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Classifying fifty-seven *Cucumis* (Cucurbitaceae) accessions into six species using leaf architectural traits

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Abstract. *Masungsong LA, Alcala AA, Buot IE Jr., Belarmino MM. 2022. Classifying fifty-seven* Cucumis *(Cucurbitaceae) accessions into six species using leaf architectural traits. Biodiversitas 23: 4006-4017.* In gene banks, there are myriads of accessions that need to be studied, grouped, classified and organized to the proper taxon and hence, be able to manage the accessions efficiently. Using leaf architecture traits, it is the objective of this study to determine the identity and classify the 57 *Cucumis* accessions in the genebank of Hortanova Farm and Research Center, East-West Seed Company, Inc., Lipa City, Batangas, Philippines. Five hundred thirteen *Cucumis* leaf samples across the 57 accessions were collected, measured, thoroughly investigated, and described using leaf architectural characters. Results of leaf architectural analyses focusing on the blade class, apex angle, secondary vein spacing, tertiary vein angle in relation to primary vein, and areole development, revealed that the 57 accessions can be grouped into six (6) species of *Cucumis*, namely, *C. melo* subsp. *agrestis* (Naudin) Pangalo, C. *melo* var. *texanus* Naudin, *C. melo* var. *flexuosus* (L.) Naudin, *C. zambianus* Widrl, J.H.Kirkbr., Ghebret. & K.R.Reitsma, *C. sativus* L., and *C. sativus* var. *hardwickii* (Royle) Gabaev. *Cucumis sativus* and *C. sativus* var *hardwickii* were delineated from the rest of the species for having a macrophyll blade class, an odd lobed acute apex angle and an obtuse tertiary vein angle in relation to the primary vein. This delineation is illustrated in the constructed dichotomous botanical key. Utilizing leaf architecture characters is an effective technique to characterize, identify and classify closely related taxa possessing confusing characters.

Keywords: Areole development, blade class, marginal ultimate venation, taxonomic tool, venation patterns

INTRODUCTION

Field gene banks or repositories house millions or billions of plant seeds for the purpose of gene pool conservation. Researchers use the gene bank accessions for continued in depth study of the nature of the material. Farmers make use of the gene bank to get propagules for planting in their farms. All of these activities are aimed at sustaining genetic diversity. There are usually huge numbers of accessions that are confusing and unidentified whether they are species of similar taxon or not, clogging these repositories. Replanting them without identifying first whether they are of similar species, will just be a waste of time and resources. Therefore, to better organize and hence, reduce cost of maintenance, there is a need to properly identify, characterize and classify accessions. After all, many of them are just duplicates and redundancies, adding an expensive cost to management and maintenance operations.

The gene bank of Hortanova Farm and Research Center, East-west Seed Company Inc., in Lipa City, Batangas, Philippines, currently houses several accessions of important economic crops, mostly leading vegetables not only in Asia but of the whole world. One of these is the genus *Cucumis* (Cucurbitaceae) collected from many countries across continents. Several species of *Cucumis* such as *C. melo* are well-known cultivated plants due to its obvious importance as food both the rich, the middle class and the poor in society. Reproductive structures are commonly used in identifying plant species but unfortunately, they are not present throughout the year, thus studying them is a challenge (da Silva et al. 2015).

A number of studies though, had reported that there is another alternative in identifying and classifying plants even in the absence of the reproductive structures (Buot 2020). Incidentally, taxonomists and systematists often neglect the leaf surface characters and other vegetative characters in the identification and classification of plant taxa. This could possibly be due to the prevailing view that these vegetative characters have high phenotypic plasticity, and hence, character states may not be that stable and reliable for taxonomic purposes. Critiques assert that use of the plastic characters could be leading to more serious misidentification consequences (Conda and Buot 2017). These claims and criticisms are true anyway to a certain extent since shapes and size often vary with varying agro climatic conditions. Nevertheless, according to Roth-Nebelsick et al. (2001), some leaf surface characters beyond the usual leaf shape and size are worth consideration. And these are the venation patterns more leaf architectural pattern which is a morphological character, is genetically fixed. Numerous studies had been following and testing the initial results of Roth-Nebelsick et al. (2001), and all reported the great potential of leaf architecture as a tool in addressing taxonomic problems (Baroga and Buot 2014; Pulan and Buot 2014; Salvaña and Buot 2014; Villareal and Buot 2015; Jumawan and Buot 2016; Conda et al. 2017; Torrefiel and Buot 2017; Conda and Buot 2018; Huiet et al. 2018; Tan and Buot 2018; Baltazar and Buot 2019b and 2019c; Masungsong et al. 2019a and 2019b; Paguntalan and Buot 2019; Averion-Masungsong and Buot 2020). Results of these studies claimed that leaf architecture can be very relevant in solving the identity of taxonomically confusing taxon, in the absence of reproductive structures which are quite seasonal. In fact, one study on the phylogeny of *Adiantum* species (Huiet et al. 2018) found out that the phylogenetic tree generated from leaf architectural data, perfectly matched with the phylogenetic tree generated from molecular data, including the identification of biogeographic provinces of the species of *Adiantum* in the study.

Hence, leaf architecture can be used in studying the identity concerns among *Cucumis* accessions at the gene bank of the Hortanova Farm and Research Center, East-West Seed Company Inc. Around seventeen (17) species of *Cucumis* each with numerous accessions are available at the said gene bank. *Cucumis* has been one of the priorities at the Center being part of the everyday meal plan of many families (Montaño et al. 2016). This study aims to elucidate the leaf architecture of the 57 accessions of *Cucumis* early on before the flowering and fruiting stages. Some of the leaf architecture studies conducted for the genus *Cucumis* are that of Rao and Rao (2015), Masungsong et al. (2019a), Masungsong et al. (2019b), and Averion-Masungsong & Buot (2020). These past investigations proved that leaf architecture is an effective taxonomic tool in classifying and identifying species and accessions of genus *Cucumis*.

The current study aims to identify which among the selected 57 *Cucumis* accessions belong to the same species using leaf architecture pattern. The diversity of leaf architectural characters had been investigated, assessed and succinctly described. A dichotomous botanical key using leaf architectural character states observed and recorded for *Cucumis* was then constructed. We envision that this work will contribute to the taxonomic identification of the extensive *Cucumis* accessions at the gene bank, and hence, may streamline management and operational protocols.

MATERIALAS AND METHODS

Collection of samples

Five hundred thirteen (513) leaf samples from 57 *Cucumis* species accessions were collected from the Screenhouse of Genetic Resource Division, Hortanova Farm and Research Center, East-west Seed Company Inc., Lipa City, Batangas, Philippines. Herbarium specimens of the *Cucumis* accessions were prepared and deposited at the Plant Biology Division Herbarium (PBDH), Institute of Biological Sciences, University of the Philippines Los Baños.

