

## RESEARCH ARTICLE

## Improving the quality of wild kiwifruit and developing superior hybrid combinations through artificial pollination

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Against the backdrop of rapid kiwifruit industry development and innovative utilization of germplasm resources, the efficient utilization and variety breeding of wild kiwifruit face challenges such as low pollination efficiency and inconsistent fruit quality. There is an urgent need to develop efficient pollination techniques to enhance fruit quality and breeding efficiency. This study selected 9 female and 9 male wild kiwifruit resources from five regions in Henan, China to systematically investigate the effects of artificial pollination on fruit quality and breeding value across different hybrid combinations. Key evaluation metrics included fruit set rate, single fruit weight, soluble solid content, vitamin content, titratable acidity, and solid acid ratio. The results showed that artificial pollination significantly increased fruit set rates with average rates reaching 75.0% to 82.0%, which represented an approximately 80.3% improvement over natural pollination rates (38.5% to 46.2%). The optimal male parent pollen (B3, Honghe germplasm) yielded fruit with a maximum weight of 105.2 g, representing a 27.1% increase over the control. Vitamin C content reached 150.0 mg/100 g, a 24.7% increase, while the solid acid ratio reached 14.0, a 54.2% increase. Comprehensive evaluation identified superior hybrid combinations. The results indicated that artificial pollination using high-viability pollen synergistically enhanced the fruit set rate, fruit quality, and breeding value of wild kiwifruit. This study provided effective strategies and theoretical basis for the resource utilization and breeding of superior varieties of wild kiwifruit.

**Keywords:** wild kiwifruit; artificial pollination; fruit quality; hybrid combination selection.

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### Introduction

The kiwifruit is hailed as the “King of vitamin C (Vc)” with China's wild germplasm resources accounting for over 70% of the global total. The Chinese kiwifruit and the wild variant of the delicious kiwifruit distributed across the Henan Plateau of China are highly favored by the breeding community for their resistance to bacterial canker disease, tolerance to low-latitude high-light conditions, and rich flavor profile [1]. However, wild kiwifruit populations

are predominantly dioecious and rely on cross-pollination. Natural pollination is constrained by low pollinator diversity, rainy flowering periods, and insufficient male plant ratios. Fruit set rates consistently hover between 35 – 45% with individual fruit weights below 90 g and soluble solid content (SSC) under 12%, which severely limits the utilization and industrial development of superior genetic resources [2]. Therefore, exploring efficient artificial pollination techniques to overcome the reproductive bottleneck of wild kiwifruit and enhance fruit

commercial quality has become a critical component in the current genetic improvement of kiwifruit and the revitalization of the specialty seed industry.

Pollen viability is the primary factor determining fertilization efficiency. Previous studies have demonstrated a highly significant positive correlation between the *in vitro* germination rate of *Prunus mume* pollen and field fruit set rates. However, due to differences in climatic adaptation strategies among male trees from distinct geographical sources, significant variation exists in pollen tube growth rates, S-gene types, and direct effects [3]. Currently, there remains a lack of systematic data on the bulk evaluation of pollen from wild male kiwifruit plants and the selection of high-viability paternal parents in China, which constrains the scientific configuration of hybrid parents. Pollination methods directly influence two key fruit traits including quantity and quality. Under natural pollination, wild kiwifruit ovules undergo incomplete fertilization, resulting in reduced seed numbers and insufficient endogenous hormone synthesis, which, in turn, limits cell division and sugar unloading, leading to the formation of “small, sour, and hard fruits” [4]. Artificial pollination significantly enhances seed plumpness and individual fruit weight by increasing effective pollen quantity and extending the pollination window. However, quantitative research on its direct effects on specific parental combinations in kiwifruit remains scarce, particularly lacking comprehensive evaluations of SSC, Vc, titratable acidity (TA), and sensory flavor attributes. Hybrid combination selection is a core step in the domestication of wild resources. Henan wild kiwifruit female plants exhibit rich phenotypic variation, but desirable traits are often linked with undesirable ones. A comprehensive evaluation system involving multiple combinations and traits is still needed to resolve the issue of how to selectively hybridize to aggregate superior traits such as high sugar content, low acidity, large fruit size, and intense aroma while avoiding the “small fruit collapse”

phenomenon caused by reproductive incompatibility [5].

This study systematically conducted pollen viability testing, artificial pollination, and comprehensive multi-trait evaluations based on wild kiwifruit germplasm collected and preserved from five prefectures in Henan, China to clarify pollen viability differences among various male plants and identify high-viability paternal lines, while quantifying the extent to which artificial pollination enhanced fruit set rate, individual fruit weight, and both internal and external quality. A weighted-score-based model for selecting superior hybrid combinations was constructed, providing theoretical foundations and parental configuration strategies for the efficient utilization of wild kiwifruit resources and the breeding of early-maturing, high-sugar new varieties. This study overcame the limitations of previous research that focused solely on a single male parent or a single trait by adopting a complete diallel cross design involving multiple regions, multiple male parents, and multiple female parents to construct a comprehensive multidimensional evaluation system to assess fruit set rate, individual fruit weight, nutritional quality including Vc, sugar solids content, total acidity, and sensory flavor.

