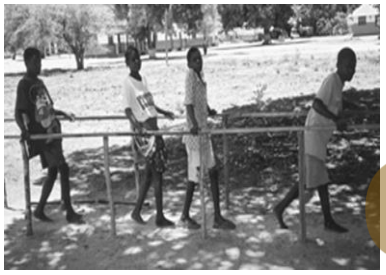
Working together to eliminate cyanide poisoning, konzo, tropical ataxic neuropathy (TAN) and neurolathyrism



CCDN News

Cassava Cyanide Diseases & Neurolathyrism Network

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Editorial

The konzo-story in Mozambique comparing to neurolathyrism

In this issue of our Newsletter we have put a shortened version of a very interesting media story on the konzo discovery and early history of konzo research in Mozambique. The key players in this story are Julie Cliff, Hans Rosling and in a later stage our founder Howard Bradbury, who spent the last 26 years of his life searching solutions to stop konzo. This story is indeed very relevant for researchers working on konzo and/or on neurolathyrism. Like in the case of neurolathyrism, konzo researchers first focused on the toxic aspects of the nutrition and only later found that nutritional deficiencies or imbalance also played a role. A better-balanced nutrition, more specific a better balance in essential amino acids limits toxicity. Like for neurolathyrism, konzo does not occur along the coastal areas where fish is part of the diet. The same is true in the coastal area of Bangladesh and among the Woito tribe in Ethiopia where fish is part of the diet together with grass pea but neurolathyrism is absent. Like for neurolathyrism, konzo affects mainly or exclusively the poorest section of the population who only can afford the cheapest food available. Both konzo and neurolathyrism should be considered socio-economic famine diseases.

In the second part of this newsletter, the abstracts of presentations at the 7th International Food Legume Research Conference (IFLRC), organised by ICARDA, are presented. Nine contributions out of 360, or merely 2.5% were on grass pea (*Lathyrus sativus*), illustrating the low status of grass pea in food legume research. Nevertheless, at the closing ceremony of this conference, Fernand Lambein received a life-time achievement award for his "lifetime support to Lathyrus research".

Delphin Diasolua Ngudi

The curse of konzo

On 21 August 1981, Australian physician Julie Cliff received the following message on her telex, a print-on-paper precursor to modern text messaging: “Polio outbreak. Memba District. 38 cases. Reflexes increased.”

The apparently routine message was sent from the Provincial Health Directorate in Nampula, a city in northern Mozambique. Cliff worked in the epidemiology department of the Mozambican Ministry of Health in Maputo, at the southern end of the country. Effective vaccines against poliomyelitis—a food and water-borne infectious disease that can damage nerves and cause paralysis—had been developed in the 1950s and 1960s, eliminating polio from many industrialized countries. However, the disease remained rife throughout sub-Saharan Africa. So the message was unremarkable—except for one thing. In the acute phase of polio, tendon reflexes are not increased. They are absent.

Dr. Cliff arrived in Nampula province shortly afterwards as part of a small Health Ministry investigation team, determined to get to the root of the mystery. Close inspection of affected individuals confirmed the disease was definitely not polio. Yet the question remained: what else could it be?

Other doctors already at the scene included a young medic from Sweden named Hans Rosling, who was working in one of the affected areas as a District Medical Officer. When first confronted with a line of women and children suffering various degrees of paralysis, he reached for the biggest neurology textbook he could find. “Their disease did not exist in that book,” he later recounted. Lacking other ideas, Rosling was soon forced to entertain a disturbing possibility: biological or chemical warfare.

In the days that followed, no evidence for any kind of biological or chemical attack emerged. Nevertheless, cases of the mysterious paralysis continued to mount. Each followed a troubling pattern: the disease usually affected women and children, almost invariably in rural villages that had already endured months of a severe drought. Typically, it would strike quickly, over a matter of days or even hours. Previously active young mothers and children would go to sleep with little inkling of anything amiss, only to wake the next morning with various degrees of muscle stiffness and contractions in the legs, and—less commonly—in the arms, too. Sometimes a bout of heavy exertion, such as collecting water for the household from a distant well, seemed to trigger symptoms the next day. Affected people would find themselves unable to walk normally or, in the worst cases, at all. It was almost as if someone—or something—had tied their legs together with invisible cord.

