DNA barcoding and nutritional profiling of some underutilized native indigenous plant species (NIPS) of Karnataka, India

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Abstract

Locally adapted native indigenous plant species (NIPS) could restore the crop diversity in sustainable agriculture. Here, we report the molecular identification and nutritional profiling of some five NIPS of Karnataka; *Musa paradisiaca* cv. Nanjangud rasabale, *Piper betle* L. cv. Mysore betel leaf, *Jasminum grandiflorum* cv Mysore mallige, *Solanum melongena* L. cv. Udupi Mattu Gulla and *S. melongena* L. cv. Erangere badane of which the first four are Geographical Indication (GI) tagged. The samples were procured, authenticated and sequenced using two standard DNA barcodes; nuclear *ITS2* and plastid *rbcl*. The phylogenetic analysis using Neighborhood joining method revealed all the *ITS2* tree topologies with higher genetic divergence than *rbcl*. All the *rbcl* tree topologies were monophyletic indicating sequence conservation. Though the concatenated *ITS2 + rbcl* trees had higher bootstrap support (> 98% except *Solanum sp*.) differences were observed because of the lack of available sequence deposition at species level. The proximate and nutritional profiling of the NIPS displayed superiority in terms of their nutritional profile and their potential application in phytopharmaceutical sector as nutritional supplements. We anticipate that if research towards the identification of NIPS species is accelerated, these nutritionally enhanced crops could be used as a safe and sustainable food in changing global climatic conditions.

Introduction

Local and traditional varieties, adapted to a particular geographical area are ideal resilient crops for climate change but are often neglected and their cultivation practices are being lost due to rapid domestication of commercial cultivars [1]. Restoring such species could empower local small farmers and provide huge benefits by improving their livelihood and alleviating poverty. The problem of malnutrition and nutritional insufficiency could also be overcome by adopting local varieties due to their higher nutritional value [2]. The conservation of such economically important underutilized varieties is critical to preserve and maintain the crop diversity. Among the many conservation strategies, exclusive community rights and registration are given to local plant varieties through Geographical Indication (GI) tags under Geographical Indications of Goods (Registration and Protection) Act 1999 (GIs Act) to protect traditional knowledge and distinguish its products based on their unique intrinsic attributes [3].

In India, more than three hundred items have been accorded with GI tags which include nearly 89 agricultural items. The states of Maharashtra and Karnataka are predominant in owning high number of GI tags especially for horticultural crops [3]. The key step involved in the conservation of local target plant species is by accurate species identification, authentication and traceability. The traditional method includes morphological characterization and taxonomic identification which is limited by environmental factors and lack of experts. In such challenging situations employing supporting tools such as DNA barcoding could be more successful in species identification. DNA barcoding require a short universal DNA sequence that exhibits a sufficient level of variation to discriminate among species [4, 5]. Some of the proposed plant DNA core barcodes by the Consortium for the Barcode of Life (CBOL) Plant Working Group comprise the chloroplast gene large subunit of ribulose bisphosphate carboxylase (*rbcl)* and matK with trnH-psbA intergenic sequence and Internal transcribed spacer (ITS), a nuclear gene as the supplement barcodes [6].

The aim of our present study is to identify and assess *rbcl* and Internal transcribed spacer 2 (*ITS2)* gene barcodes to discriminate some five local plant varieties of Karnataka, which are declining due to the promotion and cultivation of high-yielding commercial cultivars. The selected varieties include *Musa paradisiaca* cv. Nanjangud rasabale, *Piper betle* L. cv. Mysore betel leaf, *Jasminum grandiflorum* cv Mysore mallige, *Solanum melongena* L. cv. Udupi Mattu Gulla and *S. melongena* L. cv. Erangere badane of which four are GI tagged except Erangere badane. Additionally, we have performed the phylogenetic tree construction and also examined their proximate and nutritional content to determine if they are nutrient-rich so that they could be used as a sustainable source for the development of biofortified crops. The findings of this research work could provide valuable information for the classification, identification and conservation of such neglected and underutilized crop species.

