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Assessment of Genetic Diversity Using Morpho-Agronomical Traits in Horse Gram

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ABSTRACT

Horse gram [*Macrotyloma uniflorum* (Lam.) Verd.] is one of the highly nutritious minor pulse crop and has number of desirable traits like drought tolerance, high protein content, antioxidant activity, antimicrobial and medicinal properties. A total of 66 genotypes of horse gram comprising 61 accessions of indigenous collections belonging to 15 states of India, one exotic collection from Australia and four checks were evaluated in Randomized Block Design (RBD) consecutively for three years [Kharif seasons of 2013 (E1), 2014 (E2) and 2015 (E3)] at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), Regional Station, Akola, Maharashtra. Accessions were evaluated for seven each of qualitative and quantitative traits. Significant variability was observed among the accessions for most of the quantitative traits. Highest coefficient of variation was observed in seed yield per plant (g) followed by pods per plant and pod length. The phenotypic coefficients of variation (PCV) were higher than the genotypic coefficient of variation (GCV) for all the traits. The highest magnitude of PCV and GCV were recorded for seed yield per plant (g) and pods per plant followed by plant height (cm). Shannon diversity index (SDI) varied from 0.078 to 0.686 showed existence of sufficient variability among the accessions for qualitative traits. Accessions were classified on the basis of Euclidean distances using Ward's method. The dendrogram revealed two major clusters. Cluster I comprised of 17 accessions and cluster II comprised of 49 accessions. Cluster II was further classified in two sub-clusters (IIa and IIb). Most of the accessions from Tamil Nadu were confined to cluster I indicating their similarity for quantitative traits. Whereas accessions from rest of the states were well distributed in cluster II exhibiting high diversity for the traits studied. The accessions from cluster I could be used for hybridization program with the accessions from cluster II in order to develop superior horse gram genotypes/varieties for overall yield enhancement.

Keywords

GCV, PCV, Diversity analysis, Shannon Diversity index, Euclidean distances

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Introduction

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) is an important minor legume crop has

its origin in India (Bogdan, 1977). It is mainly cultivated in the localities of underprivileged resource poor farmers in central and southern region (Chhatisgarh, Jharkhand, Maharashtra,

Odisha, Telangana, Andhra Pradesh, Karnataka and Tamilnadu) of the country. It has very high nutritional value which forms a very quality protein source to the farmers in tribal areas (Dikshit *et al.*, 2014). Horse gram grains contains up to 27% protein (Kumar, 2007) high in lysine content compared to other pulses such as chick pea and red gram (Yadav, 2004). Taxonomically it belongs to the family *Fabaceae* with diploid chromosome number ($2n=20, 22, 24$). It has self-fertile cleistogamous flowers producing pods with 4-6 grains (Cook *et al.*, 2005). Clinical studies reported that horse gram has extraordinary therapeutic properties and in our traditional ayurvedic treatments it used to treat the ailments related to kidney stones, urinary diseases, piles, cardiac problems, asthma etc. It has high dietary fibre content which is very useful for good bowel movements and reduction of symptoms related to obesity (Kumar, 2007; Bhartiya *et al.*, 2015). Horse gram is also a very good source of iron and molybdenum micronutrients and overall chemical composition of grains is as good as other cultivated legumes (Vishwanatha *et al.*, 2016). This crop has also high fodder value due to its protein richness and it is majorly used to feed milch animals and horses (Cook *et al.*, 2005; Bhadana *et al.*, 2013). Agronomically, it is very important for sustainable farming with its atmospheric nitrogen fixation ability. It also helps in reduction of soil erosion with good canopy cover over the soil. Horse gram crop takes about 120-180 days to mature. It is usually cultivated in resource poor soils as a rainfed crop. In paddy growing areas it is broadcasted immediately after the harvest of paddy so as to take maximum advantage of residual soil moisture. It is very hardy and drought tolerant crop. Very few efforts have been made for the improvement of this minor but very important pulse crop. High variability existed in horse gram germplasm for various traits. The utilization of this information for

improvement is of high significance. The present investigation was targeted to study the genetic variability among the horse gram accessions collected from different states of India and to assess the correlation among the different traits of interest and drawing of inferences for utilization in the horse gram improvement.

