Role of ICARDA in improving the nutritional quality and yield potential of grasspea (*Lathyrus sativus* L.), for subsistence farmers in dry areas.

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Introduction

*Lathyrus sativus* L. or grasspea (*khesari* in India and Bangladesh, *guaya* in Ethiopia, *san li dow* in China, *pois carré* in France) has been cultivated in South Asia and Ethiopia for over 2,500 years (3) and is used as food and feed. It is a popular drought-tolerant crop as food and feedstuff in drought-prone areas of Africa and Asia (2,9). Its ability to provide an economic yield under adverse conditions has made it a popular crop in subsistence farming in many developing countries, and it offers great potential for use in marginal low-rainfall areas. Despite its tolerance to drought, grasspea is not affected by excessive rainfall and can be grown on land subject to flooding (19). In Bangladesh, India, Nepal and Pakistan, it is often broadcast into a standing rice crop, where it flourishes on the residual moisture left after the rice has been harvested. It is a very hardy crop with a penetrating root system and can be grown on a wide range of soil types, including very poor soils and heavy clays. This hardiness and its ability to fix atmospheric nitrogen make the crop one that seems designed to grow under adverse conditions.

More than 100 million people in drought-prone areas of Asia and Africa consider grasspea a traditional popular crop because of its easy cultivation, its relative resistance to drought, flood, moderate salinity and insect attack, and its good yield of tasty seeds (2,18). When other crops fail due to adverse conditions, grasspea can be the available food source for the poorest section of the population and sometimes is a survival food in times of drought-induced famine.

Since grasspea is often the cheapest food legume for low-income families, it is a common component of their traditional diet. Its seeds also contain a high amount of free L-homoarginine, which may act as a precursor of lysine in human nutrition (16). These same seeds contain a neurotoxic non-protein amino acid that can cause irreversible spastic paraparesis (paralysis) of the legs when it is consumed as a major portion of the diet over a three-to-four month period (20,21). Recent outbreaks of famine in areas where grasspea could be a promising food crop for sustainable agriculture have been followed by outbreaks of this upper-motorneurone disease in epidemic proportions: Bangladesh in 1942-45 and 1972-74 (6), China in 1973 (14), and Ethiopia in 1976-77 (5) and 1997-98 (Lambein F, personal communication, 1999).

The causative agent of neurolathyrism was confirmed as 3-((N-oxalyl)-L-2,3-diaminopropionic acid (β-ODAP) or its synonym β-N-oxalyl-L-alanine (BOAA) (17,20), and the biochemical pathway of the toxin has been elucidated (10,13). However, no biological role of β-ODAP in the plant has yet been proposed. The concentration of β-ODAP in ripe seeds is very variable and is influenced by genetic and environmental factors (1,12). Water stress can double the toxin level, whereas salinity in the soil may reduce the toxin level in the seeds (17).

Despite the obvious advantages of grasspea, until recently relatively little effort has been made towards the improvement of this very hardy pluse crop. Indeed, the history of grasspea has been one in which it has been banned by many countries due to due to its toxicity. Despite this, grasspea is still produced in significant quantities in many parts of the world. Improvement of this crop is now being addressed at the International Center for Agricultural Research in the Dry Areas (ICARDA) through its germplasm enhancement programme.

ICARDA’s role in improving the nutritional quality of grasspea

The ICARDA, which has a mandate for improving the productivity of dryland agriculture in the West Asia and North Africa (WANA) region, and more recently Central Asia and Caucasian Countries (CAC), is placing special emphasis on improving grasspea. ICARDA has a breeding programme for the improvement of cool-season food and forage legumes, including grasspea. It also holds a rich collection of *Lathyrus* spp. germplasm (1,883 accessions, including 1,560 *L. sativus*) from different parts of the world. Using this precious resource, ICARDA is collaborating with national partners to develop new grasspea lines with the objectives of improving its yield potential, adaptability, and nutritional quality through reduction of its neurotoxin β-ODAP to a safe level (<0.15%) for human consumption and animal feed.

Since 1989-90, the grasspea breeding programme at ICARDA has aimed to reduce the neurotoxin β-ODAP concentration by four approaches. These are: germplasm evaluation, genetic detoxification

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(hybridization programme), exploitation of somaclonal variation induced in response to in vitro culturing of somatic cells (an application of plant biotechnology) and effect of soil micronutrients zinc and iron on the level of neurotoxin in the grain.

**Germplasm evaluation**
An extensive screening programme was initiated in 1989-90 for five years to explore the possibility of identifying toxin-free lines from germplasm of different origins. The results indicated that no accession of any *Lathyrus* species was β-ODAP free, although in several lines the β-ODAP content was low. This appears to be species related, since samples of *L. cicera* ranges from 0.03% to 0.22%, with a mean of 0.16%. *Lathyrus sativus* showed the greatest range, from 0.02% to 24%, with a mean of 1.3% whereas *L. ochrus* lines were highest in β-ODAP, ranging from 0.46% to 2.5%, with a mean of 1.4% in the ripe seeds (8). Four IFLLS lines of *L. sativus* - 522, 588, 516, and 563 - were found to have a low β-ODAP content in the seeds, ranging from 0.02% to 0.07%. The level presumed safe for human consumption is <0.2% (4).

