Journal of Biotechnology and Crop Science
Vol. 10, No. 15, January-December, 2022, 169-175

doi: 10.5958/2582-5089.2022.00020.2

Research Article

Phytate and phosphorus contents of grains harvested from pearl millet genotypes sown on different dates

Kumari Arti, L.K. Chugh, Vinod Kumar, Sushil Nagar, Ramesh Kumar and Neeraj Kharor

ABSTRACT

For identifying low phytate-high phytase genotype(s) of pearl millet [*Pennisetum glaucum* (L.) R. Br.], forty inbreds were grown in two sets during *kharif* 2015 at Research Area of Bajra Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar in randomized block design in two rows with plot size 4m x 0.45m with 10-12 cm intra row spacing. One set of the inbreds was sown late by a month (1.7.2015) than the normal sowing date (27.5.2015). Average phosphorous (3.11 mg/g) was unaffected by late sowing. Average phytate content was 680 mg/100 g in the grains of the inbreds under normal sown condition which increased to 743 mg/100 g under late sown condition. Average phytate content of grains of normal and late sown inbreds viz., EMRL 14/223 (456 mg/100 g), EMRL 14/229 (445 mg/100 g), H 78/711 (463 mg/100 g), HFePPT-2-12-141 (492 mg/100 g) was in the intermediate range. None of the genotype exhibited low phytate content.

Keywords: Phytate, phosphorus, pearl millet, Sowing

INTRODUCTION

Phytic acid is known as the major source of phosphorus in cereal grains, comprising 1–5% by weight in cereals, legumes, oil seeds and nuts. It accounts for approximately 65–80% of the total seed phosphorus (Loewus, 2002; Verma et al., 2021a). It rapidly accumulates in seeds during the ripening period. Phytic acid is commonly known as phytate when complexed with positively charged metal ions. Phytate is of prime concern for human nutrition and health management (Kumari et al., 2020; Verma et al., 2021b). Numerous investigations have made available evidences that dietary phytate is an antinutrient. Studies on humans show that phytate has a very strong inhibitory effect on iron absorption (Brune et al., 1992). The high prevalence of iron deficiencies in developing countries has several adverse effects on the population particularly on women and children (Zimmermann & Hurrell, 2007). In India, about 56.2% of women in the age group 15-49 years, 79.2% among children aged 6-35 months, 57.9% of pregnant women and 24.3% of men aged 15-49 years suffers from iron deficiency induced anemia (IIPS, 2007). Fe deficiency leads to morbidity and mortality of mother and child at childbirth and impairs cognitive skills and physical activity. In view of these facts, reducing the phytate content in cereal grains is a desired goal for the genetic improvement of several crops.

Pearl millet is cultivated in the hot dry parts of India in regions receiving low annual rainfall ranging from 300 to 800 mm. In the country, pearl millet was grown over

Kumari Arti[1], L.K. Chugh[1](✉), Vinod Kumar[1], Sushil Nagar[1], Ramesh Kumar[2] and Neeraj Kharor[3]

[1]Department of Biochemistry, CCS Haryana Agricultural University, Hisar-125004, Haryana, India
[2]Bajra Section, [3]Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125004, Haryana, India
E-mail chughlk@yahoo.co.in

an area of 7.1 million hectares with a total production of 9.1 million tonnes and productivity of 1272 kg/ha during 2015 (Anonymous, 2015). Pearl millet also contains high content of phytic acid (Kumar et al., 2016). Developing moderate/low phytate cultivars through conventional plant breeding approaches is considered an important mean to reduce phytate content (Fanny et al., 2005). Shanmuganathan et al. (2006) reported presence of additive and non-additive genetic variation in phytate content of pearl millet and suggested to follow a population improvement programme using recurrent selection to breed for low phytate content. If a sufficient number of lines with low phytate content become available, a programme for the development of synthetics or composites can also be initiated. Identification of pearl millet genotypes with relatively low phytate and high phytase with good agronomic score would be a step towards development of pearl millet cultivars with lesser phytate content and hence better micronutrients availability. However, for final selection of the material with desired character prior knowledge about deviation in accumulation of phytate content as a consequence of production environments is of immense importance since variation in phytate content because of changing seasons has been reported (Kumar et al., 2016). Publications are not available in literature neither on comparison of growing locations nor of influence of date of sowing in respect of accumulation of phytate in pearl millet. Therefore, the present investigation was carried out for identifying low phytate pearl millet genotypes.

