Variability in total phenolic content of pearl millet genotypes: inbreds and designated B-lines

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Abstract

Total phenols of the 90 pearl millet genotypes comprising 46 each of inbreds and designated B-lines (maintainer of CMS lines) grown during *kharif-2013* was estimated and those of the 27 genotypes (9 inbreds and 18 designated B-lines) selected during *kharif-2013* further grown during *kharif-2014* and estimated total phenolic content. Across the groups, pearl millet genotypes showed a significant and marked variation during both the seasons. It varied from 175 to 435 mg catechol equi./100g during *kharif-2013* and from 191 to 387 mg catechol equi./100g during *kharif-2014*. The mean total phenolic content of two seasons (*kharif-2013* and *kharif-2014*) of selected lines varied from 199 to 400 mg catechol equi./100g. During both the seasons designated B lines showed lower mean total phenolic content than that of inbred lines. On the basis of mean performance during *kharif-2013* and *kharif-2014* HTP 94/54, LPBL 10/112, HMS 14B and HMS 21B were identified as low total phenols containing lines whereas, DPHBL-11-123, HBL 112/H12/1011, HMS 36B and HMS 53B were identified as high phenolic containing pearl millet lines.

Key Words: Pearl millet, variability, total phenolic, inbred lines

Introduction

Plant-based food products are the main staple food for human beings in many parts of the world. They constitute an important source of carbohydrates, protein, dietary fibre, vitamins and anti-nutrients (Katina et al. 2005). Pearl millet [*Pennisetum glaucum* (L) R. Br.] is an important hardy coarse cereal crop grown mostly in marginal environments in the arid and semi-arid tropical regions of south Asia and sub-saharan Africa. Pearl millet is a central component of the food and fodder security of the rural poor in dry areas with very limited rainfall. Pearl millet does not contain any condensed polyphenols such as the tannins in sorghum that can decrease digestibility. Pearl millet genotypes are very diverse in its nutritional attributes like protein, fat, carbohydrates, grain minerals contents, polyphenols, phytic acids etc. Health benefits associated with the consumption of millets have been documented (Shobana et al. 2009). Its antioxidant (Subba Rao & Muralikrishna 2002; Hegde & Chandra 2005; Varsha et al. 2009; Chandrasekara & Shahidi 2011) and antimicrobial properties (Chethan & Malleshi 2007; Varsha et al. 2009) have been reported. Phenolic extracts from millets have been reported to inhibit intestinal α -glucosidase and pancreatic α -amylase, and may play a vital role in the management of postprandial hyperglycemia (Shobana et al. 2009).

Polyphenols impart grey colour to pearl millet (Reichert 1979) and limit protein and starch utilization (Thompson and Yoon 1984; Pawar & Parlikar 1990) either by binding with proteins (Loomis 1969) or by inhibiting the digestive enzymes especially trypsin and amylase (Griffiths 1979; Singh 1984). In pearl millet, the concentration of polyphenols ranging from 608 to 788 mg catechol equi./100g has been reported (Khetarpaul & Chauhan 1991; Sharma 2006). On the contrary, lower concentration (about 300 mg catechol equi./100g) of polyphenol in the pearl millet has also been reported by Aggarwal (1992) and Yadav (2003). A range of 228-486 mg catechol equi./100g total polyphenols in pearl millet flour has been reported by Chavan and Hash (1998). Nambiar et al. (2012) reported that total phenols in raw samples ranged from 268.5-420 mg catechol equi./100g and 247.5-335 mg catechol equi./100g in cooked pearl millet. Chandrasekara et al. (2012) reported that the total phenolic content in millet ranged from 2 to 12 μ mol ferulic acid equivalents/g defatted meal. Jain & Chugh (2013) reported total phenolic contents equivalent to 276-289 mg catechol equi./100g and C-glycosylflavones equivalent to 105-125 mg/100g flour in pearl millet. Bangar et al. (1999) studied comparative distribution of phenolics in pearl millet grain fractions and reported that concentration of water soluble phenolics in the defatted pearl millet meal was lower (136 mg catechol equi./100g) than in the germ (1216 mg catechol equi./100g). Lestienne et al. 2005 also reported that bran fraction have much higher iron binding phenolic

