Physicochemical Values in Different Varieties of *Lathyrus sativus* and Their Interrelationships

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Twenty-nine varieties of *Lathyrus sativus* seeds were analyzed for trypsin inhibitory activity, BOAA, protein, moisture, and ether extractable fraction. Correlations of these biochemical parameters with size and color of the individual seeds were assessed. The results showed that these biochemical parameters did not have any significant correlation of considerable magnitude with either color or size.

The presence of antiproteolytic factors in various plant materials has been well documented (Liener, 1962; Liener and Kakade, 1969; Pusztai, 1967). The presence of trypsin inhibitor in *Lathyrus sativus* (chickling vetch or chickpea) has also been established (Roy and Rao, 1971; Roy, 1972 a,b; Roy and Bhat, 1975).

Consumption of the seeds of *Lathyrus sativus*, commonly known as Kesari dhal, as a staple is associated with neurolathyrism in man– a disease known to occur in endemic form in parts of Central India (Ganapathy and Dwivedi, 1961; Nagarajan, 1969). Seeds of *Lathyrus sativus* contain an unusual amino acid, β-*N*-oxalylamino-L-alanine (BOAA) which is believed to be the neurotoxic factor (Rao et al., 1964). The significance of the presence of either BOAA or the trypsin inhibitor in the physiology of seed is not clear (Roy and Bhat, 1975). The trypsin inhibitor, however, does not influence the neurotoxicity of BOAA in chicks (Roy, 1973).

Attempts are now being made to identify, evolve, and develop strains of *Lathyrus sativus* which have either very low levels of the toxin, or have no toxin at all. Some low toxin varieties have, in fact, been reported (Swaminathan et al., 1969; Somayajulu et al., 1975; Roy and Bhat, 1975). Although the estimation of BOAA in the seed is a relatively simple procedure, laboratory facilities are needed for this purpose. Attempts were therefore made to determine whether some of the physical characteristics of the seeds correlated with either the toxin or trypsin inhibitor content which could be used to screen large numbers of the newer varieties being developed. An attempt was also made to see whether some of the physical characteristics had any association with the protein and fat content.

EXPERIMENTAL SECTION

Materials. *Lathyrus sativus* seeds: A total of 29 varieties were studied. Six varieties were obtained from the Indian Agricultural Research Institute, New Delhi, two varieties from the Department of Social and Preventive Medicine, Medical College, Aurangabad, and three other varieties were collected locally. In addition, 18 cultivars were supplied by the Agricultural Research Station, Gujarat Agricultural University, Tauchha.

Methods. Extraction of seeds for BOAA and trypsin inhibitory activity estimation were done according to the methods reported earlier (Roy and Rao, 1971; Roy and Bhat, 1975). Protein in the extract was analyzed by the method described by Lowry et al (1951), using bovine serum albumin (Sigma, USA) as standard. Total fat was estimated by the method described in the AOAC (1965); total protein by the method described in Hawk's Physiological Chemistry (1965). Moisture was determined by heating the sample at 100 °C to constant weight. Size of the seeds was expressed as the number of seeds per gram of the seed weight and color by visual observations.

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STATISTICAL METHOD

The association between color, size, BOAA content, and trypsin inhibitory activity (TIA) of the *Lathyrus sativus* samples were assessed by calculation of correlation coefficients between color and BOAA, size and BOAA, color and TIA, and size and TIA, and fitting multiple linear regression models of BOAA and TIA with color and size as the related variables. The significance of the models built were assessed through calculation of the multiple correlation coefficients and coefficients of determination. These coefficients show the probable magnitude of all the variation existing in the variable, attributable to both color and size.

The significance of correlation coefficients and coefficients of determination were assessed through appropriate statistical tests of significance (Snedecor and Cochran, 1971; Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

The results of the study are presented in Tables I to III (see also Supplementary Material Available paragraph). Of the 29 samples studied, five were white, eight were light grey or light brown, 14 were grey or brown, and two were black. The mean and standard error values of all the biochemical parameters estimated by color of the seeds are provided in Table I.

The mean BOAA content was 0.41% in samples with white color; 0.49% in samples which were light grey or light brown; 0.58% in samples with grey or brown colors, and 0.74% in samples which were black. These differences, however, were not significant since the varieties with the same color had wide variations in their BOAA content. The size of the seeds also did not vary significantly between seeds of various colors.

The TIA either in buffer or in water extracts were not related either to color or size of the seeds. The variation in TIA was quite wide within each type of seeds as investigated by color. There was a trend for black seeds to have slightly higher TIA than those of white, grey, or brown or light grey or light brown colored seeds; however, the differences were not significant statistically.

