Diversity Among a Wide Asian Collection of Bitter Gourd Landraces and their Genetic Relationships with Commercial Hybrid Cultivars

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ABSTRACT. We report here the genetic characterization of bitter gourd (*Momordica charantia*) based on polymorphisms of 50 simple sequence repeat (SSR) loci in 114 accessions that included landraces, breeding lines, and commercial open-pollinated and F₁ hybrid cultivars widely grown in Asia. Neighbor-joining tree analysis revealed a high level of genetic variability in the collection. The 114 accessions formed three subpopulations represented by five clusters. Distribution of accessions across the five clusters reflected their geographic origin to a large extent. South Asian accessions originating from India, Bangladesh, and Pakistan were more closely related to each other than to any other geographical group. Likewise, southeast Asian accessions that originated from Cambodia, Vietnam, Indonesia, and Philippines were grouped together. Accessions that originated from Taiwan were genetically distinct and grouped separately. A landrace from Laos was genetically close to the accessions from Thailand and genetically distinct from the rest of the accessions. White-fruited genotypes were genetically distinct from green- and dark green-fruited genotypes. Low- and medium-bitter accessions were more similar to each other than to the high-bitter genotypes. Accessions with cylindrical fruit were genetically distinct from those with spindle or elongated fruit. Commercial cultivars in each cluster were closely related, which indicated a narrowing of the bitter gourd genetic base in Asia in response to market demands for uniformity and yield. Use of diverse germplasm resources in bitter gourd breeding will help in sustainable breeding and production.

Bitter gourd is a commercially and nutritionally important vegetable in Asia, where ≈340,000 ha are cultivated annually (McCreight et al., 2013). Its cultivation is becoming more popular in several African countries, such as Ghana, Zambia, Congo, and Madagascar for local consumption or for export to Europe and the Middle East to meet the demand of emigrant Asian communities. It is also cultivated in the southern United States and Australia (Northern Territory, Queensland, New South Wales, Victoria), where Asian hybrid cultivars are grown as a niche product for consumption chiefly by ethnic communities from Asia (Morgan and Midmore, 2002).

Bitter gourd fruit is a good dietary source of beta-carotene, vitamin C, folic acid, magnesium, phosphorus, and potassium (Yuwei et al., 1991). It is often used in folk medicine in Asia to manage type 2 diabetes, a noncommunicable disease that currently affects 347 million people worldwide (World Health Organization, 2016). Bitter gourd fruit contains compounds that have the potential to improve insulin sensitivity, lower blood glucose, and help regulate postprandial/intestinal glucose uptake (Krawinkel and Keding, 2006). Fiber (Uebanso et al., 2007), an insulin-like protein (Khanna et al., 1981), saponins (Klomann et al., 2010; Oishi et al., 2007), triterpenes (Tan et al.,

2008), and lipids (Klomann et al., 2010) in bitter gourd fruit have been linked to antidiabetic effects (see Tan et al., 2016 for additional health benefits of bitter gourd).

Recent biogeographic analyses show that *M. charantia* originated from Africa (Schaefer and Renner, 2010), but its place of domestication remains unknown, though southern China or eastern India have been proposed (Walters and Decker-Walters, 1988). The earliest recorded reference to cultivated forms of *M. charantia* in China was in 1370 CE (Yang and Walters, 1992). Wild or small-fruited, cultivated forms are mentioned in Ayurvedic texts written in Sanskrit from 2000 to 200 BCE by Indo-Aryan cultures (Decker-Walters, 1999).

Bitter gourd breeders have been effective in improving the bitter gourd fruit yield and quality in the last two decades (Behera et al., 2010). Consumer preferences for fruit bitterness, color, shape, skin pattern, and size vary between and within countries. For example, consumers in south Asia like highly bitter fruit, whereas less bitter fruit are preferred in southeast Asian countries; e.g., Thailand and Vietnam. Light-green and medium-long, spindle fruit are liked by consumers in Vietnam, whereas long cylindrical and smooth fruit with light-green color are preferred in Thailand. South Asian consumers like small-to-medium size, dark-green, spindle-shaped fruit with spiny exterior surfaces. White fruit are used in soups in Taiwan and India.

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Spiny fruit is dominant over smooth (Vahab, 1989). Green fruit color is dominant over white (Hu et al., 2002) and is controlled by two genes (Liu et al., 2005). Bitterness of fruit is controlled by a single gene, with more bitterness dominant to less (Suribabu et al., 1986). Saponins, momordicosides K and L, as well as momordicines I and II cause the bitter taste (Harinantenaina et al., 2006).

A major part of plant breeding success has come by employing pedigree breeding through recombination and subsequent selection during inbreeding of elite $\times$ elite crosses, and further use of derived inbreds in hybrid development programs. This approach was adopted due to the constant pursuit of two horticultural traits desired by growers, such as earliness and high marketable yield, which results from high fruit number and high fruit weight, and improvement of traits desired by consumers, such as fruit color, shape, size, and skin pattern, which became strong forces in a reduction of genetic diversity in commercially grown bitter gourd cultivars. Improved hybrid cultivars lead to further reduction in genetic diversity through displacement of landraces. For example, a significant bitter gourd area in India and Bangladesh is planted to the cultivar Palee, which is a south Asian market type: dull green, medium long with blunt spines (A. Masud, personal communication). A similar trend is developing with other market types, such as the southeast Asian type, where there is evidence of market dominance by a few hybrid cultivars. Performance-driven selection and grower preferences result in a narrow genetic base (Duvick, 2005) that can increase vulnerability to disease and insect outbreaks (Keneni et al., 2012). Plant breeders must make better use of existing bitter gourd diversity to develop improved bitter gourd cultivars that are genetically broad based. Assessment of the genetic diversity in crop collections, such as the World Vegetable Center bitter gourd holdings, is a critical component of germplasm characterization for subsequent utilization by plant breeders.

