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Inheritance of a New Trait—Twin Fused Fruit—in Cucumber

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Abstract. A new trait, twin fused fruit, was discovered in gynoecious cucumber (*Cucumis sativus* L.) line B 5263. Plants with the twin fused fruit trait had two fruit fused into a single unit. In addition to having the twin fused fruit trait, line B 5263 had fruit with necks, large tubercles (warts), and dark green skin. The inheritance of twin fused fruit was studied in populations resulting from crosses between gynoecious line B 5263 (twin fused fruit) and monoecious line B 5404 (single fruit). Research was done in 1999 to 2001 in the greenhouses of the Research Institute of Vegetable Crops, Skierniewice, Poland. The F_1 progeny developed single fruit in all cases. The observed distribution of plant phenotypes in the F_2 fitted the expected ratio of 3 with single fruit : 1 with twin fused fruit. The observed distribution of plant phenotypes in the BC_{1A} fitted the expected ratio of 1 with single fruit : 1 with twin fused fruit. Twin fused fruit occurred only in gynoecious plants, and never in monoecious plants of the cross. In the F_2 progeny, the ratio of twin fused fruit within gynoecious plants fitted the expected ratio but the gene was not expressed in monoecious plants. In the F_2 generation, the observed distribution of plant phenotypes fitted the expected ratio of 9 gynoecious single : 4 monoecious single : 3 gynoecious twin fused : 0 monoecious twin fused, indicating that there was epistasis, with twin fused fruit hypostatic to monoecious. The new gene will be named *tf* (*twin fused fruit*).

Many genes controlling the inheritance of morphological traits have been studied in cucumber (Pierce and Wehner 1990; Xie and Wehner 2001). Knowledge of the inheritance of plant traits is essential for efficient breeding procedures.

Downy mildew caused by *Pseudoperonospora cubensis* Berk. & Curt, has been the most serious disease for cucumber production in Poland since 1985 (Doruchowski and Lakowska 1992; Doruchowski and Robak 1997; Kubik et al. 2001). The highest level of resistance to downy mildew (almost immune) was observed in line B 5263. That inbred line was originally developed by Doruchowski (Doruchowski and Lakowska 1992; Kubik et al. 2001) in a downy mildew selection program. B 5263 is characterized by high combining ability for fruit yield and quality, but has an unusual trait, where twin fused fruit and a high proportion of culls (crooked and nubbined fruit) are produced. In addition to downy mildew resistance, B 5263 has gynoecious sex expression, making it a good female parent for the production of gynoecious hybrids since the twin fused fruit trait is recessive and thus not expressed in the F1 hybrid. Gynoecious sex expression results from the genotype *FF MM* (Kubicki, 1969).

The twin fused fruit trait in line B 5263 was characterized by pairs of two separate pistillate flowers with partially joined ovaries on a single peduncle at a node developing into a twin fused fruit during development (Fig. 1). The twin was the product of the fusion of two fruit on a single peduncle. One fruit of the fused pair was almost always smaller than the other. Fruit of B 5263 were characterized by a long length to diameter (LD) ratio, necked fruit, uniform (not striped) dark green, and large warts.

A second inbred line, B 5404, was also developed by Doruchowski (Doruchowski and Lakowska 1992; Kubik et al. 2001) for downy mildew resistance, and has been used widely as a male parent in hybrid production for pickling cultivars in Poland. Line B 5404 has monoecious sex expression, short fruit with light green skin, small warts, and single (not twin fused) fruit.

The twin fused fruit trait is easily studied in either the field or the greenhouse, with similar results in segregating populations. Knowledge of inheritance of the twin fused fruit trait is important, as it may have an effect on the choice of the breeding method to be used for the introduction of resistance to downy mildew from line B 5263 into susceptible genotypes such as B 5404. It may also be possible to use this trait to study the genetic control of fruit development. Thus, we were interested to study line B 5263 to determine the inheritance of the trait that made it less attractive for horticultural use. The objective of this study was to determine the inheritance of twin fused fruit trait in B 5263 and its association with sex expression.