The affliction appeared to be irreversible. As the number of paralysed people grew, the investigators worked with increasing urgency, travelling to remote parts of the province to examine potential cases, interview members of local communities to establish possible causes for the disease, and take blood samples for laboratory analysis.

Initial indications favoured a microbial origin for the condition: if not polio, then some other pathogen. Many early case reports described symptoms such as fever, headache, and diarrhoea preceding the onset of paralysis—symptoms consistent with an infectious disease. Likewise, the clear clustering of cases in small communities and family groups pointed to person-to-person transmission. After all, Africa hosted many unpleasant and little known parasites, bacteria, and viruses—such as the newly emerged Ebola virus, first identified in Sudan and Zaire in 1976.

But subsequent investigations revealed no obvious pathogens. After several days in the field Cliff’s team withdrew to Maputo to regroup and consult with international experts, including those from the World Health Organization. Telex machines from Mozambique to Geneva chattered with criss-crossing hypotheses, while blood samples were hastily despatched to specialist labs overseas, including the British biological and chemical weapons research centre in Porton Down, England.

The team perused a series of increasingly dusty and dense printed journals and textbooks. They identified two diseases with possible relevance to the Nampulan outbreak. One disease, called lathyrism, produced a very similar clinical picture: upper motor neuron damage leading to increased muscle tone and paralysis. Lathyrism typically occurred on a sporadic basis in south Asia, but was also noted among the detainees of Vapniarka, a concentration camp in Romania, during World War II. The other disease was an obscure neurological disorder named Tropical Ataxic Neuropathy, or TAN, first reported in Jamaica in 1897 but since described in a number of other countries scattered across the tropics, including another East African country, Tanzania.

Intriguingly, both diseases were nutritional, rather than infectious, in nature. With lathyrism, the illness was caused by a toxin present in certain legumes—including the livestock peas that were the sole foodstuff allowed to the unfortunate Jewish internees in Vapniarka. However, none of these legume species were grown or consumed in northern Mozambique. The purported cause of TAN was more vague — even a century after its discovery, the exact cause remains unknown. Still nutritional deficiencies have been proposed as possible factors, and experts had long linked it to a food crop widely cultivated in Mozambique and elsewhere. But the symptoms of TAN—which include blindness, hearing loss, and an unsteady gait—bore little resemblance to the distinctive paralysis the doctors saw in Nampula province.

By the end of September 1981, Cliff’s team—now expanded to include Dutch botanist Paul Jansen—was no longer looking for a contagious disease. They had considered and excluded all known infectious agents that could possibly perpetrate the perplexing plethora of paralysis. Nor had detailed analysis of various body fluids demonstrated any unknown ones.

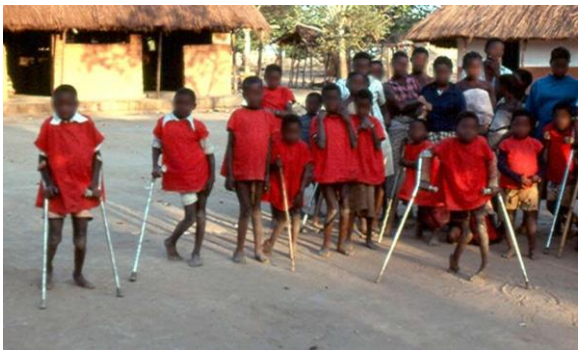

Figure 1 Stricken individuals, 1981 (via Global Health NOW)

The investigators' attention turned back towards dietary factors. Despite the drought, the problem wasn't a lack of food. While many local people had suffered, few actually starved and unlike later famines elsewhere in East Africa, mortality was low. Much of the credit for this could be attributed to a single plant species that had come to the rescue: *Manihot esculenta*, also known as 'cassava'. In rural Mozambique, as in much of Africa, people often grow cassava on a small-scale basis close to households as a "crop of last resort," for consumption when they lack other food sources in the hungry season—or in times of conflict, crisis, or drought. Cassava comes in "bitter" and "sweet" (or, more accurately, "not bitter") varieties, with the bitter types particularly valued for their resistance to locusts and other herbivores.

