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Assessment of genetic divergence in horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] using quantitative traits

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ABSTRACT

Genetic relatedness studies using 12 quantitative traits on 252 genotypes in horsegram revealed wide spectrum of variability for yield components. The genotypes were grouped into 25 clusters based on estimates of D^2 statistic. Cluster I had the largest number with 83 genotypes followed by cluster II (77 genotypes), cluster IV (33 genotypes) and cluster III (30 genotypes). On the contrary, 20 solitary clusters from cluster V to XXV were formed except cluster XIV (9 genotypes) which could be given priority for specific trait improvement. Cluster XXI recorded the highest mean value for single plant yield (65.51 g). The intra and inter cluster distance was varying in magnitude indicating the presence of larger genetic diversity among accessions. The highest intra cluster distance was noticed in cluster XIV (23.63) followed by cluster IV (22.37). Maximum inter cluster distance was observed between solitary clusters *viz*., cluster XXI & XVII followed by cluster XXI & XV and cluster XXI & VII. These clusters would offer superior segregants when employed in hybridization programme.

Key words: Cluster distance, D^2 statistic, Genetic divergence, Horsegram, Tocher's value.

INTRODUCTION

Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] is a member of leguminous family adapted to wide range of environmental regimes. It is a self-pollinated crop, having tolerance to drought (Bhardwaj and Yadav, 2012), salinity (Reddy *et al*., 1998) and heavy metal stress (Reddy *et al*., 2005). The seeds are enriched with a protein content of 18.5% - 28.5% (Savithramma and Shambulingappa, 1996), carbohydrates (50% to 70%), minerals, iron, molybdenum (Bravo *et al*., 1999) and vitamins (Sodani *et al*., 2004). Owing to its massive nutritional benefits, horsegram has been quoted as a potential therapeutic agent in Indian traditional medicine systems to treat kidney stones, urinary diseases, piles, common cold, throat infection and fever (Prasad and Singh, 2015). It is used as a fodder for milch animals because of its high protein content and also plays an important role as green manure by enriching soil fertility due to atmospheric nitrogen mineralization. Despite its role in agricultural, animal husbandry, human and soil health it is not being cultivated in ideal agronomic situations. This under-utilized legume crop will have its recognition in near future because of its potential nutritional and medicinal benefits.

Horsegram is typically a short day plant, with maturity duration of 120 to 180 days (Morris, 2008). It is a minor crop, being cultivated in low-input and marginal lands which limit the scientific efforts to enhance horsegram research and development. Knowledge on extent of genetic variability is a pre-requisite for any breeding programme. In comparison with other pulse crops, recently few studies on assessment of genetic diversity have been reported in horsegram (Gomashe *et al*., 2018; Viswanatha *et al*., 2016). Identification of diverse genotypes with significant yield and nutritional traits is a boon that could be utilized for hybridization programme to evolve superior high yielding varieties suited for diverse environments. With this view, this experimental study was conducted to understand the nature and magnitude of variability present among horsegram germplasm accessions using 12 quantitative traits.

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MATERIALS AND METHODS

Two hundred and fifty germplasm accessions retrieved from Dr. Ramiah Gene Bank, Tamil Nadu Agricultural University (TNAU) and two released varieties of horsegram *viz.,* PAIYUR-2 (released by TNAU) and CRIDA 1-18 R (released by Central Research Institute for Dry land Agriculture) comprised the experimental material. Before laying out of experiment, the field was homogenized for fertility by sufficient manuring/crop residues through soil tests for ensuring uniform crop expression. Totally, 252 genotypes were raised in randomized block design with two replications in the experimental farms of Department of Pulses, TNAU,

Coimbatore. The genotypes were sown in 4m lengthier rows with the spacing of 30 cm x 10 cm during *rabi* season of 2017. The crop was grown in pure rainfed condition. Observations were recorded on five randomly selected plants for 12 quantitative traits *viz.,* days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, pod length (cm), pod width (cm), number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, 100 seed weight (g) and seed yield per plant (g). Yield and its component traits were recorded at time of harvest except days to flowering and maturity. Assessment of genetic diversity was carried out by employing Mahalonobis D^2 statistic (Mahalanobis, 1936) using Indostat-version 7.1 software. The clustering of genotypes was done on basis of D^2 being treated as the square of generalized distance, as per method described by Tocher (Rao, 1952).

