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Evaluation of Loofah Lines for Resistance to Tomato Leaf Curl New Delhi Virus and Downy Mildew, as well as Key Horticultural Traits

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Abstract: Two loofah (*Luffa*) species, the ridge gourd (*Luffa acutangula* (L.) Roxb.) and the sponge gourd (*L. cylindrica* (L.) M. Roem.; syn. *L. aegyptiaca*), are cultivated widely in Asia by smallholder famers. Both species have significant economic and nutritional importance. However, Tomato leaf curl New Delhi virus (ToLCNDV) and downy mildew (DM) caused by *Pseudoperonospora cubensis* are important biotic constraints to loofah production throughout Asia. Loofah landrace-derived breeding lines, developed at the World Vegetable Center (WorldVeg), were evaluated at the WorldVeg East and Southeast Asia Research and Training Station, Kasetsart University, Kamphaeng Saen, Thailand—where natural epidemics of ToLCNDV and DM regularly occur. The lines were also evaluated for other commercially important horticultural traits such as days to 50% staminate and pistillate flowering, fruit color, fruit bitterness, and market segment classification. Thirteen and 59 lines of ridge gourd and sponge gourd, respectively, were determined to be resistant to both ToLCNDV and DM. These lines covered all market segments of loofah and exhibited variability for all of the evaluated horticultural traits. The results of these evaluations and their implications on loofah breeding are discussed.

Keywords: *Luffa acutangula*; *Luffa cylindrica*; Tomato leaf curl New Delhi virus; downy mildew; resistance; fruit traits; evaluation; germplasm

1. Introduction

Ridge gourd (*L. acutangula* (L.) Roxb.) and sponge gourd (*Luffa cylindrica* (L.) M. Roem.; syn. *L. aegyptiaca* Mill.) are the principal cultivated species in the genus *Luffa* [1]. Seven additional wild species of *Luffa* include *L. graveolens* Roxb. (var. *longistyla*), *L. echinata* Roxb., *L. tuberosa* Roxb., *L. umbellata* Roem, *L. quinquefda* (Hook and Arn), *L. astorii* Svans, and *L. saccata*. In Asia, the two cultivated species (hereafter referred to as loofah) are a commercially and nutritionally significant cucurbitaceous market vegetable. Immature loofah fruit have a significant role in Asian cuisines and are eaten boiled, peeled, and fried, and in curries and soups. Loofah fruits contribute calcium (20 mg/100 g Fresh Weight (FW), magnesium (14 mg/100 g FW), potassium (139 mg/100 g FW), and vitamin A (410 IU) to the human diet [2]. A 200 g serving of loofah fruit provides between 5% and 16% of the daily recommended intake, respectively, of the previously noted nutrients. In addition to their

nutritional importance, the production of these gourds in Asia provides a livelihood for resource-poor farmers. Loofah can be grown in various agro-climates and the fruits are produced during the hot and wet seasons. The immature fruits exhibit good postharvest, transportation, and storage properties. Within Asia, the sponge gourd fruit typically fall within one of two standard commercial market segments based on fruit color (light green and green), while there are three standard commercial market segments of ridge gourd based on fruit length (short, medium, and long). In addition to its edible immature fruit, the fibrous endocarp of mature fruits (scrubbing sponge) is popular with consumers in the USA, Japan, Korea, and other Asian countries.

Loofah gourds rank high in the cucurbit portfolio of private seed companies. In India alone, the estimated total loofah seed market is 490 MT (ridge gourd: F_1 hybrid seed = 80 tons, open-pollinated (OP) = 80 tons; sponge gourd: F_1 hybrid seed = 200 tons, OP = 130 tons) [3]. The current market in Bangladesh for ridge gourd and sponge gourd seed is 16 and 22 MT, respectively [4]. A recent (2019) survey of Asia Pacific Seed Association (APSA) member companies indicated that loofah should be considered a priority crop for improvement by WorldVeg. The survey predicted moderate to strong growth in the loofah seed market in the next 10 years [5]. Currently, loofah breeding research is centered in India and Bangladesh.

Tomato leaf curl New Delhi virus (ToLCNDV) is a whitefly transmitted bipartite begomovirus [6] initially reported in the Solanaceae, where it caused devastating damage to the tomato crop. This virus was first reported in India in 1995 [6], from where it was disseminated to South and Southeast Asian countries. It remained confined to Asia until 2012, when it was observed infecting cucurbits in various Mediterranean countries such as Spain [7], Tunisia [8], Italy [9], Morocco [10], Greece [11], and Algeria [12]. Very recently, this begomovirus has been reported in cucurbits in Portugal and Estonia [13]. ToLCNDV has caused significant damage to various cucurbitaceous crops, including *luffa* (*L. cylindrica* (L.) M. Roem.), wax/ash gourd (*Benincasa hispida* (Thunb.) Cogn.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* L.), melon (*C. melo* L.), bottle gourd (*Lagenaria siceraria* (Molina.) Standley), and various kinds of pumpkins/squashes (*Cucurbita* spp.) [14–18]. The symptoms associated with this virus in cucurbits are shortened internodes, distorted (upward and downward) leaf curling, severe mosaicism with chlorotic leaves, and fruit skin roughness [7]. Severe infections lead to large yield losses and to fruits with a greatly decreased market value.

Pseudoperonospora cubensis, the disease organism causing downy mildew (DM), is a member of the Peronosporaceae. It is a seed-transmitted [19] biotroph or obligate parasite that affects plants of all ages [20]. Infecting only the foliage, the subsequent reduction in the photosynthetic activity early in plant development results in stunted plants and yield reduction [20]. The host range of *P. cubensis* is extensive and includes 50 species in 20 genera within the Cucurbitaceae. Nineteen host species are in the genus *Cucumis* [21–23]. The disease is found on *Luffa* in Southeast Asia [20] and India [24]. Symptoms of DM frequently occur on the foliage, where the infection initially appears as small water-soaked lesions on the underside of the leaves. These lesions are angular and are limited by the leaf veins. They eventually turn from chlorotic to necrotic spots. Environmental conditions play a key role in the disease development [25]. The pathogen flourishes in warm humid regions. Differential test studies have detected several races of *P. cubensis* [26–30]. Six pathotypes of *P. cubensis* have been observed on the basis of their compatibility with specific host genera [31,32]. European populations of *P. cubensis* have been found to be highly variable and may contain multiple pathotypes [33].

