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Research Note

Screening of soybean germplasm for high inorganic phosphorus and low phytic acid

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Abstract
Phytic acid, is the major storage form of phosphorus in soybean [*Glycine max* (L.) Merr.] which comprises 75% of total seed phosphorus. It decreases the availability of some essential elements via bonding between the negatively charged phytic acid and the positively charged elements. Thus, diets high in phytate may lead to nutrient deficiencies. So, identification of lines with low phytic acid is of paramount importance. A germplasm survey was conducted among 250 soybean accessions to identify the accessions with low phytic acid. Phytic acid content ranged between 0.84 and 7.07 mg/ g of soy flour. The genotypes with low phytic acid content *viz.*, Williams, Williams 82, CNS – AVRDC line, RKS 52 and NRC 66 may be useful in breeding programs for the development of low phytic acid genotypes with improved nutritional value to overcome nutritional deficiency syndromes and meet the demand of biofortification.

Key words:
Soybean, Germplasm, Phytic acid, Inorganic phosphorus.

Introduction:
Phytic acid is presumed to act as a phosphate storage compound and it accounts for more than 60% of the total seed phosphate in many crops. Soybean is a very important protein source for human and is commonly used in animal feed worldwide. Like other agriculturally important crops, 60-80% of total seed phosphorus is in the form of phytic acid (myo-inositol-1, 2, 3, 4, 5, 6-hexa*kis*phosphate) (Raboy *et al.,* 1984). In cereals and legumes, phytate accumulates during seed development and reaches its higher level at seed maturity. Phytic acid can chelate with important mineral micronutrients. *viz.,* Zn, Fe and Ca rendering them virtually indigestible by humans, non-ruminants livestock and birds (poultry). Also it has a negative effect on nutritional value of protein as it binds at both acidic and basic pH. Hence the study was done to identify the germplasm accession with low phytic acid and high inorganic phosphorus.

Material and methods:
A total of 250 soybean germplasm accessions being maintained at Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University were used for screening low phytic acid lines. Previous researchers had established a significant correlation between seed inorganic phosphorus and seed phytate. Hence the entries were initially screened for High Inorganic Phosphorus (HIP) followed by phytic acid estimation.

Assay for high inorganic phosphorus in seeds: Eight seeds per plant were indirectly phenotyped for low PA content using the high inorganic Phosphorus colorimetric assay as described by Larson *et al.* (2000) with the following minor modifications. Individual seeds were soaked in 0.4 M HCl and incubated overnight for acid digestion. By next day, colorimetric assays were performed using freshly prepared Chen's reagent (Chen *et al.,* 1956). About 10 µl of the supernatant from the digested seeds was mixed with 90 µl water and 100 µl Chen's reagent. Color reactions were observed following incubation of the solution for 15- 30 minutes at room temperature. Appearance of blue color indicated the presence of relatively high levels of Pi, whereas colorless samples indicated the presence of low inorganic phosphorous. The genotypes were screened as per the following standard scores.

Phosphorus Standards for Microtiter Plate HIP assay:

Number	µl 1mM K2 HPO4	µl 0.4M HCl	µl H2O	ng P
1	0	10	90	0
2	5	10	85	155
3	15	10	75	465
4	30	10	60	930
5	45	10	45	1395

Estimation of phytic acid: Phytic acid was estimated following the method described by Davies and Reid (1979). One gram of seed material was ground and extracted with 0.5 M HNO_3 by continuous shaking, filtered and made upto 25 ml with water. To 1.4 ml of the filterate, 1 ml of ferric ammonium sulphate (21.6 mg in 100 ml water) was added, mixed and placed in a boiling water bath for twenty minutes. The contents were cooled and 5 ml of iso amyl alcohol was added and mixed. To these, 0.1 ml of ammonia solution was added, shaken thoroughly and centrifuged at 3000 rpm for 10 minutes. The alcoholic layer was separated and the color intensity was read at 465 nm against amyl alcohol blank after 15 minutes. Sodium phytate standards were run along with the sample. The results were expressed as mg phytic acid/ g dry weight.

Statistical analysis:Analysis of variance was calculated using standard statistical procedures. The mean values of the genotypes for phytic acid content over two replications (Table 1.) were subjected to descriptive statistics analysis using STATISTICA package.