Table 1. Leaf and venation characters used in the study

Leaf characters	Venation characters
Blade class	Primary vein size
Laminar shape	Primary vein category
Laminar symmetry	Primary vein course
Length-width ratio	Secondary vein category
Apex shape	Secondary vein spacing
Apex angle	Secondary vein angle
Base shape	Tertiary vein category
Base angle	Tertiary vein angle in relation to primary vein
Margin	Quaternary vein category
Tooth apex	Marginal ultimate venation
Lobation	Areole development

Leaf architecture examination

A total of five hundred thirteen leaf samples were pressed, dried, and examined. Measurements were done using a 12" ruler (Orion), 150mm caliper (Mitutoyo), and a protractor (Orion #30 & #40). The leaf architecture pattern of the leaf samples were described based on Hickey's (1973), LAWG's (1999) and Ellis' et al. (2009) leaf architectural characteristics and descriptors. In reference to similar studies (Masungsong et al. 2019a; Masungsong et al. 2019b; Averion-Masungsong & Buot 2020), eleven (11) general leaf and venation characters (Table 1) were used in examining the collected leaf samples. This was done to have a uniform characterization for all the *Cucumis* species and accessions studied. For the measured leaf characters such as blade class, primary vein size, apex and base angle, the descriptive categories were adopted from Webb (1995), Hickey's (1973) and LAWG (1999), respectively. Using Adobe Photoshop CS6 v.13.1.2, leaf architecture characters were sketched and line drawings were done based from Nikon D3100 (Tamron 90mm f/2.8 SP AF Di Macro 1: 1 lens) captured photos. With the leaf characters gathered, tables of taxonomic characters were made to which a dichotomous key for the *Cucumis* species and accessions was constructed.

RESULTS AND DISCUSSION

Leaf architectural pattern of Cucumis accessions in the study

The five hundred thirteen (513) collected leaves from the fifty-seven (57) accessions (Table 2) of the *Cucumis* species exhibited great resemblances in terms of leaf morphological features such as laminar shape, laminar symmetry, apex shape, base shape, leaf margin, tooth apex and lobation (Table 3). These *Cucumis* accessions can be grouped into six (6) species namely, *C. melo* subsp. *agrestis*, *C. melo* var. *texanus*, *C. melo* var. *flexuosus*, *C. zambianus*, *C. sativus*, and *C. sativus* var. *hardwickii* which generally have orbiculate laminar shape, symmetrical laminar symmetry, convex apex shape, lobate base shape, serrated leaf margins, spinose tooth apex and palmately lobed lobation. It was also observed that these species differed in terms of blade class and apex angle. These

Cucumis species and accessions examined in this study can be grouped into two categories based on blade class. *Cucumis zambianus*, *C. melo* var. *flexuosus*, *C. sativus* var. *hardwickii* and *C. melo* subsp. *agrestis* have mesophyll to macrophyll blade class while *C. sativus* and C. *melo* var. *texanus* have mesophyll blade class. In terms of apex angle, they can be grouped into four; *C. melo* var. *flexuosus* has an obtuse apex angle, C. *melo* var. *texanus* have an obtuse to odd lobed obtuse apex angle, *C. sativus* and *C. sativus* var *hardwickii* have odd lobed acute apex angle while *C. melo* subsp. *agrestis* and *C. zambianus* have odd lobed obtuse apex angle.

The *Cucumis* species and accessions also exhibited great similarities in terms of leaf venation patterns (Table 4). Generally, they have actinodromous suprabasal primary vein, weak primary vein size, straight branched primary vein course, craspedodromous secondary veins, two-pair acute basal secondaries secondary vein angle, alternate percurrent tertiary veins, regular polygonal reticulate quaternary vein, and looped marginal ultimate venation. Observed differences were in their secondary vein spacing, tertiary vein angle with respect to primary vein category and areolation in which these 6 *Cucumis* species and accessions can be grouped into two: *C. melo* var. *flexuosus*, *C. melo* var. *texanus* and *C. melo* subsp. *agrestis* have an increasing towards the base secondary vein spacing while the remaining species and accessions have an irregular secondary vein spacing. In terms of their tertiary vein angle with respect to primary vein, *C. sativus* and *C. sativus* var. *hardwickii* have an obtuse tertiary vein angle with respect to primary while the rest of the *Cucumis* species and accessions have an acute tertiary vein angle with respect to primary vein category. For the areole development, *C. zambianus* differ from the rest of the species and accessions for having a moderately developed areolation while others have well-developed.

Notes and leaf architectural descriptions of classified *Cucumis* species/subspecies accessions

The 57 accessions of *Cucumis* were found to be lumped into 6 species/subspecies based on leaf architecture patterns. *Cucumis melo* subsp. *agrestis* (Naudin) Pangalo (GB-00466, GB-00472, GB-00478, GB-00479, GB-00486, GB-00487, GB-00498, GB-00503, GB-00510) and *Cucumis sativus* var. *hardwickii* (Royle) Gabaev (GB-00577, GB-00578, GB-00579, GB-00580, GB-00581, GB-00582, GB-00584, GB-00585, GB-00586) are composed of nine (9) accessions each. On the other hand *Cucumis melo* var. *texanus* Naudin (GB-00400, GB-00609, GB-00610, GB-00611, GB-00612, GB-00613, GB-00614, GB-00615, GB-00616, GB-00617), *Cucumis zambianus* Widrl, J.H.Kirkbr., Ghebret. & K.R.Reitsma (GB-00652, GB-00653, GB-00654, GB-00655, GB-00656, GB-00657, GB-00658, GB-00659, GB-00660, GB-00661) and *Cucumis sativus* L. (GB-00024, GB-00027, GB-00514, GB-00029, GB-00665, GB-00737, GB-00738, GB-00739, GB-00034 and GB-00740) are comprised of ten (10) accessions each. Meanwhile, *Cucumis melo* var. *flexuosus* (L.) Naudin (GB-00596, GB-00599, GB-00600, GB-00601, GB-00602, GB-00603, GB-00604, GB-00605) includes eight (8) accessions.

Cucumis melo subsp. *agrestis* (Naudin) Pangalo (Figure 1)

PHILIPPINES • Luzon Island, Municipality of Lipa, Batangas City, Hortanova Farm and Research Center East-West Seed Company Inc., elevation 280m asl, 14 03 2020, *Exsicc.*-Masungsong and Alcala 7469 (PBDH), Masungsong and Alcala 7466 (PBDH), Masungsong and Alcala 7463 (PBDH).

Lamina orbiculate with convex apex, obtuse to odd lobed obtuse apex angle, lobate base, wide obtuse base angle and spinose tooth apex, symmetrical, mesophyll to macrophyll with 0.99 to 1.10 length to width ratio, serrated margin, and unlobed to palmately lobed. **Venation** actinodromous suprabasal; **primary vein** straight branched and weak; **secondary vein** craspedodromous with increasing towards the base spacing and two pair acute basal secondaries angle; **tertiary vein** alternate percurrent and angle with respect to primary acute; **quaternary vein** regular polygonal reticulate; **marginal ultimate venation** looped; **areoles** well developed**.**

This includes accessions GB-00466, GB-00472, GB-00478, GB-00479, GB-00486, GB-00487, GB-00498, GB-00503 and GB-00510 of the Hortanova Farm and Research Center East-West seed Company, Inc. Genetic Resource Center.

Cucumis melo var. *texanus* Naudin (Figure 2)

PHILIPPINES • Luzon Island, Municipality of Lipa, Batangas City, Hortanova Farm and Research Center East-West Seed Company Inc., elevation 280m asl, 14 03 2020, *Exsicc.*-Masungsong and Alcala 7480 (PBDH), Masungsong and Alcala 7483 (PBDH), Masungsong and Alcala 7485 (PBDH).

