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A study on sugar and mineral content of horsegram (*Macrotyloma uniflorum* (Lam) Verdc.) varieties

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Abstract
Horsegram (*Macrotyloma uniflorum* (Lam) Verdc.) is minor legume, which belongs to family *Fabaceace*. It is a potential grain legume having excellent nutritional quality with better resilience property to adapt harsh environment conditions. Seven released varieties and five advanced breeding lines of horsegram were procured from All India Coordinated Research Project – Dryland Agriculture, Vijayapur. The horsegram were assessed for sugar content and mineral content. There was significant difference ($p \leq 0.01$) for all the sugar parameters *i.e.,* total sugar, reducing and non-reducing sugars and mineral content *i.e.,* calcium, iron, zinc, manganese and copper among the varieties. While, there was no significant difference between advanced breeding lines and released varieties of horsegram for all parameters. The total, reducing and non-reducing sugar ranged from 4.96-5.92, 0.35-0.41 and 4.33-5.25 mg/100g respectively. The variety VHG15 had highest calcium content (290.00 mg/100g) and highest iron content was observed in VHG935 (9.95 mg/100g).

Keywords: Horsegram, reducing sugar, non-reducing sugar, minerals

Introduction
Food legumes constitute an important component of diet across world and are the next important food crops after cereals. As a result, pulses have significant cultural and historical significance (Roy *et al.,* 2010) [9]. Legumes are good sources of protein that are cheap and widely available for human consumption. They are staple foods for many people in different parts of the world. Legume seeds have an average of twice as much protein as cereals and nutritive value of the proteins are usually high. They are widely cultivated and distributed in Africa, Asia, West Indies, Latin America and India. The legumes can be grown in marginal soils and in arid or semi arid regions. Their deep penetrating root system enables them to withstand moisture stress (Bhokre and Joshi, 2015) [3]. Presently, attention towards underutilized legumes is increasing for finding new alternative sources to meet the ever increasing demand for vegetable protein (Bhartiya *et al.,* 2015) [2, 6]. Among legumes, horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] is a minor legume crop of India with good nutritional quality (Pal *et al.,* 2015) [6]. It belongs to family *Fabaceace,* which has potential nutritional and remedial properties with better climate resilience to adapt to harsh environmental conditions. It is one of the most important under exploited food legume being grown in almost all over the world including temperate and sub-tropical regions encompassing the countries particularly, India, China, Philippines, Bhutan, Pakistan, Sri Lanka and Australia (Durga, 2016; Bhartiya *et al.,* 2015) [2, 6]. Reports on nutritive value of horsegram indicate that it as an excellent source of protein (up to 25 per cent), carbohydrate (60 per cent), essential amino acids, energy, and low content of lipids (0.58 per cent), iron and molybdenum (Ramteke *et al*., 2016) [8]. Horsegram is relatively high in iron, but the availability of iron is reduced by the tannins, oxalic acid and phytates contents. Horsegram is also a good source of protein and appears to be good source of calcium too (Pal *et al.,* 2015) [6]. It also possesses slow digestibility starch (Siddhuraju and Manian, 2007) [10]. Improvement and release of new varieties having superior agronomical features, resistance to insect and diseases and quality parameter is a continuous process. Hence, the present study was undertaken to assess sugar content and mineral content of horse gram varieties.

Materials and Methods
Procurement of the horsegram varieties
Twelve varieties of horsegram were procured from AICRP for Dryland Agriculture RARS, Vijayapur Karnataka.

Out of twelve varieties five were advanced breeding line of University of Agricultural Sciences, Dharwad, while remaining seven varieties were released for cultivation across different regions. The details regarding the samples are given in Table 1. The samples were cleaned before the study was carried out. All estimations were carried

Table 1: Horsegram varieties selected for study

Horsegram varieties	
Released varieties	**Advanced breeding liness**
AK 42- ARJIA KULTHI 42	VHG 13-01- VIJAYAPUR HORSEGRAM -13-01
AK 44- ARJIA KULTHI 44	VHG 15- VIJAYAPUR HORSEGRAM -15
CRHG 22- CRIDA HORSEGRAM 22	VHG 44- VIJAYAPUR HORSEGRAM -44
CRHG 23- CRIDA HORSEGRAM 23	VHG 935- VIJAYAPUR HORSEGRAM -935
DHG 01- DANTEWADA HORSEGRAM 01	VHG 938-- VIJAYAPUR HORSEGRAM -938
GPM 06- GERMPLASM SELECTION 06	
KBHG 01- KARNATAKA BIJAPUR HORSEGRAM- 01	

Estimation of Mineral content

Calcium (mg/100g): Calcium was determined by titrimetric method. The calcium was precipitated as oxalate by addition of ammonium oxalate solution and was titrated with standard potassium permanganate (Raghuramulu *et al*., 2003) [7].