Figure 2 Cassava root

Early in the investigation, an elderly man in one of the affected villages told the doctors, "This disease has happened because the rain has not washed our cassava." Perhaps understandably, investigators initially ignored him. The practice of disease outbreak investigation involves an established sequence of steps concerned with relating cases to "time, place, and person." Heeding advice from grizzled old locals does not feature prominently in field epidemiology manuals.

However, as each new test failed to yield evidence for any old or new pathogens, the investigators returned to the old man's words. Cassava was an important element of the local diet in Nampula, and therefore hard for Cliff and her team to overlook if they were considering a nutritional cause for the puzzling paralysis. The doctors formulated a worrying hypothesis involving one of humanity's most feared poisons—a substance that disrupts the body's utilisation of oxygen at a cellular level, and formed the principal ingredient of the notorious Zyklon B gas used in the Nazi extermination camps: hydrogen cyanide. One clue was the fact that cassava often leaves a bitter taste in the mouth. This bitterness derives from two types of sugar molecules: *linamarin* and *lotaustralin*. These molecules are termed "cyanogenic glucosides," meaning that in certain circumstances—for example, when exposed to the enzymes and bacteria of the human intestine—they will decompose and produce hydrogen cyanide. The more bitter the cassava, the greater the potential exposure to cyanide.

Sure enough, when Jansen tested a range of foodstuffs from the disease-affected areas, he found high levels of cyanogens in the cassava samples. And when the team tested blood samples from affected people for thiocyanate—cyanide's breakdown product within the human body—the average result was 20 times normal levels. The researchers now had to entertain the possibility that the food that saved so many Mozambicans from starvation was, in some cases, paralysing them.

With a plausible hypothesis to test, the application of tried-and-tested disease outbreak investigation methods started to yield results. When Cliff and her colleagues related the pattern of cases to time, they realised the August peak of the epidemic corresponded closely to the region's main cassava harvest. When they mapped out cases by place, they saw that the disease largely spared coastal areas, which was consistent with their understanding of the local diet: access to fish and to food markets meant people depended less on cassava. Finally, when the team looked closely at "person," the preponderance of the disease in women of childbearing age and children over the age of two also made sense: in rural Mozambique, as in much of Africa, women prepare food for the household and they, with their accompanying children, would be most exposed to cassava during processing and consumption. Men are usually privileged with more diverse dietary pickings, and are spared the additional nutritional stress of pregnancy and breastfeeding. Furthermore, the extreme food shortage caused by the recent drought had forced many women to eat something that would normally be thrown away: cassava root peel, which Jansen's tests had shown to harbour particularly high levels of cyanogens.

Much of the dietary background of cyanide containing plants was familiar to Cliff and her team in 1981—so they knew that in theory, at least, the cyanide in cassava should not have posed a significant threat to human health. Furthermore, there were at least a couple of conditions already attributed to cyanide ingestion, but neither were anything like the illness the doctors saw in Nampula province. First, there was the well-established progression of symptoms seen in acute cyanide poisoning, which include vomiting, diarrhoea, seizures, coma, and ultimately, death—but not normally paralysis. Second, there was Tropical Ataxic Neuropathy, the disease that the team had read about in Maputo. TAN had long been linked to cassava cultivation, and at the time was also hypothesised to be linked to cyanide exposure. But in both cases, the entirely different pattern of symptoms threw the investigators off the bitter scent.

Doctors even lacked an established name for the

"new" disease. Initially, they used the local Mozambican word—*mantakassa*—but it was later discovered that an Italian physician, Giovanni Trolli, had identified the same pattern of symptoms in the 1930s in rural areas of Kwango province in the then-Belgian Congo. While Trolli did not establish the cause of the symptoms at the time, he collated reports from several other doctors in the region over a two-year period and recorded the local Congolese name for the condition: *khoondzo*, or "konzo." The word meant "tied legs" and was also the name given to a talisman and a trap used by people in the area to ensnare wild animals. Although separated by nearly 50 years and more than 1,000 miles, the two diseases were clearly the same. Eventually, the all-too-appropriate name "konzo" prevailed.

