RESEARCH ARTICLE

Genetic Variability Studies on Horse Gram [*Macrotyloma uniflorum* (Lam.) Verdc.] Landraces Using Agromorphological Traits

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Abstract

Horse gram, a neglected pulse crop, has been an integral component of subsistence farming in India and has the potential to significantly contribute to the global food basket under the regime of climate change. Besides being a nutritionally rich pulse, it also has the potential to treat a variety of ailments like kidney stones, throat infections, and fever due to the presence of phytic acid and phenolics. Under the present study, 155 horse gram accessions, including 150 accessions collected from 16 Indian states and five checks were evaluated to assess the extent of diversity using 27 morphological traits. The analysis indicated that seed color was the most diverse qualitative trait, having Shannon's diversity index (SDI) of 0.86, followed by plant growth habit (0.50), leaf shape (0.49), and pod beak shape (0.47), whereas seed yield/plant (0.02–22.68 g), number of pods/plant (1–44), hundred seed weight (1.6–4.1 g), primary branches/plant (2.79-8.19), plant height (29.63–88.33), and days to 80% maturity (99.4–146) were the most diverse quantitative trait. The statistical analysis has shown a positive correlation of seed yield with pod length (r = 0.54), number of pods/plant (r = 0.51), and number of seeds/pod (r = 0.49), but a negative correlation with days to 80% maturity (r = -0.72) and 50% flowering (r = -0.68). The principal component analysis validated the observed correlations. The cluster analysis showed that the accessions representing North Indian states and Madhya Pradesh were superior for yield-contributing traits and were also early maturity types.

Keywords: Horse gram, Germplasm, Landrace, Diversity, Multivariate.

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Introduction

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdcourt, Syn. *Dolichos uniflorus* Lam.] is a potential legume that has remained an integral component of subsistence farming in India. It is commonly known as Kulthi in Hindi, Kurti-kalai in Bengali, Ullavalu in Telugu, Kollu in Tamil, and Mudhira in Malayalam, and has been used as fodder for horses and cattle for centuries, deriving its English name (Watt, 1891). It is grown as a minor pulse in South Asian countries such as India, Bangladesh, Nepal, Bhutan, Myanmar, Sri Lanka, and Malaysia and as forage and green manure in Australia, Africa, and the West Indies (Jeswani and Baldev, 1990; Asha *et al.*, 2006; Witcombe *et al.*, 2008). In Uttarakhand, it is popularly known as "Gahat" and is traditionally cultivated with finger millet and as a component crop in "Barahnaaja," a traditional method of mixed cropping (Bhartiya

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et al., 2014). In India, Karnataka (34%), Tamil Nadu (18%), Maharashtra (18%), Andhra Pradesh (16%), and Odisha (16%) account for 90 to 95% of the area under horse gram, either as a sole crop or a mixed crop with millets (Kumar, 2007), whereas it is sporadically cultivated in Madhya Pradesh, Chhattisgarh, Bihar, Jharkhand, West Bengal, and the midhills of Uttarakhand and Himachal Pradesh. According to classical reports, horse grams originated in India (Vavilov, 1951; Zohary, 1970; Purseglove, 1974; Smartt, 1985), particularly in southwestern India (Arora and Chandel, 1972; Kumar et al., 2013). Two separate domestication of horse gram in Northwestern India around 4000 BP and the Indian Peninsula around 3500 BP have also been suggested based on the analysis of herbarium collections, archaeological evidence, and historical linguistics of Africa and India (Fuller and Murphy, 2018). India and the northern Himalayan region are considered rich in the genetic diversity of Macrotyloma species (Zeven and de Wet, 1982).

Tribal communities consume horse gram in various ways, including boiled or fried rice, blended with other grains in flour, and as a key ingredient in curries. Depending on the region, split horse gram seeds (dal) are consumed in various forms (Prodanov et al., 1997; Bravo et al., 1999; Bhardwaj and Yadav, 2015). Horse gram seeds contain higher protein (21.73 g/100 g) in comparison to the two most important pulses of India, chickpea (18.77 g/100 g) and pigeon pea (20.47 g/100 g), and also have equivalent levels of minerals and vitamins (Longvah et al., 2017). Besides, it also contains antinutritional compounds like phytic acid, polyphenols, and oligosaccharides, which limit its acceptability for consumers (Sreerama et al., 2012). However, the antinutritional compounds are known to have medicinal properties. The flavonoid-rich soup is used to treat common colds, throat infections, and fevers (Siddhuraju and Manian, 2007), while its ability to inhibit calcium oxalate crystallization prevents kidney stone formation (Bhardwaj and Yadav, 2015). Horse gram is also supposed to treat urinary tract infections, piles, heart diseases, asthma, bronchitis, skin disorders, leucorrhea, and an irregular menstrual cycle in women (Yadava and Vyas, 1994; Ghani, 2003; Neelam, 2007). Simple food processing techniques like dehulling (Bravo et al., 1999; Rizvi et al., 2022), cooking (Troszynska et al., 2002), germination, and roasting (Vishakha and Vibha, 2017) have been effective in lowering the antinutritional compounds. The ability of horse gram to withstand drought (Bhardwaj and Yadav, 2012), salinity (Reddy et al., 1998), heavy metal stresses (Reddy et al., 2005), and a wide range of temperatures (Smartt, 1985) makes it an ideal crop for rainfed agriculture, which covers 73 million hectares in India and accounts for 52% of the net sown area and 40% of the nation's food supply (AICRP for Dryland Agriculture, Annual Report 2017-18).

