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Diversity Analysis in Horse Gram [*Macrotyloma uniflorum* (Lam.) Verdc.] Mutants

V. Pushpayazhini[1], R. Sudhagar[2], C. Vanniarajan[1], S. Juliet Hepziba[1], J. Souframanien[3]

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ABSTRACT

Background: Horse gram is the potential rainfed legume in Indian farming. The major limitation in horse gram breeding is the narrow variability. Variability induction and its estimation would sustain food security.

Methods: Variability was induced using gamma rays, electron beam and ethyl methanesulfonate and their combinations. The induced variation for the economic traits, their inheritance and genetic gain were ascertained.

Result: The analysis of variance indicated the induction of significant variation for yield attributing traits. The population was grouped into 10 constellations by the virtue of induced variation. The groups I, II and V were the largest comprising of 38, 31 and 19 mutants respectively. The mutants exhibited significant intra and inter group variation. The mutagens induced the maximum variability for plant height (32.24%), 100 seed weight (25.42%) and number of pods per plant (19.18%). The mutants possessed high genotypic and phenotypic coefficients of variation for all the characters except flowering traits and duration. The induced variability for the yield attributing traits possessed significant breeding value as the heritability (86.66%-99.72%) and genetic advance as percent of mean (10.65-81.94) were high and the environmental influence was the minimum.

Key words: Diversity, Horse gram, Induced mutation, Mutant population.

INTRODUCTION

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] is the lifesaving legume for resource poor and marginal land farmers of India. It is known for its nutritive and therapeutic values since millennia (Kumar, 2006). Owing to its genome re-silencing capacity to various biotic and abiotic stresses it helps to sustain the nutritional security in the rainfed farming (Purushottam *et al*., 2017). The potential yield of horse gram is challenged by few undesirable agronomic traits like photo-sensitivity, fairly long duration, after duration pod shattering at maturity and susceptibility to few foliar diseases like yellow mosaic virus and powdery mildew (Dutta *et al*., 2020). Identification and utilization of donor and /or resistant parents for these negative traits would help in realizing the potential yield. Presence of variability for yield attributing traits is limited in the horse gram germplasm (Chahota *et al*., 2013). The flower morphology, pollination behavior and flower shedding after hybridization limit the success of classical plant breeding. The horse gram varieties PAIYUR 2 and CRIDA 1-18 R are the promising cultivars in Southern India. The diploid and comparatively small genome size of horse gram favours the usage of induced mutagenesis for variability evolution. The success of induced mutagenesis in variability creation was witnessed in horse gram (Priyanka *et al*., 2021a). The breeding value of the mutants can be ascertained through estimating the induced variability for yield attributing traits and their genetic structure. Mahalonobis D^2 statistic (Mahalanobis, 1936) is one such methodology that helps in drawing meaningful information and assists in predicting the breeding value including horse gram (Priyanka *et al*., 2019).Therefore, this experiment was framed with the objective of ascertaining the magnitude of variability and other associated genetic parameters in an induced mutant population.

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[1]Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai-625 104, Tamil Nadu, India.
[2]Sugarcane Research Station, Tamil Nadu Agricultural University, Melalathur, Vellore-635 806, Tamil Nadu, India.
[3]Apex Project Coordinator and Research Group Leader, Pulses Improvement Section, Bhabha Atomic Research Centre, GOI, Trombay, Mumbai-400 085, Maharashtra, India.

Corresponding Author: R. Sudhagar, Sugarcane Research Station, Tamil Nadu Agricultural University, Melalathur, Vellore-635 806, Tamil Nadu, India. Email: sudhagar.r@tnau.ac.in

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MATERIALS AND METHODS

Genetic material and data documentation

During *rabi* season 2017, the horse gram varieties PAIYUR 2 and CRIDA 1-18 R were mutated with gamma rays (G) (100, 200, 300 and 400 Gy), electron beam (EB) (100, 200, 300 and 400 Gy), G+EB (100, 200, 300 and 400 Gy) and combination of G and ethyl methanesulfonate (EMS) (G: 100-400 Gy + EMS: 0.3%). The potential M$_2$ segregants were tagged during *rabi* 2018 and the homozygosity was checked during *rabi* 2019 in M_3 for the promising mutant families. A total of 123 promising homozygous mutant families were forwarded to M_4 generation during *rabi* 2020 at Sugarcane Research Station, Tamil Nadu Agricultural University, Melalathur. The mutants were grown in the randomized block design (RBD) in three replications along with field fertility gradient. The crop was grown in an ideal cultivation condition. For estimating the breeding value, only 110 mutant families (TNAU-HG-001 to TNAU-HG-110) were considered as the other 13 mutants were suffered by inferior agronomical traits. Eleven biometrical traits *viz.*, days to first flowering, days to fifty percent flowering, days to maturity, plant height (cm), number of primary branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, single plant yield (g) and 100 seed weight (g) were documented at appropriate growth stages. For data documentation twenty plants were randomly selected at each replication. The genotypic and phenotypic coefficients of variation were estimated according to Burton (1952) and classified as per SivaSubramanian and Menon (1973). Broad sense heritability (H^2) was computed as suggested by Lush (1949) and the traits were grouped as per Johnson *et al*. (1955). The genetic advance as percent of mean was arrived and categorized (Johnson *et al*., 1955). The Mahalonobis D^2 statistic (Mahalanobis, 1936) was done through Indostat software version 7.1. The D^2 was calculated by squaring the generalized distance and thereon utilized for clustering the mutant families as suggested by Tocher method as advocated by Rao (1952).

