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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.

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 enable to digest PA and excrete a large fraction of these salts. This phenomenon can contribute to human mineral deficiency, particularly with respect

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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 10,000 r min^{-1} 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 r min^{-1} 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% $FeCl_3$, 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} $MgCl_2$ 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 r min^{-1} 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} H_2SO_4, 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 were tested for significance using 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