Lamina orbiculate with convex apex, obtuse to odd lobed obtuse apex angle, lobate base, wide obtuse base angle and spinose tooth apex, symmetrical, mesophyll with 0.97 to 1.03 length to width ratio, serrated, and palmately lobed. **Venation** actinodromous suprabasal, straight branched and weak **primary vein**; **secondary vein** craspedodromous with increasing towards the base spacing and two pair acute basal secondaries angle; **tertiary vein** alternate percurrent and angle with respect to primary acute; **quaternary vein** regular polygonal reticulate; **marginal ultimate venation** looped; **areoles** well developed.

This includes accessions GB-00400, GB-00609, GB-00610, GB-00611, GB-00612, GB-00613, GB-00614, GB-00615, GB-00616 and GB-00617 of the Hortanova Farm and Research Center East-West seed Company, Inc. Genetic Resource Center.

Table 2. List of *Cucumi*s species and accessions used in this study

Name	Accession name	Exssicata	Gb_source	Origin	Gb_species	Type
GB-00466	140858 (Songwhan Charmi)	7461	CGN, Netherlands	Southern Province, Southern Zambia	*C. melo* subsp. *agrestis*	Wild
GB-00472	140865 (Daejon Charmi)	7462	CGN, Netherlands	Ngamiland Province, Botswana	*C. melo* subsp. *agrestis*	Wild
GB-00477	140871 (LJ 90234)	7463	CGN, Netherlands	Naaldivijk, The Netherlands	*C. melo* subsp. *agrestis*	Wild
GB-00478	140872 (Freeman Cucumber)	7464	CGN, Netherlands	Kenya	*C. melo* subsp. *agrestis*	Wild
GB-00479	140873 (Ginsen Makuwa (Silver Spring)	7465	CGN, Netherlands	Tamaulipas, Mexico	*C. melo* subsp. *agrestis*	Wild
GB-00486	140883 (Snow White Honey`	7466	CGN, Netherlands	Greece	*C. melo* subsp. *agrestis*	Wild
GB-00487	140889 (Teti)	7467	CGN, Netherlands	Maine, United States	*C. melo* subsp. *agrestis*	Wild
GB-00498	CGN24624 (Local)	7468	CGN, Netherlands	Asyut, Egypt	*C. melo* subsp. *agrestis*	Wild
GB-00503	CGN24636 (IPGRI 199-8)	7469	CGN, Netherlands	Rajasthan, India	*C. melo* subsp. *agrestis*	Wild
GB-00510	CGN24657 (Kankur)	7470	CGN, Netherlands	Louisiana, United States	*C. melo* subsp. *agrestis*	Wild
GB-00596	Ames 21185 (010689-0105)	7471	USDA	Turkey, Malatya, Central market store in Mala	*C. melo* var. *flexuosus*	Wild
GB-00599	PI 525135 (Katta)	7472	USDA	Egypt, Asyut, Seed shop, Asyut	*C. melo* var. *flexuosus*	Wild
GB-00600	PI 525139 (174)	7473	USDA	Egypt, Minufiya, Seed shop, Shebin el Koum	*C. melo* var. *flexuosus*	Wild
GB-00601	PI 525140 (184)	7474	USDA	Egypt, Daqahliya, Seed shop, El Mansura	*C. melo* var. *flexuosus*	Wild
GB-00602	PI 525143 (Shamey)	7475	USDA	Egypt, Giza, Seed shop, Almohtamadeya	*C. melo* var. *flexuosus*	Wild
GB-00603	PI 614270 (USM 112)	7476	USDA	India, Rajasthan, Near Cchhappankaddu	*C. melo* var. *flexuosus*	Wild
GB-00604	PI 614271 (USM 117)	7477	USDA	India, Rajasthan, Near Suratgarh, Ganganagar	*C. melo* var. *flexuosus*	Wild
GB-00605	PI 614474 (KSM 442)	7478	USDA	India, Madhya Pradesh, Near Chhatarpur, Chhat	*C. melo* var. f*lexuosus*	Wild
GB-00400	Ames 26729 (DSW 1350)	7479	USDA	Texas, United States	*C. melo* var *texanus*	Wild
GB-00609	Ames 26695 (DSW 1230)	7480	USDA	Mexico	*C. melo* var *texanus*	Wild
GB-00610	Ames 26696 (DSW 1298)	7481	USDA	United States, Louisiana, St. John the Baptis	*C. melo* var *texanus*	Wild
GB-00611	Ames 26697 (DSW 1300)	7482	USDA	United States, Louisiana, St. James .	*C. melo* var *texanus*	Wild
GB-00612	Ames 26710 (DSW 1325)	7483	USDA	United States, Louisiana, Tensas	*C. melo* var *texanus*	Wild
GB-00613	Ames 26711 (DSW 1326)	7484	USDA	United States, Louisiana, Tensas	*C. melo* var *texanus*	Wild
GB-00614	Ames 26712 (DSW 1327)	7485	USDA	Mississippi, United States	*C. melo* var *texanus*	Wild
GB-00615	Ames 26713 (DSW 1328)	7486	USDA	United States, Mississippi, Claiborne	*C. melo* var *texanus*	Wild
GB-00616	Ames 26733 (DSW 1354)	7487	USDA	United States, Mississippi, Oktibbeha	*C. melo* var *texanus*	Wild
GB-00617	Ames 26739 (DSw 1363)	7488	USDA	United States, Texas, Jefferson	*C. melo* var *texanus*	Wild
GB-00652	PI 500416 (ZM 2442)	7508	USDA	Copperbelt, Zambia	*C. zambianus*	Wild
GB-00653	PI 500418 (ZM 2459)	7509	USDA	Copperbelt, Zambia	*C. zambianus*	Wild
GB-00654	PI 500420 (ZM 2468)	7510	USDA	Copperbelt, Zambia	*C. zambianus*	Wild
GB-00655	PI 500437 (ZM 2834)	7511	USDA	Luapula, Zambia	*C. zambianus*	Wild
GB-00656	PI 505597 (Katanda)	7512	USDA	North-Western, Zambia	*C. zambianus*	Wild
GB-00657	PI 505605 (ZM/A 5030)	7513	USDA	North-Western, Zambia	*C. zambianus*	Wild
GB-00658	PI 505606 (Katanda)	7514	USDA	North-Western, Zambia	*C. zambianus*	Wild
GB-00659	PI 505607 (ZM/A 5086)	7515	USDA	Zambia	*C. zambianus*	Wild
GB-00660	PI 505608 (Katanda)	7516	USDA	North-Western, Zambia	*C. zambianus*	Wild
GB-00661	PI 505609 (ZM/A 5105)	7517	USDA	North-Western, Zambia	*C. zambianus*	Wild
GB-00577	Ziya Dharmshala HP	7499	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00578	Chakkar mandi HP	7500	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00579	Arki 1 solan HP	7501	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00580	Sunder nagar HP	7502	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00581	Kothi Bilaspur HP	7503	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00582	Riwalsar Mandi	7504	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00584	ID-HW-00003 Hardiwickii	7505	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00585	Solan 1, HP	7506	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00586	Gaghas 1 Bilaspur HP	7507	EWIN	India	*C. sativus* var. *hardwickii*	Wild
GB-00024	OP-Green Long	7489	EWTH	India	*C. sativus*	Cult.
GB-00027	OP-Polly seed India	7490	Polly Seed Co.	India	*C. sativus*	Cult.
GB-00514	PI 167052 (Salatalik)	7491	USDA	Turkey	*C. sativus*	Cult.
GB-00029	[OP-Gr India]	7492	EWTH	India	*C. sativus*	Cult.
GB-00665	AVCU 1202 (00WGH22-1GrSA x Am	7493	AVRDC	Taiwan	*C. sativus*	Cult.
GB-00737	KE 36138 F	7494	EWINDO	Indonesia	*C. sativus*	Cult.
GB-00738	KE 36138 M	7495	EWINDO	Indonesia	*C. sativus*	Cult.
GB-00739	KE 36761 F	7496	EWINDO	Indonesia	*C. sativus*	Cult.
GB-00740	KE 36761 M	7497	EWINDO	Indonesia	*C. sativus*	Cult.
GB-00034	OP-White large	7498	EWTH	India	*C. sativus*	Cult.

