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# Effects of plant growth regulator (2,4-D) treatments on fruit development and chemical content in the kiwifruit plant (*Actinidia chinensis*)

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**Abstract**

As a dioecious fruit tree, Kiwifruit requires pollination for female plants to bear fruit. This study used 2,4-Dichlorophenoxyacetic acid (2,4-D), a plant growth regulator, to induce fruit set, improve quality and increase kiwifruit yield in *Actinidia chinensis* var. 'Donghong' without pollination. Exogenous application of 2,4-dichlorophenoxyacetic acid (2,4-D) effectively triggered parthenocarpic fruit development in 'Donghong' kiwifruit (Actinidia chinensis var. 'Donghong'), while significantly improving fruit quality. The fruit set rate of parthenocarpic fruits reached 16.95% with 2,4-D treatment at 140 days after flowering; female flowers treated with 2,4-D showed differences in pollen structure compared to those treated with water. Also, no viability was detected in female flower pollen treated with 2,4-D. At the maturity stage, the fruits from the 2,4-D treatment were slightly smaller than those from the control group. The 2,4-D treatment group showed significant differences from the control group in skin hardness, flesh firmness and core hardness. There was no significant difference in the soluble solids content between the fruits treated with 2,4-D and those from the control group at maturity. However, the dry matter content of the 2,4-D treated fruits was lower than that of the pollinated fruits. The soluble sugar content of mature fruits from 2,4-D treatment reached 18.59 g/kg, and organic acid content was 18.10 g/kg, resulting in a significantly higher sugar-acid ratio than the control group. The seeds from 2,4-D treated fruits failed to germinate normally. This technology can facilitate the induction of fruit sets in actual production, which has significant practical value and potential in kiwifruit processing. From these results, important data were obtained regarding the establishment and production of kiwifruit orchards without including pollinator varieties, which take up a significant amount of space in the orchard establishment and cause a decrease in the yield obtained from the total area.

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**Keywords** Kiwifruit, 2,4-D, Parthenocarpy, Fruit quality, germination

## Introduction

The kiwifruit is a perennial deciduous vine of the genus Actinidia, which contains 54 species and 21 varieties, 52 of which originate from China [1]– [2]. There are many varieties of kiwifruit in China, including *Actinidia chinensis* Planch. having the largest commercial cultivars and the most significant area under cultivation [3]. Kiwifruit is characterized by its high nutritional content, which is attributable to its high sugar and vitamin C content, as well as amino acids, proteins, and minerals such as calcium, phosphorus, potassium, and iron [4]. The versatility of kiwifruit is evidenced by its consumption in a variety of forms, including fresh, processed, and as part of a wide range of products, such as canned food, jams, dried fruit, fruit juice, fruit wine, and dried fruit [5].

Kiwifruit is a functional dioecious plant, with female flowers possessing both sex organs but with abortive stamens that do not produce viable pollen and male flowers with abortive pistils that do not form ovules. Female plants are much more economically productive than males, but females depend on male pollination for fruit sets [6]. The pollination of female flowers is influenced by insect activity and the natural environment in which kiwifruit trees are cultivated [7]. Pollen donor male vines or manual pollination techniques are employed to ensure fruit set. Diseased pollen can act as a vector for plant viruses, spreading to healthy plants through pollination, which adversely impacts not only the plant's health but also the fruit's quality and yield. For instance, in the case of raspberry (*Rubus idaeus* L.), the application of pollen containing Raspberry Bush Dwarf Virus (RBDV) results in the production of 'loose' fruits comprising a high proportion of immature drupes [8]. Consequently, utilizing exogenous plant growth regulators to induce unisexual fruiting in kiwifruit constitutes a contingency measure to ensure yields under extreme weather conditions and a preventive measure against tree infections caused by pollen-borne diseases. To a certain extent, this measure also solves the problem of low fruit sets caused by insufficient pollination conditions. Since kiwifruit relies on pollination of male plants, which is labor-intensive and costly, applying 2,4-D can significantly reduce labor requirements. Moreover, seedless kiwifruit offers distinct advantages in processing due to the absence of seeds. It showed seedless kiwifruit effectively prevents off-flavors caused by lipid oxidation in seeds in fermentation of kiwifruit wine [9]. Consequently, production of seedless varieties contributes to the sustainable development of the kiwifruit industry.

Parthenocarpy is defined as the formation of seedless fruits in the ovary without fertilization. There are two distinct types of parthenocarpy: natural parthenocarpy and stimulative parthenocarpy. Natural parthenocarpy is a phenomenon in which the ovary (or receptacle) develops and forms a fruit without pollination or any other stimulus [10]. In contrast, stimulated parthenocarpy refers to the phenomenon that the unfertilized ovary of a plant can produce mature seedless fruits after being subjected to certain external stimuli, which include pollination, physical stimuli (temperature, light, cloud, etc.), chemical stimuli (growth regulators, etc.), and so on [11]. The application of plant growth regulators has been demonstrated to be an effective promotional factor in the context of parthenocarpy in plants. Numerous studies have explored the effects of plant growth regulators on the induction of parthenocarpy in various horticultural crops, including grapes (*Vitis vinifera* L.) [12–14].

2,4-Dichlorophenoxyacetic acid (2,4-D), a synthetic auxin analog, has been extensively studied for its role in inducing parthenocarpy (fruit formation without pollination) in plants. Research demonstrates that 2,4-D treatment significantly increases GA4 content in pear fruits, thereby promoting ovary expansion and fruit development [15]. In Cucurbita moschata Poiret., application of 50 mg/kg 2,4-D to either styles or fruit stalks was found to enhance fruit quality parameters (including Vc and β-carotene content) while simultaneously inhibiting seed development, resulting in seedless fruit formation [16]. Furthermore, when cucumber flowers were immersed in 100 mg/L 2,4-D solution two days prior to anthesis, the treatment effectively stimulated ovary enlargement and induced phenotypic modifications in cucumber fruits [17].

Plant growth regulators (PGRs) have recently been widely used to induce parthenocarpy in various horticultural crops. However, there is a lack of comprehensive studies on the induction of parthenocarpy in kiwifruit by applying growth regulators. This study investigates the use of the plant growth regulator 2,4-D to induce parthenocarpy in Donghong kiwifruit and the subsequent comprehensive chemical content of the resulting fruits. The findings provide a scientific basis for the strategic use of plant growth regulators to improve both the fruit set rate and the overall fruit quality of kiwifruit.

## Materials and methods

### Plant materials

The experiment was conducted in 2024 at the National Kiwifruit Germplasm Repository, Wuhan Botanical Garden Chinese Academy of Sciences. Three healthy, disease-free, and five-year-old *Actinidia chinensis* var. 'Donghong' trees were selected for the study. For each

tree, 250 normally developing flower buds were chosen as experimental materials.

