Lathyrus sativus*, gene function, virus vector.

BREEDING STRATEGIES IN NIGERIA TO IMPROVE CASSAVA AGAINST CYANOGENIC GLUCOSIDES

E. H. Kwon-Ndung, A. Takyun

Plant Science and Biotechnology Unit, Dept. of Biological Sciences, Nasarawa State University, Keffi PMB 1022 Keffi, Nasarawa State (Nigeria)

OBJECTIVES: Propagation of cassava clones with very low cyanogenic glucosides.

BACKGROUND: The tuberous roots of cassava are a source of more than half the calories consumed by over 200 million people in sub-Saharan Africa. The leaves are also often used as a vegetable and are a very reliable source of protein, vitamins and minerals. Nevertheless, the cyanogenic potential of cassava has often been cited as a contributory factor of health problems, most especially in areas where cassava roots or leaves are not properly processed. Documented reports in Nigeria have

shown records of mortality arising as a result of consumption of cassava food products, commonly among the resource poor households. There is an urgent need to provide farmers with improved varieties that have low cyanogenic potential.

RESULTS and CONCLUSIONS: In a series of studies, at the Plant Science and Biotechnology program in the Department of Biological Sciences between 2006 and 2008 cropping seasons, different accessions or strains of cassava collected from different farmers in selected cassava growing areas in central Nigeria were evaluated in the field for their morphological characters and in the laboratory for their cyanogenic properties. The present results have delineated the traits in different accessions for both field and laboratory, which can be employed in conventional breeding objectives. However, in view of the constraints of conventional breeding of cassava due to its long breeding cycle, heterozygosity, and difficulties in producing seed, this study envisages that the use of marker assisted selection will be used in influencing accelerated introgression of traits from these collections so as to produce clones without any trace of cyanogenic glucosides and safe for food.

KEYWORDS: cassava, cyanogenic glucosides, breeding, clones, marker assisted selection.

RE-INTRODUCTION OF GRASS PEA (*Lathyrus sativus*) IN SERBIA

A. Mikić¹, V. Mihailović¹, B. Čupina², Đ. Krstić², B. Đurić³, M. Vasić¹

1 Institute of Field and Vegetable Crops, Novi Sad (Serbia)
2 Faculty of Agriculture, University of Novi Sad, Novi Sad (Serbia)
3 Faculty of Agriculture, University of Banja Luka, Banja Luka (Republic of Srpska, Bosnia and Herzegovina)

OBJECTIVES: Recent achievements in grass pea research in Serbia.

BACKGROUND: Vetchlings or chickling vetches (*Lathyrus* spp.) are widely distributed in both Serbia and Srpska, and represent a valuable component of local floras all over the Balkan Peninsula. Archaeological evidence witnesses that grass pea (*L. sativus* L.) and flat-podded vetchling (*L. cicera* L.) have been

cultivated for millennia. Despite this and the existence of the traditional Serbian name for grass pea, *sastrica*, and pan-Slavic name for all vetchlings, *grahor*, today they are almost forgotten crops. As a part of its long-term programme of promoting legumes in the Serbian agriculture, the joint action of the Institute of Field and Vegetable Crops and the Faculty of Agriculture is aimed at re-introducing grass pea and other vetchlings as multifunctional crops.

RESULTS and CONCLUSIONS: Within the legume collection in the Institute of Field and Vegetable Crops, a small *Lathyrus* collection has been established in 2002. In 2009, it contained about 100 accessions of 16 species, with grass pea as the most numerous one. The majority of grass pea accessions in the Novi Sad collection are advanced cultivars, mainly from Poland. Today, the most important task is to enrich the collection not only with wild populations of diverse vetchlings, such as groundnut peavine (*L. tuberosus* L.) or yellow vetchling (*L. aphaca* L.), but with local landraces of grass pea, since they still can be found in certain regions of both Serbia and Srpska, where they are used for both human consumption and medicinal purposes. The first Serbian breeding programme in Novi Sad produced two lines in the process of registration in Serbia, with forage dry matter yields of between 8 t ha⁻¹ and 9 t ha⁻¹ and grain yields of up to 5 t ha⁻¹.