The commercial loofah cultivars currently available in Asia are susceptible to both ToLCNDV and DM. Controlling these pathogens is a major challenge for loofah growers. Although loofah genetic resources naturally exhibit considerable variability for fruit color, size, and other horticultural characteristics, the diversity of the loofah germplasm held by seed companies is extremely limited. Furthermore, loofah genetic resources covering different market segments, and resistant to ToLCNDV and DM, have not been identified. ToLCNDV and DM are both endemic at the World Vegetable Center's (WorldVeg) research station at Kamphaeng Saen, Thailand. The endemic disease pressure at this site provides the WorldVeg loofah breeding program the opportunity to select for ToLCNDV and

DM resistance. Here, we report the results of the field screening of the WorldVeg loofah breeding lines (S_5 generation) for their reaction to ToLCNDV and DM, and their evaluation for key horticultural traits.

2. Materials and Methods

A field screening (two growing seasons per year) of the WorldVeg loofah germplasm collection of landraces consisting of 467 accessions of ridge gourd and 783 accessions of sponge gourd, collected from 10 Asian countries against ToLCNDV and DM, began in 2016 at the WorldVeg East and Southeast Asia Research and Training Station, Kasetsart University, Kamphaeng Saen, Thailand. Populations were initially segregated for resistance to ToLCNDV and DM, and for fruit traits such as color, shape, length, bitterness, and duration of fruit harvest. Plants tolerant to ToLCNDV and DM and belonging to different market segments (bitter vs. non-bitter fruit), were selected and hand-pollinated (selfed) to produce progeny, which were used in the subsequent growing/selection cycles (plant to progeny row). Six plants of each ridge gourd (82) and sponge gourd (65) breeding line (Tables 1 and 2), developed through this breeding approach (S_5 generation), and a susceptible check for both ToLCNDV and DM (20 plants each), were transplanted into single 9.6 m^2 plots on 25 December 2019. Natural epidemics of ToLCNDV and DM occurred on the loofah during this period.

Plants were rated in the field 60 days after transplanting for ToLCNDV severity using a 0–2 scale: 0 = no visible symptoms, 1 = mild symptoms (slight chlorosis of leaves), and 2 = severe symptoms (severe chlorosis of leaves, upward leaf curling, and stunting of plants). Severity ratings of 0, 1, and 2 corresponded to resistant, moderately resistant, and susceptible, respectively. To confirm the presence of ToLCNDV, diseased leaf tissue from individual plants of the susceptible checks were crushed in a 500 µL Tris-Ethylenediaminetetraacetic acid (TE) buffer, and DNA was extracted as described previously [34]. The quality of the DNA was evaluated on a 1% agarose gel and stored at −20 °C until further use. PCR amplifications were performed using a ToLCNDV-specifc primer pair Beg434F + ToLC1524R (developed by Dr. Orawan Chatchawankanphanich, The National Science and Technology Development Agency (NSTDA), Kasetsart University, Thailand). DNA fragments of the expected size, ca. 1.254 bp, were amplified. All of the samples of the control plants collected from the loofah experimental plot were positive, as per the PCR analysis.

DM severity was also assessed 60 days after transplanting. The genotypes were categorized into three groups, namely, immune (0%), resistant (1–10%), moderately resistant (11–30%), and susceptible (>30%), based on the percentage of symptomatic leaf area (angular lesions), as determined using a modified procedure of that described previously [35]. The presence of *Pseudoperonospora cubensis* was confirmed based on the culture morphology and microscopic examination of sporangiophores and sporangia as described by Waterhouse and Brothers [36].

The following horticultural traits were recorded: (1) days to 50% pistillate flowering after transplanting, (2) days to 50% staminate flowering after transplanting, (3) fruit color, (4) market segment of ridge gourd based on fruit length (short (up to 30 cm), medium (31–44 cm), and long (>45 cm)), and (5) market segment based on fruit length in sponge gourd (short (up to 20 cm), medium (21–30 cm), and long (>30 cm)). The number of days from transplanting to the first open pistillate and staminate flowers was recorded for each plant. Fruit bitterness was determined using three fresh fruits of each line that were harvested at marketable maturity. These were washed and cut into small (ca. 3 g) pieces (after removing the terminal three inches of each fruit) and used for organoleptic assessment by a three-person taste panel. Two categories of bitterness were recorded—non-bitter and bitter. The evaluators rinsed their mouths with water after each sample tasting.

Table 1. Summary of disease reaction of lines of ridge gourd (*Luffa acutangula*) to Tomato leaf curl New Delhi virus (ToLCNDV) and downy mildew (DM), days to 50% pistillate and staminate flowering, and fruit traits of lines field-evaluated in 2020 at the WorldVeg, Thailand.