Molecular analyses of *M. charantia* have been conducted using limited numbers of accessions that originated from either India or China (Gaikwad et al., 2008; Ji et al., 2012; Saxena et al., 2015). The genebank of the World Vegetable Center, Taiwan, has a sizeable collection of bitter gourd germplasm from more than 15 countries. Molecular and morphological characterizations of this germplasm in comparison with widely grown commercial cultivars are important for continued development of improved and genetically diverse bitter gourd cultivars. Herein, we report molecular and morphological assessments of the genetic diversity of the World Vegetable Center bitter gourd collection based on polymorphisms of 50 SSR loci in 114 landraces, breeding lines, and commercial cultivars widely grown in Asia, and five fruit traits evaluated in three seasons.

Materials and Methods

PLANT MATERIALS. One hundred and fourteen PIs comprised lines derived from accessions and landraces through inbreeding, open-pollinated lines released by the World Vegetable Center, and commercial hybrid cultivars, collectively referred to as cultigens were used for the study (Table 1). The germplasm originated from 11 countries in Asia, and represents all market types of bitter gourd based on fruit size, shape, skin pattern, and color (Table 1).

FRUIT TRAIT CHARACTERIZATION. The 114 cultigens were evaluated for five fruit traits in 2013, 2014, and 2015 at the World Vegetable Center, East and Southeast Asia/Oceania, Research and Training Station, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. Entries were planted in a single block with row spacing of 1.6 m, within-row spacing of 1 m, and five plants per plot. Five marketable fruit of each cultigen were harvested for evaluation of five fruit traits: 1) shape = cylindrical, spindle, elongated; 2) color = green, light green, medium green, dark green, white; 3) skin pattern = smooth, spiny; 4) bitterness = low, medium, high; and 5) length = long, medium, short.

Fruit bitterness was evaluated using fresh marketable fruit of each cultigen, washed and cut into small (≈ 3 g) pieces, and used for organoleptic assessments by a five-person taste panel. Three classes of bitterness were recorded: high, medium, and low. The evaluators rinsed their mouth with fresh water after each sample. We categorized the cultigens by different market segments according to the length of the fruit: short segment (<10 cm), medium segment (10–20 cm), and long segment (>20 cm). Pooled data from the 3 years were used for describing fruit shape, color, skin pattern, length, and bitterness.

SSR MARKER DESIGN. Two hundred fifty-five thousand publically available sequencing reads of a normalized *M. charantia* seed transcriptome produced from poly-A-enriched total RNA (Yang et al., 2010) were used to develop SSR markers. The raw sequence reads were cleaned from adaptor sequences using SeqClean (Dana-Farber Cancer Institute, 2005); repeats were masked (Smit et al., 1997), and the reads were assembled with CAP3 (Huang and Madan, 1999) to 20,290 contigs and 23,361 singletons. The contigs and singletons were mined for dimer, trimer, tetramer and composite SSR motifs with the SSR Locator script (da Maia et al., 2008). Primers for polymerase chain reaction (PCR) amplification of DNA fragments containing SSR motifs were designed with the Primer3 program incorporated in the SSR Locator. One hundred SSR markers were randomly chosen for amplification and polymorphism testing on 16 diverse *M. charantia* and *Momordica balsamina* accessions. The polymorphic information content (PIC) of the markers was determined in PowerMarker V3.0 (Liu and Muse, 2005), where the PIC value of an

l -allele locus was calculated as
$$PIC = 1 - \sum_{i=1}^l P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^l 2P_i P_j^2,$$

where P_i and P_j are the population frequency of the i th and j th allele (Nagy et al., 2012). From the markers that amplified reliably in the test set, 50 markers with a PIC greater than 0.1 were chosen for genotyping (Table 2).

DNA ISOLATION AND SSR GENOTYPING AND DATA ANALYSIS. Genomic DNA was isolated from fresh tissue of young leaves using the protocol of Fulton et al. (1995).

PCR amplifications were performed in 10- μ L reactions containing 0.2 μ M of each primer, 200 μ M of deoxyribonucleotides, 50 mM KCl, 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 25 ng of DNA and 0.5 units of Taq DNA polymerase. The SSR amplifications were conducted in a PTC 200 DNA engine thermal cycler (MJ Research, Waltham, MA). The temperature profile used for PCR amplification comprised 94 $^{\circ}$ C for 5 min, followed by 30 cycles of 94 $^{\circ}$ C for 30 s, 55 $^{\circ}$ C for 45 s, 72 $^{\circ}$ C for 45 s, and finally by 7 min at 72 $^{\circ}$ C for the final extension. PCR products (3 μ L) were analyzed on 6% nondenaturing polyacrylamide gel in 0.5 $\times$ Tris/Borate/EDTA buffer. After

Table 1. Cultigen code, origin, and five fruit traits of 114 bitter gourd cultigens (accessions and landraces through inbreeding, open-pollinated lines released by the World Vegetable Center, and commercial hybrid cultivars) subjected to genetic diversity analysis based on simple sequence repeat markers. Fruit characteristics based on field evaluations in 2013, 2014, and 2015 at Kamphaeng Saen, Thailand.

