



Forchlorfenuron Application Induced Parthenocarpic Fruit Formation without Affecting Fruit Quality of Cucumber

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Abstract: The plant growth regulator forchlorfenuron is often applied to promote fruit setting and development in cucumber production. However, the effect of forchlorfenuron on the appearance and nutritional quality of cucumber is unknown. In the present study, forchlorfenuron was applied to female flowers 1 day before anthesis and at the day of anthesis. The application of forchlorfenuron successfully induced parthenocarpic fruit formation in cucumber. In addition, cytokinin-responsive genes were upregulated under forchlorfenuron treatment. Fruit treated with forchlorfenuron did not differ from pollinated fruit in shape, texture and major nutrients, such as protein, total flavonoids and vitamin C, with the exception of the lower phenolic acid content. Overall, our finding suggests that forchlorfenuron could be vital in promoting the parthenocarpic fruit set without altering the quality of cucumber.

Keywords: cucumber; cytokinin; forchlorfenuron; fruit quality; parthenocarpy; texture



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1. Introduction

Cucumber (*Cucumis sativus* L.), which belongs to the Cucurbitaceae family, is an important vegetable crop that is widely cultivated worldwide. In China, the cultivated area of cucumber is 1.258 million hectares, with an annual output of 70.338 million tons, ranking first in the world (FAOSTAT 2019, <http://faostat3.fao.org>, 30 March 2021). Parthenocarpy is the development of fruit without pollination or fertilization [1]. Parthenocarpic fruit formation occurs in many horticulture crops such as tomato, grapes, watermelon and cucumber [1–4]. Parthenocarpic fruit development has become an important agronomic trait in horticulture crop breeding, which determines the fruit yield. However, the parthenocarpy in cucumber is often adversely affected by low temperature and low light, which impede flowering and pollination of cucumbers, and finally lead to low yields and abnormal fruit [5,6].

To overcome this problem, plant growth regulators are often applied to promote fruit setting and development in cucumber [1]. Forchlorfenuron, a cytokinin-like compound, is widely used in the production of various plants, including watermelon [4], tomato [2], grapes [3] and mango [7] because cytokinins have well-known effects on cell division and expansion [8]. In north China, where the major cucumber production area is located, more than 80% of cucumber were treated with forchlorfenuron to increase the fruit setting ratio. In kiwifruit, the exogenous application of *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) also significantly increased fresh weight and overall yield [9]. These results indicate that the application of plant growth regulators could enhance the parthenocarpy fruit formation

and finally increase the yield of horticulture crops. However, consumers raised concerns about the quality of fruit produced via the application of growth substances, including forchlorfenuron. For example, the CPPU treatment increased the vitamin C content of mango [7]. On the other hand, in triploid watermelon CPPU treatment resulted in lower sugar contents [4]. Similarly, reduced soluble sugar of pears post CPPU treatment was recorded [10]. In cucumber, individual treatments with CPPU, naphthaleneacetic acid and gibberellin A4+A7 (GA₄₊₇) did not affect the appearance of cucumber at harvest and after storage, while CPPU treatment decreased the phenolic acid and vitamin C contents [5]. Owing to the above concerns, it is important to investigate the effect of forchlorfenuron on cucumber parthenocarpic fruit formation and fruit quality.

Cucumber is popular for its crisper texture, flavor taste and nutrients. In the present study, we firstly examined the effect of exogenous forchlorfenuron on cucumber fruit development. Then, the effect of forchlorfenuron on fruit texture (water content and firmness), protein, vitamin C, total flavonoids phenol and phenolic acid content were analyzed. Finally, the expression level of cytokinin signaling transduction related genes was examined to reveal the possible mechanism under forchlorfenuron-induced fruit formation.

2. Materials and Methods

2.1. Plant Materials, Treatments and Growth Conditions

Cucumber line ‘Zaokang’, with a low parthenocarp ratio, was used in the present study. Seedlings were planted in a plastic greenhouse in the experimental farm of Yangzhou University. The fruit and flowers in the first six nodes of each plant were removed to unify the growth stages of the flowers.

