

# 缺铁胁迫下葡萄试管苗对外源褪黑素的生长和生理响应

陈玉鹏, 陈文绪, 孙泽东, 肖雪梅, 吴玉霞\*

(甘肃农业大学园艺学院, 兰州 730070)

**摘要:**【目的】探究在轻度缺铁胁迫下, 外源褪黑素(MT)对葡萄试管苗的生长和生理影响及对缺铁症状的缓解作用。【方法】以克瑞森无核葡萄试管苗为试验材料, 试验设置4个不同的处理组, 分别为: CK, CK+MT(MT浓度为 $140 \mu\text{mol} \cdot \text{L}^{-1}$ ), -Fe(Fe浓度为 $30 \mu\text{mol} \cdot \text{L}^{-1}$ )和-Fe+MT, 分别研究MT对CK和-Fe葡萄试管苗的生长生理响应。【结果】在缺铁胁迫下, 葡萄试管苗的株高、茎粗、干鲜质量、光合色素含量、根系活力、过氧化物酶(POD)活性和硝酸还原酶(NR)活性不同程度下降; 而膜脂过氧化产物丙二醛(MDA)含量、渗透调节物质脯氨酸(Pro)含量、可溶性糖(SS)含量、可溶性蛋白(SP)含量和超氧化物歧化酶(SOD)活性提高; 加入MT后, 植株生长形态指标、光合色素含量、根系活力、POD活性和NR活性有所上升, 渗透调节物质(Pro、SS、SP)含量, 膜脂过氧化产物MDA含量和SOD活性快速下降, 葡萄试管苗上的缺铁症状有所缓解。【结论】在轻度缺铁胁迫下,  $140 \mu\text{mol} \cdot \text{L}^{-1}$ 外源褪黑素处理可以有效缓解葡萄试管苗的缺铁症状, 保护植株在生理方面免受伤害, 保证植株的正常生长发育。

**关键词:** 葡萄试管苗; 缺铁胁迫; 褪黑素; 生理响应

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## Growth and physiological response of *in vitro* seedlings to exogenous melatonin under iron deficiency stress in grape

CHEN Yupeng, CHEN Wenxu, SUN Zedong, XIAO Xuemei, WU Yuxia\*

(College of Horticulture, Gansu Agricultural University, Lanzhou 730070, Gansu, China)

**Abstract:** 【Objective】 Exogenous melatonin (MT) can effectively alleviate stress symptoms in many crops, enabling the crop to resist adversity and be recovered from the salt-damaged or deficient environment for a short period of time. The experiment was conducted to study the effects of exogenous MT treatment on the growth and physiology of grapevines under mild iron deficiency stress conditions, so as to analyze the effect of exogenous MT on the alleviation of iron deficiency symptoms in grapevines and its alleviation efficacy. 【Methods】 The experimental materials were grape seedlings in the test tube grown in laboratory succession, and the cultivar was Crimson Seedless. Four treatments were set up as below: CK (Fe concentration of  $50 \mu\text{mol} \cdot \text{L}^{-1}$  without addition of exogenous MT), CK+MT (Fe concentration of  $50 \mu\text{mol} \cdot \text{L}^{-1}$  with MT concentration of  $140 \mu\text{mol} \cdot \text{L}^{-1}$ ), -Fe (Fe concentration of  $30 \mu\text{mol} \cdot \text{L}^{-1}$  without addition of exogenous MT), and -Fe+MT (Fe concentration of  $30 \mu\text{mol} \cdot \text{L}^{-1}$  with MT concentration of  $140 \mu\text{mol} \cdot \text{L}^{-1}$ ). The iron and MT concentrations added in the experiment were based on the previous experiments. The test was conducted by selecting healthy and uniformly growing seedlings, cutting the canes into 1.0–1.5 cm in length with a single bud, and transferring them to the B5 solid medium before starting different treatments as described above. Each treatment was transferred to 20 vials of seedlings, with 2 plantlets per vial, and replicated 3 times. The transferred seedlings were incubated at  $(27 \pm 1) \text{ } ^\circ\text{C}$