The improvement of crop plants requires continuous evaluation of genetic material comprising traditional

varieties, landraces, and wild relatives to identify trait sources. To select the desirable types, an assessment of existing diversity and genetic relatedness between the source and target populations needs to be done. A number of qualitative and quantitative traits have been used for the characterization of horse gram genotypes (Neelam *et al.*, 2014; Bhartiya *et al.*, 2017; Gomashe *et al.*, 2018; Singh *et al.*, 2019; Priyanka *et al.*, 2019; Bhavsar *et al.*, 2021; Priyanka *et al.*, 2021a; Neelima *et al.*, 2021). However, these studies have focused only on a few germplasm accessions screened against fewer descriptors, whereas the current study aimed to understand the diversity among the 155 horse gram accessions from diverse agroecological regions of India using 27 morphological descriptors.

Materials and Methods

Plant Material

A total of 155 horse gram germplasm accessions, including 150 landraces representing 16 Indian states, were procured from the Indian National Genebank at ICAR-National Bureau of Plant Genetic Resources, New Delhi. The remaining five accessions were commercially released varieties, namely CRHG-19, CRIDA-18R, VL-19, BSP 15-1, and VL-15, that were used as checks. The collection sites of 150 horsegram landraces are depicted in Figure 1, and a list of germplasm accessions is given in Supplementary Table 1.

Experimental Site and Design

The experiment was conducted under rainfed conditions at the NBPGR Regional Station, Bhowali (Uttarakhand), in augmented block design (ABD) during *Kharif*-2018 and *Kharif*-2020. The study involved five blocks of 35 entries, each with 30 test accessions and five checks. Each check

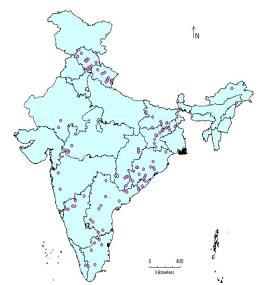


Figure 1: Map of India representing the collection sites of 150 horse gram landraces used in this study



Figure 2: Pie charts showing frequency of various qualitative traits studied in the present study

Table 1: Descriptive statistics for eleven quantitative morphological traits

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Trait*	Mean	SE#	SD⁺	%CV ^{\$}	Min. Value	Accession with min. value	Minimum check value	Max. value	Accession with max. value	Maximum check value
NPBPP	5.34	0.08	1.01	16.83	2.79	IC361651, IC273748	4.16 (VL-15)	8.19	IC373194	5.56 (CRHG-19)
LL	4.39	0.03	0.35	9.9	3.32	IC424465	4.15 (BSP-1521)	5.42	IC262880	4.67 (VL-15)
LW	3.11	0.02	0.31	9.42	2.39	IC397479	2.85 (BSP-1521)	3.79	IC394804	3.34 (VL-15)
PH	60.4	0.76	9.46	16.74	29.63	IC396007	52.75 (VL-19)	88.33	IC371987	64.77 (CRHG-19)
DF	87.68	0.79	9.88	4.15	60.2	IC280546	60 (VL-19)	118.92	IC259884	92 (BSP-1521)
DM	129.99	0.91	11.33	9.08	99.4	IC385840	118 (VL-19)	146	IC311158	138 (BSP-1521)
PL	4.07	0.04	0.44	7.46	2.82	IC569083	3.97 (BSP-1521)	5.26	IC262082	4.37 (VL-15)
NPPP	16.53	0.77	9.57	42.62	1	IC331711	15.4 (CRIDA-18R)	44	IC398780	20 (VL-19)
NSPP	5.04	0.05	0.56	7.67	3.13	IC519774	4.48 (BSP-1521)	6.33	IC278827	5.28 (VL-15)
SYPP	5.29	0.46	5.79	65.05	0.02	IC311896	1.08 (CRIDA-18R)	22.65	IC392511	10.61 (VL-15)
100SW	2.4	0.07	0.83	22.18	1.6	IC369776	1.83 (BSP-1521)	4.12	IC382656	3.34 (VL-15)
-										

*NPBPP = number of primary branches per plant; LL = leaf length (cm); LW = leaf width (cm); PH = plant height (cm); DF = day to 50% flowering; DM = days to 80% maturity; PL = pod length (cm); NPPP = number of pods per plant; NSPP = number of seeds per pod; SYPP = seed yield per plant (g); 100SW = 100 seed weight (g).#Standard Error; +Standard Deviation; \$Coefficient of variation was randomized to reduce the standard error. Accessions were sown in two lines with 45 × 15 cm spacing, with one row unsown for easy monitoring and recording of the morphological data. The crop was grown without external manure or fertilizer except for the fungicide mancozeb 75% WP at 6 g/10 L for managing anthracnose disease. Five plants were randomly selected to record morphological data on 27 morphological descriptors. Quantitative traits such as seed yield per plant and test weight were recorded from the bulked seeds of the five randomly selected plants. The mean values for the 11 quantitative descriptors were worked out using Microsoft Excel and considered for the final analysis using the statistical package "R-Studio" (R Core Team, 2023). The proportion of each descriptor state (P) was recorded for all the 16 qualitative traits, and the Shannon diversity index (H) was calculated using the following formula:

H=-Σ[P_i(logP_i)]

Where,

pi = proportion of a particular descriptor state, i.e., number of genotypes with a descriptor state/total number of individuals, i.e., 155.

