

## Characterization of horse gram accessions on the basis of morphological and biochemical characters

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**ABSTRACT** Fifty horse gram accessions were characterized based on the morphological characters viz., plant growth pattern, plant growth habit, stem color, leaf color and seed color. Significant correlation was noticed among the qualitative traits, while biochemical parameters exhibited positive and negative relationship with the morphological qualitative traits, but the relationship was non-significant. Plant growth pattern was significantly and positively correlated with plant growth habit and leaf color, while plant growth habit showed significant negative relationship with stem color and significant positive relationship with leaf color. However, biochemical parameters viz., proteins and phenols exhibited positive and negative relationship with the morphological qualitative traits, but the relationship was non-significant.

**Keywords:** *Macrotyloma uniflorum*, horse gram, characterization, morphological characters, biochemical characters

Horse gram, *Macrotyloma uniflorum* (Lam. verdc.) is an important unexploited legume suitable for tropical and sub-tropical dry land agriculture. In India, it occupies an area of 1.84 m ha and 0.022 m ha in Andhra Pradesh, with a production of 9.0 t and productivity is 400 kg ha<sup>-1</sup>, which is less than the national average (500 kg ha<sup>-1</sup>). One of the reasons for very low productivity in horse gram is limited variability [1]. Keeping in view the potential of this crop, fifty accessions collected from diverse agro-ecological regions were characterized to study the initial variability. The germplasm was evaluated for economically important traits. In order to maintain, evaluate and utilize germplasm effectively, it is important to investigate the extent of genetic diversity available. Smith and Smith [2] considered morphological characterization as an important step in description and classification of crop germplasm because a breeding programme mainly depends upon the magnitude of

genetic variability [3]. Since horse gram is a self-pollinated crop, the variability is expected to be limited and studies on variability on morpho-physiological characters are meagre.

### MATERIALS AND METHODS

#### *Morphological characterization*

The studies on characterization of horse gram accessions were taken up at National Bureau of Plant Genetic Resources Regional Station, Rajendranagar, Hyderabad. One hundred and nineteen (119) germplasm accessions of horse gram (116 accessions and 3 check varieties) were laid out in Randomized Block Design with three replications. Each accession was grown in three rows of 3 m length consisting of 45 plants, with a spacing of 60 x 20 cm. Recommended agronomic practices were followed during crop growth period. Out of 119 accessions, only 50 accessions were selected based on seed color, stem color, plant

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growth pattern, plant growth habit and leaf color for initiating screening studies and for estimation of biochemical parameters viz., proteins and phenols. Since no variation was noticed in leaf surface, flower color, pod shape and pod surface; the data is not presented in the table.

Qualitative observations viz., plant growth habit, plant growth pattern, leaf color, leaf surface, stem color, flower color, pod shape, pod surface and seed color were recorded on five randomly selected competitive plants in each germplasm accession for each replication. As per the descriptors available on horse gram all the 50 accessions of horse gram were characterized into different classes on the basis of morphological characters at specified stage.

## RESULTS AND DISCUSSION

The characterization of horse gram accessions was done for morphological and biochemical parameters. The frequency distribution of qualitative attributes of horse gram with example accessions is depicted in Table 1.

### *Plant growth pattern*

Data presented in the Table 1 revealed that 50 accessions of horse gram were classified into two categories based on plant growth pattern i.e., determinate and indeterminate. Of the 50 accessions, 30 exhibited determinate growth pattern and the remaining 20 accessions had indeterminate growth pattern (Fig. 1). A study conducted on variability in 36 accessions of *Dolichos lablab* for morphological qualitative traits observed variation regarding growth pattern (determinate and indeterminate), pigmentation of stem, leaf, flower, pod and seed, pubescence, pod shape and size [4]. Variation in pod color was noticed in different types, which were graded as light green, green, dark green and purple. A total of 107 lablab bean germplasm lines representing 20 different countries in Asia,

Africa, South America and Europe for morphological characters were studied and found variation in growth pattern (indeterminate, determinate) of lablab germplasm was observed [5].

