JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP
Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises
(Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/44/1/MRN-4045

Characterization of horse gram mutants for yield, nutrient, and anti-nutrient factors

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Received: 27.11.2021 *Revised*: 21.03.2022 *Accepted*: 24.05.2022

Abstract

Aim: This experiment assessed the potential of induced mutagenesis in evolving variability for yield, nutritional and anti-nutritional, factors in horse gram.

Methodology: The mutants were grown in three environments consisting of three sowing times and soil types and the data were pooled. D^2 analysis was used to group the mutants. Based on the magnitude of variation and character inter-relationship, single plant yield was fixed as a selection criterion to identify promising mutants from clusters. The tagged high yielding mutants were profiled for macronutrients, micronutrients and anti-nutritional factors.

Results: The induced mutagenesis evolved significant variability for yield attributing traits. It evolved the maximum variation for the traits number of pods per plant, number of clusters, plant height and single plant yield (CV= 34.80%; 33.90%; 33.80%, 30.44% and 29.60% respectively). A significant coefficient of variation was observed for nutrients (crude protein: 5.13%; crude fibre: 11.67%, crude fat: 13.44%; boron: 11.50%; magnesium: 11.52; phosphorus: 10.86; potassium: 9.53%; calcium: 18.33%; manganese: 14.25%; iron: 16.36%; copper: 12.20%; zinc: 14.50% and molybdenum: 18.41%) anti-nutritional factors (total phenol:17.24%; tannins: 10.88%; phytic acid: 16.14% and oxalic acid: 15.16%). Potential genetic stocks for yield combined with nutritional supremacy were identified for further utilization.

Rabi season 2017: Mutagenized two horse gram varieties using gamma rays, electron beam and their combinations and laid out M_1 generation

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Rabi season 2018: M_2 generation and tagged promising segregants

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Rabi season 2019: M_3 generation and selected the homogenized mutant families based on yield

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Rabi season 2020: Evaluated the homozygous mutant families over environments in M_4 generation, assessed the magnitude of induced variation and tagged the promising mutants

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Kharif season 2021: Nutrient profiling and identified useful genetic stocks

Interpretation: Induced mutagenesis evolved potential mutants for yield and nutrients. Alas, a negative relationship between yield and nutrient contents was established. To improve yield in horse gram and alleviate malnutrition, the genotypes with an average single yield potential of 55-65 g shall be prioritized.

Key words: Anti-nutritional factors, Horse gram mutants, Nutrients

How to cite : Sudhagar, R., V. Pushpayazhini, C. Vanniarajan, S.J. Hepziba, R. Renuka and J. Souframanien: Characterization of horse gram mutants for yield, nutrient, and anti-nutrient factors. *J. Environ. Biol.*, **44**, 99-107 (2023).

Introduction

Food legumes are the second most valuable plants after cereals which offer a balanced human diet as they are excellent sources of protein, dietary fibre, micronutrients, and phytochemicals. An array of legumes is though cultivated, a handful of them (chickpea, red gram, black gram, green gram etc.,) dominate the production and market chains in the developing economies (Rathi *et al.,* 2021). These legumes require ideal agricultural environments to produce a good yield; otherwise significant yield reduction is noticed under challenging situations. Few underutilized indigenous legumes offer scope for yield and nutritional security of rural, tribal, and underprivileged masses. Horse gram is one of such important crops of India primarily cultivated in the dry tropics with minimum rainfall. It is drought-resistant and adaptable to poor soil conditions unlike other pulses (Kumar, 2006). It plays an important place in human nutrition, owing to its protein, mineral, vitamin, iron, molybdenum, and calcium contents (Bhartiya *et al.,* 2015). Horse gram protein has higher lysine content than pigeon pea and chickpea.

There are several health and medicinal benefits of horse gram. It has significant non-digestible carbohydrate content which releases glucose at a slower rate thereby offering scope for diabetes management. Horse gram has a significant quantity of resistant starch which is considered an important prebiotic amongst the recent dietary fibres (Samanta *et al.,* 2011). The high dietary fibre content in horse gram flour maintains positive effects on intestine and colon physiology, besides other homoeostatic, and therapeutic functions. Different parts of horse gram plants are used for the treatment of heart conditions, asthma, bronchitis, leukoderma, urinary discharges and kidney stones (Prasad and Singh, 2015). In addition, it also supplies many bioactive substances in small quantities like phytic acid, phenolic acid, enzymatic/proteinase inhibitors and high molecular tannins which have significant metabolic and/or physiological effects (Prasad and Singh, 2015). Earlier Kaundal *et al.* (2020) and Patangare Suwarna *et al.* (2019) reported the nutritional importance of horse gram. Breeding for high yielding horse gram genotypes with better nutritional aspects is the need of the hour to sustain yield and nutritional security in the rainfed farming of India. The small flower size of horse gram restricts the utility of manual emasculation followed by pollination.