MATERIALS AND METHODS

Procurement and storage of grain samples

Forty inbreds were grown during *kharif* 2015 following the recommended package of practices at the Research Area of Bajra Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar in randomized block design in two rows with plot size 4m x 0.45m with 10-12 cm intra row spacing. The inbreds were sown on two different dates i.e., 27.5.2015 (normal sown) and 1.7.2015 (late sown). Grain samples of five hybrids, two varieties and one advance population grown during *kharif* 2015 were also procured from Bajra Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar. Mature grains harvested made free of extraneous matter were stored at -20^{o}C till further use.

Preparation of flour for chemical analysis

Flour was prepared in grinding mill (Model Cyclotech, Foss Analytical AB, Sweden) using 0.8 mm screen. Flour stored in aluminium boxes was dried to constant weight at 55^{o}C in an oven before carrying out chemical analysis. For determining enzyme activities freshly prepared flour was used.

Extraction and estimation of phytic acid

Phytic acid was extracted and estimated by employing the method of Haug & Lentzsch (1983). Finely ground sample (500 mg) was extracted with 25 ml of 0.2 N HCl for 3 h with continuous shaking in a shaker. After shaking, the contents were filtered through Whatman No. 1 filter paper. The aliquot obtained was used for estimating phytate content. An aliquot (0.4 ml) was taken in a test tube and 1.0 ml distilled water was added to it. To each test tube 1 ml ferric ammonium sulphate solution (Dissolved 0.2 g of FAS in 100 ml of 2N HCl and made the volume to 1000 ml with distilled water) was added. The tubes were placed in a boiling water bath for 30 min. The contents were centrifuged at 10,000 rpm for 5 min. One ml of the supernatant was transferred to another test tube and 1.5 ml 2, 2'-Bipyridyl solution (Ten gram of 2, 2'- Bipyridyl and 10 ml of thioglycolic acid were dissolved in distilled water and volume was made to 1000 ml.) was added. After 15 minutes, absorbance was measured on UV–Vis spectrophotometer (Thermo Scientific, EVOLUTION 201) at 519 nm against 0.2 N HCl. For plotting a standard curve, different concentrations ranging from 40 to 200 µg phytate

(Weighed 30.54 mg sodium phytate. It was dissolved in 100 ml of 0.2 N HCl which gave a solution containing 200 µg phytate per ml were taken in different tubes and volume was made to 1.4 ml with distilled water. Results were calculated as mg phytate/100g d.w.

Extraction and Estimation of phosphorus

Five hundred mg ground sample was taken in a 100 ml conical flask. To this, 20 ml diacid mixture (HNO_3:$HClO_4$ in 5:1 ratio) was added and kept it overnight. Next day, it was digested by heating till clear white precipitates settle down at bottom. The crystals were dissolved by diluting in double distilled water and made up the volume to 50 ml with double distilled water. Total phosphorous assay was determined by the method proposed by Chen et al. (1956).

