Cucurbit Genetics Cooperative

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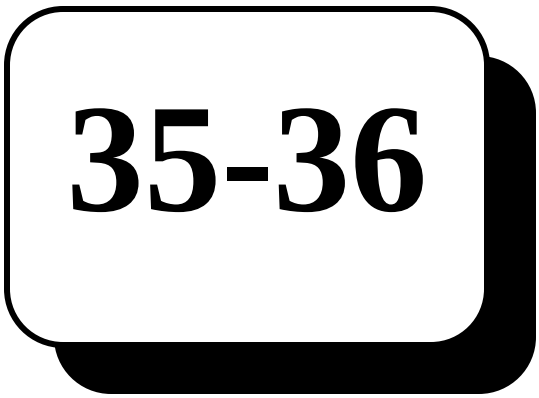

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On the front cover: *Hodgsonia heteroclita* Hook. f. & Thomson. In 1853 this member of the Cucurbitaceae was named after British civil servant Brian Houghton Hodgson who apparently provided lodging to the botanists Joseph Dalton Hooker and Thomas Thomson as they carried out field work in the Himalayas.

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Contents

Infection Process of *Sphaerotheca fuliginea* on Host Cucumber and Nonhost Wheat Leaves

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Abstract

The infection process of *Sphaerotheca fuliginea* into epidermal cells of host cucumber and nonhost wheat were studied histochemically. The percentages of germinated conidia had no significant difference on cucumber and wheat leaves at the pre-penetration stage. When *S. fuliginea* overcame the constitutive barriers of wheat leaf, papillae and hypersensitive reaction were induced to prevent the further development of *S. fuliginea*. Less than 1% haustoria could form in wheat at the post-penetration stage. However, in cucumber to *S. fuliginea* interaction, plentiful secondary hyphae and haustoria formed in cucumber leaves.

Introduction

Cucumber powdery mildew (*Sphaerotheca fuliginea*) is a severe greenhouse disease spreading worldwide and results in significant yield losses (7). *S. fuliginea*, absorbing nutrition from its host, is an obligate parasite, and its host range is very narrow. Why not colonize nonhost plants? What are their resistance mechanisms?

In recent years, much progress has been made to elucidate the mechanisms of nonhost disease resistance by utilizing biochemical, pharmacological and microscopical methods as well as functional genomic technologies (2, 9). The resistance responses of nonhost include cytoplasmic aggregation, papilla formation, accumulation of reactive oxygen species (ROS), defense-related autofluorescent materials and organelles present at around fungal penetration sites (4, 8).

Compared with other pathogens, powdery mildew fungi possess particular advantages for the study of their infection process on host and nonhost plants. Being external plant surfaces parasites, their conidium germination, appressorium and haustorium formation, in addition, hyphal growth and certain defense reactions, can be easily observed under a light microscope. The aim of this study is to obtain the preliminary cognition of infection process of *S. fuliginea* on host cucumber and nonhost wheat.

Materials and Methods

The cucumber (*Cucumis sativus* L.) 'Changchun Thorn' and wheat (*Triticum aestivum* L.) 'Shuiyuan 11', were grown from seed in organic soil in a growth chamber under fluorescent lights (60 mmol/m^2/s photon flux density), 60-70% relative humidity (RH) and 12 h photoperiod at 25°C for cucumber and 12 h photoperiod at 20°C for wheat. Third leaves of one-month-old seedlings of cucumber and primary leaves of seven-day-old seedlings of wheat were used for inoculation.

Cucumber powdery mildew fungus was maintained on the susceptible cucumber cv. 'Changchun Thorn'. For inoculation, freshly collected conidia were applied with a fine paintbrush to the upper surface of the cucumber and wheat leaves at a density of 20 conidia mm^{-2}, and inoculated leaves were incubated in a growth chamber at 20° ± 1°C, 100% RH in the dark for 12 h followed by incubation at 70-80% RH and a photoperiod of 12 h : 12 h (L:D) (60 mmol/m^2/s photon flux density).

The inoculated leaves were sampled at the indicated times and decolorized in boiling 95% ethanol. Then the tissues were clarified in saturated chloral hydrate overnight. Hypersensitive cell death was detected using the trypan blue staining method (3). At the indicated times, the inoculated leaf segments were boiled in a mixture of phenol, lactic acid, glycerol and distilled water containing 1 mg/mL trypan blue (1:1:1:1) for 3 min. Then the leaf segments were clarified overnight in saturated chloral hydrate before being stored in 50% glycerol.

For microscopical observation, the leaf tissues were rinsed in distilled water for 5 min before they were stained with an aqueous solution of 0.05% coomassie brilliant blue (Bio Basic Inc., Shanghai, China) for 5 min (3). Germination was defined when the length of germ tube was over half of maximum diameter of conidium. The treated leaf pieces were mounted on glass slides in 50% glycerol before

observation using an Olympus CX21FS1 light microscope and a Nikon 50i microscope equipped with a differential interference contrast (Japan).

Results

On cucumber leaves, the conidia of *S. fuliginea* germinated and formed 2-4 germ tubes, and the majority of conidia formed three or four germ tubes (Fig. 1A). At 8 h after inoculation (hai), most of conidia had germinated on cucumber epidermal cells, and the appressoria began to develop at the first germ tube tip. At the middle of appressoria formed primary haustoria, then primary haustoria could absorb nutrition from cucumber and develop other germ tubes, followed by the formation of elongated secondary hyphae and secondary haustoria. With the development of hyphae, clusters of conidia were detected under a light microscope and colonies were visible on the surface of cucumber leaves with naked eyes. On cucumber leaves, only a few of abnormal appressoria could be detected at 48 hai.

On wheat leaves, the conidia of *S. fuliginea* began to germinate at 10 hai, the germination of conidia on wheat was later than on cucumber, but the germination rates on wheat and cucumber leaves had no significant difference from 36 hai (data not shown). Compared with on host cucumber, the germinated conidia formed one to two germ tubes on nonhost wheat leaves, and most of them had only one germ tube (Fig. 1B). At 18 hai, the majority of germ tubes had formed matured appressoria. Some appressoria were two lobed or slender irregular appressoria. Thickened cell wall, papillae and hypersensitive cell death were observed at penetration sites of *S. fuliginea* (Fig. 1B, D). Germinated conidia rarely formed primary haustoria (<1%), and the haustoria were shriveled and elongated abnormally. No hyphae and secondary haustoria were observed on wheat leaves.

Discussion

The infection process of *S. fuliginea* on host cucumber and nonhost wheat was studied in this paper. The appressoria of conidia formed at similar rates on cucumber and wheat leaves, indicating that no resistance was expressed during the pre-penetration stage of *S. fuliginea.* The constitutive barriers, including wax layers, cell walls and antimicrobial secondary metabolites are the first line of plants against the pathogens (1, 5, 6, 10). Appressoria developed penetration pegs, from which secreted one or more sets of enzymes to degrade the cell walls, and tried to penetrate into cucumber and wheat cells. When *S. fuliginea* overcame constitutive barriers, it would face the intracellular defence response. Recent studies have emphasized the importance of host receptors for pathogen-associated molecular patterns (PAMPs) in host and nonhost resistance (12). Compare with nonhost and resistant plants, PAMP-induced defense in susceptible plants is deficient in preventing infection of pathogens (11). Therefore, the nonhost resistance exhibited by wheat towards *S. fuliginea,* such as cytoplasmic aggregation, papilla formation and hypersensitive cell death et al., resulted in cessation of *S. fuliginea* growth. Further study is required to elucidate the mechanisms in expression of nonhost resistance at molecular level.

Acknowledgements

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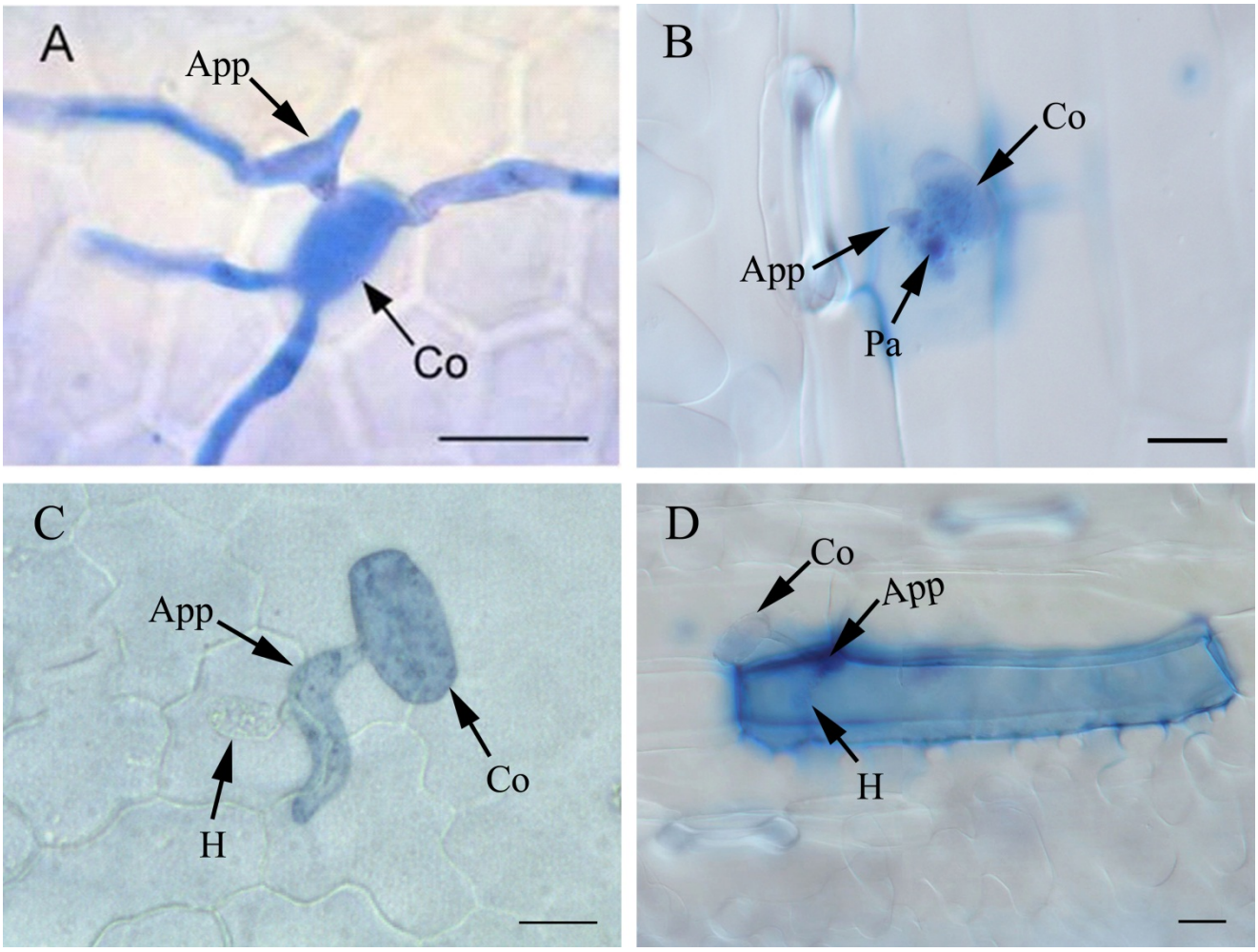

Fig. 1. Light micrographs showing of penetration by *S. fuliginea* in host cucumber (A, C) and nonhost wheat (B, D). Germinated conidium is evident by coomassie brilliant blue staining. (A) Conidium germinates with four germ tubes on cucumber leaf, 48 hai. (B) Conidium develops an appressorium on wheat, and defence reactions are shown by wheat against *S. fuliginea* with papilla formation and trypan blue staining in anticlinal cell walls, 18 hai. (C) At the middle of appressorium forms a plump rounded haustorium, 18 hai. (D) Shriveled and elongated haustorium forms in wheat epidermal cell with hypersensitive cell death, 48 hai. App, appressorium; Co, conidium; H, haustorium; Pa, papilla. Bars=20µm.

Histological Studies of *Colletotrichum orbiculare* on the Susceptible and Resistant Cucumber Cultivars

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Abstract

The infection process of *Colletotrichum orbiculare* on the leaves of susceptible and resistant cucumber cultivars were studied histologically. On both cultivars, conidia began to germinate 8 h after inoculation (hai) and formed appressoria. Then melanised appressoria formed penetration pegs at 10 hai. On the susceptible cultivar, infection vesicles formed within 24 hai and developed thick, knotted primary hyphae at 48 hai. By 72 hai, *C. orbiculare* produced highly branched secondary hyphae that invaded underlying mesophyll cells. While on the resistant cultivar, fewer germinated conidia developed biotrophic primary and invasive necrotrophic secondary hyphae than on the susceptible cultivar. These results show that the early stages of penetration process on both cultivars have no differences, and that resistance in cucumber restricts colonization by inhibiting the development of biotrophic primary hyphae and necrotrophic secondary hyphae.

Keywords: *Colletotrichum orbiculare*, cucumber, infection process, hemibiotrophic

Introduction

Colletotrichum orbiculare, belonging to ascomycete, induces fatal anthracnose disease on cucumber (Pain et al. 1992), and has become a limiting factor in commercial production (Bi et al. 2007). The cucumber plants, both in the greenhouse and field, caused roughly circular, brown to reddish lesions on all above-ground tissues including leaves, stems, petioles, and fruits after attack by *C. orbiculare* (Langston Jr et al. 1999).

Colletotrichum species have been utilized to study fungal distinction and plant-fungi interaction for many years (Perfect et al. 1999). During the colonization on plants, a series of particular structures containing germ tubes, appressoria, penetration pegs, infection vesicles, intracellular hyphae and secondary necrotrophical hyphae are developed by *Colletotrichum*, and *Colletotrichum* displayed two nutrition modes that are biotrophy and necrotrophy, respectively. *Colletotrichum* hence represents a splendid mode for studying molecular and cellular mechanisms of pathogenic fungi (Bailey and Jeger 1992; Perfect et al. 1999).

Previous research has demonstrated that the infection process of *C. orbiculare* on cucumber leaves is initiated by conidial adhesion, germination, appressorial development and growth of infectious hyphae (Jeun and Lee 2005; Jeun et al. 2008). However, the differences of infection process of *C. orbiculare* in susceptible and resistant cucumber cultivars are not well described. The objective of this study, therefore, was to elucidate the differences of the infection process and strategy adopted by *C. orbiculare* on two cucumber cultivars varying in resistance to the pathogen.

