

## PROGRESS IN PREVENTION OF TOXICO-NUTRITIONAL NEURODEGENERATIONS

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### Abstract

Grass pea (*Lathyrus sativus*) is a survival food during drought in Ethiopia and the Indian Subcontinent, producing the cheapest dietary protein and saving thousands of lives. It also is a mixed blessing as the cause of the crippling neurolathyrism after prolonged over-consumption. Cassava (*Manihot esculenta*) is a protein-poor root crop that is the staple food for over half a billion people in Africa, South America and Southeast Asia, and the cheapest source of dietary carbohydrates. Over-consumption of cassava roots in a monotonous diet can cause konzo, with clinical symptoms indistinguishable from neurolathyrism. The prominent features of both diseases are sudden onset of symmetric spastic paraparesis of the calf muscles and scissor gate. The common feature of grass pea seed and cassava roots is the low content of the essential sulphur containing amino acids methionine and cysteine. The focus of breeding has been the reduction of the neuro-excitatory amino acid  $\beta$ -ODAP ( $\beta$ -N-oxalyl-L- $\alpha$ , $\beta$ -diaminopropionic acid) in grass pea and the reduction of the cyanogenic glucosides in cassava. Developing varieties with higher content in methionine and cysteine and a better balanced diet may be more relevant in improving nutrition without jeopardizing the tolerance for biotic and abiotic stress of these crops.

**Keywords:** *Lathyrus sativus*, *Manihot esculenta*, oxidative stress, methionine deficiency, drought tolerance, spastic paraparesis

### Introduction

High dietary dependence on grass pea (*Lathyrus sativus* L.) and cassava (*Manihot esculenta* C.) with neurotoxic potential has been implicated in the aetiology of neurolathyrism and konzo, respectively. Those two neurological diseases are clinically very similar but the food plants causing these diseases are very different, belonging to different plant families. Overconsumption of the protein-rich seeds of grass pea is linked to the incidence of neurolathyrism, and overconsumption of insufficiently processed carbohydrate rich roots of cassava is linked to the occurrence of konzo. Identification of a neuroactive non-protein amino acid  $\beta$ -ODAP in grass pea seed and the presence of cyanogenic glucosides in cassava roots did not bring the aetiology of these diseases much closer. Both neurolathyrism and konzo are characterized by spastic paraparesis of the legs, leading to scissor gate with various degrees of incapacitation, up to bedridden or crawler stage.

Reliance on grass pea or on insufficiently processed bitter cassava as staple foods, combined with a diet poor in me-

thionine, has resulted in outbreaks of neurolathyrism and konzo. Epidemiology of the two diseases is more similar as both occur mainly among isolated very poor remote rural people with very little or no formal education, living as subsistence farmers on drought prone or poor soil conditions. Droughts and other determinants of food insecurity lead them to consumption of either grass pea seeds or short-cut processed bitter cassava roots as almost exclusive staple food for extended periods. Individual susceptibility to the toxic effects of these plants has been suggested to vary with subject age, gender, nutritional status, ingested quantity of the toxin and quality of the diet.

### Grass pea (*Lathyrus sativus* L., tribe *Vicieae*, family *Fabaceae*)

As a crop for human consumption grass pea has survived since the Neolithicum. The centre of biodiversity is the Eastern Mediterranean and Ethiopia. In many European countries it was a popular crop in the Middle Ages and it has a specific name in all European languages [1]. It is still grown by traditional farmers in small amounts in many European countries. Of the over 150 species of the genus *Lathyrus*, very few can be crossed by simple means. Self-pollination is the rule with limited out-crossing due to male sterility. The crop grows on marginal land and is tolerant to biological and environmental stresses better than other legumes [2]. It is also the most efficient nitrogen fixer compared to other food legume crops [3] and thus improves the fertility of the soil for subsequent crops. This property is even exploited by Ethiopian farmers to increase the economic value of land. **Figure 1** shows the cropping system in north-western of Ethiopia where grass pea is extensively cultivated. Grass pea is being used not only for human consumption but also as green manure, as fodder, as feed and for grazing. Grass pea seed has high protein content (25.6-28.4 %) and produces the cheapest protein available for the poor and is the legume consumed in largest quantities especially during periods of drought and famine when other crops fail [2]. No other legume seed is consumed as a staple food like grass pea.

