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Lathyrus

A New Grain Legume

A report for the Rural Industries Research and Development Corporation

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Foreword

This project formed part of an integrated program with GRDC and ICARDA to introduce *Lathyrus* and *Vicia* to the wheat belt of Western Australia.

Australian agriculture has drawn heavily on germplasm from the Mediterranean basin. For example, many farming systems are based on subterranean clover, the annual medics and lupins, all of which come from the region. Subterranean clover and lupins are well adapted to sandy soils of low pH and the medics to fine textured soils of high pH. However, we recognise that there is a need for pulses in particular, for fine textured soils of low to high pH. It is this niche to which *Lathyrus* seems well adapted.

Lathyrus contains neurotoxins that severely affect humans and non ruminant animals. For humans the problem occurs in developing countries in times of famine where *Lathyrus* is often one of the few food sources available.

ICARDA is breeding *Lathyrus* and appears to have identified low neurotoxin germplasm in at least one species. This has been confirmed in Australia where one line of *L. cicera* and two lines of *L. sativus* have been identified as high in yield and low in the neurotoxin ODAP (see later). These lines have been released or will be released shortly.

The project also examined the effect of environment on neurotoxin level in both field and controlled environments.

Researchers have now developed early maturing, high yielding germplasm that has low ODAP concentrations under most conditions. It is hoped that the new cultivars will have a potentially large domestic market as a high protein feed, and a potential human food export market of up to 500,000 tonnes.

This report, a new addition to RIRDC’s diverse range of over 450 research publications, forms part of our New Plant Products R&D Program, which aims to foster the development of new industries based on plants or plant products that have commercial potential for Australia.

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Peter Core
Managing Director
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Executive Summary

Lathyrus sativus and *L. cicera,* commonly referred to as the grasspea and chickling respectively, are valuable pulse crops for arid conditions and have become survival crops in areas subject to drought and famine, producing reliable yields when all other crops fail. However, they contain a water soluble neurotoxin called β-N-oxalyl-L-α-β-diaminopropionic acid (ODAP), which produces a permanent paralysis of the lower limbs, referred to as lathyrism, if consumed as a major part of the diet over a prolonged period of time.

The drought resistance of these species, together with their ability to grow on the fine textured, neutral to alkaline soils unsuited to the narrow-leafed lupin (*Lupinus angustifolius*), makes them attractive alternatives for large parts of the southern and south-western Australian cereal belt. Despite the work being done to genetically produce accessions low in ODAP, the environment also has a marked influence on neurotoxin levels and must be taken into account with the development of such accessions. The first part of this project was undertaken with the objectives of investigating certain environmental parameters on seed ODAP concentration and looking at the possible causative mechanisms. It formed part of the thesis of Mr Steven Herwig.

Sowing date was found to have a marked influence on ODAP concentration, with levels dropping as the season progressed. Significant environmental effects were also noted with three different *Lathyrus* species grown at three different sites in the West Australian wheatbelt. The greatest influence on neurotoxin levels, however, resulted from water stress. While such a stress during pre-anthesis growth did not lead to an increase in ODAP concentration, this was not the case with a post-anthesis water stress. Significant increases were noted (a doubling in one case), with the stress applied at early post-anthesis the most vulnerable stage.

What also became evident as the thesis progressed was a negative relationship between ODAP concentration and the total amount of ODAP in the plant (total seed weight x ODAP concentration). It appeared that any treatment, or stress, that resulted in an increase in ODAP concentration in the seed of the grasspea also caused a reduction in total ODAP. This led to the supposition that the effect of environment on neurotoxin levels in the seed was a secondary one. The primary effect of the environment was on the growth of the plant, which determined the number of pods, number of seeds and seed weight produced. This put either an increased or decreased demand on a finite pool of ODAP precursors, or on the process producing the toxin and translocating it to the developing seed. In short, under good conditions the plant produces more pods and seeds than it would under poor conditions and the reduced ODAP concentrations obtained as result of this are caused by toxin dilution.

Were this the case it would theoretically be possible to alter the ODAP concentration of plants grown under similar environmentally stress free conditions by controlling the number of pods, and subsequently the seed number and weight, developing on the plants. Such an experiment was undertaken and it was found that under identical unstressed conditions plants with only 10 pods produced 59% more ODAP than plants with 100 pods.

However, the possible influence of another, unknown factor cannot be discounted because ODAP/seed was found to vary across the five podding density treatments, being higher at the lower densities. If the ODAP concentration in grasspea seed was simply the result of toxin dilution this factor should not vary. Since ODAP/seed was not influenced by the stresses imposed in the other experiments it would be worthwhile repeating the podding density experiment, but with a larger number of replications and genotypes.

In conclusion, the hypothesis that environmental conditions would influence the resultant ODAP concentration in grasspea seed was confirmed. The ODAP concentration was found to increase with an increase in both sowing date and water stress. Accessions were also found to react differently to the applied environmental conditions.

In 1994, 407 *L. sativus* and 96 *L. cicera* lines were evaluated for phenology, seed yield and seed ODAP (a neurotoxin) concentration in the Mediterranean-type climate of south-western Australia. A selected number of lines from this study were grown at three sites in south-western Australia in 1995 and 1996, to examine genotypeenvironment interactions on seed yield and ODAP concentration in the seed of the two *Lathyrus* spp.

Principal components analysis showed that *L. sativus* lines grown in 1995 and 1996 could be divided into two geographical origins: Indian sub-continent and Mediterranean/European. Those lines of Mediterranean/European origin were consistently higher yielding (both in 1994 and 1995/96), with much larger seeds and later phenology. In *L. cicera* seed yield was closely associated with greater mean seed weight and to a lesser extent with early phenology. The genotype-environment study indicated that higher yielding lines of both species generally responded to favourable environments; in the case of *L. sativus* the Mediterranean/European lines and for L. *cicera* the larger seeded, earlier phenology lines.

For both species, genotype was the most important determinant of ODAP concentration and environment had less influence. Genotype-environment interactions had no effect on seed ODAP concentrations. In terms of seed yield, both species showed substantial potential in the 25 environments tested.

Further improvements in adaptation, seed yield and reduction in ODAP are possible in both species.

A new cultivar of *Lathyrus cicera* has been released. Cultivar Chalus is likely to be competitive with Dundale peas in areas where black spot is prevalent and in areas too dry for Dundale.

