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Agromorphological, nutritional and Antioxidant Properties in Horsegram [*Macrotyloma Uniflorum* (Lam) Verdc] Germplasm Collection from Diverse Altitudinal Range of North Western Himalayan hills of India

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Abstract

Horsegram is an underutilised food legume, popularly known for its resilience to adverse climatic and soil conditions as well as for possessing excellent remedial properties. In spite of nutritionally rich food/fodder, excellent nutraceutical values and ability to perform better under marginal conditions unsuitable for other crops, this food legume is neglected and benefited little in terms of genetic improvement. It is primarily grown as an indemnity in areas often facing harsh weather condition across the country. Horsegram is indigenous to India and a huge variability exists for key agro morphological and nutritional traits which is not characterised and utilised properly so far. Presently, the genetic improvement of this underutilised food legume heavily depends upon indigenous genetic resources. In the absence of their proper evaluation they are not being effectively utilized. Therefore, in the present study, an effort has been made to explore the genetic diversity in a set of horsegram accessions collected from diverse altitudes of NW Himalayan hills with respect to agro-morphological, nutritional and antioxidant traits. The study revealed that the accessions of low and high hills were more diverse among themselves as compare to the accessions of mid hills possibly due to diverse agro-ecological conditions. Accessions from mid hills (1101-1500 m asl) was found to have high mean values for key agro-morphological traits *viz*., number of pods per plant (45), biological yield per plant (16.10 g), 100 seed weight (3.31 g), grain yield per plant (7.26 g) with less maturity duration (100 days) whereas, for nutraceutical properties like free radical inhibition against DPPH (21.28 μ mol TE/g DW) and desirable nutritional traits of pulses *viz*., high protein content (20.84 mg/g) with low phytic acid (9.13 mg/g) were found in the accessions of high (>1500 m asl) and low (<1100 m asl) hills, respectively, indicating the possibility of altitudinal adaptation of accessions and influence of age old criterion of selection practiced by the peasants at varied altitudes of NW Himalayan hills.

Keywords

Altitudinal diversity; *Macrotyloma uniflorum*, N-W Himalayas; Nutritional and antioxidant properties

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Introduction

Underutilized legumes are important group of crops which has special significance in subsistence farming and attaining nutritional security for resource poor masses in developing countries. Current research efforts have identified the potential of neglected and underutilised crops to reduce food and nutrition insecurity particularly for resource poor households owing to their excellent nutritional composition as well as in expending the food basket. Among underutilized legumes, horsegram (*Macrotyloma uniflorum),* family *Fabaceae* is one of the minor or lesser known neglected legume mainly cultivated in Asian and African countries as a dual purpose crop which is endowed with excellent nutritional value comparable to commercially grown pulses [1] besides, its excellent therapeutic properties to cure kidney stones, asthma, bronchitis, leucoderma, urinary discharges, heart diseases, piles as well as posses anti-diabetic, anti-ulcer and anti-obesity activity due to the presence of beneficial bioactive compounds [2] with better climate resilience to adapt harsh environmental conditions due to ability to grow in drought and low input condition of marginal lands [3]. It is an indigenous food legume which is considered to be domesticated in Southern Indian plains and hills and due to counter migration humans it is diffused to Northern and Western part of India during Neolithic period [4]. It is grown from Himalayan region in the North to Tamil Nadu in the South and Gujarat in West to Bengal in the East generally, in areas where, very few options exist for crop diversification and as an indemnity against natural calamities. In India, it is grown as both *Rabi* and *Kharif* season and as per the national estimates horsegram constitutes about 260 thousand tons of the gross grain production from 500 thousand ha of the total acreage in the country [5]. Resilience for harsh environmental conditions and easy cultivation in hill slopes, undulating, and poorly fertile fields made it an indispensable food legume of traditional crop production systems of North Western Himalayan hills. This food legume is endemic to India and its cultivation as a pulse is little known elsewhere in the world besides, it has been very little explored for its nutritional and nutraceutical properties. Presently, the genetic improvement of the crop rely heavily upon the indigenous genetic resources. Therefore in the national horsegram genetic improvement program lot of emphasis is towards the collection, conservation and utilization of the native germplasm resources. There are indications in the literature that diversity of traditional crops is fast eroding and climate change is imposing additional threat of increasing genetic erosion of landraces [6] from traditional production systems. Realizing the urgency of these situations, priority has been given to the germplasm collection of traditional crops like horsegram at ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora. Horsegram germplasm were collected throughout the hill state of Uttarakhand over the years, where it is extensively cultivated (approx.13,000 ha) by small farmers on marginal land as well as other parts of the India. This set of horsegram germplasm collected from diverse altitudes (low, mid and high hills) of NW Himalayas were evaluated with the objective to study the diversity for agro-morphological, nutritional and antioxidant traits to expedite the genetic improvement of this underutilised food legume.

