UV-A 辐射对大豆芽苗菜中抗坏血酸含量的影响

魏圣军,耿殿祥,邬 奇,张晓燕,苏娜娜,崔 瑾

(南京农业大学 生命科学学院,江苏 南京 210095)

摘 要:以黑暗培养为对照,以白光、红光、蓝光、黄光、UV-A 和 UV-B 为处理,研究了不同光质对大豆芽苗菜生长和抗坏血酸含量的影响。结果表明:与对照相比,光照处理显著降低了大豆芽苗菜下胚轴的长度(白光除外),且显著提高了下胚轴和子叶中抗坏血酸含量。与其它光质处理相比,UV-A 连续光照 36 h 后,下胚轴和子叶中抗坏血酸含量提高最显著。进一步着重研究了 UV-A 的调节机理,与对照相比,UV-A 连续光照 36 h 后,大豆芽苗菜子叶中谷胱甘肽含量显著升高,下胚轴和子叶中 DHAR、GR 酶活性及其基因的相对表达量均显著提高。综上所述,光照有利于大豆芽苗菜中抗坏血酸含量的积累;UV-A 可能是通过提高 DHAR、GR 酶活性及基因的表达量显著提高抗坏血酸的含量。

关键词:大豆芽苗菜; UV-A;抗坏血酸;DHAR;GR

中图分类号:S565.1 **文献标识码**:A **DOI**:10.11861/j.issn.1000-9841.2015.03.0420

Effect of UV-A Irradiation on Ascorbic Acid Content in Soybean Sprout

WEI Sheng-jun, GENG Dian-xiang, WU Qi, ZHANG Xiao-yan, SU Na-na, CUI Jin

(College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China)

Abstract: Effects of light quality on the growth and ascorbic acid content of soybean sprouts were studied in this article. Dark treatment was used as the control, and white, red, blue, yellow, UV-A and UV-B irradiation were used as different treatments. The results showed that compared with the dark, light reduced the hypocotyl length of soybean sprouts except white light, and more importantly the content of ascorbic acid were significantly improved both in hypocotyl and cotyledon of soybean sprouts. Compared with other light qualities, the most striking increase in contents of ascorbic acid in hypocotyl and cotyledon of soybean sprouts were happened after continuous UV-A illumination for 36 h. Further study was emphasized on the regulation mechanism of UV-A about how it could significantly raise the content of ASA, the results indicate that, after UV-A 36 h continuous illumination, the content of GSH in cotyledon, the enzymes activities of DHAR and GR and their gene relative expression in hypocotyl and cotyledon were notability improved in contrast to the control. To sum up, light is conductive to the accumulation of ascorbic acid in soybean sprouts; and UV-A through strengthen the enzyme activity of DHAR and GR and up-regulating their gene-expression to increase the content of ascorbic acid.

Keywords: Soybean sprout; UV-A; Ascorbic acid; DHAR; GR

大豆芽苗菜,又称豆芽或黄豆芽,因其口感鲜嫩、风味独特,并且含有大量对人体健康有益的物质,如蛋白质、脂肪、大豆异黄酮、酚类物质等,深受人们喜爱。但是,由于传统黄豆芽大多是在黑暗下生产,受光照条件的限制,黄豆芽中维生素 C 的含量较低[1]。维生素 C,又名抗坏血酸,是一种重要的营养元素,在植物的生长、发育和应激反应中起到至关重要的作用[2-3],同时,维生素 C 作为人类必须的一种抗氧化物质,对人类的健康至关重要,如促进骨骼的正常发育,参与免疫应答反应,治疗过敏反应、癌症、神经性和心血管疾病等[4-6]。因此提高大豆芽苗菜中抗坏血酸的含量具有重要意义。

抗坏血酸是大豆芽苗菜中一个重要的营养指标,它的合成受到环境因子的调控,尤其是受光因子的调控[7-8]。植物体内抗坏血酸含量除了受合成调节外,也会受循环的调节[9]。在抗坏血酸循环(AsA-GSH)中,氧化型抗坏血酸(DHA)可以在脱氢抗坏血酸还原酶(DHAR)作用下还原生成还原型抗坏血酸(AsA),同时,还原型的谷胱甘肽(GSH)被氧化成了氧化型谷胱甘肽(GSSG)。GSSG 又能在谷胱甘肽还原酶(GR)作用下再生成 GSH,从而调节植物体内 AsA 的动态平衡[10]。AsA-GSH 在防止植物体中 AsA 消耗过程中有重要作用,它能够影响本生烟[11-12]和番茄果实[9,13]中内抗坏血酸的含量。在番茄中过量表达 *DHAR* 基因能够使成熟的番茄果实中的抗坏血酸提高 1.6 倍[5];过量表达 *DHAR* 基因分别使马铃薯块茎[14]和玉米籽粒[15]中的抗坏血酸含量提高了 2 ~ 4 倍和 1.5 ~ 6 倍。目前为止,有关抗坏血酸循环受光调控(尤其是 UV-A)从而影响植物体内抗坏血酸含量的报道尚不多见。