Materials

Collection of native indigenous plant species (NIPS)

A total of five native indigenous plant species (NIPS) of Karnataka; *Musa paradisiaca* cv. Nanjangud rasabale, *Piper betle* L. cv. Mysore betel leaf, *Jasminum grandiflorum* cv Mysore mallige, *Solanum melongena* L. cv. Udupi Mattu Gulla and *S. melongena* L. cv. Erangere badane were used for the present study. The samples/specimens were procured from a local market and garden areas of Mysore, Karnataka, and India on summer 2017 (Table 1) and identified as NIPS by Dr. Sampath Kumara KK, taxonomist, DOS in Biotechnology, University of Mysore, dried and stored as voucher specimen at University of Mysore.

Molecular analysis

Genomic DNA isolation, amplification and sequencing

Freshly collected specimens were used for the genomic DNA extraction. The modified CTAB (Cetyl trimethyl ammonium bromide) method previously described by **[7]** was adopted, the pellet was air-dried, 0.1X T10E1 buffer was added and stored at -20^{O}C until further use. The purity and presence of intact DNA were checked before the PCR analysis. The PCR analysis was done with nuclear *ITS2* and plastid *rbcl* specific primers recommended by CBOL plant working group and from previous studies (White et al., 1990; Levin, 2003; Supplementary Table 1). Briefly, the reaction mixture comprised (25 µl): (10 ng) DNA template, 15 µl deionized nuclease-free water, 2.5 µl 10X PCR buffer with 15 mM $MgCl_2$, 2.5 µl 2 mM dNTPs, 0.5 µl forward and reverse primer(each of 10 pmol/µl for both *ITS2* and *rbcl* gene) and 0.5 µl Taq DNA polymerase(1 U/µl) (3B BlackBio Biotech India Ltd.) under the conditions of initial denaturation at 94ºC for 3 min, denaturation at 94ºC for 1 min annealing at (54 ºC for 1 min in case *ITS2* primer and 55ºC for 1 min in case *of rbcl* primer) with an extension at 72ºC for 1 min and final extension at 72ºC for 10 min with 35 cycles of amplification. The amplicons were analyzed in 1% agarose gel pre-stained with EtBr and documented under UV A_{260nm} (Alpha Innotech, FlourChem SP imaging system, USA) and the amplicons were observed. Further, the amplified products were purified by GeneJET PCR Purification kit (Thermo Scientific, Inc., USA) and sequenced at a commercial facility (Scigenomics, Kochi, Kerala, India) using the same primers used for amplification.

Sequence data analysis

The sequenced DNA chromatograms were individually processed by removing the ambiguous base calls and background noise using BioEdit v.5.0.630 and blasted in both NCBI BLAST (Blastn) and BOLD databases (v4) (http) to determine the sequence homology with other deposited *ITS2* and *rbcl* sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with default settings. The identification of the species was based on the parameters; least Expect value (E-value), similarity percentage and highest bit (max) score. The *ITS2* and *rbcl* sequences of all the NIPS were submitted to NCBI. Phylogenetic analysis was conducted with MEGA 11 (7.0.26) software using neighbor joining tree for individual *rbcl*, *ITS2* and concatenated *rbcl* + *ITS2* regions with partial nucleotide alignments. Both the aligned gene datasets *ie.,* ITS and *rbcl* were concatenated in a simple head-to tail manner to reconstruct the phylogenetic tree. Earlier studies have reported accuracy in phylogenetic tree inference using concatenation (Ct) approach, as it minimizes variance in distance estimates due to longer sequences **[8, 9]**. The model with lowest AICc (Akaike Information Criterion, corrected) and BIC (Bayesian Information Criterion) score was selected for the tree construction **[10]**. All the trees were bootstrapped with 1000 replications to ensure the accuracy and reliability of the generated nodes. The bootstrap values above 70 % were retained in the clades of constructed tree. The plant species chosen as outgroups for the selected NIPS were based on the previous studies **[11-14]**. The barcodes were generated for all the NIPS using the BOLD database.

Proximate, mineral and metal tests

All the collected and air-dried NIPS samples were used for the proximate and metal content evaluation. The proximate parameters energy, protein, carbohydrate, fat, crude fiber, ash and moisture were estimated based on the methods described

by AOAC **[15, 16].** The analysis of minerals and metals was performed by inductively coupled plasma–optical emission spectrometer (ICP-OES Perkin Elmer. USA). The samples were made to ash and dissolved in 10% nitric acid, filtered and made up to 100 ml and fed to ICP-OES. The instrument was calibrated using multi standard elements (Perkin Elmer Life & Analytical Sciences US) with 10 % nitric acid as sample blank **[17]**. All experiments were carried out in triplicates and data were expressed as mean values ± SE.

