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Bitter gourd, *Momordica charantia* L., breeding lines differ in secondary metabolite content according to market type

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Summary

Bitter gourd, *Momordica charantia* L., is an important commercial cucurbitaceous vegetable of enormous medicinal value in Asia because of its secondary metabolite content. We report here the characterization and evaluation of open-pollinated (OP) edible South Asian and Southeast Asian types of bitter gourd breeding lines, developed at the World Vegetable Center, for horticultural traits (11 OP) and secondary metabolites (10 OP) and their comparisons with commercial OP and F_1 hybrid cultivars. Marketable yields of South Asian and Southeast Asian type breeding lines were comparable to the OP 'BARI Karella 1' and the hybrid 'Benteng', respectively.

The bitter gourd cultivars and breeding lines included in this study exhibited specific patterns for five secondary metabolites (saponins, carotenoids, chlorophyll a and b, and vitamin C): in general the two cultivars and South Asian type breeding lines contained higher levels of secondary metabolites, e.g. carotenoids, than the Southeast Asian bitter gourd breeding lines.

Some of these bitter gourd lines will be released to Asian home and school gardeners after conducting multi-location trials across Asia to improve vegetable consumption as a main task of bitter gourd breeding.

Introduction

Bitter gourd (*Momordica charantia*, Cucurbitaceae) is a commercially and nutritionally important vegetable in Asia (McCreight, 2013) and a good source for nutritious valuable metabolites, e.g., beta-carotene and vitamin C (Dhillon, 2016a). Recent clinical studies demonstrate benefits from dietary supplements of bitter gourd fruit in lowering fasting glucose in pre-diabetics (Amirthaveni M., 2018; Krawinkel, 2018). Consumption of 100 g of bitter gourd fruit provides 190% of the recommended daily allowance of vitamin C (Dhillon, 2017). Saponins are important metabolites in bitter gourd, with forms momordicoside K and L and momordicine I and II mainly responsible for the bitter taste of bitter gourd (Harinantenaina L., 2006). Saponins contribute more than bitterness, they have antidiabetic effects including the lowering of blood glucose (Habicht, 2011; Klomann, 2010; Krawinkel, 2006), and exert numerous pharmacological activities including cytotoxicity (Podolak, 2010). The carotenoid lutein is widely found in commonly consumed fruits and vegetables. Lutein exhibits biological activities that have attracted great attention in relation to human health, but associations between high intake or serum levels of lutein and lower risk for developing cardiovascular disease, several types of cancer, cataracts and age-related maculopathy have been inconsistent (Granado, 2007). Chlorophyll is said to be anti-carcinogenic, e.g., induction of liver cancer by aflatoxin, by reducing the bioavailability of carcinogens (McQuistan, 2012). Chlorophyll related compounds have anti-inflammatory activity (Lin, 2013).

Consumer preferences for bitterness, color, shape, and size differ between and within Asian countries (Fig. 1). For example, South Asian countries prefer highly bitter fruit, whereas less bitter fruits are mainly consumed in Southeast Asia (Dhillon, 2016c). About 20 horticultural types of bitter gourds are cultivated for various markets mainly for vegetable consumption without giving any attention to the nutritional value (Dhillon, 2016a). Bitter gourd breeding by the private seed sector has focused on developing hybrid cultivars for larger-scale commercial production with horticultural attributes

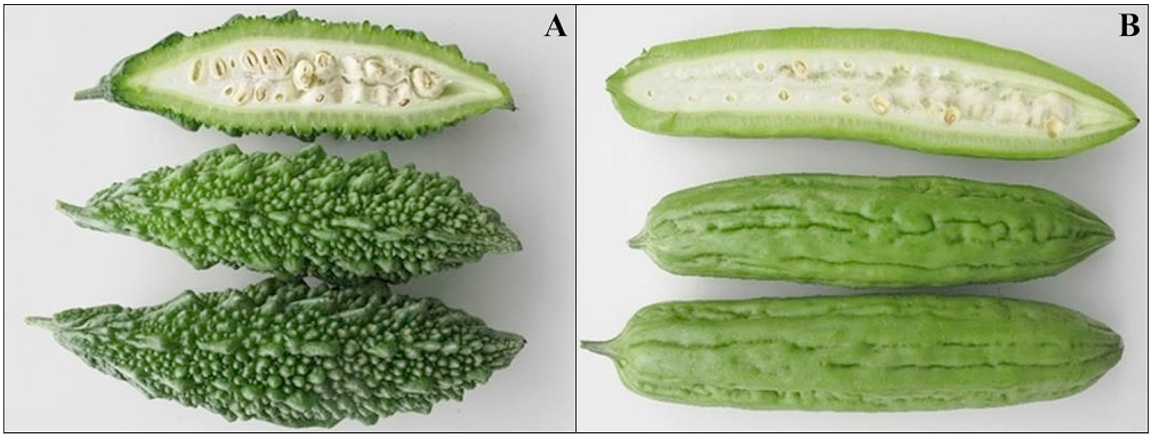

Fig. 1: South Asian (AVBG1301, A) and Southeast Asian (AVBG1313, B) bitter gourd market type fruit.

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not suitable for home and school gardens, e.g., long vines that cause problems due to space constraints in these settings. Bitter gourd cultivars with short vines (ca. 2.5 m), early and late maturity in order to extend the period of fruit availability are needed to increase vegetable consumption in private households and schools. The home and school garden grower should be able to reproduce and save the seed of these cultivars for subsequent plantings. These cultivars should also exhibit higher female:male flower ratios to increase productivity, resist prevalent diseases and insect pests, and offer high nutritional value.

A cultivar is an assemblage of plants that (a) has been selected for a particular character or combination of characters, (b) is distinct, uniform, and stable in these characters, and (c) when propagated by appropriate means, retains those characters (BRICKELL, 2009). A cultivar may be "open-pollinated," whereby it is propagated in isolation (e.g., isolated field) in order to assure purity via open-pollination, and, as in the case of cucurbits, consist of a mixture of self- and sib-pollinations, or it may be a F_1 hybrid, that results from a deliberate cross between two pure-bred/inbred lines. Growers and gardeners can save seeds of open-pollinated cultivars and breeding lines for subsequent plantings in home and school gardens, as they are not F_1 hybrids that produce highly heterogeneous offspring. In contrast, a breeding line is an assemblage of plants from the repeated self- or sib-pollination of selected plants that may have originated from a cross, whether intentional or accidental, or from a single plant selection from a landrace, farmer variety or from a seed mixture, as is often the case of cucurbit seeds purchased in markets across south and southeast Asia and that may be sold as "roasters mixes" for human consumption or for planting in a garden or field. Breeding lines are not as uniform or refined as cultivars for one or many traits, e.g., they may otherwise be horticulturally undesirable but possess a level of resistance to a particular disease. A breeding line represents a specific phase/step, i.e., generation, between the initial cross or selection for one or more traits and a finished assemblage or population that meets the definition of a cultivar. Advanced breeding lines may, in contrast, be uniform for many traits and ready for release as cultivars after field tests to characterize their production characteristics and range of adaptability.

The World Vegetable Center has developed advanced (F_7) open-pollinated bitter gourd breeding lines with improved yield (20-30 t/ha; *vs*. 12-15 t/ha from landraces and farmer varieties; (DHILLON, 2016b)), shorter vine length (ca. 2.5 m) *vs*. >3 m for landraces and farmer varieties (WORLD VEGETABLE CENTER, 2018). Some of these breeding lines are resistant to cucurbit powdery mildew incited by the fungal pathogen *Podosphaera xanthii*. Our objective was to evaluate horticultural traits (fruit weight, fruit number/plant and fruit yield), and metabolite and nutritional content (saponins, vitamin C, carotenoids, and chlorophyll a and b) of these new Southeast Asian and South Asian types of bitter gourd breeding lines bred by the World Vegetable Center and compare them with commercially available bitter gourd cultivars in order to identify the better performing breeding lines for multi-location trials with stakeholders to find the best lines for cultivation by smallholder farmers in Asia.