Among the 250 soybean germplasm accessions subjected to High inorganic Phosphorous (HIP) assay, five HIP lines viz., Williams, Williams 82, CNS- AVRDC line, RKS 52 and NRC 66 were identified by the appearance of dark blue colour equal to the standard five (Fig 1). Five improved lines Co1, Co3, JS 335, WC 31 and PK 1223-C- 52 had lowest inorganic phosphorus level equal to that of the first standard. The remaining genotypes fell between the levels of 155 to 930 ng P (standards 2 to 4) and their frequency is represented in Fig 2. Lang *et al* (2007) observed similar results in a HIP assay among rice test entries comprising of mutants, wild types, 101 improved varieties and 600 landraces.

The data recorded on phytic acid content were subjected to descriptive statistics and properties of quantitative traits such as measures of central tendency (mean, median and mode), measures of dispersion or variability (range, variance, standard deviation and standard error) and measures of symmetry (skewness and kurtosis) were analyzed and their respective values are furnished in the Table 2. The phytic acid content ranged from 0.84 mg / g (Williams) to 7.07 mg/ g (Co3) (Fig 3.) thus showing a large variability for this trait. Badigannavar and Manjaya (2012) recorded a variability of 0.16 to 4.74 mg/g among 106 soybean germplasm lines. Phytic acid content in 250 soybean germplasm accessions recorded positive Skewness value of 0.09. This indicates if selection will be made intensively in the segregating generations the gain will be faster and mild selection resulted in slower gain as explained by Snape and Riggs (1975). The frequency distribution of the soybean genotypes for phytic acid content is depicted in the Fig.4.

To understand the extent to which the observed variation was due to genetic factors, the value of genotypic and phenotypic variance, phenotypic and genotypic coefficients of variability, heritability (broad sense) and genetic advance were estimated for phytic acid and shown in the Table 3. The relative values of genotypic and phenotypic coefficient of variation provide important information on the magnitude of variation. The very narrow difference between these two coefficients of variation implies low environmental influence and predominant role of genetic factors on the expression of the character. This was further confirmed by high heritability estimates phytate. Heritability in broad sense includes both fixable (additive) and non fixable (dominant and epistatic) variances. Heritability estimates along with genetic advance would be helpful in predicting gain under selection than heritability estimates alone. In the present study, phytatic acid recorded high heritability (99.90%) as well as high genetic advance over its mean (59.30). This indicates predominance of additive gene effects for the expression of the character. So selection for improvement of the character is likely to be rewarding. These observations corroborate well with those of Arulselvi *et al* (2007) in pearl millet.

In soybean, phytic acid concentration varied considerably across the genotypes. The genetic mechanism behind the phytate level can be elucidated by hybridization between low and high phytate genotypes. The low phytate genotypes Williams (0.84 mg/g), CNS- AVRDC line (1.26 mg/g), RKS 52 (1.33 mg/g), Williams 82 (1.47 mg/g) and NRC 66 (1.50 mg/g), can be used directly for food and feed purpose and may be used in breeding program to reduce the phytic acid content of agronomically superior lines.

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Table 1. Mean performance of the soybean germplasm accessions for phytic acid content