Results And Discussion

A total of five NIPS of Karnataka, India were analyzed in the present study. The *nrITS2* and *rbcl* amplicons were analyzed on 1% agarose gel electrophoresis (Plate 1). The amplification of all the NIPS samples were performed in triplicates. The average length of the edited bar code sequences were variable (min: 497 bp and max: 565 bp) for *rbcl* and between 272 and 413 bp for *nrITS2* sequences for all the five NIPS. Subsequent analysis for species identification was done both in BOLD and BLAST databases and the details of their accession numbers are given in Table 2. The similarity results indicated differences in the percentage similarity for *Musa paradisiaca* cv. Nanjangud rasabale and *Piper betle* L. cv. Mysore betel leaf with no matching sequences with any species for *Jasminum grandiflorum* cv Mysore mallige in the BOLD database due to the lack of sequence depositions. All the other NIPS had >98% homology match to a single species in both the databases.

Phylogenetic analysis of *nrITS2* gene sequences

The Neighborhood joining (NJ) tree representations of the *nrITS2* gene sequences of all the NIPS are given in Figs (2a-2d). The tree topology of *Piper sp*. separated into two clades and both the isolates *P. betle* L. cv. Udupi and *P. betle* L. cv. Mysore betel leaf were in a single clade and closer to the *Piper betle* species of US origin. An exception was with the NIPS of *Solanum sp*. were both the native species were distantly related. Irrespective of the geographical location, the *Solanum melongena* L. cv. Erangere badane was closer to *S. torvum* and *Solanum melongena* L. cv. Udupi Mattu Gulla with *S. chrysotricum*. The mysore mallige isolate of *Jasminum grandiflorum* showed closer relation with an Indian isolate of *J. sambac*. It is to be noted that, there was a lack of deposition of *nrITS2* sequences at the species level in both the NIPS species of Solanum and Jasmine in the database. The separations of the *P. betle* species into two clades indicate genetic divergence within the species. The NIPS banana hybrids *Musa paradisiaca* cv. Nanjangud rasabale 2 and *Musa paradisiaca* cv. Nanjangud rasabale 3 were clustered together in a single clade with high bootstrap support of 99 % and the tree was delineated into two clades with poor resolution.

Phylogenetic analysis of *rbcl* gene sequences

Though the NIPS could be discriminated from other species in all the NJ trees (Figs 5-9), all the trees were monophyletic indicating *rbcl* sequence conservation and lack of diversity in rbcl sequences across species level and such observations were also made in earlier studies **[18, 19].** The *rbcl* tree of *Piper sp.* displayed closer relation for both the NIPS with good resolution similar to *nrITS2* tree topology and was closer to *P. betle* species of Thailand. Similar topologies were observed in both *Solanum sp* and the isolates were distantly related like the *nrITS2* tree topology. *S. melongena* L. cv. Erangere badane was closer to a Thailand isolate *S. wrigitti* and *Solanum melongena* L. cv. Udupi Mattu Gulla with a French isolate of *S. aethiopicum*. In case of *Jasminum grandiflorum* cv. Mysore mallige isolate, it was closer to an Indian isolate of *Jasmonium grandiflorum* CMS-BOT. Similar to *nrITS2* tree topology, both the NIPS of banana were closer and related to a *Musa* hybrid Indian isolate BSR-COB1 but supported with low resolution. The non-coding *nrITS2* gene sequences had higher sequence variability than the plastid coding marker *rbcl* but it may not be sufficient to distinguish within species variation in our present study as additional sequences are required to arrive at a conclusion.