When the researchers looked beyond Africa, another mystery became apparent: konzo's geographical distribution does not map neatly onto areas of cassava consumption. Half a billion people living across the tropics rely on cassava as an important part of their diet, yet in global terms konzo is, thankfully, a rare disease. It has never been reported in the Americas, where cassava was first domesticated, nor in Asia, where it has become an important staple crop in many areas. Cassava is widely consumed in West Africa—and grown on a commercial scale in countries such as Nigeria—but konzo has never been documented in this region.

For cassava, as with almonds and bamboo shoots, it seems that much depends on the particular variety of plant grown and the circumstances surrounding its preparation. In Asia, people tend to only cultivate the "sweet" cassava varieties, so starting concentrations of cyanogens are low. In the Americas, traditional processing methods—such as the "tipiti" plaited sleeve press used by Amazonian tribes—are highly effective at removing cyanide, although they require large volumes of water. In Africa, different areas rely on different processing techniques. In central and eastern Africa, the preferred method involves soaking peeled cassava roots in water for three days, followed by a period of sun drying, before pounding the resulting product into granules or flour. But when drought and hunger intervene, soaking and drying times are cut short.

Even then, the resulting cyanide exposure should not be sufficient to cause konzo in normal nutritional circumstances. Adequate intake of sulfur amino acid-containing protein helps the body metabolise and excrete cyanide, but cassava's low protein content—and often, a lack of access to meat, fish, and other protein-rich foods—means that cassava-dependent communities may not be able to maintain this basic dietary defence. Compounding this problem, in times of drought, bitter cassava becomes even more bitter: the water-stressed plants concentrate additional cyanogens in their leaves and roots to deter insects and other animals who might otherwise be sorely tempted by such a convenient "crop of last resort."

By the end of October 1981, Cliff and her colleagues had collected and connected the most important pieces of the Nampulan paralysis puzzle. They were certain that the disease was associated with the high levels of cyanide found in affected individuals. The cyanide came from the bitter cassava that was—thanks to an unfortunate collision of socioeconomic, botanical, and meteorological factors—the defining feature of the local diet. However, the exact biochemical and neurological chain of causation remains uncertain to this day. Konzo can be considered a form of chronic cyanide poisoning, but it is still unclear why its symptoms differ so much from those of acute cyanide poisoning—and what relationship, if any, konzo has with those other mysterious nutritional diseases, neurolathyrism and TAN. Occasionally, people in drought-stricken, cassava-dependent areas indeed display symptoms of acute cyanide toxicity. Reports of these early symptoms may have initially misdirected Cliff and her colleagues toward considering an infectious cause for the disease, but the onset of konzo itself usually occurs much later, after weeks of excess and near-exclusive cassava consumption.

Figure 3 Hans Rosling 27 7 1948 – 7 2 2017 (Jörgen Hildebrandt/Gapminder)

As the regional diet diversified in the months following the 1981 cassava harvest, the incidence of konzo in Nampula province slowed and eventually stopped. The Health Ministry investigation team dispersed. Julie Cliff returned to Maputo. Hans Rosling left his job as a District Medical Officer later that year but maintained a strong interest in konzo, going on to write his Ph.D. thesis on the disease. Meanwhile, the rains returned to northern Mozambique for the 1982-83 growing season, providing water to soak the cassava and yield a safer harvest for the local population.

Over the following decades, Cliff, Rosling, and others identified more konzo clusters in several countries in central and eastern Africa. They ultimately diagnosed more than 1,000 cases in the 1981 Mozambique epidemic—a similar number from Trolli's original 1936-37 Congolese outbreak. Researchers believe that the cumulative number of cases reported officially in Africa—around 11,000—represents a gross underestimate, largely because of poor access to health care—and hence poor case-reporting—in vulnerable regions. In the now-Democratic Republic of Congo alone, estimates go as high as 100,000 cases. Here, in particular, it remains a significant ongoing problem: decades of conflict have lumbered the population of this repeatedly re-named country with a legacy of social and economic problems, making it vulnerable to nutritional diseases like konzo.