Autogamy restricts the realization of heterosis upon hybridization. Flower drop after targeted pollination slows the progress. Low level of variability for the economic traits in the germplasm (Chahota *et al.,* 2013) warranting the usage of alternative crop improvement techniques to hasten the progress. The diploid nature of horse gram offers the scope of utility of induced mutagenesis in evolving variability for yield attributing traits. Priyanka *et al.* (2021a; 2021b) reported the success of induced mutagenesis in horse gram. The benefit of Mahalanobis D^2 statistic in estimating variability is well reported (Priyanka *et al.,* 2021c). A negative relationship between yield and quality traits has been reported by Raatz (2018) necessitating an establishment of any such relationship in horse gram for ideal genotypic selection. Khazaei *et al.* (2020) reported the utility of ICPMS in the precise quantification of micronutrients in horse gram. Estimation of breeding and nutritional potency of any population paves way for precise tagging of genotypes for utilization. Therefore, the present study aimed at quantifying the efficiency of induced mutagenesis in a horse gram mutant population for yield, nutritional and anti-nutritional factors and to establish the relationship between yield and quality parameters.

Materials and Methods

Evolution of genetic material and generation advancement: The study material comprised 110 horse gram mutants and two checks PAIYUR 2 and CRIDA 1-18 R. These mutants were evolved by mutating two-horse gram varieties CRIDA 1-18 R and PAIYUR 2 involving gamma rays, electron beam, and their combinations. The mutagenic treatments were 100Gy G (3), 200Gy G(5), 300Gy G (23), 400Gy G (0); 100Gy EB (38), 200Gy EB (7), 300Gy EB (23), 400Gy EB (0); 100Gy G+100Gy EB (7), 200Gy G+200Gy EB (1), 300Gy G+300Gy EB (1), 400Gy G+400Gy EB (0), 100Gy+0.3% EMS (7), 200Gy+0.3%EMS(0), 300Gy+0.3% EMS (0) and 400Gy+0.3% EMS (0). The values in the parenthesis are the number of useful genetic mutants evolved by respective mutagenic doses. In the M_1 generation, the mutated seeds were grown in three replications and healthy crop growth was maintained. The M_1 plants were harvested individually. During rabi season 2018, all the M_1 plants were forwarded to M_2 generation. The promising M_2 segregants were tagged, harvested individually, and forwarded to M_3 generation on the plant to row basis during *rabi* season 2019. In the M_3 generation, the mutants were checked for homozygosity and yielding potential. The homozygous and promising yield mutant families were harvested separately and utilized for raising M_4 generation during rabi season 2020.

Characterization of mutants: Horse gram is a photo-sensitive crop that requires short-day conditions for flowering and podding. Therefore in the current experiment, it was planned to characterize the potentiality of mutants over three environments (i.e. three different months of sowing and soil types). The experiments were conducted in a randomized block design with three replications at Sugarcane Research Station, Melalathur, TNAU, Vellore District, Tamil Nadu. The first environment crop was sown during October 2020 in black soil (pH: 7.1) while the second and third crops were sown during November and December in red (pH: 6.7), and sandy loam soil (pH: 6.9), respectively. Healthy crop stands were maintained by adopting all the recommended cultivation practices. A total of eleven quantitative traits days to first flowering, days to fifty per cent flowering, days to maturity, plant height, number of primary branches/plant, number of clusters/plant, number of pods/cluster, number of pods/ plant, number of seeds/pod, 100 seed weight, and single plant yield were utilized to ascertain the quantum of variability evolved by induced mutagenesis. Five plants/ genotype/ replication/ environment were randomly selected for data documentation.

The flowering and maturity traits were recorded at appropriate growth stages while all other traits were measured at harvest. The polled data of three environments was utilized for Mahalanobis D^2 statistic analysis (Rao, 1952) and the mutants were grouped based on Tocher's value. The magnitude of variation for all experimented traits was quantified. The traits with maximum variation were considered for the selection of useful mutants for nutrient profiling. During *Kharif* 2021, the selected mutants were profiled for macronutrients (crude protein, crude fibre, and crude fat), micronutrients (boron, magnesium, phosphorus, potassium, calcium, manganese, iron, copper, zinc and molybdenum), and anti-nutritional factors (ANFs) (total phenols, tannins, and phytic acid). The crude protein was estimated as per Lynch and Barbano (1999), crude fibre as suggested by Maynard (1970), and crude fat using soxhlet apparatus. The micronutrient contents were estimated by Inductively Coupled Plasma-Mass-Spectrometry (Model: Thermo Scientific™ iCAP™ RQ; Type: Single Quadrupole ICP-MS; Dynamic Range: > 9 orders of magnitude (< 1 - > 1•109 cps); Hertz: 2 MHz). The ANFs were estimated by spectrophotometer. The wavelengths for total phenols, tannins, and phytic acid were 650, 700, 480 nm respectively. The oxalic acid content was estimated by titration method against $KMnO_4$ (Ryan *et al.,* 2001). The data were analyzed using SPSS software (version 11.0) and the violin plots were arrived at using R studio version 1.0.136 to understand the ranges. The correlation between yield, nutrient, and anti-nutritional parameters was estimated using the hierarchical heat map clustering using the 'gplots' package of R studio version 1.0.136 (Warnes *et al.,* 2020).