Phylogenetic analysis of *nrITS2+ rbcl* concatenated sequences

The *nrITS2+ rbcl* regions were concatenated only with the available sequence depositions. Interestingly, all the concatenated trees were able to discriminate the NIPS and had reliable clades except *Solanum sp.* with low bootstrap support of 74 % (Figs 4a- 4d). The topology of *nrITS2+ rbcl* regions were separated to two clusters in *Piper sp.* with *P. betle* L. cv. Mysore betel

leaf forming a single clade with a Thailand isolate and *P. betle* L. cv. Udupi isolate as sister to this clade. A topological discrepancy was observed for the NIPS of *Solanum sp.* where, the topology was monophyletic and both the isolates *S. melongena* L. cv. Erangere badane and *S. melongena* L. cv. Udupi Mattu Gulla were clustered into a single clade. In case of *Jasminum sp*., *Jasminum grandiflorum* cv. Mysore mallige was sister to a clade comprising two Chinese isolates. The *Musa paradisiaca* cv. Nanjangud rasabale 2 banana isolate formed a single clade with BSR-NE1 Nendran Indian isolate and *M. paradisiaca* cv. Nanjangud rasabale 2 emerged as a sister group with high topological support of 98%. The topologies of the trees were completely different when combined; a possible reason could be due to the lack of available *nrITS2* and *rbcl* DNA sequences of same species which limited evaluation of the sample sets to arrive at meaningful barcode identification. Our findings suggest that, it is essential to have additional sequences of both *ITS2* and *rbcl* DNA regions which could provide better resolution to determine species level variation alongside morphological characterization.

Proximate and nutritional profiling of NIPS

The results of the proximate analysis and selected macro and micro nutrient evaluation of the NIPS regional to Karnataka using AOAC methods and ICP-OES technique is given in Table 3. Our results showed that the moisture content ranged from 13.78 to 83.12 %/100 g; protein 1.69 to 14.33%/100 g ash 0.62 to 7.49 %/100 g; fat 0.24 to 0.95 %/100 g; crude fiber 0.72 to 15.71 %/100 g; carbohydrate 6.94 to 77.03 %/100 g. All the NIPS had significant nutrient composition and ranged from 0.011 to 105.84 mg/100 g; 0.221 to 4023 mg/100 g; 0.053 to 2119.6 mg/100 g, 0.029 to 437 mg/100 g, 0.389 to 3.93 mg/100 g, 0.543 to 32.38 mg/100 g, 0.54 to 2.14 mg/100 g and 0.650 to 4.69 mg/100 g for Na, K, Ca, Mg, Mn, Fe, Cu and Zn respectively. The proximate and nutrient content was not evaluated for *Jasminum grandiflorum* cv. Mysore mallige as it is used for ornamental purpose **[20]**. The investigation of proximate parameters and nutritional composition could help in determining the potential health benefits provided by the consumption of local varieties.

In the ripe banana; *Musa paradisiaca cv.* Nanjangud rasabale*,* the most abundant proximate parameter was carbohydrate (77.03 g/100g) indicating it as a primarily carbohydrate staple food. The value was significantly higher than the earlier reported studies with 21.59 to 29.96 g/100 g **[21]** and 21.80 to 32.0 0 g/10 0 g. in unripe banana **[22]**. The crude fiber content was lower (0.72 g/100g) than previous studies which ranged from 0.92 to 2.79 g/100 g **[21]**; 1.58-2.42 g/100g in ripe banana cultivars Nendran, Njali poovan and Robusta **[23].** This indicates that, it has to be supplemented with fiber rich foods. Similarly, the protein content was comparable (4.03 g/100g) with an earlier study in ripe banana cultivars Nendran, Njali poovan and Robusta cultivated across Southern Indian districts ranging from 3.25 to 4.95 **[23]** and higher than the values reported for unripe Mchare cooking banana ranging from 0.61 to 1.75 g/100 g **[21]** respectively. The variation in the nutritional composition has been observed across different developmental stages of banana and also with change in climate and soil conditions.

The fat content was 0.24 g/100g and the findings were in comparable with an earlier study reporting 0.09 to 0.60 g/100 g. Contrarily, the moisture content (16.07 g/100g) was significantly lower than other reported ripe banana cultivars; 66.26-75.25 g/100g in the ripe fruits of South Indian banana cultivars **[23]**; 77.77 g/100g in case of biofertilized commercially cultivated ripe Grand Naine banana **[24]**. Our findings reveal improved shelf life and keeping quality of Nanjangud Rasabale than other commercial cultivars of banana. Among the elements, the most abundant was Potassium with 887.31 mg/100g followed by Mg with 131.9; Na with 61.96 and Ca with 41.59 mg/100g. The values of the micronutrient Ca was in agreement with a previous study reporting 31.06 to 52.60 g/100g value for the ripe banana cultivars **[23]**; the values of K, Na, Fe (7.22 mg/100g) were remarkably higher than an earlier study using ripened Nendran banana with values of 546.48, 5.41 and 3.91 mg/100g respectively. Our analyses show the potential of *Musa paradisiaca* cv. Nanjangud Rasabale as a good mineral supplement in nutraceuticals.

