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Impact of Irrigation and Artificial Pollination on *Macadamia*: Fruit Set and Yield

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Abstract

Severe flower and fruit abscission leading to low yields makes improving fruit set and increasing production critical challenges in *Macadamia* cultivation. Irrigation and artificial pollination during the flowering period significantly influence the fruiting and yield of macadamia. However, the synergistic effect of these two factors on macadamia production and yield remains unclear. This study investigated the effects of irrigation and artificial pollination on fruit set and yield using 11-year-old 'A16' *Macadamia* trees. Four treatments were applied: drought (DC), drought with artificial pollination (DC + AP), irrigation (I), and irrigation with artificial pollination (I + AP). Each treatment included three biological replicates, with a total of 12 trees. We assessed fruit set and yield, analyzing underlying mechanisms by evaluating changes in pollen viability, leaf morphology, inflorescence characteristics, and leaf/inflorescence physiology. Results revealed that DC + AP, I, and I + AP treatments exhibited significantly higher pollen viability and raceme length compared to DC. The I + AP treatment also resulted in the longest summer shoot internode length. Racemes were more sensitive to drought stress than leaves. Soluble protein and soluble sugar content in racemes were significantly higher in I + AP than in I and DC + AP, and lowest in DC. The DC treatment showed significantly higher superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) contents compared to I and I + AP. Leaf physiological traits exhibited inconsistent changes across treatments. Both artificial pollination and irrigation significantly increased fruit set. Treatment efficacy ranked as follows: I + AP (102.00% increase) > DC + AP (56.00% increase) > I (14.00% increase) > DC. Consequently, the I + AP treatment achieved significantly higher yield, fruit numbers, and fruit diameters than the other treatments. In terms of yield, treatment efficacy ranked as follows: I + AP (77.72% increase) > DC + AP (41.14 increase) > I (27.54% increase) > DC. These findings provide a scientific basis for enhancing yield in high-yield *Macadamia* cultivation systems.

Keywords: *Macadamia*; fruit set rate; yield; irrigation; artificial pollination

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1. Introduction

Macadamia (*Macadamia integrifolia* Maiden & Betche), native to the subtropical rainforests of Queensland and New South Wales, Australia, belongs to the genus *Macadamia* within the Proteaceae family [1,2]. China currently possesses the world's largest *Macadamia* cultivation area, accounting for over 50% of the global total [3]. However, severe flower and fruit abscission presents a major challenge in *Macadamia* cultivation, directly reducing yield per unit area [2,4]. Previous studies have indicated that macadamia yield is influenced by multiple factors, such as climate, soil nutrition [5], pest damage [6–8], and water availability [9,10]. Among these, water availability is considered a crucial environmental factor for tree growth, development, and achieving stable increases in yield. Specifically, water stress during flowering can lead to a lack of flowering synchrony, poor development of floral organs, reduced pollen germination rates, and impediments to pollination and fertilization [2,11]. Consequently, this results in low fruit set rates and significant yield losses [12,13]. Furthermore, water insufficiency inhibits the growth and development of young fruit, further exacerbating yield reduction [14]. Conversely, appropriate irrigation practices can effectively promote tree growth [2,14]. Irrigation during flowering, in particular, has been proven to significantly enhance nut yield and kernel yield [15]. However, some studies indicate that in regions with high rainfall, excessive irrigation may reduce yield in certain plots and lead to a decrease in individual nut weight [16].

Besides water management, pollination is another key factor determining *Macadamia* fruit set and yield [17]. Supplemental cross-pollination significantly enhances fruit set rates, overall yield, and other economic indicators [17,18]. Compared to self-pollination, cross-pollination consistently increases final nut set across multiple *Macadamia* varieties [19]. This phenomenon is closely related to the self-incompatibility present in some *Macadamia* cultivars [20]. Numerous studies have also confirmed that cross-pollination is an important method for overcoming self-incompatibility and improving fruit set rates [21–23]. This is because the stigma of *Macadamia* flowers typically exhibits peak receptivity 1–3 days after anthesis, and cross-pollination between different varieties significantly increases initial fruit set [24]. Furthermore, even though a few cultivars exhibit some capacity for self-fruitfulness, their self-pollinated fruit set rates are generally low and vary significantly between cultivars [12]. Therefore, in *Macadamia* cultivation, in addition to considering cross-compatibility among varieties, it is essential to account for the synchronization of flowering phenology between different cultivars [25]. Studies indicate that implementing supplementary artificial pollination during the full bloom stage can significantly enhance the fruit set rate in the early bearing phase, mitigate physiological fruit abscission, and ultimately improve overall fruit retention, leading to higher yields [22]. Furthermore, supplementary pollination during flowering can stimulate pollen growth and development, though its efficacy varies depending on the cultivar [26].

Most existing research focuses on the impact of single factors, specifically examining the independent effects of either flowering-period irrigation or artificial supplementary pollination on inflorescence parameters and yield during fruit development [14,19,21,26]. However, there remains a lack of systematic investigation into the comprehensive effects of combining these practices synergistically and their combined impact on *Macadamia* fruit-set and yield, despite the existence of relevant studies on other nut varieties demonstrating yield improvements. For instance, studies have indicated that timely irrigation coupled with artificial pollination can significantly enhance both yield and quality in hazelnuts [27–31]. Moreover, research on the intrinsic physiological mechanisms by which irrigation and artificial pollination—especially their synergistic effects—influence *Macadamia* fruit set and yield remain inadequate. Therefore, this study focuses on the 'A16' *Macadamia* cultivar, a major variety in Yunnan Province, China. We comprehensively

investigate two key cultivation practices: irrigation and artificial pollination. Our aim is to explore the effects of their combined application on enhancing fruit set and yield, providing theoretical support for developing efficient, high-yield cultivation techniques. Furthermore, by systematically measuring key physiological indicators under different irrigation and pollination treatments, this research will elucidate the physiological mechanisms through which water and pollination regulate fruit set and yield. This will provide a robust scientific basis for future high-yield, stable-yield, and efficient *Macadamia* cultivation practices.

