果 树 学 报 2017,34(11):1426-1434
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20170027

6-BA及氨基酸硒对葡萄叶片衰老的影响

王海波,王　帅,王孝娣,史祥宾,王宝亮,郑晓翠,王志强,冀晓昊,刘凤之*

(中国农业科学院果树研究所·农业部园艺作物种质资源利用重点实验室,辽宁兴城 125100)

摘　要:【目的】明确6-BA及氨基酸硒在激素水平上对葡萄叶片衰老的调控,为设施葡萄叶片衰老延缓技术的建立提供理论依据。【方法】在设施葡萄延迟栽培条件下,以叶片衰老速度不同的'意大利'和'无核白鸡心'2个葡萄品种为试材,分别进行叶面喷施6-BA和氨基酸硒处理,以清水为对照,测定不同处理和对照叶片衰老期间功能叶片的叶绿素含量和净光合速率(P_n)及内源激素含量与比值的变化。【结果】外源6-BA和氨基酸硒处理显著延缓了叶片叶绿素含量和净光合速率的下降,明显提高了玉米素核苷(ZR)和赤霉素(GA_3)含量和ZR/ABA(脱落酸)、GA_3/ABA、(ZR+GA_3)/ABA比值,显著降低了ABA含量。生长素(IAA)具有前期保持叶片生长发育和后期促进衰老的双重作用。2个葡萄品种间比较,'意大利'叶片衰老缓慢。【结论】6-BA和氨基酸硒通过维持较高的GA_3/ABA、ZR/ABA和(GA_3+ZR)/ABA比值,提高了葡萄叶片的叶绿素含量和净光合速率,延长了功能期,因此,外源喷施6-BA和氨基酸硒是延缓葡萄叶片衰老的重要技术措施。

关键词:葡萄;叶片衰老;6-BA;氨基酸硒;内源激素

中图分类号:S663.1　　文献标志码:A　　文章编号:1009-9980(2017)11-1426-09

Effects of 6-BA and amino acid selenium on leaf senescence and endogenous hormones content in grape

WANG Haibo, WANG Shuai, WANG Xiaodi, SHI Xiangbin, WANG Baoliang, ZHENG Xiaocui, WANG Zhiqiang, JI Xiaohao, LIU Fengzhi*

(Fruit Research Institute, Chinese Academy of Agricultural Sciences · Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Germplasm Resources Utilization), Ministry of Agriculture, Xingcheng 125100, Liaoning, China)

Abstract:【Objective】The experiment was conducted to clarify the effects of 6-BA and amino acid selenium on grape leaf senescence and to provide a theoretical basis for preventing leaves from early aging.【Methods】Two grape cultivars, 'Italia' and 'Centenial' seedless, under protected cultivation for delayed harvest were used for the study. Amino acid selenium and 6-BA were sprayed on the leaves, and spraying clear water was used as the control group (CK). The physiological indexes, such as chlorophyll content, net photosynthetic rate and endogenous hormone contents were determined during leaf senescence. The experiment materials including triennial 'Italia' and 'Centenial seedless' were planted in key grape technique demonstration orchard of Fruit Research Institute of Chinese Academy of Agricultural Sciences (Xingcheng, Liaoning province east longitude 120°06′, northern latitude 40°16′). The plants grafted on Beta rootstock were grown in a solar greenhouse. Tree and row spacing was 2.0 m×0.7 m. A tilt dragon trunk and V form trellis system was adopted with regular managements. Starting from August 1, 2013, the vines were sprayed with 50 mg·L^{-1} amino acid selenium [a patented product (ZL201010199145.0) of Fruit Research Institute of Chinese Academy of Agricultural Sciences containing 3% Se, produced by An Qiu

收稿日期:2017-03-24　　接受日期:2017-08-10

基金项目:中国农业科学院科技创新工程(CAAS-ASTIP-2015-RIP-04);国家现代农业产业技术体系建设专项(nycytx-30- zp);948重点项目"鲜食葡萄新品种及设施化生产技术引进与创新应用"(2011-G28)

作者简介:王海波,男,副研究员,主要从事果树栽培生理与技术的研究与推广工作。Tel:13591963796,E-mail:haibo8316@163.com

*通信作者 Author for correspondence. Tel:13904295109,E-mail:liufengzhi6699@126. com

Xin Hai Biofertilizer Limited Company] or 20 mg·L^{-1} 6-BA at 3:00-4:00 pm every 15 days until 15 days before leaf fall. The experimental treatments were set with 3 replicates, each with 15 trees. The concentrations of amino acid selenium and 6-BA used were based on the results of pre-experiments. Secondly, from September 8th to the end of leaf fall, representative leaf samples from shoots at consistent growth were taken at 8:00 am every half a mouth. 20 functional leaves located at the 4th to 8th nodes were collected, put into a curling stone, and brought to laboratory, where they were frozen with liquid nitrogen. Acetone soaking methods was used to extract chlorophylls. A CIRAS-2 portable photosynthesis system was used to measure net photosynthetic rate during a sunny day between 9:00am and 11:00am. The measurements were carried out at a light intensity of 1 200 μmol·m^{-2}·s^{-1}, carbon dioxide level of 360 μmol·mol^{-1} and temperature of 25 ℃. ELISA (enzyme linked immunosorbent assay for detection) was used to measure gibberellin (GA_3), zeatin riboside (ZR), auxin (IAA) and abscisic acid (ABA) contents. The ELISA kits were supplied by Crop Chemical Control Research Center, China Agricultural University. Data collected were processed with Excel 2003, and statistic analyses with SPSS 13.0.【Results】Compared with CK, amino acid selenium and 6-BA treatments significantly delayed the decreases in chlorophyll content and net photosynthetic rate. With the increase of treatment times, endogenous ZR and GA_3 contents and the ratios of ZR/ABA, GA_3/ABA and (ZR+GA_3)/ABA increased significantly, while the content of ABA decreased significantly. IAA showed maintained growth in the earlier period but promoted senescence in the later period. Before November 8th, compared with CK, amino acid selenium and 6-BA treatments significantly increased the IAA content in leaves but decreased it later. There were differences in different senescence-types of grape leaves, the leaves of 'Italia' were slower in senescence with antioxidant enzymes maintaining higher activities at the late growth stage.【Conclusion】Application of amino acid selenium or 6-BA is effective to maintain chlorophyll content and net photosynthetic rate in 'Italia' and 'Centenial seedless' grapes and changes the hormone levels and balance. The two reagents can delay leaf senescence and to some degree prolong the functional period of leaves. Therefore, amino acid selenium and 6-BA application can be an important pratice to delay leaf aging in grapes under protected cultivation for delayed harvest.