Result and Discussion

Phenotypic Diversity for Qualitative Traits

Among the 16 qualitative traits studied, seed color, having a maximum of eight classes, was the most diverse character

with a Shannon's diversity index (SDI) value of 0.856. The other diverse traits were plant growth habit (SDI = 0.50), leaf shape (SDI = 0.49), and pod beak shape (SDI = 0.47) (Supplementary Table 2). The frequency distributions of various character states have also been represented as pie charts (Figure 2). The results of the present study are consistent with the earlier study, where 13 seed color categories were observed in horse gram germplasm originating from southern India (Latha *et al.*, 2013).

Variability for Quantitative Traits

The ranges of variation and summary statistics for eleven quantitative traits are presented in Table 1. A wide range of variability was observed among the horse gram landraces for the recorded traits. The observed range for pod length was 2.82 to 5.26 cm, the number of pods per plant was 1 to 44, the mean number of seeds per pod was 3.13 to 6.4, the mean seed yield per plant was 0.0 to 22.68 g, and the 100 seed weight was 1.60 to 4.10 g. As indicated by their coefficients of variance, the seed yield per plant (65.05%), number of pods per plant (42.62%), and 100 seed weight (22.18%) were among the most diverse traits. Plant height has been found to be the most diverse morphological trait in horse gram (Bhartiya et al., 2017; Chahota et al., 2017; Gomashe et al., 2018; Singh et al., 2019; Priyanka et al., 2019; Pushpayazhini et al., 2022), though some reports suggest it to be one of the least diverse traits (Singhal et al.,

Table 2:	List of promising accessions ident	Ined
S. No.	Trait	Promising accessions identified
1	High yielding (Seed Yield/Plant) (g)	lC392511(22.68 g), lC281624(20.65 g), lC278825 (20.34 g), lC265920(19.47 g), lC263277(19.43 g)-Best check (VL-15;10.612 g)
2	Early maturity	IC273742(103 days), IC263332 & IC281835 (105 days), IC281820 & IC361651(108 days)-Best Check (VL-19; 118 days)
3	High yielding, early maturity	IC281624, IC391572, IC356050, IC278829, IC263332, IC273742
4	Bold seeded (More 100 Seed weight (g)	IC382656 (4.1 g), IC262880(3.81 g), IC281651(3.75 g), IC278830(3.73 g), IC273748(3.71 g)-Best check (VL-15; 3.344 g)

Table 3: Correlation	matrix of 11	quantitative traits
Table J. Conclation	matrix of 11	quantitative traits

Traits	NPBPP	LL	LW	PH	DF	DM	PL	NPPP	NSPP	SYPP	100SW
NPBPP	1										
LL	-0.16	1									
LW	-0.15 ^{ns}	0.75***	1								
PH	0.27***	0.09 ^{ns}	0.14 ^{ns}	1							
DF	0.31***	-0.22**	-0.04 ^{ns}	0.25**	1						
DM	0.40***	-0.24**	-0.01 ^{ns}	0.26**	0.72***	1					
PL	-0.17	0.26**	0.01 ^{ns}	-0.09 ^{ns}	-0.40***	-0.44***	1				
NPPP	0.01 ^{ns}	0.16	-0.02 ^{ns}	-0.08 ^{ns}	-0.44***	-0.30***	0.58***	1			
NSPP	-0.08 ^{ns}	0.21	-0.03 ^{ns}	-0.12 ^{ns}	-0.42***	-0.39***	0.77***	0.55***	1		
SYPP	-0.33***	0.39***	0.21**	-0.16	-0.68***	-0.72***	0.54***	0.51***	0.49***	1	
100SW	-0.32***	0.33***	0.12 ^{ns}	-0.26***	-0.57***	-0.52***	0.42***	0.36**	0.38***	0.57***	1

^{ns}= Non significant correlation; ^{**}p < 0.01; ^{***}p < 0.001; Others: p < 0.05

2010; Neelima et al., 2021). However, it emerged as the fifth most diverse attribute in our analysis, following seed yield, number of pods per plant, 100 seed weight, and number of primary branches per plant. These results corroborate earlier studies in which the seed yield per plant, number of pods per plant, and 100 seed weight were the most diverse traits (Vijayakumar et al., 2016; Singh et al., 2019; Neelima et al., 2021; Priyanka et al., 2021a; Priyanka et al., 2021b; Bhavsar et al., 2021; Pushpayazhini et al., 2022). The germplasm accessions collected from lower elevations did not perform well in terms of seed yield and related characteristics at the experimental site situated at higher elevations (1600 amsl), contributing to the vast diversity observed for these traits. The study revealed that landraces outperformed checks in most studied traits. The lowest values for days to 80% maturity within checks (for VL-19) and landraces were 118 and 103 days, respectively. Similarly, superior genotypes for 100 seed weight (4.12 g) and seed yield per plant (22.65 g) were identified, both of which were highest for VL-15 within the checks (3.34 and 10.61 g, respectively). These results suggest that bold-seeded, high-yielding, and early-maturing types can be selected from the studied germplasm (Table 2).