Prolonged over-consumption of this protein-rich seed in a monotonous diet can cause neurolathyrism, the degeneration of upper motoneurons and the irreversible paralyzing of the legs in up to 6% of the population: A free non-protein amino acid  $\beta$ -ODAP ( $\beta$ -N-oxalyl-L- $\alpha$ , $\beta$ -diaminopropionic acid) with neuro-excitatory properties is blamed as causal agent. This metabolite was identified in 1964 [4] and during the

**Figure 1.** Bird's eye view of Northern Ethiopia in December. Dark green patches are *Lathyrus sativus*, pale green is chick pea (*Cicer arietinum*), the cereals have dried out



following two generations, research to improve the dietary value of grass pea was focused on the reduction of b-ODAP. This multifunctional metabolite [5] is however highly affected by the environment. Varieties developed in Canada with very low levels of b-ODAP loose this trait when planted in the iron-rich soil (e.g. Vertisol) of volcanic origin in the highlands of Ethiopia [6].

Epidemiological surveys have now indicated risk factors and protective factors for neurolathyrism [7, 8]. These should indicate the directions for improvement of the nutritional quality of grass pea and for the prevention of neurolathyrism. Among the various culinary preparations based on grass pea seed, those that are consumed without any addition of condiments seem to be more risky than others requiring a lengthy preparation. Especially fermentation is beneficial as it can reduce the content of b-ODAP by 90% and improves the balance of essential amino acids that are deficient in unprocessed grass pea [9, 10]. Consumption of roasted seeds as snacks was found to be highly correlated with the incidence of lathyrism. This was even more significant for the consumption of the unripe green seeds picked from the plants by young boys tending the fields [8]. Young men are more affected by lathyrism than women, the ratio being about 3:1. Risk factors for the incidence of neurolathyrism include heavy physical labour and fever which are also involved in oxidative stresses that is often mentioned in the aetiology of neurodegenerative diseases [11, 12]. Recently identified protective factors are the addition of onion, ginger or garlic to the preparations, or the addition of one third cereals rich in sulphur amino acids to the grass pea [8, Bernard Senny, Pers. comm.]. In the coastal areas of Bangladesh where fish is also eaten, no case of neurolathyrism is reported, although the consumption grass pea is at similar level to the inland. Supplementing methionine to an exclusive raw grass pea seed diet fed to young broiler chicks confirmed that methionine significantly improved the growth and prevented the neurological symptoms compared to the control chicks without methionine addition in the feed [13].

Neurolathyrism is a disease of the subsistence farmers in remote rural areas. Although grass pea seed is a

popular commodity sold on the markets in the cities, the occurrence of neurolathyrism in cities is rare and mostly limited to beggars who moved from rural areas to the city under socio-economic pressure after developing neurolathyrism. The presence of antioxidants or higher levels of the sulphur amino acids in the diet may protect against oxidative stress, that ultimately can lead to apoptosis and death of motor neurons. The statistical link of the incidence of neurolathyrism with illiteracy and poverty supports our believe that lathyrism is an easily preventable disease. During the latest epidemic of neurolathyrism in Bangladesh in the 1970s, the price of grass pea was lower than any other available foodstuff [14]. Recently the price of grass pea is higher than the price of rice and no victims of neurolathyrism reported.

Because of this stigma that consumption of grass pea can cause an irreversible crippling, the market value is lower than for other legumes such as lentil (*Lens culinaris*) or chick pea (*Cicer arietinum*). The poor who cannot afford the more expensive commodities are the main consumers of grass pea. This adds an additional stigma to grass pea as being the food for the very poor. Apparently, this reputation was different when the plant got its Greek name, meaning something exciting, because of its reputation as an aphrodisiac. Also during the era of the Egyptian Pharaoh's, grass pea had a better reputation as it was present in the pyramids as royal funeral offerings [15]. During the Second World War, grass pea was the food given to horses in the Russian cavalry. When the Russian army retreated from the Ukrainian town Vapniarca, the horse food was left behind and when the German army made a forced labour camp for male Jewish prisoners in the town, the 'horse food' was given to the inmates in this camp. On a diet of 400 g boiled grass pea and 200 g of barley bread per day, the estimated intake of b-ODAP by the inmates was about 0.5 to 1 g per day. After two months on this diet, the prisoners started developing the clinical symptoms of neurolathyrism and within four months 60 % of the prisoners had developed neurolathyrism in various stages [16]. When comparing this with the daily intake of Bangladeshi farm labourers, who claim to consume at least one kg of grass pea seed (containing some 5 g of b-ODAP) per day without any symptoms, there seems to be no correlation between intake of b-ODAP and the incidence of neurolathyrism. This lack of correlation also makes it impossible to determine a threshold toxic level for b-ODAP. Other factors must have sensitised the prisoners to the grass pea toxicity. These aggravating factors might be heavy physical labour, under-nutrition or mal-nutrition of the prisoners in Vapniarca. Stress was recently identified as an aggravating factor for the incidence of neurolathyrism in experimental rats [17]. Although neurolathyrism can cripple for life, it does not affect longevity or cognitive factors [18].