Introduction

The grasspea (*Lathyrus sativus*) is related to both the chickling (*L. cicera)* and cyprus vetches (*L. ochrus*). It is not only the most agronomically important species in the *Lathyrus* genus but also the only member of the genus widely cultivated as a food crop (Jackson and Yunus, 1984). This project deals with both the grasspea and the chickling vetch.

Although its actual origin is unknown, both the grasspea and chickling vetch are believed to be natives of southern Europe and south-west Asia (Duke *et al*. 1981). The earliest known remains were discovered in India (4000-3500 BP) and West Asia (3800-3200 BP) (Saraswat 1980; Allchin 1969). Kislev (1989) postulated that the grasspea has been used as a human food since early Neolithic times and was first cultivated in the Balkan Peninsula around 6000 BP.

The forms with blue flowers and small, speckled seeds are believed to be the most primitive (Jackson and Yunus 1984; Vavilov 1951). Such forms are found in southern and south-western Asia, whereas those discovered around the Mediterranean region possess large white seed and flowers (Vavilov 1951). This suggests that Asia, along with Abyssina (a possible second centre of origin) are the origins of cultivated grasspeas and chickling vetches.

Also known as chickling vetch, khesari, sabberi or common chickling, the grasspea is a prostrate quick maturing legume grown as a food for man and both food and forage for domesticated animals. It is extremely drought tolerant, capable of yielding well even under the most adverse conditions and well adapted to cool season (winter) production in warm temperate and sub-tropical areas (Mediterranean basin, Bangladesh, India, and Pakistan). However its natural distribution is primarily centred in the eastern Mediterranean region.

The production of the grasspea has become very widespread and species are now cultivated throughout the Canary Islands, Germany, West Asia, Nepal, China, the Middle East (Iraq, Iran, Afghanistan, Syria and Lebanon), and Northern Africa (Ethiopia, Egypt, Morocco, Algeria and Libya). There is also some minor cultivation in southern Europe (France and Spain) and South America (Chile and Brazil) (Kislev 1989; Campbell *et al*. 1994).

It is widely grown because it has a high yield potential, requires very little attention during its growth cycle and can withstand both drought conditions and waterlogging. As well as this the grasspea is an excellent source of fodder, requires little fertilisation, its cost of production is low compared to other food crops and its seed is highly palatable (Kaul *et al*. 1986; Quader *et al*. 1986; Negere and Mariam 1989; Rahman *et al*. 1991). It could also be used as an alternative to fallow and in the production of high quality hay (Osman and Nersoyan 1986).

Pod shattering is much less of a problem with the grasspea than it is with either the dwarf chickling or cyprus vetch. It is sensitive to cold and its prostrate growth habit, common to members of the *Lathyrus* species, can cause harvesting problems. It suffers to some extent from botrytis and ascochyta blights, root-knot and cyst nematode attack and to powdery and downy mildews, but to a lesser extent than the dwarf chickling or cyprus vetch.

It is a very important crop in Bangladesh both in terms of a grain for human consumption and a forage source for cattle in winter. Farmers generally broadcast the seed into rice paddies prior to harvesting the rice and the grasspea is left to grow in the stubble. Cattle are grazed in the standing crop on one or two occasions before the third growth is harvested for seed and hay. Its tolerance to waterlogging during early growth, often extreme or prolonged drought at maturity and excellent forage for cattle is responsible for its great value to such farmers.

In Ethiopia farmers usually plough once or twice to help control weeds, then broadcast the seed into the field without any other cultivation. On some occasions the grasspea is intercropped with chickpea and barley or grown in rotation with tef (*Agrostis tef*). As in Bangladesh the grain is used for human consumption and the straw as animal feed. It is successful because it is tolerant to waterlogging and suffers little from insect damage. Common to the above methods of cultivating the grasspea is the ease of production. Little, if any, fertilisers are used and it does not require much in the way of management practices or pest control to produce a crop. Its tolerance to both biotic and abiotic stresses also make it a considerably more reliable crop than others that can grow in similar locations.

It has a hardy, penetrating root system which allows it to grow on a wide range of soil types such as very poor soils and heavy clays (Campbell *et al*. 1994). However, it is sensitive to acid soils below pH 5.5 (Duke *et al*. 1981). Together with its ability to fix atmospheric nitrogen, rapid growth potential and pest resistance it is obvious why the grasspea has become such a valuable plant. The wide range of ecological tolerance exhibited by the grasspea allows it to be grown in areas where few, if any, alternative grain legumes can be reliably grown.

The grasspea shows promise in the low-rainfall regions of the southern and south-western Australian cereal belt due to possessing a high degree of drought tolerance and its ability to grow well on the fine textured, neutral to alkaline soils occupying some 6.5 million hectares of the cereal belt that are unsuited to the growth of the narrow-leafed lupin. Another major advantage it has over current grain legume varieties is that it is very resistant to both abiotic and biotic stress. For instance, it does not suffer from black spot and can, therefore, grow in areas where farmers are no longer able to grow the field pea. It is also resistant to foliar diseases, broomrape (*Orobanche crenata*) parasitism and does not suffer greatly from insect attack.

The grasspea is highly palatable, a good source of protein (26-30%) and richer in iron and B-vitamins than other grain legumes. It is also low in fat, rich in lysine and contains adequate quantities of amino acids other than cystine and methionine. However, supplementation of vitamins A and C would be required for a complete diet (Spencer *et al*. 1986). Although very little research has been undertaken in the western world or, for that matter anywhere else, a breeding program was initiated in Manitoba, where the grasspea is expected to become a valuable source of animal feed on the Canadian prairies. Under good conditions test plots have yielded the equivalent of 5232 kg/ha of seed (Briggs *et al.* 1983).

Early research in Western Australia indicated good adaptation to medium rainfall environments (400 mm), with the 17 grasspea accessions tested yielding an average of 0.9 t/ha (Davies *et al.* 1993). In 1993 a trial yielded 1.6 t/ha at the best of the three sites evaluated. Across all sites yields averaged 60% of the field pea, used as a control because it is considered adapted to local conditions (Siddique *et al*. 1996).

Sixteen accessions of the grasspea were selected from 451 accessions grown in 1994 and evaluated at three sites, nine of which yielded better than the field pea (Hanbury *et al*. 1995).

In a species comparison trial at 12 sites in 1995 the yield of the grasspea variety SEL534 averaged 1.1 t/ha, with yields in excess of 2.0 t/ha measured at two sites.