KEYWORDS: grass pea, genetic resources, breeding, grain yield, forage dry matter yield.

DIFFERENT CYTOGENETIC TESTER STOCKS AND LINKAGE MAPPING IN GRASS PEA (*Lathyrus sativus* L.)

Dibyendu Talukdar

Department of Botany, University of Kalyani, Kalyani, West Bengal (India)

OBJECTIVES: Development of different cytological and genetic stocks and their use in linkage mapping in *Lathyrus sativus* L.

BACKGROUND: A comprehensive genetic linkage map comprising of different morphological, biochemical and molecular markers is necessary

for future improvement of grass pea as an ideal pulse crop with high yield and low seed neurotoxin (ODAP) content. Aneuploids including a series of trisomics, tetrasomics and double trisomics, along with different structural chromosomal aberrations and different true breeding diploid mutant lines showing contrasting phenotypic modifications, are excellent tools to study genetic control and linkage mapping of different markers. Therefore I have been trying to develop these stocks in grass pea.

RESULTS: Following treatment of seeds of seven grass pea varieties with different doses of EMS and gamma rays 87 variant plants were isolated in M₂ generation. Among these plants, 58 were diploids (2n=2x=14) which bred true and showed no chromosomal abnormalities while the rest of the plants exhibited either numerical or structural chromosomal rearrangements. In the last several years, a complete set of seven different primary trisomics (2n+1, 2n=15), several tetrasomics (2n+2, 2n=16) and double trisomics (2n+1+1=16) were cytogenetically characterized. In addition, four semi-sterile mutant lines were cytologically analysed as translocation heterozygotes. Using these primary trisomics, several genes including *df2*, *df3* (dwarfism), *wgn* (winged modification of internode), *lfc* (leaflet colour) and *cb1* (seed coat colour) were mapped on extra chromosome of different trisomics and linkage association between different isozymes and morphological trait loci on different linkage groups are now being analysed. Preliminary investigations also revealed significant variation of seed ODAP content in three primary trisomics and three respective tetrasomic lines suggesting possible involvement of number of genes on different chromosomes in governing this quantitative trait.

CONCLUSION: Different aneuploids and translocation heterozygotes as cytological tester sets and diploid mutant lines as genetic marker stocks are being developed and have the potential in mapping of different DNA-based markers along with isozyme and morphological traits in grass pea.

KEYWORDS: cytogenetic stocks, linkage map, grass pea.

TOWARDS AN AUSTRALIAN, ZERO NEURO-TOXIN LINE OF COMMON VETCH *Vicia sativa* L.

M. Tate, T. L. D. Chowdhury, D. Enneking

School of Agriculture, Food & Wine, University of Adelaide, Waite Agricultural Research Institute, Waite Rd. Glen Osmond, 5064 South Australia (Australia)

OBJECTIVE: Non-toxic seeds of common vetch (*Vicia sativa* L.).

BACKGROUND: In 1939 a paper was published (Sha, S.R.A: A note on some cases of lathyrism in a Punjab village. Indian Med. Gaz. 74: 385-388) in which a form of paralysis was noted, which resembled the lathyrism induced by the consumption of *Lathyrus sativus*. Charlotte Ressler identified the neurotoxic principle in vetch: (Ressler, C., Giza, Y.H., Nigam, S.N. (1969) "Toxic principle in vetch. Isolation and identification of γ -L-Glutamyl- β -Cyano-L-Alanine from common vetch seeds". J. Am. Chem. Soc. 91, 2758-2765). In 1992 and 1999, *Nature* papers from this laboratory reported the export of a common vetch cultivar with orange cotyledons (cv. Blanche fleur) for human consumption in third world markets. To protect its lentil industry, Australia legislated in 1999, to prescribe both vetch and lentils. Prescription ensures minimisation of vetch/lentil substitution, by inspection and obligatory phytosanitary certificates, with the correct botanical descriptions. Later Australian Government (Commonwealth Gazette, 18th June, 2003) legislation absolutely prohibits the export of split vetch from Australia, making it a criminal offence with a maximum penalty of five years imprisonment.