compounds, 1.43 vs 0.36 mg/g DM in pearl millet flour. Reichert (1979) identified 3-pH-sensitive pigments known as C-glycosylflavones viz., glucosylvitexin, glucosylorientin and vitexin. The concentration of total C-glycosylflavones was 124 mg catechol equi./100g in whole pearl millet grain. Reichert et al. (1980) reported a steep gradient in C-glycosylflavone concentration from outer to inner portion of the seeds with much higher percentage in the outer layers. C-glycosylflavones and their metabolites in grey-seeded pearl millet are goitrogenic (Birzer et al. 1987; Gaitan et al. 1989). These compounds have also been identified as the agents that cause off odour in ground pearl millet (Reddy et al. 1986). Keeping the view of the facts enumerated above, this experiment was designed to explore the variability in total phenolic content of pearl millet genotypes available at CCS Haryana Agricultural University Hisar.

Materials and methods

Grain samples of pearl millet [*Pennisetum glaucum* (L.) R. Br.] 11 hybrids/composites, 46 Inbred lines and 46 designated B lines (maintainers of CMS Lines) grown during *kharif-2013* and *kharif-2014* were procured from Bajra Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar. The grain samples freed of extraneous matter were stored at ambient temperature for further use. The flour was prepared with flour mill from the dried grains at the time of experiment.

Total phenols: Total phenols content was estimated by following the method of Malik and Singh (1980). Weighed exactly 1 g dry flour and ground it with a mortar and pestle in 10 ml of 80% (v/v) ethanol and centrifuged the homogenate at 10,000 rpm for 10 minutes. The supernatant was collected and re-extracted the residue twice with five ml 80% (v/v) ethanol. Pooled the supernatants and made final volume to 15 ml. Took one ml of the supernatant and made up the final volume to 4.0 ml with distilled water. Then added 0.5 ml of 1N *Folin-Ciocalteu* reagent and then after three minutes, 2 ml of saturated Na_2CO_3 solution was added to each tube. The contents were mixed thoroughly and placed the tube in boiling water bath for exactly one minute. Tubes were cooled and absorbance was recorded on UV-Vis spectrophotometer (Thermo Scientific, EVOLUTION 201) at 650 nm against a reagent blank. A standard curve prepared using different concentrations of catechol (0-100 mg/ml) was used to calculate phenol content. Results are expressed as mg catechol equi./100 g dry flour. All the assays were carried out in three replications and data were analyzed in completely randomized block design (CRD) using software 'Statistical Package for Agriculture Scientists', OPSTAT (available online at www.hau.ernet.in).

Results and Discussion

Data presented in tables 1 to 4 on total phenolic content of pearl millet genotypes showed a significant and marked variation from 175 to 435 mg catechol equi./100 with an

average value of 291 mg catechol equi./100g. Detailed account of phenols of different genotypes grouped under inbreds and designated B-lines is presented below. Average total phenols amounting to 310 mg/100g within a range of 217 to 435 mg catechol equi./100g of the pearl millet inbreds was noticed in grain produced during *kharif-2013* (Table 1). 78/711 (217 mg catechol equi./100g), H 77/833-2-202 (232 mg catechol equi./100g), H 0902-1 (235 mg catechol equi./100g), HTP 94/54 (240 mg catechol equi./100g), HTP 0906-3 (246 mg catechol equi./100g) and HTP 0904-1 (251 mg catechol equi./100g) were identified as low total phenols inbreds. These selected lines along with some other lines tested for total phenols content during *kharif-2014*. The mean total phenols content of two seasons (*kharif-2013* and *kharif-2014*) of selected lines varied from 224 (HTP 94/54) to 400 mg catechol equi./100g (DPHBL-11-123) (Table 2). On the basis of mean performance during *kharif-2013* and *kharif-2014*, HTP 94/54 (224 mg catechol equi./100g) and LPBL 10/112 (245 mg catechol equi./100g) identified as low total phenols containing lines whereas, DPHBL-11-123 (400 mg catechol equi./100g) and HBL 112/H12/1011 (372 mg catechol equi./100g) were identified as high total phenols containing inbreds.