Reagents

a) **10% ascorbic acid:** Dissolved10 g ascorbic acid in100 ml water. The solution was always prepared fresh.

b) **2.5% ammonium molybdate:** Dissolved 2.5 g ammonium molybdate in 100 ml water. The solution was stored at room temperature.

c) **6 NH_2SO_4:** 16.3 ml conc. H_2SO_4 was taken and volume made to 100 ml.

d) **Reagent C:** The reagent was prepared by mixing 6 N H_2SO_4, distilled water, ammonium molybdate (2.5%) and ascorbic acid in the ratio 1:2:1:1(V/V) respectively. Reagent was prepared fresh each day.

e) **Standard phosphorous solution**: Pure and dry 0.351 g anhydrous monopotassium dihydrogen orthophosphate was dissolved in a few ml of distilled water and 10 ml 10 N H_2SO_4. Then the volume was made to 1 liter with distilled water. Thus, the stock formed contained 80 µg P/ml. 25 ml stock solution was diluted to 1 liter. Now it contains 2 µg P/ml which was used as working standard solution.

An aliquot (1 ml) of mineral extract was pipetted in a test tube. The volume was made 4 ml with distilled water. Then 4 ml of colouring reagent was added and mixed well. Incubated the contents at 37°C for 90 min. Cooled the content to room temperature. Absorbance was read at 820 nm against reagent blank. For plotting a standard curve, different concentrations of standard phosphorus ranging from 40 to 200 µg phosphorus were taken in different tubes and volume was made to 4 ml with distilled water. Results were calculated as mg phosphorus/g d.w.

RESULTS AND DISCUSSION

Data on phytate content of the normal and late sown forty inbreds are presented in Table 1. Phytate content of the grains of the inbreds when sown on normal date ranged from 424 to 991 mg/100 g and from 461 to 1091 mg/100 g when sown late. Date of sowing showed maximum effect on phytate content of the three inbreds viz., HPT-10-144, H 78/711 and PT-1-10-1021. The content of these lines differed equally by 18% because of difference in sowing. For example, phytate content of H 78/711 increased by 18 % (78 mg/100 g). Similarly, phytate content of HPT-2-12-10 and S 97/120 when sown late increased by 14 %. Variation in phytate content of rest of the genotypes as a result of different dates of sowing was equal to 10 % or below. Varietal differences in phytate content of pearl millet have been documented earlier (Pelig-Ba, 2009; Suma & Urooj, 2011). Mean phytate content of the grains produced from the normal and late sown inbreds was 680 and 743 mg/100g, respectively. Average phytate content of hybrids and composites grown in Haryana and some other states of India were 592 and 608 mg/ 100 g grown during *kharif* 2013 and *kharif* 2014, respectively (Kumar, 2015). Mean phytate content (680 and 743 mg/100g, respectively) of the grains produced from the normal and late sown inbreds was higher than that reported by Kumar et al. (2016). Thus, average phytate content of grains of the tested genotypes increased significantly when sown late. Except HPT-2-12-32, phytate content of all the

Table 1: Phytate content of pearl millet inbreds sown on different dates during *kharif* 2015