Four accessions obtained from the Indian Agricultural Research Institute with very low ODAP concentrations (0.02-0.03%) were introduced into Western Australia in 1995, together with four accessions from Pakistan (0.13-0.20% ODAP) and grown out at three sites in 1996. Of these, a somoclonal accession from India (BIO L254) and a Canadian accession (LS 90278) are still being evaluated. Results to date suggest that the grasspea is better adapted to medium rainfall areas than to low rainfall ones (Hanbury and Siddique 1997).

The genus *Vicieae*, to which the grasspea belongs, contains about 42 non-protein amino acids. Some of these are toxic to mammals and fowls, causing neurological symptoms or skeletal deformations (Lambein *et al*. 1990). The compounds responsible for this toxicity are all naturally occurring water soluble chemicals known as isoxazolinones. They are major secondary metabolites and can constitute more than one per cent of the dry weight of the decotyledonized seedlings (Lambein *et al*. 1976). Although the seedlings are generally the most toxic stage, isoxazolinones can also be present in high concentrations in the seeds (Ressler 1975). The toxicity of some of these compounds represents the major stumbling block in the use of the grasspea as a food for both man and domestic animals.

Non-protein amino acids

The isoxazolinones found in the grasspea all contain a five membered isoxazolin-5-one ring that is very sensitive to both alkali and UV-irradiation. These compounds are as follows:

(I) β-(isoxazolin-5-on-2-γl)-alanine (BIA)

(VI) 2-(3-amino-3-carboxy-propyl)-isoxazolin-5-one

(VIII) 2-cyanoethyl-isoxazolin-5-one

(XI) γ-glutamyl derivative of (I)

(BAPN) β-aminopropionitrile

The majority of isoxazolinones are given names in roman numerals. This is a preference to avoid the introduction of new trivial names (Lambein *et al*. 1992)

2. Impact of stress on neurotoxins

Although considerable work has been undertaken into determining how ODAP causes lathyrism and whether its concentration is affected by changing environmental conditions (MacDonald *et al.* 1986; Ross and Spencer 1989), little work has been done on either the function of ODAP or why it varies across different sites, environments and seasons.

Many plants react to water stress by increasing the concentration of amino acids, and Lambein *et al.* (1990) postulated that since ODAP is an amino acid it may have a role in the drought tolerance that has made the grasspea such a useful species over the long period of its domestication. As such, investigating the effect of water stress is an ideal first step in looking at how certain elements of the environment can affect ODAP levels in grasspea seeds.

The effect of water stress on plant growth and yield has been investigated by many researchers. For instance, Pathak *et al.* (1988) noted that moisture stress resulted in a reduction in pod yield and other components such as flowering percentage and 100 pod weight on the ground nut. Water stress resulted in a decrease in turgor pressure in the leaves of the soybean and black gram, the reduction increasing with an increase in water stress (Morgan 1992).

Two experiments, conducted in glasshouses at the University of Western Australia in 1994 and 1995, were set up to investigate the effect of both pre- and post-anthesis water stress on ODAP concentration, total plant ODAP, ODAP/seed, seed yield and seed yield components of the grasspea. One aim of the first experiment was to examine the effect of different post-anthesis water stress regimes on the growth, yield and yield parameters of three grasspea accessions with markedly different ODAP concentrations. The main aim of this experiment, however, was to determine if such water stresses would result in an increase in the ODAP concentration in the seed and, if so, to examine the possible explanations.

The second experiment, conducted the year after the first, was run to examine whether the timing of a post-anthesis water stress was important in determining the subsequent ODAP concentration. At the same time, given that a post-anthesis water stress resulted in an increase in ODAP concentration, an experiment was run to examine whether a pre-anthesis water stress would result in a similar increase in seed ODAP concentrations.

Experiment 1: effect of post-anthesis water stress on ODAP concentration of three Lathyrus sativus accessions

Materials and methods

This experiment, and a preliminary one to determine methodology, were conducted in a glasshouse in 1994. Three grasspea accessions (526 and 80392 from Ethiopia and 455 from Germany) were obtained from ICARDA, inoculated with a commercial Group E rhizobium and germinated in jiffy pots. The accessions were chosen because they had previously exhibited ODAP concentrations ranging from moderately low to high (SEL 455 = 2.87 g/kg, SEL 526 = 4.18 g/kg, 80092 = 6.72 g/kg).

Once the plants had reached the three leaf stage they were transplanted into free draining pots made from Vinidex sewer pipe (45 cm long, 150 mm diameter) containing 7 kg of a standard potting mix. Clear plastic beads (100 g) were spread over the soil surface to reduce evaporation. The seedlings were planted three to a pot, then thinned to one representative plant once they were well established. A wire mesh support was placed around each plant to encourage it to grow in an upright fashion and to make it easier to work within a glasshouse environment. Plants were watered to field capacity (until the water drained freely from the base of the pots) every two days until 50% of the plants in each genotype had begun to flower, at which stage the stress levels were applied.

A randomised block design with four replicates was used in this study. Two stress levels were applied, mild (S_1) and severe (S_2), together with an unstressed control (S_0). The stress levels were monitored with a photosynthesis meter and a pressure bomb and were as follows:

S_0. Unstressed control (plants were watered every two days for the duration of the experiment).

S_1. Mild stress (a 30% reduction in photosynthesis, when compared to the unstressed control, and a leaf water potential of -0.8 to -1.4 Mpa)

S_2. Severe stress (a 60% reduction in photosynthesis and a leaf water potential of -1.8 to -1.9 MPa).

The leaf water potential was measured on the upper expanded leaves on sunny days between 10.30 a.m. and 2.30 p.m. using the pressure chamber technique (Scholander *et al*. 1965), with the precautions recommended by Turner (1988). Net photosynthesis rate was measured on similar leaves of the plants, and at the same time, with a portable, open gas exchange system (Model LCA3, ADC, Hoddesdon, UK). The different stresses were applied by watering the pots to field capacity and frequently measuring the photosynthesis and leaf water potential as the soil dried out. When the prescribed levels were reached the pots were re-watered to field capacity and the process repeated.

At maturity the pods were hand harvested and divided into two groups, those found at the top of the plant and those found at the bottom. The number of pods, seeds/pod, seed weight/plant, average seed size and plant dry weights were measured, and the ODAP concentration of the seeds measured using the Capillary Zone Electrophoresis method.

