Effects of Exogenous Melatonin and ABA on Photosynthetic Characteristics of Naturally Aging Kiwi Seedlings

Rongping Hu¹, Zhonghan Fan¹*, Qinghua Chen¹, Xiaoyan Xu¹, Song Chen¹, Qingdong Chen¹ and Dongsheng Yang¹

¹ Institute of Plant Protection, Sichuan Academy of Agricultural Sciences/ MOA Key Laboratory of Integrated Management of Pests on Crops in Southwest China, Chengdu, Sichuan, 610066, China;

*Corresponding author’s e-mail: 332326468@qq.com

Abstract. In this study, the annual yellow kiwi seedlings which were treated with 200μmol/L melatonin (MT), 15μmmol/L abscisic acid (ABA) and 5mmol/L sodium tungstate solution (STD) were used as experimental materials to study the changes in physiological indicators of kiwi fruit under natural aging. After comprehensive analysis, we can speculate that MT may affect the photosynthetic characteristics of aging kiwi by affecting ABA production and keeping its content at a moderate level. The experiment results provided theoretical basis and practical guidance for delaying the production and application of kiwi seedlings.

1. Introduction
Kiwifruit, belonging to Actinidia Lindl., is a deciduous vine [1]. Natural aging can seriously restrict the growth and development of kiwifruit seedlings and have adverse effects. Melatonin (N-acetyl-5-methoxytryptamine), whose main physiological function in the organisms is anti-aging [2]. Abscisic acid (ABA) is an important regulator of plant response to stress, which can promote plant bud dormancy, inhibit growth, and promote aging [3]. At present, the study of anti-aging and anti-aging effects of kiwifruit seedlings by exogenous substance is not sufficient, and the investigation of the mechanism of aging is rarely reported. This study does make sense.

In this experiment, exogenous melatonin and abscisic acid were used to explore the mechanism of kiwifruit leaves of senescence in order to clarify the role of different exogenous substances in the aging process of kiwifruit, and provide theoretical basis and practical guidance for the effects of aging on kiwifruit seedlings and production applications.

2. Materials and methods
2.1. Cultivation of plant materials
The experiment materials were annual yellow kiwifruit seedlings. After stored at 4°C for 60 days to break dormancy, seeds were treated with variable temperature to accelerate germination. Germinated seeds were sown into a nutrient bowl containing mixed matrix (the substrate was turf: coconut shell: perlite= 2: 2: 1), placed in the artificial climate chamber (25±2 °C, 12 h/12 h). When the seedlings fully unfolded a true leaf, we started pouring 1/2 Hoagland nutrient solution once a week, and replenished it with water in time. When the seedlings grew to 6 true leaves, the nutrient bowl was transferred to the outdoor shelter, and 3 seedlings in each nutrient bowl were routinely managed.
2.2. Experimental treatment

In early December, kiwifruit seedlings with consistent growth were selected, and the pretreatment group with MT were added 200 μmol / L MT 100 ml to the roots in the evening every two days. The rest of the treatment group will be root irrigated with equal amount of water at the same time. After 3 times of MT pretreatment, 15 μmol / L ABA and 5 mmol / L sodium tungstate (STD, ABA inhibitor) were sprayed on the leaves, and the remaining treatment groups were sprayed with water. The experiment was divided into 6 groups, namely(1) CK (clear water); (2)ABA (15 μmol / L ABA for foliar spray); (3)STD (5 mmol / L STD for foliar spray); (4) MT (200 μmol / L MT for root irrigation); (5)MT + ABA (200 μmol / L MT for root irrigation, and then 15 μmol / L ABA for foliar spray); (6)MT + STD (200 μmol / L MT for root irrigation, and then 5 mmol / L STD for foliar spray), 6 plants per treatment, repeated 3 times. Samples were taken at 0d, 15d, and 30d of the treatment, and relevant physiological indicators were determined.

2.3. Measurement items and methods

The leaf water potential meter (model WP4C) was used for the measurement. The determination of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) is based on the method of Li Hesheng [4]; the relative membrane permeability is determined by Chen Jianxun [5]; the relative water content of the leaves is determined by Barrs and Weatherley [6] method.