Trace minerals (mg/100g): Trace minerals like iron, zinc, copper and manganese were estimated using Atomic Absorption Spectrophotometer (AAS) (Raghuramalu *et al*., 2003) [7].

Total starch content (g/100g): About 80 per cent hot alcohol was repeatedly treated on sample to remove sugars. Then, the residue obtained was treated with perchloric acid to solubilise the residue rich in starch and filtered. The filtered extract was used to determine glucose by anthrone sulphuric acid. Glucose value was multiplied by 0.9 to convert it to starch value (McCready *et al.*, 1950) [5].

Total sugar (mg/100g): Standard Nelson- Somogyi's method was used to determine the total and reducing sugar. The principle employed was when heated with alkaline copper tartrate reduce the copper from the cupric to cuprous oxide. Then arsenomolybdic acid is treated with formed cuprous oxide, the reduction of molybdic acid to molybdenum blue takes place. Then the absorbance is measured at 620nm. Finally, non reducing sugar is computed by formula:

Non reducing sugar = (total sugar – reducing sugar) × (0.95)
Where, 0.95 = conversion factor

Statistical analysis: The experiment were carried out by following statistical methods ANOVA (Analysis of variance) was used to know the significant difference among the varieties and Independent t-test was used to know the significant difference between released varieties and advanced breeding line of horsegram varieties.

Results and Discussion

Starch and sugar content

Starch, total sugar, reducing sugar and non reducing sugar content of horsegram varieties

Starch, total sugar, reducing sugar and non reducing sugar content of horsegram varieties is presented in Table 2. There was significant difference ($p \leq 0.01$) in all parameters *i.e* Starch, total sugar, reducing sugar and non reducing sugar content of horsegram varieties. The starch content among the varieties ranged from 56.36-60.48 g/100g.The variety CRHG22 had highest starch content (60.48 g/100g) followed by KBHG01 (60.26 g/100g) and AK44 (59.78 g/100g). DHG01 (56.36g/100g) had lowest content among varieties.

The total sugar content ranged from 4.96-5.92 mg/100g. Lowest sugar content was observed in KBHG01 (4.96 mg/100g). Highest sugar content was observed in VHG938

Table 2: Starch and sugar content of horsegram varieties

Varieties	Starch (g/100 g)	Sugar (mg/100g)		
		Total sugar	Reducing sugar	Non reducing sugar
Released Varieties				
AK42	59.07 ± 0.05^{c}	5.17 ± 0.05^{de}	0.41 ± 0.00^{a}	4.52 ± 0.04^{ef}
AK44	59.78 ± 0.14^{b}	5.27 ± 0.01^{c}	0.38 ± 0.00^{d}	4.64 ± 0.01^{cd}
DHG01	56.36 ± 0.17^{g}	5.13 ± 0.15^{ef}	0.38 ± 0.00^{d}	4.51 ± 0.01^{fg}
GPM06	58.07 ± 0.11^{e}	5.34 ± 0.02^{c}	0.39 ± 0.00^{c}	4.70 ± 0.02^{bc}
KBHG01	60.26 ± 0.28^{a}	4.96 ± 0.01^{g}	0.39 ± 0.00^{c}	4.33 ± 0.00^{h}
CRHG22	60.48 ± 0.21^{a}	5.05 ± 0.02^{f}	0.39 ± 0.00^{c}	4.42 ± 0.02^{g}
CRHG23	58.44 ± 0.13^{d}	5.26 ± 0.02^{cd}	0.41 ± 0.00^{a}	4.60 ± 0.01^{def}
Advanced breeding lines				
VHG13-01	58.67 ± 0.23^{d}	5.43 ± 0.03^{b}	0.40 ± 0.00^{b}	4.77 ± 0.03^{b}
VHG15	56.86 ± 0.08^{f}	5.25 ± 0.03^{cd}	0.40 ± 0.00^{b}	4.60 ± 0.03^{de}
VHG44	58.44 ± 0.11^{d}	5.05 ± 0.03^{f}	0.38 ± 0.00^{d}	4.43 ± 0.03^{def}
VHG935	59.50 ± 0.18^{b}	5.17 ± 0.01^{de}	0.35 ± 0.00^{e}	4.57 ± 0.01^{g}
VHG938	59.66 ± 0.17^{b}	5.92 ± 0.02^{a}	0.39 ± 0.00c	5.25 ± 0.02^{a}
Mean ± SD	58.80 ± 1.24	5.25 ± 0.24	0.39 ± 0.01	4.61 ± 0.23
S.Em. ±	0.09	0.03	0.05	0.02
C.D.	0.28**	0.09**	0.01**	0.07**
F value	167.66	71.20	81.45	59.57

