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Influence of Myo-inositol Phosphate Synthase Gene Inphytic Acid contents and Superoxide Dismutase Activity (SOD) of Groundnut (*Arachis hypogaea* L.)

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ABSTRACT: Phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate, InSP6) is major storage form of phosphorus in mature cereal and legume seeds. It binds to metallic cations such as calcium, zinc, magnesium and iron to form a mixed salt called phytate. Thus, phytates act as anti-nutrients, and promote the minerals unavailable. Myo-inositol phosphate synthase (MIPS) is the major gene for phytate synthesis in legumes and it is characterized only in few major legumes such as soybean, green gram, black gram, cowpea and French bean. In groundnut, effect of MIPS gene in phytic acid contents and SOD is an important area of research. In present investigation MIPS gene and phytic acid contents was estimated in 57 groundnut genotypes. Phytic acid estimation was done in 35 days old groundnut leaf tissue and the result showed phytic acid concentration varied from 1.73 (ICGV-13264) to 2.94% (Shivpuri local42). Most of the genotypes were having phytic acid between 1.95 to 2.17 %. Total SOD ranged from 10.8 (nmol/g) for JGN 3 to 24.8 (nmol/g) forICGV-9112. Ten allele specific MIPS primers of chickpea and moong bean was used on groundnuts genotypes, three markers have shown amplification for the MIPS gene at 400 base pairs in 17 groundnut genotypes *i.e.*, Shivpuri local 37, ICGV13523, ICGV13236, ICGV7988, ICGV13245, shivpurilocal39, ICGV13549, JAGN1, DHGN4, ICGV13269, ICGV9249, ICGN13520, ICGN13573, Shivpuri local42, Shivpuri local6, Shivpuri local29 along with check variety Gangapuri. Identified groundnut genotypes with low phytic acid contents could be used for groundnut improvement programme.

Keywords: Groundnut, MIPS markers, phytic acid, SOD.

INTRODUCTION

Groundnut or peanut, is an important auto-tetraploid legume crop rich in protein and oil content grown in tropical and sub-tropical region all over the world. In addition to the huge beneficial properties, peanuts also have very high levels of phytic acid than wheat, and maize. Phytic acid content is between 0.2–4% in peanuts and a huge variability among peanut genotypes has been observed (812.3–1713.8 mg/100 g seed). Phytate is a chelator of cations such as Fe^{2+}, Zn^{2+}, Ca^{2+} and Mg^{2+}, and reduces their bioavailability in humans and monogastric animals. In developing countries where staple food is mainly seed-based, it leads to serious alimentary deficiencies in humans. Nonruminant animals are unable to digest phytic acid, and the undigested phytic acid promotes water eutrophication and environmental pollution. The Phytic acid is myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate. Phytic acid is the major storage form of phosphorous comprising 1-5% by weight in cereals, legumes, oil seeds and nuts (Vats and Banerjee 2004). It represents 50-85% of total phosphorous in plants (Reddy *et al*. 1982). Phytate rapidly accumulates in seeds during the ripening period. It is stored in leguminous seeds and oil seeds in the globoid crystal within the protein bodies. MIPS which stands for myo-Inositol-1,2,3,4,5,6-hexakisphosphate (Ins P(6)) was first described as an abundant form of phosphorus in plant seeds and other plant tissues and dubbed "phytic acid". Subsequently it was found to be a common constituent in eukaryotic cells, its metabolism a basic component of cellular housekeeping. Phytic acid chelates micronutrients which prevent their bioavailability. Superoxide dismutase activity (SOD) contributed superoxide radical dismutation. Marker assisted selection for a particular trait is one of the most important technologies for groundnut crop improvement (Adlak *et al.,* 2019; Bhawar *et al*., 2020; Pramanik *et al.,* 2019; Pramanik *et al.,* 2021; Rathore *et al.,* 2022). Bhagyawant *et al*., (2018) studied 60 chickpea genotypes for MIPS gene, antioxidant activity, mineral content and phytic acid content. They have done PCR analysis and amplified a 400 bp fragment of MIPS gene in chickpea. In our experiment we have used these markers for amplification of MIPS gene in groundnut. Objectives of groundnut crop improvement programs demand enhancement of micronutrient concentration, with low levels of phytic acid. Current study is focused on impact of MIPS gene on phytic acid

and identification of low phytic acid containing groundnut genotypes.

