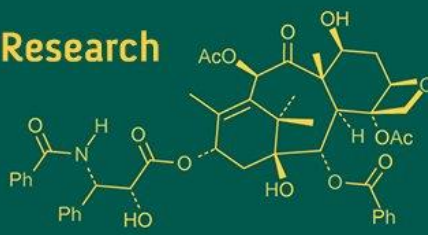
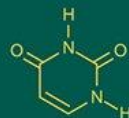
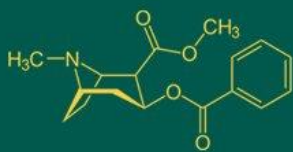


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## Morphological and Molecular Evaluation of Iron and Zinc Rich Pearl Millet Genotypes

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### Abstract

Pearl millet [*Pennisetum glaucum* (L.) R Br.] is a very nutritious and staple food crop for the poor people of Asia and Africa. It has been considered the most extensively grown cereal among the other millets from ancient times. It is very well adapted to thrive in the areas with harsh climatic conditions including drought, high temperature, high salinity, low pH and poor soil fertility. Due to its ability to sustain in extreme and adverse conditions, it is considered as an excellent dry land crop as compared to the other cereals like maize, wheat, rice and sorghum. It is also rich in various nutrients, mainly iron and zinc and thus it is very imperative to eliminate malnutrition and hidden hunger. Hence, it is very important to screen and evaluate the pearl millet genotypes which are rich in high iron and zinc so that they can be used further for developing more micronutrient rich cultivars. In this study, a total of 38 pearl millet lines rich in iron and zinc were used for screening and evaluation. Different morphological parameters including plant height, effective tiller/plant, head length, head diameter, days to 50% flowering, open pollination seed set, iron and zinc content and agronomic score were recorded. Molecular characterization was also carried out using 112 SSR markers. Out of 112 SSRs, 91 primers amplified products of varying sizes ranging from 110 to 750 bp and 20 (17.9 %) were monomorphic and 71 (63.4%) were polymorphic. A total of 194 alleles were obtained in this study and the number of alleles per locus varied between 2 to 4 with an average of 2.73 alleles. Polymorphic Information Content (PIC) varied from 0.35 to 0.78 with an average of 0.59 PIC value. This research may be helpful in developing high Fe and Zn cultivars which can further prove useful to eliminate malnutrition and hidden hunger.

**Keywords:** Biofortification, hidden hunger, iron, molecular markers, pearl millet, SSRs, zinc

### Introduction

Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is a very important and nutritious cereal among all other crops. It is a major staple food crop for more than 90 million people living in arid and semi-arid tropics of Asia and Africa. It is a C<sub>4</sub> plant having high photosynthetic efficiency and has huge potential to survive in harsh climatic conditions where other crops fail to sustain. It is also rich in carbohydrates, proteins (8-19%), fat content (3-8%), ash, dietary fibers (1.2 g/100 g), vitamins, essential amino acids, antioxidants and various minerals (296 mg/100 g P; 307 mg/100 g K; 42 mg/100 g Ca; 137 mg/100 g Mg; 75 mg/kg Fe and 40 mg/kg Zn) and can provide approximately 80-90% of calories to the poor people (Kumar et al., 2020a) [16]. It is the cheapest source of household nutrition as it can supply 361 Kcal/100 g of energy which is comparable to other major cereals such as wheat (346 Kcal/100 g), rice (345 Kcal/100 g) maize (125 Kcal/100 g) and sorghum (349 Kcal/100 g) according to the nutritive value of Indian foods (NIN, 2003) [18]. Being rich in Fe and Zn, it can endow with 19 to 63% of total iron (Fe) intake and 16 to 56% of the total zinc (Zn) intake from all the food sources and thus is a cheaper and economical source of Fe and Zn as compared to other cereals and vegetables (Satyavathi et al., 2022) [24]. Nutritional security is a major challenge these days to meet the demands of growing population across the world as people are mainly dependent on micronutrient deficient cereal based diet. Thus, in this context, pearl millet can prove very helpful for ensuring livelihood as well as nutritional security of the farmers (Satyavathi et al. 2021) [23]. Iron and zinc deficiencies are assumed among the major micronutrient deficiencies (MNDs) causing ill health, higher mortality, reduced work productivity, learning disabilities in children

