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Biochemical evaluation of some horse gram (*Macrotyloma uniflorum* L. Verde) genotypes of Himachal Pradesh

Sanjula Sharma and C.P. Awasthi
Department of Chemistry and Biochemistry, College of Basic Sciences
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India.

Abstract

Fifteen promising horse gram genotypes procured from Department of Plant Breeding and Genetics, CSK HPKV, Palampur were analysed for various biochemical parameters by following standard procedures. Significant variation in seed weight, seed yield, moisture, crude protein, non-protein nitrogen, true protein, carbohydrate, crude fibre, ash, essential limiting amino acids - methionine and tryptophan, anti-nutritional factor(s) *viz,* tannins, hydrolysable tannins and oxalates, polyphenol oxidase activity, *in vitro* protein digestibility and cooking time were observed to range from 2.48 to 3.27 g/100 seeds, 2.05 to 11.45 g/plant, 10.05 to 13.42%, 21.00 to 26.08%, 0.18 to 0.56%, 18.31 to 23.27%, 51.37 to 57.89%, 4.45 to 5.95%, 3.04 to 3.90%, 0.30 to 0.61 g/16gN protein and 0.44 to 0.76 g/16gN, 901 to 1193 mg/100g, 867 to 1162 mg/100g and 201 to 394 mg/100g, 1.07 to 3.42 units/min/g, 61.59 to 70.58% and 17 to 26 minutes among various horse gram genotypes, respectively. The study indicated that genotypes *viz,* HPKM-149 in seed weight, DMK-12 in seed yield, HPKC-2 in moisture; HPKC-5 in crude protein; HPKC-1 in carbohydrate; TRS/RKS-245 in crude fibre; HPKC-2 in ash; TRS/RKS-245 in methionine; HPKC-4 in tryptophan; SKY/SNS-510 in total tannins, HPKC-7 in hydrolysable tannins; HPKC-7 in polyphenol oxidase activity; DMK-12 in oxalates; SKY/SNS-510 for *in vitro* protein digestibility and HPKC-4 in cooking time emerged promising genotypes in that order for individual quality trait. Besides, based on varietal rating done taking into consideration performance of genotypes with regard to seed yield, protein, carbohydrate crude fibre, methionine, tryptophan, and *in vitro* protein digestibility, cooking time, total tannins and oxalate content taken together genotypes SKY/SNS-510, HPKC-7, HPKC-43, DMK-12 and HPKC-2 were identified to be over all superior multipurpose genotypes in that order.

Key words: Genotypes, *in-vitro*, protein, digestibility, tannins, polyphenol oxidase activity, oxalates.

Introduction

Horse gram (*Macrotyloma uniflorum* L. Verde.) commonly known as *kulth* or *kulthi* is a self-pollinating pulse crop coupled with medicinal value and having resistance to common diseases, biotic and abiotic stresses. The crop could be cultivated during both *kharif* and *rabi* seasons because of its wider adaptability to poor soil and adverse climatic conditions. It is considered to be originated in South East Asia and probably in Peninsular India. It is mainly grown in states of Tamil Nadu, Karnataka, Maharashtra, Madhya Pradesh, Andhra Pradesh, Bihar, Himachal Pradesh, Gujarat and Uttar Pradesh in India. It occupies an area of 1,537 thousand hectares with an average productivity of 3.69 quintal per hectare (Bhan and Mishra, 1998). In Himachal Pradesh it is the second important pulse crop next to urdbean and occupies an area of 3,028 hectares with total production of 1,475 metric tonnes (Anonymous, 2001).

Horse gram in hill agriculture is often considered as poor man's protein rich pulse coupled with medicinal value. It is mainly used as feed and fodder for cattle. However, its consumption in human diet is limited due to poor cooking quality of the dry seeds. It is one of the ingredients of an Ayurvedic medicine '*cystone*' prescribed to kidney stone patients. The powder of seed is applied to the skin to check cold sweats (Singla and Kanwar, 1985). Literature revealed inadequate systematic information on the status of biochemical constituents of nutritional significance of horse gram germplasm grown particularly in Himachal Pradesh. As such the present study was undertaken to evaluate the biochemical composition of promising horse gram genotypes to be useful for further crop improvement purposes.

