Melatonine Can Promote the Growth of *Malachium Aquaticum* Seedlings under Salt Stress

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Abstract: The effects of melatonine (MT) on the growth of *Malachium aquaticum* seedlings under salt stress were investigated through a pot experiment. The results showed that MT increased the biomass of *M. aquaticum* seedlings. With the increase of MT concentrations, the root, stem, leaf and shoot biomasses of *M. aquaticum* seedlings increased when the dose of MT was not more than 150 μmol/L, and decreased when the dose of MT was more than 150 μmol/L. MT also enhanced the photosynthetic pigment content and antioxidant enzyme activity of *M. aquaticum* seedlings. When applying MT on *M. aquaticum* seedlings, the soluble sugar content in roots decreased, and the soluble sugar content in stems and leaves increased. Therefore, MT could promote the growth of *M. aquaticum* seedlings under salt stress, and the best dose of MT was 150 μmol/L.

Introduction

Melatonin (MT) is a well-known animal hormones involved in the physiological regulation of vertebrates, including circadian rhythm and photoperiod response, and commonly used in adjusting improve sleep and treating neurasthenia [1] . In the animal field, MT is one of the hormones that can directly remove a variety of different forms of free radicals, and improve immunity [2]. MT not only presents in animals, but also presents in plants, which can be used as an effective free radical scavengers or antioxidants in plants under the abiotic stress [3]. MT also can regulate the growth and development of plants, such as promoting lateral root growth, changing florescence and delaying leaf senescence [4]. When applying MT on plant seedlings, the chlorophyll content significantly increases, and the photosynthetic ability significantly improves [5].

Malachium aquaticum is the wild vegetable of Caryophyllaceae [6]. In this study, we grew *M. aquaticum* seedlings under salt stress, and used the different concentrations of MT to treat *M. aquaticum* seedlings, to study the effects of MT on the growth of *M. aquaticum* seedlings under salt stress. The aim of the study was to screen the best MT concentration which could promote the growth of *M. aquaticum* seedlings under salt stress, and provided a reference for applying the plant hormones on other wild vegetables production under salt stress.

Materials and Methods

Materials. The *M. aquaticum* seedlings with height of 15 cm were collected from the surrounding farmland of Chengdu campus of Sichuan Agricultural University (30° 42′N, 103° 51′E) in March 2016.

Experimental Design. The vermiculites and pearlites (1:1) were put into polyethylene pot (10 cm high, 10 cm in diameter). The base of *M. aquaticum* seedlings were immersed in 5 concentrations (0, 50, 100, 150 and 200 μmol/L) of MT [7] solutions for 24 h in March 2016, respectively. Two uniform *M. aquaticum* seedlings were transplanted into each pot with 6 replicates for each treatment. After that, all of the seedlings were covered with transparent plastic film and a shade net. After 10 d, the

transparent plastic film and the shade net were removed. From the third day of transplanting, the Hogland nutrient solutions containing 50 mmol/L NaCl [8] were watered every two days, and 30 ml solutions for each pot. When *M. aquaticum* seedlings grow two month (May 2016) under salt stress, the upper mature leaves of *M. aquaticum* seedlings were collected to determine the photosynthetic pigment (chlorophyll *a*, chlorophyll *b* and total chlorophyll) contents [9]. The upper young shoots (2 cm in length) were collected to determine the superoxide dismutase (SOD) activity, peroxidase (POD) activity and catalase (CAT) activity [10]. Then, the whole plants were then gently removed. The roots and shoots were washed with tap water followed by deionized water, and the biomasses of root, stem and leaf were measured. After that, the roots, stems and leaves of *M. aquaticum* seedlings were dried at 80 °C to constant weight. The dried tissue samples were finely ground and sieved through a 0.149-mm-mesh nylon sieve for chemical analysis. The soluble sugar contents in roots, stems and leaves of *M. aquaticum* seedlings were determined by anthrone colorimetry with dry weight plant samples [11].

Statistical Analyses. Statistical analyses were conducted using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with Duncan's multiple range test (p = 0.05 confidence level).