Key words: Grape; Leaf senescence; 6-BA; Amino acid selenium; Endogenous hormones

近年来随着人民生活水平的提高与市场的需求,葡萄设施栽培发展迅速,并已成为葡萄产业发展的新方向和新趋势。其中延迟栽培作为葡萄设施栽培的一种重要形式,显著提高了我国葡萄产业的经济效益。叶片衰老是葡萄延迟栽培健康可持续发展的瓶颈,不仅导致树体贮藏养分不足,而且容易造成果实产量和品质的下降[1]。因此如何延缓叶片衰老对于葡萄延迟栽培而言,具有重要的理论价值和实践意义。随着生物学的发展,植物激素调控叶片衰老的作用已得到公认,其中细胞分裂素类物质对叶片衰老起重要作用[2],Lara等[3]研究表明,衰老伴随着叶片细胞分裂素含量的下降,通过外源施用细胞分裂素或借助转基因手段提高内源细胞分裂素的水平,均能达到延缓叶片衰老的效果。6-苄基腺嘌呤(6-BA)是一种活跃的细胞分裂素,可通过调节激素水平及平衡达到有效延缓叶片衰老的作用[4-5]。硒是谷胱甘肽过氧化物酶等的重要组分,具有极强的抗氧化作用[6],可有效延缓大豆叶片的衰老[7]。Lehotai等[8]通过试验证实亚硒酸钠能够改变拟南芥根部的内源激素水平。外源施硒及6-BA虽然能够影响植株的衰老进程,但关于外源施用该类物质对葡萄叶片衰老及其内源激素含量的影响仍有待深入研究。笔者以'意大利'和'无核白鸡心'2个叶片衰老速度不同的葡萄品种为试材,研究施硒和6-BA对叶片衰老过程中叶绿素含量、净光合速率和内源激素含量及比值等生理指标的影响,明确6-BA及氨基酸硒在激素水平上对葡萄叶片衰老的调控,为葡萄叶片抗衰老技术的建立奠定理论基础。

1 材料和方法

1.1 试材与处理

以定植于中国农业科学院果树研究所葡萄核心技术试验示范园日光温室内'贝达'嫁接的3 a生'意大利'和'无核白鸡心'为试材，行株距2.0 m×0.7 m，树体采用倾斜龙干形，叶幕采用V形叶幕，其他管理同常规。自8月1日（2013年）始至落叶前15 d结束，于下午15：00—16:00用50 mg·L^{-1}的氨基酸硒（中国农业科学院果树研究所研制，安丘鑫海生物肥料有限公司生产，专利号：ZL201010199145.0，硒元素含量3%）和20 mg·L^{-1}的6-BA对'意大利'和'无核白鸡心'2个葡萄品种进行叶面喷施，每隔15 d喷施1次，其中'意大利'喷施20 mg·L^{-1}的6-BA为处理1、喷施50 mg·L^{-1}的氨基酸硒为处理2，'无核白鸡心'喷施20 mg·L^{-1}的6-BA为处理3、喷施50 mg·L^{-1}的氨基酸硒为处理4；对照为喷等量清水，其中'意大利'对照为CK1，'无核白鸡心'对照为CK2。每处理3次重复，每重复15株树。氨基酸硒和6-BA的喷施浓度由预备试验确定。

1.2 测定方法

1.2.1 取样 从9月8日始至落叶结束，每隔15 d的上午8:00选取代表性且生长一致的葡萄新梢采样，每处理采集20枚葡萄新梢第4~8节的功能叶片，用冰壶带回实验室，经液氮速冻后置于-40 ℃冰箱保存备测。

1.2.2 净光合速率和叶绿素含量的测定 净光合速率用CIRAS-2光合作用测定系统固定参数（光强1 200 μmol·m^{-2}·s^{-1}、CO_2浓度360 μmol·mol^{-1}、温度25 ℃）于晴天上午9:00—11:00进行测定；叶绿素含量用丙酮浸提法测定[9]。

1.2.3 内源激素含量的测定 采用Elisa法（酶联免疫吸附检测法）测定GA_3、ZR、IAA和ABA等内源激素的含量。试剂盒由中国农业大学作物化学控制研究中心提供。

1.3 数据处理

用Excel 2003处理数据，采用SPSS 13.0进行统计分析。

2 结果与分析

2.1 叶喷6-BA和氨基酸硒对葡萄叶片衰老外观形态的影响

从图1可以看出，叶片喷6-BA和氨基酸硒处理叶片开始衰老时间比对照推迟30 d左右，到11月23日初现衰老特征。同时从落叶时间看，叶喷6-BA和氨基酸硒处理叶片完全落叶时间比对照推迟10~15 d，'意大利'和'无核白鸡心'的叶喷6-BA和氨基酸硒处理叶片完全落叶期分别为12月12—19日和12月1—8日。

2.2 叶喷6-BA和氨基酸硒对葡萄叶片净光合速率及叶绿素含量的影响

随着叶片发育进程的推进，'意大利'和'无核白鸡心'2个葡萄品种叶片的叶绿素含量和净光合速率呈明显下降趋势，叶片衰老缓慢的'意大利'叶片的叶绿素含量和净光合速率始终大于叶片衰老较快的'无核白鸡心'。施用6-BA和氨基酸硒处理及对照叶片的叶绿素含量9月24日之前呈缓慢上升趋势，之后呈缓慢下降趋势，至10月中下旬开始急剧下降（表1）；而处理及对照叶片的净光合速率自9月9日起开始下降，早于叶绿素含量开始下降的时间（表2）。施用6-BA和氨基酸硒处理叶片的净光合速率及叶绿素含量均极显著高于对照。

2.3 叶喷6-BA和氨基酸硒对葡萄叶片内源IAA含量的影响

施用6-BA和氨基酸硒后，'意大利'和'无核白鸡心'2个葡萄品种叶片的IAA含量变化趋势一致（图2）。11月8日之前，施用6-BA和氨基酸硒处理和对照葡萄叶片IAA含量总体呈下降趋势，但在9月24日和10月24日出现2个高值；11月8日之后，IAA含量呈迅速升高趋势。2个品种间比较，11月8日之前，叶片衰老缓慢的'意大利'叶片的IAA含量整体大于叶片衰老较快的'无核白鸡心'；之后叶片衰老较快的'无核白鸡心'叶片的IAA含量超过叶片衰老缓慢的'意大利'。施用6-BA和氨基酸硒处理叶片的IAA含量除11月23日（叶片衰老末期，对照的最后一次采样时间）低于对照外，其他时期均极显著高于对照。