Cultigen	Code	Origin ^z	Fruit				
			Shape	Color	Skin pattern	Bitterness	Market segment ^y
THMC 115	1	Cambodia	Cylindrical	Light green	Smooth	Low	Long
THMC 116	2	Cambodia	Cylindrical	Green	Smooth	Medium	Long
THMC 118	3	Cambodia	Cylindrical	Green	Smooth	Medium	Medium
THMC 122-1	4	Cambodia	Spindle	Green	Smooth	Low	Medium
THMC 126	5	Cambodia	Cylindrical	Light green	Smooth	Low	Medium
THMC 108	6	Indonesia	Elongated	Light green	Spiny	Low	Long
THMC 110	7	Indonesia	Spindle	White	Smooth	Low	Long
THMC 111	8	Indonesia	Spindle	Green	Smooth	Low	Medium
THMC 112	9	Indonesia	Spindle	White	Spiny	Medium	Medium
THMC 114	10	Laos	Spindle	Green	Spiny	Low	Short
THMC 29	11	Philippines	Spindle	Green	Smooth	Low	Medium
THMC 32	12	Philippines	Spindle	Green	Smooth	Low	Medium
THMC 33	13	Philippines	Spindle	Dark green	Smooth	Medium	Medium
THMC 35	14	Philippines	Spindle	Light green	Smooth	Low	Long
THMC 36	15	Philippines	Spindle	Medium green	Smooth	Low	Long
THMC 41	16	Philippines	Spindle	Medium green	Smooth	Low	Long
THMC 42	17	Philippines	Spindle	Medium green	Smooth	Medium	Long
THMC 44	18	Philippines	Spindle	Medium green	Smooth	Low	Long
THMC 47	19	Philippines	Spindle	Green	Spiny	Medium	Short
THMC 48	20	Philippines	Spindle	Green	Smooth	High	Short
THMC 49	21	Philippines	Spindle	Green	Spiny	Low	Short
THMC 53-2	22	Philippines	Spindle	Green	Smooth	Low	Medium
THMC 54	23	Philippines	Spindle	Medium green	Spiny	Low	Short
THMC 55	24	Philippines	Spindle	Light green	Spiny	Low	Short
THMC 101	25	Taiwan	Spindle	Light green	Spiny	Low	Short
THMC 102	26	Taiwan	Spindle	Green	Spiny	Medium	Short
THMC 103-1	27	Taiwan	Spindle	Light green	Spiny	Medium	Short
THMC 104	28	Taiwan	Spindle	Light green	Spiny	Low	Short
THMC 4	29	Thailand	Cylindrical	Green	Smooth	Medium	Medium
THMC 5	30	Thailand	Spindle	Green	Spiny	Medium	Short
THMC 9	31	Thailand	Spindle	Medium green	Smooth	Medium	Short
THMC 11	32	Thailand	Spindle	Green	Smooth	Low	Short
THMC 12	33	Thailand	Spindle	Green	Spiny	Low	Short
THMC 13	34	Thailand	Spindle	Medium green	Spiny	Low	Short
THMC 14	35	Thailand	Spindle	Green	Spiny	Medium	Short
THMC 15	36	Thailand	Spindle	Light green	Spiny	Low	Short
THMC 17	37	Thailand	Spindle	Green	Spiny	Low	Short
THMC 19	38	Thailand	Spindle	Light green	Spiny	Low	Short
THMC 22	39	Thailand	Spindle	Green	Spiny	Low	Short
THMC 23	40	Thailand	Spindle	Green	Spiny	Low	Short
THMC 25	41	Thailand	Spindle	Medium green	Smooth	Low	Medium
THMC 26-1	42	Thailand	Spindle	Medium green	Spiny	Low	Medium
THMC 58	43	Vietnam	Spindle	Green	Smooth	Medium	Medium
THMC 60-2	44	Vietnam	Spindle	White	Smooth	Low	Medium
THMC 62	45	Vietnam	Spindle	Green	Smooth	Low	Medium
THMC 63	46	Vietnam	Spindle	Green	Smooth	Low	Medium
THMC 64	47	Vietnam	Spindle	Medium green	Smooth	Medium	Medium
THMC 65	48	Vietnam	Spindle	Medium green	Smooth	Low	Medium
THMC 345	49	Vietnam	Spindle	Light green	Smooth	Medium	Long
THMC 68	50	Bangladesh	Spindle	White	Spiny	Medium	Medium
THMC 76	51	Bangladesh	Spindle	Medium green	Spiny	Medium	Medium
THMC 77	52	Bangladesh	Spindle	Medium green	Spiny	Low	Medium
THMC 81	53	Bangladesh	Spindle	Dark green	Spiny	High	Medium
THMC 87	54	Bangladesh	Spindle	Light green	Spiny	Medium	Short
THMC 89	55	Bangladesh	Elongated	Light green	Spiny	Medium	Long

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Table 1. Continued.