S.No	Entry	Phytic acid (mg/g)	S.No	Entry	Phytic acid (mg/g)	S.No	Entry	Phytic acid (mg/g)
1	AGS 746	3.04	49	GC 92811	3.63	97	Lu 22	4.38
2	AGS 747	3.64	50	HIMSO 1681	5.28	98	Lu 38	4.43
3	AMS 1	2.95	51	IC 16009	4.02	99	Lu 46	3.32
4	AMS 19	2.98	52	IC 18277	5.14	100	Lu 50	3.66
5	AMS 56	3.05	53	IC 2065	3.98	101	Lu 53	3.94
6	AMS 155	2.75	54	IC 24065	3.66	102	Lu 54	2.68
7	AMS 243	3.63	55	IC 25765	2.76	103	Lu 62	3.28
8	AMS-MB-5-18	4.23	56	IC 259-W	4.83	104	Lu 81	5.44
9	AMS-MB-5-19	4.67	57	IC 28714	4.80	105	Lu 86	2.53
10	AMSS 40	2.97	58	IC 34352-8	2.91	106	Lu 91	2.98
11	AMSS 44	4.44	59	IC 34490	2.75	107	Lu 95	5.03
12	AMSS 463	4.59	60	IC 39374	4.66	108	Lu 96	2.96
13	ASB 18	3.58	61	IC 39584	5.04	109	LUDHIANA 1	5.01
14	AVRDC 5	2.88	62	IC 55898	4.13	110	MACS 61	4.49
15	AVRDC 508	4.02	63	IC 97549	3.57	111	MACS 565	2.54
16	AVRDC 576	5.88	64	JS 20-09	4.67	112	MACS 715	5.66
17	BRAGG	2.84	65	JS 20-41	2.81	113	MACS 993	2.69
18	CLARK	2.71	66	JS 76119	3.55	114	MACS 1039	2.78
19	CNS – AVRDC	1.26	67	JS 76-1194	2.99	115	MACS 1126	5.47
20	**Co 1**	**6.77**	68	JS 87-12	3.28	116	MACS 1139	3.32
21	Co 2	4.77	69	JS 89-24	3.46	117	MACS 1140	2.60
22	**Co (SOY)3**	**7.07**	70	JS 90- 21	3.97	118	MACS 1148	5.36
23	CSB 08 04	4.56	71	JS 90-29	4.23	119	MACS 1184	2.46
24	CSB 08 06	4.25	72	JS 92 -22 -4	2.84	120	MACS 1188	4.77
25	CSB 08 08	4.20	73	JS 99-76	3.57	121	MACS 1238	3.67
26	CSB 08 09	2.46	74	JS 148	4.82	122	MACS 1254	3.97
27	CSB 08 10	4.53	75	**JS 335**	**6.59**	123	MACS 1281	3.03
28	CSB 09-08	2.88	76	JS 9868	4.96	124	MACS 1311	2.66
29	DS 2706	3.23	77	JS (SA) 93-44	5.47	125	MACS 1340	3.93
30	DS 2708	2.83	78	KB 8 - A	4.31	126	MACS 1364	4.57
31	DSb 19	2.68	79	KB 16-A	5.56	127	MACS 1366	2.95
32	DSb 20	3.46	80	KB 97	3.68	128	MAUS 2	2.98
33	DSb 21	3.92	81	KB 103	3.99	129	MAUS 61	2.00
34	EC 1024	3.43	82	KB 221	5.26	130	MAUS 61-2	3.66
35	EC 13052	3.05	83	KB 249	5.25	131	MAUS 65	3.84
36	EC 15928	4.04	84	KD 5343	5.18	132	MAUS 68	5.63
37	EC 15929	2.93	85	KDS 167-9	3.86	133	MAUS 71-07	4.30
38	EC 401408	3.57	86	KDS 344	2.84	134	MAUS 81	5.47
39	EC 41273	5.13	87	KDS 378	2.97	135	MAUS 285	5.30
40	EC 41273-A	3.87	88	KDS 699	5.07	136	MAUS 433	5.46
41	EC 4290	3.84	89	KDS 701	3.44	137	MAUS 504	4.45
42	EC 50079	4.36	90	KDS 2010	4.16	138	MAUS 611	2.74
43	EC 50082	4.05	91	KS 1	3.39	139	NRC 29	4.76
44	EC 62376-A	3.76	92	KS 3	3.47	140	NRC 56	4.50
45	EC 7048	3.26	93	KS 87-5	3.27	141	**NRC 66**	**1.50**
46	EC 73-16E	3.46	94	KS 112	3.65	142	NRC 77	3.00
47	EC 7587	2.77	95	KSO184	3.91	143	NRC 85	2.76
48	EC 799	3.96	96	L 324	3.75	144	NRC 89	2.23
145	NRC 90	5.43	181	PLS 6-B	5.27	217	SL 790	4.25
146	NRC 95-06-03	4.67	182	PLS 090	4.46	218	SL 794	3.39
147	NRC 2006-M-6	5.42	183	PS 112	3.15	219	SL 795	3.55
148	NRC 2007-A-23	5.53	184	PS 1374	3.14	220	SL 900	2.94
149	NRC 2007-B-2-2-2	2.58	185	PS 1437	4.98	221	SL 958	4.92
150	NRC 2007 - I- 3	4.00	186	PS 1466	4.76	222	TAS 40	5.49
151	NRC 2007- J-3	4.80	187	PS 1475	3.73	223	TNAU 20049	3.48
152	NRC 2007 -K-7-2	5.60	188	PS 1481	4.25	224	TNAU 20051	5.46
153	NRC 2007-L-1-5	2.68	189	PS 1499	2.83	225	TNAU 20053	3.50
154	NRC 2007-M-1-13	3.54	190	PS 1503	5.33	226	TNAU 20056	2.26
155	NRC 2008	5.18	191	PS 1505	2.80	227	TNAU 555	5.46
156	NRC 2008-B-1-9-1	2.30	192	PS 93108	3.61	228	TS 9	5.26
157	NRC 2008-B-3	2.34	193	PUNJAB -1	4.67	229	TS 40	3.33

Table 1. Contd..