and poor economic growth of any country (Bailey *et al.*, 2015) [9]. Fe and Zn deficient diets can lead to malnutrition which is a major public health issue and may affect >2 billion people worldwide (WHO, 2002) [30]. Pearl millet is highly rich in iron and Zn content and it needs focused breeding for these micronutrients along with high yield. Thus, evaluation and breeding of varieties rich in these minerals is highly required to eliminate Fe and Zn deficiency among people (Ambawat *et al.* 2024, 2025) [3, 2]. Biofortification of cereals has emerged out as a valuable approach to deal with malnutrition and enhance nutrients in major food crops. Biofortification Priority Index (BPI) and biofortification research in pearl millet has divulged that high variability for Fe and Zn content exists in pearl millet which in turn has high prospects for developing micronutrient rich pearl millet cultivars and thus it has emerged as a major target crop for iron and zinc biofortification (Satyavathi *et al.*, 2021) [23]. One meal of biofortified high-iron pearl millet variety can fulfil around 50-100% of daily requirement of iron which can help in combating iron deficiency in children, women and men (Kodkany *et al.*, 2013) [15]. Lot of genetic variation exists in pearl millet for minerals like iron and zinc and has major additive gene action for better parent heterosis as compared to other cereals. This immense genetic variation (30-140 mg/kg Fe and 20-90 mg/kg Zn) can be extensively used for developing high Fe and Zn content hybrids and varieties. Lot of efforts have been laid by India for developing biofortified crops with high nutrient contents and a number of promising donors have been also identified to generate good breeding lines possessing high yield and nutrients (Kanatti *et al.*, 2014; Kodkany *et al.*, 2013) [13, 15]. Such type of efforts and screening can be used to introgress these high iron and zinc lines into locally adapted, high-yielding cultivars and can further prove useful to eradicate Fe and Zn deficiencies. Thus, it is the need of the hour to strengthen the high-iron testing and develop research programs focusing towards biofortification of pearl millet. There are several traditional breeding approaches to meet such goals. But, the conventional approaches are very laborious and time consuming which resulted into the need of several modern biotechnological strategies to speed up the breeding programs (Ambawat *et al.*, 2020a; Prakash *et al.*, 2026) [7, 20]. SSR markers are very effective and a good choice for such studies due to their abundance in the genome, co-dominant behavior, even distribution, high-throughput, easy detection, genome specificity, multi-allelic nature and high reproducibility (Choudhary *et al.*, 2021, Ambawat *et al.*, 2020b) [11, 5]. Therefore, the use of SSR markers is very effective strategy for molecular characterization among pearl millet genotypes. Both phenotypic and molecular analysis helps to take good decisions and enables breeders to maximise heterosis and improve the efficacy of hybrid breeding programs. Hence, the present study was designed to evaluate iron and zinc rich pearl millet genotypes based on morphological parameters as well as their molecular characterization using SSRs. This can be further helpful for breeding programs focusing towards developing high Fe and Zn hybrids and varieties.

## Materials and Methods

### Plant material and morphological screening

A total of 38 pearl millet lines rich in Fe and Zn were used for morphological and molecular characterization (Table 1). This experiment was conducted at PC Unit, ICAR-AICRP on Pearl millet, Jodhpur during 2024-25. The lines were sown in

Randomized Block Design (RBD) with three replications. Each entry was sown in two rows of 4 m length with 60 cm row spacing and 15 cm plant spacing. The observations on different morphological parameters including plant height, effective tiller/plant, head length, head diameter, days to 50% flowering, open pollination seed set, iron and zinc content and agronomic score were recorded as described by Rani *et al.* (2024) [22].

**Table 1:** Pearl millet genotypes used for molecular characterization

| S. No. | Name of Pearl Millet Line | Organization     | Fe (ppm) | Zn (ppm) |
|--------|---------------------------|------------------|----------|----------|
| 1.     | PPMI 1322                 | IARI, New Delhi  | 35       | 32       |
| 2.     | PPMI 1323                 | IARI, New Delhi  | 53       | 39       |
| 3.     | PPMI 1324                 | IARI, New Delhi  | 71       | 48       |
| 4.     | PPMI 1325                 | IARI, New Delhi  | 57       | 45       |
| 5.     | PPMI 1326                 | IARI, New Delhi  | 54       | 44       |
| 6.     | HSR 24-1                  | CCS HAU, Hisar   | 30       | 25       |
| 7.     | HSR 24-2                  | CCS HAU, Hisar   | 40       | 40       |
| 8.     | HSR 24-3                  | CCS HAU, Hisar   | 41       | 30       |
| 9.     | HSR 24-4                  | CCS HAU, Hisar   | 53       | 42       |
| 10.    | 150 SB 24                 | JAU, Jamnagar    | 62       | 44       |
| 11.    | 159 SB 24                 | JAU, Jamnagar    | 33       | 33       |
| 12.    | 171 SB 24                 | JAU, Jamnagar    | 40       | 34       |
| 13.    | 217 SB 24                 | JAU, Jamnagar    | 41       | 31       |
| 14.    | 229 SB 24                 | JAU, Jamnagar    | 50       | 33       |
| 15.    | JMSB 20213                | JAU, Jamnagar    | 49       | 37       |
| 16.    | JMSB 20227                | JAU, Jamnagar    | 75       | 38       |
| 17.    | JMSB 20229                | JAU, Jamnagar    | 43       | 34       |
| 18.    | DHL CRP 01                | MPKV, Dhule      | 59       | 38       |
| 19.    | DHL CRP 02                | MPKV, Dhule      | 52       | 42       |
| 20.    | DHL CRP 03                | MPKV, Dhule      | 59       | 41       |
| 21.    | DHL CRP 04                | MPKV, Dhule      | 60       | 42       |
| 22.    | DHL CRP 05                | MPKV, Dhule      | 51       | 33       |
| 23.    | DHL CRP 06                | MPKV, Dhule      | 51       | 41       |
| 24.    | RIB 15178                 | RARI, Durgapura  | 53       | 36       |
| 25.    | RIB 15179                 | RARI, Durgapura  | 57       | 39       |
| 26.    | RIB 15180                 | RARI, Durgapura  | 44       | 35       |
| 27.    | RIB 15181                 | RARI, Durgapura  | 38       | 45       |
| 28.    | RIB 15182                 | RARI, Durgapura  | 27       | 27       |
| 29.    | RIB 15183                 | RARI, Durgapura  | 42       | 33       |
| 30.    | RIB 15184                 | RARI, Durgapura  | 57       | 36       |
| 31.    | RIB 15185                 | RARI, Durgapura  | 58       | 45       |
| 32.    | RIB 15186                 | RARI, Durgapura  | 54       | 36       |
| 33.    | RIB 15187                 | RARI, Durgapura  | 45       | 36       |
| 34.    | MIR 1117                  | PC Unit, Jodhpur | 53       | 39       |
| 35.    | MIR 1406                  | PC Unit, Jodhpur | 60       | 56       |
| 36.    | MIR 1716                  | PC Unit, Jodhpur | 37       | 35       |
| 37.    | MIR 1806                  | PC Unit, Jodhpur | 52       | 40       |
| 38.    | MIR 1907                  | PC Unit, Jodhpur | 44       | 42       |

