

# Genetic diversity assessment and gene expression analysis of prolonged shelf-life genes in Mangalore melon (*Cucumis melo* ssp. *agrestis* var. *acidulus*)

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## Research Article

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## Abstract

Mangalore melon (*Cucumis melo* ssp. *agrestis* var. *acidulus*) is a non-dessert melon, extensively grown in the coastal districts of South India, but hardly known to the rest of the World. Immature or mature fruits of Mangalore melon are used in preparation of delicious dishes such as vegetable stew, chutneys and curries. They are appreciated for nutritional values, long shelf life and biotic stress resistance. Seventy-nine accessions of Mangalore melon were collected from five states of South India and their genetic diversity was assessed using inter simple sequence repeat (ISSR) markers. Putative candidate genes of extended shelf life in Mangalore melon were studied by quantitative reverse transcription polymerase chain reaction in comparison with cantaloupe (*Cucumis melo* L.). Shelf life varied from 65 days to 300 days at room temperature. Six ISSR primers amplified 142 fragments ranging from 80 bp to 2380 bp with an average of 23.66 bands per marker on a high-resolution capillary electrophoresis system. Neighbor joining phylogenetic tree construction from the ISSR allele similarity based genetic distance revealed two major clusters with 46 and 33 accessions in each cluster. Expression of fruit ripening related genes of ethylene biosynthesis (1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase) and cell wall metabolism (polygalacturonase, xyloglucan endotransglucosylase/hydrolase and expansin) in Mangalore melons was significantly lower than the cantaloupe melon at 180 days after harvest. Mangalore melon is a promising genetic resource for enhancing the shelf life of melons and the putative candidate genes are useful in enhancing shelf life of cantaloupe following validation and conformation.

## Introduction

Culinary melons (*Cucumis melo* subsp. *agrestis* var. *acidulus*) of South India, locally referred as Mangalore melon or Sambhar Southe, is a non-dessert melon belonging to the family Cucurbitaceae with somatic chromosomal number  $2n = 2x = 24$  (Pitrat et al. 2000). It is one of the popular vegetables grown in the coastal districts of Karnataka, used for making sambar (a kind of lentil soup), hence the name Sambhar Southe. Mangalore melons are widely grown in the coastal parts of southern states of Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Telangana and Goa, referred by vernacular names Mangaluru cucumber, Mangaluru southekeyi, sambar cucumber, sambar southe, thouthe, mage-kaayi, moge-kaayi, mogem, dosakaya, budamekaya, bollari, Malabar cucumber, Madras cucumber, vellari, kanivellari, vellarikka and so on (Manchali et al. 2019; Ramesh Babu and Hariprasad Rao 2018; Silpa et al. 2019; Swamy 2017). Asian melon (*C. melo* subsp. *melo* f. *agrestis*) has been reported as the ancestor of culinary melons of South India and have been considered to be originated and domesticated in India (Endl et al. 2018; Sebastian et al. 2010).

Mangalore melons are characterized by dark green foliage, bearing monoecious flowers that produce non-climacteric fruits with varied size, shape and color (Fergany et al. 2011; Kamagoud and Shet 2018; Manohar and Murthy 2012). Flesh of Mangalore melons is white, very firm and crispy, non-sweet and slightly aromatic to non-aromatic. Seeds of Mangalore melons are 4–10 mm and are yellow colored (Fergany et al. 2011; Swamy 2017). Often Mangalore melons are referred as '*conomon*' melons (*C. melo* ssp. *agrestis* var. *conomon*) (Gondi et al. 2016; Kamagoud and Shet 2018; Lakshmi et al. 2017a; Lakshmi et al. 2017b; Ramesh Babu and Hariprasad Rao 2018; Silpa et al. 2019; Swamy 2017; Vinutha et al. 2017). But, Mangalore melons differs from their counterpart '*conomon*' melons of South East Asia with respect to larger fruits with varied shapes and size, long shelf life of more than six months and small seed size (Dhillon et al. 2012; Fergany et al. 2011; Koli and Murthy 2013; Manohar and Murthy 2012; Roy et al. 2012).

Both mature and immature fruits of Mangalore melons are cooked as vegetable. Off-white, turgid and crunchy flesh of Mangalore melon that holds crunchiness even after cooking is a delicious, yummy and low-calorie vegetable. It encompasses about 3.6% protein, 4% fat, and 2.5% carbohydrates and are also good sources of phosphorous, potassium, iron, zinc, ascorbic acid and carotenoids (Fergany et al. 2011). Consumption of Mangalore melons prevent macular degeneration in the eyes, the antioxidants in the seeds keep blood cholesterol under check and decrease the risk of cancer (Pawar 2018). Slightly bitter seeds of Mangalore melon that are usually discarded are hugely in demand by both the beauty and nutrition industries are also good sources of oils and fatty acids (Pawar 2018). Mangalore melons seeds contain 34.68% oil on dry weight basis, primarily comprises of nine fatty acids with high concentration of polyunsaturated fatty acids (80.54%). Linoleic acid (66.55%), oleic acid (13.70%), palmitic acid (11.65%) and stearic acid (7.49%) were the major fatty acids, which enhance the immunity and reduce atherosclerosis (Manohar and Murthy 2014).