Materials and Methods

Pathogen, plants and inoculation

Colletotrichum orbiculare used in this study was isolated from naturally infected field-grown cucumber (Yangling, Shaanxi Province, China) and stocked in our lab. The isolated fungus was cultured on potato dextrose agar (PDA) at 25°C. When the fungus took the shape of pink conidial pile, conidial suspensions were prepared by flooding 10-day-old culture plates with 4–5 mL of sterile distilled water, gently scraping the colony surfaces with a scalpel and filtering the suspension through sterile cheesecloth. The concentration was adjusted to 1×10^6 conidia/mL using a haemocytometer.

The cucumber (*Cucumis sativus* L.) cv. 'Changchun Thorn' (susceptible) and 'Zao qing No. 2' (resistant) were obtained from the College of Plant Protection, Northwest A&F University. The cucumber seeds were each sown in plastic pots of 20 cm in diameter filled with soil. Cucumber seedlings were grown in a growth chamber at adequate moisture with a photoperiod of 12L:12D (60 mmol/m^2/s photon flux density) at 25°C.

The inoculated cucumber leaves were incubated at 25°C and 100% relative humidity in the dark for 24 h

followed by incubation at 25°C, 60–70% relative humidity and a photoperiod of 12L:12D) (60 mmol/m^2/s photon flux density).

Sampling and observation

Leaf samples were cut into 1 cm^2 segments at 24, 48, 72, 96, 120, 144 hours after inoculation (hai). Then the samples were decolorized in boiling 95% ethanol for 10 min, cleared in saturated chloral hydrate overnight, and rinsed in distilled water for 5 min.

For light microscopy, the cleared leaf segments were stained with 0.05% (w/v) Coomassie Brilliant Blue (Bio Basic Inc., Shanghai, China) for 10 min. After staining, the tissues were rinsed in distilled water for 3 min, temporarily mounted in 50% glycerol on glass slides and examined under a Nikon 50i microscope (Tokyo, Japan) equipped with a differential interference contrast (Warwar and Dickman) optics. Percentages were calculated from 60 penetration sites on each of five cleared leaf pieces. All experiments were repeated three times.

Results

The early stage of penetration of C. orbiculare on the leaves of susceptible and resistant cultivars

Conidia of *C. orbiculare* were oval with a smooth surface and began to geminate at 8 hai on both cucumber cultivars, less than 10% of conidia had attached to and germinated on the leaf surface of susceptible and resistant cultivars. The germination of *C. orbiculare* was judged by the formation of germ tubes or the direct generation of appressoria. About 70% conidia had germinated at 12 hai and over 90% germinated conidia had formed on both cultivars at 24 hai. The germinated conidia began to form melanised appressoria from 8 hai on susceptible and resistant cultivars. Nearly all of the germinated spores formed melanized appressoria at 12 hai and 24 hai. Melanised appressoria were formed either directly from one tip of a conidium or from the tip of a germ tube. About 8% penetration pegs were observed within 10 hai on both cultivars. Over 30% melanized appressoria had formed penetration pegs on both cultivars simultaneously at 12 hai. By 24 hai, over 50% melanized appressoria had formed penetration pegs on both cultivars.

The penetration process of C. orbiculare on the leaves of susceptible and resistant cultivars

By 24 hai, the swollen and saccate infection vesicles beneath the penetration pegs were observed on both cultivars. The infection vesicles with elongated neck regions had formed in epidermal cells. Primary hyphae had formed and successfully invaded the epidermal cells at 48 hai. The primary hyphae, large-diameter and lengthy but noticeably constricted at the sites of the intercellular penetration, had expanded to develop secondary hyphae by penetrating adjacent epidermal and mesophyll cells at 72 hai on susceptible cultivar. The narrow secondary hyphae secreted cell-wall-degrading enzymes in advance of their spread and rapidly created expanding necrotic lesions. A large number of sinuous secondary hyphae had formed at 96 hai on susceptible cultivar, while the infection vesicles enlarged and formed primary hyphae and, less frequently, a few of secondary hyphae on resistant cultivar. By 120 hai, many colonies and clusters of conidia were observed on the surface of susceptible cucumber leaves, only a small number of conidia had produced secondary hyphae on resistant cultivar, significantly fewer than on the susceptible cultivar.

Discussion

Our experiment revealed that the infection process in the cucumber-*C. orbiculare* interaction is generally similar to those of other *Colletotrichum* spp. on various hosts (Ge and Guest 2011). The infection sequence of *C. orbiculare* includes conidial germination, formation of melanised appressoria and penetration pegs, then penetrate to form infection vesicles, primary hyphae, and finally necrotrophic secondary hyphae, which is similar in susceptible and resistant cultivars, like *C. lindemuthianum* on bean (O'Connell et al. 1985).

Conidia germinated and formed appressoria within 8 h after inoculation. This is earlier than *C. lindemuthianum* on French bean (O'Connell et al. 1985) or cowpea (Bailey et al. 1990). Adhesion has been implied to be required for spore germination (Kuo and Hoch 1996; Warwar and Dickman 1996; Shaw et al. 2006). Spore adhesion to the substratum is beneficial to offering the spore stability and time to receive stimuli necessary for spore germination and appressorial formation ultimately. Furthermore, it is reported that the physical and chemical structures of the epicuticular wax can also influence spore germination (Prusky and Plumbley 1992). Thus the different affinities of leaves to water may also contribute to the easier germination of *C. orbiculare* on host leaves.

Early studies indicate that the formation of large intracellular primary hyphae and necrotrophic secondary hyphae are typical characteristics of success infection with hemibiotrophic species of *Colletotrichum* (Perfect et al. 1999; Prusky and Plumbley 1992). Although almost all conidia formed appressoria on both cultivars by 24 hai, fewer developed primary and secondary hyphae on resistant cultivar. These observations suggest that the pathogen establishes the initial penetration phase rapidly in both leaves, colonises after developing invasive necrotrophic secondary hyphae in susceptible leaves,

while resistance mechanisms inhibit the transition to necrotrophy in resistant leaves, significantly. A previous study indicated that the determinants of the compatibility or incompatibility of host pathogen did not operate during the prepenetration phase of *C. gloeosporioides* f. sp. *malvae*, but was activated a few days after successful penetration (Morin et al. 1996). This point of view is supported by our present research. After the same symptom on penetration phase, studies in the *C. orbiculare*-melon interaction also discovered that infection structures transformed into a destructive, necrotrophic phase in the susceptible cultivar (Ge and Guest 2011). The reason may be linked with the secretion of depolymerases that degrade plant cell walls (Münch et al. 2008). The detailed mechanisms of the resistance in the *C. orbiculare*-cucumber interaction remain to be studied.

Acknowledgements

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Morphological Variability and Phenotypic Association among Pairs of Characters of some *Watermelon chlorotic stunt virus* Resistant Lines of *Citrullus lanatus* Thunb.

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Abstract

Twelve lines of watermelon (*Citrullus lanatus* Thunb.) with resistance to *Watermelon chlorotic stunt virus* (WCSV) were morphologically characterized and association among pairs of characteristics was estimated. These watermelon lines were developed at the National Institute for Promotion of Horticultural Exports-University of Gezira-Sudan. Wide differences exist among these lines with respect to vegetative, inflorescence, fruit and seed characteristics. High variation coefficients were obtained for seed weight (51%), number of secondary branches (45.6%), fruit weight (41.7%), rind thickness (41.2%) and seeds/kg (40.3%). Plant canopy was positively associated with male flower (0.51) and ovary length (0.56). While female flower size and ovary length was positively associated with fruit weight (0.57; 0.63), the latter was also positively associated with total soluble solids (TSS) (0.51), seed size (0.73) and seed weight (0.63). Also, male flower size was positively associated with ovary length (0.6), fruit weight (0.64), TSS (0.54) and seed size (0.52). However, flower earliness (50%) was negatively associated with female flower size (-0.61) fruit weight (-0.56) and number of seeds/kg (-0.53). These lines are being used for the development of superior hybrid lines and genetic mapping.

Introduction

Watermelon is a valuable crop species, with wide broad phenotypic diversity in seed and fruit qualities (Wehner, 2007) and there is need for genetic research to identify genes affecting fruit quality and response to diseases. Watermelon is one of the most important vegetables produced in the Sudan, and is ranked the third after onion and tomato in irrigated sectors. Under rain fed, it is grown in vast areas for seeds production and as a source of water reserve after rain fall season in drought areas in Western Sudan. Various pests and diseases attack the crop affecting both production and quality. Watermelon is prone to attacks by aphids, whiteflies or melon bugs transmitting viruses and causing direct damages. Aphids transmit cucurbit aphid-borne yellows virus (CABYV) and zucchini yellow mosaic virus (ZYMV) to watermelon. ZYMV can also be transmitted mechanically (Abass *et al.*, 2003). The whitefly-transmitted watermelon chlorotic stunt virus is the most destructive viral disease attacking the crop (Mohamed *et al.*, 2005). Watermelon chlorotic stunt virus (WCSV) belongs to the geminiviruses family with bipartite genome (DNA-a and DNA-b components). It was first reported in Yemen and Iran (Jones *et al.*, 1988; Walkely *et al.*, 1990) and Sudan (Lecoq *et al.*, 1994). The disease is transmitted by the whitefly (*Bemisia tabaci*) and by grafting, but not mechanically (Brunt *et al.*, 1990; Walkely *et al.*, 1990). It is the major disease that infects watermelon in Sudan, causing severe crop losses (Lecoq *et al.*, 1994). Efforts exerted at the National Institute for Promotion of Horticultural Exports succeeded in developing WCSV resistant lines with superior horticultural quality, some of these lines were used in this study. This project is aiming at producing WCSV resistant hybrids and to map the gene of WCSV resistance with other genes conferring vegetative and fruit characteristics in watermelon. Since WCSV resistance was found to be not simply inherited and conferred by a dominant gene with some minor genes involved (Raeed *et al.*, 2008), the priority was given to elucidate crossing among the resistant lines to select for superior hybrids. Moreover, the highest the variability among these lines for a given trait the better the chance to go for mapping gene(s) conferring this trait. Therefore, the main objective of this study was to assess variability existing among the bred lines with respect to vegetative, fruit and yield

characteristics and degree of association among pairs of characteristics.

Materials and Methods

Eighteen lines resistant to WCSV were selected and used in this study. They were characterized for agronomic, fruit, and yield characteristics. They were also analyzed for degree of similarity and simple correlation between pairs of characteristics. The genetic material used in this study could be classified according to their origin into five groups:

- Charleston R: backcrossing an F1 (UG0012 x Crimson Sweet) with Charleston Gray.
- Elite Yellow, Elite Red, Elite White, Elite Ds, B4 and line 102: RILs of the cross UG0012 x Crimson Sweet (pedigree selection).
- Sugar Baby R and Sugar Baby Ice: backcrossing the F1 (UG0012 x Crimson Sweet) with Sugar Baby.
- Crimson R and Crimson Perfect: backcrossing the F1 (UG0012 x Crimson Sweet) with Crimson Sweet.
- Commercial varieties such as Charleston Gray, Crimson Sweet and Sugar Baby (From Peto Seed Company).

The accession UG0012 belonged to the species *Citrullus colocynthis* (L.) Schrader. It was collected in Medani area since 2001. It has small rounded bitter fruits, striped out skin color of dull green with light green and small brown seeds. Resistance to WCSV in this accession might be conferred by a single dominant gene with some modifiers (Unpublished data).

Description of the experimental site: Different activities exerted in this study were conducted at the University of Gezira Research Farm at Nesheshiba, Medani, Sudan (14 º 24' N and 33 º 38' E). The climate of the area is described as arid, hot and dry. The soil is Vertisol with clay content ranging between 40 and 65%, pH value is ranging between 8 and 9.6, with less than 1% organic carbon, 300 ppm total nitrogen and 406 to 700 ppm total phosphorus (Ishag, 1994). Planting and cultural practices were done as recommended by Mohamed (1984).

Screening for WCSV resistance: Plant materials used in this study were evaluated for resistance to WCSV using a rating scale of 1-9 depending on symptoms caused by natural infection, where:

1-2= High susceptibility: The plant is completely stunted with chlorotic leaves and bears no commercial fruits.

3-4= Moderate susceptibility: Most of the plant canopy is stunted, 50-70% with clear symptoms and plants bearing few small sized fruits.

5-6=Intermediate resistance: Stunting and chlorotic symptoms are clearly observed on the top of some branches, while fruits in this area develop patches of chlorosis while older fruits remain normal.

7-8= High intermediate resistance: Plants develop slight chlorosis towards the end of the season and remain free of stunting.

9=Resistant: The plants remain healthy, vigorous and free of symptoms till the end of the season.

Parameters studied: Vegetative, inflorescence, fruit and seed descriptors were used as mentioned by watermelon descriptor list developed by the Plant Genetic Resource Unit of the Agricultural Research Corporation-Sudan (2003).

Data analysis: Collected data of characterization were subjected to statistical analysis for standard deviations and coefficients of variation. Cluster analysis of genotypes was conducted using hierarchal analysis of GENSTAT. Moreover, the standardized data matrix of characters was used to generate similarity indices based on Euclidian distances. Moreover, characterization data were subjected to statistical analysis for correlation coefficients between pairs of characters using MStat software package.

Results and Discussion

Resistance of the different lines and commercial varieties used in this study to WCSV during summer season 2010 is given in Table 1. Results showed high level of resistance of the selected lines, while the commercial varieties were severely infected by the virus causing severe crop losses. It was concluded that some minor genes were involved in WCSV resistance in these lines, since some plants showed mild disease symptoms at the end of the season. Crossing among resistant line might produce hybrids with better resistance compared by crossing with the commercial varieties. Therefore, variability existing among the resistance lines might add to the performance of the hybrids and could provide a chance of having superior quality hybrids.

Phenotypic variability and degree of similarity: High variation exists among inbred lines with respect to vegetative, inflorescence, fruit and seed characteristics, as presented in Table 2, Table 3, Table 4 and Table 5, respectively. The highest variation coefficients were obtained for 100 seeds wt (51.0), number of secondary branches (45.6), fruit wt (41.7), rind thickness (41.2) and

number of seeds/kg (40.3); whereas, no variability was observed for leaf shape.

