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Estimation of zinc and iron content of chickpea (*Cicer arietinum* L.) genotypes

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Abstract
The investigation on "Evaluation of chickpea (*Cicer arietinum* L.) genotypes for zinc and iron content'' was conducted on 18 genotypes of chickpea during *rabi* 2019-20 at Pulses Improvement Project, M.P.K.V., Rahuri. Observations were recorded for days to 50 per cent flowering, days to maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, number of pods per plant, number of seeds per pod, 100 seed weight (g), seed yield per plant (g) and wilt (%).Variation for zinc content in 18 chickpea genotypes was ranged between 2.04 to 3.65 mg/100 g. Among the 18 chickpea genotypes, the highest zinc content was recorded in the genotype Digvijay (3.65 mg/100 g) followed by Vijay (3.54 mg/100 g) and Phule Vikram (3.37 mg/100 g). The genotype AKG 1706 (2.04 mg/100 g) recorded lowest zinc content followed by JAKI 9218 (2.14 mg/100 g). Other genotypes recorded moderate zinc content. Similarly, variation for iron content in 18 chickpea genotypes was ranged between 6.15 to 8.20 mg/100 g. Among 18 chickpea genotypes, the highest iron content was recorded by the genotype AKG 1402 (8.20 mg/100 g) followed by Phule Vikrant (8.13 mg/100 g) and AKG 1702 (8.07 mg/100 g). While the genotype Phule G 1131-9 (6.15 mg/100 g) recorded lowest iron content followed by the genotype PDKV Kanchan (6.28mg/100 g). Other genotypes recorded average iron content.

Keywords: Chickpea, zinc, iron

Introduction
Pulses are one of the most sustainable crops. It takes just 43 gallons of water to produce one pound of pulses as compared with 216 gallons for Soybean and 368 gallons for peanuts. They also contribute for soil quality by fixing nitrogen in the soil.

Pulses constitute an important component in Indian agriculture since centuries. The pulse crops are also called as grain legume and have been valued as nutritious and protein rich food, fodder and feed. They have a greater role in fixing atmospheric nitrogen by symbiotic association with *Rhizobium* spp. Pulse crops are popularly considered as mini fertilizer factories which contribute to the enrichment of soil through the addition of nitrogen substantially up to 65 kg per year.

Chickpea is scientifically known as *Cicer arietinum* L. It is a self pollinated crop belongs to the family Fabaceae of the Tribe Cicereae. It is a diploid species with chromosome number 2n=2x=16. It is originated from Western Asia (Turkey).The chickpea also known as Bengal Gram, Chana in Hindi and *Harbhara* in marathi. It usually needs a tropical or subtropical climate with more than 400 mm of annual rainfall. It is rich in nutritionally important unsaturated fatty acids such as linoleic and oleic acids which are beneficial in the prevention of coronary and cardiovascular diseases. It may also lower blood cholesterol levels due to their high content of soluble fiber and vegetable protein.

In terms of both area and production, it remarkably predominates among all other pulse crops. During 2019-20, area under total pulses in India was 28.33 million ha, production was 23.15 million tonnes and productivity was 842 kg/ha. In Maharashtra, area under total pulses was 43.87 lakh ha, production was 40.27 lakh tonnes and productivity 918 kg/ha (Anonymous, 2015-16) [2, 3, 4]. India is the world's leading producer of chickpeas, accounting for 65.2 percent of total area and 65.4 percent of total production. In India, the area planted under chickpea was 10.78 million hectares, with a production of 11.35 million tonnes and a productivity of 1053 kg/ha (Anonymous, 2015-16) [2, 3, 4]. While, in Maharashtra, area under chickpea was 23.22 lakh ha, production was 25.97 lakh tonnes and productivity were 1118 kg/ha (Anonymous, 2015-16) [2, 3, 4].

The mineral nutrients are divided into two groups, viz., macronutrients and micronutrients. Macronutrients are further divided into two categories: primary and secondary nutrients.
Nitrogen, phosphorous and potassium are primary nutrients. While calcium, magnesium and sulphur are secondary nutrients. The second group of nutrients, i.e., mineral elements such as boron, copper, iron, chloride, manganese, molybdenum and zinc are examples of micronutrients. The term "micronutrient" represents a group of important nutrients that are required in relatively small quantities for plant growth. Iron and zinc are also required in small quantities by plants for their growth. The addition of iron and zinc to the fertilizer improves yield and quality. Human beings also require iron and zinc for growth and development. As a by-product of metabolic pathways, the plant accumulates and produces these minerals, vitamins and other organic molecules. In plants both iron and zinc play a crucial role in chlorophyll production and the activation of several enzymes. Soil, iron sulphate, and iron chellate are sources of iron, while soil, zinc oxides, zinc sulphate and zinc chellate are sources of zinc.
Micronutrients play a crucial role in the development of immune system. It gets weakened as a result of a lack of micronutrients placing children at risk of sickness. Malnutrition affects approximately 3000 million people worldwide, triggering metabolic disturbances. Micronutrient deficiencies which affect over two billion people globally today are the leading cause of mental retardation, preventable blindness and maternal death. Zinc is required for protein metabolism, gene expression and biomembrane integrity. With this available background information, the have been initiated with an objective to evaluate the zinc and iron content in eighteen chickpea genotypes.