Entry	Origin	Days 50% Pistillate Flowering (mean ± SE)	Days 50% Staminate Flowering (mean ± SE †)	ToLCNDV Resistance Reaction	DM Resistance Reaction	Fruit Bitterness	Fruit Color	Market Segment Based on Fruit Length
THLA 2	Bangladesh	24.3 ± 0.7	24.3 ± 0.7	Resistant	Moderately resistant	Non-bitter	Green	Medium
THLA 4	Bangladesh	26.3 ± 0.7	31.3 ± 3.5	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 20	Bangladesh	26.3 ± 0.7	25.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 31	Bangladesh	38.0 ± 5.6	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 36	Bangladesh	39.7 ± 1.7	25.7 ± 1.3	Resistant	Susceptible	Non-bitter	Green	Short
THLA 37	Bangladesh	43.7 ± 2.4	33.3 ± 1.3	Resistant	Susceptible	Non-bitter	Dark green	Short
THLA 38	Bangladesh	50.3 ± 0.3	24.0 ± 2.1	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 39	Bangladesh	31.7 ± 2.3	22.3 ± 0.7	Resistant	Resistant	Non-bitter	Green	Short
THLA 40	Bangladesh	26.3 ± 4.9	25.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 41	Bangladesh	28.7 ± 1.7	35.3 ± 1.8	Resistant	Moderately resistant	Non-bitter	Green	Long
THLA 45	Bangladesh	35.0 ± 0.6	29.3 ± 2.3	Resistant	Susceptible	Non-bitter	Green	Short
THLA 48	Bangladesh	28.7 ± 1.7	34.3 ± 11.3	Resistant	Susceptible	Non-bitter	Green	Short
THLA 51	Bangladesh	39.3 ± 2.3	23.7 ± 0.7	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 54	Bangladesh	25.0 ± 3.6	25.7 ± 0.7	Resistant	Susceptible	Non-bitter	Light green	Short
THLA 62	Bangladesh	25.0 ± 1.2	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 63	Bangladesh	25.3 ± 1.2	24.3 ± 0.7	Resistant	Susceptible	Non-bitter	Dark green	Short
THLA 64	Bangladesh	32.0 ± 2.5	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 67	Bangladesh	47.0 ± 1.5	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 70	Bangladesh	36.0 ± 1.2	23.7 ± 0.7	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 70-10	Bangladesh	24.3 ± 1.8	43.7 ± 0.3	Resistant	Susceptible	Non-bitter	Green	Short
THLA 71	Bangladesh	32.3 ± 2.7	31.0 ± 5.0	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 75	Bangladesh	29.7 ± 3.0	25.0 ± 1.2	Resistant	Susceptible	Non-bitter	Green	Short
THLA 80	Bangladesh	30.0 ± 3.0	22.0 ± 1.0	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 88	Bangladesh	28.7 ± 2.7	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 88-10	Bangladesh	28.0 ± 2.1	24.3 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 89	Bangladesh	29.7 ± 4.2	23.7 ± 0.7	Resistant	Resistant	Non-bitter	Dark green	Medium
THLA 94	Bangladesh	27.3 ± 2.6	26.0 ± 3.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 96	Bangladesh	25.0 ± 1.2	23.3 ± 1.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 108	Bangladesh	30.3 ± 2.7	29.3 ± 2.3	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 109	Bangladesh	39.7 ± 6.8	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Medium

Table 1. *Cont.*

Entry	Origin	Days 50% Pistillate Flowering (mean ± SE)	Days 50% Staminate Flowering (mean ± SE †)	ToLCNDV Resistance Reaction	DM Resistance Reaction	Fruit Bitterness	Fruit Color	Market Segment Based on Fruit Length
THLA 109-4	Bangladesh	39.7 ± 6.8	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 111	Bangladesh	23.3 ± 2.0	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 114	Bangladesh	24.3 ± 1.8	23.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Long
THLA 114-9	Bangladesh	24.3 ± 1.8	23.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 116	Bangladesh	28.3 ± 3.3	22.7 ± 0.3	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 119	Bangladesh	21.0 ± 1.0	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Dark green	Medium
THLA 120	Bangladesh	35.0 ± 0.6	23.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 121	Bangladesh	34.7 ± 0.3	33.0 ± 3.0	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 121-10	Bangladesh	38.0 ± 1.2	32.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 123	Bangladesh	36.3 ± 1.3	24.3 ± 1.2	Resistant	Susceptible	Non-bitter	Green	Short
THLA125	Bangladesh	42.3 ± 0.3	20.7 ± 0.3	Resistant	Susceptible	Non-bitter	Green	Short
THLA 126	Bangladesh	44.3 ± 1.5	23.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 127	Bangladesh	44.0 ± 2.1	21.7 ± 1.7	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 128	Bangladesh	22.7 ± 0.3	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 130	Bangladesh	44.3 ± 2.2	22.7 ± 1.2	Resistant	Susceptible	Non-bitter	Green	Short
THLA 133	Bangladesh	43.0 ± 0.0	35.3 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 134	Bangladesh	37.3 ± 6.3	23.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 136	Bangladesh	26.0 ± 3.0	23.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 137	Bangladesh	23.7 ± 0.7	31.3 ± 3.2	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 137-9	Bangladesh	29.7 ± 2.3	46.3 ± 3.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 139	Bangladesh	37.0 ± 2.0	32.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 142	Bangladesh	27.7 ± 3.2	25.0 ± 0.0	Resistant	Resistant	Non-bitter	Green	Short
THLA 143	Bangladesh	32.0 ± 2.5	28.7 ± 1.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 145	Bangladesh	36.0 ± 5.5	28.7 ± 2.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 146	Bangladesh	30.0 ± 3.0	32.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 147	Bangladesh	28.0 ± 2.1	25.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 149	Bangladesh	25.7 ± 0.7	25.7 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 151	Bangladesh	33.3 ± 0.7	21.0 ± 0.0	Resistant	Resistant	Non-bitter	Green	Medium
THLA 152	Bangladesh	31.3 ± 4.7	23.7 ± 1.5	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 154	Bangladesh	25.7 ± 3.5	24.3 ± 0.9	Resistant	Susceptible	Non-bitter	Green	Long

Table 1. *Cont.*

Entry	Origin	Days 50% Pistillate Flowering (mean ± SE)	Days 50% Staminate Flowering (mean ± SE †)	ToLCNDV Resistance Reaction	DM Resistance Reaction	Fruit Bitterness	Fruit Color	Market Segment Based on Fruit Length
THLA 155	Bangladesh	34.0 ± 4.5	25.0 ± 2.0	Resistant	Resistant	Non-bitter	Green	Medium
THLA 156	Bangladesh	26.3 ± 3.2	22.3 ± 0.7	Resistant	Resistant	Non-bitter	Green	Medium
THLA 158	Bangladesh	32.3 ± 3.8	21.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 500	Philippines	34.7 ± 1.3	36.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Medium
THLA 267	Thailand	30.0 ± 2.6	24.7 ± 1.2	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 273	Thailand	23.3 ± 2.0	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Medium
THLA 275	Thailand	28.7 ± 4.7	23.0 ± 0.0	Resistant	Resistant	Non-bitter	Green	Short
THLA 280	Thailand	26.0 ± 0.6	24.3 ± 0.7	Resistant	Resistant	Non-bitter	Green	Short
THLA 303	Thailand	36.7 ± 2.2	28.7 ± 2.7	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 310	Thailand	25.7 ± 0.7	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 311	Thailand	32.0 ± 2.6	27.3 ± 3.4	Resistant	Susceptible	Non-bitter	Green	Short
THLA 316	Thailand	33.0 ± 3.0	24.3 ± 0.7	Resistant	Moderately resistant	Non-bitter	Green	Medium
THLA 317	Thailand	35.7 ± 4.4	24.3 ± 0.7	Resistant	Susceptible	Non-bitter	Green	Short
THLA 330	Thailand	25.7 ± 1.3	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Medium
THLA 335	Thailand	27.0 ± 0.0	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Dark green	Long
THLA 359	Thailand	24.7 ± 0.9	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Medium
THLA 399	Thailand	27.0 ± 0.6	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Long
THLA 400	Thailand	27.3 ± 4.3	23.0 ± 0.0	Resistant	Susceptible	Non-bitter	Green	Short
THLA 416	Thailand	28.0 ± 2.1	25.3 ± 0.9	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 429	Thailand	34.0 ± 0.0	27.0 ± 0.0	Resistant	Susceptible	Non-bitter	Dark green	Short
THLA 449	Thailand	40.3 ± 7.8	23.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Green	Short
THLA 462	Thailand	34.7 ± 0.3	22.7 ± 1.5	Resistant	Resistant	Non-bitter	Green	Medium
THLA 456 *				Susceptible	Susceptible			