收稿日期:2014-08-31

基金项目:国家自然科学基金(31171998);国家基础人才培养基金(J1310015)。

第一作者简介:魏圣军(1989-),男,硕士,主要从事植物光生物学方面的研究。E-mail: 2012116010@njau.edu.cn。

通讯作者:崔瑾(1974-),女,博士,教授,主要从事设施栽培环境调控机理研究。E-mail: cuijin@njau.edu.cn。

本文探究了不同光质处理下大豆芽苗菜中抗坏血酸含量的变化,并着重研究了 UV-A 对抗坏血酸循环代谢的调节作用,以期为大豆芽苗菜工业化生产光调控技术的应用提供科学依据。

1　材料与方法

1.1　材料

供试大豆(*Glycine max* L. Merr.)品种为东农 690,由江苏省苏芽食品公司提供。

1.2　试验设计

选择颗粒饱满、大小均匀、无残损的大豆种子 25℃下浸泡 6 h,催芽 18 h,大豆发芽约 1 ~ 2 cm 时将大豆播种到蛭石培养基中,每盆 60 株。将蛭石培养基转移到黑暗中进行连续黑暗培养 60 h,然后对大豆芽苗菜进行不同光质连续光照处理。培养箱相对湿度(75 ± 5)%,温度为(25 ± 2)℃。

培养箱(浙江宁波赛福实验仪器厂生产)内置 LED 冷光源,光谱能量分布的主要技术参数如表 1 所示,可发出白光(W)、红光(R)、蓝光(B)、黄光(Y)及紫外光(UV-A 和 UV-B),各种光质的光谱能量分布均为 100%,由于 UV-A 和 UV-B 的 LED 产品价格昂贵,故本试验中用 UV-A (PHILIPS TL 8W/10)和 UV-B(PHILIPS,PL S9W/01/2P)紫外窄谱灯管替代 LED 灯。对照组(黑暗培养,D)普通培养箱内黑暗下培养。光强均匀分布(30 ± 3)μmol·m^{-2}·s^{-1},连续光照 12,24 和 36 h 后测定各项试验指标。

表 1　不同 LED 光谱能量分布的主要技术参数

Table 1　Major technical parameters of light spectral energy distribution under LED

光质 Light quality	波峰波长 △p/nm	波长半宽 △λ/nm
白光 W	380 ~ 750	–
红光 R	658	5
蓝光 B	460	5
黄光 Y	585	5
紫外光 UV-A	380	20
紫外光 UV-B	311	20

1.3　测定指标及方法

1.3.1　*生长指标*　大豆芽苗菜在不同光质下连续光照 36 h 后,随机取样 10 株,测定各项生长指标。可食鲜重、总鲜重用万分之一天平(Sartorius, BSA 124S)测定;可食率(%) =(可食鲜重/总鲜重)× 100;下胚轴长用直尺测定。

1.3.2　*抗坏血酸和谷胱甘肽含量*　采用紫外分光光度法测定抗坏血酸和谷胱甘肽含量,试验测定重复 3 次。还原型抗坏血酸(AsA)和总抗坏血酸(AsA + DHA)的测定参考 Law MY 等的方法[16],氧化型抗坏血酸的含量(DHA)由总抗坏血酸减去 AsA 含量获得。总谷胱甘肽(GSH + GSSG)和氧化型谷胱甘肽(GSSG)含量测定参考 Forman H J 等[17]的方法,还原型谷胱甘肽(GSH)含量由总谷胱甘肽含量减去 GSSG 含量获得。

1.3.3　*DHAR 酶和 GR 酶活性*　UV-A 连续光照 36 h后,测定大豆芽苗菜下胚轴和子叶中 DHAR、GR 酶活性,重复 3 次。脱氢抗坏血酸还原酶(DHAR)活性采用 DHAR 试剂盒(购于苏州科铭生物技术有限公司)测定。谷胱甘肽还原酶(GR)活性采用 GR 试剂盒(购于南京建成生物工程研究所)测定。酶活测定样品的处理参考试剂盒说明书。

1.3.4　*DHAR 及 GR 基因相对表达量*　总 RNA 的提取采用 Trizol 法[18],取下胚轴和子叶新鲜植物样本进行提取,琼脂糖凝胶电泳确定 RNA 完整性后反转录成 cDNA。每个处理取 8 μL 总 RNA 进行反转录,反转录参考试剂盒说明书进行(Thermo Fisher Scientific Inc., USA)。荧光定量 PCR 反应体系(20 μL)为:Bestar® SybrGreen qPCR mastermix:10 μL, PCR Forward Primer(10 μmol · L^{-1}):0.5 μL, PCR Reverse Primer(10 μmol·L^{-1}):0.5 μL, DNA 模板:1 μL, ddH_2O(灭菌蒸馏水):8.0 μL(由 DBI, Bioscience Inc., Germany 提供)。采用三步法 PCR 标准程序:第一步:预变性,95℃, 2 min;第二步:PCR 反应:95℃变性 10 s,60℃退火 34 s,72℃延伸 30 s,循环 40 次;第三步:溶解曲线:95℃, 1 min;55℃, 1 min;55 ~ 98℃(10 s/cycle 0.5℃/cycle) 86 个循环。该程序在荧光定量 PCR 仪(Eppendorf, Hamburg, Germany)上进行。