Materials and Methods

A total of 66 horse gram genotypes comprising 61 accessions of indigenous collections from about 15 states of India, one exotic collection from Australia and four checks (Table 1) were evaluated at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), Regional Station, Akola, Maharashtra in Randomized Block Design (RBD) consecutively for three years [Kharif seasons of 2013 (E1), 2014 (E2) and 2015 (E3)]. Recommended package of practices were followed to raise a good crop. Sowing was done with 90×30 cm crop geometry. Each genotype was sown in 3 rows of 3 meter length. The observations on seven quantitative traits *viz.*, plant height (cm), primary branches per plant, pods per plant, pod length (cm), seeds per pod, 100 seed weight (g) and seed yield per plant (g) were recorded on five random plants. For qualitative traits visual scoring was done using standard descriptors for horse gram. Quantitative data was analysed using Windostat software to work out variability parameters. Diversity analysis using Euclidean distances (Wards' method) was carried out with Paleontological Statistics Software Package for Education and Data Analysis (PAST) (Hammer *et al.*, 2001) software (Ver. 3.14) freely available at www.folk.uio.no/ohammer/past/.

Results and Discussion

Horse gram with its importance as functional food is gaining attention worldwide

specifically in under developed and developing countries. In developed nations it is an important food for dietary fibres. There are various advantages of growing horse gram for its capabilities to fix atmospheric nitrogen, drought tolerance and cover crop to check soil erosion. It is mainly grown on resource poor soils with negligence of improved production technologies. Farmers use the remnant seeds to cultivate this crop which results in poor performance and reduction in overall productivity. Keeping this in view, a well-planned strategy is required to assess the genetic variability in horse gram for various important agronomic attributes, identification of association between the traits and effect of numerous traits on yield levels. The present investigation was also carried out to address these issues. A total 66 horse gram accessions were evaluated for three years and mean performance and variability parameters were assessed for further utilization in crop improvement programme. Significant differences were observed among the accessions for most of the traits except pod length (cm) and seeds per pod (Table 2). Highest coefficient of variation was observed in seed yield per plant followed by pods per plant and pod length (Parameshwarappa and Kumar 2002; Gupta *et al.*, 2010; Kulkarni and Mogle, 2011). Among the 66 genotypes, IC 277625 (7.00), IC 277630 (6.70) and IC 145250 (6.63) recorded more primary branches per plant. For pods per plant, IC 53627 (49.60), IC 26132 (49.43) and IC 9623 were superior. Pod length (cm) and seeds per pod were almost similar in all the accessions studied. Tall plant types was observed in the accessions IC 277630 (90.65 cm) followed by IC 277677 (87.04 cm) and IC 277625 (83.49 cm). Superior genotypes for seed yield per plant (g) were IC 341308 (19.51 g), IC 145250 (13.66 g) and IC 22783 (13.29 g). Accessions, IC 139527 (3.59g), IC 15735 (3.57g) and IC277677 (3.36g) recorded highest 100 seed weight (g) (Table 2). Similar observations on

agro-morphological traits were reported by Prakash and Khanure (2000); Prakash *et al.*, 2008; Prakash *et al.*, 2010; Sunil *et al.*, 2014 and Vishwanatha *et al.*, 2016 in horse gram.

Genetic variability estimates

The phenotypic coefficients of variation (PCV) were higher than the genotypic coefficient of variation (GCV) for all the traits. The characters seed yield per plant, pods per plant, plant height and 100 seed weight exhibited high estimates of GCV, PCV and ECV (Sunil *et al.*, 2008; Gupta *et al.*, 2010). The heritability estimates were also high for seed yield per plant, 100 seed weight and plant height. Genetic advance % of mean at 5% was high for seed yield per plant and pods per plant (Table 3).