* Susceptible check; † Standard Error. Note: THLA stands for Thailand *Luffa acutangula* and it is followed by line number registered in WorldVeg cucurbit breeding database.

Table 2. Summary of disease reaction of lines of sponge gourd (*Luffa cylindrica*) to ToLCNDV and DM, days to 50% pistillate and staminate flowering, and fruit traits of lines field evaluated in 2020 at the WorldVeg, Thailand.

Entry	Origin	Days 50% Pistillate Flowering (Mean ± SE)	Days 50% Staminate Flowering (Mean ± SE)	ToLCNDV Resistance Reaction	DM Resistance Reaction	Fruit Bitterness	Fruit Length Segment	Market Segment Based on Fruit Color
THLC 1	Bangladesh	35.3 ± 0.9	35.3 ± 0.7	Resistant	Moderately resistant	Non-bitter	Long	Light green
THLC 3	Bangladesh	43.7 ± 0.9	44.3 ± 1.2	Resistant	Resistant	Non-bitter	Medium	Light green
THLC 43	Bangladesh	29.7 ± 2.2	34.7 ± 0.7	Resistant	Resistant	Non-bitter	Medium	Light green
THLC 43-9	Bangladesh	27.0 ± 0.0	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Long	Green
THLC 45-7-2	Bangladesh	33.7 ± 0.3	34.0 ± 0.0	Resistant	Resistant	Non-bitter	Medium	Light green
THLC 45-7-5	Bangladesh	33.7 ± 0.3	34.0 ± 0.0	Resistant	Resistant	Non-bitter	Medium	Green
THLC 45-8	Bangladesh	36.3 ± 0.7	40.0 ± 2.0	Resistant	Resistant	Non-bitter	Medium	Light green
THLC 46	Bangladesh	32.0 ± 2.0	34.3 ± 0.3	Resistant	Moderately resistant	Non-bitter	Long	Light green
THLC 46-5	Bangladesh	26.3 ± 0.3	34.0 ± 0.0	Resistant	Susceptible	Non-bitter	Short	Green
THLC 56	Bangladesh	30.3 ± 1.9	31.7 ± 2.3	Resistant	Moderately resistant	Non-bitter	Medium	Light green
THLC 57	Bangladesh	33.7 ± 0.3	35.3 ± 1.3	Resistant	Resistant	Non-bitter	Medium	Dark green
THLC 60	Bangladesh	27.7 ± 1.9	31.3 ± 2.7	Resistant	Moderately resistant	Non-bitter	Medium	Dark green
THLC 62	Bangladesh	26.3 ± 0.3	28.0 ± 3.1	Resistant	Susceptible	Non-bitter	Long	Green
THLC 70	Bangladesh	25.0 ± 1.2	25.0 ± 0.6	Resistant	Susceptible	Non-bitter	Long	Dark green
THLC 73	Bangladesh	24.0 ± 0.0	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Long	Green
THLC 83	Bangladesh	26.7 ± 0.3	31.7 ± 2.3	Resistant	Moderately resistant	Non-bitter	Short	Dark green
THLC 87	Bangladesh	27.3 ± 0.3	35.0 ± 0.6	Resistant	Resistant	Non-bitter	Medium	Green
THLC 96	Bangladesh	28.7 ± 2.7	32.3 ± 1.7	Resistant	Moderately resistant	Non-bitter	Medium	Green
THLC 97	Bangladesh	28.0 ± 1.0	28.7 ± 0.3	Resistant	Susceptible	Non-bitter	Long	Dark green
THLC 99	Bangladesh	24.3 ± 1.5	26.3 ± 1.8	Resistant	Susceptible	Non-bitter	Medium	Light green
THLC 99-4	Bangladesh	24.3 ± 1.5	26.3 ± 1.8	Resistant	Resistant	Non-bitter	Medium	Dark green
THLC 104	Bangladesh	27.3 ± 0.3	29.0 ± 0.0	Resistant	Resistant	Non-bitter	Medium	Green
THLC 113	Bangladesh	25.7 ± 1.3	30.7 ± 1.7	Resistant	Moderately resistant	Non-bitter	Long	Green
THLC 115	Bangladesh	24.7 ± 0.7	24.3 ± 1.3	Resistant	Moderately resistant	Non-bitter	Medium	Dark green
THLC 119	Bangladesh	27.0 ± 1.5	32.3 ± 1.7	Resistant	Susceptible	Non-bitter	Medium	Green
THLC 120	Bangladesh	26.3 ± 0.7	32.3 ± 1.7	Resistant	Susceptible	Non-bitter	Medium	Light green
THLC 124	Bangladesh	34.3 ± 0.3	35.0 ± 0.6	Resistant	Moderately resistant	Non-bitter	Long	Light green
THLC 125	Bangladesh	34.0 ± 0.6	39.7 ± 2.8	Resistant	Moderately resistant	Non-bitter	Long	Light green
THLC 134	Bangladesh	28.3 ± 1.2	34.0 ± 0.0	Resistant	Susceptible	Non-bitter	Medium	Dark green
THLC 134-6	Bangladesh	35.7 ± 1.7	32.7 ± 1.3	Resistant	Moderately resistant	Non-bitter	Medium	Light green
THLC 136	Bangladesh	27.7 ± 1.2	34.7 ± 0.7	Resistant	Moderately resistant	Non-bitter	Medium	Dark green
THLC 138	Bangladesh	35.0 ± 1.2	36.7 ± 1.3	Resistant	Moderately resistant	Non-bitter	Medium	Green