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Materials and Methods

Crosses were made in the greenhouse in 1999, and research was done in 2000 to 2001. Although the trait can be studied in the field, greenhouse-grown plants are easier to classify individually, so this research was done in the greenhouses of the Research Institute of Vegetable Crops, Skierniewice, Poland. The gynoecious line B 5263 (parent A, twin fused fruit) was crossed with monoecious line B 5404 (parent B, single fruit). The F_1 progeny was self pollinated to obtain the F_2 generation and backcrossed to each parent to produce the BC_{1A} ($F_1 \times P_A$) and BC_{1B} ($F_1 \times P_B$). All pollinations were made by hand.

Seeds of the parental lines, F_1, F_2, BC_{1A} and BC_{1B} were sown on May 2000 and 2001. The number of plants tested for each generation was 30 each of parents A and B, 50 of the F_1, 80 of the BC_1 to parents A and B, and 200 of the F_2. Plants were grown in the greenhouse, with temperatures set at 25/20 °C day/night. Relative humidity exceeded 90% during most of the growth season, and conditions were generally good for plant growth (although with some cloudy days). Plants were grown in a soilless mix (1 peat : 1 pine bark) in pots. Plants were watered and fertilized daily using a drip irrigation system. For the study of inheritance, there were 3 sets of 6 generations each, with a total (after pooling over sets) of 30 plants of each parent, 50 F_1, 40 of each backcross, and 200 F_2.

Plants from each generation were evaluated for sex expression and fused fruit. Sex expression was evaluated at full flowering stage. Plants were considered gynoecious if most of

Fig. 1. Cucumber line B 5263 showing the twin-fused fruit (*tf tf*) trait.

Table 1. Segregation of twin fused fruit in populations derived from crosses between B 5263 and B 5404.

Generation	Observed distribution: Single fruit	Observed distribution: Twin fused fruit	Observed distribution: Total plants	Expected ratio[z] (assuming single recessive gene): Single fruit	Expected ratio[z] (assuming single recessive gene): Twin fused fruit	χ^2	df	*P*
P_A (B 5263)	0	30	30	0	1	---	---	---
P_B (B 5404)	30	0	30	1	0	---	---	---
F_1	50	0	50	1	0	---	---	---
F_2	154	46	200	3	1	0.427	1	0.514
BC_{1A}	22	18	40	1	1	0.400	1	0.527
BC_{1B}	40	0	40	1	0	---	---	---

[z]Expected ratio assumes that twin fused fruit is the result of a single recessive gene.

Table 2. Segregation of twin fused fruit and sex expression in populations derived from crosses between B 5263 and B 5404.[z]

Generation	Observed distribution: Single fruit gynoecious	Observed distribution: Single fruit monoecious	Observed distribution: Twin fruit gynoecious	Observed distribution: Twin fruit monoecious	Observed distribution: Total plants	Expected ratio[z]: Single fruit gynoecious	Expected ratio[z]: Single fruit monoecious	Expected ratio[z]: Twin fruit gynoecious	Expected ratio[z]: Twin fruit monoecious	χ^2	df	*P*
P_A (B5263)	0	0	30	0	30	0	0	1	0	---	---	---
P_B (B5404)	0	30	0	0	30	0	1	0	0	---	---	---
F_1	50	0	0	0	50	1	0	0	0	---	---	---
F_2	107	47	46	0	200	9	4	3	0	2.996	3	0.392
BC_{1A}	22	0	18	0	40	1	0	1	0	0.4	3	0.940
BC_{1B}	21	19	0	0	40	1	1	0	0	0.1	3	0.992

[z]Expected ratio assumes that plants having twin fused fruit along with monoecious sex expression do not express the twin fused fruit trait, but are grouped with the plants having single fused fruit and monoecious sex expression.

the flowers on the plant were pistillate, and monoecious if fewer than half of the flowers on the plant were pistillate.