引物设计片段:*DHAR* (NM_001250000.1) F: 5′-AGGAAGGGTTGGGAAGC-3′, R: 5′-AAGGAGGTCACAGTCGTTG-3′;*GR* (L11632.1) F: 5′-CGGTGCCTCAAGGGAAGAAAT-3′, R: 5′-TTCACTCGACTTCCAGGCTC-3′。

1.4　数据分析

采用 Excel 2007 进行数据整理,DPS 8.50 进行方差分析,Origin 8.5 进行绘图,显著性分析采用 Duncan 新复极差法($P < 0.05$)。

2　结果与分析

2.1　光质对大豆芽苗菜生长的影响

表 2 显示,不同光质连续光照 36 h 后,与对照(黑暗)相比,光照显著降低了大豆芽苗菜下胚轴的长度(白光除外);大豆芽苗菜的总鲜重和可食鲜重在光照下均显著低于对照组,且在 UV-A 和 UV-B

处理下下降得较为显著;与对照(黑暗)相比,光照　　处理对大豆芽苗菜的可食率影响不显著。

表 2　光质对大豆芽苗菜生长的影响

Table 2　Effects of light quality on the growth of soybean sprouts

光质处理 Light treatment	下胚轴长度 Hypocotyl length/cm	总鲜重 Total fresh weight/g	可食鲜重 Edible fresh weight/g	可食率 Edible rate/%
黑暗 D	11.57 ± 1.01 a	0.59 ± 0.03 a	0.46 ± 0.06 a	0.78 ± 0.06 ab
白光 W	10.77 ± 0.54 a	0.51 ± 0.04 b	0.39 ± 0.04 b	0.76 ± 0.02 b
红光 R	9.37 ± 0.10 b	0.49 ± 0.03 bc	0.38 ± 0.02 b	0.77 ± 0.01 b
蓝光 B	8.33 ± 0.01 c	0.48 ± 0.03 bc	0.37 ± 0.03 bc	0.76 ± 0.01 b
黄光 Y	8.8 ± 0.12 bc	0.48 ± 0.07 bc	0.38 ± 0.05 b	0.79 ± 0.01 ab
UV-A	7.3 ± 0.12 d	0.42 ± 0.03 cd	0.34 ± 0.02 bc	0.80 ± 0.04 ab
UV-B	5.1 ± 0.07 e	0.35 ± 0.07 d	0.29 ± 0.06 c	0.83 ± 0.02 a

同列不同小写字母表示在 0.05 水平上差异显著。下同。

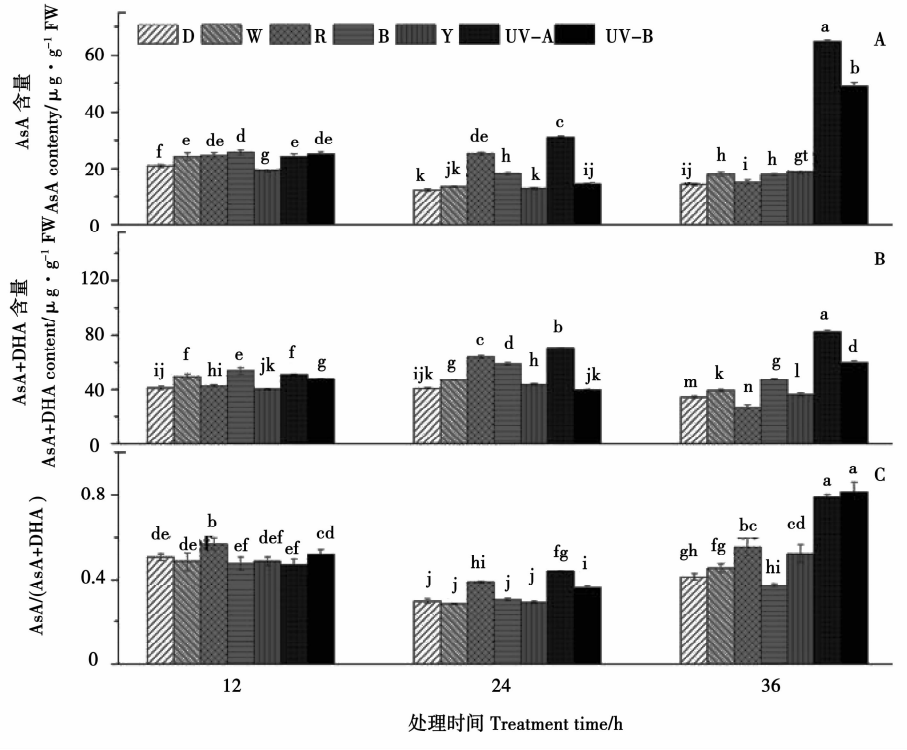
Values within a column followed by different lowercase letters are significantly difference at 0.05 level. The same below.

