J. Phytol. Res. **18**(1) : 53-57, 2005

EVALUATION OF NUTRITIVE VALUE OF PROMISING SOMACLONES OF PUSA-24 VARIETY OF KHESARI (*LATHYRUS SATIVUS L.*)

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Four somaclones along with their parent Pusa-24 and two local genotypes of Khesari (*Lathyrus sativus)* were chemically analysed for proximate composition, vitamin-C, β-N-oxalyl, L-α, β-diamino propionic acid (ODAP) content and contents of amino acids and nutritionally important mineral elements. The somaclones showed significant increase in methionine (0.71 - 0.75 g/16 g N), methionine-S as per cent of total sulphur (20.94 - 21.50 %), chemical score of methionine (20.82 - 22.06) and potassium content (1893 - 1930 mg %) as against the parent (P-24). Protein content (28.61%), energy value (344.5 K cal/100 g), tryptophan (0.59 g/16 g N) and iron contents (6.87 mg %) were also increased in some of the somaclones. The content of vitamin C (5.70 - 6.84 mg %), magnesium (118 - 133 mg %) and total sulphur (0.200 - 0.216 %) of the somaclones did not differ significantly. Somaclonal variation however did not show beneficial effect on crude fibre (3.23 - 3.70%), phosphorus (402 - 499 mg %) and tryptophan (0.49 - 0.51 mg %) contents. The ODAP content was significantly reduced in all the somaclones (0.085%) as compared with their parent (0.291 %) and local genotypes (0.396 and 0.597%). The somaclone L-208 appeared to be superior in respect of protein, calcium, sulphur and methionine while, the somaclone L-212 showed it's superiority in respect of iron and tryptophan contents.

Keywords : Amino acid score; β-N-oxalyl; Elemental composition; L-α,β-diamino propionic acid (ODAP); *Lathyrus sativus;* Proximate composition; Vitamin C.

Introduction

Grain legumes are the major source of proteins in Indian vegetarian diets. They complement cereals in terms of amino acid balance. *Lathyrus sativus* provides a nourishing diet of good quality proteins and carbohydrates but the presence of neurotoxin (ODAP) makes it unsuitable for human consumption[1,2]. In recent years, somaclonal variation has been successfully exploited for crop improvement with respect to low toxin content and better nutritive value with higher yield potentials[3]. The present study was therefore undertaken to evaluate the nutritive value of somaclones in comparison with their parent P-24 and the local cultivars.

Material and Methods

The somaclones viz., L-208, L-212, L-54 and L-63 of *Lathyrus sativus* along with their parent Pusa-24 were procured from Biochemistry Division, IARI, New Delhi and two local genotypes designated as Local-1 and Local-2 were procured from Chandrapur and Bhandara districts of Maharashtra state respectively. The grains were cleaned, finely ground to 80 mesh and used for chemical analysis.

Moisture, crude fibre, Ash and crude protein contents were estimated according to the standard methods[4]. Crude fat was estimated by soxhlet method, carbohydrate by substraction method and energy value by calculation[5]. Methionine and tryptophan were estimated as per the methods described by Kendurkar *et al.*[6] and their chemical score was calculated according to the formula given by Singh *et al.*[7]. The diacid extracts (HNO_3 and $HClO_4$) were used for estimation of phosphorus spectrophotometrically by vanadomolybdate yellow colour method and potassium by flame photometer. Calcium and magnesium in diacid extracts were estimated titrimetrically[8].Triacid extracts were used for estimation of sulphur turbidimetrically[9] and iron spectrophotometrically[10] by using o-phenanthroline reagent. The ascorbic acid in m-phosphoric acid extracts was estimated by employing DCPIP method.

β-N oxalyl, L-α, β-diaminopropionic acid (ODAP) was estimated according to method developed by Rao[11] with slight modifications. The ODAP from 25 mg flour was extracted with 5 ml of double distilled water in boiling water both for 30 minutes. The volume was made to 5 ml and the extract was centrifuged (4000 rpm, 10 mins). An aliquot (0.2 ml) from the supernatant was mixed with 5 ml of 3 N KOH in duplicate. One tube was kept for hydrolysis in boiling water bath for 30 minutes while, another tube was kept at room temperature (without hydrolysis). After hydrolysis, the tube was cooled at room temperature. The volume in both the tubes was made to one ml with distilled water. Two ml of OPT-reagent (0.1 g o-pthalaldehyde, 1 ml of ethanol, 99 ml of 0.5 M $Na_2B_4O_7$, pH 9.7 and 0.2 ml of 2-mercaptoethanol) were added to each tube. The intensity of yellow colour was measured after 30 minutes at 420 nm in SL-150 Elico-

Spectrophotometer. The blank was prepared by using 0.2 ml distilled water instead of extract. The difference between the absorbance readings with and without hydrolysis (O.D.) was taken for calculating ODAP content using the standard value of 117.25 nmoles (22.2775 µg) of ODAP per O.D. by employing the following formula.

$$\%ODAP = \frac{O.D. \times 22.2775 \times \text{total vol. of extract} \times 100}{10,00,000 \times \text{Aliquot taken for colour development} \times \text{Weight (g) of flour}}$$

The statistical analysis of the data were done as per the standard statistical methods[12].