RESULTS AND DISCUSSION

Horsegram is extensively cultivated in southern states *i.e*. Karnataka, Andhra Pradesh and Maharashtra and to some extent in parts of West Bengal, Bihar, Himachal Pradesh, Orissa, Chhattisgarh and foot hills of Uttar Pradesh (Purushottam *et al.,* 2017). In India, horsegram covers an acreage of 0.5 million hectares with production and productivity of 0.26 million tones and 520 kg/ha respectively (Anonymous, 2015). Horsegram cultivating lands are resource challenging/demanding which requires development of climatic resilience genotypes. The climatic challenges include extremities of drought and cold, salinity and diseases. Because of these constraints, the yield potential of horsegram is not fully realized. Additionally, it is being grown extensively in *rabi* season due to photosensitivity nature. Development of multiple tolerant and photo-insensitive varieties with improved yield would extend its cultivation in non-traditional areas thereby yield gap can be compensated.

Presence of wide spectrum of genetic variability is essential for any crop improvement strategy. Genetic diversity can be exploited through collection and evaluation of germplasm lines and genotypes of a crop, which is pre-requisite for any breeding programme (Ramakrishnan *et al*., 2014). The analysis of variance revealed highly significant differences for all the traits included in the study (Table 1). However, it is not a reliable measure for prediction of divergence among genotypes (Geetha *et al*., 2011). Knowledge on quantitative divergence of the traits contributing to yield is of primary concern for a plant breeder, which was widely fulfilled by multivariate (D^2) analysis.

D^2 statistic is a powerful tool to measure the genetic divergence within set of genotypes (Murty *et al*., 1966; Dasgupta *et al*., 2005). Based on estimates of D^2 statistic, 252 accessions were grouped into 25 clusters (Table 2) with generalized distance ranging from 0.00 to 52.79. Traits *viz*., number of pods per plant (34.14%), hundred seed weight (18.82%), days to fifty per cent flowering (16.97%),

Table 1: Analysis of variance for quantitative traits.

Source of Variation	Days to 50 % flowering	Days to maturity	Plant height (cm)	Pod length (cm)	Pod width (cm)	Number of clusters per plant	Number of primary branches per plant	Number of pods per plant	Number of pods per cluster	Number of seeds per pod	Hundred seed weight (g)	Single plant yield (g)
Replication	3.335**	0.198	92.364**	0.018	0.000	4.960	23.573**	114.286**	0.012	0.084**	0.102**	28.667**
Treatment	18.430**	19.992**	382.717**	0.821**	0.018**	416.213**	5.911**	5451.947**	1.124**	1.104**	0.409**	254.828**
Error	0.2596	0.5649	21.0764	0.0370	0.0006	6.4264	0.6810	34.2937	0.01368	0.0393	0.0070	2.5407

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

Table 2: Clustering pattern of 252 genotypes in horsegram.

