



Genetic diversity in fresh fruit pulp mineral profile of 100 Indian *Musa* accessions

Ramajayam Devarajan^{*}, Jeyabaskaran Kandallu Jayaraman,
Saraswathi Marimuthu Somasundaram, Sivasankari Ragupathy, Pitchaimuthu Raman,
Kalpana Sathiamoorthy, Uma Subbaraya

ICAR-National Research Centre for Banana, Tiruchirapalli 620 102, Tamil Nadu, India

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ABSTRACT

Evaluation of 100 Indian *Musa* accessions (IMA) for nine elements in their fresh fruit pulp (FFP) revealed genetic variability of 4.7-fold for K & Mg to 111.1-fold for Ca but, only with either highly or moderately positively skewed distribution. The descending order of mineral concentrations (MC) was $K > Ca > Na > Mg > Fe > Mn > B > P > Zn$. 100 g FFP contributes fairly about 5 (Fe) to 10% (Mn, Ca & Mg) of daily mineral requirement of Indians. Calcium (97%) and Fe (96%) showed the highest heritability while Zn exhibited lowest (85%). Significantly positive correlation was observed for all minerals. Magnesium had maximum direct effect on Fe content followed by Mn, Zn and Na in path analysis. Both principal component analysis and cluster analysis failed to group the IMA according to their ploidy/genome/subgroups. Twenty commercial cultivars were placed in top 10 positions based on their MC. Besides Ca and Mg, IMA were richer for all micronutrients than the world's *Musa* gene-pool.

1. Introduction:

Bananas (Banana and Plantain) are important staples, ranking next only to rice, wheat and maize in terms of their importance and consumption in >150 producing countries (<https://www.bananalink.org.uk/all-about-bananas/>). Currently, the world total production is 166.80 million tonnes and India the largest producer in the world, contributes 30.81 million tonnes. However, India exports only about 0.12 million tonnes, and the rest of bananas (over 99.6%) is consumed locally as they are cheap and available throughout the year (FAOSTAT, 2020). Accordingly, the per capita consumption of bananas in India was highest in the world during the past decade and is projected to remain plateau of 23.5 Kg till 2029 (OECD/FAO, 2020). Cultivating micronutrient-rich banana varieties has the potential to significantly reduce micronutrient deficiencies afflicting more than half of the world's population, especially the women and preschool children in most countries of Africa and Asia who are vulnerable to this 'hidden-hunger' (FAOUN, 2015). The most cost-effective approach for mitigating micronutrient malnutrition is to introduce banana varieties selected and/or bred for increased mineral nutrients through plant breeding. Though the transgenic approach is currently employed to develop Fe- and Zn-rich

bananas (Kumar et al., 2011; ICAR-NRCB, 2020), attempts to breed enhanced fruit nutrient contents for other minerals are still in their infancy.

India is a home to wide range of banana cultivars belonging to different ploidy/genomic groups/subgroups (PGS) in addition to 34 wild taxa from two genera out of the three in the *Musaceae* Family viz. *Ensete* and *Musa* (Joe et al., 2014). Though, the number of banana cultivars grown in India is hard to pin down owing to synonyms and homonyms, over 50 cultivars are cultivated commercially in different Indian states (http://nhb.gov.in/report_files/banana/BANANA.htm). 'Grand Naine' occupy the first position in production share (63%), followed by 'Poo-van' (17%) and 4% each of 'Nendran', 'Rasthali', 'Bluggoe', 'Pome' and other cultivars (Vision 2050, 2015)

Significant differences for a given mineral contents may not only be due to the influence of banana cultivars (Davey et al., 2009; Pillay & Fungo, 2016; Sulaiman et al., 2011; Leterme et al., 2006; Gibert et al., 2009; Deshmukh et al., 2009) but also due to soil, climate, agricultural practice, quality of water for irrigation as well as storage conditions (Tahvonon, 1993; Bugaud et al., 2009; Cano et al., 1997; Forster et al., 2002; Arvanitoyannis & Mavromatis, 2009). Further, the composition of banana may vary depending on diverse sampling and analytical

^{*} Corresponding author at: ICAR-National Research Centre for Banana, Tiruchirapalli 620 102, Tamil Nadu, India
E-mail address: Ramajayam.Devarajan@icar.gov.in (R. Devarajan).

strategies employed (Davey et al., 2009), fruit developmental stage and growing conditions (Obiageli et al., 2016). Despite the historical, economic and social importance of bananas, there have been no systematic studies on the macro- and micronutrient content of Indian *Musa* diversity to date, and have only been limited to few cultivars (Deshmukh et al., 2009; Narayana et al., 2017). To our knowledge, globally few reports exist for pulp mineral contents, up to maximum of 47 genotypes (Davey et al., 2009; Pillay & Fungo, 2016) despite the vast diversity of over 6500 accessions and > 1000 varieties subdivided into 50 groups (<https://www.bananalink.org.uk/all-about-bananas/>).

Thus, this study aims (i) to assess the genetic variability of nine fruit pulp mineral (FPM) contents (Ca, P, K, Mg, Na, B, Fe, Mn, and Zn) in 100 indigenous banana genotypes among our strategic field gene-bank collections, comprising of six genomes and more than one dozen subgroups, representing both diploids and triploids, and (ii) identify promising accessions/cultivars with higher content of different FPMs that can be used in crosses, genetic studies, molecular-marker development, parent-building, etc. or for direct commercialization.

2. Materials and methods

2.1. Plant material and study site description:

From the available 374 banana germplasm (ICAR-NRCB, 2020), only 100 Indian *Musa* accessions (IMA) belonging to six genomic groups viz., AA (2 genotypes), BB (2), AB (6), AAA (7), AAB (47) and ABB (36) were utilized for this study based on their bunch availability. All the 374 genotypes were field planted during January 2017 and grown under a standard package of practices (Uma et al., 2020) at 2.0 × 2.0 m spacing in the Research Farm of the ICAR-National Research Centre for Banana (11.50°E latitude and 74.50°E longitude, 90 m in altitude) with four plants per accession. The predominant climate is warm and humid, with a relatively medium rainfall of 800 mm. Average monthly maximum and minimum temperature ranges were 30–41 °C and 21–29 °C, respectively with average monthly relative humidity of 34 to 82%.

Soils are silty clay loam (*Typic Ustropept*, mixed, hyperthermic) with the following chemical characteristics at a depth of 0–20 cm: pH (1:2.5), 8.7; EC, 0.2 dS/m; organic carbon, 0.23%; CaCO₃, 5.2%; CEC, 11.5 cmol/kg; N, 230 kg/ha; P₂O₅, 8.5 kg/ha; K₂O, 150 kg/ha; Ca, 924 kg/ha; Mg, 471 kg/ha; B, 1.6 kg/ha; Cu, 0.9 kg/ha; Fe, 1.2 kg/ha; Mn, 3.1 kg/ha; and Zn, 2.9 kg/ha. The suckers were planted in a one cubic foot pit with basal application of well decomposed cattle manure (2 kg/pit) with full dose of P (30 g per plant as single super phosphate), after proper sucker treatments. The Nitrogen (as Urea), 200 g and Potassium (as KCl), 400 g per plant were given as soil application in three equal splits at 3rd, 5th and 7th month after planting (MAP). The plants were also foliar sprayed with 2% of 'Banana Shakti' (a micronutrient mixture, containing 2.50% B, 2.40% Cu, 4.75% Fe, 4.50% Mn and 5.25% Zn developed by our Centre) with wetting agent at 4th, 5th and 6th MAP.