Correlation Analysis

The results of the correlation analysis are presented in Table 3, which shows many combinations of obvious correlations such as pod length and number of seeds per pod (r = 0.77, p < 0.001), leaf length and leaf width (r = 0.75, p < 0.001), and days to 50% flowering and 80% maturity (r = 0.72, *p* < 0.001) which are self-explanatory. Here, we report a positive correlation of seed yield with leaf length (r = 0.39, p < 0.001) and leaf width (r = 0.21, p < 0.01), which may be attributed to the higher production and mobilization of photosynthates leading to higher grain yield. This correlation may be used as an important marker for the identification of genotypes having higher yield potential. Studies showing a direct relationship between the seed yield and leaf surface area, particularly in horse gram, are lacking. However, there is ample evidence of a positive association between the area of flag leaf and yield in cereals (Rahman et al., 2013). Additionally, the strong negative correlation observed between the seed yield and days to 80% maturity (r = 0.72, p < 0.001) indicated that late-maturing types had lower yields. These findings are consistent with the earlier reported values by Alle et al. (2016) and Singh et al. (2020). However, the possible cause of this negative association might be the shattering habit of the crop since late-maturing types would have faced significant yield loss due to shattering.

Principal Component Analysis

Principal component analysis (PCA) is a method that reduces the dimensionality of datasets and enhances interpretability with minimal loss of information (Jolliffe and Cadima, 2016). The analysis showed that the first three principal

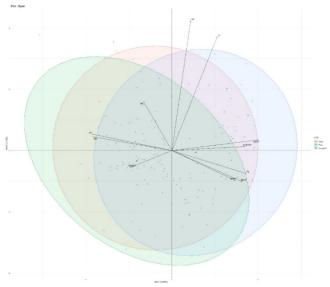


Figure 3: PCA bi-plot showing the distribution of accessions according to early plant vigour (EPV) gram landraces used in this study

components (PCs) explained 69.57% of the total variation, with the first PC alone contributing to 40.55% of the total variation (Table 4). The traits contributing most to the first principal component were seed yield per plant (0.86), days to 50% flowering (-0.79), days to 80% maturity (-0.78), pod length (0.74), 100 seed weight (0.73), number of seeds per pod (0.70), and number of pods per plant (0.63). The leaf width (0.91) and leaf length (0.80) contributed most to the second principal component. The number of primary branches per plant (0.63) and plant height (0.54) had the maximum loadings in the third principal component. The

 Table 4: The proportion of variance explained by the principal components

components			
Particulars	PC-1	PC-2	PC-3
Eigen value	4.46	1.75	1.44
Percent variance	40.55	15.92	13.11
Cumulative percent variance	40.55	56.47	69.57
Character weightage			
NPBPP	-0.41	-0.12	0.63
LL	0.45	0.80	0.11
LW	0.19	0.91	0.02
PH	-0.28	0.34	0.54
DF	-0.79	0.12	0.21
DM	-0.78	0.10	0.30
PL	0.74	-0.16	0.41
NPPP	0.63	-0.21	0.47
NSPP	0.70	-0.22	0.45
SYPP	0.86	0.07	-0.03
100SW	0.73	0.03	-0.18

two-dimensional bi-plot of the first two PCs is presented in Figure 3, and the scree plot is provided in Supplementary Figure 1.

Cluster Analysis: A Comparison of HCA and KCA

The study, we compare the efficacy and accuracy of hierarchical cluster analysis (HCA) and K-means cluster analysis (KCA), the two most common methods used for grouping the test entries (Yoo et al., 2020). The gap statistic method identified three optimal clusters (Supplementary Figure 2), which were considered in the K-means cluster analysis (Figure 4) (Tibshirani et al., 2001). Ward's method was used to generate a hierarchical clusterbased dendrogram using squared Euclidean distances (Figure 5). The HCA and KCA methods produced clusters with a strong correspondence, with Cluster-I containing the same set of 58 accessions, but slight deviations were observed in the compositions of the other two clusters (Supplementary Table 3). Since the KCA is a simpler method, we have discussed the cluster attributes of this method. Cluster-I, with 58 accessions, included all the accessions from the states of Uttarakhand (29) and Madhya Pradesh (10), and the majority of the accessions from Himachal Pradesh (12 out of 15). Cluster II contained 29 accessions, comprising all the accessions from Karnataka (8) and a significant number of accessions from Odisha (6 out of 22), Andhra Pradesh (6 out of 23), and Tamil Nadu (3 out of 6). Cluster III was the largest one with 68 accessions, containing the majority of the accessions from Andhra Pradesh (17 out of 23), Odisha (15

SN	Trait	1	11	<i>III</i>
1	No. of primary branches per plant (NPBPP)	5.18	5.50	5.59
2	Leaf length (LL)	4.61	4.40	4.32
3	Leaf width (LW)	2.89	3.17	2.81
4	Plant height (PH)	56.49	65.29	59.67
5	Day to 50% flowering (DF)	77.02	95.03	91.16
6	Days to 80% maturity (DM)	115.59	137.79	134.31
7	Pod length (PL)	4.32	3.62	4.15
8	No. of pods per plant (NPPP)	23.41	14.32	24.55
9	No. of seeds per pod (NSPP)	5.34	4.62	5.10
10	Seed yield per plant (SYPP)	8.84	0.49	1.73
11	100 seed weight (100-SW)	3.04	2.10	2.33

