Genetic variation for phytic acid content in mungbean (Vigna radiata L. Wilczek)

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Abstract

Mungbean (Vigna radiata L. Wilczek) is a short-duration legume crop cultivated for seeds that are rich in protein and carbohydrates. Mungbeans contain phytic acid (PA), an anti-nutritional factor that is the main storage form of organic phosphorus in seeds. It is a strong inhibitor against the absorption of nutrients including iron, zinc, calcium and magnesium in monogastric animals. Genotypes with low phytic acid (lpa) in seed may show increased assimilation of nutrients and be useful in breeding lpa cultivars. The present study was conducted to identify lpa sources, genetic variation, heritability, and association with seed coat color, inorganic phosphorus (IP), and seed size in 102 mungbean genotypes including released varieties, land races, mutants, and wild species grown in two seasons: summer 2011 and rabi 2012. PA and IP in dry seeds were estimated by modified colorimetric method and Chen’s modified method, respectively. PA, IP, and 100-seed weight differed significantly in the two seasons. PA content in 102 genotypes ranged from 5.74 to 18.98 mg g⁻¹ and 5.85 to 20.02 mg g⁻¹ in summer 2011 and rabi 2012, respectively. High heritability was found for PA (0.87 and 0.86) and seed size (0.82 and 0.83) but low heritability for IP (0.61 and 0.60). A negative correlation was found between PA and seed size (r = -0.183 and -0.267). Yellow and green seed coat genotypes contained significantly less PA than black seed coat genotypes. Cluster analysis revealed the distinctness of wild species, land races and cultivated varieties on the basis of PA content. The genotypes YBSM (6.001 mg g⁻¹) and JL-781 (6.179 mg g⁻¹) showed lowest PA. These lpa sources can be used to develop high-yielding mungbean cultivars with low phytic acid.

Keywords: Mungbean, Phytic acid, Inorganic phosphorus, Cluster analysis

1. Introduction

Mungbean (Vigna radiata L. Wilczek), an important short-duration grain legume crop, is cultivated for its dry seeds, which are a rich source of easily digestible protein, carbohydrates, vitamin C, folic acid, thiamin, iron, zinc, potassium, magnesium, copper, manganese, and phosphorus [1-3]. However, mungbean also contains phytic acid (PA, myo-inositol hexakisphosphate), an anti-nutritional factor that is the main storage form of organic phosphorus (P). As an effective chelator of positively charged cations, PA binds to nutritionally important mineral cations such as calcium, iron, and zinc. Phytate also inhibits trypsin [4]. Humans as well as other non-ruminants such as poultry, swine and fish lacking the enzyme phytase are unable to digest PA and excrete a large fraction of these salts. This phenomenon can contribute to human mineral deficiency, particularly with respect...
to iron and zinc, and also causes eutrophication of waterways [5-7]. Vegetarian populations in developing countries such as India are at greatest risk of mineral deficiencies caused by dietary PA, particularly children and child-bearing women in rural communities that depend on cereals, and legumes as staple foods [8]. Recently there has been increasing interest in the development of crops with low phytic acid (lpa) content to enhance the bioavailability of minerals and other nutrients. Earlier breeding efforts have identified several lpa mutants, resulting in reduction of seed phytic acid phosphorus (PAP) by 50 to >95% in crops such as barley, wheat, maize, soybean, and common bean [9-13]. But no lpa mutant was identified in mungbean. PAP typically represents from 65% to 85% of seed total P [14]. Mungbean seeds contain 6.17–9.90 mg g⁻¹ of PA [3,15]. PAP and inorganic phosphorus (IP) contents in mungbean seeds ranged between 1.77 and 5.79 mg g⁻¹ and 0.25 to 0.73 mg g⁻¹ respectively [16]. PA content was reported to be lower in yellow than in green seed coat mungbean cultivars [15]. If a source is available, genes for lpa can be transferred into improved high-yielding varieties, as the heritability of PA is high (0.80) [16]. For the improvement of any trait, genetic variation is a prerequisite. Very few reports on the PA content in mungbean germplasm are available. Since mungbean is native to India, natural genetic variation is much higher in Indian germplasm than the rest of the world collection hence need to be evaluated for identification of lpa sources. Once a source is available, genes for lpa can be transferred to high-yielding varieties. For effective transfer of genes, information on heritability as well as on the correlation of PA with important traits such as IP and 100-seed weight is needed to avoid negative correlated response to selection. Differences in PA content among yellow, green and black seed coat mungbean genotypes should be evaluated to identify possible associations of PA with seed coat color.