Results and Discussion

Horse gram is the promising cool-season legume in the semi-arid tropics of India which needs genetic restructuring to sustain productivity. To rectify few undesirable characteristics like photosensitivity, indeterminate growth habit, and duration in horse gram an exclusive induced mutagenesis program involving varieties PAIYUR 2 and CRIDA 1-18 R was undertaken from 2016

Table 1: Details of variability evolved by induced mutagenesis in horse gram

Statistical parameter	Traits										
	DFsF	DFfF	DM	PH	NPB	NCR	NPC	NPP	NSP	HSW	SPY
Range	32.33-58.00	35.67-64.00	88.67-127.33	42.67-193.47	3.00-10.67	28.27-237.83	2.00-5.00	105.33-673.67	3.00-6.67	2.24-4.07	17.52-90.02
C.D.	0.92	1.32	1.63	4.32	0.65	3.85	0.65	48.13	0.92	0.13	2.55
SE (m)	0.33	0.58	0.58	1.88	0.33	1.53	0.00	4.58	0.33	0.06	1.15
SE (d)	0.33	0.33	0.58	1.10	0.00	1.20	0.33	23.85	0.33	0.02	0.58
SD	3.50	3.76	6.77	34.53	1.84	41.23	0.67	110.52	0.72	0.40	15.50
CV	6.92	6.52	5.76	33.80	33.44	33.90	21.92	34.80	14.23	11.91	29.60

DFsF – Days to first flowering; NPB - Number of primary branches per plant; NSP - Number of seeds per pod; DFfF- Days to 50% flowering; NCR- Number of clusters per plant; HSW - Hundred seed weight (g); DM - Days to maturity; NPC- Number of pods per cluster; SPY – Single plant yield (g); PH - Plant height (cm); NPP- Number of pods per plant

Table 2: Pooled mean values of various quantitative traits of selected horse gram mutants

Cluster	Genotypes	Mutant with SPY (g)	CM: SPY(g)	DFsF	DFfF	DM	PH	NPB	NCR	NPC	NPP	NSP	HSW
Cluster I	38	TNAU-HG-089(59.46)	41.12	51.44	58.90	118.92	109.98	5.46	88.31	3.00	254.71	5.36	3.32
Cluster II	31	TNAU-HG-070(78.86)	56.53	50.85	57.94	120.41	143.10	7.04	120.33	2.94	332.69	4.88	3.73
Cluster III	7	TNAU-HG-007(90.17)	75.04	50.24	57.90	119.95	154.49	10.05	148.85	3.52	502.81	5.00	3.41
Cluster IV	11	TNAU-HG-028(47.44)	39.50	52.30	59.09	120.55	124.23	4.79	117.37	3.18	349.88	4.76	2.87
Cluster V	19	TNAU-HG-081(57.41)	29.85	48.95	56.14	110.18	73.79	4.74	57.06	3.16	176.33	4.91	3.63
Cluster VI	1	TNAU-HG-039(57.25)	57.25	53.00	59.00	118.33	197.63	8.67	100.17	4.00	400.67	4.33	3.77
Cluster VII	1	TNAU-HG-059(53.74)	53.74	51.67	60.67	122.00	175.03	7.33	112.33	2.00	224.67	6.33	4.08
Cluster VIII	1	TNAU-HG-011(57.31)	57.31	49.00	56.67	119.00	138.60	6.33	144.67	4.00	578.67	4.67	2.54
Cluster IX	1	TNAU-HG-018(69.26)	69.26	51.00	58.00	122.33	178.59	9.00	245.83	2.00	491.67	4.67	3.48
Cluster X	2	TNAU-HG-078 (31.62)	25.65	32.50	35.83	90.50	49.20	4.17	41.18	3.50	136.17	5.67	3.39
Parent 1		PAIYUR 2(34.12)	41.12	49.67	58.00	109.00	98.03	5.67	93.67	3.00	281.00	4.67	3.88
Parent 2		CRIDA 1-18R(26.16)	29.85	40.33	45.33	96.00	76.17	5.00	67.17	2.67	173.00	4.67	3.42

CM: SPY – Cluster Mean value for single plant yield; DFsF – Days to first flowering; NPB-Number of primary branches per plant; NSP - Number of seeds per pod; DFfF- Days to 50% flowering; NCR- Number of clusters per plant; HSW - Hundred seed weight (g); DM - Days to maturity; NPC- Number of pods per cluster; SPY – Single plant yield (g); PH - Plant height (cm); NPP- Number of pods per plant

Table 3: Estimation of macronutrient contents (%) in the selected horse gram mutants

