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Kaushal Kumar
M.Sc. Research Scholar, Department of Agricultural Biotechnology and Molecular Biology, Dr. R.P.C.A.U., Pusa, Samastipur, Bihar, India

Lokesh Thakur
Ph.D. Scholar, Department of Biotechnology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh

Kumari Anjani
Assistant Professor, Department of Agricultural Biotechnology and Molecular Biology, Dr. R.P.C.A.U., Pusa, Samastipur, Bihar, India

SK Singh
Assistant Professor, Department of Soil Science, Dr. R.P.C.A.U., Pusa, Samastipur, Bihar, India

Corresponding Author:
Kaushal Kumar
M.Sc. Research Scholar, Department of Agricultural Biotechnology and Molecular Biology, Dr. R.P.C.A.U., Pusa, Samastipur, Bihar, India

Biochemical characterization of grain iron and zinc content in little millet genotypes

Kaushal Kumar, Lokesh Thakur, Kumari Anjani and SK Singh

Abstract
Little millet is an important climate resilient crop and a rich source of micronutrients particularly iron and zinc, thus can be helpful to combat iron and zinc malnutrition. Iron and Zinc are two of the most important micronutrients. The present study was carried out for estimation of grain iron and zinc content of 26 little millet genotypes using atomic absorption spectrophotometer (AAS). The results depicted significant variation in iron concentrations ranging from 21.03 ppm to 60.23 ppm whereas zinc content ranging from 22.28 ppm to 30.37 ppm. Among the different genotypes of little millet, the highest grain iron content was found in genotype OLM-203 (60.233±0.145 ppm) and the lowest in genotype WV-156 (21.033±0.088 ppm). Similarly, significant variations in the grain zinc content of little millet were also obtained. The highest zinc content was found in the genotype TNPS-173 (30.377±0.011 ppm) and the lowest zinc content was found in genotype WV-168 (22.28±0.024 ppm). The grouping of the genotypes showed that most of the genotypes (17 for zinc and 18 for iron content) were found to contain medium range of grain iron and zinc. The study thus showed the presence of significant amount of grain iron and zinc in the little millet genotypes which can be useful for malnutrition mitigation problem.

Keywords: Iron, zinc, little millet, climate resilient

Introduction
Iron (Fe) and zinc (Zn) are two of the most essential micronutrients for human growth and development, especially for the infants. The most severe symptoms of deficiency of iron are presented in females, particularly anemia throughout puberty and pregnancy periods. According to Oliver and Gregory (2015) [13] deficit in Fe impairs children's mental development until they reach puberty, as well as their vulnerability to infectious illness and mortality. Fe and Zn are required in trace amount by both animals and plants and are crucial for a variety of metabolic processes (Welch and Graham, 2004) [17]. Fe is a major recognized component of myoglobin and hemoglobin. Likewise, zinc is an important co-factor for numerous regulatory proteins and enzymes and it is crucial in gene expression, synthesis of DNA and RNA. Zn regulates absorption of iron (Fe) in the intestines, Zn insufficiency appears to be linked to anemia associated with Fe shortage (Graham *et al*., 2012) [6]. The deficiency of these nutrients can lead to many health impairments. Micronutrient malnutrition has been identified as one of the most severe human health related problems around the whole world. Iron deficiency is the most noticeable of these having 9 th rank, whereas zinc deficiency is at 11th rank among the top twenty risk factors, which contribute to global disease burden. Although there are many adequate sources for these nutrients, still insufficiency is an issue mainly in poors. So, it is important to find the low-cost cereals having high iron and zinc content along with other essential nutrients. Millets may be a viable answer to this issue.
Little millet (*Panicum sumatrense*) is a self-pollinated allotetraploid crop species. It can be grown in a wide range of conditions, most notably dry, semi-arid, and sub-humid drought-prone agro habitats. Little millet is known as "Miracle grains/Nutria-cereals", which is regarded to be nutritionally superior to cereals. It is rich source of mineral nutrients such as iron, zinc, calcium, potassium and phosphorus. Little millet is a promising food ingredient that may be used on a wide scale and can help to ensure food security. It can also help in mitigation of iron and zinc deficiency. The genotypes of little millet have high content of zinc and iron but elaborate information is still lacking (Tiwari *et al*., 2018) [16]. Thus, there is a need of characterization of the genotypes based on their iron and zinc content.

Material and Methods

We have taken 26 genotypes of Little millets, collected from the Hill Millet Research Station, Navsari Agricultural University, Gujarat for iron and zinc analysis (Table 1). The grain samples of little millet were used for estimation of grain iron and zinc content by using Atomic Absorption Spectroscopy (AAS) using Di-acid method (Gupta, 2020) [8] in the Soil Science Laboratory, RPCAU, Pusa. The observations were taken in three replications for each of the genotypes. The samples' value was calculated by comparing them to a blank sample.