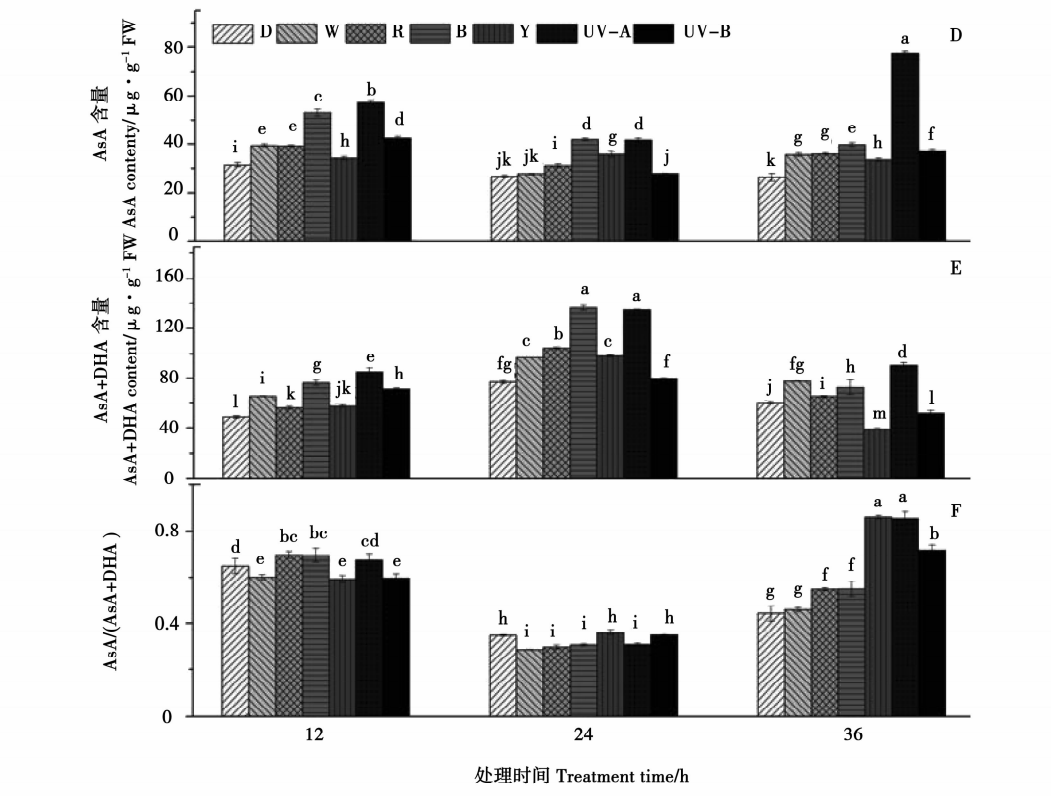
2.2　光质对大豆芽苗菜中抗坏血酸含量的影响

由图 1-A/D 可知,与对照(黑暗)相比,光照处理均能显著提高大豆芽苗菜子叶和下胚轴中 AsA 含量;且与其它光质相比,UV-A 连续光照 36 h,大豆芽苗菜下胚轴和子叶中 AsA 含量提高最为显著。图 1-B/E 表明,不同光质处理下,总抗坏血酸(AsA + DHA)含量在子叶和下胚轴中的变化趋势不同,在下胚轴中总抗坏血酸的含量在 UV-A 连续光照 36 h 最高;子叶中总抗坏血酸含量在蓝光和 UV-A 照射 24 h 达到最高值,UV-A 照射 36 h 稍微低于 24 h 照射。图 1-C/F 显示,不同光质之间,还原型抗坏血酸在总抗坏血酸中所占的比例[AsA/(AsA + DHA)],随着光照时间呈现先下降后升高的趋势,UV-A 连续光照 36 h,AsA 在总抗坏血酸中的比例最高。总之,与对照相比,光照处理显著提高了下胚轴和子叶中抗坏血酸含量;与其它光质处理相比,UV-A 连续光照 36 h 后,下胚轴和子叶中抗坏血酸含量提高最显著。

2.3　UV-A 对大豆芽苗菜中谷胱甘肽含量的影响

图 2-A/B/C 显示,与对照(黑暗)相比,UV-A 连续光照 36 h,对大豆芽苗菜下胚轴中 GSH、GSH + GSSG 及 GSH/(GSH + GSSG)的影响不显著。图 2-D/E/F 表明,与黑暗相比,UV-A 连续光照 36 h,显著提高了大豆芽苗菜子叶中 GSH 含量和 GSH 在总谷胱甘肽的比例,但对总谷胱甘肽含量影响不显著。



