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Short Communication

Status of phytic acid content in Indian barley (*Hordeum vulgare* L) varieties and its correlation with other grain parameters

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Phytic acid, myo-inositol 1,2,3,4,5,6-hexakisphosphate (InsP6), is the major form of stored phosphate in food grains and reported to contain 50-80% of total phosphorus in the grain (Gupta *et al.*, 2015; Silva *et al.*, 2021). Besides binding the phosphorus, phytic acid is also known as chelator of cations like Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+} and K^{+} (Liu *et al.* 2005). Barley grains contain appreciable amount of minerals, but their availability is low because of the formation of insoluble complexes with phytate, a salt of phytic acid, which is also one of the main inhibitors for iron and zinc absorption in humans (Ockenden *et al.*, 2004). Therefore, phytic acid is also regarded as 'anti-nutritional' factor as it reduces the bioavailability of important minerals. Although the direct use of barley as food is very limited, but the proper availability of minerals is very important for balanced nutrition specially for poor and underdeveloped populations. The major portion of barley production (60-70%) goes as animal feed and there also poor bioavailability of minerals may lead to under nutrition and thereby its consequences in animals as well. Further, chelated phosphorus ends up in the faecal matter led environmental pollution. Around 25-30% barley goes for malt industry. During malt production also different energetic and biosynthetic processes require phosphorus and other minerals (Lee, 1990). Phytic acid also show the ability to form complexes with proteins affecting their solubility and ultimately their degradation into smaller peptides. So, lower values of phytic acid and/or higher activity of phytase enzyme (which breaks down phytic acid into inositol and phosphates) are highly desirable. After barley malting, larger proportion of malt is used for brewing where yeasts do the fermentation of wort (malt extract). For fermentation as well, besides various other nutrients, sufficient availability of zinc and magnesium has been shown to improve the fermentation efficiency (Lee 1990; Rimsten *et al.*, 2002; Dai *et al.*, 2007; Edney *et al.*, 2011). Therefore, irrespective of the end use of barley, be it feed, malt or food, lower levels of phytic acid is one of the important and desirable trait. This study was conducted with two objectives; first to know the status of phytic acid in Indian barley varieties and second to see if any correlation of this parameter exists with other physical traits of barley grain.

A total of 71 varieties were grown at ICAR-Indian Institute of Wheat & Barley Research (IIWBR) at Karnal in Haryana state of India, in *rabi* season using recommended package and practices for the region in three replications. The grains were harvested at full maturity, cleaned and grains from three replications were mixed to make a composite sample. Around 10g of composite sample was grounded in a Cyclotech Mill (Foss, Denmark) to pass through 0.5 mm screen. Phytic acid content was determined by the method of McKie and McCleary (2016) using Megazyme Phytic Acid Assay Kit in the barley flour. Thousand kernel weight was estimated by counting thousand grains on a grain counter (Pfeuffer, Germany) and then weighing the grains on electronic weighing balance. Grain plumpness was determined using Sortimat machine (Pfeuffer, Germany), where 100 g grains were separated over 2.8 mm, 2.5 mm and 2.2 mm screens; the grains retained over 2.8 and 2.5 mm were considered plump ones. Protein content was measured on dry weight basis using NIR (Infratec 1241, Grain Analyser, Foss, Denmark). All these parameters were determined in single sample of each variety to get preliminary information since statistical analysis was not performed. However, the correlation between different traits was worked out using the software OPSTAT (Sheoran *et al.*, 1998).