2. Materials and Methods

2.1. Experimental Materials

This study was conducted from January to August 2024 at the Yunnan Institute of Tropical and Subtropical Cash Crops (24°58′22″ N, 98°52′51″ E), situated in Lujiangba Town, Longyang District, Baoshan City, Yunnan Province. The area exhibits a typical plateau monsoon climate with distinct three-dimensional features. The average annual temperature ranges from 15 to 17 °C, and annual precipitation averages 900–1200 mm. Characterized by pronounced wet and dry seasons, significant diurnal temperature fluctuations, and an average altitude of 645.5 m. The experimental material comprised 11-year-old 'A16' *Macadamia* trees displaying uniform and vigorous growth. Key growth indicators—including tree height, north–south crown spread, east–west crown spread, diameter at breast height (DBH), basal diameter, and branch-free—were measured for 12 sample trees. Statistical analysis revealed no significant differences in these growth metrics among the selected trees (Table S2).

2.2. Experimental Design

This experiment utilized a two-factor design involving pollination and water treatment. Pollination consisted of two methods: natural pollination and artificial pollination. Water treatments included normal irrigation (maintaining 55–60% field water holding capacity) and drought treatment (no irrigation). This resulted in four distinct treatment combinations: drought + natural pollination, drought + "951" artificial pollination, irrigation + natural pollination, and irrigation + "951" artificial pollination. Each treatment combination had three replicates.

The maternal line selected for the experiment was 'A16', while the paternal line was '951', chosen for its strong compatibility. Artificial pollination was conducted via cross-pollination using test tubes: collected "951" pollen was applied directly to the stigmas of "A16" sample trees. Drought treatment was implemented by covering the ground with plastic film to exclude natural rainfall. Conversely, the irrigation treatment involved watering once every 7 days at 18:30. Supplemental cross-pollination was performed on sample trees within 24–26 h after floret opening, specifically between 9:00 AM and 11:00 AM [32].

2.3. Sample Collection

Raceme sampling: Sampling was conducted at two key stages: 7 days after initial flowering and at full bloom. At each stage, ten raceme clusters were collected per sample tree using pruning poles. During the initial flowering stage, sampling targeted pre-marked branches distributed throughout the plant canopy that exhibited consistent raceme size and flower count. At full bloom, racemes located in the middle section of the plant with over 50% of flowers open were selected.

Leaf sampling: Leaf sampling was uniformly conducted between 8:00 AM and 9:00 AM. The target tissue consisted of the 2nd to 3rd most recent functional leaves on branches within the middle canopy. Sampling occurred at three distinct stages: initial

flowering (late February), full bloom (early to mid-March), and initial fruit drop (mid to late March). At each stage, ten leaves per tree were collected, comprising two leaves from each of five selected fruiting shoots. The sampled flowers and leaves were immediately wrapped in aluminum foil and stored at −80 °C for subsequent biochemical analysis.

Fruit abscission dynamics survey: Surveys were conducted at 1, 2, 4, 6, and 20 weeks after flowering. By week 20, fifty marked fruiting clusters per tree exhibiting consistent initial fruit set were selected from all directions to serve as the final survey subjects. Based on these samples, the following parameters were determined: final fruit number per raceme; green-skinned fruit weight, transverse diameter, and longitudinal diameter of green-skinned fruits; as well as fruit setting rate, incremental yield, incremental yield ratio, fruit shape index, and kernel yield. Specifically, using the fruit count recorded 1 week after flowering as the baseline, the patterns of fruit set rate changes in subsequent periods were systematically recorded and analyzed.

2.4. Determination of Fruit Economic Indicators

The fruit economic traits measured in this study comprised fruit setting rate, fruit abscission rate, initial fruit set, cumulative fruit abscission rate, relative fruit abscission rate, yield increase value, yield increase ratio, fruit shape index, and kernel yield. The calculation formulas are as follows.

$$\text{The fruit setting rate (\%) = the average number of fruits per raceme/the average number of flowers per raceme} \times 100\% \quad (1)$$

$$\text{Fruit abscission rate (\%) = (Initial fruit set number per raceme} - \text{Final fruit set number per raceme)/Initial fruit set per raceme} \times 100\% \quad (2)$$

$$\text{Initial fruit setting quantity = Final fruit count + Cumulative number of dropped fruits} \quad (3)$$

$$\text{Cumulative fruit abscission rate (\%) = Cumulative number of dropped fruits up to the survey date/Initial fruit setting quantity} \times 100\% \quad (4)$$

$$\text{Yield increase value (kg) = Average yield per plant (Treatment group)/Average yield per plant (Control group)} \quad (5)$$

$$\text{Yield increase ratio (\%) = Yield increase value/Average yield per plant (Control group)} \times 100\% \quad (6)$$

$$\text{Fruit shape index = Nut longitudinal diameter/Nut transverse diameter} \quad (7)$$

$$\text{Kernel yield = Nut kernel mass/fresh fruit (in-hull) mass} \times 100\% \quad (8)$$

2.5. Pollen Viability Determination

Pollen viability was assessed using the agar culture method [18]. At the full flowering stage, flowers with arched styles and freshly dehisced anthers were collected. Two flower spikes were sampled from each tree. The anthers were carefully removed to release the pollen, which was immediately cultured. The culture medium consisted of 0.5% agar and 10% sucrose, with a pH adjusted to 5.8–6.0. Pollen grains were transferred onto glass slides pre-coated with the agar medium and placed in a Petri dish. To maintain humidity, the dish lid was lined with moist filter paper. The samples were then incubated in a light-controlled growth chamber at 25 °C for 10 h. After incubation, pollen germination was examined under a microscope at 10 × 16 magnification. For each treatment, three replicate slides were prepared, and five random fields of view were observed per slide to calculate the pollen germination rate.

2.6. Physiological Index Determination

Relative water content of leaf was measured using the oven-drying and gravimetric method [33]. Key osmotic adjustment substances, including soluble sugars, soluble proteins, and proline, were quantified. Additionally, the activities of antioxidant enzymes—SOD, POD, and CAT—were also assessed. Soluble sugar content was determined via the anthrone colorimetric method [34], while soluble protein levels were measured using the Coomassie Brilliant Blue assay [35]. Free proline content was analyzed by the ninhydrin colorimetric method [36]. The activities of SOD, POD, CAT, and the concentration of MDA were quantified using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; Cat# A001-3-2, A084-3-1, A007-1-1, and A003-1-2) following the manufacturer's instructions. All analyses were performed on whole floral organs (excluding pedicels) and leaves, with three biological replicates included for each experiment.