The next NIPS; *Piper betle L.* cv. Mysore betel leaf*,* leaves possess many valuable bioactivities and have been used in traditional medicinal systems **[25]**. The *P. betle* has been reported as potent antimicrobial **[26],** antioxidant [**27**], and anthelmintic [**28**] herb **[29].** It is primarily consumed in South Asia and by certain Asian emigrants worldwide as betel quid or

paan, in combination with areca nut or tobacco **[30, 31].** The macro and micro minerals in mysore betel leaf were in the order K > Ca > Mg > Na > Fe > Zn > Mn > Cu respectively (Table 3). The results were congruent with an earlier study reporting the nutritional composition of dehydrated leaves of Kariyele and Ambadiyele varieties **[32]**. The higher moisture content of 81.86 %/100g indicates the perishability upon storage and necessitates possible alternative measures such as dehydration and freeze drying for long-term storage. Though very little attention is given for this crop plant, our results suggest the richness of nutrients and its excellent potential as a cash crop for its application as a nutritional supplement.

The protein, carbohydrate, moisture and crude fiber content of the edible part was very variable and significant between the two varieties with *S. melongena* L. cv. Erangere badane displaying higher values (Table 3). Similarly, there was also wide variability in the micro and macronutrient composition between both the varieties. The high values of Na, K, Ca and Zn were seen in Erangere badane variety. The Fe content was significantly higher in *S. melongena* L. cv. Udupi Mattu Gulla and with 32.38 compared to 6.74 mg/100g in Erangere badane variety. The nutrient profiles of both the varieties had considerable differences. The protein content was lower in both the varieties than previously reported values of 30.65 and 28.49 ±0.058 respectively [**33]**. However, low values of micro and macro nutrients were reported by **[34]** in a native variety of brinjal found in North-eastern India. The high amount of protein, crude fiber and fair amount of macro and micronutrients suggest Erangere badane as an excellent source of easily digestible protein and nutrient rich food.

The role of mineral and microelements in regulating the physiology of human body and their deficiencies is well documented in the literature. Potassium is crucial to heart function and plays a vital role in skeletal and smooth muscle contraction **[35]**. The potassium/sodium balance is fine-tuned and important for the transmission of electrical impulse in the heart **[36]**. The Recommended Dietary Allowance (RDA) of Potassium is 1600 to 2000 mg (40 to 50 milliequivalents [mEq]) per day for adults. Among the NIPS, Mysore betel leaf followed by Udupi Mutta Gula and Erangare badne eggplant could be used as a plant derived dietary supplements for K. Calcium is another important element required for bone formation, blood clotting, growth, cell metabolism and heart function **[37]**. The daily body requirement is about 450 mg/100g. Mg plays an important role in a variety of metabolic processes, including oxidative phosphorylation and muscle contraction. It is also acting as an important co-factor for many enzymes **[38]**. The RDA of magnesium varies according to age and gender and is about 300–400 mg for men and 270–310 mg for women. Our findings indicate mysore betel leaf and Erangare badne eggplant as rich sources for Ca and Mg.

Manganese (Mn) is an essential nutrient involved in the formation of bone and in amino acid, cholesterol, and carbohydrate metabolism. It is also essential for many metalloenzymes **[39]**. The RDA for Mn is 2.5 to 3 mg/day. Our results indicate Nanjangud Rasabale and mysore betel leaf as good dietary sources of Mn. Iron (Fe) is an essential dietary mineral used to support vital human functions, such as erythropoiesis, cellular energy metabolism, and immune system development and function. Fe deficiency results in anemia and is the world's most common nutrient disorder. The RDA for adults 19-50 years is 8 mg daily for men, 18 mg for women, 27 mg for pregnancy, and 9 mg for lactation (https://www.hsph.harvard.edu/nutritionsource/iron/). Mysore betel leaf and Udupi Mutta Gulla are good dietary sources of Fe. Cu is an important cofactor for oxidative balance. The RDA for adult men and women is 900 µg/day and MBL is a good source of Cu. The results of the present study demonstrate mysore betel leaf could be used as an excellent source for plant based dietary supplement as it fulfills the RDA of micro and macro nutrients to combat nutrient deficiency and hidden hunger.