Table 2. *Cont.*

Entry	Origin	Days 50% Pistillate Flowering (Mean ± SE)	Days 50% Staminate Flowering (Mean ± SE)	ToLCNDV Resistance Reaction	DM Resistance Reaction	Fruit Bitterness	Fruit Length Segment	Market Segment Based on Fruit Color
THLC 139	Bangladesh	29.0 ± 1.5	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Long	Green
THLC 141	Bangladesh	25.0 ± 1.5	30.7 ± 1.7	Resistant	Susceptible	Non-bitter	Medium	Light green
THLC 143	Bangladesh	32.3 ± 2.2	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Medium	Light green
THLC 147	Bangladesh	28.0 ± 0.0	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Medium	Light green
THLC 152	Bangladesh	29.7 ± 1.8	35.3 ± 1.3	Resistant	Moderately resistant	Non-bitter	Medium	Green
THLC 153	Bangladesh	30.7 ± 1.2	35.0 ± 1.0	Resistant	Moderately resistant	Non-bitter	Medium	Light green
THLC 156	Bangladesh	34.0 ± 0.0	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Medium	Dark green
THLC 158	Bangladesh	29.3 ± 2.3	32.3 ± 1.7	Resistant	Moderately resistant	Non-bitter	Short	Green
THLC 168	Bangladesh	36.0 ± 1.5	36.3 ± 1.5	Resistant	Susceptible	Non-bitter	Medium	Green
THLC 169	Bangladesh	32.3 ± 1.7	34.7 ± 0.7	Resistant	Susceptible	Non-bitter	Medium	Dark green
THLC 170	Bangladesh	45.5 ± 2.9	43.0 ± 2.9	Resistant	Resistant	Non-bitter	Medium	Green
THLC 181	Bangladesh	30.7 ± 3.3	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Long	Light green
THLC 190	Bangladesh	37.0 ± 0.6	34.7 ± 0.7	Resistant	Resistant	Non-bitter	Long	Light green
THLC 193	Bangladesh	34.0 ± 0.0	35.0 ± 0.6	Resistant	Resistant	Non-bitter	Short	Light green
THLC 195	Bangladesh	37.7 ± 0.7	35.7 ± 1.7	Resistant	Moderately resistant	Non-bitter	Long	Light green
THLC 197	Bangladesh	29.3 ± 0.7	28.3 ± 0.7	Resistant	Moderately resistant	Non-bitter	Long	Dark green
THLC 198	Bangladesh	38.3 ± 1.8	38.3 ± 1.8	Resistant	Moderately resistant	Non-bitter	Long	Dark green
THLC 203	Bangladesh	41.3 ± 0.3	34.0 ± 0.0	Resistant	Moderately resistant	Non-bitter	Long	Light green
THLC 204	Bangladesh	38.3 ± 2.3	36.3 ± 1.5	Resistant	Susceptible	Non-bitter	Long	Light green
THLC 843	Bangladesh	34.3 ± 0.3	35.0 ± 0.6	Resistant	Resistant	Non-bitter	Long	Dark green
THLC 213	Cambodia	35.7 ± 0.9	35.3 ± 1.3	Resistant	Resistant	Non-bitter	Long	Green
THLC 765	Indonesia	33.3 ± 0.3	38.0 ± 2.3	Resistant	Resistant	Non-bitter	Long	Green
THLC 387	Thailand	35.7 ± 1.7	35.3 ± 1.3	Resistant	Moderately resistant	Non-bitter	Long	Green
THLC 406	Thailand	36.7 ± 2.7	35.3 ± 0.7	Resistant	Resistant	Non-bitter	Long	Green
THLC 414	Thailand	34.0 ± 0.0	34.0 ± 3.1	Resistant	Resistant	Non-bitter	Long	Dark green
THLC 424	Thailand	36.3 ± 3.8	34.0 ± 0.0	Resistant	Resistant	Non-bitter	Medium	Green
THLC 455	Thailand	30.0 ± 1.5	36.0 ± 1.0	Resistant	Resistant	Non-bitter	Long	Green
THLC 459	Thailand	50.3 ± 1.7	36.3 ± 1.2	Resistant	Resistant	Non-bitter	Short	Light green
THLC 463	Thailand	27.0 ± 0.6	32.3 ± 1.7	Resistant	Moderately resistant	Non-bitter	Long	Dark green
THLC 833	Thailand	37.7 ± 2.6	34.7 ± 0.7	Resistant	Resistant	Non-bitter	Short	Green
THLC 781	Vietnam	39.7 ± 3.4	34.0 ± 0.0	Resistant	Resistant	Non-bitter	Long	Green
THLC 798	Vietnam	32.3 ± 0.3	34.0 ± 0.0	Resistant	Resistant	Non-bitter	Long	Green
THLC 799	Vietnam	35.3 ± 1.2	37.3 ± 2.0	Resistant	Susceptible	Non-bitter	Medium	Green
THLC 684 *				Susceptible	Susceptible			

* Susceptible check. Note: THLC stands for Thailand *Luffa cylindrica* and it is followed by line number registered in WorldVeg cucurbit breeding database.

3. Results

3.1. Evaluation of Luffa acutangula Lines

"THLA 456" (susceptible check) was susceptible to ToLCNDV (mean rating = 2) and DM (>41% symptomatic leaf area). Eighty-two lines of *Luffa acutangula* evaluated in this study were resistant to ToLCNDV (mean rating = 0; Table 1). A majority (62/76.8%) of the ToLCNDV-resistant lines were derived from landraces originating in Bangladesh, while 20 (24.1%) of the resistant lines were developed from the landraces originating in Thailand. A single virus-resistant line originated in the Philippines. These ToLCNDV-resistant lines were categorized into three commercial market segments based on fruit length, namely: short (47/57.3%), medium (30/36.6%), and long (5/6.1%; Figure 1). The fruits of these lines were non-bitter. Three distinct fruit skin colors were observed in the ToLCNDV-resistant lines, namely: green (75/91.4%), light green (1/1.2%), and dark green (6/7.4%). Days to 50% pistillate and staminate flowering (after transplanting) ranged from 21–50 and 21–46, respectively, among these ToLCNDV-resistant lines. The earliest (<25 days to 50% flowering) pistillate flowering lines originated in Bangladesh.