2.7. Statistical Methods

Each treatment in this study contains three replicates, calculates the mean value and standard deviation of the three replicates. One way ANOVA and multiple comparisons are performed between different treatments. All data were analyzed by R4.3.2 program, ANOVA and Duncan test were used for multi-group comparison between different treatments, the statistical significance was set as $p < 0.05$ (bilateral test), and the quantitative data was expressed as [mean ± standard deviation].

3. Results

3.1. Effects of Different Treatments on Morphology of Macadamia Flowers, Leaves, and Summer Shoots

To investigate the effects of irrigation and artificial pollination on the fruit setting rate of *Macadamia*, four treatments were established during the flowering period of mature *Macadamia* trees: drought, drought + artificial pollination, irrigation, and irrigation + artificial pollination. The results showed that compared with drought treatment alone, pollen germination rate was significantly higher under irrigation + artificial pollination (+29.78%), irrigation (+27.12%), and drought + artificial pollination (+14.60%) treatments (Figure 1a,b). Similarly, the raceme length was significantly greater under irrigation + artificial pollination (+5.51%), irrigation (+6.31%), and drought + artificial pollination (+7.80%) treatments than under drought treatment alone (Figure 1c). There was no significant difference in the number of flowers per raceme among the 4 treated sample trees (Figure 1d). These findings suggest that drought significantly suppress pollen activity and raceme development.

Furthermore, the influence of these treatments on *Macadamia* leaf and summer shoot growth was examined. Statistical analysis indicated no significant differences in leaf length or width across the four treatments, implying that leaf development remained unaffected. However, significant variations were observed in the internode length of summer shoots. Specifically, the internode length in the irrigation + artificial pollination treatment was significantly greater than that in the other three treatments (Table S1). This suggests that the combined effect of irrigation and artificial pollination may promote the elongation growth of new shoots.

3.2. Effects of Different Treatments on Physiology of Macadamia Racemes

To investigate the physiological responses of *Macadamia* racemes to different treatments, the contents of osmotic adjustment substances, antioxidant enzyme activities, and the accumulation of oxidative products were measured. The results revealed that soluble protein content in racemes under irrigation + artificial pollination was significantly higher than that under drought and drought + artificial pollination treatments. In addition, com-

pared with drought treatment alone, irrigation + artificial pollination significantly increased the soluble sugar content in racemes at 8 and 10 weeks of drought stress. Additionally, soluble protein content exhibited a continuous increase from week 8 to week 10 under drought stress (Figure 2a,b). Drought stress had a pronounced effect on the antioxidant enzyme system. As the duration of drought progressed, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were significantly higher in the drought and drought + artificial pollination treatments than in the irrigation and irrigation + artificial pollination treatments (Figure 2c–e).

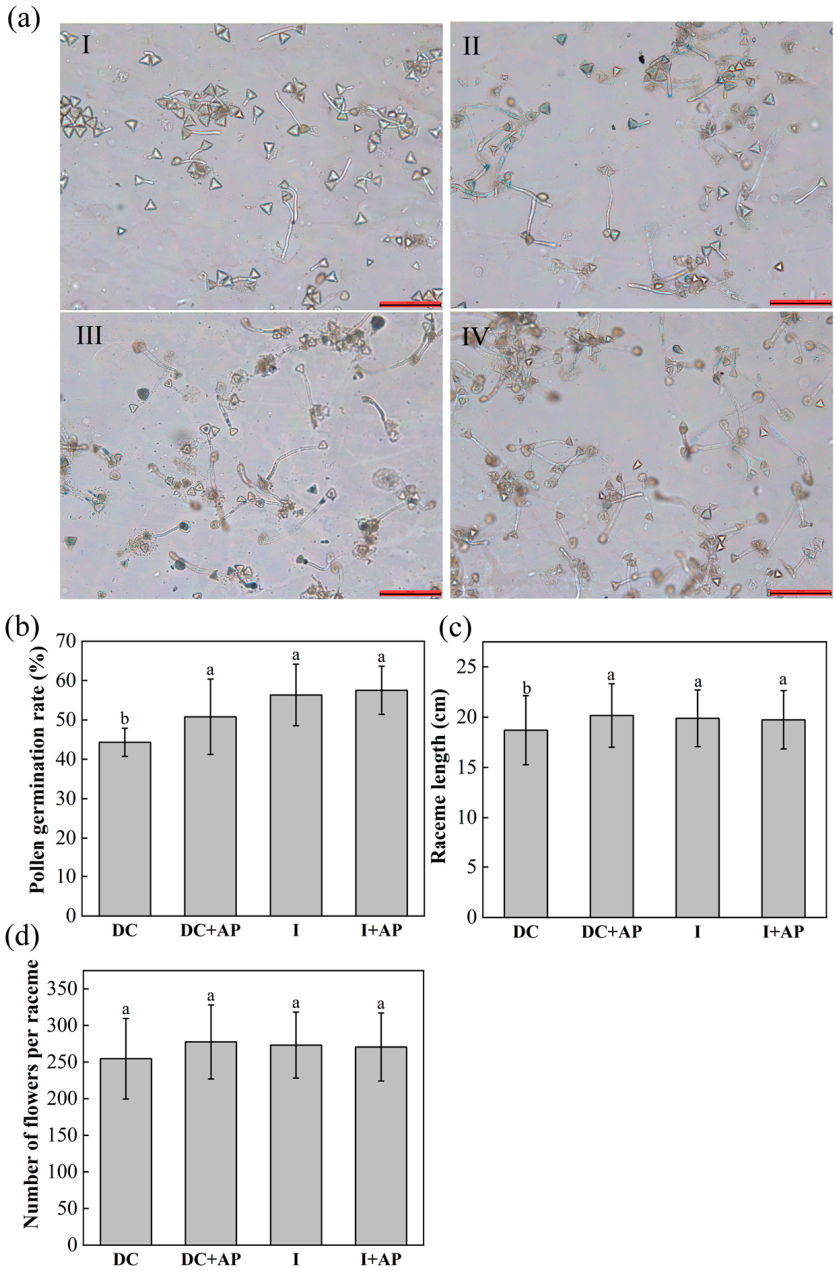


Figure 1. Effects of different treatments on *Macadamia* pollen germination and raceme. (**a**) Microscopic views of pollen grains. Panels (I)–(IV) represent: drought treatment, drought + artificial pollination, irrigation treatment, and irrigation + artificial pollination, respectively. Scale bar: 200 μm (red, lower right). (**b**) Pollen germination rate (%). (**c**) Raceme length (cm). (**d**) Number of flowers per raceme. Data represent means ± SD (n = 3). Different letters (a, b) indicate significant differences among groups ($p < 0.05$, Duncan's test). DC, DC + AP, I, and I + AP represent drought treatment, drought + artificial pollination, irrigation treatment, and irrigation + artificial pollination, respectively.

