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RESEARCH ARTICLE

Collection, Characterization and Identification of Elite Genotypes of Wild/Semi-Domesticated Bitter Gourd (*Momordica charantia* var. *muricata*) for Utilization and Conservation

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Abstract

Fifty genotypes of wild/semi-domesticated bitter gourd collected from major bitter gourd growing tracts of India were characterized for various qualitative, quantitative and biochemical traits to record the existing variability and genetic resources available for utilization and conservation. Wide variability was observed for fruit and seed characteristics. The preliminary screening of accessions under natural field conditions for their reaction to powdery mildew and viral infection resulted in the identification of five accessions *viz.*, IC 213312, AC-16/1, AC-16/4, AC-16/9, and AC-16/21 as potent resistant sources. Principal component analysis grouped the entire collection into four genetically diverse clusters irrespective of the origin or place of collection of accessions. The four better performing wild/semi-domesticated genotypes of bitter gourd *viz.,* AC-16/1, AC-16/16, IC 467681 and JJNS-15/65 should be further tested in large-scale yield trials and recommended for commercial cultivation, especially for homestead farming.

Keywords: Wild/semi-domesticated genotypes, Characterization, Principal component analysis, Screening, Resistant sources.

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Introduction

Bitter gourd/bitter melon/balsam pear (*Momordica charantia* L.), belonging to the family Cucurbitaceae, is an important commercial vegetable crop grown in India, Sri Lanka, Philippines, Thailand, Malaysia, China, Japan, Australia, tropical Africa, South America and the Caribbean. The genus *Momordica* is indigenous to the Paleotropics (Robinson and Decker-Walters, 1997). *Momordica charantia,* the most widely cultivated species in this genus, has been highly valued for its nutritive and medicinal properties. Bitter gourd has been used for centuries in the traditional systems of medicine of India, China, Africa, and Latin America. The fruits are known to possess antioxidant, anti-microbial and anti-diabetic properties (Raman and Lau,1996).

Bitter gourd was originally described by Linnaeus (1753) from Peninsular India and it is now found naturalized in all tropical and subtropical regions. Two varieties of *M. charantia* are reported in India *viz.*, *M. charantia* var. *charantia and M. charantia var. muricata* (Chakravarty, 1990). The wild variety, *M. charantia* var. *muricata*, is considered as the progenitor of the cultivated type (Degner, 1947) with small and round fruits having markedly sculptured seeds and *M. charantia* var. *charantia* is the cultivated type which produces large fusiform fruits having feebly sculptured seeds (Chakravarty, 1990). The first authentic document on the Flora of Malabar, Hortus

Malabaricus, illustrated four *Momordica* entities, of which "Pavel" and "Pandi-Pavel" refer to small-fruited bitter gourd and are reported to have been extensively cultivated by the natives of India in the past (Roxburgh, 1832). The large-fruited *charantia* types replace this because of its large or heavy fruits and high-yielding potential. Now, the cultivation of small-fruited bitter gourds is restricted to few homestead gardens and under wild conditions in disturbed open forest and have practically escaped the attention of farmers and breeders alike (John and Antony, 2009).

Compared with the highly vulnerebale cultivated types, wild/semi-domesticated *muricata* types are endowed with resistance/tolerance to some of cucurbits' common pests and diseases (Bharathi and John, 2013). The *muricata* types are even reported to have potent antioxidant and free radical scavenging activities (Wu and Ng, 2008). Since both botanical varieties are readily crossable, chance mating between them may result in semi-domesticated cultivars. Thus, the small-fruited wild bitter gourd is on the verge of extinction in Kerala and rest of Southern peninsular India. In view of their excellent nutritional quality and tolerance to biotic and abiotic stresses, these wild types need to be collected and conserved from the whole range of their distribution across India and evaluated for the identification of promising lines to create new cultivars with commercial value and adaptability to adverse growing conditions.

In this context, the present study was taken up to showcase the genetic wealth and existing variability of wild/semi-domesticated bitter gourd in India and thereby identify elite wild types with promising quality attributes that can be out-rightly recommended for cultivation or further crop improvement programs in bitter gourd. Multivariate data analysis, being a powerful statistical technique for analyzing genetic relationships from morphological traits, was used to discover the contribution of phenotypic traits to the total diversity in a germplasm collection. This article gives an insight into the extent of domestication in the wild genotypes, which may facilitate better conservation of this underutilized vegetable.

Materials and Methods

Plant Material

Fifty accessions of wild/semi-domesticated bitter gourd (*M. charantia* var. *muricata*) collected from the Regional Station of ICAR-National Bureau of Plant Genetic Resources (NBPGR), Thrissur, farmer's fields and wild habitats across Kerala, Karnataka and Tamil Nadu formed the material for the study. The plant materials from NBPGR comprised 24 accessions, of which five each were collected from Madhya Pradesh and Mizoram, two each from Rajasthan and Punjab, four from Kerala and three from Andaman and Nicobar (Figure 1). During the study period, a preliminary survey was conducted in Kerala and 12 sites of districts *viz.*, Thrissur, Palakkad, Kannur, Kottayam, Wayanad and Idukki were visited and 16 indigenous genotypes of wild/semi-domesticated bitter gourd were collected (Figure 1). Six collections from Tamil Nadu and four from Karnataka were added to cover a potentially higher variability (Figure 1).