Interestingly, the metabolite b-ODAP in grass pea held responsible for neurolathyrism is also present in the longevity promoting Ginseng root (*Panax ginseng*) where it is named dencichine in Chinese herbal medicine and considered as a haemostatic agent [19, 20]. b-ODAP is a multifunctional plant metabolite for which over 30 different physiological or biochemical activities have been described in the plant or in animals [5]. Remarkable ac-

tivities are the facilitation of the uptake of zinc ions by the plant during zinc deficiency [21] and the protection of enzymes of photosynthesis against high light intensity [22]. Besides the well known excitation of the AMPA-receptors on the neurons it also affects other receptors and transporters in brain cells [23] [24] and  $Ca^{2+}$  homeostasis in neuronal cells [25].

The prolonged consumption of a diet deficient in methionine and cysteine can deplete glutathione from the body, one of our main metabolic defences against oxidative stress. Any additional stress such as heavy physical labour, micronutrient imbalance [26] or the neuro-excitation by b-ODAP can contribute to this oxidative stress and jeopardize the integrity of motor neurons.

### **Cassava (*Manihot esculenta* Cranz, Family *Euphorbiaceae*)**

The root crop cassava originated in South America and was already used as food plant for 5000 years when it came to Africa in the 17<sup>th</sup> century. It became the typical staple food crop of Africa, feeding over half a billion people. It is estimated that in D.R. Congo about 60% of dietary energy is derived from cassava roots [27]. It is a popular crop also in South America and Southeast Asia, where it may be the cheapest source of dietary carbohydrates. It is being used for human consumption under many traditional preparations from tapioca in 'pearl soup' to chips and the thick porridge 'luku' or 'fufu' that is the staple food in many African countries. Besides being used for human consumption it has also many industrial applications, and is even considered an economic source of bio-fuel. No other member of the *Euphorbiaceae* plant family is consumed in such quantities as cassava. Cassava is a very reliable crop that is tolerant to drought. The underground roots can retain their nutritional value even when the above ground parts are dry. A changing climate with increasing atmospheric  $CO_2$  can however negatively affect the productivity of the plant [28].

Cassava is a perennial plant that is rarely left to develop into flowering stage because the young leaves are used as vegetable. Propagation is by vegetative multiplication using cuttings. The underground roots are harvested any time of the year, peeled and soaked in ponds or slow running streams for retting. During the retting processing, the integrity of the root cells is broken and the enzyme linamarase, stored in the cytoplasm, comes into contact with the cyanogenic glucoside linamarine that in intact cells is stored in the vacuole and separated from the linamarase enzyme. Linamarine was first described in linseed (*Linum usitatissimum*). The enzymatic breakdown will release the volatile hydrogen cyanide, a highly toxic gas interfering with respiration. An insufficient period of retting will result in residual cyanogens and higher intake of cyanide than the maximum tolerated level of 10 mg per kg of cassava flour [27]. To some extent the human body can detoxify the residual cyanide into the less toxic thiocyanate that is excreted with the urine. However, when the body is depleted of methionine this process is interrupted and cyanate is produced instead of thiocyanate. The resulting cyanate has neuro-excitatory activity that in turn can again contribute to oxidative stress [29].