The present study was conducted to identify genetic variation for phytic acid in 102 mungbean genotypes and its correlation with seed coat color, IP, and 100-seed weight.

2. Material and methods

2.1. Plant materials and field experiments

Material consisted of 102 diverse mungbean germplasm lines, including released varieties, mutants, newly developed genotypes, land races, and wild species (Table S1 of supplementary information). These lines were selected on the basis of their origin; morphological traits including plant type, seed size, and seed coat color; resistant/susceptible reaction to major diseases and pests; and molecular diversity studies performed earlier using SSR and ISSR markers [17-19]. These genotypes were grown in a randomized complete block design with two replications at the experimental field facility section, Bhabha Atomic Research Centre, Mumbai during summer 2011 and rabi 2012. Single plants were harvested at maturity and seeds were dried in an oven for 72 h at 50 °C. The 100-seed weight was recorded in grams (g). Seeds from the two seasons were used to estimate PA and IP contents. Samples of 20 to 30 randomly selected seeds from each genotype and replication were ground to fine powder and sieved through 40 mesh to remove seed coat particles.

2.2. Measurement of PAP and PA

In a 2 mL microcentrifuge tube, 50 mg of the fine powder was thoroughly mixed with 1 mL of 2.4% HCl. The tubes were shaken overnight in a Lab-Line Incubator Shaker (Lab-Line Instruments Inc., Melrose Park, IL, USA) and centrifuged at 8,000 rpm in a tabletop centrifuge (Eppendorf, Hamburg, Germany) at 25 °C for 20 min. Crude acid extracts were transferred to 1.5 mL microcentrifuge tubes containing 100 mg NaCl. The contents were vortexed to dissolve the salt and incubated at -20 °C for 20 min to precipitate remaining matrix components that could interfere with the colorimetric reaction. The mixtures were then centrifuged at 10,000 rpm for 20 min at 25 °C to yield clear supernatant. The supernatant was diluted 25 times by addition of deionized distilled water and 750 μL of the diluted supernatant was mixed with 250 μL of modified Wade reagent (0.03% FeCl₃ and 0.3% sulfosalicylic acid). The supernatant was then collected for the determination of PAP using the colorimetric method [20,21]. A series of calibration standards containing 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.5, 10.0, and 12.0 mg PAP mL⁻¹ were also prepared from the sodium salt of phytic acid (Sigma, St Louis, MO, USA) and treated in the same way as described above. The phosphorus content of sodium phytate was 18.38%. The absorbance of color reaction products for both samples and standards was measured at 500 nm on a UV/V spectrophotometer (Jasco, Cambridge, UK). The pink color of the Wade reagent is due to the reaction between ferric ion and sulfosalicylic acid with absorbance maxima at 500 nm. PA content was calculated as PA = 3.552 PAP [16].

2.3. Measurement of IP

In a 2 mL microcentrifuge tube, 400 μL of 12.5% trichloroacetic acid with 25 mmol L⁻¹ MgCl₂ was added to 50 mg of the powdered seed sample and vortexed. The suspension was shaken overnight at room temperature (25 °C) for complete extraction, and then centrifuged at 10,000 rpm for 20 min. The supernatant was diluted with deionized distilled water (1:2). Then, 100 μL of the diluted supernatant was mixed with 900 μL of Chen’s reagent (6 mol L⁻¹ H₂SO₄, 2.5% ammonium molybdate, 10% ascorbic acid, and water, in 1:1:1.2 proportions) and incubated in a water bath at 50 °C for 1 h. A series of standards containing 0.15, 0.31, 0.46, 0.62, 0.77, 0.93, 1.08, 1.24, 1.39, and 1.55 mg IP mL⁻¹ of sodium dihydrogen phosphate were prepared and processed in the same way as described above. The absorbance of color reaction products for both samples and standards was measured at 660 nm. Total IP was estimated by Chen’s modified method [22].