Material and methods

Plant material

Fourteen edible bitter gourd entries (11 breeding lines and three commercially available cultivars; Tab. 1) were grown in fields of the World Vegetable Center East and Southeast Asia/Oceania Research and Training Station, Kasetsart University Thailand from May to August in 2014 and 2015. The mean maximum and minimum temperature ranged from 34.1° to 36.8 °C and 21.2° to 23.9 °C, respectively during the trial period in 2014 and the range during the trial period in 2015 was 34.4° to 37.5 °C and 25° to 25.8 °C, respectively. The mean maximum and minimum relative humidity (%) ranged from 94% to 95.1% and 53.3% to 61.5%, respectively during the trial period in 2014 and from 89.2% to 91.8% and 46.6% to 56%, respectively, during the 2015 trial period. Each field trial was planted in a randomized complete block design with three replications of 10 plants per plot. Entries were planted on raised, 1.6 m wide beds covered with black plastic mulch. Plots were 10-m long on a single bed; each consisted of 10 transplants spaced 1 m apart. Plot size was 16 m^2. Plants were trellised on plastic netting erected on vertical bamboo poles. Horticultural traits evaluated included yield and yield contributing traits (fruit number/plant and mean fruit weight),

Tab. 1: Means of three yield parameters, and four fruit quality characteristics of 11 South Asian and Southeast Asian type bitter gourd breeding lines and three cultivars, field evaluations in 2014 and 2015 at Kamphaeng Saen, Thailand.

Type and entry	No. fruit per plant		Fruit weight (g)		Yield (t/ha)		Fruit quality characteristics			
	2014	2015	2014	2015	2014	2015	Shape	Bitterness	Skin color	Skin pattern
South Asian type										
AVBG1301	31	34	149	176	21.6	25.5	Spindle	Medium	Medium green	Spiny
AVBG1310	16	22	248	174	26.7	22.2	Spindle	Low	Green	Spiny
AVBG1323	34	35	145	115	21.0	20.3	Spindle	Medium	Green	Spiny
AVBG1324	39	38	163	146	25.6	25.7	Spindle	Low	Green	Spiny
AVBG1325	30	31	161	186	20.7	22.8	Spindle	Low	Green	Spiny
AVBG1330	29	32	113	114	16.1	16.6	Spindle	High	Green	Spiny
AVBG1334	26	41	113	110	15.2	21.0	Spindle	High	Green	Spiny
BARI Karella 1	26	22	221	182	26.2	19.3	Spindle	High	Dark green	Spiny
Palee	25	30	263	247	35.0	37.0	Elongated	Medium	Green	Spiny
Southeast Asian type										
AVBG1311	11	19	414	457	22.8	33.6	Cylindrical	Low	Light green	Smooth
AVBG1313	13	17	426	265	27.7	29.4	Cylindrical	Low	Light green	Smooth
AVBG1314	14	18	337	282	26.1	27.5	Cylindrical	Low	Light green	Smooth
AVBG1327	11	17	392	330	21.8	24.1	Cylindrical	Low	Light green	Smooth
Benteng	14	14	449	317	30.5	24.1	Cylindrical	Low	Light green	Smooth
LSD ($P = 0.05$)	6.1	10	101	133	6.1	11.1				
CV (%)	16.1	23.5	23.6	36	15.2	26.6				

and four fruit traits (color, shape, skin pattern, and bitterness). Ten marketable fruits of each entry were harvested in each replication for fruit traits assessment. Fruit bitterness was evaluated using fresh marketable fruit of each entry, washed and cut into small (ca. 3.0 g) pieces, and assessed for organoleptic quality by a five-person taste panel. The evaluators rinsed their mouth with water after each sample. Three classes of bitterness were recorded: low, medium, and high (DHILLON, 2012).

Saponin, vitamin C, carotenoid, and chlorophyll a and b determinations were done on the 13 bitter gourd entries (10 breeding lines and three commercially available cultivars; Fig. 2) grown in a greenhouse in Karlsruhe, Germany, October 2014 to February 2015. The lines were seeded in a peat-based substrate (low fertilized, Gramoflor, Vechta, Germany) in 10 L pots and kept in the greenhouse with a temperature range between ca. 20 °C (night temperature) and 30 °C (day temperature in the summer) without humidity control. Due to seed germination issues with breeding line AVBG1334 analytical analyses of secondary metabolites were not possible. Photoperiod followed the natural diurnal pattern for the location (49° 0'50.976"N 8° 25'35.832 E), but light intensity was supplemented 10 h daily with artificial light (Philips SON-T AGRO 400). The plants were trellised as their vines elongated. Two plants of each line were cultivated in order to obtain ca. 10 fruits for metabolite analyses. Fruits were harvested ca. 20 days post-pollination.

Saponin determination

Fruits were cut and freeze-dried for 72 h. The dried fruits were ground for 60 s at 30 Hz with a Retsch MM 200 mill (Retsch GmbH, Haan, Germany) using 10 mm steel balls. Ground samples of the different bitter gourd lines were kept at -80 °C until measured with LC-MS. Saponin determination was carried out as follows: extraction of the saponins momordicoside L and momordicoside G was done according to the method of Wang et al. (WANG Y.H., 2008) modified as follow. Each ground sample (100 mg) was mixed with 50 µL internal standard (0.5 mM Soyasaponin II in DMSO; ChromDex, Irvine, CA, USA). Then 2.5 mL of a methanol-water mixture (9/1, v/v) was added and extraction was done in an ultrasonic bath for 25 min at 35 °C. The suspension was centrifuged at 16,000 × *g* for 5 min and the supernatant collected. The residue was extracted further three times and the four supernatants of each sample were pooled. The extract was subsequently evaporated to a volume of approx. 1.5 mL using a SpeedVac (SPD131; Thermo Electron LED GmbH, Langenselbold, Germany) and then was filled up exactly to 2.0 mL with a methanol-water mixture (9/1, v/v). The solution was filtered using 15 mm 0.45 µm PTFE syringe filters and centrifuged at 16,100 × *g* for 5 min. The supernatant was collected and analyzed by LC-MS.

The LC-MS analyses were performed on a TripleTOF 5600 mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with a 1290 Infinity LC system (Agilent, Waldbronn, Germany), which consisted

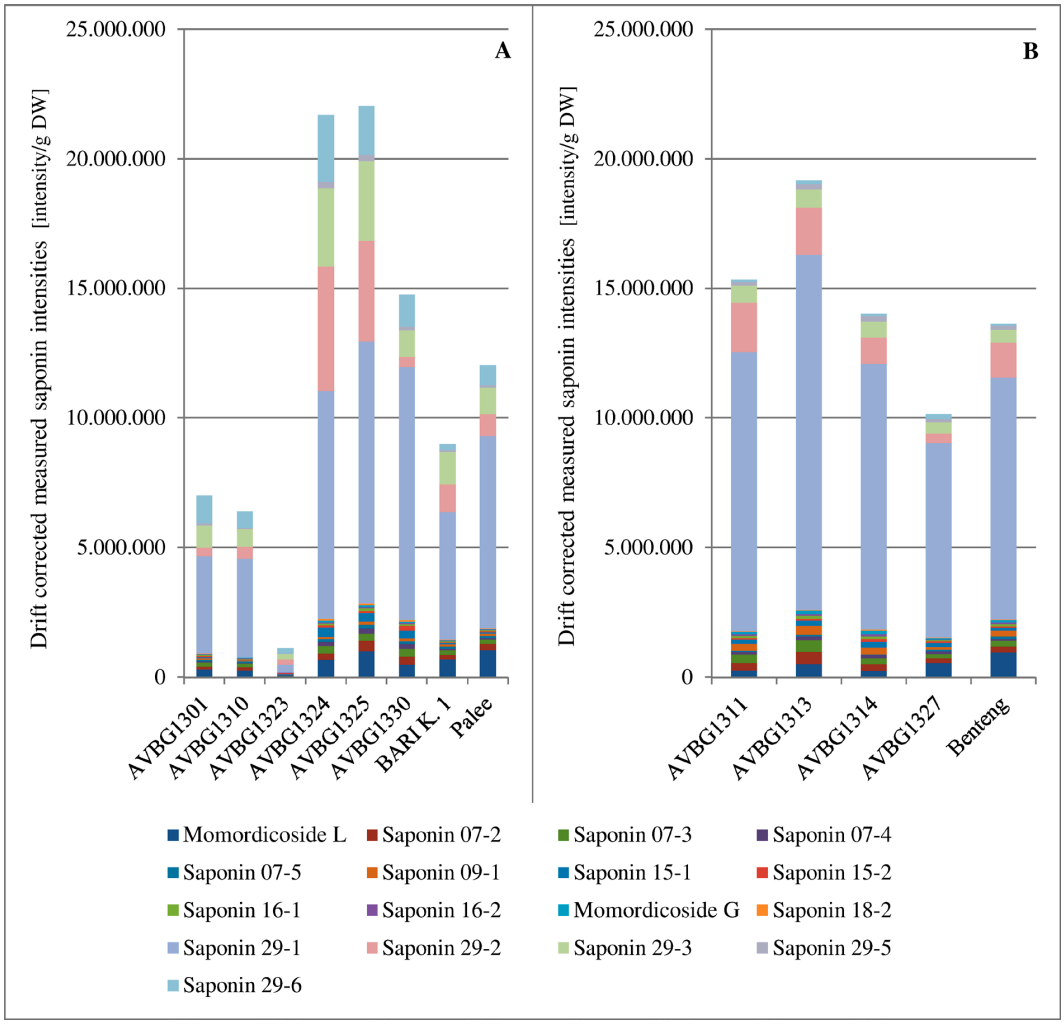

Fig. 2: LC-MS intensity distributions of saponins in eight South Asian (A) and five Southeast Asian (B) type bitter gourd entries cultivated in a greenhouse in Karlsruhe, Germany. BARI K. 1 = 'BARI Karella 1', DW = dry weight. Momordicoside denotations are based on a laboratory internal numbering with the exceptions of L and G. Means of 10 fruits per entry.

