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Screening of Low Bitterness Bitter Gourd (*Momordica charantia*) Parental Lines based on Low Calcium Contents and Other Morpho-physio and Nutritional Attributes

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ABSTRACT

The bitter taste of *Momordica charantia* due to its high level of calcium content limits people from consuming it, although it has enough various beneficial nutrients. Parental line evaluation is prerequisite for any desired improvement activities through breeding. In this regard *M. charantia* parental lines were evaluated based on bitterness (high Ca content) and morpho-nutritional attributes to augment additional value and opportunity to be commercialized. Field and laboratory experiments were carried out for three open-pollinated *M. charantia* varieties (V1: GW-105; V2: Leckat 921 microgreen and V3: Japan Long-Everrgreen) to screen low bitterness parental lines based on low calcium contents and other morpho-nutritional qualities, targeting to generalize this important vegetable crops for all types of consumers from child to older age. The experiment was arranged in Randomized Complete Block Design (RCBD) with 3 replications. From the overall results of the study, Variety 2 showed better quality for numbers of fruits per plant, fruit weight (g), moisture content (%), chlorophyll content (mmol/m^2), stomatal conductance (mmol/m^{2}s) and nutrients content such as calcium (Ca), nitrogen (N), magnesium (Mg) and iron (Fe). Variety 3 had good quality for morphological parameters like plant height (cm), numbers of leaves per plant, leaf area (cm^2), fruit length (cm), phosphorus (P), potassium (K), aluminium (Al) and copper (Cu). Variety 1 had the highest values only for number of branches per plant and amount of sodium (Na) content. After that, Variety 2 was identified for having lowest calcium (Ca) contents compared to the other two varieties. Thus, Variety 2 was selected as better parents to hold good promise for hybridization based breeding programs for varietal improvement in obtaining low bitterness bitter gourd but rich in other nutrient contents.

Keywords: *Momordica charantia*, bitter vegetables, parental lines, bitterness and calcium, mineral nutrients

INTRODUCTION

Momordica charantia belongs to the Cucurbitaceae family and known as different names like bitter gourd, bitter melon, balsam pear (Krawinkel and Kedig, 2006; Choo et. al, 2014), kerala, or peria since it grows in tropical regions such as India, Malaysia, China, tropical Africa, Middle East, America (Kubola and Siriamornpun, 2008) and Thailand. There are two groups of cultivated *M. charantia;* var. minima with fruits diameter less than 5 cm and var. maxima with fruits diameter more than 5cm (Choo et al., 2014). They are commonly grown for their edible fruits and leaves for some regions like in India or Bangladesh also for medicines.

As a medicinal plant, it is reported that bitter gourd possess antilipolytic, analgesic, abortifacient, antiviral, cytotoxic, hypoglycemic, antimutagenic (Singh et al., 1998; Kubola and Siriamornpun, 2008), anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial, antitumor and antidiabetic properties (Paul and Raychaudhuri, 2010). They are rich sources of carotene, ascorbic acid, folic acid, vitamin C, vitamin A and minerals like calcium, iron and phosphorus (Ullah et al., 2011). The bitter flavour of bitter guard is due to the alkaloid momordicine produced in fruits and leaves (Paul and Raychaudhuri, 2010) and has strong correlation with calcium content of vegetables, based on USDA Nutrient Database values (Tordoff et al., 2009). Due to its taste, bitter gourd is quite less consumed especially by younger generations. According to Statistic of Agro Food Production book 2014, published by Ministry of Agriculture Malaysia, bitter gourd production (17,510 tonnes) is much lesser than other vegetables such as long bean (53,483 tonnes) and brinjal (54,443 tonnes). Looking at bitter gourd with various nutritional benefits, it deserves higher production and development. However, the bitter taste of *Momordica charantia* which due to its high level of calcium content (reference?) may be the main cause making people back out from consumption, although it has many medicinal benefits. Furthermore, open pollinated bitter gourd varieties are very low yielder and cannot attract customer preferences due to poor fruit quality.