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作者简介: 陈玉鹏, 男, 在读硕士研究生, 研究方向为园艺植物栽培生理。Tel: 15348003424, E-mail: 2278547690@qq.com

\*通信作者 Author for correspondence. Tel: 13893118451, E-mail: wuyx@gsau.edu.cn

with a light intensity of 3000 lx and 2:1 ratio of light to dark period, and the grapevine growth was observed regularly. At day 50 after inoculation, grape seedlings in the test tube were removed from triangular vials and the plant height, stem thickness, plant dry and fresh weight, leaf area and root system indicators were measured. Leaf area and root system indicators were scanned with an Epson root system scanner (STD 4800) and analyzed with a software (Win RHIZO 5.0, Canada). Moreover, those indicators were also determined, including leaf chlorophyll, malondialdehyde, proline, soluble protein and soluble sugar contents, SOD, POD and NR activities as well as root vigor. The data were comprehensively analyzed to study the effects of exogenous MT on the growth of grapevine *in vitro* seedlings under mild Fe-deficiency stress, and to investigate the alleviating effect of melatonin on Fe-deficiency stress at the physiological level. 【Results】 In terms of growth indexes, grapevines showed poor growth with yellowing of leaves, dwarf vines and stunted root development under Fe deficiency stress. After the addition of MT, plant growth recovered better and all indexes were significantly better improved than those with Fe deficiency treatment. It was suggested that exogenous melatonin may improve the growth environment of grapevine roots, promote plant growth and improve the resistance of grapevines to iron deficiency adversity by participating in the synthesis pathway of essential substances and regulating the amount of phytohormone synthesis. In terms of physiological indicators, the chlorophyll a, chlorophyll b, carotenoids and total chlorophyll contents in leaves significantly increased by the addition of exogenous MT compared with CK and -Fe treatments, and the most significant effect was relieved by -Fe treatment, indicating that exogenous MT had a good protective effect on chlorophyll structure of grapevine leaves under Fe deficiency stress. Under Fe deficiency stress, the SOD activity as well as Pro and MDA contents of grapevines increased significantly compared with CK treatment, but POD activity decreased; after adding exogenous MT, POD activity increased, and MDA content and SOD activity decreased rapidly. The content of osmoregulatory substances was significantly higher in leaves with -Fe treatment compared with CK, while the addition of MT to CK and -Fe effectively reduced the Pro, soluble protein and soluble sugar contents with the stress treatment (-Fe). Compared with the Fe deficiency treatment, root vigor and NR activity were significantly enhanced by adding exogenous MT. 【Conclusion】 Under mild Fe deficiency stress, the root volume and leaf area of grape seedlings increased and the vines grew well after the addition of MT. Leaf photosynthetic pigment content, POD and NR activity as well as root vigor increased significantly, while MDA, Pro, soluble protein and soluble sugar contents as well as SOD activity decreased significantly. The  $140 \mu\text{mol} \cdot \text{L}^{-1}$  MT treatment was effective in alleviating the symptoms of iron deficiency with *in vitro* seedlings, protecting the plants from physiological damage and ensuring normal growth and development.