MATERIAL AND METHODS

Plant Material. In present investigation, five check varieties including JGN3, GPBG4, SunOleic95R, KDG128, Gangapuri and fifty-one groundnut germplasm lines collected from Junagarh, Gujarat, Shivpuri, Dhar, Badwani and Jhabua Madhya Pradesh were evaluated for phytic acid, SOD and MIPS gene identification.

Methodology

Phytic Acid Estimation. Phytic acid estimation was done using 25 mg groundnut leaf sample of 35 days old and grinded it in liquid nitrogen. Wilcox *et al.* (2000) method was used for seed extraction with HCL (0.4mM) for phytic acid evaluation. To get the clear supernatant Chen's reagent (3M sulphuric acid, 2.5% ammonium molybdate, 10% ascorbic acid and deionized water in the ratio of 1:1:1:2 was used. The mixture was incubated for 15 minutes at room temperature and then absorption was taken at 650 nm in a spectrophotometer.

Assaying for superoxide dismutase activity (SOD) Estimation. The total SOD activity was measured by taking 25mg of leaf sample at 35 DAS and crush it in liquid nitrogen. Added 250 microliters of 0.1% trichloro acetic acid to it in aneppendorf tube. Vortex for 10 min and centrifuge for 20 minutes at 10000rpm. Taken 160 microliters supernatant in an Eppendorf tube and add 160 microliters of phosphate buffer. Added 680 microliters of 1M potassium iodide. Keep the reaction mixture in dark for 1 hour and then take absorption at 390 nm. Taxonomic distance measured by phytic acid and SOD were analysed using Jaccard's similarity index, calculated by NTSYSpc v2.1 software (Rohalf 1998).

Molecular Characterization. Genomic DNA was isolated from 20-30 days young leaves of groundnut germplasms by modified CTAB method (Murray and Thompson 1980; Tiwari *et al*., 2017; Tiwari *et al*., 2021). Ten MIPS markers (Bhagyawant *et al*., 2018)were used for allele specific gene amplification, out of these markers three have generated amplification of 400 bp in 56 genotypes included germplasm lines and check varieties. Polymerase chain reaction was accomplished in 10µl reaction mixture encompassing of 1X PCR buffer, 0.1 U Taq DNA polymerase (Fermentas), 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pM) and 20 ng/µl of genomic DNA in a thermocycler (Bio-Rad, USA). The PCR protocol comprised of initial denaturation step of 94°C for 3 min tracked by 35 cycles of 94°C for 1 min, annealing at 55°C for 30 sec, elongation at 72°C for 1 min with final extension at 72°C for 10 min. The PCR products were resolved on 3% agarose gel at 120V for 2-3 hrs and documented using Syngene, Gel Documentation System (USA).

RESULTS AND DISCUSSIONS

Phytic acid concentration varied from 1.73 (ICGV-13264) to 2.94% (Shivpuri local-42). Most of the genotypes were having phytic acid between 1.95 to 2.17 % (Fig 1). Total SOD ranged from 10.8 (nmol/g) for JGN 3 to 24.8 (nmol/g) forICGV-9112. No significant correlation was found between phytic acid and SOD. Total 4 groups were formed between SOD and phytic acid as shown in 2D diagram (Fig. 2).