S.No.	Genotype	Phytate (mg/100g)			Difference in phytate content (mg/100g)	Per cent deviation in phytate content
		Normal sown	Late sown	Mean		
1	A5RLT 14/106	945	968	957	23	2
2	AC 04/13	854	861	858	7	1
3	EMRL 14/115	956	998	977	42	4
4	EMRL 14/116	991	1091	1041	100	10
5	EMRL 14/121	810	886	848	76	9
6	EMRL 14/127	625	665	645	40	6
7	EMRL 14/208	783	840	812	57	7
8	EMRL 14/222	945	1001	973	56	6
9	EMRL 14/223	433	478	456	45	10
10	EMRL 14/226	505	523	514	18	4
11	EMRL 14/229	428	461	445	33	8
12	EMRL 14/243	576	629	603	53	9
13	EMTR-11-104	597	652	625	55	9
14	H 78/711	424	502	463	78	18
15	H12/011	732	761	747	29	4
16	HFePPT-2-12-123	732	772	752	40	5
17	HFePPT-2-12-141	475	509	492	34	7
18	HFIT-3-11-125	664	731	698	67	10
19	HPT -1-10-1131	687	731	709	44	6
20	HPT-10-129	648	679	664	31	5
21	HPT-10-144	473	560	517	87	18
22	HPT-1-12-34	944	1002	973	58	6
23	HPT-1-12-44	919	1006	963	87	9
24	HPT-1-12-84	780	791	786	11	1
25	HPT-2-12-10	597	682	640	85	14
26	HPT-2-12-32	988	950	969	-38	-4
27	HTP 03/13-901-78-3	694	750	722	56	8
28	HTP 93/104-1	636	665	651	29	5
29	MP 293-4 -1	854	921	888	67	8
30	MP 293-4	544	574	559	30	6
31	PT -1-10-1021	519	613	566	94	18
32	PT-1-10-1002	838	898	868	60	7
33	PT-1-10-1047	721	801	761	80	11
34	RAJ-3	552	604	578	52	9
35	S 97/120	614	703	659	89	14
36	SGP-10-120	590	651	621	61	10
37	TCH 26-1	630	686	658	56	9
38	TCP-10-110	765	805	785	40	5
39	TPT-A2 -1-11-121	606	672	639	66	11
40	TPT-A2 -1-11-155	644	681	663	37	6
	Mean	680	743			
	C.D.	4.7	4.0			
	SE(m)	1.6	1.4			
	SE(d)	2.3	1.9			
	C.V.	3.5	3.0			

Table 2: Total phosphorus (P) content of pearl millet inbreds sown on different dates during *kharif* 2015

S.No.	Genotype	P (mg/g)			Difference in P (mg/g)	Per cent deviation in P (mg/g)
		Normal sown	Late sown	Mean		
1	A5RLT 14/106	3.67	3.62	3.65	-0.1	1
2	AC 04/13	3.31	3.05	3.18	-0.3	8
3	EMRL 14/115	3.71	3.63	3.67	-0.1	2
4	EMRL 14/116	3.82	3.79	3.81	0.0	1
5	EMRL 14/121	3.01	3.50	3.25	+0.5	16
6	EMRL 14/127	3.04	3.15	3.09	+0.1	4
7	EMRL 14/208	2.73	3.46	3.09	+0.7	27
8	EMRL 14/222	2.98	3.60	3.29	+0.6	21
9	EMRL 14/223	2.96	2.89	2.92	-0.1	2
10	EMRL 14/226	2.93	3.08	3.00	+0.1	5
11	EMRL 14/229	2.96	2.86	2.91	-0.1	3
12	EMRL 14/243	2.98	3.36	3.17	+0.4	13
13	EMTR-11-104	3.11	3.21	3.16	+0.1	3
14	H 78/711	2.93	2.82	2.88	-0.1	4
15	H12/011	3.16	3.28	3.22	+0.1	4
16	HFePPT-2-12-123	3.13	3.28	3.21	+0.1	5
17	HFePPT-2-12-141	2.98	3.00	2.99	0.0	0
18	HFIT-3-11-125	3.09	3.31	3.20	+0.2	7
19	HPT -1-10-1131	3.09	3.31	3.20	+0.2	7
20	HPT-10-129	2.92	3.31	3.11	+0.4	13
21	HPT-10-144	2.97	2.94	2.96	0.0	1
22	HPT-1-12-34	3.04	3.06	3.05	0.0	1
23	HPT-1-12-44	3.06	3.06	3.06	0.0	0
24	HPT-1-12-84	3.42	3.44	3.43	0.0	1
25	HPT-2-12-10	3.48	3.20	3.34	-0.3	8
26	HPT-2-12-32	3.73	3.78	3.75	+0.1	1
27	HTP 03/13-901-78-3	3.09	3.27	3.18	+0.2	6
28	HTP 93/104-1	2.66	3.17	2.92	+0.5	19
29	MP 293-4 -1	3.35	3.40	3.38	+0.1	2
30	MP 293-4	2.96	3.13	3.04	+0.2	6
31	PT -1-10-1021	2.96	3.12	3.04	+0.2	5
32	PT-1-10-1002	3.47	3.40	3.44	-0.1	2
33	PT-1-10-1047	3.11	3.28	3.19	+0.2	6
34	RAJ-3	2.97	3.33	3.15	+0.4	12
35	S 97/120	3.04	3.15	3.09	+0.1	4
36	SGP-10-120	3.05	3.36	3.21	+0.3	10
37	TCH 26-1	2.62	3.16	2.89	+0.5	21
38	TCP-10-110	3.24	3.31	3.27	+0.1	2
39	TPT-A2 -1-11-121	3.01	3.23	3.12	+0.2	7
40	TPT-A2 -1-11-155	2.73	3.19	2.96	+0.5	17
	Mean	3.11	3.26	3.19		
	C.D.	0.168	0.301			
	SE(m)	0.059	0.105			
	SE(d)	0.083	0.148			
	C.V.	2.664	4.542			