2.2. Sample collection and preparation:

Owing to the diversity of germplasm, sampling could not be made at the same time per season over seasons, but bunches were selected as consistently as possible for a given genotype. List of IMA sampled in this study and their details as recorded in the ProMusa website (<http://www.promusa.org/Banana> + cultivar + checklist) were given in the Supplementary table (ST). 1. For each genotype, three replicate bunches were sampled between January and March of 2018. Selected bunches were harvested at unripe fully mature stage (stage 1) and the second hand in each bunch was kept in the ripening chamber (Blue star, 2 tonnes capacity) and infused 100 ppm of ethylene for 24 h (18 ± 2 °C; RH: 85 ± 2%). Then the hands were shifted to a storage chamber (Blue star, 2 tonnes capacity) for ripening at 22 ± 2 °C with relative humidity (%) of 85 ± 2. Fruit maturity stage was estimated according to peel colour, as described by Dadzie & Orchard, (1997). Four fruits were sampled from

the middle of the second hand, at the fresh ripe stage corresponding to stage 6, when fruit colour turned yellow and the full flavor and adequate conditions for its consumption were observed.

2.3. Sample processing and analysis of mineral nutrients contents:

For each sample, 5 g homogenized pooled fresh fruit pulp (FFP) was quickly weighed in 150 ml conical flask, and dried overnight at 60 °C in a thermostatted oven. The FFP samples were then digested in tri-acid (Sulphuric acid: Nitric acid: Perchloric acid in a ratio of 7:5:3) overnight. The samples were digested with care on a hot plate at 70 °C kept in a fume hood chamber until the solution was colourless or nearly so. When the digestion was completed, the samples were cooled, transferred to a 100 ml volumetric flask with distilled water and the final volume was made to the mark. The solution was filtered through Whatman No.42 filter paper and stored in airtight polypropylene containers before use. Blank digestion in triplicate was carried out in the same way (Tandon, 1995).

Mineral contents were analyzed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Prodigy 7, Serial No.: P70174, Teledyne Leeman Labs, USA), using proprietary protocols (Wavelength range = 165 to 1100 nm; RF (solid state) power: 40 MHz; Spectrometer = High power Echelle polychromator; Plasma view = Dual (axial and radial); Gas = Argon for plasma and Nitrogen for purging; sample introduction by variable speed 4 channel peristaltic pump; Cyclone spray chamber with concentric glass nebulizer). The manufacturer's standards were analyzed to verify the accuracy of the calibration before, during and after sample analysis. The results were corrected for the dilution factor resulting from the digestion procedure and the mineral content was then converted into mg/Kg on fresh weight (f.w.) basis.

2.4. Statistical analysis:

The experiment was carried out following randomized complete block design with three bunches as biological replicates (blocks). The data set of mineral contents were submitted to one-way analysis of variance (one-way ANOVA), followed by the Tukey HSD at 1% for mean comparison among the genotypes. The one-way ANOVA, correlation, frequency distribution, boxplot of the data set were performed using SAS software hosted at ICAR-IASRI, New Delhi (<http://stat.iasri.res.in/SASLogon/index.jsp? sasapp = Information + Delivery + Portal + 4.3&>). The path coefficient analysis was done online using <http://14.139.232.166/opstat/>, and agglomerative cluster analysis and PCA using the algorithms (correlation matrix method is adopted for data analysis) of the statistical software STAR (Statistical Tool for Agricultural Research), Version: 2.0.1, International Rice Research Institute (IRRI) 2013–2020. The mean values of mineral contents were input as variables and the accession numbers were input as cases. A Ward's method was followed using the squared Euclidean distance as a measure of interval. A dendrogram was then generated to cluster the accessions into groups.

3. Results and discussion

3.1. Mineral concentration and genetic diversity in the FFP nutrients of Indian *Musa* accessions:

The mean FPM content (mg/Kg) of 100 IMA along with standard deviation (SD), as estimated by ICP-OES, are summarized in ST.2a (macro-) & 2b (micro- nutrients). The means of all the 100 IMA for each minerals were compared post hoc using the Tukey's HSD test at 1%. Wide variability and highly significant differences (P < 0.0001) were observed for all the FPM contents among the 100 IMA selected for this study. ANOVA revealed highly significant genotypic differences for all FPM contents. Moreover, highly significant mean squares were recorded for all FPM contents as shown in ST.3. Similarly, substantial variation for

nine different FPM concentrations in 100 IMA is presented in the form of box-plot in [Supplementary Figure \(ST\)0.1](#). This indicates that IMA have high variability for all FPM contents.

The frequency distribution of 100 IMA, at concentration intervals (mg/Kg), according to their mean FPM concentrations is summarized in [Fig. 1](#) (a-i). for all the nine elements studied. Highly positively skewed distribution (PSD) (skewness > 1.0) was observed for most of the minerals (B, Ca, Fe, Mg, Mn, Zn and Na) except for K and P which showed moderately PSD (skewness > 0.5 and < 1.0). None of the FPM showed either symmetric (-0.5 and 0.5) or negatively skewed distribution (>-0.5) ([Table 1](#)). PSD among the minerals indicate that majority of the IMA have low magnitude in FPM contents. In other words, it is difficult to find accessions with values for these minerals high enough to be included in the breeding programme.

Based on the skewness patterns, it is possible to infer the magnitude and nature of alleles governing the traits. It is evident that there are more mineral-decreasing-alleles that are dominant for B, Ca, Fe, Mg, Mn, Zn and Na, minerals which are highly positively skewed (PS). For minerals with moderately PSD i.e., K and P, this implies that mineral decreasing alleles are in slight excess and dominant. [Hardisson et al. \(2001\)](#) also observed a similar PSD for most minerals in bananas from the island of Tenerife. However, this contrasts the results reported by [Davey et al. \(2009\)](#) for the 47 genotypes analysed where, Fe and Zn contents showed normal distribution. This is indeed possible as they obtained the fruit samples from very different locations and grown in different soil types. The concentration as well as the degree of variation in fruit pulp (FP) Fe and Zn contents was slightly lower than that

measured in our present study, since the values expressed were on a d.w. basis.

The extent of variability ([Table 1](#)) among IMA was also analyzed in terms of range, coefficient of variation (CV), and genotypic and phenotypic coefficient of variation (GCV & PCV). Substantial variability for all the nine FPM was evident from the estimates of range and coefficient of variation (CV) which was highest for Ca (101.1%) followed by B (94.8%), Fe (76.8%), Zn (54.9%), Mn (52.6%) and lowest for K (24.7%). Similarly, Ca, B, Fe, Zn and Mn exhibited comparatively higher estimates of GCV as well as PCV ($\geq 50\%$). The magnitude of PCV was slightly higher than the corresponding GCV for all the mineral contents which reflects the influence of environment on the accumulation of these minerals in the FFP. This finding was similar to what [Singh et al. \(2009\)](#); [Upadhyaya et al. \(2011\)](#); [Gerrano and Tejada Moral \(2017\)](#) found in germplasm of cabbage, finger millet and okra, respectively. The minerals with high GCV (Ca, B, Fe, Mn and Zn) possess a higher magnitude of variability and, thus, present a possibility of improvement through different breeding strategies ([Singh et al., 2009](#); [Upadhyaya et al., 2011](#)).