Note: Cult.: cultivated

Table 3. General leaf architecture characters of selected accessions in six *Cucumis* species

Species	Leaf organization	Laminar shape	Laminar symmetry	L:W Ratio	Blade class	Apex shape	Apex angle	Base shape	Base angle	Margin	Tooth apex	Loba Tion
C. melo subsp. *agrestis*	simple	orbiculate	symmetrical	0.99-1.10	Mesophyll to macrophyll	convex	Odd lobed obtuse	lobate	Wide obtuse	serrate	spinose	Palmately lobed
C. melo var. *texanus*	simple	orbiculate	symmetrical	0.97-1.03	mesophyll	convex	Obtuse to odd lobed obtuse	lobate	Wide obtuse	serrate	spinose	Palmately lobed
C. melo var. *flexuosus*	simple	orbiculate	symmetrical	0.99-1.06	Mesophyll to macrophyll	convex	obtuse	lobate	Wide obtuse	serrate	spinose	Palmately lobed
C. zambianus	simple	orbiculate	symmetrical	0.92-1.03	Mesophyll to macrophyll	convex	Odd lobed obtuse	lobate	Wide obtuse	serrate	spinose	Palmately lobed
C. sativus	simple	orbiculate	symmetrical	0.95-1.17	mesophyll	convex	Odd lobed acute	lobate	Wide obtuse	serrate	spinose	Palmately lobed
C. sativus var. *hardwickii*	simple	orbiculate	symmetrical	1.03-1.12	Mesophyll to macrophyll	convex	Odd lobed acute	lobate	Wide obtuse	serrate	spinose	Palmately lobed

Table 4. Detailed leaf architecture venation characters of selected accessions in six *Cucumis* species

Species and accessions	Primary vein (1°)			Secondary vein (2°)			Tertiary vein (3°)		Quaternary vein category (4°)	Marginal ultimate venation	Areole development
	Category	Size	Course	Category	Spacing	Angle category	Category	Angle to 1°			
C. melo subsp. *agrestis*	Actinodromous suprabasal	weak	Straight branched	Craspedodromous	Increasing towards the base	Two pair acute basal secondaries	Alternate percurrent	acute	Regular polygonal reticulate	Looped	Well developed
C. melo var. *texanus*	Actinodromous suprabasal	Weak	Straight branched	Craspedodromous	Increasing towards the base	Two pair acute basal secondaries	Alternate percurrent	acute	Regular polygonal reticulate	Looped	Well developed
C. melo var. *flexuosus*	Actinodromous suprabasal	Weak	Straight branched	Craspedodromous	Increasing towards the base	Two pair acute basal secondaries	Alternate percurrent	acute	Regular polygonal reticulate	Looped	Well developed
C. zambianus	Actinodromous suprabasal	Weak	Straight branched	Craspedodromous	Irregular spacing	Two pair acute basal secondaries	Alternate percurrent	acute	Regular polygonal reticulate	Looped	Moderately developed
C. sativus	Actinodromous suprabasal	Weak	Straight branched	Craspedodromous	Irregular spacing	Two pair acute basal secondaries	Alternate percurrent	Obtuse	Regular polygonal reticulate	Looped	Well developed
C. sativus var. *hardwickii*	Actinodromous suprabasal	Weak	Straight branched	Craspedodromous	Irregular spacing	Two pair acute basal secondaries	Alternate percurrent	Obtuse	Regular polygonal reticulate	Looped	Well developed