2.4 叶喷6-BA和氨基酸硒对葡萄叶片内源GA_3、ZR含量的影响

'意大利'和'无核白鸡心'2个葡萄品种叶片的GA_3含量变化均呈现先升高后降低的趋势，施用6-BA和氨基酸硒处理及对照的峰值出现时间均在10月24日左右，之后呈逐渐降低趋势（图3）。叶片的ZR含量变化趋势与GA_3相似，同样呈先升高后降低的趋势，对照叶片ZR含量在9月24日达到最高，以

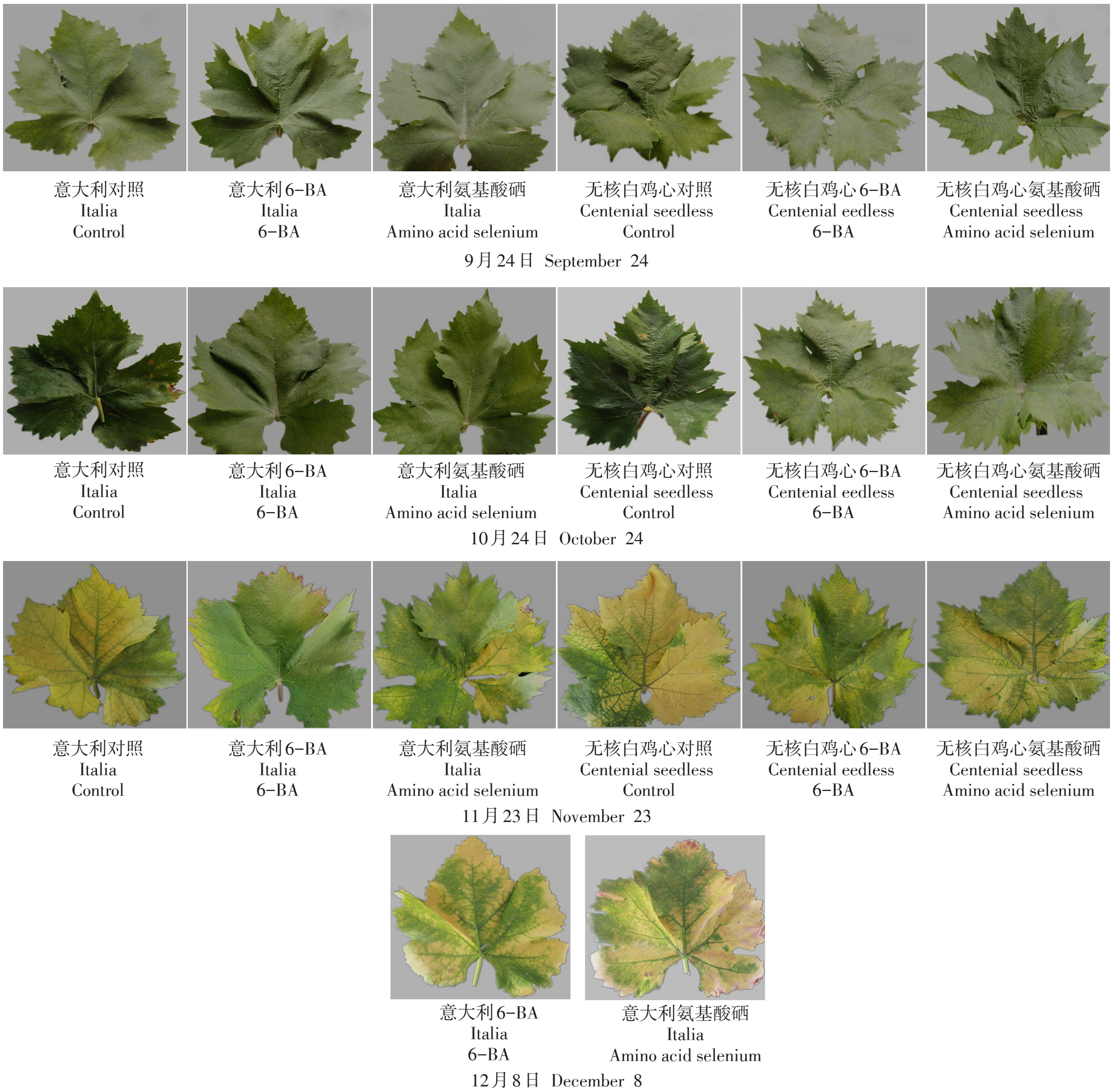

图1　叶喷6-BA和氨基酸硒对‘意大利’和‘无核白鸡心’葡萄叶片外观形态的影响

Fig. 1 Effects of 6-BA and amino acid selenium on leaf morphology in ‘Italia’ and ‘Centenial seedless’ grapes

表1　6-BA和氨基酸硒处理对‘意大利’和‘无核白鸡心’葡萄叶片叶绿素含量的影响

Table 1 Effects of 6-BA and amino acid selenium on content of chlorophylls in ‘Italia’ and ‘Centenial seedless’ grape leaves

($mg \cdot g^{-1}$)

日期 Date	对照1 CK1	处理1 Treatment 1	处理2 Treatment 2	对照2 CK2	处理3 Treatment 3	处理4 Treatment 4
2013-09-09	2.329±0.009 Bb	2.670±0.044 Aa	2.725±0.023 Aa	2.463±0.039 Bb	2.668±0.018 Aa	2.689±0.021 Aa
2013-09-24	2.500±0.006 Cc	2.863±0.002 Aa	2.813±0.002 Bb	2.515±0.002 Cc	2.891±0.004 Aa	2.740±0.034 Bb
2013-10-09	2.373±0.020 Cc	2.789±0.023 Aa	2.728±0.003 Bb	2.295±0.004 Cc	2.728±0.003 Aa	2.659±0.004 Bb
2013-10-24	2.156±0.020 Cc	2.518±0.002 Aa	2.479±0.007 Bb	2.044±0.013 Cc	2.305±0.012 Bb	2.375±0.003 Aa
2013-11-08	1.601±0.002 Cc	1.892±0.015 Bb	2.016±0.016 Aa	1.514±0.008 Cc	1.838±0.009 Aa	1.795±0.002 Bb
2013-11-23	0.921±0.020 Cc	1.597±0.007 Aa	1.457±0.007 Bb	0.771±0.006 Cc	1.371±0.012 Aa	1.222±0.004 Bb
2013-12-08	–	0.936±0.005 Aa	0.873±0.005 Bb	–	–	–

注：不同大写字母代表同一品种不同处理间差异达1%显著水平，不同小写字母代表同一品种不同处理间差异达5%显著水平。下同。

Note：The letters in the same row show signifancance of difference among treatmenrs in the same variety. Different capital letters indicate significant difference at the 0.01 levels; different small letters indicate significant difference at the 0.05 levels. The same below.