### 2,4-D treatment of kiwifruit flower buds

The experiment was conducted as follows: flower buds at the small bud stage (approximately 5 mm in diameter) were treated with 0.5 mg/L 2,4-D growth regulator. For each treatment, 250 flower buds of *Actinidia chinensis* var. 'Donghong' was selected, and the buds were sprayed twice daily for three consecutive days; the working solution was 250 mL. Two control groups were established: (1) Water treatment (The process was carried out using purified water without adding chemical reagents to ensure a strict experimental control between the treated groups.): 250 flower buds of *Actinidia chinensis* var. 'Donghong' was selected and sprayed twice daily, with the same bagging procedure during full bloom to prevent stigma exposure; (2) Pollination treatment: Pollen from 'Moshan Xiong 2' (*Actinidia chinensis*) was uniformly applied to the stigmas of 250 *Actinidia chinensis* var. 'Donghong' flowers during full bloom. The flowers were then bagged, and the experiment was set up as a hand-pollination group.

### Scanning electron microscopy observation of pollen structure and pollen viability analysis

The bellflowers, just before opening, were collected on a sunny day. The anthers were removed and placed flat in an oven (25 °C, 24 h). After the pollen grains had dispersed, they were sieved through a fine mesh and transferred into clean centrifugal tubes. The pollen was then stored in a refrigerator at −20 °C for future use. The test pollen was carefully applied to a conductive adhesive using a sterile cotton swab, and the conductive film was coated with an ion-sputtering coater. The samples were examined and photographed using a desktop scanning electron microscope (SEM). Flowers of water treatment (C), 2,4-D treatment (2,4-D) and male flowers were collected at bellflower stage.

Pollen viability was assessed by observing pollen tube germination. Pollen was dispensed onto a slide using fine hair, and the slide was placed in a petri dish. The petri dish was then incubated in a thermostat at 25 °C for five hours. After incubation, the slide was removed and observed under a tabletop microscope (Nikon SMZ25), where it was also photographed. When examining a field of view under a microscope, the number of germinated pollen grains is counted. Pollen is considered germinated only if the pollen tube length exceeds the diameter of the pollen grain. Calculation: Pollen Germination Rate = (Total number of germinated pollen grains)/(Total number of germinated + non-germinated pollen grains) × 100%.

### Paraffin sectioning

Flower samples from different developmental stages were collected and fixed in FAA (50% ethanol) fixative, then stored at 4 °C. The conventional paraffin sectioning technique was employed, where the fixed tissue blocks were dehydrated, clarified, and embedded in wax. Subsequently, sections were cut using a rotary microtome (Servicebio PM-24), followed by drying, dewaxing, staining with toluidine blue, clearing, and mounting. Finally, the samples were examined under a Leica DM6B orthogonal fluorescence microscope, and images were captured. Flowers of water treatment (C) and 2,4-D treatment (2,4-D) were collected at bellflowers (F1) and full bloom (F2), and fruits of pollination treatment (P) and 2,4-D treatment (2,4-D) were collected at 3 days after flowering (3 DAF).

### Morphological observation of flower and fruit development and dynamic measurement of fruit phenotype

During the study, flowers of water treatment (C) and 2,4-D treatment (2,4-D) were collected at F1 and F2, and fruits of pollination treatment (P) and 2,4-D treatment (2,4-D) were collected at 3 DAF, 20 DAF, 50 DAF, 100 DAF, 140 DAF. A motorized somatic fluorescence microscope imaging system and a Nikon D7500 camera were used to observe and capture images of the flowers and fruits during development. After fruit set, ten fruits were measured at each stage. The transverse, lateral, and longitudinal diameters of the fruits were recorded using Vernier calipers.

### Determination of fruit quality

Fruits of pollination treatment (P) and 2,4-D treatment (2,4-D) were collected at 100 DAF and 140 DAF for analysis, with 5 fruits randomly chosen at each time point. The weight of a single fruit was measured using an ME1002T/02 electronic balance, and the soluble solids content was determined with a PAL-1 refractometer. The dry matter content was measured using an LT-82 dry fruit machine. Additionally, the texture parameters of Actinidia chinensis var. 'Donghong' were evaluated using a texture analyzer by performing a puncture test, including peel hardness, peel rupture distance, flesh brittleness, core hardness, and fruit compactness.

### Extraction and quantification of soluble sugars and organic acids

The soluble sugars and organic acid components were quantified using High-Performance Liquid Chromatography (HPLC). Fruits of pollination treatment (P) and 2,4-D treatment (2,4-D) were collected at 100 DAF and 140 DAF. Fruits of pollination treatment (P) and 2,4-D treatment (2,4-D) were collected at 100 DAF and 140

DAF, with 10 fruits randomly chosen from each time. The fruits were cut into small pieces and homogenized in liquid nitrogen, and approximately 1 g of each sample was placed in a 15 mL centrifuge tube. 5 mL of ultrapure water was added to each tube, followed by shaking and ultrasound treatment for 30 min. The samples were then centrifuged at 7500 × g for 10 min, and the supernatant was collected and filtered using a 0.22 × 0.13 mm syringe filter. The conditions for determining soluble sugar components and their contents were as follows: The column temperature was set to 90 °C, with ultrapure water as the mobile phase, and a flow rate of 0.5 mL/min. The injection volume was 20 μL per sample. The conditions for organic acid components and their contents were as follows: An Athena C18-WP column (4.6 × 250 mm, 5 μm, CNW) was used, with a column temperature of 40 °C and a flow rate of 0.8 mL/min. The injection volume was 10 μL, with detection at a wavelength of 210 nm. The mobile phase was a 0.02 mol/L $KH_2PO_4$ solution (pH 2.4).

### Seed germination

Mature seeds were collected and rinsed 5–6 times with distilled water. After washing, the seeds were placed on filter paper to remove surface moisture. Fifty seeds were then selected and placed in Petri dishes for germination. Once germination occurred, seedling morphology was photographed using a Nikon D7500 camera and the germination rate was recorded. Calculation: Germination rate = (Number of germinated seeds/Total number of seeds) × 100%.

### Data analysis

Data analysis and chart creation were performed using Excel 2021, while significant differences were analyzed using GraphPad Prism 8 and SPSS 20.0.

## Results

### Effects of 2,4-D treatment on flower development in kiwifruit

#### *Floral organ morphology*

2,4-D treatment was applied to 'Donghong' kiwifruit during the F0 stage, resulting in a fruit set rate of approximately 17%. No significant differences in floral organ development were observed at the F1 stage compared to the control group. The longitudinal section of the ovary in the F3 stage of the control group was nearly elliptical. However, after 2,4-D treatment, the upper portion of the ovary, near the stigma, was noticeably larger than the lower part near the receptacle. This phenomenon became more obvious after the 3 DAF period (Fig. 1).

### Pollen morphology and pollen viability

A comparison was made between the morphology of pollen grains from normal male flowers of *Actinidia chinensis* and those from female flowers in the control group. It was observed that the pollen exhibited a trilobate-round shape in the polar view, while there were significant differences in the equatorial view. Pollen from normal male flowers was oblong, whereas the pollen from female flowers in the water control group displayed a nearly globose shape. In the 2,4-D treatment group, the pollen exhibited a trilobate-round shape in the polar view. In contrast, in the equatorial view, the pollen appeared nearly globose compared to the normal male flowers of *Actinidia chinensis*. The water control and 2,4-D treatment groups exhibited pollen with a trilobate-round morphology in the polar view and subglobose morphology in the equatorial view. However, the pollen from the 2,4-D treatment group appeared fuller (Fig. 2A a-c). The size of the pollen grains was measured, and it was found that the pollen from the 2,4-D treatment group was smaller than that from the water control group (Fig. 2B).

