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Genetic variation for seed phosphorus and yield traits in Indian sorghum landraces and varieties

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ABSTRACT

Phytic acid is the major storage form of phosphorus in cereals. It binds with nutritionally important metals and affects mineral bioavailability. The present study analyzed phytic acid, inorganic phosphorus (IP) content, seed weight, and grain yield in 98 sorghum landraces and varieties grown in two environments to evaluate genotypic and environmental effects and to determine trait stability. Genotypic effects and genotype × interaction were significant for phytic acid concentration and yield components. A promising landrace, Malkhed-1, had the lowest phytic acid (0.015 mg g^{-1}) concentration, with a higher yield (70.02 g $plant^{-1}$), than the check variety M-35-1 in both environments. Similarly, among the varieties, Phule Maulee showed the lowest phytic acid (0.07 mg g^{-1}) and a higher grain yield of 53.15 g $plant^{-1}$ in both environments. Phytic acid and IP were negatively correlated ($r = -0.34$), whereas grain yield and seed weight were positively correlated ($r = 0.20$). Cluster analysis based on seed phosphorus traits and yield components identified five and six clusters, respectively. Genotypes containing low phytic acid with high yield identified in this study would be helpful for increasing the bioavailability of mineral nutrients.

1. Introduction

Sorghum is an important cereal grown mainly for food, feed, dietary fiber, and biofuel in subtropical and tropical Asia and Africa. In India it is cultivated on 7.89 Mha, of which 4.88 Mha is cultivated during the post-rainy season with a production of 4.18 Mt [1]. Cultivation of sorghum is concentrated mainly in peninsular and central India as a post-rainy season crop contributing 50% of total cereal intake. Sorghum is nutritionally superior to rice, as it supplies minerals, vitamins, protein, and micronutrients essential for health, growth, and development [2]. The presence of antinutritive factors, such as trypsin and amylase inhibitors and phytic acid, is known to interfere with protein, carbohydrate, and mineral metabolism. To improve the nutritional quality of sorghum and effectively exploit its potential as a food and feed crop, efforts should be made to reduce these antinutritive factors.

Phytic acid (myo-inositol hexaphosphoric acid, IP6) is the major phosphorus storage compound of most seeds and cereal grains, accounting for more than 70% of total

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phosphorus. Phytic acid (PAP) has a strong ability to chelate multivalent metal ions, especially zinc, calcium and iron. This binding can result in very insoluble salts with poor bioavailability of minerals [3]. Phytic acid is hydrolyzed enzymatically by phytases or chemically to lower inositol phosphates during storage, fermentation, germination, food processing, and digestion in the human gut [4]. The effects of phytic acid in human and animal nutrition are associated with the interaction of phytic acid with proteins, vitamins and minerals, thereby restricting their bioavailability [5]. Several methods have been employed to improve the nutritional quality of sorghum. Some of these methods, such as germination or sprouting, fermentation, soaking, dehulling, and cooking can drastically reduce the phytic acid content [6]. Low-phytic acid (*lpa*) mutants have been reported in soybean, maize, barley and rice [7].

Indian sorghum landraces possess moderate to high genetic variability, but their utilization in breeding programs for improving yield and seed quality has not been realized [8]. Assessment of genetic variability has accordingly become an essential component of identifying potential parents for recombination breeding. Quantitative traits such as phytic acid, inorganic phosphorus (IP), seed weight, and grain yield tend to differ from one environment to another. The interaction between genotype and environment has an important influence on the breeding behavior of the genotype. There is a need for extensive testing of these genotypes in varied agroclimatic conditions, for reducing the environmental influence. In this context, the present study aimed to estimate genetic variability for phytic acid, inorganic phosphorus, seed weight, and grain yield among sorghum landraces and popular varieties over two locations.