			/	
Cluster	C-I	C-II	C-III	
C-I	2.43			
C-II	5.03	2.74		
C-III	3.36	2.88	2.20	

out of 22), Jharkhand (13 out of 14), Chhattisgarh (9 out of 11), and Bihar (5 out of 7). The demarcation of clusters has been superimposed on the map of India (Supplementary Figure 3). On the basis of the mean values of clusters (Table 5), it was revealed that Cluster-I exhibited superior yieldrelated traits like pod length, seeds per pod, and seed weight. Surprisingly, the early maturing genotypes also dominated this cluster, contrary to the usual pattern of early maturing genotypes producing lower yields (Narayanan *et al.*, 2018). The development of short-durational and highyielding varieties has always been the prime objective of pulse improvement in India (Pooniya *et al.*, 2015). Thus,

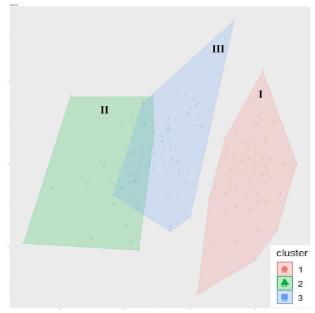


Figure 4: Clusters formed by K-means cluster analysis (k = 3)

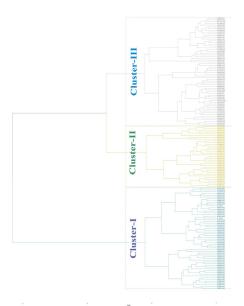


Figure 5: Dendrogram generated by hierarchical cluster analysis labelled with three clusters (k = 3)

the accessions from this cluster may be best suited for developing high-yielding and early-maturing cultivars, particularly for northern and central India.

The intra-cluster (diagonal) and inter-cluster (vertical) centroid distances calculated using KCA are shown in Table 6. The three clusters formed by the cluster analysis almost grouped the accessions according to their site of collection, with some random grouping in the third cluster. The widest inter-cluster distance observed between clusters I and II (5.03) suggests that the hybridization program between accessions from these two clusters would yield superior hybrids. The minimum inter-cluster distance was recorded between clusters II and III (2.88), which was almost equal to the intra-cluster distance recorded for cluster II (2.74). Thus, the accessions from clusters II and III, despite having in different clusters, were closer to each other in comparison to those of cluster I.

Conclusion

The study revealed wide variability for both quantitative and qualitative traits among the horse gram germplasm which could be exploited for genetic improvement of this crop. The correlation studies suggested that while selecting for the development of high-yielding genotypes, importance to leaf area may also be given in addition to the traits directly contributing to yield. Accessions primarily from Uttarakhand, Himachal Pradesh, and Madhya Pradesh exhibited superiority in many yield-related traits. They grouped separately into a single cluster and the remainder of accessions, though in another two clusters were still close to each other, giving support to the hypothesis of two separate domestications of horse gram. The superior accessions identified in the study can be recommended for release after conducting multilocation trials.

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Supplementary material

S. No.	Accession No.	Vernacular name	Passport data				
<i>3. NO.</i>	Accession No.	ventacular hame	village	District	State		
1	IC424465	Kulthi	Sonpiya	West Singhbhum	Jharkhand		
2	IC369776	Pallavulvalu	Gunjarada	Vizianagaram	Andhra Pradesh		
3	IC519774	Ulavalu	Darimalli	Srikakulam	Andhra Pradesh		
4	IC546210	Ulavalu	Niluvai	Adilabad	Andhra Pradesh		
5	IC394804	Khulti Kalai	Saralpara	Assam	Assam		
6	IC417809	Kulthi	Govindchak	Chapra	Bihar		
7	IC398699	Kulthi	Nima	Siwan	Bihar		
8	IC398698	Kulthi	Nayagaon	Saran	Bihar		
9	IC396011	Hirnwa	Pali/Raigarh	Raipur	Chhattisgarh		
10	IC398780	Kurthi	Khurtha/Rana	Lakhisarai	Bihar		
11	IC139570	-	Tissa	Chamba	Himachal Pradesh		
12	IC139571	-	Labh-jewali	Kangra	Himachal Pradesh		
13	IC331711	-	Tabo/Bandhgaon	West Singhbhum	Jharkhand		
14	IC392329	Kulthi	Torai	Pakud	Jharkhand		
15	IC520827	Kulthi	Dhenukanta/ Palajori	Deogarh	Jharkhand		
16	IC424461	Kulthi	Kumardiha	West Singhbhum	Jharkhand		
17	IC425077	Lokapur local-1	Lokapur	Bagalkot	Karnataka		
18	IC425081	Mudenoor local-2	Mudenoor	Belgaum	Karnataka		
19	IC333129	Kulthi	Osada	Barwani	Madhya Pradesh		
20	IC432138	-	Ghoti	Nashik	Maharashtra		
21	IC432011	-	Ahmednagar	Ahmednagar	Maharashtra		
22	IC432059	-	Wai	Satara	Maharashtra		
23	IC372065	Kulthi	Damodar beda	Malkangiri	Odisha		
24	IC311892	Kulthi	Gajinaju	Kandhamal	Odisha		
25	IC311893	Kulthi	Kotagarh	Kandhamal	Odisha		
26	IC311894	Kulthi	Kotagarh	Kandhamal	Odisha		
27	IC311895	Kulthi	Bariguda	Nowrangpur	Odisha		
28	IC311896	Kulthi	Bilungia	Kandhamal	Odisha		
29	IC311897	Kulthi	Podapadar	Koraput	Odisha		
30	IC311898	Kulthi	Pradhaniguda	Nowrangpur	Odisha		
31	IC278831	Kulathi	Bhatnaul	Sirmour	Himachal Pradesh		
32	IC278832	Kulathi	Shamoga	Sirmour	Himachal Pradesh		
33	IC546209	Ulavalu	Mancherial	Adilabad	Andhra Pradesh		
34	IC311900	Kulthi	Janbai	Nowrangpur	Odisha		
35	IC139540	-	Kadwal	Udaipur	Rajasthan		
36	IC351714	Gad	Legship	West Sikkim	Sikkim		
37	IC393391	-	Papparapatti	Dharmapuri	Tamil Nadu		
38	IC410169	Kanapayir	Azhagarkoil	Madurai	Tamil Nadu		
39	IC405710	Kollu	Thamarakkarai	Erode	Tamil Nadu		
40	IC383494	Kollu	Mangalam Yelagiri	Vellore	Tamil Nadu		
41	IC383470	Gahat	Mansingh kanda	Pithoragarh	Uttarakhand		