RESULTS AND CONCLUSION: The % γ -L-Glutamyl- β -Cyano-L-Alanine content in a white-seeded common vetch (Jericho White = 1.88%) was reduced by two hybridizations via a white-seeded PBR protected line (Love 2 = 0.9%). Recurrent selection has provided white- and dark-seeded lines with toxin < 0.4%. Cyano-alanine toxin (mg) per seed is a more reliable indicator of progress than the environmentally-sensitive variation of % toxin values.

KEYWORDS: *Vicia sativa*, nitrile-neurotoxin, new crop development, single-seed IR selection, plant breeding.

AGRONOMIC, QUALITATIVE (ODAP) AND MOLECULAR VARIABILITY IN GRASSPEA POPULATIONS OF THE MARCHE REGION (CENTRAL ITALY).

S. Tavoletti, L. Iommarini, L. Mogliani

Dipartimento di Scienze Alimentari, Agro-Ingegneristiche, Fisiche, Economico-Agrarie e del Territorio (S.A.I.F.E.T), Università Politecnica delle Marche, Ancona (Italy)

OBJECTIVES: Characterisation of grass pea (*Lathyrus sativus* L.) accessions collected in the Marche Region (Central Italy) to start a breeding program for the valorization of this under-utilized crop.

BACKGROUND: Grass pea is an interesting crop to be included in rational crop rotations in inner areas of the Marche Region, since it is characterised by a wide adaptation to different environmental conditions. However, breeding efforts are needed to improve its agronomic performance and to lower as much as possible ODAP seed content. Since year 2000, a germplasm collection and evaluation program has been carried out. Accessions were evaluated by field trials for morphological and agronomic traits and for seed ODAP content. Moreover, AFLP molecular markers were applied to investigate the level of genetic variation characterising the populations included in the collection. In year 2008 a breeding plan has been started using two household populations showing the lowest average ODAP content and the highest agronomic performance, respectively.

RESULTS and CONCLUSIONS: Significant variability among population was detected for agronomic traits. In particular, household populations, collected in local farms, showed a higher seed production compared with unadapted accessions. Concerning ODAP content, a range of variation between 0.26% and 0.53% was detected. Based on these results, a within population analysis for this

trait will be started using the accession with the lowest ODAP content. Interesting information was also gathered using AFLP molecular markers, since a wide range of genetic variation supported the possibility of starting a mapping project aimed at the identification of genes involved in the ODAP biochemical pathway.

KEYWORDS: ODAP, AFLP markers, grass pea breeding.

TRIGGERING TIME OF NON-HYDRAULIC ROOT-SOURCED SIGNAL ACCOUNTS FOR THE INTENSITY OF ABSCISIC ACID SIGNAL AND THE GENERATION OF TOXIN ODAP IN GRASS PEA SEEDLINGS

Y.C. Xiong, F.M. Li[†], C.J. Jiao, C.Y. Wang, H.Y. Kong, P.F. Li, Y.F. Wang, Z.X. Li, X.W. Fan

MOE Key Laboratory of Arid and Grassland Ecology, Lanzhou University, Lanzhou 730000 (P. R. China)

OBJECTIVES: Roles of exogenous Eu^{3+} application in regulating drought adaptation of grass pea seedlings.