Results and Discussion

The chemical analysis of proximate principles revealed that the somaclone L-208 recorded the highest protein content of 28.61 per cent which was significantly higher than all other genotypes (Table 1).

The protein content of somaclones L-54 and L-212 was at par and somaclone L-63 showed significantly lesser protein content than the parent (27.32%). On the whole, the somaclones were having protein content (27.03%) significantly higher than local genotypes (24.73%). These values were closely related to the protein values already reported[13]. Thus, the *Lathyrus* genotypes under study showed a large variation of 4.87 per cent in protein content. The fat content of all the somaclones (0.76%) and their parent P-24 (0.80%) were at par with each other and significantly lower than the fat content of local genotypes (1.77%). The fat content of somaclones was also in fair agreement with the values reported by Williams *et al.*[13]. The somaclone L-54 recorded the highest ash content (3.44%) which was significantly higher than the ash content of all other genotypes except parent (P-24). The crude fibre content was significantly decreased in somaclones (3.46%) as compared to P-24 (4.51%). The ash content of *Lathyrus* genotypes was well confirmed and the crude fibre content was lower than the reported values[14]. The lower carbohydrate content in the improved genotypes appeared to be due to relatively higher content of protein and mineral matter. Among the somaclones, L-208 had higher energy value (344.5 K cal/100 g) than the parent (337.7 K cal/100 g). These values were found to be fairly good and agreed well with those reported by Williams *et al.*[13].

Vitamin C (ascorbic acid) is generally absent in pulses[15]. It was however interesting to note that the different genotypes of *Lathyrus* under study showed vitamin C content ranging from 5.70 to 6.84 mg per 100 g. The differences in the genotypes with respect to vitamin C content were however not significant. These values of vitamin C compared well with the values reported by Patil[16] for moth bean.

β-N oxalyl L-α, β-propionic acid (ODAP) is a neurotoxin found in *Lathyrus* pulse. The nutritive value of *Lathyrus* genotypes is set backed if the ODAP content is higher than 0.2 per cent - a limit prescribed by National Institute of Nutrition, Hyderabad[1] and Tedesse[2]. Keeping this in view, it is observed that the ODAP content in all the somaclones (0.076 to 0.094%) was much below the safer limit (0.2%). The ODAP content of Pusa-24 was 0.291 per cent while the Local genotypes showed much higher levels of ODAP (0.396% in Local-1 and 0.597% in Local-2). Gadkari and Singh[1] have reported that most of the commercially available cultivars of *Lathyrus* contain 0.6 to 0.7 per cent ODAP. On comparing the ODAP content of somaclones grown under Konkan agro-climatic conditions with the ODAP content of these somaclones grown under Delhi agro-climatic conditions, it is seen that the ODAP content at both the locations did not vary much and compared well with the results obtained by Mehta *et al.*[3]. This indicates the genotypic stability of somaclones for ODAP content.

The amino acid content of *Lathyrus* indicated (Table 2) that the somaclones contained significantly higher amount of methionine (0.73 g / 16 g N) as compared with their parent (0.63 g / 16 g N) and local genotypes (0.65 g /16 g N). The somaclonal variation however was not beneficial with respect to content of tryptophan in the somaclones. On the contrary, the tryptophan content was significantly decreased in the somaclones except L-212 (0.59 g / 16 g N) which was statistically at par with the parent (0.57 g / 16 g N). The amino acid score for methionine showed a slight variation and ranged from 20.82 to 22.06, while that of tryptophan showed a variation of about 7.0 units in the tryptophan score and ranged from 32.27 to 39.29 in the different somaclones. The methionine and tryptophan scores of Pusa-24 were 18.56 and 38.00 respectively. While in local genotypes, it was 19.15 for methionine and 34.53 for tryptophan. In general, the values of amino acids support the earlier observations of Chatterjee and Abrol[17]. But the amino acid scores were comparatively lower than the values reported[15, 17]. On the basis of methionine and tryptophan scores, it could be revealed that all the genotypes under study were highly deficient in both the amino acids and of the two essential amino acids methionine appeared to be the first limiting amino acid in *Lathyrus* grain protein. The sulphur content in different somaclones ranged from 0.200 to 0.216 per cent and did not differ significantly with their parent. The sulphur content in the locals was significantly lower. The somaclonal variation was found to be beneficial on methionine-S as per cent of total sulphur and resulted in about 3 per cent increase as compared to their parent P-24 (18.33 %). These results are in accordance with the results of methionine content of somaclones. These values of total-S were in good agreement with earlier findings[16].