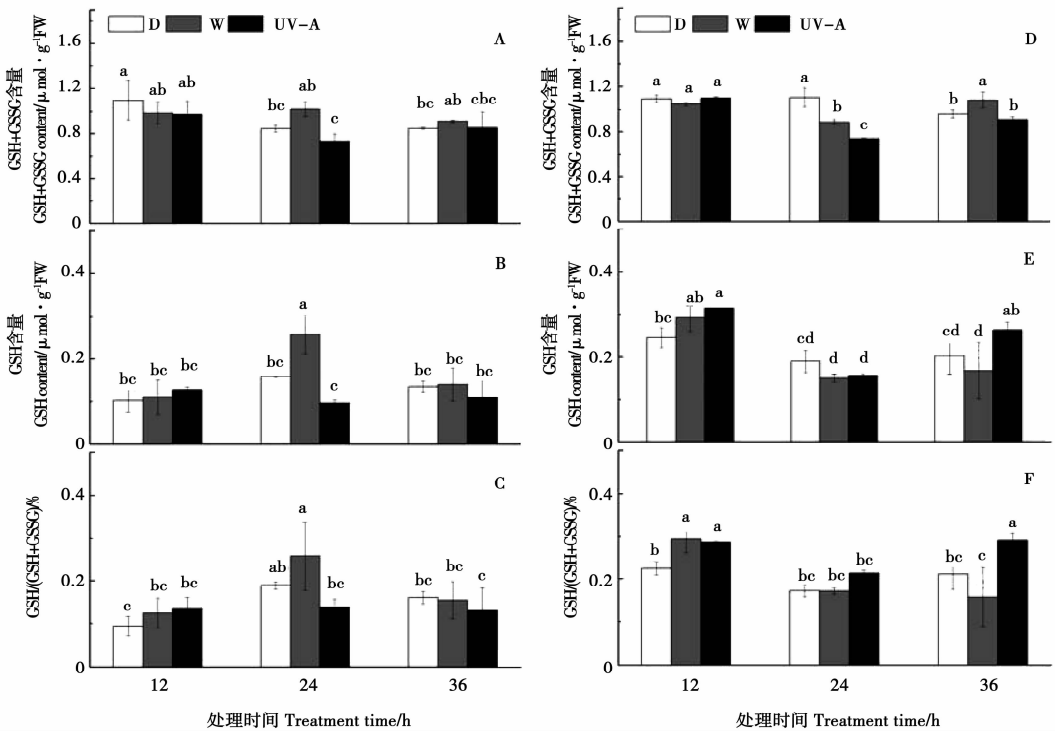


A ~ C 为下胚轴;D ~ F 为子叶。

A-C are hypocotyl; D-F are cotyledon.

图 1　光质对大豆芽苗菜中 AsA、AsA + DHA 及 AsA/(AsA + DHA)的影响

Fig. 1　Effects of light quality on AsA, AsA + DHA and AsA/(AsA + DHA) in soybean sprouts



A ~ C 为下胚轴;D ~ F 为子叶。

A-C are hypocotyl; D-F are cotyledon.

图 2　UV-A 对大豆芽苗菜中 GSH,GSH + GSSG 及 GSH/(GSH + GSSG)的影响

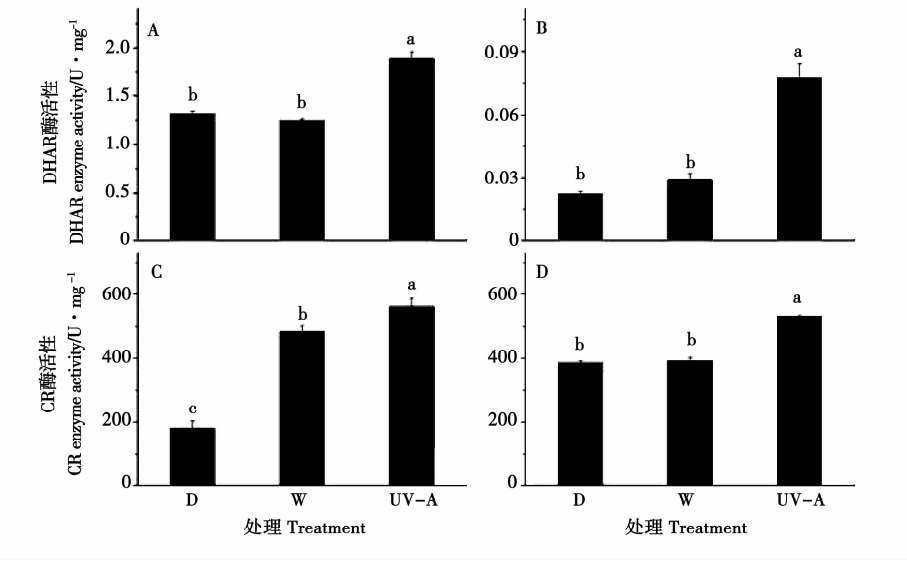
Fig. 2　Effects of UV-A on GSH, GSH + GSSG and GSH/(GSH + GSSG) in soybean sprouts

2.4 UV-A 对大豆芽苗菜中 DHAR 酶及 GR 酶活性的影响

图 3 表明,与对照(黑暗)相比,UV-A 连续光照 36 h,大豆芽苗菜下胚轴和子叶中 DHAR、GR 酶活性均显著高于对照及白光处理;下胚轴中 DHAR 酶活性是子叶中 DHAR 酶活性的 24 倍,下胚轴中 GR 酶活性是子叶中酶活性的 1.06 倍。

2.5 UV-A 对大豆芽苗菜中 *DHAR*、*GR* 基因相对表达量的影响

如图 4 所示,与对照(黑暗)相比,UV-A 连续光照下,大豆芽苗菜下胚轴和子叶中 *DHAR*、*GR* 基因相对表达量均显著提高;下胚轴中 *GR* 基因的相对表达量提高了 5 倍,*DHAR* 基因的相对表达量提高了近 20 倍;子叶中 *GR* 和 *DHAR* 基因的相对表达量分别提高了 1.2 和 1.7 倍。

