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

CCDN News

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Second workshop on toxico-nutritional neurodegenerations konzo and neurolathyrism
Recent advances, innovations and engagement
Democratic Republic of Congo – Kinshasa, (dates to be notified soon)

Most universities are founded with a civic purpose. Most donors expect changes in people's lives. We have a fundamental obligation to apply our skills, resources, and energy to address the most challenging issues in our society (DTK & TRUCEN). Toxic diseases associated with Cassava (*Manihot esculenta)* i.e. konzo and ataxic polyneuropathy, and grass pea (*Lathyrus sativus*) i.e. neurolathyrism pose serious threats to human life and development under the tropics; this has been documented as a "fact" in Africa, Asia, and South America.

Both cassava (*Manihot esculenta)* and grass pea (*Lathyrus sativus*) are important crops for millions of people around the globe. Both crops are adaptable to variable environments, tolerant to drought and are sustainable crops for human nutrition with little agronomic inputs. Cassava roots can be harvested all year around whenever necessary and need no storage facilities. Post harvest processing is required to remove the toxic cyanogenic glycosides and to yield the cheapest staple food rich in carbohydrates.

Grass pea is the most drought tolerant commercial legume and also an outstanding fixer of atmospheric nitrogen, adaptable to marginal soils and the source of the cheapest dietary protein. However, a neuro-excitatory amino acid beta-ODAP is present in the seeds in variable amounts, making this survival food during droughts and famine a mixed blessing.

For several years, Cassava Cyanide Diseases and Neurolathyrism Network (CCDNN) has shared information and concerns about food security and safety in the tropics as well as toxicities and disabilities associated with the consumption of poorly processed cassava or grass pea. For the first time in 2009, konzo and neurolathyrism researchers, and various stakeholders, gathered in Ghent (Belgium) to share research findings and discuss strategies to tackle the two seemingly neglected but preventable diseases. *It is time to discuss our research and meeting outcomes have to be translated into policies or research advances. It is time to think about strategy to affect policy and serve as vectors of change and impact on people's lives.*

Africa shares the highest burden of cassava and grass pea related disabilities. African scientists have engaged in cassava and grass pea research, with support from international scientists, to achieve highest levels of education and research skills in various fields of nutrition, medicine (basics, clinical, and epidemiology), agriculture, and social sciences. The benefits of this type of global engagement and support have to be demonstrated. This can be done on the African continent. We intend to follow on the Ghent workshop with a "**Second workshop on toxico-nutritional neurodegenerations konzo and neurolathyrism: Recent advances, innovations, and engagement**" in Kinshasa, Democratic Republic of Congo (DRC), dates and place to be decided and communicated soon.

Recent advances, innovations, and engagement will be discussed around the following tentative sessions: (1) nutritional epidemiology and toxicity, (2) cassava food safety for an urbanistic Africa, (3) bringing grass pea and cassava together: risks and benefits, (4) breeding for crop improvement and increased safety, (5) interventions and policy: changing the environments and improve people's lives. A round table discussion will consider the need for additional research and the way forward to set up an international committee to advice governments and funding agencies. Welcome to Kinshasa 2014.

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Removal of poisonous cyanogens from pounded cassava leaves at ambient temperature

Cassava leaves are used particularly by the Congolese population of central Africa and in Liberia, Sierra Leone and Guinea and there is moderate use in other tropical African countries. The traditional method of removing cyanogens from cassava leaves is by pounding in a pestle and mortar followed by boiling in water for about 30 minutes.[1] On boiling, the bright green colour of the leaves becomes dull green and there is considerable loss of vitamins and protein, including S-containing amino acids present in the protein in the leaves. The S-containing amino acids (methionine and cysteine/cystine) are needed for detoxification in the body of poisonous cyanide (CN) which is converted to thiocyanate (SCN) and is removed in the urine. Large cyanide intake from a monotonous diet of bitter cassava has long been associated with the occurrence of konzo[2,3] and the recent prevention of konzo in seven villages in the Democratic Republic of Congo (DRC)[4-6] by use of the wetting method that greatly reduces cyanogen intake, has confirmed excessive cyanide intake as the source of the konzo problem.

Konzo is an irreversible paralysis of the legs that occurs mainly in children and young women, who live on a monotonous low protein diet of bitter cassava. The importance of animal protein in preventing the occurrence of konzo with prevalence rates up to 7% was shown in three different konzo outbreaks in Mozambique, Tanzania and the DRC. People of the same ethnic group as those who got konzo, who lived only 5 km away, did not get konzo because in Mozambique they had fish from the sea,[7] in Tanzania they had fish from Lake Victoria[8] and in the DRC they had animal protein from the forest.[9] Thus konzo can be prevented by reduced cyanide intake and/or adequate nutrition, in particular, an adequate supply of S-containing amino acids to detoxify ingested cyanide.

We have now found that cyanogens are virtually completely removed from cassava leaves by following three simple consecutive steps as follows: (1) pounding, (2) standing for 2 hours in the sun or 5 hours in the shade and (3) washing three times in water. Using four different cassava cultivars, we found that the mean residual total cyanide content after steps 1, 2 and 3 were 28%, 12% and 1% respectively. The pounded, washed leaves which are free of cyanogens retain their bright green colour and texture.[10] It is hoped that this mild method of removing cyanogens from cassava leaves, may be a useful alternative to boiling pounded leaves in water, which would save on fuel for cooking and much more importantly would improve the nutritional status of the cassava eating population of tropical Africa.

References

1 Achidi, AU, Ajayi OA, Bokanga M, Maziya-Dixon B. (2005) The use of cassava leaves as food for Africa. Ecology of Food and Nutrition 44, 423-435.

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

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

4 Banea JP, Nahimana G, Mandombi C, Bradbury JH, Denton IC, Kuwa N. (2012) Control of konzo in DRC using the wetting method on cassava flour. Food Chem. Toxicol. 50, 1517-1523.