Results

Fig. 1 shows the rate of photosynthesis until 25 November for the three grasspea accessions at the three water stress levels, and Fig. 2 shows the mean leaf water potential until 2 December, the results of both indicating that the stress levels were successfully achieved.

Leaf water potentials were maintained at -0.3 to -0.5 MPa at zero stress, reached -0.9 to -1.1 MPa at mild stress, and -1.2 to -1.5 MPa at severe stress. Similarly, the rate of photosynthesis was 12-16 μmol $CO_2/m^2/s$ at zero stress, dropped to 9-13 μmol $CO_2/m^2/s$ at mild stress, and 2-5 μmol $CO_2/m^2/s$ at severe stress. Note that the photosynthesis rate fell with time, even at zero stress.

After four to five weeks the plants began to mature and their leaves started to senesce, causing both stress indicators to decrease. This made it difficult to distinguish between treatments so the stress levels were continued from this point until the end of the experiment on 23 December by maintaining the same watering frequencies as were used between 18 November and 2 December. At each marked spot in Figure 1 the plants were re-watered and allowed to dry naturally until their predetermined stress levels were reached. It should be noted that the applied stresses were measured arbitrarily, not absolutely. For instance, the severe stress was not twice that of the mild one. However, the results indicate that three different stress levels were achieved (Table 1).

			Leaf-water potential			**Photosynthesis**	
	SEL 455						
	Date	Mild	Severe		Date	Mild	Severe
	28-Oct	650	650		28-Oct	19.4	19.4
	4-Nov	840	840		4-Nov	11.7	11.7
	11-Nov	1050	1160		11-Nov		5.8
	18-Nov	890	1415		18-Nov	5.3	
	25-Nov	1000	1000		25-Nov	6.2	0.1
	30962						

Figure 1. Mean photosynthesis rates (mmol $CO_2/m^2/s$) of three grasspea accessions under mild (—) and severe (- -) stress regimes.

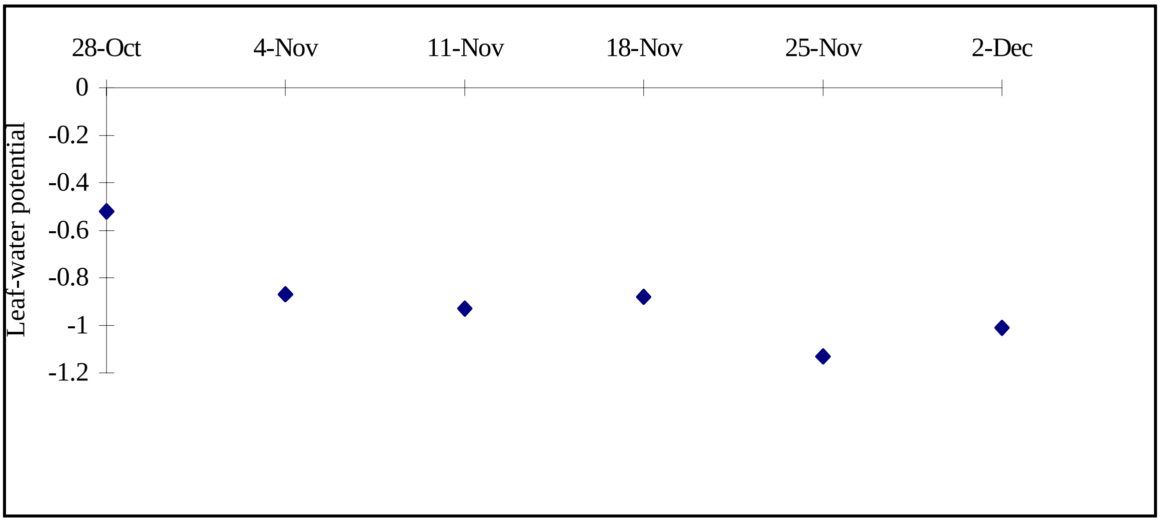

Figure 2. Mean leaf water potentials (Mpa) of three grasspea accessions under mild (—) and severe (- -) stress regimes.

The ODAP concentration for all three accessions was found to vary significantly with an increasing water stress. In all cases the severely stressed plants had significantly higher ODAP concentrations ($P<0.01$) than the unstressed controls (Table 1). For example, the ODAP concentration of selection 455 varied from 1.50-3.12 g/kg for the unstressed and severely stressed plants respectively. Accessions 455 and 80092 also showed a difference between both the unstressed and mildly stressed plants, and the mildly and severely stressed ones. There were also significant differences between accessions, with 80092 having the highest ODAP concentration overall. No accession x stress interaction was observed).

Table 1. Means of ODAP concentration (g/kg) for the three grasspea accessions grown under three water stress regimes.

(Unstressed control (S_0), mild stress (S_1), severe stress (S_2))

Stress level	SEL 455	80092	SEL 526	Mean
S_0	1.50	2.45	1.76	1.90
S_1	2.10	3.03	1.98	2.37
S_2	3.12	3.77	2.63	3.17
Mean	2.24	3.09	2.13	2.48
L.S.D	0.50	0.50	0.64	0.36

Table 2 shows how total plant ODAP was affected by water stress. Acc. 80092 showed no significant differences, while acc. 526 only showed a difference between the zero and severe stresses. Acc. 455 was the only variety that showed significant differences between all three stresses. Analysis of variance for total ODAP showed that not only were there significant differences between stress and accessions, there were also significant accession x environment interactions. The means of ODAP/seed (Table 3) indicate no significant difference exists for all three accessions at the zero and mild stress, and although the severe stress of all accessions other than acc. 80092 was higher than the first two stresses, an analysis of variance shows that overall there was no significant effect of stress on ODAP/seed. A small, although significant, accession difference was noted with 80092 being higher than the other accessions.

Table 2. Means of total ODAP (g) for the three grasspea accessions grown under the stress regimes.

(Unstressed control (S_0), mild stress (S_1), severe stress (S_2))

Stress level	SEL 455	80092	SEL 526	Mean
S_0	70.13	30.14	103.76	68.01
S_1	33.46	24.50	46.61	34.86
S_2	20.36	21.98	29.28	23.87
Mean	41.32	25.54	59.88	42.25
L.S.D	8.87	10.30	17.86	10.25

Seed yield, seed yield components and plant dry weights were influenced by the imposed water stresses (Table 4). An increase in stress led mainly to a decrease in yield, expressed through a decrease in pod number. Plant dry weight was reduced for accessions 455 and 526 ($p<0.01$). Mean seed weight was affected to a lesser extent for accession 455 ($p<0.05$). Seeds/pod were unaffected by the stresses for all accessions.