Diversity for qualitative traits

Shannon diversity index varied from 0.078 to 0.686 showed existence of sufficient variability among the accessions (Table 4). High values for Shannon Diversity Index (SDI) were reported for the traits growth pattern, leaf surface, stem colour and pod surface. This indicated richness of this population for diverse phenotypes available for these traits. Sankar *et al.*, 2015 reported high SDI for Per cent disease Index (PDI) trait in horse gram.

Diversity in quantitative traits

Accessions were classified in to different clusters on the basis of Euclidean distances using Ward's method. The dendrogram revealed two major clusters. Cluster I comprised of 17 accessions and cluster II comprised of 49 accessions. Cluster II was further classified in two sub-clusters (IIa and IIb). Most of the accessions from Tamil Nadu were confined to cluster I indicating their similarity for quantitative traits.

Table.1 List of horse gram accessions with passport information

SN	IC/EC No.	State	Country	SN	IC/EC No.	State	Country
1	IC 9623	Sikkim	India	34	IC 89010	Tamil Nadu	India
2	IC 9624	Sikkim	India	35	IC 105588	Jharkhand	India
3	IC 9626	Sikkim	India	36	IC 121640	Himachal Pradesh	India
4	IC 10938	Karnataka	India	37	IC 123030	Tamil Nadu	India
5	IC 15735	Jharkhand	India	38	IC 123033	Bihar	India
6	IC 15775	Manipur	India	39	IC 139331	Maharashtra	India
7	IC 19552	Odisha	India	40	IC 139527	Andhra Pradesh	India
8	IC 22750	Madhya Pradesh	India	41	IC 139544	Uttarakhand	India
9	IC 22783	Chhattisgarh	India	42	IC 145245	Himachal Pradesh	India
10	IC 23444	Chhattisgarh	India	43	IC 145250	Himachal Pradesh	India
11	IC 23510	Chhattisgarh	India	44	IC 145268	Gujarat	India
12	IC 26132	Karnataka	India	45	IC 264704	Kerala	India
13	IC 26135	Manipur	India	46	IC 277625	Karnataka	India
14	EC 28842	-	Australia	47	IC 277630	Karnataka	India
15	IC 45748	Tamil Nadu	India	48	IC 277659	Karnataka	India
16	IC 47119	Andhra Pradesh	India	49	IC 277670	Karnataka	India
17	IC 47129	Andhra Pradesh	India	50	IC 277671	Karnataka	India
18	IC 49290	Maharashtra	India	51	IC 277677	Karnataka	India
19	IC 49555	Maharashtra	India	52	IC 277687	Karnataka	India
20	IC 53627	Chhattisgarh	India	53	IC 282589	Jharkhand	India
21	IC 55065	Manipur	India	54	IC 321300	Tamil Nadu	India
22	IC 68593	Kerala	India	55	IC 336492	Bihar	India
23	IC 71725	Tamil Nadu	India	56	IC 341308	Maharashtra	India
24	IC 71730	Tamil Nadu	India	57	IC 343105	Jharkhand	India
25	IC 71743	Tamil Nadu	India	58	IC 343190	Chhattisgarh	India
26	IC 71763	Tamil Nadu	India	59	IC 347181	Jharkhand	India
27	IC 71766	Tamil Nadu	India	60	IC 385839	Jharkhand	India
28	IC 71770	Tamil Nadu	India	61	IC 561038	Jharkhand	India
29	IC 71781	Tamil Nadu	India	62	IC 561040	Jharkhand	India
30	IC 71783	Tamil Nadu	India	63	AK-21	Maharashtra	India
31	IC 71803	Tamil Nadu	India	64	AK-26	Maharashtra	India
32	IC 71817	Tamil Nadu	India	65	HGGP	-	India
33	IC 71825	Tamil Nadu	India	66	PHG-9	-	India