Results and Discussion

Biomass of *M. aquaticum* Seedlings. After the treatments of different concentrations of MT, the root biomass of *M. aquaticum* seedlings increased compared with the control under salt stress (Table 1). With the increase of MT concentrations, the root biomass of *M. aquaticum* seedlings increased when the dose of MT was not more than 150 μmol/L, and decreased when the dose of MT was more than 150 μmol/L. At 50, 100, 150 and 200 μmol/L MT, the root biomass increased by 5.51% ($p > 0.05$), 16.91% ($p < 0.05$), 22.91% ($p < 0.05$) and 5.70% ($p > 0.05$) respectively, compared with the control. When applying MT on *M. aquaticum* seedlings under salt stress, the stem, leaf and shoot biomasses of *M. aquaticum* seedlings increased compared with the control (Table 1). With the increase of MT concentrations, the trends of stem, leaf and shoot biomasses were the same as the root biomass. At 50, 100, 150 and 200 μmol/L MT, the shoot biomass increased by 15.04% ($p > 0.05$), 30.89% ($p > 0.05$), 52.75% ($p < 0.05$) and 2.04% ($p > 0.05$) respectively, compared with the control. So, MT could promote the growth of *M. aquaticum* seedlings, and the 150 μmol/L MT was the best concentration.

Table 1 The biomass of *M. aquaticum* seedlings

MT concentration (μmol/L)	Roots (g/plant FW)	Stems (g/plant FW)	leaves (g/plant FW)	Shoots (g/plant FW)
0	1.017±0.028b	6.419±1.378a	4.193±0.873b	10.612±2.260b
50	1.073±0.054b	6.876±1.307a	5.332±0.865ab	12.208±0.441ab
100	1.189±0.016a	7.379±1.331a	6.511±0.692ab	13.890±2.022ab
150	1.250±0.010a	8.895±0.417a	7.315±1.563a	16.210±1.980a
200	1.075±0.047b	6.551±1.766a	4.278±1.021b	10.829±2.787ab

Values are means (±SE) of six replicate pots. Different lowercase letters within a column indicate significant differences (one-way analysis of variance followed by Duncan's multiple range test; $p < 0.05$).

Photosynthetic Pigment Content of *M. aquaticum* seedlings. The same as the biomass of *M. aquaticum* seedlings, MT increased the contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll under salt stress (Table 2). With the increase of MT concentrations, the contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll increased when the dose of MT was not more than 150 μmol/L, and decreased when the dose of MT was more than 150 μmol/L. At 50, 100, 150 and 200 μmol/L MT, the chlorophyll *a* content increased by 24.12% ($p < 0.05$), 59.22% ($p < 0.05$), 67.06% ($p < 0.05$) and

9.02% ($p > 0.05$) respectively, the chlorophyll *b* content increased by 15.95% ($p < 0.05$), 60.78% ($p < 0.05$), 68.97% ($p < 0.05$) and 10.34% ($p < 0.05$) respectively, the total chlorophyll content increased by 22.51% ($p < 0.05$), 59.46% ($p < 0.05$), 67.28% ($p < 0.05$) and 9.18% ($p > 0.05$) respectively, compared with the respective control. For the chlorophyll a/b, 50 μmol/L MT increased the chlorophyll a/b of *M. aquaticum* seedlings, and the other contrations of MT decreased chlorophyll a/b of *M. aquaticum* seedlings (Table 2).

Table 2 The photosynthetic pigment content of *M. aquaticum* seedlings

MT concentration (μmol/L)	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b
0	1.020±0.016c	0.232±0.008c	1.253±0.023c	4.397
50	1.266±0.018b	0.269±0.002b	1.535±0.020b	4.706
100	1.624±0.051a	0.373±0.004a	1.998±0.056a	4.354
150	1.704±0.059a	0.392±0.005a	2.096±0.064a	4.347
200	1.112±0.009c	0.256±0.008b	1.368±0.017c	4.344

Values are means (±SE) of six replicate pots. Different lowercase letters within a column indicate significant differences (one-way analysis of variance followed by Duncan's multiple range test; $p < 0.05$).