### Genomic DNA isolation and quantification

Genomic DNA was extracted from young and fresh leaves of 12 days old plantlets of 38 genotypes of pearl millet from using cetyl trimethyl ammonium bromide (CTAB) method along with some modifications as described by Ambawat *et al.* (2020c) [6]. The extracted DNA was analyzed on 0.8% agarose gel and diluted to a final concentration of 10 ng/μl for amplification with SSR primers.

### Molecular characterization and SSR analysis

A total of 112 SSRs including 6 primers (Ppmsb1 to Ppmsb 6) which were reported to be specific for Fe and Zn characterization (Singhal *et al.*, 2021, Ambawat *et al.*, 2022, 2024) [28, 4, 3] were used for PCR amplification with 38

genotypes. The reaction was performed in a 96-well thermal cycler (Agilent Technologies) in a volume of 10 µl consisting of 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 mM each dNTP, 0.4 µM 10-mer primer, 1 unit Taq DNA polymerase (GeNei, India) and 10 ng of DNA as template. It was programmed to 1 cycle of 5 mins at 94 °C for initial denaturation followed by 35 cycles of 30s at 94 °C for denaturation, 30 s at 58 °C for annealing and 1 min at 72 °C for primer extension. Finally, 1 cycle at 72 °C for 10 mins was used for final extension followed by hold at 4 °C (Ambawat *et al.* 2020b) [5]. The amplified products were run on 2.5% agarose gel.

## Results and Discussion

### Morphological evaluation

Performance of 38 pearl millet genotypes under field conditions were evaluated and observations were recorded for days to 50% flowering, effective tillers per plant, plant

height, head diameter, head length etc. Days to 50% flowering ranged from 48 (PPMI 1323) to 65 (RIB 15181 and RIB 15186). Plant height ranged from 85 cm (JMSB 20227 and DHL CRP 01) to 163 cm (JMSB 20229). Effective tillers per plant was 1.5 (PPMI 1322, HSR 24-2, 217 SB 24, RIB 15178, RIB 15179 and RIB 15183) to 4 cm (MIR 1716). Head length ranged from 11 cm (HSR 24-4, DHL CRP 01) to 35 cm (JMSB 20229). Head girth ranged from 1.8 cm (MIR 1117) to 4 cm (HSR 24-2). The seed set (%) under open pollination condition ranged from 33 (RIB 15183) to 88 (MIR 1907). Agronomic score ranged from 1.8 (HSR 24-4) to 4.5 (MIR 1907). Iron content ranged from 27 (RIB 15182) to 75 ppm (JMSB 20227) while zinc content ranged from 25 (HSR 24-1) to 56 ppm (MIR 1406) (Table 2). Similar kind of investigations were also made by other researchers (Singh *et al.*, 2016; Abubakar *et al.* 2019; Khandelwal *et al.*, 2022; Gunguniya *et al.* 2023; Rani *et al.*, 2024; Paulos and Kindie, 2025) [27, 1, 14, 12, 22, 19].