To develop high nutritious value with low bitterness varieties, only selective hybridization can do the miracle. Hybridization is the process of crossing two genetically different parents to produce new offspring's or genotypes with different set of traits. Thus, to get low bitterness offspring is not impossible. However, information about the morpho-physiology and nutritional attributes are essential for selection of diverse parents upon which hybridization can result in productive hybrids. The parental information on identification of better parents for better F1 production is needed to improve bitter gourd variety to be widely consumed by all types of consumers from children to old age groups. For this purpose, an investigation was done with three open pollinated varieties to compare morpho-physiological and nutritional attributes (especially Ca) for selecting high yield potential with low bitterness (low calcium content) parental lines for future varietal improvement program.

MATERIALS AND METHODS

Momordica charantia seed collection and experiment set up

In market most of the available commercial varieties are hybrid so it was very tough to find out open-pollinated bitter gourd variety seeds. However, only three open-pollinated (OP) varieties of *Momordica charantia* (V1 = GW-105; V2 = Leckat 921 microgreen and V3 = Japan Long-Everrgreen) seeds were collected from local market at Besut, Terengganu, Malaysia. After collection the seeds were soaked overnight to break dormancy and boost up germination then planted into seed tray. After two (2) weeks**,** the grown seedlings were transplanted into large size poly bags contained 3:2:1 (topsoil:compost:sand) medium, with planting space of 1.0m X 0.5m plant-to-plant and row-to-row at farm field of Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA), Besut Campus, Besut, Terengganu. As to ensure all better growth, those *M. charantia* plants were fertilized right after transplanting and every two weeks interval, and irrigated manually twice per day - early morning and late evening. Recommended agronomic practices were followed to raise a good crop. Trellis system was made using bamboo and net as bitter gourd requires a trellis support for vine climbing and there are several methods of trellising (Behera et al., 2010).

Morphological Data Collection

From varied morphological parameters plant height (cm), number of branches, number of leaves per plant, leaf area (cm^2) using leaf area meter (CI-202 Portable Laser Area Meter, Bio-Science, Inc. USA), fruit length (cm), fruit weight (g), number of fruits per plant, fresh weight of plants and dry weight of plants were recorded. The data collection and analysis was done following the methods described by Singh et al. (2013) and Singh et al. (2014).

Physiological data collection

Among different physiological parameters; relative moisture content (%), total chlorophyll content ($mmol/m^2$) and stomatal conductance ($mmol/m^2s$) were measured.

Total chlorophyll content or greenness of leaves was determined after 30 days of transplanting (SPAD30) using portable SPAD meter (Minolta TM SPAD-502, Minolta Camera Co., Osaka, Japan). Five leaf SPAD readings were taken and averaged to have the mean SPAD reading for each replicate. Chlorophyll meter (Minolta) uses light sources and detects the light transmitted by a plant leaf at two wavelengths (red and infrared regions of the spectrum) (Biljana and Aca, 2009). Stomatal conductance is the rate at which either water vapor or carbon dioxide passes through the stomata, which are the small pores of a plant. It was determined by using leaf porometer (Decagon SC-1, USA). Relative moisture content were determined from the variation of fresh weight and dry weight of the fruit samples and converted into percentage.

Major micro and macro nutrients analysis – Spectroscopy

Sample Preparation

The harvested healthy and fresh bitter gourd edible fruits for each variety was washed thoroughly until no extraneous material remained. They were blotted till the excess moisture absorbed, and air dried. The fresh fruits used for nutrients analysis were washed using deionised water. Then the fruits were cut into small pieces, the seeds were removed and only peel were collected. The samples were then kept in oven at 80 ºC till a constant weight is obtained. The oven dried plant materials were then randomly mixed and powdered to a fine powder by using an electric grinder. The sample was packed into airtight sample plastic and used for the nutrient analysis.