Correlation coefficients (Table 4) indicate high stress is associated with a reduction in seed and pod number, which in turn reduces seed weight (yield). ODAP concentration increases with an increase in stress, while total ODAP, seeds/pod and plant dry weight all decrease. ODAP/seed and average seed size were not significantly influenced by water stress. Correlations in Table 6 indicate seed yield depends mainly on pod and seed number. There was also a significant positive correlation between seed yield and average seed size, although seeds/pod was not correlated with yield.

Table 3. Means of ODAP/seed (mg) for the three grasspea selections grown under three stress regimes.

(Unstressed control (S_0), mild stress (S_1), severe stress (S_2))

Stress level	SEL 455	80092	SEL 526	Mean
S_0	0.39	12.10	0.60	4.36
S_1	1.59	3.89	2.02	2.50
S_2	4.83	6.83	7.97	6.54
Mean	2.27	7.61	3.53	4.47
L.S.D	1.23	12.57	4.04	4.45

** Significant at P<0.01.

Table 5. Correlation coefficients between stress and selected plant characters.

Character	Correlation
Pod number	-0.67 **
Seed number	-0.70 **
Seed weight	-0.66 **
Average seed size	-0.06 n.s.
Seeds / pod	-0.25 **
Plant dry weight	-0.38 **
ODAP concentration	0.51 **
ODAP total	-0.57 **
ODAP / seed	0.09 n.s.

** Significant at P<0.01, n.s. not significant at P<0.05.

Table 4. Means of plant dry weights, seed yield and selected seed yield components of the three grasspea accessions grown under three water stress regimes.

(Unstressed control (S_0), mild stress (S_1), severe stress (S_2))

Character	S_0	S_1	S_2	Mean	L.S.D
SEL 455					
Seed yield (g/plant)	48.13	15.91	6.93	23.66	4.42
Pod number	153.63	55.19	32.63	80.48	14.63
Seed number	420	144	79	214	42
Seeds/pod	2.74	2.66	2.39	2.60	0.31
Av. seed size (mg)	120	120	90	110	23
Plant dry weight (g)	30.51	13.53	9.85	17.97	3.37
Acc. 80092					
Seed yield	13.84	8.37	5.85	9.26	4.80
Pod number	42.13	31.13	23.88	32.17	13.89
Seed number	128	88	60	92	41
Seeds/pod	2.82	2.83	2.54	2.73	0.36
Average seed size	100	100	100	100	23
Plant dry weight	9.11	7.64	6.68	7.78	3.29
SEL 526					
Seed yield	62.70	25.93	11.73	32.83	8.61
Pod number	135.60	60.00	30.75	74.17	16.64
Seed number	333	142	63	179	46
Seeds/pod	2.48	2.36	2.10	2.31	0.42
Average seed size	190	190	190	190	23
Plant dry weight	58.93	35.52	29.14	40.05	10.27

Table 6. Correlation coefficients between seed yield (g/plant) and seed yield components.

Character	Correlation
Pod number	0.91 **
Seed number	0.89 **
Seeds / pod	0.13 n.s.
Average seed size	0.44 **

** Significant at P<0.01, n.s. not significant at P<0.05.

Experiment 2: pre-anthesis and two post-anthesis water stress effects on ODAP concentration in seeds of **Lathyrus sativus**

Materials and methods

The *L. sativus* accession 455 used in Experiment 1 was selected for this experiment because of its growth habit. Not only did it grow and yield well, it was easy to work with in a glasshouse environment as it not produce an excessive amount of vegetative material. Other than the seed being directly sown into the pots the general methodology of this experiment, conducted in 1995, was the same as that used in the previous experiment up until the time the seedlings were well established.

A randomised block design with four replicates was used. Five water stresses were applied:

S_0. Unstressed control

S_1. Pre-anthesis water stress

S_2. Early post-anthesis water stress

S_3. Late post-anthesis water stress

S_4. Stressed from anthesis to maturity

The unstressed controls were watered every two days for the duration of the experiment whereas the plants stressed from anthesis to maturity were put under the severe stress determined in the previous experiment from the time the plants had reached the 50% flowering stage up until the time the plants had matured and their pods collected. A severe pre-anthesis water stress was applied once the plants had reached the four leaf stage and were growing well. It continued until the plants had reached the 50% flowering stage, at which time the plants were watered at the frequency used for the unstressed controls.

Immediately following 50% flowering the early post-anthesis water stress plants were put under a severe stress, which was discontinued after a period of six weeks, at which time the pods were either just beginning to form or were incompletely filled. A severe stress was then placed on the late post-anthesis plants and continued until the end of the season.

All fully open flowers and developing pods were tagged on both the early and late post-anthesis stress plants just prior to the latter stress being applied. This was to ensure only the pods developing under each stress would be included in the statistical analyses and ODAP measurement.

At maturity the pods were collected, but while those from the control (S_0), the plants stressed from anthesis to maturity (S_4) and the pre-anthesis water stress plants (S_1) were bulked, those from the post-anthesis stresses were divided into two groups, tagged and untagged. Pod number, seeds/pod, seed weight/plant, mean seed weight and plant dry weights were then calculated and the ODAP concentration of the seeds chemically determined using the Capillary Zone Electrophoresis method.

Results

The mean ODAP concentrations measured for the five water stresses are presented in Table 7. The analysis of variance indicates the differences noted in the first table were significant.

The plants placed under a pre-anthesis water stress (S_1) did not show any significant increase in ODAP concentration when compared to the unstressed controls.

The plants stressed from anthesis to maturity (S_4) and the early post-anthesis water stressed plants (S_2) had significantly higher ODAP concentrations than the unstressed controls (S_0) ($p<0.01$). The first figure in Table 9 for the late post-anthesis stress (S_3) indicates the ODAP concentration of the seeds that developed prior to the water stress being applied. As such the plants were still being watered at the same frequency as the unstressed controls and their ODAP concentrations did not differ significantly. The second figure, in parentheses, is the ODAP concentration from the seed that developed after the water stress was applied. It was higher, although not significantly so, than that of the unstressed controls.