The pollen viability of male flowers from *Actinidia chinensis* was 43.12%, whereas the pollen from female flowers in both the water control and 2,4-D treatment groups was found to be non-viable. Comparison of pollen viability between male and female flowers in the control group and female flowers in the treatment group revealed that plant growth regulators had minimal effects on pollen viability of female flowers in *Actinidia chinensis* (Fig. 2A d-f).

### Histological observation of flower development

The ovary and ovules were examined through paraffin sections to investigate further the effect of 2,4-D treatment on the histomorphology of flower development. The results showed that the ovules within the ovary of the 2,4-D treatment group underwent gradual degeneration after the full bloom stage and detached from the placenta three days after flowering, in contrast to those in the pollination treatment group. In contrast, the ovules of the pollination treatment group developed normally. (Fig. 3A a-f). Moreover, the thickness of the ovary wall increased after 2,4-D treatment, reaching 1000 μm at full bloom and 1500 μm three days after flowering, which was significantly greater than that observed in the pollination treatment group at the corresponding stages (Fig. 3C). A comparison of the cell size in the ovary wall revealed that the average cross-sectional area of the cells was more significant in the 2,4-D treatment group than in the pollination treatment group. However, the difference was not statistically significant (Fig. 3D). At 150× magnification within a fixed field area (34,457.6 $\mu m^2$), the 2,4-D-treated group exhibited significantly fewer cells compared to the pollinated group, a phenomenon potentially associated with reduced cell density due to increased cell volume (Fig. 3E).

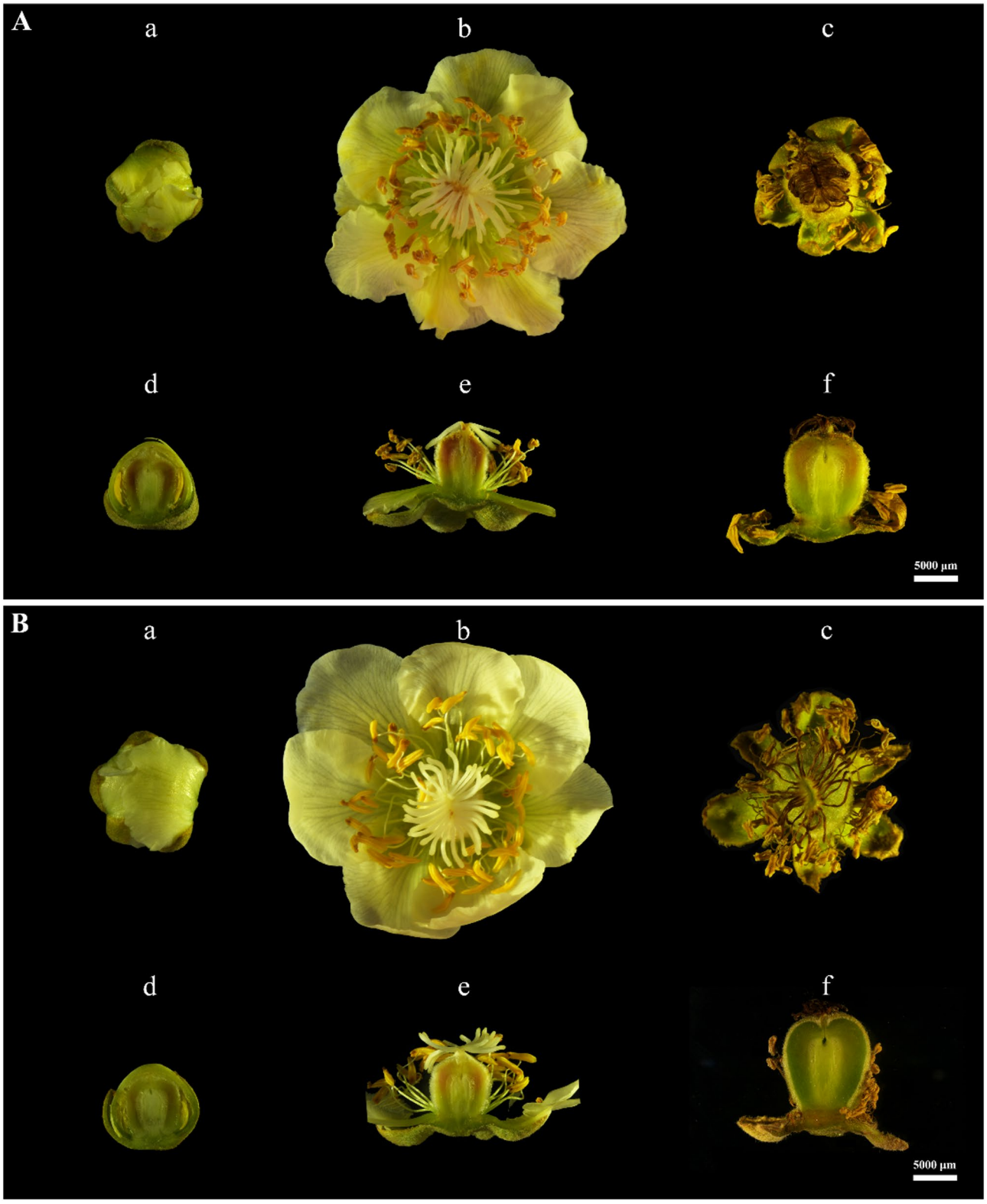



**Fig. 1** Morphological diagram of flower development stage after pollination treatment and 2,4-D treatment of 'Donghong' kiwifruit. (**A**) Control treatment groups; (**B**) 2,4-D treatment groups; (a) intact flower morphology in F1; (b) intact flower morphology in F2; (c) intact fruit-sitting morphology in 3 DAF; (d) flower longitudinal cut morphology in F1; (e) flower longitudinal cut morphology in F2; (f) fruit-sitting longitudinal cut morphology in 3 DAF