Characterization Trials

Morphological characterization

The 50 *M. charantia* var. *muricata* accessions were raised in an augmented block design (Federer, 1956) along with three

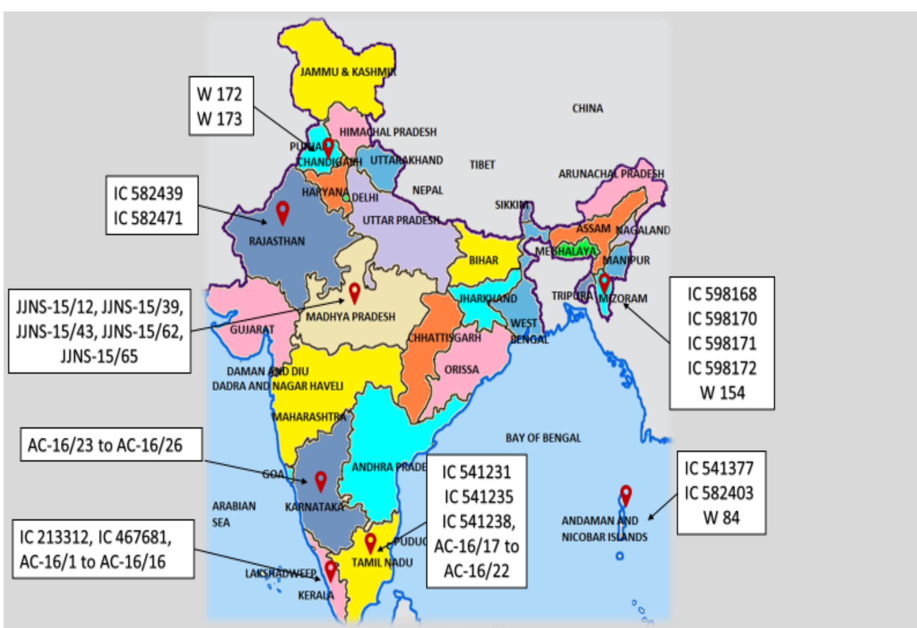

Figure 1: Map showing the collection sites of bittergourd accessions

check varieties *viz.,* Preethi, Priyanka and Pusa Purvi. Preethi and Priyanka are the two most popular varieties of bitter gourd, belonging to *M. charantia* var. *charantia*, released by Kerala Agricultural University (KAU) whereas Pusa Purvi is the first small fruited bitter gourd variety developed by ICAR-Indian Agricultural Research Institute (IARI), New Delhi. It is the only variety of *muricata* type released from India. Data on the germplasm were recorded during two seasons of 2015 and 2016 in the experimental fields of the Department of Plant Breeding and Genetics, KAU, Vellanikkara, Thrissur, Kerala. There were 10 blocks and each block had 5 accessions of wild/semi-domesticated bitter gourd and three check varieties. The collected accessions were planted in pits 60 x 60 x 30 cm in size. The spacing between the pits and row was 3 × 3 m. Five plants were maintained per accession. All the recommended agronomic practices were followed as per the Kerala Agricultural University's package of practices (KAU, 2011) for raising a good and healthy crop. The plants were supported by installing pandals with bamboo and nets.

Plant characters and data recording

The data were recorded on 28 traits, of which 11 *viz*., days to first pistillate flower, days to first fruit harvest, number of pistillate flowers, number of fruits per plant, a yield of fruits per plant (g), fruit weight, length and width, flesh thickness (cm), cavity size (cm) and number of seeds per fruit were measured quantitatively, while another 17 traits viz., early plant vigor (EVP), plant growth habit (GH), the twining tendency (TT), leaf margin, leaf shape, leaf size (LS), fruit shape (FSH), fruit color (FC), fruit size (FS), fruit ends (FE), nature of tubercles (NT), seed color (SC), seed luster (SL), seed shape (SSH) and seed size (SS) were assessed qualitatively on different scales. All the traits were measured/assessed based on the descriptor list developed by NBPGR (2001).

All the accessions were scored for their susceptibility to biotic stresses like powdery mildew and viral infection. The disease severity of powdery mildew was recorded by using 0–9-point scale of TNAU (1980), where (0) no lesions, (1) Small sized powdery specks infecting < 1% leaf area, (2) Enlarged irregular powdery growth covering > 1 to 5% leaf area, (3) Powdery growth to form big patches covering > 5 to 25% leaf area, (4) Powdery growth covering > 25 to 50% leaf area followed by yellowing, (5) >50 to 100% leaf area covered with powdery growth, yellowing and dropping of infected leaves. A modified 0 to 5 scale of Arunachalam *et al.* (2002) based on disease symptoms, was used to score the viral infection, where (0) no symptoms, (1) Minute chlorotic/mosaic specks on leaf, (2) Wide area of mosaic symptoms on whole leaf without distortion, (3) Mosaic symptom with reduction of 0 to 25% of leaf area, (4) Mosaic symptom with reduction of >25 to 75% of leaf area, and (5) Mosaic symptom with reduction of >75 to 100% of leaf area. Based on the disease score, level of resistance/susceptibility of the accessions to the disease were determined by percent disease severity (PDS) following the formula of McKinney (1923). The accessions were grouped into six categories based on the PDS of powdery mildew disease (Naik and Kulkarni, 2018) and viral infection (Arunachalam *et al.*, 2002) as given in Table 1. Each accession that showed resistance was tested twice to confirm the reaction.

Biochemical characterization

The collected genotypes were evaluated using standard procedures for four biochemical parameters: vitamin C, iron, fiber, and total antioxidant capacity. The vitamin C and crude fibre content of the fruits of bitter gourd accessions were estimated by volumetric, acid, and alkaline digestion methods, respectively, as Sadasivam and Manickam (1996) suggested. The fruits of 53 samples of bitter gourd were dried, powdered and digested with concentric nitric acid in a microwave digester. Iron content of the digest was then estimated in AAS using the hydride vapor generator method suggested by Jackson (1973). The phosphomolybdenum method (Prieto *et al.,* 1999) was used to estimate bitter gourd's total antioxidant capacity (TAC).