Figure 1. Fruit color and length variability among ridge gourd breeding lines.

Resistance to ToLCNDV in ridge gourd is conditioned by a single dominant gene [37]. A dark green fruit color is dominant over a green and light green color, whereas a green fruit color is

dominant over light green [37]. The identified ToLCNDV-resistant lines provide ample opportunity for the development of early maturing, virus-resistant F_1 hybrid cultivars of various market segments possessing the dark green and green fruit colors preferred by consumers.

Six and three DM-resistant lines originated in Bangladesh and Thailand, respectively. All were non-bitter. The fruit colors of eight and one DM-resistant lines were green and dark green, respectively. Ridge gourd cultivars resistant to DM are not currently available. Hence, this germplasm is a potential source of resistance for DM in ridge gourd and is of importance to public and private sector breeding programs in Asia.

3.2. Evaluation of Luffa cylindrica Lines

The sponge gourd susceptible check line "THLC 684" was susceptible to both ToLCNDV (mean rating = 2) and DM (>41% symptomatic leaf area). All 65 breeding lines were resistant to ToLCNDV (mean rating = 0; Table 2). These lines originated in Bangladesh (52/80%), Thailand (8/12.3%), Vietnam (3/4.6%), Cambodia (1/1.5%), and Indonesia (1/1.5%). Three distinct fruit skin colors were observed, namely: green (26/40%), light green (23/35.4%), and dark green (16/24.6%). The following three categories of fruit length were observed: short (6/9.2%), medium (31/47.7%), and long (28/43.1%; Figure 2). The fruit of all of the lines were non-bitter. Days to 50% pistillate and staminate flowering (after transplanting) ranged from 24–50 and 24–44), respectively. All of the earliest (<25 days to 50% flowering) pistillate flowering lines originated in Bangladesh.

Figure 2. Fruit color and length variability among sponge gourd breeding lines.

Resistance to ToLCNDV in sponge gourd was determined to be governed by a single dominant gene [38]. Commercial sponge gourd cultivars resistant to ToLCNDV are currently unavailable in the global seed market. Therefore, the ToLCNDV-resistant lines described here provide an opportunity for the development of virus resistant F_1 hybrid cultivars.

Although none of the sponge gourd lines were immune to DM, 23 lines were resistant (1–10% symptomatic leaf area). Resistant lines (36) originated in Bangladesh and Southeast Asia (22).

Sponge gourd cultivars resistant to DM are unavailable in all sponge gourd market segments. Hence, this germplasm could be of use in the development of DM-resistant cultivars, so as to fill the various market segments.

4. Discussion

ToLCNDV infecting cucurbits results in major economic losses among cucurbits, including loofah, in Asia. The disease can result in 100% crop loss under epidemic conditions [39–41]. A sponge gourd accession "DSG-6" from India was previously reported to be resistant to ToLCNDV. That resistance was determined to be governed by a single dominant gene [38]. However, the seed industry has not been able to exploit that resistance to develop F_1 hybrids, as the resistance failed to persist during multi-location trials across India [37]. In addition, the fruits of "DSG-6" are dark green with superficial stripes. However, the sponge gourd market in India, Pakistan, and Bangladesh is dominated by cultivars producing green or light green fruit without stripes. "DSG-6" originated in West Bengal (India). Interestingly, the majority of the sponge gourd lines found to be resistant to ToLCNDV in this study originated in Bangladesh, which was part of the West Bengal state of India before the partition of British India in 1947. The Indo-Burma region and India (including Bangladesh) are considered the primary centers of diversity of sponge gourd and ridge gourd, respectively [42], although the exact area of domestication of sponge and ridge gourds is disputed [43]. Sponge gourd material from Bangladesh also provided the earliest pistillate flowering lines. Ridge gourd lines resistant to ToLCNDV have not been previously reported, although Premchand et al. [44] evaluated germplasm from India for resistance to this begomovirus. The first commercial ridge gourd F_1 hybrid cultivar "Arti", resistant to ToLCNDV, was released in India by VNR Seeds Private Limited in 2008 [37]. However, the resistance proved to be unstable across locations in India.

Downy mildew was observed in sponge gourd fields of commercial farmers in the Odisha state of India in 2014 [24]. Sources of DM resistance in ridge gourd and sponge gourd have not been reported. The current study has identified sources of DM resistance in the short and medium fruit length market segments of ridge gourd, and in the short, medium, and long fruit length market segments of sponge gourd. These include green, light green, and dark green fruit skin colors. These resistant lines provide a basis for the development of new DM-resistant elite ridge gourd and sponge gourd F_1 hybrids. Multiple genes for DM resistance have been reported in cucumber and melon [45,46]. Information on the genetic mechanisms of resistance to DM in loofah is lacking in the literature.

ToLCNDV- and DM-resistant lines will be showcased during the WorldVeg's Loofah Open Field Day in 2021. These lines are being further evaluated for ToLCNDV and DM in multi-location trials across Asia, in partnership with seed companies and public institutes. SNP-based genetic diversity analysis of these lines, and a study of their relationship with commercial hybrid cultivars currently popular with the farmers in Asia, is in progress.

5. Conclusions

Ridge gourd and sponge gourd fruit contribute to human nutrition, and their production and sale provides a livelihood for resource-poor farmers in Asia. These cucurbits are also important members of home, school, and community gardens in the tropics. ToLCNDV and DM are the major production constraints faced by growers in Asia, and commercial cultivars resistant to both these economic diseases are currently unavailable. The adoption of disease-resistant cultivars is an economically sound approach for disease management. The very few currently available hybrid cultivars of loofah are popular with farmers. However, these cultivars have a narrow genetic base. The current work utilized the genetically diverse landraces of loofah maintained in the WorldVeg genebank to identify and develop loofah inbred lines resistant to ToLCNDV and DM. This assortment of genetically broad-based breeding lines includes those producing fruits displaying horticultural traits associated with various commercial segments of loofah. Hence, these lines provide an opportunity for the global loofah seed industry to develop new F_1 hybrids resistant to ToLCNDV and DM to meet the needs of both

growers and consumers. The majority of the loofah lines resistant to ToLCNDV and DM originated in Bangladesh. Thus, future loofah germplasm collection and conservation efforts should focus on the loofah landraces present in the different agro-climatic regions of that country.

