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The contents of the neuro-excitatory amino acid β-ODAP (β-*N*-oxalyl-L-α,β-diaminopropionic acid), and other free and protein amino acids in the seeds of different genotypes of grass pea (*Lathyrus sativus* L.)

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Abstract

The free and protein amino acids of nine different genotypes of grass pea (*Lathyrus sativus* L.) seeds were analysed by HPLC with pre-column PITC (phenyl isothiocyanate) derivatisation. Among the free amino acids, homoarginine was quantitatively the most important (up to 0.8% seed weight) and stable while the neuro-excitatory amino acid β-ODAP (β-*N*-oxalyl-L-α,β-diaminopropionic acid) showed highest variation (0.02–0.54%) in the nine genotypes examined. Among protein amino acids, glutamic acid was quantitatively most significant, followed by aspartic acid, arginine, leucine, lysine and proline. The sulphur amino acid, methionine, showed the lowest concentration in all the *L. sativus* genotypes, and also in lentil (*Lens culinaris*) and in soybean (*Glycine max*) seeds analysed at the same time.

Keywords: Free and protein amino acids; Methionine; Homoarginine; Drought tolerance; Food legume; Neurolathyrism

1. Introduction

Grass pea (*Lathyrus sativus* L.) is perhaps the most environmentally resistant legume crop with high tolerance to drought, flooding and insect attacks (Campbell, 1997). In Ethiopia, grass pea is the third most important legume crop after faba bean and chick pea. It is used, not only for human food, but also for cattle feed and green forage. During drought and famine in Ethiopia, grass pea is the survival food for the poor when other crops fail to grow. However, over-consumption of grass pea as the only staple food during 2–3 months might cause the crippling disease of neurolathyrism in up to 6% of the rural population. A non-protein amino acid, β-ODAP (β-*N*-oxalyl-L-α,β-diaminopropionic acid), found in grass pea seeds was suggested to be the causal agent of this upper motorneurone disease. Recent reports have shown that the mechanism of neurotoxicity of grass pea leading to neurolathyrism is very complex and oxidative stress might be involved in the pathogeneses (Lambein, Kuo, Kusama-Eguchi, & Ikegami, 2007).

The β-ODAP content of grass pea varies widely, both among genotypes and environments (Campbell, 1997; Dahiya & Jeswani, 1975; DZARC Report, 2003; Leakey, 1979; Ramanujam, Sethi, & Rao, 1980). Hanbury, Siddique, Galwey, and Cocks (2000), based on the evaluation from three Australian locations growing 407 lines of *L. sativus* and 96 lines of *Lathyrus cicera* collected from Ethiopian, Mediterranean and European origins, reported that, for both species, genotype was the most important determinant of β-ODAP concentration while environment had less influence. However, Wuletaw (2003) studied the stability of β-ODAP content in *L. sativus* and reported the significance of genotype with environment interaction. Debre Zeit

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Agricultural Research Centre in Ethiopia did a repeated multi-location trial with growing conditions varying from vertisol soil at high altitude (2200 m above sea level (asl)) and low temperature (18 ± 2 °C) to light soil at low altitude (1600 m asl) and higher temperature (25 ± 2 °C) (Annual Report, 2003). The results of this trial and the specific observation from Campbell (1997), both indicated that the β-ODAP concentration doubles or more, for the same grass pea cultivar, when varying the growing environment to higher stress conditions.

There appear to be clear differences, not only for β-ODAP, but also for other anti-nutritional constituents of grass pea, such as tannins, phytic acid and trypsin inhibitor activities when it is grown in different agro-ecological locations of Ethiopia (Urga, Fufa, Biratu, & Hussain, 2005). Concentrations of total phenolics and condensed tannins were reported to be positively correlated and they are determined by genotypes rather than by environment in grass pea (Wang et al., 1998a). The same authors also reported that trypsin inhibitor activities in grass pea did not differ among cultivars or environments (Wang et al., 1998b).

The β-ODAP content of grass pea seeds analysed by different laboratories is not always consistent. The variation might be caused by the different analytical methods used which include OPT (*o*-phthalaldehyde) colorimetric assay, automatic amino acid analyser, HPLC (high performance liquid chromatography), CZE (capillary zone electrophoresis), NIR (near infrared reflectance spectroscopy) and GC–MS (gas chromatography–mass spectrometry). Some of these methods can not differentiate between α- and β-ODAP. α-ODAP was present naturally in minor amounts (<5%) and was reported to be without neuro-excitatory effect. For this study, we have collected grass pea seeds from different origins and analysed their contents of β-ODAP and other free amino acids by HPLC with pre-column PITC (phenyl isothiocyanate) derivatisation, as reported previously (Kuo, Ikegami, & Lambein, 2003).