Organoleptic evaluation

Immediately after harvest, sensory evaluation of mature fruits from each genotype was done to identify the most accepted accession among them. Freshly harvested mature fruits of each accession were cut into small pieces and steamed for 5 minutes after adding a pinch of salt. Minimum processing was done in order to retain the flavor, color, taste and other qualities of the bitter gourd genotypes. Organoleptic parameters, namely, appearance, color, texture, flavor, odor, taste, after taste and overall

Table 1: Classification of accessions based on PDS of powdery mildew and mosaic diseases

Classification based on PDS of powdery mildew disease		*Classification based on PDS of mosaic disease*	
PDS	*Disease response*	*PDS*	*Disease response*
0	Immune (I)	0–5	Highly resistant (HR)
< 1	Highly resistant (HR)	>5–10	Resistant (R)
>1–10	Resistant (R)	>10–20	Moderately resistant (MR)
>10–25	Moderately resistant (MR)	>20–40	Moderately susceptible (MS)
>25–50	Moderately susceptible (MS)	>40–70	Susceptible (S)
>51–100	Highly susceptible (HS)	>70–100	Highly susceptible (HS)

acceptability of all the 53 bitter gourd genotypes, were evaluated by 20 semi-trained panel members between the age group of 18 to 40 years as suggested by Jellinek (1985) using a nine-point hedonic scale which arranged from 1 to 9. Hedonic ratings were then converted to rank scores, and rank analysis was done using Kendall's coefficient of concordance. Cumulative organoleptic score (COS) was also calculated for all the accessions by adding the mean rank recorded for each parameter.

Identification of better-performing wild/semi-domesticated genotypes

A modified method of Arunachalam and Bandyopadhyay (1984) was used to rank the genotypes, where each character's score was allotted based on its importance. The value of each accession for each trait was worked out by multiplying the score with the mean value of each character. These values were added across characters to provide a final/cumulative score for each accession. Based on the final scores, the entries were ranked on their performance over a set of characters. Thus, the better-performing bitter gourd genotypes among 50 wild/semi-domesticated accessions with respect to economically important characters were identified by ranking them using the selection index (SI) as enumerated below.

$$SI= [Y*10] + [FW*8] + [COS*6] + [NV*5]-[PM*8]-[M*8]$$

Where, Y-yield/plant, FW-fruit weight, COS-cumulative organoleptic score, NV-nutritive value, PM-percent disease severity of powdery mildew, M-percent disease severity of mosaic

Statistical Analysis

The germplasm accessions were evaluated in an augmented block design with three standard check varieties in 10 blocks. Analysis of variance was done for each of the quantitative characters observed using the free online Statistical Package for Augmented Designs (SPAD) developed by Indian Agricultural Statistical Research Institute (IASRI).The data obtained on various traits were further analyzed by principal component analysis (PCA) (Eriksson *et al.*, 1999), using the R based statistical software GRAPES version 1.14.02 (Gopinath, 2020) to quantify the contribution of each variable towards total genetic divergence in bitter gourd.

Results

Frequency Distribution for Qualitative and Quantitative Traits

Wide variability was present among the accessions for all the traits measured qualitatively. Early plant vigor recorded 30 days after sowing, which determines the subsequent growth of plant, was poor to good for 85% accessions. Long viny growth habit was observed for 79% of accessions, indicating the wild nature and 21% were medium viny type. The twin tendency was found to be slight in 53% of accessions and intermediate in the remaining ones. Leaf margin and leaf shape was uniform in all the genotypes, multifid and cordate, respectively, as reported by Sidhu and Pathak (2016). More than 50% of accessions had smaller leaves with intermediate pubescence.

The qualitative characteristics of fruits, like shape, end shape, size, color, nature of tubercles etc. showed variability among the accessions. About 66% of the accessions produced small fruits, while 28% were natural intermediates with medium-sized fruits. Only 6% of the genotype produced large fruits. The fruit shape of bitter gourd was disc in 2%, rhomboid in 13%, cylindrical in 4%, spindle-shaped in 7.5%, elliptical in 36%, oblong in 30%, and globular in 7.5% accessions. In all the accessions evaluated, fruit ends were pointed either at both ends or only at the blossom end, except in one (AC-16/25), where both ends were round (blossom end shape is blunt). The surface of the fruit was highly tubercled with long green ridges. Sharp and pointed tubercles were found in 48% of accessions, followed by soft and raised tubercles. Green-colored fruit was present in all the accessions with different light-to-dark green intensities. In the present investigation, remarkable variability was observed with respect to the qualitative characteristics of bitter gourd seeds. A wide range of seed colors was obtained: Straw-colored, black, black and brown patched, brownish tan and whitish brown. However, straw-colored seeds (60%) with intermediate glossiness (58%) were predominant. The seeds of all the genotypes evaluated had a squarish oval shape, except 4% of accessions, which are round in shape. More than 50% of genotypes produced very small-sized seeds.