**Key words:** Grape seedlings; Iron deficiency stress; Melatonin; Physiological response

葡萄 (*Vitis vinifera* L.) 是一种富含各类维生素、膳食纤维等较高营养物质的浆果类水果<sup>[1]</sup>, 在世界五大洲均有种植, 2018 年全球葡萄总产量达 7780 万 t, 其中鲜食葡萄占 36%, 而中国鲜食葡萄产量已位居世界首位<sup>[2]</sup>。在植物的新陈代谢中, 铁是植物生长必需的微量元素之一, 参与叶绿素及呼吸酶的合成、氮代谢、活性氧代谢等<sup>[3]</sup>过程。褪黑素 (*N*-乙酰基-5-甲氧基色胺, Melatonin, MT) 是色氨酸吲哚类衍生物, 为新型植物生长调节剂<sup>[4]</sup>。然而不合理的施肥

方式、栽培管理措施导致设施土壤盐渍化<sup>[5-6]</sup>, 许多的微量元素不能被足量吸收, 不能满足作物生长的需求, 导致植物发生缺铁性失绿症, 影响植物正常生长及发育<sup>[7-8]</sup>。近年来, 外源供给能够提高植物细胞的抗逆性并缓解逆境胁迫, 这已成为全球农业研究的热点<sup>[9]</sup>。探索分析其他领域中的外源供给物质和使用剂量及在葡萄试管苗缺铁胁迫上的生长表现和生理响应, 为葡萄抗逆境胁迫的外源供给物质库添砖加瓦, 成为研究的指南针。目前研究调控植物逆境

的外源物质众多<sup>[10]</sup>。如:Noor等<sup>[11]</sup>研究的茉莉酸酯等在短生长周期作物的抗逆境生物学响应,Mfarrej等<sup>[12]</sup>研究了外源NO和H<sub>2</sub>S对小麦内涝的缓解作用,外源曲霉提高多年生黑麦草的耐旱性和耐热性<sup>[13]</sup>及外源邻苯二甲酸对西瓜种子萌发、根系生理特性及矿质元素吸收的影响<sup>[14]</sup>等。但是在果树的抗逆境研究中外源物质的使用较少,存在某外源物质对提高葡萄的逆境抗性不明显。油菜素内酯(BR)、水杨酸(SA)、NO、外源硅、外源Ca<sup>2+</sup>和MT能够通过提高叶片中叶绿素的含量,降低作物叶片的黄化面积,促进抗氧化酶<sup>[15]</sup>(SOD等)的活性,降低超氧阴离子累积效应的损伤程度,促进葡萄果实中可溶性糖和可溶性蛋白的合成<sup>[16]</sup>,提高在氨基酸上的代谢、合成水平,协助作物提高渗透调节物质(Pro、SS和SP)含量以抵御逆境,有效缓解作物(华山松<sup>[17]</sup>、番茄<sup>[18]</sup>、小白菜<sup>[19]</sup>、黄瓜<sup>[20-21]</sup>、葡萄<sup>[16,22-23]</sup>等)缺铁黄化症状,减轻逆境对植株生长的伤害,但未见明确描述外源MT对葡萄试管苗缺铁逆境的响应。缺铁胁迫下植株会表现出叶片黄化的现象,影响单株的生物合成量及长势,外源MT处理可以有效缓解许多作物的胁迫症状,使作物能够抵抗逆境并短时间脱离盐害或缺素环境。然而在葡萄试管苗的缺铁胁迫研究中,并未明确外源褪黑素对植株的生长表现影响和生理响应如何。笔者在本试验中以实验室组培苗繁殖技术提供的克瑞森无核葡萄试管苗为材料,在缺铁胁迫下添加外源褪黑素,观察各处理葡萄的生长情况,并从生理层面上研究褪黑素对缺铁胁迫的缓解效应,确定外源褪黑素对缺铁胁迫下的葡萄缓解效果是否显著,以期增添农业生产中葡萄抗缺铁胁迫调控的外源物质种类。

## 1 材料和方法

### 1.1 试验材料

试验材料为继代保存的克瑞森无核葡萄试管苗,采用改良B5固体培养基扩繁,选择长势均匀且生长健壮的试管苗,剪成长1.0~1.5 cm的单芽茎段,转接至添加了不同处理的B5培养基上,置于温度(27±1)℃,光照度3000 lx,光、暗期比2:1的环境下培养。