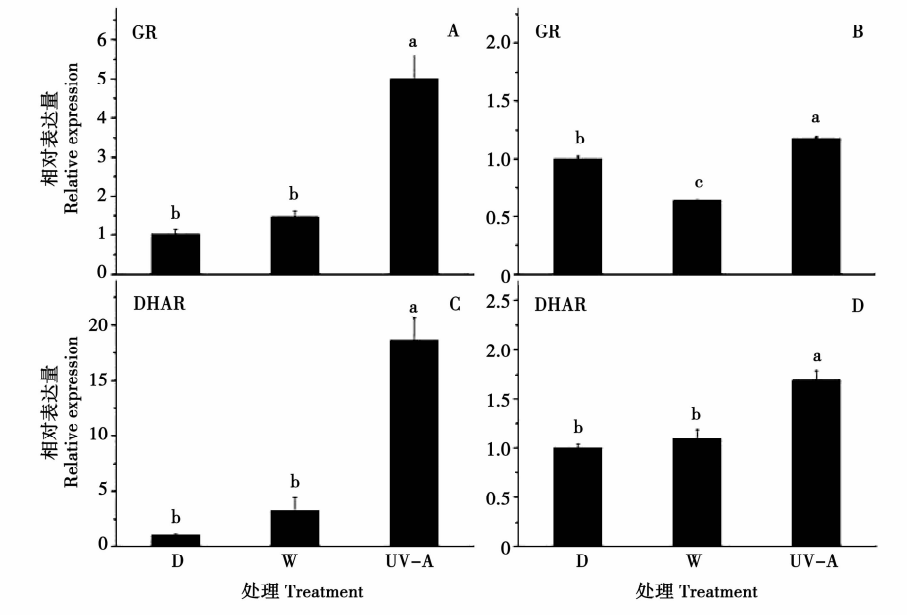


A 和 C 为下胚轴;B 和 D 为子叶。

A and C are hypocotyl; B and D are cotyledon.

图 3 UV-A 对大豆芽苗菜中 DHAR、GR 酶活性的影响

Fig. 3 Effects of UV-A on the activities of DHAR and GR enzymes in soybean sprouts



A 和 C 为下胚轴;B 和 D 为子叶。

A and C are hypocotyl; B and D are cotyledon.

图 4 UV-A 对大豆芽苗菜中 DHAR、GR 基因相对表达量的影响

Fig. 4 Effects of UV-A on the expression of DHAR and GR genes in soybean sprouts

3　结论与讨论

3.1　光质对大豆芽苗菜生长的影响

本试验结果表明,与对照(黑暗)相比,光质处理显著地降低了大豆芽苗菜下胚轴的长度(白光除外)。Lee 等[19]的研究表明,单一光质会引起光能量在光系统Ⅰ和Ⅱ的不均匀分布,从而影响植物的生长,降低下胚轴的长度,而下胚轴的降低可能是光质处理下大豆芽苗菜总鲜质量和可食鲜质量降低的主要原因。

3.2　光质对大豆芽苗菜中抗坏血酸和谷胱甘肽含量的影响

光不仅是植物生长发育、形态建成的重要环境因子,同时也决定了植物体内的抗坏血酸含量[20]。有报道指出,在番茄和拟南芥中,光照和环境胁迫能诱导抗坏血酸合成相关基因的表达并提高抗坏血酸含量[21-22]。Smirnoff[23]发现,光照能导致拟南芥叶片中抗坏血酸的积累,且拟南芥中抗坏血酸的合成具有光依赖性。与弱光下生长的拟南芥相比,高光强下植物体内的抗坏血酸含量较高,当拟南芥从光下转移到黑暗下后,叶片中抗坏血酸含量显著下降。本试验发现,与对照(黑暗)相比,还原型和总抗坏血酸在不同的光质照射下均有显著提高;与其它光质相比,UV-A 连续光照 36 h,AsA 含量的上升最为显著,并且显著提高了 AsA 在总抗坏血酸中的比例,这与 Younis 等[24]在蚕豆幼苗中的研究结果相似。

氧化型抗坏血酸可以通过抗坏血酸 - 谷胱甘肽循环途径被还原,生成还原型的抗坏血酸,这个过程有还原型的谷胱甘肽的参与[10]。已有研究表明,抗坏血酸 - 谷胱甘肽循环能够有效地阻止 AsA 的过多消耗[9,12-13]。本研究还发现,UV-A 连续光照 36 h 对大豆芽苗菜中下胚轴中 GSH、GSH + GSSG 及 GSH/(GSH + GSSG)的影响不显著,但提高了大豆芽苗菜子叶中 GSH 的含量和 GSH 在总谷胱甘肽中的比例。由此认为,稳定的 GSH 含量和较高的 GSH 比例可以维持植物体内较高的 AsA-GSH 循环效率,使植物体在 UV-A 照射下维持较高的 AsA 水平。