2.4. Data analysis

The data were subjected to analysis of variance for each year and combined over both years, where replication and years were fitted as random effects and genotypes as fixed effects. Analysis of variance and means separation were based on PROC GLM of SAS 9.3.1 (SAS Institute Inc., Cary, NC). Pearson’s correlation coefficients and cluster analysis were estimated with SAS 9.3.1 separately for each season. The mixed model was used to identify significant differences between seasons among the three seed coat
color genotypes for PA, IP, and 100-seed weight. Genotypic and phenotypic variances were estimated using means of two replications of PA, IP, and 100-seed weight. Broad-sense heritability ($h^2_{bs}$) was estimated for PA, IP, and 100-seed weight according to the standard method [23].

3. Results

3.1. Variation between genotypes and seasons

The 102 mungbean genotypes were evaluated in two seasons with different environmental conditions. Genotype × season interaction was nonsignificant for PA and 100-seed weight but significant for IP. PA, IP and 100-seed weight differed significantly between the two seasons (Table 1). The PA accumulation in seed was greater in rabi 2012 (8.26 mg g$^{-1}$) than in summer 2011 (8.01 mg g$^{-1}$). In contrast, IP (0.99 mg g$^{-1}$) and 100-seed weight (5.18 g) were higher in summer 2011 than IP (0.92 mg g$^{-1}$) and 100-seed weight (4.78 g) in rabi 2012. This observation indicated the influence of environment on PA, IP, and 100-seed weight. Among the 102 genotypes, 42 and 31 genotypes showed less than 7.5 mg g$^{-1}$, 28 and 39 genotypes showed between 7.5 and 8.5 mg g$^{-1}$, and 32 genotypes showed more than 8.5 mg g$^{-1}$ of PA in summer 2011 and rabi 2012, respectively (Table 3). Seasonwise analysis of variance indicated that considerable genetic variation was present between genotypes for all three traits (Table 2). The PA content varied significantly among genotypes and ranged from 5.74 mg g$^{-1}$ in VC-6379 (58-97) to 18.98 mg g$^{-1}$ in Thokalwadi wild in summer 2011, but 5.85 mg g$^{-1}$ in YBSM to 20.02 mg g$^{-1}$ in Thokalwadi wild in rabi 2012. The lowest IP content was found in VC-6379 (58-97) (0.99 mg g$^{-1}$) in summer 2011 and the yellow seed coat genotype YBSM (0.89 mg g$^{-1}$) in rabi 2012. The mean PA of black seed coat genotypes was highest, owing to one genotype, Thokalwadi wild (18.98 and 20.02 mg g$^{-1}$) which showed almost two times higher PA than the rest of the genotypes. The black seed coat color genotype Alibag local had 6.83 and 7.66 mg g$^{-1}$ of PA in summer 2011 and rabi 2012 respectively, similar to the means of yellow and green seed coat genotypes.

3.2. Heritability, and correlations among PA, IP, and 100-seed weight

Broad-sense heritability was high for PA (0.87 and 0.86) and 100-seed weight (0.82 and 0.83) but low for IP (0.61 and 0.60) in both summers 2011 and rabi 2012, respectively (Table 3). Significant negative correlations were observed between PA and 100-seed weight ($r = -0.183$ and $-0.267$) in both summer 2011 and rabi 2012 (Table 4). Some of the largest seed size genotypes (100-seed weight greater than 6.5 g) showed lowest PA in both summer 2011 and rabi 2012: JL-781 (5.886 and 6.472 mg g$^{-1}$), VC-6379 (58-97) (5.740 and 6.289 mg g$^{-1}$), VC-6357 (44-55-2) (5.997 and 6.780 mg g$^{-1}$), and SML-668 (6.602 and 7.440 mg g$^{-1}$). These genotypes can be used as donors for lpa genes in mungbean.