of a controller, a degasser, a binary pump, an autosampler, a column oven, and a DAD. The LC-MS system was controlled by the software Analyst TF 1.6.0. LC separation was carried out on a Waters Cortecs UPLC C18 (100 × 2.1 mm, particle size 1.6 µm) equipped with a Phenomenex Security Guard Ultra C18. Eluent A was an aqueous 5 mM ammonium acetate buffer (pH 7), and eluent B was an acetonitrile-methanol mixture (1/1, v/v). A linear gradient was used with a flow rate of 0.45 mL/min and the following elution profile: 0.0-18.0 min from 5% to 95% B, 18.0-20.0 min isocratic with 95% B, 20.0-20.5 min from 95% to 5% B and 20.5-25.0 min isocratic with initial conditions. The column oven was adjusted to 40 °C. The injection volume was 2 µL. The DAD recorded data from 190 to 400 nm with a sampling rate of 10 Hz. The DuoSpray source was operated in positive ESI mode using the following source parameters: Curtain gas (CUR) 45 psi, ion spray voltage (IS) 5500 V, ion source gas-1 (GS 1) 70 psi, ion source gas-2 (GS 2) 80 psi, ion source gas-2 temperature (TEM) 650 °C. The declustering potential (DP) was adjusted to 80 V. The MS full scans (TOF MS) were recorded from m/z 100-1000 with an accumulation time of 150 ms and a collision energy voltage (CE) of 10 V. The MS/MS spectra (Product Ion) were recorded from m/z 50-1000 in the high sensitivity mode with an accumulation time of 45 ms, a collision energy voltage (CE) of 45 V, and a collision energy spread of 25 V. Nitrogen was used as collision gas. All used organic solvents and reagents were in LC grade quality and the water was taken from an in-house ultrapure water system (LaboStar; Siemens, Erlangen, Germany) at 0.055 µS/cm.

Analysis of data was performed with PeakView 2.2.0, MasterView 1.1 and FormulaFinder 2.2.0. (AB Sciex, Darmstadt, Germany). Analytes were identified by retention time, accurate mass, isotope pattern and MS/MS spectrum. Extracted ion chromatograms (XIC) based on the accurate mass of the molecular ions of the compounds (10 mDa extraction width) was used to monitor and quantify the analytes. In detail, momordicoside L and momordicoside G were quantified by an external calibration using a reference fruit sample with known contents of momordicoside L and momordicoside G. For the reference fruit sample (quality control (QC) sample), all lyophilized and ground bitter gourd samples in the study were mixed together (approx. 1 g of each sample) and the concentrations of momordicoside L and momordicoside G were determined by the method of standard addition using commercial reference compounds of momordicoside L (purity 88.9%, determined by HPLC-DAD at 205 nm; Quality Phytochemicals LLC, New Jersey, USA) and momordicoside G (purity 69.2%, determined by HPLC-DAD at 205 nm; Biorbyt, Cambridge, UK), respectively. The quantified concentrations of momordicoside L and momordicoside G were corrected by the purity of the commercial reference compounds.

Fifteen saponins were detected in the LC-MS analysis, but only tentative structures based on accurate masses, isotopic patterns, MS/MS spectra and literature search could be determined, because no commercial reference compounds were available (Tab. S1). Only MS intensities for these 15 saponins are, therefore, reported and their denotation is based on a laboratory internal numbering (Fig. 2).

One hundred ninety-one samples were assayed (131 study samples from 13 cultivars and 60 Quality control samples (QC)) during the 15-day measurement data acquisition period. Ten study samples and 4 QC samples were measured per day, on average. Instrumental signal intensity drift was observed during the 15 (non-consecutive) measurement days. In order to compensate the analytical variation, the signal intensity drift was subsequently corrected by use of a local linear QC-based drift regression method analogous to the method described in literature (Dunn, 2012). Twelve (71%) of the 17 saponins had a coefficients of variation (CV) < 20% before correction and 94 % after correction, respectively. The total and intra-day CV-values before and after correction are given in the supplemental material (Tab. S2 and S3).

Vitamin C determination

Fruit flesh samples (6-8 g) were taken at the equatorial area of each fruit and cut in small pieces. Pieces were weighed out in a 100 mL, high-grade steel top part of a laboratory blender (Waring Commercial, New Hartford, Connecticut, USA) and mixed with a threefold amount of 1.5% meta-phosphoric acid. 20 g of the solution were weighed out in a 100 mL beaker, pH was adjusted with the titrator titroline easy (Schott Instruments, Mainz, Germany) to 3.5 to 4 with 10 M potassium hydroxide, filled up to 50 ml and filtered through a 3 hw pleated filter (neoLab Migge, Heidelberg, Germany). Vitamin C content was determined enzymatically according to the description for L-ascorbic acid (R-Biopharm, Darmstadt, Germany) with an additional reduction of dehydroascorbic acid to L-ascorbic acid. The reduction of dehydroascorbic acid to L-ascorbic acid was achieved using 1.4-dithiothreit in 0.5 mol/l phosphate buffer pH 7.5. All chemicals were purchased from Merck, Darmstadt, Germany.

Carotenoid determination

Samples for carotenoid determination were prepared as described above (saponin determination). Seven to nine milligram lyophilized grounded material was used for extraction. Carotenoids and chlorophylls were extracted by adding 500 µL methanol: tetrahydrofuran (1:1, v/v) shaking for 5 min at 1400 rpm. After centrifugation for 5 min at 4000 × *g*, the supernatant was transferred into a new tube. The pellets were re-extracted until colorless. After concentrating, the combined supernatant was vaporized under N_2 to dryness; it was re-dissolved in 50 µL dichloromethane and 200 µL 2-propanol. The solution was filtered (0.2 µm, PTFE) and kept at 8 °C in the autosampler. Separation was performed on a C30-column (YMC Co. Ltd., Kyoto, Japan, YMC C30, 100 × 2.1 mm, 3 µm) on an Agilent Technologies 1290 Infinity UHPLC. The column temperature was maintained at 20 °C. The mobile phases were (A) methanol/water (96/4, v/v) and (B) methanol/tert-butyl methyl ether/water (6/90/4, v/v/v). To increase the ionization, 20 mM ammonium acetate was added to the mobile phases. The flow rate was 0.2 mL/min. Elution was carried out with the following gradient: 100% A for 10 min, 100% A to 80% A in 7 min, 80% A for 28 min, 80% A to 0% A in 10 min, and 0% A to 100% A in 2 min. The samples were analyzed using an Agilent Technologies 6530 QTOF LC/MS equipped with an APCI ion source in positive ionization mode. The gas temperature was set to 300 °C at a flow rate of 8 L/min, the vaporizer to 350 °C, and the nebulizer pressure to 35 psig. The voltage was set to 3500 V and a fragmentor voltage of 175 V was applied at a corona current of 4 µA. Standards were prepared and the contents were determined spectrophotometrically and used for external calibration. Quantification was performed at a detection wavelength of 450 nm.

Statistics

Horticultural evaluation data were subjected to analysis of variance using SAS General Linear Model (GLM) procedure (SAS Institute, Cary, N.C.).The saponin and carotenoid mean data were analyzed using Mann-Whitney-U-test with the statistic programme IBM SPSS Statistics Version 20.0 on a significance level p= 0.05.