Note: Values are mean of three replications, S.Em.: Standard Error of Mean, C.D.: Critical difference, **Significant @1%

(5.92 mg/100g) followed by VHG13-01 (5.43 mg/100g) and GPM06 (5.34 mg/100g). The reducing sugar and non reducing sugar content ranged from 0.35-0.41 mg/100g and 4.33-5.25 mg/100g respectively. Highest reducing sugar content was observed in CRHG23 and AK42 (0.41 mg/100g) followed by CRHG22 (5.05 mg/100g) and GPM06 (0.39 mg/100g). Lowest reducing sugar content among the horsegram varieties was observed in VHG935 (0.35 mg/100g). Highest non reducing sugar was observed in VHG938 (5.25 mg/100g) followed by VHG13-01 (4.77 mg/100g) and GPM06 (4.71 mg/100g). Lowest non reducing sugar content was observed in KBHG01 (4.33 mg/100g) among the horsegram varieties. Thilagavathi *et al.* (2015) [11] showed similar value for non reducing sugars (4.00 per cent) and total sugars (4.89 per cent), slightly lower values for starch. This difference may due to the varietal difference.

Comparison of starch and sugar content of released varieties and advanced breeding lines of horsegram

Table 3 presented the comparison of starch and sugar content of released varieties and advanced breeding lines of horsegram. There was no significant difference between the released varieties and advanced breeding lines of horsegram. Total sugar content and non reducing sugar content was higher in advanced breeding lines compared to released varieties whereas starch and reducing sugar were higher in released varieties, when compared to advanced breeding lines of horsegram.

Table 3: Comparison of starch and sugar content of released varieties and advanced breeding lines of horsegram varieties

Varieties	Starch (g/100 g)	Sugars (mg/100g)		
		Total sugar	Reducing sugar	Non reducing sugar
Released varieties	58.92 ± 1.37	5.17 ± 0.13	0.39 ± 0.01	4.53 ± 0.12
Advanced breeding lines	58.62 ± 1.04	5.36 ± 0.31	0.38 ± 0.01	4.70 ± 0.29
t value	NS	NS	NS	NS

Note: Values are mean of three replications, NS-Non Significant

Mineral content

Mineral content of horsegram varieties

Calcium, iron, zinc, manganese and copper content of horsegram varieties are presented in Table 4**.** There was significant difference ($p \leq 0.01$) in all the parameters of horsegram varieties. The calcium content among the varieties ranged from 210.33-290.00 mg/100g. The variety VHG15 had highest calcium content (290.00 mg/100g) followed by CRHG22 (282.00 mg/100g) and GPM06 (279.00 mg/100g). Least calcium content was observed in CRHG23 and VHG938 (210.33 mg/100g).

Iron content of horsegram varieties ranged from 8.06-9.95 mg/100g. Lowest iron content was observed in KBHG01 (8.04 mg/100g). Highest iron content was observed in VHG935 (9.95 mg/100g) followed VHG44 (9.43 mg/100g) and DHG01 (8.94 mg/100mg). Zinc content varied from 2.13-3.73 mg/100g. VHG13-01 showed highest zinc content

Table 4: Mineral content of horsegram varieties (mg/100 g)