In spite of its high nutritional and medicinal values, cultivation of culinary cucumber is limited to humid tropics of Southern India and are available for about 5 to 6 months during the spring and kharif season in the South Indian markets (Dhillon et al. 2012). Exploration of genetic diversity is crucial for broadening the genetic base for sustainable crop improvement. Molecular markers are invaluable tools for assessing the genetic variation within and among the plant genetic resources. Characterization of South Indian

melon landraces with SSR markers (Fergany et al. 2011), RAPD markers (Lakshmi et al. 2017a; Manohar and Murthy 2011) and AFLP markers (Lakshmi et al. 2017b) have revealed substantial genetic variation among the culinary melons collected from different parts of South India. However, genetic relatedness among the landraces from different south Indian states need to be explored with large collections.

Long shelf of Mangalore melons up to one year is a breeding treasure for enhancing the shelf life of melons. Contrarily, dessert type cantaloupe melons (*Cucumis melo* L.) that are widely cultivated and consumed all over the world have short shelf life of less than 15 days (Burger et al. 2010). Enhancing the shelf life of cantaloupes for long distance transport is one of the major objectives of cantaloupe breeding (Nunez-Paleniuss et al. 2008). Opportunistically Mangalore melons have same chromosome numbers that of cantaloupe and are readily crossable with each other with crossability index ranging from 50–70% (Subramanian 2008)(Dr. Ratnakar Shet, personal communication). Thus, Mangalore melons with long shelf life with firm fruits grant an opportunity to exploit Mangalore melons in enhancing the shelf life of cantaloupe. But non-dessert type Mangalore melons, characterized with low sweetness, unacceptable flesh color and flavour and other undesirable agronomic traits, pose risk of linkage drag. Identification of genes linked to shelf life is crucial for employing Mangalore melons in breeding of cantaloupe for enhanced shelf life. Candidate genes of enhanced shelf life in most of the fruits belong to the fruit ripening biochemical pathways such as cuticle function, cell-wall integrity, ethylene biosynthesis and upstream components that regulate ripening (Friedman 2019).

In this study, 79 accessions of Mangalore melon were collected from the five states of Southern India and the diversity in the fruit shape, skin color and shelf life were evaluated, and their genetic diversity was assessed using six inter simple sequence repeat (ISSR) markers. Putative candidate genes of shelf life were identified by assessing the expression levels of key shelf life related genes of ethylene biosynthesis and cell wall metabolism using quantitative reverse transcriptase PCR (qRT-PCR) in comparison with the cantaloupe.

## Material And Methods

### Plant material

Seventy-nine Mangalore melon accessions were collected from different parts of Karnataka (42), Kerala (15), Andhra Pradesh (13), Telangana (05), Tamil Nadu (04) (Table 1). Additionally, seeds of cantaloupe variety 'Hara Madhu' were procured from the market. Seeds were sown in trays and plantlets were transplanted to the field at College of Horticulture Farm-Sirsi, Uttara Kannada, Karnataka, India during October-December 2018 for assessing fruit shape, fruit epicarp color and shelf life. Observations on fruit shape and color were recorded as per the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, melon descriptors on five randomly selected plants in each accession (Srivastava et al. 2001). For assessing the shelf life, five fruits of each accession were hung to the roof with the help of banana fiber and examined on weekly interval for the spoilage (Fig. 1).

Table 1  
List of South Indian landraces of Mangalore melon and their diversity in fruit shape,  
color and shelf life

<b>Genotype</b>	<b>State</b>	<b>Fruit color</b>	<b>Fruit shape</b>	<b>Shelf life (days)</b>
MS1	Kerala	green	ovate	149
MS2	Kerala	yellow	elongated	130
MS3	Kerala	green	elongated	75
MS4	Kerala	orange	elongated	134
MS5	Kerala	striped	Elongated	90
MS6	Kerala	striped	pyriform	290
MS8	Kerala	yellow	ovate	80
MS9	Kerala	striped	oblate	195
MS10	Kerala	orange	oblate	135
MS11	Kerala	striped	oblate	170
MS12	Kerala	striped	ovate	281
MS13	Kerala	striped	oblate	120
MS14	Kerala	yellow	globular	165
MS15	Kerala	yellow	globular	150
Mudicode	Kerala	striped	ovate	106
IIHR381	Karnataka	yellow	oblate	150
IIHR386	Karnataka	striped	Elongated	130
Manbhagi	Karnataka	striped	ovate	120
MS16	Karnataka	striped	oblate	65
MS17	Karnataka	striped	pyriform	171
MS18	Karnataka	striped	oblate	65
MS19	Karnataka	striped	ovate	95
MS21	Karnataka	Dark green	oblate	210
MS22	Karnataka	green	oblate	97
MS23	Karnataka	striped	ovate	163
MS24	Karnataka	Dark green	oblate	78
MS25	Karnataka	green	ovate	165
MS26	Karnataka	striped	ovate	126
MS27	Karnataka	green	oblate	205
MS28	Karnataka	Dark green	oblate	195
MS29	Karnataka	striped	ovate	145
MS30	Karnataka	striped	oblate	300
MS31	Karnataka	striped	oblate	70
MS32	Karnataka	striped	oblate	185