Results and discussion

Characterization and evaluation of horticultural traits

A combined ANOVA over the two years revealed significant differences among lines for all horticultural traits. Genotype × year interactions were significant (P=0.05) for yield, fruit number/plant and mean fruit weight (data not shown). Mean year 1 and year 2 marketable yields of Southeast Asian type lines AVBG1313 (27.7 and 29.4 t/ha, respectively), AVBG1311 (22.8 and 33.6 t/ha, respectively) were

comparable to 'Benteng' (30.5 and 24.1 t/ha, respectively) (Tab. 1). Mean year 1 and year 2 marketable yields of South Asian type lines AVBG1324 (25.6, 25.7 t/ha, respectively), AVBG1301 (21.6 and 25.5 t/ha, respectively) were comparable to 'BARI Karella 1' (26.2, 19.3 t/ha, respectively). Mean vine length of these two market types ranged 2.0-2.5 m (data not shown) among the four breeding lines. These breeding lines have potential usefulness for commercial cultivation, as well as home and school garden cultivation.

Fruit of Southeast Asian type bitter gourd lines and cultivars are light green, smooth, cylindrical, and less bitter. All South Asian type lines and 'BARI Karella 1' produced spindle-shaped fruit with spiny skin, and one of two distinct fruit skin colors in the breeding lines: green and medium green. Three classes of bitterness among lines were noted: low, medium and high. Mean fruit weight of Southeast Asian type lines were higher than that of the South Asian lines, but they produced fewer fruit per plant than the South Asian type lines.

Saponin content

Relative intensities of 17 saponins were determined (Fig. 2, Tab. S1), but absolute amounts could be determined only for momordicosides L (Fig. 3) and G (Tab. 2) because of availability of appropriate references. The saponin with the highest intensity in South Asian and Southeast Asian types was saponin 29-1. Referring to LC-MS data and literature, the tentative structure of saponin 29-1 might be 3β-Hydroxycucurbita-5,24-dien-19-al-7,23-di-O-beta-glucopyranoside (supplemental material Tab. S1; (Keller et al., 2011; Ma, 2010). Saponin 29-2 and 29-3 were among the leading five saponins in this set of South Asian and Southeast Asian bitter gourd breeding lines and cultivars, and might momordicosides M and N, or isomers of them, according to the LC-MS data and literature (supplemental material Tab. S1, (Li, 2007)). Momordicoside K, one of the bitter taste-inducing saponins (Murakami, 2001), occurred at low intensity in this group of bitter gourd entries (Fig. 2, saponin 9-1). Momordicoside L was one of the five most intense saponins, while momordicoside G one of the least intense saponins in this set of lines and cultivars.

Saponin momordicoside L showed about a tenfold higher intensity than momordicoside G in both bitter gourd types. Mean contents of momordicoside G in the South Asian types ranged from 0.07 nmol/g DW (AVBG1323) to 0.71 nmol/g DW (AVBG1325). In the Southeast Asian types momordicoside G ranged from 0.43 nmol/g DW (AVBG1327) to 1.41 nmol/g DW (AVBG1314), almost double the leading mean values of the South Asian types (Tab. 2) with the exception of Southeast Asian type line AVBG1327, which had a lower content than South Asian type lines AVBG1324 and AVBG1325.

South Asian type lines AVBG1324, AVBG1325, and AVBG1330 had significantly ($P = 0.05$) higher momordicoside G contents than 'Palee' and 'BARI Karella 1' (Tab. 2), while AVBG1323 and AVBG1310 had significantly lower momordicoside G values. Furthermore, the mean momordicoside G content of the South Asian type AVBG1301 was not significantly different from 'BARI Karella 1' and 'Palee', but had a significant higher mean value than AVBG1310 and AVBG1323. AVBG1310 had a significantly higher momordicoside G content than AVBG1323.

Among Southeast Asian types 'Benteng' had significantly lower momordicoside G content than AVBG1314, but was not different from AVBG1311 and AVBG1313. In contrast, AVBG1327 had significantly lower momordicoside G content than AVBG1314, AVBG1311, AVBG1313, and 'Benteng'. Lines AVBG1314 and AVBG1313 did not differ significantly for momordicoside G content (Tab. 2).

Mean momordicoside L contents of South Asian type bitter gourd entries ranged from 0.62 nmol/g DW (AVBG1323) to 18.64 nmol/g DW ('Palee') (Fig. 3A). Momordicoside L content in 'Palee' was significantly higher than in AVBG1330, AVBG1301, AVBG1310, and AVBG1323. Lines AVBG1323, AVBG1310, and AVBG1301 had a significantly lower momordicoside L content than 'BARI Karella 1', AVBG1324, and AVBG1325. Furthermore, AVBG1330 had a significant higher mean momordicoside L content than AVBG1301, AVBG1310, and AVBG1323 but a significant lower level of momordicoside L than 'Palee'.

Momordicoside L contents of Southeast Asian type bitter gourds ranged from 4.43 nmol/g DW (AVGB1314) to 16.37 nmol/g DW ('Benteng') (Fig. 3B). 'Benteng' had significantly higher momordicoside L content than AVBG1311, AVBG1313, and AVBG1314, but not AVBG1327. Among the Southeast Asian types, AVBG1327 had

Tab. 2: Secondary metabolite contents of South Asian and Southeast Asian type bitter gourd fruits from 10 breeding lines and cultivars grown in a greenhouse, Karlsruhe, Germany, October 2014 to February 2015.

Type and entry	Momordicoside (nmol/g DW)		Vitamin C	Lutein	β-Carotene	Chlorophyll (ng/mg DW)	
	G	L	(mg/100g FW)	(ng/mg DW)	(ng/mg DW)	a	b
South Asian							
AVBG1301	0.25 ± 0.06 b	5.46 ± 2.39 c	84.28 ± 28.00 bcd	229.40 ± 51.57 a	59.00 ± 13.91 b	3050.78 ± 675.15 a	655.30 ± 147.40 a
AVBG1310	0.16 ± 0.08 c	4.94 ± 2.04 c	65.56 ± 8.54 d	158.12 ± 32.48 b	38.53 ± 5.72 c	2165.27 ± 404.52 c	515.96 ± 95.82 b
AVBG1323	0.08 ± 0.04 d	0.62 ± 0.24 d	58.16 ± 11.99 d	56.91 ± 22.04 c	19.24 ± 6.46 d	759.55 ± 268.17 d	167.48 ± 70.08 c
AVBG1324	0.67 ± 0.33 a	11.83 ± 5.03 ab	71.60 ± 19.69 cd	44.31 ± 29.23 c	16.50 ± 7.42 d	684.22 ± 383.07 d	154.86 ± 122.04 c
AVBG1325	0.72 ± 0.27 a	16.89 ± 10.92 ab	89.37 ± 15.25 bc	59.61 ± 24.18 c	19.84 ± 5.90 d	852.02 ± 378.41 d	158.91 ± 88.77 c
AVBG1330	0.54 ± 0.19 a	8.80 ± 2.97 b	73,94 ± 18.62 cd	168.90 ± 31.77 b	40.77 ± 5.37 c	2210.46 ± 487.61 bc	469.00 ± 116.14 b
BARI K. 1	0.33 ± 0.28 b	12.61 ± 12.07 ab	147.21 ± 23.94 a	234.64 ± 65.69 a	82.95 ± 26.04 a	2855.35 ± 911.91 ab	530.29 ± 165.49 ab
Palee	0.27 ± 0.09 b	18.64 ± 8.90 a	114.05 ± 25.68 b	196.64 ± 57.21 ab	57.13 ± 13.92 b	2537.22 ± 854.06 abc	627.94 ± 174.58 a
Southeast Asian							
AVBG1311	0.94 ± 0.48 b	4.63 ± 2.33 c	72.58 ± 11.87	27.35 ± 10.60 ab	10.99 ± 3.18 b	549.62 ± 161.28 ab	103.91 ± 37.66 ab
AVBG1313	1.14 ± 0.45 ab	8.53 ± 9.50 bc	79.69 ± 16.07	29.66 ± 13.90 ab	11.18 ± 4.37 b	548.56 ± 219.82 ab	99.44 ± 39.91 ab
AVBG1314	1.41 ± 0.47 a	4.43 ± 2.08 c	78.37 ± 19.42	24.82 ± 6.14 b	11.31 ± 2.72 b	406.53 ± 150.67 b	83.05 ± 18.54 b
AVBG1327	0.44 ± 0.22 c	9.42 ± 6.10 ab	76.60 ± 14.20	41.07 ± 17.75 a	15.67 ± 4.43 a	684.13 ± 254.14 a	130.51 ± 47.64 a
Benteng	0.95 ± 1.00 b	16.37 ± 10.40 a	64.40 ± 13.01	34.40 ± 12.53 ab	11.52 ± 1.96 b	536.28 ± 213.11 ab	108.82 ± 30.52 a

DW = dry weight, FW = fresh weight. Mean ± standard deviation; breeding lines and cultivars with same letters are not significantly different ($p > 0.05$) within their respective type; 10 fruits per entry, except for vitamin C, which varied from 3 to 10 fruits per entry.