Supplementary Table 1: List of 155 horse gram accessions used in the present study with passport data

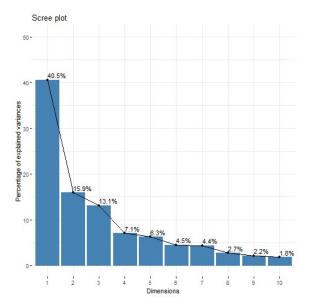
S. No.	Accession No.	Vernacular name	Passport data village	District	State
42	IC406524	Ghath	Jhagigaon	Rudraprayag	Uttarakhand
42 43	IC400524	Gahat	Dhayna	Almora	Uttarakhand
44	IC469855	Gahat	Mostabora	Champawat	Uttarakhand
45	IC469794	Gahat	Tamali	Champawat	Uttarakhand
46	IC263263	Gahat	Dhasper	Almora	Uttarakhand
47	IC263264	Gahat	Dhasper	Almora	Uttarakhand
48	IC263277	Gahat	Lonthi	Pithoragarh	Uttarakhand
49	IC263294	Gahat	Gumood	Champawat	Uttarakhand
50	IC356050	Gahat	Mango	Chamoli	Uttarakhand
51	IC382656	Gahat	Gwar Thapli	Rudraprayag	Uttarakhand
52	IC561027	Kulthi	Nautanwa/Madhupur	Giridih	Jharkhand
53	IC561023	Kulthi	Sukhuwadiha/Dumri	Giridih	Jharkhand
55 54	IC561025	Kulthi	Chapuadiha/Bengabad	Giridih	Jharkhand
54 55	IC561029	Kulthi	Sidraul/Namkum	Ranchi	Jharkhand
55 56	IC280546	Kulthi	Banmalipur	Nayagarh	Odisha
50 57	IC280546	Kulthi	Janisahi	Nayagarh	Odisha
57	IC280584	Horsegram	Kolthur	Khammam	Andhra Pradesh
59	ELS-65	Ulavulu	Kotthui		Andhra Pradesh
59 60	IC371910	Kolatha	- Gaudaguda	Vizayanagarum Malkangiri	Odisha
61	IC371910	Kolatha	-	Malkangiri	Odisha
62		Kultho	Katraguda Kubdi	Rayagara Kalahandi	Odisha
	IC373194	Kultho Kalatha			Odisha
63 64	IC372087		Na. pada	Navrangpur	
	IC373255	Kultho	Tingharia	Gajapati	Odisha
65 	IC371987	Kolatta	Nandiguda	Koraput	Odisha
66	IC364123	Vellaikana payir	Pudupatti	Dindugul	Tamil Nadu
67	IC382218	Kolth	Bajaura	Kullu	Himachal Pradesh
68	IC376253	Huruli	Sirnoor	Gulbarga	Karnataka
69	IC376307	Chalmitanda local-1	Chalmitanda	Bijapur	Karnataka
70	IC376308	Chalmitanda local-2	Chalmitanda	Bijapur	Karnataka
71	IC361651	Kulth	Garola	Chamba	Himachal Pradesh
72	IC310660	Karrat	Amet	Rajsamand	Rajasthan
73	IC278825	Kulathi	Kyar	Sirmour	Himachal Pradesh
74	IC278827	Kulathi	Bagol	Sirmour	Himachal Pradesh
75	IC278829	Kulathi	Didag	Sirmour	Himachal Pradesh
76	IC278830	Kulathi	Chhogtali	Sirmour	Himachal Pradesh
77	IC283434	Kulthi	Shashibhusan gram/ Mohanpur	Deogarh	Bihar
78	IC283442	Kulthi	Rampur/Banka	Ahmedabad	Bihar
79	IC281653	Gahat	Thalal	Uttarkashi	Uttarakhand
80	IC281651	Gahat	Thalal	Uttarkashi	Uttarakhand
81	IC281624	Gahat	Nagarkotiya	Chamoli	Uttarakhand
82	IC280768	Kanam	Ayyappan Kovil	Idukki	Kerala
83	IC283868	Ulavulu	Punganur	Chittoor	Andhra Pradesh
84	IC281835	Gahat	Dyangan	Bageshwar	Uttarakhand
85	IC281820	Gahat	Dhamus	Almora	Uttarakhand