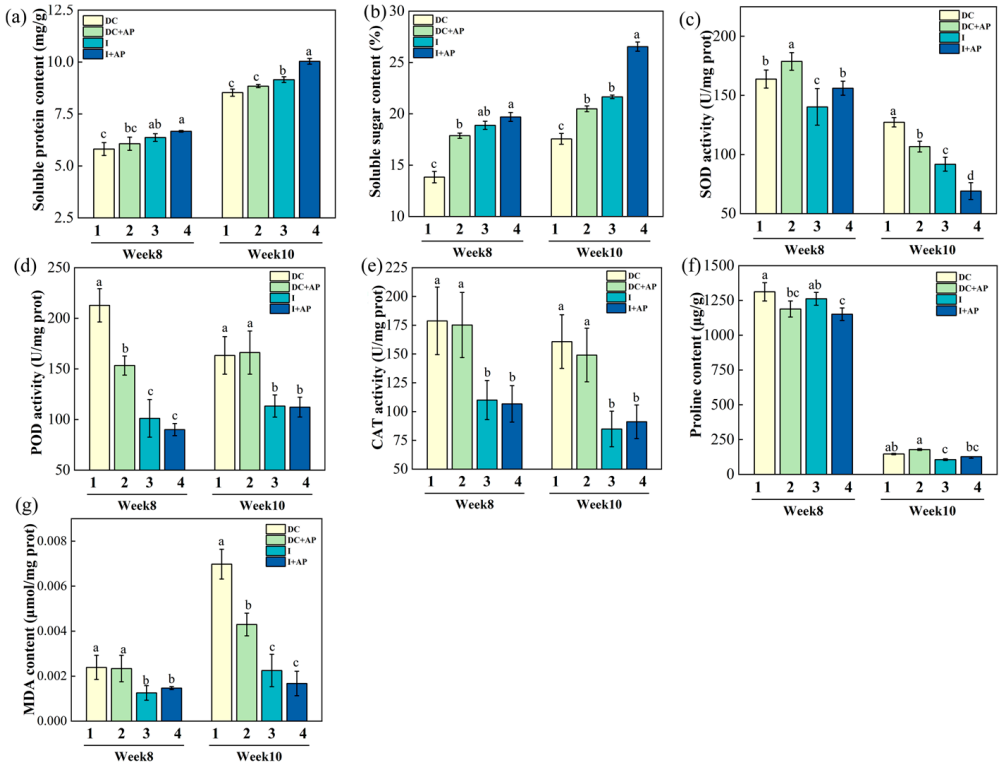


Figure 2. Effects of different treatments on physiology of *Macadamia* raceme. (**a**) Soluble protein content (mg/g). (**b**) Soluble sugar content (mg/g). (**c**) SOD activity (U/mg prot). (**d**) POD activity (U/mg prot). (**e**) CAT activity (U/mg prot). (**f**) Proline content (μg/g). (**g**) MDA content (μmol/mg prot). DC, DC + AP, I, and I + AP represent drought treatment, drought + artificial pollination, irrigation treatment, and irrigation + artificial pollination, respectively. Data represent means ± SD (n = 3). Different letters (a, b, c, d) indicate significant differences among groups ($p < 0.05$, Duncan's test).

Proline content analysis showed that, at 8 weeks of drought stress, racemes subjected to the drought treatment accumulated significantly more proline than those under the drought + artificial pollination and irrigation + artificial pollination treatments. By week 10, proline levels remained significantly higher in the drought treatment compared to the irrigation treatment. Furthermore, at this stage, the drought + artificial pollination treatment also displayed significantly higher proline content than the irrigation + artificial pollination treatment (Figure 2f). In terms of oxidative products, malondialdehyde (MDA) content was significantly elevated in racemes under the drought and drought + artificial pollination treatments relative to the irrigation treatments (irrigation and irrigation + artificial pollination) at 8 and 10 weeks of drought stress (Figure 2g). There results indicated that the different treatments significantly influenced the accumulation of osmotic adjustment substances, the activities of key antioxidant enzymes, and the levels of oxidative products in *Macadamia* racemes.

3.3. Effects of Different Treatments on Physiology of Macadamia Leaves

To assess physiological changes in *Macadamia* leaves under different treatments, the relative water content of leaf was measured. The results showed that leaves under irrigation maintained significantly higher relative water content compared to those under drought conditions. Furthermore, the relative water content of leaf progressively decreased as drought stress continued (Figure 3a). Regarding soluble protein content, the drought treatment and the drought + artificial pollination treatment exhibited significantly higher levels than the irrigation and irrigation + artificial pollination treatments at week 8. By week 12,

however, no significant differences were observed among the treatments, although soluble protein content in all groups had decreased relative to week 8 (Figure 3b). For soluble sugar content, the drought + artificial pollination treatment showed significantly higher levels than the other three treatments at week 8. By week 12, no significant differences were detected among treatments (Figure 3c).