'Perfect Crimson' and 'Crimson R', showed close phenotypic similarity to the commercial 'Crimson Sweet', with respect to agronomic and fruit characteristics; whereas, 'Sugar Baby R' showed close similarity to the commercial 'Sugar Baby'. These results were depicted in the dendrogram based on phenotypic markers (Figure 1). In this dendrogram, The 'Elite (ds)', 'Charleston Gray' and 'Charleston R2' belong to one group, while the resistant lines and commercial 'Crimson Sweet' and 'Sugar Baby' belong to a separate group. The RILs 'Elite Yellow', 'Elite Red', 'Elite White', B4 and line 102 were clustered together in a separate sub-group with 'Sugar Baby Nice'. In this figure, lines were divided into two major groups at a similarity index corresponding to 0.65. Phenotypic data collected in the second season were in the same trend as in the first season presented in Figure 1. At a similarity index corresponding to 0.85 these groups were further divided into five distinct subgroups.

Correlation among pairs of characters: Traits having significant associations, at probability level of 0.05, are presented in Table 6. Plant vine length (cm) is associated with plant canopy size (0.57) and number of secondary branches (0.6). Plant canopy is associated with male (0.51) and ovary length (0.56), whereas number of primary branches is associated with number of secondary branches (0.53) and negatively associated with fruit wt (-0.54). Number of secondary branches was negatively associated with female flower size (-0.66) while positively associated with number of seeds/kg (0.52) Earliness of flowering (50%) was negatively associated with female flower size (-0.61) and fruit wt (-0.56). Fruit weight is associated with female flower size (0.57) and male flower size (0.64).

Ovary length is associated with fruit wt (0.63), size of male flower (0.6), TSS(0.51), seed size (0.73) and wt of 100 seeds (0.63), while it is negatively associated with number of seeds/kg (-0.53). Likewise, size of male flower was positively associated with fruit wt (0.64), TSS (0.54) and seed size (0.52).

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Table 1. Evaluation of lines and commercial varieties for resistance to WCSV, summer 2010

Entry	Number of plants in each level of the rating scale*								
	1	2	3	4	5	6	7	8	9
Charleston R						1	2	8	9
Elite Yellow							5	3	12
Elite Red						2	1	3	14
Elite White						4	6	1	9
Elite Ds						1	1	6	12
B4							2	5	13
Line 102							7	2	11
Sugar Baby R							2	3	15
Sugar Baby Nice								3	17
Crimson R							1	7	12
Crimson Perfect							2	9	9
Charleston Gray (commercial variety, P.S.)	20								
Crimson Sweet (commercial variety, P.S.)	16	4							
Sugar Baby (commercial variety (P.S.)	10	4	3	3					

* Twenty plants of each entry were used to screen for WCSV resistance in this season.

Table 2. Standard deviation and coefficient of variation for vegetative characters in the first season

Vegetative characters	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation
Length of main branch (cm)	131.0	361.4	227.1	52.9	23.3
Number of primary branch (No)	2.4	5.0	3.4	0.7	20.5
Number of secondary branch (No)	3.0	22.2	11.8	5.4	45.6
Leaf shape (Score (1,2,3))*	1.0	2.2	1.6	0.5	29. 2
Leaf color (Score (1,3,5,7,9))*	1.0	7.0	4.9	1.6	32.4
Leaf size (Score (3,5,7))*	5.0	7.0	6.4	0.9	13.7
Leaf pubescence density(Score (1,3,5,7))*	3.0	4.8	5.0	0.5	10.9
Leaf pubescence texture(Score (3,5,7))*	4.6	7.0	5.5	0.9	16.6
Stem pubescence density (Score (1,3,5,7)) *	3.0	7.0	5.8	1.2	20.7

Two *Cucurbita moschata* Bush-Parthenocarpic Breeding Lines Through an Interspecific Cross

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Introduction

Cucurbita moschata varieties in Korea have soft flesh when their immature fruits are panbroiled. Their vine growth habit hinders wide cultivation in the greenhouse. Zucchini varieties belonging to *Cucurbita pepo* have bush growth habit but a little hard flesh when their immature fruits are done. These species are generally non-parthenocarpic although the latter species has larger differences in parthenocarpy than the former species (2,3).

In Korea, summer squash growers in the greenhouse put much labor on a daily basis into fruit set and arranging the vines at 3-4 day intervals. To reduce labor requirements, the interspecipic cross between *C. pepo* 'Ford Zucchini' and *C. moschata* 'Seoulmadi' was made in 1988. In 1997 'Wonye 401' and 'Wonye 402' with semi-bush growth habit and good quality of immature flesh were bred(1).

The present paper reports the development of novel squash types with better expression of bush growth habit than these previously released varieties possessing parthenocarpy bred through the interspecific cross between Cheongma Zucchini (*C. pepo*) and Wonye 402 (*C. moschata*).

Materials and Me*thods*

The interspecific cross was made between the cultivar hybrid, Cheongma Zucchini *(C. pepo)* and the breeding line Wonye 402 *(C. moschata*) in 2001. Five seeds from the three-way cross made without in vitro embryo culture techniques required were obtained. They were seeded in late autumn and four plants grown in the greenhouse during the winter. Approximate 60 selfed seeds were obtained and 54 plants were grown in spring, 2002. One strongly bush growth habit individual and another semi-bush individual with good fruit shape and green immature fruit in the segregating population were sib–mated. The progenies were then selected for good fruit shape and green immature fruit, and selfed to near homozygosity (Fig.1).

Results and Discussion

Selection was made based on good fruit shape, green immature fruit and bush growth habit with the two lines finally selected strongly expressing these traits. In addition they showed a high degree of parthenocarpy, and averaged 10 seeds per fruit after selfing. The two breeding lines were named 'Bush Jadong No. 1' and 'Bush Jadong No. 2 '.

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Cheongma Zucchini hybrid (*C. pepo*) × Wonye 402 (*C. moschata*)

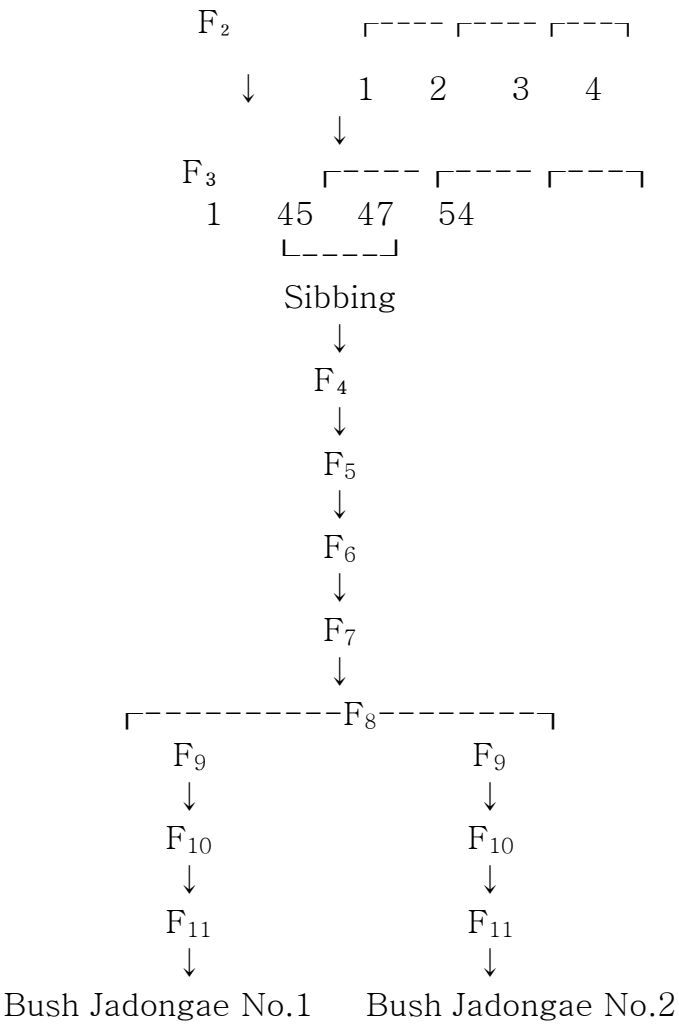

Fig.1. Pedigree of two bush- parthenocarpic breeding lines, 'Bush Jadongae No.1' and 'Bush Jadongae No.2.'

Identification of Natural Sources of Resistance to Powdery Mildew, *Zucchini yellow mosaic virus* (ZYMV) and Fruit Flies Infestation in the Indigenous Pumpkin (*Cucurbita moschata*) Germplasm

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Introduction

Pumpkin (*Cucurbita moschata*) is ranked as one of the least economic important vegetable crops in Sudan despite having high nutritive values and great industrial uses. It is grown in small holdings along rivers banks. The crop is threatened by damage caused by powdery mildew, Zucchini yellow mosaic virus (Sid Ahmed *et al.*, 2003) and fruit flies (Gesmalla, 2000). The main objective of this study is to obtain natural sources of resistance to powdery mildew, ZYMV and/or fruit flies infestation among the indigenous pumpkin germplasm.

Materials and Methods

This project was started with surveys and collection missions in Gezira, Kassala, Gedarif and Sinnar states, since rainfall season of 2008. One hundred and twenty three accessions were collected in the surveyed fields and markets. Then, the collected accessions were grown at the University of Gezira research farm in winter season of 2008-09. Thirty plants of each accession were planted in a randomized complete block design (RCBD), with three replications. Each plant was screened for powdery mildew and zucchini yellow mosaic virus (ZYMV) and fruit flies infestation. Two selected accessions were subjected to three cycles of purification to obtain uniform lines during rainfall season 2009, winter season 2009-010 and rainfall season 2010. At the end of field experimentation; the two accessions were subjected to proximate analysis.

Results and Discussion

Field surveys showed the great threat to pumpkin production caused by powdery mildew, ZYMV and fruit flies infestation in the different states. Reduction in yield was estimated to be 10-20% for powdery mildew (PM), 25% for ZYMV and 45-55% for fruit flies (FFs). Results of screening the collected germplasm during winter 2008-09 for resistance to PM, ZYMV and FFs are given in Table 1. It was found that resistance against powdery mildew, ZYMV and fruit flies was not common among the indigenous Sudanese cultivated pumpkin with only eight, five and three accessions segregating for resistance to PM, ZYMV and FFs, respectively, and one uniform accession for ZYMV resistance. Results also indicated that only one accession (UG 002) which was collected in ElShowak area, Eastern Sudan, showed resistance to PM, ZYMV and FFs. While another accession (UG 042), which was collected in El-Haj Abdalla, 40 km south of Medani, showed resistance to powdery mildew and fruit flies while susceptible to ZYMV infection. The general characteristics and percentage of resistance for PM, ZYMV and FFs of UG 002 and UG 042 are given in table 2 and 3, respectively. Results of screening UG 012 and UG 042 plants in the three cycles of purification concluded that resistance against PM, ZYMV and FFs were independent and simply inherited (Table 4). Results encourage more efforts to be exerted in the breeding project to obtain homozygous resistant lines with known genetics of resistance. The proximate analysis of the line UG 012 is presented in Table 5. It was rich in energy, carbohydrates, protein and fiber contents and comparable to pumpkin cultivars used commercially worldwide. The collected accessions with complete passport data are being prepared to be conserved at the Plant Genetic Resource Unit of the Agricultural Research Corporation at Wad Medani Research Station.

Acknowledgements

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Table 1. General evaluation of the collected accessions against powdery mildew, ZYMV and fruit flies

Total number of accessions	Powdery mildew resistant accessions		ZYMV resistant accessions		Fruit flies resistant accessions	
	Uniform	segregating	uniform	Segregating	Uniform	Segregating
123	-	8	1	5	-	3

Note: Number of screened plants of each accession was 30 plants.

Table 2. General characteristics of the promising accessions UG 002 and UG 042

Accession	Plant canopy	Number of fruits/plant	Fruit size	Fruit out skin color	Rind thickness	Cavity size	Flesh colour
UG 002	medium	8-10	large	Uniform light brown	medium	small	orange
UG 042	large	12-16	Medium to small	Striped green with light green	thin	large	yellow

Table 3. Percentage of resistance for powdery mildew, ZYMV and fruit flies in the two accessions UG 012 and UG 042

Accession number*	Powdery mildew	ZYMV	Fruit flies	Percentage of plants with combined resistance
UG 012	65%	68%	27%	7%, only two plants were selected
UG 042	43%	-	18%	13%, only four plants were selected

Table 4. Progress of resistance (in percentage) against powdery mildew, ZYMV and fruit flies in the pedigree of UG 012 and UG 042

Accession	UG012			UG 042	
	Powdery mildew	ZYMV	Fruit flies	Powdery mildew	Fruit flies
First season	73	68	71.5	53.7	34
Second season	85	87	76	66	39
Third season	94	93	85	79	57.3

Table 5. Proximate analysis of flesh of the line UG012

Content	
Moisture %	92.1
Kcals/100gm	
Calories content	25.5
Content in gm/100g	
Protein content	1.05
Fat content	0.12
Ash	0.9
Carbohydrates	6.5
Fibre content	1.5

Inheritance of Rind Color and Reverse Striping in a *Cucurbita pepo* (subsp. *texana*) Cross

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Introduction

Several loci determining exterior fruit color have been characterized in *C. pepo* (5), among which are the *L-1* and *L-2* loci and the *D* gene. Interactions of multiple alleles at the *L-1* and *L-2* loci confer varying degrees of dark or light pigmentation, with the dominant alleles *L-1* and *L-2* conferring dark green coloration and their recessive counterparts *l-1* and *l-2* conferring a light green rind color (6). In addition, the $l\text{-}1^{BSt}$ allele causes fruit to exhibit broad, contiguous dark stripes in combination with the *L-2* allele, and is recessive to *L-1* but dominant to *l-1* (3). A recently identified allele, $l\text{-}2^{R}$, reverses the stripes (a phenotype known as "reverse striping") in the presence of any striping allele—such as $l\text{-}1^{BSt}$—at the other locus, such that the broad stripes in between the fruit's vein tracts are lighter than the background color, which remains darker over the vein tracts (4). The *D* gene affects both fruit and stem color, with the dominant *D* allele conferring dark coloration to stem and fruit beginning at about 15-18 days after anthesis (6). Epistatic to *l-1* and *l-2*, the *D* allele can cause fruits which would otherwise be light colored (i.e. *l-1/l-1*, *l-2/l-2*) to be dark.