All the 53 accessions of bitter gourd were screened for their response to two important biotic stresses in Kerala like powdery mildew and viral infection based on standard disease score charts. PDS values were calculated during two consecutive crop seasons and resistant genotypes were identified. Based on the preliminary screening for powdery mildew resistance, two genotypes (Preethi and Priyanka) were found to be highly susceptible (PDS- 51.84 and 59.67, respectively), 24 were resistant, 19 were moderately resistant, three were moderately susceptible and five were highly resistant (PDS- 0.29–0.85). Whereas one (AC-16/4) was found to be highly resistant, five were resistant, nine were moderately resistant, 25 were moderately susceptible, 11 were susceptible and two were highly susceptible to viral infection. Five wild/semi-domesticated genotypes, IC 213312, AC-16/1, AC-16/4, AC-16/9 and AC-16/21, with high resistance to both powdery mildew and viral infection, were identified.

Frequency distribution graphs for quantitative traits depicted in Figure 2 showed that more than 50% of accessions fall in the medium group of days to first pistillate flower (50–60 days) and days to first fruit harvest (50–70 days). The number of pistillate flowers and the number of fruits per plant revealed wide variation ranging from 179.71 to 17.78% and 14.32 to 168.39%, respectively. The highest range of fruit length (10.1–20.0 cm) and fruit width (4.1–5.0 cm) was observed in less than 10% of accessions. High variability was recorded for fruit yield per plant (124.05–8568.61 g). The average weight of individual fruit varied significantly among accessions and ranged from 3.53 to 420.61 g where only 4% of accessions produced the heaviest fruit (>100 g). The flesh thickness was 0.11 to 0.50 cm for 68% of the accessions, while the cavity size was 1.01 to 2.0 cm for 51% of the accessions. The majority of accessions had 10 to 20 number of seeds per fruit.

Principal Component Analysis

The principal component analysis was performed based on the nine yield contributing characters *viz.,* number of pistillate flowers per plant, number of fruits per plant, fruit yield per plant, fruit weight, fruit length, fruit width, flesh thickness, cavity size and number of seeds per fruit. The cumulative variance, factor scores and contribution

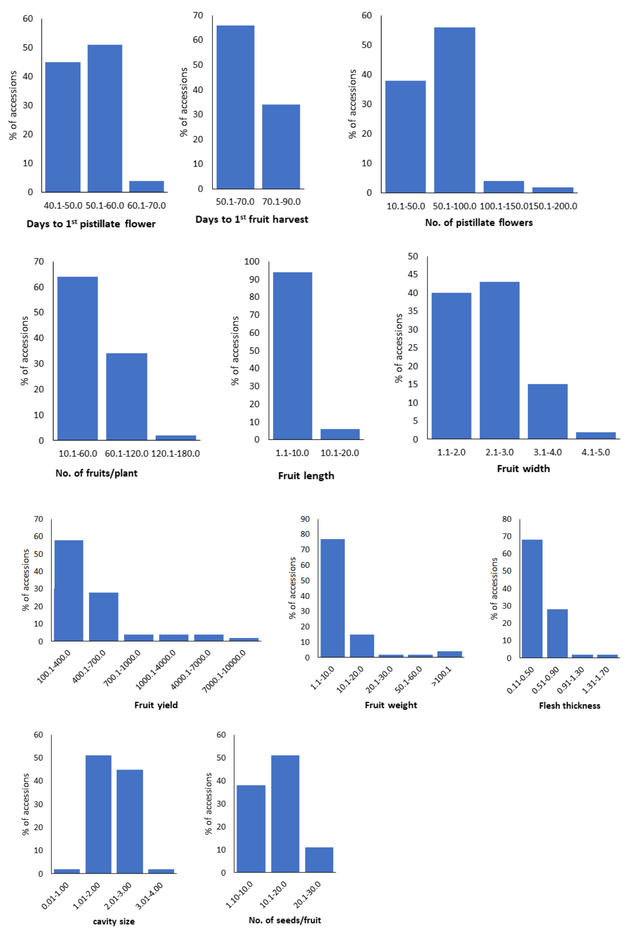

Figure 2: Frequency distribution of bitter gourd accessions for quantitative traits

Table 2: Eigen values, factor loadings and contribution of variations by yield attributing characters in bitter gourd

Variables	*PC1*	*PC2*	*PC3*
No. of pistillate flowers	-0.225	-0.654	0.022
No. of fruits per plant	-0.216	-0.664	0.047
Fruit yield plant	0.342	-0.193	0.489
Fruit weight	0.356	-0.089	0.505
Fruit length	0.402	-0.043	-0.016
Fruit width	0.376	-0.157	-0.324
Flesh thickness	0.379	-0.031	0.191
Cavity size	0.327	-0.22	-0.45
No. of seeds per fruit	0.323	-0.102	-0.399
Variance (%)	58.62	18.73	12.66
Cumulative variance (%)	58.62	77.35	90.01

of variation of each of the characters is presented in Table 2. In response to principal components, the scree plot revealed that the first three principal components had eigen eigenvalue > 1, accounting for 90.01% of the total variation. The first principal component (PC1) comprised of fruit yield per plant, fruit weight, fruit length, fruit width and flesh thickness and explained 58.62% of the total variation, whereas the PC2 comprised of a number of pistillate flowers and a number of fruits per plant and explained 18.73% of total variation. The PC3 accounted for 12.66% of variability and featured cavity size and number of seeds per fruit (Table 2).

The clustering of 53 accessions of bitter gourd based on the first two principal components formed four major distinct clusters (Figure 3). Cluster IV included the highest number of collections (46) and cluster I and III contained two accessions each. The score plot indicated that cluster I (Priyanka, Preethi), and II (AC-16/1, AC-16/16, IC467681) positioned at the first quadrant on the positive side of PC1 and PC2, exhibited their superiority over other clusters. The accessions grouped as cluster I belong to var. *charantia* and were superior to other clusters with preferred yield contributing attributes. Cluster II consisted of three collections that stand as elite wild/semi domesticated bitter gourd lines with good fruit yield per plant and weight. The two accessions in cluster III were superior with respect to number of pistillate flowers and fruits per plant.

