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Phytic acid in Indian soybean: genotypic variability and influence of growing location

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Abstract: Phytic acid, the heat-stable anti-nutritional factor, was determined in 80 cultivars/strains of Indian soybean to identify genotypes that possess low concentrations of phytic acid. Variation of values of 28.6–46.4 g kg^{-1} soy flour was observed. Information on the influence of growing locations with widely differing soil types on phytic acid content being scarce, phytic acid in the mature dry seeds of eight Indian soybean cultivars grown over four locations was evaluated. Variation in different varieties at different locations was 27.8–45.0 g kg^{-1} soy flour. Averaged over eight genotypes, the maximum mean value for phytic acid was observed at Pantnagar and the minimum at Palampur. These differences in locational mean values for phytic acid can be explained on the basis of characteristics of the soils and environment. The higher mean value at Pantnagar may be attributed to higher soil organic phosphorus, nearly neutral pH and favorable temperature from flowering to maturity. However, the lower value observed at Palampur can be explained by the acidic nature of its soil, with lower maximum and minimum temperatures prevailing from flowering to maturity. Locational and genotypic × locational effects were found to be significant ($p < 0.01$). The results indicated that soil characteristics and soil environment play a significant role in the accumulation of phytic acid in soybean seeds.

Keywords: phytic acid; soil; soybean; growing location

INTRODUCTION

Soybean is fast emerging as one of the most economical and nutritious foods in developing countries and recently was also described as the 'functional food of the century', owing to its nutraceutical applications in reducing the risk of cardiovascular diseases, breast cancer, osteoporosis, diabetes and reducing menopausal symptoms.[1] However, soybean does possess a heat-stable anti-nutritional factor, phytic acid. Phytic acid, 1,2,3,4,5,6-inositol hexaphosphoric acid, is the principal source of phosphorus in soybean seeds, and its presence is much higher in soybeans than in other legumes.[2] Phytic acid binds with nutritionally important metals, especially zinc, calcium and magnesium, forming phytic acid–metal complexes that are not absorbed readily in the intestine. Thus, the presence of phytic acid affects mineral bioavailability, contributing to the deficiency of these nutrients.[3–5] Furthermore, the heat-stable nature of phytic acid means it remains active even after cooking. Phytic acid is also capable of forming complexes with negatively charged protein molecules at alkaline pH through calcium and magnesium binding mechanisms and with positively charged protein molecules at pH values below their isoelectric point by charge neutralization. As a consequence of this non-selective binding to proteins, phytate has been shown not only to inhibit the action of a number of enzymes important in digestion,[6] but also to affect the isoelectric point, solubility and functionality of soy proteins.[7]

In tofu, a relatively large amount of coagulants, viz $CaSO_4$ and $MgCl_2$, is required to compensate for the effect of phytic acid on tofu quality.[8] The hard–to-cook phenomenon has also been suggested to be associated with the presence of phytic acid in legumes.[9,10]

A few reports pertaining to the genotypic variability of phytic acid in soybean have appeared.[11–14] However, information on the influence of growing locations with widely differing soil types on accumulation of phytic acid in soybean seeds is scarce. In the present studies, apart from investigating the variability of phytic acid among 80 released cultivars/strains of Indian soybean, the phytic acid content of eight cultivars grown over four locations widely differing in latitude (12–32 °N) and soil type was also evaluated to study the effect of soil environment on phytic acid accumulation in soybean seeds.

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MATERIAL AND METHODS

The field experiment was laid out in a randomized complete-block design with three replications of 80 released cultivars/strains of Indian soybean at the National Research Centre for Soybean, Indore (22 °N) during the cropping season in 2002. Multilocational trials were also conducted with eight elite cultivars, viz Hardee, Hara Soya, JS 335, Kalitur, KhSb2, NRC 37, Pb1 and Shilajeet, laid out in a split plot arrangement of a randomized complete-block design with three replications in the fields of the National Research Centre for Soybean, Indore (22 °N), HP *Krishi Vishva Vidyalaya*, Palampur (32 °N), GB Pantnagar University of Agriculture and Technology, Pantnagar (29 °N) and the University of Agricultural Sciences, Bangalore (12 °N) during the 2002 cropping season. Each plot consisted of two rows which were 5 m long and 45 cm apart. Freshly harvested seeds from these cultivars/strains from all four locations were oven-dried at 60 °C for 5 days until they were moisture-free. The dried seeds were ground to pass through a 0.5 mm sieve and subjected to phytic acid analysis following the standard method.[15] Phytate content was calculated from the iron concentration in ferric chloride by assuming a constant Fe:P molecular ratio of 1:1.5 in the precipitate of the extracts. The values given in Tables 1 and 2 are means of phytic acid determined in triplicate samples.

Surface soil samples (0–15 cm) were also collected from each growing location for subsequent organic phosphorus and pH analysis employing the standard procedure.[16]

Statistical differences among locations were estimated from the ANOVA test using MSTAT-C programme. A pair-wise comparison of means by least significant difference was carried out at $p = 0.01$.