## Materials and methods

### Testing time, location, and material selection

The study was conducted in the Forest Breeding Laboratory of the School of Water Resources and Modern Agriculture, Nanyang Normal University, Nanyang, Henan, China. 9 wild kiwifruit female plants and 9 male plants were collected from 5 production areas across Henan, China including Jiyuan, Songxian, Gushi, Xixia, and Xingyang. According to morphological identification, all germplasms were Chinese kiwifruit. To ensure the accuracy of the materials, the gender of all female and male plants was confirmed through floral structure during the flowering period. The main botanical traits of each germplasm were recorded and documented. The female plants

were uniformly numbered from F1 to F9, corresponding to 9 different female plants, while male plants were numbered A1, A2 from Jiyuan, B3, B4 from Songxian, C5, C6 from Gushi, D7, D8 from Xixia, and E9 from Xingyang. The experiment started with artificial pollination during the flowering period in March 2022. Fruit samples from each hybrid combination were collected during the ripening period from August to September 2022 to measure quality indicators including individual fruit weight, longitudinal and transverse diameters, SSC, and Vc. To improve the representativeness and reliability of the data, each hybrid combination was set up with 3 biological replicates. Three experimental fields were located within the supporting experimental base of the College Forestry Breeding Laboratory with the geographical coordinates of N33°01'25.3"/E112°31'18.7", N33°01'28.6"/E112°31'22.1", N33°01'30.2"/E112°31'15.4" and spaced 50 – 80 m apart with an altitude difference of  $\leq 10$  m. The soil type was yellow brown with no significant differences in basic physical and chemical properties including pH value and organic matter content. 25 fruits were randomly collected from each hybrid combination in each experimental field, and a total of 75 fruits were used for quality determination and data analysis. Different hybrid combinations were evaluated for the effect of improving the quality of wild kiwifruit.

#### **Design of artificial pollination and hybrid combination selection trials**

A systematic experiment encompassed phenotypic diversity evaluation, pollen viability testing, assessment of the fruit set rate of artificial pollination, and observation of fruit growth patterns was designed. This exploratory experiment consisted of five steps. Wild kiwifruit pollen was first collected and prepared. The large buds in the pre-bloom stage of 9 male wild kiwifruit plants were collected [6, 7]. Anthers were taken and spread evenly on 10 × 10 cm<sup>2</sup> tracing paper and placed in a cool, dry, well-ventilated indoor environment to allow natural pollen shedding for 24 hours. Fresh pollen was collected and packed in sealed 15 mL centrifuge

tubes and stored at -20°C for future use. The storage time for all pollen should not exceed 30 days [8]. Before pollination, the stored pollen was placed at 4°C for 12 hours to ensure that the pollen vitality remained stable during the thawing process [9]. Pollen viability testing was then carried out using *in vitro* culture method to determine pollen germination rates [10]. Pollen from each male plant was evenly spotted onto the solid medium consisted of 1% agar, 12% sucrose, and 0.01% boric acid and incubated in darkness at 28°C in HPX-9052MBE constant-temperature incubator (Shanghai Boxun Industrial Co., Ltd, Shanghai, China). After 8 hours, three random view fields were selected under a microscope to count the total number of pollen grains and the number of germinated grains (defined as pollen grains with pollen tube lengths exceeding their diameter). The germination rate was then calculated [11]. Simultaneously, pollen viability was determined by using the Sherdakov staining (biphenylamine- $\alpha$ -naphthol) method. Pollen grains stained red or purplish red indicated viability, while colorless or yellow grains indicated non-viability. The percentage of viable pollen was statistically calculated. Male plants exhibiting optimal pollen quality were selected as the preferred paternal parents for artificial pollination [12]. Artificial hybrid pollination was then processed. One day before the female parent's flowers opened, when petals appeared yellowish-white, well-developed inflorescences were selected. The excess flower buds were thinned out, and only the central flowers were retained. The stamens were gently removed by using tweezers and immediately covered with a 15 × 20 cm<sup>2</sup> sulfur paper bag (Longji Plastic Industry Co., Ltd, Taizhou, Zhejiang, China) for isolation. When the stigma exhibited shiny and sticky mucilage in the following day (the optimal pollination window), a handheld pollinator tool (Huangyan Fengrun Plastic Co., Ltd, Taizhou, Zhejiang, China) was used to dip into pre-prepared paternal pollen and gently dab it onto the stigma [13]. After completion, the isolation bag was replaced, and a plastic label was hung indicating the hybrid combination (female × male) and pollination

date. The hybrid combinations constituted the experimental groups, while the naturally pollinated combinations served as the control group (CK). Each combination received pollination on at least 50 flowers. Concurrently, 50 flowers on each maternal parent were marked for natural pollination as a control. Fruit-sitting rate statistics and fruit maintenance were carried out thereafter. Fifteen days after pollination, the isolation bags were removed, the number of set fruits was counted, and the fruit-sitting rate was calculated as follows.