2.4. Data analysis

Data were expressed as mean ± standard deviation (SD), and significant analysis was performed using one-way ANOVA and Duncan methods of SPSS 20.0.

3. Results and discussions

3.1. Effects of exogenous MT and ABA on photosynthetic characteristics of kiwi seedlings

During the experiment, photosynthesis parameters of kiwifruit seedlings gradually decreased (Table 1). At 0 days of natural aging, melatonin pretreatment significantly increased the net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs) of kiwifruit seedlings. At 15 days, the net photosynthetic rate of kiwi seedlings in the MT + STD treatment group was significantly higher than that in the other treatment groups. At 30 days, the net photosynthetic rate of the MT + ABA treatment group was the highest, but it was not significantly different from the other treatment groups. Based on it, we can speculate that MT can promote the increase of net photosynthetic rate of leaves by affecting the production of ABA.

| Table 1. Effects of MT and ABA on photosynthetic characteristics of kiwifruit seedlings. |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|
| Treatments                        | Pn(μmolCO₂m⁻²s⁻¹) | Tr(mmolH₂O m⁻²s⁻¹) | GS(mmolH₂O m⁻²s⁻¹) | Ci(μmolCO₂m⁻²s⁻¹) |
| CK                                | 10.89±0.312bc    | 1.91±0.498bc     | 0.18±0.025b       | 281.08±5.539a    |
| MT                                | 16.09±2.049a     | 3.50±0.279a      | 0.28±0.016a       | 271.06±14.276abc |
| CK                                | 9.05±0.631cd     | 0.80±0.141c      | 0.11±0.027cd      | 239.06±29.502de  |
| ABA                               | 9.15±1.108cd     | 1.12±0.278c      | 0.12±0.035cd      | 249.81±18.936ab  |
| STD                               | 8.58±2.820cd     | 0.80±0.266c      | 0.14±0.059bc      | 278.76±9.238ab   |
| MT                                | 9.08±2.074cd     | 1.20±0.291c      | 0.14±0.037bc      | 276.21±19.099ab  |
| MT+ABA                            | 9.50±1.617cd     | 0.99±0.095c      | 0.14±0.025bc      | 269.21±35.152ab  |
| MT+STD                            | 12.80±0.082b     | 0.73±0.090c      | 0.08±0.010d       | 243.52±40.134ede |
| CK                                | 7.26±0.983d      | 0.74±0.102c      | 0.09±0.010cd      | 233.68±5.364e    |
| ABA                               | 8.40±0.477d      | 1.08±0.251c      | 0.11±0.008cd      | 234.95±11.673e   |
| STD                               | 7.90±0.969d      | 0.84±0.085c      | 0.08±0.006d       | 244.00±7.208ede  |
| MT                                | 8.06±0.525d      | 0.96±0.070c      | 0.11±0.022cd      | 238.12±3.662e    |
| MT+ABA                            | 8.82±0.722d      | 0.88±0.176c      | 0.11±0.014cd      | 240.04±9.432de   |
| MT+STD                            | 8.02±0.546d      | 0.90±0.144c      | 0.10±0.018cd      | 236.88±8.019e    |

Note: The data shown are the averages ± SE of three replicates. The means denoted by the same letter do not significantly
differ at a P<0.05 (LSD test).

3.2. Effects of exogenous MT and ABA on fluorescence parameters of kiwi seedlings

During the experiment, the maximum photochemical quantum yield (Fv/Fm) and non-photochemical quenching coefficient (qN) of kiwi seedlings showed a downward trend, while the PSII photon quantum transfer efficiency (ΦPSII), photochemical quenching coefficient (qP), and apparent electron transfer rate (ETR) is gradually increasing (Table 2). At 15 days of natural senescence, the Fv/Fm of leaves of STD treatment group and qN of leaves of ABA treatment group were significantly higher than those of other test groups, and qP of leaves of ABA treatment group was significantly lower than those of other test groups. At 30 days, the Fv/Fm of the STD group and the MT + ABA group was significantly higher than the other experimental groups. Based on it, we can speculate that MT can affect leaf fluorescence parameters by affecting the production of ABA.