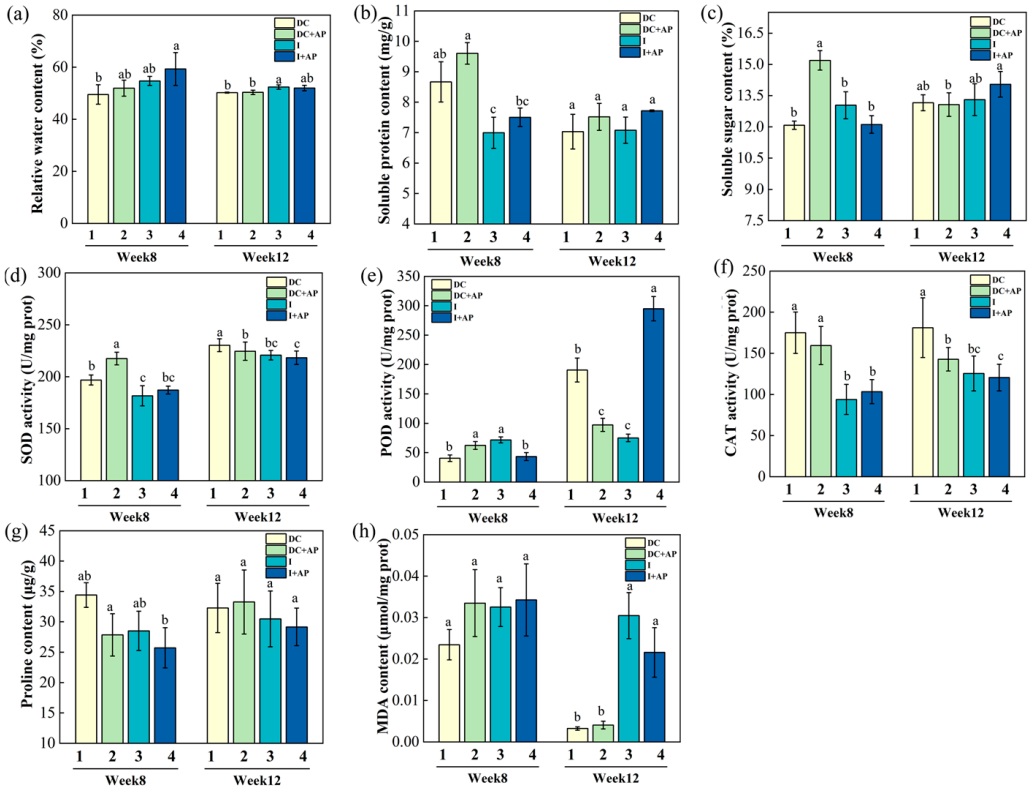


Figure 3. Effects of different treatments on physiology of *Macadamia* leaves. (**a**) Relative water content (%). (**b**) Soluble protein content (mg/g). (**c**) Soluble sugar content (mg/g). (**d**) SOD activity (U/mg prot). (**e**) POD activity (U/mg prot). (**f**) CAT activity (U/mg prot). (**g**) Proline content (μg/g). (**h**) MDA content (nmol/mg prot). DC, DC + AP, I, and I + AP represent drought treatment, drought + artificial pollination, irrigation treatment, and irrigation + artificial pollination, respectively. Data represent means ± SD (n = 3). Different letters (a, b, c) indicate significant differences among groups ($p < 0.05$, Duncan's test).

Analysis of antioxidant enzyme activity revealed that SOD activity was significantly higher in the drought treatment than in the corresponding irrigation treatment at both week 8 and week 12. Similarly, the drought + artificial pollination treatment displayed significantly higher SOD activity than the irrigation + artificial pollination treatment (Figure 3d). POD activity was significantly higher only in the drought + artificial pollination treatment compared to the irrigation + artificial pollination treatment at week 8 (Figure 3e). CAT activity was significantly elevated in the drought treatment and the drought + artificial pollination treatment relative to the irrigation treatment at week 8. By week 12, CAT activity in the drought treatment was significantly higher than in all other treatments (Figure 3f). In osmotic adjustment substances, proline content was significantly higher in the drought + artificial pollination treatment than in the irrigation + artificial pollination treatment at week 8, but no significant differences were observed by week 12 (Figure 3g). For MDA content, no significant differences were detected among treatments at week 8. However, by week 12, MDA levels in the two irrigation treatments were significantly higher than in the drought treatments (Figure 3h). These find-

ings demonstrate that the different treatments significantly influenced leaf physiological responses, with patterns similar to those observed in inflorescence treatments.

3.4. Effects of Different Treatments on Macadamia Fruit Setting Rate

To investigate the effects of different treatments on the fruit setting rate of *Macadamia*, fruit setting rates at various developmental stages were examined. The results demonstrated that both irrigation and artificial pollination significantly increased the number of fruit set per raceme. Specifically, the final number of fruit abscission per raceme was significantly lower in the irrigation + artificial pollination treatment compared to the drought treatment. However, differences in fruit abscission rates among the four treatments did not reach statistical significance (Figure 4a–d). All treatments exhibited a sharp decline in fruit setting rates from week 1 to week 6, followed by stabilization thereafter (Figure 4i). Both irrigation and artificial pollination significantly influenced the final fruit setting rate. At different fruit development stages, the fruit setting rate under irrigation + artificial pollination was significantly higher than under drought and irrigation alone, though no significant difference was observed compared to the drought + artificial pollination treatment (Figure 4e–h). Relative to the drought treatment, the final fruit setting rates increased significantly by 55.85% in the drought + artificial pollination treatment, 12.74% in the irrigation treatment, and 102.00% in the irrigation + artificial pollination treatment.