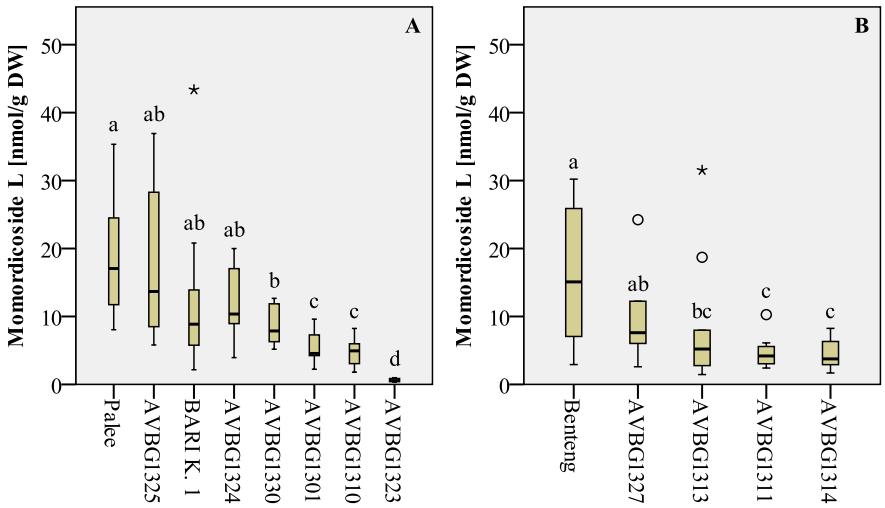

Fig. 3: Momordicoside L contents of eight South Asian (A) and five Southeast Asian (B) type bitter gourd entries cultivated in a greenhouse in Karlsruhe, Germany. Stars stand for extreme values, open circles for outliers. Extreme values and outliers were also included in statistical analyses. BARI K. 1 = 'BARI Karella 1', DW = dry weight. Plots with same letters are not significant different ($P > 0.05$). Means of 10 fruits per entry.

a significantly higher momordicoside L content than AVBG1311 and AVBG1314. AVBG1327 and AVBG1313 did not significantly differ from each other for momordicoside L content. Also, there were no differences for momordicoside L content among AVBG1313, AVBG1311, and AVBG1314.

This is the first report to our knowledge of individual saponin data in bitter gourds of comparable genotypes. Some publications mention, however, total saponin content only of bitter gourd (Macusi, 2009).

Vitamin C content

Vitamin C content was determined only if there were enough fruit and freeze-dried material, thus fruit number per entry for vitamin C analyses ranged from three to 10. The South Asian type 'BARI Karella 1' had the highest vitamin C content followed by 'Palee'. All South Asian type breeding lines had significantly lower vitamin C contents than 'BARI Karella 1' (Tab. 2). Mean vitamin C content of the South Asian types ranged from 58.16 mg/100g FW (AVBG1323) to 147.21 mg/100g FW ('BARI Karella 1'). Similar mean vitamin C contents were reported in bitter gourds of different origins (Dhillon, 2016a; Raj, 2005). South Asian type 'Palee' had significantly more vitamin C than AVBG1330, AVBG1324, AVBG1310, and AVBG1323, but did not differ from AVBG1325 or AVBG1301. In addition, AVBG1325 contained significantly higher vitamin C content than AVBG1310 and AVBG1323.

Mean vitamin C content of Southeast Asian type bitter gourds ranged from 64.40 mg/100g FW ('Benteng') to 79.69 mg/100g FW (AVBG1313) (Tab. 2). Comparable vitamin C levels were found in fresh bitter gourds subjected to different drying treatments and blanching (Mehta, 2017). The four Southeast Asian type breeding lines did not differ significantly in their mean vitamin C content among each other or in comparison to 'Benteng'.

Carotenoid content

Eight different carotenoids were determined in this set of bitter gourds. Intraspecific variability in carotenoids has rarely been investigated, even though recent studies confirm the impact of genetic background and breeding on content of this metabolite (Mageney, 2016; Schroter, 2018). AVBG 1301 had the highest mean carotenoid content among the South Asian type entries, including 'Palee' and 'BARI Karella 1'. All South Asian bitter gourds have in common the highest intensity for the carotenoid lutein, followed by β-carotene and α-carotene (Fig. 4A). Previously reported lutein determinations of bitter gourd also showed this carotenoid as the dominant type (Dhillon, 2016a; Saini, 2017). AVBG1323, AVBG1324, and AVBG1325 had the lowest mean carotenoid contents, but their distribution of the carotenoids was similar to the other lines of this type of bitter gourd. 'Palee' exhibited slightly higher mean zeaxanthin content than the other South Asian type bitter gourds.

Mean carotenoid content of Southeast Asian type bitter gourds was similar among the four breeding lines and 'Benteng'. The carotenoid lutein was found in the highest mean intensity among the detected carotenoids in this type of bitter gourd (Fig. 4B). Lutein and β-carotene were analyzed in South Asian and Southeast Asian lines more intensively due to their wide distribution and health-promoting importance (Cuong, 2017).

Mean lutein values of the South Asian type bitter gourds ranged from 44.31 ng/mg DW (AVBG1324) to 234.64 ng/mg DW ('BARI Karella 1') (Fig. 5A). Lutein content in lines AVBG1323, AVBG1324, and AVBG1325 was significantly the lowest in comparison with the other three lines and two cultivars (Fig. 5A). Significantly highest mean lutein values were detected in 'BARI Karella 1' and AVGB1301 than in lines AVBG1310, AVBG1330, and 'Palee'. Lines AVBG1330 and AVBG1301 did not differ from 'Palee' for lutein content.

Mean lutein content was lower in the Southeast Asian type bitter gourds than in the South Asian type entries (Fig. 5B). Mean values of the Southeast Asian type bitter gourds ranged from 24.82 ng/mg DW (AVBG1314) to 41.07 ng/mg DW (AVBG1327). Comparable lutein levels were found in another study (Cuong, 2017). AVBG1327 and AVBG1314 differed significantly from each other for lutein content, but neither differed from the other three Southeast Asia type entries, including 'Benteng'.

Mean β-carotene contents of the South Asian type breeding lines ranged from 16.5 ng/mg DW to 82.95 ng/mg (Tab. 2). 'BARI Karella 1' had the highest content (82.95 ng/mg DW), AVBG1324 had the lowest mean content (16.5 ng/mg DW). The South Asian type bitter gourds differ significantly ($P < 0.05$) in their β-carotene content in the following decreasing order: 'BARI Karella 1' > AVBG1301 = 'Palee' > AVBG1330 = AVBG1310 > AVBG1325 = AVBG1323 = AVBG1324.

In contrast, mean β-carotenoid content in the Southeast Asian types were lower, ranging from 11 (AVBG1311) to 15.6 ng/mg DW (AVBG1327) (Tab. 2), and were comparable to Chinese bitter gourds,

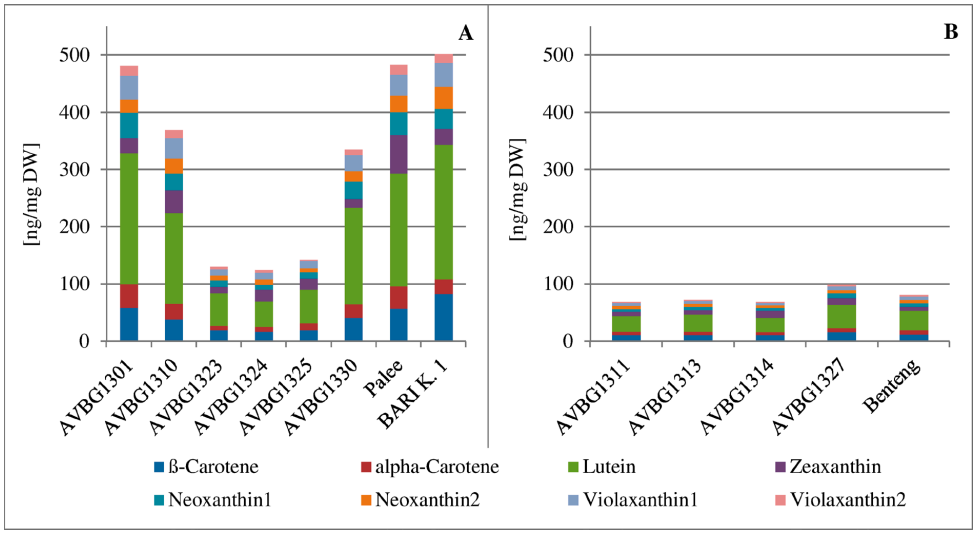

Fig. 4: Intensity distributions of carotenoids in eight South Asian (A) and five Southeast Asian (B) type bitter gourd entries cultivated in a greenhouse in Karlsruhe, Germany. BARI K. 1 = 'BARI Karella 1'. 10 fruits per entry.