Current evidence points to two possible systems of reverse striping inheritance in *C. pepo,* (1,4) In Paris' model (4), complete or partial RS phenotype in delicata x spaghetti squash results from the presence of at least one copy of the $l\text{-}1^{BSt}$ allele and the $l\text{-}2^{R}$ allele, independent of any other loci such as the *D* gene. Loy's more recently proposed system (1), based on crosses involving egg gourd, is the following: RS phenotype results from the presence of the dominant *D* allele, at least one copy of the $l\text{-}1^{BSt}$ allele, and an *L-2/l-2* or *l-2/l-2* genotype, without a separate $l\text{-}2^{R}$ allele. The heterozygous genotype *L-2* /$l\text{-}2^{R}$ is responsible for "partial" RS in the first system and heterozygous *L-2/l-2* for a similar "Type 2 RS" in the second.

Crosses between delicata and acorn squash are often used for the aesthetic of rind patterns on winter squash. The goal of this study was to initiate an evaluation of the two proposed genetic systems in these types of cultivars.

Materials and Methods

A cross between a delicata squash (*Cucurbita pepo* subsp. *texana* cv. Bush Delicata) expressing reverse striping and a dark green acorn squash (*Cucurbita pepo* subsp. *texana* cv. Sweet Reba) was performed. The F_1 was self-pollinated, and the F_2 population grown in the field in the summer of 2012 at the Homer C. Thompson Vegetable Research Farm in Freeville, NY, alongside the parents and F_1. Fruits were harvested at maturity , and photographs of fruit from parents, F_1 and135 F_2 plants were used for phenotyping.

Results and Discussion

The cross segregated for exterior fruit color (Figure 1). Fruit from 122 of the F_2 plants fell into easily identifiable RS ('Bush Delicata') or dark ('Sweet Reba') phenotypic categories, while 13 plants did not fit these categories and had fruit that showed light-green splotchiness or orange mottling. Of these 122 plants, 68 were RS and 54 were dark with respect to rind color. Fruit were not further separated into completely or partially reversed phenotypic classes due to difficulty in discerning differences.

The predictions of each of the afore-mentioned systems of RS inheritance with respect to the cross were compared against the obtained results. Acorn squash is known to have genotype *DD, l-1/l-1, L-2/L-2* (2). Following the model of Paris, delicata has genotype *DD,* $l\text{-}1^{BSt}/l\text{-}1^{BSt}$, $l\text{-}2^{R}/l\text{-}2^{R}$ (4). Using the system of inheritance proposed by Loy, delicata is assigned the genotype *DD,* $l\text{-}1^{BSt}/l\text{-}1^{BSt}$, *l-2/l-2*. In either system the F_1 would be heterozygous at the *L-2* locus and confer a partially reverse striped phenotype, which we observed (Figure 1). The expected F_2 phenotypic ratio for this cross predicted by both models is a 9:7 ratio of RS to dark exterior fruit color (Table 1). The $L\text{-}1^{BSt}/\_\_$, *L-2/L-2* genotype would ordinarily confer a broad, normal striping phenotype, but because of epistatic interaction in the presence of *D*, the stripes would not be apparent and the fruit are simply dark. Of the 122 classifiable plants, 68

were RS and 54 were dark—results consistent with either model (χ^2 = 0.013, p= 0.9092).

The difference between the two systems involves the *D* gene and the existence of the *l-2*^{R} allele. Loy, in his crosses, found that the dominant *D* allele was necessary to confer the RS phenotype (1), whereas Paris found RS exhibited even in crosses involving only light-stemmed *(dd)* plants (4). Differences in these systems may be a result of the different parents used for crossing (H. Paris, personal communication).

Because of the nature of our cross, especially its lack of segregation for the *D* gene, clarification regarding the inheritance of RS and the role of the *D* gene in reverse striping in *C. pepo* cannot be further elucidated using our current data. Additional crosses and plantings are planned.

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Table 1. Expected genotypic frequencies and associated phenotypes of the F_2 population given two systems of reverse striping

Genotype: *D*-Independent Model	Genotype: *D*-Dependent Model	Frequency	Phenotype
DD, L-1^{BSt}/__, L-2/L-2	*DD, L-1^{BSt}/__, L-2/L-2*	3/16	dark
DD, L-1^{BSt}/__, L-2/l-2^R	*DD, L-1^{BSt}/__, L-2/l-2*	6/16	RS (partial/Type 2)
DD, L-1^{BSt}/__, l-2^R/l-2^R	*DD, L-1^{BSt}/__, l-2/l-2*	3/16	RS (complete/Type 1)
DD, l-1/l-1, L-2/L-2	*DD, l-1/l-1, L-2/L-2*	1/16	dark
DD, l-1/1-1, L-2/l-2^R	*DD, l-1/1-1, L-2/l-2*	2/16	dark
DD, l-1/l-1, l-2^R/l-2^R	*DD, l-1/l-1, l-2/l-2*	1/16	dark

Figure 1. Delicata with complete reverse striping (left), acorn with dark green rind color (middle), and F_1 with intermediate/partial/"Type 2" reverse striping (right)

Patterns of Broad Normal Striping in Egg Gourd

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Introduction

There are two general patterns of striped pigmentation patterns in *Cucurbita pepo*, reverse striping (3, 7), characterized by green narrow stripes over the vein tracts (vasculature) and wide white stripes between the vein tracts, and normal striping (5, 6, 8), characterized by narrow white to blue-gray stripes over vein tracts and wide green stripes between the vein tracts. There are different phenotypes of normal striping conferred by multiple alleles at the *l-1* locus (5, 6), and two systems for reverse striping (3, 7). Striped alleles are dominant to *l-1*, but recessive to *L-1*.

I have developed bush breeding lines of egg gourd (*C. pepo* L. ssp. *ovifera* (L.) D.S. Decker (1) which carry the allele $l\text{-}1^{BSt}$ for broad normal striping, a phenotype characterized by distinct, fairly broad blue-gray stripes over the vein tracts with intervening dark green pigmentation (3). We have also developed additional BNS lines carrying the *B* gene for precocious orange or yellow pigmentation , as well as incorporated the *D* gene into BNS lines. This paper describes those phenotypes, along with inheritance data illustrating BNS phenotypes that can be generated in populations segregating for *D*/*d* and *Wf*/*wf*.

Materials and Methods

During the summer months, June through October 1, gourds were grown at the Kingman Research Farm in Madbury, NH, USA. Plants were grown on raised beds mulched with black polyethylene, and supplied with drip irrigation. Plants were either direct seeded or grown in 50-cell plug trays and then transplanted. Standard fertility and pesticide practices were used according to New England Vegetable Management Guide (2). In the greenhouse during the months of January through May, plants were grown in 8.7 L plastic nursery pots in a soil-less mix (Pro-mix, Griffin Greenhouse Supply, Tewksbury, MA, US). Daytime temperatures were 24 ^{o}C (16 h) and nighttime temperatures were 18 ^{o}C. Phenotypes of gourds segregating for fruit color and pattern traits were typically evaluated at anthesis or shortly thereafter, at 18 to 25 days after pollination (DAP) and at maturity (45 to 55 DAP).

Results and Discussion

Two populations segregating for broad normal stripes (BNS) were generated by crossing a green-fruited line (G344-22) with dark stems (*L-1*/*L-1*, *L-2*/*L-2*, *D*/*D*) to a BNS line (G424-25-3-11) with light stems, ($l\text{-}1^{BSt}$/$l\text{-}1^{BSt}$, *L-2*/*L-2*, *d*/*d*). The F_2 population segregated 46 dark stem (*D*/_) to 15 light stem (*d*/*d*) and 44 green fruit (*L1*/_) to 17 BNS ($l\text{-}1^{BSt}$/$l\text{-}1^{BSt}$) fruit (Table 1), both ratios conforming to a 3:1 segregation ratio, and in agreement with results of Paris and Burger (8) that non-striped or green pigmentation (*L-1*/_) is dominant to BNS ($l\text{-}1^{BSt}$/$l\text{-}1^{BSt}$) in plants homozygous for *L-2*/*L-2*. The backcross population segregated 40 dark stem to 33 light stem plants, and 32 plants with green fruit and 41 with BNS fruit, ratios close to the expected 1:1. Segregation in both populations conformed to independent assortment for the two loci in question, with χ^2 probabilities of 0.78 and 0.57, respectively, for F_2 and BC populations (Table 1). The results also confirm that plants homozygous for the dominant *L-2* allele do not express the reverse stripe trait in the presence of the *D* allele. However, the *D* allele alters the BNS phenotype, producing BNS fruit with narrow green and broad dark-green stripes (Fig. 1D) as opposed to *d*/*d* fruit with narrow blue-gray stripes and broad green stripes (Fig. 1A). Because the green-fruited line (G344-22) carries the recessive *wf* allele for orange flesh and G424-25-3-11 is *Wf*/*Wf* for white flesh, 3 out of 61 plants (1/16 expected) in the F_2 progeny had orange /green BNS fruit at maturity (Fig. 1E, F).

Table 2 shows segregation of both reverse and broad normal stripes in two F_2 populations derived from reciprocal F_1 crosses in which all plants were homozygous for the $l\text{-}1^{BSt}$ allele and segregating for *D/d* and *L2/l-2*. These populations are exceedingly small to reflect correct ratios of the six expected phenotypes, but data fit expectations and Chi-square probabilities were relatively high. Data show four different phenotypes displaying broad normal striping. Two phenotypes are conferred when *L-2* is homozygous and in combination with either *D/_* or *d/d* as in Table 2 above. The other two phenotypes occur when plants are recessive for the *d* allele and have either the genotype *L-2/l-2*, producing fruit with narrow white/wide light green stripes, or are *l-2/l-2*,

producing the wh/wh BNS fruit phenotype. In the latter phenotype, fruit appear white, but expression of homozygous *l-1*^{BSt} alleles can be detected by slightly raised ridges over the vein tracts. Type 1 RS plants show the complete reverse stripe phenotype, narrow dark green stripes over the vein tracts and white pigmentation between vein tracts. In Type 2 RS, there are various degrees of mottled green and white pigmentation between the vein tracts.

As shown in Table 1, when plants are homozygous for *L-2*, then plants with either genotypes *L-1*/*l-1*^{BSt} or *L-1*/*L-1* have dark green fruit, regardless of whether plants are *D*/_ or *d*/*d* as illustrated in Table 2, and no striping or at most very minimal striping is evident in any of the fruit. However, when the *L-2* gene is heterozygous in plants carrying a *D* allele, some degree of intermediate reverse striping (some degree of green-white mottling between narrow dark green stripes) occurs regardless of whether *L-1* is homozygous or heterozygous (*L-1*/*l-1*^{BSt}), and the degree of white-green mottling varies with maturity of fruit.

The *B* gene in combination with *L-2* produces orange fruit (4), and when combined with BNS (*l-1*^{BSt}), can produce attractive striped fruit. The range of phenotypes is further expanded by expression of genes which result in bicolor pigmentation patterns, whereby the *B* gene is expressed only in a portion of the fruit. Genes which affect the degree of expression of the *B* gene have been designated as *Ep* genes (9); the inheritance of these genes has not been determined in egg gourd. Figures 2A and 2B show a BNS breeding line (G14-2-1-10) homozygous for the *B* gene but segregating for white (*WF/_)* versus orange (*wf/wf*) flesh. When the *Wf* allele is present, stripping is white/yellow when *B* is expressed; when fruit is *wf/wf*, stripping is yellow/orange. Figure 2C shows a BNS egg gourd line with the *B* gene in combination with orange flesh and the *D* gene. Fruit with the *D* gene show darker yellow/orange and orange/green stripping than fruit which are *d/d*, in either *B*/_ or *b/b* backgrounds.

Acknowledgement

This work was supported by Hatch Grants from the New Hampshire Agricultural Experiment Station.

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Table 1. Dihybrid segregation of progeny with either broad normal stripes (BNS), *l-1*^{BSt}/*l-1*^{BSt}) or green (*L-1*/_) fruit and with either light (*d/d*) or dark green (*D*/_) stem color. G07-1, F_2, was derived from G424-25-3-11 (*l-1*^{BSt}*/l-1*^{BSt}*, L-2/L-2* , d/d) x G344-22 *L-1*/*L-1*, *L-2*/*L-2*, *D*/*D*) ⊗, and G07-2 was derived from G424-25-3-11 (male parent) backcrossed to the above F_1.

	Distribution of phenotypes				Expected		
Parental	gr fr/dk st[z]	gr fr/lt st	BNS/dk st	BNS/lt st	ratio	χ^2	P
G07-1 F_2	35	9	12	5	9:3:3:1	1.09	0.78
G07-2 BC	17	15	23	18	1:1:1:1	1.99	0.57

[z]gr – green; dk st – dark stem; lt st = light stem; BNS/dk st = narrow green/wide dark-green stripes, dark stem; BNS/lt st = blue-gray narrow/wide green stripes, light stem.

Table 2. Segregation of reverse and broad normal stripes in F_2 populations derived from G424-25-3-11 x G12194-3 (G10-126) or its reciprocal (G10-110). Plants are homozygous for l-1^{BSt}, but segregating for *L-2*/*l-2* and *D*/*d*. Plants in the first three columns carry the *D* allele; plants in the last three columns are *d*/*d*.

	Distribution of phenotypes[z]								
Parental	Type 2 RS	Type 1 RS	BNS gr/dk gr	BNS wh/lt gr	BNS gray/gr	BNS wh/wh	Expected Ratio	χ^2	P
G10-126	35	17	8	11	8	5	6:3:3:2:1:1	5.99	0.31
G10-110	22	10	10	6	3	0	6:3:3:2:1:1	3.65	0.60
G10-110 +126	57	27	18	17	11	5	6:3:3:2:1:1	5.17	0.39

[z]Type 2 RS = narrow green /wide mottled gr/wh stripping; Type 1 RS = narrow green /wide white striping; BNS:gr/dk gr = green/dark green striped fruit, dark stem; BNS:wh/lt gr = white/light green striped fruit, light stem; BNS:gray/gr = blue-gray narrow stripes/wide green stripes, light stem; BNS: wh/wh = narrow white/wide off-white stripes, light stem.