Antioxidant Enzyme Activity of *M. aquaticum* seedlings. After the treatment of MT under salt stress, the antioxidant enzyme activity of *M. aquaticum* seedlings enhanced (Table 3). So, MT could improve the resistance of *M. aquaticum* seedlings to salt stress. At 50, 100, 150 and 200 μmol/L MT, the SOD activity increased by 83.06% ($p < 0.05$), 126.61% ($p < 0.05$), 206.53% ($p < 0.05$) and 42.52% ($p < 0.05$) respectively, the POD activity increased by 10.03% ($p > 0.05$), 19.16% ($p < 0.05$), 30.46% ($p < 0.05$) and 1.90% ($p > 0.05$) respectively, the CAT activity increased by 7.85% ($p > 0.05$), 16.15% ($p < 0.05$), 22.82% ($p < 0.05$) and 7.77% ($p > 0.05$) respectively, compared with the respective control.

Table 3 The antioxidant enzyme activity of *M. aquaticum* seedlings

MT concentration (μmol/L)	SOD activity (U/g)	POD activity (U/g)	CAT activity (U/g)
0	31.23±1.80e	1210.74±26.88d	79.76±4.55c
50	57.17±4.52c	1332.14±51.52bc	86.02±4.59bc
100	70.77±0.22b	1442.68±58.55b	92.64±3.81ab
150	95.73±3.80a	1579.59±14.84a	97.96±2.62a
200	44.51±2.93d	1233.80±52.15cd	85.96±1.69bc

Values are means (±SE) of six replicate pots. Different lowercase letters within a column indicate significant differences (one-way analysis of variance followed by Duncan's multiple range test; $p < 0.05$).

Soluble Sugar Content in *M. aquaticum* seedlings. When applying MT on *M. aquaticum* seedlings, the soluble sugar content in roots of *M. aquaticum* seedlings decreased, and the soluble sugar content in stems and leaves increased under salt stress (Table 4). At 50, 100, 150 and 200 μmol/L MT, the soluble sugar content in roots decreased by 9.16% ($p < 0.05$), 10.74% ($p < 0.05$), 14.60% ($p < 0.05$) and 19.46% ($p < 0.05$) respectively, the soluble sugar content in stems increased by 6.70% ($p < 0.05$), 22.32% ($p < 0.05$), 47.05% ($p < 0.05$) and 2.95% ($p < 0.05$) respectively, the soluble sugar content in leaves increased by 14.37% ($p < 0.05$), 14.93% ($p < 0.05$), 33.56% ($p < 0.05$) and 14.03% ($p < 0.05$) respectively, compared with the respective control. So, MT could adjust the distribution ratio of soluble sugar in source, stream and sink of plants.

Conclusions

Under salt stress, MT increased the biomass of *M. aquaticum* seedlings. With the increase of MT concentrations, the root, stem, leaf and shoot biomasses of *M. aquaticum* seedlings increased when

the dose of MT was not more than 150 μmol/L, and decreased when the dose of MT was more than 150 μmol/L. MT also enhanced the photosynthetic pigment content and antioxidant enzyme activity of *M. aquaticum* seedlings. When applying MT on *M. aquaticum* seedlings, the soluble sugar content in roots decreased, and the soluble sugar content in stems and leaves increased. Therefore, MT could promote the growth of *M. aquaticum* seedlings under salt stress.

Table 4 The soluble sugar content in *M. aquaticum* seedlings

MT concentration (μmol/L)	Roots (mg/g DW)	Stems (mg/g DW)	leaves (mg/g DW)
0	18.35±0.58a	11.20±0.50c	8.91±0.24b
50	16.67±0.76b	11.95±1.04c	10.19±0.07b
100	16.38±0.87bc	13.70±0.74b	10.24±0.78b
150	15.67±0.27bc	16.47±0.49a	11.90±1.15a
200	14.78±0.48c	11.53±0.17c	10.16±0.21b

Values are means (±SE) of six replicate pots. Different lowercase letters within a column indicate significant differences (one-way analysis of variance followed by Duncan's multiple range test; $p < 0.05$).

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