5 Banea JP, Bradbury JH, Mandombi C, Nahimana D, Denton IC, Kuwa N, Tshala Katumbay D. (2013) Control of konzo by detoxification of cassava flour in three villages in the Democratic Republic of Congo. Food Chem. Toxicol., 60, 506-513.

6 Banea JP, Bradbury JH, Mandombi C, Nahimana D, Denton IC, Foster MP, Kuwa N, Tshala Katumbay D. (2014) Cyanogen removal from cassava flour

using the wetting method prevents konzo in the DRC. Food Chem Toxicol., submitted for publication.
[7] Ministry of Health Mozambique (1984) Mantakassa; an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area in Mozambique. 1. Epidemiology and clinical laboratory findings in patients. Bull. WHO, 62: 477-484.
[8] Howlett WP, Brubaker G, Mlingi N, Rosling H. (1992) A geographical cluster of konzo in Tanzania. J Trop Geogr Neurol 2, 102-108.
[9] Banea Mayambu JP. (1993), Cassava processing, dietary cyanide exposure and konzo in Zaire. Masters Thesis, Uppsala Univesity, Uppsala, Sweden, p.55.
[10] Bradbury JH, Denton IC. (2014) Mild method for removal of cyanogens from cassava leaves with retention of vitamins and protein. Food Chem., submitted for publication.

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Lathyrus in Poland – origin, breeding, research status and consumption

Introduction

The fact that the human population has recently passed 7 billion and is expected to approach 9 billion by 2050, considered together with likely adverse impact of climate change on agricultural production is increasing awareness of issues related to global food security[1]. From the early 1900s till now, wheat productivity has increased from an average of 1.2 t ha-[1] to 4 t ha-[1] in Europe (and over 10 t ha-1 has been recorded in some countries) and about half of this increase is estimated to be due to breeding activities[2,3]. Modern varieties are bred to be genetically uniform to maximize production efficiency under those inputs that make the environment best suited to the crop (irrigation, fertilization, pest control, etc) as well as to meet increasing demands of mechanized harvesting and handling, and meet supermarket quality control. The high yielding modern cultivars represent most spectacular success of genetics applied to agriculture and have contributed to alleviating the historical rural poverty[3]. Although only a few staple crops produce the majority of our food supply, the contribution of at least 6000 minor cultivated species should not be underestimated. Agricultural research has traditionally focused on these few staples while relatively little attention has been given to minor (or neglected) crops. More than most staples many of these neglected species are adapted to various marginal growing conditions such as highland areas, arid areas and salt-affected soils. From nutritional point of view many or these neglected species are particularly important at a national or regional level as food supply in small regions and at certain periods of the year[4]. A few species in the genus *Lathyrus* are neglected crops important from agricultural point of view. The paucity of Polish research papers on toxic aspects of *Lathyrus* can be explained by the absence of human neurolathyrism cases in Polish history.

Origin

In Poland grass pea is grown on a small scale in eastern Poland. It is cultivated as vegetable for consuming and feeding purposes. According to data given by grass pea “father” in Poland, Prof. Milczak et al.[5] this species was introduced to Poland (Region Podlasie) in XVII century, probably with settlement of Tatars by Polish king Jan III Sobieski, and today the Tatars form an interesting social and cultural minority. Probably the Tatars brought to Poland the seeds of grass pea as contamination of lentils. It is fascinating that with time grass pea as contaminant showed markedly better adaptation to unfavorable areas (salt-affected soils, high rainfall level, sandy soils). Thanks to the “plasticity” for growing in different environmental conditions, grass pea became more popular than lentils and local ecotypes of grass pea (landraces) gained in popularity. Grass pea is now mainly cultivated in farmer’s vegetable gardens for increasing of human consumption of seeds and seldom used for animal feeding.

The above mentioned hypothesis about East-European origin of Polish grass pea genotypes is confirmed by molecular experiments (PCR- ISSR and RAPD) indicating a high level of genetic similarity between Polish, Russian and Ukrainian genotypes, unlike accessions from Mediterranean countries[6]. In addition, Polish grass pea and genotypes from Russia, Ukraine and Central Asia are typical small-seeded forms[7] that differ markedly from large-seeded genotypes from Mediterranean countries[4].

Breeding history and actual status

In traditional agriculture, farmers used to save seeds from the previous crop for sowing and cultivation. With different environments, many landraces (local ecotypes, primitive varieties) developed and improved within a certain region after domestication or introduction[3]. The “Podlasie region” in Eastern Poland is characterized by small farms and farmers often use old landraces (marginal, or underutilized or neglected crops) and traditional farming system. This is particularly true for local ecotypes of grass pea present in Podlasie and other East-Poland regions since XVII century. These forms of grass pea are known and sold under local names as “Podlaski lentil, great lentil, Russian lentil or white pea” indicating an old history in this region[5]. The first breeding experiments were performed in the region of Gdańsk in 1932-1935. After the WWII experiments with *Lathyrus* continued at the Scientific Institute of Agriculture in Bydgoszcz and IUNG Institute in Bydgoszcz, Minikowo and Poświętne. The goal of this work was the use of *L. tingitanus* and *L. sativus* as forage crops. Because of the higher biomass yield in comparison to *L. sativus* the main experiments concentrated on *L. tingitanus*. As a result, in 1950 a commercial cultivar of *L. tingitanus* “Bydgoski” (with amaranth flowers) was released[8]. In 1960 *Lathyrus* was removed as an agricultural crop from the Register of Polish Varieties, and breeding programs were stopped.

In the opinion of Negri et al.[3] each landrace in itself is highly genetically diverse. This diversity had been a key to agricultural food security for generations and allowed the harvest whether the year is dry or wet, or whether there is a pest or disease attack. In 1990 the first attempts to introduce grass pea into Polish agriculture were made by Milczak et al.[5] from Agricultural Academy in Lublin (today: Live University). In 1991, a small field trial was conducted in Radzyń Podlaski using old local landraces that had been cultivated by small farmers for many years as a vegetable for family consumption. Further experiments showed that grass pea, known in Polish literature as a typical forage crop, could be interesting for the production of seed with high nutritional value, taste preference, high seed yield (also on sandy soils) and great tolerance to abiotic stresses, especially to long term drought. The two most promising landraces selected in 1992-1996 were named ”Derek” and “Krab” after the villages of origin and in 1997 registered as Original Varieties in Poland.