Fruit-sitting rate = number of set fruits/number of pollinated flowers × 100%

Subsequently, routine orchard management was performed including fruit thinning (retaining 25 fruits per cluster with uniform growth), irrigation, fertilization, and pest and disease control to ensure normal fruit development [14]. Observations and sampling were eventually conducted on fruit growth patterns. During physiological ripening (when SSC ceased significant increase), fruits were collected according to combination markers. For each combination, 25 undamaged fruits of uniform ripeness were randomly picked from different orientations, immediately stored in ice box, and transported back to the laboratory. All quality index measurements were completed within 48 hours [15].

#### **Methods for evaluating kiwifruit phenotypic diversity**

This study systematically evaluated the phenotypic diversity of kiwifruit quality through a multidimensional assessment employing seven measurement methods. Single fruit weight (g) was measured through 3 repetitions of direct weighing using a Sartorius electronic balance (accuracy 0.01 g) with the average value as the result. Fruit length and width (mm) were measured through the transverse diameter at the fruit's equatorial plane and the longitudinal diameter from the fruit apex to the pedicel using a Guanglu vernier caliper (accuracy 0.01 mm). The fruit shape index (longitudinal

diameter/transverse diameter) was then calculated [16]. SSC (%) was determined by using an ATAGO PAL-1 digital refractometer (ATAGO, Bellevue, WA, USA). A sample pulp from the middle of the fruit was taken, squeezed to extract juice, and dropped onto the prism surface. The reading was taken directly with three repetitions per fruit and the average value recorded [17]. Vc content (mg/100 g) was determined by using 2, 6-dichlorophenol titration method with slight modifications. 5.0 g of fruit pulp homogenate was accurately weighed and diluted to 100 mL in a volumetric flask using a 2% oxalic acid solution. After mixing thoroughly, the solution was filtered. 10 mL of the filtrate was transferred to a conical flask and titrated with a standard solution of 2, 6-dichlorophenol indole-3-carboxylic acid of known concentration until a stable pink color developed and persisted for 30 seconds without fading. The volume of titrant consumed was recorded. The Vc content in the sample was calculated based on the titration results. TA content (%) was determined by using NaOH neutralization titration method [18]. The results were expressed as a percentage based on citric acid content with the calculation formula as follows.

TA content = (NaOH concentration × consumed volume × citric acid conversion factor × dilution factor)/sample mass × 100%

Solid acid ratio was determined by calculating the ratio of SSC to TA (SSC/TA). Flavor sensory evaluation was performed by 10 evaluators who had undergone no less than 20 hours of specialized systematic training on kiwi flavor sensory evaluation including recognition of typical kiwi flavor characteristics, sensory intensity calibration, and common deviation control methods. During the evaluation process, each fruit sample was evaluated twice by the evaluator in an independent sensory interval of no less than 1 hour between the two evaluations to reduce subjective errors. A percentage blind evaluation method was employed to comprehensively evaluate sweetness, acidity, aroma, flavor intensity, and overall acceptance.

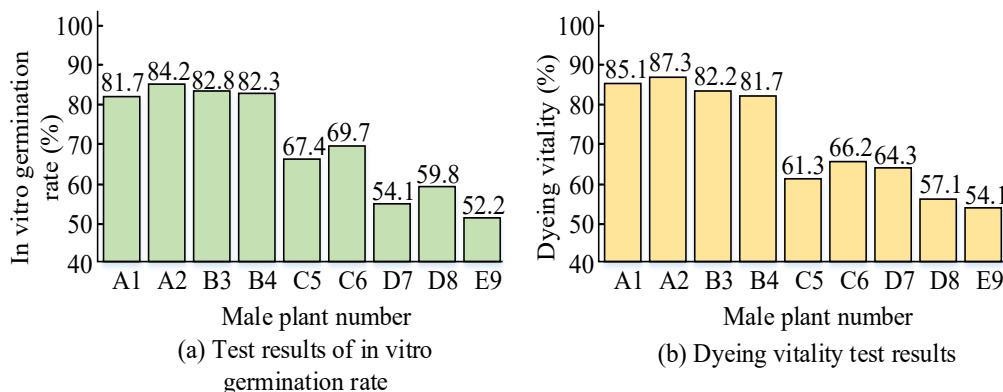


Figure 1. Pollen viability test results for male plants from different germplasm sources.

### Data processing and statistical analysis

Data collected during the experiment including SSC, TA, Vc, soluble sugars, sugar-acid ratio, individual fruit weight, fruit diameter, peel hardness, and color parameters were preliminarily organized using Excel 2019 (Microsoft, Redmond, WA, USA). The mean  $\pm$  standard deviation (SD) for each hybrid combination indicator was calculated. Subsequently, by using the hybrid combinations as the classification variable, the data were imported into SPSS 25.0 (IBM, Armonk, NY, USA) and R 4.2.1 (<https://www.r-project.org>) as independent sample sets. One-way ANOVA was applied with the hybridization combination being set as the factor and the target trait as the dependent variable. *P* value less than 0.05 was defined as statistically significant. Duncan's Multiple Range test was further applied to identify specific levels of difference among combinations.