### *Plant growth habit*

Based on plant growth habit, horse gram accessions were classified into 4 groups viz., bushy, spreading, semi-spreading and trailing (Table 1). Out of 50 accessions, 18 accessions were bushy type, 5 were spreading type, 12 were semi-spreading type and 15 were having trailing type growth habit (Fig. 1). 484 accessions of black gram were evaluated for qualitative traits and a large variation in plant growth habit (erect, semi-erect and spreading) was reported [6]. The conducted studies on variability in 36 accessions of *Dolichos lablab* for morphological qualitative traits was conducted [4] of the 36 types, Indore Choudi and CBS No. 32 had bushy habit of growth; others were stem twiners. A review of some preliminary efforts on assessment of genetic variability and its use in country bean exhibited great diversity for phenotypic traits like plant habit. Out of 107 lablab bean germplasm lines studied. The variation for plant type (dwarf, small spreading vine, medium spreading vine and large spreading vine) was noticed [5].

### *Stem color*

On the basis of stem color, horse gram accessions were distinguished into 2 categories i.e., purple and green colored stems (Fig. 1). Among 50 accessions, 18 had purple stem while 32 accessions were green colored (Table 1). Similar studies conducted earlier on variability in 36 accessions of *Dolichos lablab* for morphological qualitative traits and reported variation regarding pigmentation of stem. Leaf shape and stem colour was used for identification and classification of buckwheat cultivars efficiently [8]. Studied a total of 107 lablab bean germplasm lines

Table 1. Qualitative attributes of horse gram accessions

S.No.	Plant descriptor	Range in expression	No. of accessions	Accession no.
1	Plant growth pattern	Determinate Indeterminate	30 20	NS/05/94, KSAS/06/280, SKN-88, NS-74, NS/05/02, NSM-125, NS/05/85, NSJ/NAIP/031-130, NS/04/124, NS/05/101, NS/05/84, NS/05/103, PSRJ-12997, PSRJ-13006-1, NS/05/16, NS/04/57, PSRJ-13030, NS/05/86, NS/05/113, PSRJ-13150-2, NSB-10, BAR-231-1, NS/05/93, PSRJ-13068, PSRJ-13089-1, RJR-94, NSJ/NAIP/140-239, NSJ/NAIP/BD/ADB-35-1, NS/05/14, Palem-1(check)
2	Plant growth habit	Bushy	18	KSAS/06/391, NS/05/13, NS/05/97, NSM-201, NS/04/107, HG-35, NDS-259, NSB-27, SK-73, NS/05/136, NSM-147, PSRJ-13089, NS/05/116, NS/05/87, NS/05/105, SK-28, NS/05/42, NSJ/NAIP/006-105, Palem-2 (check), AK-21 (check)
3	Stem color	Spreading Semi-spreading Trailing Purple Green	5 12 15 18 32	NS/05/94, KSAS/06/280, SKN-88, NS/05/85, NSJ/NAIP/031-130, PSRJ-12997, PSRJ-13006-1, NS/04/57, PSRJ-13030, NS/05/113, NSB-10, NS/05/93, PSRJ-13068, PSRJ-13089-1, RJR-94, NSJ/NAIP/140-239, NSJ/NAIP/BD/ADB-35-1, Palem-1 (check) KSAS/06/391, NSM-201, NSM-125, PSRJ-13150-2, NSJ/NAIP/006-105 NS/05/02, NS/04/124, NS/05/101, NS/05/84, NS/05/103, SK-73, NS/05/136, NS/05/16, PSRJ-13089, NS/05/86, BAR-231-1, NS/05/14 NS-74, NS/05/13, NS/05/97, NS/04/107, HG-35, NDS-259, NSB-27, NSM-147, NS/05/116, NS/05/87, NS/05/105, SK-28, NS/05/42, Palem-2 (check) NS/05/94, KSAS/06/280, SKN-88, NS/05/02, NS/05/85, NS/04/124, NDS-259, NS/05/101, PSRJ-12997, PSRJ-13006-1, NS/05/113, NSB-10, BAR-231-1, NS/05/93, NS/05/105, RJR-94, NS/05/14, Palem-1(check),
4	Leaf color	Green	45	KSAS/06/391, NS-74, NS/05/13, NS/05/97, NSM-201, NS/04/107, NSM-125, HG-35, NSJ/NAIP/031-130, NS/05/84, NSB-27, NS/05/103, SK-73, NS/05/136, NS/05/16, NS/04/57, NSM-147, PSRJ-13030, PSRJ-13089, NS/05/86, NS/05/116, NS/05/87, PSRJ-13150-2, PSRJ-13068, SK-28, PSRJ-13089-1, NS/05/42, NSJ/NAIP/BD/ADB-35-1, NSJ/NAIP/006-105, Palem-2 (check), AK-21 (check), NSJ/NAIP/140-239 NS/05/94, KSAS/06/391, KSAS/06/280, SKN-88, NS-74, NS/05/13, NS/05/97, NS/05/02, NS/04/107, NSM-125, HG-35, NSJ/NAIP/031-130, NS/04/124, NDS-259, NS/05/101, NS/05/84, NS/05/85, NSJ/NAIP/031-130, NS/04/124, PSRJ-12997, SK-73, PSRJ-13006-1, NS/05/136, NS/05/16, NS/04/57, PSRJ-13030, PSRJ-13089, NS/05/86, NS/05/113, NS/05/116, PSRJ-13150-2, NSB-10, BAR-231-1, NS/05/93, PSRJ-13068, NS/05/105, SK-28, PSRJ-13089-1, NS/05/42, RJR-94, NSJ/NAIP/140-239, NSJ/NAIP/BD/ADB-35-1, NSJ/NAIP/006-105, NS/05/14, Palem-1 (check), AK-21 (check)