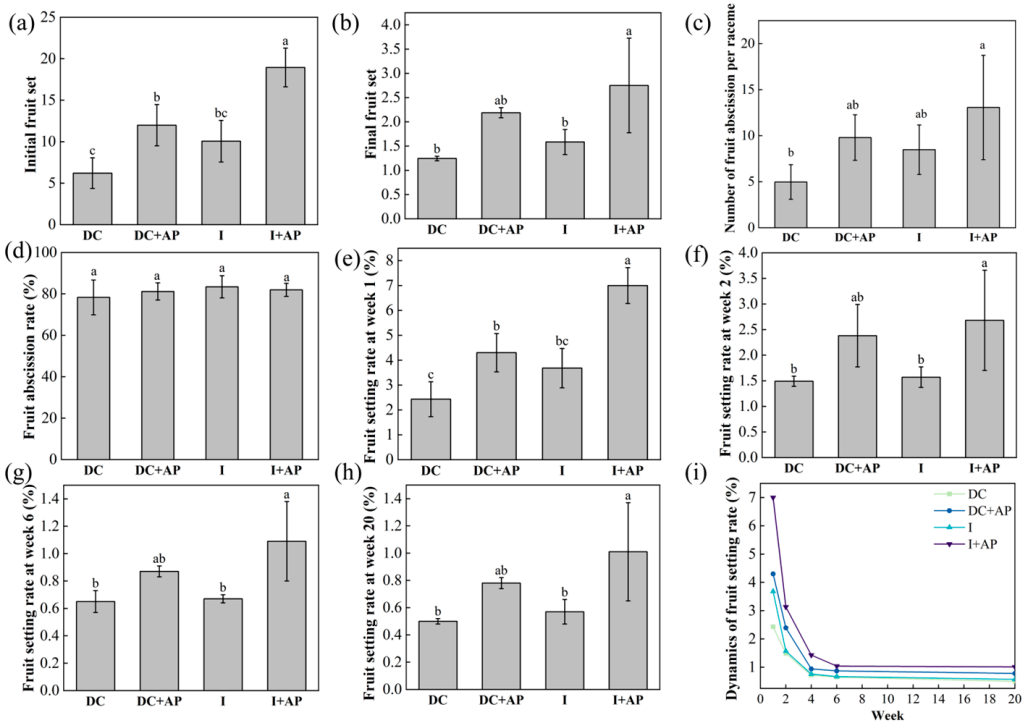


Figure 4. Effects of different treatments on fruit set per raceme, fruit abscission, and fruit setting rate in *Macadamia*. (**a**) Initial fruit set. (**b**) Final fruit set. (**c**) Number of fruit abscission per raceme. (**d**) Fruit abscission rate. (**e**) Fruit setting rate at Week 1. (**f**) Fruit setting rate at Week 2. (**g**) Fruit setting rate at Week 6. (**h**) Fruit setting rate at Week 20. (**i**) Temporal changes in fruit setting rate across treatments. DC, DC + AP, I, and I + AP represent drought treatment, drought + artificial pollination, irrigation treatment, and irrigation + artificial pollination, respectively. Data represent means ± SD (n = 3). Different letters (a, b, c) indicate significant differences among groups ($p < 0.05$, Duncan's test).

3.5. Effects of Different Treatments on Macadamia Yield

To investigate the effects of different treatments on *Macadamia* yield, we analyzed the yield data from each experimental group. The results demonstrated that the

irrigation + artificial pollination treatment significantly enhanced both yield and fruit count compared to all other treatments. However, no significant difference in yield was observed between the irrigation-only treatment and the drought + artificial pollination treatment. Regarding the yield increase rate, the irrigation and irrigation + artificial pollination treatments exhibited significantly higher values than the drought treatment (Figure 5). Specifically, the irrigation + artificial pollination and drought + artificial pollination treatments resulted in greater average yields, higher fruit counts, larger yield increases, and superior yield increase rates compared to the irrigation-only or drought-only treatments. Among all treatments, irrigation + artificial pollination achieved the highest values across all measured parameters, including yield increase (+77.68%), yield increase rate (+77.68%), average yield (+77.72%), and average fruit count (+75.42%). Compared to the drought treatment, the yield increase rates were as follows: 29.82% for drought + artificial pollination, 27.52% for irrigation, and 77.68% for irrigation + artificial pollination. These results suggest that the combined application of irrigation and artificial pollination proved to be the most effective strategy for enhancing *Macadamia* yield.

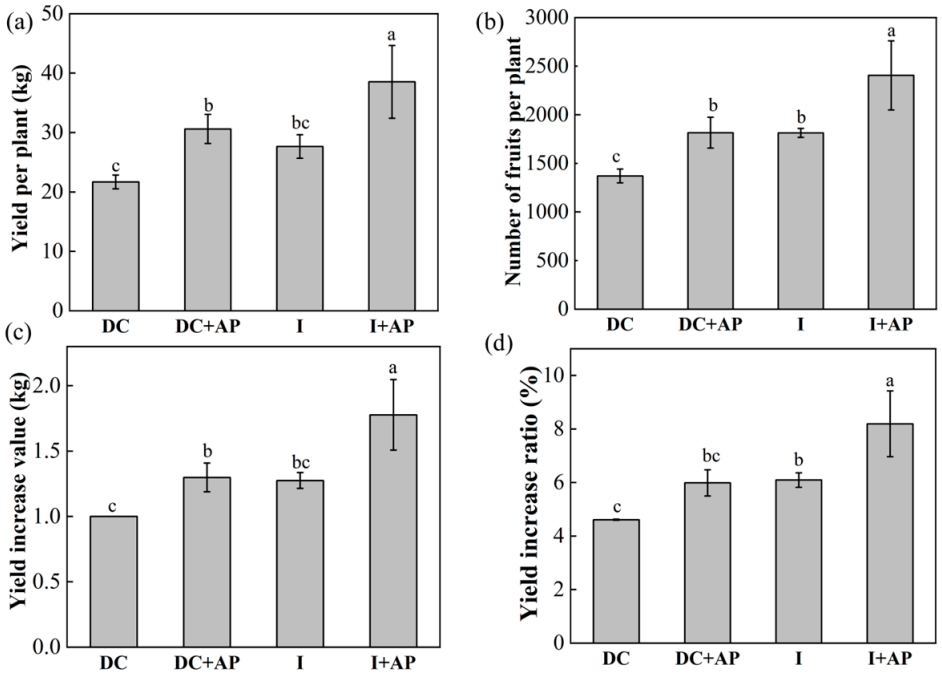


Figure 5. Effects of different treatments on *Macadamia* yield. (**a**) Yield per plant (kg). (**b**) Number of fruits per plant. (**c**) Yield increase value (kg). (**d**) Yield increase ratio (%). Data represent means ± SD (n = 3). DC, DC + AP, I, and I + AP represent drought treatment, drought + artificial pollination, irrigation treatment, and irrigation + artificial pollination, respectively. Data represent means ± SD (n = 3). Different letters (a, b, c) indicate significant differences among groups ($p < 0.05$, Duncan's test).