Table.2 Mean performance of horse gram genotypes for various traits

SN	IC/EC No.	PRI_BRN	POD_PLT	POD_LT	PLT_HGT	SED_POD	YLD_PLT	SED_WGT
1	IC 9623	5.90	48.47	4.34	57.13	4.43	7.65	3.03
2	IC 9624	5.03	28.20	3.98	47.74	3.90	4.26	2.53
3	IC 9626	5.53	19.67	4.08	65.39	4.03	3.76	2.83
4	IC 10938	5.97	24.27	4.21	58.56	4.00	8.52	2.64
5	IC 15735	5.90	31.97	4.34	64.93	4.33	3.66	3.57
6	IC 15775	5.63	30.97	4.05	62.86	3.90	3.16	3.29
7	IC 19552	5.03	26.10	4.35	61.22	4.13	7.93	2.96
8	IC 22750	5.97	26.23	4.09	57.30	4.20	5.44	2.64
9	IC 22783	6.23	46.43	4.10	61.25	4.35	13.29	3.07
10	IC 23444	5.27	29.90	4.24	66.69	4.27	5.93	3.26
11	IC 23510	6.22	35.23	4.27	70.15	4.63	9.71	3.14
12	IC 26132	5.83	49.23	4.40	66.36	4.23	9.93	2.89
13	IC 26135	6.03	29.50	4.16	63.80	4.33	6.21	3.11
14	EC 28842	5.57	23.60	4.65	74.33	4.53	4.95	3.08
15	IC 45748	5.13	28.33	4.32	60.32	4.50	5.23	2.89
16	IC 47119	5.80	39.40	4.25	60.59	4.37	5.96	3.28
17	IC 47129	5.50	29.57	5.66	65.33	4.33	6.04	3.15
18	IC 49290	5.57	43.03	4.05	63.90	3.97	3.92	2.30
19	IC 49555	5.37	31.60	4.20	53.16	4.30	5.11	2.39
20	IC 53627	5.47	49.60	4.27	57.45	3.97	6.50	3.04
21	IC 55065	5.60	46.07	4.24	64.23	4.83	6.38	2.88
22	IC 68593	5.93	36.97	4.46	68.03	4.47	7.03	3.01
23	IC 71725	6.47	41.70	3.80	68.32	3.93	3.26	3.18
24	IC 71730	5.87	24.93	4.52	60.90	4.50	3.67	2.60
25	IC 71743	6.13	33.43	4.17	54.63	4.07	8.75	2.97
26	IC 71763	5.93	39.63	4.35	55.30	4.57	5.88	3.12
27	IC 71766	5.47	32.27	4.59	55.68	4.53	3.24	2.52
28	IC 71770	5.17	31.60	4.53	59.97	4.50	3.68	2.44
29	IC 71781	5.13	31.60	4.22	50.47	4.37	3.40	2.59
30	IC 71783	5.13	26.43	4.55	55.15	4.13	3.35	2.50
31	IC 71803	5.63	22.27	4.43	52.75	4.20	3.73	2.96
32	IC 71817	6.00	27.23	4.76	51.27	4.63	3.48	2.52
33	IC 71825	5.10	35.53	4.64	51.98	4.27	4.46	2.68
34	IC 89010	5.63	22.50	4.56	71.60	4.30	3.29	3.05
35	IC 105588	5.60	24.53	4.41	59.79	3.80	6.06	2.61
36	IC 121640	5.67	24.43	4.02	46.39	3.90	3.45	2.82
37	IC 123030	5.43	23.57	3.96	52.96	3.87	3.75	3.01
38	IC 123033	5.92	34.03	4.40	57.71	4.20	7.81	2.85
39	IC 139331	6.43	32.33	4.23	61.17	4.50	8.09	3.34
40	IC 139527	6.50	44.60	4.24	74.37	4.77	11.71	3.59