### Results and discussion

#### Differential analysis of pollen viability from different germplasm sources

The pollen vitality test results of 9 male plants showed that the *in vitro* germination rates of pollen from male plants A1, A2, B3, and B4 from Jiyuan and Songxian were relatively superior in pollen viability with the germination rates above 80% and robust and uniform pollen tube growth.

These four male plants were suitable candidates for cultivating high-quality kiwifruit as high-viability pollen parents ensured efficient artificial pollination. However, the pollen vitalities of male plants C5, C6, D7, D8, and E9 were relatively low as 67.4%, 69.7%, 54.1%, 59.8%, and 52.2%, respectively, and the germination rate was less than 70% (Figure 1a). The results indicated reproductive adaptation differentiation among wild kiwifruit male plants from different geographical populations in this region. The staining activity test results of male pollen from different sources showed that the staining activities of male plants A1, A2, B3, and B4 were 85.1%, 87.3%, 82.2%, and 81.7%, respectively, while the pollen staining activities of male plants C5, C6, D7, D8, and E9 were relatively low as 61.3%, 66.2%, 64.3%, 57.1%, and 54.1%, respectively (Figure 1b). The trends observed in both methods were consistent, further validating the authenticity of the results. Based on the dual determination results of pollen germination rate and staining activity *in vitro*, male plants A1, A2, B3, and B4 were selected as high vitality male parents for subsequent artificial pollination.

#### Analysis of the effect of artificial pollination on fruit set rate and average fruit weight

Artificial pollination was conducted by using male plants A1, A2, B1, and B1 selected through pollen viability tests, and the results are compared with those of natural pollination. The fruit set rates of all four artificial pollination treatments (A1, A2,

**Table 1.** Comparison of fruit set rates (%) under different pollination methods.

Mother plant	CK (natural pollination)	A1 × mother version	A2 × mother version	B3 × mother version	B4 × mother version
F1	42.7 ± 3.1 <sup>c</sup>	78.4 ± 2.5 <sup>a</sup>	76.8 ± 3.0 <sup>a</sup>	74.6 ± 2.8 <sup>ab</sup>	75.2 ± 2.6 <sup>ab</sup>
F2	38.5 ± 2.8 <sup>d</sup>	81.3 ± 2.2 <sup>a</sup>	79.5 ± 2.7 <sup>a</sup>	77.1 ± 2.4 <sup>ab</sup>	78.0 ± 2.9 <sup>ab</sup>
F3	45.0 ± 3.5 <sup>c</sup>	80.1 ± 2.6 <sup>a</sup>	78.9 ± 2.3 <sup>a</sup>	76.5 ± 3.1 <sup>ab</sup>	77.3 ± 2.7 <sup>ab</sup>
F4	41.2 ± 3.0 <sup>c</sup>	79.6 ± 2.4 <sup>a</sup>	77.2 ± 2.9 <sup>a</sup>	75.8 ± 2.6 <sup>ab</sup>	76.5 ± 2.8 <sup>ab</sup>
F5	39.8 ± 2.7 <sup>d</sup>	82.0 ± 2.1 <sup>a</sup>	80.4 ± 2.5 <sup>a</sup>	78.9 ± 2.3 <sup>ab</sup>	79.1 ± 2.6 <sup>ab</sup>
F6	43.5 ± 3.2 <sup>c</sup>	78.9 ± 2.8 <sup>a</sup>	77.6 ± 3.0 <sup>a</sup>	75.0 ± 2.9 <sup>ab</sup>	76.0 ± 2.5 <sup>ab</sup>
F7	40.3 ± 2.9 <sup>c</sup>	80.7 ± 2.3 <sup>a</sup>	79.1 ± 2.6 <sup>a</sup>	77.4 ± 2.5 <sup>ab</sup>	78.2 ± 2.7 <sup>ab</sup>
F8	44.1 ± 3.4 <sup>c</sup>	81.5 ± 2.0 <sup>a</sup>	80.0 ± 2.4 <sup>a</sup>	78.0 ± 2.7 <sup>ab</sup>	78.8 ± 2.4 <sup>ab</sup>
F9	46.2 ± 3.3 <sup>c</sup>	79.8 ± 2.7 <sup>a</sup>	78.3 ± 2.8 <sup>a</sup>	76.9 ± 3.0 <sup>ab</sup>	77.5 ± 2.9 <sup>ab</sup>