3.6. Effects of Different Treatments on the Economic Traits of Macadamia Nuts

To investigate the effects of different treatments on the economic traits of *Macadamia* nuts, we first analyzed changes in fruit transverse diameter across various growth stages. The results indicate that both irrigation and artificial pollination treatments promoted fruit growth. The ranking of fruit transverse diameter among the treatment groups was as follows: irrigation + artificial pollination (+2.34%) > drought + artificial pollination (+1.67%) > irrigation (+1.34%) > drought. During the early fruit stage (weeks 1 to 6), the fruit transverse diameter exhibited a significant increasing trend; after week 6, the rate of increase markedly slowed. Compared to the drought treatment, the irrigation + artificial pollination treatment significantly increased fruit transverse diameter (Table 1).

Table 1. Effects of different treatments on the transverse diameter of *Macadamia* green-skinned fruits.

Fruit Diameter (cm)	Week 1	Week 2	Week 4	Week 6	Week 20
Drought	0.30 ± 0.06 c	0.44 ± 0.05 c	1.09 ± 0.11 d	2.79 ± 0.08 c	2.99 ± 0.17 c
Drought + artificial pollination	0.32 ± 0.04 b	0.46 ± 0.04 b	1.17 ± 0.06 c	2.86 ± 0.07 b	3.04 ± 0.07 ab
Irrigation	0.32 ± 0.03 b	0.47 ± 0.04 b	1.19 ± 0.04 b	2.86 ± 0.19 b	3.03 ± 0.07 b
Irrigation + artificial pollination	0.36 ± 0.05 a	0.49 ± 0.04 a	1.24 ± 0.05 a	2.96 ± 0.08 a	3.06 ± 0.07 a

Note: Data represent means ± SD (n = 3). Different letters (a, b, c, d) indicate significant differences among groups ($p < 0.05$, Duncan's test).

In addition, multiple post-harvest traits of fruits were also measured under different treatments, including transverse diameter of green-skinned fruits, longitudinal diameter of green-skinned fruits, aspect ratio of green-skinned fruits, green-skinned fruit weight, transverse diameter of shell, longitudinal diameter of shell, aspect ratio of shell, and kernel yield. However, the results showed no statistically significant differences in these indicators among the treatments (Table 2).

Table 2. Effects of different treatments on economic indexes of *Macadamia*.

Treatment	Drought	Drought + Artificial Pollination	Irrigation	Irrigation + Artificial Pollination
Transverse diameter of green-skinned fruits (cm)	2.99 ± 0.16 a	3.00 ± 0.14 a	2.98 ± 0.14 a	3.03 ± 0.13 a
Longitudinal diameter of green-skinned fruits (cm)	3.43 ± 0.17 a	3.47 ± 0.17 a	3.42 ± 0.18 a	3.44 ± 0.18 a
Aspect ratio of green-skinned fruits (cm)	1.15 ± 0.04 a	1.14 ± 0.03 a	1.15 ± 0.04 a	1.15 ± 0.03 a
green-skinned fruit weight (g)	16.63 ± 2.44 a	17.30 ± 2.32 a	16.65 ± 2.07 a	17.28 ± 2.34 a
Transverse diameter of shells (cm)	2.33 ± 0.13 a	2.38 ± 0.18 a	2.33 ± 0.13 a	2.35 ± 0.15 a
Longitudinal diameter of shells (cm)	2.59 ± 0.16 a	2.61 ± 0.14 a	2.57 ± 0.13 a	2.65 ± 0.14 a
Aspect ratio of shells (cm)	1.11 ± 0.04 a	1.1 ± 0.04 a	1.1 ± 0.04 a	1.13 ± 0.04 a
Shell weight (g)	7.94 ± 1.56 a	8.31 ± 1.43 a	7.78 ± 1.08 a	8.10 ± 1.41 a
Kernel yield (%)	47.52 ± 2.51 a	47.84 ± 2.39 a	46.69 ± 2.70 a	46.71 ± 2.90 a

Note: Data represent means ± SD (n = 3). Different letters indicate significant differences among groups ($p < 0.05$, Duncan's test).

4. Discussion

Drought stress significantly inhibits the growth and development of *Macadamia* trees, manifesting as reduced pollen fertility, decreased fruit set, diminished yield, and inferior fruit quality [37,38]. Numerous previous studies have confirmed that artificial pollination can effectively mitigate these adverse effects [15,39,40]. However, due to variations in cross-compatibility between parental cultivars, fruit set, yield, and fruit quality following artificial pollination remain highly variable [23,40]. Furthermore, previous studies have predominantly focused on the impact of single factors (such as irrigation or artificial pollination) on fruit set or development [17,21,41–43]. Therefore, using major *Macadamia* cultivars (A16) cultivated in Yunnan Province as test materials, this study systematically investigates the interactive effects of irrigation and artificial pollination simultaneously on fruit set and yield, providing a new theoretical basis for efficient *Macadamia* cultivation.

Macadamia exhibits partial self-incompatibility [20,21]; consequently, cross-pollination during flowering significantly enhances fruit set and yield [37,44]. Research shows that irrigation during flowering significantly improves pollen fertility, thereby increasing fruit set and yield [20]. The final number of fruits dropped per inflorescence for the four treatment sample trees was as follows: irrigation + artificial pollination > drought + artificial pollination > irrigation > drought. However, there was no significant difference in the fruit drop rates. A possible explanation is that the first two treatments (Irrigation + Artificial Pollination and Drought + Artificial Pollination) resulted in higher initial fruit sets. Yet, due to the absence of fruit preservation measures in later stages, they ultimately experienced

greater fruit drop. Nevertheless, because these two treatments began with higher fruit set rates, they still maintained relatively high final yields. Under the combined treatments in this study, the fruit setting rate and yield exhibited a gradient pattern—irrigation + artificial pollination > irrigation > drought + artificial pollination > drought. Notably, the irrigation and pollination treatments did not significantly affect fruit morphological indices (including transverse and longitudinal diameters, weight, and aspect ratio). Potential explanations include the use of a single paternal cultivar ('951') or the application of treatments solely during flowering without extension into the fruit development stage. Previous studies confirm significant differences in fruit size among pollination combinations [9,45], suggesting these traits may be regulated by maternal genetic characteristics. Future research should focus on experimenting with cross-pollination among different macadamia varieties. This will help identify superior parental combinations for large-scale cultivation, ultimately enhancing yield more effectively.

