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## Cumulative studies on the micronutrients of soybean (*Glycine max*) genotypes in response to charcoal rot incidence

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#### Abstract

Soybean being a short duration legume crop it is an ideal for various intercropping as well as sequential cropping system. Despite being one of the most economical source of protein and packed with several nutraceutical components, soybean does suffer from shortfalls that limit its utilization in food uses. Given the fluctuating production of soybean in the recent years due to several biotic and abiotic stresses, it has become important to breeding for quality traits in tandem with overcoming other biotic/ abiotic stress that affect crop productivity. However, its productions and productivities are challenged by a number of abiotic and biotic (diseases and insects + pests) stresses which ultimately hampers the complete biochemical and nutritional content of the crop. Among the biotic challenges, charcoal rot disease is the most serious one. It is caused by fungus *Macrophomina phaseolina* (Tassi) Goid. a soil borne pathogen. In general, 30-50 % losses occur every year in soybean worldwide and recorded up to 77% in India considering the losses it has become important to have a complete study covering all the spheres of promising genotypes thoroughly. In the present study micronutrient content was evaluated. The lowest calcium content was recorded in the genotype AMS MB 5-18 (73.3 mg/100g) and the highest level was recorded in AMS 475 (90.58 mg/100g) genotype. Highest content of iron was found in AMS MB 5-19 (15.20 mg/100g) and lowest amount was found in JS 335 (8.14 mg/100g). Highest boron was found in JS 93-05 (11.98 mg/100g) and lowest amount was found in AMS MB 5-19 (6.7 mg/100g). Highest zinc content was found in AMS MB 5-19 (4.58mg/100g) and lowest amount was found in AMS 243 (3.62 mg/100g). Highest copper content was found in JS-93-05 (1.93 mg/100g) and lowest amount was found in AMS MB 5-19 (1.3mg/100g). Highest manganese content was found in AMS 38-24 (3.23 mg/100g) and lowest amount was found in AMS 353 and AMS 358 (2.13 mg/100g).

**Keywords:** Soybean, pulse crop, biotic stress resistance, charcoal rot, micronutrients analysis

#### Introduction

Soybean (*Glycine max* (L.) Merrill) designated as miracle bean established its potential as an industrially vital and viable oilseed crop in many areas of India. It is the cheapest source of vegetable oil and protein. It contains about 40 percent protein, well balanced in essential amino acids, 20 percent oil rich with poly unsaturated fatty acid specially omega 6 and Omega 3 fatty acids, 6-7 percent total mineral, 5-6 percent crude fiber and 17-19 percent carbohydrates (Chauhan and Opena, 1988) [2]. It has innumerable uses as food, feed and fuel (biodiesel) besides limitless industrial and pharmaceutical applications. The soybean oil is used as cooking oil as well as a main ingredient in margarine, salad oils, salad dressings, mayonnaise and shortening. The soybean meal is used primarily as a protein source for swine, poultry, dairy and fish. It is also used to make protein concentrate, texturized protein and protein-isolates that are used in food products for human consumption. On account of its multifarious uses and limitless benefits, soybean is rightly called as “golden bean”, “miracle bean” or “wonder crop” (Orf, 2010) [5]. Soybean plays a very important role in the economy and foreign earnings of our country as it contributes 37% and 25% to the national oilseeds and edible oil production, respectively. Export of deoiled cake (DOC) of soybean earned India more than 6585 crores of foreign exchange during 2012-13 (Anonymous, 2013) [1]. Therefore, developing soybean cultivars with higher yield, resistance to major pests and diseases and improved quality has become the major objective of soybean breeding program across the country. For that a complete study covering all the spheres of promising genotypes should be studied thoroughly. Micronutrients analysis was done so as to understand the metabolic behavior of the crop under different

conditions. Genotypes showing good nutrient potential may be useful in breeding programme through hybridization and selection.

## Material and Methods

### Plant material

A set of 14 diverse soybean genotypes were used for

screening. The collected genotypes included promising varieties, indigenous, mutants, few pre released collections, advanced breeding lines as well as obsolete varieties. It varied in maturity, seed color, flower colour, seed size, and reaction to charcoal rot disease as well as other yield attributing traits. Specific features of the genotypes are presented in Table 1.

**Table 1:** Soybean genotypes included in the study

S.N	Genotypes	Parents	Remarks
1	AMS MB 5-19	Mutant of Bragg	Developed by Mutation breeding and characteristically fixed at M8 generation.
2	AMS MB 5-18	Mutant of Bragg	Developed by Mutation breeding and characteristically fixed at M8 generation.
3	AMS – 1001	Mutants	Pre released variety
4	AMS – 77	Mutant of JS 93-05	Developed by Mutation breeding and characteristically fixed at M5 generation.
5	AMS – 353	Mutants	Pre released variety
6	AMS – 358	Mutant of JS 93-05	Developed by Mutation breeding and characteristically fixed at M5 generation.
7	BRAGG	Parental genotype	Parental genotypes
8	AMS – 243	Mutant of Bragg	Developed by Mutation breeding and characteristically fixed at M8 generation.
9	JS - 93-05	Parental genotype	Parental genotypes
10	AMS 99-33	Mutants	Pre released variety
11	AMS 38-24	TAMS 38 x RKS 24	Recombinant breeding, entry fixed at F2 generation.
12	AMS -475	Mutant of JS 93-05	Developed by Mutation breeding and characteristically fixed at M5 generation.
13	JS – 335 (R)	(Check-Resistant)	High yielding variety, most popular
14	TAMS -38 (S)	(Check-Susceptible)	Highly susceptible variety