**Note:** Different lowercase letters within the same column indicated significant differences ( $P < 0.05$ ).

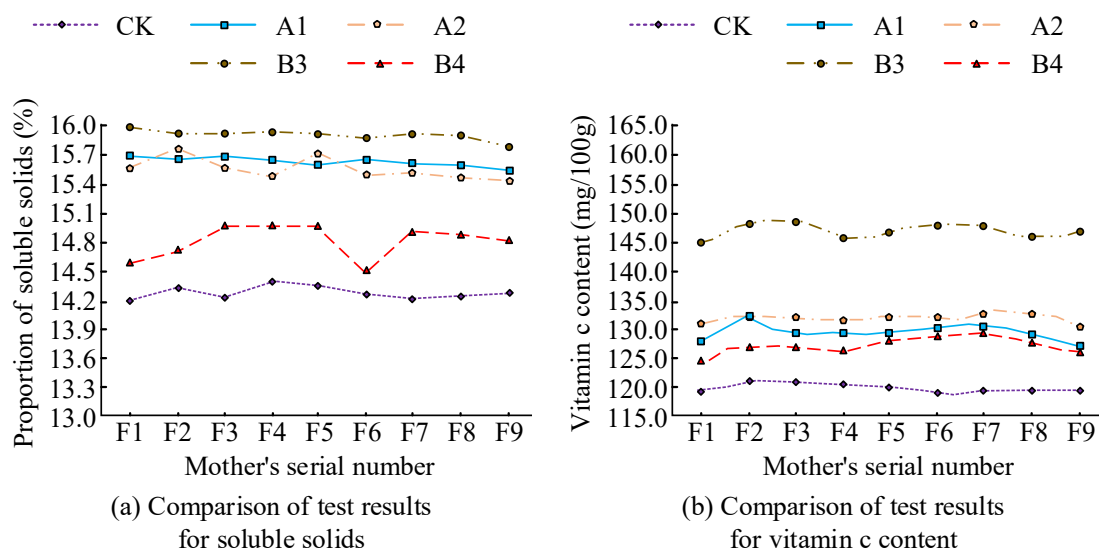
B3, B4) for all nine maternal lines (F1 - F9) were significantly higher than their corresponding natural pollination CK (Table 1). The average fruit set rate for artificial pollination ranged from 75.0% to 82.0%, while the average for natural pollination was only between 38.5% and 46.2%, which indicated that artificial pollination was an effective measure to overcome the low pollination efficiency under natural conditions and improve the fruit set rate of wild kiwifruit. Within the artificial pollination group, there were some differences in pollination effectiveness among different paternal varieties of A1, A2, B3, B4 when pollinating the same maternal variety. However, according to Duncan's multiple range test results, these differences were not statistically significant ( $P > 0.05$ ). This result demonstrated that all four pre-selected high-viability pollen sires (A1, A2, B3, B4) effectively completed pollination and fertilization, yielding consistently high and stable fruit set rates. Additionally, under natural pollination conditions, fruit set rates were significantly different among various maternal lines (F1 - F9), ranging from 38.5% to 46.2%, which indicated the diversity in self-fertility among female wild kiwifruit plants from different germplasm sources. The average fruit weight under different pollination methods showed that artificial pollination significantly affected single fruit weight, but the effect varied considerably depending on the paternal and maternal genotypes. Most artificial pollination

combinations had significantly higher single fruit weight than the natural pollination CK. Among them, paternal line A1 had the most outstanding and stable performance with its single fruit weight from 98.5 g to 105.2 g in hybrids with all maternal lines, which was significantly higher than the control with increases ranging from 18.4% to 27.1%. However, the performance of the paternal line B4 exhibited extreme genotype-specific interactions. When crossed with maternal lines F1 and F3, it demonstrated outstanding single-fruit weight of 103.5 g, but when crossed with F2, F4, F5, F6, F8, and F9, the increase in single-fruit weight (87.0 - 89.2 g) was limited. Particularly concerning the combination with maternal line F7, the single fruit weight (42.7 g) dropped sharply, even falling significantly below that of its open-pollinated CK, which indicated the presence of severe reproductive incompatibility or recessive genetic interaction (Table 2). This result indicated that artificial pollination was an effective method for increasing individual fruit weight. However, its effectiveness strongly depended on paternal selection and specific parental combinations, exhibiting a pronounced phenomenon of pollen receptivity. To enhance the rigor of the conclusion, the study separately added reproductive biology validation for the B4 × F7 combination. Fluorescence microscopy observation showed that B4 pollen exhibited typical pollen tube terminal swelling and growth arrest in the F7 style, failing to reach the ovule,

