LR-5052
[1-7]

RESEARCH ARTICLE **Legume Research- An International Journal**

Organization of Genetic Variability and Trait-specific Accessions in a Diverse Set of Horse Gram [*Macrotyloma uniflorum* (Lam.) Verdc.] Germplasm

R. Kirankumar, S. Ramesh, B.R. Chandana, G. Basanagouda, C.B. Siddu, M.P. Kalpana

10.18805/LR-5052

ABSTRACT

Background: Mere presence of phenotype-based quantitative traits (QT's) variability is of less significance in breeding crops including horse gram. A dependable knowledge on relative contribution of genetic and non-genetic sources on the QT's variability and organization of variability in terms of similar and diverse groups of genotypes is useful in selection of most desired parents to generate breeding populations and in formulating appropriate selection strategies to breed improved horse gram cultivars. The objectives of the study were to (i) quantify QT's variability and (ii) identify trait-specific accessions in a diverse set of germplasm accessions.

Methods: A set of 196 horse gram germplasm accessions along with two checks were evaluated in simple lattice design with two replications at two locations, representing southern and eastern dry zones of Karnataka, India during 2020 and 2021 late rainy seasons for five quantitative traits.

Result: The germplasm accessions differed significantly and variance attributable to genotypes contributed over 95% to total phenotypic variance for all the traits. The germplasm accessions could be grouped into four clusters. The quantitative trait means and variances of accessions distributed among the four clusters differed significantly. The trait-specific accessions identified in our study are suggested for preferential use in generating variability followed by selection in F_2/BC_1 populations for economically important traits.

Key words: Germplasm, K-means clustering, Quantitative traits, Trait-specific accessions, Variability.

INTRODUCTION

Horse gram is one of the important climate resilient indigenous grain legume crops grown in India. It is the fifth most widely grown legume crop in India. It is self-pollinated crop with 2n=20 chromosomes (Halder, 2012). It is one of the good sources of protein to a large number of people, especially to those depending on vegetarian diet for source of energy (Morris, 2008). It is predominantly grown in marginal soils in rainfed ecosystems by resource-poor farmers (Chandana *et al.* 2022). The productivity of horse gram in farmers' fields is rather low compared to that of other legumes such as soybean, cowpea, green gram, black gram, pigeon pea, *etc.* Limited efforts to breed horse gram compared to other legumes (among others) is attributed to low horse gram productivity (Chandana *et al.* 2022). Further, breeding populations are most often developed by crossing elite parents in horse gram as well, as is true in other self-pollinated crops. Use of such elite parents leads to decreased genetic diversity in breeding populations and in the cultivars that are released and used. In major crops such as maize, rice, wheat, soybean, tomato, *etc.,* breeding programmes are differentiated largely by the germplasm that they have and use instead of by the breeding methods that they use. Breeding programmes therefore need a steady infusion of new germplasm to ensure long-term genetic gains (Bernardo, 2020). Introduction of farmer- and consumer-preferred traits in crops through breeding has led to an increase in genetic diversity in crop cultivars (Schouten *et al.* 2019). Increased and continued use of genetic resources (GR) is the key to enhance the genetic diversity within breeding programmes and to enhance productivity of crops including horse gram. This requires eliciting dependable information on the extent and organization of variability, relative contribution of genotypes and genotype × environment interaction towards total phenotypic variability for economically important quantitative traits. Identification of trait-specific and multiple trait-specific GR is yet another prerequisite for breeding horse gram cultivars with improved productivity and desired combination of traits (Upadhyaya *et al.* 2013).

Short-term, medium and long-term strategies were and are being adopted to use GR in breeding crops (Bhandari *et al.* 2017; Swarup *et al.* 2021). As a short-term strategy,

Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India.

Corresponding Author: R. Kirankumar, Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Bangalore-560 065, Karnataka, India.
Email: kirankumarrotti1996@gmail.com

How to cite this article: Kirankumar, R., Ramesh, S., Chandana, B.R., Basanagouda, G., Siddu, C.B. and Kalpana, M.P. (2022). Organization of Genetic Variability and Trait-specific Accessions in a Diverse Set of Horse Gram [*Macrotyloma uniflorum* (Lam.) Verdc.] Germplasm. Legume Research. doi: 10.18805/LR-5052.