Cluster Number	Number of genotypes	Genotypes
I	83	PLS 6007, PLS 6196, PLS 6199, PLS 6229, PLS 6008, PLS 6232, PLS 6040, PLS 6206, PLS 6039, PLS 6038, PLS 6025, PLS 6019, PLS 6036, PLS 6041, PLS 6037, PLS 6179, PLS 6013, HG 19, HG 119, HG 14, PLS 6001, PLS 6213, PLS 6063, PLS 6023, 8606/2-1, PLS 6060, PLS 6073, PLS 6048, PLS 6172, PLS 6074, PLS 6068, PLS 6197, PLS 6208, PLS 6164, PLS 6184, PLS 6006, PAIYUR 2, PLS 6131, PLS 6052, PLS 6089, PLS 6193, PLS 6190, PLS 6177, HG 68, PLS 6216, PLS 6234, PLS 6140, PLS 6231, PLS 6194, PLS 6099, PLS 6186, PLS 6154, PLS 6169, PLS 6181, PLS 6161, PLS 6083, PLS 6107, PLS 6115, PLS 6104, PLS 6175, PLS 6119, PLS 6168, PLS 6141, PLS 6110, PLS 6114, PLS 6081, PLS 6185, PLS 6049, PLS 6165, PLS 6151, PLS 6109, PLS 6230, PLS 6192, PLS 6120, PLS 6102, PLS 6092, PLS 6062, PLS 6112, PLS 6103, PLS 6135, PLS 6085, PLS 6118, PLS 6050.
II	77	PLS 6242, HG 35, HG 50, PLS 6279, PLS 6262, HG 5A, PLS 6096, PLS 6266, PLS 6018, HG 86, PLS 6237, PLS 6268, PLS 6236, HG 57, HG 58, PLS 6278, PLS 6272, HG 41, PLS 6281, PLS 6280, PLS 6132, 8602/1-2, HG 28, 8514/4-1, PLS 6275, HG 94, PLS 6245, 8605/2-1, 8601/2-5, HG 59, PLS 6117, HG 36, PLS 6240, HG 473, PLS 6263, 12, HG 23, HG 21, HG 63, PLS 6055, 8602/2-2, HG 31, HG 121-4, PLS 6282, HG 4, HG 122, 8515/4-1, HG 37, 8515/2-1, 8606/2-3, 8606/2-2, HG 79, HG 18, HG 115, 8606/1-3, PLS 6246, HG 30, HG 125, PLS 6260, HG121, 8515/1-2, PLS 6252, HG 204, HG 72, HG 9A, PLS 6070, 8605/2-2, PLS 6244, HG 5, PLS 6251, HG 96, HG 93, HG 47, HG 92, PLS 6247, 8605/2-4, PLS 6078.
III	30	PLS 6125, PLS 6061, PLS 6142, PLS 6077, PLS 6071, PLS 6113, PLS 6106, PLS 6150, PLS 6116, PLS 6047, PLS 6046, PLS 6183, PLS 6090, PLS 6121, PLS 6097, PLS 6088, PLS 6072, PLS 6082, PLS 6201, PLS 6080, PLS 6095, PLS 6051, PLS 6035, PLS 6064, PLS 6270, PLS 6094,PLS 6014, PLS 6009, PLS 6002, HG 67.
IV	33	HG 78, HG 61, PLS 6034, HG 80, HG 43, HG 95, HG 38, PLS 6016, PLS 6003, LS 6021, 8601/2-1, PLS 6200, PLS 6212, HG 8, 8516/1-1, HG 9, 8606/2-4, PLS 6261, PLS 6043, HG 101, HG 54, PLS 6111, HG 27, PLS 6069, PLS 6005, PLS 6015, PLS 6256, PLS 6258, HG 116, PLS 6217, PLS 6218, PLS 6253, HG 376.
V	1	PLS 6255
VI	1	PLS 6227
VII	1	HG 34
VIII	1	PLS 6066
IX	1	CRIDA 1-18 R
X	1	PLS 6030
XI	1	PLS 6224
XII	1	PLS 6219
XIII	1	HG 114
XIV	9	HG 112, PLS 6202, PLS 6205, PLS 6228, HG 120, PLS 6211, PLS 6059, PLS 6233, PLS 6226
XV	1	HG 90
XVI	1	PLS 6250
XVII	1	8512/2/1
XVIII	1	PLS 6221
XIX	1	8513/4-3
XX	1	HG 85
XXI	1	PLS 6105
XXII	1	PLS 6004
XXIII	1	PLS 6269
XXIV	1	PLS 6033
XXV	1	HG 2

Table 3: Mean intra (bold) and inter cluster distance in horsegram.