**Table 2:** Performance of different pearl millet genotypes under field conditions

| S. No. | Name of Pearl Millet Line | Days to 50% Flowering | Plant Height (cm) | Effective Tillers/Plant | Head Length (cm) | Head Dia Meter (cm) | Open Pollination Seed set % | Agronomic Score (1-5), 1 = Poor, 5 = Excellent | Fe (ppm) | Zn (ppm) |
|--------|---------------------------|-----------------------|-------------------|-------------------------|------------------|---------------------|-----------------------------|------------------------------------------------|----------|----------|
| 1      | PPMI 1322                 | 61                    | 118               | 1.5                     | 22               | 1.9                 | 73                          | 3.3                                            | 35       | 32       |
| 2      | PPMI 1323                 | 48                    | 148               | 2.5                     | 21               | 2.3                 | 73                          | 3.3                                            | 53       | 39       |
| 3      | PPMI 1324                 | 61                    | 110               | 1.8                     | 15               | 2.3                 | 55                          | 2.3                                            | 71       | 48       |
| 4      | PPMI 1325                 | 56                    | 150               | 2.5                     | 21               | 3.2                 | 65                          | 3.5                                            | 57       | 45       |
| 5      | PPMI 1326                 | 62                    | 135               | 1.8                     | 21               | 2.4                 | 55                          | 2.5                                            | 54       | 44       |
| 6      | HSR 24-1                  | 52                    | 122               | 3.0                     | 13               | 1.9                 | 73                          | 4.3                                            | 30       | 25       |
| 7      | HSR 24-2                  | 61                    | 105               | 1.5                     | 16               | 4.0                 | 76                          | 3.0                                            | 40       | 40       |
| 8      | HSR 24-3                  | 59                    | 105               | 2.3                     | 14               | 3.2                 | 68                          | 2.3                                            | 41       | 30       |
| 9      | HSR 24-4                  | 58                    | 105               | 2.0                     | 11               | 2.6                 | 48                          | 1.8                                            | 53       | 42       |
| 10     | 150 SB 24                 | 55                    | 145               | 3.5                     | 17               | 3.0                 | 60                          | 3.3                                            | 62       | 44       |
| 11     | 159 SB 24                 | 55                    | 123               | 2.0                     | 18               | 2.1                 | 48                          | 2.3                                            | 33       | 33       |
| 12     | 171 SB 24                 | 52                    | 135               | 2.0                     | 21               | 2.3                 | 63                          | 2.3                                            | 40       | 34       |
| 13     | 217 SB 24                 | 61                    | 108               | 1.5                     | 20               | 2.4                 | 47                          | 2.3                                            | 41       | 31       |
| 14     | 229 SB 24                 | 55                    | 148               | 2.3                     | 27               | 2.9                 | 53                          | 2.0                                            | 50       | 33       |
| 15     | JMSB 20213                | 60                    | 117               | 2.8                     | 21               | 2.9                 | 60                          | 2.3                                            | 49       | 37       |
| 16     | JMSB 20227                | 64                    | 85                | 3.0                     | 16               | 3.8                 | 80                          | 3.3                                            | 75       | 38       |
| 17     | JMSB 20229                | 64                    | 163               | 2.8                     | 35               | 3.2                 | 70                          | 2.3                                            | 43       | 34       |
| 18     | DHL CRP 01                | 55                    | 85                | 2.0                     | 11               | 2.7                 | 40                          | 2.0                                            | 59       | 38       |
| 19     | DHL CRP 02                | 61                    | 138               | 2.5                     | 17               | 3.1                 | 44                          | 2.5                                            | 52       | 42       |
| 20     | DHL CRP 03                | 63                    | 143               | 2.5                     | 20               | 3.4                 | 63                          | 4.3                                            | 59       | 41       |
| 21     | DHL CRP 04                | 62                    | 148               | 2.3                     | 21               | 3.5                 | 50                          | 3.3                                            | 60       | 42       |
| 22     | DHL CRP 05                | 63                    | 153               | 2.0                     | 23               | 3.6                 | 55                          | 2.3                                            | 51       | 33       |
| 23     | DHL CRP 06                | 60                    | 153               | 3.0                     | 23               | 3.5                 | 55                          | 2.3                                            | 51       | 41       |
| 24     | RIB 15178                 | 58                    | 105               | 1.5                     | 15               | 2.6                 | 50                          | 2.3                                            | 53       | 36       |
| 25     | RIB 15179                 | 64                    | 105               | 1.5                     | 16               | 3.0                 | 80                          | 2.3                                            | 57       | 39       |
| 26     | RIB 15180                 | 57                    | 103               | 2.3                     | 17               | 3.0                 | 50                          | 2.3                                            | 44       | 35       |
| 27     | RIB 15181                 | 65                    | 133               | 2.5                     | 19               | 3.1                 | 48                          | 2.3                                            | 38       | 45       |
| 28     | RIB 15182                 | 51                    | 135               | 2.5                     | 19               | 3.2                 | 73                          | 2.3                                            | 27       | 27       |
| 29     | RIB 15183                 | 64                    | 128               | 1.5                     | 19               | 2.4                 | 33                          | 2.3                                            | 42       | 33       |
| 30     | RIB 15184                 | 59                    | 155               | 2.5                     | 24               | 2.9                 | 75                          | 3.3                                            | 57       | 36       |
| 31     | RIB 15185                 | 64                    | 113               | 1.8                     | 19               | 3.8                 | 85                          | 3.8                                            | 58       | 45       |
| 32     | RIB 15186                 | 65                    | 100               | 2.0                     | 18               | 3.9                 | 75                          | 3.0                                            | 54       | 36       |
| 33     | RIB 15187                 | 57                    | 138               | 2.3                     | 20               | 3.6                 | 65                          | 2.8                                            | 45       | 36       |
| 34     | MIR 1117                  | 64                    | 125               | 3.0                     | 18               | 1.8                 | 85                          | 3.3                                            | 53       | 39       |
| 35     | MIR 1406                  | 55                    | 103               | 2.3                     | 12               | 3.3                 | 70                          | 3.5                                            | 60       | 56       |
| 36     | MIR 1716                  | 54                    | 130               | 4.0                     | 19               | 1.9                 | 83                          | 4.0                                            | 37       | 35       |
| 37     | MIR 1806                  | 53                    | 120               | 3.8                     | 22               | 2.0                 | 80                          | 4.0                                            | 52       | 40       |
| 38     | MIR 1907                  | 53                    | 105               | 2.8                     | 12               | 3.4                 | 88                          | 4.5                                            | 44       | 42       |
|        | C.D.                      | 0.149                 | 0.666             | 0.02                    | 0.15             | 0.02                | 0.462                       | 0.023                                          | 0.347    | 0.196    |
|        | SE(m)                     | 0.053                 | 0.236             | 0.007                   | 0.053            | 0.007               | 0.164                       | 0.008                                          | 0.123    | 0.070    |
|        | SE(d)                     | 0.075                 | 0.334             | 0.01                    | 0.075            | 0.01                | 0.232                       | 0.012                                          | 0.174    | 0.098    |
|        | C.V.                      | 0.155                 | 0.327             | 0.53                    | 0.49             | 0.424               | 0.447                       | 0.501                                          | 0.430    | 0.317    |