3.3　UV-A 对大豆芽苗菜 DHAR、GR 酶活性和 *DHAR*、*GR* 基因表达量的影响

本试验结果显示,与黑暗相比,UV-A 照射下 DHAR、GR 酶活性和 *DHAR*、*GR* 基因的表达量均显著提高。有研究发现,在番茄中过量表达 *DHAR* 基因能够使成熟的番茄果实中的抗坏血酸提高 1.6 倍[5];过量表达 *DHAR* 基因分别使马铃薯块茎[14]和玉米籽粒[15]中的抗坏血酸含量提高了 2 ~ 4 倍和 1.5 ~ 6 倍;Chen 等[25-26]发现过量表达小麦的 *DHAR* 基因能提高本生烟中抗坏血酸含量。Capucine 等[27]也发现高温能够降低 GR 酶活性限制 AsA 循环效率,降低了番茄果实中 AsA 的含量。由此推测,UV-A 对大豆芽苗菜抗坏血酸含量的影响主要是通过提高 DHAR、GR 酶活性和促进 DHAR、GR 基因的表达进行调控的。

综上所述,适当的 UV-A 辐射可以通过影响抗坏血酸 - 谷胱甘肽循环,提高 DHAR、GR 酶活性和促进 DHAR、GR 基因的表达,显著提高大豆芽苗菜中抗坏血酸的含量。

参考文献

[1] 蔡梦珊,李江滨,林锦琼,等. 毛豆、黄豆、黄豆芽中蛋白质和 VC 含量及营养价值评价[J]. 食品科学,2013(14):270. (Cai M S, Li J B, Lin J Q, et al. Determination and nutritional value evaluation of protein and vitamin c in edamame, soybeans and bean sprouts[J]. Food Science, 2013(14):270.)

[2] Conklin P L, de Paolo D, Wintle B, et al. Identification of *Arabidopsis* VTC3 as a putative and unique dual function protein kinase: Protein phosphatase involved in the regulation of the ascorbic acid pool in plants[J]. Journal of Experimental Botany, 2013, 64(10):2793-804.

[3] Mastropasqua L, Borraccino G, Bianco L, et al. Light qualities and dose influence ascorbate pool size in detached oat leaves[J]. Plant Science, 2012, 183(2):57-64.

[4] Zhang Y Y, Han L, Ye Z B, et al. Ascorbic acid accumulation is transcriptionally modulated in high-pigment-1 tomato fruit[J]. Plant Molecular Biology Reporter, 2014, 32:52-61.

[5] Liu W, An H M, Yang M. Overexpression of rosa roxburghii l-galactono-1, 4-lactone dehydrogenase in tobacco plant enhances ascorbate accumulation and abiotic stress tolerance[J]. Acta Physiologiae Plantarum, 2013, 35(5):1617-1624.

[6] Haroldsen V M, Chi-Ham C L, Kulkarni S, et al. Constitutively expressed DHAR and MDHAR influence fruit, but not foliar ascorbate levels in tomato[J]. Plant Physiology and Biochemistry, 2011, 49(10): 1244-1249.

[7] Chen Z, Gallie D R. The ascorbic acid redox state controls guard cell signaling and stomatal movement[J]. The Plant Cell, 2004, 16:1143-1162.

[8] Dowdle J, Ishikawa T, Gatzek S, et al. Two genes in *Arabidopsis* encoding GDP-l-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability[J]. The Plant Journal, 2007, 52:673-689.

[9] Stevens R, Page D, Gouble B, et al. Tomato fruit ascorbic acid content is linked with monodehydroascorbate reductase activity and tolerance to chilling stress[J]. Plant Cell Environment, 2008, 31: 1086-1096.

[10] Foyer C H, Noctor G. Ascorbate and Glutathione: The heart of the redox hub[J]. Plant Physiology, 2011, 155:2-18.

[11] Chen Z, Ling J, Chang S C, et al. Increasing vitamin C content of plants through enhanced ascorbate recycling[J]. Proceedings of the National Academy of Sciences, 2003, 100:3525-3530.

[12] Eltayeb A E K N, Badawi G H, Kaminaka H, et al. Overexpres-

sion of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses[J]. Planta, 2007, 225:1255-1264.

[13] Stevens R, Buret M, Duffe P, et al. Candidate genes and quantitative trait loci affecting fruit ascorbic acid content in three tomato populations[J]. Plant Physiology, 2007, 143:1943-1953.

[14] Young-Min Goo, Hyun Jin Chun, Tae-Won Kim, et al. Expressional characterization of dehydroascorbate reductase cDNA in transgenic potato plants[J]. Journal of Plant Biology, 2008, 51: 35-41.

[15] Naqvi S, Zhu C, Farre G, et al. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways[J]. Proceedings of the National Academy of Sciences of the United States of America, 2009, 106:7762-7767.

[16] Law M Y, Charles S A, Halliwell B. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and of Paraquat [J]. Biochemical Journal, 1983, 210:899-903.

[17] Forman H J, Zhang H Q, Rinna A. Glutathione: Overview of its protective roles, measurement, and biosynthesis[J]. Molecular Aspects of Medicine, 2009, 30:1-12.

[18] Su N N, Wu Q, Liu Y Y, et al. Hydrogen-rich water reestablishes ros homeostasis but exerts differential effects on anthocyanin synthesis in two varieties of radish sprouts under UV-A irradiation [J]. Journal of Agricultural and Food Chemistry, 2014, 62: 6454-6462.

[19] Lee S J, Ahn J K, Khanh T D, et al. Comparison of isoflavone concentrations in soybean (*Glycine max* L. Merrill) sprouts grown under two different light conditions[J]. Journal of Agricultural and Food Chemistry, 2007, 55(23): 9415-9421.

