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Biochemical analysis of *Lathyrus sativus*, collected from Six different districts of Madhya Pradesh and Chhatissgarh.

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Abstract: Among all Indian pulses *Lathyrus sativus* contains highest amount (26 to 30 g per 100g)of protein and is fairly rich in vitamins and minerals. This pulse crop is highly resistant to drought, insect-pest and plant diseases. The main objective of the present was to evaluate the protein, aminoacid profile and toxin β-Oxalyl-amino alanine (BOAA) content of *Lathyrus sativus* seeds collected from the six different district of Madhya Pradesh and Chhattisgarh namely Bhopal, Rewa, Jabalpur, Sagar, Bilaspur and Raipur and to study the correlation between protein, aminoacids and the toxin β-Oxalyl-amino alanine (BOAA).Our results indicate that the *Lathyrus* seeds were found to be deficient in sulphur containing amino acids. An inverse relationship was observed between protein and toxin contents in all the six samples. Hence, simply by finding out protein content any particular variety can be declared as low BOAA (toxin)containing variety. Another salient finding was that the sample from low rainfall regions viz. Rewa, Raipur and Jabalpur showed higher concentration of BOAA and low concentration of protein.
Keywords: *Lathyrus sativus*, BOAA

INTRODUCTION

Lathyrus sativus, popularly known as khesari dal, tohraor grasspeas cultivated during the Rabi season (October to April) in arid and semi-arid tracts of Indian sub-continent. It is not only resistant to insects and pest, but also grows well in moisture depleted soils and under flooded conditions. Being a leguminous crop it improves soil fertility through nitrogen fixation [1]. *Lathyrus* has been reported to add as high as 67 kg of nitrogen per hectare to the soil as a result of symbiotic nitrogen fixation by *Rhizobium* species [2,3].

It is highly nutritious legume crop containing 41 percent carbohydrate, 26 to 31 percent protein and 17 percent of total dietary fibre [4]. It provides a nourishing and inexpensive component of the human diet among poor people, mainly in villages. The digestibility of khesari protein is has been reported as 90 percent at 10 percent level of protein intake [5]. Its consumption is restricted or limited due to presence of a neuro-toxic compound, BOAA (β-Oxalyl-Amino Alanine) in seeds of *Lathyrus sativus* [6,7].

In view of the above, the present study was undertaken to investigate protein content, amino acid profile and BOAA (toxin) content and their co-relation in *Lathyrus sativus* seeds cultivated in different climatic conditions.

MATERIAL AND METHODS

Collection of samples

Depending upon the availability, 29 samples of *Lathyrus sativus* were randomly collected, from six different districts of Madhya Pradesh and Chhattisgarh namely Sagar (5), Rewa (4), Bilaspur (4), Raipur (3), Jabalpur (6) and Bhopal (7). Attempts were made to collect information about exact field in which *Lathyrus sativus* was grown. And also information was collected of the year of the cultivation of the sample and the climate condition with particular reference to the rainfall of the district. All the samples were appropriately labelled and stored in the laboratory for analysis.

Analytical Methods

All samples were analysed for protein, BOAA contents and amino acid profile.

Determination of Protein

Total nitrogen content was determined by the Kjeldahl method as per AOAC [8].

Determination of Amino acids and BOAA

The amino acids were analysed by High Performance Liquid Chromatography (BIORAD).

Extraction of sample

One g of powder of *Lathyrus* seed was thoroughly macerated in 80 percent aqueous ethanol. The content was then filtered through G-3 sintered

filter. The filtrate was then evaporated until dryness and finally dissolved in 1 ml of distilled water.

Procedure

BOAA(β-Oxalyl-Amino Alanine) was estimated by the method of Khan *et al.* [9]. using reverse phase high performance liquid chromatography (HPLC) after pre-column derivatization with Edman's reagent (Phenyl isothiocynate)

The chromatography system

The Biorad HPLC system at Central Institute of Agricultural Engineering, Bhopal was used for estimation.

Derivatization reaction

The derivatization and separation of BOAA-PITC complex was based on the method described by Heinrikson and Meredith [10].

Statistical Analysis

Simple, descriptive statistical tools were used to analyze the data. Quantitative variables were expressed as mean±SD (Standard Deviation). Students t-test was applied for group comparison and $p<0.05$ was considered as statistically significant.

RESULTS

The results in Table -1 show the mean values, standard deviation and standard error for protein and BOAA contents. The mean values of protein in samples collected from Rewa, Raipur and Jabalpur were 26.08±1.25, 27.49±1.07 and 26.29±1.02 respectively while the respective average BOAA content of these samples was 0.528±0.05, 0.467±0.09 and 0.476±0.052.

Statistical analysis shows the negative correlation between protein and BOAA in all samples.

The p-values showing significant comparison ($p<0.05$) in protein and BOAA contents in *Lathyrus sativus* samples collected from different regions (Table-2).

In this study we have also measured the aminoacids profile of *Lathyrus sativus* seeds and their correlation to toxin content. These results are shown in Table-3. The direct relationship was observed between BOAA level and methionine, cysteine, threonine, phenylalanine and histidine.