(Myo-Inositol-1,2,3,4,5,6-hexakisphosphate (Ins P (6) (MIPS) gene is responsible for phytic acid in plant seeds and plant tissue. The molecular markers used for the identification of MIPS gene shows a band of 400 base pairs after PCR amplification. When the markers earlier used by the study done by (Bhagyawant *et al.*, 2018) in chickpea 10 primers was used on groundnuts selected 36 germplasms out of which three markers have shown amplification for the MIPS gene at 400 base pairs in some of the genotypes (Fig. 3). Total 17 genotypes *i.e.*, Shivpuri local-37, ICGV-13523, ICGV-13236, ICGV-7988, ICGV-13245, shivpurilocal-39, ICGV-13549, JAGN-1, DHGN-4, ICGV-13269, ICGV-9249, ICGN-13520, ICGN-13573, Shivpuri local-42, Shivpuri local6, Shivpuri local-29 along with check variety Gangapuri were having MIPS gene. Although, marker assisted selection is one of the promising approaches of crop improvement, conventional methods of selections are also equally important. Trait specific molecular markers are being used widely for characterization in many crops including groundnut (Mishra *et al.*, 2020; Mishra *et al*., 2021; Upadhyay *et al*., 2020; Shaym *et al.*, 2020; Baghel *et al.*, 2020; Tiwari *et al.*, 2014; Sahu *et al*., 2020; Choudhary *et al.*, 2020; Makwana *et al*., 2021; Tiwari *et al*., 2017; Verma *et al*., 2021; Rajpoot *et al*., 2020).

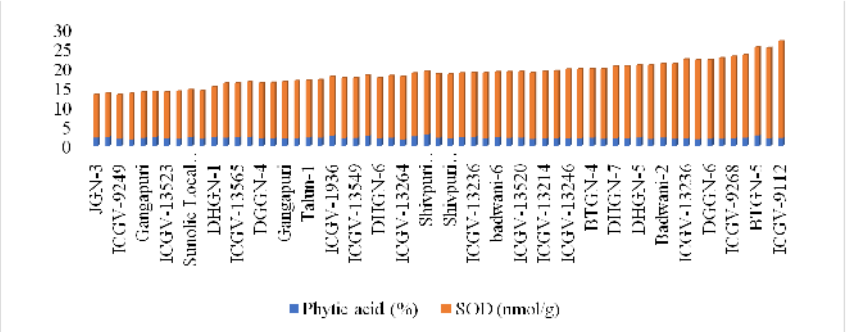


Fig. 1. Bar diagram presenting clustering of groundnut genotypes for phytic acid and SOD.

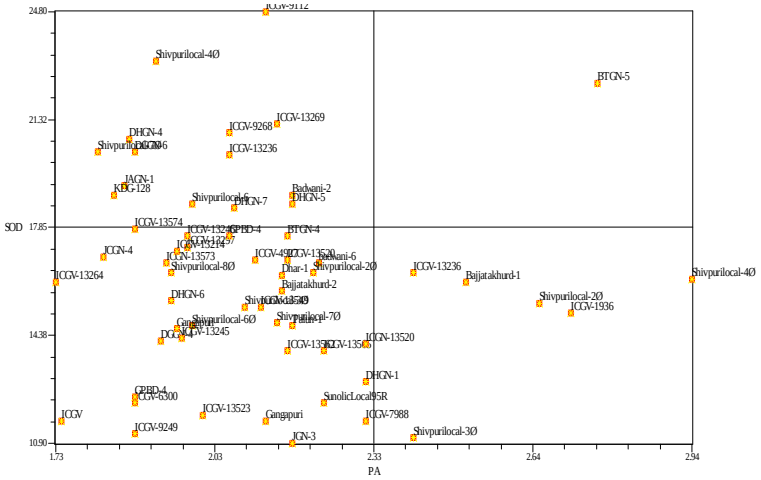
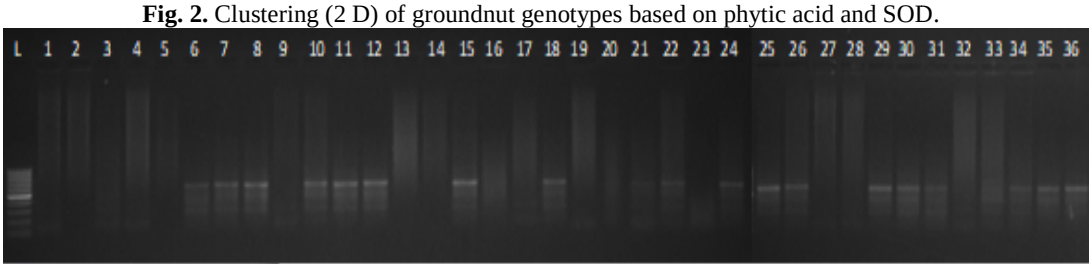
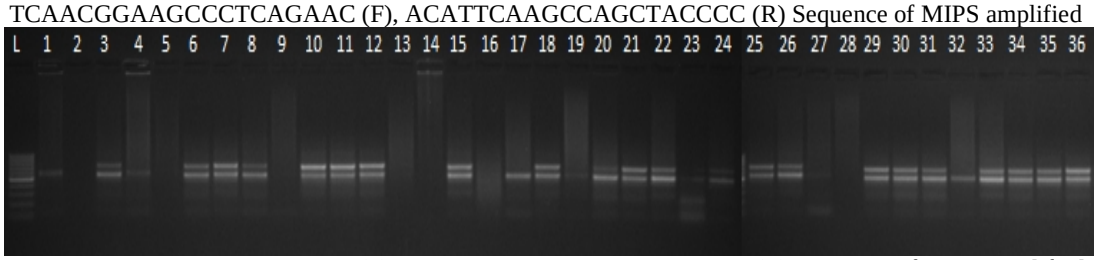