Conclusion

The cultivation of local plant varieties is often a neglected topic in modern agriculture. The cultivation of such neglected species could improve the economic livelihood of local community and promote genetic diversity of local ecosystem in the rapidly challenging global climatic conditions [40]. In this view, it is essential to properly identify and conserve such local species. In our study, the phylogenetic analysis of some five NIPS species of Karnataka; *Musa paradisiaca* cv. Nanjangud rasabale, *Piper betle* L. cv. Mysore betel leaf, *Jasminum grandiflorum* cv Mysore mallige, *Solanum melongena* L. cv. Udupi

Mattu Gulla and *S. melongena* L. cv. Erangere badane showed varied topologies among *ITS2, rbcl* and *ITS2 + rbcl* concatenated DNA barcodes due to the non-availability of sequences at species level and warrants further investigation in this field of research. The present study reveals the tremendous potential of such neglected location specific species as functional foods and nutrient supplements in phytopharmaceutical sector. The high nutritive value of such NIPS shows that they could be used as biofortified crops to overcome malnutrition and hidden hunger. Further, it is also important to promote the cultivation of such underutilized species in urban and semi-urban regions of specific geographical origin and improve the livelihood of farmers and sustain food security. The information we presented in this study could also interest breeders to develop varieties adapted to local environmental conditions which could restore the genetic diversity and conserve the ecosystem.

Declarations

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Author contributions statement

RMK wrote the final manuscript, prepared all the figures and tables. BTR performed all the laboratory experiments, prepared draft manuscript and PHS revised and approved the final manuscript.

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Ethics approval

Not applicable

Informed consent

Not applicable as the research did not involve any human participants/ animals

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Tables

Table 1 Sampling location and geographical distribution of all the native indigenous plant species (NIPS) of Karnataka

S. No.	Specimen	Family	Tissue sample	Collection site	Geographical Co-ordinates	Process IDs in BOLD	Institutional voucher number
1	*Piper betle* L. cv. Mysore betel leaf	*Piperaceae*	Leaf	Yelthota, Mysore	12.2834485986132 76.6588522733658	BSRUG003-22	UOMBTBSR006A
2	*Solanum melongena* L. cv. Udupi Mattu Gulla	*Solanaceae*	Fruit	Devaraja Market, Mysore	12.311985015132 76.651802059811	BSRUG004-22	UOMBTBSR005A
3	*S. melongena* L. cv. Erangere badane	*Solanaceae*	Fruit	Kini stores, Mysore	12.3411606335661 76.6385548629406	BSRUG005-22	UOMBTBSR004A
4	*Jasminum grandiflorum* cv Mysore mallige	*Oleaceae*	Flower	Devaraja Market, Mysore	12.311985015132 76.651802059811	BSRUG002-22	UOMBTBSR002A
5	*Musa paradisiaca* cv. Nanjangud rasabale	*Musaceae*	Fruit	Devaraja Market, Mysore	12.311985015132 76.651802059811	BSRUG001-22	UOMBTBSR001A

All the sample specimens were collected by Dr. Bharathi

Table 2 Species identification results (homology comparison) based on GenBank and BOLD of *nrITS2* and *rbcL* genome regions of all the five NIPS of Karnataka

Sample name	Genome region	Sequence length (BP)	Genbank accession number	GENBANK	BOLD
				% similarity to nearest genera	% similarity to nearest genera
Musa paradisiaca cv. Nanjangud rasabale	nrITS	267	MT547697	93.23% *Musa AAB group*	85.83% *Musa acuminata*
	rbcL	547	MT498321	98.02% *Musa ornata*	98.48% *Musa acuminate sub sp. malaccensis*
Piper betle L. cv. Mysore betel leaf	nrITS	350	MT556475	100% *Piper betel*	90.22% *Piper excelsum*
	rbcL	612	MT498318	99.49% Piper betel	98.01% *Piper laetispicum*
Jasminum grandiflorum cv Mysore mallige	nrITS	265	MT531411	87.64% *Jasminum sambac*	Unable to match with any species in bold
	rbcL	609	MT498317	98.58% *Jasminum sambac*	98.23% *Jasminum sambac*
Solanum melongena L. cv. Udupi Mattu Gulla	nrITS	350	MT5477705	100% *Solanum melongena*	98.31% *Solanum aculeatissimum*
	rbcL	607	MT476301	98.34% *Solanum melongena*	98.5% *Solanum*
S. melongena L. cv. Erangere badane	nrITS	315	MT556478	98.44% *Solanum virginianum*	98.18% *Solanum*
	rbcL	566	MT498320	98.16% *Solanum melongena*	98.48% *Solanum*