### Molecular characterization and SSR analysis

Researchers need to screen and evaluate the diverse germplasm available for developing micronutrient rich hybrids and varieties. Both phenotypic and genotypic evaluation is useful as phenotypic diversity offers valuable insights into the different traits whereas molecular characterization using DNA markers provides a more precise understanding of the underlying genetic structure and relationships among the lines available which in turn help in developing good cultivars. Conventional breeding techniques are laborious and time consuming and so molecular tools are preferred by the researchers to overcome these limitations (Bollam *et al.*, 2018; Ambawat *et al.*, 2020a) [10, 7]. In this study, a total of 112 SSR primers were used for PCR amplification among 38 pearl millet genotypes. Out of these, 91 primers amplified generating amplicons of varying sizes ranging from 110 to 750 bp and 20 (17.9 %) were observed to be monomorphic while 71 (63.4%) were polymorphic depicting a good amount of polymorphic markers which can be used in pearl millet improvement programs (Fig. 1, 2). A total of 194 alleles were acquired in this investigation and the number of alleles per locus ranged between 2 to 4 with an average of 2.73 alleles. Similar types of findings were also observed by other researchers (Shaikh, 2015; Choudhary *et al.*, 2021; Ambawat *et al.*, 2022, 2024) [25, 11, 4, 3]. Polymorphic

Information Content (PIC) was ranging from 0.35 to 0.78 with an average of 0.59 PIC value (Table 3). The highest PIC values were recorded for the marker IPES0185 and PSMP2074 (0.78) followed by IPES0009 (0.77) and Ppmsb5 (0.76), while it was lowest for the markers PSMP2076 and PSMP2202 (0.35) and PSMP2080 and ICMP 4014 (0.38) indicating that PSMP2074 was the best and the most informative marker for these genotypes whereas PSMP2076 and PSMP2202 were the least powerful marker. An average PIC value of 0.59 observed in this study is comparable to other studies (Anuradha *et al.*, 2017; Kumar *et al.*, 2020b; Ambawat *et al.*, 2022, 2025) [8, 17, 4, 2]. SSR markers are considered as the most efficient markers and are desired by researchers owing to their easiness, multiallelic and co-dominant nature, higher reproducibility and polymorphism with immense aptitude to discriminate the genotypes (Verma *et al.*, 2021) [29]. Similar kind of results for efficacy of SSRs in characterizing the genotypes were also accounted in other studies (Singh *et al.*, 2017; Radhika Ramya *et al.*, 2017; Kumar *et al.*, 2020b; Choudhary *et al.*, 2021; Ambawat *et al.*, 2024, 2025; Prakash *et al.*, 2026) [26, 21, 17, 11, 3, 2, 20]. The findings of this study can be used to develop higher Fe and Zn cultivars which will prove useful in eradicating malnutrition and hidden hunger.

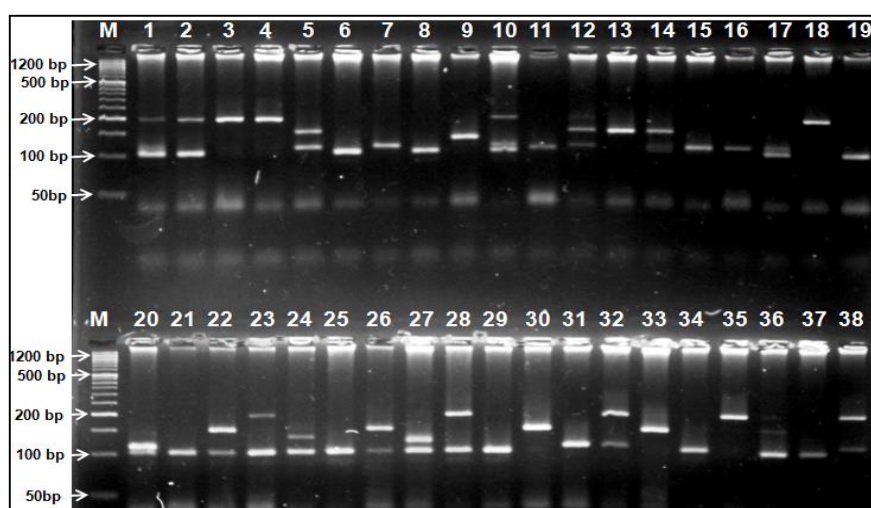


**Fig 1:** Agarose gel illustrating amplification profiles of pearl millet lines using the primer IPES 0185. Lane M-50 bp ladder, Lane 1-38 pearl millet lines