Cluster	Mutants	Crude protein	Crude fibre	Crude fat
Cluster I	TNAU-HG-089	24.95*	2.44*	0.78
Cluster II	TNAU-HG-070	25.16*	2.18	1.14*
Cluster III	TNAU-HG-007	23.74	2.14	1.15*
Cluster IV	TNAU-HG-028	21.94	2.16	1.08
Cluster V	TNAU-HG-081	23.72	2.29	1.22*
Cluster VI	TNAU-HG-039	23.73	2.36*	0.92
Cluster VII	TNAU-HG-059	23.94	1.76	0.86
Cluster VIII	TNAU-HG-011	21.44	1.83	1.19*
Cluster IX	TNAU-HG-018	23.69	2.56*	0.96
Cluster X	TNAU-HG-078	21.98	2.18	0.94
Parent 1	PAIYUR 2	22.81	1.80	1.00
Parent 2	CRIDA 1 -18R	22.24	2.15	1.02
Grand mean		23.28	2.15	1.02
Standard error (SE)		0.34	0.07	0.04
Standard deviation (SD)		1.20	0.25	0.14

Table 4: Estimation of micronutrient contents (ppm) in the selected horse gram mutants

Cluster	Mutants	B	Mg	P	K	Ca	Mn	Fe	Cu	Zn	Mo
Cluster I	TNAU-HG-089	7.34*	410.59*	1210.52*	2090.30	710.15*	10.19*	2.89*	2.71*	6.63	0.28
Cluster II	TNAU-HG-070	6.67	336.22	1019.99	1860.36	610.52	7.78	2.00	2.62	6.94	0.36
Cluster III	TNAU-HG-007	5.95	324.32	1078.05	1838.65	511.45	8.43	2.18	2.29	6.04	0.28
Cluster IV	TNAU-HG-028	6.27	344.48	1073.22	1897.65	533.16	7.05	2.13	2.72*	5.88	0.46*
Cluster V	TNAU-HG-081	4.77	290.75	867.88	1496.78	434.11	7.23	1.94	2.01	5.71	0.35
Cluster VI	TNAU-HG-039	5.42	357.67	1188.52*	2136.91*	547.29	9.21	2.63*	2.98*	8.28*	0.40*
Cluster VII	TNAU-HG-059	6.72	352.78	1062.79	2129.08*	810.36*	9.53*	3.17*	2.71*	8.13*	0.25
Cluster VIII	TNAU-HG-011	5.65	326.14	981.60	1939.87	512.45	8.14	2.36	2.11	6.03	0.30
Cluster IX	TNAU-HG-018	6.93*	365.38	1117.53	2080.19	587.22	9.56*	2.02	2.56	5.82	0.28
Cluster X	TNAU-HG-078	6.38	404.59*	1256.36*	2097.06	471.13	10.08*	2.15	2.67	7.71*	0.39*
Parent 1	PAIYUR-2	6.58	400.12*	1221.33*	2069.08	625.89	6.69	2.48	2.70	8.07*	0.35
Parent 2	CRIDA 1 -18R	6.76*	429.90*	1259.63*	2138.51*	523.74	7.86	2.13	2.12	7.51	0.39*
Grand mean		6.29	361.91	1111.45	1981.20	573.12	8.48	2.34	2.52	6.90	0.34
Standard deviation (SD)		0.72	41.68	120.72	188.77	105.03	1.21	0.38	0.31	1.00	0.06
Standard error (SE)		0.21	12.03	34.85	54.49	30.32	0.35	0.11	0.09	0.29	0.02

B- Boron; Mg-Magnesium; P-Phosphorus; K-Potassium; Ca-Calcium; Mn-Manganese; Fe-Iron; Cu-Copper; Zn-Zinc; Mo-Molybdenum

to 2020. The standard plant breeding procedures related to fixation of LD_{50}, mutagenic treatments, plant selection, and generation advancements were followed till M_2 generation. This dedicated program evolved a total of 123 promising mutants for yield (113), photo-insensitivity (6), and reduced duration (4). Owing to strict self-pollinating behaviour of horse gram, the homozygosity of mutants was tested in M_3 generation.

Complete homozygous mutant families with good yield potential totalling 110 were alone tagged and utilized for the current investigation along with two checks. Among the mutagenic treatments, EB 100 Gy evolved the maximum useful mutants (38) followed by EB 300 Gy and G 300 Gy (23). For the identification of potential mutants from a population, a multi-environment based breeding experiment assumes importance. Therefore, the current experiments were conducted in three different environments. The analysis of pooled quantitative data revealed that the induced mutagenesis evolved noteworthy variability for yield attributing traits like number of pods per plant, number of clusters, plant height and single plant yield (CV= 34.80%; 33.90%; 33.80%, 30.44% and 29.60% respectively) while narrow variation was observed for flowering and maturity traits (Table 1). The Mahalanobis D^2 statistics categorized the mutants into ten different groups. A significant variation in cluster wise mutant grouping was observed. The number of mutants in a cluster varied from 1 to 38 (Table 2).