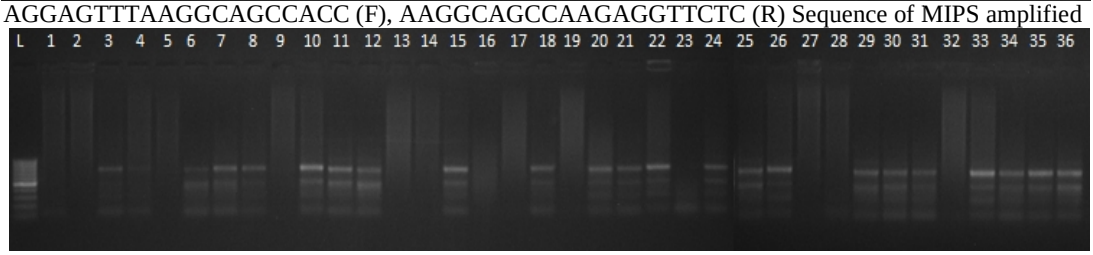
Fig. 2. Clustering (2 D) of groundnut genotypes based on phytic acid and SOD.



TCAACGGAAGCCCTCAGAAC (F), ACATTCAAGCCAGCTACCCC (R) Sequence of MIPS amplified



AGGAGTTTAAGGCAGCCACC (F), AAGGCAGCCAAGAGGTTCTC (R) Sequence of MIPS amplified



CAGAGAGGGTATTTCATGGGCA (F), GGCTTGATACCAGCTCCCAC (R) Sequence of MIPS amplified

Fig 3. Allelic specific amplification of MIPS markers in groundnut germplasm.

Although groundnut molecular breeding is being applied widely, it has certain limitation of low polymorphism (Adlak *et al.*, 2021; Pramanik *et al.*, 2021). Breeding for low phytate peanut genotypes promises to be cost-effective intervention in the fight against micronutrient deficiencies in developing economies. However, tools and genomic resources are still not available to develop such varieties. Our study has identified groundnut genotypes with low phytic acid that can be used in molecular breeding to reduce the phytate content in peanuts.

CONCLUSION

Current study identified groundnut genotypes having MIPS gene and their phytic acid content. Marker assisted selection is important tool for high-speed crop improvement programme. MIPS gene was identified by amplification of 400 bp amplicon. Identified genotypes could further be used for hybridization programme of groundnut and for getting improved varieties with low phytic acid contents.

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Conflict of Interest. None.

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