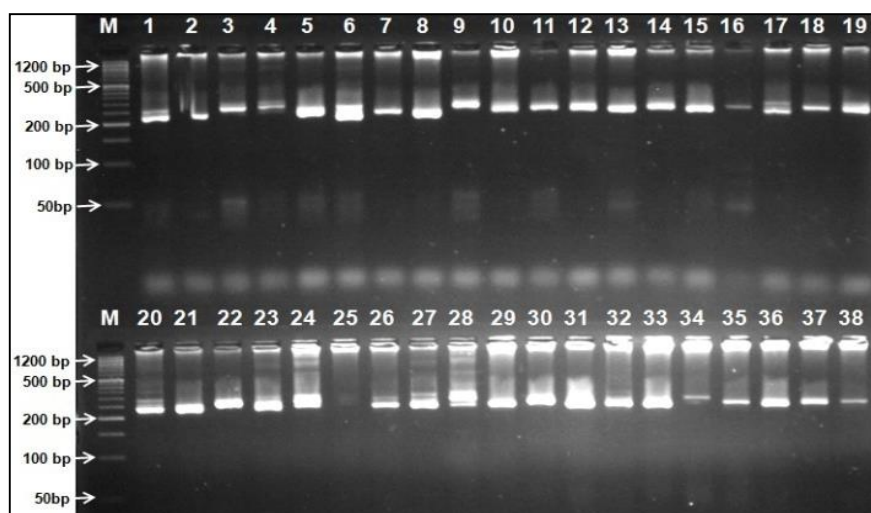


**Fig 2:** Agarose gel illustrating amplification profiles of pearl millet lines using the primer IPES 0203. Lane M-50 bp ladder, Lane 1-38 pearl millet lines