Submitted: 01-10-2022 **Accepted:** 19-12-2022 **Online:** 24-01-2023

desired traits can be transferred to elite popular cultivars defective in such traits through conventional/marker-assisted backcross breeding programs. As a medium term strategy, GR with desired traits' combination could be crossed with elite genotypes to generate breeding populations for subsequent selection of recombination inbred line (RILs) for use as pure-line cultivars. Pre-breeding *i.e.*, conversion of unadapted/less adapted and immediately unusable GR harbouring one/few desired traits into usable ones for subsequent use in pure-line cultivar development is a long-term strategy (Swarup *et al.* 2021). Under these premises, the objective of our investigation were to assess and explore organization of variability for quantitative traits and to identify trait-specific accessions from the diverse set of horse gram germplasm.

MATERIALS AND METHODS

The material for the study consisted of 196 germplasm accessions originating from different geographical locations in India including two check varieties *viz.,* PHG 9 and BGM 1 (Fig 1). The checks, PHG 9 and BGM 1 are high-yielding pure-line varieties released by University of Agricultural Sciences (UAS), Bangalore, India for commercial horse gram production in southern and eastern dry agro-climatic zones of Karnataka, India. The seeds of 196 germplasm accessions were sown in simple lattice design with two replications at two locations, namely Gandhi Krishi Vignana Kendra (GKVK), Bengaluru and Zonal Agricultural Research station (ZARS), VC Farm, Mandya during 2020 and 2021 late rainy seasons. While GKVK, Bengaluru represents eastern dry zone (EDZ), ZARS, Mandya represents southern dry zone (SDZ) of Karnataka. Each accession was sown in a single row of 3 m length with row-to-row spacing of 0.45 m. Fifteen-days after sowing, the seedlings were thinned to maintain plant-to-plant spacing of 0.15 m. A total of 15 to 16 plants survived to maturity in each accession. Recommended crop management practices were followed during the crop growth period to raise a healthy crop.

Sampling of plants and data collection

Data were recorded on ten randomly chosen plants (avoiding border ones) in each accession on five quantitative traits namely days to 50% flowering, primary branches plant^{-1} pods plant^{-1}, pod yield plant^{-1} (g) and grain yield plant^{-1} (g).

Statistical analysis

Replication-wise and location-wise quantitative trait means of each accession computed across two years were used for statistical analysis. Analysis of variance (ANOVA) was performed separately for each location data. Combined ANOVA was also performed to detect genotype × location interactions (GLI) and to quantify relative contribution of genotype, location and GLI towards total variation for all the five quantitative traits. For all the traits, best linear unbiased predictors (BLUPs) using linear mixed model and components of phenotypic, genotypic, location and GLI variability were estimated using restricted maximum likelihood (REML) methods (Piepho *et al.* 2008). All these statistical analysis was implemented using META-R software version 6.0. Descriptive statistics such as traits mean, absolute range (Highest – Lowest mean) standardized range (Absolute range/mean), phenotypic coefficient of variation (PCV) were estimated using statistical analysis option available in MS Excel software. The 196 accessions were classified into four clusters following model-based "K means" clustering approach (MacQueen 1967) to unravel organization of variability. These four clusters were determined by minimizing and maximizing "within-cluster" and "between-cluster" variances' respectively. As an obvious consequence of this approach, the accessions with comparable quantitative trait means were grouped into a single cluster and those with different trait means were grouped into a different cluster. As a corollary, we hypothesized that quantitative trait means and variances of accessions classified into different clusters would differ significantly. To test this hypothesis, the significance of differences in trait means and variances of the accessions

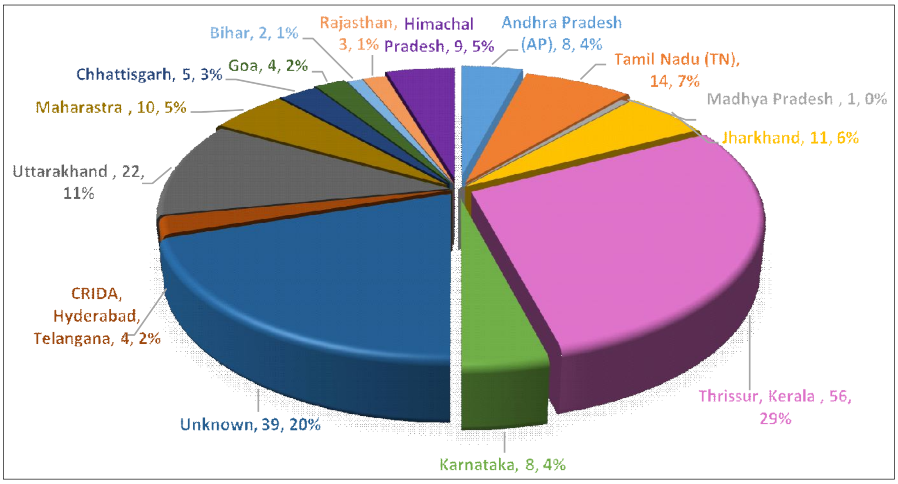

Fig 1: Pie chart showing geographical origin of horse gram germplasm accessions.