Significant variations for different FPM contents in banana were reported by various workers earlier ([Davey et al., 2009](#); [Deshmukh et al., 2009](#); [Fungo et al., 2010](#); [Pillay & Fungo, 2016](#)). A high heritability (>85%) was observed for all the FPM contents which indicated that a large portion of phenotypic variance is contributed through genotypic variance and, therefore, reliable selection for higher mineral content in banana is possible through breeding approaches. Similarly, the magnitude of GA as percentage of mean was high ($\geq 95\%$) for Ca, B, Fe, Mn and

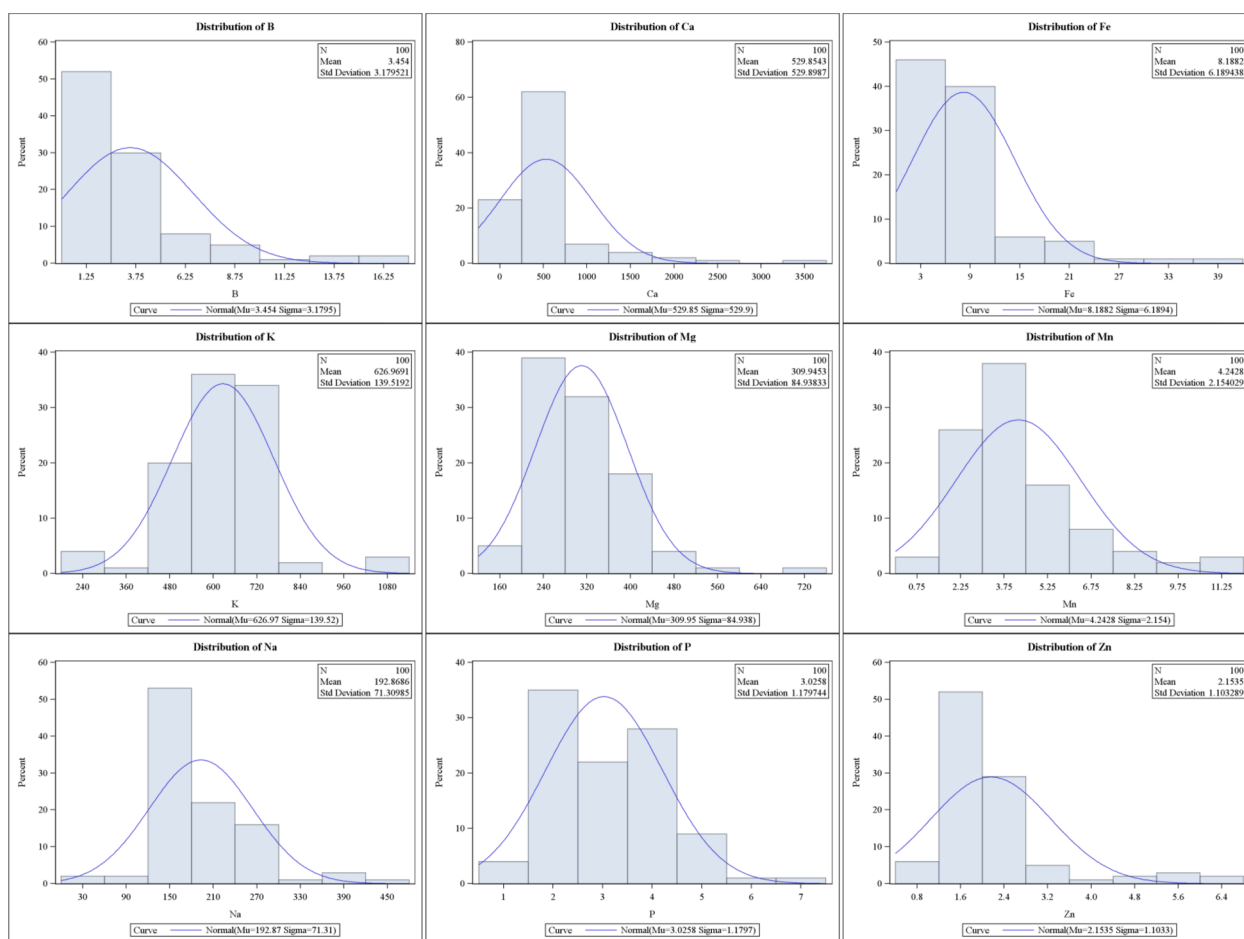


Fig. 1. Univariate frequency distribution pattern of 100 Indian *Musa* accessions for fruit pulp concentrations of the following nutrients: (a) Boron, (b) Calcium, (c) Iron, (d) Potassium, (e) Magnesium, (f) Manganese, (g) Sodium, (h) Phosphorous and (i) Zink. *x*-axis denotes percentage of genotypes, while *y*-axis represents concentrations (ppm).

Table 1

Estimates of descriptive statistics (including the range, mean, SD, SE, CV (%), Skewness and Kurtosis), variance, heritability and genetic advance for nine different fruit pulp mineral contents of 100 Indian *Musa* accessions (n = 300).

Minerals	Range(mg/Kg f. w.)	Mean (mg/ Kg f.w.)	SD	SE	CV (%)	Skewness	h ² (%)	GCV (%)	PCV (%)	GA	GA as % means	Over all range of the published mineral content of bananas in the literatures outside India on wet & dry weight basis	mg/ Kg d.w.
Ca	28.0–3986.0	529.9	535.5	30.9	101.1	3.1	97.0	99.5	101.0	1069.5	201.9	1.0–686.0	5.3–950.0
Mg	133.7–871.1	309.9	91.4	5.3	29.5	1.8	91.0	27.0	28.3	164.2	53.0	100.0–680.0	34.3–1530.0
P	0.5–7.8	3	1.3	0.1	41.7	0.8	85.7	38.0	41.0	2.2	72.4	20.0–943.0	78.1–2679.0
K	209.8–1293.3	627	155	8.9	24.7	0.7	87.8	21.8	23.2	263.3	42.0	590.0–7500.0	100.0–14747.0
Na	34.1–534.7	192.9	74.6	4.3	38.7	1.5	94.6	36.6	37.7	141.5	73.4	8.0–1100.0	1.5–1325.0
B [#]	0.0–19.1	3.5	3.3	0.2	94.8	2.3	91.9	90.7	94.6	6.2	179.1	0.8–3.2	0.3–8.1
Cu ^{*,*}	0.0–2.8	0.4	–	–	–	–	–	–	–	–	–	–	–
Fe	0.7–45.8	8.2	6.3	0.4	76.8	2.7	96.3	75.1	76.6	12.4	151.8	0.2–26.2	1.0–45.0
Mn	0.7–13.7	4.2	2.2	0.1	52.6	1.5	93.4	50.2	51.9	4.2	99.9	0.6–24.0	0.3–62.6
Zn	0.6–9.4	2.2	1.2	0.1	54.9	2.6	85.1	49.8	54.0	2.0	94.6	0.0–12.2	0.1–11.4

[#] Value of limit of quantification (LOQ) for B and Cu is 0.10 mg/Kg and 0.035 mg/Kg, respectively;

* Based on only 13 out of 100 accessions and not subjected for any statistical analysis; CV = Coefficient of variation indicating variability among genotypes; SD = Standard deviation; SE = Standard error; h² = heritability; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; GA = Genetic advance.

Zn (Table 1). In the present study, a high heritability accompanied with high GCV and GA for Ca, B, Fe, Mn and Zn suggest the role of additive gene action, and hence a high genetic gain is expected from selection for these FPMs; while non-additive gene action was responsible for the rest of the minerals (K, Mg, P and Na) as revealed by low to moderate GA (42.0 – 73.4%) along with high heritability (>85%) which could be enhanced in the FP through heterosis breeding.