### 1.2 试验处理

在前期试验的基础上,设置铁浓度为30 μmol·L<sup>-1</sup>(轻度胁迫),外源褪黑素处理浓度为140 μmol·L<sup>-1</sup>,

试验处理如表1所示。每个处理转接20瓶试管苗,共3次重复,总计60瓶。

表1 试验处理

Table 1 Experimental treatments

处理 Treatment	c(铁) Iron content/(μmol·L <sup>-1</sup> )	c(褪黑素) Melatonin content/(μmol·L <sup>-1</sup> )
CK	50	0
CK+MT	50	140
-Fe	30	0
-Fe+MT	30	140

### 1.3 测定指标与方法

处理后定期观察葡萄长势,接种后50 d时,采样测定相关指标。

将植株从三角瓶中取出,测定株高、茎粗、植株干鲜质量、叶面积及根系指标。叶面积和根系指标用Epson根系扫描仪(STD 4800型)扫描,用根系分析软件(Win RHIZO 5.0, Canada)进行分析。

叶绿素含量采用95%乙醇浸提法测定,叶片丙二醛含量采用硫代巴比妥酸比色法测定,脯氨酸含量用茚三酮法测定,SOD活性用氮蓝四唑光化学还原法测定,POD活性用愈创木酚法<sup>[24]</sup>测定;根系活力采用氯化三苯基四氮唑法<sup>[25]</sup>测定;可溶性蛋白含量采用考马斯亮蓝G-250染色法<sup>[26]</sup>测定;可溶性糖含量采用蒽酮法<sup>[27]</sup>测定;硝酸还原酶活性采用磺胺比色法<sup>[28]</sup>测定。

### 1.4 统计分析

数据用IBM SPSS 24软件进行差异显著性分析(Duncan法),用Origin 9.1软件制图。

## 2 结果与分析

### 2.1 外源褪黑素对缺铁胁迫下葡萄生长的影响

2.1.1 外源褪黑素对缺铁胁迫下葡萄植株形态及生物量的影响 各处理葡萄植株形态如图1所示,CK+MT处理植株叶片鲜绿,茎秆粗壮,长势最强;-Fe处理植株矮小,茎秆纤细,长势最弱,在幼叶部位出现了明显的黄化现象;然而较-Fe处理植株,-Fe+MT处理植株长势略显优势,茎秆较粗,黄叶数量显然减少。由表2可知,试管苗的株高和茎粗,对照组显著高于缺铁处理,但-Fe和-Fe+MT处理间差异不显著;CK+MT株高较CK、-Fe和-Fe+MT分别高21.98%、75.88%、51.53%,茎粗分别高21.96%、75.85%、51.52%。全株鲜质量CK+MT较CK高19.09%,差异显著;-Fe+MT较-Fe高33.29%,差异显著;CK和-Fe+