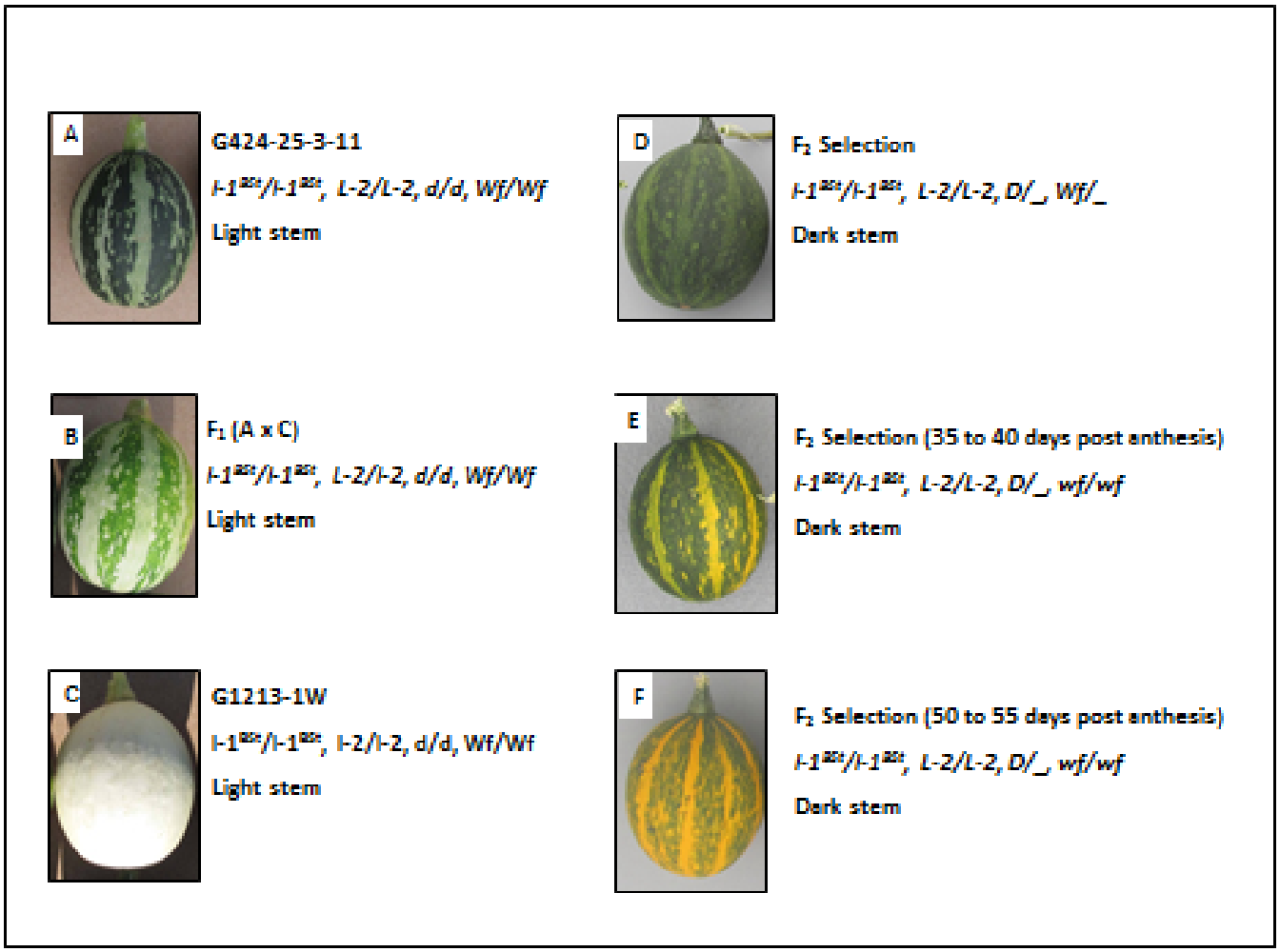

Figure 1. Illustrations and genotypes of different broad normal stripe (BNS) phenotypes.

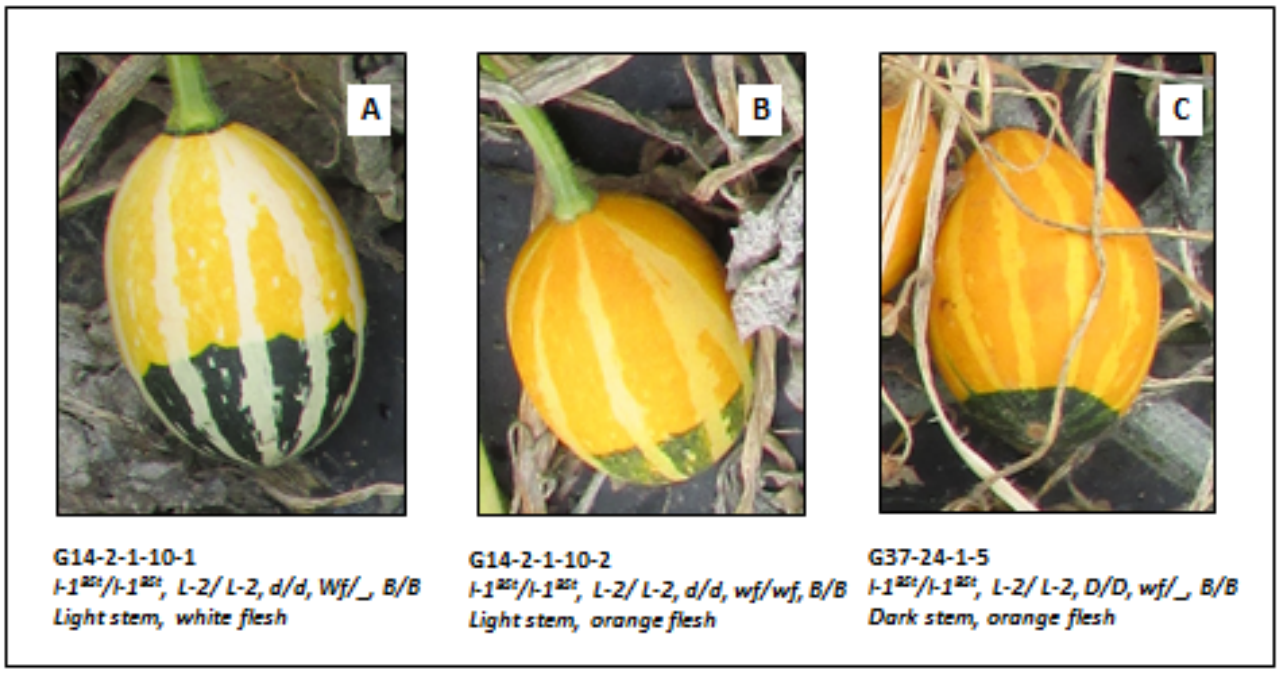

Figure 2. Interaction of the 'B' gene with the D/d alleles and Wf/wf alleles in pigmentation of mature striped egg gourds.

Interaction of Three Loci, *l-1*, *l-2*, and *d*, in Conferring the Reverse Stripe Phenotype in Egg Gourd

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Introduction

There are more than a dozen described genes that govern rind color and rind color patterns in fruit of *Cucurbita pepo* L. (9), and expression of such traits is complex because colors and patterns can change at different stages of fruit development (11) and many of the gene loci affecting fruit pigmentation have multiple alleles. In addition, two or more genes affecting fruit color may interact to produce novel patterns of fruit coloration. Three such independently assorting loci are *l-1*, *l-2* and d (10). When both *l-1* and *l-2* are recessive, fruit are pale green. When either allele is dominant, the fruit are green; when both alleles are dominant, the fruit are darker green. There are additional alleles at the *l-1* locus which in the presence of *L-2*/*L-2* result in different degrees of striped fruit characterized by narrow stripes of light pigmentation over the vein tracts and green pigmentation between the vascular tissue (6, 8). One such pattern that I have introgressed into egg gourd is referred to as broad normal stripes (BNS), and is conditioned by the $l\text{-}1^{BSt}$ allele (6). The allele $l\text{-}1^{BSt}$ is recessive to *L-1*, but dominant to *l-1*. There is also a reverse striping pattern characterized by narrow green stripes over the vein tracts, with white pigmentation between the narrow stripes, a striping pattern conferred by the complementary interaction of alleles at two loci, $l\text{-}1^{BSt}$ together with a newly described allele, $l\text{-}2^{R}$, at the *l-2* locus (7). The *D* gene affects both stem and fruit color, but is not expressed until about 15 to 18 days after pollination (10). Plants carrying recessive *d*/*d* have light stems and peduncles throughout development; whereas, those with dominant *D* develop dark stems and peduncles (10). Also, *D* is epistatic to *l-1* and *l-2*, resulting in a change in fruit pigmentation from pale green to moderately dark green. When *l-1* and *l-2* alleles are combined with the *Wf* allele for white flesh, the fruit rind becomes white early in development (5).

Bailey (1) described a monotypic white egg gourd (*C. pepo* L. ssp. *ovifera* (L.) D.S. Decker (2), having a vining growth habit and fruit size ranging from about 50 to as large as 120 g. As a result of breeding work initiated in 1995 at the University of New Hampshire, several bush (*Bu*) lines of egg gourd have been developed that are homozygous for genes conferring different patterns of fruit pigmentation (4). Based on observations of segregation for fruit color in early breeding populations, the genotype of the original white egg gourd appears to be *l-1*/*l-1*, *l-2*/*l-2*, *Wf*/*Wf*, *d*/*d*. Bush egg gourd germplasm has proven especially suitable for genetic studies of fruit pigmentation because bush gourds are adapted to relatively close plant spacing and each plant produces several fruit, allowing for convenient observation of fruit at several stages of development. This paper describes inheritance of a reverse stripe (RS) trait in egg gourd involving the interaction of three loci, *l-1*, *l-2* and *d*.

Materials and Methods

During the summer months, June through 01 October, gourds were grown at the Kingman Research Farm in Madbury, NH, USA. Plants were grown on raised beds mulched with black polyethylene, and supplied with drip irrigation. Plants were either direct seeded or grown in 50-cell plug trays and then transplanted. Standard fertility and pesticide practices were used according to New England Vegetable Management Guide (3). In the greenhouse during the months of January through May, plants were grown in 8.7 L plastic nursery pots in a soil-less mix (Pro-mix, Griffin Greenhouse Supply, Tewksbury, MA, US). Plants were grown under natural daylight with 24 ^{o}C day (16 h) and 18 ^{o}C night (8 h) temperatures. Phenotypes of gourds segregating for fruit color and pattern traits were typically evaluated at anthesis or shortly thereafter, at 18 to 25 days after pollination (DAP) and at maturity (45 to 55 DAP).

The cv. Lil-Pump-Ke-Mon (Harris Seeds, Rochester, NY, USA) was used as the source of the reverse strip (RS) phenotype. All other germplasm used in the study was developed at the University of New Hampshire Experimental Research Farms. For pollination, staminate and pistillate flowers were closed the day before anthesis, using 10 cm 'Twist-ems' ties (Griffin Greenhouse Supply), and pollinations were performed before 9:00 h on the following day. Immediately after pollination, pistillate

flowers were re-closed with Twist-ems, tagged with the date, and if necessary, covered with a poly mesh bag. Fruit were harvested 50 to 60 DAP, and the seeds were subsequently removed, cleaned, and dried at 30 °C in a forced air dryer.

Results and Discussion

Beginning in 2004, a RS trait derived from an ornamental pumpkin, 'Lil-Pump Ke-Mon', was introgressed into egg gourd germplasm (4). By spring of 2009, two related RS bush egg gourd lines, $G1213F_6$ and $G1219F_6$, had been selected. However, both lines were segregating for reverse stripe and white fruit in a 3:1 ratio. Further selfing produced line G1213-1W, homozygous for white fruit, and line G12194-3, homozygous for the RS trait. The white-fruited line displayed ghost stripes in the form of narrow white stripes over slightly raised vein tracts, with the intervening wider stripes being slightly off-white. Lines with this phenotype were identified and genotyped earlier in the NH breeding program, and are designated as wh/wh BNS, with the genotype *l-1^{BSt}/l-1^{BSt}, l-2/l-2.* Prior to spring of 2010 it was assumed that the genotype of the RS lines was *l-1^{BSt}/l-1^{BSt}*, *l-2^{R}/l-2^{R}*, according to inheritance of the RS trait reported by Paris (7). Crosses of the RS line to a green-fruited line, G344-22 (*L-1/L-1*, *L-2/L-2*), and to a BNS line, G424-25-3-11 (*l-1^{BSt}/l-1^{BSt}, L-2/L-2*) produced F_1 plants with intermediate reverse striping, narrow green and wide mottled green and white stripes, results expected for plants heterozygous for the semi-dominant *l-1^{R}* allele. Intermediate reverse striping in the two F_1s differed; plants with the *L-1/l-1^{BSt} genotype* had very narrow green stripes (Fig 1C); whereas, plants homozygous for *l-1^{BSt}* had wider, more prominent green stripes (Fig. 1B). A cross of G1213-1W to G424-25-3-11 produced BNS F_1 plants with narrow white and wide light green stripes, results expected for BNS plants heterozygous (*L-2/l-2)* at the *l-2* locus. Unexpectedly, however, F_1 plants derived from G1213-1W x G344-22 and having the genotype *L-1/l-1^{BSt}, L-2/l-2* produced intermediate RS fruit identical to fruit derived from the cross of 344-22 to the RS line G12194-3. These results suggested that another dominant gene was involved in reverse striping in egg gourd. A small testcross population was grown during the summer of 2010, resulting from a cross of a wh/wh BSN line (G424-10-1) x an intermediate RS F_1 plant (G424-25-3-11 x G1219-4). If RS was due to the *l-2^{R}* allele, there should have been 1:1 segregation for intermediate RS (*l-1^{BSt}/l-1^{BSt}, l-2^{R}/l-2)* and white/green BNS (*l-1^{BSt}/l-1^{BSt}, L-2/l-2*). However, in a population of 43 plants, 10 were wh/wh BNS, 13 were gray or white/green BNS, 10 were RS and 10 were intermediate RS, a testcross ratio suggesting segregation of two loci (*L-2/l-2* and a dominant gene conferring RS), and indicating that G1219-4 carries a dominant allele conferring RS in combination with homozygosity for *l-2* and *l-1^{BSt}*.