For individual konzo sufferers, treatment options are

minimal. The muscles in the legs tighten and contract to a varying extent ("spastic paraparesis," or "tetraparesis" if all four limbs are affected). In mild cases, people can still walk unaided, albeit with the tiptoeing "tied legs" gait that gives the disease its name. In moderate cases, crutches or walking sticks are needed. In severe cases, people's legs are completely paralysed ("spastic paraplegia"). Physical therapy can help people manage their symptoms, but the motor neuron damage is irreversible. This is particularly devastating in societies with very little formal health or social support—where physiotherapy, for example, is not widely available, and where income is often earned via physical labour.

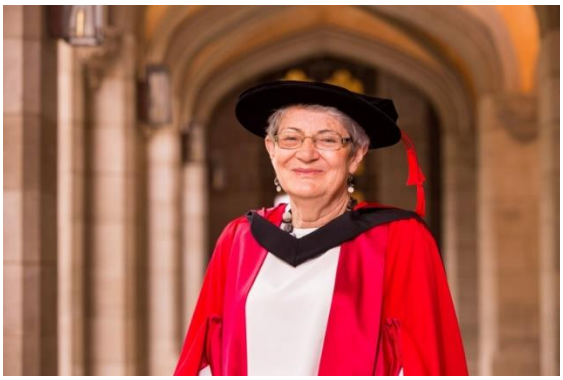

Figure 4 Julie Cliff ca. 2015 (University of Melbourne)

However, in 2004, it was discovered that the disease is completely and easily preventable. A recently retired Australian plant scientist named Howard Bradbury (1926-2016) (who spent the last 26 years of his life frantically seeking ways to prevent konzo) discovered that an additional step in cassava processing—namely, wetting cassava flour with water and leaving the resulting paste to stand for five hours—would greatly reduce cyanogen levels. As long as the gloop contains sufficient linamarase, a natural enzyme that should be present in the flour anyway, and there is adequate ventilation to allow the safe outgassing of hydrogen cyanide, the procedure—not too dissimilar, of course, from the "rain-washing" the old man in Nampula had described—will nearly always reduce human cyanide exposure to safe levels. Subsequently, Bradbury discovered that in direct sunlight, the enzyme works even faster: just two hours will suffice. The water requirements are modest, and field trials in east and central Africa have shown this "wetting method" to be practical, effective, and widely welcomed by women in vulnerable villages.

The story of cassava and konzo should not alarm global connoisseurs of tapioca pudding, bamboo shoots, and sweet almonds. It demonstrates that well-nourished, well-educated, and wealthy people generally have very little to fear from eating potentially cyanide-containing food plants. Only a sustained period of consumption of large amounts of inadequately prepared bitter cassava—which only occurs in conjunction with a collection of other disagreeable social and environmental circumstances—causes the disease. In short: only poor people suffer the curse of konzo.

Original text by: ***Matt Castle*** (https://www.damninteresting.com/the-curse-of-konzo/)

Grass pea Abstracts from the 7th International Food Legumes Research Conference in Marrakech/ Morocco 2018

Grasspea (*Lathyrus sativus* L.) –boon or bane?

Grass pea has survived as a crop for at least 8 millennia and was already part of funeral offerings to Pharaohs. The drought tolerance of grass pea has made it a survival crop during droughts and famines. In drought-prone areas of Ethiopia and the Indian Subcontinent, farmers consider grass pea a life insurance crop that gives them stamina for working in the field, and improves soil fertility thanks to nitrogen fixation. An unbalanced overconsumption of grass pea for extended periods can cause neurodegeneration with crippling paraparesis (neurolathyrism) of the legs in up to 6% of the population with varying severity. A neuro-excitatory amino acid β-N-oxalyl-α,β-diaminopropionic acid (ODAP) is often blamed as cause of this crippling, while the deficiency of essential amino acids is mostly overlooked. Research has mainly focused on toxicity while socio-economic aspects and the total diet received less attention. Dietary balancing of the essential amino acids by cereals and foodstuffs rich in sulphur-amino acids and anti-oxidants are effective protective factors in the epidemiology of neurolathyrism. The presence of ± 1% of homoarginine in the seeds is interesting in phyto-pharmacy. This is an alternative precursor for nitric oxide (NO) that has multiple physiological functions including vaso-dilatation, and has positive effects on cardio-vasculature.