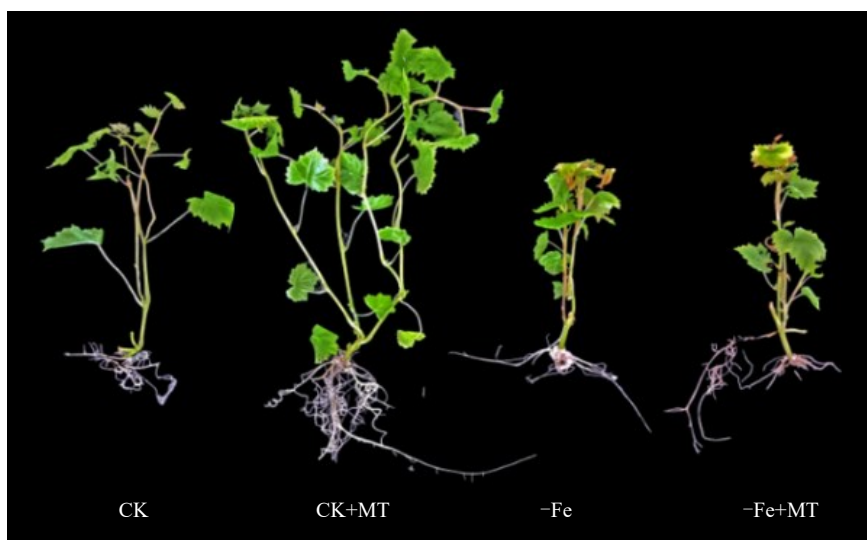


图1 不同处理葡萄的植株形态

Fig. 1 Plant morphology of grapes under different treatments

表2 外源褪黑素对缺铁胁迫下葡萄地上部生长及生物量的影响

Table 2 Effect of exogenous melatonin on above-ground growth and biomass of grapes under iron deficiency stress

处理 Treatment	株高 Plant height/cm	茎粗 Stem thickness/mm	鲜质量 Fresh mass/g	干质量 Dry mass/mg
CK	10.767±0.825 b	0.979±0.075 b	1.425±0.020 b	50.233±4.931 ab
CK+MT	13.133±1.014 a	1.194±0.092 a	1.697±0.038 a	65.873±6.967 a
-Fe	7.467±0.203 c	0.679±0.018 c	0.763±0.052 c	23.400±4.443 c
-Fe+MT	8.667±0.203 bc	0.788±0.018 bc	1.017±0.050 b	37.867±4.001 bc

注:不同小写字母表示在  $p < 0.05$  差异显著。下同。

Note: Different small letters indicate significant difference at  $p < 0.05$ . The same below.

MT 差异不显著。对照组植株干质量显著大于缺铁处理, -Fe 干质量较 CK 低 53.63%, 但 CK 和 CK+MT 间、-Fe 和 -Fe+MT 间差异不显著。可见, MT 对植株生长具有一定促进作用, 对于轻度缺铁胁迫下的植株有良好的保护作用, 使植株胁迫症状得到缓解。

2.1.2 外源褪黑素对缺铁胁迫下葡萄根系和叶片生长的影响 由表3可知, CK+MT 的总根长、平均根系直径、根系表面积、根系投影面积、根系总体积和叶面积显著高于其他处理, 平均值最高, -Fe 最低。-Fe+MT 较 CK 平均根系直径、根系表面积、根

系投影面积、根系总体积和叶面积差异显著, 分别下降 8.60%、39.84%、42.70%、26.00% 和 26.80%; -Fe+MT 的叶投影面积较 -Fe 显著上升 147.78%。通过分析发现, 加入外源 MT, 对 CK 的根系建成和叶面积增加有促进效果, 对 -Fe 地上地下的胁迫症状有缓解作用, 保证植株的正常生长。

## 2.2 外源褪黑素对缺铁胁迫下葡萄生理指标的影响

2.2.1 外源褪黑素对缺铁胁迫下葡萄叶绿素含量的影响 叶绿素 a、叶绿素 b 和类胡萝卜素含量, CK 和

表3 外源褪黑素对缺铁胁迫下葡萄根系和叶片生长的影响

Table 3 Effect of exogenous melatonin on root and leaf growth of grapes under iron deficiency stress

处理 Treatment	总根长 Length/cm	平均根系直径 Average diameter/mm	根系表面积 Surface area/cm <sup>2</sup>	根系投影面积 Root projected area/cm <sup>2</sup>	根系总体积 Root volume/cm <sup>3</sup>	叶投影面积 Leaf projected area/cm <sup>2</sup>
CK	63.847±10.131 ab	0.388±0.015 a	7.624±1.092 a	2.548±0.274 a	0.050±0.008 b	10.361±0.740 b
CK+MT	81.991±3.524 a	0.428±0.030 a	8.548±0.820 a	2.948±0.148 a	0.081±0.006 a	13.406±0.609 a
-Fe	30.497±0.591 c	0.300±0.012 b	3.126±0.527 b	0.995±0.168 b	0.027±0.004 c	3.061±0.478 d
-Fe+MT	50.171±5.528 bc	0.302±0.011 b	4.587±0.817 b	1.460±0.260 b	0.037±0.006 bc	7.584±0.829 c