Table 1. Total antioxidant activity (DPPH) ($\mu\text{g vit.C equi. /g}$) and total phenols (mg catechol equi./100g) of pearl millet inbreds grown during *kharif-2013*

S.No.	Pedigree	Total Phenols	S.No.	Pedigree	Total phenols
1	B08/2013	383	24	HBL-0854	273
2	Brs-10-2	290	25	HBL-0902-1	235
3	Brs-10-6	252	26	HBL-0902-5	292
4	Brs-10-7	259	27	HBL-0904-1	251
5	DPHBL-11-123	419	28	HBL-0904-2	347
6	EBLT-11-101	333	29	HBL-0906-2	313
7	EBLT-11-114	273	30	HBL-0906-3	246
8	HBL- 0703	259	31	HBL-1108	273
9	HBL-0508	325	32	HBL-112/H12/1011	357
10	HBL-0510-2	362	33	HBL-1120	285
11	HBL-0547	372	34	HBL-34	264
12	HBL-0561	300	35	HBL-72	365
13	HBL-0620	281	36	HBL-828-1	339
14	HBL-0706	360	37	ICMB-88006	304
15	HBL-0802	343	38	LPBL-10/112	261
16	HBL-0809	435	39	LPBL-10/120	342
17	HBL-0825-1	415	40	TPBL-11-109	333
18	H-1305	301	41	94/54-1	282
19	HBL-0828-2	322	42	G-73/107	316
20	HBL—0832	339	43	HTP-94/54	240
21	HBL-0843-2	333	44	HBL-11	300

22	HBL-0843-4	317	45	H-77/833-2-202	232
23	HBL-0847-3	341	46	78/711	217
				Mean	310
	C.D. (p <0.05)	32.9		C.D. (p <0.05)	32.9
	SE(d)	16.3		SE(d)	16.3
	C.V. (%)	5.2		C.V. (%)	5.2

Table 2. Total phenolic content (mg catechol equi./100g) of selected pearl millet Inbred Lines grown during *kharif-2013* and *kharif-2014*

S.No.	Pedigree	Total Phenol		Mean
		K-13	K-14	
1	LPBL-10/112	261	229	245
2	HTP 94/54	240	208	224
3	HBL-11	300	321	311
4	H-1305	301	326	314
5	G-73/107	316	287	302
6	HBL-0843-4	317	348	332
7	LPBL-10/120	342	285	314
8	HBL-112/H12/1011	357	387	372
9	DPHBL-11-123	419	382	400
10	H-77/833-2-202	232	287	310
11	78/711	217	308	257
	Mean		327	
	C.D. (p <0.05)	32.9	24.45	
	SE(d)	16.3	12.8	
	C.V. (%)	5.2	2.87	

Total phenolic content of the designated B-lines (CMS lines) also varied significantly ranging from 175 (HMS 61B) to 388 mg catechol equi./100g (HMS 53B) with an average content of 282 mg catechol equi./100g (Table 3) grown during *kharif-2013*. HMS 61B (175 mg catechol equi./100g), HMS 14B (190 mg catechol equi./100g), HMS 21B (206 mg catechol equi./100g), HMS 40B (206 mg catechol equi./100g), HMS 18A (223 mg catechol equi./100g), HMS 28B (231 mg catechol equi./100g) and HMS 16B (231 mg catechol equi./100g) were identified as promising B lines for low total phenols content. These selected lines with some other lines were grown during *kharif-2014* for knowing changes, if any, in the contents of total phenolics. In grains of these and other genotypes harvested during *kharif-2014* total phenolic content varied from 191 to 359 mg catechol equi./100g with an

average content of 278 mg catechol equi./100g. HMS 14B and HMS 21B both having about 200 mg catechol equi./100g and HMS 36B and HMS 53B having about 370 mg catechol equi./100g total phenolics (average of *kharif-2013* and *kharif-2014*) were identified as contrasting (Table 4).