distributed among the clusters was examined using "one-way ANOVA" and Levene's test (Levene 1960), respectively.

Identification of trait-specific accessions

Based on early flowering time (Mean-1SD) *i.e.* ≤40.35 days from date of sowing and higher (Mean+2 SD) expression of the accessions for other four traits, the promising accessions for each of the five traits and those with combination of traits (multiple traits-specific) were identified.

RESULTS AND DISCUSSION

ANOVA

Variability attributable to genes controlling target traits is referred to as genetic variation. As genes cannot express in vacuum and require appropriate environment such as spatial environment (location) in the present study, the degree of expression of traits controlled by genes is impacted by environment. Selection is effective only on variation attributable to genotype *per se* and/or non-crossover genotype × environment interaction. It is therefore imperative to detect and quantify genetic and its interaction variation in breeding populations and/or germplasm accessions.

ANOVA is a diagnostic step to detect significance of different desired sources of variability for target quantitative traits. The accessions (genotypes), location and genotype × location interaction (GLI) contributed significantly to total phenotypic variability for all the traits except GLI for days to 50% flowering (Table 1). Significance of mean squares attributable to locations not only suggested differential expression of accessions in two locations, but also justified the selection of locations for evaluation of the accessions. However, substantially lower variance attributable to GLI compared to that attributable to genotype (Table 2) suggest comparable performance ranks of accessions in the two locations for all the five traits (Bernardo, 2020). BLUPs allow the comparison of accessions over time (generation, year) and space (location, block) by minimizing their effects (Bernardo, 2020; Tajalifar and Rasooli, 2022). Hence, traits BLUPs were estimated within and across locations for all the germplasm accessions. In both the locations, the accessions varied widely as could be inferred from the estimates of absolute and standardized range and PCV for all the traits (Table 3; Fig 2). The estimates of range provide clues about the occurrence of accessions with extreme trait expression. The estimates of unit-independent standardized range of the accessions were higher for primary branches plant^{-1}, pods plant^{-1}, pod yield plant^{-1} (g) and grain yield plant^{-1} (g) compared to that for days to 50% flowering, which were

Table 1: Pooled analysis of variance of horse gram germplasm accessions for five quantitative traits.

Sources of variation	Df	Mean sum of squares				
		Days to 50% flowering	Primary branches plant^{-1}	Pods plant^{-1}	Pod yield plant^{-1} (g)	Grain yield plant^{-1} (g)
Location	03	732.46**	11.72**	2803.87**	86.13**	26.43**
Replication (Location)	04	473.67*	03.54*	1725.71*	54.68*	17.22**
Block (Replication × Location)	52	01.86	0.01	0.99*	0.04*	0.01*
Genotypes	195	92.74**	10.71**	1665.52**	77.23**	13.53**
Genotypes × Location	585	02.79	0.01**	31.73**	1.33**	0.20**
Error	728	02.89	0.01	0.41	0.02	0.01

Df- Degrees of freedom, ** Significant at P=0.01; * Significant at P=0.05.

Table 2: Estimates of components of phenotypic variability among horse gram germplasm accessions for five quantitative traits.

Components	Days to 50% flowering	Primary branches plant^{-1}	Pods plant^{-1}	Pod yield plant^{-1} (g)	Grain yield plant^{-1} (g)
Genotype variance (σ^2_g)	11.24	01.34	204.21	09.49	01.66
Environment variance (σ^2_e)	00.66	00.02	002.67	00.08	00.02
Genotype × Location variance ($\sigma^2_{g\times l}$)	00.01	00.01	015.61	00.65	00.10
Residual variance ($\sigma^2_{residual}$)	02.81	00.01	000.41	00.01	00.04
Heritability (h^2) (%)	96.97	99.96	098.10	98.28	98.52

Table 3: Descriptive statistics for five quantitative traits of horse gram germplasm accessions.