CK+MT之间、-Fe和-Fe+MT及CK和-Fe+MT之间差异均显著(表4)。叶绿素a、叶绿素b和类胡萝卜素含量对比中,CK+MT较CK分别上升18.46%、142.94%、16.24%;-Fe+MT较-Fe分别上升55.07%、

57.14%、63.77%;-Fe+MT较CK分别下降28.20%、33.94%、21.35%。而在总叶绿素含量中,CK与CK+MT之间、-Fe与-Fe+MT之间差异不显著,-Fe+MT较CK下降了23.37%,差异显著。

表4 外源褪黑素对缺铁胁迫下葡萄叶绿素含量的影响

Table 4 Effect of exogenous melatonin on chlorophyll content of grapes under iron deficiency stress

处理 Treatment	$\rho$ (叶绿素a) Chlorophyll a content/( $\text{mg}\cdot\text{L}^{-1}$ )	$\rho$ (叶绿素b) Chlorophyll b content/( $\text{mg}\cdot\text{L}^{-1}$ )	$\rho$ (类胡萝卜素) Carotenoids content/( $\text{mg}\cdot\text{L}^{-1}$ )	$\rho$ (总叶绿素) Total chlorophyll content/ ( $\text{mg}\cdot\text{L}^{-1}$ )
CK	0.986±0.002 b	0.333±0.042 b	0.431±0.001 b	1.750±0.040 ab
CK+MT	1.168±0.001 a	0.809±0.454 a	0.501±0.010 a	2.473±0.445 a
-Fe	0.454±0.001 d	0.140±0.002 d	0.207±0.001 d	0.801±0.003 c
-Fe+MT	0.708±0.003 c	0.220±0.004 c	0.339±0.001 c	1.341±0.001 bc

2.2.2 外源褪黑素对缺铁胁迫下葡萄MDA和渗透调节物质含量的影响 由表5可知,-Fe处理的MDA、Pro、可溶性糖和可溶性蛋白含量明显高于其他处理。在CK与CK+MT和-Fe与-Fe+MT之间,MDA、可溶性糖和可溶性蛋白含量差异不显著,-

Fe+MT较CK分别上升9.68%、3.08%、9.38%,差异显著;CK+MT脯氨酸含量较CK显著下降36.56%,-Fe较CK显著上升29.37%。综上可知,MDA、Pro、可溶性糖和可溶性蛋白含量由高到低均为:CK+MT>-Fe>-Fe+MT>CK。

表5 外源褪黑素对缺铁胁迫下葡萄MDA和渗透调节物质含量的影响

Table 5 Effect of exogenous melatonin on the content of MDA and osmoregulatory substances in grapes under iron deficiency stress

处理 Treatment	$b$ (丙二醛) MDA content/( $\mu\text{mol}\cdot\text{g}^{-1}$ )	$w$ (脯氨酸) Proline content/( $\mu\text{g}\cdot\text{g}^{-1}$ )	$w$ (可溶性糖) Soluble sugar content/( $\text{mg}\cdot\text{g}^{-1}$ )	$w$ (可溶性蛋白) Soluble protein content/( $\text{mg}\cdot\text{g}^{-1}$ )
CK	0.031±0.001 bc	9 569.757±601.536 b	24.360±0.056 bc	3.230±0.053 bc
CK+MT	0.028±0.001 c	6 070.824±401.046 c	24.031±0.296 c	2.981±0.080 c
-Fe	0.035±0.001 a	12 379.847±948.437 a	25.336±0.137 a	4.519±0.154 a
-Fe+MT	0.033±0.001 ab	11 790.790±663.529 ab	25.110±0.412 ab	3.533±0.076 ab