A maximum of 40 per cent of the clusters were solitary indicating the potential of induced mutagenesis in the evolution of trait-specific mutants. Earlier, Varma *et al.* (2013) reported the formation of significant solitary clusters. It is proved that the genotypes of a cluster are genetically related. From a breeding

Table 5: Estimation of anti-nutritional factors ($mg\ g^{-1}$) in the selected horse gram mutants

Cluster	Mutants	Total phenols	Tannins	Phytic acid	Oxalic acid
Cluster I	TNAU-HG-089	0.73	9.37	8.56*	5.32
Cluster II	TNAU-HG-070	0.65*	6.93*	9.58*	5.02
Cluster III	TNAU-HG-007	0.64*	9.16	9.43*	4.38
Cluster IV	TNAU-HG-028	0.75	7.81*	8.23*	4.38
Cluster V	TNAU-HG-081	0.64*	8.71	10.01	3.83
Cluster VI	TNAU-HG-039	0.62*	8.81	10.58	3.99
Cluster VII	TNAU-HG-059	0.70	7.20*	13.24	3.19*
Cluster VIII	TNAU-HG-011	0.88	9.16	12.47	4.28
Cluster IX	TNAU-HG-018	0.55*	9.06	11.78	3.54*
Cluster X	TNAU-HG-078	0.85	8.11	12.78	3.56*
Parent 1	PAIYUR 2	0.95*	9.83	12.96	4.42
Parent 2	CRIDA 1 -18R	0.89	9.69	10.87	4.87
Grand mean		0.74	8.65	10.87	4.23
Standard error (SE)		0.13	0.94	1.76	0.64
Standard deviation (SD)		0.04	0.27	0.51	0.19

Table 6: Details of coefficient of variation observed for nutrient and anti-nutritional factors in the horse gram mutants

	Micronutrients									
	Boron	Magnesium	Phosphorus	Potassium	Calcium	Manganese	Iron	Copper	Zinc	Molybdenum
CV(%)	11.5	11.52	10.86	9.53	18.33	14.25	16.36	12.2	14.5	18.41

	Macronutrients			Anti-nutritional factors			
	Crude protein	Crude fibre	Crude fat	Total phenols	Tannins	Phytic acid	Oxalic acid
CV(%)	5.13	11.67	13.44	17.24	10.88	16.14	15.16

CV - Coefficient of variation

perspective, when more genotypes are grouped in a cluster, it is suggested to select one or two best genotypes to evolve heterotic segregants. Previously, Alle *et al.* (2016) and Singh *et al.* (2020) reported a positive and significant association between single plant yield and its attributing traits *viz.,* number of pods per plant, number of clusters per plant, number of primary branches, and plant height. The same results were obtained in the current investigation as well (Table 1). Based on these findings, the mutants were selected for nutrient profiling based on their single plant yield potential. Accordingly, from each large cluster having different mutants, one mutant with the highest single plant was selected. The selected mutants were TNAU-HG-089 (cluster I), TNAU-HG-070 (cluster II), TNAU-HG-007 (cluster III), TNAU-HG-028 (cluster IV), TNAU-HG-081 (cluster V), TNAU-HG-039 (cluster VI), TNAU-HG-059 (cluster VII), TNAU-HG-011 (cluster VIII), TNAU-HG-018 (cluster IX) and TNAU-HG-078 (cluster X).

The supremacy of selected mutants for yield attributing traits is furnished in Table 2. The single plant yield of the tagged mutants was significantly higher than the respective cluster mean value. All the selected mutants possessed considerably higher values for various yield attributing traits indicating a positive and linear interrelationship. Statistically, no variation was observed for the trait 100 seed weight between parents and mutants. These results are in agreement with the previous findings of Alle *et al.* (2016) and Singh *et al.* (2020) and justify mutant selection for nutrient profiling. From a functional food perspective, horse gram has the following multiple utilities, (i) it exhibits astringent, diuretic and antioxidant properties, (ii) used in the treatment of diarrhoea, haemorrhage and haemorrhoids, (iii) cures menstrual problems, leucorrhea and bleeding during pregnancy, (iv) flushes out the worms on regular consumption, (v) regulates the digestive system and wards off acidity and flatulence, and (vi) lowers the cholesterol level (Ramteke *et al.,* 2016). Nutrient profiling of earmarked horse gram mutants is warranted to identify nutrient specific genetic stocks thereby the development of various genomic resources for functional food research become feasible.

The estimates of macronutrients like crude protein, crude fibre, and crude fat are furnished in Table 3. A higher coefficient of variation was observed for crude fat (13.44%) while a narrow variation was observed for protein content (5.13%) (Table 6). The crude protein content ranged from 21.44% to 25.16%. Two mutants TNAU-HG-070 (25.16) and TNAU-HG-089 (24.95%) had the highest and significant protein content than the grand mean (23.28 %). The mutant TNAU-HG-070 had significant protein (25.16%) and single plant yield (78.86 g) (Table 2 and 3) and hence shall be considered as a donor for protein