RESULTS AND DISCUSSION

Phytic acid ranged from 28.6 to 46.4 g kg^{-1} soy flour with a mean value of 33.1 g kg^{-1} soy flour among the 80 released soybean varieties and strains evaluated (Table 1). The ratio between the lowest and the highest value for phytic acid observed was 1:1.63, which could be attributed to genotypic differences. The highest value for phytic acid was observed in LSb 1 and the lowest in Bhatt yellow. In comparison with the ranges of 3.9–23.0, 18.8–21.9 and 17.4–32.8 g kg^{-1} soy flour observed in earlier reports,[12–14] our results indicated higher levels than the ranges reported in the literature.

Table 2 shows that phytic acid for different cultivars at different locations ranged from 27.8 to 45.0 g kg^{-1} soy flour. The highest and lowest values for phytic acid were observed in Shilajeet from Pantnagar (29 °N) and Punjab 1 from Palampur (32 °N). All the cultivars showed maximum values for phytic acid at Pantnagar (29 °N). Averaged over four locations, Shilajeet showed the maximum mean value while KhSb2 showed the lowest value. Locational and genotypic × locational effects were found to be highly significant ($p < 0.01$). JS 335, a cultivar with wide adaptability all over India, was found to be the least sensitive as it showed a difference of only 9.6% in phytic acid level between the location showing the highest value and that showing the lowest value. Shilajeet was affected the most as it showed a difference of 48%, followed by Punjab 1, showing a difference of 42.4% between the highest and lowest values over the four location. Averaged over eight genotypes, Pantnagar (29 °N) showed the maximum mean value for phytic acid followed by Indore, Bangalore and Palampur.

Soil analysis data (Table 3) revealed that Palampur (32 °N) and Bangalore (12 °N) soils were acidic in nature (pH 5.5 and 5.6, respectively), while soils at Indore (22 °N) and Pantnagar (29 °N) were alkaline

Table 1. Phytic acid (g kg^{-1} soy flour on dry weight basis) evaluation among released cultivars/strains of India

Genotype	Phytic acid	Genotype	Phytic acid
ADT 1	34.8 ± 1.22	MAUS 1	29.0 ± 1.11
Alankar	33.2 ± 2.34	MAUS 2	32.9 ± 1.31
Ankur	34.4 ± 0.99	MAUS 32	34.6 ± 1.26
Bhatt black	29.3 ± 1.36	MAUS 47	33.3 ± 1.59
Bhatt yellow	28.6 ± 0.78	MAUS 61–2	35.1 ± 1.57
Bhawali bold	34.3 ± 0.88	MAUS 71	28.8 ± 0.88
Bragg	35.2 ± 0.96	Monetta	35.3 ± 0.99
Birsa soya 1	33.5 ± 1.76	NRC 2	32.2 ± 0.88
CO 1	34.2 ± 0.75	NRC 7	34.0 ± 0.99
CO 2	34.4 ± 2.3	NRC 12	30.8 ± 0.53
Hardee	34.3 ± 1.35	NRC 37	33.5 ± 0.63
Hara soya	32.4 ± 1.45	Palamsoya	31.4 ± 1.87
His 1	34.5 ± 1.64	PK 262	33.4 ± 2.0
GS 1	32.8 ± 1.76	PK 308	35.5 ± 1.42
GS 2	34.9 ± 1.55	PK 327	33.0 ± 1.31
Improved pelican	33.4 ± 1.32	PK 416	33.6 ± 1.33
IS 9	31.0 ± 1.39	PK 471	31.8 ± 1.26
JS 2	34.6 ± 0.99	PK472	31.1 ± 1.44
JS 71–05	32.8 ± 0.76	PK 564	33.3 ± 1.31
JS 72–44	32.2 ± 0.89	PK 1029	30.0 ± 1.63
JS 75–46	37.6 ± 1.23	PK 1092	35.0 ± 1.22
JS 72–280	32.2 + 1.65	Pb 1	35.3 ± 1.11
JS 76–205	32.7 ± 1.87	Pusa 16	32.0 ± 1.39
JS 79–81	32.6 ± 2.01	Pusa 20	31.3 ± 1.19
JS 80–21	31.4 ± 1.41	Pusa 22	32.9 ± 2.1
JS 90–41	35.2 ± 1.31	Pusa 24	30.0 ± 2.04
JS 93–05	33.9 ± 1.41	Pusa 37	31.0 ± 1.31
JS 93–06	30.9 ± 1.31	Pusa 40	33.1 ± 1.83
JS 335	34.3 ± 1.67	RAUS 5	33.1 ± 1.99
Kalitur	34.7 ± 1.31	SAMRAT	32.1 ± 1.32
KB-79	33.1 ± 0.79	Shilajeet	33.5 ± 1.36
KhSb2	34.0 ± 1.99	Shivalik	33.1 ± 1.13
Lee	32.6 ± 1.38	SL 96-N	30.9 ± 1.21
LSb1	46.4 ± 1.23	SL 295	35.3 ± 1.45
MACS 13	32.4 ± 1.88	SL 459	30.0 ± 1.32
MACS 124	37.0 ± 0.76	T49	31.8 ± 1.49
MACS 57	37.2 ± 0.86	VLS 1	35.5 ± 1.29
MACS 58	34.2 ± 1.32	VLS 2	34.3 ± 1.31
MACS 330	32.4 ± 1.22	VLS 21	32.7 ± 1.29
MACS 450	32.4 ± 1.11	VLS 47	33.2 ± 0.87

Each value is mean of phytic acid determination in three samples.