41	IC 139544	5.57	26.00	4.45	61.55	4.10	7.69	2.86
42	IC 145245	6.03	17.73	4.05	50.65	3.72	6.39	2.85
43	IC 145250	6.63	41.47	4.50	60.66	3.77	13.66	2.99
44	IC 145268	5.77	28.60	4.36	58.80	4.33	10.44	3.11
45	IC 264704	6.70	32.73	4.41	63.80	3.80	9.13	2.88
46	IC 277625	7.00	27.83	4.33	83.49	4.33	4.13	3.26
47	IC 277630	6.97	38.53	4.32	90.65	4.57	6.53	3.10
48	IC 277659	6.57	19.53	4.26	81.32	4.13	3.75	2.98
49	IC 277670	5.87	16.13	4.20	73.26	3.57	2.96	2.81
50	IC 277671	6.67	19.40	4.24	77.29	4.33	5.52	3.18
51	IC 277677	6.13	22.17	4.12	87.04	4.18	2.97	3.36
52	IC 277687	5.93	25.00	4.11	76.27	4.30	4.01	3.12
53	IC 282589	5.87	31.73	4.35	61.60	4.43	5.85	2.76
54	IC 321300	6.00	24.83	4.27	66.20	4.50	4.57	3.13
55	IC 336492	5.90	21.73	4.16	75.21	4.13	4.16	3.20
56	IC 341308	5.83	44.67	4.42	73.11	4.37	19.51	3.40
57	IC 343105	5.50	22.30	4.47	66.91	4.17	4.37	3.04
58	IC 343190	5.73	26.77	4.28	56.54	3.93	6.40	3.01
59	IC 347181	5.73	30.17	4.02	63.07	4.00	5.00	2.69
60	IC 385839	5.97	25.90	4.20	72.96	4.00	5.65	3.06
61	IC 561038	5.93	15.47	4.28	65.94	3.97	3.22	2.93
62	IC 561040	6.07	27.67	4.06	72.81	4.17	4.69	2.76
63	AK-21	6.37	33.70	4.36	71.33	4.23	3.79	3.08
64	AK-26	5.27	26.07	4.43	56.64	4.47	11.45	3.17
65	HGGP	5.90	34.73	4.15	63.28	4.37	3.70	2.90
66	PHG-9	5.57	15.30	4.27	74.32	4.37	6.23	3.14
	Mean	5.84	30.35	4.31	63.88	4.23	6.02	2.96
	Minimum	5.03	15.30	3.80	46.39	3.57	2.96	2.30
	Maximum	7.00	49.60	5.66	90.65	4.83	19.51	3.59
	C.D. 5%	0.90	15.11	NS	14.81	NS	3.57	0.40
	C.D. 1%	1.19	19.90	NS	19.51	NS	4.71	0.53

PRI_BRN = Primary branches per plant; POD_PLT= Pods per plant; POD_LT= pod length (cm); PLT_HGT= Plant height (cm); SED_POD= Seeds per pod; YLD_PLT = Seed yield per plant (g); SED_WGT = 100 seed weight (g)

Table.3 Variability estimates for different traits in horse gram

Parameters	PRI_BRN	POD_PLT	POD_LT	PLT_HGT	SED_POD	YLD_PLT	SED_WGT
ECV	13.63	43.83	13.65	20.41	16.31	52.25	11.88
GCV	5.45	21.65	1.64	11.92	2.28	46.18	7.97
PCV	14.68	48.89	13.75	23.64	16.15	69.73	14.30
h ² (B S.)	0.13	0.19	0.01	0.25	-0.02	0.43	0.31
GA as % of Mean 5%	4.17	19.75	0.40	12.39	-0.66	63.00	9.15

PRI_BRN = Primary branches per plant; POD_PLT= Pods per plant; POD_LT= pod length (cm); PLT_HGT= Plant height (cm); SED_POD= Seeds per pod; YLD_PLT = Seed yield per plant (g); SED_WGT = 100 seed weight (g)