<b>Genotype</b>	<b>State</b>	<b>Fruit color</b>	<b>Fruit shape</b>	<b>Shelf life (days)</b>
MS33	Karnataka	striped	oblate	144
MS34	Karnataka	striped	oblate	82
MS35	Karnataka	orange	oblate	169
MS36	Karnataka	striped	oblate	80
MS37	Karnataka	striped	Elliptic	174
MS38	Karnataka	striped	oblate	280
MS39	Karnataka	green	oblate	290
MS40	Karnataka	striped	ovate	129
MS41	Karnataka	orange	oblate	181
MS42	Karnataka	dark_green	oblate	288
MS43	Karnataka	striped	Elongated	123
MS44	Karnataka	striped	oblate	144
MS45	Karnataka	Yellow striped	oblate	95
MS46	Karnataka	striped	pyriform	117
MS80	Tamil Nadu	striped	oblate	271
MS81	Karnataka	green	ovate	150
SID1	Karnataka	green	Elliptic	152
SID2	Karnataka	striped	ovate	160
SS1	Karnataka	orange	elongated	70
SS11	Karnataka	striped	pyriform	207
SS14	Karnataka	striped	ovate	129
SS16	Karnataka	striped	Ovate	157
SS7	Karnataka	striped	ovate	148
SS9	Karnataka	striped	ovate	150
MS49	Andhra Pradesh	orange	globular	86
MS50	Andhra Pradesh	Golden yellow	globular	137
MS51	Andhra Pradesh	orange	globular	141
MS52	Andhra Pradesh	Golden yellow	globular	75
MS53	Andhra Pradesh	yellow	globular	199
MS54	Andhra Pradesh	yellow	globular	84
MS63	Andhra Pradesh	orange	oblate	156
MS64	Andhra Pradesh	striped	globular	191
MS66	Andhra Pradesh	yellow	globular	165
MS68	Andhra Pradesh	yellow	globular	80
MS69	Andhra Pradesh	striped	globular	127
MS71	Andhra Pradesh	orange	globular	153

Genotype	State	Fruit color	Fruit shape	Shelf life (days)
MS72	Andhra Pradesh	yellow	globular	101
MS48	Telangana	orange	globular	73
MS56	Telangana	orange	globular	127
MS57	Telangana	orange	globular	132
MS59	Telangana	Golden yellow	globular	174
MS60	Telangana	orange	globular	78
MS73	Tamil Nadu	Golden yellow	oblate	80
MS74	Tamil Nadu	striped	elliptic	226
MS75	Tamil Nadu	striped	pyriform	90

## Plant genomic DNA isolation

Leaf samples from five plants per accession were collected and immediately frozen in liquid nitrogen and stored at -80 °C for further genomic DNA extraction. Total genomic DNA was isolated from collected leaves using a modified cetyl-trimethyl-ammonium-bromide (CTAB) method (Clarke 2009). Briefly, 100 mg of leaf was homogenized by grinding in 0.8 ml of extraction buffer (100mMTris-HCl pH8.0, 20 mM EDTA, 1.4 M NaCl, 3.5% CTAB, 0.2%  $\beta$ -mercaptoethanol) with sterile mortar and pestle and then incubated at 600 C for 30 min. Extracted genomic DNA was purified using chloroform: isoamyl alcohol (24:1), DNA was pelleted in cold isopropanol, and re-suspended in nuclease free sterile water. DNA quality was analysed using gel electrophoresis and nanodrop Spectrometer (ND-1000, ThermoFisher, MA, USA). Equal quantity (100 ng) of DNA from five plants of each accession was pooled and used in polymerase chain reaction (PCR)

## DNA fingerprinting of culinary cucumber using ISSR markers

Seventy-nine Mangalore melons were fingerprinted with six ISSR markers developed at University of British Columbia (Table 2). Polymerase chain reaction (PCR) was carried out in 15  $\mu$ l reaction mix, containing 100 ng of DNA, 1X PCR master mix (Ampliqon Co, Denmark), 0.5  $\mu$ M ISSR primer (New England Biolabs, Ipswich, MA, USA). PCR programme was set with initial denaturation at 94 °C for 5 min and 35 cycles of denaturation at 94 °C for 30 s, annealing for 30 s, extension at 72 °C for 60 s, followed by final extension at 72 °C for 5 min. in a thermocycler (Eppendorf Mastercycler ® nexus; Eppendorf, Hamburg, Germany). Amplified PCR products were diluted 1: 3 (PCR product: dilution buffer) and resolved on a QIAxcel genetic analyser (Qiagen, Hilden, Germany), using OM800 method in preassembled QIAxcel DNA high-resolution cartridge, with QX Size Marker 25 bp-500 bp and QX alignment Marker 15 bp-600 bp. Prominent ISSR bands were manually scored for band presence (1) or absence (0) for each accession and a binary qualitative data matrix was constructed.

Table 2  
List of ISSR primers used for genetic diversity analysis of South Indian landraces of Mangalore melon

ISSR primer	Primer sequence (5'-3')	Number of bases	Annealing temperature
Prime809	AGAGAGAGAGAGAGAGYG	18	55 °C
UBC-808	AGAGAGAGAGAGAGAGC	17	55 °C
UBC-856	ACACACACACACACACCTA	19	55 °C
UBC-866	CTCCTCCTCCTCCTC	18	55 °C
UBC820	GTG TGT GTG TGT GTG TC	22	55 °C
UBC835	AGA GAG AGA GAG AGA GYC	23	55 °C

## Statistical analysis and phylogenetic tree construction

Polymorphic information content (PIC) and major allele frequency of each marker was calculated using PowerMarker (Liu and Muse 2005). The allele frequency was calculated as the ratio between the number of amplified fragments at each locus and the total number of accessions (excluding missing data). The PIC of each primer was calculated using average PIC value from all loci of each primer. Analysis of molecular variance (AMOVA), genetic distance and principal co-ordination (PCoA) were calculated using GenAlEx-6.503 (Peakall and Smouse 2006). Phylogenetic tree was drawn using MEGAX (Kumar et al. 2018) from the genetic distance calculated using GenAlEx-6.503.