Traits	Mean±SE	Range		Standardized range	PCV (%)
		Min	Max		
Days to 50% flowering	43.89±0.25	39.04	58.63	0.44	08.06
Primary branches plant^{-1}	03.92±0.08	01.50	06.20	1.19	29.50
Pods plant^{-1}	36.06±1.01	10.50	81.25	1.96	39.25
Pod yield plant^{-1} (g)	07.34±0.22	02.62	17.44	2.02	41.61
Grain yield plant^{-1} (g)	03.42±0.09	001.2	07.43	1.82	37.41

amply reflected by the estimates of PCV (Table 3). The magnitude of PCV varied substantially even among the former four traits. Similar results were reported by Priyanka *et al.* (2021) and Visakh and Bindu (2022) in horse gram. Given that GR evolve as a result of natural selection and/or human selection, substantial variability among the accessions could be attributed to accumulations of mutant alleles at loci controlling traits relevant to fitness (under natural selection pressure) and those relevant to domestication (Swarup *et al.* 2021).

Organization of variability

Hybridization is the most convenient and hence popular method of generating variability. Selection of the most suitable parents for use in hybridization is the key for generating useful and exploitable variability. Quantitative genetic theory suggests genetically diverse genotypes (though phenotypically similar) preferably those in which desirable alleles are dispersed between them are most likely to uncover high frequency of transgressive RILs. The rationale for maximizing the frequency of transgressive RILs is that plant breeding was/is being successful owing to the occurrence of transgressive RILS. Without the occurrence of transgressive RILs, plant breeding would not work (Mackay *et al.* 2021). Such genetically diverse genotypes can be easily identified by classifying the genotypes into different clusters by maximizing the variability between clusters and minimizing variability within clusters. In the present study, we could classify the accessions into four clusters.

The quantitative trait mean differences among the four clusters were significant for all the traits (Table 4). The trait variances among the four clusters were also significant for all the traits, except for primary branches plant^{-1} (Table 5). These results suggested effectiveness of 'K-means'

Table 4: Estimates of quantitative traits means of horse gram germplasm accessions classified into different clusters.

Traits	C_1	C_2	C_3	C_4	'F' Statistic	Probability
Size of the cluster	38	20	56	82		
Days to 50% flowering	46.53	49.32	43.60	43.84	029.89	0.001
Primary branches plant^{-1}	04.43	05.05	03.15	03.94	022.73	0.001
Pods plant^{-1}	47.74	65.42	21.29	33.58	572.66	0.000
Pod yield plant^{-1} (g)	09.52	12.59	04.48	07.00	124.07	0.000
Grain yield plant^{-1} (g)	04.15	05.59	02.13	03.44	117.13	0.000