Homoarginine stays in the bloodstream longer than arginine, the normal precursor for NO. Grass pea is the only legume that, in abnormal situations of drought-triggered famines, is consumed as a staple food whereas in normal situations, when taken as part of the diet, it has considerable advantages.

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Lathyrus diversity: Available resources with relevance to crop improvement

Some highly resilient *Lathyrus* species, although currently neglected as crops, regain interest in the immense challenges agriculture is facing. Grass pea (*Lathyrus sativus*) and chickling pea (*L. cicera*) are two of these species used for food or feed since ancient times. Under a long term joint collaboration effort, we have been characterizing Iberian landraces for resistance against a number of diseases and parasitic weeds in both species, what is being complemented now with pest resistance screenings, using more extended *L. sativus* collections. We have been also generating mapping populations in both species that are being used to study the genetic control of several complex traits, with the integration of genomic and transcriptomic approaches. Species specific as well as cross-species transferable molecular markers have been developed and used in these studies, allowing comparative mapping. Gene expression analysis complemented these approaches by assessing the function of putative

genes thought to be involved on the identified resistance responses on these *Lathyrus* sp. In an attempt to increase the variability, interspecific crosses were made between *L. sativus* and *L. cicera* generating valuable pre-breeding material. This work is contributing to the development of grass pea and chickling pea cultivars with improved adaptation, yield, resistance and quality, meeting farmers' and consumers' expectations.

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Indian perspective on lentil and lathyrus improvement

Lentil and lathyrus are important secondary winter legumes altogether grown on about 1.84 million ha in India. The improvement work on these crops has started in 1967. Over a period, superior cultivars of lentil (53) and lathyrus (4) have been released for general cultivation. The germplasm received from ICARDA has suitably used in development of so far 11 cultivars of lentil. In lentil, shorter growing period after rice harvest and rust in eastern India and poor biomass and wilt in Central India are serious production constraints. Systematic breeding efforts led to development of appropriate varieties for each growing situation. Recent breeding efforts have been directed to develop short duration (<100 days) and biofortified lentil with high iron and zinc and resultantly such varieties could be released for commercial cultivation. Lathyrus was grown in the country on about 1.0 million ha area in 1970s, however, the area got declined subsequently up to 0.48 million ha and farmers tended to shift from lathyrus to that of other alternative crops owing to high content of ODAP. Sincere efforts have been made in developing lathyrus varieties with low ODAP and resultantly four such varieties were developed. Bio L 212, a somaclonal derivative with lowest ODAP content (0.07%) became more popular among farmers. With the use of this in a cross combination, a low ODAP variety, Mahateora has been developed. Efforts are being made to replace local selections with improved varieties with low ODAP in traditional areas of lathyrus cultivation.

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Advances in Lentil and grasspea improvement in Bangladesh

Lentil and grass pea are the key pulses in the rice-based cropping system of Bangladesh, occupying >60% of the total pulses grown in the country. Lentil and grass pea area and production are declining since the nineties due to priority given to spring rice production for food security. Bangladesh currently produces 270,400 tonnes of lentil with a productivity of 1.3 t/ha and 301,800 tonnes of grass pea with a productivity of 1.1 t/ha. To provide sustainability in the cropping systems and to ensure protein supply to the people, the country's agriculture policy is currently shifted to crop diversification with pulses. Pulses research in Bangladesh in collaboration with international organizations, especially ICARDA has developed 19 high yielding, disease resistant and micronutrients-rich lentil and five low-ODAP grass pea varieties along with production technologies. Relay cropping, conservation agriculture in rice field is popular, where farmers get 64% yield advantage over sole crop due to timely sowing and use of residual soil moisture. With several initiatives in technology dissemination, 92% and 35% of the area is covered by adoption of improved technologies lentil and grass pea, respectively. The development of super-early maturing lentil to fit the <90-day window between two rice crops can provide a sustainable rice-based system, increasing cropping intensity and support house-hold nutritional security. Farmers are also giving priority to lentil and grass pea cultivation due to higher yield leading to gradual area increases in the country. Thus, through international collaboration and national efforts, Bangladesh is approaching towards reducing pulse import, and providing nutritional support to its people from domestic production.