Biochemical Characterization

Biochemical evaluation of 53 genotypes of bitter gourd based on vitamin C, iron, fiber content and antioxidant activity were done following standard procedures. A significant difference was observed among the accessions based on the analysis of variance. The average vitamin C content among 53 genotypes of bitter gourd was 94.14 mg/100 g, ranging from 76.53 mg/100 g (AC-16/25) to 125.67 mg/100 g (W 154). W 172 had the lowest (1.48 mg/100 g)

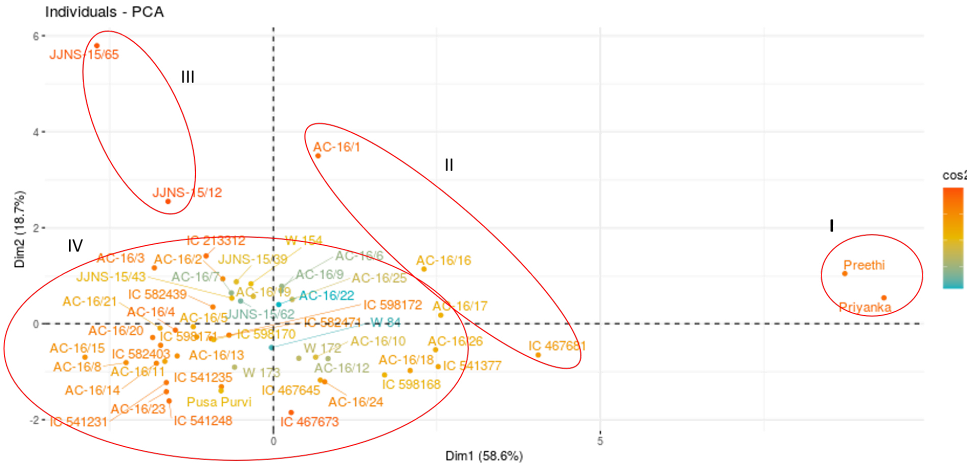

Figure 3: Score plot based on first two principal components of bitter gourd accessions

and AC-16/6 had the highest iron content (3.31 mg/100 g). The crude fibre content ranged from 12.17% in IC 598170 to 30.44% in IC 582403. The antioxidant capacity varied from 41.88 to 92.01 mg AAE/g.

Organoleptic Evaluation

The sensory profile of most preferred bitter gourd accessions is presented in Figure 4. Priyanka, Preethi, AC-16/1, IC467645, JJNS-18/43 and IC598168 ranked higher for their texture, taste, flavor, odor, after taste, appearance, color and overall acceptability. The maximum score for all the organoleptic parameters except taste and flavor were observed for Priyanka. The maximum taste and flavor scores were observed for AC-16/24 (43.75) and IC 598168 (43.40), respectively. Among the wild/semi-domesticated genotypes, the maximum score for appearance was found in AC-16/12 (43.75). Similarly, the accessions AC-16/18, AC-16/1, AC-16/21, and W 154 scored maximum for texture, color, odor and aftertaste, respectively. A cumulative score was also calculated by adding the scores obtained for all eight parameters. The overall acceptability was high for the cultivated types like Priyanka (52.05) and Preethi (47.10) followed by the wild accession AC-16/1(39.25) with a cumulative score of 288.60. The cumulative organoleptic score (COS) was the lowest in W 173 (130.25). Therefore, when the accessions were ranked based on organoleptic evaluation, first rank was recorded for Priyanka, followed by Preethi and AC-16/1 and last rank was recorded for W 173.

Identification of Elite Wild/Semi-domesticated Bitter Gourd Genotypes

All the 50 wild genotypes were ranked based on the selection index (Table 3) and better performing wild/semi-domesticated genotypes were identified with respect to higher yield, fruit weight, acceptability, nutritive value and resistance to biotic stresses. When the wild/semi-domesticated accessions were ranked based on yield, fruit weight, organoleptic score, nutritive value and resistance,

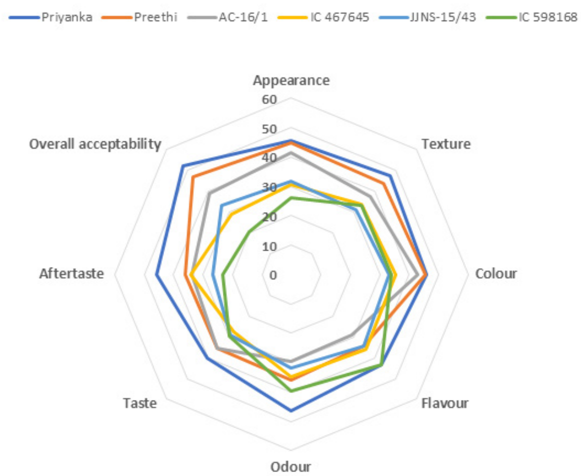

Figure 4: Organoleptic evaluation of most preferred bitter gourd collections

the best genotypes were AC-16/1, followed by AC-16/16, IC 467681 and JJNS-15/65. So, these accessions can be further tested in large-scale yield trials and recommended for commercial cultivation, especially for homestead farming.