Materials and Methods

Experimental details

A total of 73 accessions collected from varied altitudinal range *viz*., low (<1100 m asl), mid (1101-1500 m asl) and high (>1501 m asl) were evaluated under mid-hill condition at ICAR-VPKAS experimental farm, Hawalbagh, Almora (29.36^{0}N and 79.30^{0}E situated at an elevation of 1,250 m above mean sea level) during *Kharif* 2015 in augmented block design with four checks VL *Gahat* 8, VL *Gahat* 10, VL *Gahat*15 and VL *Gahat*19. The checks were randomized evenly in each block. Each germplasm and check was grown in rows of 3 m length and spaced 45 cm apart. Distance of plants within row was maintained at 10 cm. All the recommended cultural practices were followed to raise a healthy crop.

Morphological characterization

Observations on principal phenological stages were recorded at 50% of flowering and 80% maturity, respectively on whole plot basis. The remaining quantitative traits *viz*., plant height (cm), number of pods per plant, dry matter weight per plant (g) and seed yield per plant (g) were recorded on three randomly selected plants in each genotype following standard procedures whereas, pod length (cm) was recorded on 10 pods sampled at random within each accession and seed weight (g) was recorded in hundred randomly selected seeds.

Determination of nutritional and antinutritional parameters

Nutritional properties were estimated by performing biochemical analysis for protein content by Bradford method [7], total carbohydrate content by Phenol–Sulfuricracid method [8] in which glucose was used as the standard in the range of 0-100 µg concentration for calculation of total carbohydrate content. For the determination of total lipid content gravimetric method by Bligh and Dyer [9] was used whereas, phytic acid content was calculated from a calibration curve using phytate phosphorus salt in the range of 10–50 µg using defatted horsegram flour as per the method described by Haug and Lantzsch [10].

Determination of total polyphenols and scavenging effects on DPPH radicals

Fine powders of clean dry raw samples (1.0 g) was extracted by stirring with 20 ml of 85% methanol at 35°C, 150 rpm/min for 12 h and filtered through Whatman filter paper No. 1. The extraction was repeated again as described earlier. The extracts were mixed, filtrated and diluted to 100 ml with 85% methanol. The extract solution stored in amber bottles at 4°C served as the working solution (10 mg/ml) for determination of total phenolics, and antioxidant activities. The total polyphenolic (TPP) compounds were determined by Folin Ciocalteu reagent [11] and calculated from a standard calibration curve based on tannic acid (0– 0.1 mg/ml) and the results were expressed as gallic acid equivalents in mg per g dry weight (mg GAE/g DW). Scavenging effects on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals by horsegram methanolic extract was measured following standard methods of Brand-Williams et al. [12]. A standard curve of trolox (10–100 µM) was prepared and free radical inhibition was expressed as µM trolox equivalent/gram dry weight (µM TE/g DW).