3.3. Variation between yellow, green, and black seed coat genotypes

Among 102 germplasm lines, 4, 93, and 5 genotypes had yellow, green, and black seed coats, respectively (Table 5). Lowest mean PA (7.648 mg g$^{-1}$) was found in yellow seed coat genotypes, followed by green (8.016 mg g$^{-1}$) and black (10.715 mg g$^{-1}$) over the seasons (summer 2011 and rabi 2012). The yellow and green seed coat genotypes showed significantly less PA than the black seed coat genotypes. However, differences in PA content were not significant between yellow and green seed coat genotypes. The lowest PA contents were shown by the green seed coat genotype VC-6379 (58-97) (5.74 mg g$^{-1}$) in summer 2011 and the yellow seed coat genotype YBSM (5.85 mg g$^{-1}$) in rabi 2012. The mean PA of black seed coat genotypes was highest, owing to one genotype, Thokalwadi wild (18.98 and 20.02 mg g$^{-1}$) which showed almost two times higher PA than the rest of the genotypes. The black seed coat color genotype Alibag local had 6.83 and 7.66 mg g$^{-1}$ of PA in summer 2011 and rabi 2012 respectively, similar to the means of yellow and green seed coat genotypes.

3.4. Cluster analysis

On the basis of two seasons of data for PA, IP, and 100-seed weight, 102 mungbean genotypes were grouped into two major clusters. Cluster I contained 101 genotypes and cluster II only one (Fig. S1 of supplementary information). The first major cluster showed four subclusters containing cultivated varieties belonging to V. radiata var. radiata. Thokalwadi wild, which belongs to V. radiata var. sublobata, fell into the second cluster. Genotypes containing the highest PA were grouped in subclusters II (9.994 mg g$^{-1}$) and IV (8.215 mg g$^{-1}$), whereas subclusters I (7.534 mg g$^{-1}$) and III (7.214 mg g$^{-1}$) contained genotypes with low PA (Table 6). The mean IP was highest in subcluster IV (0.984 mg g$^{-1}$) followed by subclusters II (0.981 mg g$^{-1}$), I (0.935 mg g$^{-1}$), and III (0.892 mg g$^{-1}$). Genotypes with large seed size were grouped in subcluster I (6.27 g per 100 seeds), those with medium size in subcluster III (4.56 g per 100 seeds), and those with small size in subcluster II (4.11 g per 100 seed).

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Table 1 – Mean sums of squares for PA, IP, and 100-seed weight over two seasons.

<table>
<thead>
<tr>
<th>df</th>
<th>MS (PA)</th>
<th>F-Value</th>
<th>MS (IP)</th>
<th>F-Value</th>
<th>MS (100-seed weight)</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1</td>
<td>9.76</td>
<td>33.09 **</td>
<td>1.75</td>
<td>107.43 **</td>
<td>7.81</td>
</tr>
<tr>
<td>Genotype</td>
<td>101</td>
<td>9.74</td>
<td>33.04 **</td>
<td>0.07</td>
<td>4.48 **</td>
<td>5.17</td>
</tr>
<tr>
<td>Genotype × year</td>
<td>101</td>
<td>6.03</td>
<td>2.27</td>
<td>0.86</td>
<td>27.79 **</td>
<td>3.75</td>
</tr>
<tr>
<td>Error</td>
<td>101</td>
<td>0.29</td>
<td>–</td>
<td>0.02</td>
<td>–</td>
<td>0.18</td>
</tr>
</tbody>
</table>