**Table 3:** List of SSR primers showing polymorphism with pearl millet genotypes

| S. No. | Oligo Name | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') | Product range | No. of alleles amplified | PIC  |
|--------|------------|---------------------------------|---------------------------------|---------------|--------------------------|------|
| 1      | Ppmsb1     | GGGAAGGTGTTGCTGACTTC            | GGGCTGTTGTTCCATGACA             | 510-650       | 3                        | 0.56 |
| 2      | Ppmsb2     | TGTTTTGATCCATGGTTTGC            | AACGAACAGGCTCAGTGTC             | 500-700       | 4                        | 0.59 |
| 3      | Ppmsb5     | GCGGAAGTGAACACCATTTT            | CTCGCCACCAGGATCGTC              | 350-750       | 4                        | 0.76 |
| 4      | Ppmsb6     | CTGCCAGCAGTGACGTGTT             | CTTTCTGGCTCCTCGTTGT             | 200-650       | 3                        | 0.68 |
| 5      | CTM8       | GCTGCATCGGAGATAGGGAA            | CTCAGCAAGCAGCTGCTCT             | 140-230       | 3                        | 0.62 |
| 6      | CTM12      | GTTGCAAGCAGGAGTAGATCGA          | CGCTCTGTAGGTTGAACCTCT           | 230-290       | 2                        | 0.75 |
| 7      | CTM25      | GCGAAGTAGAACACCGCGCT            | GCACTTCCCTCGCCGT                | 200-260       | 2                        | 0.53 |
| 8      | CTM26      | GCAAGTGATCCATGACATTACGA         | GCGAAGTAGAACACCGCGCT            | 190-500       | 4                        | 0.59 |
| 9      | Xcump007   | GAGGGATTCCAGGCGGTTTC            | GCGAGGAGCACATTCGATGAA           | 120-200       | 2                        | 0.47 |
| 10     | ICMP4014   | TTCTTCAATACACAGTTGTTGG          | ACCATAGGACCTTGACCAG             | 230-250       | 2                        | 0.38 |
| 11     | ICMP3017   | CACCAAAACAGCATCAAGCAG           | AGGTAGCCGAGGAAGGTGAG            | 160-190       | 2                        | 0.51 |
| 12     | ICMP3086   | ACCAAACTGCTCAAAACAGAG           | ATATCTTCTCGCTGCGGTGT            | 140-170       | 2                        | 0.47 |
| 13     | ICMP3002   | AAGATGGATGATGGATTGATGA          | TACACACACATTGCCACACG            | 180-210       | 2                        | 0.63 |
| 14     | ICMP3058   | CGGAGCTCCTATCATTCCAA            | GCAAGCCACAAGCCTATCTC            | 240-410       | 4                        | 0.68 |
| 15     | ICMP3080   | CAAACAGCATCAAGCAGGAG            | GCGTAGACGGCGTAGATGAT            | 200-240       | 2                        | 0.57 |
| 16     | ICMP3092   | GTTGCTGTATGTCGTCTGG             | CATCATGCCTGTGAGCAATG            | 210-450       | 4                        | 0.73 |
| 17     | ICMP3056   | ACGGAGCTACGGTTGGAATA            | CACAAGGGACCCACGATA              | 120-190       | 3                        | 0.71 |
| 18     | ICMP3021   | GCCGACAGGAAGATTACGAT            | AGCAAAACGCAGAACACAG             | 120-170       | 2                        | 0.56 |
| 19     | ICMP3048   | CGGAAGTCTGAGGTGAAAT             | GCGACTTCGACCGACTTTT             | 200-290       | 3                        | 0.58 |
| 20     | ICMP3066   | AGGCCCAAAGTACTTCCCTA            | TGTCAGACACAGATGCCACA            | 190-220       | 2                        | 0.55 |
| 21     | ICMP3063   | TCCGGTAGAGACCGTAATGG            | GGCACTCCCTAGCAAAATGA            | 170-210       | 2                        | 0.45 |
| 22     | ICMP3081   | ACGCCGTTTTCGTGTAGTCT            | TCCACAAGGTGACCTCACTG            | 200-330       | 3                        | 0.74 |
| 23     | ICMP3020   | GTTCCATGTTGAGCTGGAAGTC          | GCTAGACAGGGCCGTTACA             | 190-240       | 2                        | 0.54 |
| 24     | ICMP3014   | TGCTTCACAGCCTCTCCATA            | CCACCATGCAACAGCAATAA            | 200-290       | 3                        | 0.66 |
| 25     | ICMP3050   | ATGTCCAGTGTGACGGTGA             | CGGGGAAGAGACAGGCTACT            | 250-300       | 3                        | 0.67 |
| 26     | IPES0185   | TCTGCTTGTGTTTTACCCCC            | CGTTGGTACCCATGATTTTCA           | 110-230       | 4                        | 0.78 |
| 27     | IPES0203   | CCCTCGAAGAGATCGAAGTG            | CTGAAACAACAGCCTGCAAA            | 210-260       | 3                        | 0.65 |
| 28     | IPES0001   | ACATTGACCTCCACAGCCTC            | TTCTTCCATGTTTGGAATG             | 120-200       | 2                        | 0.42 |
| 29     | IPES0181   | CTCCTCAAACACATCCAGCA            | AAGGTGCTGCTCTTGTAGCC            | 200-650       | 4                        | 0.72 |
| 30     | IPES0098   | ATCAAGCTTCATACCCCTGC            | CATCTTCTCATCATCTTCGC            | 120-140       | 2                        | 0.49 |
| 31     | IPES0233   | TCCATCATCCATGTGAGACC            | TCAAAGATGAATGTATTACACAACT       | 240-270       | 2                        | 0.50 |
| 32     | IPES0221   | TAGCCAGCGCTTCTCTAGC             | TCAGGAACCCACCTAGC               | 230-270       | 2                        | 0.47 |
| 33     | IPES0214   | CTGATGGTGATGCAATGGAC            | CTTCTCCCTTCAGTGGCTG             | 130-310       | 4                        | 0.75 |
| 34     | IPES0042   | GATAGAAGCAGATGGGCCTG            | CTCGTCATCTCTCGCCAC              | 210-410       | 3                        | 0.71 |
| 35     | IPES0176   | TGGTGCAAGAATGACCATGT            | CGCAGGATTACAAACATCCA            | 110-250       | 2                        | 0.66 |
| 36     | IPES0027   | TGCTTGGGACAAAAGGCT              | TAAGTCAAGTGAGCGCAAGG            | 250-500       | 4                        | 0.67 |
| 37     | IPES0217   | TCAGTTCCGTCCACTGTGAG            | CCATAATTTCCGGGCTTG              | 220-550       | 3                        | 0.58 |
| 38     | IPES0236   | GGCCAGCTCGCCTAGAT               | AGATCCACCGCCTAATGAAA            | 200-290       | 3                        | 0.69 |