## RNA isolation and shelf-life gene expression analysis

Expression of melon shelf life related genes of ethylene biosynthesis; 1-aminocyclopropane-1-carboxylate synthase (*CmACS1*), 1-aminocyclopropane-1-carboxylate oxidase (*CmACO1*), and cell wall modification; polygalacturonase (*CmPG1*), Xyloglucan endotransglucosylase/hydrolase (*CmXTH1*) and expansin (*CmExp1*) that were differentially expressed between climacteric and non-climacteric melons was studied with previously reported primers (Saladié et al. 2015) (Table 3). Melon cyclophilin (*CmCYP7*) gene was used as endogenous control.

Table 3

Primer sequences of the melon shelf life related genes used for the expression of analysis in South Indian landraces of Mangalore melon

Gene	Name	Forward primer sequence 5'-3'	Reverse primer sequence 5'-3'
MELO3C025848	Cyclophilin ( <i>CmCYP7</i> )	CGATGTGGAAATTGACGGAA	CGGTGCATAATGCTCGGAA
MELO3C021182	1-aminocyclopropane-1-carboxylate synthase ( <i>CmACS1</i> )	GATTGATCATAAGCTAAGGGTTTGGT	GGATAGCTAACCTTTGGGAACACTT
MELO3C014437	1-aminocyclopropane-1-carboxylate oxidase ( <i>CmACO1</i> )	AGAGGGCTTGTCTTTGTGTTTG	ATTTAGTTGAAAAGTCAAACCCAA
<i>CmPG1</i>	Polygalacturonase	AAGCATTAGCATAGGTTAAATGTGTGTTA	TGATGATTGAACGAACCAGTC
<i>CmXTH1</i>	Xyloglucan endotransglucosylase/hydrolase	TCGTTTTACTTTTTTTTTTCAATCTCCTATA	TGTGGCATGCTTTTAACTTATTTTAAC
<i>CmExp</i>	Expansin	GAGAGAGCTCAGACTCATTTCCTCC	CCCCTACTCCAACTTTCCCGGG

Mangalore melon accessions MS21, MS28 and MS30 that exhibited higher fruit firmness at 180 days after harvest were selected for studying the gene expression. Total RNA was extracted from the pulp and skin of three fruits the of the cantaloupe variety, Hara Madhu at 15 days after harvest and from three fruits each of Mangalore melon accessions MS21, MS28 and MS30 at 180 days after harvest using Spectrum™ Total Plant RNA Kit (Sigma Aldrich) as per the user guide protocol and treated with DNase I (Invitrogen). Purified RNA (500 ng from each sample) was reverse transcribed using Invitrogen cDNA synthesis kit (Invitrogen). Two microliters of 40x-diluted cDNA were used in a quantitative real-time PCR (qPCR) reaction using KAPA SYBR FAST qPCR Master Mix (2x) Universal (KAPA Biosystem, Wilmington, MA, U.S.A.) in QuantStudio® 5 Real-Time PCR system (Applied Biosystems). PCR programme was set at initial denaturation at 94 °C for 3 min, followed by 94 °C for 30 s; 60 °C for 45 s with 40 cycles. Fold change in differential expression of genes was calculated by  $2^{-\Delta\Delta CT}$  method with cantaloupe as the control sample (Livak and Schmittgen 2001).

## Results

### Variability in fruit traits

Substantial variation was found in the Mangalore melon accessions with respect to fruit shape, fruit skin color and shelf life. As per the NBPGR melon descriptors, six different shapes with seven skin colors were observed in 79 accessions of Mangalore melon (Fig. 2). Fruit shapes observed in the Mangalore melon accessions were oblate (28), Globular (19), ovate (17), elongated (07), pyriform (05) and elliptical (03). Observed skin colors were green striped (40), orange (13), yellow (10), green (08), four each of golden yellow and dark green. Shelf life fruits at room temperature varied from 65 days to 300 days. Four accessions from Karnataka MS30, MS39,

MS42 and MS38 exhibited much longer shelf life of 300, 290, 288 and 280 days respectively. Most of the fruits remained with firm skin throughout their storage time. Fruits ponged fruity aroma, one to two weeks before deterioration.

## ISSR markers diversity in Mangalore melon accessions

Six ISSR primers amplified 142 fragments ranging from 80 bp to 2380 bp with an average of 23.66 bands per marker. The ISSR marker UBC808 amplified highest (31 bands) with average PIC of 0.13, followed by UBC856 (30 bands, PIC = 0.06), Prime809 (29 bands, PIC = 0.11), UBC835 (24 bands, PIC = 0.09), UBC866 (15 bands, PIC = 0.04), and UBC820 (13 bands, PIC = 0.05). All the fragments were polymorphic, differentiating at least one genotype, thus exhibiting 100% polymorphism. A total of 864 bands were scored in 79 accessions of Mangalore melon with an average of 10.93 bands per genotype. Amplification percentage ranged from 1.2% (amplified in one accession) to 89.9% (71 accessions) in UBC835\_900. Accession MS31 from Karnataka exhibited the highest number of bands (23) followed by MS72 from Andhra Pradesh (21 bands) and MS24 from Karnataka (20). Least number of bands were amplified in MS49 of Andhra Pradesh (3 bands) followed by MS42 and MS45 of Karnataka with 04 bands each.