In general, K was highest in the IMA, followed by Ca, Mg, Na, Fe, Mn, B, P and Zn. The results obtained in our study are in line with the previous quantification reports in banana for all the minerals except for P content on fresh weight (f.w.) basis, which is much lower than those previously reported both in India (Siji & Nandini, 2017; Longvah et al., 2017) as well as from other countries (ST.4). Interestingly, values obtained for micronutrients (B, Fe, Mn and Zn) on a f.w. basis in this study fell within the ranges reported in large number of previous studies published in other countries which had expressed on a d.w. basis (ST.4), although ripe banana fruit is considered to have a moisture content of 60–80% (Mayer, 1997; Wall, 2006; Hapsari & Lestari, 2016; Narayana et al., 2017). Therefore, to know the nutritious value of IMA, the ranges obtained in this study for different FPM contents were compared with the previously published 28 literatures from other countries (ST.4.) which focused mainly on the FPM contents of banana both on f.w. and d. w. basis.

3.1.1. Macronutrients

3.1.1.1. Calcium. In this study, calcium is the second most abundant mineral found in banana FFP. Values for Ca content ranged from 32 to 3523 mg/Kg with an average of 530 mg/Kg and a CV of 101.1%, representing a range of 111.2-fold estimated in this study. This suggests that there is potential for the genetic improvement of IMA for enhanced Ca level by breeding. The distribution patterns for Ca also revealed that 74 IMA had lower than the average Ca content (530 mg/Kg) recorded in this study while seven genotypes had higher than the average Ca content i.e. 3.2-fold (1681 mg/Kg) to 6.7-fold (3523 mg/Kg). These Ca content ranges correspond well with the values reported in literature on banana on f.w. basis (see ST.4 and references (ST4R) 1–6, 8 & 12), as well as on d.w. basis (ST4R 13–28, except 15 & 24). However, a couple of studies on f.w. (ST4R 10) as well as d.w. basis (ST4R 15 & 24) reported lower Ca contents than our range.

3.1.1.2. Magnesium. Magnesium concentrations in IMA ranged from 158 to 749 mg/Kg, representing a range of 4.7-fold with a general mean of 310 mg/Kg and low CV (29.5%). Ninety-eight IMA had an Mg concentration of <460 mg/Kg, and the remaining two accessions had 590

mg/Kg and 749 mg/Kg. The values correspond well with the values in the range of 100–680 mg/Kg reported in literature (ST4R 1–12). However, the mean Mg contents reported on d.w. basis in few studies are higher (ST4R 18, 19, 24, 26 & 28) similar (ST4R 13–16 & 22) and /or much lower than the present study (ST4R 21 & 25).

3.1.1.3. Phosphorus. Values for P content ranged from 0.60 to 7.27 mg/Kg with a general mean of 3.03 mg/Kg and a high CV of 41.7%, which is much lower than all the previously published literature reports (ST4). The distribution patterns for P revealed that >50 percent of IMA (57 accessions) had lower than the average P content recorded in this study. Only five genotypes recorded higher than 5.0 mg/Kg. This contrasts with all previously published studies who reported > 2 to 118-fold and > 78 to 2519-fold more than our values on f.w. and d.w. basis, respectively.

3.1.1.4. Potassium. Bananas are a good source of K in human diet (Wall, 2006), and our study recorded mean content of 627 mg/Kg, but representing a narrow range of 4.7-fold with lowest CV (24.7%) and skewness (0.7) than the rest of the minerals analyzed in this study. As with Mg concentration, it seems that the degree of genotypic variation in K content is also limited, indicating that no IMA possesses extremely high or low K content. The distribution patterns for K content followed near normal, with almost 85 IMA falling between 480 and 720 mg/Kg. Seven accessions had values lower than 480 mg/Kg and eight accessions recorded higher than 720 mg/Kg. Some earlier studies have provided similar data for FP potassium content expressed on f.w. (ST4R 3) as well as on d.w. basis (ST4R 15, 21 & 27). However, the degree of variability for K content in our study (241.1 ± 32.6–1134.3 ± 153.4 mg/Kg) is much lower than those previously reported for bananas both on fresh (59–4000 mg/100 g) and d.w. basis (0.1–15900 mg/Kg). Only Khawas et al. (2014) from India and Obiageli et al. (2016) from Nigeria reported lower K range even on d.w. basis than our range.

The reasons for deviation of both P and K contents in the present study from that of previously reported could be due to many factors like use of different banana cultivars (Forster et al., 2002; Davey et al., 2009; Pillay & Fungo, 2016; Sulaiman et al., 2011; Leterme et al., 2006; Gibert et al., 2009; Deshmukh et al., 2009), differences in growing climatic conditions, agricultural practices, soil and water quality (Cano et al., 1997; Bugaud et al., 2009; Arvanitoyannis & Mavromatis, 2009;), and even maturity stages, diverse sampling and analytical strategies followed (Forster et al., 2003; Davey et al., 2009; Obiageli et al., 2016).

3.1.1.5. Sodium. The Na concentration recorded for the 100 IMA ranged from 40 to 467 mg/Kg (mean of 193 mg/Kg), representing a

range of 11.7-fold with a low CV of 38.7%. Majority of the IMA (92) had a Na concentration between 119 and 298 mg/Kg. Only three accessions, namely 'Boddida Bukkisa' (40 mg/Kg), 'Kunnan' (51 mg/Kg) and 'Pidi Monthan' (75 mg/Kg), recorded low Na concentration. The values obtained in this study fall within (ST4R 1, 3, 5, 6 & 10) and/or even below the ranges reported for bananas on f.w. basis (ST4R 2, 4, 8 & 12) except the range reported by Siji & Nandini (2017). However, few studies also report much lower values even on d.w. basis (ST4R 13, 21 & 22).

3.1.2. Micronutrients

3.1.2.1. Boron: The B concentration in the FFP of IMA ranged substantially from 0.00 (Limit of Quantification (LOQ) = 0.10 mg/Kg) to 17.16 mg/Kg with a general mean of 3.45 mg/Kg and agrees well with the published results for *Musa* on f.w. (ST4R 3 & 6), as well as on d.w. basis (ST4R 15 & 18). There is a >17-fold variation in B levels with a high CV (94.8%) of IMA. However, the univariate distribution patterns for B revealed that most of the IMA (71 genotypes) had low B content (<3.75 mg/Kg) and only 5 genotypes recorded > 10 mg/Kg.

3.1.2.2. Copper: In our study, only 13 IMA (ST.2b) comprising commercial cultivars (7 numbers) and other accessions (6 numbers) out of 100 IMA showed the presence of Cu (0.04 to 2.08 mg/Kg), and no Cu (LOQ = 0.035 mg/Kg) was detected in rest of the IMA. Majority of the previous works, reported no Cu (Sulaiman et al., 2011; Adamu et al., 2017) while some did on f.w. (ST4R 2–6 & 12) and d.w. basis (ST4R 13–16, 18, 24 & 28) which corroborate our values.