Additional backcross and F_2 populations were generated in 2010 and 2011 from G1219-4, G1213-1W, G424-25-3-11 and G344-22 which indicated that RS plants were homozygous for *l-1^{BSt}* and *l-2*, and carried an additional dominant gene that conferred reverse striping. However, results were difficult to interpret because of deficiencies of green progeny, an excess of progeny showing an intermediate RS phenotype, and in certain populations, variability in expression of BNS patterns. Fortuitously, in spring of 2012 it was observed that stems and peduncles of the wh/wh BNS line (G1213-1W) were light green (*d/d*; Fig. 1F), and stems of F_1 RS plants resulting from a cross of the white fruited line G212-349 (*l-1/l-1, l-2/l-2, d/d*) to the RS line 121943-4 (*l-1^{BSt}/l-1^{BSt}, l-2/l-2*) were dark green, indicating that the RS line was homozygous for the *D* gene (4). A later planting in spring of 2012 revealed that G424-25-3-11 plants have light peduncles (Fig. 1E), whereas G344-22-1 (Fig. 1D) and G121943-4 (Fig. 1A) plants have dark peduncles (*D/D*), suggesting that the '*D*' allele was the dominant factor contributing to the reverse stripe trait. As a result of observations on the association of stem color with striping phenotypes, we made appropriate crosses with the breeding material that was on hand in the greenhouse to further test the hypothesis that *l-1^{BSt}* and *l-2* act in a complementary manner with the dominant *D* gene to produce reverse striping, and to obtain a better understanding of the genetics of broad normal striping (BNS) patterns. In addition, we had remnant seed of (G1213-1W x G1219-4)⊗.

The F_2 population derived from G1213-1 x G1219-4 segregated 3:1 (19:7) for RS/dark stem and wh/wh BNS/light stem progeny, corroborating that dominant *D* together with recessive l-1^{BSt} and l-2 alleles confers reverse striping. Two populations were grown in summer of 2012 that were segregating for *l-1* versus *l-1^{BSt}* and *D* versus *d*, but homozygous for *l-2* (Table 1). The RS phenotype is not fully expressed until about two weeks after pollination (Fig. 2) coinciding with the delayed expression of *D* on stem pigmentation (10). Therefore, data on reverse striping were not taken until several fruit had reached at least 15 to 18 DAP. The F_2 population (G12-3) segregated 3:1 for *D/_* (54) versus *d/d* (16). All reverse stripe plants had dark stems and the proportion of RS progeny was consistent with the expectation that plants with either the l-1^{BSt}/l-1 or l-1^{BSt}/l-1^{BSt} genotypes in combination with the *D* gene displayed the RS phenotype. Plants with green fruit also had dark

stems, and the proportion of progeny with green fruit was consistent with the expectation that the *D* gene was epistatic to *l-1* and *l-2* (10), producing green fruit in plants homozygous for *l-1* and *l-2* alleles. In the second population, G12-5, there was 1:1 segregation of D/_ (41) versus d/d (44) as expected, and the segregation of RS, green and white fruit fit the expected 3:1:4 model (χ^2 P = 0.23).

In crosses, involving segregation for *L-1*/*l-1*^{BSt}, *L-2*/*l-2*, and *D*/*d*, solid green fruit are only produced when *L-2* is homozygous. Various degrees of intermediate reverse stripe fruit are produced with the following genotypes: *L-1/L-1*, *L-2/l-2*, *D*/_; *L-1*/*l-1*^{BSt}, *L-2/l-2*, *D*/_; l-1^{BSt}/*l-1*^{BSt}, *L-2/l-2*, *D*/_, and *L-1/L-1*, *L-2/l-2*, *d/d*. An example of different degrees of intermediate RS striping in a backcross population segregating 1:1 (44:41) for intermediate RS striping (*L-1/L-1*, *L-2/l-2, L-1/l-1*^{BSt}, *L-2/l-2* and) and green fruit (*L-1/L-1*, *L-2/L-2* and *L-1/l-1*^{BSt}, *L-2/L-2*) is shown in Fig. 3.

Conclusions

1. In egg gourd, reverse striping is conferred by complementary interaction of the *D* allele, and homozygous *l-2* alleles with either *l-1*^{BSt}/*l-1*^{BSt} , *L-1*/*l-1*^{BSt} or *l-1*^{BSt}/*l-1* allelic combinations.
2. Several genotypes, but most noticeably those heterozygous for *L-2/l-2* in the presence of the *D* allele and either L-1/L-1, L-1/l-1^{BSt} or l-1^{BSt}/l-1^{BSt}, can produce intermediate reverse stripe phenotypes characterized by narrow green stripes over the vein tracts and various degrees of mottled white and green wide striping between vein tracts.

Acknowledgements

This work was supported by Hatch Grants from the NH Agricultural Experiment Station.

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Table 1. Inheritance of the reverse stripe trait in egg gourd, based on segregation in two populations homozygous for *l-2*: (1) G12-3, an F_2 population derived from crossing a white line, G212-349 (*l-1*/*l-1*, *l-2*/*l-2*, *d*/*d*) x a reverse stripe line, G12194-3 (*l-1*^{BSt}/*l-1*^{BSt}, *l-2*/*l-2*, *D*/*D*), and (2) G12-5, a population created by crossing two F_1 plants, G212-349 x G2123-1W (*l-1*^{BSt}/*l-1*, *l-2*/*l-2*, *d*/*d*) x G212-349 x G12194-3 (*l-1*^{BSt}/*l-1*, *l-2*/*l-2*, D/*d*).

	Distribution of phenotypes[z]			Expected		
Parental population	RS/dk st	Gr/dk st	Wh/lt st	ratio	χ^2	P
G12-3	45	11	16	9:3:4	0.88	0.64
G12-5	26	15	44	3:1:4	2.97	0.23

[z]RS/dk – reverse stripe, dark stem; Gr/lt st = green, dark stem; Wh/lt st = white, light stem. White class included *l-2*/*l-2*, *d*/*d* plants carrying either *l-1* or *l-1*^{BSt} alleles; reverse stripe class included two genotypes, *L-1*/*l-1*^{BSt}, *D*/_ and *l-1*^{BSt}/*l-1*^{BSt}, *D*_; and green class was *l-1*/*l-1*/*D*/_.

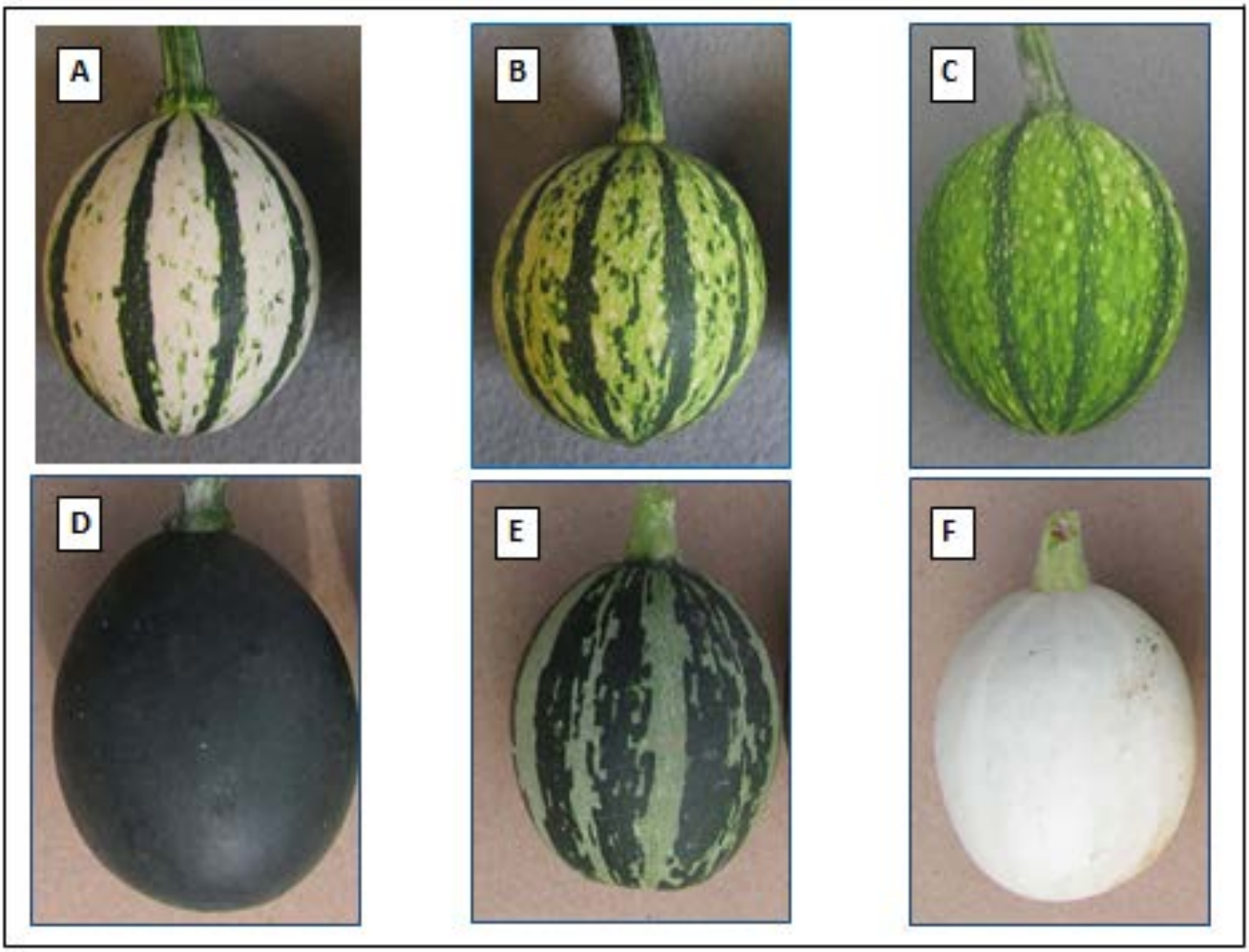

Fig. 1. Different striped phenotypes and genotypes for egg gourd lines and selections. A = reverse stripe (RS; *l-1*^{BSt}/*l-1*^{BSt}, *l-2*/*l-2*, *D*/*D*), B = RS wide intermediate (*l-1*^{BSt}/*l-1*^{BSt}, *L-2*/*l-2*, *D*/_); C = RS narrow intermediate (*L-1*/*l-1*^{BSt}, *L-2*/*l-2*, *D*/_); D = green (*L-1*/L-1, *L-2*/*L-2, D/D*),; E = broad normal stripe (BNS; *l-1*^{BSt}/*l-1*^{BSt}, *L-2*/*L-2, d/d);* F = wh/wh BNS (*l-1*^{BSt}/*l-1*^{BSt}, *l-2*/*l-2, d/d)*.

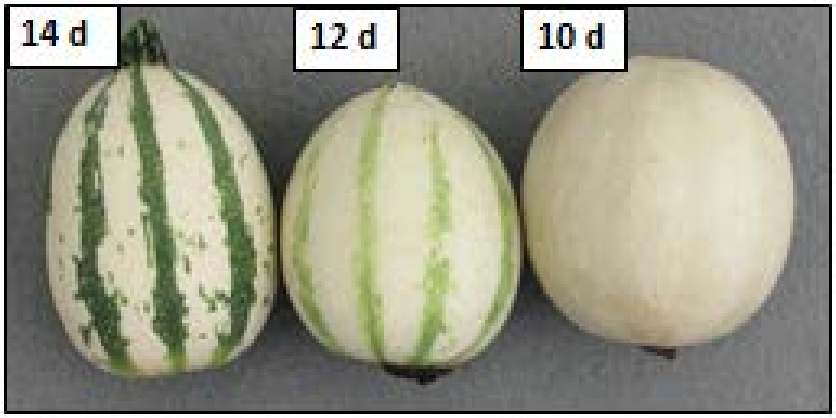

Fig. 2. Transition to reverse striping during development of egg gourd, expressed as days after pollination (DAP).

Fig. 3. Variability in intermediate reverse striping in a population derived from G344-22 x (G1213-1W x G344-22), segregating 1:1 for green fruit (*L-1*/*L-1*, *L-2*/*L-2* or *L-1*/*l-1*^{BSt}, *L-2*/*L-2*), represented by four fruit on right, or intermediate RS fruit (*L-1*/*L-1*, *L-2*/*l-2* or *L-1*/*l-1*^{BSt}, *L-2*/*l-2*). Plants were either *D/D* or *D/d*.

Tendril Morphology in Bush and Vine Genotypes of Squash and Pumpkin

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Introduction

Tendrils are threadlike, coiling organs differentiated from specialized lateral shoots initiated at leaf axils, and which function in mechanical support of plants with a vining habit of growth. Morphologically, they are commonly comprised of a basal stem or shoot axis of variable length which branches into tendrils which are thigmotropic, coiling around objects in which they come in contact. They are a ubiquitous organ within the Cucurbitaceae family (5, 7), but do not appear to confer any substantial advantage to domesticated cucurbits mostly grown in row cultural systems employed in modern agricultural practice.

In the genus *Cucurbita*, many of the new modern cultivars have a bush or semi-bush habit of growth, and it was stated in the classical book on Cucurbits by Whitaker and Davis (9) that bush squashes "differ from the common trailing varieties by their much-shortened internodes and lack of tendrils." At the time the "Cucurbits" book was published in 1962, most bush varieties of squash were types grown for consumptions of immature squash, and characterized by extremely shortened internodes, a phenotype likely conferred by several genes, and not the single incompletely dominant *Bu* gene described by Shifriss in 1947 (8).

In the current breeding germplasm of ornamental pumpkin and winter squash at the University of New Hampshire, both bush and vine forms are represented among the three major economic species, *C. pepo* L., *C. moschata* Duch., and *C. maxima* Duch., and we have found that presence of tendrils is the rule and not the exception in bush plants. This report summarizes the relationship of the basal shoot or stem length in tendrils to internode length in several breeding lines representing bush and vine genotypes among the three economically important species of squash and pumpkin.

Materials and Methods

Plants were seeded into 50-cell plug trays during the first week of January, 2013, and transplanted into 8.7 L plastic nursery pots in a soil-less mix (Pro-mix, Griffin Greenhouse Supply, Tewksbury, MA, US) during the first week of February. Temperatures were maintained at 24 ^{o}C day and 18 ^{o}C night, and plants grown under natural daylight. Data on mature internode and tendril length were recorded on 01 April, 2013, using bush and vine breeding accessions.