表2 6-BA和氨基酸硒对‘意大利’和‘无核白鸡心’葡萄叶片净光合速率的影响

Table 2 Effects of 6-BA and amino acid selenium on P_n in ‘Italia’ and ‘Centenial seedless’ grape leaves

($\mu mol \cdot m^{-2} \cdot s^{-1}$)

日期 Date	对照1 CK1	处理1 Treatment 1	处理2 Treatment 2	对照2 CK2	处理3 Treatment 3	处理4 Treatment 4
2013-09-09	6.433±0.058 Bb	7.033±0.153 Aa	6.933±0.153 Aa	6.833±0.058 Cc	7.500±0.100 Aa	7.233±0.058 Bb
2013-09-24	6.067±0.153 Cc	6.867±0.153 Aa	6.467±0.058 Bb	6.533±0.058 Bc	7.067±0.115 Aa	6.833±0.058 Ab
2013-10-09	4.933±0.153 Bc	5.900±0.100 Aa	5.667±0.058 Ab	4.833±0.058 Cc	5.833±0.153 Aa	5.467±0.155 Bb
2013-10-24	4.367±0.153 Bb	5.067±0.153 Aa	5.133±0.153 Aa	4.267±0.153 Bb	5.000±0.100 Aa	5.133±0.115 Aa
2013-11-08	3.767±0.115 Bb	4.700±0.100 Aa	4.567±0.058 Aa	3.700±0.100 Bb	4.533±0.153 Aa	4.400±0.200 Aa
2013-11-23	-0.533±0.153 Cc	1.933±0.058 Aa	1.333±0.058 Bb	-0.733±0.058 Cc	1.467±0.058 Aa	0.967±0.058 Bb
2013-12-08	-	1.300±0.100 Aa	1.067±0.058 Bb	-	-	-

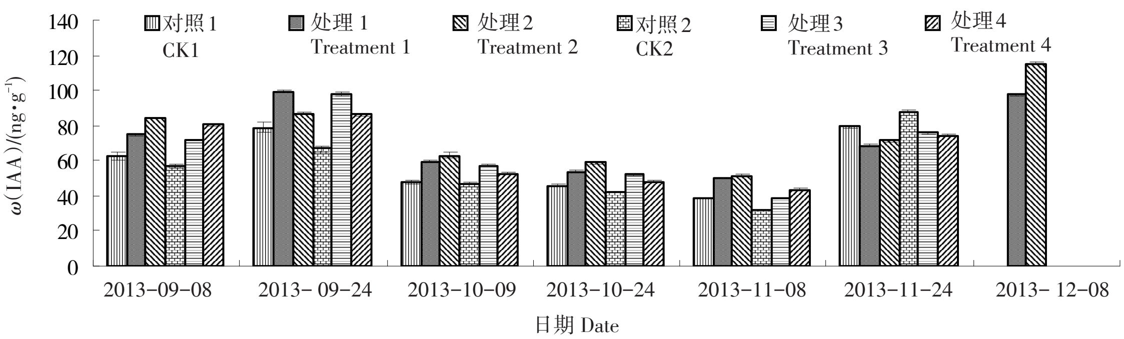

图2 6-BA和氨基酸硒对‘意大利’和‘无核白鸡心’葡萄叶片IAA含量的影响

Fig. 2 Effects of 6-BA and amino acid selenium on content of IAA in ‘Italia’ and ‘Centenial seedless’ grape leaves

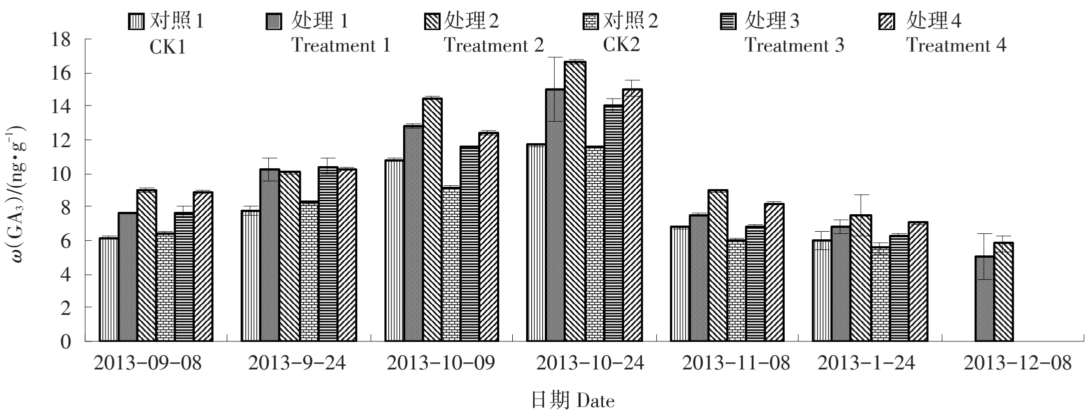

图3 6-BA和氨基酸硒对‘意大利’和‘无核白鸡心’葡萄叶片GA_3含量的影响

Fig. 3 Effects of 6-BA and amino acid selenium on content of GA_3 in ‘Italia’ and ‘Centenial seedless’ grape leaves

后呈逐渐降低的趋势,施用6-BA和氨基酸硒处理叶片ZR含量在10月9日达到最高,之后含量逐渐降低(图4,表3、表4)。施用6-BA和氨基酸硒处理极显著提高了叶片的GA_3和ZR含量,且随处理时间延长,与对照差异越明显。自10月9日以后,叶片衰老缓慢的‘意大利’叶片的GA_3和ZR含量开始高于叶片衰老较快的‘无核白鸡心’。

2.5 叶喷6-BA和氨基酸硒对葡萄叶片内源ABA含量的影响

‘意大利’和‘无核白鸡心’2个葡萄品种叶片ABA含量呈先升后降再升趋势,即9月9—24日叶片的ABA含量迅速升高,9月24日—10月9日又有所下降,之后呈持续上升的趋势(图5)。在此过程中,叶片衰老较快的‘无核白鸡心’叶片ABA的积累速率大于叶片衰老缓慢的‘意大利’。与对照比较,外源施用6-BA和氨基酸硒处理叶片的ABA含量均极显著地低于对照。