3.1.2.3. Iron: Of all microelements estimated, Fe content was found to be high in the FFP of IMA. Significantly highest Fe content of 41.3 mg/Kg was recorded in 'Rajapuri' followed by 'Eathen' (32.2 mg/Kg), 'Anaikomban' (26.2 mg/Kg) which was on par with 'Kapur' (22.6 mg/Kg) while the lowest Fe was detected in 'Hybrid Co-1' (0.8 mg/Kg). This represents a 51.6-fold range of variation, very similar or lower (Fungo et al., 2010) to all the previously reported results obtained on f.w (ST4R 1–7 & 10–12), as well as even on d.w. basis (ST4R, except 28), except for the report of Goswami & Borthakur (1996) and Borges et al. (2020) from India and Brazil, respectively who reported higher range than the present study. The distribution of mean Fe contents across the 100 IMA clearly shows that Fe concentrations are non-normally distributed (i.e. PS), with most accessions (77) having a mean Fe content of <10 mg/Kg. In fact, the number of IMA having Fe contents higher than 10 mg/Kg decreases exponentially, and only 8 accessions had values higher than 15 mg/Kg whilst the accessions with the top 10 highest Fe contents, cover a range from 14.0 to 41.3 mg/Kg. This contradicts with the report of Davey et al. (2009) which shows normal distribution for the FP Fe contents within the 47 genotypes. Moreover, the concentration as well as the degree of variation in FP Fe contents was slightly lower than our present study, considering that the values expressed were on a d.w. basis. This is possible as they have obtained the fruit samples from very different locations with very different soil types. Generally, plant tissue mineral micronutrient contents are strongly dependent on the soil availability of Fe and Zn, which itself depends on factors such as the soil pH, redox status, cation exchange capacity, water content, plant root architecture, and the presence of mycorrhizal fungi (Davey et al., 2007).

3.1.2.4. Manganese: The Mn concentration in the FFP of IMA ranged substantially from 0.79 to 11.36 mg/Kg, representing a 14.4-fold range with a general mean of 4.24 mg/Kg. Frequency distribution for Mn concentration presented in Fig. 3F shows that sixty IMA had less than mean Mn concentration, and only ten accessions recorded >7.28 mg/Kg. The Mn ranges obtained in this study are in line with all the previous quantification reports obtained both on fresh as well as d.w. basis (ST4R) except, Forster et al. (2002) and Adamu et al. (2017) who reported slightly lower Mn content (<0.76 mg/Kg) on d.w. basis.

3.1.2.5. Zinc: Zinc values for 100 IMA ranged from 0.67 to 6.59 mg/Kg with an average of 2.15 mg/Kg. These values are, again, very similar to all the previously reported results obtained on fresh (ST4R 3–7 & 9–12) as well as d.w. basis (ST4R 13–16, 18, 20, 22 & 25–27) except, Obiageli et al. (2016) who has reported slightly lower values. However, the mean Zn contents reported on d.w. basis by Narayana et al. (2017) from India and couple of studies from other countries are higher (ST4R 17 & 28) than the present study. Among the 100 IMA, 'Kari Bontha' had the highest Zn concentration on par with 'Bluggoe', 'Chinali483', 'Chinia' and 'Thenkali' (5.36 to 6.59 mg/Kg). There is 9.8-fold variation in Zn level with a high CV of 54.9% recorded, however the distribution pattern revealed that ninety IMA recorded below 3.2 mg/Kg (i.e. PS). Similar to Fe, this contrasts with the results of Davey et al. (2009) for the distribution of FP Zn contents, which again is statistically 'normally' distributed.

Although, fruit sampling was done in the gene bank located within our Research Farm, the large degree of variation in FPM contents for a given accession indicates that even establishing baseline levels under standardized growth conditions is not a trivial problem. Whilst the degree of variability among the 100 IMA is slightly higher for few macro- (Ca, Mg & Na) and all the microelements than that measured in most of the previous works (ST. 2 a & 2b), the degree of variation for the FFP macro- and microelement contents is still low, considering that fruit samples were obtained from different PGS backgrounds. This indicates a lack of genetic diversity in the mechanisms of some of the macro- and micro- nutrients uptake, sequestration and transport (Frossard et al., 2000). Generally, most present day banana cultivars are derived from two wild diploid ancestral species: *Musa acuminata* Colla and *Musa balbisiana* Colla. In the course of evolution, there have been haploid contributions from two more other species viz. *Musa schizocarpa* N.W. Simmonds and *Musa textilis* Née, either through pollen or ovary (Christelová et al., 2017). However, this does not detract from the fact that certain accessions are still able to accumulate 4.7- to 111.1-fold higher levels of macro- and micronutrients in their FFP under the same 'optimal' growth conditions there in this study. This indicates that IMA have high variability for all FPM contents.

3.1.3. Ranking of IMA, and contribution of commercial banana cultivars to Recommended dietary allowances (RDA) values of Indians

For each of the FPM, the highest 10 IMA were selected except for Na, for which the lowest 10 accessions were selected (ST.5). Out of 100 IMA, 51 were rich (or low for Na) at least in one FPM, 20 were rich at least in ≥ 2 FPM and 11 accessions rich at least in ≥ 3 FPM. Few of the IMA were also significantly higher for four ('Borchampa' and 'Rasthali') or five ('Bluggoe' and 'Kapur') FPM contents. Interestingly twenty IMA which are cultivated commercially in different parts of India are also placed in the list of top 10 accessions selected based on their FPM contents (ST.5). These 51 unique IMA high in one or more nutrients could be exploited for developing nutrient-rich and well-adapted high yielding banana hybrids.

Based on the average FPM contents calculated for all the commercial cultivars (39 out of 100) used in this study (Table 3), consumption of 100 g of banana pulp is shown to contribute fairly to the Indian adults' (men and women with sedentary/moderate/heavy physical activity) daily requirement in the ascending order of Mn (11.3%) > Ca (10.3%) > Mg (9.5% & 10.4%) > Fe (4.6% & 3.8%) > Cu (3.3) > Zn (1.8%&2.2%) > K (1.7% & 2.0%) > Na (0.9% & 1.0%) and > P (0.05%), according to the revised RDA values for Indians (ICMR, 2010). Therefore, even if these minerals were fully bioavailable, bananas by itself is unlikely to contribute significantly to the daily macro- and micro-mineral requirements of the population at normal consumption quantities. However, the contribution of Indian banana cultivars to RDA is either well-superior for Ca, Na, Fe, and Zn minerals, equal (Mg, Cu & Mn) or much lower for P, K & Cu minerals than the reported values of Canary/Ecuador (Forster et al., 2002) and Tenerife (Hardisson et al., 2001) bananas based on RDA/Estimated safe and adequate daily dietary intake

range' and 'Estimated daily requirements', respectively. While, these contents were either equal (Ca, Mg, Mn, Na, Fe & Zn) or lower (K, P & Cu) than the banana cultivars grown in Malaysia (Sulaiman et al., 2011) or Hawaii (Wall, 2006) based on 'Dietary reference intakes', and Indonesia (Hapsari & Lestari, 2016) based on 'Nutrient values daily need'.

3.1.4. Impact of PGS on accumulation of FPM contents:

ANOVA with post hoc comparison using Tukey's HSD for the means of all the ploidy/genomic as well as subgroups (ST.6) revealed no significant differences for all FPMs. More importantly, overall, there do not appear to be clear cut divisions among PGS, and that the genetic basis for high FPM content is widely distributed among all the IMA. The proportions of individual mineral nutrients in all IMA of their respective ploidy/genomic groups (Fig. 2.a & b) and subgroups (Fig. 2.c & d) revealed that about 99% of the FPMs estimated are macro nutrients. Potassium/Calcium and Fe were dominating in the genomic groups containing more B and A genome, respectively. Noteworthy, genomic groups containing more A genome (AA, AAA and AAB) tend to have the highest proportion of cations (Fig. 2d) controlled by ZIP gene family (Fe, Mn and Zn) than the genomic group containing more B genome (ABB and BB). Similarly, 'Silk' recorded the highest proportion of Ca (48%) followed by 'Ney poovan' & 'Mysore' (44%) while 'Kunnan' and 'Plantains' had the lowest (18–20%) but with highest K (47%) (Fig. 2c). In case of micronutrients, 'Plantains' possessed highest proportion of Fe (64%) while 'Cavendish', 'Silk' and 'Unique' subgroups had highest Zn (28%) (Fig. 2d). Clearly these mean proportions can only provide a broad indication of the possible importance of PGS composition, and should be interpreted with caution as the numbers of genotypes analysed within each PGS vary widely. Moreover, the cultivars may not be entirely 'representative' of that group. Nonetheless, Fig. 2c. confirms the findings of Pillay & Fungo, (2016) that the presence of the A genome in the genotypes used in this study may have influenced the uptake and transport of cations controlled by the ZIP gene family.