The size and color did not significantly correlate with TIA in water extracts and TIA in buffer extracts in the samples investigated ($P > 0.05$). The correlation coefficient of TIA with size was less than 0.15 and with color 0.18 (Table II).

The ether extractable fractions and moisture contents of *Lathyrus sativus* samples did not vary by color or size

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Table I. Mean ± Standard Error (Sample Size) of Biochemical Values (%) of *Lathyrus sativus* by Color and Size of the Seed

Color	Size, no./g wt	Total protein, %	BOAA content, %	Ether extractable fraction, %	Moisture, %	Trypsin inhibitory activity[a] (TIA), sp act., TUI/mg of protein	
						In buffer extract	In water extract
White	9.4 ± 1.91 (5)	28.9 ± 0.89 (5)	0.41 ± 0.065 (5)	1.42 ± 0.073 (5)	9.41 ± 0.078 (5)	179.8 ± 9.05 (5)	244.3 ± 19.12 (5)
Light grey or light brown	14.4 ± 0.92 (8)	26.4 ± 0.31 (8)	0.49 ± 0.066 (8)	1.47 ± 0.079 (8)	10.72 ± 0.312 (8)	177.3 ± 17.65 (8)	203.1 ± 27.85 (8)
Grey or brown	15.6 ± 2.52 (14)	27.0 ± 0.35 (14)	0.58 ± 0.039 (14)	1.29 ± 0.050 (14)	10.52 ± 0.293 (14)	198.6 ± 18.93 (13)	230.9 ± 15.97 (14)
Black	13.0 (2)	25.7 (1)	0.74 (2)	1.60 (1)	10.50 (1)	245.0 (1)	334.0 (1)
All colors	14.0 ± 1.32 (29)	27.1 ± 0.29 (28)	0.54 ± 0.032 (29)	1.38 ± 0.039 (28)	10.38 ± 0.189 (28)	188.7 ± 11.92 (27)	229.0 ± 12.32 (28)
"*F*" ratio between colors:	0.96	4.22	2.87	2.16	2.23	2.53	1.45
Level of significance	$P > 0.25$	$P < 0.05$	$P < 0.10$	$P > 0.10$	$P > 0.10$	$P < 0.10$	$P < 0.25$

[a] TIA, trypsin inhibitory activity; TUI, trypsin units inhibited; protein in the extracts analyzed by the method of Lowry et al. (1951). For TIA assay: incubation mixture consists of 0.5 mL of trypsin solution, 1.0 mL of phosphate buffer, 0.4 mL of HCl solution (0.001 M), 2 mL of 2% casein solution, 0.1 mL of the extract (buffer or water) to make the total volume 4.0 mL.

Table II. Coefficients of Correlation between Color, Size, BOAA, and Trypsin Inhibitory Activity[a,e]

	Color	Size	BOAA	Trypsin inhibitory activity (TIA)	
				Buffer	Water
Color	1.0000	0.2394	0.4942[d] (0.9499)[b]	0.1747	0.1585
Size		1.0000	−0.0428 (0.2090)[c]	−0.1178	−0.1549

[a] Trypsin inhibitory activity (TIA). [b] The correlation coefficient for the data of Dahiya (1976) was calculated by the authors. [c] Dahiya (1976). [d] $P < 0.01$. [e] The following scores (or ranks) were given for various colors in finding out the coefficients of correlation: white, 1; light brown or light grey, 2; grey or brown, 3; black, 4.

of the seeds investigated ($P > 0.05$, Table I).

The multiple regression models for BOAA and TIA were fitted with color and size as independent variables to assess whether both color and size are related. The models built by the method of least squares and the multiple correlation coefficients showing the relationship of BOAA/TIA with color and size are provided in Table III. The BOAA was significantly correlated with color and size ($R = 0.52$, $P < 0.01$). The coefficient of determination was, however, about 27%, indicating that of the total variation existing in BOAA content in samples, only about 27% could be attributed to color and size put together. The remaining 73% variation existing in BOAA content of the samples was due to other factors.

The coefficient of determination explaining the variation existing in TIA attributable to color and size put together was less than 10% (Table III).