**Table 2.** Comparison of single fruit weight (g) among different pollination methods.

Mother plant	CK (natural pollination)	A1 × mother version	A2 × mother version	B3 × mother version	B4 × mother version
F1	82.8 ± 4.3 <sup>c</sup>	105.2 ± 5.1 <sup>a</sup>	101.5 ± 4.7 <sup>a</sup>	102.7 ± 4.8 <sup>ab</sup>	103.5 ± 4.1 <sup>ab</sup>
F2	81.5 ± 3.9 <sup>d</sup>	104.1 ± 4.9 <sup>a</sup>	103.8 ± 5.3 <sup>a</sup>	102.7 ± 4.8 <sup>a</sup>	88.5 ± 4.4 <sup>ab</sup>
F3	80.2 ± 4.5 <sup>c</sup>	102.3 ± 5.0 <sup>a</sup>	99.2 ± 5.5 <sup>a</sup>	101.5 ± 4.7 <sup>a</sup>	103.5 ± 4.1 <sup>a</sup>
F4	83.5 ± 3.7 <sup>c</sup>	101.9 ± 4.6 <sup>a</sup>	100.8 ± 5.3 <sup>a</sup>	99.2 ± 5.5 <sup>a</sup>	88.5 ± 4.4 <sup>ab</sup>
F5	82.0 ± 4.0 <sup>d</sup>	102.3 ± 5.0 <sup>a</sup>	101.5 ± 4.7 <sup>a</sup>	100.8 ± 5.3 <sup>a</sup>	89.2 ± 5.5 <sup>ab</sup>
F6	84.8 ± 4.2 <sup>c</sup>	90.2 ± 5.5 <sup>a</sup>	98.5 ± 4.4 <sup>ab</sup>	100.8 ± 5.3 <sup>ab</sup>	89.0 ± 3.8 <sup>a</sup>
F7	81.0 ± 3.8 <sup>c</sup>	104.1 ± 4.9 <sup>a</sup>	100.8 ± 5.3 <sup>a</sup>	101.5 ± 4.7 <sup>a</sup>	42.7 ± 4.8 <sup>ab</sup>
F8	83.2 ± 4.6 <sup>c</sup>	98.5 ± 4.4 <sup>a</sup>	87.0 ± 3.8 <sup>ab</sup>	88.5 ± 4.4 <sup>ab</sup>	88.5 ± 4.4 <sup>ab</sup>
F9	82.5 ± 4.1 <sup>c</sup>	102.3 ± 5.0 <sup>a</sup>	89.2 ± 5.5 <sup>ab</sup>	90.8 ± 5.3 <sup>ab</sup>	89.2 ± 5.5 <sup>ab</sup>

Note: Different lowercase letters within the same column indicated significant differences ( $P < 0.05$ ).

**Figure 2.** Test results for the proportion of SSC and Vc content in fruit.

which excluded the influence of environmental accidental factors and confirmed a typical phenomenon of reproductive incompatibility.

#### Analysis of the effect of artificial pollination on internal quality indicators of fruit

The results for the proportion of SSC and Vc content in fruits under different pollination methods demonstrated that artificial pollination significantly enhanced fruit SSC. All artificial pollination combinations exhibited significantly higher SSC than the natural pollination CK ( $P < 0.05$ ). Paternal parent B3 demonstrated the most outstanding performance, achieving the highest

SSC values of 15.7 - 16.0% in crosses with all maternal parents, which represented a 14.3 - 23.7% increase compared to the natural pollination group. The performance of paternal lines A1 and A2 was inferior but still significantly superior to the control, while paternal line B4 exhibited a relatively weaker enhancement effect (Figure 2a). The results indicated that artificial pollination, particularly when using B3 as the paternal parent, significantly promoted fruit sugar accumulation and conversion, effectively enhancing fruit sweetness. The Vc content of all artificial pollination combinations was extremely significantly higher than that of

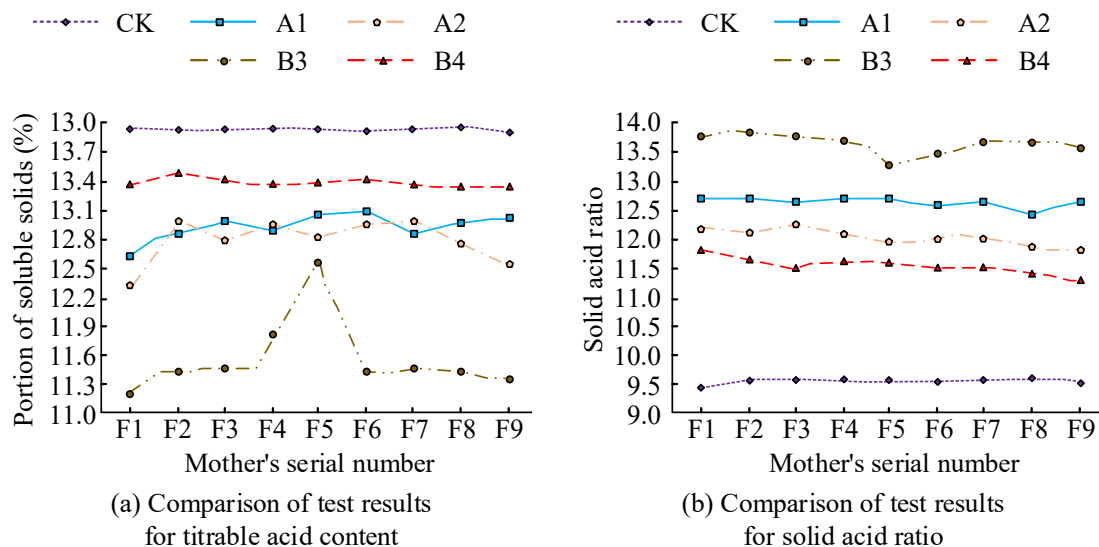


Figure 3. TA content and solid acid ratio test results of the fruit.