Materials and Method

The present investigation on "Evaluation of chickpea (*Cicer arietinum* L.) genotypes for zinc and iron content" was carried out during *rabi* 2019-20 at Pulses Improvement Project, M. P. K. V., Rahuri. Evaluation of eighteen genotypes for zinc and iron content is done in laboratory of Department of Biochemistry, PGI, M. P. K. V., Rahuri. The reagents were used for biochemical analysis of chickpea seeds were Concentrated sulphuric acid (H_2SO_4), Perchloric acid, Standard solutions of zinc and iron.

Diacid digestion of samples for evaluation of chickpea genotypes for zinc and iron Content

A finely powdered sample of 0.2 g of dried seeds of chickpea was taken in a digestion tube and 15 ml solution of diacid digestion mixture was added which is prepared by mixing solutions of conc. sulphuric acid (H_2SO_4) and perchloric acid in the ratio of 9:4. The digestion tubes were kept in digestion chamber and allowed to digest for 48 hrs at temperature of 160 to 180 ^{0}C. The digestion of seed sample along with diacid mixture was continued until it turns to yellow color and finally becomes colorless. After cooling of digested samples, a volume of 50 ml was made with distilled water after filtering them to glass wool fibres. The blank tubes consist of 15 ml of nitric acid and perchloric acid (9:4) was also digested with the same that served as blank. After those readings were taken on Atomic Absorption Spectrophotometer at wavelength of 515 nm for iron and 470 nm for zinc which is then converted by using following formula:

$$\text{Zinc or iron content (mg/100 g)} = \text{Sample OD} \times \text{Multiplication factor} \times \frac{\text{Volume made}}{\text{Weight of sample}} \times 100$$

Estimation

Zinc or iron content in the sample was estimated in the sample by using atomic absorption spectrophotometer (Perkin Elmer).For the estimation of desired element, specific hollow cathode lamp for that particular element was inserted into the lamp slots of atomic absorption spectrophotometer. Instrument optimization, calibration and elemental analysis were carried used to calibrate standard curve of particular element.

Atomic absorption spectrophotometer works on the following principle

1. In the analysis employing atomic absorption spectrophotometer (AAS), the sample in the form of a homogeneous liquid aspirated into a flame where "free" atoms of the element to be analyzed were created.
2. A light source (hollow cathode lamp) used to excite the free atoms formed in the flame by absorption of the electromagnetic radiation
3. The decrease in energy (absorption) was then measured which follows the Beer-Lambert law, i. e., the absorbance is proportional to the number of free atoms in the ground state (Baker and Suhr 1982) [17].

For preparation of standard stock solutions

Iron (Fe)

7.022 g of analytical grade (NH_4) Fe $(SO_4)_2$.6 H_2O was dissolved in 400 ml of distilled water. 5 ml of conc. H_2SO_4 was added to make final volume with distilled water to 1 liter. This would give a stock solution of Fe 1000 mg L^{-1} or 1000 ppm. Pipetted out 0, 0.5, 1, 2, 3, 4 and 5 ml of stock solution of 100 mg/L separately into 100 ml volumetric flask and the volume was made with water and shaked well gave 0, 0.5, 1, 2, 3, 4 and 5 mg of Fe L^{-1} respectively and read on flame photometer. A standard curve was drawn by plotting flame photometer reading i. e. absorbance on Y axis and Fe mg L^{-1} on X axis.

Zinc (Zn)

4.4398 g of $ZnSO_4$. $7H_2O$ was dissolved in distilled water and final volume of 1 liter was made. This solution gave concentration of 1000 mg Zn/L. After preparation dilute 1000 ppm to 100 ppm diluting, it 10 times. Pipetted out 0, 1, 5, 10 and 20 ml of standard zinc solution separately into 50 ml volumetric flask, 10 ml of vanadomolybdate reagent was added. The volume made to 50 ml with distilled water and that gave 0-20 mg Zn L^{-1} or 0-20 µg Zn mL^{-1} concentration respectively. The intensity of yellow color read on spectrophotometer at 470 nm wavelength after 30 minutes. A standard curve drawn by plotting absorbance on Y axis and Zn concentrations Zn mL^{-1} solution on X axis.