Discussion

Bitter gourd is the highly nutritious and most popular vegetable throughout the tropics and subtropics of Asia (Reshmika *et al*., 2019). The economic and breeding gains from germplasm collections can be easily derived by morphological characterization (Bekele *et al.*, 2006). Dey *et al*. (2006) and Paul *et al.* (2010) have also studied the genetic diversity of bitter gourd based on morphological characters. Chakravarty (1990) reported that plants belonging to different species of *Momordica* showed differences in morphological characters such as flower color, fruit shape, fruit color, fruit size and leaf shape. The simplified phenotypic approach has been found useful for preliminary characterization and discrimination of accessions to understand the level of genetic diversity (Szilagyi *et al*., 2011).

Table 3: Ranking of accessions based on selection index

Accessions	*FY*	*FWt*	*COS*	*VIT C*	*FE*	*FIBRE*	*TAC*	*PM*	*VI*	*Rank*
AC-16/1	1493.88	14.19	288.6	98.89	2.79	19.5	70.03	0.29	6.95	1
AC-16/16	1058.62	19.11	226.55	83.65	2.19	15.7	48.32	12.17	27.83	2
IC 467681	969.44	50.15	225.4	99.56	2.18	13.6	86.93	38.82	14.34	3
JJNS-15/65	900.38	5.35	195.35	100.51	2.04	15.71	81.33	16.24	6.67	4
AC-16/9	642.33	9.38	205.4	86.21	2.73	19.52	41.88	0.46	5.84	5
IC 213312	570.27	6.94	178.75	85.11	3	17.39	67.47	0.365	7.34	6
JJNS-15/12	561.91	5.42	187.75	94.25	2.18	14.36	86.45	7.44	18.84	7
W 84	504.69	11.89	233.15	94.37	2.54	13.17	92.01	11.14	32.34	8
AC-16/3	485.33	5.87	236.85	95.58	2.9	13.11	78.83	6.57	13.84	9
AC-16/12	472.72	14.99	237.75	120.63	2.16	14.65	86.43	5.71	39	10
AC-16/5	508.05	8.18	214.65	92.44	2.67	20.15	83.72	1.89	50.84	11
AC-16/2	496.94	7.09	218.85	101.23	2.16	14.3	69.73	28.37	37.17	12
AC-16/6	454.88	6.91	233.15	96.37	3.31	15.03	54.83	4.46	10.17	13
JJNS-15/39	477.15	6.83	195.85	86.75	2.19	15.06	70.13	11.28	16.67	14
IC 598168	441.48	22.82	250.4	95.3	3.05	14.35	83.22	10.52	44.17	15
W 154	437.27	6.21	234.45	125.67	2.94	21.5	64.39	16.48	36.5	16
AC-16/25	455.42	7.36	189.35	76.53	2.27	17.39	78.21	7.02	30.17	17
JJNS-15/62	421.94	6.92	218.45	88.5	2.94	16.21	84.52	12.54	33	18
JJNS-15/43	401.79	6.24	251.25	84.43	2.05	17.83	87.33	21.95	27.67	19
AC-16/10	391.21	10.46	206.8	90.57	2	21.04	84.04	16.98	12.5	20
AC-16/17	399.85	10.57	198.6	100.54	2.74	21.58	73.9	13.34	35.17	21
AC-16/22	420.03	7.29	188.15	93.38	2.18	19.88	49.33	13.38	36.83	22
AC-16/19	393.84	6.09	194.3	97.12	2	19.46	90.36	7.32	44.17	23
IC 598172	359.43	7.09	230.15	80.47	2.58	15.64	63.37	3.41	29.67	24
AC-16/26	367.92	13.27	233.7	80.51	2.16	16.3	64.1	4.13	50.84	25
IC 582471	331	6.62	245.4	104.52	2.57	20.92	65.19	5.11	40.67	26
W 173	374.06	8.19	130.25	95.5	2.51	14.39	75.13	19.82	14.84	27
IC 598171	354.51	6.56	165.5	82.94	3.15	18.69	60.91	8.26	25.67	28
W 172	300.69	7.29	211.75	106.71	1.48	24.83	84.74	8.29	28.34	29
IC 598170	310.36	5.97	238.15	112.43	2.99	12.17	64.9	13.98	37.67	30
AC-16/4	283.26	5	212.5	100.06	2.55	19.25	71.6	0.36	3.51	31
AC-16/11	317.96	6.05	203.45	98.79	2.53	12.94	75.11	4.52	39.34	32
AC-16/13	315.42	6.44	194.15	110.47	2.02	19.62	64.29	3.11	36	33
IC 582439	303.03	4.77	236.6	78.93	2.95	19.33	49.62	4.04	28.17	34
AC-16/20	288.61	4.77	221.1	99.7	3.07	15.43	68.37	5.31	24.67	35
IC 541377	289.11	13.04	178.3	91.64	2.14	17.61	80	6.89	15.5	36
AC-16/24	268.7	9.08	240.05	92.51	2.85	15.83	70.49	1.45	41.5	37
AC-16/21	220.31	3.59	245.95	100.1	2.17	14.58	82.7	0.85	10	38
AC-16/8	254.06	4.97	195.3	85.49	3.17	20.33	87.38	14.09	24.33	39
IC 467645	220.14	8.19	261.05	89.88	1.94	19.82	64.92	3.81	73.84	40
AC-16/7	246.75	4.1	193.65	83.82	3.07	16.47	59.41	23.67	32.67	41
IC 582403	203.46	3.53	159.65	91.45	2.11	30.44	68.45	2.69	24.17	42
AC-16/18	192.79	9.29	225.15	97.94	2.4	15.1	49.63	33.78	42.67	43
AC-16/14	193.03	4.01	148.05	102.48	2.89	15.6	83.18	11.57	34	44