Table 1: List of Little millet genotypes collected from Navsari Agriculture University, Gujarat

S. No.	Genotype	Sl. No.	Genotype	S. No.	Genotype	S. No.	Genotype
1	WV- 108	8	WV- 124	15	WV- 155	22	WV- 199
2	WV- 110	9	WV- 126	16	WV- 156	23	WV- 207
3	WV- 112	10	WV- 130	17	WV- 159	24	TNPS- 164
4	WV- 114	11	WV- 142	18	WV- 164	25	TNPS- 173
5	WV- 115	12	WV- 145	19	WV- 167	26	OLM- 203
6	WV- 119	13	WV- 146	20	WV- 168		
7	WV- 122	14	WV- 151	21	WV- 179		

Procedure for extraction of micronutrients

1. The seeds were crushed using mortar and pestle to make it in a powdered form. 0.5g of powdered seed was taken in a 100ml conical flask.
2. 10 ml of di-acid mixture (Nitric acid and Perchloric acid in the ratio of 9:4) was carefully added and kept for pre-digestion. After pre-digestion, the flask was kept on hot plate in acid-proof digestion chamber having fume exhaust system and heated at 100 °C for 1hr and then the temperature was raised to about 200 °C. The digestion was continued until the solution becomes colourless and only white dense fumes appear.
3. The acid content was reduced to about 2-3 ml by continuous heating. The flask was removed from hot plate and cooled.
4. Distilled water was added to make the volume 50 ml. The content was filtered through Whatman No. 42 filter paper into a volumetric flask. The reading was taken using Thermo scientific Atomic Absorption Spectrophotometer against a blank having only di-acid mixture.

The reading of blank sample obtained from AAS was subtracted from sample reading and it was multiplied with dilution factor to obtain the amount of micronutrients in the sample. The Fe and Zn content reading were taken in three replications and evaluated using a completely randomized design at a 5% level of significance to test the significance of the results. The genotypes were grouped into three categories, high, medium and low based on their iron and zinc content using the scoring method as given in Table 2.

Table 2: Scoring method for the genotypes

Value of the parameter	CLASS
< MEAN - SD	Low
MEAN-SD – MEAN + SD	Medium
> MEAN + SD	High

Results and Discussion

Micronutrient analysis of grain was performed in twenty-six little millet genotypes to determine iron and zinc concentration. The elemental analysis of little millet results depicted significant variation in iron concentrations ranging from 21.03 ppm to 60.23 ppm whereas zinc content ranging from 22.28 ppm to 30.37 ppm. Among the different genotypes of little millet, the highest grain iron content was found in genotype OLM-203 (60.233±0.145 ppm) followed by WV-199 (56.10±0.173 ppm), WV-119 (55.80±0.115 ppm) and WV-126 (52.167±0.120 ppm). The lowest iron content was found in genotype WV-156 (21.033±0.088 ppm) followed by WV-168 (21.533±0.176 ppm), WV-130 (23.70±0.153 ppm) and WV-146 (27.833±0.176 ppm). Similarly, significant variations in the grain zinc content of little millet were also obtained. The grain zinc content among different genotypes ranged from 22.28 ppm to 30.49 ppm. The highest zinc content was found in the genotype TNPS-173 (30.377±0.011 ppm) followed by WV-145 (29.88±0.012 ppm), WV-142 (28.777±0.015 ppm) and OLM-203 (28.487±0.008). The lowest zinc content was found in genotype WV-168 (22.28±0.024 ppm) followed by WV-146 (22.38±0.018 ppm), WV-156 (23.20±0.021 ppm), WV-114 (23.583±0.008) and WV-155 (23.873±0.03 ppm) (Table 3).

The critical difference (CD) found in twenty-six genotypes of little millet in relation with zinc content is 0.558 and in relation with iron content is 0.408. The mean standard error was 0.196 and 0.143 in relation with zinc and iron content respectively where as standard error of difference is 0.277 and 0.203 respectively. The coefficient of variation (CV) in zinc content is 1.294 and in iron content is 0.619.

The genotypes were grouped in 3 classes i.e. low, medium and high based on the grain iron and zinc content of twenty-six genotypes of little millet (Fig 1). The value of grain zinc content ranged from 22.28 to 23.87 ppm for the genotypes in low group. This group includes five little millet genotypes namely WV-168, WV-146, WV-156, WV-114 and WV-155. Seventeen genotypes were categorized in the medium group having grain zinc content ranging from 24.38 to 28.29 ppm. The high zinc content group had a value ranging from 28.48 to 30.37 ppm and included four genotypes. Similarly, three categories based on iron content were low (21.03 to 27.83 ppm), medium (32.13 to 47.33 ppm) and high (52.17 to 60.23 ppm) which included four, eighteen and four genotypes respectively (Table 4). Most of the genotypes evaluated in the present study had zinc and iron content in the medium range.