Research area

Grass pea is cultivated mainly in East Poland as a marginal crop. It is not a popular research topic, but increased popularity of this plant in the last years convinced a small group of Polish researchers to include Polish grass pea material in their researches. A short survey of selected investigations focused on cultivation, mutagenesis, in vitro culture, nutrients and anti-nutritional factors, animal feeding and human consumption is given below.

Cultivation

About 60 % of Polish arable lands are light – sandy soils, typical for rye, potato, lupine and grass pea cultivation but too poor for field pea, common vetch, chick pea or broad bean. Its high tolerance to drought and to a wide range of soil types, including soils with low water capacity, make grass pea ideal for growing in this environment. Basic information for farmers in terms of soil preparation, sowing, fertilization, plant protection and harvest was elaborated from field trials by Dziamba9. These were published and popularized, but unfortunately not any national agricultural centre continues creative grass pea breeding. Earlier released cultivars developed by Vegetable Plant Breeding Centre in Nochowo, seeds are available on the national market.

Mutagenesis

To induce new variation as well as for widening existing variability in grass pea traits, Rybiński10 used two chemical mutagens (N-nitroso-N-methyl urea and sodium azide) and Polish cultivars as initial material. Three promising mutants were tested in a field trial on sandy soil with water shortage. The seed yield was higher in comparison with their initial cultivars Derek and Krab11. Unfortunately mutants with the desired determinate growth were not found. One spontaneous mutant appeared, probably as a result of the Tshernobyl catastrophe showed spherical seed shape, unlike the typical angular shape12. Molecular analyzes indicated the close relationship of this small-seeded mutant with lower β-ODAP content with cv Krab. This suggests a spontaneous mutation inside the population from which cv. Krab was released13. Other obtained mutants had improved seed yield14, seed quality15,16, decreased anti-nutritional factors17, changed seed microstructure18, better resistance on mechanical loads19, or had positive effect in feeding experiment with broiler chickens20.

In vitro experiments

In 2007 began the first study in Poland on somaclonal variation in grass pea. Callus tissue could easily be obtained from some Polish varieties[21]. Histological examination revealed intensive differentiation in callus with numerous meristematic centres. More importantly was obtaining embryogenic callus lines with numerous somatic embryo-like structures (ELSs)22. Furthermore, we are trying to optimize protoplast culture procedures of different *Lathyrus* species with the intention to carry out protoplasts fusion in the future. Another result of research is well established protocols of mesophyll protoplasts isolation23. Some difficulties in obtaining dividing protoplasts of Polish varieties led to search for the causes of their recalcitrance. We suspect that it may be correlated with disturbed/improper biosynthesis of a new cell wall24. Recently we started in vitro selection of different *Lathyrus* accessions for drought resistance.

Nutrients and antinutritional factors

Legume species are an irreplaceable source of dietary proteins and other nutrients for humans. A survey of nutrient composition of small- middle and large-seeded forms of local landraces showed that the seeds are rich in protein (23,8-28.4 % DM basis) and lysine (6 g/100 g protein) and have an interesting fatty acid composition (linoleic acid 38-56%, linolenic acid 6-8%) and relatively low fat content (0.66-1.51%)25. Nutrient content of used ecotypes from the Radom Region ranges as follows: crude protein 310-360 g kg-1, ether extract 8.2-9.2 g kg-1, crude fibre 55-56 g kg-1, NDF 154.5-226.5 g kg-1, ADF 78.3-86.2 g kg-1 and crude ash 35.7-38 g kg-1. The small-seeded forms contained higher linolenic (18:3) acid and lower linoleic (18:2) acid. This confirms that grass pea is a good quality food and feed26.

The wider utilization of grass pea is limited by anti-nutritional factors (ANFs): The neuro-active β-ODAP, tannins and trypsin inhibitors. Both the environment and genotype can affect ANFs content in seed. Seeds of Polish grass pea contained 9.2%, respectively 15.3% more β-ODAP than accessions from Russia, resp. Ukraine, but 23.7% less than accessions from Czechia. In general, seeds from South Central Europe (Czechia, Hungary, Slovakia) contained more β-ODAP than those from East-Central Europe (Poland, Russia, Ukraine)27. Seeds originating from the Mediterranean basin contain12.3% more β-ODAP than accessions from West-Central Europe28. When the Polish seeds were cultivated in central-east Poland the seeds contained about 40% more β-ODAP, 2.4 g kg-1tannins, 3.1 g kg-1 phytate phosphorus and 17.5 TUI g-1kg of DM[29]. Polish grass pea seeds contained an average of 3 g tannins in 1 kg DM, this is similar in seeds originated from Ukraine but lower than in seeds from Slovakia and Russia (3.46 and 3.43 respectively). Heat processing methods were used for grass pea as feed. Extrusion cooking technique of grass pea seeds reduces considerably the level and activities of some ANFs[27]. The effect of 14 to 30% moisture and three levels of temperature on the content of β-ODAP, tannins, phytic phosphorus and trypsin inhibitors activity in extrudates were determined by Grela et al[30]. Extrusion cooking resulted in lower content of β-ODAP and trypsin inhibitor activity. Higher moisture extrudate gave a higher decrease of β-ODAP and trypsin inhibitor activity and smaller decrease of the tannin content[31,32]. Autoclaving of grass pea seeds at different times and temperature affected the properties of the protein depended on heating intensity[33].