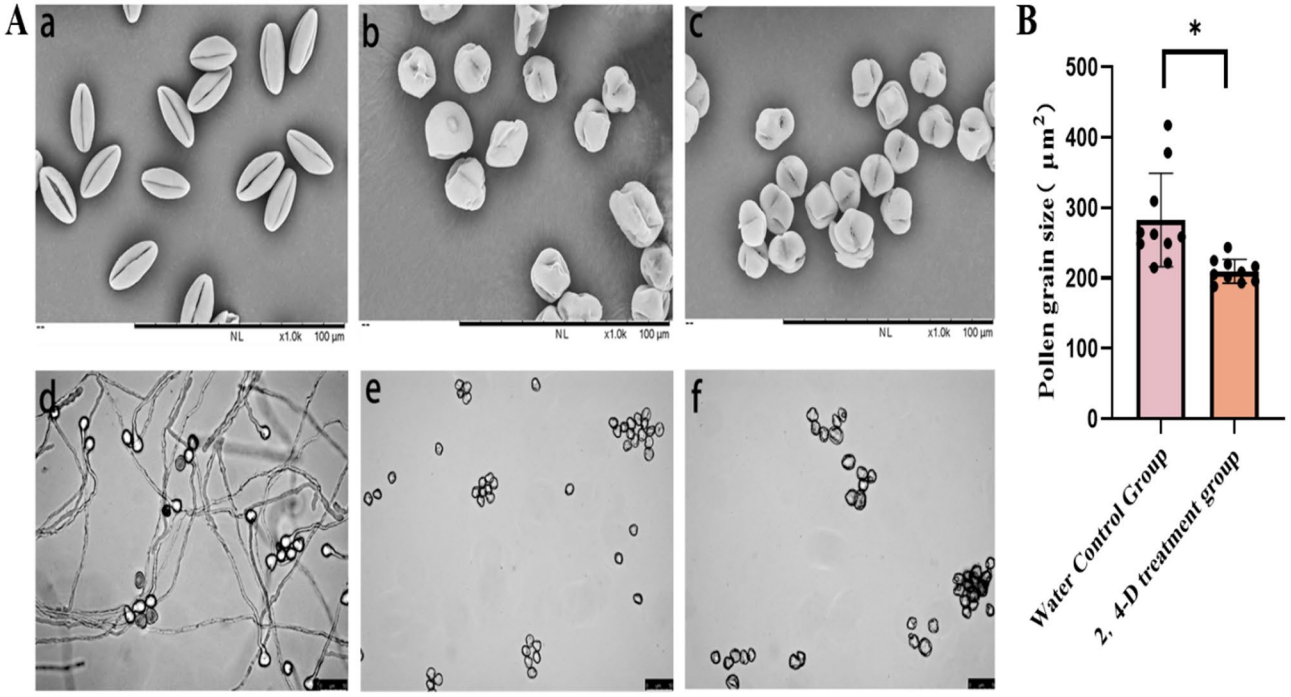



**Fig. 2** Pollen morphology and pollen viability of *Actinidia chinensis* (**A**), Pollen grain size (**B**). (a) male pollen grains of *Actinidia chinensis*; (b):'Donghong'kiwifruit pollen (water control group); (c):'Donghong'kiwifruit pollen grains (2,4-D treatment group); (d): Pollen tube germination of male flowers of *Actinidia chinensis*; (e):'Donghong 'kiwifruit pollen tube germination (water control group); (f): 'Donghong 'kiwifruit Pollen tube germination (2,4-D treatment group); the statistical methods used One-way ANOVA; * represent significant difference at p<0.05. ** represent extremely significant difference at p<0.01

## The effect of parthenocarpy induction on the fruit quality of 'donghong' kiwifruit

### *Fruit formation and development*

This study focused on the impact of 2,4-D treatment on fruit development, with observations of fruit morphology and characteristics at various stages. Fully developed black seeds were observed in normally pollinated fruits 100 days after flowering, while only a few seeds were present in 2,4-D-treated fruits. However, the number of seeds in the 2,4-D-treated fruits increased as the fruit developed toward maturity (Fig. 4A-B). Additionally, the lateral, transverse, and longitudinal diameters and fruit shape and weight revealed no significant differences between the 2,4-D and pollination treatments (Fig. 4C-F). Nevertheless, 2,4-D-treated fruits were slightly smaller than those from the pollination treatment at the ripening stage (140 days after flowering). When Fig. 4G is examined, it is seen that at 140 DAF, the average fruit weight in the 2,4-d treatment group was significantly smaller than that of the pollination treatment.

### Analysis of fruit weight, dry matter, soluble solids content, and texture during the mature period

There is no significant difference in the soluble solids content of fruits between the 2,4-D and pollination treatments. However, at 140 days after flowering, the dry matter content of fruit from the 2,4-D treatment was lower than that of the pollination treatment, with values of 13% and 17%, respectively (Fig. 5a). Furthermore, differences in fruit texture were noted, particularly in peel hardness and fruit firmness, which varied significantly at 100 days and 140 days after flowering between the 2,4-D treatment and the control. Peel hardness gradually declined as the fruit matured, being lower in the 2,4-D treatment compared to the control. Additionally, the Compactness of the pollination treatment was lower than that of the 2,4-D treatment 100 days after flowering. In contrast, 140 days after flowering, the compactness of the pollination treatment was higher than that of the 2,4-D treatment. No significant differences were observed in flesh firmness or flesh brittleness. However, a significant difference in core firmness was noted at 140 days after flowering, with the 2,4-D treatment exhibiting lower core firmness than the pollination treatment (Fig. 5b-f).

### Composition and content of soluble sugars and organic acids in fruits

By analyzing the soluble sugars and organic acids in the fruits during the ripening period, it was found that the contents of sucrose, glucose, and total sugars in the fruits with 2,4-D treatment were higher than those in the pollinated fruits at 100 days after flowering. Moreover, besides the sucrose, glucose, and total sugar contents, the contents of fructose in the fruits with 2,4-D treatment