The average protein content in legume seeds is 21–25%. These proteins are rich in most of the essential amino acids, particularly lysine, but are usually poor in the sulphur-containing amino acids, methionine and cysteine. In this study, besides the free amino acids, we also determined the profile of protein amino acids of the same batch of *Lathyrus* seeds from different origins. The objective was to compare the contents of free amino acids, as well as the protein amino acids, among different grass pea genotypes, with special attention to the neuro-excitatory β-ODAP and to the essential sulphur amino acid methionine.

2. Materials and methods

2.1. Plant materials

Nine genotypes of *L. sativus* seeds collected from different parts of the world, including Ethiopia (wild types purchased from markets at Debre Zeit, Gonder, Wollo & Bahirdar), India (received from R.L. Pandey, Indira Gandhi Agricultural University, Raipur), China (black eye variety, received from Yu Jin-Zhong, Soil and Fertility Institute, Yangling, Shaanxii province), Poland (Derek variety, obtained from Marian Milczak, Lublin) and Canada (LS 82046 & LS 87124, both were low β-ODAP lines bred by C. Campbell, Agriculture Canada, Morden). Two other grain legume seeds, lentil (*Lens culinaris*) from Turkey and soybean (*Glycine max*) from Canada, obtained from commercial sources, were also analysed at the same time as references for comparison.

Dry seeds were powdered in an electric mill and sieved. The seed powders were used for further analysis.

2.2. Preparation of seed extracts for free amino acids analysis

Two hundred milligrams of seed powder were weighed and suspended in 10 ml of 70% (v/v) aqueous ethanol. In each sample, 50 μl of DL-allylglycine (100 μmol/ml, Sigma) were added as internal standard. The mixture was allowed to stand at 4 °C overnight before centrifugation at 34,800*g* for 20 min. The supernatant was collected and the pellet was washed with 2 ml of 70% (v/v) aqueous ethanol and centrifuged as above. The pooled supernatant was concentrated to 0.5 ml of extract with a rotatory evaporator under vacuum at 45 °C. An aliquot of 50 μl was used for derivatisation with PITC for HPLC analysis.

2.3. Preparation of seed extract for protein amino acid analysis

One hundred milligrams of seed powder were weighed in a 5 ml glass ampoule designed for hydrolysis under vacuum ('Vacules', Wheaton, USA). In each ampoule, 2 ml of 6 N HCl, 0.01% mercaptoethanol and 100 μl internal standard, norleucine (100 μmol/ml, Sigma) were added. The mixture was allowed to freeze by placing the ampoule in dry ice (carbon glass) for 5–8 min. The ampoule was then connected to a vacuum system and shaken gently during the application of the vacuum. The evacuated ampoule was then sealed in a gas flame and placed in an oven for hydrolysis at 110 °C for 18 h.

The hydrolysate was transferred to an Eppendorf tube and centrifuged in an Eppendorf centrifuge (Hawksley MBC, England). The supernatant was transferred to a conical evaporatory flask and dried with a rotatory evaporator under vacuum at 45 °C and this procedure was repeated twice by adding distilled water after drying to remove the HCl; 2 ml of distilled water were added to the flask to dissolve the dried samples and the mixture was centrifuged. The supernatant was collected and a 50 μl aliquot was used for derivatisation with PITC for HPLC analysis.

2.4. PITC (phenyl isothiocyanate) derivatisation

Fifty microlitres samples were firstly dried in an Eppendorf concentrator (5301) at 45 °C. To each dried sample,

20 μl of coupling buffer (methanol/water/triethylamine:2/2/1 by volume) were added, and the whole mixed and dried in the concentrator. Finally, 30 μl of the PITC reagent (methanol/water/triethylamine/phenyl isothiocyanate:7/1/1/1 by volume) were added and reacted at room temperature for 20 min before concentrating to dryness.

To each PITC derivatised sample 500 μl of buffer A (0.1 M ammonium acetate, pH 6.5) were added, mixed well and the whole centrifuged. The supernatant was filtered through a Millipore Millex filter (0.45 μm) and a 20 μl aliquot was injected into the HPLC for analysis.