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Lentil and grass pea improvement in Ethiopia

Lentil and grass pea are the two important cool-season food legumes grown by small holder in the cereal-based cropping system in the highlands during the main and small rainy season. The two crops are key sources of food, incomes and animal feed as well as The production of lentil was fluctuating over years due to insect pests (aphids) and diseases (rust, wilt root rot and currently Ascochyta blight), waterlogging and terminal drought. Lentil and grass pea improvement was started in early 1970's and able to release 13 high yielding and diseases resistant lentil cultivars and one grass pea cultivar for production. Major successes were recorded in the scaling of lentil cultivars like Alemaya and Adaa) with high yield (over 2t/ha), disease resistance, low decortication losses and amenable for August planting. Area of grass pea production has increased over years due to its tolerance to drought, waterlogging and diseases. More research efforts are needed to improve lentil and grass pea productivity by improving resistance to foliar and root diseases as well as management practices to reduce the impact of parasitic weeds and insect pests. Since Ethiopia has different agro-ecologies, ICARDA and NARS partners can develop elite germplasm that can cater other agro-ecologies in Eritrea, Yemen and other East African countries.

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Adaptation of exotic grass pea germplasm for rainfed farming in Central India

Grasspea (Lathyrus sativus L.) is grown in India in about 550,000 ha for human food and animal feed mainly in eastern Indian states in rice-based cropping systems. It is a hardy crop, adapted to harsh edapho-climatic conditions and has tolerance to multiple biotic and abiotic stresses. But grass pea has an ambivalent reputation due to presence of a toxin compound, β-N-oxalyl-α,β-diaminopropionic acid (β-ODAP) in its plant parts. Efforts are underway to develop high yielding, early to medium maturity and low-toxin/toxin-free grass pea varieties for safe human consumption.

In this study, 25 grass pea genotypes originated from Greece (5), Morocco (2), Turkey (1), Algeria (1), Bulgaria (1), Germany (1), Syria (2), Pakistan (1), Nepal (1), Bangladesh (9) and India (1) were evaluated for phenological and yield traits at ICARDA research station, Amlaha, India during 2015-2016 cropping season. The results showed significant genetic variability for days to maturity (112 – 158 days), plant height (26.7 – 73.7 cm), biological yield per plant (0.9 – 4 kg), grain yield per plant (up to 1.3 kg) and 100-seed weight (5.10 – 8.5 g) ($p \leq 0.01$). Genotypes from Greece showed minimum (27.3cm), biomass (0.87 kg) and 100-seed weight (5.10 g). Maximum biomass (4 kg) and 100-seed weight (8.5 g) were noted in Indian and Syrian genotypes, respectively. Indian variety, Ratan was the earliest maturing genotype (112 days) with maximum grain yield (1.3 kg) as compared to exotic germplasm. Significant positive correlation was observed between days to first flower and 50% flowering (r=0.802**); days to first flowering and days to maturity (r=0.290*), seed yield and biomass (r=0.48**), seed yield and plant height (r = 0.74**). However, seed yield showed significant negative correlation with days to first flower (r = - 0.54**), 50% flowering (r = - 0.55**) and maturity (r = -0.38**).

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Towards development of zero/low β-N-Oxalyl-L-α,β-diaminopropionic acid (β-ODAP) varieties of grass pea (*Lathyrus sativus* L.)