BACKGROUND: Previous work showed that addition of exogenous Eu^{3+} significantly affected drought tolerance and stomatal sensitivity in grass pea (*Lathyrus sativus* L.) seedlings, both of which were regulated by the start and closure of non-hydraulic root-sourced signal (nHRS). To further understand the triggering mechanism of nHRS and its eco-physiological effect, the dynamics of plasma membrane H^+ -ATPase (PM H^+ -ATPase) in root system, and abscisic acid (ABA) and β -N-oxalyl-L- α,β -diaminopropionic acid (ODAP, a toxic amino acid induced by drought stress) in leaves were simultaneously quantified along with a soil drying gradient from -0.21 to -6.94 MPa in *Lathyrus sativus* seedlings grown in soil with (+ Eu^{3+} treated) and without (- Eu^{3+} treated) added Eu^{3+} solution. At the soil water potentials (Ψ_{soil}) of -0.75 and -1.75 MPa respectively, the nHRS was successively triggered and ceased in - Eu^{3+} treated seedlings, while addition of Eu^{3+} significantly altered these two Ψ_{soil} to

-0.45 and -2.29 MPa respectively, a wider threshold range.

RESULTS: This result was evidently supported by the dynamics of PM H⁺-ATPase activity and ABA biosynthesis. Addition of Eu³⁺ induced earlier increase of PM H⁺-ATPase activity in roots and ABA content in leaves, coupled with ODAP production in leaves. With prolonged drought stress, PM H⁺-ATPase activity and ABA content were observed to be generally higher in +Eu³⁺ treated seedlings than in -Eu³⁺ treated seedlings, however, under the same condition, the event of ODAP accumulation was reversed. The accumulation of ODAP might be up-regulated by early onset of root-sourced signal, and the latter might be correlated with earlier activation of PM H⁺-ATPase. This result can be explained by an "X" model hypothesis of ABA signal intensity (decrease ratio of Gs / increase ratio of ABA content). Our study provides a preliminary evidence or mechanism for our previously proposed "weakening hypothesis of root-sourced signal" under water deficit, and a potential agronomic approach to lower ODAP production through modulating root-sourced signal behavior in grass pea.

KEYWORDS: plasma membrane H⁺-ATPase, abscisic acid, drought stress, grass pea, ODAP.

**Workshop on
Toxico-Nutritional
Neurodegenerations Konzo
and Lathyrism
Ghent, 21-22 September
2009**

**Recommendations from the
Cassava/konzo-Grass
pea/neurolathyrism Group**

Because of their ability to perform well in marginal soils and under harsh agro-climatic conditions, cassava (*Manihot esculenta*) and grass pea (*Lathyrus sativus*) offer ample opportunities for sustainable agriculture and food security for the poorest of the poor in the context of climate change. These crops have also been associated with health problems of konzo, tropical ataxic neuropathy (TAN) and neurolathyrism, which are

irreversible neurodegenerations, that need to be addressed. The poorest populations of subsistence farmers mostly depend on these crops for their food and nutritional security in years of environmental extremities and are most at risk of these incurable but preventable paralysis of the lower limbs. A two-day "International Workshop on Toxico-Nutritional Neurodegenerations – Konzo and Lathyrism" was organized at Ghent University, Belgium on 21 and 22 September 2009 in which 55 scientists from five continents presented 18 oral presentations and 27 posters, highlighting various aspects of these two important crops and their health problems related to over-consumption. During the panel and round-table discussions, the following recommendations were made:

1. There is an **urgent need to better understand the etiology of these two diseases** with very similar epidemiology and the same clinical symptoms, but from consuming completely different food (konzo from cassava roots and neurolathyrism from grasspea seeds). There is a need to explore the molecular pathways of both diseases and to find the common biochemical and neurobiological events. From this common ground, effective prevention strategies can be designed.
2. We need to more correctly evaluate the nutritional quality of these two most hardy lifesaver crops and their contribution to food security as well as to dispel the misgivings about the toxico-nutritional problems (konzo and neurolathyrism) associated with these crops. The presence of toxins (neuro-active amino acids and nitriles) as well as the deficiency of essential nutrients need to be considered. Especially the sulfur amino acids that are deficient in both crops need special consideration. With genetic enhancement of the crops and optimized post-harvest processing, the nutritional status of the populations depending on these crops can be much improved.
3. It is crucial to understand the genetics of the unique traits (drought tolerance, disease resistance, and high nitrogen fixation in the case of grass pea) of these life-saving crops for harvesting the benefit of molecular tools. For the **genetic improvement** of these crops, research efforts should be directed towards better nutritional quality, higher yield and resistance to key diseases, drought and high temperature and also better nitrogen fixing ability or fertilizing. This requires creating and strengthening of plant breeding and molecular research at national and international levels involved in grass pea and cassava improvement research programs. These traits are of global importance and can also benefit other crops.
4. Research on better **agronomic practices** and the environmental effect (drought, CO₂) on the toxin content and the nutritional quality are required for improving their adaptation to various crop production systems and new niches. Introduction of cassava and grass pea into new niches needs to be accompanied by knowledge transfer on production agronomy, post-harvest processing, risks and opportunities. Introduction of both crops in the same cropping systems and niches might have a synergistic effect on the incidence of konzo and/of neurolathyrism and needs preliminary studies with experimental animals.
5. There is an urgent need to standardize the **food processing methods** and food preparations for lowering toxins in cassava roots and grass pea seeds for safe consumption. Fermentation and bio-fortification of cassava and grass pea foods needs focused research for optimized nutrition and a better balanced diet.
6. **Multi-disciplinary efforts** involving nutritionists, toxicologists, neuropathologists, biochemists, plant scientists and social scientists are required for solving these global problems of konzo and neurolathyrism. A formal research forum with Fernand Lambein, IPBO, Ghent

University as its coordinator is recommended to establish a network of researchers for effective sharing of information on these two crops and on the prevention of konzo and neuropathy.

7. **Konzo and Neuropathy are seen as clinical symptoms of abject poverty and malnutrition.** Making konzo and neuropathy **reportable diseases** can make emergency food aid more effective and will help to direct agricultural development to the most neglected and the really needy. Prevention of malnutrition through diversification of diets, alleviation of poverty and improved education needs to be **effectively communicated** among the different stakeholders in order to give cassava and grass pea the right place in a healthy diet. The poorest of the poor subsistence farmers who survive on grass pea and cassava as a source of staple food and an insurance crop should be identified and mapped. Involvement of policy makers, education institutions, NGOs, self-help groups and different stakeholders in communicating about the benefits and risks of cassava and grass pea needs to be ensured. Local knowledge associated with these two orphan crops needs to be documented for the benefit of society. This has to be coupled with rehabilitation programs for the affected people.

8. In order to activate these recommendations, there is a need to develop **research project proposals** for financial assistance from national and international donors (FAO, WHO, BM Gates Foundation, EU, JICA, ACIAR, USAID, DFID, DANIDA). There is also need for a **Konzo—lathyrism (or Cassava-Grass pea) Newsletter** as a regular periodical as a tool to speed up communication and information.

Coordinator's Note: It is hoped that part of Recommendation 8 has been adequately fulfilled, by expansion of the Cassava Cyanide Diseases Network News into the Cassava Cyanide Diseases & Neuropathy News.

CCDN News is the Newsletter of the Cassava Cyanide Diseases and Neuropathy Network (CCDN). The CCDN is a free, worldwide network commenced in June 2001, which is working towards the elimination of konzo, TAN, other cassava cyanide diseases and neuropathy.

CCDN News will consider for publication short articles and letters (1-3 pages A 4 double spaced) written in English. Because CCDN News is a newsletter, full-size original papers or reviews cannot be considered for publication. Material published in CCDN News may be freely reproduced, but please always indicate that it comes from CCDN News. Please send all correspondence to the CCDN Coordinator, Dr J Howard Bradbury, Botany and Zoology, Research School of Biology, Australian National University, Canberra, ACT 0200, Australia.

Dr. Dulce Nhassico from Mozambique first produced the 'Poison Poster' that gives instructions (developed by Dr. Howard Bradbury in Australia) on how to process cassava flour to reduce the cyanide poison in it to a safe level.