were categorized into brown, creamy, black and grey colored seeds (Fig. 1). Among 50 horse gram accessions, 27 resulted in to brown colored seeds, 17 with cream colored seeds, 3 showed black colored seeds and 3 with grey colored seeds (Table 1). In soybean, varietal characterization was made and significant variation among cultivars was observed for seed morphological traits like seed colour [10]. A high degree of variation in seed coat color of pea germplasm evaluated at different vegetative stages for genetic variation was reported [11]. The performance of 23 advanced chickpea mutants/lines for morphological components was evaluated and it was reported that mutant CPM-825 (gr) had distinct greenish seed coat color, which could serve as a genetic marker for identification of developed chickpea accessions [12]. The evaluation of 484 accessions of black gram for qualitative traits revealed that small variation was observed for seed color and spots on seed coat. The studies on variability in 36 accessions of *Dolichos lablab* for morphological qualitative traits were conducted [4]. Variation in pod color was noted in different types, which were graded as light green, green, dark green and purple. The variation in morphological characters of pod (white and green) and seed coat (yellowish white, yellowish brown, mixed, brown chocolate and black), size of leaf and pod was observed among 107 lablab bean germplasm lines of Asia, Africa, South America and Europe [5]. Great diversity for phenotypic traits including pod color, seed color, seed mosaic and seed luster was observed in country bean [7].

Morphological variation among the 50 horse gram accessions for various qualitative attributes was depicted in Fig. 1 may serve as an important aid for the identification of different accessions of horse gram based on plant growth pattern, plant growth habit, stem color, leaf color and seed color. This will also

serve as a useful guide for preparation of DUS manual for horse gram.

Horse gram being a self-pollinated crop, characterization of different accessions would be difficult with a single morphological character. Therefore, a better classification could be obtained with a group of morphological characters. Similar findings, were reported in previous studies [13] who classified all the Buckwheat varieties as individual type with morphological characters alone and reported that they had more relevance for characterization of cultivars. Hence, morphological characters in combination with more reliable methods such as biochemical methods and molecular markers may prove to be fruitful for varietal characterization in horse gram.