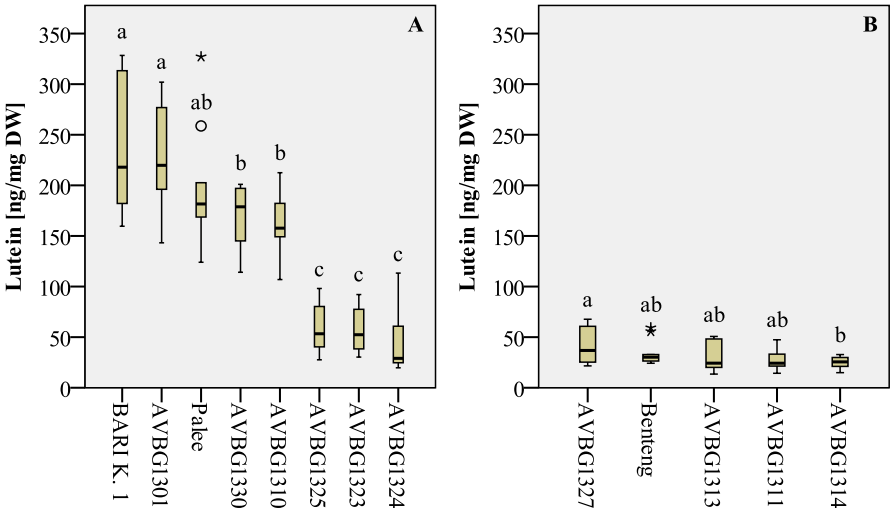

Fig. 5: Lutein contents in eight South Asian type (A) and five Southeast Asian (B) type bitter gourd entries cultivated in a greenhouse in Karlsruhe, Germany. Plots with same letters are not significantly different ($P > 0.05$). Stars stand for extreme values, open circles for outliers. BARI K. 1 = 'BARI Karella 1'. 10 fruits per entry.

which were Southeast Asian types (Cuong, 2017). The Southeast Asian type bitter gourd breeding lines included in this study had β-carotenoid contents similar to 'Benteng', except for AVBG1327, which had significantly higher β-carotenoid content.

Contents of β-carotene and lutein in these bitter gourd lines and cultivars are comparable to other vegetables such as pak choi, broccoli, cauliflower, or Chinese cabbage (Reif, 2013), and are also a valuable source for pro-vitamin A and other carotenoids. All these species may promote health in humans since they have the potential to protect against cardiovascular diseases, certain types of cancer, eye-related diseases, and light-induced skin damage (Fiedor, 2014). There is evidence that lutein reduces the incidence and progression of age-related macular degeneration (AMD) (Chew, 2013; Landrum, 1999; Seddon, 1995).

Chlorophyll a and b contents

Carotenoids and chlorophylls have common precursors in their biosynthesis and thus their accumulation is linked. Previous studies showed strong correlations between carotenoids and chlorophyll contents (Kopsell, 2004). Mean chlorophyll a and b values of South Asian and Southeast Asian bitter gourd types (Fig. 6 and Tab. 2) showed the same patterns, respectively, as lutein and β-carotene (Tab. 2). South Asian type AVBG1301 had the highest mean chlorophyll a content (3050 ng/mg DW) followed by 'BARI Karella 1' and 'Palee' with 2855 and 2537 ng/mg DW, respectively (Fig. 6A). Lines AVBG1323, AVBG1324, and AVBG1325 had the lowest mean chlorophyll a values, and differed significantly from 'BARI Karella 1', 'Palee', AVBG1301, AVBG1330, and AVBG1310. Mean chlorophyll a content of AVBG1301 was significantly higher than the means of the other five South Asian type breeding lines. 'BARI Karella 1' had significantly higher chlorophyll a content than AVBG1310, AVBG1323, AVBG1324, and AVBG1325 but not 'Palee' or AVBG1330.

Mean chlorophyll a content of the Southeast Asian types was lower than mean of the South Asian types once (Fig. 6B). Mean chlorophyll a content in the Southeast Asian types ranged from 407 ng/mg (AVBG1314) to 684 ng/mg DW (AVBG1327). The only significant difference in this group was between AVBG1327 and AVBG1314.

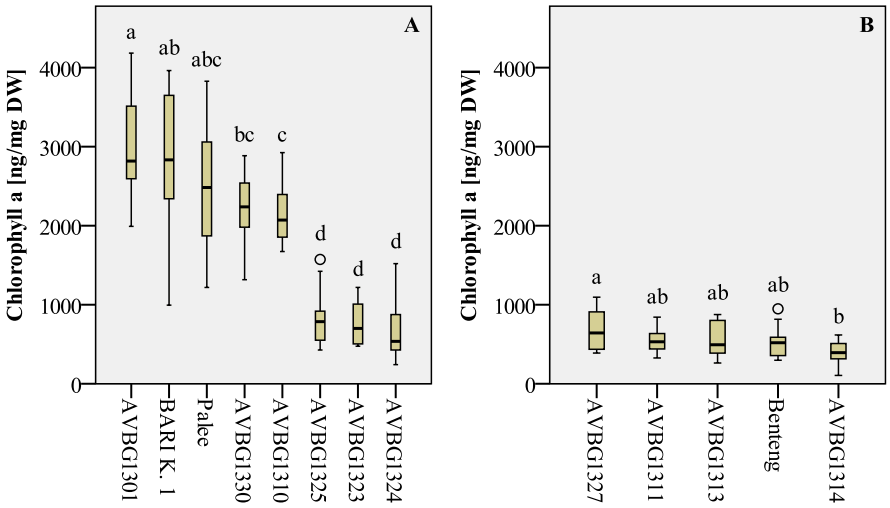

Fig. 6: Chlorophyll a contents of eight South Asian type (A) and five Southeast Asian (B) type bitter gourd entries cultivated in a greenhouse in Karlsruhe, Germany. Plots with same letters are not significantly different ($P > 0.05$). BARI K. 1 = 'BARI Karella 1'. 10 fruits per entry. Open circles stand for outliers. Outliers were included in statistical analyses.

'Benteng' did not differ from any of the Southeast Asian type breeding lines.

Chlorophyll b contents of South Asian type bitter gourds showed similar patterns as their chlorophyll a contents but were much lower (Tab. 2). Chlorophyll b contents ranged from 155 ng/mg (AVBG1324) to 655 ng/mg DW (AVBG1301). 'Palee' (627 ng/mg DW) and 'BARI Karella 1' (530 ng/mg DW) were lower than AVBG1301, but not significantly. AVBG1324, which had the lowest content of chlorophyll b did not differ from AVBG1325 or AVBG1323. 'Palee', 'BARI Karella 1', AVBG1301, AVBG1310, and AVBG1330 had significantly higher chlorophyll b content than AVBG1323, AVBG1324, and AVBG1325. Chlorophyll b values of AVBG1301 and 'Palee' were significantly greater than those of AVBG1310 and AVBG1330. 'BARI Karella 1' did not differ from 'Palee', AVBG1301, AVBG1310, or AVBG1330 for chlorophyll b content.