Statistical analysis

Statistical analysis was performed for 14 quantitative descriptors and range and mean were calculated for each group of the collection and variation within group expressed as coefficient of variation [13]. Principal Component Analysis [14] was performed to examine the percentage contribution of each trait to total genetic variation; correlation coefficients between principal component axis scores and the original mean values performed to know traits contributing maximum variability using computer software XL STAT 12 version. First and second principal component axes scores were plotted to aid visualization of origin group differences and to detect morphological variation within collection using SYSTAT 13 version.

Results and Discussion

The mean coefficient of variation with in groups revealed that maximum per cent variation was recorded from group 'A' (17.73%), followed by group 'C' (17.17%) and group 'B' (14.81%)as compared to whole collection (17.96%). The variation within group expressed as per cent coefficient of variation gives rough estimate of diversity and variability within group [15]. The farming situation at lower and higher hills was more diverse than mid hills, this could be one of the reasons for the presence of high CV% at lower and higher hills. The high variability in agronomic traits among accessions of different origin groups indicates that horsegram accessions are adapted to specific zone [16]. Group 'B' (1101-1500 m asl) recorded least mean values for phenological traits *viz*., days to 50% flowering and days to maturity and the maximum mean values for most of the agro-morphological traits *viz*., number of pods per plant (45), biological yield per plant (16.10 g), 100 seed weight (3.31 g), grain yield per plant (7.26 g) as well as nutritional traits *viz*., total carbohydrate (54.41mg/g) and total lipids (1.49 mg/g) (Table 1). Although, horsegram is generally grown under sub humid to semi arid climate up to an altitude 1800 m asl but it's cultivation is mainly distributed in mid hills [2] and the productive and nutritionally superior quality germplasm accessions of horsegram with less reproductive phase might have resulted due to age old practice of farmer's selection in mid hills (Table 1). High mean values for total polyphenols (2.73 mg GAE/g) was also found in the accession of mid hills. Antioxidant properties and their uses in health care phenolics have attracted the attention of food and health scientists in recent times [4]. There is ample proof of health benefits as anti-inflammatory, cicatrizant and anti-HIV functions together with their role in protection against environmental stresses (drought, UV-B radiation and atmospheric pollution), microbial pathogens, harmful insects and other herbivores in plants [18]. Although, Phenolic compounds are considered one of the biggest groups of non essential dietary compounds but the antioxidant properties of phenolics occur mainly due to their potential for ox-reduction, which enables them to act as reducing agents, donating hydrogen and neutralizing free radicals [19]. Pulses with high total phenolic content and exert the highest antioxidant capacity assessed by 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging capacity [20]. Maximum mean values for DPPH radical scavenging activity (21.28 µ mol TE/g DW) was found for group 'C' (>1500 m asl) whereas, group 'A' had maximum mean values for protein content (20.84 mg/g) with minimum values for phytic acid (9.13 mg/g). In general, group 'B' which comprise the accessions from mid hills was having maximum mean values for grain yield and it's component traits whereas, high (Group 'A') and low hills (Group 'C') collection had desirable nutritional and nutraceutical properties.

Data on all the traits were subjected to Principal Component Analysis, six of the 14 principal components axis (PCA) had eigen value more than one were selected as proposed by Jeffers. The first two principal component axes accounted for 33.71% and the per cent variation explained by the first six PCs were most informative principal components and accounted for 70.28% of the total variance (Table 2).

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Table 1: Altitudinal diversity in horsegram collection for different agro-morphological, nutritional and antioxidant properties.