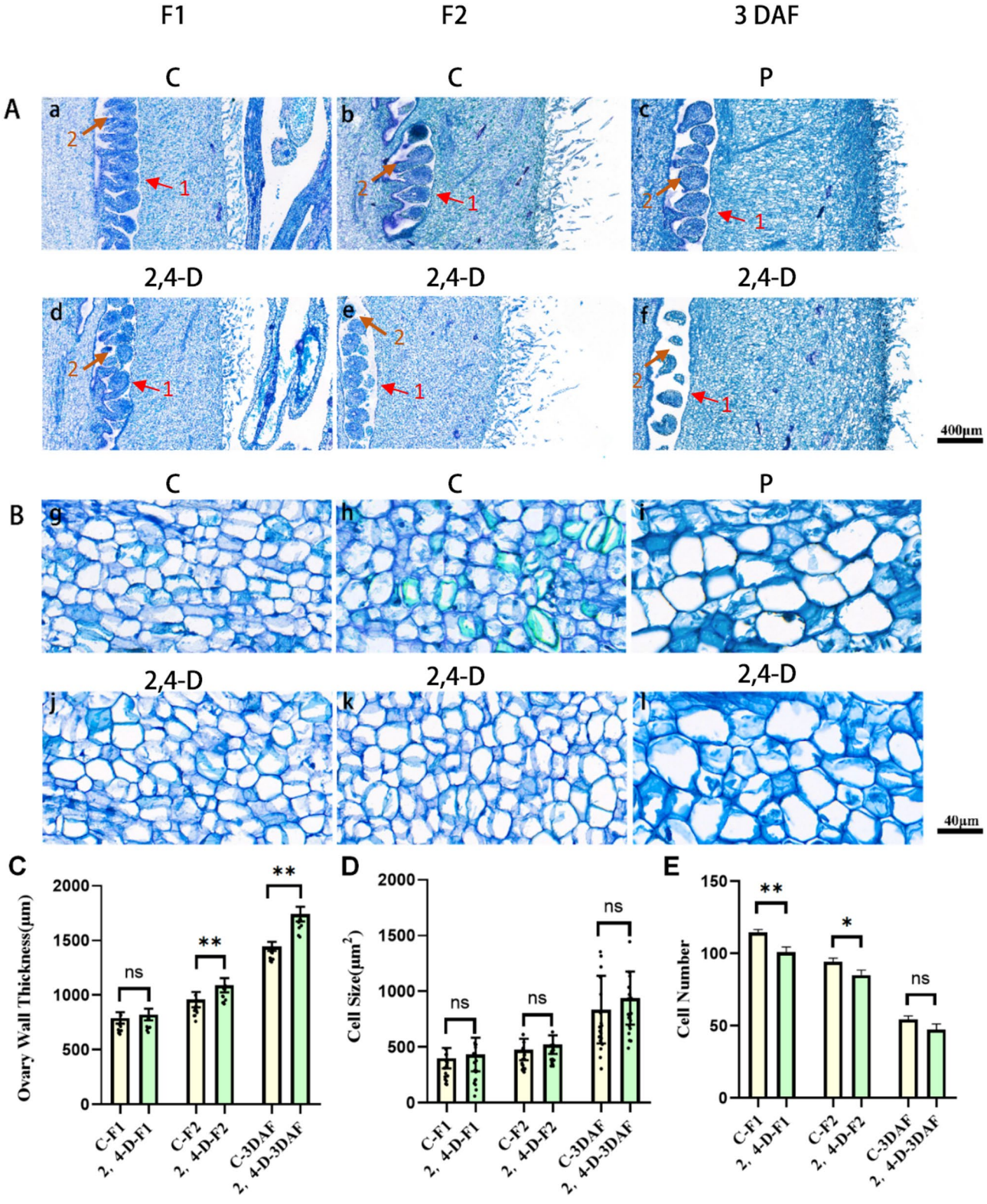



**Fig. 3** (See legend on next page.)

(See figure on previous page.)

**Fig. 3** Histological observation of different treatments at various stages of flower development of 'Donghong' kiwifruit. (**A**) 'Donghong' kiwifruit ovary microscopic transverse section; (a) ovary microscopic transverse section in control group at F1; (b) ovary microscopic transverse section in control group at F2; (c) ovary microscopic transverse section in control group at 3 DAF; (d) ovary microscopic transverse section in 2,4-D treatment group at F1; (e) ovary microscopic transverse section in 2,4-D treatment group at F2; (f) ovary microscopic transverse section in 2,4-D treatment group at 3 DAF; (**B**) the microscopic transverse section of the ovary wall cells; (g) control group F1 period; (h) control group F2 period; (i) control group 3 DAF period; (j) 2, 4-D treatment group F1 period; (k) 2,4-D treatment group F2 period; (l) 2,4-D treatment group 3 DAF period; (**C**) ovary wall thickness; (**D**) cell size; (**E**) number of cells; water treatment was control at F1 and F2, pollination treatment was control at 3 DAF; C: water treatment; P: pollination treatment; 2,4-D: 2,4-D Treatment; 1: ovules; 2: ovary; the statistical methods used One-way ANOVA; * represent a significant difference at $p<0.05$. ** represent extremely significant difference at $p<0.01$

the fruits were higher than those in the pollinated fruits at 140 days after flowering, with the fructose content increasing by approximately 2 g/kg (Fig. 6 A). Regarding organic acids, significant differences were observed in the contents of quinic acid and citric acid between the 2,4-D treatment and pollinated fruits at 100 days after flowering. However, no significant difference was found in the total acid content between the two treatments. At 140 after flowering, no significant differences in quinic acid and malic acid content were observed between the 2,4-D and pollination treatments, while citric acid content was significantly lower in the 2,4-D treatment compared to the pollination treatment. The organic acid content at 140 DAF was reduced in both treatments compared to 100 DAF, with a decrease of 6 g/kg in the 2,4-D treatment and 4 g/kg in the pollination treatment, respectively (Fig. 6B). The sugar-acid ratio was significantly higher in the 2,4-D-treated the fruits than in the pollinated fruits at 140 days after flowering, reaching a value greater than 1 (Fig. 6 C).

#### Comparison of seed germination rates

Seeds were collected and cleaned at 140 DAF for germination tests. Fifty seeds were selected and placed in Petri dishes for germination. Once germination occurred, seedling morphology was photographed using a Nikon D7500 camera and the germination rate was recorded. Seed germination was observed 20 days after germination treatment. The results indicated that the seeds of kiwifruit from pollination treatment resulted in 81% seed germination, while the seeds of fruit with 2,4-D treatment presented 0% germination (Fig. 7A-B). Seeds from 2,4-D-treated fruits exhibited signs of septicity and did not show any tendency to germinate.

## Discussion

Endogenous plant hormones and external environmental factors regulate fruit development. Exogenous plant growth regulators have been shown to replace the signals typically released during pollination and fertilization, thereby regulating fruit set and early fruit development. In loquat (*Eriobotrya japonica*) production, applying gibberellic acid ($GA_3$) to flowers has been proven to induce parthenocarpy, resulting in seedless fruit [18]. CPPU treatment effectively promotes tomato parthenocarpic fruit development by increasing gibberellin and auxin levels [19]. Among these regulators, 2,4-D is commonly used to induce parthenocarpy, resulting in seedless or seed-abortive fruits in species such as pears [20], watermelons [21], and sweet chili pepper 'Shishito' (*Capsicum annuum*) [22]. Parthenocarpy occurred in *Actinidia deliciosa* var. 'Miliang No1' is made by female flowers spraying a mixture of gibberellic acid, 2,4-D, amino acids, and calcium at full bloom [23]. In addition, a level of 31.8% fruit set with 8 mg/L treatment of 2,4-D on female flowers of *Actinidia argute* [24]. A cotton swab saturated with a 2,4-D solution at a concentration of 20 ppm was carefully applied to the ovary of the flower bud, which effectively induced parthenocarpy in a Japanese Pungency-variable Sweet Chili Pepper 'Shishito' (*Capsicum annuum*) [25]. Moreover, 2,4-D has been shown to promote ovary enlargement, which was especially significant at low concentrations [26]. We attempted to research the main cultivated species of kiwifruit, aiming to explore the effects of 2,4-D treatment on Actinidia chinensis. Previous studies have found that 2,4-D can affect the parthenocarpy of kiwifruit, but there is a lack of research on the development and quality of fruits resulting from parthenocarpy. This study conducted comprehensive research on both the external attributes, including fruit weight and size, and the internal qualities, such as sugar and acid content, throughout the fruit's developmental process. Also, it offers valuable insights into the scientific selection of growth regulators for enhancing kiwifruit fruiting. Research indicated that the application of 2,4-dichlorophenoxyacetic acid (2,4-D) on kiwifruit (*Actinidia deliciosa* 'Hayward') significantly enhances fruit size, while exerting negligible effects on fruit quality parameters such as soluble solids content and acidity [27]. However, in this study, no statistically significant difference in fruit size was observed between 2,4-D-treated fruits and the control group. The sugar-acid ratio of kiwifruit is a critical determinant of its sensory qualities. Several studies indicate that the sensory qualities of kiwifruit are primarily shaped by the balance between sweetness and acidity, with the sugar-acid ratio playing a pivotal role. This harmonious balance, combined with a complex array of volatile compounds, defines the fruit's flavor and enhances its overall appeal [28]. For instance, research has indicated that flavor acceptability increases with higher Brix levels,