the natural pollination group ( $P < 0.01$ ) with an average increase of 18.6%. Paternal parent B3 also demonstrated the best performance with its Vc content of 145.0 - 150.0 mg/100 g significantly higher than other paternal parents, showing a 21.3 - 24.7% increase compared to the natural pollination group. The enhancement effects of male parents A1 and A2 were comparable, while that of male parent B4 was relatively weaker (Figure 2b). The results indicated that artificial pollination promoted the biosynthesis and accumulation of Vc in fruits by optimizing the fertilization process, thereby enhancing fruit nutritional quality and antioxidant capacity. The TA content and solid acid ratio results of fruits under different pollination methods demonstrated that the TA content of all artificial pollination combinations was significantly lower than that of the natural pollination group ( $P < 0.05$ ) with an average reduction of 13.2%. The reduction effect of paternal line B3 was the most pronounced with TA content of 1.10 - 1.25%, showing a decrease of 16.5 - 18.6% compared to the natural pollination group. The effects of paternal lines A1 and A2 were the next most pronounced, while paternal line B4 exhibited a relatively weaker effect (Figure 3a). The results indicated that artificial pollination, by regulating fruit metabolic processes, reduced organic acid

accumulation, laying a crucial foundation for enhancing fruit flavor. The solid acid ratio of all artificial pollination combinations was significantly higher than that of the natural pollination group ( $P < 0.01$ ) with an average increase of 41.3%. The performance of paternal line B3 was most prominent, showing a 48.7 - 54.2% increase in solid acid ratio (13.5 - 14.0) compared to the natural pollination group, significantly higher than other paternal lines. The effects of paternal lines A1 and A2 were comparable, while paternal line B4 showed relatively weaker effects (Figure 3b). The substantial increase in solid acid ratio indicated that artificial pollination effectively coordinated fruit sugar-acid metabolism, significantly improving fruit flavor quality and making the sweet-acid ratio more palatable.

#### Effect of artificial pollination on sensory evaluation indicators of fruit flavor

The assessment results for different kiwifruit varieties showed that the B3 parental combination had the highest scores for sweetness (85.0), aroma (83.2), flavor intensity (84.5), and overall acceptance (85.3), while scoring the lowest for acidity (48.5). The performance of the two parental lines A1 and A2 was very similar with all indicators significantly

**Table 3.** Sensory evaluation results of fruits from different pollination methods (on a 100-point scale, n = 10).

Treatment combination	Sweetness	Acidity	Aroma	Flavor intensity	Overall acceptance
CK	68.2 ± 4.1 <sup>c</sup>	65.5 ± 3.8 <sup>a</sup>	66.0 ± 4.0 <sup>c</sup>	65.8 ± 3.9 <sup>c</sup>	66.4 ± 3.7 <sup>c</sup>
A1 × mother version	82.5 ± 3.2 <sup>a</sup>	52.3 ± 3.1 <sup>b</sup>	80.1 ± 3.5 <sup>a</sup>	81.0 ± 3.3 <sup>a</sup>	82.0 ± 3.4 <sup>a</sup>
A2 × mother version	81.0 ± 3.0 <sup>a</sup>	53.0 ± 3.2 <sup>b</sup>	79.5 ± 3.7 <sup>a</sup>	80.2 ± 3.5 <sup>a</sup>	81.5 ± 3.2 <sup>a</sup>
B3 × mother version	85.0 ± 2.8 <sup>a</sup>	48.5 ± 2.9 <sup>c</sup>	83.2 ± 3.1 <sup>a</sup>	84.5 ± 3.0 <sup>a</sup>	85.3 ± 2.9 <sup>a</sup>
B4 × mother version	78.3 ± 3.4 <sup>b</sup>	55.1 ± 3.4 <sup>ab</sup>	76.8 ± 3.6 <sup>b</sup>	77.5 ± 3.4 <sup>b</sup>	78.0 ± 3.3 <sup>b</sup>

**Note:** Different lowercase letters within the same column indicated significant differences ( $P < 0.05$ ).

superior to CK and B4, but slightly inferior to B3. They significantly reduced fruit tartness while enhancing sweetness and aroma, making them reliable superior male parents. The B4 combination performed the weakest among all cross-pollination groups with scores significantly lower than B3, A1, and A2, yet still significantly higher than CK (Table 3). The results indicated that while its pollen viability was adequate (ensuring fruit set), its genetic material exhibited a less pronounced direct effect on regulating fruit sugar, acidity, and aroma compound synthesis compared to the other three parents.