Material and Methods

Fifteen promising horse gram genotypes were procured from Department of Plant Breeding and Genetics, CSK HPKV, Palampur during the month of June. 2005. Dry mature seeds of horse gram were cleaned and freed of any extraneous substances and dried in a hot air oven at $60^0 \pm 5^0$ C. The dried samples were ground in pestle and mortar and stored in polythene packets. These samples were properly labelled and kept at room temperature for further analysis. The biochemical parameters *viz,* moisture, crude protein, crude fibre and ash were analysed by the method described in AOAC (1970). Non-protein nitrogen was determined by Pellet and Young (1980). True protein was computed as difference between crude protein and non-protein nitrogen. Carbohydrate content was determined by following the procedure of Yemm and Wills (1954). The limiting amino acids (being those amino acids which are present in limited amounts in horse gram protein as compared to those found in reference (egg/milk) protein) *viz.* methionine and tryptophan were estimated by following the procedure of Horn *et al.* (1946) and Spies and Chambers (1949), respectively. Hydrolysable tannins and total tannins were analysed by the method described by Makkar *et al.* (1993). Polyphenol oxidase activity was determined by the method given by Bastin and Ulner (1972). Oxalate content was analysed by following the procedure of Abaza *et al.* (1968). *In vitro* protein digestibility was determined by the method described by Akeson and Stahman (1964) as modified by Singh and Jambunathan (1981). Cooking time was recorded by the method described by Sharma *et al.* (2004). The data were analysed statistically by using analysis of variance as given by Panse and Sukhatme (1984).

Results and Discussion

The data showing variability in the biochemical attributes of various hose gram genotypes are presented in Table 1.

Table 1. Variation in biochemical constituents of dry mature seeds of horse gram genotypes of Himachal Pradesh

Genotypes	100-Seed weight (g)	Seed yield (g / plant)	Moisture (%)	Crude protein (%)	NPN (%)	True protein (%)	Carbohydrates (%)	Crude fibre (%)	Ash (%)
HPKC-1	3.03	10.75	11.50	21.35	0.34	19.23	57.89	4.45	3.51
HPKC-2	3.05	8.00	10.05	22.75	0.18	21.61	56.33	5.58	3.90
HPKC-4	3.01	2.05	10.90	24.50	0.56	21.00	53.21	5.35	3.49
HPKC-5	2.71	2.50	12.77	26.08	0.45	23.27	51.37	5.42	3.82
HPKC-6	3.12	8.25	11.19	22.23	0.21	20.92	56.68	5.21	3.04
HPKC-7	3.01	9.75	10.73	24.50	0.40	22.00	54.05	5.40	3.67
TRS/RKS-245	2.48	5.50	11.11	21.00	0.43	18.31	57.38	5.95	3.38
SKY/SNS-510	2.53	8.10	10.71	24.38	0.35	22.20	54.42	5.69	3.72
HPKC-43	2.72	10.90	13.30	22.40	0.52	19.16	54.04	5.75	3.62
DMK-12	2.48	11.45	13.42	22.57	0.26	20.92	54.42	5.16	3.49
HPKC-18	3.20	5.65	11.19	24.33	0.49	21.27	54.08	5.12	3.37
IC-18985	2.94	9.50	10.71	21.53	0.34	19.41	57.41	5.40	3.43
IC-14350	2.49	7.85	11.14	25.55	0.39	23.12	52.63	5.39	3.51
HPK-4*	2.83	5.85	10.67	21.88	0.49	18.81	57.41	5.10	3.39
HPKM-149	3.27	5.35	13.42	24.15	0.22	22.79	52.63	5.32	3.23
SE (± m)	0.061	1.40	0.33	0.26	0.033	0.20	0.34	0.049	0.089
CD (P=0.05)	0.12	2.98	0.69	0.53	0.070	0.44	0.70	0.099	0.180

* Local check-Released variety of the State of Himachal Pradesh

Significant variation in 100-seed weight was observed from 2.48 to 3.27 g with the highest value in HPKM-149 and the lowest in TRS/RKS-245 and DMK-12. The trend of variation with regard to this parameter in the present investigation was in agreement with those reported by Hiremath *et al.* (1978) reporting variation from 2.66 to 4.75g in horse gram genotypes.HPKC-4 (2.05 g) and

DMK-12 (11.45 g) exhibited the minimum amd maximum seed yield per plant.

The moisture content was found to vary from 10.05 to 13.42 per cent with the lowest and highest value for this parameter noticed in HPKC-2 and HPKM-149. The range of variation in moisture content in the present study was in accordance with the findings of Sudha *et al.* (1995) in horse gram genotypes (10.2 to 13.0 per cent).