A standard amino acid mixture (AA-S-18, Sigma), L-(+)-homoarginine (99+%, Janssen Chimica) and synthetic β-ODAP (a gift from Dr. Rao S.L.N., India) were also derivatised and prepared as above and injected into the HPLC as standard.

2.5. High performance liquid chromatography (HPLC) for amino acid analysis

A Waters 625 LC system with Waters 991 photodiode array detector was used, as reported previously (Kuo, Ikegami, & Lambein, 2003). A gradient system with buffer A (0.1 M NH_4OAc, pH 6.5) and buffer B (0.1 M NH_4OAc, containing acetonitrile and MeOH; 44/46/10 by volume, pH 6.5) with flow rate of 1 ml/min was used for the separation of amino acids during 50 min. An Alltima C18 column (250 × 4.6 mm I.D., 5 μm particle size, protected by a guard-column of 7.5 × 4.6 mm, Alltech, USA) was used with column temperature at 43 °C during analysis. The absorbance at 254 nm was used for calculations. The results were analysed by Millennium software (Waters, version 1.10).

2.6. Statistics

All the experiments were repeated twice and each sample was injected twice into the HPLC. The results were expressed as means ± SD. The data were analysed with ANOVA using SPSS 12 software. Statistical significance of differences in mean values was calculated with a confidence level of 95%.

3. Results and discussion

3.1. Free amino acids profile

The grass pea seeds collected from different origins showed high variability in morphology, especially size, shape and colour. In this study we found that the non-protein amino acid, homoarginine (Har), was the most abundant amino acid among all the genotypes of *L. sativus* seeds analysed (Table 1). In addition, this high concentration (0.68–0.86%) was rather stable among all the geno-

Table 1
Free amino acids (% seed weight; means ± SD, $n = 4$) in *Lathyrus sativus* seeds of different genotypes

	D. Zeit, Eth	Gonder, Eth	Wollo, Eth	B. Dar, Eth	India	China	Poland	LS82046	LS87124
Har	0.68 ± 0.11^{a}	0.78 ± 0.16^{a}	0.69 ± 0.09^{a}	0.86 ± 0.18^{a}	0.79 ± 0.05^{a}	0.68 ± 0.20^{a}	0.80 ± 0.32^{a}	0.74 ± 0.10^{a}	0.69 ± 0.15^{a}
β-ODAP	0.35 ± 0.11^{ab}	0.28 ± 0.09^{bc}	0.35 ± 0.07^{ab}	0.48 ± 0.18^{ab}	0.54 ± 0.06^{a}	0.38 ± 0.13^{ab}	0.18 ± 0.09^{c}	0.02 ± 0.01^{d}	0.02 ± 0.01^{d}
Asparagine	0.04 ± 0.01^{b}	0.08 ± 0.04^{b}	0.06 ± 0.10^{b}	0.05 ± 0.04^{b}	0.03 ± 0.02^{b}	0.07 ± 0.03^{b}	0.15 ± 0.06^{a}	0.06 ± 0.05^{b}	0.03 ± 0.01^{b}
Arginine	0.05 ± 0.07^{ab}	0.03 ± 0.02^{bc}	0.05 ± 0.01^{ab}	0.03 ± 0.03^{bc}	0.06 ± 0.04^{a}	0.04 ± 0.01^{bc}	0.03 ± 0.01^{bc}	0.03 ± 0.01^{bc}	0.01 ± 0.00^{c}
Aspartic acid	0.02 ± 0.02^{b}	0.03 ± 0.03^{ab}	0.04 ± 0.03^{a}	0.03 ± 0.03^{ab}	0.04 ± 0.04^{a}	0.04 ± 0.02^{a}	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.01 ± 0.00^{c}
Glutamic acid	0.031 ± 0.03^{b}	0.08 ± 0.06^{a}	0.07 ± 0.04^{a}	0.04 ± 0.03^{b}	0.03 ± 0.03^{b}	0.04 ± 0.02^{b}	0.08 ± 0.02^{b}	0.04 ± 0.01^{b}	0.04 ± 0.02^{b}

Values in a row followed by the same letter are not significantly different ($p > 0.05$).