RESULTS AND DISCUSSION

Horse gram is the arid *rabi* legume provides ray of scope of nutrient sustainability in the Indian marginal lands. The yield of horse gram is hampered by few inherent agronomic traits linked to growth and duration. The tune of variability is the basic requirement of crop improvement program (Bhandari *et al.,* 2017), horse gram is not an exception. Horse gram improvement is slowed by limited variability (Chahota *et al*., 2013). Induced mutagenesis widens the variability (Priyanka *et al*. 2021a and b). Ascertaining the breeding value of a mutant population would strengthen the breeding program. The analysis of variance (ANOVA) is used to understand the divergence for traits in a population. In the current study it displayed statistically significant difference for all the eleven quantitative traits (Table 1) which indicated the presence of wide variability. Earlier Priyanka *et al*. (2019) also reported significant difference among the yield attributing traits and utility of D^2 statistic. Significant variation due to replication was witnessed which was possibly due to variation in fertility gradient at blocks as the experiment was laid out along with field fertility gradient and large number of genotypes.

The 110 mutants and two checks were grouped into ten clusters (Table 2). The cluster I was the largest with 38 mutants followed by II (31 mutants), V (19 mutants), IV (11 mutants) and III (7 mutants). The number of solitary clusters was quite high (Table 2) which indicating the potential of induced mutagenesis in evolving unique mutants as the study material were not collected from different geographical regions. An array of useful trait specific mutants was also identified for further utilization. Priyanka *et al*. (2021b) established the potential of induced mutagenesis in variability evolution and formation of solitary clusters by Varma *et al.* (2013).

The induced mutagenesis created the maximum variability for plant height (32.24%), 100 seed weight (25.42%) and number of pods per plant (19.18%) while it failed to induce variability for days to maturity (Fig 1). Therefore, the probability is high to improve the test traits except days to maturity by classical hybridization and selection thereon. This is in confirmatory with the earlier findings of Priyanka *et al*. (2021b).

The highest intra cluster distance exhibited by cluster V (19.94) and cluster IV (19.93) (Table 3) revealed the wide genetic base and thus could be utilized for genetic advancement (Puneet, 2004). Zero intra cluster distance was observed between the clusters VI, VII, VIII and IX, indicating the close relatedness. The measurements of inter cluster distance is an indicative selection tool for identification of promising clusters. The maximum inter cluster distance was noticed between the clusters X and VIII (67.18), followed by clusters X and IX (65.57) and clusters X and III (63.94) mooted the idea of hybridization between these diverse clusters to evolve heterotic segregants. On contrary, the minimum distance was observed between the clusters VII and II (20.60) followed by cluster VI and II (21.21) indicating the close relatedness of mutants (Durga Kanaka, 2012).

Table 1: Analysis of variance for quantitative traits in horse gram mutants.

Source of variation	DF	DFF	DM	PH	NPB	NCR	NPC	NPP	NSP	TW	SPY
Replication	2.431**	27.645**	6.717	28.693**	0.145	578.609**	0.413**	74.098	4.83**	0.02**	66.551**
Treatment	36.696**	42.486**	137.692**	3590.993**	10.283**	4856.612**	1.5928**	32446.038**	1.564**	0.475**	716.517**
Error	0.909	0.654	14.134	14.138	0.283	59.937	.053	90.485	0.208	0.0027	6.395

**Significant at 1% level;*Significant at 5% level.

DF – Days to first flowering; DFF - Days to fifty percent flowering; DM - Days to maturity; PH - Plant height (cm); NPB - Number of primary branches per plant; NCR- Number of clusters per plant; NPC- Number of pods per cluster; NPP- Number of pods per plant; NSP - Number of seeds per pod; TW- 100 seed weight (g); SPY – Single plant yield (g).