Different genotypes of horse gram varied significantly in crude protein, non-protein nitrogen and true protein content from 21.00 (TRS/RKS-245) to 26.08 (HPKC-5) per cent, 0.18 (HPKC-2) to 0.56 (HPKC-4) per cent, 18.31(TRS/RKS-245) to 23.27 (HPKC-5) per cent, respectively. The promising genotypes for seed protein content were identified as HPKC-5, IC-14350, and HPKM-149 in that order. The findings of the study on this aspect were in agreement with those reported by other workers (Yadav *et al.*, 2004).

Variation in carbohydrate content of horse gram genotypes was noticed from 51.37 to 57.89 per cent. The maximum and minimum value with regard to carbohydrate content were found in HPKC-1 and HPKC-5 in that order. Similar pattern for carbohydrate content in horse gram genotypes have also been reported by other investigators (Sudha *et al.*, 1995).

The study revealed significant variation in crude fibre and ash content as well to range from 4.45 (HPKC-1) to 5.95 (TRS/RKS-245) per cent and 3.04 (HPKC-6) to 3.90 (HPKC-2) per cent, accordingly. The findings of the study are in close proximity with the values reported by other researchers (Diwakar *et al.*, 1996; Arora, 1997).

It is evident that methionine and tryptophan content of horse gram genotypes showed wide variation to range from 0,30 (HPKC-7, HPKC-18, IC-14350) to 0.61 (TRS/RKS- 245) mg/16gN and 0.44 (IC-14350) to 0.76 (HPKC-4) mg/16gN, respectively. The range of variation in respect of these quality attributes observed in the present investigation is in consonance with the findings of other investigators on this crop and other food legumes. (Dhiman, 1996 and Mandape *et al.*, 2003). Variation in essential limiting amino acids, anti-nutritional components, *in vitro* protein digestibility and cooking time of dry mature seeds of horse gram genotypes of Himachal Pradesh are presented in Table 2.

Table 2. Variation in essential limiting amino acids, anti-nutritional components, *in vitro* protein digestibility and cooking time of dry mature seeds of horse gram genotypes of Himachal Pradesh

Genotypes	Methionine (g/16gN)	Tryptophan (g/16gN)	Total tannins (mg / 100g)	Hydrolysable tannins (mg / 100g)	Oxalates (mg / 100g)	*In vitro* protein digestibility (%)	Cooking time (In minutes)
HPKC-1	0.60	0.47	1000	984	272	67.62	26
HPKC-2	0.53	0.56	974	951	345	66.35	25
HPKC-4	0.51	0.76	1146	1115	227	63.40	17
HPKC-5	0.34	0.59	1193	1158	204	62.07	21
HPKC-6	0.32	0.53	984	958	270	66.42	24
HPKC-7	0.30	0.65	904	867	367	66.52	20
TRS/RKS-245	0.61	0.53	1132	1113	343	63.02	18
SKY/SNS-510	0.53	0.53	901	870	229	70.58	20
HPKC-43	0.48	0.60	1000	984	394	67.38	22
DMK-12	0.48	0.57	1157	1118	201	68.33	25
HPKC-18	0.30	0.65	1139	1072	379	61.59	24
IC-18985	0.31	0.55	1086	1050	349	64.01	23
IC-14350	0.30	0.44	1188	1162	368	62.89	19
HPK-4*	0.59	0.55	1089	1052	210	67.98	23
HPKM-149	0.36	0.49	1025	987	368	64.77	26
SE (± m)	0.022	0.025	4.32	4.39	2.78	-	2.73
CD (P=0.05)	0.046	0.050	8.82	8.97	5.68	NS	5.82

Local check-Released variety of the State of Himachal Pradesh

The status of anti-nutritional factors *viz,* total tannins, hydrolysable tannins, polyphenol oxidase activity and oxalate content in horse gram genotypes was also evaluated. Among different genotypes of horse gram, total tannins content showed variation from 901 (SKY/SNS-510) to 1193 (HPKC-5) mg/100g and hydrolysable tannin content ranged from 867 (HPKC-7) to 1162 (HPKC-5) mg/100g. Sudha *et al.* (1995), Srivastava and Srivastava (2003) and Chopra and Sankhala (2004) have also observed variation in tannin content from 763.7 to 895.9 mg/100g in different pulses including horse gram. The data revealed that PPO activity varied from 1.07 to 3.42 unit /min/g in dry mature seeds of horse gram. The minimum activity of this enzyme was noticed in the genotype HPKC-7, whereas the highest in IC-18985. However, apparently no extensive data on this aspect have been documented so far in relation to genotypic variability in dry mature seeds of horse gram. Values in respect of oxalate content in horse gram genotypes varied significantly from 201 (DMK-12) to 394 (HPKC-43) mg/100g. Variations noticed in different genotypes in this parameter were by and large in close conformity with the values observed by Pore (1979) in this crop (i.e. 182 to 419 mg/100g).