The plant was classified as a twin fused fruit plant when at least one twin fused fruit was developed at one node. The other fruit on a twin fused fruit plant were often irregular as a consequence of the effect of the second undeveloped ovary on main ovary. Segregation ratios for phenotypic classes were summarized and checked for expected ratios with chi-square tests (Srb and Owen 1955). Linkage analysis was not performed for gynoecy vs. twin fused fruit, since there was complete association.

Results and Discussion

The inheritance of twin fused fruit and its association with sex expression was studied in segregating populations resulting from crosses between gynoecious line B 5263 (twin fused fruit) and monoecious line B 5404 (single fruit). All plants of the F_1 progeny were 100% gynoecious and developed single fruit in two years of studies. Results indicated that B 5263 has *MMFF* genotype described by Kubicki (1969).

For the twin fused fruit trait, the distribution of F_2 plants was 154 with single fruit, 46 with twin fused fruit, and fitted a ratio of 3:1 (χ^2 = 0.427, *P* = 0.514) (Table 1). The distribution of BC_{1A} plants was 22 with single fruit, 18 with twin fused fruit, and fitted a ratio of 1:1 (χ^2 = 0.400, *P* = 0.527). All plants of the BC_{1B} generation developed only single fruit without the tendency toward twin fused fruit. Analysis of the F_1, F_2, BC_{1A}, and BC_{1B} generations indicated that the inheritance of twin fused fruit was controlled by a single recessive gene, for which we propose the symbol *tf*.

We observed that twin fused fruit occurred only in gynoecious plants, and never in monoecious plants of the crosses (Table 2). In the F_2 generation, the observed distribution of plant phenotypes was 107 gynoecious single : 47 monoecious single : 46 gynoecious twin fused : 0 monoecious twin fused. Thus, it appeared that two classes were combined, with plants having the twin fused fruit and single fruit traits along with monoecious sex expression being indistinguishable phenotypically. The observed distribution fitted the expected ratio 9:4:3:0 (χ^2 = 2.996, *P* = 0.392). The results indicated that there was epistasis, with *tftf* hypostatic to *ff*. The segregating generations also were grown in the field to determine the ease of selection against the twin fused fruit trait, and similar results were obtained (data not shown).

Nandgaonkar and Baker (1981) reported that a single recessive gene (*mp*) was responsible for multiple pistillate flowering. This may be the same gene that Fujieda et al. (1982) later labeled as *pf* for plural pistillate flowering. However, they indicated that three different alleles were responsible, with single pistillate being incompletely dominant over multiple pistillate. Thus, the allele *Pf Pf* produced plants with single pistillate flowering, *pfd pfd* produced plants with double pistillate flowering, and *pfm pfm* produced plants with multiple pistillate flowering (more than two flowers per node).

Plants with the multi-pistillate flower trait (*mp*) have separate fruit produced on separate peduncles per node. The twin fused fruit trait is similar to multi-pistillate flowering in cucumber, except that twin fused fruit is always characterized by pairs of pistillate flowers with ovaries (but not perianth) partially joined at the base on a single peduncle per node, which then develops into a twin fused fruit. Another difference between twin fused fruit and multi-pistillate flowering is that twin fused fruit is associated with gynoecious sex expression, whereas multi-pistillate flowering is not. Also, twin fused fruit has two ovaries on a single peduncle, whereas multi-pistillate flowering has multiple ovaries each on its own peduncle. It may be that the twin fused fruit line B 5263 also possesses the *mp* gene, since it has two or more peduncles with fruit at each node. We are not able to explain why twin fused fruit only develop on gynoecious plants. Perhaps twin fused fruit will develop only under the conditions where the plant is producing hormones that result in the gynoecious type.

The new gene will be named *tf* (*twin fused fruit*), and seeds of the type line (B 5263) will be sent to the curators for the cucumber gene mutants.

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