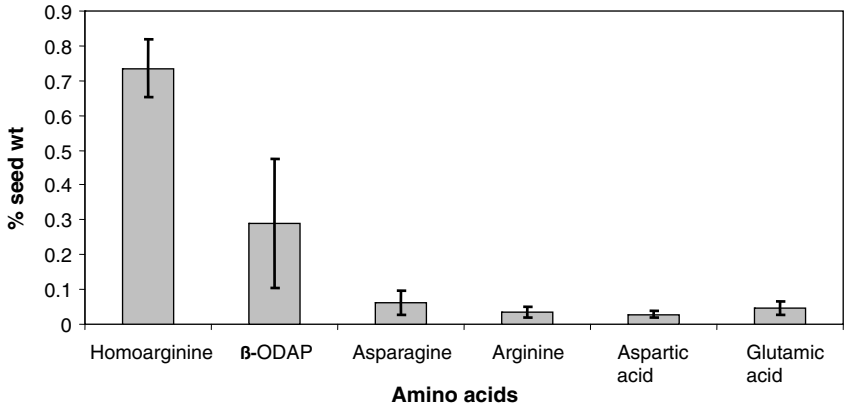

Fig. 1. Free amino acid profile in *Lathyrus sativus* seeds (mean of nine genotypes).

types analysed, with no significant differences. This is a unique feature, specific for grass pea seeds. Har has also been found in the dry seeds and 4-day-old seedlings of some lentil species but the amount was very low and negligible (Rozan, Kuo, & Lambein, 2001). The study of free amino acids profile of some other common food legumes, including pea, bean and lentil (Kuo, Rozan, Lambein, Frias, & Vidal-Valverde, 2004) also showed that Har was undetectable in the dry seeds while, quantitatively, the most important free amino acids were arginine (in pea and bean) or glutamic acid (in lentil). Haque (1997) reported that the concentration of Har in the seeds increased dramatically when *L. sativus* LS-82046 was growing under stress conditions (doubling the concentration of trace elements, Zn^{2+}, Fe^{2+}, $B(OH)_4^-$ and Co^{2+}, in the standard Hoagland hydroponic media, respectively, or with Al^{3+} at 2×10^{-6} M in the media, or with increasing salinity up to 0.8% NaCl).

Both Har and β-ODAP were detected in the blood plasma and in the urine after human volunteers ingested a single meal of 200 g cooked grass pea seeds (Nunn, Perera, Bell, & Lambein, 1994). Har has been reported to antagonise the neurotoxic action of β-ODAP fed to 1-day-old chicks (Shamim, Hossain, Islam, Yusuf, & Lambein, 2002). This might be explained by the modulating effect of L-Har on the nitric oxide synthase (Jyothi & Rao, 1999) and its bearing on oxidative stress.

β-ODAP is quantitatively the second most concentrated free amino acid in *L. sativus* seeds but with greatest variation from 0.20 mg/g dry seed in Canadian lines to 5.4 mg/g dry seed in a sample from Raipur (India). Earlier studies, on the influence of nutrient supply on the β-ODAP content in grass pea seeds growing in hydroponic media, indicated that reduction of the macro-nutrients such as NO_3^-, Mg^{2+}, or K^+ in the Hoagland solution resulted in a sharp increase of β-ODAP content of the ripe seeds (Kebede, Haque, Kuo, & Lambein, 1994). Jiao et al. (2006) reported that the content of β-ODAP in grass pea might be related to the level of total free nitrogenous compounds and that nitrogen and phosphate may be the crucial nutrient factors influencing β-ODAP content under field conditions. Environmental factors, such as drought, zinc deficiency, iron oversupply and the presence of heavy metals in the soil, can considerably increase the level of β-ODAP in the seeds (Haque, 1997).

The presence of β-ODAP in grass pea seeds was blamed for causing the crippling disease neurolathyrism and the toxicity is possibly mediated by collective effects of L-β-ODAP on the AMPA-type receptor, metabotropic glutamate receptors, and NO (nitric oxide) production (Kusama-Eguchi et al., 2006). Mixing grass pea with cereals richer in methionine or with condiments rich in antioxidants was proposed as a protective factor against neurolathyrism (Getahun, Lambein, Vanhoorne, & Van der Stuyft, 2003). β-ODAP was found to chelate copper and zinc (Davies, Nunn, O'Brien, Pettit, & Wang, 1990), which might interfere with the redox homeostasis in the body

Table 2
Protein amino acids (% seed weight; means ± SD, $n = 4$) in the seeds of *Lathyrus sativus* of different genotypes and two other food legumes *Lens culinaris* and *Glycine max*