The Southeast Asian type bitter gourds also had a much lower mean chlorophyll b than chlorophyll a content. Their mean chlorophyll b content was lower than the mean of the South Asian types (Tab. 2). Chlorophyll b content in this type of bitter gourd ranged from 83 ng/mg DW (AVBG1314) to 130 ng/mg DW (AVBG1327). AVBG1327 and 'Benteng' were significantly higher for chlorophyll b content than AVBG1314, but not AVBG1311 or AVBG1313. High chlorophyll contents were also described in various green vegetables such as broccoli, spinach (*Spinacia oleracea*), asiatic pennywort (*Centella aiatica*), and betel vine (*Piper betel*) (Hsu, 2013; Nartnampong, 2016; Park, 2014; Turkmen, 2006). Chlorophyll contents of the investigated breeding lines and cultivars are comparable to literature data (Behera, 2013). Chlorophylls exhibit also antioxidant and anti-inflammatory functions, too even though less studied compared to carotenoids (Hsu, 2013; Park, 2014). The ratio of chl a/chl b is slightly greater than the expected factor of three (Gross, 2012), however such values have been published for green tissues before e.g. *Arabidopsis thaliana* (Zhang, 2008) or for vegetables grown in greenhouses (Samuolien, 2012). The higher chl a/chl b ratios might be the result of a limited rate of photosynthesis in the bitter gourds grown in green houses. Under suboptimal light conditions chl b may be converted into chl a, thus resulting in the increased chl a/chl b ratios.

Conclusion

The South Asian and Southeast Asian breeding lines were comparable or even better to their check varieties. This can be concerned for horticultural traits and secondary metabolites. Some South Asian breeding lines and cultivars had higher contents of secondary metabolites than Southeast Asian ones did. Additionally, some of these breeding lines have exhibited resistance to cucurbit powdery mildew (*Podosphaera xanthii*) in various trials across Asia. The variation in fruit quality characteristics among the different breeding lines and cultivars reflects their origins, and provides interesting variation for further breeding purposes, small growers, and an improved food supply by home and school gardens.

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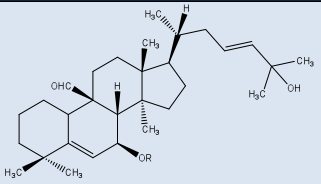
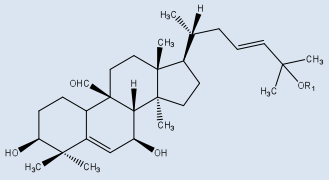
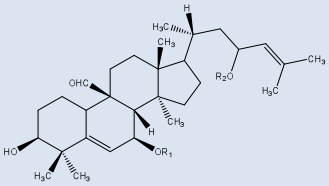
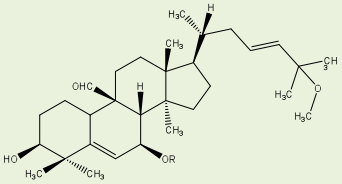
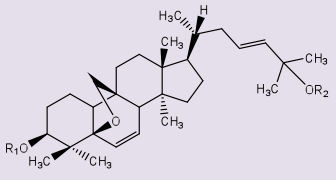
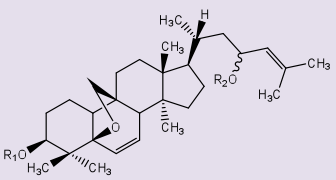
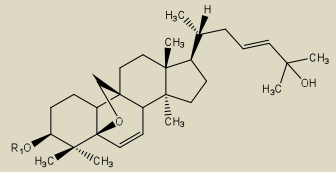
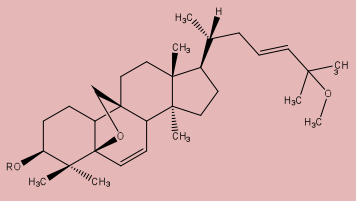
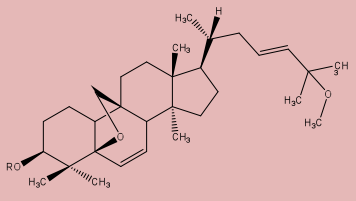
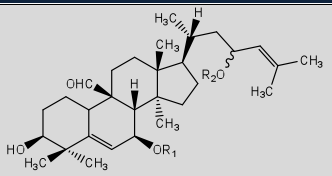
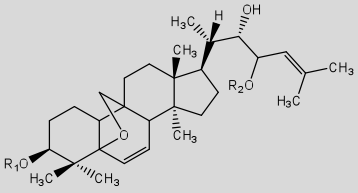
Tab. S1: Compound ID and the corresponding determined chemical formula based on LC-MS data (accurate mass, isotopic pattern) as well as possible structures of the measured bitter gourd saponins, derived from the LC-MS data (accurate mass, isotopic pattern, MS/MS) and literature. Furthermore retention time (RT) as well as detected ions (positive mode) in full scan and MS/MS are given. The same background color in the cells indicates the same chemical formula.

Compound ID	Chemical Formula	RT [min]	Detected Ions (Full Scan; MS/MS) [m/z]	Possible Structures	Reference
Momordicoside L	C36 H58 O9	13.00	652.44 ($M+NH_4^+$); 455, 437, 419, 409, 391	 Momordicoside L[1,2]: R=Glucose	[1] Li et al., 2007, Magn Reson Chem 45:451-456 [2] Nakamura et al., 2006, Chemical and Pharmaceutical Bulletin 54:1545-1550
7-2	C36 H58 O9	12.78	652.44 ($M+NH_4^+$); 437, 419, 409, 391	 Momordicoside U[3]: R_1=Glucose	[3] Ma et al., 2010, Planta Med 76:1758-1761 [4] Keller et al., 2011, Phytomedicine 19:32-37 [5] Comprehensive Natural Products II: Chemistry and Biology, Vol.1:Natural Products, Elsevier 2010
7-3	C36 H58 O9	13.64	652.44 ($M+NH_4^+$); 437, 419, 409, 391		
7-4	C36 H58 O9	11.62	652.44 ($M+NH_4^+$); 455, 437, 419, 409, 391	 Momordicine II[3,4]: R_1=H, R_2=Glucose Momordicine IV[5]: R_1=Glucose, R_2=H	
7-5	C36 H58 O9	14.22	652.44 ($M+NH_4^+$); 455, 437, 419, 409, 391		

Compound ID	Chemical Formula	RT [min]	Detected Ions (Full Scan; MS/MS) [m/z]	Possible Structures	Reference
9-1	C37 H60 O9	14.98	666.456 ($M+NH_4^+$); 437, 419, 409, 391	 Momordicoside K[6,2]: R=Glucose	[6] Murakami et al., 2001, Chemical and Pharmaceutical Bulletin 49:54-63 [2] Nakamura et al., 2006, Chemical and Pharmaceutical Bulletin 54:1545-1550
15-1	C42 H68 O13	12.76	781.47 ($M+H^+$); 619, 439, 421, 409, 403, 391	 Goyaglycoside e[6]: R_1=Allose, R_2=Glucose	[6] Murakami et al., 2001, Chemical and Pharmaceutical Bulletin 49:54-63
15-2	C42 H68 O13	12.96	781.47 ($M+H^+$); 619, 439, 421, 409, 403, 391	 Goyaglycoside f[6] : R_1=Glucose, R_2=Allose	

Compound ID	Chemical Formula	RT [min]	Detected Ions (Full Scan; MS/MS) [m/z]	Possible Structures	Reference
16-1	C36 H58 O8	14.69	619.42 ($M+H^+$); 457, 439, 421, 409, 403, 391	 Momordicoside I[6,2]: R_1=Glucose Momordicoside F2[6,2]: R_1=Allose	[6] Murakami et al., 2001, Chemical and Pharmaceutical Bulletin 49:54-63 [2] Nakamura et al., 2006, Chemical and Pharmaceutical Bulletin 54:1545-1550
16-2	C36 H58 O8	14.81	619.42 ($M+H^+$); 457, 439, 421, 409, 391		
Momordicoside G	C37 H60 O8	16.81	633.436 ($M+H^+$); 471, 439, 421, 409, 403, 391	 Momordicoside G[2]: R=Allose	[6] Murakami et al., 2001, Chemical and Pharmaceutical Bulletin 49:54-63 [2] Nakamura et al., 2006, Chemical and Pharmaceutical Bulletin 54:1545-1550
18-2	C37 H60 O8	16.71	633.435 ($M+H^+$); 471, 439, 421, 409, 403, 391	 Momordicoside F1[6,2]: R=Glucose	

Compound ID	Chemical Formula	RT [min]	Detected Ions (Full Scan; MS/MS) [m/z]	Possible Structures	Reference
29-1	C42 H68 O14	10.70	814.495 ($M+NH_4^+$); 437, 419, 409, 391	 3beta-Hydroxycucurbita-5,24-dien-19-al-7,23-di-O-beta-glucopyranoside : R_1=R_2=Glucose	[3] Ma et al., 2010, Planta Med 76:1758-1761 [4] Keller et al., 2011, Phytomedicine 19:32-37
29-2	C42 H68 O14	9.25	797.47 ($M+H^+$); 635, 455, 437, 425, 419, 407, 389	 Momordicoside M[1]: R_1=Glucose, R_2=Allose Momordicoside N[1]: R_1=Allose, R_2=Allose	[1] Li et al., 2007, Magn Reson Chem 45:451-456
29-3	C42 H68 O14	9.32	797.47 ($M+H^+$); 635, 455, 437, 425, 419, 407, 389		
29-6	C42 H68 O14	9.09	797.47 ($M+H^+$); 635, 455, 437, 425, 419, 407, 389		
29-5	C42 H68 O14	12.60	797.47 ($M+H^+$); 635, 439, 421, 409, 403, 391	No structure suggestion ca be made	

Tab. S2: Intra-day (n = 4) and total (n = 60) repeatability as CV values of saponin compounds in QC samples before signal intensity drift/batch correction.