AC-16/23	159.87	4.17	171.6	95.14	1.94	17.46	88.51	3.76	18.83	45
AC-16/15	182.53	3.17	152.35	99.57	2.05	15.68	58.01	9.67	23.34	46
IC 541235	124.27	4.06	212.8	83.32	1.94	22.21	73.8	7.87	33.84	47
IC 541231	140.06	3.73	182.25	106.73	2.63	15.67	83.84	24.87	40.84	48
IC 467673	124.05	7.23	233.75	86.59	2.04	20.13	71.11	3.75	76.33	49
IC 541248	127.67	3.97	221.35	80.46	1.99	14.22	70.61	19.72	45	50

*FY-Fruit yield, FWt- Fruit weight, COS- Cumulative organoleptic score, VitC- Vitamin C content, Fe- Iron content, Fibre- Fibre content, TAC- Total antioxidant capacity, PM- PDS of Powdery mildew, M- PDS of viral infection

This study describes one of the large-scale phenotypic characterization of wild/semi-domesticated bitter gourd landraces of India. The germplasm collected in this study presented wide range of genetic variability for 30 traits among 53 accessions. The frequency distribution obtained for these characters showed the presence of maximum possible range of variability expected in the Western Ghats of Kerala. This survey showed that wild/semi-domesticated bitter gourd co-exists with cultivated varieties in adjacent habitats or under wild conditions in disturbed open forests. Specific cases of home garden cultivation of small-fruited wild genotypes were also noticed. In West Bengal, Andaman Island and Tamil Nadu, the small-fruited *muricata* types were even cultivated commercially for market supply (John and Antony, 2009).

Plant growth habits were found to vary from medium viny nature in cultivated varieties to long viny in wild cultivars. The short internodal length and a greater number of nodes per plant in the wild/semi-domesticated genotypes have given a more compact mat-like growth habit in which the vines spread like a carpet and easily cover the support (Asna, 2018). Even though the plants grow as an annual crop, the long viny wild *muricata* types have longer lifecycle and can extend beyond one season. The robustness and size of leaves and stem were greater in *M. charantia* var. *charantia* and progressively reduced to *M. charantia* var. *muricata*. These observations are in agreement with studies conducted by Bharathi and John (2013).

Among different traits, fruit characteristics are the major determinant of consumer's preference. In this study, marked variation was observed in fruit size ranging from very small to large with green color in varying tonalities. The preference for fruit size and color was found to be region-specific. The north Indians prefer long or medium long spindle-shaped glossy green fruits, whereas south Indians require long but white fruits. In eastern parts of the country, small and dark green-colored wild *muricata* types are preferred (Dey *et al*., 2010). Similar observations on fruit bitterness, color, shape and size preference have been recorded worldwide (Dhillon *et al*., 2014; Rao *et al.,* 2021). This suggests that differences exist between different regional cultivar groups in bitter gourd, reflecting the distinct consumer preferences in different countries and introgression from cultivars back to wild accessions and vice-versa. Population resequencing revealed the divergence between wild and South Asian cultivars about 6,000 years ago, followed by the separation of the Southeast Asian cultivars about 800 years ago, with the latter exhibiting more extreme trait divergence from wild progenitors and stronger signs of selection on fruit traits (Matsumura *et al*., 2020).

Rajput *et al.* (1996) and Rasul *et al.* (2004) found a wide range of variation among the genotypes of bitter gourd in respect of flowering habits. Bitter gourd is a monoecious plant, naturally inducing a greater number of staminate flowers than the pistillate flowers. This is a common problem in bitter gourd cultivation, since this flowering behavior results in lower fruit set and yield. In order to have higher yield, the staminate and pistillate flower ratio needed to be synchronized (Ram *et al.,* 2000). So, the germplasm lines *viz.,* JJNS-16/65 having higher sex ratio than cultivated varieties have an important role in breeding programs for developing high-yielding crop varieties. The days to first fruit harvest were the least in the accession AC-16/25 (57 days) because of the earlier initiation of the first pistillate flower (40 days).

The wild, semi-domesticated and cultivated types have fallen in different classes of performance for yield per plant. Yield per plant itself is not a trait but a product of complex interaction of fruit traits such as fruit length, diameter, weight, number, and flesh thickness (Dey *et al.,* 2005; Rani *et al.,* 2015). In this study, the yield components like, fruit length ranged from 2-16 cm, fruit width from 1 to 4 cm, fruit weight from 3 to 420 g and the number of fruits per plant from 14 to 168. The flesh thickness of the fruit ranged from 0.15 to 1 cm, reflecting the quality and edible portion of bitter gourd fruit.

Apart from fruit traits, the variation found in number of seeds per fruit was significantly wider in the collected accessions. The seed size can easily identify the wild and cultivated genotypes. Wild bitter gourd produces small seeds resembling cockroach eggs whereas, genotypes belonging to *charantia* develop comparatively large, broadly rectangular, heavily or feebly sculptured seeds with sub-tridentate ends (Bharathi and John, 2013). It was noticed that the wild/semi-domesticated genotypes are more highly seeded than the *charantia* types. Seediness is a wild character that helps in surviving and producing more offspring. The cultivated check varieties like Preethi and Priyanka, with long fusiform fruits and high fruit weight, produced only 21.58 and 19.81 seeds, respectively. While even the accession with lowest weight *i.e.,* IC 582403 (3.53 g), produced 9.57 seeds per fruit. The smaller size and highly seeded nature of *muricata* types may be the reason for the consumers' low acceptability of this highly medicinal variety of bitter gourd. Reduced seed content of fruits is desirable in bitter gourd (Islam *et al.*, 2009). Even though, the number and size of seeds of the wild/semi-domesticated genotypes was lower than that of the var. *charantia*, the proportion of the seeds to the total fruit biomass was higher. So, when the seeds are removed the consumable portion of the fruit becomes very small in *muricata* types. Hence, these fruits are preferred for cooking during immature stage itself along with the seeds.