The phytic acid content varied from 0.676% to 1.997 % showing a good variability in Indian varieties (Table 1). Out of total 71 varieties screened for phytic acid content, 11 varieties had value of < 0.8%; 39 varieties were having value of > 0.8 to 1.0 %; 18 were having values of > 1.0 % to 1.5 % and 3 were having > 1.5% (Fig 1). Three varieties with lowest phytic acid content were NDB 1173 (0.676%), BH 393 (0.686 %) and BCU 73 (0.698 %). The three varieties with highest phytic acid were HBL 276 (1.997 %), Dolma (1.997 %) and HBL 113 (1.737 %). Lee (1990) reported phytic acid content of nine North American six- and two-rowed barley varieties ranging from 0.95 to 1.11%. Dai *et al.* (2007) found phytic acid content of 100 barley varieties from 3.85 mg/g to 9.85 mg/g, with a mean of 7.01 mg/g. In our study the range is quite wide and upper values is touching 2%, the differences could probably be because of environment, genetic variability or the procedure employed for phytic acid estimation. Since, in this study only single location has been used for growing varieties, the absolute values may change depending upon the environment and cultural practices. The environment has been shown to affect phytic acid content to a large extant (Dai *et al.*, 2007). Despite all these possibilities, the results indicate a good amount of variability among the varieties investigated. As per our knowledge, this is the first report on phytic acid content in Indian barley varieties and thus can provide important preliminary information to scientific community regarding further basic and applied studies on this trait in barley and barley-based food, malt and feed improvement programme.

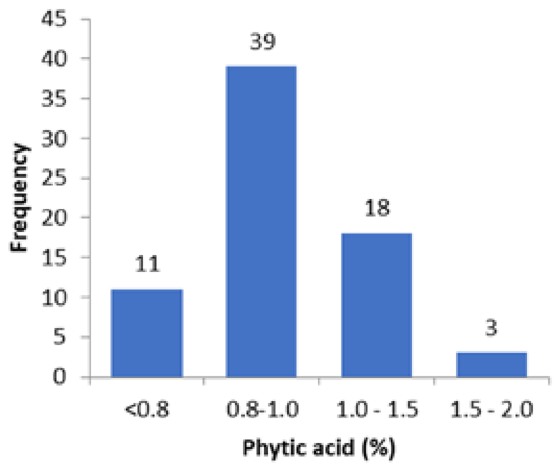

Fig 1. Frequency distribution of barley varieties with respect to their phytic acid content

Table 1. Phytic acid (%) and other parameters in grains of barley varieties

Variety	Parentage (Kumar *et al.*, 2017)	Hull type	Phytic acid (%)	TW (Kg/hl)	BG (%)	TG (%)	TGW (g)	PR (%)
Alfa 93	Aurora/Queen//Beka	H	0.784	65.6	76.9	3.8	44.9	11.1
Amber	K12/CN294	H	0.897	56.4	48.6	18.5	43.9	8.5
Azad	K12/K19	H	0.869	59.9	27.2	11.0	37.9	10.0
BCU 73	WUM 143 (YAGAN)	H	0.698	62.8	78.7	6.7	50.6	9.6
BG 105	C141/MONTLESSO	H	0.814	53.5	46.5	1.6	50.4	8.5
BG 25	C138/CN170	H	0.839	52.8	43.1	2.3	49.7	8.7
BH 393	California Mariout/Ratna	H	0.686	54.6	61.7	11.7	39.2	7.3
BH 75	RD150/AHOR31/68	H	0.720	53.8	79.4	3.7	47.5	7.7
BH 902	BH495/RD2552	H	0.824	56.6	46.4	2.6	44.1	9.4
BHS 169	Kailash/Briggs	HL	0.855	56.5	63.0	13.8	37.1	8.5
BHS 352	HBL240/BHS504//VLB129	HL	1.031	70.9	11.0	68.1	31.0	10.7
BHS 380	Voilet/MJA/7/ABN-B6	HL	0.909	57.1	39.7	23.3	32.3	9.3
BHS 46	BHS37-37/BHS 14-88//Kailash	HL	0.997	52.3	70.7	8.2	38.9	7.1