Over-consumption of cassava roots in a monotonous diet can cause an irreversible crippling disease konzo. Insufficient processing which leaves residual cyanogens (mainly the glucosinolate linamarin) is blamed for this disease with clinical symptoms indistinguishable from neurolathyrism. The popular processing of cassava in the rural villages includes retting during three nights (60-70 hrs), drying in the sun, pounding and adding to boiling water to make a stiff paste. This is consumed with sauce, made from available cooked vegetables that often are the young fresh cassava leaves. The roots are very poor in protein (2.4 % of dry weight) and this can be balanced by using the protein-rich young leaves (28.6 %). But both roots and leaves are poor in methionine and cysteine [30]. This deficiency of sulphur containing amino acids was suspected to be part of the aetiology of konzo as these amino acids promote cyanide detoxification [31]. In villages where no other crops are available, long periods of monotonous consumption of cassava diets can lead to a crippling disease characterised by sudden onset of symmetric spastic paraparesis and scissor gate. The disease is called 'konzo' by the rural people in the Kwango area of Bandundu province, D.R. Congo, meaning 'bound legs'. Epidemiological surveys did not include studies of the complete diet, but observations in the field showed that villages where corn is mixed with the cassava have a much lower incidence of konzo. Also the consumption of onion seems to have a protective effect [Bernard Senny, pers. comm.]. Higher incidences occur during the dry season when other food is available in low amount [31]. Increased incidence also occurs after merchants come to the village to buy processed cassava roots and the villagers sell their ready-to-eat food. The next two days they are forced to eat insufficiently processed roots containing higher levels of cyanogens and are at higher risk of developing konzo. In urban areas the people eat the same staple cassava preparations with no incidence of konzo. The reason for this may be the better selection for quality and availability of other food ingredients in the market.

### **Similarities between neurolathyrism and konzo and possible worst case scenario**

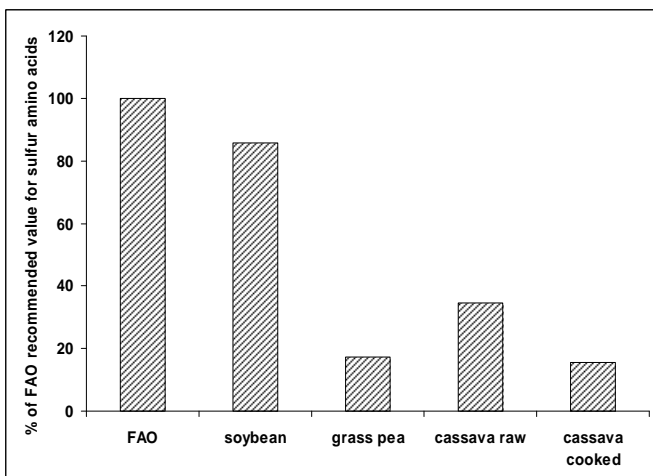
The two crops grass pea and cassava are cheap crops requiring minimal inputs and considered a reliable food supply during drought. The prominent similarities of the two crops are the resistance to biotic and abiotic stress, the tolerance to drought and the easy cultivation on marginal soil. Both crops are the food for the poor and when impoverished by environmental disaster such as drought or by military conflict these crops become the only available or affordable food. Both diseases neurolathyrism and konzo can be considered neglected orphan diseases of the remote rural areas and are virtually absent from urban areas [29, 32]. Both diseases occurred in areas where drought, poverty, illiteracy and malnutrition are prevalent and both are under-reported. The reason for under-reporting may be due to the remoteness of the locations, the illiteracy and poverty of the victims giving them little or no socio-economic or political voice, and the fact that both diseases are not infectious or transmissible.

As described above, both *neurolethyrism* and *konzo* are characterised by sudden onset of symmetric spastic paraparesis and scissor gate, after a prolonged period of monotonous unbalanced diet containing mainly the protein-rich grass pea or the protein-poor cassava roots. A common risk factor is heavy physical labour, while a common protective factor is the consumption of onion or cereals. In both cases there is a significant link with poverty and illiteracy. Remote areas with subsistence farming are more vulnerable especially during periods of drought. In urban areas where grass pea or cassava roots are available both diseases are virtually absent. Both diets of prolonged monotonous consumption of grass pea seeds or cassava roots may induce an increased oxidative stress due to the deficiency of the essential amino acids methionine and cysteine (**Figure 2**), and the physiological effects of a neuro-excitatory amino acid *b*-ODAP in grass pea or a methionine depleting detoxification process of cyanide after consuming cassava. For both diseases, a number of epidemiological risk factors can be explained as aggravating this oxidative stress while protective factors can be explained as counteracting oxidative stress.