Acid digestion

For the analysis of inorganic constituents the acid digestion methods of Toth et al. (1948) was followed. Five (5) gm of oven dried and blended powder of fruit was transferred into 150 ml clean borosil beaker and 10 ml concentrated HNO_3 was added. It was covered with watch glass and kept for an hour till the primary reactions subsided. Then, it was heated on hot plate till all the material was completely dissolved and allowed to cool to room temperature and then 10 ml of Perchloric acid (60%) was added to it and mixed thoroughly. It was then heated strongly on the hot plate until the solution became colourless and reduced to about 2-3 ml. After cooling, it was transferred to 100 ml volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day the extract was filtered through Whatman No. 44 (Ashless) filter paper. The filtrate was stored properly and used for analysis of inorganic constituents.

Spectroscopic analysis

The nutrient contents in fruits were analyzed through spectroscopy following the methods described by Cindric et al. (2012) with slight modifications. ICP-AES measurements performed using a Prodigy High Dispersive ICP spectrometer working in a simultaneous mode. The filtrates obtained from acid digestion methods of bitter gourd fruits samples were diluted 1:20 with 2% HNO3 in triplicate. For the direct determinations of the minor and major elements from bitter gourd, each sample was diluted (v/v 1:20) with 2% HNO3 in triplicate. Furthermore dilutions 1:5 and 1:10 were tested for their applicability. For the microwave assisted digestion approx. 5 mL of each sample (in duplicate) and 3 mL HNO3, conc. underwent the following procedure (time [min]/power [W]/T [°C]: 3/700/85; 5/500/125; 4:30/1000/160; 17:30/1000/160; ventilation 18 min). The resulting clear solutions were then brought to 20 mL with double distilled water. The ICP-AES operating conditon was maintained as; RF-Generator - 40 MHz "free-running"; Output power - 1.1 kW; Argon flow - Coolant: 18 L min^{-1}, Auxiliary: 0.8 L min^{-1}, and Nebulizer: 1 L min^{-1}; Peristaltic pump - 1.0 mL min^{-1}; Nebulizer - Pneumatic (glass concentric); Spray chamber - Glass cyclonic; Plasma viewing – Axial; 3 replicates for each analysis run and 30 s delay for sample uptake (Cindric et al., 2011). In this study, all samples of bitter gourd fruits were analyzed for their micro and macro element contents (P, K, Mg, Na, Fe, Mn, Zn and Cu), including calcium (Ca) and all elements were estimated in triplicate.

Nitrogen analysis

Total nitrogen content in fruits was estimated according to the method used by Hawk et al. (1948) with slight modifications. One gram powder of fruit samples for each variety was weighed and put into Kjeldahl's digestion tubes along with two tablets of catalyst Kjeltabs Cu 3.5 to accelerate the digestion and to avoid bumping of solution in flask, respectively. Then, to each digestion tubes 15 ml concentrated H_2SO_4 was carefully added in the fume hood and gently shaken. The rack was loaded with exhaust system into digestion block. The exhaust system was attached to digestion tubes in the rack. Tap water was turned on, scrubber unit and control unit were switched on next. Temperature was set at 400 ºC. Digestion was carried out till a clear with green/blue solution was obtained. After cooling to room temperature, the digestion tubes with clear solutions were transferred to distillation unit. Receiver solution (25 ml of 2% boric acid with 5 drops of indicator) was prepared into conical flask and was put into distillation unit. The desired program was selected where 70 ml of distilled water was dispensed into tube followed by 50 ml of 32% NaOH automatically. The distillate green receiver solution for each sample then was titrated with standardized 0.1N HCl until they turned to pink/red. The volume of HCl used for all samples and blank were recorded and used for calculation to find amount of N content.

Experimental design and statistical analysis

This study was conducted in Randomized Complete Block Design (RCBD) with 3 replications for the varieties and measurements as well. Recorded data was analyzed by using one way ANOVA using SPSS statistical software version 17.0. Every data was recorded and presented as mean ± standard error (±SE), and $p<0.05$ was considered as significant. Differences of mean among varieties were determined by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Morphological attributes

Results are presented in Table 1. The plant height of Variety 3 bitter gourd plant was the tallest with 300.0±22.9 cm compared to V2, while the shortest was in Variety 2 with 195.7±36.7 cm. The mean differences of plant height showed significant differences between Variety 2 and Variety 3 at $p < 0.05$ level, where plant height of V3 bitter gourd plant was 1.2 times higher than the variety 2 (Table 1).

