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Full Length Research Paper

Genetic identification of a dwarf mutant in cucumber (*Cucumis sativus* L.)

Ming Xin[1], Zhiwei Qin[1]*, Lei Wang[2], Tao Wu[1] and Xiuyan Zhou[1]

[1]Horticultural Department, Northeast Agricultural University, 59 Mucai Street District，Harbin 150030，China.
[2]State Key Laboratory of Urban Water Resources and Environment, Harbin Institute of Technology, Harbin, 150090, China.

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The dwarf (compact) plant architecture is an important trait in cucumber (*Cucumis sativus* L.) breeding. A dwarf type mutant was selected from the cucumbers. The morphological and reproductive characteristics of the dwarf were compared with the vine plants. The dwarf type of cucumbers is characterized by its short internodes, underdeveloped tendril and small flower, while the dwarfs possessed more female flowers, more fruits and shorter growing period than the vine plants. The phenotype of hybridization and genetic analysis indicated that the dwarf plants have a monogenic inheritance, in which the vine genotype is dominant (CP) to the dwarf genotype (cp). Based on cell-size analysis, the microstructure of internodes was recognized. The microscopic observation revealed that this dwarf appearance was the result of cell elongation inhibition.

Key words: Cucumber, genetic analysis, cell elongation, internodes.

INTRODUCTION

Cucumber, (*Cucumis sativus* L., 2n = 14) is an important specialty vegetable worldwide. However，the yield of this vegetable has been stagnant since the 1980s (Gusmini and Wehner, 2008). In order to solve the problem, great effort has been made to select high yield components with a higher heritability (Wehner, 1989; Cramer and Wehner, 2000). Reduced plant height is an important agronomic trait in cucumber breeding that has potential to be used in once-over mechanical harvest (Li et al., 2011; Mondal et al., 2011). Moreover, such traits permit higher plant populations, more pistillate flowers, less branches per plant and more concentrated maturity, which facilitates production (Wu et al., 2010; Maynard et al., 2002; Ma et al., 2004). The dwarf trait of plants, such as vegetables and crops represented a key step to make sure the spectacular increase in food production (Hedden, 2003; Sakamoto and Matsuoka, 2004; Fambrini et al., 2011). Moreover, it was reported that dwarf phenotype could reduce disease incidences. Ando and Grumet (2006) found that the dwarf mutant cucumber possess a tendency to hold young fruits off the ground, could resist the disease caused by *Phytophthora capsici* and stabilize yield. The vine cucumber, particularly of irrigated one, has increased plant height in relation to a relatively restricted root system. Therefore, when it was grown in field where strong winds blew during the fruit setting period, plants were subjected to lodging. The reduction in plant height could lead to enhanced lodging resistance. In recent years, the cultivation of short fruit cucumber has become increasingly fashionable and would be also used as houseplants if height could be effectively controlled.

In this present study, we selected a dwarf mutant cucumber D0460, a typical dwarf mutant, exhibited significantly reduced internodes length (super dwarf). F_1 generation was obtained by a cross between the dwarf and the vine lines, and experiments were designed to analyze the morphology and inheritance of the dwarf trait.

MATERIALS AND METHODS

Plant material

The dwarf cucumber D0460 was used as the female parent in this present study (Figure 1A). This line was obtained from the USDA

*Corresponding author. E-mail: qzw303@126.com. Tel: +86 (0) 451 55190588. Fax: +86 (0) 451 55190058.

Figure 1. Young vine (left) and dwarf (right) plants in cucumber (20 days).

North Central Regional Plant Introduction Station (Ames, IA, USA). This monoecious dwarf plant was self-fertilize and all of its progenies were dwarf in appearance, suggesting homozygosis. The vine (Figure1B) parent was a typical pure line in plant habit.

The single F_1 plant from the cross between the dwarf (female) and the vine (male) was self-pollinated to produce F_2 progeny. In addition, F_1 families were backcross to both parents to produce the reciprocal backcross progenies, BC_{11} (F_1 × vine) and BC_{12} (F_1 × dwarf).

Genetic analysis and measurements

Field plot design was a 6 × 10 simple rectangular lattice consisting of two replications of field plots located at the Institute of Vegetable Science of Northeast Agricultural University in 2005. Each field plot included 10 plants grown in a row, with 42 cm between plots.