Among different states, highest number of bands were amplified in accessions from Karnataka (94 bands), of which 25 were private bands, followed by Kerala with 72 bands and 24 private bands (Fig. 3). Less number of private bands were amplified in the Mangalore melon accession from Andhra Pradesh, Telangana, and Tamil Nadu, indicating less diversity among these accessions. Mean percent polymorphism varied from 64.79% in the Karnataka accessions to 20.42% in Telangana and Tamil Nadu accessions. Kerala and Andhra Pradesh accessions exhibited 50.70% and 42.25% polymorphism respectively. Mean expected heterozygosity ranged from 0.047 in Telangana accessions to 0.073 in Tamil Nadu. Analysis of molecular variance (AMOVA) in Mangalore melon accessions using six ISSR markers revealed that 98% variation is within the accessions of each state and merely 2% variation was observed among the accessions from different states (Table 4).

Table 4  
Analysis of molecular variance of South Indian landraces of Mangalore melon using ISSR markers

Source	df	SS	MS	Est. Var.	%
Among Pops	4	38.33	9.58	0.18	2%
Within Pops	74	540.52	7.30	7.30	98%
Total	78	578.86		7.49	100%

df: degrees of freedom, SS: Sum of Squares, MS: Mean sum of squares, Est. var.: Estimated variance

## Principal Co-ordinate (PCoA) analysis

Mangalore melons accessions were grouped based on the principal co-ordinates contributing to first and second axes, calculated from the genetic distance of ISSR band similarities. Well-defined clustering of Mangalore melon accessions according to geographical region was not observed as per the ISSR marker analysis (Fig. 4). First two coordinates explained a total variation of 32.23% variation with co-ordinate 1 and 2 explaining 17.34% and 14.89% variation respectively. Sixteen Karnataka accessions were clustered together with negative eigen values in both the coordinates. A small cluster of Kerala accessions were clustered within the Karnataka accessions. When the Mangalore melon accessions were plotted according to the fruit skin color, most of the accessions with striped skin (green and yellow stripes) were clustered together along with the green, dark green and yellow skin colored accessions. No such obvious grouping was observed for fruit shape and shelf life.

## Phylogenetic analysis of Mangalore melons

Neighbor joining phylogenetic tree was constructed based on the genetic distance derived from the ISSR band similarities. Seventy-nine Mangalore accessions were clustered into two major clusters with total branch length of 412.78 and overall mean genetic distance of 14.84 (Fig. 5). Clad I consisted 46 accessions with total subtree distance of 245.46 and clad II consisted 33 accessions with subtree distance of 167.09. Like PCoA, distinct clustering of accessions according to the geographical region (states) was not observed. Intra-state distance (15.60, 13.57, 16.82, 13.0, 19.33 respectively for Kerala, Karnataka, Andhra Pradesh, Telangana, Tamil Nadu respectively) was higher than the between state distance (13.60 to 19.13). Largest divergence was observed between Tamil Nadu accessions and Andhra Pradesh accessions (19.13). Accessions of Telangana and Karnataka were proximal with a genetic distance of 13.60. Like PCoA, majority of the Karnataka and Kerala genotypes were grouped in the clad I.

## Differential expression of shelf life genes



Differential expression of seven shelf life related genes, 1-aminocyclopropane-1-carboxylate synthase (CmACS1), 1-aminocyclopropane-1-carboxylate oxidase (CmACO1) of ethylene biosynthesis and polygalacturonase (PG), xyloglucan endotransglucosylase /hydrolase (XTH) and expansin (EXP) of cell wall modification in the flesh and skin of three Mangalore melon accessions (MS21, MS28 and MS30) was studied by qPCR in comparison to cantaloupe variety 'Hara Madhu' at 180 days after storage. Expression levels of four genes, except ACO1 was significantly lower than that of the cantaloupe in both skin and flesh (Fig. 6a and 6b). Expression of ACS1 was 57.7 times lower in the flesh of MS30 than the flesh of cantaloupe, followed by 47.3- and 46.2-times lower in the skin of MS21 and MS28 respectively. Expression of ACO1 was higher in flesh of MS21 (46.81 times), flesh (9.0) and skin (21.3) of MS28, but its expression was significantly lower in MS30 (-8.0 times) that exhibited longest shelf life of 300 days.

Like ethylene biosynthesis and perception genes, expression of cell wall modification genes was also significantly lower in Mangalore melons accessions than cantaloupe. Expression of EXP1 was more than 1000 times lower in the skin of MS30 that exhibited highest shelf life of 300 days. Expression of PG was significantly lower in the flesh of MS30 (-292 times) and skin of MS28 (-60.9 times). Expression of XTH also exhibited lower expression in the skin of Mangalore melon accessions with 18.3, 26.2- and 1.9-times lower expression respectively in MS21, MS28 and MS30.

## Discussion

Mangalore melon, endemic to tropical humid regions of southern India is a unique non-dessert melon, mostly consumed as a cooked vegetable in preparation of South Indian vegetable stew. It is a staple ingredient in the Mangalorean cuisine (coastal district of Karnataka) known for its exceptional taste and undocumented nutritional and medicinal values (Balachander 2012; Pawar 2018). In spite, the Mangalore melons are less known to the rest of the world, except the coastal districts of South India. It remains to be an orphan with respect to genetic and molecular studies. Previously culinary melon landraces were collected from two states, Kerala and Tamil Nadu belonging to two groups: var. *acidulus* Naudin and var. *Momordica* (Roxb.) Duthie et Fuller (Fergany et al. 2011; Koli and Murthy 2013; Manohar and Murthy 2012) and Karnataka (Gondi et al. 2016; Lakshmi et al. 2017a; Manohar and Murthy 2012). However, *acidulus* melons are cultivated in at least six states of South India; Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Telangana and Goa (personal survey). This is the first study on collection and genetic diversity assessment of exclusive *acidulus* melons from five states of South India.