Table 1. Means (±SE) of morphological attributes of 3 different bitter gourd varieties

	Plant Height (cm)	No. of Branches Plant^{-1}	No. of Leaves Plant^{-1}	Leaf Area (cm^2)	No. of Fruits Plant^{-1}	Fruit Length (cm)	Fruit Weight (g)
V1	250.0 ± 18.0^{ab}	23.00 ± 2.65^{a}	212.67 ± 15.18^{b}	25.79 ± 1.91^{b}	9.33 ± 0.57^{ab}	14.07 ± 0.60^{b}	140.56 ± 7.99^{ab}
V2	195.7 ± 36.7^{b}	12.67 ± 2.52^{b}	281.70 ± 38.80^{ab}	31.30 ± 2.90^{ab}	11.33 ± 0.57^{a}	12.53 ± 0.96^{b}	188.12 ± 31.30^{a}
V3	300.0 ± 22.9^{a}	15.00 ± 2.00^{b}	341.30 ± 32.90^{a}	45.20 ± 3.01^{a}	7.67 ± 1.15^{b}	19.14 ± 0.21^{a}	124.78 ± 7.26^{b}

Note, here mean values (±SE) followed by different letters differ significantly according to Duncan's multiple range test at $p < 0.05$.

Meanwhile for number of branches per plant, bitter gourd Variety 1 had the highest reading among those three varieties with 23.00±2.65, which is significantly higher at $p < 0.05$ level from the other two varieties. Variety 2 had the lowest number of branches with 12.67±2.52 per plant (Table 1).

Although Variety 3 had medium number of branches, but it had the highest number of leaves with 341.30±32.90 leaves per plant unlike Variety 1, in which earlier had abundance of branches and had the lowest number of leaves per plant with only 212.67±15.18. There had significant differences for number of leaves between those two Variety 3 and Variety 1 at $p < 0.05$ level (Table 1).

Not only having large numbers of leaves, Variety 3 also had the largest leaf area 45.20±3.01 cm^2. Same went to Variety 1 which not only had lowest number of leaves but smallest leaf area among all, with 25.79±1.91 cm^2 which was significantly lower compared to V3 (Table 1).

The highest numbers of fruits (11.333±0.577) was produced by Variety 2 which also weighed the largest with 188.12±31.30 g and tailed by Variety 3 with the lowest numbers of production per plant and fruit weight as well, with 7.67±1.155 and 124.78±7.26 g, respectively. These two varieties showed significantly different between each other at $p < 0.05$ for both parameters, where V2 bitter gourd plant produced 1.48 times higher fruit's than the variety 3 but the fruit weight was 1.51 times higher in V2 compared to V3 (Table 1).

Although Variety 2 led by both parameters for number of fruits and fruit weight produced per plant, it seemed to have the shortest fruit length with 12.53±0.961 cm. However, Variety 3 with lowest production of fruits per plant and fruit weight, produced the longest fruit with means 19.14±0.21 cm and showed significant differences with other varieties (Table 1).

Physiological attributes

The moisture content of fruit is in equilibrium with air humidity such that when the air is dry, the fruit loses moisture and split open to dehiscent (Schmidt, 2007). Thus, fruits with higher moisture content need higher temperature or longer time for dehiscence to occur. For *Momordica charantia*, it is consumed at young and edible stage. High moisture content and late dehiscence were the best for the variety.

Table 2. Means (±SE) of physiological attributes of 3 different bitter gourd varieties

Variety	Moisture Content (%)	Chlorophyll Content ($mmol/m^2$)	Stomatal Conductance ($mmol/m^2s$)
V1	92.76 ± 1.03^{ab}	11.76 ± 0.57^{ab}	190.7 ± 27.4^{b}
V2	93.47 ± 1.1^{a}	12.20 ± 1.49^{a}	271.4 ± 84.5^{a}
V3	91.20 ± 1.6^{b}	10.53 ± 0.58^{b}	243.0 ± 61.8^{ab}

Note; here mean values (±SE) followed by different letters differ significantly according to Duncan's multiple range test at $p < 0.05$.