Varieties	Calcium	Iron	Zinc	Manganese	Copper
Released Varieties					
AK42	259.60 ± 0.57^{c}	8.45 ± 0.03^{g}	2.62 ± 0.00^{gh}	1.53 ± 0.00^{f}	0.10 ± 0.00^{k}
AK44	211.00 ± 1.00^{h}	8.55 ± 0.01^{f}	2.55 ± 0.00^{j}	1.53 ± 0.00^{f}	0.12 ± 0.00^{i}
DHG01	239.67 ± 0.57^{e}	8.94 ± 0.00^{a}	2.88 ± 0.00^{d}	1.67 ± 0.00^{b}	0.16 ± 0.00^{d}
GPM06	279.00 ± 0.73^{b}	8.26 ± 0.01^{h}	2.15 ± 0.00^{a}	1.55 ± 0.00^{ef}	0.18 ± 0.00^{b}
KBHG01	221.00 ± 0.00^{g}	8.06 ± 0.00^{i}	2.13 ± 0.00^{c}	1.63 ± 0.00^{cd}	0.17 ± 0.00^{c}
CRHG22	282.00 ± 0.64^{i}	8.10 ± 0.00^{d}	3.55 ± 0.00^{j}	1.10 ± 0.00^{h}	0.16 ± 0.00^{e}
CRHG23	210.33 ± 0.57^{h}	8.65 ± 0.03^{h}	3.62 ± 0.00^{g}	1.87 ± 0.00^{a}	0.21 ± 0.00^{a}
Advanced breeding lines					
VHG13-01	250.08 ± 1.00^{d}	8.52 ± 0.00^{f}	3.73 ± 0.11^{b}	2.13 ± 0.05^{g}	0.13 ± 0.00^{h}
VHG15	290.00 ± 1.00^{a}	8.40 ± 0.00^{c}	3.17 ± 0.02^{f}	1.61 ± 0.00^{d}	0.14 ± 0.00^{g}
VHG44	230.33 ± 0.52^{f}	9.43 ± 0.03^{e}	3.12 ± 0.01^{h}	1.67 ± 0.00^{b}	0.15 ± 0.00^{f}
VHG935	230.00 ± 1.00^{f}	9.95 ± 0.02^{b}	2.60 ± 0.00^{i}	1.65 ± 0.00^{bc}	0.13 ± 0.00^{h}
VHG938	210.33 ± 0.57^{h}	8.10 ± 0.00^{de}	2.65 ± 0.00^{e}	1.57 ± 0.00^{e}	0.11 ± 0.00^{j}
Mean ± SD	234.36 ± 30.28	8.62 ± 0.55	2.90 ± 0.52	1.63 ± 0.23	0.15 ± 0.03
S.Em. ±	0.72	0.00	0.01	0.00	0.00
C.D.	0.10**	0.01**	0.05**	0.01**	0.01**
F value	1.87	30.40	766.49	606.39	534.25

Note: Values are mean of three replications, S.Em.: Standard Error of Mean, C.D.: Critical difference, **Significant @1%

(3.73 mg/100g) followed by CRHG23 (3.62 mg/100g) and CRHG22 (3.55 mg/100g), Least zinc was seen in KBHG01 (2.13 mg/100g).

Manganese content among the horsegram varieties ranged from 1.10-2.13 mg/100mg. Highest manganese content was observed in VHG13-01 (2.13 mg/100g) followed by DHG01 and VHG44 (1.67mg/100g) and VHG935 (1.65 mg/100g). Lowest manganese content was observed in CRHG22 (1.10 mg/100g). Copper content of horsegram varieties varied from 0.10-0.21 mg/100g. Lowest copper content among varieties was observed in AK42 (0.10 mg/100g) and highest was observed in CRHG23 (0.21 mg/100g) followed by GPM06 (0.18 mg/100g) and KBHG01 (0.17 mg/100g). The study conducted by Thilagavathi *et al.* (2015) [11] showed similar values with slight variations to above mentioned minerals. The study conducted by Ahmed *et al.* (2016) [1] showed

similar values for copper, iron, manganese and zinc and slightly lower values for calcium. These differences in mineral content may be due to availability of mineral content in soil and the ability of the root to absorb minerals.

Comparison of mineral content of released and advanced breeding line of horsegram

Table 5 depicts the comparison of mineral content between released varieties and advanced breeding lines of horsegram. There was no significant difference between the released varieties and advanced breeding lines with regard to calcium, iron, zinc, manganese and copper content. Higher content of calcium, iron, zinc and manganese was observed in advanced breeding lines of horsegram varieties compared to released varieties, whereas higher copper content was in released varieties compared to advanced breeding lines of horsegram varieties.

Table 5: Comparison of mineral content (mg/100 g) of released varieties and advanced breeding lines of horsegram varieties

Varieties	Calcium	Iron	Zinc	Manganese	Copper
Released varieties	128.81 ± 31.28	8.43 ± 0.30	2.79 ± 0.575	1.56 ± 0.22	0.16 ± 0.03
Advanced breeding lines	142.13 ± 27.98	8.88 ± 0.71	3.06 ± 0.425	1.73 ± 0.21	0.13 ± 0.01
t value	NS	NS	NS	NS	NS

Note: Values are mean of three replications, NS-Non Significant

Conclusion

There was significant difference in total starch, sugar, reducing and non-reducing sugar among the varieties. The starch content among the varieties ranged from 56.36-60.48 g/100g. The total, reducing and non-reducing sugar ranged from 4.96-5.92, 0.35-0.41 and 4.33-5.25 mg/100g respectively. While, there was no significant difference in all the parameters between released varieties and advanced breeding lines of horsegram varieties. Similarly, there was significant difference among the varieties for calcium, iron, zinc, manganese and copper content. While there was no significant difference between the released varieties and advanced breeding lines of horsegram varieties. Thus, the advanced breeding lines were had better starch, sugar and mineral content compared to released varieties of horsegram.

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