Table 2. Effect of growing location on phytic acid (g kg^{-1} soy flour) content in soybean[a]

Cultivar	Palampur (32 °N)	Pantnagar (29 °N)	Indore (22 °N)	Bangalore (12 °N)	Mean
Hardee	31.7efgh	38.8 bc d	34.2 bc defg	35.0 bc defg	34.9a
Hara soya	33.4 defgh	38.6 bc d	32.4efgh	32.1efgh	34.1a
JS 335	33.3defgh	36.5bc de	34.3bc defg	34.7 bc defg	34.7a
Kalitur	31.9efgh	36.00bcdef	34.7 bc defg	33.7c defg	34.1a
KhSb2	31.6efgh	38.4bc d	34.0bc defg	30.2gh	33.6a
NRC 37	33.3efgh	39.3 bc	33.5c defg	35.3 bc defg	35.4a
Pb1	27.8h	39.6a b	35.3 bc defg	35.7 bc defg	34.6a
Shilajeet	30.4d	45.0a	33.5c defg	32.0efgh	35.2a
Mean	31.7c	39.0a	34.0b	33.6b	

[a] Values with same letters are not statistically different, Least significant difference ($p = 0.01$). Genotype, NS. Location, 1.5. Genotype × location, 5.6.

Table 3. Temperature and soil characteristics at different soybean growing locations during the cropping season, 2002

Temperature (°C) and soil characteristics	Palampur (32 °N)	Pantnagar (27 °N)	Indore (22 °N)	Bangalore (12 °N)
Maximum temperature[a]				
I	27.2	31.4	31.5	27.7
II	25.8	30.2	31.7	27.0
Minimum temperature				
I	15.8	21.8	22.7	17.7
II	12.2	18.5	21.6	16.2
Soil characteristics				
Group	Alfisol	Molllisol	Vertisol	Alfisol
pH	5.5	7.3	8.1	5.6
Organic phosphorus (ppm)	135.5	145.2	66.6	95.0

[a] I, flowering to maturity; II, sowing to maturity.

(8.3) and neutral (pH 7.3), respectively. It is also evident that total organic phosphorus was maximum (145.2 ppm) in Pantnagar soils followed by Palampur, Bangalore and Indore soils.

Phytic acid accounts for more than two-thirds of total phosphorus in soybean seed and nearly all the phosphorus translocated to developing soybean seeds is incorporated into phytic acid from third week of flowering to maturity.[17] Thus accumulation of phytic acid depends upon factors that affect the uptake of phosphorus, viz the differential phosphorus status of the soils,[18,19] soil pH and temperature; the latter two affect the activity of the phosphate-mineralizing microorganisms in the soils.[20] The activity of phosphate mineralizing bacteria is maximum at neutral pH and at higher soil temperature.[21,22] The higher mean value for phytic acid observed in the soybean seeds at Pantnagar (Table 2) can be explained by soil characteristics and temperature. Pantnagar soils, which are mollisols, were observed to possess a higher content of organic phosphorus and nearly neutral pH (Table 3), the former indicating the level of mobile phosphorus and the latter a congenial soil reaction for phosphate-mineralizing bacteria. Since mean maximum air temperature is positively correlated with temperature of surface soil layers,[22] the higher ambient temperature from flowering to maturity in Pantnagar (Table 3) might have led to higher soil temperature as well, a congenial condition for biological activity required for mineralization of phosphorus. In contrast, the soils of Palampur (32 °N), which are alfisollic in nature (Table 3), showed the lowest phytic acid (Table 2), and did not possess congenial conditions for accumulation of phytic acid in soybean seed grown on them. These soils are acidic in reaction, with pH value of 5.5 (Table 3) and not only possess limited biological activity, but the presence of oxides of iron and aluminum in these soils also restricts the mobilization of phosphorus for uptake by plants.[23] Moreover, lower levels of maximum and minimum atmospheric temperatures prevailing from flowering to maturity at Palampur (32 °N) than other locations (Table 3) might also have led to lower soil temperature, leading to poor biological activity and resulting in decreased mineralization of organic phosphorus and hence poor uptake of phosphorus by the plants. Variation in the sensitivity of the cultivars to the effects of growing conditions may be attributed to genotypic variation in phosphorus uptake by crop plants as a consequence of changes in root surface area, rhizosphere acidification and rhizodeposition pattern, as reported earlier.[24,25]

The results obtained indicate that the accumulation of phytic acid, a heat-stable antinutritional factor found in soybeans, is not only affected by the genetic make-up of the genotype, but also depends upon soil characteristics and the soil environment. Hence, soil characteristics should be considered while breeding for low phytic acid.

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