Bilara 2	RS17/C251	H	0.925	55.6	47.6	24.0	37.7	7.0
Clipper	Introduction from Australia	H	1.075	61.1	44.0	5.0	39.8	10.5
DL 88	BG 1/MEX5-13	H	1.301	54.3	40.7	3.8	39.5	8.3
Dolma	Selection from USA-115	HL	1.997	68.8	5.1	76.1	31.4	9.6
DWR 28	BCU73/PL172	H	0.873	65.0	91.2	1.9	56.1	10.4
DWRB 73	PL710/DWR17	H	1.071	63.1	87.4	1.9	54.9	11.8
DWRUB 52	DWR17/K551	H	1.203	65.6	84.2	2.8	47.9	11.0
DWRUB 64	DL472/PL705	H	1.249	61.3	78.7	2.4	47.4	8.5
Geetanjali	K12/K572/10//EB410	HL	1.331	66.8	18.3	42.3	49.7	8.8
HBL 113	Selection from Zyphzee	HL	1.737	56.7	76.1	7.2	41.4	8.4
HBL 276	HBL233/HBL238	HL	1.997	69.2	3.7	72.9	50.3	9.1
HBL 316	Mutant of HBL98	HL	1.165	59.4	52.9	17.6	34.8	8.3
Himani	EB489/Kailash//BHS 15-88	H	0.746	59.4	61.1	13.0	32.9	7.7
Jagriti	K138/P103	H	0.887	57.9	69.0	7.1	44.5	7.3
JB 58	RD2615/DL70/BG105	H	0.772	56.3	54.6	12.2	36.0	7.2
Jyoti	K12/C251	H	0.855	56.7	54.0	17.1	40.5	10.7
K 141	K18/IB254	H	0.875	52.0	21.2	33.6	36.1	11.6
K 409	Jyoti/DL85	H	0.824	58.6	72.5	8.6	41.6	8.5
Pragati	K394/K141	H	0.839	55.1	50.4	19.6	30.4	8.7
K 551	P464/Jyoti	H	0.897	58.2	78.4	3.2	40.8	9.7
K 560	K404/DL479	H	0.921	55.5	57.8	12.5	42.0	9.3
K 603	K257/C138	H	0.899	57.8	80.0	5.7	46.3	8.9
Kailash	EB438/NP100	HL	0.891	65.1	48.1	10.7	36.1	10.1
Karan 16	Azam (DWARF)1/EB7576	HL	1.079	68.0	11.8	47.0	27.9	12.1
Lakhan	K12/IB226	H	1.087	55.9	65.1	9.5	42.0	8.1
LSB 2	Introduction as USA 94	H	1.127	56.9	58.6	16.8	39.3	8.6
Manjula	K4126/SOHAN	H	1.057	52.9	17.2	35.6	54.6	9.9
NB 1	Karan15/P408	H	0.843	54.2	38.9	26.3	35.8	8.1
NB 2	DL470/RD2035	H	0.939	56.3	64.5	11.2	35.6	7.8
NB 3	K425/Jyoti	H	0.917	59.4	67.3	10.7	34.6	8.3
NDB 1173	BYTLRA3 (94-95)/NDB217	HL	0.676	60.8	62.8	11.0	35.2	10.0
PL 172	RD178/DW472	H	0.867	50.3	79.2	3.5	49.0	8.8
PL 419	PL101/BH182	H	0.828	55.9	87.0	3.5	44.3	8.3
PL 426	Karan92/PL101	H	0.831	56.6	86.1	3.3	44.4	9.2
PL 56	Mutant of C164	H	0.905	54.3	54.0	15.7	36.9	9.9
PL 751	K226/PL226	H	0.843	54.8	49.2	21.2	31.5	9.2
Raj Kiran	RDB1/MORROCAINE	H	0.820	54.7	65.7	10.2	44.5	8.9
Ranjit	BG1/MEX5-13	H	0.929	53.3	77.1	5.8	40.4	9.1
Ratna	Selection from local material	H	0.923	54.4	70.7	5.9	43.8	8.3
RD 103	RDB1/MORROCAINE	H	0.845	55.4	78.2	6.4	42.9	8.5
RD 2035	RD137/PL101	H	0.748	59.7	71.6	6.8	39.5	6.8
RD 2052	Api-CM-67/SO-727//PL101	H	0.814	56.6	74.6	4.9	41.1	8.1
RD 2503	RD103/BH153//RD2046	H	0.770	56.9	77.7	5.3	36.5	7.2
RD 2508	RD2035/P409	H	0.871	55.2	71.2	9.4	37.1	9.8