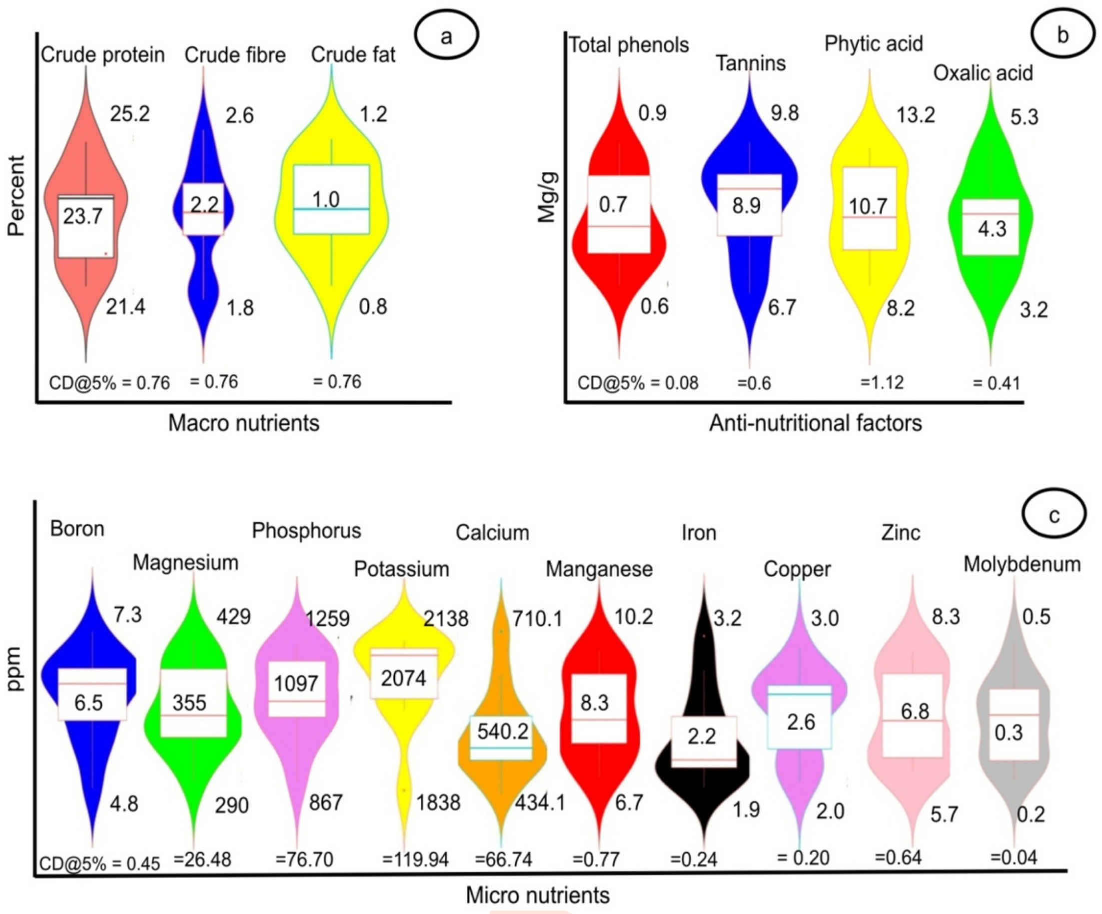
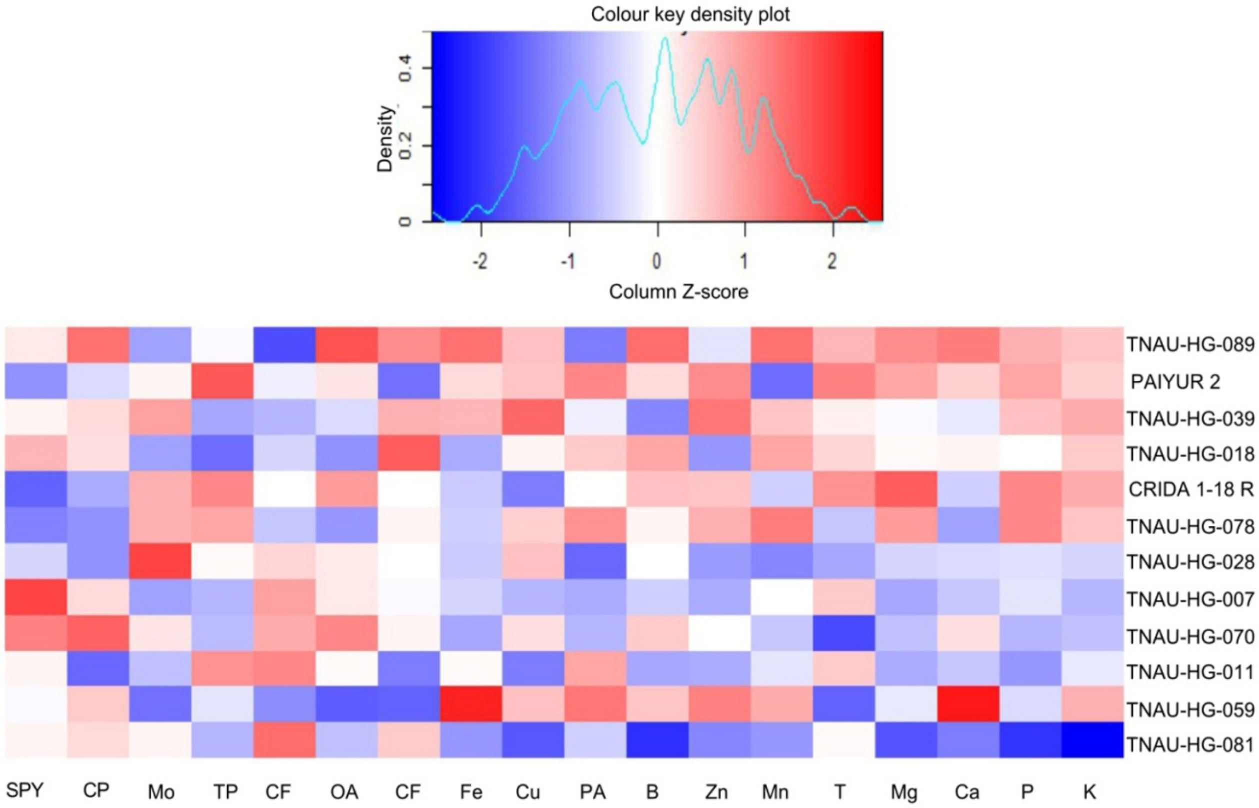