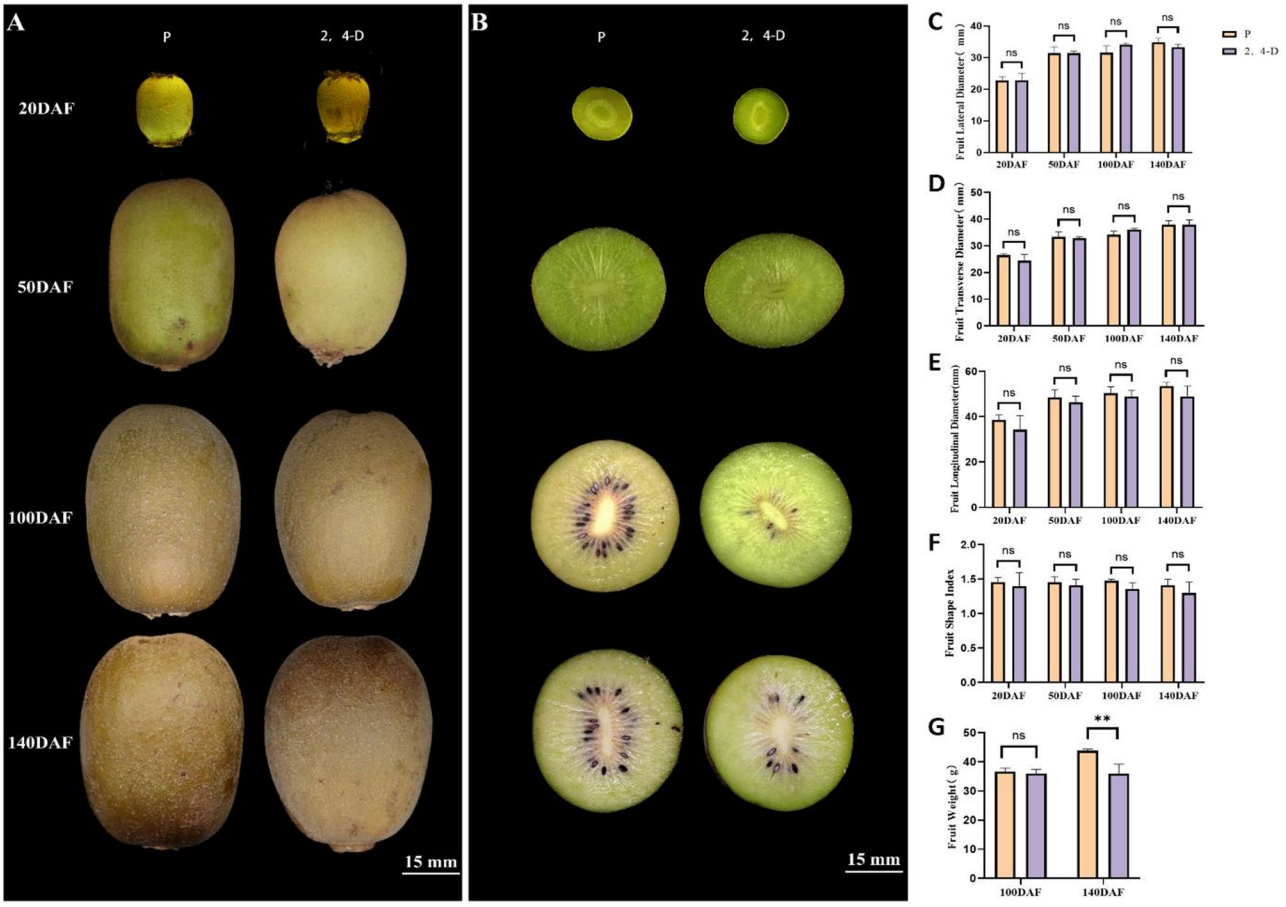



**Fig. 4** Morphological map of parthenocarpy of 'Donghong' kiwifruit in different periods and fruit transverse diameter, longitudinal diameter, lateral diameter, fruit shape index and single fruit weight. (**A**) Appearance figure; (**B**) cross-section morphological map; (**C**) fruit transverse diameter; (**D**) fruit lateral diameter; (**E**) the fruit longitudinal diameter; (**F**) fruit shape index; single fruit weight(**G**); P: pollination treatment; 2,4-D: 2,4-D treatment; the statistical methods used One-way ANOVA; * represents a significant difference at $p<0.05$. ** represents extremely significant difference at $p<0.01$

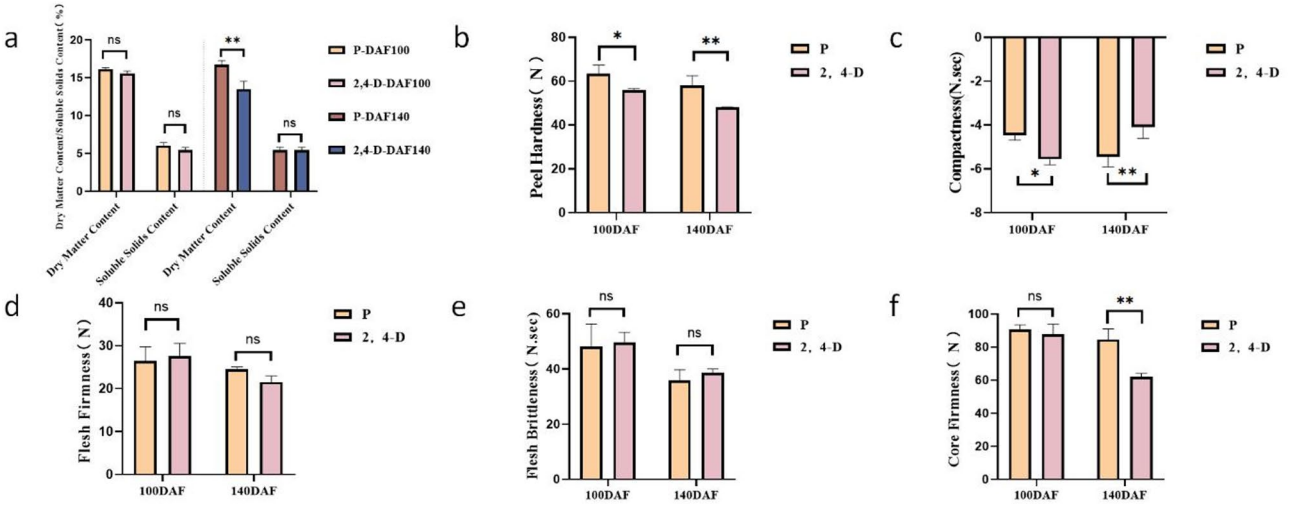



**Fig. 5** Single fruit weight, dry matter, soluble solids (**a**) and texture analysis (**b**-**e**). P: pollination treatment; 2,4-D: 2,4-D treatment; the statistical methods used One-way ANOVA; * represents a significant difference at $p<0.05$. ** represents extremely significant difference at $p<0.01$