** Significant at the 0.01 probability level.
On the basis of two seasons of data for PA, IP, and 100-seed weight, 102 mungbean genotypes were grouped into two major clusters. Cluster I contained 101 genotypes and cluster II only one (Fig. S1 of supplementary information). The first major cluster showed four subclusters containing cultivated varieties belonging to V. radiata var. radiata. Thokulwadi wild, which belongs to V. radiata var. sublobata, fell into the second cluster. Genotypes containing the highest PA were grouped in subclusters II (9.994 mg g$^{-1}$) and IV (8.215 mg g$^{-1}$), whereas subclusters I (7.534 mg g$^{-1}$) and III (7.214 mg g$^{-1}$) contained genotypes with low PA (Table 6). The mean IP was highest in subcluster IV (0.984 mg g$^{-1}$) followed by subclusters II (0.981 mg g$^{-1}$), I (0.935 mg g$^{-1}$), and III (0.892 mg g$^{-1}$). Genotypes with large seed size were grouped in subcluster I (6.27 g per 100 seeds), those with medium size in subcluster III (4.56 g per 100 seeds), and those with small size in subcluster II (4.11 g per 100 seed) and subcluster IV (4.26 g per 100 seed).

4. Discussion

The present study identified significant differences due to environment in PA, IP, and 100-seed weight. Temperature and humidity were high in the summer but low in the rabi season. In the summer season, plant growth was fast and mungbean matured early with good seed development, whereas in the rabi season, plant growth was slow with late maturity and seed was not developed to its maximum potential. The seasonal difference was also reflected in accumulation of PA and IP in seeds. PA content was greater in the rabi than in the summer season. The PA content reported in the present study was comparable to those in earlier reports, whereas the IP content was slightly higher [3,15,16]. The main objective of the present study, to identify lpa sources, was achieved with the identification of two lpa genotypes: YBSM and JL-781. These genotypes showed almost 25% lower PA than the mean PA of the 102 genotypes over the two seasons, 40% lower PA than the mean of subcluster II with the highest mean PA, which contained the cultivated mungbean genotypes, and 70% lower than Thokulwadi wild, which contained the highest PA.

The high heritability found in the present study was also reported earlier for PA and 100-seed weight, but heritability for IP content was slightly lower than those reported earlier [16,24]. This slight difference may be due to the type of population used in the present study (germplasm or pure lines) in contrast to the F2 population used in the previous study [16]. The high heritability values also suggested that there is low environmental effect on PA content and that the trait is controlled either by major genes or by polygenes with additive gene action. PA content in mungbean has been reported to be controlled by dominant genes with duplicate recessive epistasis, and three quantitative trait loci were identified using molecular markers [16,25]. High heritability with stable values across environments (summer 2011 and rabi 2012) for PA and 100-seed weight further indicated that selection in the desired direction can be made efficiently. No significant correlation of IP with PA and 100-seed weight was observed in either season, whereas a significant negative correlation was observed between PA and 100-seed weight.

Table 2 – Mean sums of squares for PA, IP, and 100-seed weight in summer 2011 and rabi 2012.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PA</td>
<td>IP</td>
<td>100-seed weight</td>
<td></td>
<td>PA</td>
<td>IP</td>
<td>100-seed weight</td>
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<tr>
<td>Replication</td>
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<td>MS</td>
<td>F-Value</td>
<td>MS</td>
<td>F-Value</td>
<td>MS</td>
<td>F-Value</td>
<td>MS</td>
<td>F-Value</td>
</tr>
<tr>
<td>Genotypes</td>
<td>101</td>
<td>5.1415</td>
<td>0.0004</td>
<td>5.530</td>
<td>0.0036</td>
<td>4.03</td>
<td>0.040</td>
<td>0.317</td>
<td>0.737</td>
</tr>
<tr>
<td>Error</td>
<td>101</td>
<td>0.377</td>
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<td>0.113</td>
<td></td>
<td>0.28</td>
<td></td>
<td>0.0089</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at the 0.05 probability level.

Table 3 – Estimates of genetic components of variance and broad-sense heritability ($h^2_{BS}$) for PA, IP, and 100-seed weight in summer 2011 and rabi 2012.