#### Proteins

Protein content in 50 horse gram accessions varied from 12.74 to 25.20% (Table 2). The results revealed that less protein content was observed in the accessions PSRJ-13089 (12.74) followed by the accessions viz., NS/05/86 (13.82%), PSRJ-12997 (14.14%), PSRJ-13150-2 (14.26%), PSRJ-13089-1 (14.62%), NS/05/84 (15.12%), NSB-10 (15.16%), NS/05/85 (15.30%), RJR-94 (15.40%) and NS/04/57 (15.56%) and were significantly superior and found to be on par with each other (Fig. 2).

However, more protein content was present in the accessions NS/05/14 (25.20%) followed by NSM-147 (24.5%), SK-73 (24.20%), NS/05/101 (24.20%), NS/05/02 (24.26%), NSJ/NAIP/006-105 (24.12%), HG-15 (23.78%) and NS/05/105 (23.88%) which were found to be on par with each other (Fig 2).

#### Phenols

The results presented in the Table 2 and Fig. 3 revealed that the phenol content varied from 17.04 mg (KSAS/06/280) to 75 mg (NSJ/NAIP/031-130). The data pertaining to phenols showed more phenol content in NSJ/

Table 2. Studies on biochemical parameters of horse gram accessions

S.No.	Accession no.	Protein content (%)	Phenol content (mg/100g sample)	S.No.	Accession no.	Protein content (%)	Phenol content (mg/100g sample)
1	Palem-2 (Check)	18.02	54.24	29	BAR-231-1	23.24	40.16
2	NS/04/124	23.44	55.24	30	HG-35	22.40	37.36
3	SKN-88	17.22	34.08	31	NS/05/136	16.62	43.44
4	KSAS/06/391	19.08	39.84	32	PSRJ-13150-2	14.26	38.80
5	NSB-10	15.16	25.84	33	PSRJ-13089	12.74	43.12
6	NS/05/103	17.96	23.04	34	NSJ/NAIP/ 140-239	22.76	34.80
7	NS/05/105	23.88	26.72	35	SK-28	22.36	30.88
8	NS/05/93	20.24	20.40	36	NS/05/87	23.36	30.08
9	KSAS/06/280	15.58	17.04	37	NS/05/14	25.20	40.08
10	NSM-125	22.82	45.80	38	PSRJ-13006-1	22.34	29.20
11	NS/05/16	22.28	47.00	39	NS/05/116	22.10	31.12
12	PSRJ-13089-1	14.62	24.48	40	NS/05/94	22.76	18.40
13	NS-74	22.04	21.12	41	NSJ/NAIP/ 006-105	24.12	28.24
14	NS/04/57	15.56	29.92	42	NS/05/97	20.30	33.28
15	RJR-94	15.40	32.88	43	NS/05/13	23.42	27.60
16	Palem-1 (Check)	16.04	59.48	44	PSRJ-13068	16.88	38.80
17	Palem-3 (Check)	17.44	39.36	45	NS/05/85	15.30	27.84
18	NSB-27	22.38	65.68	46	NS/05/101	24.20	39.60
19	NS/05/113	16.12	59.08	47	NS/04/107	23.36	37.60
20	NDS-259	17.20	67.08	48	PSRJ-12997	14.14	36.08
21	NS/05/84	15.12	54.88	49	NS/05/42	22.74	30.08
22	NSM-147	24.50	50.64	50	NSJ/NAIP/ BD/ABD-35-1	21.82	43.80
S.No.	Accession no.	Protein content (%)	Phenol content (mg/100g sample)	S.No.	Accession no.	Protein content (%)	Phenol content (mg/100g sample)
23	PSRJ-13030	23.52	62.28	51	NSJ/NAIP/ 031-130	21.20	75.00
24	HG-15	23.78	52.16		SEm±	2.11	2.88
25	NS/05/86	13.82	34.32		CD(5%)	5.93	8.11
26	SK-73	24.20	34.56				
27	NSM-201	17.24	43.04				
28	NS/05/02	24.26	33.04				