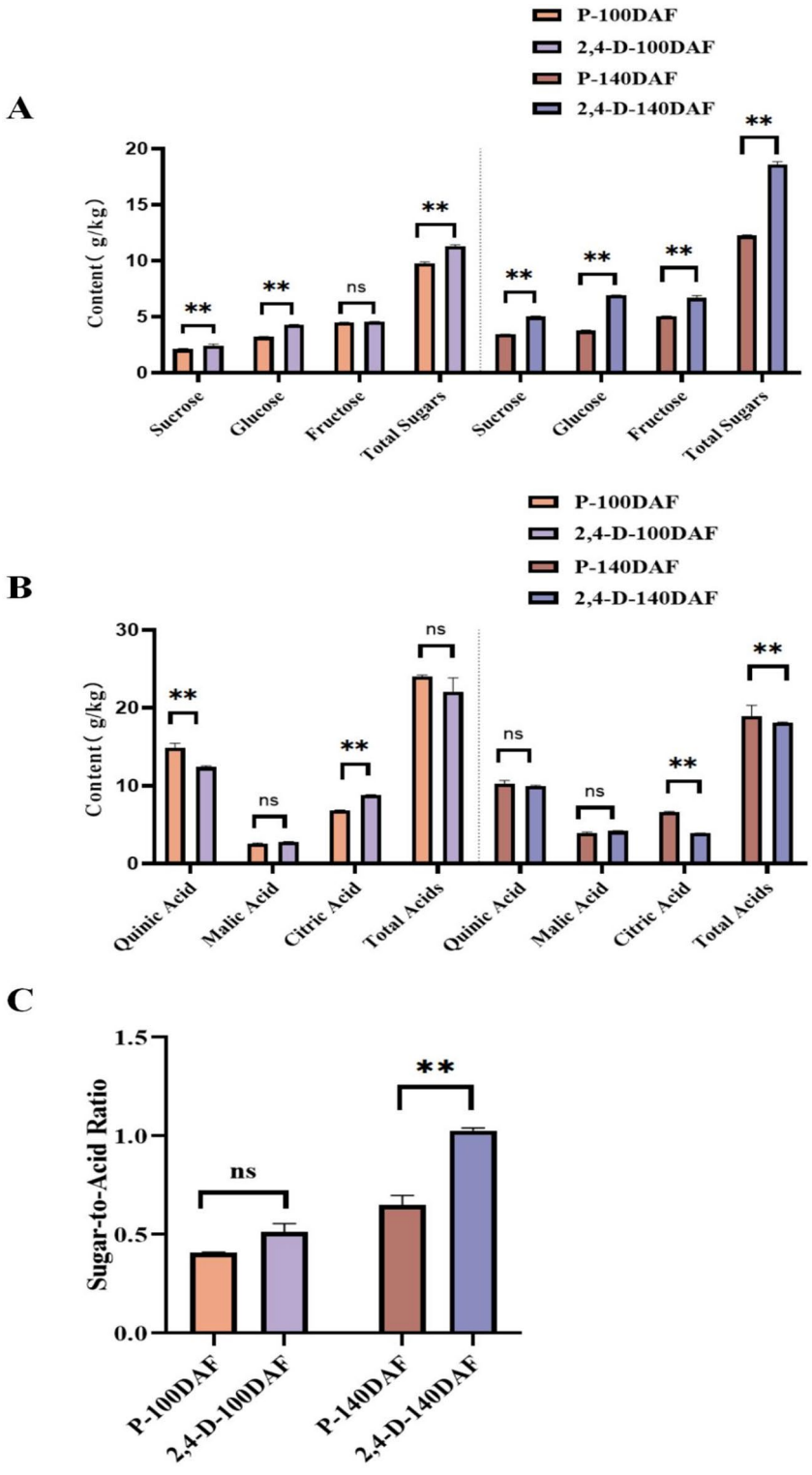



**Fig. 6** Determination of soluble sugar (**A**) and organic acid (**B**) content and ratio of sugar to acid (**C**) at mature stage. P: pollination treatment; 2,4-D: 2,4-D treatment; the statistical methods used One-way ANOVA; * represents a significant difference at $p<0.05$. ** represents extremely significant difference at $p<0.01$.

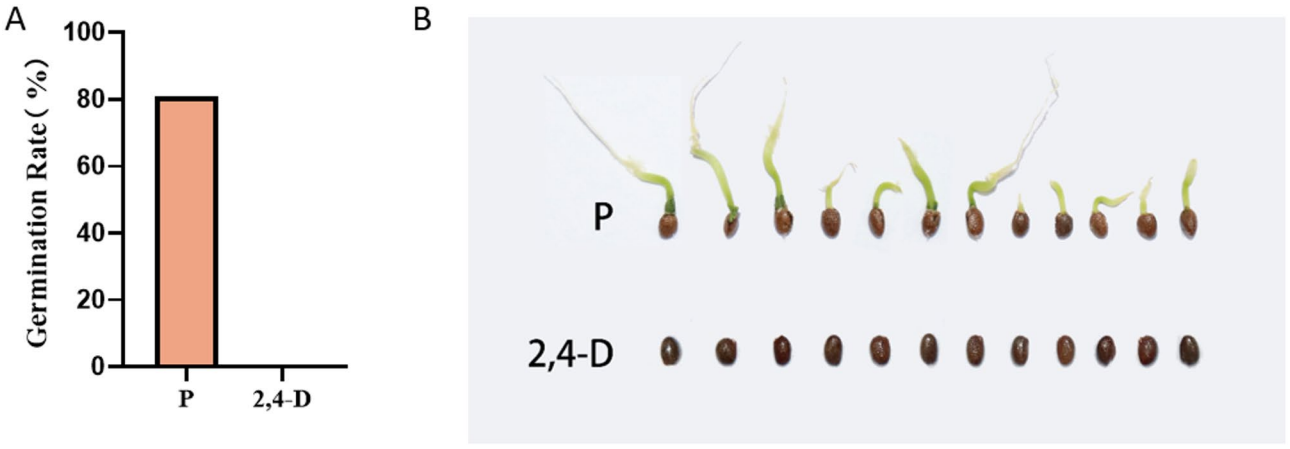



**Fig. 7** Seed germination rate (**A**) and seed germination morphology (**B**)

correlating with perceived sweetness intensity. The sugar-acid ratio is recognized as a critical factor affecting fruit flavor, with a higher ratio often associated with a sweeter taste [29]– [30]. In this study, the sugar-acid ratio of the 2,4-D-treated 'Donghong' kiwifruit exceeded 1 at maturity, while the ratio in pollination-treated fruits was less than 0.6. These findings suggest that 2,4-D treatment can significantly increase the sugar-acid ratio, suggesting the flavor quality of the kiwifruit will be improved.