In feeding experiments with cattle and pigs, 15 % of grass pea seeds in concentration mixtures for beef cattle did not have negative influence on gains. For pigs, feed utilization and meat quality with 10 % extruded grass pea seeds into the mixture either or without lysine and methionine supplementation produced the desirable performance. Grela et al. concluded that grass pea seeds can be a good component of concentrate mixtures for beef cattle and at lower scale for pigs and can be an alternative for faba bean or rapeseed meal[34,35]. Apart from the content of anti-nutritional factors in *Lathyrus* (i.e. trypsine inhibitors, neurotoxins), up to 20 % of grass pea meal in formulated feed, is a reasonable ingredient[36].

Consumption

Ensuring high-quality protein in human and animal nutrition is an important issue in EU countries. In Poland this requires ca. 1 mln t of protein per annum, 80% of this being covered by import of soybean meal. A greater part of that requirements could be covered from own cultivation of grain legume crops include grass pea adapted to local climate. In Poland and in Europe the area of cultivation of such crops is only1 and 3% respectively of total crops. Consumption of grain legumes in Poland amounted to only 10-12 g per day and person. According to oral information from older citizens from Podlasie region reported by Milczak and Masłowski[37], moderate doses of grass pea seeds of local landraces were consumed since many, many years with no any accident of neurolathyrism. Grass pea seeds were consumed occasionally as supplement in a well balanced vegetable-rich diet. Due to the culinary experience of local people, many cooking recipes were elaborated in Podlasie region and these were published locally as “Recipes for Grass pea Dishes”[38]. Some popular recipes with use of grass pea are: soup, meat chop, cakes, mushroom filling, grass pea-soybean pie and many kinds of salads.

Unripe grass pea is also used for canning[39]. Fermentation of grass pea flour by Lactobacillus plantarum resulted in the lowering of ODAP, trypsin inhibitors and inositol phosphates contents, as compared to raw material[40]. For the food industry, seeds at milk-wax maturity or not fully mature seeds are used for freezing and canning and the mineral components studied[41].

A little known property of grass pea is its honey production, with a nectar secretion of 0.37-1.44 mg per flower grass pea can yield 3.9-6.9 kg honey per 1 ha[42]. The recent trend for ecological healthy food production may benefit from the many interesting ecological and agronomic properties of grass pea.

References

1. Shehadeh A, Amri A, Maxted N. Ecogeographic survey and gap analysis of Lathyrus L. species. Genet. Resour. Crop Evol. 2013, 60: 2101-2113.
2. Grigg D. Storia dell’ Agricoltura in Occidiente. Basil Blackwell, Oxford, II Mulino, Bologna, Italy, 1994.
3. Negri W, Maxted N, Vetelainen M. European landraces conservation: an introduction. In: European landraces on-farm conservation, management and use. Bioversity Technical Bulletin 15. 2009: 1-22.
4. Campbell C G. Promoting the conservation and use of underutilized and neglected crops. 18. Institute of Plant Genetics and Crop Plant Research. Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.1997.
5. Milczak M, Pędziński M, Mnichowska H, Szwed-Urbaś K, Rybiński W. Creative breeding of grasspea (*Lathyrus sativus* L.) in Poland. Lathyrus Lathyrism Newsletter 2001, 2: 18-23.
6. Pankiewicz K. Variability of chosen species of *Lathyrus* genus on phenotypic and molecular level. Institute of Plant Genetic Polish Academy of Sciences, Poznań. Doctor thesis. 2012.
7. Rybiński W, Bocianowski J, Dziamba Sz. Variation of quantitative traits in Polish local grasspea accessions (*Lathyrus sativus* L.). Biuletyn IHAR. 2012, 264: 195-209.
8. Mackiewicz Z. Lathyrus tingitanus. Państwowe Wydawnictwo Rolnicze i Leśne. Warszawa, 1956: 3-34.
9. Dziamba SZ. The biology and agrotechnics of chickling vetch. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human”. Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 27-33.
10. Rybiński W. Mutagenesis as a tool for improvement of traits in grasspea (*Lathyrus sativus* L.). Lathyrus Lathyrism Newsletter. 2003, 3: 27-31.
11. Cichy H, Rybiński W. The yielding ability of grasspea (*Lathyrus sativus* L.) mutants and cultivars in field trials. Zeszyty Problemowe Postępów Nauk Rolniczych. 2007, 522: 177-185.
12. Milczak M, Sawicki B, Masłowski J. Breeding of unique genotype of grasspea for human consumption. Annales Universitatis Marie Curie-Skłodowska, Lublin – Polonia. 2001, LVI 5: 37-41.
13. Pankiewicz K, Rybiński W, Kaczmarek Z. Estimation of variability and molecular level of spherical form of grasspea (*Lathyrus sativus* L.) Biuletyn IHAR. 2011, 260/261: 351-365.
14. 14 Rybiński W, Bocianowski J, Pankiewicz K. Variability of quantitative traits of two mutated