Fig. 1: Violin plot depicting the range for nutritional factors in horse gram: (a) Macronutrients; (b) Anti-nutritional factors and (c) micronutrients.

improvement programs. Similarly, Marimuthu *et al.* (2013) reported higher protein content in horse gram (22.12%). Patangare Suwarna *et al.* (2019) also reported a protein content of 21.87% in horse gram. Kaundal *et al.* (2020) reported protein content ranging between 18.15% and 28.8% in horse gram. The estimation of crude fibre presumes significance in functional food viewpoint, especially diabetic formulations.

The fibre rich food slows the sugar release in the blood upon consumption. A moderate level of variability (11.67%) was observed for crude fibre content. Three mutants TNAU-HG-018 (2.56%), TNAU-HG-089 (2.44%), and TNAU-HG-039 (2.36%) possessed high and significant mean crude fibre content. Of them, TNAU-HG-018 (2.56%) had a single plant yield of 69.26g (Table 2 and 3) and can be considered for further exploitation. Sudha *et al.* (1995) reported fibre contents of whole and dehulled seeds as 5% and less than 2% respectively. Similarly, Patangare Suwarna *et al.* (2019) witnessed a fibre content of 3.9% ± 0.04. The beneficial unsaturated fatty acid content in horse gram is 72.49 per cent (Mishra and Pathan, 2011), and therefore it is suggested to select the genotypes with the highest crude fat content in a breeding program. The maximum fat content was observed in the mutant TNAU-HG-081 (1.22%) with the single plant yield of 57.41g (Table 2 and 3) followed by TNAU-HG-011 (1.19%), TNAU-HG-007 (1.15%), and TNAU-HG-070 (1.14%) and their significance were tested at 5% probability level.

The variability for macronutrients are presented in the Fig. 1a. Earlier, Sreerama *et al.* (2012) reported a fat content of 1.4% and Kaundal *et al.* (2020) reported a fat content range of 1.10 to 1.9%. On the contrary, Patangare Suwarna *et al.* (2019) documented a low-fat content range of 0.45% in horse gram. Though horse gram has nutritional and health-promoting effects, the presence of few anti-nutritional factors (ANFs) restricts its regular consumption as a human food because of few inhibitory effects. In low quantity, ANFs are considered as bioactive compounds (Prasad and Singh, 2015). On the contrary, high levels of ANFs impair the absorption of other essential nutrients during digestion. In the present quest, the ANFs total phenols, tannins, phytic acid, and oxalic acid were estimated (Table 5). The maximum coefficient of variation was observed for total phenols (17.24%) while the minimum was tannins (10.88%) (Fig. 1b). The ranges of ANFs are depicted in Fig. 1b. The minimum values of ANFs are considered for genotype selection. No mutant was



SPY: Single plant yield
CP: Crude protein
Mo: Molbdenum
TP: Total phenols
CF: Crude fibre
OA: Oxalic acid
CF: Crude fat
Fe: Iron
Cu: Copper
PA: Phytic acid
B: Boron
Zn: Zinc
Mn: Manganese
T: Tannins
Mg: Magnesium
Ca: Calcium
P: Phosphorus
K: Potasium

Fig. 2: Relationship between yield, nutrient factors, and anti-nutritional factors in horse gram.