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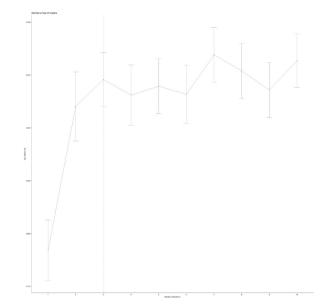
S. No.	Accession No.	Vernacular name	Passport data villaae District State				
86	IC311158		village Hiranpur	<i>District</i> Mayurbhanj	State Odisha		
80 87	IC553492	- Ulavalu	Atmakur	Kurnool	Andhra Pradesh		
	IC553492	Ulavalu	Kurchintalabhai		Andhra Pradesh		
88 89		Gahat		Mahbubnagar	Uttarakhand		
	IC469752		Polop	Champawat			
90	IC469766	Gahat	Polop	Champawat	Uttarakhand		
91	IC469827	Gahat	Chami	Champawat	Uttarakhand		
92	IC425144	Belawadi Local	Bailhongal	Belgaum	Karnataka		
93	IC396009	Hirnwa/Kulthi	Janjgeer/Tamnar	Raipur	Chhattisgarh		
94	IC396012	Hirnwa	Chimtapani/ Gharghoda	Raipur	Chhattisgarh		
95	IC396007	Hirnwa	Bhagaura/Tamnar	Raipur	Chhattisgarh		
96	IC561021	Kulthi	Bhakhora/Kuju	Hazaribag	Jharkhand		
97	IC263319	Gahat	Gaina	Pithoragarh	Uttarakhand		
98	IC263325	Gahat	Gadapani	Almora	Uttarakhand		
99	IC263332	Gahat	Sata	Pithoragarh	Uttarakhand		
100	IC569083	Kolatha	Mohana	Gajapati	Odisha		
101	IC382789	Nalla vulavalu	Devukona	Vizianagaram	Andhra Pradesh		
102	IC382817	Vulavalu	Aluwada	Vizianagaram	Andhra Pradesh		
103	IC382854	Vulavalu	Bellidi	Vizianagaram	Andhra Pradesh		
104	IC415687	Kulthi	Bhamragad	Gadchiroli	Maharashtra		
105	IC400120	Kala kolatha	Nanajhar	Bolangir	Odisha		
106	IC385840	Kulthi	Mohanpur/mohanpur	Deogarh	Jharkhand		
107	IC385919	-	Nagripur	Dumka	Jharkhand		
108	IC385920	-	Mahua	Dumka	Jharkhand		
109	IC385923	-	Basmata	Giridih	Jharkhand		
110	IC391416	Gahat	Palsau	Champawat	Uttarakhand		
111	IC391572	Gahat	Baijgaon	Champawat	Uttarakhand		
112	IC426454	Ulavalu	Nakrekal	Nalgonda	Andhra Pradesh		
113	IC426472	Ulavalu	Sukumamidi	Khammam	Andhra Pradesh		
114	IC426475	Ulavalu	Bachluru	East Godavari	Andhra Pradesh		
115	IC426507	Ulavalu	Dekathipalem	Visakhapatnam	Andhra Pradesh		
116	IC398783	Kurthi	Samachaque/Lakhimp	Jamui	Bihar		
117	IC397423	Kulthi	Chandenkhedi	Dhar	Madhya Pradesh		
118	IC397441	Kulthi	Kikarwas	Dhar	Madhya Pradesh		
119	IC397443	Kulthi	Kikarwas	Dhar	Madhya Pradesh		
120	IC397461	Kulthi	Dharamrai	Dhar	Madhya Pradesh		
121	IC397465	Kulthi	Sewariapani	Barwani	Madhya Pradesh		
122	IC397479	Kulthi	Silawad	Barwani	Madhya Pradesh		
123	IC397511	Kulthi	Awalda	Barwani	Madhya Pradesh		
124	IC397588	Kulthia	Mehalgaon	Jhabua	Madhya Pradesh		
125	IC397611	Kulthia	Walpur	Jhabua	Madhya Pradesh		
126	IC397809	Kollu	Perungolathur	Thiruvannamalai	Tamil Nadu		
120	IC423505	Kulath	Nagarli	Shimla	Himachal Pradesh		
127	IC423538	Kulath	Thalog	Shimla	Himachal Pradesh		
120		Nuluti	malog	Jiiilia	rinnachai riauesil		