Table-1: Protein and BOAA contents and their co-relation in *Lathyrus sativus* seeds

Sample Nos.	Name of the Districts	Protein (g per 100 g	BOAA (g per 100 g)	Correlation Coefficient (r)
1.	Sagar	28.67±0.24	0.282±0.041	-0.928
2.	Rewa	26.08±1.259	0.528±0.057	-0.808
3.	Bilaspur	27.54±0.663	0.380±0.067	-0.952
4.	Raipur	27.49±1.070	0.467±0.099	-0.810
5.	Jabalpur	26.29±1.026	0.476±0.052	-0.736
6.	Bhopal	27.20±1.010	0.430±0.086	-0.606

Table-2: Comparison of protein and BOAA in *Lathyrus sativus* samples

Samples Compared	p-values (Protein)	p-values (BOAA)
1 and 2	<0.001	<0.001
1 and 3	0.020	<0.05
1 and 4	<0.001	0.01
1 and 5	<0.001	<0.01
1 and 6	<0.01	<0.02
2 and 3	<0.01	<0.01
2 and 4	NS	NS
2 and 5	NS	NS
2 and 6	NS	0.02
3 and 4	NS	NS
3and 5	<0.05	<0.05
3 and 6	NS	NS
4 and 5	<0.05	NS
4 and 6	NS	NS
5 and 6	NS	NS

Table-3: Amino acid contents in *Lathyrus sativus* (g per 100 g)

Amino acids	Mean	Range	Std.dev.	% CV	(r) b/w
Asp	1.03	1.02- 1.04	± 0.009	0.920	-0.815
Glu	0.91	0.88- 0.97	± 0.040	4.480	-0.870
Arg	1.92	1.86- 1.99	± 0.067	3.480	-0.036
His	0.68	0.66-0.70	± 0.018	2.750	+0.336
Lys	1.55	1.54- 1.57	± 0.012	0.833	-0.500
Trp	0.50	0.26- 1.18	± 0.455	9.105	-0.296
Phe	1.07	0.60- 1.30	± 0.037	29.410	+0.267
Tyr	0.50	0.34-0.56	± 0.011	21.400	-0.211
Met	0.34	0.33- 0.34	± 0.005	1.720	+0.219
Cys	0.33	0.31- 0.34	± 0.012	3.840	+0.030
Thr	0.92	0.85- 0.98	± 0.072	7.870	+0.016
Leu	2.72	2.68- 2.78	± 0.055	2.010	-0.173
Ile	1.23	1.11- 1.32	± 0.100	8.170	-0.072
Val	1.17	1.09- 1.21	± 0.054	4.621	-0.182
BOAA	**0.038**	**0.288 -0.582**	**± 0.067**	**0.434**	**1.000**

DISCUSSION

In our study the possibility to quantify samples of *Lathyrus sativus* from different places has been attempted**.** The biochemical composition of *L. sativus* seeds reveals that it is highly nutritious and contains 26 to 30 % high protein and considerable quantities of vitamins and minerals [11].

The results in Table -1 indicate that samples collected from Rewa, Raipur and Jabalpur showed higher concentration of BOAA viz. 0.528,0.467 and 0.476% respectively. The averageprotein contents in these samples were 26.08, 26.47 and 26.29% respectively which are in the lower range. These regions are considered to be low rainfall region.

The result for coefficient of correlation b/w protein and BOAA content indicate significant inverse correlation meaning thereby that with an increase in protein contents in *Lathyrus sativus* seeds there is fall in the concentration of toxin BOAA or vice versa. Our results are in agreement with other studies [12,13] those reported highly significant negative correlation in different strains of *Lathyrus sativus.*

The result for comparison of protein and toxin contents between samples collected from different region is shown in Table-2. Rewa, Raipur and Jabalpur samples when compared with samples collected from Sagar, Bilaspur and Bhopal showed significant difference in protein as well as toxin content ($p<0.05$). Our results with respect to BOAA and protein contents and their correlation coefficients are in agreement of other studies [14,15,16,17].who have confirmed that water stress conditions are directly responsible for increase in toxin (BOAA) accumulation in *Lathyrus sativus* seeds and simultaneous lower level of total protein.

Analysis of *L. sativus* seeds for aminoacid profile (Table-3) indicate that the concentration of sulphur amino acids viz. methionine and cysteine were low while lysine, leucine, isoleucine, arginine and valine were in appreciable quantity. Our results are in accordance with other studies [5,18,19].

Further the correlation of different aminoacids with BOAA content indicates negative correlation with aspartic acid and glutamic acid. The findings in this investigation are in agreement with earlier studies [16,19,20] who have reported similar trend.

CONCLUSION

It is known that the BOAA of *Lathyrus sativus* is neurotoxin amino acid but is not present in other biomolecules. It is thus kept under the category of heat stable anti-nutritional principle. The variability in the BOAA content in could be due to various environmental factors or their combination. In conclusion, the present study revealed that the pulse was being cultivated. If the nutritional value of this pulse can be utilized effectively, it may become a good source of protein and some of the essential and non-essential aminoacids.

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