## Diversity in the underexploited Mangalore melon

Mangalore melons exhibited high phenotypic variability with respect to fruit shape, color and shelf life (Table 1, Fig. 2). Observed variability for fruit shape, skin color and shelf life were observed in all the regions, not specific to any region. Accordingly, seventy-nine accessions from five states didn't cluster according the geographical regions from where the accessions were collected as per the ISSR marker data (Figs. 4 and 5). Possibly, most of the Mangalore melons have the common ancestry as they are considered to be originally cultivated in the Mangalore district of Karnataka and spread to its neighboring states with similar food habit (Balachander 2012) (personal communication with growers and tradesman). Striped skin accessions might have originated from the crosses between and yellow and green types as all the three were grouped together (Fig. 4b). Genetics of phenotypic variability need to be studied through investigations on their ancestry.

Mangalore melon is still a genomic orphan and arbitrary primed RAPD and AFLP markers and SSR markers from melon (*C. melo* L.) have been successfully utilized analyzing the genetic diversity of Mangalore melons (Fergany et al. 2011; Lakshmi et al. 2017a; Lakshmi et al. 2017b; Manohar and Murthy 2011). In this study, arbitrary primed ISSR markers that allows the analysis of multiple loci were used to study the genetic diversity of 79 Mangalore accessions. High genetic diversity was observed within geographical regions (Table 4, Fig. 5). Similarly, moderate to high genetic diversity was reported with RAPD, AFLP and ISSR markers (Fergany et al. 2011; Lakshmi et al. 2017a; Lakshmi et al. 2017b; Manohar and Murthy 2011). Co-dominant SSR and SNP markers were useful in differentiating melon landraces different geographical regions including the conomon and *acidulus* melons of East India and South India respectively (Fergany et al. 2011; Nimmakayala et al. 2016). The SSR markers were also able to partially group the accessions from different states. Hence, development of reproducible SSR and SNP markers in Mangalore melon and diversity analysis using such markers is necessary for precise genetic diversity analysis and investigating their ancestry.

## Treasure for enhancing the shelf life of melon

Poor keeping quality of most of the commercial cantaloupe varieties, which produce climacteric fruits limits long distance transport and export. Enhancing the shelf life of melons is a priority in melon breeding (Burger et al. 2010; Nunez-Palenius et al. 2008). Non-dessert melons such as makua, conomon and acidulus melons are the genetic treasure for enhancing shelf life of dessert melons (Perpiná et al. 2017; Sripongprapai and Tira-Umphon 2014). Acidulus melons of South India have exhibited the longest shelf life up to 300 days (Table 1). Crossability of Mangalore melon (acidulus) with other melon groups (Singh et al. 2013) and cantaloupe (Subramanian 2008), Dr. Ratnakar Shet, personal communication) have exhibited wide heterosis for early maturity and enhanced shelf life respectively. In addition, Mangalore melons have also exhibited greater variability for resistance to biotic stresses such as cucumber mosaic virus, zucchini yellow mosaic virus, different races of fusarium wilt and powdery mildew (Fergany et al. 2011) and tolerance to fruit fly, downy mildew (Gondi et al. 2016), leaf minor and thrips infestation (Vinutha et al. 2017). Thus, Mangalore melons are exceptional genetic resources for the melon breeding, especially for enhancing the shelf life of cantaloupe.

## **Mangalore melons exhibit non-climacteric fruit ripening**

Increased ethylene synthesis at the onset of fruit ripening is required for the normal ripening of both climacteric and non-climacteric fruits (Barry and Giovannoni 2007; Cazzonelli et al. 1999). Significant lower expression of ethylene biosynthesis and perception genes in Mangalore melon accessions at 180 days after harvest compared to ripened cantaloupe melon (Fig. 6a) is suggestive of potential role of ethylene in delayed ripening in Mangalore melons. Delayed onset of ethylene biosynthesis is a characteristic of non-climacteric fruits (Ezura and Owino 2008; Pech et al. 2008). Thus, Mangalore melons may be classified as non-climacteric type of fruit ripening, similar to 'inodorus' and 'conomon' group (Saladié et al. 2015).

## **Putative candidate genes for enhancing shelf life of melons**

Fruit ripening has a major impact on the shelf life of fruits, which is governed by overlapping molecular mechanisms such as ethylene biosynthesis, perception and signaling (Stepanova and Alonso 2009) and cell wall metabolism (Goulao and Oliveira 2008). Significantly lower expression of CmACS1, CmACO1 of ethylene biosynthesis and CmPG1, CmXTH1 and CmExp of cell wall metabolism indicate delayed ripening of Mangalore melon accessions. Contrastingly, higher expression of genes of both the pathways were observed in the climacteric fruits of cantaloupe compared to the non-climacteric fruits of 'inodorus' and 'conomon' melons (Saladié et al. 2015).

Conversion of S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) is the first and committal step in the ethylene biosynthesis pathway and ACC oxidase (ACO) is involved in the final step of ethylene production in plant tissues (Pech et al. 2012). Of the multiple ACS and ACO enzymes reported in melon genome, CmACS1 and CmACO1 are specific to fruit ripening as its expression increases in climacteric fruit after the burst of ethylene (Miki et al. 1995; Saladié et al. 2015; Yamamoto et al. 1995). Good shelf life in climacteric fruits such as cantaloupes is associated with a slow break down of firmness of mature fruit at room temperatures, which in turn is controlled by the internal ethylene concentration (Giovannoni 2001). Low expressed CmACS1 and CmACO1 of Mangalore melon are potential candidates for enhancing the shelf life of melon.