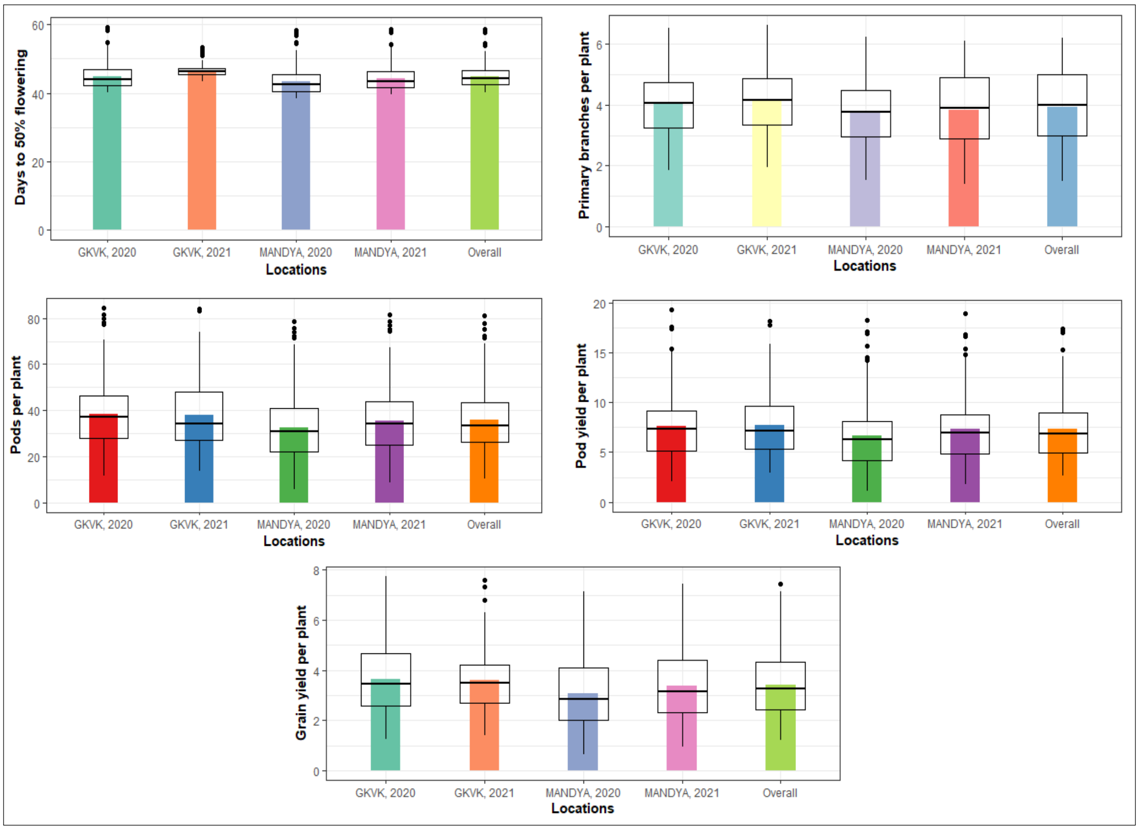

Fig 2: Box-Whisker and bar plots showing mean variation for five quantitative traits within and across locations.

Note: The length of bar plots represents the trait means, while total length of box and whisker line represents absolute range of traits.

clustering approach to classify the accessions into different clusters. Effective classification of accessions into different clusters enable reliable identification of genetically diverse genotypes for use as parents for generating breeding populations. The estimates of the mean of the five quantitative traits are contrasting among the accessions included in Clusters II and III. It is therefore desirable to choose accessions from among those included in Cluster II and III for various applications in horse gram breeding research and for developing improved cultivars. Through the increased use of these accessions, the efficiency of horse gram breeding for widely/specifically adapted and highly stable high-yielding pure-lines could be maximized. Several researchers classified varying number of horse gram accessions into different clusters. For example, Dasgupta *et al*. (2005) classified 50 accessions into ten clusters, Singhal *et al*. (2010) classified 88 accessions into five clusters, Geetha *et al*. (2011) classified 100 accessions into sixteen clusters and Kanaka *et al.* (2015) classified 38 accessions into two clusters respectively.

Trait-specific accessions

The use of trait-specific accessions help meet short-term objectives, as very often breeders are forced to meet immediate requirement of farmers, consumers and end-users. In the present study, several trait-specific accessions were identified (Table 6). A few of these accessions were

Table 5: Estimates of quantitative traits variances among horse gram germplasm accessions classified into different clusters.

Traits	C$_1$	C$_2$	C$_3$	C$_4$	'F' Statistic	Probability
Size of the cluster	38	20	56	82		
Days to 50% flowering	10.86	24.56	02.76	05.29	7.35	0.001
Primary branches plant^{-1}	00.87	00.82	00.96	01.14	1.20	0.310
Pods plant^{-1}	19.49	54.43	19.13	13.82	7.72	0.000
Pod yield plant^{-1} (g)	05.24	05.61	01.43	02.96	4.23	0.006
Grain yield plant^{-1} (g)	00.79	00.83	00.27	00.65	4.24	0.006

Table 6: Promising trait-specific accessions identified from horse gram germplasm collections.

Traits	Selection criteria	Range	Germplasm accessions
Days to 50% flowering	Earliness (Mean-1SD)≤40.35	39.04-40.35	IC 56130, IC 45748, IC 383374, IC 277670, NBPGRT-192, IC 27767, NBPGRT-629, HPKM-319, Birsa-Kulthi, IC 3612 90, IC 383475, IC 391576, NBPGRT- D107, IC 383202
Primary branches plant^{-1}	High (Mean+2SD) ≥6.10	6.10-6.20	IC 1 39419, IC 105785, IC 139556
Pods plant^{-1}	High (Mean+2SD) eH64.36	64.36-81.25	IC 139556, IC 15728, CRHG-19, IC 105785, IC 139329, Paiyur 1, IC 78605761, Paiyur 2, NBPGRT-D179
Pod yield plant^{-1} (g)	High (Mean+2SD) eH13.44	13.44-17.44	IC 321300, Paiyur 1, Paiyur2, Palem 2, NBPGRT-239, IC 392329, Palem 1, CRHG-19, IC 139329
Grain yield plant^{-1} (g)	High (Mean+2SD) eH5.98	5.98-7.43	NBPGRT-D179, IC 139556, Palem 2, CRIDA-18R, CRHG-19, CRHG-22, IC 78605761

Table 7: Estimates of quantitative traits means of the horse gram germplasm accessions promising for multiple traits.