grasspea (*Lathyrus sativus* L.) cultivars. Biuletyn IHAR. 2012, 264: 211-225.
15. 15.Starzycki M, Rybiński W. Estimation of chemical composition of seeds and chosen phenotypic traits in accessions material of grasspea (*Lathyrus sativus* L.). Zeszyty Problemowe Postępów Nauk Rolniczych. 2010, 555: 399-407.
16. 16.Piotrowicz-Cieślak AI, Rybiński W, Michalczyk DJ. Mutations modulate soluble carbohydrates composition in seed of *Lathyrus sativus*. Acta Societatis Botanicorum Poloniae. 2008, 77 (4): 281-287.
17. 17. Rybiński W, Grela ER. Genetic variation of traits and chemical composition of seeds in grasspea mutants (*Lathyrus sativus* L.). Zeszyty Problemowe Postępów Nauk Rolniczych. 2007, 517: 613-627.
18. Rybiński W, Błaszczak W, Fornal J. (2006). Seed microstructure and genetic variation of characters in selected grasspea mutants (*Lathyrus sativus* L.). Int. Agrophysics. 2006, 20 (4): 317-326.
19. Rybiński W, Szot B, Pokora L. Estimation of genetic variation of traits and physical properties of seeds for grass pea mutants (*Lathyrus sativus* L.). Int. Agrophysics. 2004, 18 (4): 339-346.
20. Smulikowska S, Rybiński W, Czerwiński J, Taciak M, Mieczkowska A. Evaluation of selected mutants of grasspea (*Lathyrus sativus* L.) var. Krab as an ingredient in broiler chicken diet. Journal of Animal and Feed Sciences. 2008, 17: 75-87.
21. Piwowarczyk B, Pindel A. Callus induction and proliferation in in vitro culture of grasspea (*Lathyrus sativus* L.) 'Derek'. Adv Agric Sci Probl Iss. 2009. 534: 223-229. (in Polish).
22. Piwowarczyk B, Pindel A. Influence of the media on the differentiation of grass pea (*Lathyrus sativus* L.) callus tissue. Biotechnologia. 2012, 93(2): 158.
23. Piwowarczyk B, Rybiński W. Protoplasts of *Lathyrus* sp. – optimization of isolation procedure. Eurobiotech 8-11.10.2013, Kraków. 2013, Abstracts: 133
24. Wiszniewska A, Piwowarczyk B, Pindel A. The influence of isolation stress on the (re)organization of cell walls in protoplasts of in vitro recalcitrant plants. Biotechnologia. 2012, 93(2): 102-108.
25. Grela ER, Winiarska A. Chemical composition and nutritive value of flat pea (*Lathyrus sativus* L.) seeds. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 49-55.
26. Grela ER, Skórnicki H. Chemical composition of flat pea (*Lathyrus sativus* L.) seeds in local cultivars from Radom region. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 59-67.
27. Grela ER, Rybiński W, Matras J, Sobolewska S. Variability of phenotypic and morphological characteristics of some *Lathyrus sativus* L. and *Lathyrus cicera* L. accessions and nutritional traits of their seeds. Genet. Resour. Crop Evol. 2012, 59: 1687-1703.
28. Grela R, Rybiński W, Klebaniuk R, Matras J. (2010). Morphological characteristics of some accessions of grass pea (*Lathyrus sativus* L.) grown in Europe and nutritional traits of their seeds. Genetic Resources and Crop Evolution. 2010, 57 (5) : 693-701.
29. Grela ER, Studzinski T, Matras J. Antinutritional factors in seed of *Lathyrus sativus* cultivated in Poland. Lathyrus Lathyrism Newsletter. 2001, 101-104.
30. Grela ER, Truchliński J, Rzedzicki Z, Pallauf J, Winiarska A. Influence of different conditions of extrusion on the nutritional factors content in flat pea (*Lathyrus sativus* L.) seeds. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 80-85.
31. Fiołka M, Sadurska A., Piedra JV, Grela ER. Effects of dietary supplementation of raw and extruded flat pea (*Lathyrus sativus* L.) seeds on hematological parameters and non-specific immunity in guinea pigs (*Cavia porcella*). Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 90-94.
32. Śliwa E, Grela E, Sadurska A, Kałkowska E. Effect of raw and extruded flat pea seeds (*Lathyrus sativus* L.) in the guinea pigs diets on biochemical parameters of the limb bones. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 114-117.
33. Szmigielski M, Matyka S. Comparison of selected methods applied for the evaluation of thermal processing efficiency of chickling vetch seed. Polish Journal of Food and Nutrition. 2004, 13/54 (3): 243-248.
34. Grela ER, Matras J, Florek M, Litwińczuk A, Barłowska J, Winiarska A, Wacław D. Effect of flat pea (*Lathyrus sativus* L.) seeds in beef cattle feeding on performance and carcass traits. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 134-139.
35. Grela ER, Winiarska A, Krusiński R, Krasucki W, Rabos A. Influence of flat pea (*Lathyrus sativus* L.) in growing-finishing pig diets on performance and carcass traits. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 142-148.
36. Lipiec A, Grela ER. *Lathyrus* seeds (*Lathyrus sativus* L.) in animal feeding. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 130-133.
37. Milczak M, Masłowski J. Variability and correlation of some features in flat pea, a

potential vegetable plant. Zeszyty Naukowe Akademii Rolniczej we Wrocławiu. 1993, Agriculture LVIII, 223:341-346
38. Lesisz M. Use of grasspea for human consumption. Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-9. 1997, 154-160.
39. Milczak M, Janoszuk E. Potentially possibilities of use Polish grasspea seeds in household kitchen and agricultural processing. Conference "Quality of Vegetable Raw Materials for Food Industry". Skierniewice, 19-20 October, 1995, 73-79.
40. Starzyńska-Janiszewska A, Stodolak A. Effect of inoculated lactic acid fermentation on antinutritional and antiradical properties of grasspea (Lathyrus sativus cv. Krab.) flour. Polish J. Food Nutr. Sci. 2011, 61(4): 245-249.
41. Korus A. Content of mineral components in fresh and preserved seeds of two grass pea (*Lathyrus sativus* L.) cultivars at the not fully mature stage. Acta Technologia Alimentaria. 2002, 37-46.
42. Wróblewska A. The apiarian value and insect visit of chickling vetch (*Lathyrus sativus* L.). Int. Symposium „*Lathyrus sativus* – cultivation and nutritive value in animals and human". Lublin-Radom, 9-10 June, 1997. Proceedings- ISBN83-86779-17-91997, 161-164.