The female flowers above the sixth nodes were pinched by clips at one day before anthesis to prevent pollination (unpollinated treatment). Then, a 100 mg/L forchlorfenuron solution containing 0.2% Tween 100 was sprayed onto the pinched flowers 1 day before anthesis and on the day of anthesis. The pollinated fruit were used as control. To evaluate their appearance, the fruit were harvested at 0, 1, 2, 4, 6, 8 and 10 days after anthesis. Nutrient characters were evaluated at 8 days after anthesis. The expression of cytokinin signaling transduction genes was analyzed at 0, 1 and 2 days after anthesis. Twenty fruit were selected for each treatment.

2.2. Measurement of Fruit Length, Weight, Transverse Diameters and Flesh Firmness

Fruits were weighed at indicated stages. Fruit length and the transverse diameter were measured. Pulp firmness was measured with a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalming, UK) according to the method of Qian et al. [5].

2.3. Measurement of Water Content

Each group of samples was weighed before and after drying. Water content was calculated as $(M_i - M_f) \times 100 / M_i$, where M_i and M_f are the initial and final weights after 48 h of drying.

2.4. Measurement of Protein, Vitamin C, Total Flavonoids, Total Phenol and Phenolic Acid

Protein content was measured by the method of Bradford [11]. Vitamin C was measured as described by Cruz-Castillo et al. [9]. Total flavonoids and phenol were measured according to Dewanto et al. [12]. Phenolic acid was determined according to Qian et al. [13].

2.5. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Analysis

The total RNA extraction and qRT-PCR analysis were performed as described by Qi et al. [14]. Gene-specific primers were designed according to the reference UniGene sequences by using Primer Premier 5.0 (Supplementary Table S1).

2.6. Data Analysis

The data were expressed as mean $\pm$ standard deviation (SD) for at least 3 biological replications. ANOVA was performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The difference between the means was analyzed by Duncan's new multiple range test. The different lower case letter indicated the significance at 0.05 level. The * and ** indicated the significance at 0.05 and 0.01 level, respectively.

3. Results and Discussion

3.1. Effects of Forchlorfenuron on Cucumber Fruit Development

Fluctuation in environmental conditions often has a negative influence on the development of cucumber fruit. In the present research, both forchlorfenuron application and pollination successfully induced fruit development, while the growth of untreated fruit ceased 2 days after anthesis (Figure 1). The transverse diameter and weight of forchlorfenuron-treated fruit were greater than the pollinated fruit at 8 and 10 days after anthesis (Figure 1B,C), while the two types of fruit had similar lengths (Figure 1A). Our results confirmed the role of forchlorfenuron in inducing fruit formation.