The original version was in Portuguese and linked the poison with the danger of getting konzo.

Since the original version was produced, the poster has been translated into a number of languages and the wider effects of the poison mentioned, not just konzo.

When the poster first came out, a simple cassava flour processing method which took 5 hours to complete was available to reduce the poison. A modified method is now available which takes 2 hours. The posters are being changed to include both options.

Versions of the poster below are currently available in Amharic, Ateso, English, French, Hausa, Kifuluru, Kiswahili, Macua and Portuguese.

Portuguese

Como tirar o veneno da farinha de mandioca

O QUE É QUÊ, O VENENO DA FARINHA DA MANDIOCA AMARGA PROVOCA?
Quando você come a farinha feita de mandioca amarga que não foi correctamente processada, pode ter:

- Envenenamento logo depois de comer a farinha; com aparecimento de tonturas, dor de cabeça, dor do estômago, diarreia, vômitos e algumas vezes pode chegar a morrer
- **Konzo**; que é uma paralisia definitiva das pernas e que não deixa a pessoa andar. Esta paralisia acontece algumas semanas depois de comer a farinha da mandioca amarga não processada.

COMO TIRAR O VENENO?

- 1** É muito fácil: Medir a quantidade de farinha que você quer cozinhar.
- 2** Colocar a farinha numa panela ou bacia, alisar a superfície da farinha e de seguida marcar a altura da mesma com a ponta de uma faca.
- 3** Deitar água limpa aos poucos e mexendo, até a farinha ficar molhada e o seu nível ser igual ao da farinha seca (como você marcou por dentro). A farinha deve ficar completamente molhada, mas **NÃO** em papa e também **SEM** boias de farinha seca.
- 4** Espalhar a farinha numa peneira, esteira limpa ou tabuleiro, usando uma colher ou a mão, de maneira que a altura dessa farinha espalhada não passe a altura da unha do dedo da sua mão. Depois, deixar a farinha na sombra durante cinco horas.
- 5** Colocar água na panela, deixar ferver e deitar a farinha já tratada até obter a consistência desejada. É importante usar menos água do que o habitual porque a água usada para molhar a farinha também conta, porque ela não seca.
- 6** Agora você tem certeza de estar a dar comida boa à sua família e sem causar paralisia.