RD 2552	RD2035/DL472	H	0.704	52.3	71.5	8.3	40.5	8.0
RD 2592	RD2503/UBL9	H	1.077	51.6	68.1	10.9	35.9	7.2
RD 2624	Bilara2/RD2508	H	0.812	49.8	50.3	21.3	33.8	8.9
RD 2660	RD2052/RD2566	H	1.155	49.7	72.2	8.8	39.2	8.1
RD 2668	RD 2035/BCU 73	H	0.859	57.0	55.5	12.4	42.9	9.6
RD 2715	RD 387/BH 602//RD 2035	H	1.251	46.8	59.3	16.6	32.3	8.8
RD 31	RS17/PRIOR	H	1.009	55.8	62.1	12.2	42.5	8.4
RD 57	RS17/PRIOR	H	0.949	55.0	67.8	8.8	43.9	7.6
RS 6	RS17/NP21	H	0.875	58.7	58.9	11.3	37.8	8.6
Sonu	Selection from EB233/GIZA117	H	0.776	54.1	69.1	9.9	39.4	8.4
UPB 1008	Higo/Lino/3/Chanico/Tocte// Congona/4/…	H	0.937	54.9	63.9	10.3	41.2	9.7
Vijaya	K12/C251	H	1.329	53.0	67.1	10.5	33.9	9.2
VLB 1	NP109/HBL62	H	0.881	57.0	72.9	7.9	42.2	12.0
VLB 56	Morocco/VLB1	H	1.011	54.7	49.0	25.4	30.8	8.3

H = Hulled; HL = Hulless; TW – Test Weight; BG – Bold Grains; TG – Thin Grains; TGW – Thousand grain weight; PR – Protein content on dry weight basis

The protein content varied from 6.8% to 12.1% (dwb) with a non-significant correlation with phytic acid (Table 2). However, Dai *et al.* (2007) has reported a very strong and significant correlation (0.412), between these traits. Again, our study being very limited, may have scanty information on this aspect even though the correlation is confirmed to be positive. Now a days malting industry is demanding high protein varieties coupled with good malt extract, in that situation this parameter needs to be looked into carefully with the malting quality and nutrition perspective.

The bold grain percentage (> 2.5 mm in size) varied from 3.7% to 91.2%, with a negative correlation (-0.4**) with phytic acid content (Table 2). This is quite obvious also, as plump grains have greater contribution from endosperm and phytic acid is reported to be mainly concentrated in aleurone layer and embryo (O'Dell *et al.,* 1972). So, in malt barley varieties, selection of bold or plump grains can be one of the major criteria and same can also be followed for feed or food barley. However, there is need to further understand the factors affecting the phytic acid concentration in the grain towards the development of low phytate varieties. The thin grain percentage (< 2.2 mm in size) had a range of 1.6 to 76.1%. As expected, a positive correlation (0.56**) was obtained between phytic acid content and thin grains (Table 2). Thin grains have lower starch content leading to the relative effect on phytic acid concentration.

The test weight values varied from 46.8 to 70.9 kg/hl, where hulless grains had higher test weights. A significant positive correlation was observed between phytic acid content and test weight (0.33**). The flour extraction rate was found also positively correlated with the test weight. The results thereby suggest that for food purpose, the low phytic acid grains of barley with higher test weight must be preferred.

Table 2: Pearson Correlation of Phytic Acid with other traits

	TW	**BG**	**TG**	**TGW**	**Protein**
PA	0.332**	-0.402**	0.557**	0.000^{NS}	0.095^{NS}

*PA = Phytic Acid; TW = Test weight; BG = Bold grain percentage; TG = Thin grain percentage; TGW = Thousand grain weight

Though this was a preliminary investigation, but has provided important clues for further in-depth studies on Indian barley improvement programme. Multilocation studies are necessary to establish the effect of location and growing conditions on the phytic acid content. Hulless varieties have shown the highest values of phytic acid in the present study. For food purpose, hulless varieties with high test weight and protein content are preferred. Further, based on this study, new breeding programmes can be developed for food barley with an aim to develop hulless varieties with low phytic acid. This study also highlights the need of conducting investigations to identify barley varieties with high Fe, Zn and phytase levels for their future use in biofortification programmes.

Conflict of Interest

Authors declare that they have no conflict of interest

Ethical Compliance Statement

NA

Author's Contribution

Dinesh Kumar (Planning, execution and writing of MS); Sneh Narwal (Planning and editing of MS); Ramesh Pal Singh Verma (Editing of MS); Gyanendra Pratap Singh (Planning and Guidance)

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