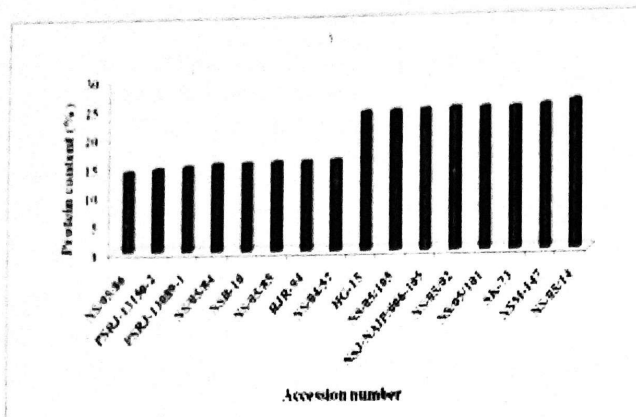


Fig. 2. Percent protein content of selected horse gram accessions

NAIP/031-130 (75 mg), which is significantly different and superior to the next best accession viz., NDS-259 (67.08 mg). The other accessions, which possessed high phenol content were NSB-27 (65.68 mg), PSRJ-13030 (62.28 mg), Palem 1 (59.48 mg), NS/05/113 (59.08 mg), NS/04/124 (55.24 mg), NS/05/84 (54.88 mg), Palem-2 (54.24 mg) and HG-15 (52.16 mg).

On the other hand, low phenol content was recorded in the accession KSAS/06/280 (17.04 mg) followed by NS/05/94 (18.40 mg), NS/05/93 (20.40 mg), NS-74 (21.12 mg), NS/05/103 (23.04 mg) and PSRJ-13089-1 (24.48 mg), which were found to be at par with each other.

#### Correlations

Correlations between qualitative traits and biochemical parameters for 50 horse gram accessions are presented in Table 3. From the data, it is interesting to note that significant correlations are noticed among the qualitative traits, while biochemical parameters exhibited positive and negative relationship with the morphological qualitative traits, but the relationship was non-significant.

Plant growth pattern exhibited significant positive relationship with plant growth habit (0.769) and leaf colour (0.410) while a significant negative relationship was noticed between plant growth pattern and

stem color (-0.462). Plant growth habit showed significant negative relationship with stem color (-0.471) and significant positive relationship with leaf color (0.408). However, plant growth pattern, plant growth habit and leaf color exhibited non-significant positive relationship with protein content. Similarly, stem color exhibited non-significant negative relationship with protein content. Plant growth habit had no correlation with phenol content, indicating no relationship between these two parameters. Plant growth pattern, stem color and leaf color exhibited non-significant negative correlation with phenol content. On the other hand, seed color showed non-significant positive relationship with phenol content. Thus, it was found that none of the morphological traits studied could serve as morphological markers for identifying high or low protein and phenol containing accessions.

#### CONCLUSION

In characterization of horse gram studies, 50 horse gram accessions collected from diverse agro-ecological regions of Andhra Pradesh were classified into five groups based on the morphological characters viz., plant growth pattern, plant growth habit, stem color, leaf color and seed color. Based on plant growth pattern, 30 accessions exhibited determinate growth pattern while 20 accessions had indeterminate growth pattern. Based on plant growth habit, 18 accessions were bushy type, 5 were spreading type, 12 were semi-spreading type and 15 possessed trailing type growth habit. On the basis of stem color, 18 had purple stem while 32 accessions were green colored. Based on leaf color, 45 accessions possessed green colored leaves and five accessions possessed dark green colored leaves. Classification of horse gram accessions on the basis of seed color revealed that 27 resulted in to brown colored seeds, 17 with cream colored seeds, 3 showed black colored seeds while 3 with grey colored seeds.



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