Cultigen	Code	Origin ^z	Fruit					Market segment ^y
			Shape	Color	Skin pattern	Bitterness		
THMC 92	56	Bangladesh	Spindle	Light green	Spiny	High	Long	
THMC 94	57	Bangladesh	Spindle	Light green	Spiny	Low	Medium	
THMC 98	58	Bangladesh	Spindle	Green	Spiny	High	Short	
THMC 134	59	India	Spindle	Green	Spiny	Low	Medium	
THMC 136-2	60	India	Spindle	Green	Spiny	High	Medium	
THMC 137	61	India	Spindle	Green	Spiny	Low	Medium	
THMC 141	62	India	Spindle	Medium green	Spiny	Low	Medium	
THMC 143	63	India	Spindle	Medium green	Spiny	Low	Medium	
THMC 144	64	India	Spindle	Green	Smooth	High	Medium	
THMC 145	65	India	Spindle	Medium green	Spiny	High	Medium	
THMC 152	66	India	Spindle	Green	Spiny	Low	Medium	
THMC 153	67	India (Thailand) ^x	Spindle	Green	Spiny	Low	Short	
THMC 167	68	India	Spindle	Dark green	Spiny	High	Medium	
THMC 170	69	India (Taiwan) ^x	Spindle	Green	Spiny	Low	Short	
THMC 177	70	India (Philippines) ^x	Spindle	White	Spiny	High	Medium	
THMC 187	71	India	Elongated	Light green	Spiny	High	Long	
THMC 281	72	India	Spindle	Green	Smooth	Low	Short	
THMC 304	73	India	Elongated	Medium green	Spiny	High	Long	
THMC 132	74	Pakistan	Spindle	Dark green	Spiny	Medium	Medium	
THMC 131	75	Sri Lanka	Spindle	White	Spiny	Medium	Long	
AVBG1301	76	World Vegetable Center	Spindle	Medium green	Spiny	Medium	Medium	
AVBG1304	77	World Vegetable Center	Elongated	Dark green	Smooth	Low	Long	
AVBG1305	78	World Vegetable Center	Spindle	Medium green	Spiny	Low	Long	
AVBG1308	79	World Vegetable Center	Spindle	Medium green	Spiny	Low	Medium	
AVBG1310	80	World Vegetable Center	Spindle	Green	Spiny	Low	Long	
AVBG1311	81	World Vegetable Center	Cylindrical	Light green	Smooth	Low	Medium	
AVBG1312	82	World Vegetable Center	Cylindrical	Light green	Smooth	Low	Long	
AVBG1313	83	World Vegetable Center	Cylindrical	Light green	Smooth	Low	Long	
AVBG1314	84	World Vegetable Center	Cylindrical	Light green	Smooth	Low	Long	
AVBG1320	85	World Vegetable Center	Spindle	Medium green	Spiny	Medium	Long	
AVBG1323	86	World Vegetable Center	Spindle	Green	Spiny	Medium	Medium	
AVBG1324	87	World Vegetable Center	Spindle	Light green	Spiny	Low	Medium	
AVBG1325	88	World Vegetable Center	Spindle	Green	Spiny	Low	Long	
AVBG1327	89	World Vegetable Center	Cylindrical	Light green	Smooth	Low	Long	
CT 108	90	Chia Tai Seeds	Spindle	Green	Spiny	Medium	Medium	
BGCT 485	91	Chia Tai Seeds	Spindle	Dark green	Spiny	Low	Long	
BGCT 532	92	Chia Tai Seeds	Cylindrical	Light green	Smooth	Low	Long	
Bonito	93	East-West Seed	Spindle	Dark green	Smooth	Low	Short	
Best	94	East-West Seed	Cylindrical	Light green	Smooth	Low	Long	
Galactica	95	East-West Seed	Cylindrical	Dark green	Smooth	Medium	Long	
Galaxy	96	East-West Seed	Cylindrical	Dark green	Smooth	Medium	Long	
Mestisa	97	East-West Seed	Cylindrical	Light green	Smooth	Medium	Long	
Palee	98	East-West Seed	Spindle	Dark green	Spiny	Low	Long	
Prachi	99	East-West Seed	Spindle	Dark green	Smooth	Medium	Short	
Kohinoor	100	Indo-American Hybrid Seeds	Spindle	Green	Spiny	High	Medium	
NS 1018	101	Namdhari Seeds	Spindle	Dark green	Spiny	High	Short	
Amanshiri	102	Nunhems India	Spindle	Dark green	Spiny	High	Medium	
Chaman	103	Nunhems India	Spindle	Dark green	Spiny	High	Medium	
Racer	104	Nunhems India	Spindle	Dark green	Spiny	Medium	Medium	
Abhishek	105	Seminis	Spindle	Green	Spiny	Medium	Medium	
Arjuna	106	Rasi Seeds	Spindle	Green	Spiny	Medium	Medium	
Chayan	107	Rasi Seeds	Spindle	Dark green	Spiny	Medium	Medium	
Parijat	108	Rasi Seeds	Spindle	Dark green	Spiny	Medium	Short	
Raja	109	Rasi Seeds	Spindle	Dark green	Smooth	Medium	Medium	
US 390	110	US Agri Seeds	Spindle	Green	Spiny	Medium	Short	
US 6214	111	US Agri Seeds	Spindle	Dark green	Spiny	Medium	Medium	

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Table 1. Continued.

Cultigen	Code	Origin ^z	Fruit					Market segment ^y
			Shape	Color	Skin pattern	Bitterness		
VNR 28	112	VNR Seeds	Spindle	Green	Spiny	High		Short
VNR 32	113	VNR Seeds	Spindle	Green	Smooth	Medium		Short
Sagar	114	VNR Seeds	Spindle	Dark green	Spiny	Medium		Medium

^zChia Tai Seeds (Kanchanaburi, Thailand), East-West Seed (Chiang Mai, Thailand), Indo-American Hybrid Seeds (Bangalore, India), Namdhari Seeds (Bangalore, India), Nunhems India (Bangalore, India), Seminis (Bangalore, India), Rasi Seeds (Attur, India), U.S. Agri Seeds (Bangalore, India), VNR Seeds (Raipur, India).

^yMarket segment is the common terminology used by the bitter gourd breeders globally for classes of bitter gourd based on fruit length.

^xGermplasm of these accessions was procured from India, and the country in brackets represents its origin.

electrophoresis, the gels were stained with 5 µg·mL⁻¹ ethidium bromide and the bands were visualized under ultraviolet light using the alpha imager system. The details of SSR primer pairs used for the analysis are provided in Table 2. Allele number and frequency were determined in Powermarker V3.0. Diversity analysis based on SSR data was performed in Darwin 6.0.10 (Perrier and Jacquemoud-Collet, 2006) using the Jaccard coefficient $J = a/(n - d)$, where a and d are the number of matches and mismatches, respectively, and n is the total number of samples, and a phylogenetic tree was constructed by neighbor-joining analysis. The genetic distance between groups with common geographic origin, fruit color, shape, and taste was analyzed in Popgene1.32 (Yeh et al., 1999) using Nei's (1978) unbiased genetic identity and genetic distance. The population structure was analyzed based on 50 SSR loci using the software STRUCTURE 2.3.4 (Pritchard et al., 2000). The optimal k value for population structure was determined by the method described by Evanno et al. (2005).

