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 farmers. 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. quinqueflora (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, 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]. Infesting 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 (S5 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 (S5 generation), and a susceptible check for both ToLCNDV and DM (20 plants each), were transplanted into single 9.6 m² 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-specific 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       | 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                                |
| THLA 125  | 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                   | Susceptible            | 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                   | Susceptible            | 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                                 |
| 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 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                                | Moderately resistant        | 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                   | Susceptible            | Non-bitter        | Long                 | Dark green                         |
| THLC 60  | Bangladesh| 27.7 ± 1.9                                 | 31.3 ± 2.7                                | Resistant                   | Moderately resistant   | Non-bitter        | Long                 | 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                 | 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                   | Susceptible            | Non-bitter        | Medium               | Dark green                         |
| THLC 104 | Bangladesh| 27.3 ± 0.3                                 | 29.0 ± 0.0                                | Resistant                   | Susceptible            | 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                   | Non-bitter             | Medium            | Green                 |                                     |
| THLC 181 | Bangladesh  | 30.7 ± 3.3                               | 34.0 ± 0.0                               | Resistant                   | Moderately resistant   | Non-bitter       | Medium               | 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 208 | Bangladesh  | 34.3 ± 0.3                               | 35.0 ± 0.6                               | Resistant                   | Susceptible            | Non-bitter       | Long                 | Dark green                          |
| THLC 213 | Cambodia    | 35.7 ± 0.9                               | 35.3 ± 1.3                               | Resistant                   | Non-bitter             | Non-bitter       | Long                 | Green                               |
| THLC 218 | Indonesia   | 33.3 ± 0.3                               | 38.0 ± 2.3                               | Resistant                   | Non-bitter             | 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                   | Non-bitter             | Non-bitter       | Short                | Green                               |
| THLC 781 | Vietnam     | 39.7 ± 3.4                               | 34.0 ± 0.0                               | Resistant                   | Non-bitter             | Non-bitter       | Long                 | Green                               |
| THLC 798 | Vietnam     | 32.3 ± 0.3                               | 34.0 ± 0.0                               | Resistant                   | Non-bitter             | 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 F1 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.

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 F1 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 F1 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 F1 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 F1 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 F1 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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