Table 3: Zinc and Iron content in different little millet genotype (in ppm)

Sl. No.	Genotype	Zn in ppm	Fe in ppm
1	WV- 108	27.56±0.031	36.167±0.088
2	WV- 110	26.063±0.027	34.467±0.134
3	WV- 112	27.29±0.026	41.3±0.153
4	WV- 114	23.583±0.008	35.2±0.115
5	WV- 115	26.473±0.018	32.7±0.115
6	WV- 119	26.47±0.02	55.8±0.115
7	WV- 122	26.47±0.014	45.633±0.089
8	WV- 124	27.453±0.037	32.133±0.133
9	WV- 126	27.037±0.023	52.167±0.12
10	WV- 130	25.46±0.031	23.7±0.153
11	WV- 142	28.777±0.015	37.767±0.186
12	WV- 145	29.88±0.012	44.133±0.089
13	WV- 146	22.38±0.018	27.833±0.176
14	WV- 151	25.667±0.994	44.5±0.153
15	WV- 155	23.873±0.03	33.233±0.088
16	WV- 156	23.2±0.021	21.033±0.088
17	WV- 159	24.387±0.008	46.233±0.145
18	WV- 164	27.073±0.029	46.067±0.033
19	WV- 167	24.48±0.016	40.467±0.088
20	WV- 168	22.28±0.024	21.533±0.176
21	WV- 179	25.993±0.009	45.333±0.145
22	WV- 199	26.077±0.015	56.1±0.173
23	WV- 207	27.28±0.018	39.367±0.233
24	TNPS- 164	28.293±0.014	47.333±0.167
25	TNPS- 173	30.377±0.011	43.433±0.233
26	OLM- 203	28.487±0.008	60.233±0.145
	C.D.	0.558	0.408
	SE (m)	0.196	0.143
	SE (d)	0.277	0.203
	C.V.	1.294	0.619

Table 4: List of Low, Medium and High iron and zinc containing little millet genotypes

Category	Iron	Zinc
Low	WV-156, WV-168, WV-130 and WV-146	WV-168, WV-146, WV-156, WV-114 and WV-155
Medium	WV- 108, WV- 110, WV- 112, WV- 114, WV- 115, WV- 122, WV- 124, WV- 142, WV- 145, WV- 151, WV- 155, WV- 159, WV- 164, WV- 167, WV- 179, WV- 207, TNPS- 164 and TNPS- 173	WV- 108, WV- 110, WV- 112, WV- 115, WV-119, WV- 122, WV- 124, WV-126, WV-130, WV- 151, WV- 159, WV- 164, WV- 167, WV- 179, WV-199, WV- 207 and TNPS- 164
High	OLM-203, WV-199, WV-119 and WV-126	TNPS-173, WV-145, WV-142 and OLM-203

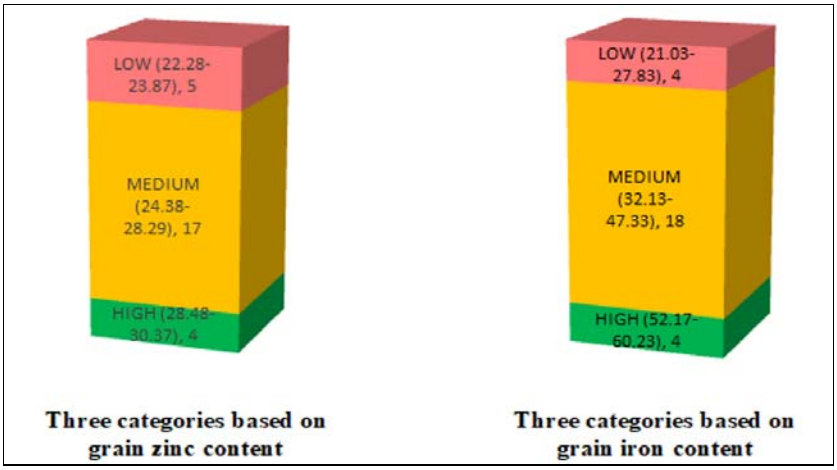

Fig 1: Schematic representation of the three categories based on grain iron and zinc content

Conclusion

The elemental analysis of little millet depicted significant variation in iron concentrations ranging from 21.03 ppm to 60.23 ppm with an average of 40.15 ppm whereas zinc content ranging from 22.28 ppm to 30.37 ppm with an average of 26.24 ppm. This variation of Fe and Zn content in little millet grains is nearly consistent with the results obtained by Chandel *et al*., 2014 [2]; Manimozhi *et al*., 2015

[11] and Mani *et al*., 2021 [10]. Average iron and zinc contents in little millet genotypes in this study were more than the major cereals like rice (Fe 13 ppm and Zn 24 ppm) and comparable with the average iron and zinc content present in wheat (Garcia-Oliveira *et al*., 2018; Graham *et al*., 1999) [4, 7]. The genotypes were grouped in 3 classes i. e. low, medium and high based on the grain zinc and iron content of twenty-six genotypes of little millet. The low grain zinc content ranging from 22.28 to 23.87 ppm which includes five little millet genotypes, the medium grain zinc content ranging from 24.38 to 28.29 ppm and includes seventeen genotypes and high zinc content ranging from 28.48 to 30.37 ppm and it includes four genotypes. Similarly, three categories based on iron content are low (21.03 to 27.83 ppm), medium (32.13 to 47.33 ppm) and high (52.17 to 60.23 ppm) which includes four, eighteen and four genotypes respectively. The genotype OLM-203 was found to be the best among all the little millet genotypes in terms of iron and zinc content since it was categorized in high group for both the nutrients.

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