Trait	Altitude	Mean	Range	Std. deviation	CV%
Days to 50% flowering	A	54.44	49-60	3.48	6.40
	B	54.13	51-59	2.23	4.12
	C	55.08	49-63	3.37	6.12
Days to maturity	A	101.11	96-107	3.27	3.23
	B	100.13	97-105	2.56	2.56
	C	101.50	96-109	3.21	3.16
Plant height (cm)	A	75.67	54-93	12.21	16.14
	B	73.53	56-87	9.16	12.45
	C	72.60	47-90	10.48	14.44
Pod length (cm)	A	5.13	4.77-5.43	0.17	3.35
	B	5.10	4.68-5.33	0.18	3.60
	C	5.14	4.63-5.43	0.17	3.30
Number of pods/plant	A	42.22	18-60	13.40	31.73
	B	45.20	30-55	7.07	15.65
	C	43.63	23-71	11.67	26.75
Biological yield/plant (g)	A	12.97	5.04-20.07	4.42	34.12
	B	16.10	8.32-27.57	4.52	28.06
	C	13.73	7.89-25.27	3.97	28.95
100 seed weight (g)	A	3.28	2.59-4.06	0.37	11.24
	B	3.31	2.79-4.01	0.34	10.14
	C	3.29	2.64-4.61	0.39	11.88
Grain yield/plant (g)	A	6.57	1.36-10.75	2.65	40.28
	B	7.26	4.19-12.44	2.17	29.90
	C	6.72	2.30-13.23	2.36	35.11
Total carbohydrate (mg/100 mg)	A	52.47	49.21-60.90	2.89	5.50
	B	54.41	50.68-60.56	3.03	5.57
	C	53.96	49.04-62.21	3.89	7.21
Protein (mg/100mg)	A	20.84	16.98-24.88	2.33	11.18
	B	20.30	17.08-26.49	2.20	10.82
	C	20.31	17.50-25.18	2.07	10.18
Total lipid (mg/100mg)	A	1.36	0.86-1.95	0.27	20.14
	B	1.49	1.28-2.50	0.30	19.94
	C	1.33	0.70-2.20	0.31	22.90
α, α-diphenyl-β-picrylhydrazyl (DPPH) (μ mol TE/g DW)	A	21.10	19.01-25.63	1.61	7.65
	B	20.83	18.70-22.70	1.32	6.34
	C	21.28	11.61-24.76	2.22	10.41
Total polyphenols (mg GAE/g)	A	2.07	1.14-4.15	0.94	45.31
	B	2.73	1.31-4.64	1.10	40.51
	C	2.30	0.91-3.97	0.98	42.56
Phytic acid (mg/g)	A	8.64	6.94-10.49	1.03	11.94
	B	9.13	6.59-12.83	1.61	17.63
	C	8.68	4.72-11.67	1.49	17.14
Altitude (A:<1100m asl, B:1101-1500m asl& C:>1500m asl)					

Correlation coefficient between PC score and adjusted mean value of the traits revealed that in PC I (20.78%), the traits that accounted for most of the observed variability among horsegram accessions included grain yield per plant (0.899), biological yield per plant (0.850), number of pods per plant (0.848) and plant height (0.509). Likewise, in PC II (12.94%) important traits with greater allowance was total polyphenols; PC III (10.02%) and PC IV (9.76%) were explained by phytic acid (0.729) and pod length (0.647), respectively whereas, PC V (8.85%) was accounted by total lipids (0.642) and days to 50% flowering similarly, PC VI (7.93%) was explained by 100 seed weight (0.624) and protein content (0.586). Principal Component Analysis revealed that agro morphological traits *viz.*, days to 50% flowering, plant height, number of pods per plant, pod length, 100 seed weight, biological yield per plant, grain yield per plant and quality traits *viz.*, total

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Table 2: Correlation coefficients between the first six principal components (PCI, PCII, PCIII, PCIV, PCV & PCVI) and different agro morphological, nutritional & antioxidant properties of horsegram accessions.