Table 3 Proximate and nutrient content evaluation of selected native indigenous plant species (NIPS) of Karnataka

S.N	Parameter	*Musa paradisiaca* cv. Nanjangud rasabale	*Piper betle* L. cv. Mysore betel leaf	*Solanum melongena* L. cv. Udupi Mattu Gulla	*S. melongena* L. cv. Erangere badane
1.	Energy (Kcal)	326.33±0.08	48.64±0.1	320.39±0.01	304.92±0.02
2.	Protein (%)	4.03±0.01	3.69±0.01	5.14±0.03	14.32±0.01
3.	Carbohydrate (%)	76.96±0.08	7.38±0.05	72.80±0.01	59.80±0.01
4.	Fat (%)	0.23±0.01	0.68±0.02	0.95±0.01	0.92±0.02
5.	Crude Fiber (%)	0.71±0.01	1.83±0.03	15.67±0.06	11.80±0.01
6.	Ash (%)	2.60±0.02	2.82±0.01	7.32±0.01	7.49±0.01
7.	Moisture (%)	16.04±0.03	81.81±0.05	13.79±0.01	17.43±0.02
8.	Sodium as Na (mg)	61.94±0.04	31.72±0.02	62.61±0.06	104.9±0.1
9.	Potassium as K (mg)	887.30±0.01	4023.11±0.19	2658.27±0.06	2829.86±0.28
10.	Calcium as Ca (mg)	41.59±0.01	2119.4±0.35	208.42±0.22	579.70±0.6
11.	Magnesium as Mg (mg)	131.90±0.01	437.04±0.17	216.06±0.61	279.3±0.01
12.	Manganese as Mn (mg)	2.77±0.1	3.93±0.11	1.42±0.05	2.07±0.01
13.	Iron as Fe (mg)	7.21±0.01	22.11±0.01	32.32±0.06	6.69±0.05
14.	Copper as Cu (mg)	0.53±0.01	2.13±0.02	0.71±0.01	0.71±0.01
15.	Zinc as Zn (mg)	1.15±0.01	4.68±0.02	1.72±0.03	2.78±0.05

Range units are Kcal/100 g , %/100 g, mg/100 g dry weight. All the values are represented as mean ± S.E. of three replicates.

Plate 1

Plate 1 is available in Supplementary Files section.

Figures

Figure 1

Representative images of four different genus of native indigenous plant species (NIPS) collected **a.** *Solanum melongena* L. cv. Udupi Mattu Gulla **b.** *S. melongena* L. cv. Erangere badane **c.** *Piper betle* L. cv. Mysore betel leaf **d.** *Jasminum grandiflorum* cv Mysore mallige, and **e.** *Musa paradisiaca* cv. Nanjangud rasabale

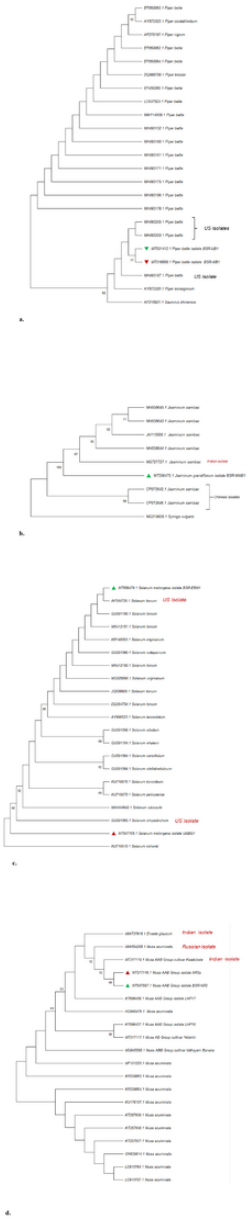

Figure 2

a-dshows the phylogenetic Neighborhood joining tree resulting from the MEGA v11.0.13 analysis of nuclear *ITS2* sequences of selected species; **a.** *Piper sp.* **b.** *Solanum sp.* **c.** *Jasminum sp.* and **d.** *Musa sp.* with 1000 bootstrap replicates. The native indigenous plant species (NIPS) species used in the present study are represented in a triangle taxon marker.