found promising for more than one ANF. The tagged mutants were TNAU-HG-018 (total phenols: 0.55 mg g^{-1}), TNAU-HG-070 (tannins: 6.93 mg g^{-1}), TNAU-HG-028 (phytic acid: 8.23 mg g^{-1}), and TNAU-Hg-059 (oxalic acid: 3.19 mg g^{-1}). Sreerama *et al.* (2012) reported a phytic acid content of 10.2 mg g^{-1}. Thirukkumar and Sindumathi (2014) reported a significant level of phytic acid and tannin in horse gram. Ojha *et al.* (2020) estimated the ranges of the phytate, tannin, and oxalic contents (10.22 mg g^{-1}, 11.75 mg g^{-1} and 3.13 mg g^{-1}). Pagar *et al.* (2021) observed a high tannin content of 319 mg $100g^{-1}$. The importance of micronutrients *viz.,* boron (Nielsen and Eckhert, 2020), magnesium (Rosanoff, 2021), phosphorus (Chen *et al.,* 2016), potassium (Filippini *et al.,* 2020), calcium (Pravina, 2013), manganese (Santamaria, 2008), iron (Briguglio, 2020), copper (Taylor, 2020), zinc (Santos, 2020), and molybdenum (Bell, 2021) in human health is well documented which necessitated the micronutrient profiling of mutants in the current investigation.

The estimated micronutrient contents were compared with the checks and grand mean to identify potential genetic stocks (Table 4). A noticeable amount of variability was detected for all the micronutrients (Fig. 1c). The maximum coefficient of variation was observed for molybdenum (18.41%) followed by calcium (18.33%), iron (16.36%), zinc (14.50%), manganese (14.25%), copper (12.20%), magnesium (11.52%), boron (11.50%), phosphorous (10.86%), and potassium (9.53%). The above listed micronutrients ranged from 4.77 to 7.34 ppm, 290.75 to 429.90 ppm, 867.88 to 1259.63 ppm, 1496.78 to 2138.51 ppm, 434.11 to 810.36 ppm, 6.69 to 10.19ppm, 1.94 to 3.17ppm, 2.01 to 2.98 ppm, 5.71 to 8.28 ppm, 0.25 to 0.46 ppm, respectively (Fig. 1c).

Micronutrient profiling helps in identifying promising mutants. The earmarked mutants were TNAU-HG-089 (boron, magnesium, phosphorous, calcium, manganese, iron, and copper), TNAU-HG-078 (magnesium, phosphorus, manganese, zinc, and molybdenum), TNAU-HG-039 (copper, potassium, iron, zinc, and molybdenum), TNAU-HG-059 (potassium, calcium, manganese, iron, copper, and zinc), TNAU-HG-018 (boron and manganese), and TNAU-HG-028 (copper and molybdenum). Therefore, the selected mutants offer scope for micronutrient biofortification programs. Patil and Kasturiba (2019) reported horse gram genetic stocks for calcium, iron, manganese, and copper. Pagar *et al.* (2021) reported significant calcium and iron contents in raw horse gram. To select potential mutants, the relationship between single plant yield, macronutrient, anti-nutritional factors were ascertained by hierarchal cluster analysis (Fig. 2).

The high yielding genotypes like TNAU-HG-007 (90.17g), and TNAU-HG-070 (78.86g) exhibited a negative relationship with nutrient factors. The mutants with a medium yield potential otherwise had a positive relationship with macro and micronutrients, excepting TNAU-HG-081. By the virtue of yield, and nutrient factors with less ANF, the mutants TNAU-HG-018 (69.26g), TNAU-HG-039 (57.25g), and TNAU-HG-059 (53.74g) were tagged as useful genetic stocks for further utilization. Earlier, Raatz (2018) established a negative relationship between yield and nutritional content. In the high yielding genotypes, a dilution effect on micronutrient contents was reported due to significant translocation of carbohydrate to the grain (Diapari *et al.,* 2014).

Induced mutagenesis evolved significant variability for yield and nutrient contents in horse gram. It is suggested (i) to select genotypes with medium yield potential to alleviate malnutrition, and (ii) the identified mutants with high macro and micronutrients with low anti-nutritional factors can further be utilized as the donor for trait-specific introgression or MAGIC population development. Considering yield, nutritional and anti-nutritional factors, the mutants TNAU-HG-018, TNAU-HG-039, and TNAU-HG-059 offer scope for further exploitation. This experiment helped in quantification of variability for quantitative and nutritional traits in horse gram which presumes importance in nutri-genomic studies.

Acknowledgments

The scientific support rendered by Dr. S.D. Sivakumar, Dr. Leelavathi, Dr. Vellaikumar, Dr. Mini, Dr. Jasmine Juliet, and Dr. S. Rajkumar are highly appreciated.

Add-on Information

Authors' contribution: R. Sudhagar: Conceptualization, experimentation, data analysis, and manuscript preparation; **V. Pushpayazhini:** Experimentation, data documentation and analysis; **C. Vanniarajan:** Conceptualization, and manuscript correction; **S.J. Hepziba:** Supervision and manuscript correction; **R. Renuka:** Experimentation, and data analysis; **J. Souframanien:** Conceptualization, manuscript correction and funding.

Research content: The research content of manuscript is original and has not been published elsewhere.

Funding: This is an independent research, did not receive any funding support.

Ethical approval: Not applicable.

Conflict of interest: The authors express no conflict of interest.

Data from other sources: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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