Results and Discussion

As described for Echinocystis lobata (5), the axillary bud complex differentiates on one side of a node with tendrils differentiated at the most basal portion of a node, the flower bud(s) at the most distal portion, and the axillary shoot, when present, initiated between the tendril and flower organ (Fig 1). Tendril morphology of the examined breeding lines is shown in Fig. 2 and 3, and quantitative data on internode length and length of the basal shoot of tendrils is given in Table 1, along with branching characteristics of the tendrils. Breeding lines were chosen so as to represent the wide variation in internode length that exists in both bush and vine cultigens of *Cucurbita* (6). Length of tendril shoots was positively correlated with mean internode length among breeding lines of all three species (Table 1). However, occasionally a bush accession with moderately long internodes, had an extremely short tendril shoot, as exemplified by NH29-1-27 (Table 1). Genetic dwarfism in *C. pepo* (4) and *C. maxima* (3) is reversed by application of gibberellins and so any deficiency of this hormone or a deficiency in perception of this hormone in a plant would be expected to elicit a similar response in both internodes and tendril shoots. There also appeared to be a general tendency for reduced branching and elongation of tendrils in bush plants (Fig. 2 & 3), and in bush plants of NH199-30-5-2 (C. *moschata*), tendrils were not present at all nodes. A similar deficiency of tendrils was observed in bush breeding lines of egg gourds (*C. pepo*) and the basal shoot of tendrils was often barely visible (Fig. 2E). Therefore, gibberellins may be involved in both elongation and differentiation of tendril shoots. The results of Ameha et al. (2) lend some support to this postulate. They grew

plants from seeds and cotyledon segments of cucumber *in vitro* with different growth regulator treatments. In cultures supplemented with GA_{4+7}, shoots were highly elongated and developed tendrils but not flower buds; whereas, cultures supplemented with either BA or GA_3, developed flower buds but not tendrils at leaf nodes. Contrary to the results of Ameha et al. (2), it is normal for nodes of *Cucurbita* plants to differentiate both flowers and tendrils.

There were no vine genotypes of *C. maxima* grown in the greenhouse in winter-spring of 2013, but one breeding line segregated for extreme bush plants, permitting a comparison of tendril length in two bush genotypes having significantly different internode lengths. Internodes of NH31-7-134 were more than double the length of those of NH46-4-11-2164-5, and the basil tendril shoot of NH31-7-134 was three times longer than that of NH46-4-11-2164-5 (Table 1; Fig. 3D, E). However, both bush *C. maxima* lines averaged 5 tendril branches, a number not out of line with branching reported in vine cultigens (1).

Acknowledgements

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Table 1. Variation in tendril morphology among bush and vine forms of squash and pumpkin among the three major economic species of *Cucurbita*.[z]

Species and accession	Genotype	Internode length	Tendril stem length	Tendril branching
Cucurbita pepo				
NH123-12-428	bush	3.2±0.8	1.0±0.3	2 + 1[y]
NH29-1-27	bush	7.9±1.9	1.1±0.2	2 - 3
NH24-6-1	bush	10.9±1.5	6.3±1.0	4 (5)[x]
NH.HO2-113	vine	18.1±1.2	17.3±4.1	4
Cucurbita moschata				
WBN1-4-10	vine	16.4±1.3	13.2±1.3	3
NH204-3-9-16-1	vine	9.6±1.1	6.7±0.6	3
NH199-30-5-2	bush	7.1±1.8	2.2±0.5	2
Cucurbita maxima				
K31-7-134	bush	11.0±1.4	5.0±1.0	5
K46-4-11-2164-5[w]	bush	4.9±1.2	1.7±0.1	5 (6)

[z]Data are means ± SD of 8 mature internodes (4 per plant) and 8 tendrils which subtended the distal node of each internode. Data were taken with calipers to hundredths of a cm and rounded to tenths.
[y]Two tendril branches + occasionally a compound tendril which branched into two tendrils.
[x]Occasionally 5 tendril branches.
[w]Extreme bush segregant; mean values of 5 internodes and tendrils; occasionally 6 tendril branches.

Figure 1. Node on a bush plant of *C. pepo* showing positions of tendril, axillary shoot and flower bud.

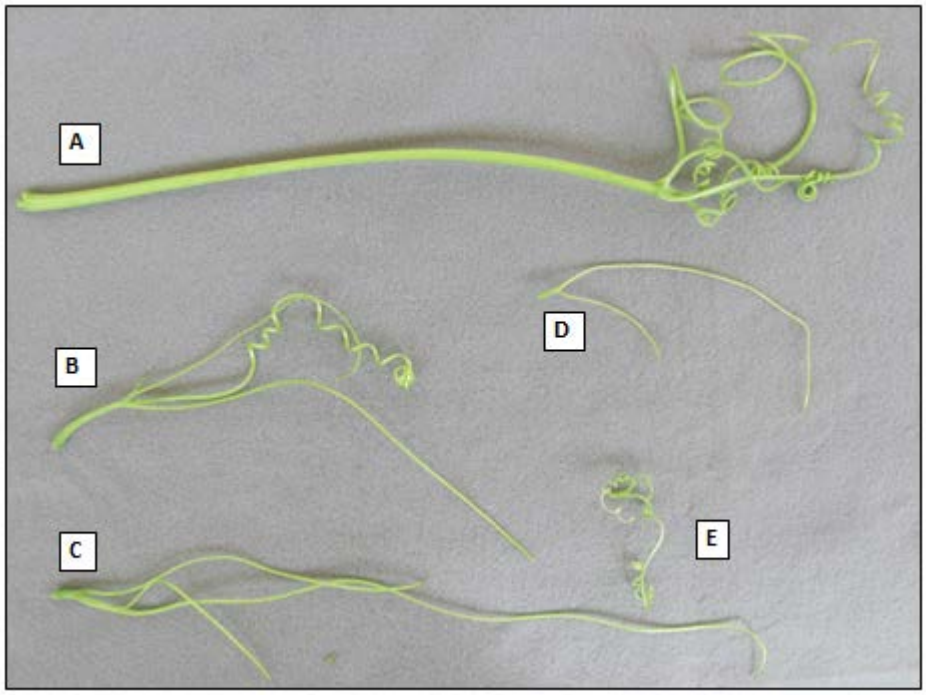

Figure 2. Tendril morphology in *C. pepo*: NH.HO2-113V (A), NH24-6-1V (B), NH29-1-27Bu (C), NH123-12-4-2-8Bu (D), and bush egg gourd (E).

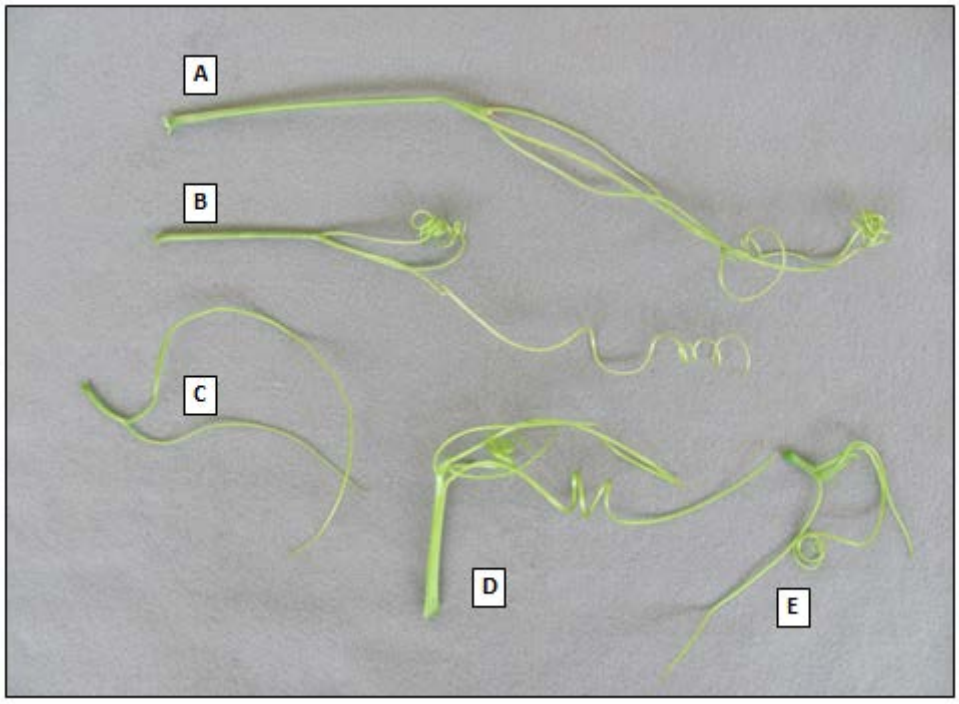

Figure 3. Tendril morphology in *C. moschata*: A = WBN1-4-10V; B = NH204-3-9-16-1V; C = NH199-30-5-2Bu; and *C. maxima*: (D = NH31-7-1-3-4Bu; E = NH46-4-11-2164-5Bu).

A Recessive Tendrilless Mutant in Ornamental Pumpkin

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Introduction

Tendrils are borne at leaf nodes and are a common morphological feature of most cucurbits. Reports of tendrilless mutants are rare in the cucurbit family. In a perusal of recent gene lists for the major cucurbits two tendrilless mutants were reported, one in cucumber (4) and the other in watermelon (3). In spring of 2010 we discovered several breeding lines of ornamental pumpkin derived from the same breeding population that were either segregating for a tendrilless trait or were homozygous for the trait. Here we report on the inheritance of this tendrilless trait.

Materials and Methods

Plant material used for all experiments consisted of ornamental pumpkin breeding lines developed at University of New Hampshire, and populations generated from these lines. During the summer months, June through October 1, pumpkins were grown at the Kingman Research Farm in Madbury, NH, USA. Seeds were sown on raised beds mulched with black polyethylene, and supplied with drip irrigation. Standard fertility and pesticide practices were used according to New England Vegetable Management Guide (2). In the greenhouse during the months of January through May, plants were grown in 8.7 L plastic nursery pots in a soil-less mix (Pro-mix, Griffin Greenhouse Supply, Tewksbury, MA, US). Plants were grown under natural daylight with 24 ^{o}C day (16 h) and 18 ^{o}C night (8 h) temperatures.

Results and Discussion

The orginal tendrilless trait in our breeding program can be traced back to a bush breeding line, NH1182Bu, developed in the late 1990s. Another bush breeding line developed from NH1182 was crossed to a vine line with resistance to powdery mildew, and in an F_2 population derived from this cross, several lines were developed that were either homozygous or segregating for the tendrilless trait. In crosses of plants with tendrils (T) to plants which are tendrilless (t), F_1 plants had normal tendrils, regardless of the direction of the cross (Table 1). In a small backcross population of tendrilless x (tendrils x tendrilless) plants segregated 1:1 (15 T to 11t). Likewise, a small segregating population derived from a plant identified as possibly heterozygous for the tendrilless trait, segregated 3:1 (19:7). In a larger F_2 population of bush plants derived from NH128-5Bu (T) x NH43-1472Bu (t), 61 plants had tendrils and 20 were tendrilless. In a second F_2 population derived from NH128-5Bu, (T) x 27-2-12-2V (t), 64 plants had tendrils and 16 were tendrilless. Both F_2 populations fit a Chi square ratio of 3:1 (tendrils: tendrilless). Thus, segregation data indicate that a single recessive gene confers tendrilless. I suggest that the tendrilless mutant be designated as *td*, following the cucumber nomenclature (1). In addition, the results indicate that expression of tendrils occurred with equal frequency on bush and vine plants. The dihybrid ratio of (NH128-5Bu x 43-147V)⊗ was 55 bush/tendrils:12 bush/tendrilless:9 vine/tendrils: 4 vine/tendrilless, with a χ^2 probability of 0.15 for an expected 9:3:3:1 ratio. The rather low probability can probably be attributed to a deficiency (13) of vine segregants. Tendril shoots on bush plants in the field were shorter than those on vine plants, agreeing with results in a greenhouse study (see accompanying article in CGC). Most bush and vine lines in our acorn breeding germplasm are tendrilless, and we are in the process of determining if those plants share the same tendrilless allele with our ornamental pumpkin lines.

Acknowledgements

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Table 1. Segregation of a tendrilless trait in backcross and F_2 populations of *Cucurbita pepo*.

	Tendrils	Tendrilless	Exp. Ratio	χ^2	P
NH128-5Bu x 27-472V[z]	18	0	1:0		
NH43-1472Bu[z] x HO2-126V	10	0	1:0		
NH6-11-12H, V⊗[z]	19	7	3:1	0.05	0.82
NH27-4-7V[z] x NH6-11-12H,V[z]	15	11	1:1	0.61	0.43
(NH128-5Bu x NH43-1472Bu[z])⊗	61	20	3:1	0.004	0.95
(NH128-5Bu x 27-2-12-11V[z])⊗	64	16	3:1	1.07	0.30

[z]Indicates parents which are tendrilless or heterozygous (H) for tendrilless trait. Bu = bush and V = vine phenotypes.

Figure 1. Example of tendrilless plant (NH6-11-12-1) (A) and a plant with compound branching tendrils (B) in *Cucurbita pepo*.

Inheritance of Lethal Yellow Seedling in *Cucurbita moschata* Duch.

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Introduction

The lethal yellow seedling phenotype has been observed in seedlings of the genus *Cucurbita*. It was first reported in *C. pepo* (1) and more recently in *C. maxima* (2). In both cases a single recessive gene was reported to control the trait. It is reported here the occurrence and the inheritance of the lethal yellow seedling in *C. moschata*. To our knowledge, this is the first report on the occurrence of this trait in *C. moschata*.

Materials and Methods

The lethal yellow seedling phenotype was first observed in seedlings of an inbred line of *C. moschata* derived from the Japanese cv. Futtu. Thirty (23.4%) out of one hundred and twenty eight seedlings were observed to show the lethal yellow seedling phenotype. Seedlings were raised in Styrofoam trays under plastic house at Sakata's Bragança Paulista Research Station, Brazil, in the fall of 2012. All seedlings showing the lethal yellow seedling phenotype died at cotyledonal stage within approximately ten days from seed emergence. Forty four out of the ninety eight left normal green seedling plants were self-pollinated for the inheritance study. Seeds of each individual family were sown and raised in Styrofoam trays under plastic house at Sakata's Bragança Paulista Research Station, Brazil, in the spring of 2012. Normal green and yellow seedlings were counted. A chi-square test was run for each segregating family, pooled family data and also for the original segregating line. The chi-square test was run considering a single recessive gene (3:1 normal green to lethal yellow seedling ratio) inheritance hypothesis (H0 hypothesis).