Values for *in vitro* protein digestibility of dry mature seeds of horse gram genotypes ranged from 61.59 (HPKC-18) to 70.58 (SKY/SNS-510) per cent. The results of the present study were found in close proximity to those reported by Osama *et al.* (2002) and Oliverira *et al.* (2004), who observed the range of variation on this aspect from 59.2 to 62.0 per cent and 76 to 78 per cent in that order. The cooking time of dry mature seeds of horse gram varieties / genotypes was observed to vary from 17 to 26 minutes for desired cooking. The genotypes HPKC-4, TRS/RKS-245, IC-14350, HPKC-7 and SKY/SNS-510 required comparatively less time for cooking over others. The findings of this study are almost in agreement with the trend of variation noticed in cooking time of various food legumes (Vimla and Pushpamma, 1985).

Besides identifying genotypes superior in individual quality trait (s) to be useful for further breeding and crop improvement purposes, attempts were made to carry out overall genotypic rating in respect of various quality attributes *viz,* seed yield, protein, carbohydrate, essential limiting amino acids- methionine and tryptophan, anti-nutritional factors *viz,* tannins and oxalates, *in vitro* protein digestibility, cooking time taken together in order to identify overall nutritionally superior multipurpose horse gram genotypes and the genotypes SKY/SNS-510, HPKC-7, HPKC-43, DMK-12 and HPKC-2 were thus identified as versatile nutritionally superior genotypes in that order (Table 3).

Table 3. Varietal grading of horse gram genotypes in search of versatile multipurpose genotypes

Parameters	HPKC-1	HPKC-2	HPKC-4	HPKC-5	HPKC-6	HPKC-7	TRS/RKS-245	SKY/SNS-510	HPKC-43	DMK-12	HPKC-18	IC-18985	IC-14350	HPK-4	HPKM 149
Seed yield*	3	8	15	14	6	4	12	7	2	1	11	5	9	10	13
Proteins*	13	7	3	1	10	3	14	4	9	8	5	12	2	11	6
Carbohydrates*	1	5	10	12	4	8	3	6	9	6	7	2	11	2	11
Crude fibre*	14	4	8	5	10	6	1	3	2	11	12	6	7	13	9
Methionine*	2	4	5	8	9	11	1	4	6	6	11	10	11	3	7
Tryptophan*	10	6	1	4	8	2	8	8	3	5	2	7	11	7	9
In vitro protein digestibility*	4	8	11	14	7	6	12	1	5	2	15	10	13	3	9
Cooking time**	10	9	1	5	8	4	2	4	6	9	8	3	3	7	10
Total tannins**	5	3	11	14	4	2	9	1	5	12	10	7	13	8	6
Oxaiates**	7	9	4	2	6	11	8	5	14	1	13	10	12	3	12
Total	69	63	69	79	72	57	70	43	61	61	94	72	92	67	92
Cumulative grading	6	4	6	10	8	2	7	1	3	3	12	9	11	5	11

*Genotypes graded in the descending order for nutritionally desirable characters
** Genotypes graded in ascending order for nutritionally undesirable attributes

In all the study indicated wide variation in individual biochemical parameter(s) of nutritional significance to be useful to the plant breeders and those engaged in horse gram improvement. Moreover, the multipurpose genotypes identified in the study based on genotypic rating might be used for dietary intake purpose for optimum human nutrition.

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References

Abaza RH, Blake JF and Fisher EJ 1968. J. Association of Official Analytical Chemists **51**: 963.

Akeson WE and Stahman MA 1964. A pepsin–pancreatin digest index of protein quality evaluation. J. Nutr. **83**: 257-59.

Anonymous 2001. Annual Season and Crop report. Government of Himachal Pradesh.

AOAC 1970. *Official Methods of Analysis*. Association of Official Analytical Chemists. 11th edition. Washington D.C.

Arora N 1997. A preliminary study of horse gram (*Dolichos biflorus*). Indian Vety. J. **74** (9): 802-03.