Results and Discussion

The present research entitled "Evaluation of chickpea (*Cicer *arietinum* L.) genotypes for zinc and iron content" was conducted at Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri during *rabi* 2019-20 to evaluate the zinc and iron contents from eighteen chickpea genotypes, variability, correlation and path analysis among the various characters such *viz*., days to 50% flowering, no. of primary branches per plant, no. of secondary branches per plant, no. of pods per plant, 100 seed weight, no. of seeds per pod, seed yield per plant, wilt % were noted significantly.

Analysis of seed zinc and iron content in chickpea seeds during r*abi* 2019-20 were carried out with eighteen chickpea genotypes to meet one of the objectives of present investigation which is given in the Table1.

The seed zinc content varied from 2.04 to 3.65 mg/100 g. Among the eighteen chickpea genotypes, the highest zinc content was recorded in genotypes Digvijay (3.65 mg/100 g) followed by Vijay (3.54 mg/100 g) and Phule Vikram (3.37 mg/100 g). Similarly, the variation in seed iron content was ranged between 6.15 to 8.20 mg/100 g. Among eighteen chickpea genotypes, the highest iron content was recorded in genotypes AKG 1402 (8.20 mg/100 g) followed by Phule Vikrant (8.13 mg/100 g) and AKG 1702 (8.07 mg/100 g).

The results obtained for the present investigation are similar with the findings of Carla *et al*. (2013) [7]. According to this research findings, the zinc content in chickpea genotypes varied from 2.52 mg/100 g to 4.11 mg/100 g and observed significant varietal differences in total zinc among chickpea cultivars. The similar results were obtained in the earlier findings of Grewal *et al*. (2020) [8] under investigation of understanding genotypic variation and identification of promising genotypes for zinc and iron content in chickpea. Zinc and iron content ranged from 1.10 to 5.91 mg/100 mg and 0.50 to 8.54 mg/100 g, respectively.

Seed is the major reservoir of both organic as well as inorganic nutrients in crop plants and seed reserve concentration vary across the species and genotypes. Environment and the efficiency of the plant to take up, utilize and translocate nutrients to the seed or to the edible parts influence growth, development and yield.

Table 1: Mean zinc and iron content of eighteen chickpea genotypes

Sr. No.	Name of genotype	Mean zinc content (mg/100 g)	Mean iron content (mg/100 g)
1	Phule G 15109	2.62	6.67
2	Phule G 1131-4	2.46	6.34
3	Phule G 1131-9	2.93	6.15
4	Phule G 1010-14	2.66	6.90
5	AKG 1402	2.84	8.20
6	AKG 1702	2.54	8.07
7	AKG 1706	2.04	7.95
8	BDNG 2017-23	2.27	7.26
9	BDNG 2017-44	3.17	7.18
10	BDNG 2017-49	2.86	7.58
11	Vijay	3.54	7.46
12	Digvijay	3.65	7.84
13	JAKI 9218	2.14	7.86
14	PDKV Kanchan	2.49	6.28
15	BDN 9-3	2.28	7.17
16	BDN 797	2.16	7.22
17	Phule Vikrant	3.31	8.13
18	Phule Vikram	3.37	7.65
	General Mean	2.74	7.33
	Range	2.04 - 3.65	6.15 – 8.20
	SE	0.01	0.02
	CD at 5%	0.04	0.05

Conclusion

Analysis of chickpea seed zinc content of eighteen chickpea genotypes revealed genetic variation in seed zinc content, from 2.04 to 3.65 mg/100 g. Among eighteen chickpea genotypes, the highest zinc content was recorded in genotypes Digvijay (3.65 mg/100 g) followed by Vijay (3.54 mg/100 g) and Phule Vikram (3.37 mg/100 g). While the genotype AKG 1706 (2.04 mg/100 g) having lowest zinc content followed by JAKI 9218 (2.14 mg/100 g). Other genotypes contained moderate zinc content.

The variation in seed iron content of eighteen chickpea genotypes was ranged between 6.15 to 8.20 mg/100 g. Among eighteen chickpea genotypes, the highest iron content was recorded in genotypes AKG 1402 (8.20 mg/100 g) followed by Phule Vikrant (8.13 mg/100 g) and AKG 1702 (8.07 mg/100 g). While the genotype Phule G 1131-9 (6.15 mg/100 g) having lowest iron content followed by PDKV Kanchan (6.28 mg/100 g). Other genotypes contained average iron content.

The chickpea genotypes containing high zinc and iron content in chickpea seeds would be used in the crossing programmes so as to develop genotypes with high zinc and iron content.

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