Based on Table 2, moisture content in bitter gourd Variety 2 was the highest (93.47%) while the lowest was observed in Variety 3 with 91.20%. The mean differences of moisture content showed there was significant different between Variety 2 and Variety 3 (Table 2).

Reduction in stomatal conductance would cause inhibition of stomatal aperture. Oxygen deficiency generally induces a rapid reduction in the rate of photosynthesis which is generally considered as the result of reduced stomatal conductance or aperture and decrease in leaf chlorophyll content (Rao et al., 2016). From the other words, Stomatal conductance estimates the rate of gas exchange and transpiration through the leaf stomata as determined by the degree of stomatal aperture. Hence, it is a function of the density, size and degree of opening of the stomata; with more open stomata allowing greater conductance, and consequently indicating that photosynthesis and transpiration rates are potentially higher. High chlorophyll content also indicate high rate of photosynthesis and high oxygen (Pietragalla and Pask, 2012).

On the other hand Variety 2 had the highest reading for stomatal conductance with 271.4±84.5 $mmol/m^2s$ while Variety 1 had the lowest stomatal conductance with 190.7±27.4 $mmol/m^2s$ (Table 2). There was significant different for stomatal conductance at $p < 0.05$ level between Variety 1 and 2. For total chlorophyll content, bitter gourd Variety 2 still had the highest among those three varieties with 12.20±1.49 $mmol/m^2$ and significantly different from Variety 3 which had the lowest chlorophyll content of 10.53±0.58 $mmol/m^2$.

Major micro and macro nutrient contents

Based on results presented in Table 3, Variety 1 of *M. charantia* showed the highest calcium (Ca) content (35.0±2.0 mg/100g DW) and varied significantly (p < 0.05) with the other two varieties, though Variety 2 showed the lowest Ca (27.87±2.14 mg/100g DW) content but was not differed significantly with V3 (Table 3). On the other hand, Variety 2 possesses the highest nitrogen (2.33±0.14%) content which significantly (p < 0.05) differed with other two varieties and the Variety 3 showed the lowest N (0.14±0.02%) content (Table 3).

Table 3. Major mircro and macro elements (mg/100 g DW) determined from 3 varieties of bitter gourd fruits.

	Ca	N (%)	P	Cu	Na	Mg	Fe	K	Al	Zn
V1	35.0 ± 2.0[a]	1.05 ± 0.02[b]	53 ± 0.12[b]	1.47 ± 0.12[b]	53.64 ± 0.37[a]	25.87 ± 1.21[b]	3.87 ± 0.42[a]	240 ± 2.25[a]	2.6 ± 0.22[a]	0.95 ± 0.01[a]
V2	27.87 ± 2.14[b]	2.33 ± 0.14[a]	60 ± 0.23[ab]	1.60 ± 0[a]	49.50± 2.8[a]	43.05 ± 1.08[a]	4.01 ± 0.31[a]	293 ± 2.25[a]	2.8 ± 0.19[a]	0.89 ± 0.03[a]
V3	28.07 ± 0.81[b]	0.14 ± 0.02[c]	73 ± 0.15[a]	1.53 ± 0.12[ab]	51.30± 2.1[a]	38.53 ± 1.06[a]	3.73 ± 0.50[a]	387 ± 1.72[a]	3.1 ± 0.11[a]	1.0 ± 0.01[a]

Note; here mean values (±SE) followed by different letters differ significantly according to Duncan's multiple range test at p < 0.05.

For phosphorus (P); Variety 3 had the highest concentration (73.0±0.15 mg/100g DW) in bitter gourd with the lowest concentration (53.0±0.12 mg/100g DW) in Variety 1, respectively (Table 3). P content in bitter gourd varieties was also significantly (p<0.05) different between Variety 1 and 3. Furthermore, significantly (p<0.05) highest Copper (Cu) concentration (1.60±0.0 mg/100g DW) was recorded in Variety 2 while the lowest (1.47±0.12 mg/100g DW) was seen in Variety 1, respectively (Table 3).