The estimate of average cluster mean helps in selection of trait specific mutants (Table 4). Cluster X had the lowest cluster mean values for days to first flowering (32.50), days to fifty percent flowering (35.83) and days to maturity (90.50) and hence favorably be considered to evolve genotypes with early duration. Cluster III (75.04), cluster IX (69.26) and cluster VI (57.25) possessed the highest mean values pronounced their suitability to generate high yielding genotypes. The solitary cluster VI had the maximum mean value for plant height (197.63). The other solitary clusters VII, VIII and IX possessed the highest mean values for number of seeds per pod (6.33), number of pods per plant (578.67) and number of clusters per plant (245.83) respectively. These clusters shall be considered for specific trait improvement.

In the quest, high estimates of GCV and PCV were observed for all the experimented quantitative traits except days for flowering and maturity and that indicated the existence of adequate variability (Table 5). Earlier, Priyanka *et al*. (2019) reported wide variability for these traits. However Alle *et al*. (2015) reported the lowest GCV and PCV for flowering and maturity. The PCV was found to be slightly

Table 2: Clustering pattern and promising horse gram mutants.

Clusters with more than one mutant

Cluster	Number of mutants	Trait specific promising mutants*	
		High yield (grams)	Early duration (days)
I	38	TNAU-HG-089(59.46), TNAU-HG-008(56.34) TNAU-HG-064(55.75), TNAU-HG-033(54.53) and TNAU-HG-084(52.27)	TNAU-HG-095 (105.33), TNAU-HG-084 (111.33) TNAU-HG-110 (116.00), TNAU-HG-091 (116.00) and TNAU-HG-060 (116.67)
II	31	TNAU-HG-070 (78.86), TNAU-HG-049 (71.33) TNAU-HG-062 (68.47), TNAU-HG-073 (68.24) andTNAU-HG-083 (67.54)	TNAU-HG-030 (116.67), TNAU-HG-073 (117.00) TNAU-HG-075 (117.33), TNAU-HG-025 (117.67) and TNAU-HG-053 (117.67)
III	7	TNAU-HG-007 (90.17), TNAU-HG-031 (79.95) and TNAU-HG-034 (77.63)	TNAU-HG-016 (115.33) and TNAU-HG-027 (117.33)
IV	11	TNAU-HG-028 (47.44), TNAU-HG-004 (45.80) TNAU-HG-048 (45.54), TNAU-HG-029 (43.75) and TNAU-HG-063 (43.32)	TNAU-HG-107 (117.67), TNAU-HG-014 (118.33) TNAU-HG-013 (118.67), TNAU-HG-106 (119.00) and TNAU-HG-063 (119.33)
V	19	TNAU-HG-081 (57.41), TNAU-HG-061 (50.63) TNAU-HG-035 (50.46), TNAU-HG-088 (42.55) and TNAU-HG-041 (38.52)	TNAU-HG-099 (101.67), TNAU-HG-104 (102.00) TNAU-HG-096 (102.33), TNAU-HG-103 (102.67) and TNAU-HG-102 (106.00)
X	2	TNAU-HG-078 (31.62)	TNAU-HG-078 (88.67)
Solitary clusters			
		Mutant	Trait
VI	1	TNAU-HG-039	Plant height (197.63cm)
VII	1	TNAU-HG-059	Number of seeds per pod (6.33) and 100 seed weight (4.08g)
VIII	1	TNAU-HG-011	Number of pods per plant (578.67)
IX	1	TNAU-HG-018	Number of clusters per plant (245.83)

*Mutants with more than cluster mean value.

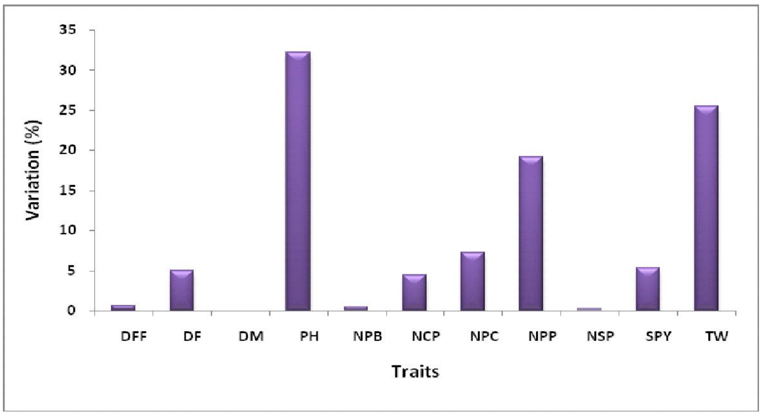

Fig 1: Per cent variation created for yield attributing traits by induced mutagenesis in horse gram.

higher than GCV indicating the less environmental influence and therefore phenotypic based selection could be relied upon for improvement.