Table 3. Total phenolic content (mg catechol equi./100g) of pearl millet designated B-lines (CMS lines) grown during *kharif-2013*.

S.No.	Pedigree	Total phenols	S.No.	Pedigree	Total phenols
1	HMS 7B	289	24	HMS 42B	278
2	HMS 7B-1	292	25	HMS 44B	298
3	HMS 13B	303	26	HMS 45B	242
4	HMS 14B	190	27	HMS 46B	336
5	HMS 16B	231	28	HMS 49B	308
6	HMS 18B	251	29	HMS 50B	250
7	HMS 20B	289	30	HMS 51B	327
8	HMS 21B	206	31	HMS 52B	300
9	HMS 22B	273	32	HMS 53B	388
10	HMS 23B	251	33	HMS 55B	341
11	HMS 26B	277	34	HMS 56B	255
12	HMS 28B	231	35	HMS 57B	280
13	HMS 29B	252	36	HMS 58B	353
14	HMS 30B	261	37	HMS 59B	255
15	HMS 32B	332	38	HMS 60B	253
16	HMS 33B	314	39	HMS 61B	175
17	HMS 34B	320	40	HMS-18A	223
18	HMS 36B	375	41	ICMB-89111	348
19	HMS 37B	280	42	ICMB-95222	314
20	HMS 38B	243	43	ICMB-97111	264
21	HMS 39B	272	44	ICMB-94555	342
22	HMS 40B	206	45	ICMB-94222	283
23	HMS 41B	297	46	ICMB-843-22	322
			Mean		282
C.D. (p <0.05)		28.20	C.D. (p <0.05)		28.20
SE(d)		13.98	SE(d)		13.98
C.V. (%)		4.96	C.V. (%)		4.96

The present findings of polyphenol content of pearl millet are in agreement with the observations of Khetarpaul & Chauhan (1991). In pearl millet, the concentration of polyphenols ranging from 608 to 788 mg catechol equi./100g has been reported (Khetarpaul & Chauhan 1991; Sharma 1994). A range of 228-486 mg catechol equi./100g total

polyphenols in pearl millet has been reported by Chavan and Hash (1998). Bangar et al. (1999) studied comparative distribution of phenolics in pearl millet grain fractions and reported that concentration of water soluble phenolics in the defatted pearl millet meal was lower (136 mg catechol equi./100g) than in the germ (1216 mg catechol equi./100g).

Table 4. Total phenolic content (mg catechol equi./100g) of selected pearl millet designated B-lines (CMS lines) grown during *kharif-2013* and *kharif-2014*

S. No.	Pedigree	Total phenols		Mean
		K-13	K-14	
1	HMS 14B	190	213	202
2	HMS 21B	206	191	199
3	HMS 40B	206	240	223
4	HMS 16B	231	256	244
5	HMS 38B	243	213	228
6	HMS 18B	251	227	239
7	HMS 59B	255	231	243
8	HMS 39B	272	280	276
9	HMS 26B	277	265	271
10	HMS 7B	289	311	300
11	HMS 7B-1	292	303	298
12	HMS 52B	300	270	285
13	HMS 13B	303	323	313
14	HMS 33B	314	323	319
15	HMS 32B	332	347	340
16	ICMA89111	348	303	326
17	HMS 36B	375	359	367
18	HMS 53B	388	353	371
Mean		-	278	
C.D. (p <0.05)		28.20	27.82	
SE(d)		13.98	12.53	
C.V. (%)		4.96	4.68	

In whole grain, it was 80 mg catechol equi./100g. C-glycosylflavones and their metabolites in grey seeded pearl millet are also goiterogenic (Birzer et al. 1987; Gaiton et al. 1989). These compounds have also been identified as the agents that cause off odour in ground pearl millet (Reddy et al. 1986). In spite of these compounds having some medicinal properties as antioxidants, but due to their role in off odour generation and goiterogenic effects, in pearl millet low phenolic as well as C-glycosylflavone contents are desirable.

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