Cluster	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25
C1	**15.52**	26.04	20.07	22.25	26.88	19.60	34.50	18.09	20.32	20.01	18.25	20.40	29.29	25.45	31.33	30.23	32.72	19.41	32.99	28.52	31.01	27.18	26.41	25.95	26.63
C2		**15.73**	35.31	22.60	23.41	30.34	19.32	28.75	32.52	29.37	31.58	32.23	26.86	33.75	21.91	20.56	21.85	29.53	22.32	21.67	43.30	39.95	24.63	30.13	28.03
C3			**17.26**	30.02	36.26	22.76	43.99	22.26	23.19	21.97	21.59	25.71	37.99	29.43	41.28	39.08	41.65	24.03	42.46	35.76	26.96	22.89	35.09	27.80	33.56
C4				**22.37**	24.43	25.21	29.07	24.23	25.81	26.41	24.67	24.90	25.68	28.46	26.28	27.90	26.53	24.77	28.06	27.88	40.17	34.90	25.09	29.49	29.07
C5					**0.00**	22.39	22.68	31.41	31.32	29.35	25.73	25.33	18.33	31.07	23.02	23.86	27.28	24.06	16.17	35.30	40.97	43.15	24.23	38.37	37.45
C6						**0.00**	35.40	24.46	19.97	19.81	13.51	17.64	26.30	23.79	32.94	33.19	35.21	13.69	31.58	37.89	26.20	28.99	30.19	32.48	36.99
C7							**0.00**	36.59	39.31	37.73	40.31	40.38	27.35	39.62	21.31	14.27	21.62	36.39	13.02	31.94	50.97	48.52	34.05	39.77	38.09
C8								**0.00**	14.18	29.98	25.34	24.24	28.28	22.37	28.43	29.37	29.92	27.56	34.15	32.50	37.68	33.01	33.59	33.05	26.42
C9									**0.00**	30.05	20.68	19.74	25.44	17.67	29.53	34.18	32.20	25.46	35.09	38.44	38.51	32.32	35.37	36.04	33.65
C10										**0.00**	17.39	23.87	37.73	33.93	40.73	36.97	40.23	12.33	38.63	29.59	24.74	19.66	25.16	17.49	31.16
C11											**0.00**	9.73	28.37	24.42	35.78	38.04	37.96	12.53	36.46	34.94	29.18	26.38	23.77	28.78	33.44
C12												**0.00**	25.30	23.34	33.93	36.87	37.43	16.28	35.22	36.18	36.39	32.42	24.06	33.95	33.51
C13													**0.00**	24.98	16.69	26.40	21.44	32.24	17.42	38.42	48.52	46.70	29.80	43.75	40.94
C14														**23.63**	29.87	35.61	32.78	29.42	34.53	40.11	41.36	37.97	35.44	39.80	37.52
C15															**0.00**	19.70	16.10	36.95	16.85	32.41	50.97	50.64	31.54	43.97	34.40
C16																**0.00**	25.85	35.02	15.69	29.56	47.42	48.14	33.35	41.21	32.96
C17																	**0.00**	38.82	20.88	33.36	52.79	46.14	34.61	38.53	38.13
C18																		**0.00**	35.36	33.87	28.46	25.75	24.98	26.37	31.16
C19																			**0.00**	35.93	50.13	49.90	32.39	43.72	41.42
C20																				**0.00**	42.63	40.24	22.14	26.82	21.39
C21																					**0.00**	31.49	40.60	35.69	43.25
C22																						**0.00**	40.30	17.89	39.50
C23																							**0.00**	30.53	28.24
C24																								**0.00**	30.51
C25																									**0.00**

number of clusters per plant (10.21%) contributed maximum towards genetic divergence whereas, least contribution was made by days to maturity (0.10%) and number of primary branches per plant (0.35%). It was noted that most of the yield related traits had exhibited considerable contribution towards cluster divergence hence, probability of improving these traits turns to be feasible by hybridization and selection (Fig 1). The results were in conformity with the findings of Geetha *et al*., (2011).

Among the 25 clusters, maximum accessions were grouped under cluster I (83 genotypes) followed by cluster II (77 genotypes), cluster IV (33 genotypes) and cluster III (30 genotypes). On the contrary, 20 solitary clusters *viz*., cluster V to XXV except cluster XIV were formed, which could be given priority for improvement of specific traits. Clusters with single genotype were high owing to reason that experimental accessions were collected through various explorations across Indian States over years. These geographical variations might have yielded more number of solitary clusters. The intra and inter cluster distance among 25 clusters were presented in Table 3. The highest intra cluster distance was noticed in cluster XIV (23.63) followed by cluster IV (22.37), cluster III (17.26), cluster II (15.73) and cluster I (15.52) while the remaining 20 clusters were found to be unique cluster possessing no intra cluster value. The genotypes present within cluster tend to be genetically less diverse compared to other clusters. Maximum inter cluster distance was observed between solitary clusters *viz*., cluster XXI and XVII (52.79) followed by cluster XXI and XV (50.97) and cluster XXI and VII (50.97). Promoting hybridization between diverse genotypes would be highly heterotic thereby evolve superior varieties (Singhal *et al*., 2010). The least inter cluster distance was recorded between cluster XIX and VII, cluster XVIII and X and cluster XVIII and XII with a distance of 13.02, 12.33 and 12.53 respectively.