The figure in brackets for the early post-anthesis water stress (S_2) is the ODAP concentration in the seed that developed after the stress was removed. It continued to increase, although not significantly so. Given that the ODAP concentration was higher than for the other stress regimes it appears a water stress applied immediately following the onset of flowering is the most critical stage in determining the final ODAP concentration in the seed.

The means of dry weights, yield and yield components for the plants placed under the five water stresses are shown in Table 8. The pre-anthesis stressed plants were not found to be significantly different than the unstressed controls in any of the measured parameters. However, at the time the stress was removed (when 50% of the plants had at least one fully open flower) the plants were visibly smaller than the unstressed controls and flowered earlier than the other treatments. Growth of these plants was very rapid following the removal of the stress and by the time they were ready to be harvested they were similar to the unstressed controls in all growth parameters.

Table 8 shows that the plants stressed from anthesis to maturity had a significantly lower seed yield than all treatments other than the two post-anthesis water stresses, and a lower pod number than all except the late post-anthesis water stress ($p<0.01$). The plants under this stress had fewer seeds/pod than for the pre-anthesis water stress, and smaller seed than the fully stressed control plants. Plant dry weight was lowest for the plants stressed from anthesis to maturity, although the early post-anthesis stressed plants were not significantly different.

Table 7. Mean ODAP concentrations (g/kg) measured under the five water stresses regimes.

(Unstressed controls (S_0), pre-anthesis stress (S_1), early post-anthesis stress (S_2), late post-anthesis stress (S_3), stressed from anthesis to maturity (S_4)).

Treatment	ODAP concentration (g/kg)
S_0	2.02
S_1	2.05
S_2	3.27 (3.53) [A]
S_3	2.16 (2.38) [B]
S_4	2.84
L.S.D	0.74

[A] Value in brackets indicates ODAP concentration found in the seeds of the pods that developed after the water stress was removed.

[B] Value in brackets indicates ODAP concentration found in the seeds of the pods that developed after the water stress was applied.

Table 8. Means of seed yield (g/plant), pod number, seeds/pod, av. seed size (mg) and plant dry weight (g) for the five stresses

(S_0 = unstressed controls; S_1 = pre-anthesis stress; S_2 = early post-anthesis stress; S_3 = late post-anthesis stress, S_4 = stressed from anthesis to maturity).

Character	S_0	S_1	S_2 *	S_3*	S_4	Average	L.S.D
Seed yield	48.27	46.06	18.64	11.56	8.81	26.67	10.12
Pod no.	121.83	126.50	55.92	39.92	22.17	73.27	23.37
Seeds/pod	2.02	2.25	1.95	1.72	2.00	1.99	0.35
Seed size	178	166	170	144	201	172	37
Dry wt	55.36	50.53	34.05	42.80	23.65	41.28	14.91

* These figures include both the tagged and untagged proportions.

Discussion

Other than a preliminary study undertaken by Swarup and Lal (1993) this is probably the first project to investigate the effect of water stress on the concentration of ODAP in the seeds of the grasspea. It not only demonstrates that water stress can cause an increase in ODAP concentration but determines that the timing of the stress is critical. From the results obtained in both experiments it appears that ODAP concentration is affected by phenology as well as by both genetic control and environmental conditions. While a positive relationship was noted between total ODAP and plant dry weight, the negative relationship found between plant dry weight and ODAP concentration indicates the control of ODAP concentration by environment is not direct.

In the first experiment, in which the effects of three levels of post-anthesis water stress were compared, it was observed that the ODAP concentration was increased in the accessions in which mean seed weight was reduced by stress. An increased post-anthesis stress resulted in a decreased yield, mainly caused by a decrease in the pod number per plant. This agrees with related studies that demonstrated a close relationship between number of pods per plant and seed yield. For example, Kaul *et al.* (1986) noted in an experiment with 127 grasspea accessions that, of the characters he measured, pods/plant was the most highly correlated with grain yield (0.94). The number of pods/plant and plant height were found to be the main yield determinants in another experiment with the grasspea (Shaikh 1989). Similar results to these were found by Filipetti (1990) with a collection of chickpeas and by Rao and Suryawanshi (1988) with the mung bean (*Vigna mungo*).

The positive relationship noted between total ODAP and plant dry weight could be due to larger plants producing more pods and seeds, thereby causing an overall increase in the total ODAP in the plant. Correlations between plant dry weight and yield and yield components (results not presented) support this view. For example, the correlation between plant dry weight and total seed weight was 0.71 (significant at $P<0.01$). Conversely, the negative correlation found between plant dry weight and ODAP concentration is indicative of the extra seed produced by larger plants placing either an increased demand on a finite assimilate pool of ODAP precursors, or on the process producing the toxin and translocating it to the developing seed. Significant negative correlations between ODAP concentration and pod number, seed number and total seed weight are evidence of this (results not presented). Furthermore, the seed on the top half of the plant was found to have a lower ODAP concentrations than that on the bottom half (1.8 and 2.1 g/kg respectively for the unstressed plants). This helps support the view that a pool of assimilates is involved, since in the early stages of flowering, when fewer pods are forming on the plants, there is less of a drain on an assimilate pool and, therefore, the seeds develop a higher ODAP concentration.

Caution, however, is required as growing this normally trailing plant in an upright position with the use of wire supports may have had some influence on the ODAP concentration.

A water stress applied from the four leaf stage of development to the onset of flowering resulted in reduced growth and earlier flowering, but once the stress was removed the growth rapidly caught up to match that of the unstressed controls and no significant difference was noted in any of the measured growth parameters.

Both of the post-anthesis water stresses showed a seed yield and pod number reduction, and the early post-anthesis stress plants had a significantly lower dry weight than the unstressed controls. As with Experiment 1, seed yield was mainly controlled by pod number.

A post-anthesis water stress resulted in an increase in ODAP concentration, but the timing of the applied stress was critical. The application of such a stress immediately following flowering led to an ODAP concentration 62% higher than that of the unstressed controls, whereas a similar stress applied six weeks later led to no appreciable difference in ODAP concentration. Such a stress applied prior to flowering showed no significant difference in ODAP concentration when compared to the unstressed controls.

A significant negative correlation was noted between ODAP concentration and seed yield (results not presented). It agrees with the results of experiment 1 and although not proving conclusively the existence of a finite assimilate pool of ODAP precursors does provide evidence that the ODAP concentration is not affected directly by the environment.