Results

FRUIT TRAIT EVALUATION. The 114 bitter gourd cultigens were highly diverse for the five fruit traits (Table 1). Three types of fruit shape were identified in the germplasm: cylindrical, spindle, and elongated. The majority of the cultigens produced spindle-shaped fruit (82.5%); cylindrical and elongated fruit were produced by 13.2% and 4.3% of the cultigens, respectively. Five distinct fruit skin colors were observed in the cultigens: green (36.9%), light green (21.9%), medium green (18.4%), dark green (17.5%), and white (5.3%). Two fruit skin patterns were found in the cultigens: smooth (39.5%) and spiny (60.5%). Three classes of bitterness among the cultigens were noted: low (51.8%), medium (34.2%), and high (14%). The cultigens were categorized into three market segments based on the fruit length: long (27.2%), medium (43.9%), and short (29.9%).

CHARACTERIZATION OF SSR LOCI. The 50 SSR markers detected a total of 202 alleles (with a mean of four alleles per locus) in the 114 cultigens. The major allele frequency ranged from 43.5% to 97.4% [mean 75.1% (Table 2)]. Rare alleles were present exclusively in landraces and in one F₁ hybrid cultivar (Bonito). All but two of the detected alleles were present in at least one landrace, and 92 of the 202 alleles were exclusively present in the landraces. The allele diversity of the commercial cultivars (60 of the 202 alleles present) was on average, larger than in the landraces (54 of the 202 alleles), as most cultivars were hybrids. The largest number of alleles (and highest degree of heterozygosity) was found in landrace THMC 101. Interestingly, the commercial cultivar Best, an F₁ hybrid,

was homozygous at all tested alleles. Also, commercial cultivars US 390, NS 1018, Palee, Sagar, US 6214, Chayan, Galactica, Galaxy, and BGCT 532 were comparable to landraces for degree of homozygosity at the tested loci (<59 alleles per 50 loci).

We assessed correlations of molecular marker genotypes in terms of genetic distance and the geographical origin among the landraces (Fig. 1A), and the relationship of the molecular diversity with fruit phenotypic characteristics among all entries. It showed that genotypes from India, Bangladesh, and Pakistan were genetically more related to each other than to any other geographical group, with Nei's genetic distance below 0.1. Similarly, genotypes from Cambodia, Vietnam, Indonesia, and the Philippines clustered together at genetic distances smaller than 0.05, indicating a common origin and selection history for these landraces. The landrace from Laos was genetically related to the lines from Thailand, but genetically distinct from the rest of the cultigens. The genotypes from Taiwan and Sri Lanka were quite distinct from each other and also from the other countries. Genotypes with spindle or elongated fruit were more related to each other (Nei's genetic distance of 0.2) than to the entries with cylindrical fruit (Fig. 1B), landraces with white fruit color were genetically distinct from the green-colored fruit types (Nei's genetic distance greater than 0.3), and the dark-green genotypes were well separated from the other green genotypes (Fig. 1C). Similarly, low and medium-bitter types were genetically more similar to each other than the bitter genotypes (Fig. 1D).

GENETIC STRUCTURE OF THE BITTER GOURD COLLECTION. Population structure analysis revealed three major subpopulations based on 202 alleles detected by the 50 SSR markers (Fig. 2). All accessions with cylindrical fruit belonged to subpopulation 1, and all accessions of subpopulation 2 had spindle-shaped fruit. Spindle fruit form also was found in subpopulations 1 and 3. The elongated fruit form appeared in subpopulations 1 and 3.

The population structure was reflected by the separation of the entries on a phylogenetic tree (Fig. 3). The three subpopulations (Fig. 2) were represented by three major clusters (Fig. 3). The distribution of cultigens was largely consistent with their geographic origin (Fig. 3). For example, 86% (24 of 28) of the cultigens from India were in subcluster B; 90% (19 of 21) of the cultigens from Philippines were in subcluster A; 71% (10 of 14) of the cultigens of Thai origin were in subcluster C; and 80% (4 of 5) of the cultigens from Taiwan grouped in subcluster D. All the cultigens from three other south Asian countries (Bangladesh, Pakistan, and Sri Lanka) grouped together along with Indian cultigens in subcluster B. All the cultigens from Indonesia, Vietnam, and Cambodia grouped

Table 2. Characteristics of the 50 simple sequence repeat (SSR) markers used to characterize genetic diversity in 114 bitter melon cultivars.