#### Comprehensive evaluation ranking of superior hybrid combinations

To achieve balanced and practical screening of hybrid combinations from multiple key dimensions, the study referred to the core objectives and industry standards of kiwifruit quality breeding. SSC and TA, which determined flavor quality, were given the highest weight with a total of 40%, and the ratio of solid to acid was used as an independent indicator for 10% to highlight the key impact of sugar acid balance on flavor. This was consistent with the principle of "flavor first" in commercial breeding. In addition, by fully considering the unique advantages of wild kiwifruit germplasm resources, a high weight of 20% was assigned to Vc with the aim of screening and strengthening its outstanding nutritional traits as the "King of Vc". Further, considering both yield and commodity basis, a 10% weight was allocated to the fruit setting rate and single fruit weight to ensure that the optimal combination had actual production value. The 10% weight of sensory evaluation was used to

verify the consistency between physical and chemical indicators and actual human perception. Based on weighted indicators including fruit set rate for 10%, single fruit weight for 10%, SSC for 20%, Vc for 20%, TA for 20%, solid acid ratio for 10%, and sensory evaluation for 10%, comprehensive scores were calculated for different hybrid combinations to facilitate selection. The results demonstrated that parental line B3 exhibited the most outstanding overall performance with combinations involving this parent occupying eight of the top ten positions. Among them, F5 × B3, F1 × B3, and F9 × B3 were the top three. These combinations exhibited balanced and excellent performance across all indicators, particularly showing significant effects in enhancing Vc content, reducing TA, and improving flavor. The performance of male parent A1 was the second best with its combinations primarily ranking between 4<sup>th</sup> and 17<sup>th</sup> place, indicating that it was also a relatively excellent male parent, particularly demonstrating stable performance in improving fruit set rate and single fruit weight. Paternal lines A2 and B4 were relatively weaker overall with most combinations ranking between 18<sup>th</sup> and 36<sup>th</sup>. Paternal line B4 exhibited severe reproductive incompatibility with certain maternal lines, causing significant declines in performance metrics and substantially impacting its overall score (Figure 4).

#### Conclusion

The study made use of wild kiwifruit germplasm from various regions in Henan, China as research

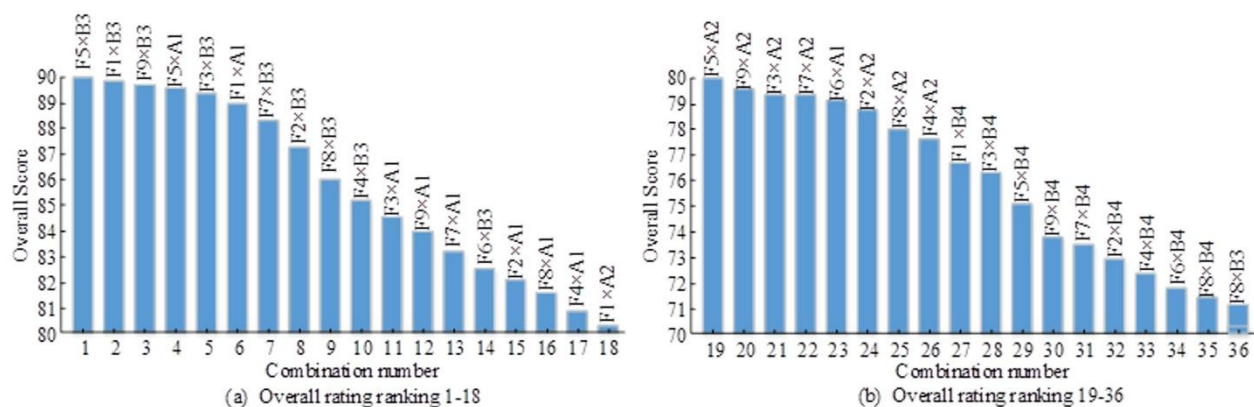


Figure 4. Comprehensive evaluation ranking of superior hybrid combinations.

subjects and systematically explored the regulatory effect of artificial pollination on the quality of wild kiwifruit and the matching rules of excellent hybrid combinations. The results confirmed that the technology system for selecting high-vitality male parents for artificial pollination could effectively overcome the reproductive bottleneck caused by the low natural pollination efficiency of wild kiwifruit. This system could also achieve a synergistic improvement in the fruit-setting rate, single-fruit weight, intrinsic nutritional quality, and flavor sensory quality. Moreover, the direct effect of male parent pollen and the reproductive affinity between parents were key factors affecting the improvement of fruit quality. The constructed multi-index weighted evaluation model also provided a reliable quantitative screening method for wild kiwifruit hybrid breeding. This research still had certain limitations, which included that the vitality of pollen from wild male plants was easily affected by environmental and storage conditions, and existing preservation methods made it difficult to maintain the long-term stability of pollen activity. In addition, the selected excellent hybrid combination F1 generation seeds had a low germination rate, which restricted the rapid domestication and breeding process of wild germplasm. Future studies would further optimize the pollen collection, preservation, activation technology system and establish a long-term stable preservation plan suitable for wild kiwifruit

pollen. In addition, future study could simultaneously combine biotechnology methods such as embryo rescue and backcross breeding to improve the germination and seedling rates of excellent combinations. Multigenerational trait identification might also be needed to ensure the stable inheritance of excellent agronomic traits.

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