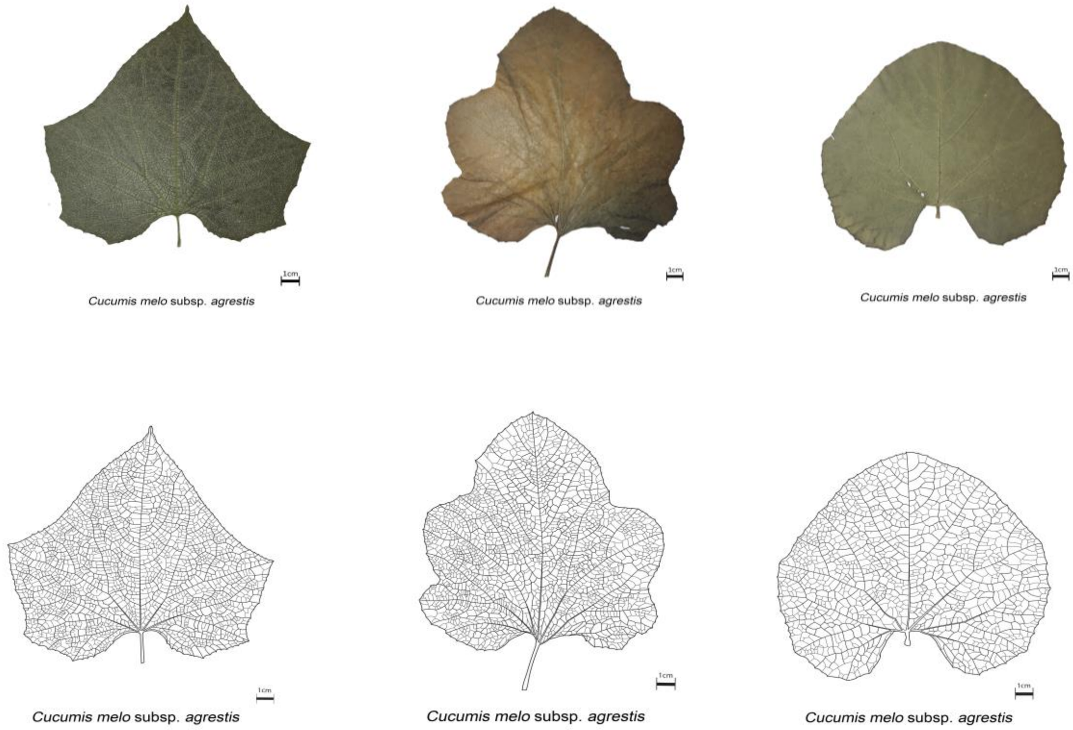

Figure 1. Leaf samples and venation patterns of *Cucumis melo* subsp. *agrestis*

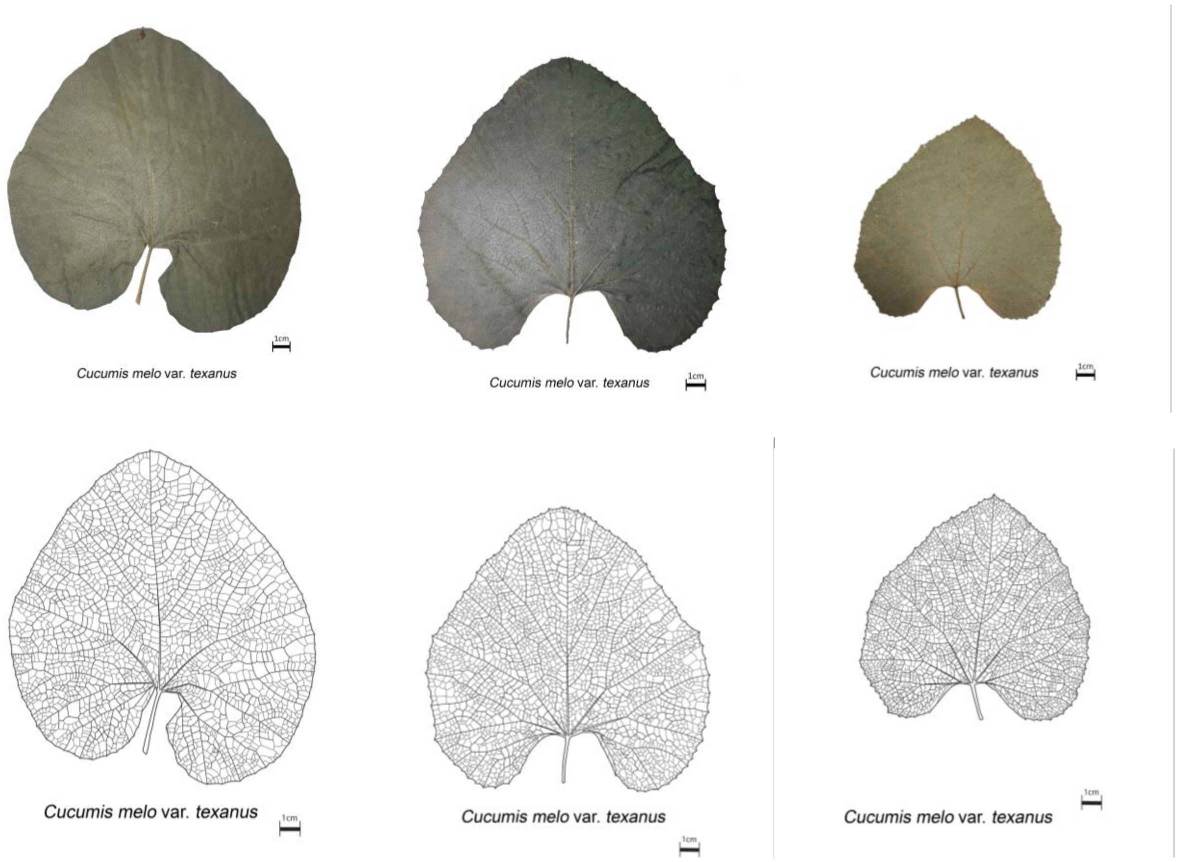

Figure 2. Leaf samples and venation patterns of *Cucumis melo* var. *texanus*

***Cucumis melo* var. *flexuosus* (L.) Naudin (Figure 3)**

PHILIPPINES • Luzon Island, Municipality of Lipa, Batangas City, Hortanova Farm and Research Center East-West Seed Company Inc., elevation 280m asl, 14 03 2020, *Exsicc.*-Masungsong and Alcala 7472 (PBDH), Masungsong and Alcala 7477 (PBDH), Masungsong and Alcala 7471 (PBDH)

Lamina orbiculate with convex apex, obtuse apex angle, lobate base, wide obtuse base angle and spinose tooth apex, symmetrical, mesophyll to macrophyll with 0.99 to 1.06 length to width ratio, serrated, and palmately lobed. **Venation** actinodromous suprabasal, straight branched and weak **primary vein**; **secondary vein** craspedodromous with increasing towards the base spacing and two pair acute basal secondaries angle; **tertiary vein** alternate percurrent and angle with respect to primary acute; **quaternary vein** regular polygonal reticulate; **marginal ultimate venation** looped; **areoles** well developed.

This includes accessions GB-00596, GB-00599, GB-00600, GB-00601, GB-00602, GB-00603, GB-00604 and GB-00605 of the Hortanova Farm and Research Center East-West seed Company, Inc. Genetic Resource Center.