Synchronous analysis of the six lines, parents, F_1, BC_{11}, BC_{12} and F_2 was carried out and the plants were classified as either of dwarf or vine phenotype based on their main stem length. The length of internode was measured on day 60 after emergence from matured plant (Figure 2). The number of male and female flowers was observed daily during the florescence. Total number of fruits was investigated when the fruits matured. Anthesis was determined as a period from the time of planting to the appearance of the first pistillate flowers in half plants. The genic pattern of inheritance by the goodness-of-fit test was estimated after evaluating the height of plant.

Microscopy

Materials for microscopy were extracted from five-week-old plant internodes of dwarf and vine-types, respectively. Tissues of internode were fixed overnight in 50% ethanol, 5% formaldehyde, and 5% acetic acid, v/v (FAA). The samples were dehydrated in a graded ethanol series and were then embedded in paraffin after a transition through xylene. Thin sections (5 mm) of specimens from each sample for microscopic observation were cut by an ultramicrotome (PowerTome-XL, RMC, USA), stained with hematoxylin. The cell sizes and shapes were examined under a light microscope (CX21-CV320, OLYMPUS, Japan) at 20× magnifications.

RESULTS

Morphology of dwarf and vine plans in the cucumber

Compared with vine plants, the dwarf showed obvious

Figure 2. Mature vine (left) and dwarf (right) plants in cucumber (60 days)

difference in morphology. The dwarf line (D0460) has remarkable phenotypes including smaller flowers, less and shorter internodes, earlier flowering, shorter tendrils (Figures 1A and B). Expression of the compact gene was generally not affected by environment. Thus dwarf plants could be relatively easy to recognize by its vine characteristics. Actually, the reduced hypocotyl length of dwarf plant, indicating the restricted growth was represented in the seedling stage. The morphology of vine and dwarf plants is compared in Table 1. Dwarf plants had shorter

Table 1. Morphological difference between vine and dwarf plants in cucumber (60 days).

Measurement	Dwarf (X±S.D.)	F_1 (X±S.D.)	Vine (X±S.D.)
Plant height (cm)	37.50 ± 1.32**	236.50 ± 37.86	238.70 ± 20.01
Number of internode	16.80 ± 4.06	16.40 ± 2.1	14.46 ± 2.8
Intermode length (cm)	15.57 ± 1.96	14.86 ± 1.56	3.37 ± 0.67**
Number of female flowers	31.63 ± 2.58	22.07 ± 0.67**	21.83 ± 0.31**
Anthesis (d)	10.70 ±1.01**	13.76 ± 1.02	13.06 ± 0.74
Total number of fruits	21.43 ±1.16	10.57 ± 0.6**	11.13 ± 0.35**

Significance *p ≤ 0.05 and **p ≤ 0.01. 10 plants were sampled from each type.

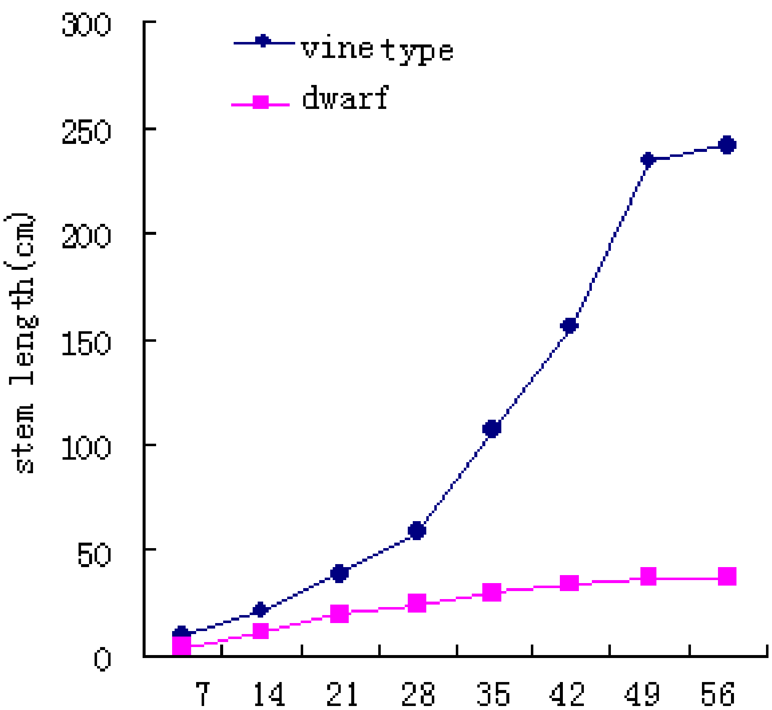


Figure 3. Growth of vine and dwarf cucumber plants (the values on the x-axis are in days).