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References

1. Dhillon, N.P.S.; Sanguansil, S.; Singh, S.P.; Masud, M.A.T.; Kumar, P.; Bharathi, L.K.; Yetisir, H.; Huang, R.; Canh, D.X.; McCreight, J.D. Gourds: Bitter, Bottle, Wax, Snake, Sponge and Ridge. In *Genetics and Genomics of Cucurbitaceae*; Grumet, R., Katzir, N., Garcia-Mas, J., Eds.; Springer International Publishing AG: Cham, Switzerland, 2017; pp. 155–172.
2. US Department of Agriculture (USDA). National Nutrient Database for Standard Reference. Available online: http://www.ars.usda.gov/Services/docs.htm?docid=8964 (accessed on 1 March 2020).
3. Chawda, V.; (VNR Seeds Private Limited, Raipur, Chhattisgarh, India); Anil, M.; (Acsen HyVeg Private Limited, Bangalore, Karnataka, India); Hegde, G.; (Noble Seeds Private Limited, Bangalore, Karnataka, India). Personal communication, 2020.
4. Masud, A. (Bangladesh Agricultural Research Institute, Gazipur, Bangladesh). Personal communication, 2020.
5. Lin, M.L. (World Vegetable Center, Shannhua, Taiwan). Personal communication, 2020.
6. Srivastava, K.M.; Hallan, V.; Raizada, R.K.; Chandra, G.; Singh, B.P.; Sane, P.V. Molecular cloning of Indian tomato leaf curl virus genome following a simple method of concentrating the supercoiled replicative form of viral DNA. *J. Virol. Methods* **1995**, *51*, 297–304. [CrossRef]
7. Juarez, M.; Tovar, R.; Fiallo-Olive, E.; Aranda, M.A.; Gosalvez, B.; Castillo, P.; Moriones, E.; Navas-Castillo, J. First detection of tomato leaf curl New Delhi virus infecting zucchini in Spain. *Plant Dis.* **2014**, *98*, 857–858. [CrossRef] [PubMed]
8. Mnari-Hattab, M.; Zammouri, S.; Belkadhi, M.S.; Bellen Dona, D.; Ben Nahia, E.; Hajlaoui, M.R. First report of tomato leaf curl New Delhi virus infecting cucurbits in Tunisia. *New Dis. Rep.* **2015**, *31*, 21. [CrossRef]
9. Panno, S.; Iacono, G.; Davino, M.; Marchione, S.; Zappardo, V.; Bella, P.; Tomassoli, L.; Accotto, G.P.; Davino, S. First report of tomato leaf curl New Delhi virus affecting zucchini squash in an important horticultural area of southern Italy. *New Dis. Rep.* **2016**, *33*, 6. [CrossRef]
10. Sifres, A.; Saez, C.; Ferriol, M.; Selmani, E.; Riado, J.; Pico, B.; Lopez, C. First report of tomato leaf curl New Delhi virus infecting zucchini in Morocco. *Plant Dis.* **2018**, *102*, 1045. [CrossRef]
11. Orfanidou, C.G.; Malandraki, I.; Beris, D.; Kektsidou, O.; Vassilakos, N.; Varveri, C.; Katis, N.I.; Maliogka, V.I. First report of tomato leaf curl New Delhi virus in zucchini crops in Greece. *J. Plant. Pathol.* **2019**, *101*, 799. [CrossRef]
12. Kheireddine, A.; Sifres, A.; Saez, C.; Pico, B.; López, C. First report of tomato leaf curl New Delhi virus infecting cucurbit plants in Algeria. *Plant Dis.* **2019**, *103*, 3291. [CrossRef]
13. European and Mediterranean Plant Protection Organization. Available online: https://www.eppo.int/QUARANTINE/Alert_List/viruses/ToLCNDV.htm (accessed on 20 November 2019).
14. Sohrab, S.S.; Mandal, B.; Pant, R.P.; Varma, A. First report of association of tomato leaf curl virus-New Delhi with yellow mosaic disease *of Luffa cylindrica* in India. *Plant Dis.* **2003**, *87*, 1148. [CrossRef] [PubMed]