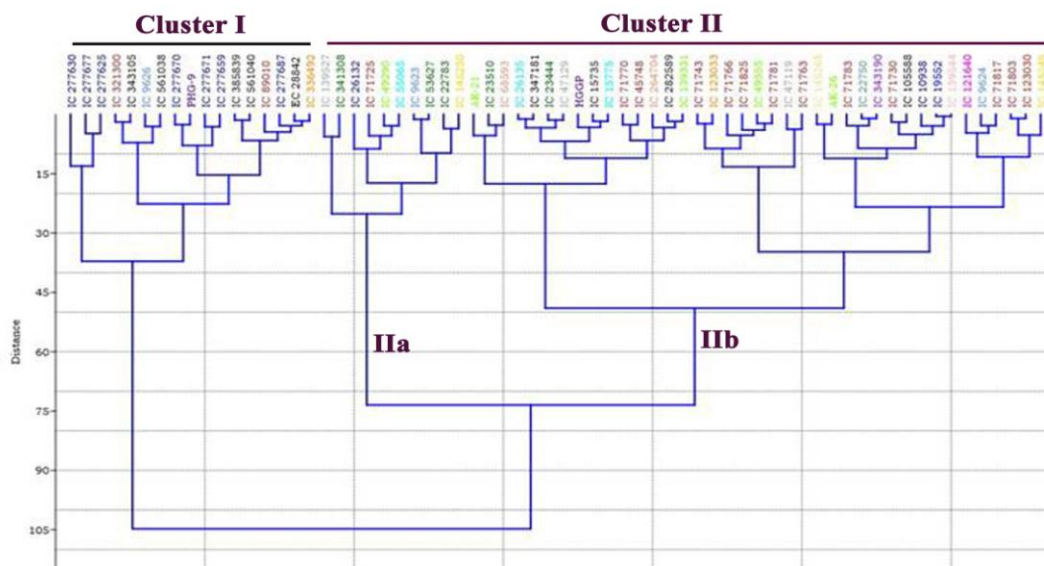
Table.4 Variability in qualitative traits among 66 horsegram accessions

Descriptors	Descriptor states	Frequency	Percent	SDI
Plant vigour	Poor	2	3.03	0.501
	Good	8	12.12	
	Very good	56	84.85	
Growth habit	Bushy	1	1.52	0.078
	Semi spreading	65	98.48	
Growth Pattern	Determinate	26	39.39	0.670
	Indeterminate	40	60.61	
Leaf surface	Glabrous	45	68.18	0.625
	Pubescent	21	31.82	
Stem colour	Green	50	75.76	0.554
	Purple	16	24.24	
Pod surface	Glabrous	16	24.24	0.554
	Pubescent	50	75.76	

Table.5 Promising accessions identified for different traits in horse gram

SN	Trait	Promising accessions
1	Primary branches per plant	IC 277625, IC 277630, IC 145250
2	Pods per plant	IC 53627, IC 26132, IC 9623
3	Plant height (cm)	IC 277630, IC 277677, IC 277625
4	Seed yield per plant (g)	IC 341308, IC 145250, IC 22783
5	100 seed weight (g)	IC 139527, IC 15735, IC 277677

Fig.1 Grouping of 66 horse gram accession on the basis of Euclidean distances



Whereas accessions from rest of the states were well distributed in cluster II exhibiting high diversity for the traits studied. The accessions from cluster I could be used for hybridization program with the accessions from cluster II in order to develop high yielding genotypes/varieties for overall yield enhancement (Fig. 1). Sunil *et al.*, 2008 and Vishwanatha *et al.*, 2016 also reported clustering of horse genotypes into different clusters and also reported important and distant accessions for utilization in horse gram crop improvement (Gupta *et al.*, 2010). Promising accessions for different traits are reported (Table 5) and these can be used for improvement of the specific traits in horse gram.

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