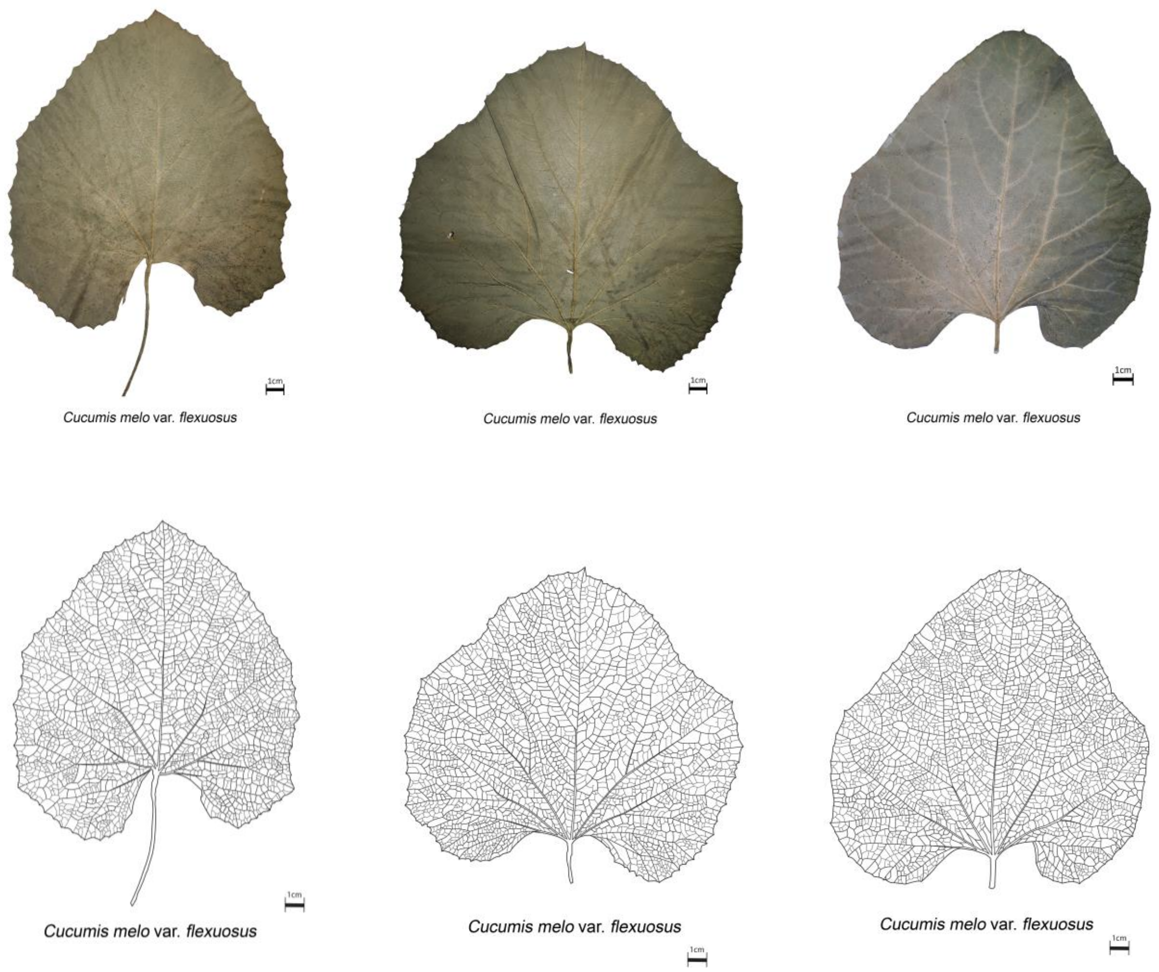

Figure 3. Leaf samples and venation patterns of *Cucumis melo* var. *flexuosus*

***Cucumis zambianus* Widrl., J.H.Kirkbr., Ghebret. & K.R.Reitsma (Figure 4)**

PHILIPPINES • Luzon Island, Municipality of Lipa, Batangas City, Hortanova Farm and Research Center East-West Seed Company Inc., elevation 280m asl, 14 03 2020, *Exsicc.*-Masungsong and Alcala 7515 (PBDH), Masungsong and Alcala 7510 (PBDH), Masungsong and Alcala 7508 (PBDH)

Lamina orbiculate with convex apex, odd lobed obtuse apex angle, lobate base, wide obtuse base angle and spinose tooth apex, symmetrical, mesophyll to macrophyll with 0.92 to 1.03 length to width ratio, serrated, and palmately lobed. **Venation** actinodromous suprabasal, straight branched and weak **primary vein**; **secondary vein** craspedodromous with irregular vein spacing and two pair acute basal secondaries angle; **tertiary vein** alternate percurrent and angle with respect to primary acute; **quaternary vein** regular polygonal reticulate; **marginal ultimate venation** looped; **areoles** moderately developed.

This includes accessions GB-00652, GB-00653, GB-00654, GB-00655, GB-00656, GB-00657, GB-00658, GB-00659, GB-00660 and GB-00661 of the Hortanova Farm and Research Center East-West seed Company, Inc. Genetic Resource Center.

***Cucumis sativus* L. (Figure 5)**

PHILIPPINES • Luzon Island, Municipality of Lipa, Batangas City, Hortanova Farm and Research Center East-West Seed Company Inc., elevation 280m asl, 14 03 2020, *Exsicc.*-Masungsong and Alcala 7491 (PBDH), Masungsong and Alcala 7497 (PBDH), Masungsong and Alcala 7495 (PBDH)

Lamina orbiculate with convex apex, odd lobed acute to odd lobed obtuse apex angle, lobate base, wide obtuse base angle and spinose tooth apex, symmetrical, mesophyll with 0.95 to 1.17 length to width ratio, serrated, and palmately lobed. **Venation** actinodromous suprabasal, straight branched and weak **primary vein**; **secondary vein** craspedodromous with irregular vein spacing and two pair acute basal secondaries angle; **tertiary vein** alternate percurrent and angle with respect to primary obtuse; **quaternary vein** regular polygonal reticulate; **marginal ultimate venation** looped; **areoles** well developed.

This includes accessions GB-00024, GB-00027, GB-00514, GB-00029, GB-00665, GB-00737, GB-00738, GB-00739, GB-00034 and GB-00740 of the Hortanova Farm and Research Center East-West seed Company, Inc. Genetic Resource Center.

***Cucumis sativus* var. *hardwickii* (Royle) Gabaev (Figure 6)**

PHILIPPINES • Luzon Island, Municipality of Lipa, Batangas City, Hortanova Farm and Research Center East-West Seed Company Inc., elevation 280m asl, 14 03 2020, *Exsicc.*-Masungsong and Alcala 7501 (PBDH), Masungsong and Alcala 7504 (PBDH), Masungsong and Alcala 7507 (PBDH)

Lamina orbiculate with convex apex, odd lobed acute to odd lobed obtuse apex angle, lobate base, wide obtuse base angle and spinose tooth apex, symmetrical, mesophyll to macrophyll with 1.03 to 1.12 length to width ratio, serrated, and palmately lobed. **Venation** actinodromous suprabasal, straight branched and weak **primary vein**; **secondary vein** craspedodromous with irregular vein spacing and two pair acute basal secondaries angle; **tertiary vein** alternate percurrent and angle with respect to primary obtuse; **quaternary vein** regular polygonal reticulate; **marginal ultimate venation** looped; **areoles** well developed.