3.2. Correlation among FPMs:

Pearson correlation analysis was carried out to determine the strength of the relationship between the FPMs (Table 2). Boron was positively correlated with Ca, K and Mg. Iron had highly significant

positive correlations (SPC) of lower/moderate magnitude with Ca, Mg, Mn, Na and Zn (Pillay & Fungo, 2016). Manganese recorded positive and highly SPC of higher magnitude with Mg and Zn. It also recorded moderately SPC with Ca and Fe, and low magnitude correlations with Na and P. The present findings are in agreement with those of (Forster et al., 2002), who reported SPC of Mn with all the metals studied, except Fe. However, highly SPC of moderate/higher magnitude was observed for Zn with Ca, Fe, Mg and Mn, and SPC of lower magnitude with Na (Pillay & Fungo, 2016; Anyasi et al., 2018). For breeding purposes, the correlation between Mg and Zn is of interest, because direct selection for high Zn concentration could be difficult due to high $G \times E$ interactions. Thus, if efforts are focused on the easier selection for high Mg concentration, some indirect progress for Zn concentration can also be expected.

Calcium showed highly SPC of high magnitude with Mg and Na, while it was negatively correlated with P in conformity with the findings of others (Alkarkhi et al., 2009; Anyasi et al., 2018). Calcium also had SPC of moderate magnitude with B, Fe and Zn. Similarly, K recorded SPC of lower magnitude with B, Mg and P, and of moderate magnitude with Na (Forster et al., 2002). Magnesium showed no correlation with P, but it had highly SPC with all the other seven minerals. It also recorded an association of higher magnitude with Ca, Mn, Na and Zn, of moderate magnitude with Fe, and of low magnitude with B and K as reported by (Deshmukh et al., 2009). Sodium showed no correlation with B and P, while low/moderately SPC were seen with Fe, K, Mn and Zn. However, a highly SPC of higher magnitude was observed for Na with Ca and Mg agreeing with the findings of Alkarkhi et al. (2009). P had highly SPC of lower magnitude with K and Mn, and significant negative association with Ca (Anyasi et al., 2018).

All the correlations between the FPMs were positive, which indicated that, when the concentration of one nutrient increases, the concentration of the other also increases, helping identify accessions with high mineral nutrient values. However, correlations between Ca-Mg, Ca-Na, Mg-Mn, Mg-Na, Mg-Zn, and Mn-Zn can be highlighted because of the relatively high correlation coefficients ($r = 0.50$ to 0.77 at $p < 0.0001$) among the analyzed IMA. Similar results were reported by Deshmukh et al. (2009), Alkarkhi et al. (2009) and Forster et al. (2002). Highly significant PC of lower/moderate magnitude of Fe with Ca, Mg, Mn, Na and Zn and that of highly significant PC of moderate/higher magnitude for Zn with Ca, Fe, Mg and Mn suggest better prospects of combining higher micronutrients with higher macronutrients for the development

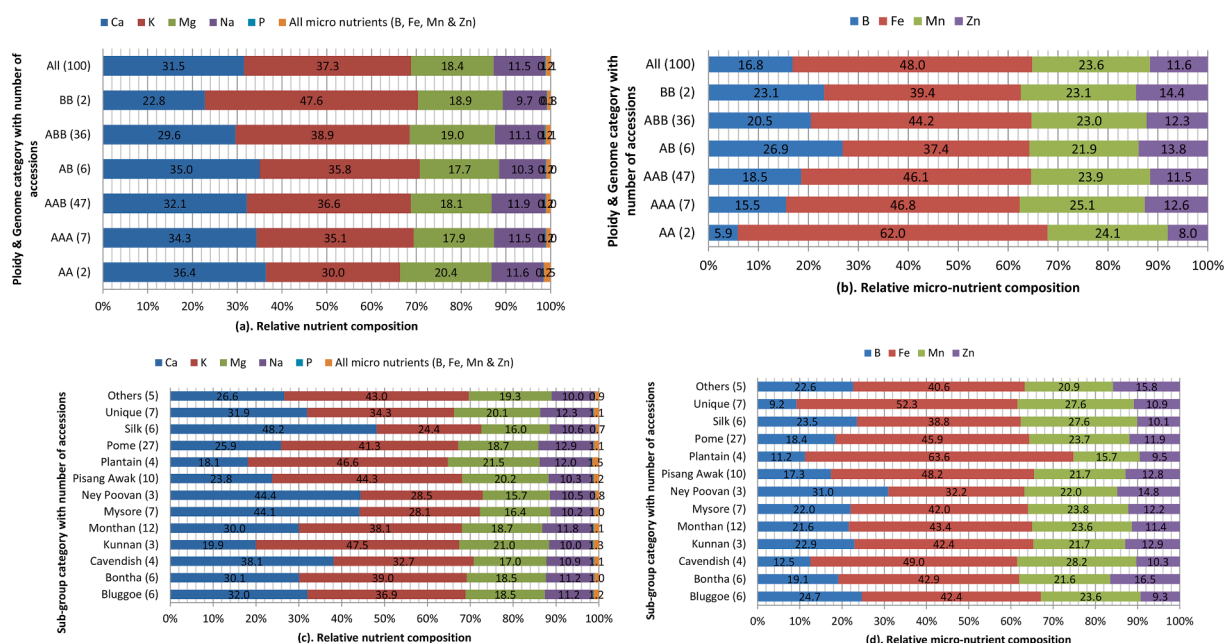


Fig. 2. Relative macro- and micronutrient composition (%) of 100 banana genotypes belonging to different genome/ploidy (a&b) and sub-group (c&d) category.