Variables	PC I	PC II	PC III	PC IV	PC V	PC VI	Eigen value	Variability (%)
Days to 50% flowering (DFF)	-0.290	-0.357	0.333	-0.273	**0.498****	0.171	2.91	20.78
Days to maturity (DM)	-0.219	**-0.600****	0.303	0.275	0.233	0.387	1.81	12.94
Plant height (PH)	**0.509***	**-0.515****	0.242	0.090	-0.232	0.101	1.40	10.02
Pod length (PL)	0.307	0.214	-0.089	**0.647****	0.155	-0.028	1.37	9.76
Number of pods/plant(PPP)	**0.848****	-0.073	0.139	-0.164	0.115	-0.093	1.24	8.85
Biological yield/plant (BY)	**0.850****	-0.061	-0.054	-0.013	0.257	-0.088	1.11	7.93
100 seed weight (100SW)	0.133	-0.111	**-0.472***	0.144	-0.276	**0.624****	0.90	6.40
Grain yield/plant (GY)	**0.899****	-0.097	0.037	0.052	0.037	-0.079	0.72	5.15
Total carbohydrate (TC)	0.054	-0.406	-0.195	**-0.601****	-0.149	0.098	0.67	4.81
Total polyphenols (TP)	0.072	**0.645****	-0.148	-0.357	0.304	0.114	0.59	4.23
Total lipid (TL)	-0.152	-0.346	-0.397	-0.043	**0.642****	-0.260	0.47	3.33
α, α-diphenyl-β-picrylhydrazyl (DPPH)	-0.078	0.317	0.323	0.344	0.328	0.261	0.40	2.85
Protein (P)	0.329	0.286	-0.184	-0.222	0.228	**0.586****	0.22	1.59
Phytic acid (PA)	0.097	0.318	**0.729****	-0.284	-0.130	0.084	0.19	1.36