ODAP concentrations have long been known to be influenced by both environmental and genetic factors (Dahiya 1986, Barat *et al*. 1989, Siddique *et al*. 1996), but the mechanism by which the environment acts is not clear. Lambein *et al*. (1990) suggested that ODAP is produced as a means of combating water stress. Our alternative explanation is that the lower concentration found in good environments is the result of toxin dilution, suggesting a restricted ‘pool’ of toxin is distributed over a larger number and weight of seed. Plant growth, leading to pod and seed production, is primarily affected by changing environmental conditions, which in turn affects the resultant ODAP concentration in the seed.

Most of the stressed plants in both experiments were found to contain more than the arbitrary safe level of 3 g/kg ODAP (Hanbury *et al*. 1995), while the control plants were considered by plant breeders to be safe. Such a result indicates, regardless of whether ODAP concentration is directly affected by the environment or not, breeding for low ODAP concentration in the seed of *L. sativus* needs to take environmental factors into consideration.

3. Genotype-environment interaction for seed yield and ODAP concentration of *Lathyrus sativus* L. and L. *cicera* L. in Mediterranean-type environments

The text for this component is attached as a paper in *Euphytica* (1999). The text has been faxed separately.

4. New Cultivar

Chalus *– a high yielding, high quality Lathyrus cicera variety for low and medium rainfall areas of Australia*

Chalus was selected by Colin Hanbury and Kadambot Siddique from the line IFLA 1279 supplied by the International Centre for Agricultural Research in the Dry Areas (ICARDA). It flowers 4-6 days earlier than Lath-BC, a variety released in South Australia, and about 20 days later than Dundale fie;ld pea. Chalus finishes flowering before Dundale and has rapid seed filling. Maturity is reached at approximately the same time as Dundale.

Chalus shows good tolerance to a wide range of herbicides, including Spinnaker, Diuron, 2,2 DPA, Broadstrike, Simazine, Brodal and Bladex for broad leafed weeds and a range of grass selective herbicides. Weed management packages will be developed shortly.

Bean yellow Mosaic Virus has been observed to infect Chalus but no other diseases have been recorded. Chalus is not susceptible to black spot of field pea and hence can be sown early. It can also be used as another crop legume to widen rotations where blackspot is a serious problem in field pea.

Chalus is a high yielding variety. It has consistently performed well over 15 trial sites across southern Australia, yielding on average 5% more than Lath-BC. At dry sites in Western Australia, its average yield has been equivalent to or greater than Dundale field pea.

ODAP concentration in the seed of Chalus is considerably lower (44%) than Lath-BC and average seed quality is slightly greater. Protein levels are good, around 27% which is slightly lower than narrow-leafed lupins but greater than field peas or faba bean. Its lysine content is high, 5.8 g/16 g nitrogen compared with 4.7 g/16 g nitrogen in narrow-leafed lupins. Fat and fibre contents are less than narrow-leafed lupins. The *In sacco* protein degradability of Chalus is 93%, approximately twice the maintenance level for cows. This compares with 94% for chickpeas, faba beans and field peas and 90% in narrow-leafed lupins. It has a quickly soluble fraction of 53% compared with 35% for narrow-leafed lupins. This suggests that Chalus is a source of readily available protein in the rumen and presumably the rest of the animal.

Apart from ODAP there are several other antinutritional factors in Chalus common to many grain legumes. These are generally in higher concentrations than narrow-leafed lupins.

There is no intellectual property in this cultivar because it is based on public germplasm from ICARDA.

5. Bibliography

Allchin, F.R. (1969). Early cultivated plants in India and Pakistan. *In 'The domestication and exploitation of plants and animals*'. (Eds. P.J. Ucko and G.W. Dimbleby). Duckworth, London.

Barat, G.K., Ghose, C. and Singh, J. (1989). Methods for the estimation of BOAA. In '*The Grasspea - Threat and Promise*'. Proceedings of the International Network for the Improvement of *Lathyrus sativus* and Eradication of Lathyrism. May 1988, London, U.K. (Ed. P.S. Spencer). pp. 122-127. (Third World Medical Research Foundation: New York and London).

Briggs, C.J., Parreno, N. and Campbell, C.G. (1983). Phytochemical assessment of *Lathyrus* species for the neurotoxic agent, β-N-oxalyl-L-α-β-diaminopropionic acid. *Planta Medica* **47**, 188-190.

Campbell, C.G., Mehra, R.B., Agrawal, S.K., Chen, Y.Z., Abd El Moneim, A.M., Khawaja, H.I.T., Yadov, C.R., Tay, J.U. and Araya, W.A. (1994). Current status and future strategy in breeding grasspea (*Lathyrus sativus*). *Euphytica* **73**, 167-175.

Dahiya, B.S. (1986). Genetics and stability analysis in grasspea (*L. sativus* L.). Its implications in future breeding programmes. In '*Lathyrus and Lathyrism*'. (Eds. A.K. Kaul and D. Combes). pp. 161-168. (Third World Medical Research Foundation, New York, USA). Proceedings of the International Symposium 9-13 September 1985, Pau, France.

Davies, C.L., Siddique, K.H.M. and Perry, M.W. (1993). Seed introduction and initial evaluation of alternative crop legume species. Grain Research and Development Corporation (W.A.), Final report, March 1993.

Duke, J.A., Reed, C.F. and Weder, J.K.P. (1981) *Lathyrus sativus* L. *In 'Handbook of legumes of world economic importance*'. (Ed. J.A. Duke). pp. 107-110. (Plenum Press, New York and London).

Filippetti, A. (1990). Variability of plant and seed characteristics in a collection of chick-pea (*Cicer arietinum* L.) *Legume Research*, **13 (1)**, 36-46.

Hanbury, C.D. and Siddique, K.H.M. (1997). Adapatation of *Lathyrus sativus* and *L. cicera* to Western Australian mediterranean-type environments. Co-operative Research Centre for Legumes in Mediterranean Agriculture, 1997, Occasional Publication No. 16.

Hanbury, C., Sarker, A., Siddique, K.H.M. and Perry, M.W. (1995). Genetic evaluation of *Lathyrus* species in a Mediterranean-type environment of Western Australia. *CLIMA Occasional Publication*, No. 8, p 23. (C2)

Jackson, M.T. and Yunus, G. (1984). Variation in the grass pea (*Lathyrus sativus* L.) and wild species. *Euphytica* **33**, 549-559.