The elemental composition of *L. sativus* grain (Table 3) revealed that the phosphorus content (463 mg %

Table 1. Proximate composition, vitamin C and ODAP content of different genotypes of *L. sativus*

Genotypes	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Carbohydrate (%)	Energy (K cal/100g)	Vitamin C (mg/100 g)	ODAP (g/100g grain)
L-208	8.31	28.61	0.73	3.26	3.23	55.86	344.5	6.84	0.076
L-212	9.13	26.33	0.63	3.16	3.70	57.05	339.2	5.70	0.081
L-54	9.33	27.22	0.86	3.44	3.28	55.87	340.1	6.27	0.087
L-63	9.25	25.95	0.80	3.26	3.63	57.11	339.4	6.27	0.094
Mean (Somaclones)	—	27.03	0.76	3.28	3.46	56.47	340.8	6.27	0.085
P-24	8.72	27.32	0.80	3.35	4.51	55.29	337.7	7.41	0.291
Local-1	7.61	25.72	1.58	2.62	3.27	59.20	353.9	5.70	0.396
Local-2	8.41	23.74	1.96	2.32	3.22	60.34	354.0	6.84	0.597
SE±	0.22	0.31	0.06	0.03	0.11	—	—	0.609	0.008
C.D. at 1%	0.91	1.28	0.26	0.11	0.45	—	—	N.S.	0.035

Table 2. Methionine, tryptophan and sulphur content of different genotypes of *Lathyrus sativus*

Genotypes	Methionine (g/16g N)	Tryptophan (g/16g N)	Amino acid score		Total sulphur (g/100 g grain)	Methionine-S as % of total sulphur
			Methionine	Tryptohan		
L-208	0.72	0.49	21.24	32.27	0.216	21.15
L-212	0.71	0.59	20.82	39.27	0.202	21.50
L-54	0.72	0.50	21.18	32.73	0.200	21.09
L-63	0.75	0.51	22.06	33.87	0.200	20.94
Mean (Somaclones)	0.73	0.52	21.33	34.54	0.205	21.17
P-24	0.63	0.57	18.56	38.00	0.201	18.33
Local-1	0.64	0.52	18.94	34.53	0.175	20.13
Local-2	0.66	0.52	19.35	34.53	0.144	23.28
SE±	0.019	0.007	—	—	0.005	—
C.D. at 1%	0.08	0.03	—	—	0.022	—

Table 3. Elemental composition of different genotypes of *Lathyrus sativus*

Genotypes	P	K	Ca	Mg	Fe
	(mg/100g)				
L-208	499	1930	197	123	5.74
L-212	482	1920	145	118	6.87
L-54	468	1893	179	128	5.80
L-63	402	1923	162	133	6.40
Mean (Somaclones)	463	1917	171	126	6.20
P-24	527	1870	145	123	5.99
Local-1	300	1997	145	113	7.20
Local-2	316	2003	162	118	6.26
SE±	3.48	4.98	11.17	6.43	0.12
C.D. at 1%	15	21	47	N.S.	0.51

was significantly decreased in all the somaclones as compared with their parent (527 mg %). The phosphorus content in locals was also significantly lower than Pusa-24 and its somaclones. On the contrary, the potassium content in all the somaclones was significantly higher than their parent (P-24). Among the somaclones, L-208 had the highest amount of potassium and calcium whereas the magnesium content did not differ significantly. The somaclone L-212 was found to be superior with respect to iron content. The values of phosphorus, calcium, magnesium and iron observed in present study are in fair agreement with those reported by Shobhana *et al.*[14] for *Lathyrus*.

The present investigation revealed that the somaclonal variation in *Lathyrus sativus* was found to be highly beneficial as it not only improved the nutritive value of the grain but also decreased the neurotoxin levels (ODAP) significantly, too much below the safer limit. Somaclone L-208 was adjudged to be nutritionally much superior as it contained much higher amount of protein, calcium, sulphur and methionine while, the somaclone L-212 showed it's superiority with respect to iron and tryptophan contents.

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