[20] Yabuta Y, Mieda T, Rapolu M, et al. Light regulation of ascorbate biosynthesis is dependent on the photosynthetic electron transport chain but independent of sugars in *Arabidopsis*[J]. Journal of Experimental Botany, 2007, 58: 2661-2671.

[21] Huang C, He W, Guo J, et al. Increased sensitivity to salt stress in an ascorbate-deficient *Arabidopsis* mutant[J]. Journal of Experimental Botany, 2005, 56:3041-3049.

[22] Ioannidi E, Kalamaki M S, Engineer C, et al. Expression profiling of ascorbic acid-related genes during tomato fruit development and ripening and in response to stress conditions[J]. Journal of Experimental Botany, 2009, 60: 663-678.

[23] Smirnoff N. Ascorbate biosynthesis and function in photoprotection [J]. Philosophical Transactions of the Royal Society of London, 2000, 355:1455-1464.

[24] Younis M B, Hasaneen M N, Abdel-Aziz H M. An enhancing effect of visible light and UV radiation on phenolic compounds and various antioxidants in broad bean seedlings[J]. Plant Signaling & Behavior, 2010, 5:1197-1203.

[25] Chen Z, Todd E, Young J L, et al. Increasing vitamin C content of plants through enhanced ascorbate recycling[J]. Proceedings of the National Academy of Sciences, 2003, 100:3525-3530.

[26] Chen Z, Gallie D R. Increasing tolerance to ozone by elevating foliar ascorbic acid confers greater protection against ozone than increasing avoidance [J]. Plant Physiology, 2005, 138:1673-1689.

[27] Capucine M, Doriane B, Félicie L L, et al. High temperature inhibits ascorbate recycling and light stimulation of the ascorbate pool in tomato despite increased expression of biosynthesis genes[J]. PLOS ONE, 2013, 8(12).

(上接第 419 页)

[16] Hansen P M, Schjoerring J K. Reflectance measurement of canopy biomass and nitrogen status in wheat crops using normalized difference vegetation indices and partial least squares regression[J]. Remote Sensing of Environment, 2003, 86(4): 542-553.

[17] Schmidtlein S, Sassin J. Mapping of continuous floristic gradients in grasslands using hyperspectral imagery[J]. Remote Sensing of Environment, 2004, 92(1): 126-138.

[18] Ferrio J P, Villegas D, Zarco J, et al. Assessment of durum wheat yield using visible and near-infrared reflectance spectra of canopies [J]. Field Crops Research, 2005, 94(2-3): 126-148.

[19] Huang Z, Turner B J, Dury S J, et al. Estimating foliage nitrogen concentration from HYMAP data using continuum removal analysis [J]. Remote Sensing of Environment, 2004, 93(1-2): 18-29.

[20] Nguyen H T, Lee B W. Assessment of rice leaf growth and nitrogen status by hyperspectral canopy reflectance and partial least square regression[J]. European Journal of Agronomy, 2006, 24 (4): 349-356.

[21] Brás L g P, Bernardino S A, Lopes J A, et al. Multiblock PLS as an approach to compare and combine NIR and MIR spectra in calibrations of soybean flour[J]. Chemometrics and Intelligent Laboratory Systems, 2005, 75(1): 91-99.

[22] Kovalenko I V, Rippke G R, Hurburgh C R, et al. Determination of amino acid composition of soybeans (*Glycine max*) by near-infrared spectroscopy[J]. Journal of Agricultural and Food Chemistry, 2006, 54(10): 3485-3491.

[23] Luna A S, da Silva A P, Pinho J S A, et al. Rapid characterization of transgenic and non-transgenic soybean oils by chemometric methods using NIR spectroscopy[J]. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2013, 100: 115-119.

[24] Wang D, Dowell F E, Ram M S, et al. Classification of fungal-damaged soybean seeds using near-infrared spectroscopy[J]. International Journal of Food Properties, 2004, 7(1): 75-82.

[25] 王惠文. 偏最小二乘回归方法及其应用[M]. 北京:国防工业出版社, 1999: 150-177. (Wang H W. Partial least squares regression method and applications[M]. Beijing: National Defense Industry Press, 1999: 150-177.)

[26] Loague K, Green R E. Statistical and graphical methods for evaluating solute transport models: Overview and application [J]. Journal of Contaminant Hydrology, 1991, 7(1-2):51-73.

[27] Aparicio N, Villegas D, Araus J L, et al. Relationship between growth traits and spectral vegetation indices in durum wheat[J]. Crop Science, 2002, 42(5): 1547-1555.

[28] Dunn Iii W J, Scott D R, Glen W G, et al. Principal components analysis and partial least squares regression[J]. Tetrahedron Computer Methodology, 1989, 2(6): 349-376.

[29] 张宁, 齐波, 赵晋铭, 等. 应用主动传感器 GreenSeeker 估测大豆籽粒产量[J]. 作物学报, 2014, 40(4): 657-666. (Zhang N, Qi B, Zhao J M, et al. Prediction for soybean grain yield using active sensor greenseeker[J]. Acta Agronomica Sinica, 2014, 40 (4): 657-666.)