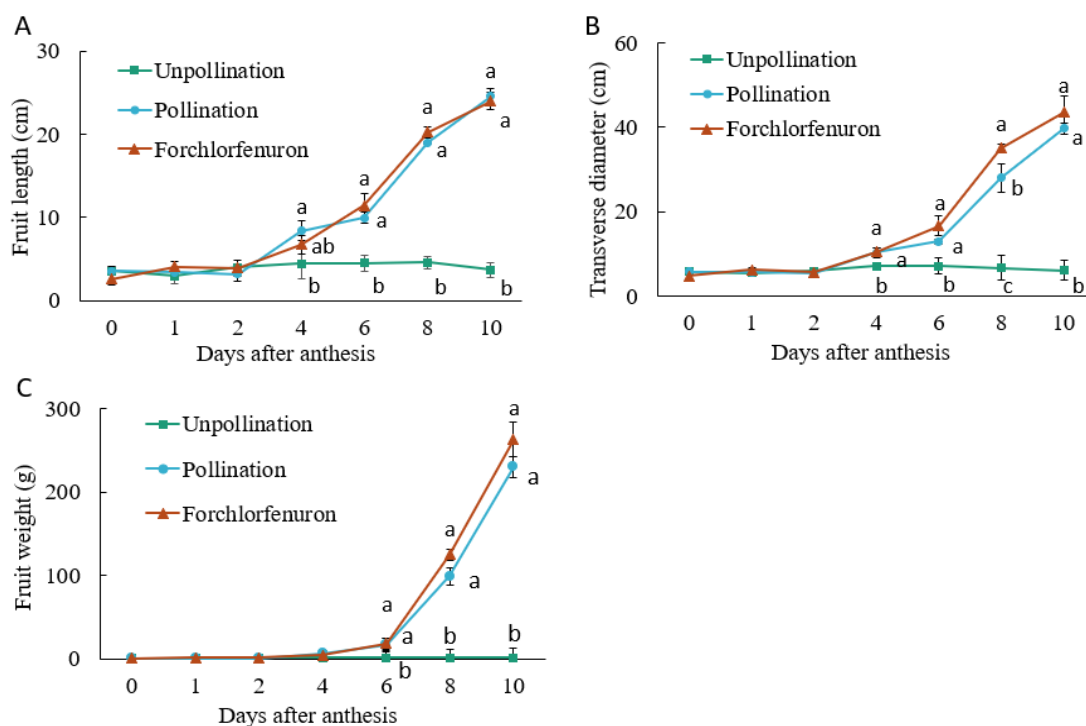


Figure 1. Effects of forchlorfenuron, pollination and non-pollination on fruit length (A), transverse diameter (B) and weight of cucumber fruit (C). Values followed by a different lower case letter are significantly different ($p < 0.05$).

Numerous studies have shown that CPPU can induce parthenocarpic fruit formation in watermelon and pear [10,15]. In watermelon, the growth rate of pollinated fruit exceeded that of most watermelons treated with CPPU at 10 to 20 days after flowering, but the final fruit sizes under these two treatments were similar [15], which is in agreement with the presented results. In pears, CPPU and GA₄ increased the fruit core ratio (transverse diameter of core/transverse diameter of fruit) [10]. In the present study, the fruit shape was not significantly affected by forchlorfenuron. Our results further indicate that the application of forchlorfenuron did not alter the shape or the harvesting time of cucumber.

3.2. Effects of Forchlorfenuron on Fruit Texture

The worldwide popularity of cucumber is largely due to its crisp texture. We evaluated water content and flesh firmness because of their importance in determining cucumber

texture. Forchlorfenuron treatment increased the water content and flesh firmness, but a significant difference was only observed for water content (Figure 2). These results are in agreement with previous reported studies in pears and cucumber [5,10], whose flesh firmness was also increased by CPPU. The increase in water content and flesh firmness can reduce the respiration rate of the fruit; thus, maintaining its freshness during storage and transportation, sustaining the freshness, and is conducive to commodity processing and longer trading.

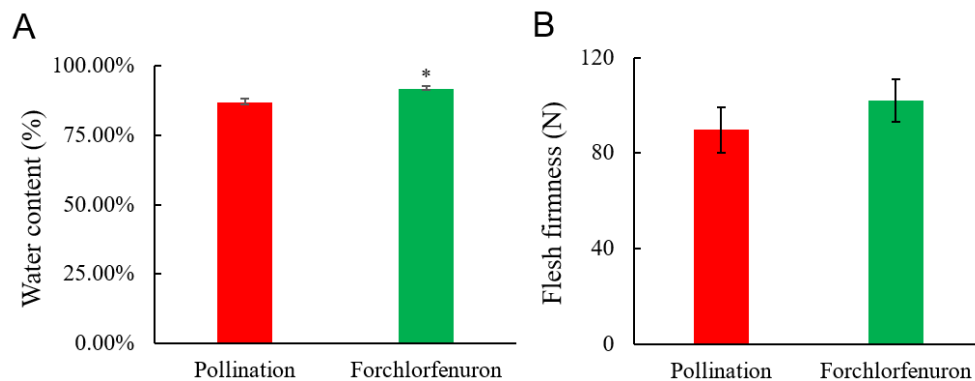


Figure 2. Effects of forchlorfenuron and pollination on water content (A) and flesh firmness (B) of cucumber fruit. * $p < 0.05$.