R=Check Resistant; S=Check Susceptible

The Calcium, Iron, Boron, Zinc, Copper and Manganese traits were analyzed using the Atomic Absorption spectrophotometer (Spectra AA 220) by following steps. To analyze the micronutrients, seed samples were taken of the selected genotypes.

**Collection of samples:** The seeds were collected after harvesting the crop in the required quantity. The seeds were finely crushed using mortar and pestle.

**Di-acid digestion of the samples:** The objective of digestion is to destroy the organic compounds and bring the elements to soluble form.

**Determination of nutrients:** Calcium was analyzed by titration with EDTA (Ethylene Di-amine Tetra acetic acid) using Sodium hydroxide buffer and calcon indicator. EDTA is a powerful ligand and forms a stable metal complex with many metals at different pH and the nutrient can be analyzed by using a standard indicator. Iron, Zinc, Copper, Manganese and Boron: present in the seeds were measured using the Atomic Absorption spectrophotometer (AAS).

### Process

First 0.5 g of seed samples were weighed and placed in labeled conical flasks. 10 ml-12 ml of di-acid was added to each flask. Di-acid is a mixture of nitric acid and per chloric acid in the ratio of 9:4. The flasks were placed on a hot plate and set at 150°-200° C till the residue turned colorless then after flasks were allowed to cool at room temperature. Later filtration was done using whattman filter paper no.42 in a 50 ml volumetric flask. The final volume was made up to 50 ml using double distilled water.

## Result and Discussion

### Micronutrients traits

Micronutrients are essential for plant growth and play an

important role in balanced crop nutrition. They include boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), nickel (Ni) and chloride (Cl). They are as important to plant nutrition as primary and secondary macronutrient. The present study was aimed to assess nutritional composition of all the selected soybean genotypes. Seven nutrient traits were analyzed at mature stage. The nutrients contents viz., calcium, iron, zinc, boron, copper, and manganese contents were estimated and are given in Table 2.

### 1. Calcium (mg/100g)

Calcium is a secondary nutrient that is critical to crop development. It is needed in large amounts by all plants for the formation of cell walls and cell membranes. Due to the immobility of calcium in the soil and plant tissues, a continuous supply must be present for plants to access. In the present study, the calcium content present in the mature stages of selected legume seeds were analyzed through EDTA Titration method. In R8 stage it ranged from 73.3 mg/100g (AMS MB 5-18) to 90.58 mg/100g (AMS -475). Similar results were reported by Keatinge *et al.* (2011)<sup>[4]</sup> and Garg *et al.* (2014)<sup>[3]</sup>.

### 2. Iron (mg/100g)

Iron (Fe) is essential for crop growth and food production. Plants take up Fe as the ferrous (Fe<sup>2+</sup>) cation. Iron is a component of many enzymes associated with energy transfer, nitrogen reduction and fixation, and lignin formation. In the present study, the iron content present in selected soybean genotypes was analyzed using Atomic Absorption Spectroscopy. The Iron content in all the genotypes varied significantly. Highest iron content was recorded in AMS MB 5-19 (15.2 mg/100g) and the lowest iron content was found in JS – 335 (8.14 mg /100 g). These results are in accordance with Garg *et al.* (2014)<sup>[3]</sup>.

**Table 2:** Estimated micronutrients from selected soybean genotypes

S. No	Genotype	Calcium	Iron	Boron	Zinc	Copper	Manganese
1	AMS MB 5-19	76.24	15.2	6.7	4.58	1.3	2.86
2	AMS MB 5-18	73.3	13.55	9.57	4.19	1.32	2.73
3	AMS - 1001	86.66	13.1	10.3	4.02	1.53	2.63
4	AMS - 77	85.48	12.41	9.82	3.86	1.48	2.5
5	AMS - 353	79.23	8.18	9.83	4.55	1.41	2.13
6	AMS - 358	78.9	10.21	7.61	4.5	1.39	2.13
7	BRAGG	85.48	11.14	8.64	4.46	1.36	2.58
8	AMS - 243	81.3	12.15	7.98	3.62	1.36	2.67
9	JS - 93-05	80.46	12.76	11.98	3.88	1.93	2.72
10	AMS 99-33	88.96	14.74	8.53	4.21	1.79	2.88
11	AMS 38-24	89.3	11.05	9.2	4.22	1.76	3.23
12	AMS -475	90.58	14.21	9.82	4.28	1.34	2.94
13	JS – 335 (R)	80.1	8.14	9.61	3.79	1.47	2.43
14	TAMS -38 (S)	89.3	10.36	7.86	4.24	1.55	3.14
•	GM	82.54	11.88	9.15	4.19	1.52	2.69
•	Range	73.3 - 90.58	8.14 - 15.2	6.7 - 11.98	3.62 - 4.58	1.3 - 1.93	2.13 – 3.23
•	SE(m)	2.17	0.78	0.22	2.11	0.03	0.19
•	CD @5%	6.38	2.31	0.65	7.14	0.1	0.57
•	F Test	S	S	S	S	S	S