Promising germplasm accessions	Days to 50% flowering	Primary branches plant^{-1}	Pods plant^{-1}	Pod yield plant^{-1} (g)	Grain yield plant^{-1} (g)
IC 105785	44.39	6.20	72.37	12.84	5.52
IC 139556	46.69	6.10	81.25	11.27	7.14
CRHG-19	46.45	5.00	75.36	13.92	6.32
IC-139329	48.03	4.50	71.68	13.63	5.76
Paiyur 1	58.57	5.81	68.86	17.20	4.49
Paiyur 2	54.57	5.74	68.25	17.05	4.57
NBPGRT-D179	43.42	5.50	67.69	12.40	7.43
IC 78605761	53.84	5.50	68.50	10.54	6.15
Palem 2	58.33	5.31	61.26	15.30	6.59
Yield checks					
PHG 9	47.66	4.69	45.32	11.31	4.08
BGM 1	57.72	5.04	53.29	13.30	5.44
SEm ±	00.25	0.08	01.01	00.22	0.09
CD @ 5%	01.73	0.18	05.91	01.20	0.48
CV (%)	03.73	1.01	01.77	01.72	1.90

desirable for a combinations of multiple traits (Table 7). While a few these accessions (IC 105785, Paiyur 1, Paiyur 2 and IC-139329) were comparable to checks, others (IC 139556, CRHG-19, NBPGRT-D179, IC 78605761 and Palem 2) were significantly better than both the checks (Table 7). Several previous researchers have also identified trait-specific accessions in different legumes. To quote a few, Kulkarni and Mogle (2011) in horse gram, Meena and Kumar (2014) in chickpea and Cobbinah *et al.* (2011) in cowpea have identified trait-specific accessions.

CONCLUSION

The traits-specific accessions identified in the present study are suggested for preferential use in crossing programs to generate variability for developing farmer-acceptable varieties with consumer/end-user-preferred traits. To enhance the pace of horse gram breeding, these traits-specific accessions may be recombined with elite advanced breeding genotypes/check varieties followed by selection in segregating populations for transgressive RILs for use as pure-line cultivars. The selection may be practiced in generations derived from F_2 or back cross (BC) breeding populations. The expected mean of RILs is higher if they are selected from F_2 and BC generations derived from parents with comparable performance and those with substantial differences in their performance, respectively. Under these premises, it is desirable to develop new pure-line varieties from BC population derived from check varieties (PHG 9 and BGM 1) × IC 139556 and NBPGRT-D179 as there exists large difference in mean grain yield between them. On the other hand, F_2 generations is preferable breeding population if the breeders' plan is to use CRHG-19, IC 78605761 and Palem 2 in breeding horse gram for developing improved pure-line varieties as these GR and check varieties are comparable for grain yield.

ACKNOWLEDGEMENT

The author gratefully acknowledges the Department of Sciences and Technology (DST), Government of India for providing financial support in the form of INSPIRE-fellowship DST/INSPIRE Fellowship/IF190235 dated:21/11/2020 for conducting thesis research for partial fulfillment for the award of PhD degree by the University of Agricultural Sciences, Bangalore, India.

Conflict of interest: None.

REFERENCES

Bernardo, R. (2020). Breeding for Quantitative Traits in Plants. Third Edition. Stemma Press. Woodbury, Minnesota, USA.

Bhandari, H.R., Bhanu, A.N., Srivastava, K., Singh, M.N. and Shreya, H.A. (2017). Assessment of genetic diversity in crop plants-an overview. Advances in Plants and Agriculture Research. 7(3): 279-286.

Chandana, B.R., Ramesh, S., Kirankumar, R. and Basanagouda, G. (2022). Predicting the frequency of transgressive RILs and minimum population size required for their recovery in horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.]. Plant Genetic Resources: Characterization and Utilization. 20(1): 73-75.