2.2.3 外源褪黑素对缺铁胁迫下葡萄叶片酶活性和根系活力的影响 从表6可知,-Fe与-Fe+MT、CK与CK+MT的叶片SOD活性差异不显著,CK+MT较-Fe的下降5.12%,差异显著( $p<0.05$ );CK与CK+MT的POD活性差异不显著,-Fe较-Fe+MT的显著下降36.56%。-Fe较-Fe+MT和CK较CK+MT的根系活力差异显著,其中CK+MT的根系活力值

最高,-Fe的最低,CK与CK+MT较-Fe与-Fe+MT的都高。-Fe的NR活性最低,较CK、CK+MT和-Fe+MT呈现显著下降的趋势,分别下降了47.51%、53.2%和21.49%;在MT处理后,CK+MT与-Fe+MT较CK与-Fe, NR活性整体有明显的上升趋势,同比于未施MT的处理CK与-Fe分别上升了12.15%、27.37%。NR活性由高到低为:CK+MT>CK>-Fe+MT>-Fe。

表6 外源褪黑素对缺铁胁迫下葡萄叶片酶活性和根系活力的影响

Table 6 Effect of exogenous melatonin on enzyme activity and root vigor of grape leaves under iron deficiency stress

处理 Treatment	SOD活性 SOD activity/( $\text{U}\cdot\text{g}^{-1}$ )	POD活性 POD activity/( $\text{U}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ )	硝酸还原酶活性 NR activity/( $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ )	根系活力 Root viability/( $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ )
CK	244.395±0.346 bc	407.001±22.904 ab	1 005.555±14.699 b	240.468±3.3446 b
CK+MT	240.631±2.956 c	427.333±32.739 a	1 127.778±30.932 a	294.644±10.085 a
-Fe	253.863±1.366 a	209.556±13.844 c	527.778±33.7932 d	134.018±7.573 d
-Fe+MT	251.607±4.120 ab	330.333±20.764 b	672.222±38.889 c	182.573±6.4074 c

### 3 讨 论

#### 3.1 缺铁胁迫下葡萄响应外源褪黑素处理的生长效应

有研究者发现,在轻度缺铁胁迫下,葡萄顶叶表现失绿或黄白色,光合代谢受到抑制;MT能调节细胞壁延伸的物理过程以诱导植物根系生长,使根系投影面积,总根长和根系总体积增加<sup>[29]</sup>。本试验中,缺铁胁迫下,葡萄呈现长势不良、株高不均,叶片黄化,茎秆细小,根系发育不良等状况;添加MT后,植株生长状况有较好的恢复,各项指标明显优于缺铁处理。外源褪黑素可能通过参加必需物质的合成途径、调节植物激素的合成量和充当各类酶的辅基,促进蛋白质的表达<sup>[30]</sup>,改善葡萄根系的生长环境,促进正常处理的植株生长,提高葡萄对逆境的抗性。

#### 3.2 缺铁胁迫下葡萄响应外源褪黑素处理的生理效应

植物生长发育的基础是光合作用,叶片是植物光合作用的主要器官,光合色素是评价植株光合特性的重要指标之一,光合色素的含量直接影响光合作用的强弱以及植物的生长<sup>[31]</sup>。试验表明:在CK与-Fe均加入外源褪黑素后,叶绿素a、叶绿素b、类胡萝卜素和总叶绿素含量都有明显回升,对-Fe处理的缓解效果最明显,说明外源褪黑素可能会加速受损的叶绿素分解,促进合成吡啶胺延缓叶绿素分解,增强了叶绿素的从头合成<sup>[32-34]</sup>,对缺铁胁迫下葡萄叶片的叶绿素结构有很好的保护作用。