	D. Zeit, Eth	Gonder, Eth	Wollo, Eth	B. Dar, Eth	India	China	Poland	LS82046	LS87124	*Lens culinaris*	*Glycine max*
Aspartic acid	1.40 ± 0.53^{a}	1.98 ± 0.57^{b}	1.50 ± 0.45^{de}	1.75 ± 0.63^{cd}	1.92 ± 0.57^{bc}	1.63 ± 0.55^{d}	1.95 ± 0.65^{bc}	1.54 ± 0.38^{de}	1.39 ± 0.46^{e}	1.18 ± 0.45^{f}	3.36 ± 1.30^{a}
Glutamic acid	2.3 ± 0.91^{ef}	3.07 ± 1.10^{bc}	2.43 ± 0.83^{de}	2.90 ± 1.19^{cd}	3.02 ± 1.13^{cd}	2.67 ± 0.93^{ef}	2.98 ± 1.04^{cd}	2.64 ± 0.92^{e}	2.48 ± 0.95^{e}	1.94 ± 0.76^{f}	5.26 ± 2.29^{a}
Serine	0.85 ± 0.34^{cd}	0.91 ± 0.04^{cd}	0.75 ± 0.03^{e}	0.95 ± 0.24^{b}	0.94 ± 0.11^{bc}	0.88 ± 0.05^{cd}	0.93 ± 0.04^{bc}	0.86 ± 0.07^{cd}	0.63 ± 0.21^{f}	0.76 ± 0.09^{de}	1.56 ± 0.51^{a}
Glycine	0.69 ± 0.31^{cd}	0.71 ± 0.08^{bc}	0.62 ± 0.10^{cd}	0.76 ± 0.17^{b}	0.72 ± 0.03^{bc}	0.74 ± 0.08^{b}	0.79 ± 0.04^{b}	0.72 ± 0.03^{bc}	0.52 ± 0.18^{e}	0.65 ± 0.02^{cd}	1.21 ± 0.38^{a}
Histidine	0.45 ± 0.22^{cd}	0.50 ± 0.17^{bc}	0.41 ± 0.18^{e}	0.51 ± 0.18^{bc}	0.51 ± 0.12^{bc}	0.49 ± 0.21^{d}	0.52 ± 0.17^{b}	0.50 ± 0.13^{c}	0.43 ± 0.08^{cd}	0.43 ± 0.09^{cd}	0.89 ± 0.27^{a}
Arginine	1.32 ± 0.45^{cd}	1.47 ± 0.33^{bc}	1.21 ± 0.27^{cd}	1.51 ± 0.58^{bc}	1.56 ± 0.43^{b}	1.43 ± 0.28^{c}	1.55 ± 0.36^{b}	1.41 ± 0.33^{b}	1.04 ± 0.30^{d}	1.19 ± 0.29^{d}	2.26 ± 1.00^{a}
Threonine	0.52 ± 0.23^{cd}	0.62 ± 0.14^{bc}	0.52 ± 0.13^{cd}	0.68 ± 0.20^{b}	0.61 ± 0.11^{bc}	0.61 ± 0.21^{bc}	0.66 ± 0.14^{b}	0.59 ± 0.08^{c}	0.48 ± 0.10^{d}	0.56 ± 0.03^{c}	1.06 ± 0.49^{a}
Alanine	1.03 ± 0.40^{b}	0.81 ± 0.04^{def}	0.83 ± 0.19^{d}	0.99 ± 0.43^{cd}	0.81 ± 0.14^{de}	1.02 ± 0.28^{bc}	0.97 ± 0.16^{cd}	1.01 ± 0.14^{c}	0.76 ± 0.18^{f}	1.04 ± 0.27^{b}	1.31 ± 0.62^{a}
Proline	0.90 ± 0.32^{cde}	0.93 ± 0.16^{cd}	0.80 ± 0.07^{e}	1.01 ± 0.07^{bc}	1.04 ± 0.27^{b}	0.94 ± 0.21^{bcd}	1.06 ± 0.24^{b}	0.95 ± 0.24^{c}	0.83 ± 0.26^{d}	0.67 ± 0.04^{e}	1.53 ± 0.78^{a}
Tyrosine	0.44 ± 0.22^{cd}	0.49 ± 0.17^{c}	0.43 ± 0.16^{d}	0.53 ± 0.16^{b}	0.51 ± 0.10^{bc}	0.49 ± 0.20^{c}	0.51 ± 0.15^{bc}	0.48 ± 0.12^{c}	0.42 ± 0.08^{e}	0.41 ± 0.08^{d}	0.96 ± 0.55^{a}
Valine	0.73 ± 0.30^{cd}	0.79 ± 0.08^{c}	0.70 ± 0.12^{e}	0.84 ± 0.37^{b}	0.78 ± 0.0^{c}	0.81 ± 0.06^{bc}	0.81 ± 0.04^{bc}	0.81 ± 0.06^{bc}	0.66 ± 0.08^{e}	0.72 ± 0.09^{d}	1.30 ± 0.77^{a}
Methionine	0.11 ± 0.09^{e}	0.10 ± 0.03^{d}	0.10 ± 0.03^{d}	0.12 ± 0.04^{bc}	0.09 ± 0.02^{e}	0.12 ± 0.07^{bc}	0.10 ± 0.05^{cd}	0.13 ± 0.03^{b}	0.09 ± 0.02^{e}	0.10 ± 0.02^{d}	0.24 ± 0.09^{a}
Isoleucine	0.48 ± 0.16^{cd}	0.58 ± 0.38^{b}	0.49 ± 0.33^{cd}	0.57 ± 0.42^{bc}	0.55 ± 0.35^{bc}	0.53 ± 0.38^{c}	0.56 ± 0.36^{bc}	0.52 ± 0.34^{cd}	0.44 ± 0.28^{d}	0.44 ± 0.29^{d}	0.99 ± 0.67^{a}
Leucine	0.96 ± 0.55^{d}	1.17 ± 0.36^{b}	0.97 ± 0.29^{d}	1.15 ± 0.31^{bc}	1.15 ± 0.38^{bc}	1.07 ± 0.19^{cd}	1.13 ± 0.32^{c}	1.05 ± 0.32^{cd}	0.92 ± 0.30^{e}	0.91 ± 0.33^{e}	2.08 ± 0.61^{a}
Phenylalanine	0.82 ± 0.32^{cd}	0.91 ± 0.28^{b}	0.78 ± 0.26^{e}	0.91 ± 0.26^{b}	0.91 ± 0.31^{b}	0.82 ± 0.19^{de}	0.88 ± 0.23^{c}	0.88 ± 0.17^{c}	0.78 ± 0.17^{e}	0.83 ± 0.21^{d}	1.66 ± 0.45^{a}
Lysine	0.91 ± 0.59^{d}	1.13 ± 0.22^{b}	0.94 ± 0.23^{cd}	1.13 ± 0.23^{b}	1.10 ± 0.25^{bc}	1.10 ± 0.19^{bc}	1.21 ± 0.02^{b}	1.01 ± 0.11^{cd}	0.96 ± 0.12^{cd}	0.88 ± 0.16^{d}	1.81 ± 0.14^{ab}