Grasspea (*Lathyrus sativus* L.) is an important pulse crop known for having climate resilient traits. However, presence of neurotoxin β-ODAP in seeds and other plant parts renders grass pea unfit for consumption. One of the potential approaches to reduce β-ODAP content is by downregulating/ knocking down ODAP biosynthesis pathway related genes by Genetic engineering/ Genome-editing technology. For harnessing the potential of these technologies, an in vitro regeneration system amenable to genetic transformation is pre-requisite. Initial, regeneration studies on grass pea indicated high responsiveness to regeneration. Notably, embryonic axis explant appears to be the most suitable explant in grass pea for Agrobacterium-mediated transformation in terms of de novo regeneration of shoots, sensitivity of explant for selection agent and high transient GUS expression. Further, to identify gene for ODAP manipulation, we looked back the previously reported ODAP biosynthesis pathway. Last two steps of ODAP biosynthesis are catalysed by Oxalyl CoA synthetase and ODAP synthase enzymes. However, so far no gene encoding these enzymes has been identified in grass pea. Here, we identified the full length gene sequence of Oxalyl CoA synthetase enzyme in grass pea based on sequence information in homologous species, publically available transcriptome of grass pea and through PCR. Studies on 171 gene expression analysis in response to varied ODAP content, presence of different isoforms and allele mining strategies being followed to establish the role of this gene in ODAP biosynthesis. Identifying candidate gene in the ODAP biosynthesis pathway and their manipulation through Genetic-engineering/Genome-editing shall results in realization of low/zero ODAP grass pea variety fit for safe consumption.

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Breeding low neurotoxin, high yielding grass pea (*Lathyrus sativus* L.) genotypes for rice based cropping system of Chhattisgarh

Grass pea (*Lathyrus sativus* L.) is the most important pulse crop of Chhattisgarh State in India covering maximum rabi season pulse area (348000 ha) and production of 207,000 tonnes with productivity 595 kg/ha). It is a drought tolerant, hardy, nutritionally rich and in some parts of the Indian sub-continent, the pods are boiled, salted and sold by street vendors, as tasty snacks. Two potential low ODAP cultivars viz. Prateek and Mahateora have been evolved by IGKV Raipur which are very popular among farmers in the state. In this study, 14 newly developed genotypes with 3 low ODAP checks varieties were evaluated over 8 locations across the state covering three agro-climatic zones in Chhattisgarh in 2015-16. Three genotypes namely RLS 2010-1 (1156.5 kg/ha), RLS 2010-6 (1129.5 kg/ha), RLS 3006 (1125.4 kg/ha) and RLS 2011-4 (1117.7 kg/ha) gave promising yield across locations and will be used in variety development or promoted for national level evaluation for release as new variety.

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Identifying promising sources of quantitative resistance to *Fusarium oxysporum* f. sp. pisi in grass pea

Grass pea (*Lathyrus sativus*) is a promising source of protein in drought-prone areas, and an interesting

alternative for cropping systems diversification in marginal lands in Europe. However, yield inconsistency due to sensitivity to specific diseases strongly limits its cultivation. Fusarium wilt, caused by the soil-borne fungus Fusarium oxysporum f. sp. pisi (Fop), is one of the most destructive diseases affecting pea (*Pisum sativum*) worldwide, and is now becoming a threat for grass pea production in Portugal. Variable responses to fusarium wilt under field conditions have been recorded previously among grass pea accessions. This supported the search for Fop resistance sources using an extended collection of grass pea accessions with representatives not only from the Iberian Peninsula but also from distant grass pea production regions in the world. Until now, 131 grass pea accessions were characterized under controlled conditions for Fop race 2 resistance. Disease rating over time and the area under disease progression curve revealed great variability among the collection, with the identification of highly resistant to susceptible accessions. These results support also the existence of quantitative resistance mechanisms in our collection. To better describe these mechanisms, the fungus colonization extension was also characterized in the most contrasting accessions. This study represents the first steps on breeding grass pea varieties resistant to fusarium wilt and may, due to the phylogenetic closeness to pea, contribute as a resistance source to future pea improvement. Upcoming work will involve the unravelling of the genetic basis of resistance through genome wide association analysis.

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