3.3. Effect of Forchlorfenuron on Nutrient Quality of Fruit

Forchlorfenuron treatment did not cause any significant changes in the contents of protein, vitamin C, total flavonoids and phenol (Figure 3A–D). CPPU treatment also did not affect protein, vitamin C and total flavonoids of cucumber [5]. However, CPPU treatment significantly reduced the total phenol content in potato [16]. On the other hand, CPPU treatment augmented the total phenol content of grapes, making them more astringent [3]. CPPU did not affect the vitamin C content of kiwifruit [9], while it increased the vitamin C content of mango [7]. Phenolic acid content was significantly lower in forchlorfenuron-treated cucumber fruit than in pollinated fruit (Figure 3E). Similarly, CPPU reduced the level of phenolic acid in cucumber fruit kept in cold storage, but not in fresh fruit [5].

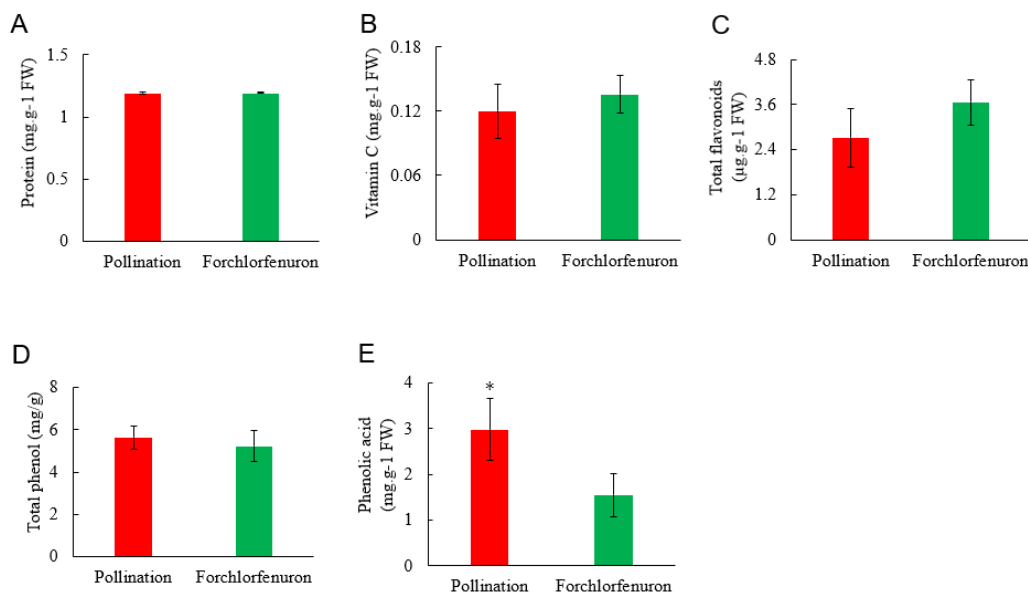


Figure 3. Effects of forchlorfenuron on the content of protein (A), vitamin C (B), flavonoids (C), total phenol (D) and phenolic acid (E) of cucumber fruit. * $p < 0.05$.

3.4. Expression of Cytokinin-Responsive Genes

Response regulator genes (*CsRR3/4a*, *CsRR3/4b*, *CsRR8/9a*, *CsRR8/9b*, *CsRR8/9c*, *CsRR8/9d*, *CsRR8/9e* and *CsRR16/17*) are markers of cytokinin signaling [2]. Application of forchlorfenuron one day before anthesis significantly up-regulated the expression levels of eight *CsRR* genes at the day of anthesis (Figure 4), which indicated that the inner cytokinin level was induced by forchlorfenuron treatment. It was reported that the parthenocarpic ability between different cucumber varieties is related to inner CK concentration and RR genes expression. It is also speculated that CK biosynthesis and signal transduction are involved in the induction of parthenocarpic fruit formation of cucumber [17]. Therefore, the enhanced cytokinin signaling transduction under forchlorfenuron treatment is one important cause for cucumber fruit initiation and development. In tomato, cell division before flowering is the stage that determines fruit size [2]. In the present study, *CsRRs* were highly up-regulated in forchlorfenuron-treated fruit at the day of anthesis. In addition, the difference among forchlorfenuron-treated and unpollinated fruit decreased two days after anthesis. This indicated that the day of anthesis is the key stage of cell division that regulates fruit development.