Values in a row followed by the same letter are not significantly different ($p > 0.05$).

after heavy consumption. However, this multifunctional compound, named as dencichine in traditional Chinese herbal medicine, was also detected in the longevity-promoting ginseng root (Kuo et al., 2003), and recognised for its haemostatic and platelet-increasing properties (Xie et al., 2007).

Efforts, in plant breeding research, to eliminate β-ODAP from the seeds have produced large numbers of "low toxin" varieties but have not yet resulted in "toxin-free" varieties. The potential role of β-ODAP in the stress tolerance of the plant can therefore not be assessed. In grass pea plants, the biosynthetic precursor of β-ODAP was identified as β-(isoxazolin-5-on-2-yl)-L-alanine, which is formed by cysteine synthase (Kuo, Ikegami, & Lambein, 1998). This biosynthetic link with cysteine might present a handle for the genetic improvement of the plant by reduction of β-ODAP and increasing the sulphur amino acids. The post-harvest processing of grass pea seeds by solid state fermentation with *Aspergillus oryzae* and *Rhizopus microsporus* dramatically reduced the level of β-ODAP to a minimum amount of around 0.1 g kg^{-1} but could not remove this secondary metabolite completely, even with the low β-ODAP lines (Kuo, Bau, Quemener, Khan, & Lambein 1995; Kuo, Bau, Rozan, Chowdhury, & Lambein, 2000).

Besides Har and β-ODAP, the other free amino acids, asparagine, glutamic acid, arginine and aspartic acid, were found in much lower concentrations. The free amino acids profile of *L. sativus* (average of the analysis of nine grass pea genotypes) is shown in Fig. 1.