Table 2. Effects of MT and ABA on fluorescence parameters of kiwifruit seedling leaves.

| Treatments | Fv/Fm | ΦPSII | qP | qN | ETR |
|------------|-------|-------|----|----|-----|
| 0d         |       |       |    |    |     |
| CK         | 0.78±0.05a | 0.54±0.024abc | 0.80±0.019bcd | 0.40±0.051ab | 32.27±1.428e |
| MT         | 0.77±0.010a | 0.50±0.055bcd | 0.79±0.042cde | 0.50±0.128a | 29.69±3.256e |
| CK         | 0.72±0.006def | 0.48±0.043cd | 0.77±0.071de | 0.40±0.062ab | 40.31±3.603cd |
| ABA        | 0.72±0.001cede | 0.44±0.087d | 0.73±0.086e | 0.48±0.155a | 36.78±7.301de |
| 15d        |       |       |    |    |     |
| STD        | 0.75±0.003b | 0.57±0.009ab | 0.84±0.021abc | 0.31±0.046bc | 48.02±0.786bc |
| MT         | 0.71±0.008f | 0.59±0.031ab | 0.87±0.025ab | 0.20±0.025c | 49.14±2.635b |
| MT+ABA     | 0.70±0.0008f | 0.56±0.108abc | 0.89±0.039a | 0.32±0.170bc | 47.09±8.986bc |
| MT+STD     | 0.71±0.004ef | 0.56±0.044abc | 0.84±0.056abc | 0.23±0.081c | 46.86±3.680bc |
| CK         | 0.72±0.013def | 0.57±0.016ab | 0.88±0.015a | 0.30±0.009bc | 47.70±1.457bc |
| ABA        | 0.72±0.016def | 0.59±0.048a | 0.88±0.008a | 0.24±0.018c | 49.42±3.994ab |
| 30d        |       |       |    |    |     |
| STD        | 0.73±0.006ecd | 0.60±0.023a | 0.88±0.018a | 0.27±0.034bc | 49.84±1.915b |
| MT         | 0.71±0.009ef | 0.53±0.016abc | 0.84±0.022abc | 0.33±0.061bc | 44.38±1.362bc |
| MT+ABA     | 0.73±0.013c | 0.57±0.043ab | 0.85±0.058abc | 0.30±0.043bc | 47.68±3.594bc |
| MT+STD     | 0.70±0.011f | 0.58±0.030ab | 0.88±0.024a | 0.26±0.034bc | 61.06±10.604a |

3.3. Effects on photosynthetic pigment content in kiwi leaves

Leaf chlorosis and yellowing are typical appearance characteristics of leaf senescence [7]. During the experiment, the chlorophyll content and carotenoid content of kiwi seedling leaves showed a downward trend (Figure 1). At 0 days of natural senescence, MT pretreatment increased leaf chlorophyll and carotenoid content by about 5%. At 15 days, the chlorophyll content and carotenoid content of the ABA treatment group were the lowest. The chlorophyll content of leaves in the STD and MT + ABA treatment groups was higher than that in the control group, but the difference was not significant. At 30 days, the chlorophyll content and carotenoid content in the leaves of the ABA and STD treatment groups were significantly higher than those in the other treatment groups, while the chlorophyll content and carotenoid content in the leaves of the MT and MT + STD treatment groups were significantly lower than those of the other treatment groups. Based on it, we can speculate that MT can affect the changes of leaf photosynthetic pigment content by affecting the production of ABA.
Figure 1. Effects of MT and ABA on Chlorophyll and Carotenoid contents in kiwifruit seedlings.

4. Discussion and conclusion
Aging is a progressive and irreversible state of functional decline, which is a comprehensive manifestation of various functional decline of the body [8]. During this process, chlorophyll content and photosynthetic rate will decrease [9-10]. By comprehensively considering the changes in photosynthetic characteristics of each treatment group in the experiment, we can speculate that MT may improve the photosynthesis of aging kiwi by affecting the production of ABA and keeping its content at a moderate level. However, the role of ABA in natural aging of plants and the mechanism of MT affecting ABA need to be further studied.

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