Author and E-mail Index

Name	e-mail address	Page			
Ahmed S.		9	Llorens J.	jllorens@ub.edu	15
Balegamire J. M.		6	Lola J. M.		6
Balegamire S.		6	Lund O. S.		19
Balol'ebwami S. Z.		6	Lyddiard J. R. A.		13
Banea-Mayambu J. P.	jpbanea@yahoo.fr	5, 6, 8, 12	Maheshe B.		6
Bejiga G.	G.Bejiga@cgiar.org	17	Malela Pingudi		12
Bonnet M. J.		6	Masheka O. C.	oliviermasheka@yahoo.fr	6
Bradbury J. H.	Howard.Bradbury@anu.edu.au	4, 5, 8	Masika Y. D.		11
Brimer L.	leon.brimer@gmail.com	9, 11	Mba C.		18
Callewaert G.		16	Mihailović V.		20
Chabwine J. N.	jo_nsimy@yahoo.fr	6	Mikić A.	mikic@ifvcns.ns.ac.rs	20
Chitundu M.		9	Minoura A.	phai09018@g.nihon-u.ac.jp	16
Chiwona-Karlton L.	Linley.Chiwona.Karlton@sol.slu.se	9, 11	Mlingi N.L.V.	nmlingi@yahoo.co.uk	5
Chowdhury T. L. D.		21	Mogliani L.		21
Ciglenecki I.	lza.ciglenecki@geneva.msf.org	6	Mulumba Nkuadi A.	mulnkuadi@yahoo.fr	5, 12
Cliff J.	julie.cliff@gmail.com	4, 8	Muquinque H.		4
Constantin G. D.		19	Mutendela K.		6
Ćupina B.		20	Mwanza J. C.	jcmwanza@hotmail.com	15
Dadi L.		12	Nahimana Gitebo D.	damenahimana@yahoo.fr	5, 8
De Bock M.		16	Nakweti K.		18
De Vuyst E.		16	Nambisan B.	balanambisan@yahoo.co.uk	17
Decrock E.		16	Ndiku L.		18
Denton I. C.		8	Ndofunsu D.	aimediamuini@yahoo.fr	18
Dereje K. G.	kassagetu@yahoo.com	18	Nemery B.		6
Diasolua Ngudi D.	ddiasolu@yahoo.com	6	Ngude H.		5
Dufour D. L.	darna.dufour@colorado.edu	4	Nhassico D.		4
Đurić B.		20	Nkya S.		5
Enneking D.	enneking@westnet.com.au	15, 21	Nunga Matadi R.		8
Eshete M.		9	Nunn P. B.	shoeman_r@yahoo.co.uk	13
Eyema R.		6	Nyamabo L. K.		15
Fan X.W.		21	Nyirenda D. B.	bandanyirenda@gmail.com	9, 11
Feleke A.	felekeam@yahoo.com	7	Nzwalo H.		4
Fikre A.	fikreasnake@yahoo.com	7, 9, 19	Otono B.		18
Gebre-Medhin M.		8	Pandey R. L.		13
Geda A. K.		13	Rao S. L. N.	slnrao213@rediffmail.com	3
Getahun H.	getahunh@who.int	9	Rosling H.	hans.rosling@ki.se	8
Girma A.	antegirma@yahoo.com	12	Rutega B.		6
Girma D.	doju12@yahoo.com	19	Shangalume O.		6
Haque M. R.	dr_rabiul@yahoo.com	10	Sundaresan S.		17
Ikegami F.		13, 16	Takyun A.		19
Iommarini L.		21	Talukdar D.		20
Jamckowicz J.		18	Tatala S.R.		5
Jiang J.L.		11, 18	Tate M.	max.tate@adelaide.edu.au	21
Jiao C.J.	jiaochj02@st.lzu.edu.cn	11, 18, 21	Tavoletti S.	s.tavoletti@univpm.it	21
Johansen I. E.	e.johansen@dias.kvl.dk	19	Tefera B.		12
Kabanda C.		6	Tekle-Haimanot R.		7
Kabena O. N. G.		11	Terryn N.		19
Ke L.M.		11, 18	Tshala-Katumbay D.	tshalad@ohsu.edu	14, 15
Kejuni K.		18	Tylleskar T.	Thorkild.Tylleskar@cih.uib.no	5, 8
Kolsteren P.		6	Taafo F.		6
Kong H.Y.		21	Urbaniak V.		6
Krstić Đ.		20	Van Den Bosch L.		16
Kuo Y.H.	yuhaeykuo@ugent.be	9, 10, 19	Van Moorhem M.	Marijke.VanMoorhem@UGent.be	14, 16
Kusama T.		13, 16	Vasić M.		20
Kusama-Eguchi K.	kusama.kuniko@nihon-u.ac.jp	13, 16	Wang C.Y.	wangcy@lzu.edu.cn	11, 18, 21
Kwon-Ndung E. H.	kwon_ndung@yahoo.com	19	Wang N.		16
Lambein F.	fernand.lambein@gmail.com	6, 7, 9, 10, 13, 14, 16, 19	Wang X.Y.		11, 18
Leybaert L.	Luc.Leybaert@UGent.be	14, 16	Wang Y.F.		21
Li C.		11, 18	Watanabe K.		16
Li F.M.		11, 18, 21	Xiong Y.C.	xiongyc@lzu.edu.cn	11, 18, 21
Li P.F.		21	Yamazaki Y.		13
Li Z.X.		11, 18, 21	Yandju D. L. M. C.	mcyandju@yahoo.fr	11
			Yoshino N.		16