3.2. Protein amino acids profile

In seed hydrolysate of all grass pea genotypes, glutamic acid was found to have the highest concentration, followed by aspartic acid, arginine and lysine (Table 2). In the lentil and soybean seeds examined, a similar pattern was found, except that leucine showed a higher concentration than did lysine. The sulphur amino acid methionine ranked the lowest among all the amino acids in all genotypes of *L. sativus*, as well as in lentil and soybean. The average concentration of methionine in *L. sativus* (ca. 0.1% of the seed weight) was similar to that in lentils (Table 2), but only half of that in soybean (ca. 0.2%) which is known as the richest protein source among the legume crops. The protein amino acids profile of grass pea (average of nine genotypes), lentil and soybean is summarised in Fig. 2.

The protein-bound methionine content seems rather stable among nine grass pea genotypes in spite of the high variation of β-ODAP; there is no significant difference in protein methionine levels between grass pea from India with high β-ODAP content and Canadian LS 87124 with low β-ODAP content. Thus, the protein methionine concentration seems not to be correlated with the concentration of β-ODAP in the seeds of the genotypes of *L. sativus* examined.

Gatta, Polignano, and Bisignano (2002) reported that protein content of 161 accessions in the Bari (Bangladesh Agricultural Research Institute) *Lathyrus* germplasm collection ranged from 23% to 29.9% (mean 26.3%) with lowest coefficient of variation (4%). Monsoor and Yusuf (2002) reported that the crude protein in grass pea seeds ($29.9 \pm 1.26\%$) was higher than that of lentil ($23.2 \pm 1.07\%$) and chickpea ($20.4 \pm 2.27\%$). The survey of Urga et al. (2005) also showed that the protein content of grass pea seeds collected from the major production area of Ethiopia was 28–32%. Grass pea seeds are the major protein source for the poor and the survival food during drought and famine in rural areas. However, Nunn, Lydd-

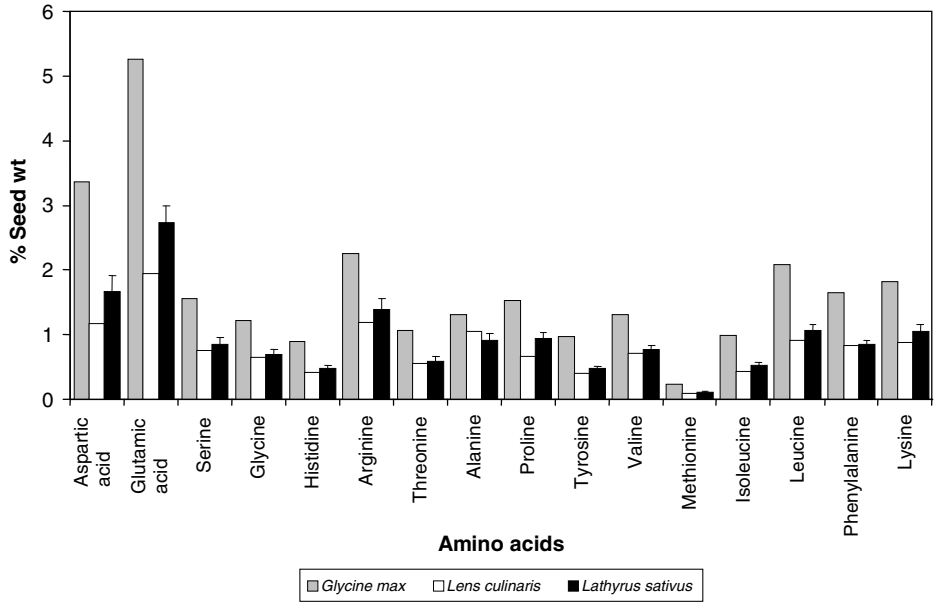

Fig. 2. Protein amino acid profile in *Lathyrus sativus* (mean of nine genotypes), lentil (*Lens culinaris*) and soybean (*Glycine max*).

iard, Perera, and Bell (2005) reported that consumption of these seeds, limiting in methionine, as staple food for long period, leads to the deprivation of methionine in plasma. Methionine is uniquely concentrated in rat motor neurons. If this is also true for the human motor neurons, the long term deficiency of methionine in plasma and thus reduced methionine flux into motor neurone cells might lead to the higher susceptibility of these cells to the excitatory effects of β-ODAP (Nunn et al., 2005).

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