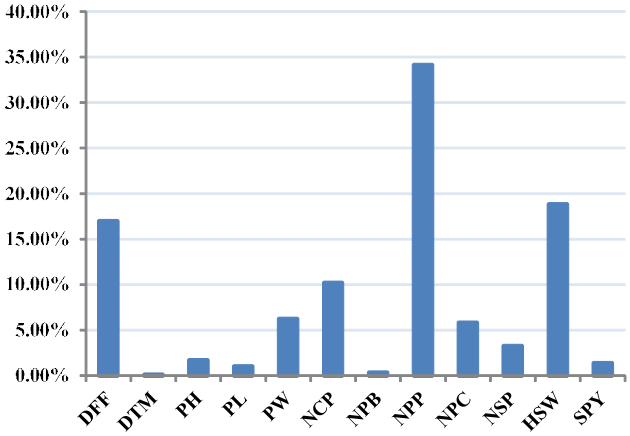
The average cluster mean values for 12 traits would help to identify genotypes for improving specific yield components (Table 4). Cluster XIII with single genotype and Cluster XIV with 9 genotypes recorded lower mean value for days to fifty percent flowering whereas, cluster IX and XIII for days to maturity. These genotypes could be utilized as a donor for evolving short duration types. Among the germplasm accessions, cluster XXI (PLS 6105) was found to be high yielding line with mean yield of 65.51 g. Cluster XXII (PLS 6004) recorded the highest group mean for yield components *viz*., number of clusters per plant, number of pods per plant and pod width. Being a unique cluster, the

Table 4: Cluster mean values obtained by Tocher's method for 12 quantitative traits in horsegram.

Clusters	Days to 50 % flowe--ring	Days to maturity	Plant height (cm)	Pod length (cm)	Pod width (cm)	Number of clusters per plant	Number of primary branches per plant	Number of pods per plant	Number of pods per cluster	Number of seeds per pod	Hundred seed weight (g)	Single plant yield (g)
Cluster 1	56.89	106.83	61.41	5.37	0.53	49.37	5.52	137.01	2.81	5.73	3.90	27.38
Cluster 2	58.01	108.36	55.57	4.57	0.59	36.07	5.78	63.73	1.79	5.04	4.54	12.91
Cluster 3	57.25	107.00	68.46	5.73	0.51	64.95	6.60	193.15	3.01	5.82	3.82	38.36
Cluster 4	56.12	106.31	60.38	4.76	0.61	42.21	6.43	97.84	2.54	5.30	4.14	18.73
Cluster 5	55.00	106.00	33.13	5.20	0.63	21.50	4.00	73.50	3.39	6.40	4.91	21.08
Cluster 6	54.50	105.00	62.95	5.64	0.74	45.50	7.50	168.00	3.68	6.78	4.50	44.63
Cluster 7	57.00	106.50	36.56	5.18	0.73	26.50	4.50	35.50	1.35	5.95	5.13	9.20
Cluster 8	52.50	102.00	64.81	6.01	0.49	62.00	4.50	120.50	1.89	5.67	3.65	23.89
Cluster 9	50.50	98.50	64.04	5.03	0.61	52.50	8.50	158.50	2.99	6.01	3.54	30.47
Cluster 10	62.00	113.00	50.38	5.20	0.67	50.50	10.00	159.00	3.33	6.17	4.05	34.88
Cluster 11	56.50	106.50	71.88	4.89	0.63	38.00	7.50	165.00	4.35	5.80	3.92	34.75
Cluster 12	54.50	105.00	84.45	5.05	0.63	30.00	6.50	129.50	4.33	6.59	3.71	27.78
Cluster 13	49.50	99.00	74.86	4.80	0.63	26.00	6.00	86.50	3.35	5.34	4.87	17.49
Cluster 14	49.78	100.28	76.12	4.94	0.61	44.83	6.50	150.11	3.28	5.52	3.96	29.90
Cluster 15	50.50	100.50	49.65	4.32	0.53	23.50	5.50	47.00	2.00	4.78	4.57	10.16
Cluster 16	55.50	105.50	48.96	6.34	0.56	23.50	4.50	31.00	1.32	6.66	4.80	9.81
Cluster 17	51.50	101.50	54.03	3.76	0.64	55.00	5.50	73.00	1.33	3.90	4.84	11.01
Cluster 18	59.00	109.50	51.85	5.52	0.76	37.00	7.50	135.00	3.67	7.12	3.87	34.62
Cluster 19	53.00	103.50	46.38	5.16	0.63	21.50	4.50	50.50	2.35	6.13	5.32	12.37
Cluster 20	62.50	113.50	83.79	4.20	0.37	35.00	8.50	58.50	1.67	4.31	4.11	9.60
Cluster 21	61.00	112.00	63.43	5.97	0.47	63.50	6.50	229.50	3.63	6.31	4.76	65.51
Cluster 22	61.50	111.00	64.30	4.72	0.76	84.50	7.00	233.50	2.92	5.54	3.59	41.24
Cluster 23	60.50	111.50	73.50	4.65	0.47	17.00	9.50	61.50	3.68	4.53	4.28	11.72
Cluster 24	64.00	115.00	55.74	4.22	0.67	72.00	8.00	159.50	2.16	4.36	3.80	24.16
Cluster 25	60.50	111.50	46.65	5.59	0.46	31.00	3.50	46.00	1.47	4.68	2.87	5.86