MDA是细胞膜脂质过氧化程度的重要诊断指标,其含量体现植物对缺铁的反应程度大小<sup>[35]</sup>;而植物遭受逆境胁迫,细胞膜氧化程度升高,细胞的膜透性增强。与徐宁等<sup>[4]</sup>研究的外源褪黑素对硝酸盐的缓冲机制相似,逆境使细胞内部的氧化能力提高,过氧化物和超氧离子单位面积含量等急剧升高,机体将会打开防御机制,对逆境进行主动或被动调节,抗氧化防御系统(SOD-POD抗氧化防御系统)被激活,会引起POD活性下降,SOD活性上升<sup>[36]</sup>,主要清除细胞体内的超氧自由基和过氧化产物<sup>[35]</sup>。本试验中,缺铁胁迫下,葡萄的SOD活性、Pro和MDA含量较CK处理显著升高,POD活性下降;加入外源MT后,POD活性有所上升,膜脂过氧化产物MDA含量和SOD活性快速下降。说明缺铁胁迫下添加MT,可激活SOD-POD抗氧化防御系统,减轻盐胁迫下

过量的自由基对植物细胞的盐害作用<sup>[37]</sup>,提升了葡萄的抗逆性能。

Pro、SP和SS是植物逆境渗透调节的重要物质,反映植物对逆境的抵抗能力。在轻度缺铁胁迫下,CK与-Fe相比,-Fe处理下叶片的渗透调节物质含量显著高于CK,而CK与-Fe添加MT后,能有效降低胁迫处理(-Fe)的Pro、可溶性蛋白和可溶性糖含量,CK的降幅不大。外源褪黑素可能通过促进氨基酸转运、调节蛋白质和糖类的合成,提高葡萄的抗逆境能力<sup>[37]</sup>,提高了渗透调节的能力,保护细胞膜的完整性<sup>[4,38]</sup>,减少内含物质的丢失,也是为SOD-POD防御机制开启第二道防线,有效地缓解了缺铁胁迫对细胞膜的损伤。

根系活力表示植株的根系细胞活性,即对土壤营养元素的吸收能力<sup>[39]</sup>。且NR是一类关于氮元素在植物体内相互转化的活性酶,其作用是催化NO<sub>3</sub><sup>-</sup>转化为NO<sub>2</sub><sup>-</sup>来进行氮代谢的调控<sup>[40]</sup>,其活性反映氮代谢的强弱,活性越强,硝酸盐的累积量就越少,植物氮代谢能力越强。本试验中,-Fe处理的NR活性较低,但较其他处理试管苗的硝酸盐累积量高,-Fe+MT处理NR活性值上升。缺铁胁迫可能导致NR活性值降低,从而降低了植物对硝酸盐的利用率<sup>[40]</sup>,导致植株对氮元素的吸收减少而体内累积硝酸盐,根系的生长和吸收能力受阻,导致植株长势不良。硝酸盐的累积又会导致根系活力的降低,细胞膜结构被破坏,植物体电解质渗透率提高,细胞膜稳定指数越来越低,活性氧代谢加强,MDA含量上升,叶绿素分解加剧,在双胁迫的压力下,植株长势矮小,茎秆纤细。再加入MT后,由于自身亲水,进入细胞通畅,进一步提高了幼苗叶片中可溶性糖、可溶性蛋白、脯氨酸和游离氨基酸含量,从维持细胞膜结构的稳定和叶片中渗透调节物质的运输能力,同时缓解缺铁胁迫和硝酸盐累积胁迫<sup>[4,41]</sup>。

### 4 结 论

在轻度缺铁胁迫下,加入MT后,葡萄试管苗根系体积和叶面积增大,植株生长状态良好;叶片光合色素含量、POD活性、NR活性和根系活力显著上升,MDA含量、Pro含量、SOD活性、SS含量和SP含量显著下降。140 μmol·L<sup>-1</sup> MT处理能有效缓解葡萄试管苗的缺铁症状,保护植株在生理方面免受伤害,保证植株的正常生长发育。

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