15. Ito, T.; Sharma, P.; Kittipakorn, K.; Ikegami, M. Complete nucleotide sequence of a new isolate of tomato leaf curl New Delhi virus infecting cucumber, bottle gourd and muskmelon in Thailand. *Arch. Virol.* **2008**, *153*, 611–613. [CrossRef]
16. Singh, A.K.; Mishra, K.K.; Chattopadhyay, B.; Chakraborty, S. Biological and molecular characterization of a begomovirus associated with yellow mosaic vein mosaic disease of pumpkin from Northern India. *Virus Genes* **2009**, *39*, 359–370. [CrossRef]
17. Chang, H.H.; Ku, H.M.; Tsai, W.S.; Chien, R.C.; Jan, F.J. Identification and characterization of a mechanical transmissible begomovirus causing leaf curl on oriental melon. *Eur. J. Plant Pathol.* **2010**, *127*, 219–228. [CrossRef]
18. Roy, A.; Spoorthi, P.; Panwar, G.; Bag, M.K.; Prasad, T.V.; Kumar, G.; Gangopadhaya, K.K.; Dutta, M. Molecular evidence for occurrence of tomato leaf curl New Delhi virus in ash gourd (*Benincasa hispida*) germplasm showing a severe yellow stunt disease in India. *Indian J. Virol.* **2013**, *24*, 74–77. [CrossRef] [PubMed]
19. Cohen, Y.; Rubin, A.E.; Galperin, M.; Ploch, S.; Runge, F.; Thines, M. Seed transmission of *Pseudoperonospora cubensis*. *PLoS ONE* **2014**, *9*, e109766. [CrossRef] [PubMed]
20. Colucci, S.J.; Holmes, G.J. Downy mildew of cucurbits. *Plant. Health Instr.* **2010**. [CrossRef]
21. Palti, J.; Cohen, Y. Downy mildew of cucurbits (*Pseudoperonospora cubensis*). The fungus and its hosts, distribution, epidemiology and control. *Phytoparasitica* **1980**, *8*, 109–147. [CrossRef]
22. Lebeda, A. Screening of wild *Cucumis* species against downy mildew (*Pseudoperonospora cubensis*) isolates from cucumbers. *Phytoparasitica* **1992**, *20*, 203–210. [CrossRef]
23. Lebeda, A.; Widrlechner, M.P. A set of cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J. Plant Dis. Prot.* **2003**, *110*, 337–349.
24. Nayak, A.K.; Bandamaravuri, K.B. First report of downy mildew caused by *Pseudoperonospora cubensis* on *Luffa cylindrica* in India. *J. Plant Pathol.* **2018**, *101*. [CrossRef]
25. Cohen, Y. The combined effects of temperature, leaf wetness and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. *Can. J. Bot.* **1977**, *55*, 1478–1487. [CrossRef]
26. Palti, J. The significance of pronounced divergences in the distribution of *Pseudoperonospora cubensis* on its crop hosts. *Phytoparasitica* **1974**, *2*, 109–115. [CrossRef]
27. Bains, S.S.; Jhooty, J.S. Host-range and possibility of pathological races in *Pseudoperonospora cubensis*—Cause of downy mildew of muskmelon. *Indian Phtopathol.* **1976**, *29*, 214–216.
28. Inaba, T.; Morinaka, T.; Hamaya, E. Physiological races of *Pseudoperonospora cubensis* isolated from cucumber and muskmelon in Japan. *Bull. Natl. Inst. Agro-Environ. Sci.* **1986**, *2*, 35–43.
29. Angelov, D.; Krasteva, L. Dominant inheritance of downy mildew resistance in melons. *Acta Hortic.* **2000**, *510*, 273–276. [CrossRef]
30. Shetty, N.V.; Wehner, T.C.; Thomas, C.E.; Doruchowski, R.W.; Shetty, V.K.P. Evidence for downy mildew races in cucumber tested in Asia, Europe and North America. *Sci. Hortic.* **2002**, *94*, 231–239. [CrossRef]
31. Thomas, C.E.; Inaba, T.; Cohen, Y. Physiological specialization in *Pseudoperonospora cubensis*. *Phytopathology* **1987**, *77*, 1621–1624. [CrossRef]
32. Cohen, Y.; Meron, I.; Mor, N.; Zuriel, S. A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in Israel. *Phytoparasitica* **2003**, *31*, 458–466. [CrossRef]
33. Lebeda, A.; Urban, J. Disease impact and pathogenicity variation in Czech populations of *Pseudoperonospora cubensis*. In *Progress in Cucurbit Genetics and Breeding Research, Proceedings of the Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Olomouc, Czech Republic, 12–17 July 2004*; Lebeda, A., Paris, H.S., Eds.; Palacky University in Olomouc: Olomouc, Czech Republic, 2004; pp. 267–273.
34. Doyle, J.J.; Doyle, J.L. Isolation of plant DNA from fresh tissue. *Focus* **1983**, *12*, 13–15.
35. Jenkins, S.F.; Wehner, T.C. A system for the measurement of foliar diseases in cucumbers. *Cucurbit Genet. Coop. Rep.* **1983**, *6*, 10–12.
36. Waterhouse, G.M.; Brothers, M.P. The taxonomy of *Pseudoperonospora*. *Mycol. Pap.* **1981**, *148*, 1–28.
37. Chawda, V.; (VNR Seeds Private Limited, Raipur, Chhattisgarh, India). Personal communication, 2020.
38. Islam, S.; Munshi, A.D.; Mandal, B.; Kumar, R.; Behera, T.K. Genetics of resistance in *Luffa cylindrica* Roem. against Tomato leaf curl New Delhi virus. *Euphytica* **2010**, *174*, 83–89. [CrossRef]
39. Singh, R.P.; Mohan, J.; Singh, D.P. Symptomatology and distribution of ridge gourd mosaic virus. *Agric. Sci. Digest.* **2001**, *21*, 149–152.

40. Nagendran, K.; Mohankumar, S.; Aravintharaj, R.; Balaji, C.G.; Manoranjitham, S.K.; Singh, A.K.; Rai, A.B.; Singh, B.; Karthikeyan, G. The occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu state, India. *Crop. Protect.* **2017**, *99*, 10–16. [CrossRef]
41. Kumar, R.; Esakky, R.; Acharya, S. Molecular evidence of Tomato leaf curl New Delhi virus infecting cucurbits in several states in India. *Arch. Phytopath. Plant. Prot.* **2019**, *52*, 895–905. [CrossRef]
42. Whitaker, T.W.; Davis, G.N. *Cucurbits. Botany, Cultivation, and Utilization*; Interscience Publishers, Inc.: New York, NY, USA, 1962; p. 250.
43. Filipowicz, N.; Schaefer, H.; Renner, S.S. Revisiting *Luffa* (Cucurbitaceae) 25 years after C. Heiser: Species boundaries and application of names tested with plastid and nuclear DNA sequences. *Syst. Bot.* **2014**, *39*, 205–215. [CrossRef]
44. Premchand, V.; Shankarappa, K.S.; Mamatha, A.; Mesta, R.K. Screening of *Luffa acutangula* (L.) Roxb. genotypes for resistance against Tomato leaf curl New Delhi virus, the causal agent of yellow mosaic disease of ridge gourd. *Environ. Ecol.* **2017**, *35*, 2280–2284.
45. Criswell, A.D.; Call, A.D.; Wehner, T. Genetic Control of Downy Mildew Resistance in Cucumber—A Review. Available online: https://pdfs.semanticscholar.org/93d7/dae64056608d65835cd807eca0b6c4c6d949.pdf (accessed on 10 March 2020).
46. Epinat, C.; Pitrat, M. Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis*) in muskmelon (*Cucumis melo*). I. Analaysis of a 8 × 8 diallel table. *Agronomie* **1994**, *14*, 239–248. [CrossRef]