Firmness of melon fruits decreases as they ripen in both non-climacteric (inodorus and conomon) as well as in climacteric cantaloupe varieties, but localized fruit softening is observed earlier in climacteric fruits than non-climacteric ones (Saladié et al. 2015). Analogously, expression of CmPG1, CmXTH and CmExp was significantly lower in Mangalore melon compared to cantaloupe. Polygalactouranases are one of the major classes of enzymes involved in degradation of complex cell wall polysaccharide pectin, leading to skin softening and tissue deterioration (Giovannoni 2001). Low expression polygalactouranases has been evident in melons with extended shelf life (Saladié et al. 2015). Xyloglucan endotransglucosylase/hydrolases (XTHs) are cell wall-modifying enzymes that hydrolyze one more cell wall polysaccharide xyloglucan during fruit ripening (Saladié et al. 2006). Similarly, expansins also co-operatively hydrolyze the complex cell wall polymers along with polygalactouranase and Xyloglucan endotransglucosylase/hydrolases (Rose and Bennett 1999) and, accordingly, higher expression of CmExp was enhanced with the onset of ethylene production, concomitant with the initiation of the softening (Nishiyama et al. 2007).

Crossability of Mangalore melon with cantaloupe melon offers an opportunity to enhance the shelf life of cantaloupe (Subramanian 2008), either through development of introgression lines similar to MAK-10 Charentais breeding line developed by introgression of a genomic region from Makuwa melon (Perpiná et al. 2017) or through marker assisted backcrossing (Behera et al. 2011). In either case, identification of genes/markers linked to will hasten precise transfer of shelf life genes. However, shelf life is not only governed by ethylene and cell wall metabolism, instead they are also governed several transcriptional factors and epigenetic modification. Moreover, ripening also influence the organoleptic properties of the fruit, such as aroma, flavour, sweetness, acidity, colour and

firmness of the fruit and delayed expression of fruit ripening may alter the quality of fruits. Hence, their biochemical cross talk between enhancing the shelf life and fruit quality deterioration need to be studied and further validation and conformation of candidate genes is required before they are utilized in breeding.

## Conclusion

Mangalore melon is a low-calorie vegetable, rich in vitamin, minerals and antioxidants, is also being used as a folklore medicine for constipation and curing piles and fistula (personal communication). Additionally, seeds of Mangalore melon are rich source of human health beneficial polyunsaturated fatty acids. Mangalore melons restricted to South India needs globalization for worldwide cultivation to supplement the global nutritional security. Mangalore melons are also potential genetic resources for multiple pests and disease resistance. The exceptional feature of Mangalore melons is their shelf life of up to one year at room temperature. Crossability of Mangalore melon with the cantaloupe afford prospect for cantaloupe breeders to enhance the shelf life and breeding for resistance to important pests and diseases. Identification of candidate genes for the specific traits and adaption of genomics assisted breeding and genome editing would minimize or overcome the transfer undesirable traits into cantaloupe.

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**Availability of data and material:** Included as tables. Seeds and raw ISSR genotyping data is available on request

**Code availability:** Not applicable

**Authors' contributions:** **RG** conceived and designed research, wrote the manuscript. **RMS** collected, evaluated and maintaining the germplasm. **AL** and **DHR** conducted experiments. **RCJ** Funding acquisition and project administration. All authors read and approved the manuscript.

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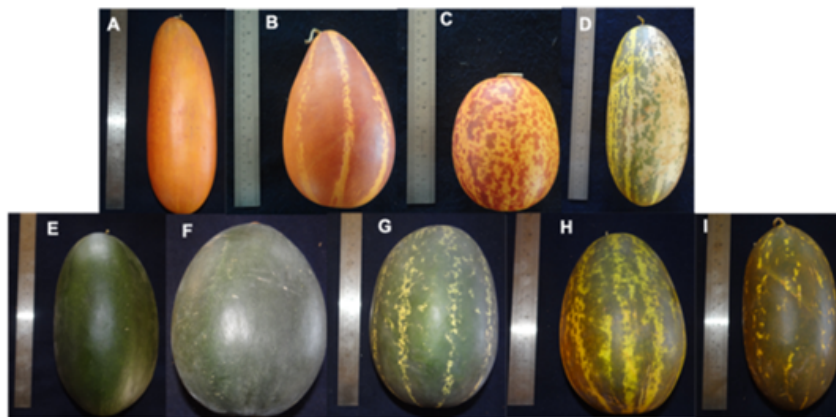
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## Figures