Analytes	Intra-day															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Momordicoside L	*4.3*	*7.2*	*2.3*	*7.1*	*14.8*	*6.2*	*3.8*	*5.5*	*11.8*	*7.6*	*7.6*	*4.5*	*5.8*	*6.4*	*6.3*	**13.0**
Saponin 07-2	*2.0*	*9.9*	*9.9*	*10.1*	*9.0*	*2.9*	*5.8*	*12.8*	*10.5*	*6.9*	*8.6*	*16.7*	*9.2*	*7.2*	*14.4*	**16.4**
Saponin 07-3	*6.6*	*7.4*	*9.1*	*7.8*	*23.0*	*2.7*	*11.2*	*15.5*	*2.5*	*9.3*	*15.8*	*5.0*	*15.2*	*14.5*	*15.1*	**16.7**
Saponin 07-4	*14.5*	*7.8*	*17.3*	*10.8*	*10.8*	*12.3*	*18.7*	*5.1*	*23.8*	*15.9*	*4.9*	*13.1*	*19.2*	*13.4*	*13.3*	**17.9**
Saponin 07-5	*11.6*	*10.0*	*13.5*	*6.3*	*7.8*	*10.5*	*7.7*	*5.5*	*9.4*	*9.0*	*3.9*	*18.5*	*9.4*	*5.7*	*4.9*	**13.1**
Saponin 09-1	*15.9*	*15.7*	*11.7*	*18.8*	*20.3*	*10.4*	*9.6*	*15.4*	*24.7*	*15.1*	*15.1*	*21.1*	*31.3*	*19.5*	*11.7*	**29.7**
Saponin 15-1	*6.3*	*8.6*	*16.9*	*6.1*	*15.1*	*15.0*	*10.9*	*16.8*	*23.6*	*8.7*	*7.4*	*5.5*	*7.9*	*9.2*	*7.5*	**18.3**
Saponin 15-2	*16.8*	*18.2*	*6.3*	*35.4*	*38.8*	*4.3*	*17.0*	*55.2*	*9.2*	*20.7*	*4.9*	*35.8*	*15.2*	*15.9*	*8.8*	**27.5**
Saponin 16-1	*23.4*	*3.6*	*12.7*	*6.4*	*14.9*	*8.7*	*11.0*	*23.6*	*13.7*	*8.7*	*17.5*	*14.8*	*10.0*	*4.1*	*10.7*	**14.6**
Saponin 16-2	*10.1*	*23.8*	*13.0*	*14.3*	*24.0*	*11.0*	*7.2*	*14.5*	*20.2*	*19.9*	*18.5*	*4.1*	*16.5*	*13.6*	*16.4*	**20.4**
Momordicoside G	*16.2*	*7.4*	*22.8*	*9.4*	*13.6*	*12.3*	*9.4*	*13.7*	*13.8*	*19.3*	*12.0*	*15.0*	*15.4*	*4.7*	*18.2*	**26.9**
Saponin 18-2	*8.8*	*9.3*	*12.4*	*10.6*	*23.1*	*26.4*	*12.7*	*11.9*	*23.9*	*20.5*	*8.2*	*22.1*	*12.5*	*9.5*	*11.6*	**27.2**
Saponin 29-1	*12.1*	*16.6*	*18.2*	*9.8*	*19.5*	*8.8*	*13.4*	*15.8*	*12.7*	*6.5*	*6.8*	*22.5*	*4.2*	*11.8*	*8.4*	**15.2**
Saponin 29-2	*6.9*	*12.7*	*10.2*	*7.0*	*16.4*	*18.2*	*12.8*	*11.2*	*5.8*	*16.7*	*7.7*	*9.4*	*16.8*	*11.8*	*8.0*	**15.6**
Saponin 29-3	*7.3*	*7.7*	*8.0*	*14.1*	*5.2*	*7.8*	*12.5*	*12.9*	*3.6*	*16.8*	*12.7*	*19.2*	*6.7*	*17.5*	*5.9*	**15.1**
Saponin 29-5	*14.2*	*8.6*	*7.5*	*17.8*	*24.0*	*5.6*	*9.2*	*11.2*	*9.2*	*12.0*	*15.2*	*20.8*	*6.5*	*13.7*	*9.9*	**19.6**
Saponin 29-6	*6.8*	*12.8*	*10.9*	*13.2*	*7.7*	*3.2*	*11.0*	*4.6*	*4.1*	*6.4*	*8.3*	*21.2*	*9.6*	*14.2*	*6.9*	**15.7**

Tab. S3: Intra-day (n = 4) and total (n = 60) repeatability as CV values of saponin compounds in QC samples after signal intensity drift/batch correction

Analytes	Intra-day															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Momordicoside L	3.2	2.3	1.3	6.4	14.6	2.1	3.7	0.6	11.4	4.6	5.5	4.1	1.5	4.0	6.3	***5.3***
Saponin 07-2	2.0	7.3	9.8	10.1	8.4	2.8	1.9	12.6	6.3	5.1	8.6	11.4	6.6	3.7	8.2	***6.7***
Saponin 07-3	5.3	6.8	9.1	4.8	19.0	2.7	4.5	14.8	2.3	9.3	7.7	4.0	13.9	14.5	10.7	***8.6***
Saponin 07-4	14.3	4.9	12.0	10.5	4.7	8.4	4.7	4.3	18.1	8.5	4.0	12.6	11.2	13.3	10.4	***9.0***
Saponin 07-5	6.6	8.9	12.2	5.7	7.3	7.6	7.5	5.0	7.9	8.7	0.4	2.3	8.3	5.6	4.8	***6.2***
Saponin 09-1	3.6	10.0	8.8	8.2	8.6	7.5	5.8	5.7	1.3	10.2	10.2	10.0	9.3	5.3	7.7	***6.9***
Saponin 15-1	3.8	2.8	12.6	3.3	8.3	14.7	10.9	16.2	16.2	7.1	7.4	2.7	7.3	9.2	4.4	***8.4***
Saponin 15-2	16.9	15.1	5.9	36.7	38.5	3.6	9.4	57.4	7.7	13.5	1.2	32.4	15.0	15.6	4.7	***20.9***
Saponin 16-1	22.4	3.6	12.1	6.4	6.1	8.5	7.6	24.1	8.1	8.6	15.2	1.9	9.4	3.6	4.3	***9.9***
Saponin 16-2	7.3	20.3	12.2	9.7	18.3	10.8	4.6	7.5	19.5	19.6	15.4	4.0	15.0	12.5	11.9	***11.9***
Momordicoside G	12.2	6.3	2.8	4.0	9.0	8.0	6.8	6.7	5.7	9.9	2.1	13.0	6.6	2.0	3.0	***6.4***
Saponin 18-2	8.4	4.4	5.2	10.5	15.6	6.5	12.4	8.4	16.7	12.6	7.6	18.2	7.4	9.4	10.4	***9.6***
Saponin 29-1	9.1	4.0	4.3	9.8	6.9	7.5	13.3	9.9	12.8	5.2	2.6	10.8	4.1	10.2	5.1	***7.3***
Saponin 29-2	6.9	12.4	8.3	4.1	11.0	16.0	8.2	8.9	2.6	13.2	4.0	9.4	16.3	8.3	8.0	***8.7***
Saponin 29-3	4.6	7.5	7.6	7.7	2.9	7.0	11.0	11.7	2.2	11.8	11.8	18.1	5.8	17.6	4.0	***8.7***
Saponin 29-5	13.5	7.9	6.9	9.4	6.3	5.0	9.2	5.6	3.6	11.7	13.4	17.4	4.9	11.8	8.2	***8.5***
Saponin 29-6	2.6	11.7	9.6	10.0	7.6	1.4	8.6	2.1	3.6	6.3	6.3	19.5	9.6	11.6	6.2	***7.8***