Results and Discussion

Nineteen out of forty four families segregated for normal green and lethal yellow seedlings. The ratio of normal to lethal yellow seedlings of each segregating family and its respective chi-square test value are presented in Table 1. Only for family number 43, the qui-square test value was significant at 5%, rejecting the H0 hypothesis. For the rest of the evaluated families, pooled family data and also for the original segregating line data confirmed the expected 3:1 genetic segregation hypothesis. These results indicate that, similar to what is observed in *C. pepo* and in *C. maxima*, a single recessive gene is responsible for the lethal yellow seedling phenotype in *C. moschata*. According to the rules for gene nomenclature, we propose to name this gene as *ys* as defined for *C. pepo* and *C. maxima*.

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Table 1: Expected and observed normal green to lethal yellow seedlings for *C. moschata* families derived from the Japanese cv. Futtu and their respectively chi-square test values.

Family number	Expected number of seedlings		Observed number of seedlings		X^2	P-value (%)
	Green	Lethal Yellow	Green	Lethal Yellow		
1	18	6	20	4	0.89	34.58 ns
2	15	5	13	7	1.07	30.17 ns
3	15	5	17	3	1.07	30.17 ns
7	15	5	16	4	0.27	60.56 ns
10	14.25	4.75	12	7	1.42	23.32 ns
12	15	5	16	4	0.27	60.56 ns
13	12.75	4.25	15	2	1.59	20.76 ns
17	17.25	5.75	20	3	1.75	18.54 ns
22	18	6	15	9	2.00	15.73 ns
23	18	6	21	3	2.00	15.73 ns
26	18	6	18	6	0.00	100.0 ns
28	18	6	17	7	0.22	63.73 ns
31	12.75	4.25	13	4	0.02	88.86 ns
33	18	6	17	7	0.22	63.73 ns
36	11.25	3.75	12	3	0.20	65.47 ns
38	18	6	18	6	0.00	100 ns
39	15	5	16	4	0.27	60.56 ns
42	18	6	16	8	0.89	34.58 ns
43	18	6	13	11	5.55	1.84 *
Pooled Data	305.25	101.75	305	102	0.01	97.72 ns
O. L.[1]	96	32	98	30	0.17	68.31 ns
Total	401.25	133.75	403	132	0.03	86.13 ns

[1]= Original Line; ns= no significant at 5%; *= significant at 5%.

Pictures: Normal and lethal yellow seedlings in *C. moschata*.

2013 Gene List for Other Genera of Cucurbitaceae

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The Cucurbitaceae includes many important vegetables species, including cucumber, melon and watermelon. Those are major crop species originally from the Old World: cucumber from India; melon and watermelon from Africa (Wehner and Maynard, 2003). However, there are other important species originally from Africa such as gherkin (*Cucumis anguria*), African horned melon (*Cucumis metuliferus*), bottle gourd (*Lagenaria siceraria*); and species originally from India such as sponge gourd (*Luffa* spp.), Melothria (*Melothria maderaspatana*) and bitter melon (*Momordica charantia*). They have fruit that are used for food, decoration, containers, utensils or sponges. The exception is *Melothria*, which has medicinal uses (Iman *et al.*, 2006).

Gene List Update

The following list is the latest version of the gene list for the other genera of the Cucurbitaceae, those that are not covered in the CGC gene lists. The genes originally were organized and summarized by Robinson (1979, 1982). The list was subsequently updated by Taja and Wehner (2009). This current gene list provides an update of the known genes, with 27 total mutants grouped by species.

Researchers are encouraged to send reports of new genes, as well as seed samples of lines having the gene mutant to the gene curator (Mark G. Hutton), or the assistant curator (Thomas C. Andres). Please inform us of omissions or errors in the gene list. Scientists should consult the list as well as the rules of gene nomenclature for the Cucurbitaceae (Cucurbit Gene List Committee, 1982; Robinson *et al*., 1976) before choosing a gene name and symbol. Please choose a gene name and symbol with the fewest characters that describes the recessive mutant, and avoid use of duplicate gene names and symbols. The rules of gene nomenclature were adopted in order to provide guidelines for naming and symbolizing genes. Scientists are urged to contact members of the gene list committee regarding rules and gene symbols. The gene curators for other genera of the Cucurbit Genetics Cooperative are collecting seeds of the type lines for use by interested researchers, and would like to receive seed samples of any of the lines listed.

This gene list has been modified from previous lists in that we have made editorial corrections, and added genes not previously described: *F1,2y* and *pia* (*Cucumis anguria*), *pm (Lagenaria siceraria*), *Rf-1* and *Rf-2* (*Luffa acutangula*), *Tlcy* and A^{dgn} (*Luffa aegyptiaca* or *L. cylindrica*).

Previous Gene Lists

Robinson, 1979: 13 genes added, 13 genes total
Robinson, 1982: 1 gene added, 14 genes total
Taja and Wehner, 2009: 6 genes added, 20 genes total
VandenLangenberg and Wehner, 2013: 7 genes added, 27 genes total

West Indian Gherkin (*Cucumis anguria*)

Six gene loci have been described so far for West Indian gherkin. A single dominant gene produces bitter fruit: *Bt* (Koch and Costa, 1991). Another dominant gene controls resistance to *Cucumber green mottle mosaic virus*: *Cgm* (den Nijs, 1982). Two loci control fruit spininess: *S and P* (Koch and Costa, 1991). A single dominant gene controls resistance to *Fusarium oxysporum* f. sp. *melonis* race 1,2y: *F1,2y* (Matsumoto and Miyagi, 2012). The resistant type line was PI 320052. A single recessive gene controls alleviation of pollen-pistil incongruity: *pia* (Matsumoto and Miyagi, 2012).

African Horned Melon (*Cucumis metuliferus*)

Watermelon mosaic virus resistance in African horned melon or kiwano is controlled by a single dominant gene *Wmv* (Provvidenti and Robinson, 1972). Another single dominant gene, *Prsv* controlled resistance to *Papaya ringspot virus* (Provvidenti and Gonsalves, 1982). The resistant type line was PI 292190, and the susceptible type line was Acc 2459.

Bottle Gourd (*Lagenaria siceraria*)

Red pumpkin beetle (*Aulacophora foveicollis*) resistance is controlled by a single dominant gene *Af* (Vashishta and Choudhury, 1972). Different genes affect shape and color of the fruit in bottle gourd. The genotype

bb produces bottle-shaped fruit, and *BB* produces disk-shaped fruit. The genotype *rr* produces round fruit shape that is also recessive to the genotype *RR*, with disk-shaped fruit. The gene *db* interacts with *b* to produce an F2 of 9 club: 3 round: 4 dumbbell-shaped fruit (Tyagi, 1976). Dark green fruit color is controlled by the genotype *GG* which is dominant to the genotype *gg* with light green fruit color (Tyagi, 1976). The genotype *lb lb* controls the light brown seed coat color, but it is recessive to the genotype *Lb Lb* with brown seed coat color (Tyagi, 1976).

Four normal-leaf parents (Pusa Naveen, PBOG 13, PBOG 22 and PBOG 61) were crossed with segmented-leaf parents (PBOG 54) of bottle gourd to study the inheritance of segmented leaf shape. Normal-leaf shape parents showed true breeding normal-leaf shape plants. However, the segmented-leaf parent (PBOG 54) surprisingly segregated in a ratio of 3 segmented: 1 normal-leaf plants. Moreover, F_1 also segregated in 1 segmented: 1 normal leaf shape suggesting that the parental cultivar PBOG 54 was heterozygous for the leaf shape gene and the segmented leaf was dominant over normal type. The segregation in the backcrosses of 1 segmented: 1 normal-leaf type confirmed that a single dominant gene *S* is responsible for the segmented leaf shape character in bottle gourd (Akhilesh and Ram, 2006).

Powdery mildew resistance was reported by Wang *et al*., (2011) as being under the control of a single recessive gene. We suggest the gene symbol *pm*, with the recessive type line J083 as the source of resistance.

Luffa Gourd (*Luffa* spp.)

Luffa species (also spelled loofah) include luffa sponge gourd or smooth luffa (*Luffa aegyptiaca or L. cylindrica*), luffa ridge gourd or angled luffa (*Luffa acutangula*). The gynoecious gene *g* (Choudhury and Thakur, 1965) interacts with andromonoecious gene *a* to produce the following phenotypes: monoecious or trimonoecious (*AA GG*), andromonoecious (*aa GG*), gynoecious (*AA gg*), or hermaphroditic (*aa gg*) plants. A single dominant gene, A^{dgn}, was reported by Singh *et al.,* (2012) to control the expression of androgynous inflorescence in *Luffa cylindrica*. The landrace Androgyn-K was used to create three inbreds, and subsequent crosses with monoecious line NDSG-5 resulted in androgynous monoecious F_1 offspring. The F_2 and BC_1 segregation data suggested inheritance was under the control of a single dominant gene (Singh *et al.,* 2012). Their report also indicated that A^{dgn} may control the number of organs produced on vine nodes. Further research should be conducted to determine the relationship of A^{dgn} to previously reported genes, including those that alter vine node organ number.

Two dominant genes restore male fertility in the presence of sterile cytoplasm in *Luffa acutangula*: *Rf-1* and *Rf-2* (Pradeepkumar *et al*., 2012).

A single dominant gene controls resistance to *Tomato leaf curl New Delhi virus* (ToLCNDV) in *Luffa cylindrica*. We suggest the gene symbol: *Tlcv*. Resistance sources include inbred lines DSG-6 and DSG-7 (Islam *et al*., 2010).

Melothria (*Melothria maderaspatana*)

Small seed size (3.0 mm) is controlled by the gene *s* (Sing, 1972) that is recessive to *SS* for large seed size (3.6 mm). White seed coat color is controlled by the gene *w.* Homozygous recessive *ww* produces a white seed coat, heterozygous *Ww* produces an ash-colored seed coat, and homozygous dominant *WW* produces a black seed coat (Sing, 1972).

Bitter Melon (*Momordica charantia*)

Light brown seeds *lbs* (Ram *et al*., 2006) is inherited as a single gene that is recessive to dark brown seeds *Lbs*. Large seed size is controlled by the gene *ls*, which is recessive to small seed size (Srivastava and Nath, 1972). White immature fruit skin is inherited as a single gene *w* for white epicarp that is recessive to *W* for green epicarp (Srivastava and Nath, 1972).

Ram *et al*. (2006) reported that gynoecism in Gy263B was controlled by a single recessive gene *gy-1*. The gynoecious plants of Gy263B had significantly longer (2000 mm) vine length than their monoecious counterparts (1275 mm).

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Table 1. The morphological and resistance genes of the miscellaneous genera and species of the Cucurbitaceae.

Symbol	Gene description and type lines	References
Cucumis anguria		
Bt	*Bitter fruit.* Fruit with bitter flavor due to a single dominant gene determined in the segregating populations of *Cucumis anguria* x *C. longipes*.	Koch and Costa, 1991
Cgm	*Cucumber green mottle resistance.*	den Nijs, 1982
F1,2y	*Fusarium oxysporum f. sp. melonis race 1,2y resistance*. Resistance controlled by a single dominant gene.	Matsumoto and Miyagi, 2012
P	*Spined fruit.* The fruit spininess is determined in the segregating populations of *Cucumis anguria* x *C. longipes* by two pairs of independent genes.	Koch and Costa, 1991
pia	*pollen-pistil incongruity alleviation*. Pollen-pistil incongruity alleviation is controlled by a single recessive gene.	Matsumoto and Miyagi, 2012
S	*Spined fruit*. The fruit spininess is determined in the segregating populations of *Cucumis anguria* x *C. longipes* by two pairs of independent genes.	Koch and Costa, 1991
Cucumis metuliferus		
Prsv	*Papaya ringspot virus resistance.* Resistance to *Papaya ringspot virus*; dominant to susceptibility.	Provvidenti and Gonsalves, 1982
Wmv	*Watermelon mosaic virus resistance.* Resistance to *Watermelon mosaic virus*; dominant to susceptibility.	Provvidenti and Robinson, 1972
Lagenaria siceraria		
Af	*Aulacophora foveicollis resistance.* Resistance dominant to susceptibility to the red pumpkin beetle.	Vashishta and Choudhury, 1972
b	*bottle.* Bottle-shaped fruit recessive to disk.	Tyagi, 1976
db	*dumbbell*. Interacts with b to produce F2 of 9 club: 3 round: 4 dumbbell-shaped fruit.	Tyagi, 1976
G	*Green.* Dark green fruit color; dominant to light green.	Tyagi, 1976
lb	*light brown seed*. Light brown seed coat color recessive to dark brown.	Tyagi, 1976
pm^z	*Powdery mildew resistance.* A single recessive gene controls resistance.	Wang *et al*., 2011
r	*round.* Round fruit; recessive to disk-shape fruit.	Tyagi, 1976
S	*Segmented leaves.* A single dominant gene which is responsible for segmented leaf shape in bottle gourd from PBOG 54 (heterozygous for segmented leaf shape).	Akhilesh and Ram, 2006

Luffa spp.		
A^{dgn}	*Androgynous*. A single dominant gene controls the expression of androgynous monoecious inflorescence.	Singh *et al*., 2012
g	*gynoecious.* Pistillate flowers only; interacts with *a* to produce monoecious or trimonoecious (*AA GG*), andromonoecious (*aa GG*), gynoecious (*AA gg*), or hermaphroditic (*aa gg*) plants.	Choudhury and Thakur, 1965
Rf-1	*Restorer of fertility 1*. One of two dominant genes having complimentary action govern fertility restoration.	Pradeepkumar *et al*., 2012
Rf-2	*Restorer of fertility 2*. One of two dominant genes having complimentary action govern fertility restoration.	Pradeepkumar *et al*., 2012
Tlcv[z]	*Tomato leaf curl New Delhi virus resistance.* A single dominant gene controls resistance to ToLCNDV.	Islam *et al*., 2010
Melothria maderaspatana		
s	*small seeds.* Small (3.0 mm) seeds recessive to large (3.6 mm) seeds.	Sing, 1972
w	*white seeds.* White seed coat if *ww*, ashy if *Ww*, and black if *WW.*	Sing, 1972
Momordica charantia		
gy-1	*gynoecious.* Recessive gene for a high degree of pistillate sex expression from Gy263B (100% gynoecious line).	Ram *et al*., 2006
lbs	*light brown seed.* Light brown seed coat color; recessive to dark brown.	Srivastava and Nath, 1972
ls	*large seed.* Large seed size; recessive to small seed size.	Srivastava and Nath, 1972
w	*white epicarp*. White immature fruit skin; recessive to green.	Srivastava and Nath, 1972

[z] Suggested gene name according to the rules of gene nomenclature for the Cucurbitaceae (Robinson *et al.,* 1976).