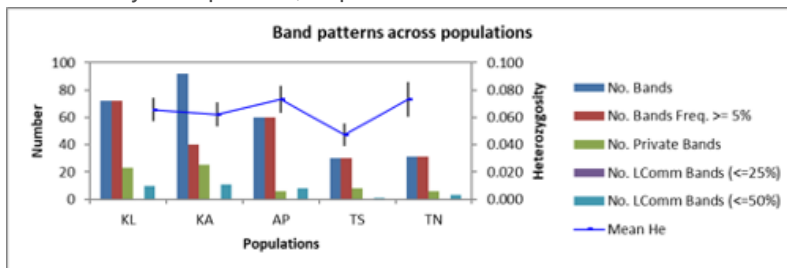
**Figure 1**

Preservation of Mangalore melons by hanging to roof at room temperature



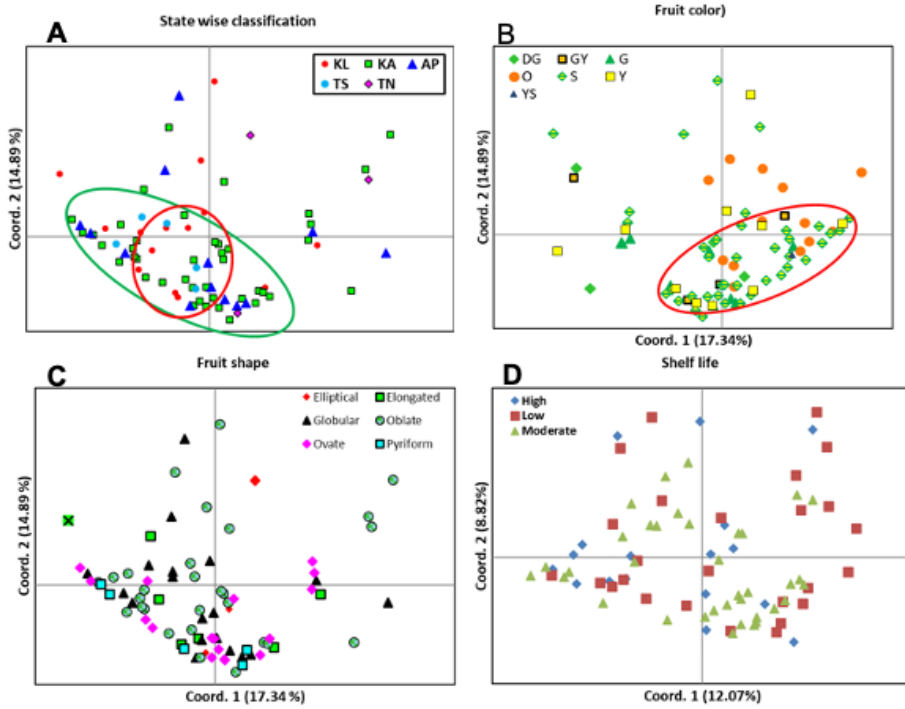
**Figure 2**

Variability in shape and color of Mangalore melon fruits A: Orange Elongated, B: Yellow Pyriform, C: Yellow Round, D: Green and white patches elliptical, E: Green oblate, F: Light green round G: Patchy green with white stripes, ovate, H: Green and yellow stripes, ovate I: Green with yellow patches, elliptical



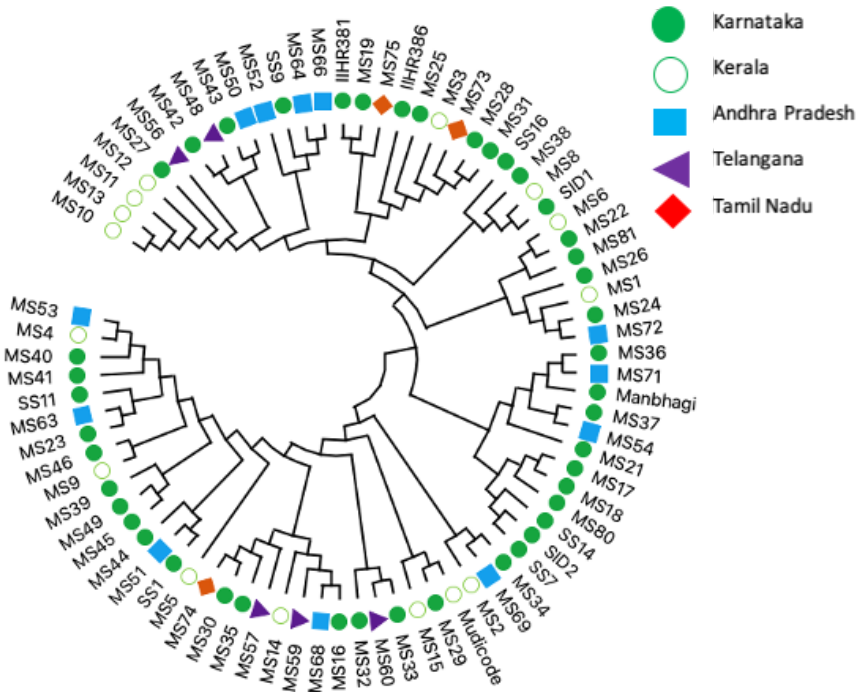
**Figure 3**

State wise banding pattern of ISSR markers in Mangalore melon KL: Kerala, KA: Karnataka, AP: Andhra Pradesh, TS: Telangana, TN: Tamil Nadu



**Figure 4**

Well-defined clustering of Mangalore melon accessions according to geographical region was not observed as per the ISSR marker analysis



**Figure 5**

Expression of shelf-life related genes in Mangalore melons (MS21, MS28, MS30) in comparison to cantaloupe melon A: Ethylene biosynthesis (ACS1: 1-aminocyclopropane-1-carboxylate synthase, ACO1: 1-aminocyclopropane-1-carboxylate oxidase). B: Cell wall metabolism (EXP: Expansin, PG1: Polygalacturonase, XTH1: Xyloglucan endotransglucosylase /hydrolase)

Image not available with this version

**Figure 6**

Expression of shelf-life related genes in Mangalore melons (MS21, MS28, MS30) in comparison to cantaloupe melon A: Ethylene biosynthesis (ACS1: 1-aminocyclopropane-1-carboxylate synthase, ACO1: 1-aminocyclopropane-1-carboxylate oxidase). B: Cell wall metabolism (EXP: Expansin, PG1: Polygalacturonase, XTH1: Xyloglucan endotransglucosylase /hydrolase)