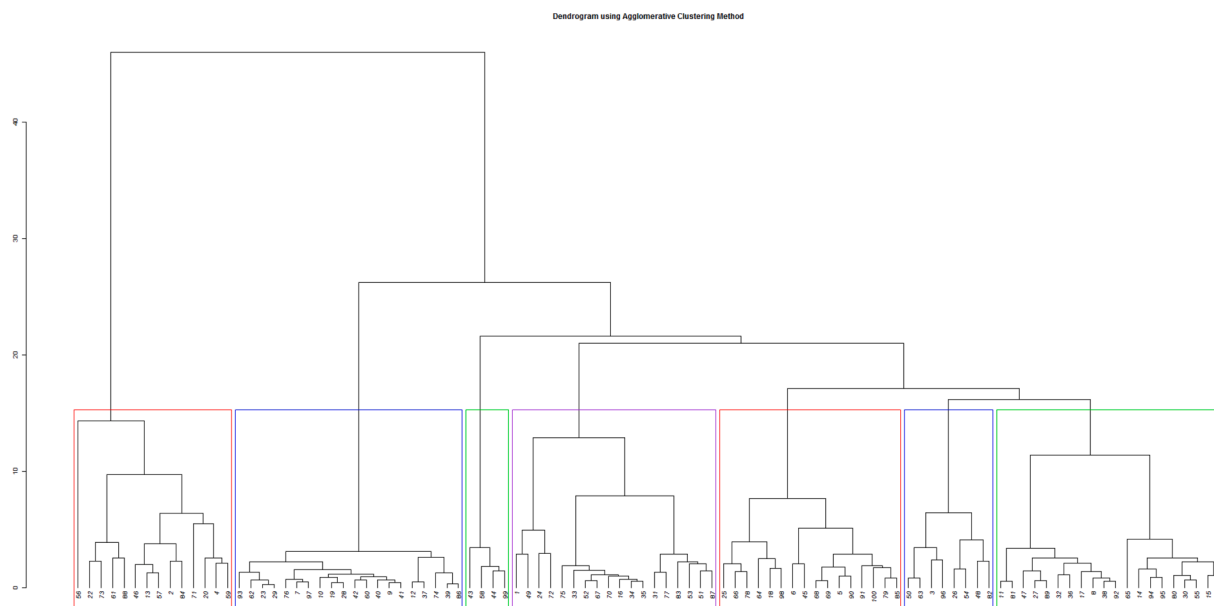


Fig. 3. Dendrogram showing clustering pattern of 100 Indian banana accessions based on their fruit pulp mineral contents.

Table 2
Correlation coefficients for fruit pulp mineral contents in 100 Indian banana accessions.

Minerals	Type	Boron	Calcium	Iron	Magnesium	Manganese	Potassium	Zink	Sodium
Calcium	PCC	0.256****							
	GCV	0.256**							
	PCV	0.249**							
Iron	PCC	-0.070NS	0.211***						
	GCV	-0.068NS	0.204**						
	PCV	-0.082NS	0.204**						
Magnesium	PCC	0.226****	0.774****	0.456****					
	GCV	0.211**	0.804**	0.444**					
	PCV	0.206**	0.787**	0.446**					
Manganese	PCC	0.011NS	0.340****	0.479****	0.561****				
	GCV	0.002NS	0.338**	0.469**	0.540**				
	PCV	-0.006NS	0.332**	0.470**	0.541**				
Potassium	PCC	0.125*	0.070NS	0.003NS	0.183**	0.041NS			
	GCV	0.122*	0.027NS	-0.081NS	0.015NS	-0.073NS			
	PCV	0.096NS	0.043NS	-0.038NS	0.088NS	-0.022NS			
Zink	PCC	-0.016NS	0.351****	0.336***	0.499****	0.506****	-0.052NS		
	GCV	-0.083NS	0.382**	0.343**	0.486**	0.499**	-0.167**		
	PCV	-0.037NS	0.342**	0.323**	0.471**	0.489**	-0.131*		
Sodium	PCC	0.103NS	0.506****	0.207***	0.568****	0.194***	0.385****	0.168**	
	GCV	0.083NS	0.502**	0.176**	0.524**	0.139*	0.308**	0.123*	
	PCV	0.082NS	0.502**	0.187**	0.536**	0.160**	0.330**	0.127*	
Phosphorus	PCC	-0.088NS	-0.144**	0.067NS	-0.013NS	0.226****	0.233****	0.022NS	0.108NS
	GCV	-0.075NS	-0.183**	0.004NS	-0.114*	0.194**	0.130*	0.001NS	0.033NS
	PCV	-0.112NS	-0.166**	0.046NS	-0.078NS	0.198**	0.176**	-0.018NS	0.062NS

The upper, middle and lower numbers refer to the PCC (Pearson correlation coefficients), GCV (Genotypic coefficient of variation) and PCV (Phenotypic coefficient of variation), respectively.

Asterisks indicate significance at

* P < 0.05,

** P < 0.01,

*** P < 0.001 and

**** P < 0.0001; NS = Non significant at P < 0.05.

of nutri-rich bananas.

3.3. Path coefficient analysis (PA):

The PA at the genotypic level was done to partition the correlation coefficient into direct and indirect effects by taking Fe as a dependent variable as it is one of the most important mineral nutrients associated with dietary deficiencies (ST.7). PA revealed that Mg made maximum direct contribution to Fe content followed by Mn, Zn and Na which are

higher or fairly close to its highly significant correlation coefficients. Though Ca content is more desirable in banana, its contribution directly and indirectly to Fe content is negative. Likewise, K also had a negative contribution towards Fe enrichment. Therefore, during selection, weightage should be given to these minerals for the development of Fe rich banana cultivars. However, the high magnitude of residual effect (0.666) at genotypic level indicates that the attributes of Fe included in the present study could account for only 33.4 percent of the variation in Fe and the rest was accounted by factors not included in this study.

Table 3

Contribution of 100 g of banana pulp to RDA (Recommended dietary allowances)/AI (Adequate intake) requirement of different minerals for the Indian adults calculated based on the mean value of Indian commercial cultivars (39 out of 100) used in this study.

Minerals	RDA [#] or AI [#] (Values given are for sedentary/moderate/heavy work men; women) (mg/day)	Mineral contents obtained in this study based on only 39 commercial cultivars out of 100. (mg/Kg f.w.)			Contribution of banana pulp (100 g) to the % of RDA		
		Mini.	Maxi.	Mean	Mini.	Maxi.	Mean
Ca	600	191.5	3523.3	617.7	3.2	58.7	10.3
Mg	340;310	197.6	749.2	323.4	5.8;6.4	22.0;24.2	9.5;10.4
P	600	1.5	5.8	3.1	0.03	0.1	0.05
K	3750;3225	241.1	1128.2	628.0	0.6; 0.8	3.0; 3.5	1.7; 2.0
Na	2100;1900	50.9	466.5	197.2	0.2; 0.3	2.2; 2.5	0.9;1.0
B	ND	0.2	17.2	4.1	-	-	-
Cu*	1.7 (AI)	0.17	2.08	0.56	1.0	12.2	3.3
Fe	17; 21	1.4	32.2	7.9	0.8;0.7	18.9;15.3	4.6; 3.8
Mn	4.0 (AI)	0.8	11.4	4.5	2.0	28.5	11.3
Zn	12; 10	0.7	6.1	2.2	0.6; 0.7	5.1; 8.7	1.8; 2.2

* Based on only 7 out of 39 commercial cultivars used in this study. ND = Not determinable.

Nutrient Requirements and Recommended Dietary Allowances for Indians (ICMR 2010).

Despite a wealth of literature on the mineral content of bananas, to our knowledge, there are no available reports on PA for mineral content.

3.4. Cluster analysis (CA):

The CA of 100 IMA based on 9 FPM contents produced a dendrogram with seven main clusters (Fig. 3). The dissimilarity level ranged from 1 to 40, revealing that there was a great degree of similarity/dissimilarity among IMA. Furthermore, the clustering pattern also revealed that the accessions belonging to the same PGS did not necessarily cluster together. For example, in the first cluster accessions belonging to five different PGS (AA, AB, AAA, AAB & ABB) were grouped, similarly even a smallest cluster-III comprises of accessions from two different genomic groups (AAB & ABB), indicating a wide genetic diversity in FPM contents within these PGS which confirms the findings of Deshmukh et al. (2009).