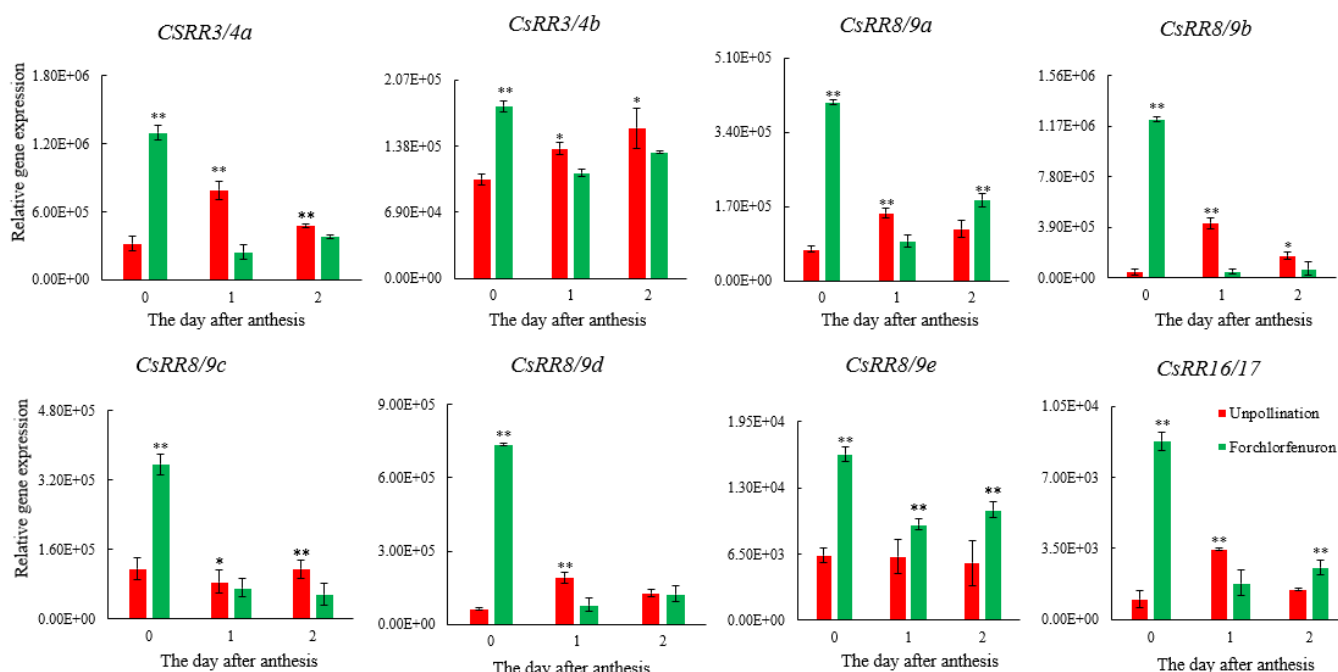


Figure 4. Expression of cytokinin-responsive genes in forchlorfenuron-treated and the control plant at 0, 1 and 2 days after anthesis. * $p < 0.05$, and ** $p < 0.01$.

4. Conclusions

The exogenous application of forchlorfenuron over the unpollinated ovaries of cucumber induced parthenocarpic fruit setting by up-regulating the cytokinin signaling genes. Forchlorfenuron treatment did not affect the shape, texture or major nutrients such as protein, total flavonoids and vitamin C content, but it did decrease phenolic acid content.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7060128/s1>, Table S1: Primer sequences used for qRT-PCR analyses.

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Abbreviations

CPPU: N-(2-chloro-4-pyridyl)-N'-phenylurea; GA: gibberellin A; qRT-PCR: quantitative real-time polymerase chain reaction; RR: Response regulator.

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