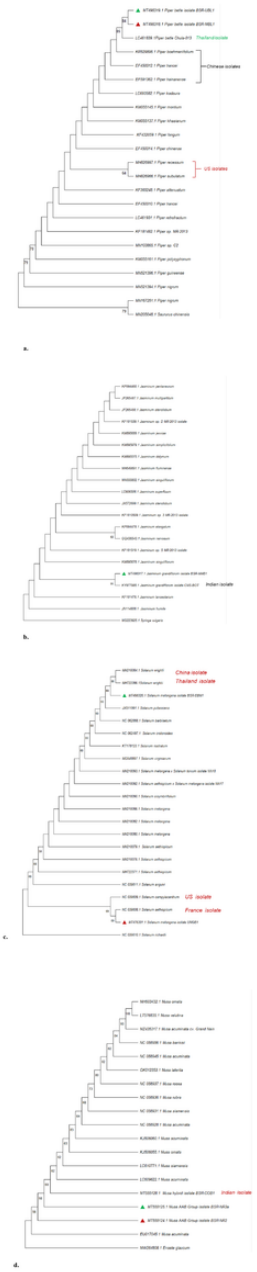

Figure 3

a-d shows the phylogenetic Neighborhood joining tree resulting from the MEGA v11.0.13 analysis of plastid *rbcl* sequences of selected species; **a.** *Piper sp.* **b.** *Solanum sp.* **c.** *Jasminum sp.* and **d.** *Musa sp.*with 1000 bootstrap replicates. The native indigenous plant species (NIPS) species used in the present study are represented in a triangle taxon marker.

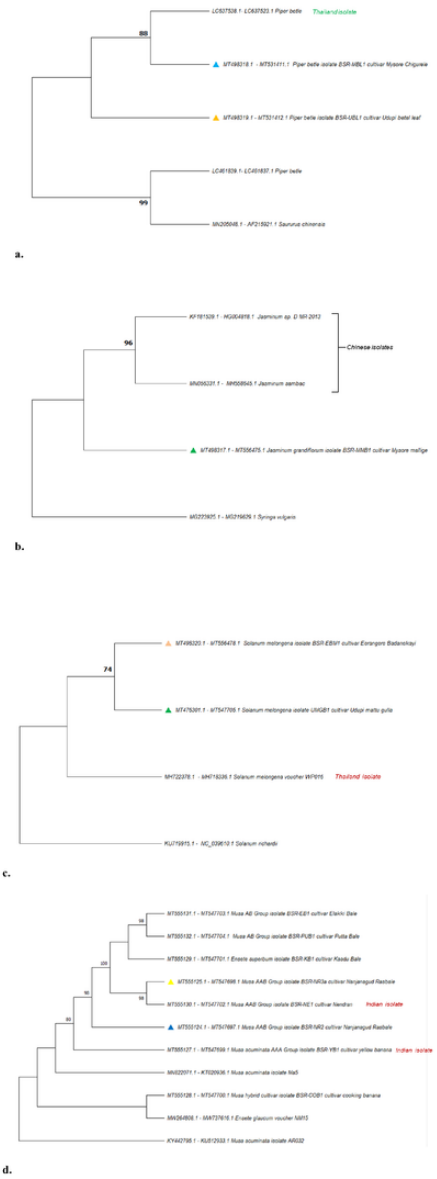

Figure 4

a-d Neighborhood joining tree inferred by the concatenation of nuclear *ITS2* and plastid *rbcl* genetic sequences; **a.** *Piper sp.* **b.** *Solanum sp.* **c.** *Jasminum sp.* and **d.** *Musa sp.* with the Genbank accession IDs and the species binomial name as generated in MEGA v11.0.13. The native indigenous plant species (NIPS) species used in the present study are represented in a triangle taxon marker.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Plate.1.png
- SupplementaryTables.docx