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Detoxification of cassava leaf for utilization in food or feed

Cassava (*Manihot esculenta* Crantz) tubers provide the staple food for millions of people in tropical countries. Unlike the roots which are rich in carbohydrates, the leaves are a good source of protein and vitamins which can provide a valuable supplement to a carbohydrate diet. The nutrient content of cassava leaves compares favorably with other green leaves and vegetables[1.] The leaves contain approximately 7% protein on fresh weight basis and 30% on dry weight basis, other major nutrients include carotene 10mg and ascorbic acid 300 – 500mg/100g fresh weight, Ca 300mg and Fe 7.6mg/100gdw, these levels being higher than other leafy vegetables[2,3]. The leaves constitute a major component of the diet in some regions of Africa, Asia and S. America[4,5] and also have utility as a livestock feed component. However, in spite of high protein content its potential as food and feed is limited due to the presence of cyanogenic glucosides, linamarin and lotaustralin, which by the action of an endogenous enzyme linamarase, release toxic hydrocyanic acid. The leaves also contain tannins which can interfere with protein digestion. In order to utilize cassava leaves, optimum processing methods need to be developed, which would result in maximum removal of cyanogenic glucosides and phenols, and at the same time, retain most of the nutrients such as carotenoids, proteins and carbohydrates. The two major mechanisms by which cyanogens can be eliminated from cassava are i) solubilisation of linamarin in the processing medium and ii) hydrolysis of linamarin by endogenous linamarase to acetone cyanohydrins/cyanide which are lost by volatilization The safe limit of cassava cyanogenic glucoside for human consumption, as prescribed by the WHO is 10mg cyanide equivalent/kg dry product[6].

Most of the techniques generally employed for processing cassava leaves cause significant reduction but not complete removal of cyanogens. Simple boiling or cooking is generally used to process cassava leaves but the process is insufficient in removing cyanide completely and small residual amounts always persist[7]. Chopping and crushing the leaves prior to boiling is also recommended[8]. It was found that 97% reduction in total cyanide was achieved after cooking leaves in boiling water for 60 - 90 min[9]. Sun drying the whole leaves or shredding and sun drying for 2-3 days was also effective in reducing cyanogens, the percentage of retention being 3-6% [10].

The most common traditional method of processing leaves is to pound them in a wooden mortar and pestle followed by boiling in water [11]. Pounding fresh leaves for 15 min, followed by boiling in twice the weight of water for 15 min could reduce the CNG level from 1000mg to between 4 and 11mg/kg, while pounding alone reduced CNG to between 300 to 450 mg/ kg[4]. However this process results in loss of nutrients. Recently, mild methods of processing cassava leaves which remove cyanogens and preserve nutrients were developed[12,13]. One method involved maceration of leaves for 10 min, followed by washing the pounded leaves twice with water, which reduced the cyanide to 8%, or washing 4 times which reduced the cyanide to 3%. Since boiling was not involved, soluble nutrients were not lost. The same authors had earlier developed a wetting method for reducing cyanide to safe levels[14].

We developed a simple and cost effective process for detoxification of cassava leaves. Leaves are harvested along with intact petioles, air dried for 48h and the dried leaves are coarsely powdered. This process removes 95 % of the cyanogens. The powdered leaf is washed in water for 10 min, strained to remove water and the leaf is again dried. The resulting product was found to contain 4 – 6mg CN /kg dw, 30 mg carotene, 2-3% phenols, 30% crude protein and 50% carbohydrate /100gdw.

The cyanogen content of this leaf product is within the safe limits recommended by the Codex Alimentarius Committee for cassava flour. Such a low retention has not been reported by any processing method. During the process of drying, tissue disruption occurs, and hydrolysis of linamarin by linamarase is facilitated because the temperature is optimum and moisture content is retained over a longer period. The process consumes little energy and gives a nutrient rich storable product. Another advantage of this process is that it reduces exposure to cyanide during processing. The process of pounding fresh leaves releases large amounts of free cyanide which is inhaled by the processor, and this is a major health hazard.

The significant reduction in phenols during this process, with little loss in carotenoids and protein improved the quality of the product. The assayable tannin content of cassava leaves was reduced markedly by drying[15,16]. Phenols present in cassava leaves have the property of binding with proteins, thereby reducing their bioavailability. During processing phenols can undergo polymerization or oxidation, or could be hydrolyzed to simple phenols. Drying could also reversibly fix tannin to other cell polymers thereby reducing the total assayable tannins.

. The detoxification process described above ensures a storable, cassava leaf product with safe levels of cyanide, moderate phenolic content, along with significant amounts of carotenoids, protein and carbohydrates. Detoxified cassava leaf preparations have enormous potential as a nutrient supplement in food and feed.

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References

1. Lancaster, P.A., Brooks, J.E.1983. Cassava leaves as human food. Econ Bot, 37, 331 – 348.
2. West, C.E., Pepping, F., Temalilwa, C.R. 1988. The composition of foods commonly eaten in East Africa. Wageningen Agricultural University, The Netherlands.
3. Bokanga, M. 1994. Processing of cassava leaves for human consumption. Acta Hort, 375, 203 – 207.
4. Awoyinka, A.F., Abegunde, V.O., Adewusi, S.R.1995. Nutrient content of young cassava leaves and assessment of their acceptance as a green vegetable in Nigeria Pl Food Hum Nutr, 47, 21 – 28.
5. Siqueira, E.M.de A., Arruda, S.F., deVargas, R.M., deSouza, E.M.T.2007. β-carotene from cassava (*Manihot esculenta* Crantz) leaves improves Vit A status in rats. Comp Biochem and Physiol. 235 – 240.
6. FAO/WHO 1995. Codex standard for edible cassava flour. Codex Standard 175-1989. Rome, Italy; Food and Agriculture Organization and World Health Organisation of the United Nations.
7. Johnson, R.M., Raymond, W.D. 1968.The chemical composition of some tropical food plants IV Manioc. Trop Sci, 7, 109 – 115.
8. De Bruijn, G.H. 1973. In B. Nestel and R. Macintyre (Ed), The cyanogenic character of cassava (*Manihot esculenta*) Chronic cassava toxicity IDRC monograph 010e, Ottawa,pp 43 – 48.
9. Essers, A.J.A., Bosveld,M., Van der Grift, R.M.,Voragen, A.G.J.1993.Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen . J.Sci Food Agric, 63, 287 – 296.
10. Fasuyi, A.O.2005.Nutrient composition and processing effects on cassava leaf (Manihot esculenta Crantz) antinutrients. Pak J of Nutr, 4, 37 – 42.
11. Ngudi, D.D., Kuo, Y.H., Lambein, F.2003. Cassava cyanogens and free aminoacids in raw and cooked leaves. Food Chem Toxicol, 41, 1193-1197.
12. Bradbury, J.H., Denton,I. 2010.Correct and incorrect ways to process cassava leaves- warning. CCDN news, No 15, p2-3.
13. Bradbury, J.H., Denton, I.C. 2011. Mild methods of processing cassava leaves to remove cyanogens and conserve key nutrients. Food Chem, 127,1755-1759
14. Bradbury, J.H., Denton, I.C. 2010a. Rapid wetting method to reduce cyanogen content of cassava flour. Food Chem, 121, 591 – 594..
15. Padmaja, G.1989. Evaluation of techniques to reduce assayable tannins and cyanide in cassava leaves. J Agric Food Chem, 37, 712 – 716.
16. Ravindran,V.1993. Utilisation of cassava leaves in animal nutrition. J Nat Sci Council of Sri.