The one unexplained difference between *konzo* and *neurolethyrism* is the higher incidence of *konzo* among women at childbearing age, while young boys are more vulnerable to *neurolethyrism* [33]. In some cases the physical effort of childbirth can trigger the onset of *konzo*, which gives rise to dramatic situations when the young mother can no longer get up from the bed to nurse the child [Lieve Van Wijmeersch, pers. comm.].

As of now, there is no geographical overlap of the production of grass pea and cassava. Considering the present interest in drought tolerant crops, demographic pressure and climate change conditions may be responsible for the increasing cultivation and consumption of both grass pea and cassava. There is a real risk that these crops spread and overlap. While the exact aetiology of *neurolethyrism* and *konzo* is not completely understood, the result of over-consumption of either grass pea or cassava in a diet poor in the essential sulphur

**Figure 2.** Grass pea seed and cassava leaf preparations (raw and cooked) are both deficient in methionine and cysteine



amino acids methionine and cysteine is so similar that, when poor consumers would start mixing a cheap protein-rich crop with a cheap carbohydrate-rich crop that both have the same deficiency in essential amino acids could be predicted as a 'worst case scenario'. Education and dietary information on the risks of these crops would be extremely important in order to prevent such a worst case scenario. Also the consumption of cassava together with other legume seeds such as cowpea as is the case in D.R. Congo is not advised, because only soybean has an acceptable level of methionine and cysteine albeit not enough to balance the deficiency in cassava. Three approaches can reduce the incidence of these two disorders: i) better diversification of the diet, ii) development by plant breeding or transgenic technology of new cultivars with low toxin content and iii) development of new cultivars with higher content of methionine/cysteine and of micronutrients.

### Conclusion

The obvious road for making grass pea and cassava based diets more healthy is the improvement of the amino acid balance and the addition of antioxidant-rich condiments into the diet. Improving the nutritional quality of grass pea and cassava crops by increasing the content of sulphur amino acids methionine and cysteine may ultimately be the cheapest solution for the subsistence farmers surviving on those crops in environments where other crops are less productive, provided that unreasonably expensive bio-safety regulations are adapted to the real needs of the poor. The drought tolerance of these two ancient crops, that have retained their popularity during several thousand years, can make them even more important for a future affected by global warming and water shortages. Under certain conditions of over-consumption and malnutrition, both crops can have deleterious and irreversible effects on the consumer. However, while a single meal of unprocessed bitter cassava can be suicidal, large amounts of grass pea are needed to be consumed as a monotonous diet during several months to have a 6% chance of developing *neurolethyrism*. Because no other legume is consumed in such quantities it is not possible to predict whether a monotonous diet of an alternate legume would be safer than grass pea. A practical and cheap animal model for *neurolethyrism* or *konzo* does not exist but is urgently needed. Without such a model it is not possible to guarantee the safety of new varieties of grass pea with lower levels of *b*-ODAP or new varieties of cassava with lower levels of linamarine.

Very recently, a workshop on the two diseases was held in Ghent University (Belgium) with the aim to be an interface between researchers of *neurolethyrism* and *konzo* in facilitating the identification of common ground, stimulating communication between researchers of different areas, and preventing the two diseases through integrated approaches. One consensus was that both diseases are the clinical symptoms of abject poverty and malnutrition and are neglected by the authorities.

## Main recommendations from the workshop on **neurolethyrism and konzo**

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 been associated with health problems of konzo, tropical ataxic neuropathy (TAN) and neurolethyrism, 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 this incurable but preventable paralysis of the lower limbs. A two-day "International Workshop on Toxic-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 neurolethyrism from grass pea 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 neurolethyrism) 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 involving grass pea and cassava. **These traits are of global importance and can also benefit other crops.**
4. Research on better **agronomic practices** and the environmental effect (drought, CO<sub>2</sub>) 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 in agronomy, postharvest, 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 neurolethyrism 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 need a 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 neurolethyrism.
7. **Konzo and Neurolethyrism are seen as clinical symptoms of abject poverty and malnutrition.** Making konzo and neurolethyrism **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.

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