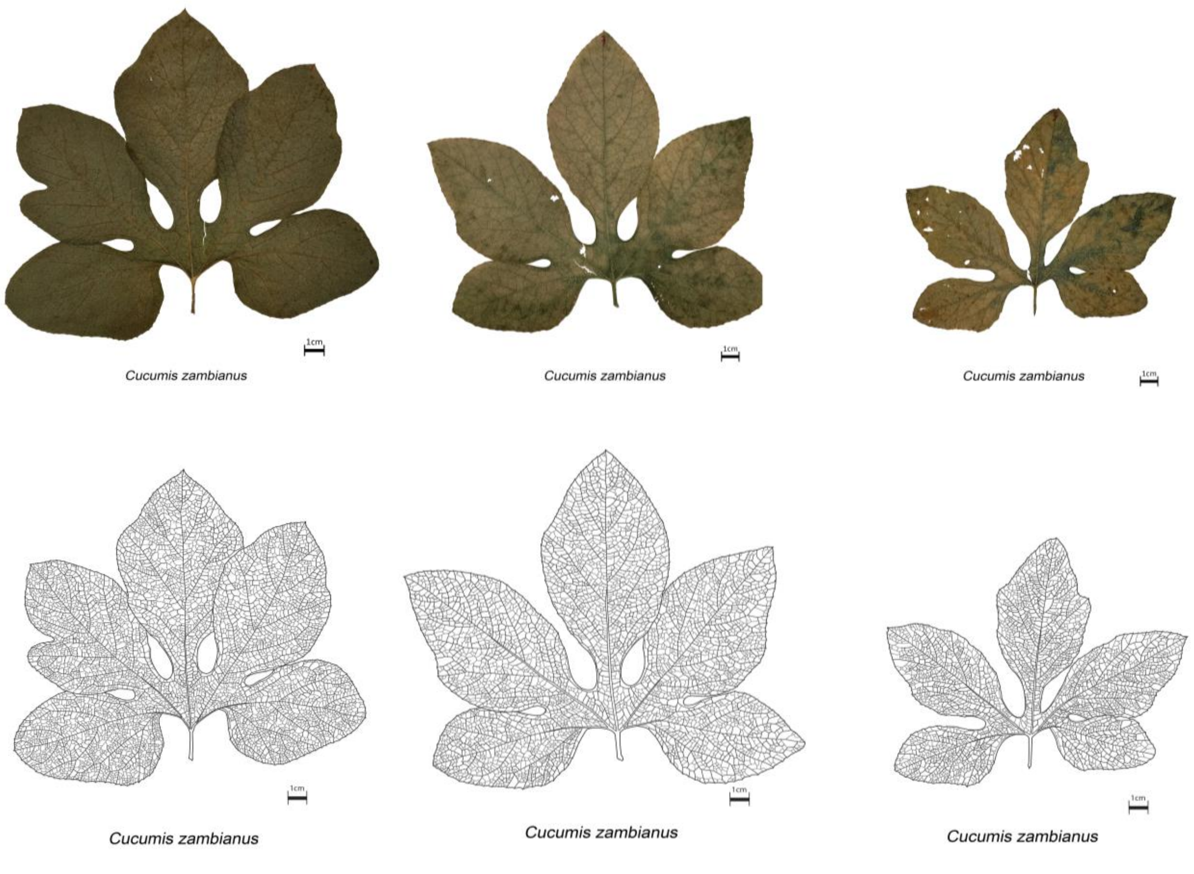

Figure 4. Leaf samples and venation patterns of *Cucumis zambianus*

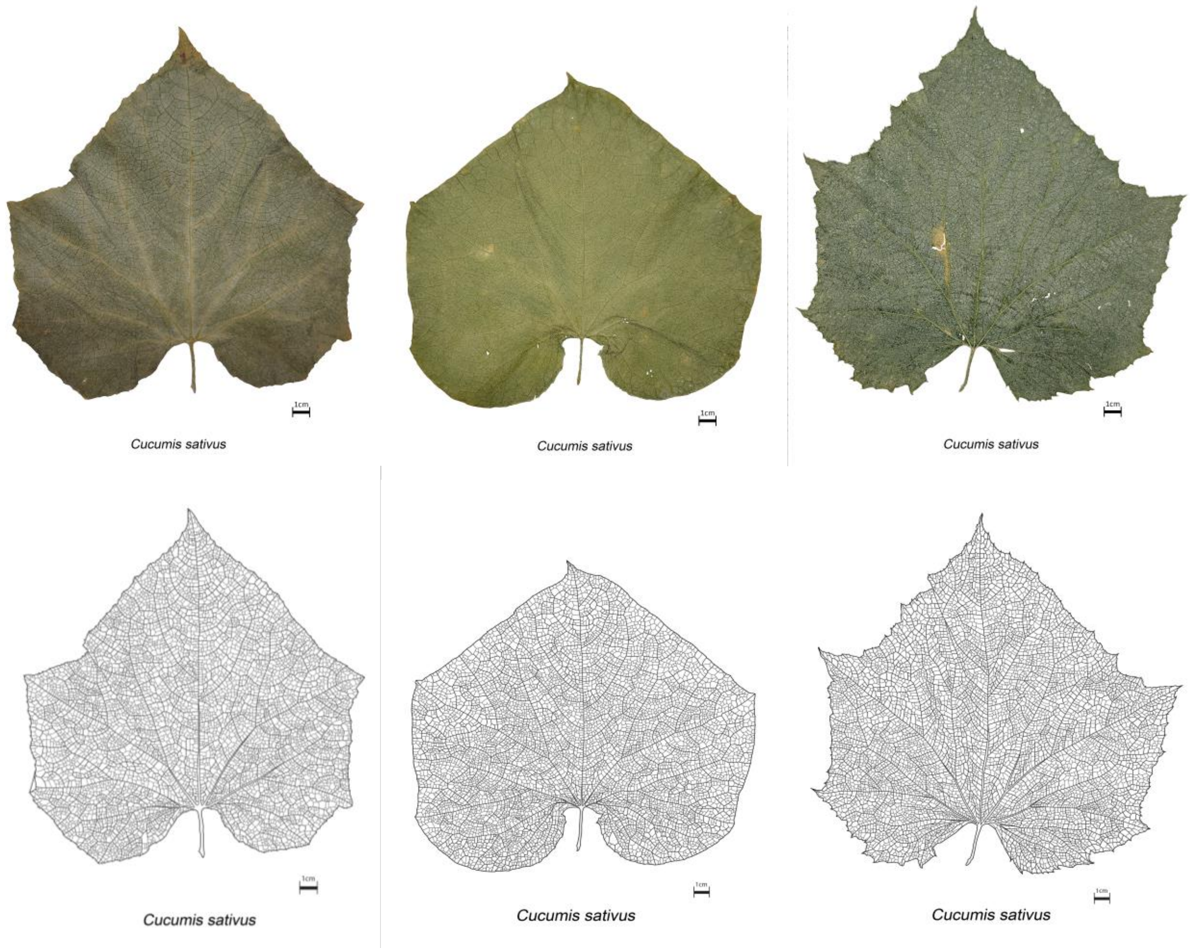

Figure 5. Leaf samples and venation patterns of *Cucumis sativus*

This includes accessions GB-00577, GB-00578, GB-00579, GB-00580, GB-00581, GB-00582, GB-00584, GB-00585, and GB-00586 of the Hortanova Farm and Research Center East-West seed Company, Inc. Genetic Resource Center.

It was observed that the 57 accessions of the 6 species of *Cucumis* examined in this study vary in terms of blade class and apex angle. In the study of Masungsong et al. (2019a), blade class delineated *C. anguria* from *C. anguria* var. *longaculeatus*. However, two accessions of this species have different blade class which caused them to cluster with *C. anguria* var. *longaculeatus.* In some leaf architecture studies, blade class was also used in delineating species in a particular genus and infraspecific taxa (Baroga and Buot 2014; Kpadehyea and Buot 2014; Tan and Buot 2018; Antonio and Buot 2021). In other economically important crops, like *Glycine max*, blade class was used as a tool in the identification of all accession of this species. In the study of Nandyal et al. 2013, base and apex angle were used as an important basis for their proposed method of medicinal plant classification and identification.

There were 4 character states described for apex angle namely odd lobed obtuse, obtuse to odd lobe obtuse, obtuse, and odd lobed acute which separated the 6 species and its accessions examined in this study. Diversity of apex angle character state was also observed by Masungsong et al. (2019a & 2019b) in other *Cucumis* species and there is agreement on the result of this study. The degree of lobation observed in *Cucumis* leaves can be represented by apex angle of odd lobed leaves and leaf surface area. These characters, thus, are associated to leaf shape. In addition, leaf measurements like apex angle are part of numerical taxonomic approaches used to describe species. The numerical values are represented by qualitative characters. Numerical and measured characters are more or less definite which supports the use of apex angle as an important taxonomic character (Nandyal et al. 2013). In the study of Viacrucis and Buot (2021), apex angle served as one of the unifying leaf characters for the examined *Syzygium* species.