2.6 叶喷6-BA和氨基酸硒对葡萄叶片内源激素比值的影响

叶片衰老不仅与内源激素含量的变化有关,还

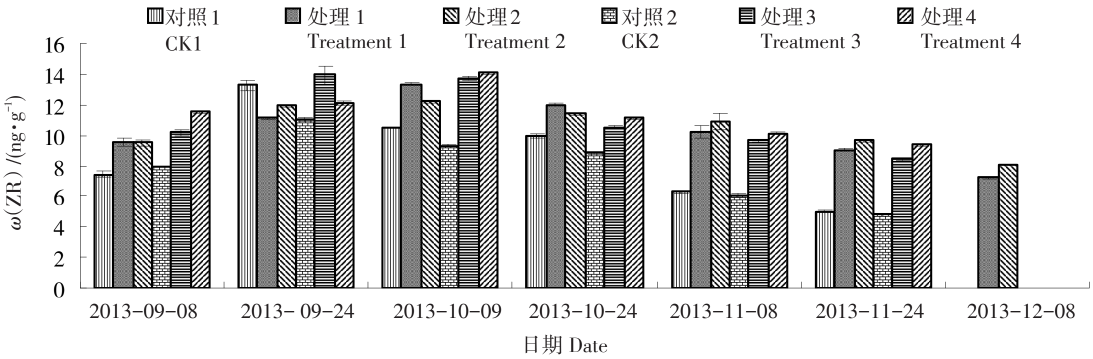

图4　6-BA和氨基酸硒对'意大利'和'无核白鸡心'葡萄叶片ZR含量的影响

Fig. 4 Effects of 6-BA and amino acid selenium on content of ZR in 'Italia' and 'Centenial seedless' grape leaves

表3　6-BA和氨基酸硒对'意大利'和'无核白鸡心'葡萄叶片 GA_3/ABA的影响

Table 3 Effects of 6-BA and amino acid selenium on ratio of GA_3/ABA in 'Italia' and 'Centenial seedless' grape leaves

日期 Date	对照1 CK1	处理1 Treatment 1	处理2 Treatment 2	对照2 CK2	处理3 Treatment 3	处理4 Treatment 4
2013-09-09	0.080±0.001 Bb	0.112±0.002 Aa	0.112±0.003 Aa	0.086±0.001 Cc	0.119±0.003 Bb	0.134±0.001 Aa
2013-09-24	0.100±0.003 Cc	0.131±0.001 Aa	0.114±0.001 Bb	0.097±0.001 Cc	0.136±0.006 Aa	0.117±0.001 Bb
2013-10-09	0.103±0.002 Cc	0.151±0.002 Aa	0.128±0.001 Bb	0.087±0.001 Bc	0.136±0.002 Aa	0.133±0.001 Ab
2013-10-24	0.090±0.002 Cc	0.118±0.002 Aa	0.111±0.002 Bb	0.079±0.002 Cc	0.098±0.001 Bb	0.103±0.001 Aa
2013-11-08	0.054±0.000 Bc	0.095±0.004 Ab	0.104±0.005 Aa	0.050±0.001 Bc	0.086±0.001 Ab	0.088±0.001 Aa
2013-11-23	0.040±0.001 Bc	0.079±0.001 Ab	0.081±0.001 Aa	0.032±0.001 Cc	0.066±0.001 Bb	0.071±0.001 Aa
2013-12-08	-	0.054±0.001 Bb	0.057±0.001 Aa	-	-	-

表4　6-BA和氨基酸硒对'意大利'和'无核白鸡心'葡萄叶片ZR/ABA的影响

Table 4 Effects of 6-BA and amino acid selenium on ratio of ZR/ABA in 'Italia' and 'Centenial seedless' grape leaves

日期 Date	对照1 CK1	处理1 Treatment 1	处理2 Treatment 2	对照2 CK2	处理3 Treatment 3	处理4 Treatment 4
2013-09-09	0.066±0.001 Cc	0.089±0.002 Bb	0.106±0.001 Aa	0.070±0.002 Cc	0.090±0.004 Bb	0.103±0.002 Aa
2013-09-24	0.070±0.002 Bb	0.101±0.007 Aa	0.096±0.001 Aa	0.073±0.001 Bb	0.101±0.005 Aa	0.095±0.001 Aa
2013-10-09	0.107±0.002 Cc	0.144±0.001 Bb	0.152±0.001 Aa	0.086±0.001 Bb	0.120±0.001 Aa	0.123±0.002 Aa
2013-10-24	0.106±0.001 Cc	0.147±0.001 Bb	0.163±0.003 Aa	0.104±0.002 Bc	0.131±0.004 Ab	0.139±0.005 Aa
2013-11-08	0.058±0.001 Cc	0.070±0.001 Bb	0.085±0.001 Aa	0.050±0.001 Cc	0.061±0.000 Bb	0.072±0.001 Aa
2013-11-23	0.047±0.001 Cc	0.060±0.001 Bb	0.063±0.000 Aa	0.038±0.001 Cc	0.049±0.001 Bb	0.053±0.001 Aa
2013-12-08	-	0.038±0.002 Bb	0.041±0.001 Aa	-	-	-

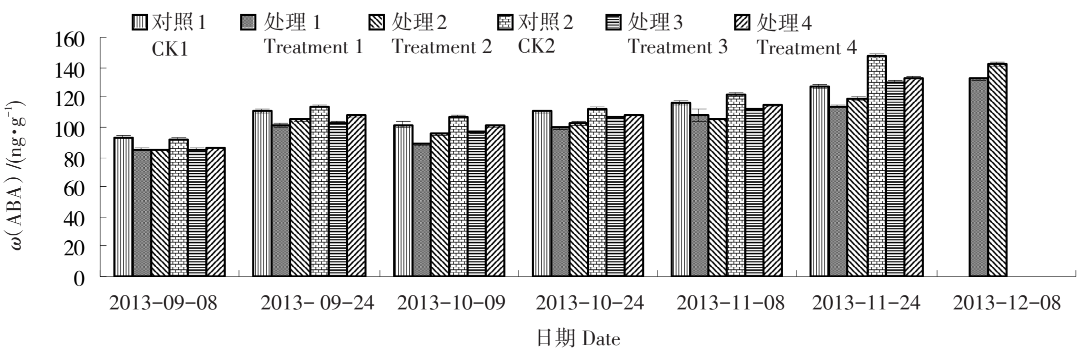

图5　6-BA和氨基酸硒对'意大利'和'无核白鸡心'葡萄叶片ABA含量的影响

Fig. 5 Effects of 6-BA and amino acid selenium on content of ABA in 'Italia' and 'Centenial seedless' grape leaves