genotypes increased with late sowing. Phytate content of most of the genotypes tested during the present investigation also exceeded the values reported earlier by other investigators (Lestienne et al., 2005; Osman 2011; Suma & Urooj, 2011). Thus, none of the inbred was low in phytate. Four of the inbreds viz., EMRL 14/223, EMRL 14/229, H 78/711 and HFePPT-2-12-141 had phytate content less than 500 mg/100 g. These were termed as intermediate phytate inbreds which could be useful for breeding programme.

Results on phosphorous of the normal and late sown forty inbreds are presented in Table 2. Among all the tested genotypes for phosphorous content in only three genotypes more than 20 % variation was seen as a consequence of varying sowing time which were EMRL 14/208, EMRL 14/222 and TCH 26-1. EMRL 14/121, HTP 93/104-1, and TPT-A2-1-11-155 showed variation between 15-20% as a consequence of sowing time. Rest of the genotypes showed less than 15% variation in their phosphorus content as a result of different sowing time. Phosphorus content of the grains of the inbreds when sown on normal date ranged from 2.62 to 3.82 mg/g and from 2.82 to 3.79 mg/g when sown late. Mean phosphorous content (3.11 mg/g and 3.26 mg/g, of the grains produced from the normal and late sown inbreds respectively) was within the range (1.18 mg/g to 4.56 mg/g) reported by Pelig-Ba (2009) and by Ravindran (1991). Sade (2009) also reported similar average phosphorus content in raw millet flour. Gemen et al. (2015) found out mean phosphorous content of more than 100 genotypes grown under low and high phosphorous conditions equivalent to 2.26 and 2.48 mg/g, respectively. Thus, average phosphorous content of the tested genotypes improved slightly when sown late. Phosphorous content of EMRL 14/116 was highest (3.82 mg/g when sown timely or late). Four other genotypes viz., A5RLT 14/106, EMRL 14/115, HPT-2-12-32 and PT-1-10-1002 showing about 3.5 mg/g phosphorus were rich in phosphorus.

A strong positive correlation (r= 0.739**) was found between total phosphorus and phytate content of the genotypes. Simultaneous increase in phosphorus and phytate contents of the genotypes when sown late indicates better uptake and utilization of the element. Four of the intermediate phytate-inbreds viz., EMRL 14/223, EMRL 14/229, H 78/711 and HFePPT-2-12-141 could be useful for breeding programme.

REFERENCES

Anonymous (2015). Accessed from *agricoop.nic.in.*

Brune, M., Rossander-Hultén, L., Hallberg, L., Gleerup, A., & Sandberg, A.S. (1992). Iron absorption from bread in humans: Inhibiting effects of cereal fibre, phytate and inositol phosphates with different numbers of phosphate groups. *Journal of Nutrition, 122*, 442–449.