Table 5: Promising accessions identified.

Traits	Range	Accessions (Cluster)
Days to 50% flowering	49.50 - 64.00	HG 114 (Cluster XIII)
Days to maturity	98.50 - 115	CRIDA -18 R (Cluster IX)
Plant height (cm)	33.13 - 84.45	PLS 6255 (Cluster V)
Number of primary branches per plant	3.5 - 10	PLS 6030 (Cluster X)
Pod length (cm)	3.76 - 6.34	PLS 6250 (Cluster XVI)
Pod width (cm)	0.37 - 0.76	PLS 6221 (Cluster XVIII) and PLS 6004 (Cluster XXII)
Number of clusters per plant	17 - 84.5	PLS 6004 (Cluster XXII)
Number of pods per cluster	1.32 - 4.35	PLS 6224 (Cluster XI)
Number of pods per plant	31 - 233.5	PLS 6004 (Cluster XXII)
Number of seeds per pod	3.9 - 7.12	PLS 6221 (Cluster XVIII)
100 seed weight (g)	2.87 - 5.32	8513/4-3 (Cluster XIX)
Seed yield per plant (g)	5.86 - 65.51	PLS 6105 (Cluster XXI)



DFF- Days to 50% flowering, **DTM**- Days to maturity, **PH**- Plant height, **PL**- Pod length, **PW**- Pod width, **NCP**- Number of clusters per plant, **NPB**- Number of primary branches per plant, **NPP**- Number of pods per plant, **NPC**- Number of pods per cluster, **NSP**- Number of seeds per pod, **HSW**- Hundred seed weight, **SPY**- Single plant yield.

Fig 1: Contribution of quantitative traits towards cluster divergence.

genotype can be used in crossing programme to evolve high yielding varieties as it exhibits superior *per se* performance for several yield components. A solitary cluster XI (PLS 6224) recorded the highest mean for number of pods per cluster (4.35). Neelam *et al*. (2014) reported significant correlation between number of pods per plant and single plant yield. Hence, accession PLS 6224 can be utilized for yield improvement by breeding for increased number of pods per plant. Several other unique clusters were also contributed for yield components *viz*., cluster XIX (8513/4-3) recorded the highest mean yield with 5.32 g for hundred seed weight, cluster XVIII (PLS 6221) possessed highest mean value for number of seeds per pod (7.12 g) and cluster X (PLS 6030) for number of primary branches per plant (10). These genotypes can be used as a donor to derive agronomically superior varieties. On a nutshell, it was concluded that horsegram accessions taken for study revealed wide range of genetic diversity and many unique clusters with promising accessions were formed contributing to the improvement of specific yield components (Table 5). These unique clusters can be given due importance in the future breeding programme to evolve superior types. These diverse genotypes shall be utilized in different research institutes.

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