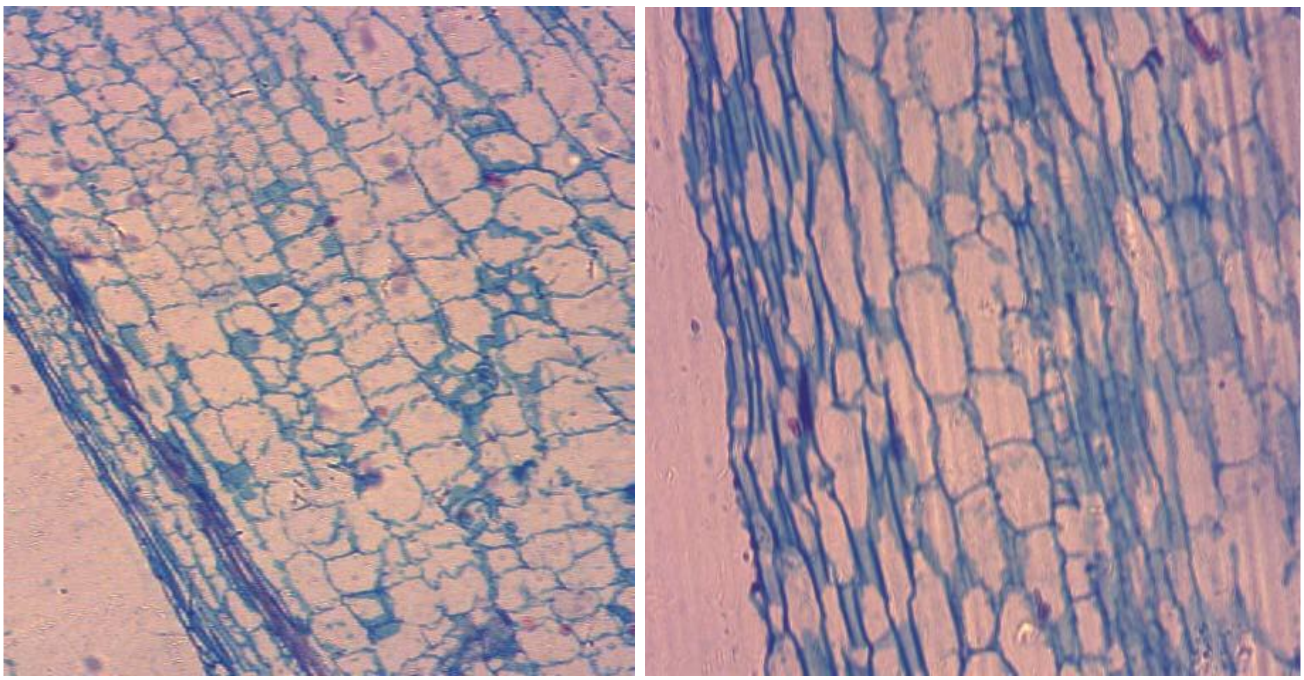
internodes, much more pistillate flowers and less internode than vine plants. Adult dwarf plants in general had a height less than 50 cm while the vine usually exceeded 200 cm (Figure 3). However, the dwarf had more female flowers (31.63 ± 2.58) per plant than the vine (21.83 ± 0.31). Also the extreme dwarf plants flowered three days earlier than vine plants. In addition, dwarf cucumber had a higher rate of fruit abortion than the vine type (data not shown), thereby had a lager total number of fruit than the vine plants in a plant basis. The morphology of F_1 plants was also investigated. There were no significant difference of vegetative characteristics found in the vine plants (homozygosis) and F_1 generation (heterozygosis) crosses between the dwarf and the vine.