R=Check Resistant; S=Check Susceptible

### 3. Boron (mg/100g)

Boron (B) exists primarily in soil solutions as the  $\text{BO}_3^{-3}$  anion – the form commonly taken up by plants. One of the most important micronutrients affecting membrane stability, The highest boron content was recorded in the genotype JS - 93-05 (11.98 mg/100g) and the lowest level was recorded in AMS MB 5-19 (6.7 mg/100g) genotype, respectively. Similar results were reported by Garg *et al.* (2014)<sup>[3]</sup>.

### 4. Zinc (mg/100g)

Zinc (Zn) is taken up by plants as the divalent  $\text{Zn}^{+2}$  cation. It was one of the first micronutrients recognized as essential for plants and the one most commonly limiting yields. Although Zn is required only in small amounts, high yields are impossible without it. The highest zinc content was recorded in the genotype AMS MB 5-19 (4.58 mg/100g) and the lowest level was recorded in AMS – 243 (3.62mg/100g) genotype, respectively. Similar results were reported by Garg *et al.* (2014)<sup>[3]</sup>.

### 5. Copper (mg/100g)

Copper (Cu) activates enzymes and catalyzes reactions in several plant growth processes. The presence of copper is closely linked to vitamin A production, and it helps ensure successful protein synthesis. It ranged from 1.3 mg/100g (JS - 93-05) to 1.93 mg/100g (AMS MB5-19). Similar observations were reported by Garg *et al.* (2014)<sup>[3]</sup>.

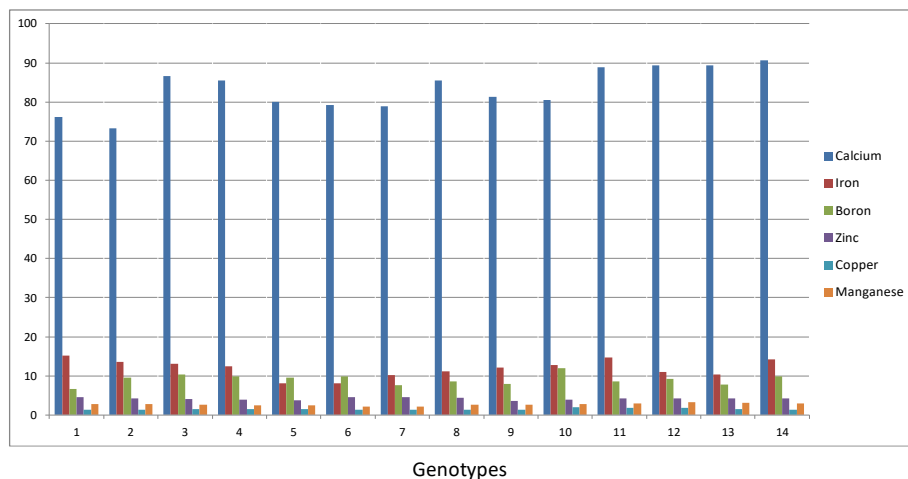
### 6. Manganese (mg/100g)

Manganese (Mn) functions primarily as part of enzyme

systems in plants. It activates several important metabolic reactions and plays a direct role in photosynthesis. Manganese accelerates germination and maturity while increasing the availability of phosphorus (P) and calcium (Ca) The manganese The highest manganese content in was recorded in AMS 38-24 (3.23 mg/100g) followed by TAMS -38 (3.14mg/100g). However, the genotypes AMS – 353 and AMS – 358 simultaneously had lowest manganese content (2.13mg /100g). Similar results were depicted by Garg *et al.* (2014)<sup>[3]</sup>.

### Conclusions

Micro-nutritional studies included the micronutrients like calcium, boron, iron, zinc, copper and manganese. It was observed that the four genotypes *viz.* AMS MB 5-18, AMS MB 5-19, AMS -77 and AMS -1001 have highest contents of nearly all the nutritional factors (Fig 1). On an average 82.54mg/100g of calcium was estimated, 11.88mg/100g of iron was estimated, 9.15mg/100g of boron was estimated, 4.19mg/100g of zinc was estimated, 1.52 mg/100g of copper and 2.69mg/100g of manganese was estimated from the selected genotypes. In this study only the micronutrient contents were evaluated of the mentioned promising genotypes but in future taking advantage of current tools in genomics, proteomics, genetic engineering, as well as forward and reverse genetics, studies helping in significant progress in our understanding the mode of pathogenesis, host response, and identifying gene(s) associated with host resistance to charcoal rot should be made.



**Fig 1:** Cumulative micronutrients of soybean genotypes in response to charcoal rot incidence

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