Pollen viability directly influences pollination, fertilization, and yield [46]. Its manifestation is governed by genetic control and influenced by developmental environments [47,48]. Moderate irrigation effectively maintains pollen viability, promoting pollen germination and pollen tube elongation [42,49]. Conversely, water stress inhibits inflorescence development and reduces pollen activity [42,50]. Previous studies primarily concentrated on physiological responses of *Macadamia* seedlings under water stress, measuring leaf-related indicators [51]. This study extends the perspective to adult trees, systematically measuring physiological responses of racemes under water stress. Plant drought resistance mechanisms primarily involve osmoregulatory substance accumulation and membrane system stability maintenance [51,52]. Under drought, *Macadamia* trees enhance antioxidant enzyme activity to scavenge reactive oxygen species while accumulating osmoregulatory substances like soluble proteins, sugars, and free proline [53]. This experiment found that with increasing water stress intensity, MDA content significantly increased in both racemes and leaves. However, osmoregulatory substance responses exhibited tissue specificity: soluble protein and sugar contents in drought-treated raceme were significantly lower than in irrigated ones, while the opposite trend occurred in leaves. This discrepancy may relate to differing organ sensitivity [54]. Previous studies have indicated that the levels of osmoregulatory substances, such as soluble sugars and soluble proteins, typically increase in plants under external stress conditions. However, research focusing on the physiological responses of macadamia nuts under stress has demonstrated that, as the intensity of external stress increases, the content of soluble proteins in the leaves actually decreases. This phenomenon may be due to water stress leading to the active hydrolysis of starch, which reduces the osmotic potential within the plant and hinders the transport of photosynthetic products away from the leaves. Additionally, the damage caused by stress can disrupt normal physiological processes, resulting in the accumulation of soluble sugars [55]. Further studies have shown that the changes in soluble sugar content vary among different macadamia nut varieties under increasing stress levels: some varieties exhibit an increase, while others may initially rise before subsequently decreasing. This variation is attributed to the differences in stress resistance among the varieties. The results of this experiment support this conclusion [56].

The antioxidant enzyme system exhibits a dynamic response to stress intensity: SOD activity increases continuously, while POD activity rises under mild stress but declines under severe stress [57,58]. Based on the observed POD activity increase in this study, it can be inferred that test plants experienced mild water stress. Furthermore, significantly higher levels of free proline and MDA in racemes of drought-stressed plants confirm that water stress during flowering disrupts normal physiological metabolism [59,60]. Regarding the variability of physiological indicators in the leaf blades of the experimental sample

trees, previous studies have shown that fluctuations in the activity of the protective enzyme system under stress conditions are associated with the drought resistance of plant species or varieties [61]. Drought-resistant varieties, in particular, can maintain higher levels of protective enzyme activity under adverse stress conditions. Existing research also indicates that during prolonged drought, POD activity in plant leaves tends to increase initially and then decrease—a response influenced by both the genetic background of the variety and the physiological state of the plant [62,63]. Accordingly, the content of relevant biomarkers may fluctuate under stress, a finding consistent with our experimental results [64]. As for the variability in MDA content observed in the treated leaves, it may be attributed to the physiological condition of the trees at the time of sampling, as well as inherent limitations in the sampling methodology. This also highlights the constraints of the current laboratory-based evaluation system. Future research should aim to incorporate dynamic monitoring technologies and field-based phenotyping for more comprehensive validation. These findings align with established theory: proline accumulates to enhance cellular water retention capacity [65], while MDA, a key biomarker of membrane lipid peroxidation, directly reflects oxidative damage to cell membranes [66,67].

Research has shown that under drought stress, fruit trees can initiate drought resistance responses through coordinated physiological and molecular mechanisms. At the physiological level, trees rapidly close stomata to reduce water loss, accumulate osmoprotectants such as proline to maintain cellular osmotic balance and activity, and activate antioxidant enzyme systems to scavenge excess reactive oxygen species (ROS) and mitigate oxidative damage. At the molecular level, abscisic acid (ABA) acts as a key signaling molecule that activates transcription factor networks such as DREB and AREB, which in turn induce the expression of functional genes related to LEA proteins, antioxidant enzymes, and other protective compounds. This multi-level response mechanism—from stress perception to gene expression regulation and subsequent physiological adaptation—collectively enhances the overall drought tolerance of fruit trees [68–71]. Although this study reveals the patterns of fruit set and physiological traits of macadamia under drought and artificial pollination, the molecular basis for these patterns is not yet understood. Therefore, future work should focus on elucidating the related molecular mechanisms. In summary, water stress significantly affected the physiological regulation processes of *Macadamia* trees. This study lays a scientific foundation for understanding the physiological mechanisms underlying their stress response.

5. Conclusions

Physiological fruit abscission in *Macadamia* cultivation exhibits distinct stage-specific characteristics, primarily concentrated during the first six weeks of fruit development. To ensure yield, it is recommended to systematically implement blossom and fruit retention practices within this initial six-week period following fruit set. Research indicates that implementing moderate irrigation combined with artificial pollination during the flowering stage produces a significant synergistic effect. Simultaneously, it enhances pollen viability (by 29.78%) and extends inflorescence length (by 5.51%). Additionally, it promotes floral organ development, increasing fruit diameter by 1.67–2.34%, while also significantly stimulating summer shoot growth (by 72.27%). This integrated management approach can increase the fruit setting rate by 102.00%, ultimately achieving an overall yield increase of 77.68%. This study has effectively improved the fruit set rate and yield of macadamia nuts through scheduled irrigation and artificial pollination. In future macadamia production, considering both labor costs and practical feasibility, it is recommended to adopt drip irrigation systems. For pollination, dedicated pollinator trees can be planted in combination with

beekeeping within the orchards to enhance pollination efficiency, thereby further increasing the economic returns of nut production.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae11091111/s1, Table S1: Effects of different treatments on the morphology of leaves and summer shoots; Table S2: Growth status of *Macadamia* sample trees.

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Abbreviations

The following abbreviations are used in this manuscript:

SOD	Superoxide Dismutase
POD	Peroxidase
CAT	Catalase
MDA	Malondialdehyde

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