Genetic analysis

To make use of dwarf-type plant architecture, it was imperative to know its mode of inheritance. Table 2 shows the genetic segregation of plant habit in cucumber plants. All F_1 plants were vine phenotype. The F_1 and the vine reciprocal BC_{11} families were all vine plant. While the BC_{12} families, reciprocal across between the F_1 and dwarf plants were segregated approximately one vine : one dwarf plant. The F_2 progeny behaved as expected on the basis of independently segregating genes for plant type, which fitted a 3:1 ratio. These results confirm the previous conclusion that compact growth habit was controlled by a simply inherited recessive gene (Kauffman and Lower, 1976).

Table 2. Number of plants for each category and goodness-of-fit test for a monogenic inheritance in cucumber.

Generation	Number of plant		Expected ratio	x^2	P-value
	Dwarf	Vine			
Vine parent	0	40	-	-	-
Dwarf	40	0	-	-	-
F_1	0	40	0:1	-	-
F_1	0	40	0:1	-	-
F_2	82	258	3:1	1.67	0.1-0.25
BC_{11} (F_1 × vine)	0	180	0:1	-	-
BC_{12} (F_1 × dwarf)	79	101	1:1	2.45	0.1-0.25



A B

Figure 4. Microstructure of the mature internode of dwarf and vine cucumber plants (5 weeks). (A) The longitudinal sections of the dwarf culture; (b) the longitudinal sections of the vine culture.

Cell length reduction possibly leading to growth restriction in dwarf plants

The reduced growth of dwarf plant could be due to less cells in the dwarf line, defect in cell elongation or cell division (Wu, et al., 2007; Fambrini et al, 2011). To distinguish between these possibilities, the internodes of the dwarfism and the vine were investigated at the cellular level. As an internode was constituted by cell ranks arranged longitudinally (Figure 4), its length was a function of the mean number of cells per rank and the mean cell size. Microscopic observation showed that the number of cells of internode was much less in dwarf plants than in vine plants (Figure 3), whereas no differences were detected in the number of cells along the length of all organs between the dwarf and vine plants (data not shown). In addition, the size of cell decreased in mature internodes of the dwarf plants (Figure 4). It suggests that the restricted growth of dwarf cucumber plants is probably caused by the failure in cell elongation

DISCUSSION

There are different views on the inheritance of stem length. Seven genes affecting stem length were identified (Pierce et al., 1990). The Crops Research Institute for

Ukraine found that the height of stem was controlled by two major genes and polygenes. In this study, the results of the genetic analysis were in agreement with the views of Kauffman and Lower (1976) that the strait of determinate growth was controlled by a recessive locus. The F_1 (vine × dwarf) and the reciprocal BC_{11} families were of normal phenotype (vine), while BC_{12} were segregated 1:1 (vine: dwarf) in this study (Table 2), indicating that the expression of dwarf phenotype was recessive. In the F_2 generation, 258 plants were vine and 82 plants were dwarf. The segregation ratio was approximately 3:1. The genetic analysis of dwarf plants in cucumber indicated that the mode of inheritance of plant habit could be considered monogenic, and dwarf (cp) was recessive to vine-type (CP).

Le´colier (2009) found that the number of cells was fixed as early as the first internode in five-month-old dwarf Bourbon, limiting the growth of internodes; whereas a compensatory process by cell division existed in vine mutant Bourbon pointu during the internodal growth. To the contrary, measurements of internode and stem length, as shown in Table 1 and Figure 3, indicates that the growth of dwarf plants was restricted immediately after germination. The number of cells in internode showed little difference between the two cultivars. The cell size of the internode was reduced in dwarf as compared to that in vine plants, suggesting a reduction in cell elongation in the dwarf cucumber line (Figure 3). Thus, the trait of dwarf plants might be due to a failure of individual cells to elongate.

Generally, dwarf cucumbers have some valuable advantages, which is, they allow higher plant densities, facili-tate cultivation, and have a more concentrated maturity than vine counterparts (Sun et al., 1990; Maynard et al., 2002). Even though the compact form produces smaller fruits than the vine, it could produce more fruits and increase yield per hectare. Therefore, the knowledge of the mode of inheritance of dwarf form in cucumber was essential in breeding procedures.

Conclusion

The morphology of vine and dwarf plants in cucumber was compared in this report. Genetic analysis of plant habit showed that a monogenic inheritance was expressed and dwarf-type (cp) was recessive to vine (CP). Microscopy observations indicated that dwarf was the result of cell elongation inhibition rather than a reduction in cell number.

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