Cobbinah, F.A., Add-Quaye, A.A. and Asante. I.K. (2011). Characterization, evaluation and selection of cowpea [*Vigna unguiculata* (L). Walp] accessions with desirable traits from eight regions of Ghana. ARPN Journal of Agricultural and Biological Science. 6(7): 21-32.

Dasgupta, T., Mukherjee, K., Roychoudhury, B. and Nath, D. (2005). Genetic diversity of horse gram germplasms. Legume Research. 28(3): 166-171.

Geetha, K., Mani, A.K., Hepziba, M.J., Latha, R. and Shanthi, P., (2011). Studies on genetic diversity among germplasm accessions of horse gram [*Macrotylom auniflorum* (Lam) Verde]. Legume Research. 34(1): 14-19.

Halder, S., Datta, A.K., Mandal, A. and Ghosh, B.K. (2012). *Macrotyloma uniflorum* (Lam.) Verdc. (Leguminosae)-A note on chromosomal studies. Cytologia. 77(4): 447-451.

Kanaka, D.K., Ankaiah, R. and Ganesh, M. (2015). Characterization of horse gram cultivars using plant morphological characters. Indian Journal of Agricultural Research. 49(3): 215-221.

Kulkarni, G.B. and Mogle, U.P. (2011). Characterization and evaluation of horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt] genotypes. Science Research Reporter. 1(3): 122-125.

Levene, H. (1960). Robust tests for equality of variances. In: Contributions to Probability and Statistics: Essays in Honour of Harold Hotelling, [(ed.) Olkin, *et al.*] Stanford University Press, Stanford, Connecticut. 278-292.

Mac Queen, J.B. (1967). Some Methods for classification and analysis of multivariate observations. Proceedings of 5th Berkeley Symposium on Mathematical Statistics and Probability. University of California Press, Berkeley, California. 1: 281-297.

Mackay, I.J., Cockram, J., Howell, P. and Powell, W. (2021). Understanding the classics: The unifying concepts of transgressive segregation, inbreeding depression and heterosis and their central relevance for crop breeding. Plant Biotechnology Journal. 19(1): 26-34.

Meena, H.P. and J. Kumar. (2014). Variability for qualitative characters in the mini-core collection of chick pea (*Cicer arietinum* L.). Agriculture for Sustainable Development. 2(2): 149-152.

Morris, J.B. (2008). *Macrotyloma axillare* and *Macrotyloma uniflorum*: Descriptor analysis, anthocyanin indexes and potential uses. Genetic Resources and Crop Evolution. 55: 5-8.

Piepho, H.P., Mohring, J., Melchinger, A.E. and Buchse, A. (2008). BLUP for phenotypic selection in plant breeding and variety testing. Euphytica. 161(1): 209-228.

Priyanka, S., Sudhagar, R., Vanniarajan, C. and Ganesamurthy, K. (2021). Assessment of genetic divergence in horse gram [*Macrotyloma uniflorum* (Lam) Verdc.] using quantitative traits. Legume Research. 44: 261-267.

Schouten, H.J., Tikunov, Y., Verkerke, W., Finkers, R., Bovy, A., Bai, Y. and Visser, R.G. (2019). Breeding has increased the diversity of cultivated tomato in the Netherlands. Frontiers in Plant Science. 10: 1606.

Singhal, H.C., Tomar, S.S., Baraiya, B.R., Sikarwar, R.S. and Tomar, I.S. (2010). Study of genetic divergence in horse gram (*Macrotyloma uniflorum* L.). Legume Research. 33(2): 119-123.

Swarup, S., Cargill, E.J., Crosby, K., Flagel, L., Kniskern, J. and Glenn, K.C. (2021). Genetic diversity is indispensable for plant breeding to improve crops. Crop Science. 61(2): 839-852.

Tajalifar, M. and Rasooli, M. (2022). Importance of BLUP method in plant breeding. Journal of Plant Science and Phytopathology. 6: 40-42.

Upadhyaya, H.D., Dronavalli, N., Dwivedi, S.L., Kashiwagi, J., Krishnamurthy, L., Pande, S., Sharma, H.C., Vadez, V., Singh, S., Varshney, R.K. and Gowda. C.L.L. (2013). Mini core collection as a resource to identify new sources of variation. Crop Science. 53: 2506-2517.

Visakh, R.L. and Bindu, M.R. (2022). Investigation on genetic variability in horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] under moisture stress condition. Journal of Pharmaceutical Innovation. 11(1): 600-603.