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C No	Accession No.	Vernacular name	Passport data					
S. No.		vernacular name	village	District	State			
130	IC418359	Kulthi	Suran	Pithoragarh	Uttarakhand			
131	IC261295	Natu Ulavalu	Reddivaripalle	Chittoor	Andhra Pradesh			
132	IC261283	Ulavalu	Diguvarpalli	Anantapur	Andhra Pradesh			
133	IC259884	Kolatha	Bhatapura	Raigad	Odisha			
134	IC261300	Ulavalu	Rekkalgunta	Anantapur	Andhra Pradesh			
135	IC261296	Ulavalu	Enmulauarpally	Anantapur	Andhra Pradesh			
136	IC261303	Ulavalu	Y. Ramapuram	Anantapur	Andhra Pradesh			
137	IC273740	Kulthi	Bakawand	Bastar	Chhattisgarh			
138	IC273741	Kulthi	Bakawand	Bastar	Chhattisgarh			
139	IC273742	Kulthi	Lamker	Bastar	Chhattisgarh			
140	IC273743	Kulthi	Lamker	Bastar	Chhattisgarh			
141	IC273750	Kulthi	Sakargaon	Bastar	Chhattisgarh			
142	IC273748	Kulthi	Sakargaon	Bastar	Chhattisgarh			
143	IC265920	Kulth	Manjhangan	Mandi	Himachal Pradesh			
144	IC317402	Gahat	Bharwari	Mayurbhanj	Odisha			
145	IC276108	-	Karko	Upper Siang	Arunachal Pradesh			
146	IC425075	Jumnal local-3	Jumnal	Bijapur	Karnataka			
147	IC425079	Lokapur local-3	Lokapur	Bagalkot	Karnataka			
148	IC262082	Gahat	Kafadkhan	Almora	Uttarakhand			
149	IC262106	Gahat	Koirali	Nainital	Uttarakhand			
150	IC262880	Kulthi/Kolth	Saroa	Mandi	Himachal Pradesh			
151	CRHG-19	Released variety	-	Hyderabad	Andhra Pradesh			
152	CRIDA-18R	Released variety	-	Hyderabad	Andhra Pradesh			
153	VL-19	Released variety	-	Almora	Uttarakhand			
154	BSP-1521	Released variety	-	Bilaspur	Chhattisgarh			
155	VL-15	Released variety	-	Almora	Uttarakhand			



Supplementary Figure 1: PCA Scree plot showing relative contribution of PCs



Supplementary Figure 2: GapStat method showing the optimum number of clusters (k=3)

<u>S.N.</u> 1	Descriptor Seed color (SC)	Descriptor state Black Brown	Frequency 16 35	Percent 10.32	Shannon diversity index (SDI) 0.856
		Brown			
		Croom	JJ	22.58	
		Cream	8	5.16	
		Grey	23	14.84	
		Greyish brown	32	20.65	
		Redish brown	12	7.74	
		Light yellow	17	10.97	
		Others (Mixture)	12	7.74	
2	Plant growth habit (PGH)	Bushy	46	29.68	0.498
		Semi-spreading	74	47.74	
		Spreading	31	20.00	
		Trailing	2	1.29	
		Others (Intermediate)	2	1.29	
3	Leaf shape (LSh)	Deltoid	1	0.65	0.486
		Elliptical	30	19.36	
		Lanceolate	2	1.29	
		Ovate	75	48.39	
		Ovate-lanceolate	47	30.33	
4	Pod beak shape (PBSh)	Curved	54	34.84	0.468
		Slightly-curved	63	40.65	
		Straight	38	24.56	
5	Seed shape (SSh)	Globose	47	30.33	0.464
		Kidney	69	44.56	
		Ovoid	39	25.16	
6	Stem pubescence density (SPD)	Dense	36	23.23	0.464
		Intermediate	67	43.23	
		Sparse	52	33.55	
7	Early plant vigour (EPV)	Poor	7	4.52	0.365
		Good	81	52.26	
		Very good	67	43.23	
8	Leaf Color (LC)	Dark green	67	43.23	0.365
		Green	81	52.26	
		Pale green	7	4.52	
9	Stem Color (SCI)	Green	116	74.84	0.320
		Light yellow	17	10.97	
		Purple	22	14.19	
10	Leaf Surface (LSf)	Glabrous	89	57.42	0.296
		Pubescent	66	42.58	
11	Plant Growth Pattern (PGP)	Determinate	53	34.19	0.279
		Indeterminate	102	65.81	
12	Shattering Habit (SH)	Absent	49	31.61	0.271
		Present	106	68.39	
13	Flower Color (FC)	Cream	4	2.58	0.242

Supplementary Table 2: Diversity indices for qualitative traits recorded during the study

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S.N.	Descriptor	Descriptor state	Frequency	Percent	Shannon diversity index (SDI)
		Light yellow	126	81.29	
		Yellow	25	16.13	
14	Pod Pigmentation (PP)	Absent	127	81.94	0.205
		Present	28	18.07	
15	Pod Shape	Flat	20	12.90	0.167
		Cylindrical	135	87.10	
16	Pod Surface	Glabrous	3	1.94	0.041
		Pubescent	152	98.07	

Supplementary Table 3: Comparison of Cluster membership by the HCA and KCA

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Charles .	Cluster-l		Cluster-II	Cluster-II		Cluster-III	
State	HCA	КСА	НСА	КСА	НСА	КСА	— Total
Uttarakhand (UK)	29	29	0	0	0	0	29
Himachal Pradesh (HP)	12	12	2	2	1	1	15
Madhya Pradesh (MP)	10	10	0	0	0	0	10
Odisha (OD)	1	1	7	6	14	15	22
Maharashtra (MH)	2	2	0	0	2	2	4
Chhattisgarh (CG)	2	2	0	0	9	9	11
Rajasthan (RJ)	1	1	0	0	1	1	2
Jharkhand (JH)	1	1	1	0	12	13	14
Karnataka (KA)	0	0	8	8	0	0	8
Tamil Nadu (TN)	0	0	5	3	1	3	6
Assam (AS)	0	0	1	1	0	0	1
Arunachal Pradesh (AR)	0	0	1	1	0	0	1
Andhra Pradesh (AP)	0	0	8	6	15	17	23
Bihar (BR)	0	0	2	2	5	5	7
Kerala (KL)	0	0	0	0	1	1	1
Sikkim (SK)	0	0	0	0	1	1	1
Total	58	58	35	29	62	68	155



Supplementary Figure 3: Map of India showing Indian states covered by cluster I and III formed by KCA