Bastin M and Ulner 1972. Effect of actinomycin-D on the formation of enzymes in Jeruselum artichoke tuber discs. Planta **102**: 357-61.

Bhan VM and Mishra JS 1998. Efficient use and management of agrochemicals for increasing pulse production. Agric. Situation India **55** (4): 187-96.

Chopra S and Sankhala A 2004. Effect of soaking and sprouting on tannin, phytate and *in vitro* iron availability in under utilized legumes-horsegram (*Dolichos biflorus* L.) and moth bean (*Phaseolus aconitifolius* L.). J. Food Sci. Tech. **41**(5): 547-50.

Dhiman A 1996. Influence of phenolic compounds on nitrate assimilation, biochemical constituents and yield of broad bean (*Vicia faba* L.). M Sc Thesis, Himachal Pradesh Krishi Vishvavidyalaya, Palampur (unpublished).

Diwakar P, Kushwah A and Kushwah HJ 1996. Biochemical composition of some local varieties of horse gram in M.P. Indian J. Nutr. Dietet. **33**: 188-92.

Hiremath SR, Shivashankar G and Chikkadevaiah 1978. Cooking characters of horse gram. Indian J. Agric. Sci. **48** (7): 399-401.

Horn MJ, Jone DD and Blum AE 1946. Colorimetric determination of methionine in proteins and foods. J. Biol. Chem. **116**: 313-20.

Makkar HPS, Blummel M, Borowy NK and Becker K 1993. Gravimetric determination of tannins and their correlation with chemical and protein precipitation method. J. Sci. Food Agri. **61**: 161-65.

Mandape RC, Fattepurkar SC, Ghodeswar, R.C., Kolhe, C.D. and Bedse, T.J. 2003. Evaluation of proximate composition and limiting amino acids in grains of green gram. Annals of Plant Physiol. **17**(1): 70-74.

Oliverira JTA, Pinto VPT, Vasconcelos IM, Fernandes CF, Ramos MV, Fereira FVA and Rios FJB 2004. *In vitro* and *in vivo* digestibility of the albumin and globulin fractions of eight Brazilian cow pea (*Vigna ungiculata* L.) cultivars. J. Sci. Food Agri. **85**: 1823-30.

Osama OAS, Abdullahi HT, Wahab AA and Elkhalifa AE 2002. Proximate composition, minerals, tannins, *in vitro* protein digestibility and effect of cooking on protein fractions of hyacinth bean (*Dolichos lablab*). J. Food Sci. Agri. **39**(2): 111-15.

Panse VG and Sukhatme PV 1984. *Statistical Methods for Agricultural Workers*. ICAR, New Delhi.

Pellet LP and Young VR 1980. Nutritional evaluation of protein foods. UN. Univ. Publ.

Pore HS 1979 Mineral composition and oxalic acid in horse gram. Indian J. Agric. Sci. **49** (9): 712-14.

Sharma S, Saxena AK and Dhillon SK 2004. Physico-chemical and cooking quality characteristics of soybean (*Glycine max* L.). J. Food Sci. Tech. **41**(5): 554-56.

Singh U, Raju SM and Jambunathan R 1981. Studies on Desi and Kabuli chickpea (*Cicer arietinum* L.) cultivars II seed protein fractions and amino acid composition. J. Food Sci. Tech. **18**: 86.

Singla SK and Kanwar K 1985. Inhibitors of calcification from *Dolichos biflorus*. Proceedings of 3rd National Conference of Urolithiasis Society of India. Udaipur, Feb 27-28, 1985, p51.

Spies JR and Chambers DC 1949. Chemical determination of tryptophan in protein. Analyt. Chem. **21**: 1249.

Srivastava RP and Srivastava GK 2003. Nutritional value of Pulses. Indian J. Agric. Biochem. **16** (2): 57-65.

Sudha N, Begum JM, Shambulingappa KG and Babu CK 1995. Nutrients and some anti-nutrients in horse gram (*Macrotyloma uniflorum* (Lam.) Verde). Food Nutr. Bull. **16** (1): 81-83.

Vimla V and Pushpamma P 1985. Effect of improved storage methods on cook-ability of pulses stored for one year in different containers. J. Food Sci. Tech. **22**: 327-29.

Yadav S, Negi KS and Mandal S 2004. Protein and oil rich wild horse gram. Genetic Resources and Crop Evolution **51**: 629-33.

Yemm EW and Wills AJ 1954. The estimation of carbohydrate in plant extract by anthrone reagent. J. Biochem. **57**: 508-14.