SSR name	Forward primer sequence	Reverse primer sequence	SSR motif	Alleles (no.)	Major allele frequency (%)	PIC ^z
AVRDC-BG1	CAAGGAACGCAGAAATCCTA	GAGGTCTGCCTCTTCCAAAA	(AC)12	6	43.5	0.7426
AVRDC-BG2	GAGCACACAGAAAATTGGGT	TGATCCACTCCCAATCTTAGC	(AC)8	4	70.4	0.552
AVRDC-BG3	GACGGGTCTGTGTAAGGTTT	CCCTGGAAATCAGATGAAGG	(AG)12	3	61.7	0.4387
AVRDC-BG15	AGGGCTACCAAAAGCAGAAA	TACGTTGTCATTCCCAAGC	(AG)7	4	93.9	0.1674
AVRDC-BG25	TCGAGATCACGATAGGGACA	CATCACGGCTACTCTTTTCG	(AG)8	6	86.1	0.3173
AVRDC-BG26	CAGGGACGACGATTATCTGA	GACCGTCTTTTCGATTTCCA	(AG)9	3	88.7	0.2956
AVRDC-BG27	GGGGACCAAAAGCATTGAA	CAGCTCTGTGAAAGGACCAA	(AG)9	3	67.0	0.4006
AVRDC-BG29	GGACATCACACCCAGCAGT	CCAAAAGCAAGAAAGGAAGG	(CA)8	2	90.4	0.1585
AVRDC-BG30	CAATTCTACGCGCAACTCAT	GGCTCAGAAAGGAAGCTCAC	(CT)10	4	92.2	0.217
AVRDC-BG32	GGAGTTTGTGCGTATGATGG	CCGTCCGATCACCTAAAAAT	(CT)12	7	60.0	0.5819
AVRDC-BG33	CATTGACTGAAGTTGGCGTT	CATCCACAACGTCTCACACA	(CT)12	5	79.1	0.3884
AVRDC-BG35	CACCATGGCCGGTTCTTA	TTCGAAGGCTATTACAGAGGC	(CT)8	3	48.7	0.5614
AVRDC-BG37	ATTTCTCCATGTTCTTCCGC	AAGAGAGAGAGAAAGCGA	(CT)8	2	74.8	0.2831
AVRDC-BG41	TTTACCCTTCCATTACTCGC	CTTGGTGAATCTGGAGAGCA	(CT)9	6	80.0	0.4232
AVRDC-BG48	GCAAAAACACTGTCACCCAC	TTCGCTTCTTCCCTCTTCAT	(GA)8	3	66.1	0.4775
AVRDC-BG49	CTGAAGGGCAGTGTAACGAA	CACTCCCCATTCTCAACTT	(GA)8	2	62.6	0.3705
AVRDC-BG50	TTAATCCAACCCGTAGGAGC	TGGCCTTTTGCTTCTTAGGT	(GA)8	4	85.2	0.2943
AVRDC-BG51	CCCGTTCCCTTGAACTAAA	TTGGGTGGTGATGAAGTTGT	(GA)9	2	67.8	0.3604
AVRDC-BG54	GCAGTTGGTCCACCTTCATA	TTCATAATCAGTCGCTCGG	(TA)9	2	93.0	0.1326
AVRDC-BG55	CCGGGATCTTCTTCCCTTTA	TCGTAGCGAAGATGTGAAGC	(TC)10	4	80.0	0.3156
AVRDC-BG56	ATCACCATGGACAAAACCCCT	GCACCATCTGTATCGGTTG	(TC)10	3	93.9	0.1254
AVRDC-BG57	GGGACAACACACACCCAAA	CCCATGGAGAAATTCAGGT	(TC)11	2	93.9	0.1316
AVRDC-BG58	GGCTCCTTTTCCCAAATCT	AGATTATGAATTCGCGGTCC	(TC)12	3	87.0	0.2251
AVRDC-BG59	GGGGAAAGACAAAGGTAGCA	TCGGACATTTTGTAGCAGAG	(TC)8	3	64.3	0.4563
AVRDC-BG66	AGAGGTCTGCCTCTTCCAAA	CAAGGAACGCAGAAATCCTA	(TG)9	5	48.7	0.7062
AVRDC-BG67	ACCGTGTGAACCTCTGTCAA	ACCGGTTGTGAAGTGGAAGT	(AAG)8	4	91.3	0.1794
AVRDC-BG70	CATAAGGCCTTCCTCTGCTC	CGGGGATTCCACTCTTCTT	(AGA)12	4	84.3	0.3348
AVRDC-BG71	TGGACTTGGAAGTGGTGA	TCACGACACAATCCACCTTT	(AGA)8	6	77.4	0.4135
AVRDC-BG73	GAACGACAAAGGGAAGGAAA	CTTCTTTGCCATCATCCTCA	(AGA)8	4	62.6	0.4029
AVRDC-BG74	AACACCTTCTGACTCCACCC	CGTTCAATCCTCTCCTCCTC	(AGA)9	7	76.5	0.5084
AVRDC-BG75	AGACTTCCGGTACGAAAACG	TTCTCTCACATGGGAATCCA	(AGA)9	5	86.1	0.2728
AVRDC-BG83	TATGCAGGGAAGACTGATGG	TTTTGCTGGCTAAGGTGTTG	(GAA)8	6	78.3	0.4574
AVRDC-BG85	TGCAACCACTTGGGTCTAA	CACGCCAGTAGCTTCAACAT	(GAA)8	5	55.8	0.625
AVRDC-BG86	AAGGACAGAAGCACAAACCC	TTCCCGAAGCTTCATTCTCT	(GAA)9	3	60.9	0.4952
AVRDC-BG90	TGTCTTGGAATTGCTTCTCG	GGAGGAGAAAATGATCGGAA	(TCT)12	6	61.7	0.5955
AVRDC-BG92	GGGGAATATTTGCAATCCA	TGGGGAGTCATAGAACGAGA	(TCT)8	4	87.8	0.4272
AVRDC-BG93	CCACTATGACGAATCCGTTG	TTCTTCAAGTCGCTGCTGTT	(TCT)8	3	90.4	0.1751
AVRDC-BG94	GGGAGAACACGTTTGGATTT	ATACCCACACAAAAAGGGA	(TCT)9	6	85.2	0.292
AVRDC-BG97	GGTAAAGGAGGCAAGGATGA	GGGGGTAAAGGGATTCAATT	(TTA)8	4	85.2	0.2717
AVRDC-BG98	ACTCTTGACCGGCTCGTAGT	CCATGTTTGACGACCTTGAG	(TTC)8	3	87.8	0.2238
AVRDC-BG99	TGAAGGCAAATGCTCCTGTA	CCTTCTGGTTGAACAAATGC	(TTC)9	3	66.1	0.5
AVRDC-BG100	CGTCTGTTTCTCCATCGAAT	GATCAGAACAAAGAGCGCAG	(TTC)9	4	63.5	0.5308
AVRDC-BG101	AACCCCATATTAGACGGTG	CCAGGTTAAGCAATTTCAAG	(GGT)4-(GAA)6	2	97.4	0.1048
AVRDC-BG104	GTAAACGGCTCTTTAGGGTT	CTCTCTCTGTTCTTCTCTCTC	(AG)9-(AG)6	10	58.3	0.6708
AVRDC-BG109	CCCGTAAGGTTTATTGCAT	TCCTTCTCTTCTTCTCTTC	(AGA)8-(GAA)4	4	48.7	0.4654
AVRDC-BG111	GAACAAGACTAATCACCCCA	CCAACCACAAGAAGAAGAAG	(CTT)6-(CTT)4	2	87.0	0.2255
AVRDC-BG112	ACGACGATTATCTGATTGCT	GACCGTCTTTCGATTTCC	(GA)6-(AG)9	3	88.7	0.3053
AVRDC-BG125	CGGAAGAGGCTTCGAAAT	TTCAGGCTGCTGATTTTAC	(TC)9	4	82.6	0.3178
AVRDC-BG135	GCTCCTAACCATCACCTGT	GGACACAGAATTCCAAAGCC	(TGAG)6	4	57.4	0.4905
AVRDC-BG136	TCGCAGTCTCATTTCTCAAG	AGTGGCAGAGCGTTTTACCT	(TTC)7	5	55.7	0.6101
AVERAGE				4	75.1	