2. Materials and methods

The material used in this study comprised 83 sorghum landraces and 15 varieties including the popular check variety M-35-1, adapted to the post-rainy season in Karnataka, Maharashtra, and Andhra Pradesh states of India (Table S1). These genotypes were grown in two replications in a randomized complete block design at the Experimental and Gamma Field Facility, Bhabha Atomic Research Centre (E1), Trombay, Mumbai (19°03′ N; 72°93′ E) during the post-rainy season, 2013 and the Agricultural Research Station (E2), Gulbarga (17°36′ N; 76°81′ E), Karnataka state during the 2012 crop season. All agronomic practices were followed to produce an optimally healthy crop. Four quantitative characters: phytic acid, IP, grain yield per plant (g) and 1000-seed weight (g) were recorded for five randomly selected plants in both replications and locations. Plant yield was measured as the weight of the seed threshed from individual panicles. One thousand seeds were counted and weighed for each accession and recorded as the seed weight. For seed phosphorus estimation, selfed seeds from each genotype in each location were used.

2.1. Determination of phytic acid (PAP)

Phytic acid in sorghum was estimated by a modified colorimetric method [9]. A sample of 30–40 mg of ground seed was prepared from selfed seeds in each location in two replications. Ground samples (30 mg) were placed in an Eppendorf tube and 1 mL of 0.2 mol L^{-1} HCl extraction buffer was added and left overnight. Crude acid extracts were transferred to fresh tubes containing 20 mg NaCl. The contents were shaken at 3500 r min^{-1} for 20 min to dissolve the salt and allowed to settle at −20 °C for 20 min. The mixtures were centrifuged (8000 r min^{-1}) at 10 °C for 20 min and the clear supernatant was diluted 25 times by mixing with distilled water. Of this diluted sample, 750 μL was combined with 250 μL of modified Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid) in an Eppendorf tube, thoroughly mixed by vortexing, and centrifuged at 8000 r min^{-1} at 10 °C for 10 min. A series of calibration standards containing 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7.5, 10, and 12 μg mL^{-1} of PAP were prepared from sodium phytate (Sigma, St. Louis, MO). The pink color of the Wade reagent is produced by the reaction between ferric ion and sulfosalicylic acid, with an absorbance maximum at 500 nm measured with a UV spectrophotometer (Thermo Electron Corporation). In the presence of phytate, iron is bound to the phosphate ester and is unavailable to react with sulfosalicylic acid, resulting in differential pink color intensity. The delta absorbance values were used to estimate phytic acid content and expressed in mg g^{-1} of the flour sample [10].

2.2. Determination of IP

Inorganic phosphorus (IP) was estimated colorimetrically using 30–50 mg of ground sample in two replications for each location separately. Ground samples were placed in an Eppendorf tube and incubated in extraction buffer [12.5% (v/v) trichloroacetic acid and 25 mmol L^{-1} $MgCl_2$] [11]. These samples were centrifuged at 10,000 r min^{-1} and the supernatant was diluted in a 1:2 ratio with distilled water. A 100-μL aliquot of the diluted sample was mixed with Chen's reagent [prepared by mixing 6 N H_2SO_4:2.5% ammonium molybdate:10% ascorbic acid:distilled water in a 1:1:1:2 (v/v/v/v) ratio] and incubated in a water bath at 50 °C for 1 h. After incubation, samples were cooled and absorbance was measured at 660 nm in a UV–vis spectrophotometer. A standard curve was plotted with the absorbance of known solutions of disodium hydrogen phosphate. Based on the calibration curve of the standard IP, the OD values of samples were converted to concentrations of IP and expressed in mg g^{-1} of sorghum flour.

2.3. Statistical analysis

Analysis of variance for PAP and IP concentrations, 1000-seed weight and grain yield per plant among the genotypes tested were computed with the SAS [12] procedure PROC GLM. Replication and locations were fitted as random effects and the fixed effects of genotypes were tested for significance. Summary statistics, genotypic and phenotypic coefficients of variation [13], heritabilities [14] and correlation coefficients were calculated for each of the traits. Cluster analysis was performed to evaluate associations among the genotypes based on the seed "P" and yield traits. All the above statistical analyses were performed using SAS.