与激素间的平衡密切相关。比较叶片衰老速度不同的'意大利'和'无核白鸡心'2个葡萄品种叶片衰老期间施用6-BA和氨基酸硒处理条件下叶片的内源激素比值变化后发现,GA_3/ABA、ZR/ABA和(GA_3+ZR)/ABA的值均随着叶片衰老呈不同程度的下降趋势,且叶片衰老缓慢的'意大利'叶片内GA_3/ABA、 ZR/ABA 和(GA_3+ZR)/ABA 的值稍大于叶片衰老较快的'无核白鸡心'。施用6-BA 和氨基酸硒处理对 GA_3/ABA、ZR/ABA 和(GA_3+ ZR)/ABA 的值有明显影响,施用6-BA 和氨基酸硒处理的2个葡萄品种叶片内源 GA_3/ABA、ZR/ABA 和(GA_3+ZR)/ABA 的值均显著高于对照(表3~表5)。

表5 6-BA 和氨基酸硒对'意大利'和'无核白鸡心'葡萄叶片 (ZR+GA_3)/ABA 的影响

Table 5 Effects of 6-BA and amino acid selenium on ratio of (ZR+GA_3)/ABA in 'Italia' and 'Centenial seedless' grape leaves

日期 Date	对照1 CK1	处理1 Treatment 1	处理2 Treatment 2	对照2 CK2	处理3 Treatment 3	处理4 Treatment 4
2013-09-09	0.146±0.001 Cc	0.201±0.004 Bb	0.218±0.004 Aa	0.156±0.003 Cc	0.210±0.007 Bb	0.237±0.003 Aa
2013-09-24	0.171±0.005 Cc	0.231±0.008 Aa	0.210±0.002 Bb	0.170±0.001 Cc	0.237±0.002 Aa	0.213±0.001 Bb
2013-10-09	0.210±0.003 Cc	0.295±0.003 Aa	0.280±0.001 Bb	0.172±0.002 Bb	0.256±0.002 Aa	0.256±0.003 Aa
2013-10-24	0.196±0.002 Cc	0.265±0.001 Bb	0.274±0.005 Aa	0.182±0.002 Cc	0.229±0,004 Bb	0.242±0.005 Aa
2013-11-08	0.112±0.001 Cc	0.165±0.003 Bb	0.189±0.004 Aa	0.099±0.001 Cc	0.148±0.001 Bb	0.160±0.002 Aa
2013-11-23	0.087±0.001 Cc	0.139±0.002 Bb	0.144±0.001 Aa	0.070±0.000 Cc	0.113±0.001 Bb	0.123±0.002 Aa
2013-12-08	-	0.092±0.002 Bb	0.098±0.001 Aa	-	-	-

3 讨 论

叶片衰老是叶片生长发育周期中一个复杂的高度被调控的过程。大量研究表明,叶绿素含量和净光合速率的下降可作为叶片衰老的重要生理指标[10-11]。本研究结果显示:随着葡萄叶片生长发育进程的推进,其净光合速率和叶绿素含量整体呈下降趋势。这与陆定志等[12]的研究结果一致。同时笔者发现,外源6-BA 和氨基酸硒处理显著提高了'意大利'和'无核白鸡心'2个品种叶片的净光合速率和叶绿素含量,增强了葡萄叶片的光合能力。已有研究表明,外源6-BA 能够促进叶绿素前体δ-氨基乙酰丙酸的合成,从而提高叶片的叶绿素含量[13];硒可能通过促进呼吸速率和呼吸链的电子传递速率进一步加速叶绿素的生物合成[14],因此,6-BA 和氨基酸硒可能通过调控叶绿素的代谢,提高叶绿素含量,达到增强葡萄叶片光合的效果。

植物内源激素对叶片的衰老起着重要的调控作用,多数研究认为CTK、IAA、GA_3具有延缓衰老、抑制成熟的作用,而ABA 则能够加速叶片衰老[15-18]。外源施用激素类物质可通过改变内源激素水平来调节植物体内生理代谢的各个环节。生长素参与植物生长发育的诸多过程,例如根茎发育和芽伸长等,但关于其在叶片衰老中的确切作用较为复杂并存在争议[19-20]。Lim 等[21]通过对拟南芥突变体的研究认为生长素对叶片衰老有一定的延缓作用。然而Ghanem 等[22]在研究番茄叶片内源激素含量的变化时发现,衰老的叶片内IAA 含量很高,并由此认为IAA 可能促进叶片衰老。本试验发现,11月8日之前,'意大利'和'无核白鸡心'2个葡萄品种叶片的IAA 含量均呈双峰趋势变化,但总体呈下降趋势;11月8日之后(叶片衰老后期),IAA 含量迅速升高。说明IAA 在叶片中具有双重作用的特殊性,在前期可提高植株的生理活性,而后期则促进叶片的衰老,其加速衰老的作用可能是通过提高乙烯的释放量实现的。因为有研究表明IAA 能够调控乙烯的生物合成,并且具有促进乙烯产生的作用[23-24]。由此也表明生长素在调控衰老方面可能不是一个主要因素,因此还要结合其他植物内源激素进行深入讨论。本试验中,随着生育时期的推进,不同处理和对照的叶片IAA 含量各不相同,11月8日之前,6-BA 和氨基酸硒处理的叶片IAA 含量极显著地高于对照;11月8日之后,对照的IAA 含量逐渐超过各处理条件下的叶片IAA 含量。结合前文论述的IAA 具有双重作用的观点进行分析讨论,认为6-BA 和氨基酸硒能够通过调控IAA 的水平起到延缓叶片衰老的功效。

Rosenvasser 等[25]研究认为,GA_3不仅可以有效阻止叶绿素的降解,还能够抑制衰老相关基因SAGs 的表达。Skas 等[26]在康乃馨切花上的研究发现,内源赤霉素含量的下降与衰老的发生密切相关。有研究认为CTK 对保持细胞膜结构的完整、清除自由基及延缓衰老等有一定的作用,并在转录水平上抑制与衰老相关酶的活性[27-28]。Dodd[29]认为ABA 能够诱导气孔关闭降低光合作用从而加快衰老的发生。赵平等[30]对烤烟叶片的研究指出,随着烤烟中位叶片的成熟,CTK 含量呈下降趋势,ABA 含量呈直线上升趋