^zPolymorphic information content.

with cultivars from Philippines in subcluster A, and 58% (7 of 12) and 42% (5 of 12) of bitter melon lines released by the World Vegetable Center were scattered in subcluster A and B,

respectively. A single cultivar of *M. balsamina* comprised a separate subcluster, E, and was genetically divergent from the gene pool of *M. charantia*.

Fig. 1. Genetic diversity of 114 bitter melon cultivars grouped by (A) country of origin, (B) fruit shape, (C) fruit skin color, and (D) fruit bitterness.

Discussion

Bitter melon is an important commercial cucurbitaceous cash crop that is cultivated mainly by smallholder farmers in Asia. The current bitter melon seed market in Asia is annually worth ≈16 million Euros (N.K. Singh, personal communication). An understanding of the genetic diversity in a given collection of a species is important for curators, geneticists and breeders. We employed SSR analysis to characterize genetic diversity in 114 bitter melon cultivars that originated from 11 countries that constitute the major bitter melon growing areas of south and southeast Asia.

The 114 cultivars were divided into three subpopulations based on the SSR analysis (Fig. 2), and were subdivided into

five subclusters (Fig. 3). There are clear genetic distinctions between bitter melon gene pools of southeast Asia (Philippines, Thailand, Vietnam, Cambodia, Indonesia)—subclusters A and C; south Asia (India, Bangladesh, Pakistan, Sri Lanka)—subcluster B; and east Asia (Taiwan)—subcluster D. Short- and medium-fruit segment cultivars were found in the collection that originated from Thailand; short-fruited cultivars were clustered in subcluster C, whereas medium-fruit length cultivars clustered in subcluster A. Cultivar THMC 153 (Code 67), grouped in subcluster C, though collected from India, originated in Thailand (M. Anil, personal communication). Similarly, THMC 114 (Code 10) collected from Laos clustered along with Thai cultivars, and in all probability originated from Thailand because seed of bitter melon cultivars grown in Laos is imported mostly from Thai seed companies, and human traffic between the two countries is intense and regular. Laos shares 1835 km of its border with Thailand and does not have a local vegetable seed industry. Thus, it was not unexpected when both THMC 153 and THMC 114 tended to cluster with small-fruited Thai cultivars in cluster C. An active breeding program for small-fruit segment bitter melon does not exist in Thailand; these cultivars are probably farmer-developed landraces. These small-fruited landraces, locally named as *Kee Nuk* (“bird droppings”) are grown in home gardens in Thailand; farmers selected disease-resistant variants from these populations. Entries originating from eastern Asia (Taiwan) were grouped in subcluster D (Fig. 3). Cultivar THMC 170 (Code 69) was collected from an Indian source but its origin is Taiwan (M. Anil, personal communication). South Asian sub-cluster B comprises entries from India, Bangladesh, Pakistan, and Sri Lanka (Fig. 3). Within this cluster, landraces originating from eastern India such as THMC 143 (Code 63), THMC 145 (Code 65), THMC 167 (Code 68), THMC 152 (Code 66) were grouped separately and diverged from other

cultivars of Indian origin. Cultivars THMC 143 and THMC 167 were resistant to local isolates of cucurbit powdery mildew incited by *Podosphaera xanthii* in trials conducted in 11 locations in Thailand, Vietnam, Taiwan, Philippines, and India (N.P.S. Dhillon, unpublished data).

Bitter melon landraces that originated from three eastern states of India, Odisha (formerly Orissa), West Bengal, and Jharkhand (formerly part of Bihar state) have been reported to be genetically distinct from bitter melon collections of other agro-ecological zones of northern and southern parts of India (Gaikwad et al., 2008). Cultivars that originated from Bangladesh were genetically diverse and grouped in three subgroups in subcluster B (Fig. 3). The popular, high-yielding bitter melon hybrid cultivar Chaman (Nunhems India, Bangalore, India) has

in its pedigree parents from eastern and southern India (R. Hegde, personal communication). It is a common belief among Asian bitter gourd breeders that crosses between lines derived from bitter gourd gene pools of southern and eastern India, and Bangladesh provide heterosis for early and high yield in many instances (A. Kapur, personal communication).