High H^2 (86.66 % - 99.72%) was observed for all traits which indicated the non-significant influence of environment. Combined estimate of H^2 and GAM provides an opportunity to predict genetic gain (Table 5). High heritability coupled with high GAM was observed for all traits indicating the preponderance of additive gene action. Therefore, these traits could be improved by phenotype based selection. High H^2 and GAM were reported for yield attributing traits except flowering and maturity traits (Joshi *et al*., 2007). It can be

Table 3: Details of mean intra (bold) and inter cluster distance in horse gram mutants**.**

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**15.54**	22.65	35.84	22.43	23.44	32.63	26.85	41.47	39.87	44.69
II		**16.71**	26.76	26.27	32.87	21.21	20.60	38.43	28.67	51.33
III			**16.84**	31.47	48.42	22.30	36.67	25.04	24.16	63.94
IV				**19.93**	34.08	31.54	34.75	30.97	35.56	52.01
V					**19.94**	45.08	34.77	54.35	52.05	37.05
VI						**0.00**	23.36	35.28	25.74	62.95
VII							**0.00**	51.31	35.55	54.80
VIII								**0.00**	35.66	67.18
IX									**0.00**	65.57
X										**17.56**

Table 4: Cluster mean values obtained by Tocher's method for quantitative traits in horse gram mutants.

Cluster	DF	DFF	DM	PH	NPB	NCR	NPC	NPP	NSP	TW	SPY
I	51.44	58.90	118.92	109.98	5.46	88.31	3.00	254.71	5.36	3.32	41.12
II	50.85	57.94	120.41	143.10	7.04	120.33	2.94	332.69	4.88	3.73	56.53
III	50.24	57.90	119.95	154.49	10.05	148.85	3.52	502.81	5.00	3.41	75.04
IV	52.30	59.09	120.55	124.23	4.79	117.37	3.18	349.88	4.76	2.87	39.50
V	48.95	56.14	110.18	73.79	4.74	57.06	3.16	176.33	4.91	3.63	29.85
VI	53.00	59.00	118.33	197.63	8.67	100.17	4.00	400.67	4.33	3.77	57.25
VII	51.67	60.67	122.00	175.03	7.33	112.33	2.00	224.67	6.33	4.08	53.74
VIII	49.00	56.67	119.00	138.60	6.33	144.67	4.00	578.67	4.67	2.54	57.31
IX	51.00	58.00	122.33	178.59	9.00	245.83	2.00	491.67	4.67	3.48	69.26
X	32.50	35.83	90.50	49.20	4.17	41.18	3.50	136.17	5.67	3.39	25.65
PAIYUR 2 (P1)	49.67	58.00	109.00	98.03	5.67	93.67	3.00	281.00	4.67	3.88	34.13
CRIDA 1-18 R (P2)	40.33	45.33	96.00	76.17	5.00	67.17	2.67	173.00	4.67	3.44	26.16

DFF- Days to first flowering; DFF - Days to fifty per cent flowering; DM - Days to maturity; PH - Plant height (cm); NPB - Number of primary branches per plant; NCR- Number of clusters per plant; NPC- Number of pods per cluster; NPP- Number of pods per plant; NSP - Number of seeds per pod; TW- 100 seed weight (g); SPY- Single plant yield (g). P1: Parent 1; P2: Parent 2.

Table 5: Estimates of genetic parameters for quantitative traits in horse gram mutants.

Quantitative trait	GCV	PCV	H^2	GAM
Days to first flowering	6.84	6.92	97.52	13.91
Days to 50% flowering	6.47	6.52	98.46	13.23
Days to maturity	5.46	5.76	89.73	10.65
Plant height (cm)	29.18	29.24	99.61	59.99
Number of primary branches per plant	30.13	30.55	97.24	61.20
Number of clusters per plant	40.03	40.28	98.77	81.94
Number of pods per cluster	23.34	23.74	96.65	47.27
Number of pods per plant	35.59	35.64	99.72	73.22
Number of seeds per pod	13.28	14.27	86.66	25.47
100 seed weight (g)	11.50	11.54	99.41	23.62
Single plant yield (g)	33.58	33.74	99.11	68.88

GCV – Genotypic coefficient of variation; PCV – Phenotypic coefficient of variation; H^2- broad sense heritability; GAM- Genetic advance as percent of mean.

concluded that the induced mutagenesis has the capacity to evolve significant variation in horse gram and it is highly heritable. The improved traits are governed by additive gene action and further they can be improved using phenotypic selection.

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