势。本研究结果表明，随着叶片衰老，'意大利'和'无核白鸡心'2个葡萄品种叶片的GA_3和ZR呈下降趋势，而叶片中ABA含量逐渐升高，说明功能叶片中GA_3、ZR含量下降及ABA浓度升高加速了叶片的衰老过程，这一结果与史国安等[31]在研究芍药衰老过程中内源激素含量的变化动态中得出的结论类似。本试验中，喷施6-BA和氨基酸硒改变了内源激素水平，外源6-BA和氨基酸硒均显著提高了叶片的GA_3和ZR含量，降低了叶片的ABA含量，并可在较长时期内使叶片GA_3和ZR含量维持在较高水平。本文与前人研究6-BA处理对棉花衰老过程中叶片内源激素含量的影响时得出的结论相一致[32]。而关于硒与内源激素之间关系的研究较少，王宁宁等[33]指出亚硒酸钠对烟草冠瘿组织生长的作用与其对内源激素的影响是密切相关的。

植物体中的某一生理过程，往往不是一种激素单独作用，而是多种激素的协同作用的结果，因此内源激素间的平衡可能对叶片衰老起更大的调节作用。植物生长促进物质与生长抑制物质比值的变化可以作为调控叶片衰老过程的重要生理信号[29]。本研究表明，随着葡萄叶片发育进程的推进，'意大利'和'无核白鸡心'2个品种叶片的GA_3/ABA、ZR/ABA和(GA_3+ ZR)/ABA比值均有所下降，叶面喷施6-BA和氨基酸硒显著提高了2个葡萄品种GA_3/ABA、ZR/ABA和(GA_3+ ZR)/ABA比值，表明6-BA和氨基酸硒通过维持较高的GA_3/ABA、ZR/ABA和(GA_3+ ZR)/ABA比值延缓了叶片的衰老，这可能是6-BA和氨基酸硒在激素水平上延缓叶片衰老的重要原因。植物激素对叶片衰老的调控作用十分复杂，本研究虽然初步揭示了外源6-BA和氨基酸硒处理条件下，葡萄叶片衰老进程中内源激素的变化规律，为生产上叶片衰老的调控奠定了理论基础，但各激素及其互作在叶片衰老调控方面的具体分子生理机制仍有待进一步研究。

4 结 论

在葡萄延迟栽培条件下，6-BA和氨基酸硒通过调控ZR、GA_3、ABA和IAA等激素的水平和平衡，维持较高的GA_3/ABA、ZR/ABA和(GA_3+ ZR)/ABA比值，提高了葡萄叶片的叶绿素含量和净光合速率，有效延缓了'意大利'和'无核白鸡心'葡萄叶片的衰老，延长了叶片的功能期，因此，外源喷施6-BA和氨基酸硒是延缓葡萄叶片衰老的重要技术措施。

参考文献 References：

[1] 王帅，王海波，王孝娣，史祥宾，王宝亮，郑晓翠，刘凤之. 施硒和6-BA对葡萄叶片衰老与活性氧代谢的影响[J]. 果树学报，2015，32(2): 206-214.
WANG Shuai, WANG Haibo, WANG Xiaodi, SHI Xiangbin, WANG Baoliang, ZHENG Xiaocui, LIU Fengzhi. Effects of selenium and 6-BA on leaf senescence and active oxygen metabolism in grape[J]. Journal of Fruit Science, 2015, 32(2): 206-214.

[2] ZIMMERMANN P, ZENTGRAF U. The correlation between oxidative stress and leaf senescence during plant development[J]. Cellular and Molecular Biology Letters, 2005, 10(3): 515.

[3] LARA M E B, GARCIA M C G, FATIMA T, EHNE B R, LEE T K, PROELS R, TANNER W, ROITSCH T. Extracellular invertase is an essential component of cytokinin-mediated delay of senescence[J]. The Plant Cell Online, 2004, 16(5): 1276-1287.

[4] ZAVALETA-MANCERA H A, LOPEZ-DELGADO H, LOZA-TAVERA H, MORA-HERRERA M, TREVILLA-GARCIA C, VARGAS-SUAREZ M, OUGHAM H. Cytokinin promotes catalase and ascorbate peroxidase activities and preserves the chloroplast integrity during dark-senescence[J]. Journal of Plant Physiology, 2007, 164(12): 1572-1582.

[5] CHEN J G, ZHAO H Y, ZHOU X, MAO L S, CHEN X X. Fluctuation in levels of endogenous hormones after decapitation and 6-benzyl amino purine treatment in azalea, and their relationship to apical dominance[J]. Scientia Horticulturae, 1997, 71(1): 49-58.

[6] AGAR G, TURKER M, BATTAL P, EREZ M E. Phytohormone levels in germinating seeds of *Zea mays* L. exposed to selenium and aflatoxines[J]. Ecotoxicology, 2006, 15(5): 443-450.

[7] DJANAGUIRAMAN M, DEVI D D, SHANKER A K, SHEEBA J A, BANGARUSAMY U. Selenium-an antioxidative protectant in soybean during senescence[J]. Plant and Soil, 2005, 272(1/2): 77-86.

[8] LEHOTAI N, KOLBERT Z, PETO A, FEIGL G, ORDOG A, UMAR D, TARI I, ERDEI L. Selenite- induced hormonal and signalling mechanisms during root growth of *Arabidopsis thaliana* L.[J]. Journal of Experimental Botany, 2012, 63(15): 5677-5687.

[9] 邹琦. 植物生理学实验指导[M]. 北京: 中国农业出版社，2000: 72-75.
ZOU Qi. Experimental guidance of plant physiology[M]. Beijing: China Agriculture Press, 2000: 72-75.

[10] PROCHAZKOVA D, WILHELMOVA N. Leaf senescence and activities of the antioxidant enzymes[J]. Biologia Plantarum, 2007, 51(3): 401-406.

[11] FORD D M, SHIBLES R. Photosynthesis and other traits in relation to chloroplast number during soybean leaf senescence[J]. Plant Physiology, 1988, 86(1): 108-111.

[12] 陆定志，潘裕才，马跃芳，林宗达，鲍为群，金逸民，游树鹏. 杂交水稻抽穗结实期间叶片衰老的生理生化研究[J]. 中国农业

科学,1988,21(3): 21-26.
LU Dingzhi, PAN Yucai, MA Yuefang, LIN Zongda, BAO Weiqun, JIN Yimin, YOU Shupeng. Physiological and biochemical studies on leaf senescence at heading and grain formation stage in hybrid rice[J]. Scientia Agricultura Sinica, 1988, 21(3): 21-26.