Chen, P.S., Toribara, T.T., & Warner, H. (1956) Microdetermination of phosphorus. *Analytical Chemistry, 28*, 1756–1758.

Gemenet, D.C., Beggi, F., Hash, C.T., Sy, O., Sanogo, M.D., Zangre, R.G., Falalou, H., Buerkert, A., & Haussmann, B.I. (2015). Towards understanding the traits contributing to performance of pearl millet open-pollinated varieties in phosphorus-limited environments of West Africa. *Plant & Soil, 407*, 243-259.

Haug, W., & Lantzsch, H.J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. *Journal of the Science of Food and Agriculture, 34*, 1423-1426.

IIPS (2007). National Family Health Survey-3 India 2005-06. Mumbai, India and ORC Macro, Calverton, Maryland, USA.

Kumar, M. (2015). Biochemical investigations on nutritional properties of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. PhD thesis, CCS HAU, Hisar, India.

Kumar, M., Chugh, L.K., & Kumar, R. (2016). Possibility of using leaf *in vivo* nitrate reductase activity as a biochemical marker for predicting grain protein content of pearl millet. *International Journal of Current Microbiology and Applied Sciences, 4*, 630-634.

Kumari, A., Chugh, L., Kumar, V., Nagar, S., & Kharor, N. (2020). Phytase from pearl millet: its partial purification and characterization. *Forage Research, 46*(1), 78-83.

Lebouc, F., Dez, I., & Madec, P.J. (2005). NMR study of the phosphonomethylation reaction on chitosan. *Polymer, 46*, 319-325.

Lestienne, I., Besancuon, P., Caporiccio, B., Lullien-Pellerin, V.R., & Treche, S. (2005). Iron and zinc *in vitro* availability in pearl millet flours (*Pennisetum glaucum*) with varying phytate, tannin, and fiber contents. *Journal of Agricultural and Food Chemistry, 53*, 3240-3247.

Loewus, F. (2002). Biosynthesis of phytate in food grains and seeds. In N. R. Reddy and S. K. Sathe (Eds.), Food phytates. Boca Raton, Florida, USA: CRC Press. pp. 53–61.

Osman, M.A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Sciences, 10(1*), 1–6.

Pelig-Ba, K.B. (2009). Assessment of Phytic Acid Levels in Some Local Cereal Grains in Two Districts in the Upper East Region of Ghana. *Pakistan Journal of Nutrition, 8*, 1540-1547.

Ravindran, G. (1991). Studies on millets: proximate composition, mineral composition, and phytate and oxalate contents. *Food Chemistry, 39*, 99-107.

Sade, F.O. (2009). Proximate, Antinutritional Factors and Functional Properties of Processed Pearl Millet (*Pennisetum glaucum*). *Journal of Food Science and Technology, 7*(3), 92-97.

Shanmuganathan, M., Gopalan, A., & Mohanraj, K. (2006). Genetic analysis of pearl millet for phytic acid content. *Journal of Agricultural Science, 2*(2), 1–5.

Suma, P.F., & Urooj, A. (2011). Influence of germination on bioaccessible iron and calcium in pearl millet (*Pennisetum typhoideum*). *Journal of Food Science and Technology, 51*, 976-981.

Verma, M., Bora, R.S., Sheikh, I., Kumar, V., Sangwan, P., & Dhaliwal, H.S. (2021a). Effect of gibberellins and ascorbic acid treatment on phytic acid and micronutrients dialyzability in germinated biofortified wheat seeds. *Indian Journal of Community Health, 33*(1), 123-129.

Verma, M., Saxena, A., Sangwan, P., Sheikh, I., Kumar, V., & Dhaliwal, H.S. (2021b). Phytase mediated beneficial impact on nutritional quality of biofortified wheat genotypes. *Current Nutrition & Food Science, 17*(5), 490-500.

Zimmerman, M.B., & Hurrell, R.F. (2007). Nutrition iron deficiency. *Lancer, 370*, 511-520.