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Recent and Historical Status of grass pea (*Lathyrus sativus* L.) in Turkey

The genus *Lathyrus* (Fabacea) includes 160 annual or perennial species grown in the Old World and New World. Turkey is an important center for Lathyrus with 65 species, 23 of them endemic (Gunes and Ozhatay 2000). Grass pea (*Lathyrus sativus*) is agronomically the most important *Lathyrus* species and widely cultivated as a food (Jackson and Yunus, 1984). Grass pea was first cultivated in the Balkan Peninsula around 6000 BP (Kislev, 1989). However, its natural distribution is primarily centred in the Eastern Mediterranean region (Cocks et al., 2000). In archaeological excavations in Turkey and Iraq, *Lathyrus* seeds were found as collected or cultivated items dated at BC 8000 (Turk et al., 2007).

Grass pea attracts interest as it has a number of biological and agronomic advantages including drought and water logging tolerance, low input requirements, insect and pest resistance, adaptation to a variety of climates and soils. It can provide economic seed yield even under low rainfall. Grass pea seeds are also nutritionaly important with up to 35% protein content (Rosa et al., 2000). Additionally, pod shattering which can cause harvesting problems in many legumes is much less of a problem in grass pea. Despite its many agronomic and biological advantages, overconsumption of grass pea seeds during a period of 3-4 months can cause a motor neuron disease known as neurolathyrism. β-ODAP (β-N-oxalyl-L-α,β-diaminopropionic acid) found in grass pea seed is believed to be the causative agent for neurolathyrism (Rao et al., 1964; Spencer et al., 1986). Throughout history, cultivation or the sale of grass pea was banned by governments in some countries due to its toxic effects, however, it is still cultivated in many countries. Because grass pea is an insurance crop in some regions prone to extreme environmental conditions, its cultivation continuous. Over the recent decades, grass pea has received renewed interest in drougt- prone and marginal agro environments in the world (Vaz patto et al., 2006). For this reason, many breeding programmes are in progress worldwide to develop genotypes combining high yield with high protein content and low or no neurotoxin (β-ODAP) (Hanbury et al. 2000).

As in most of the world, *Lathyrus sativus* (grass pea) has the greatest economical importance and is widely cultivated in Turkey. The other species, for example, *L. ochrus, L. cicera*, and *L. clymenum* (Cetin, 2006; Basaran et al., 2011) are cultivated in very limited areas of Aegean and Mediterranean Regions to meet household consumption and for feed. Nearly 40 years ago two other species were cultivated in Turkey , *L. hirsutus* as forage (Tosun 1974) and *L. odoratus* as ornemantal (Davis, 1970), but we have not any evidence of this today.

This plant arouses interest in Turkey in recent years as forage bedause of goverment support for forage production. Today, its planting area have reached about 35.000 ha (28.000 ha for forage and 7.000 ha for seed) (TUIK, 2012) by increasing over 2000% during the last decade.

This increasing suggests that grass pea culture is very variable in Turkey, mainly depending on economic reasons. However, grass pea has still received little attention and there is no well established trade for this crop. Although it was widely cultivated in the past as forage, feed and food, grass pea is now rarely grown in Turkey. There are three registered varieties of grass pea in Turkey and, two of them were released in 2013. So, almost all the seeds cultivated until now are landrace type.

Local names for grass pea are mürdümük, culban, fasıl and feslek in different regions. These different local names may suggest a long history of grass pea cultivation in Turkey. In fact, we know that grass pea cultivation has a very long history in Turkey. But, interestingly, it was not reported in detailed in scientific papers. So, we do not have comprehensive knowledge about historical process of grass pea cultivation and uses as well. However, the current situation can shed light on the past.

During our extensive expedition covering all regions of Turkey in 2007, we observed that grass pea is still widely grown in many parts of the country but in small amount, with low or no inputs especially in marginal areas. Senior farmers over the age of 50 are knowledgeable about grass pea very well both as feed and as food, but young farmers are not, which indicates a significant decrease in cultivation of grass pea over the last 40 years. This is probably due to the economically more interesting alternative crops such as vetch, lentil and chickpea which can grown in the same ecology, and also due to mechanisation of farming. This crop is mainly grown for forage. However, its seeds are also used for animal feed by partly grinding, and less for human consumption. Although not generally, grass pea is known as poor men food and is also seen as an indicator of low social status. This is quite strange because farmers know nothing about its toxic effect and also they say that it is very tasty crop. In addition, Turkish scientific literature does not include any case of neurrolathyrism in humans or in animals. For this reason, the registration process of grass pea does not include ODAP analysis in Turkey. One interesting thing we encountered during our seed collection studies is that grass pea is known as vetch (Vicia sp.) by some farmers. Vicia species are exclusively used for animal feeding. Perhaps, this is why people who consumed grass pea experience social marginalisation.