S.No	Entry	Phytic acid (mg/g)	S.No	Entry	Phytic acid (mg/g)	S.No	Entry	Phytic acid (mg/g)
158	NRC 2008 F-1	2.40	194	PUSA 9703	3.71	230	TS 94	5.24
159	OS 612	2.57	195	RAUS 5	4.05	231	UGM 70	5.58
160	PK 25	4.37	196	RAUS 97-1	5.45	232	UGM 73	2.72
161	PK 257	4.94	197	RKS 18	2.61	233	UGM 74	5.07
162	PK258	5.34	198	RKS 48	4.37	234	UGM 75	3.35
163	PK 292	2.41	199	**RKS 52**	**1.33**	235	UGM 77	3.65
164	PK 731	4.34	200	RKS 63	3.35	236	URSM 259	5.49
165	PK 1000	5.56	201	RKS 66	1.75	237	VCB 201	5.07
166	PK 1028	4.24	202	RKS 68	2.12	238	VLS 41	5.03
167	PK 1029	3.01	203	RSC 1	2.79	239	VLS 53	3.65
168	PK 1029-A	3.81	204	RVS 2001-18	5.24	240	VLS 62	5.14
169	PK 1038	3.35	205	SL 46	2.64	241	VLS 69	5.01
170	PK 1125-A	4.86	206	SL 64-A	2.57	242	VLS 70	1.66
171	PK 1146	4.75	207	SL 295	3.07	243	VLS 74	3.18
172	PK 1158	4.39	208	SL 432	4.56	244	VLS 75	3.04
173	PK 1223-A	5.49	209	SL 443	3.27	245	VLS 81	1.84
174	PK 1223-B	3.34	210	SL 518	3.41	246	**WC 31**	**6.43**
175	**PK 1223-C-52**	**6.06**	211	SL 525	4.69	247	WC 37	5.04
176	PK 1224	5.29	212	SL 679	2.35	248	WC 67	2.63
177	PK 1225	5.08	213	SL 682	4.02	249	**Williams**	**0.84**
178	PK 1243	4.49	214	SL 710	3.45	250	**Williams 82**	**1.47**
179	PK 1303	5.42	215	SL 744	2.74			
180	PLS 6	3.54	216	SL 752	4.01			
Grand mean								**3.90**
SE								**0.07**
CD (P=0.05)								**0.06**
CD (P=0.01)								**0.08**

Table 2. Descriptive statistics and Estimate of variance, coefficients of variation, heritability, genetic advance as per cent of mean for phytic acid in 250 soybean genotypes

Parameters studied	Values
Mean	3.90
Median	3.79
Mode	4.67
Maximum	0.84
Minimum	7.07
Range	6.23
SE	0.07
Skewness	0.09
Kurtosis	-0.43
PCV	28.81
GCV	28.80
ECV	0.75
Heritability	99.90
GA as per cent of mean	59.30