| 39     | IPES0210   | ATTCTCTGTATGCCGAAGAC            | GCACCATGACCACAAAATG             | 170-250       | 2                        | 0.44 |
| 40     | IPES0045   | CAGCACCATTAGTGGCAAAA            | CGTAACCTTTGGTCAGGCATACA         | 210-280       | 2                        | 0.56 |
| 41     | IPES0105   | GGGGGCTCACAGAACAAGTA            | CCGAAGTCCCACAGAATGT             | 150-190       | 2                        | 0.54 |
| 42     | IPES0126   | CCAGCAGGGAAGTCTTTTAC            | AAAGGCGCTTGTGATTTT              | 110-190       | 3                        | 0.42 |
| 43     | IPES0009   | TTGATCGATCGTCTACGGTT            | TATCTCACTCACGGCAGCG             | 140-370       | 4                        | 0.77 |
| 44     | IPES0127   | TGTACAAATGATACTTGATATCCAAA      | TGCAGAATTACACTGCCCTG            | 220-280       | 2                        | 0.68 |
| 45     | IPES0076   | ATCCTTAGCAGGACAAGCGA            | CCAAGCTTTTGGTTCAGGAG            | 180-240       | 3                        | 0.51 |
| 46     | IPES0226   | CACCAACAGCATCAAGCAG             | AGGAGGGTAAACACACGCAC            | 360-500       | 3                        | 0.60 |
| 47     | IPES0079   | GTTGGACAGCGCAACGATAC            | AGCTCTCTGCATTTTTCGTG            | 210-430       | 3                        | 0.64 |
| 48     | IPES0007   | ACACCTCGTGCACCTCTA              | GCAACACAGATGAGACTGGC            | 130-180       | 2                        | 0.46 |
| 49     | IPES0197   | GTGTTCTTCCGAATCCGTGT            | CGCTTTGCATTGAACACAGT            | 230-280       | 2                        | 0.57 |
| 50     | IPES0146   | CATCAGAATACGGACGCCTT            | CATCAGCTTTGGAGTCAGCA            | 130-360       | 3                        | 0.56 |
| 51     | IPES0101   | CCTGGAAGGAGGGAGAACAG            | GATAGCCCAAAGGCAACAAA            | 300-500       | 4                        | 0.75 |
| 52     | IPES0103   | CATGCCAAATCATCTCGATCT           | CTGAACCCGGAATTGCATAC            | 180-280       | 3                        | 0.58 |
| 53     | PSMP2236   | ATAAGTGGGACCCACATGCAGCAC        | CGAAAGACTAGCAAAATGCGCCTTC       | 250-500       | 4                        | 0.74 |
| 54     | PSMP2076   | GGAATGATATATTGGCAAAATGTG        | ATACTACACTGTAAGCATTGTC          | 140-170       | 2                        | 0.35 |
| 55     | PSMP2080   | CAGAATCCCCACATCTGCAT            | TGCAACTGAGCGAAGATCAA            | 190-200       | 2                        | 0.38 |
| 56     | PSMP2251   | TCAAACATAGATATGCCGTGCCTCC       | CAGCAAGTCGTGAGGTTTCGGATA        | 200-250       | 2                        | 0.53 |
| 57     | PSMP2237   | TGGCCTTGGCCTTTCCACGCTT          | CAATCAGTCCGTAGTCCACACCCCA       | 190-270       | 3                        | 0.61 |
| 58     | PSMP2008   | GATCATGTTGTCATGAATCACC          | ACACTACCTACATACGCTCC            | 170-290       | 3                        | 0.64 |
| 59     | PSMP2063   | GAGCACATGAAATAGGAAGCAG          | AAGGTAGTTATAGTTAGCTTGATC        | 180-220       | 2                        | 0.56 |
| 60     | PSMP2261   | AATGAAAATCCATCCATTTTCGCC        | CGAGGACGAGGAGGGCGATT            | 190-400       | 4                        | 0.75 |
| 61     | PSMP2248   | TCTGTTGTTTGGGTGAGGTCCTTC        | CGAATACGTATGGAGAAGTGCATC        | 150-180       | 2                        | 0.55 |
| 62     | PSMP2074   | AGGACTGTAGGAGTGTGGACAAC         | ACAACCAGACCTACCAGTGAATGAGA      | 110-600       | 4                        | 0.78 |
| 63     | PSMP2040   | CATTACACGTTTCTTCAAACGC          | TCTTCGGCCTAATAGCTCTAAC          | 130-180       | 2                        | 0.44 |
| 64     | PSMP2227   | ACACCAAGACCAACCATAAAG           | TCGTACGCAATCACTAATGACC          | 170-280       | 2                        | 0.47 |
| 65     | PSMP2249   | CAGTCTCTAACAAACAAACACGGC        | GACAGCAACCAACTCCAACTCCA         | 140-150       | 2                        | 0.49 |
| 66     | PSMP2202   | CTGCCTGTTGAGAATAAATGAG          | GTTCCGAATATAGAGCCCAAG           | 140-180       | 2                        | 0.35 |
| 67     | PSMP2263   | AAAGTGAATACGATACAGGAGCTGAG      | CATTTACGCCGTTAAGTGAGACAA        | 220-290       | 3                        | 0.57 |
| 68     | PSMP2086   | CGCTTGTCTTCTTCTTGCTGTT          | CCTTCTCAGATCCTGTGCTTTCTT        | 110-170       | 3                        | 0.68 |
| 69     | PSMP2266   | CAAGGATGGCTGAAGGCTATG           | TTTCCAGCCACACAGTAATC            | 180-290       | 3                        | 0.56 |
| 70     | PSMP2229   | CCACTACCTTCGTCTTCTCCATTC        | GTCCGTTCCGTTAGTTGTTGCC          | 130-270       | 3                        | 0.74 |
| 71     | PSMP2232   | TGTTGTTGGGAGAGGGTATGAG          | CTCTCGCCATTCTTCAAGTTCA          | 220-260       | 2                        | 0.58 |

## Conclusion

Micronutrients are a very important component in our diet and increasing their concentration in staple crops is very essential. Biofortification of cereals is a very effective strategy to develop high-yielding and micronutrient rich cultivars and the researchers must collaborate to breed for these micronutrient traits. The multidisciplinary approach can play a vital role in this direction and can help to eradicate malnutrition and also ensure nutritional security. Joint efforts of nutrition missions, millet missions and other government-sponsored missions and agencies can also play a significant role to overcome the hurdles and issues faced by researchers and production organizations so that we can get the maximum benefits from the biofortified crops. The findings of this investigation will prove helpful to develop zinc and iron rich pearl millet hybrids and varieties which can further eradicate hidden hunger and malnutrition. Bioavailability studies for these micronutrients are a very important and future thrust area which needs to be explored to get maximum benefits out of this staple crop.

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