For sodium (Na) content statistically non-significant (p>0.05) variation was observed among all those 3 varieties, but independently Variety V1 showed the highest Na concentration (53.64±0.37 mg/100g DW), followed by variety V3 (51.30±2.1 mg/100g DW) and Variety V2 (49.50±2.8 mg/100g DW), respectively (Table 3). Different from Na, Variety V2 and V3 showed non-significant variation for magnesium (Mg) content but significantly differed with V1, where the highest Mg (38.53±1.03 mg/100g DW) was recorded in Variety V2 and the lowest (25.87±1.21 mg/100g DW) was found in V1, respectively (Table 3). After that, same as Na content, statistically non-significant (p>0.05) variation was noted for iron (Fe) content among 3 bitter gourd varieties (Table 3), among which V2 possessed the highest reading for iron (4.01±0.31 mg/100g DW) and the lowest (3.73±0.50 mg/100g DW) was in variety V3, respectively (Table 3).

Meanwhile for potassium (K) and aluminum (Al) content it is observed that the highest reading for K (387±1.72 mg/100g DW) and Al (3.1±0.11 mg/100g DW) in Variety 3, respectively. The lowest reading was seen for Variety 1 with 240±2.25 mg/100g DW for K and 2.6±0.22 mg/100g DW for Al (Table 3).

Different from the other nutrient contents, zinc (Zn) concentration was found statistically non-significant (p>0.05) for all those 3 varieties with the highest (0.95±0.01 mg/100g DW) in V1 and the lowest (0.89±0.03 mg/100g DW) was recorded in V2 (Table 3).

Parental line selection

V1 had the highest values for two parameters out of 20 which were number of branches per plant and amount of sodium (Na) content. V2 showed better quality for almost half from 20 attributes which were number of fruits per plant, fruit weight, moisture content, chlorophyll content, stomatal conductance and nutrients contents such as calcium (Ca), nitrogen (N), magnesium (Mg) and iron (Fe). For the third variety (V3), it showed good quality for most of morphological parameters like plant height, number of leaves per plant, leaf area, fruit length, phosphorus (P), potassium (K), aluminum (Al) and copper (Cu).

As V1 had very few best of quality thus it is excluded to be considered as best parental lines among those three varieties. However, if the aim is **to** get medium plant height, then V1 can be selected as a parent to produce hybrid with others good quality parental lines.

V2 and V3 seemed to have almost equal quality with different attributes. V3 was monopoly for morphological and nutritional attributes with 4 out of 7 and 5 out of 10 parameters, respectively. Meanwhile, V2 was monopoly for all other additional values of physiological attributes and some of nutritional content level (3 out of 7 nutrients).

As our main target was to select parental lines based on lowest Ca content (low bitterness) in combination with other morpho-physio and nutritional attributes. Thus, from the results it is observed that among the 3 bitter gourd varieties V2 and V3 had almost similar Ca content and they can be selected for hybridization through crossing and reciprocal crossing to produce new F1 with lowest Ca content compared to both of the parents along with other desired characteristics.

CONCLUSION

Screening of bitter gourd varieties based on their morpho-physio and nutritional attributes had been done where V3 was monopoly for morphological and nutritional attributes while V2 was monopoly for all other additional values of physiological attributes with some of nutritional benefits. After that, in order to accomplish the main objective of this study V2 has been identified for having lowest calcium (Ca) content with other better qualities, such as numbers of fruits per plant, fruit weight, moisture content, chlorophyll content, stomatal conductance and nutrients content such as nitrogen (N), magnesium (Mg) and iron (Fe). It also produced the largest number of fruits with the highest weight that indicates more flesh. So, from the present study, the variety V2 (Leckat 921 microgreen) hold good promises to produce a suitable F1 through hybridization with V1 for obtaining low bitterness bitter gourd (low Ca content) and other attributes for future varietal improvement program, to make the bitter gourd as a general consumable vegetables for all types of consumers especially for children and old age groups without fear of bitterness.

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