Analysis of a large number of germplasm accessions of *L. sativus* revealed that samples originating from Bangladesh, Ethiopia, India, Nepal, and Pakistan were high in β-ODAP content in the dry seeds, in a range from 0.7% to 2.4%. Whereas samples from North Africa, Syria, Turkey, and Cyprus had significantly lower β-ODAP, ranging from 0.02% to 1.2%.

**Genetic detoxification (hybridization programme)**
Because the low-neurotoxin lines have undesirable traits, such as late flowering, susceptibility to insects and diseases, and low yields, a hybridization programme was initiated in 1991-92. The objective was to improve the yield potential and adaptability, and to increase the nutritional quality by transferring the low-neurotoxin character to locally adapted germplasm originating from grasspea-producing countries, such as Bangladesh, Ethiopia, and Pakistan.

The ICARDA breeding programme has made significant progress in selecting low-neurotoxin, high-yielding lines that can be locally adapted. This work is carried out by a multidisciplinary team involving the breeder, pathologist, entomologist, biotechnologist, and animal nutritionist. The major avenue of dissemination for the elite lines and segregated populations developed by the breeding programme for selection under target environments is through the ICARDA International Legume Nursery Program.

Research activities with the Ethiopian National Program commenced in the 1998-99 growing season. ICARDA supplied the Ethiopian National Program with 100 improved lines of *L. sativus*. These lines were planted at Holetta Research Station for quarantine in 1997-98. In 1998-99, these lines were tested at two locations, Inewari and Molale. Eight high-yielding lines were selected at Inewari, and 14 lines with high yield and cold tolerance were selected at Molale. Nine lines were also selected with a β-ODAP content 40% less than the local checks (from Adet, Ginchi, Inewari and Molale). ICARDA also supplied the Ethiopian National Program with 120 samples of segregated populations of crosses between Ethiopian landraces and ICARDA's low β-ODAP lines for selection under Ethiopian conditions.

In 1999 the UK/Consultative Group on International Agricultural Research (CIGAR) Competitive Research Facility (CRF) of the Department for International Development (DFID) funded ICARDA to implement a project with the Ethiopian Agricultural Research Organization (EARO) on “Improving Yield Potential and Quality of Grasspea (L. sativus): a Dependable Sources of Protein for Subsistence Farmers in Ethiopia”. The project's goals are to alleviate malnutrition, reduce shortages of dietary protein, and increase food quality and quantity for rural subsistence farm households in Ethiopia. This project promotes grasspea as a safe source of dietary protein, thereby removing the stigma of neurolathyrism associated with this hardy and promising crop.

Through this project ICARDA supplies National Programs (NARs) with segregated populations for selection of lines with reduced concentrations of β-ODAP, combined with disease and insect resistance.

**Exploitation of somaclonal variation**
Biotechnological methods are being applied to develop toxin-free lines of *L. sativus*. Recently, exploitation of somaclonal variation from landraces of Ethiopia and Pakistan has helped in isolating some somaclones different in various characters, such as flower colour, leaf size, seed colour, pod length, and number of seeds per pod. Somaclones with a low β-ODAP content (less than 0.1%) have been developed. These somaclones are being tested in different environments to study the stability of the neurotoxin content in the ripe seeds.

**Soil micronutrients: Zn$^{2+}$ and Fe$^{2+}$**
The neurotoxin of *L. sativus* is hypothesized to function as a carrier molecule for zinc ions (11). Soils that have been depleted in micronutrients from flooding by monsoon rains (Indian subcontinent) or that are otherwise poor in available zinc and with high iron content (Ethiopian vertisols) may be responsible for the high level of neurotoxin in ripe seeds and subsequently for the high incidence of human lathyrism. This may explain why landraces originating from Bangladesh, Ethiopia, India, Nepal, and Pakistan have a higher β-ODAP content than those from North Africa, Turkey, Syria, and Cyprus.
Zinc deficiency in the soil is an agronomic problem in Bangladesh, especially in the monsoon-washed soils where grasspea is grown during the dry winter. Zinc deficiency in humans is also a widespread phenomenon in Bangladesh and Ethiopia, leading to a number of symptoms such as loss of hair, nail deformation, diarrhoea, and mental retardation (15). ICARDA, through the DFID project in collaboration with EARO, is placing more emphasis on the soil and other environmental conditions that can lead to a solution for neurolathyrism. More balanced fertilization of the soil may reduce toxin-increasing stress factors for the plants and at the same time increase productivity.

Conclusions
The Grasspea Improvement Program at ICARDA will continue to develop high yielding, adapted lines containing very little or none of the neurotoxin β-ODAP. The feasibility of introgression of the low-neurotoxin character from other closely related species such as underground chickling (Lathyrus ciliolatus L.) will also be addressed. Our attempts to apply plant biotechnological methods to develop toxin-free lines of grasspea will continue. Analysis of somaclonal variation in landraces from Bangladesh, Ethiopia, and Pakistan will be continued in order to identify new genotypes with zero or near-zero levels of neurotoxin. Development of low-or zero-neurotoxin lines will lead to grasspea consumption at a safe level and remove the stigma of neurolathyrism from this hardy crop.

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References