There is a strong correlation between hormone-induced parthenocarpy and embryo failure. Plant hormones regulate fruit formation and seed development by influencing the developmental processes of the ovary and ovule. Hormones promote fruit development by modulating the plant's hormonal balance while simultaneously inhibiting the normal development of the ovule [31]. In mangoes (*Mangifera indica* L.), the degradation of embryo sac cells is a primary cause of embryo failure, and this degradation is closely linked to hormonal regulation [32]. Fruit treated with 2,4-Dichlorophenoxyacetic acid (2,4-D), which induces parthenocarpy, developed with a morphology identical to that of pollinated fruit, and the corresponding seeds developed despite being unfertilized but without embryos [33]. As an auxin analog, 2,4-D initiated parthenocarpic fruit development by stimulating the endogenous auxin signaling cascade, evidenced by the upregulation of auxin response factors (ARFs) and auxin/indole-3-acetic acid (IAA) genes. This process possibly operates in concert with gibberellin (GA) signaling to coordinately enhance both cell division and cell expansion, ultimately leading to ovary enlargement and seedless fruit formation [34]. Similarly, 2,4-D has been shown to negatively impact seed development, with studies indicating that it inhibits seed development, leading to reduced embryo formation and even necrosis in Padi Indica (*Oryza sativa* L. var. Ciherang) [35]. In this study, a few seeds were observed in kiwifruit at maturity. However, seed germination experiments revealed that seeds from 2,4-D-treated fruits showed no germination. Therefore, hormone-induced parthenocarpy in kiwifruit may be attributable to ovule abortion and seed necrosis.

Parthenocarpy enables the successful development of fruit without pollination, which is particularly significant under conditions where pollination is limited. This study elucidates that parthenocarpy enables fruit set and development without pollination and fertilization, thereby ensuring stable production under suboptimal conditions. The potential of parthenocarpy lies in its ability to enhance fruit sets under adverse environmental conditions, thereby improving crop quality and yield, especially when pollinator services are limited or unavailable [36]. Studies have shown that low concentrations of 2,4-D (5 and 10 ppm) can successfully induce parthenocarpy in tomatoes, leading to high-quality seedless fruits [37]. The fruits formed through parthenocarpy are seedless and of superior quality, making them an ideal trait for consumers [38]. As the experimental material kiwifruit is a dioecious plant, traditional pollination methods require manual intervention, resulting in excessive labor resource consumption. To address this issue, the present study employed 2,4-D to induce parthenocarpy in kiwifruit, effectively preventing fruitlessness caused by unpollinated female flowers. The experimental results demonstrated that 2,4-D treated fruits successfully developed and exhibited significantly fewer seeds than manually pollinated fruits, along with a notable improvement in fruit quality. Therefore, hormone treatments to induce parthenocarpy can overcome pollination challenges and produce seedless fruits, which are important for commercial cultivation.

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conditions. The potential of parthenocarpy lies in its ability to enhance fruit sets under adverse environmental conditions, thereby improving crop quality and yield, especially when pollinator services are limited or unavailable [36]. Environmental factors such as light intensity, photoperiod, temperature, and humidity can influence 2,4-D-induced parthenocarpy in kiwifruit. Modified light conditions in closed or shaded environments may interact with 2,4-D by altering endogenous auxin metabolism, either enhancing or inhibiting parthenocarpic fruit development depending on the cultivar. Additionally, stable temperature and humidity in controlled environments help maintain consistent hormonal activity and optimize fruit set [39]. Further study could be conducted to explore optimal environmental conditions (light, temperature, humidity) in controlled cultivation systems to enhance 2,4-D-induced parthenocarpy while ensuring fruit quality. The underlying mechanisms of environmental regulation of parthenocarpy remained to be investigated. Studies have shown that low concentrations of 2,4-D (5 and 10 ppm) can successfully induce parthenocarpy in tomatoes, leading to high-quality seedless fruits [37]. The fruits formed through parthenocarpy are seedless and of superior quality, making them an ideal trait for consumers [38]. As the experimental material kiwifruit is a dioecious plant, traditional pollination methods require manual intervention, resulting in excessive labor resource consumption. To address this issue, the present study employed 2,4-D to induce parthenocarpy in kiwifruit, effectively preventing fruitlessness caused by unpollinated female flowers. The experimental results demonstrated that 2,4-D treated fruits successfully developed and exhibited significantly fewer seeds than manually pollinated fruits, along with a notable improvement in fruit quality. Therefore, hormone treatments to induce parthenocarpy can overcome pollination challenges and produce seedless fruits, which are important for commercial cultivation.

This study systematically investigates the mechanism by which 2,4-D influences the development and quality of seedless fruits in *Actinidia chinensis* var. 'Donghong'. Building upon previous findings demonstrating 2,4-D was able to induce seedless fruit formation in kiwifruit, our research focuses on elucidating the regulatory effects of exogenous 2,4-D treatment on fruit developmental dynamics, quality formation. The findings clarified the role of plant growth regulators in seedless kiwifruit development, and established a 2,4-D treatment strategy that provided essential physiological insights for breeding novel seedless kiwifruit varieties. This research holds significant practical value for enhancing the efficiency and sustainability of the kiwifruit industry.

## Conclusion

The present study demonstrated the effectiveness of 2,4-D treatment in inducing parthenocarpic fruit set in *Actinidia chinensis* var. 'Donghong'. Both the external attributes, including fruit weight and size, and the internal qualities, such as sugar and acid content, throughout the fruit development were studied. The treatment with 0.5 mg/L 2,4-D notably enhanced the fruit's sensory qualities by increasing its sweetness and decreasing its acidity. Therefore, the sugar-acid ratio of the fruit was significantly increased, improving its overall quality. However, there are significant differences in fruit weight and dry matter content between the 2,4-D-treated and pollinated fruits. This study provided valuable insights into parthenocarpy and promoting fruit quality in kiwifruit. This application can be an alternative, especially for kiwifruit growers who want to obtain fruits without using male (pollinator) varieties and without artificial pollination to obtain more yield in their orchards.

#### Abbreviations

| | |
|---|---|
| F1 | Bellflowers |
| F2 | Full bloom |
| 3 DAF | 3 days after flowering |
| 20 DAF | 20 days after flowering |
| 50 DAF | 50 days after flowering |
| 100 DAF | 100 days after flowering |
| 140 DAF | 140 days after flowering |
| C | control group |
| P | Pollination treatment |
| 2,4-D | 2,4-D treatment group |

#### Author contributions

Conceptualization, L.W. and Q.Z.; methodology, L.W. and J.D.; software, L.W. and J.D.; validation, H.Z., L.Y. and A.A.; review and editing, D.Q., Q.Z., C.Z. All authors have read and agreed to the published version of the manuscript.

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#### Data availability

Data will be available on request.

## Declarations

#### Ethics approval and consent to participate

All methods and experimental research were carried out in compliance with local and national guidelines.

#### Competing interests

The authors declare no competing interests.
Clinical trial number.
It is not applicable.

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