Kaul, A.K., Islam, M.Q. and Hamid, A. (1986) Screening of *Lathyrus* germplasm of Bangladesh for BOAA content and some agronomic characters. In '*Lathyrus and Lathyrism*'. (Eds. A.K. Kaul and D. Combes). pp. 130-141. (Third World Medical Research Foundation, New York, USA). Proceedings of the International Symposium 9-13 September 1985, Pau, France.

Lambein, F., Kuo, Y.H., Ikegami, F. and Murakoshi, I. (1990). Toxic and non-toxic nonprotein amino acids in the *Vicieae*. *In 'Amino Acids, Chemistry, Biology and Medicine*'. (Eds. G. Lubec and Rosenthal). pp 21-28. (ESCOM Science Publication B.V.).

Lambein, F., Ongena, G. and Kuo, Y.H. (1990). β-isoxazolinone-alanine is involved in the biosynthesis of the neurotoxin, β-N-oxalyl-L-α,β-diaminopropionic acid. *Phytochemistry*, **29 (12)**, 3793-3796.

Lambein, F., Khan, J.K., Becu, C. and De Bruyn, A. (1992). Characterization of γ-Glutamyl-β-(Isoxazolinonyl)-Alanine from *Lathyrus sativus* and its decarboxylation product from *Lathyrus odoratus*. *Phytochemistry* **31 (3)**, 887-892.

MacDonald, J.F., Morris, M.E. and Miljkovic, Z. (1986). Mechanism of excitation of central mammalian neurones by L-3-oxalylamino-2-amino-propionic acid (isolated from *Lathyrus sativus*). In '*Lathyrus and Lathyrism'*. (Eds. A.K. Kaul and D. Combes) pp. 306-314. (Third World Medical Research Foundation: New York). Proceedings of the International Symposium 9-13 September 1985, Pau, France.

Morgan, J.M. (1992). Adaptation to water deficits in three grain legume species. Mechanisms of turgor maintenance. *Field Crops Research* **29**, 91-106.

Negere, A. and Mariam, S.W. (1989). An overview of grass pea (*Lathyrus sativus*) production in Ethiopia. In *'The Grasspea - Threat and Promise'*. Proceedings of the International Network for the Improvement of *Lathyrus sativus* and Eradication of Lathyrism. May 1988, London, U.K. (Ed. P.S. Spencer). pp. 67-71. (Third World Medical Research Foundation: New York and London).

Osman, A.E. and Nersoyan, N. (1986). Effect of the proportion of species on the yield and quality of forage mixtures, and on the yield of barley in the following year. *Experimental Agriculture* **22**, 345-351.

Pathak, S.R., Patel, M.S., Quereshi, A.U. and Ghodasara, G.V. (1988). Effect of water stress on yield and diurnal changes of biophysical paramater of groundnut. *Legume Research* **11 (4)**, 193-195.

Quader, M., Ramanujam, S. and Barat, G.K. (1986) Genetics of flower colour, BOAA content and their relationship in *Lathyrus sativus* L. In '*Lathyrus and Lathyrism'*. (Eds. A.K. Kaul and D. Combes). pp. 93-97. (Third World Medical Research Foundation: New York). Proceedings of the International Symposium 9-13 September 1985, Pau, France.

Rahman, M.M., Quader, M. and Kumar, J. (1991). Status of khesari breeding and future strategy. In '*Advances in pulse research in Bangladesh*'. (Eds. J. Kumar, B.B. Sahni and U. Raman). pp. 25-28. (ICRISAT, Patancheru, India). Proceedings of the Second National Workshop on Pulses 6-8 June 1989, Joydebpur, Bangladesh.

Rao, S.K. and Suryawanshi, R.K. (1988). Analysis of yield factors in urdbean (*Vigna mungo*). *Legume Research* **11 (3)** 134-138.

Ressler, C. (1975). Plant neurotoxins (Lathyrogens and Cyanogens). *Recent Advances in Phytochemistry* **9**, 155-166.

Ross, S.M. and Spencer, P.S. (1989). β-N-oxalylamino-L-alanine: potential mechanisms of neurotoxicity. In *'The Grasspea - Threat and Promise'*. Proceedings of the International Network for the Improvement of *Lathyrus sativus* and Eradication of Lathyrism. May 1988, London, U.K. (Ed. P.S. Spencer). pp. 97-108. (Third World Medical Research Foundation: New York and London).

Scholander, P.S., Hammel, H.T., Bradstreet, E.D. and Hemmingsen, E.A. (1965). Sap pressure in vascular plants. *Science* **148**, 339-346.

Shaikh, M.A.Q. (1989). Pulses improvement through nuclear and conventional techniques. In '*Advances in Pulses Research in Bangladesh*'. (Eds. J. Kumar, B.B. Sahni and U. Raman). pp. 35-41. (ICRISAT, Patancheru, India). Proceedings of the Second National Workshop on Pulses 6-8 June 1989, Joydebpur, Bangladesh.

Siddique, K.H.M. and Khan, T. (1996). Improved chickpea lines ready for release. In *'Cooperative Research Centre for Legumes in Mediterranean Agriculture Annual Report 1995-96'*. p 28. Perth, Western Australia.

Siddique, K.H.M., Loss, S.P., Herwig, S.P. and Wilson, J.M. (1996). Growth, yield and neurotoxin (ODAP) concentration of three *Lathyrus* species in mediterranean-type environments of Western Australia. *Australian Journal of Agricultural Research* **36**, 209-218.

Spencer, P.S., Roy, D.N., Palmer, V.S. and Dwivedi, M.P. (1986). *Lathyrus sativus* L: The need for a strain lacking human and animal neurotoxic properties. In '*The Grasspea - Threat and Promise'*. Proceedings of the International Network for the Improvement of *Lathyrus sativus* and Eradication of Lathyrism. May 1988, London, U.K. (Ed. P.S. Spencer). pp. 297-305. (Third World Medical Research Foundation: New York and London).

Swarup, I. and Lal, M.S. (1993). Effect of moisture stress on *Lathyrus* production - a preliminary study (abstract). In '*Second Internation Colloquium on Lathyrism in Bangladesh: Lathyrus sativus and human lathyrism, Progress and Prospects*'. p 31. Dhaka, 10-12 December.

Vavilov, N.I. (1951). Phytogeographic basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants. *Chronica Bot*. **13**, 13-54.