<table>
<thead>
<tr>
<th>Genetic parameter</th>
<th>PA (mg g$^{-1}$ of seed)</th>
<th>IP (mg g$^{-1}$ of seed)</th>
<th>100-seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>8.01</td>
<td>8.26</td>
<td>0.99</td>
</tr>
<tr>
<td>Range</td>
<td>5.74–18.98</td>
<td>5.85–20.02</td>
<td>0.69–1.39</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.46</td>
<td>7.63</td>
<td>9.52</td>
</tr>
<tr>
<td>$V_g$</td>
<td>2.39</td>
<td>2.57</td>
<td>0.01</td>
</tr>
<tr>
<td>$V_p$</td>
<td>2.75</td>
<td>2.96</td>
<td>0.02</td>
</tr>
<tr>
<td>$h^2_{BS}$</td>
<td>0.87</td>
<td>0.86</td>
<td>0.61</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; $V_g$, genotypic variance; $V_p$, phenotypic variance; GCV, genotypic coefficient of variation; and $h^2_{BS}$, broad-sense heritability.
The possibility of negative correlation between seed size and PA will correspond to the proportion of seed coat in the sample. Smaller seeds have a lower proportion of seed coat. In earlier reports of mungbean, no correlation was found between PA and 100-seed weight [24, 25]. This finding was probably due to the size and type (F2) of the population used in the previous studies, which may have shown lower variation for seed size. In the present study, 102 diverse genotypes were used in which 100 seed weight ranged from 2.38 to 8.16 g and 2.27 to 7.47 g in summer 2011 and rabi 2012, respectively. The large sample size along with variation for seed size yielded a reliable estimate of negative correlation between PA and 100-seed weight. We infer that simultaneous improvement is possible for large seed size and lpa. At the same time, we can improve IP content without affecting PA content, given that no correlation was found between them.

Seed coat color is one of the important parameters for consumer preference. Green seed coat mungbean cultivars are most preferred, while black seed coat cultivars are least preferred by consumers. Three seed coat colors, yellow, green, and black, were found among the 102 genotypes in the present study. The range of PA within each of the three seed coat color genotypes suggested that PA content is not always associated with seed coat color, although a lower mean PA content was observed in yellow than in black and green seed coat genotypes in both seasons. Lower PA content in yellow seed coat color mungbean has been reported earlier [15]. Mean IP content was highest in green followed by yellow and black seed coat genotypes, with no significant differences. During domestication of mungbean, yellow and green seed coat color were preferred for consumption and cultivation over black, which was assumed to be associated with some antinutritional factors [15].

The cluster analysis revealed genetic variation present in the mungbean genotypes used in the present study. The two major clusters separated cultivated and wild genotypes. The wild species Thokalwadi wild is separated from the cultivated mungbean genotypes mainly by its PA content, which was almost twice the mean PA. The subclusters of the major cluster I differed from one another for PA, IP and seed size. We infer that simultaneous improvement is possible for large seed size and lpa. At the same time, we can improve IP content without affecting PA content, given that no correlation was found between them.

5. Conclusions

In mungbean, considerable genetic variation and high heritability were observed for PA content. Negative correlation was found between seed size and PA, indicating that simultaneous improvement in different directions is possible for these traits. No significant differences in PA content were observed between different clusters. The cluster analysis revealed genetic variation present in the mungbean genotypes used in the present study. The two major clusters separated cultivated and wild genotypes. The wild species Thokalwadi wild is separated from the cultivated mungbean genotypes mainly by its PA content, which was almost twice the mean PA. The subclusters of the major cluster I differed from one another for PA, IP and seed size. Low PA with high 100-seed weight in one subcluster and high PA with low 100-seed weight in another subcluster are the result of negative correlation between these traits. This information is useful for mungbean breeders to select lpa genotypes for hybridization with high yielding genotypes from different clusters so as to develop lpa mungbean cultivars with broad genetic bases. The lpa genotype YBSM and JL-781 identified in the present study can be used as a source for lpa genes.
between yellow and green seed coat genotypes, but black contained significantly greater PA than yellow and green seed coat genotypes. The sources for lpa genes identified in the present study can be utilized for breeding high-yielding lpa mungbean cultivars with broad genetic bases by use of cluster analysis information. Consumption of mungbean grains with lpa will increase the assimilation of nutrients in monogastric animals.

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Supplementary material

Supplementary material to this article can be found online at http://dx.doi.org/10.1016/j.cj.2014.12.002.

REFERENCES