Comparison of cluster means showed variation in all the FPMs among the seven groups (ST.8). Among the nine FPMs studied, cluster-I & -IV exhibited the highest means for Ca, Fe, Mg and Zn, while the lowest values for these minerals were recorded in cluster-VI except for Zn which was observed in cluster-III. Highest content for B was recorded in cluster V, for K, Na and P in cluster-III, and Mn in cluster-I. Cluster-I & -III appear to be most important since they showed higher mean values for all the FPMs except for B and from these two clusters improved genotypes could be directly selected or could be used as parents to complement the deficit of other important parents present in distant groups. Interestingly, most of the minimum and maximum mean values were distributed in relatively distant clusters. Therefore, hybridization between accessions falling in different clusters may provide ample scope for the development of desirable cultivars with higher FPM contents. The results of the present study suggested that hybridization between genotypes in cluster-I & -III could provide a wide spectrum of variation for FPMs in the segregating generation which might provide an opportunity for isolating elite lines.

3.5. Principal component analysis (PCA):

The PCA results showed a high variation of nine different FPM contents among the 100 IMA. For each factor, a principal component (PC) loading value (boldface type) of >0.36 was considered as being significant for FPMs (ST.9). Therefore, grouping of IMA after the PCA were mainly based on the first three PC (eigenvalue > 1.0), which accounted for 65.3% of the variability observed (ST.10). In the first component (PC1), accounting for 34.2% of the total variance, Ca, Mg, Mn, and Zn were prominent, while the less important variables integrated by PC1 were B, K and P. In the second component (PC2)

representing 17.0% of the total variance, B, K, and Na were the most significant variables. The third component (PC3) representing 14.1% of the total variance has been influenced by K and P, although this component was less significant than PC1 and PC2. Iron fails to load significantly on retained PCs. It is more negligible in PC3 than in PC1 and PC2.

PCA did not show any difference between the FPM compositions and IMA of different ploidy/genome, and the genotypes were distributed on all four sides of the plot (Figs. 4a & b). Loading in the bi-plot (Fig. 4a) of the first two PC determined three main groupings of FPMs: the first group was composed of K, B, Na, Ca and Mg; the second consisted of Zn, Mn and Fe; and the third had a single element P. This was in accordance with correlation analysis results that showed SPC among all the FPMs. The association among Fe, Zn and Mn is unambiguous, stronger and more significant in PCA bi-plot than in correlation analysis agrees with the results of Alkarkhi et al. (2009) and Deshmukh et al. (2009).

PCA plot was used to visualize the dispersion of IMA based on their ploidy/genome (Fig. 4b). The points representing the IMA are very scattered and there is no clear orientation. Also, it is not possible to detect groups of genotypes that belong to the same ploidy/genomic group, implying that they have a mixed type of features with respect to their FPM contents. Therefore, it could be concluded that these minerals have no significant influence on the attained classification of the IMA

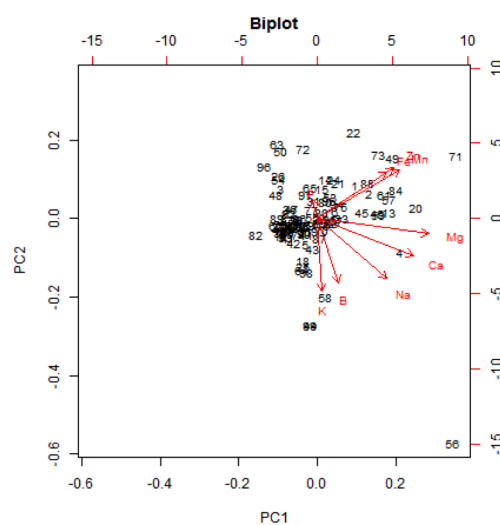


Fig. 4a. Principal component analysis of nine fruit pulp mineral concentrations recorded on 100 Indian Banana accessions. Biplot vectors are trait factor loadings for PC1 and PC2.

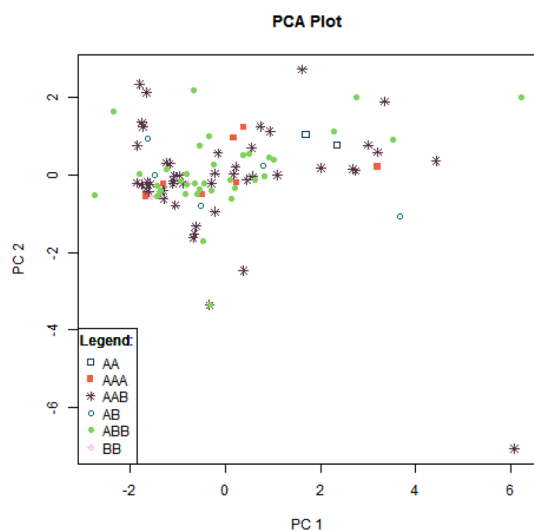


Fig. 4b. Scatter-plot of first versus second principal component showing six genomic grouping of 100 Indian *Musa* genotypes.

which agrees with the results of Deshmukh et al. (2009) and Forster et al. (2002). However, both genotypes belonging to the BB genome were tendentially discriminated to negative scores concerning PC1 and PC2. On the other hand, both genotypes belonging to the AA genome were discriminated for positive scores for PC1 and PC2. The separation of these two *Musa* genomic groups is attributed to the parental pedigree of the accessions and differences in their species level. The association of IMA of other ploidy/genome (AB, AAA, AAB & ABB) in close proximity to each other can be explained by the direct as well as indirect involvement of two ancestral species of banana in the evolution of most banana accessions. Also due to recombination combined with sexual and vegetative propagation that occurred over a long period of time, as well as uncontrolled spread of planting materials within India.

4. Conclusion

The results obtained demonstrate that, within the IMA pool, there is substantial genetic variability in FPM contents. And further confirmed with the ANOVA, PCA and CA analyses which fail to group/classify them according to their PGS. Also, highly SPC among the six minerals viz., Ca, Mg, Na, Fe, Mn and Zn, suggest that these minerals can be improved concurrently for the development both macro- and micronutrient combined bananas. Therefore, this diversity could be exploited to identify accessions potentially suitable for direct introduction as cultivar in afflicted regions, and/or as donor parent in breeding programmes to enhance the nutrient contents in commercially cultivated bananas. However, it is evident from our present study that the degree of genotypic variation in *Musa* FPM content is clearly limited with low concentrations (PS). Further, the influence of environment on the accumulation of minerals in FP, as the magnitude of PCV was slightly higher than the corresponding GCV for all the FPM contents. The results also suggest that there is little potential for mineral bio-fortification using IMA through conventional breeding unless new fertile accessions having very high mineral accumulating ability are identified via screening of more and more world *Musa* accessions. If not, then the strategy has to be shifted to genetic transformation which showed some promising results of 5.5-fold and 3.0-fold increase in Fe levels in FP of transgenic lines of 'Grand Naine' and 'Rasthali', respectively (ICAR-NRCB, 2020). Twenty commercial cultivars are placed in the top 10 list based on their FPM contents. However, even if these minerals were fully made bio-available to the consumers, it is clear that one has to consume considerable quantum of banana FFP to meet the RDA values for these FPMs which is practically not possible at the present consumption levels

in vogue in India.

CRediT authorship contribution statement

Ramajayam Devarajan: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Jeyabaskaran Kandallu Jayaraman:** Methodology, Validation, Investigation, Supervision. **Saraswathi Marimuthu Somasundaram:** Writing - review & editing. **Sivasankari Ragupathy:** Investigation, Writing - review & editing. **Pitchaimuthu Raman:** Investigation, Writing - review & editing. **Kalpana Sathiamoorthy:** Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization, Supervision. **Uma Subbaraya:** Conceptualization, Methodology, Validation, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.130080>.

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