Though there is no formal infraspecific system of classification, the extensive work done by Walters and Decker-Walters (1988) and Yang and Walters (1992) have given an insight into the difference between wild and domesticate types of bitter gourd as fruits of wild plants are 2 to 7 cm long, pointed at both ends and have small grey to nearly black seeds that often have scattered darker black patches whereas the fruits of domesticate are upto 60 cm long, are sometimes flattened at the proximal end, and have larger brown seeds. In the present study, 42 collections had fruit lengths of 2 to 7 cm and can be considered wild types. Eight accessions with a fruit length above 7 cm and seed characters similar to cultivated types represent the degree of domestication in bitter gourd genotypes.

Although beneficial to health, dietary phytonutrients in bitter gourd are bitter, acidic and/or astringent in taste, reducing consumer choice and acceptance of bitter gourd during food selection (Snee *et al.*, 2011). But the role of this bitter melon in treating diabetes increases its acceptability. Since higher fruit yield leads to decrease in ascorbic acid, iron and calcium content (Tendulkar, 1997; Dey *et al.,* 2005), small fruited accessions (*M. charantia* var. *muricata*) should be preferred over large fruited ones (*M. charantia* var. *charantia*) for better availability of ascorbic acid, iron and calcium content with increased fruit number. The small size of fruits and the belief that the bitterness increases with the intensity of green color can be a reason for low acceptability of *muricata* types, even though it is endowed with high nutritional values. Organoleptic scoring aided in selecting the most superior genotypes with better appearance, texture, color, flavor, odor, taste and aftertaste. Even though, the cultivated types surpassed wild genotypes in organoleptic evaluation, AC-16/1 with good COS and better organoleptic qualities can be selected as the best wild/semi-domesticated bitter gourd genotype which can be commercialized in future.

Grouping large number of germplasm accessions into few numbers of homogenous clusters facilitates the selection of diverse parents. Similarity indices and pattern of relationships obtained through genetic diversity and PCA are useful to evalute potential breeding value of germplasm through traits loaded on various components (Keneni *et al.*, 2005). Principal component analysis grouped the cultivated types separately from wild/semi-domesticated types. The wild/semi-domesticated accessions *viz.,* AC-16/1, AC-16/16 and IC 467681, having preferred attributes as that of cultivated stypes stand as elite collections for utilization and conservation.

Besides, understanding the nature and magnitude of genetic diversity obtained through different traits, it is desirable to identify the accessions having resistance against most prevalent pests and diseases. The bitter gourd has been reported to be the natural host of many viruses (Tiwari *et al.,* 2010) like cucumber mosaic virus (CMV), potyvirus and bitter gourd distortion mosaic virus (BDMV) (Ashwini *et al.,* 2016). The simultaneous occurrence of different viruses in bitter gourd plants resulted in the development of a mosaic complex. Here we simultaneously screened the accessions for powdery mildew disease, which cause yield loss up to 80% during December – January (AICRP on vegetable crops, 2014) and viral infection. Although bitter gourd is vulnerable to many pest and disease problems demanding high chemical control and consequent pesticide residues, wild genotypes of this crop is endowed with resistance/ tolerance to some of the common diseases and pests of bitter gourd (Bharathi and John, 2013). PDS at peak disease level distinguished the resistant from the susceptible ones well. Duration of the crop is yet another factor that influences the susceptibility/tolerance reaction level. It was observed in the present study that early-maturing genotypes were relatively more susceptible to the disease than late-maturing genotypes, in conformity with the earlier reports by Kolte (1985) and Hiremath (1976). The five wild/semi-domesticated bitter gourd accessions, namely, IC 213312, AC-16/1, AC-16/4, AC-16/9 and AC-16/21, showed tolerance/resistance response to both powdery mildew and viral infection during two consecutive crop seasons were identified as good resistant sources for future breeding programs.

Wild/semi-domesticated materials were found to be at the verge of extinction due to competition from large fruited bitter gourds, destruction of homestead ecosystem, changed consumer culture, and reduction in a number of traditional farmers and farm holdings. The bird-mediated dispersal of seed is also much reduced as they are harvested at the immature stage. Thus, the identification of lines suitable for the eco-friendly production of this valuable vegetable is necessary. Four accessions (AC-16/1, AC-16/16, IC 467681 and JJNS-15/65) with superior yield, nutritional value and resistance can be profitably grown in organic way as an ideal homestead vegetable in every homestead across extreme environments and diverse ecosystems.

Conclusion

Wild/semi-domesticated bitter gourd is a relict vegetable of southern Peninsular India with tremendous potential for commercial cultivation in India. Great variability exists in both morphological and biochemical attributes with excellent culinary traits and perceived tolerance to abiotic and biotic stresses. The superior types satisfying consumer quality filtered out through cluster analysis and selection index need to be conserved for further confirmation studies and location trials. Thus, the study revealed that the wild landraces are comparable with the best-known varieties and even excel in nutritive values and resistance. This heritage crop needs to be popularized to diversify the diet and conserve biodiversity and nutritional security.

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