Short-fruited cultigens were restricted to subclusters B, C, and D, whereas subcluster A contained medium- and long-fruited cultigens (Fig. 3). Smooth-skinned cultigens that originated from the Philippines, Thailand, Vietnam, and Cambodia are grouped together in subcluster A (Fig. 3). Cambodia, Vietnam, and Indonesia do not have indigenous bitter gourd breeding programs, and farmers in these countries rely on bitter gourd hybrids introduced by companies such as East-West Seed (EWS), Bulacan, Philippines, and in some instances farmers have developed open-pollinated cultivars from these hybrids through repeated inbreeding and selection. This was observed

by two authors of this paper (N.P.S. Dhillon and S. Sanguansil) during their comprehensive farmers' field visits in southeast Asia and in interactions with growers and seed company breeders. Initially, EWS breeders based their bitter gourd breeding program on a collection of landraces from the Philippines. Thus, in hindsight, it is obvious that cultigens of Philippines origin and hybrid cultivars released by EWS, such as Galactica, Galaxy, Best, Palee, Bonito, Prachi, and Mestisa, and the other cultigens collected from Cambodia, Vietnam, and Indonesia would cluster together in subcluster A (Fig. 3). All the cultigens possessing light-green, smooth, medium-to-long fruit, and low-to-medium bitterness are grouped in subcluster A, whereas green-to-dark green, spiny, medium-to-high bitter and short-fruited cultigens are restricted to south Asian subcluster B (Fig. 3). Wild type bitter gourds are green-to-dark green, spiny, highly bitter and short, and abundantly found in the fields in northeastern India (Gaikwad et al., 2008). Thus, it

appears that south Asia is the center of domestication of bitter gourd, and that other types (long, cylindrical, smooth, light green, less bitter) available in southeast Asia are the result of mutations followed by farmer selection according to the needs of the local consumer. Cultigen THMC 281 (Code 72) is genetically distinct from rest of the cultigens used in this study, but it clustered together with accessions of subpopulation 2 (Fig. 3). It is

Fig. 2. Population structure analysis of 114 bitter gourd cultigens based on 50 simple sequence repeat markers.

Fig. 3. Phylogenetic tree of 114 bitter gourd cultigens coded in Table 1.

M. balsamina and it is extremely difficult to obtain hybrids between *M. charantia* × *M. balsamina* (Bharathi et al., 2012). This cultigen is found growing wild in northern and eastern states of India.

Repeated cycling of a relatively small number of genetically closely related bitter gourd lines in commercial breeding has narrowed the genetic diversity within the commercial bitter gourd crop. For example, VNR 28, a high-yielding bitter gourd cultivar in the short-fruit segment, was released by VNR Seeds (Raipur, India) in 2004. The short fruit segment accounts for 10% of the Indian bitter gourd seed market, which was estimated at 200 t in 2015 (P. Agarwal, personal communication). Using this germplasm, three other Indian seed companies developed and released three hybrid cultivars in the short fruit segment: Parijat, Racer, and US 390 (Codes 108, 104, and 110, respectively) (M. Anil, personal communication; S. Thippeswamy, personal communication). These three hybrid cultivars are tightly linked together with VNR 28 (Code 112) in subcluster B (Fig. 3), confirming their close genetic relatedness. Similarly, in subcluster A, commercial cultivars Galactica (Code 95) and Galaxy (Code 96) from EWS are grouped together in a single subgroup. Commercial cultivars Prachi, Mestisa, and Bonito (Codes 99, 97, and 93, respectively), released by EWS, are closely related and placed together in subcluster A (Fig. 3). Thus, a narrowing of the bitter gourd genetic base is underway in Asia. Likewise, it has been observed that genetic diversity among commercial cultivars of other cucurbit species such as melon, watermelon and bottle gourd is much less than the overall diversity within these cucurbit crops (Decker-Walters et al., 2001; Levi et al., 2001; Nakata et al., 2005; Sheng et al., 2012; Solmaz et al., 2016). Seed companies are under constant competition and also pressure from the growers to provide new cultivars after a gap of 3 to 4 years. To help ensure their companies will survive in a competitive market, seed company breeders are tempted to use sister lines of a common pedigree to bring new cultivars to market to satisfy demands from growers. Breeders focus mainly on elite × elite crosses to sustain the breeding progress they have achieved in previous years. This has resulted in genetic uniformity among commercial cultivars of each of the various market segments of bitter gourd, but rendered the bitter gourd crop vulnerable to biotic and abiotic stresses, and limited long-term yield improvement. This trend can be reversed by a conscious effort to introduce additional genetic diversity. Breeders need to evaluate and include wild types, landraces, heirloom cultivars and obsolete cultivars in their hybridization programs. These additional sources of bitter gourd genetic diversity are conserved in situ in various genebanks in Asia. For example, the national genebanks of India store ≈1000 bitter gourd accessions, and the genebanks of the World Vegetable Center and Kasetsart University, Thailand, have 430 and 250 accessions, respectively, available (A. Ebert, personal communication). The Chinese Academy of Agricultural Sciences has 177 bitter gourd accessions stored in several provinces, such as Guangdong, Guangxi and Yunnan (K.-L. Hu, personal communication). Additional bitter gourd landraces should be collected for research and breeding. The germplasm from India, China, Bangladesh, and Myanmar, considered important reservoirs of bitter gourd diversity, must be preserved before it is eroded by elite hybrid cultivars. This could be accomplished by an international team of scientists sponsored by one or more international organizations, such as

The Crop Trust, with the approval of germplasm regulators in the source countries. These diverse germplasm resources would then be available for sustainable bitter gourd breeding and production.

In conclusion, our study revealed that the commercial cultivars of bitter gourd for different market segments such as south Asian and southeast Asian types have distinct, narrow genetic bases. Bitter gourd genetic resources originating from south Asia (India, Bangladesh, Sri Lanka), southeast Asia (Philippines, Vietnam, Cambodia, Thailand), and east Asia (Taiwan) are molecularly different from each other. Thus, the bitter gourd germplasm bases of these regions can be broadened by strategic mating among cultigens of different origin.

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