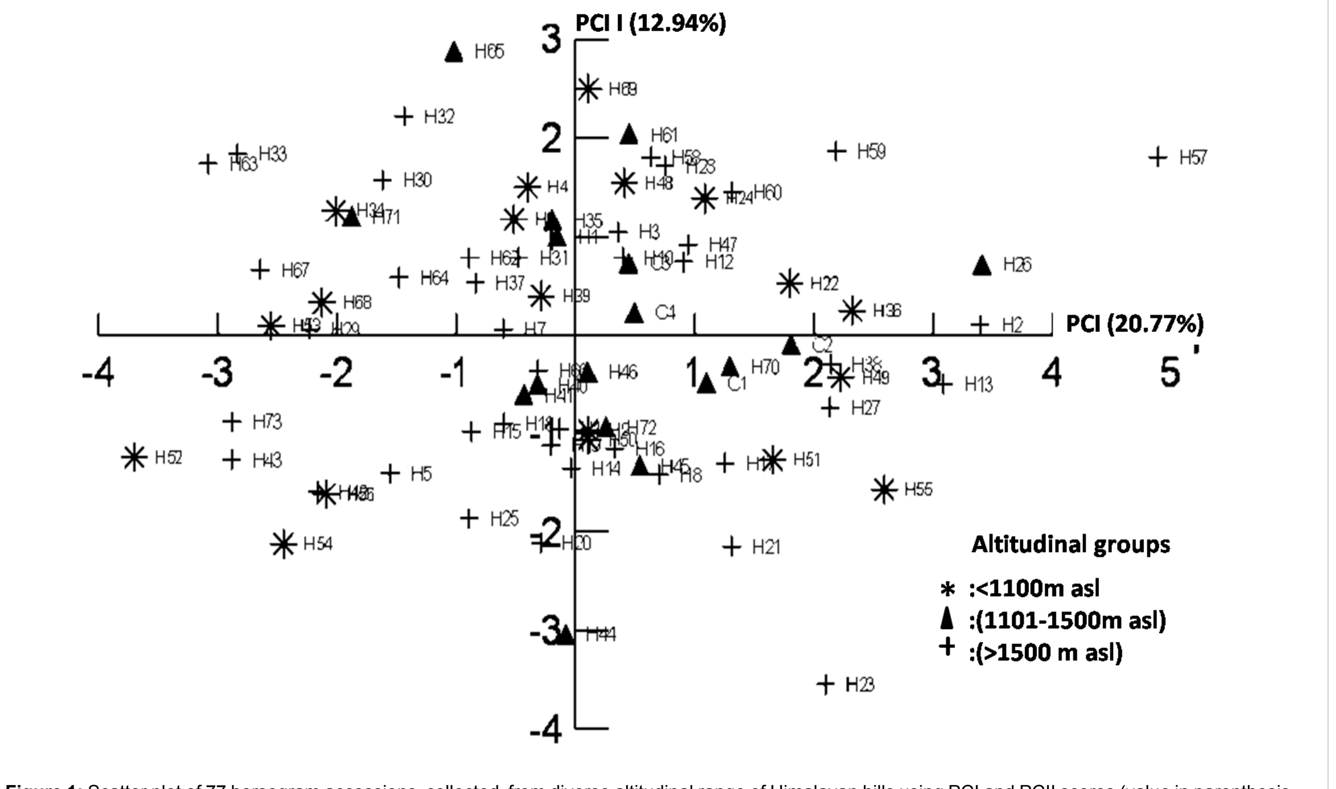

Figure 1: Scatter plot of 77 horsegram accessions collected from diverse altitudinal range of Himalayan hills using PCI and PCII scores (value in parenthesis explains percentage of variation).

polyphenols, phytic acid, total lipids and protein content contributed mostly to genetic diversity of hill horsegram collection. The biplot of PCI and PCII scores distributed the accessions and confirmed the presence of variability among the accessions collected from different altitudes with some overlapping among accessions (Figure 1) which could arise due to altitudinal influences as well as differential selection pressure practiced by the hill farmers as well as photo and thermo sensitivity due to altitudinal differences [21]. Accessions from low and high hills exhibited more dispersion than the landraces collected from mid hills which could be due to the fact that horsegram is entirely grown under rainfed condition and farmer's selection for adaptation to unfavourable weather under similar climatic conditions had reduced the diversity in the horsegram accessions collected from mid hills [16] and prevailing high levels of stress factors in agro-ecological zones tend to exhibit more homogenous genotypes and less degree of variation [22,23].

Table 3: Promising accessions of horsegram for 14 quantitative traits from diverse altitudes of NW Himalayan hills.

Quantitative Traits	Promising Accessions	Altitude (m asl)
Days to 50% flowering (< 50 days)	VRB-PH 1770-1 VRB-PH 1443-1 VRB-PH 1773	1020 1620 1020
Days to maturity (<100 days)	VRS -PH -969 VRB -PH -1770 VASHM-PH 3218 VRS-PH 987	1720 1020 1852 920
Plant height (< 50 cm)	VASHM-PH 3287	1928
Pod length (> 5 cm)	VASHM-PH 3210	1658
Number of pods/plant (>70)	VRS -PH -969 VRB -PH -1683 VRB -PH -1770	1720 1370 1020
Biological yield/plant (> 25 g)	VLG 5 VASHM-PH 3210	1250 1668
100 seed weight (>4g)	VRB-PH 1741-1	1520
Grain yield/plant (>12 g)	VRS-PH 958-1 VLG 5	1910 1250
Total carbohydrate (>62mg/g)	VASH-M-PH 3262 VRB-PH 1741-2	1555 1520
Total polyphenols (>4.5 mg GAE/g) (<1 mg GAE/g)	VLG 10 VRB-PH 1443	1250 1620
Total lipid (>2 mg/g)	VRB -PH -1741 VRB -PH -1756	1520 1480
DPPH (>25 μ mol TE/g DW)	VRS -PH -1011	970
Protein (>25 mg/g)	VASHM-PH 3216	1852
Phytic acid (<5 mg/g)	VRB-PH 1443	1620

The presence of the broad spectrum of genetic diversity for different key traits of agronomic and nutritional importance in horsegram accessions from NW Himalayan hills implies ample opportunity for its genetic improvement. Accessions namely, VRB-PH 1770, VRB-PH 1741 and VRB-PH 1443 were found promising for multiple traits and can be utilised as ready use material for the genetic improvement of horsegram (Table 3) [24]. Furthermore, there are still immense possibilities exist for this legume to be explored for its innate health-promoting aspects and many undiscovered phytochemicals to make the most use of this indigenous underutilized legume to address food and nutritional security issues [25].

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