In nine varieties of *L. sativus* seeds, using a completely randomized block design, Dahiya (1976) has a close relationship between BOAA content of the seed and its color ("*F*" ratio test, $P < 0.01$). Dahiya's data was analyzed by us and models fitted as was done for our data, and the results are presented in Table III. BOAA content of varieties of *L. sativus* seeds studied by Dahiya (1976) was found to have a close association with color as well as size, the multiple correlation coefficient being as high as 0.96 with a coefficient of determination of 91.5% ($P < 0.001$). Color alone was closely associated with BOAA having a coefficient of determination of 90.23% ($P < 0.001$). Addition of another factor, size of the seeds, slightly improved the coefficient of determination to a value of 91.5% ($P < 0.001$). The reasons for the differences between the results presented here and those of Dahiya (1976) are not clear. They might be due to differences in the sample size, maturity of seeds, soil conditions, and varieties of seeds investigated by size and color. The relevance of each of these factors to the BOAA content may need further investigation.

The results of this study, therefore, suggest that physical characteristics are of little value in screening seeds of *L. sativus* for their toxin content. A further study with more

Table III. Multiple Regression Equations of BOAA and TIA on Color and Size[a]

Source of data	S. no.	Multiple regression equation	Coefficient of determination (R^2)	Multiple correlation coefficient (R)	Level of significance
National Institute of Nutrition, Hyderabad	1.	BOAA = 0.319956 + 0.105557 color, −0.001926 size	26.78	0.5175	$P < 0.01$
	2.	TIA (water) = 170.7000 + 16.3422 color, −1.4773 size	5.94	0.2438	$P > 0.05$
	3.	TIA (buffer) = 219.9455 + 33.5899 color, −3.7992 size	6.68	0.2585	$P > 0.05$
Dahiya[b] (1976)		BOAA = −1.57790 + 0.13528 color, +0.001778 size	91.46	0.9563	$P < 0.001$

[a] (i) The following scores or ranks were given for various colors in fitting the models: white, 1; light brown or light grey, 2; grey or brown, 3; black, 4. (ii) In fitting the equations, the pairs of observations were 29 for BOAA, 28 for TIA (water) and 27 for TIA (buffer). [b] The number of samples studied were nine.

varieties may be of interest to supplement the findings on chemical characteristics.

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Supplementary Material Available: Analytical values of some of the *Lathyrus sativus* varieties and cultivars with the content of the toxins–BOAA and "trypsin inhibitory activity" (3 pages). Ordering information is given on any current masthead page.

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Studies on the Proteins of the Mutants of Barley Grain. 3. Fractionation and Characterization of the Glutelin Fraction

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Mutational effects were observed in the glutelin fraction of the barley grain protein. Fractionation of the glutelin of the parent variety, NP-113, its mutants, Notch-1 and Notch-2, and the nutritionally superior Hiproly barley on Sephadex columns indicated that the fraction II (glutelin II) was considerably enriched in the mutants, and this was comparable with glutelin of the Hiproly barley. Data on the amino acid composition also indicated a favorable change for this fraction from the point of nutrition. The in vitro digestibility study of this fraction adduced support for this nutritionally favorable change and suggests that the glutelin II fraction could serve as an index of superior grain type from breeding point of view for nutritional purpose.

Fractionation of the isolated classical protein fractions of barley mutants into subfractions could help in identification of the changes induced by mutagenesis in the strategy of improving the grain protein quality. Earlier work (Singh and Sastry, 1977b) on the alcohol-soluble proteins of these mutants revealed an increase (compared to the parent) in the content of the 35% ethanol-soluble subfraction, which showed relatively superior amino acid composition and in vitro digestibility. These changes were consistent with those in the Hiproly barley of established nutritive quality (Munck et al., 1970; Singh and Sastry, 1977b). The present investigation has been extended to the proteins of the glutelin fractions isolated from the mutants, Notch-1 and Notch-2, their parent, NP-113, and the Hiproly barley, an isolate of Ethiopian origin from the World Barley Collection with superior nutritive quality of its protein.

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MATERIALS AND METHODS

The grain samples, namely, NP-113, Notch-1, Notch-2, and Hiproly, used in this investigation were obtained as described earlier (Singh and Sastry, 1977a). The glutelin fraction from each of the barleys was obtained by extraction with a solvent system comprising acetic acid (0.1 M), urea (3 M), and cetyltrimethylammonium bromide (0.01 M) as described earlier (Singh and Sastry, 1977a). The solvent system was designated as AUC solvent system and the protein AUC–glutelin (Singh and Sastry, 1977a). The determination of this protein content, amino acid composition, and electrophoresis of this protein fraction was also described.

Gel Filtration of Glutelin. Twenty milligrams of the glutelin protein was dissolved in 4 mL of the AUC solvent system. The solution was centrifuged and, after measuring

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