There may be three reasons for the absence of neurolathyrism in Turkey. The first is low consumption, which is a very strong possibility.The second is the consumption form as a suplementary food in the diet. The third may be the low content of of ODAP in Turkish grass pea genotypes.

The ecology of Turkey is uitable for the growth of many crops. So people are able to access a wide range of different foods during all seasons. This is why long-term dependency on a single crop is unlikely. Additionaly, daily diets of Turkish people include a high portion of vegetable, anion and garlic that are known as protective factors against neurolathyrism. Grass pea is consumed as soup, pilaf (rice cooked in seasoned broth), snack and as a mixture with some other foods by the people. All these consumption types include a cooking process. For animal feeding, seeds are used by soaking in water or grinding and mixing with cereals. Maybe this processing is the reason why neurolathyrism is not observed in animals in Turkey. Neuro-active ODAP is a water-soluble amino acid that can be leached from seed by soaking in water (Akalu et al., 1998).

Previous studies have shown that grass pea genotypes originating from Turkey had lower ß-ODAP content in seeds than the 0.2 % which some researchers propose as upper limit presumed safe for human and animal consumption, whereas samples originating from Bangladesh, Ethiopia, India, Nepal, and Pakistan had high ß-ODAP content ranging from 0.7 to 2.4% (Abd El Moneim et al., 1999). Basaran et al (2011), who investigated 51 landraces and 1 released variety (Gurbuz-2001) of grass pea and one *L. clymenum* landrace, all of which are still cultivated in Turkey, reported lower values for ß-ODAP (from 0.135 to 0.386 % and average 0.22%) in seeds. The same authors reported that most of the Turkish landraces had lower ß-ODAP content than 0.2 % (Abdel Moneim et al., 1999).

All these data clearly indicate that Turkey can be an important center for grass pea breeding with high variation and low ß-ODAP genotypes. Moreover, the lack of enough research in this area should make it attractive for scientists.

Figure1. Different views from Turkish grass pea landraces.

References

1. Abd El Moneim, AM., Van Dorrestein B., Baum, M., Mulugeta W., 1999. Role of ICARDA in improving the nutritional quality and yield potential of grass pea (*Lathyrus sativus*) for subsistence farmers in developing countries CGIAR-wide conference on Agriculture-Nutrition, 5–6.
2. Akalu, G., Johansson, G., Nair, B.M., 1998. Effect of processing on the content of β-N-oxalyl-α,β-diaminopropionic acid (β-ODAP) in grass pea (*Lathyrus sativus*) seeds and flour as determined by flow injection analysis. Food Chem. 62 (2), 233–237.
3. Cetin T., 2006 — Karyotype Analysis of some species of Lathyrus L. (Master Thesis), Süleyman Demirel University, Enstitute of sciences, Isparta, Turkey.
4. Basaran, U., Onal-Asci, O, Mut, H., Acar, Z., Ayan, I., 2011. "Some quality traits and neuroroxin ß-N-oxalyl-*L*-α,ß-diaminopropionic acid (ß-ODAP) contents of *Lathyrus sp*. Cultivated in Turkey". African Journal of Biotechnology, 10(20): 4072-4080.
5. Cocks, P., Siddique, K., Hanbury, C., 2000. Lathyrus A New Grain Legume. A report for the Rural Industries Research and Development Corporation (RIRDC) Publication No 99/150.
6. Davis, P. H., 1970. Flora Of Turkey and East Aegean Islands. Edinburgh, 328-369.
7. Gunes F. and Ozhatay, N., 2000 — Lathyrus L. In: A. Güner, N. Özhatay, T. Ekim and K.H.C. Baser (eds), "Flora of Turkey and East Aegean Islands" 11 (supplement 2): 92-94, University Press, Edinburgh.
8. Hanbury, C.D., White, C.L., Mullan, B.P., Siddique, K.H.M., 2000. A review of the potential of *Lathyrus sativus* L. and *L. cicera* L. grain for use as animal feed. Anim. Feed Sci. Technol. 87: 1–27.
9. Jackson, M.T. and Yunus, A.G. 1984. Variation in the grass pea (*Lathyrus sativus* L.) and wild species. Euphytica 37: 549-559.
10. Kislev, M.E., 1989. Origins of the cultivation of *Lathyrus sativus* and *L. cicera* (Fabaceae). Econ. Bot. 43:262-270.
11. Rao, S.L.N., Adiga, P.R., Sarma, P.S., 1964. Isolation and characterization of *β*-oxalyl- L-*α*,*β*-diaminopropionic acid: A neurotoxin from the seeds of *Lathyrus sativus*. Biochemistry 3: 432–436.
12. Rosa, M. J. S., Ferreira, F. B., Teixeira, A. R., 2000. Storage proteins from *Lathyrus sativus* seeds. J. Agric.Food Chem.*, 48*, 4432-5439.
13. Spencer, P.S., Roy, D.N., Ludolph, A., Hugon, J., Dwivedi, M.P., Shaumburg, H.H., 1986. Lathyrism. Evidence for role of neuroexcitatory amino acid BOAA. Lancet ii, 1066-1067.
14. Tosun, F. 1974. Forage Legumes and Grasses Culture, Ataturk Univ. Pres. No:242:180-181.
15. TUIK, 2012, Turkish Statistical Institute, Main statistics, Agriculture, Plant Production. http://www.turkstat.gov.tr
16. Tuk, M., Albayrak, S., Celik N., 2007. Estimates of Broad-Sense Heritability for Seed Yield and Yield Components of Grass pea (*Lathyrus sativus* L.), Turk J Agric For. 31,155-158.
17. Vaz Patto, M.C., Skiba, B., Pang, E.C.K,. Ochatt, S.J,. Lambein, F., Rubiales, D. 2006. *Lathyrus* improvement for resistance against biotic and abiotic stresses: From classical breeding to marker assisted selection, Euphytica, 147: 133–147.

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