[13] DOWNES B P, CROWELL D N. Cytokinin regulates the expression of a soybean β- expansin gene by a post- transcriptional mechanism[J]. Plant Molecular Biology, 1998, 37(3): 437-444.

[14] GERM M, KREFT I, OSVALD J. Influence of UV- B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (*Cucurbita pepo* L.)[J]. Plant Physiology and Biochemistry, 2005, 43(5): 445-448.

[15] ANANIEVA K, MALBECK J, KAMINEK M, STADEN J V. Changes in endogenous cytokinin levels in cotyledons of *Cucurbita pepo* (zucchini) during natural and dark-induced senescence [J]. Physiologia Plantarum, 2004, 122(1): 133-142.

[16] ZHU Y X, DAVIES P J. The control of apical bud growth and senescence by auxin and gibberellin in genetic lines of peas[J]. Plant Physiology, 1997, 113(2): 631-637.

[17] JACKSON D, YAMAGUCHI J, BANOWETZ G M, HAKE S, JUAREZ M T. Leaf senescence is delayed in tobacco plants expressing the maize homeobox gene knotted1 under the control of a senescence-activated promoter[J]. The Plant Cell, 1999, 11(6): 1073-1080.

[18] HUNG K T, KAO C H. Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid[J]. Journal of Plant Physiology, 2003, 160(8): 871-879.

[19] KIM J I, MURPHY A S, BAEK D, LEE S W, YUN D J, BRESSAN R A, NARASIMHAN M L. YUCCA6 over-expression demonstrates auxin function in delaying leaf senescence in *Arabidopsis thaliana*[J]. Journal of Experimental Botany, 2011, 62(11): 3981.

[20] DAVIES P J. The plant hormones: their nature, occurrence, and functions[M]. Springer Netherlands: Plant Hormones, 1995: 1-12.

[21] LIM P O, LEE I C, KIM J, KIM H J, RYU J S, WOO H R, NAM H G. Auxin response factor 2 (ARF2) plays a major role in regulating auxin-mediated leaf longevity[J]. Journal of Experimental Botany, 2010, 61(5): 1419-1430.

[22] GHANEM M E, ALBACETE A, MARTINEZ- ANDUJAR C, ACOSTA M, ROMERO-ARANDA R, DODD I C, LUTTS S, PEREZ- ALFOCEA F. Hormonal changes during salinity- induced leaf senescence in tomato (*Solanum lycopersicum* L.)[J]. Journal of Experimental Botany, 2008, 59(11): 3039-3050.

[23] TONUTTI P, CASSON P, RAMINA A. Ethylene biosynthesis during peach fruit development[J]. Journal of the American Society for Horticultural Science, 1991, 116(2): 274-279.

[24] OHMIYA A, HAHI T. Promotion of ethylene biosynthesis in peach mesocarp discs by auxin[J]. Plant Growth Regulation, 2002, 36(3): 209-214.

[25] ROSENVASSE S, MAYAK S, FRIEDMAN H. Increase in reactive oxygen species (ROS) and in senescence- associated gene transcript (SAG) levels during dark- induced senescence of *Pelargonium* cuttings, and the effect of gibberellic acid[J]. Plant Science, 2006, 170(4): 873-879.

[26] SAKS Y, VAN S J. Evidence for the involvement of gibberellins in developmental phenomena associated with carnation flower senescence[J]. Plant Growth Regulation, 1993, 12(1/2): 105-110.

[27] SHIBAOKA H, THIMANN K V. Antagonisms between kinetin and amino acids experiments on the mode of action of cytokinins [J]. Plant Physiology, 1970, 46(2): 212-220.

[28] 郑莎莎,孙传范,孙红春,刘连涛,赵金峰,李存东. 不同外源激素对花铃期棉花主茎叶生理特性的影响[J]. 中国农业科学, 2009, 42(12): 4383-4389.
ZHENG Shasha, SUN Chuanfan, SUN Hongchun, LIU Liantao, ZHAO Jinfeng, LI Cundong. Effects of different exogenous hormones on physiological characteristics of main stem leaves at flower and boll stage in cotton[J]. Scientia Agricultura Sinica, 2009, 42(12): 4383-4389.

[29] DODD I C. Root-to-shoot signalling: assessing the roles of 'up' in the up and down world of long- distance signalling in planta [M]. Springer Netherlands: Root Physiology, 2005: 251-270.

[30] 赵平,林克惠,郑毅. 氮钾营养对烟叶衰老过程中内源激素与叶绿素含量的影响[J]. 植物营养与肥料学报, 2005, 11(3): 379-384.
ZHAO Ping, LIN Kehui, ZHENG Yi. Effect of N and K nutrition on chlorophyll content and endogenous hormones in the process of tobacco senescence[J]. Journal of Plant Nutrition and Fertilizer, 2005, 11(3): 379-384.

[31] 史国安,郭香凤,张国海,包满珠. 芍药花开放与衰老过程中生理指标的变化[J]. 西北植物学报, 2008, 28(3):506-511.
SHI Guo'an, GUO Xiangfeng, ZHANG Guohai, BAO Manzhu. Physiological changes during florescence and flower senescence of chinese penoy[J]. Acta Botanica Boreali-Occidentalia Sinica, 2008, 28(3): 506-511.

[32] 刘敬然,刘佳杰,孟亚利,王友华,陈兵林,张国伟,周治国. 外源 6-BA 和 ABA 对不同播种期棉花产量和品质及其棉铃对位叶光合产物的影响[J]. 作物学报, 2013, 39(6):1078-1088.
LIU Jingran, LIU Jiajie, MENG Yali, WANG Youhua, CHEN Binglin, ZHANG Guowei, ZHOU Zhiguo. Effect of 6-BA and ABA applications on yield, quality and photosynthate contents in the subtending leaf of cotton with different planting dates[J]. Acta Agronomica Sinica, 2013, 39(6): 1078-1088.

[33] 王宁宁,刘汝,朱亮基,汤兆达. 亚硒酸钠对烟草冠瘿组织生长的影响及其与内源激素的关系[J]. 植物生理学报, 1992, 18(2):160-166.
WANG Ningning, LIU Ru, ZHU Liangji, TANG Zhaoda. The relationship between the effect of sodium selenite on the growth of nicotiana tabacum crown gall tissue and the level of endogenous hormones[J]. Plant Physiology Journal, 1992, 18(2):160-166.