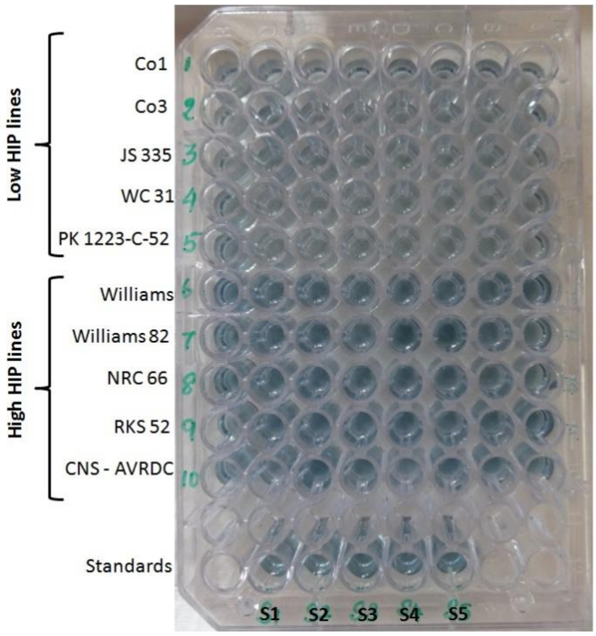

Fig1. High inorganic phosphorus assay with high and low HIP lines

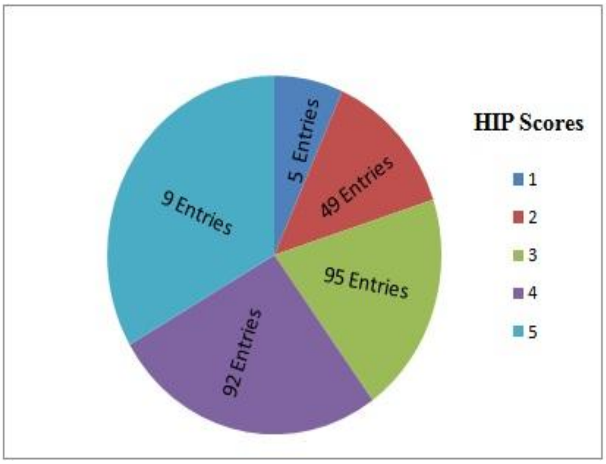

Fig 2. Frequency distribution of 250 soybean germplasm lines for HIP assay

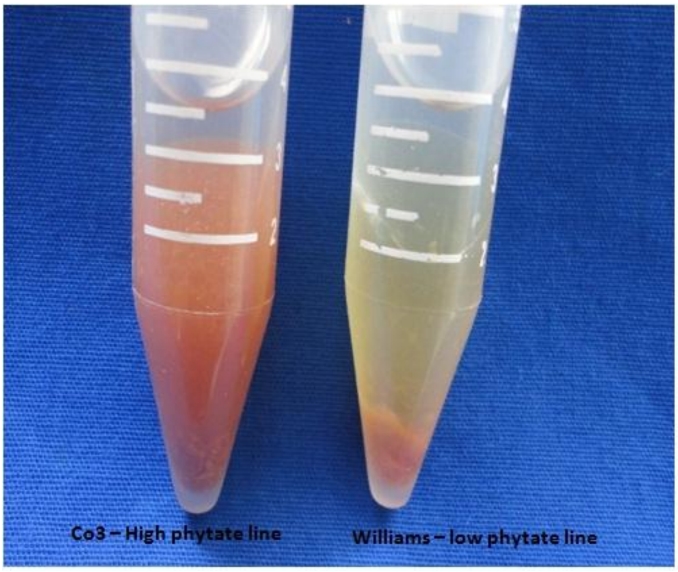

Fig 3. Variation in phytate sedimentation between high and low phytate lines

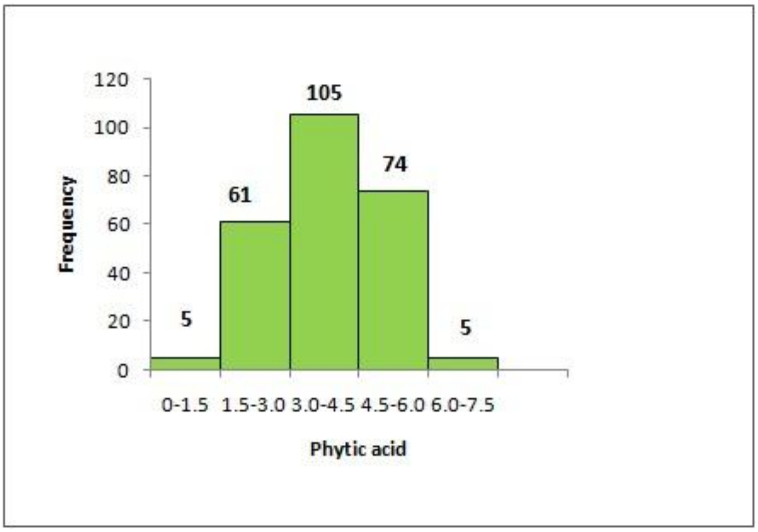

Fig 4. Frequency distribution of 250 soybean germplasm lines for phytic acid