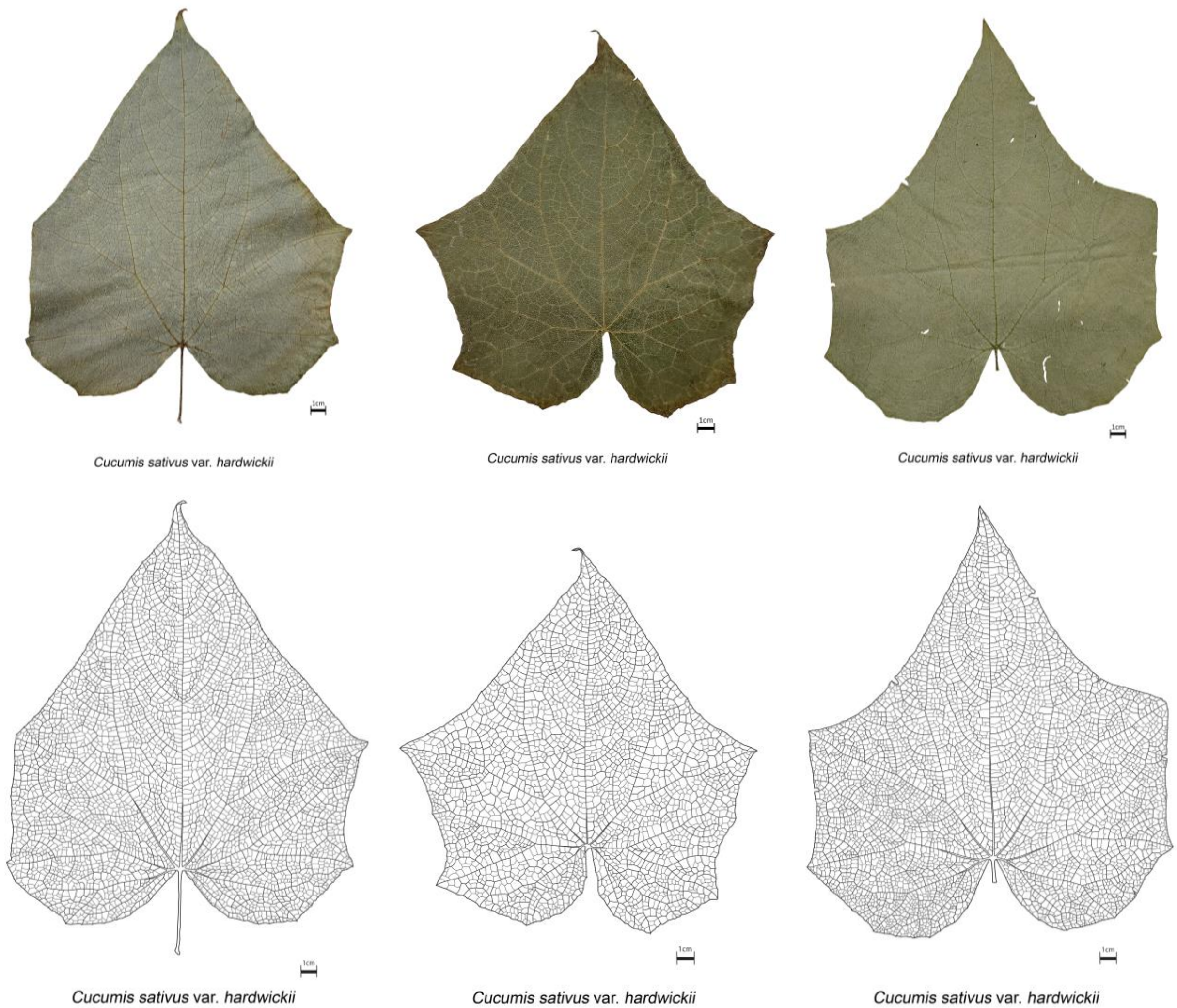

Figure 6. Leaf samples and venation patterns of *Cucumis sativus* var. *hardwickii*

Secondary vein spacing also differed among species and accessions examined. There were two character states observed; increasing towards the base and irregular spacing. These two character states are also observed in other *Cucumis* species (Masungsong et al. 2019a). Apex angle and secondary vein spacing are both linked to lobation. Species with unlobed leaves have higher surface area than the palmately lobed species. Lobations in the leaves are essential to discriminate specific vein patterns and can display accurate homologous and/or ancestral relationships (Yang et al. 2022).

Acute and obtuse tertiary vein angle with respect to primary vein were observed in examined species and accessions. This character had delineated *Cucumis metuliferus* from *C. myriocarpus* (Masungsong et al. 2019a). Differences in the leaves of intra-and interspecific taxa can be seen in higher vein orders like tertiary veins (Nelson and Dengler 1997). Areole development also differ among species and accessions examined in this study exhibiting a moderately developed to well-developed areoles which were also seen in the study of Masungsong et al. (2019b). Sack and Scoffoni (2013) stated that high number of areoles per unit leaf area can be correlated with high vein length per unit area, and may provide the advantages of having high gas exchange rates across closely related species.

Dichotomous key to the resulting 6 species/subspecies of the 57 accessions of *Cucumis* based on leaf architecture

A dichotomous key, using leaf architectural traits, was constructed based on the descriptions for each species and accessions examined. Based on the descriptions made together with the key, it is apparent that leaf architecture, especially venation characters, is a very useful marker in describing *Cucumis*. Similar findings were reported by Celadiña et al. (2012) in Philippine *Cinnamomum* species, Salvaña and Buot (2014) in Hoya species and Pulan and Buot, (2014) in Philippine *Shorea* species. Moreover, leaf architecture was also used by Conda and Buot (2017) in describing selected *Diplazium* species where they were able to produce an Illustrated Manual of Leaf Architecture of Pteridophytes based on the descriptions they have generated.

1. Leaf simple, palmately lobed, with mesophyll blade class …2
1. Leaf simple, palmately lobed, with mesophyll to macrophyll blade class ……………………………………………………… 3
   2. Straight 2° vein course with increasing towards the base 2° vein spacing …………….... *Cucumis melo* var. *texanus* (GB-00400, GB-00609, GB-00610, GB-00611, GB-00612, GB-00613, GB-00614, GB-00615, GB-00616, GB-00617)
   2. Straight 2° vein course with irregular 2° vein spacing ……. …………………………………………….... *Cucumis sativus* (GB-00024, GB-00027, GB-00514, GB-00029, GB-00665, GB-00737, GB-00738, GB-00739, GB-00740, GB-00034)
      3. Tertiary vein alternate percurrent with obtuse 3° vein angle to 1° vein …… *Cucumis sativus* var. *hardwickii* (GB-00577, GB-00578, GB-00579, GB-00580, GB-00581, GB-00582, GB-00584, GB-00585, GB-00586)
      3. Tertiary vein alternate percurrent with acute 3° vein angle to 1° vein …………………………………… 4
         4. Convex apex shape with obtuse apex angle ……........………..... *Cucumis melo* var. *flexuosus* (GB-00596, GB-00599, GB-00600, GB-00601, GB-00602, GB-00603, GB-00604, GB-00605)
         4. Convex apex shape with odd lobed obtuse apex angle …………..……………………………… 5
            5. Marginal ultimate venation looped with well-developed areolation ……….……… …….. ……………….. *Cucumis melo* subsp. *agrestis* (GB-00466, GB-00472, GB-00477, GB-00478, GB-00479, GB-00486, GB-00487, GB-00498, GB-00503, GB-00510)
            5. Marginal ultimate venation looped with moderately developed areolation ……….…. …………………………. *Cucumis zambianus* (GB-00652, GB-00653, GB-00654, GB-00655, GB-00656, GB-00657, GB-00658, GB-00659, GB-00660, GB-00661)

In conclusion, the dichotomous key constructed with the distinct leaf characters obtained from the 57 accessions of 6 *Cucumis* species revealed that leaf architecture is a reliable tool in describing, delineating and classifying numerous species and accessions of a closely related taxa with confusing characters. This successful delineation, description and classification of *Cucumis* accessions into 6 species proved that leaf architecture is very essential in species identification and classification especially in gene banks. Even though the studied accessions of the *Cucumis* species have great similarities in terms of general leaf features, it can still be delineated in terms of venation characters where differences commonly occurred (Table 4). This is in agreement with the observations of Rao and Rao (2015) that vein characters such as actinodromous suprabasal primary veins and looped marginal ultimate venation are unique characteristics of *Cucumis* representative species. This implies that venation patterns are consistent in delineating accessions and species of *Cucumis*.

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