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6-苄(基)腺嘌呤和抗坏血酸对渗透胁迫时杨树光合作用光抑制的影响*

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【摘要】 研究了6-BA和AsA对渗透胁迫时杨树幼苗叶片光合作用光抑制和活性氧代谢的影响. 结果表明, 渗透胁迫时杨树叶片净光合速率(Pn)和表观量子效率(AQY)降低, 光合作用光抑制加剧, 超氧化物歧化酶(SOD)活性升高, 抗坏血酸过氧化物酶(APX)活性降低, $O_2^{\bar{\cdot}}$ 产生加快, H_2O_2 和膜脂过氧化产物丙二醛(MDA)含量升高. 6-BA和AsA预处理使胁迫时叶片SOD和APX活性升高, $O_2^{\bar{\cdot}}$ 生成减少, H_2O_2 和MDA含量降低, 同时缓解了光合作用的光抑制. 相关分析表明, 杨树叶片活性氧水平和MDA含量与Pn和AQY呈负相关. 胁迫时杨树叶片活性氧的积累与光合作用光抑制有一定关系, 6-BA和AsA对光抑制的缓解作用与其对活性氧清除系统的促进作用有关.

关键词 杨树无性系 渗透胁迫 光合作用光抑制 活性氧 保护酶 6-BA AsA
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Effects of 6-BA and AsA on photosynthesis photoinhibition of attached poplar leaves under osmotic stress of root. FENG Yulong[1,2], MA Yongshuang[2], FENG Zhili[1] ([1] *Kunming Division, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming* 650223, *China*; [2] *College of Life Science, Hebei University, Baoding* 071002, *China*). -*Chin. J. Appl. Ecol.*, 2004, **15**(12): 2233~2236.

In order to know more about the relationships between photosynthesis photoinhibition and reactive oxygen species metabolism, the effects of 6-benzyladenine (6-BA) and ascorbate (AsA) on net photosynthetic rate (Pn), apparent quantum yield (AQY), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities, $O_2^{\bar{\cdot}}$ generation rate, and H_2O_2 and malondialdehyde (MDA) contents were studied with attached leaves of poplar clone seedlings under osmotic stress of root. Under osmotic stress, the photosynthesis photoinhibition of attached poplar leaves, judged by the significant decrease of Pn and AQY, was aggravated, and the balance of reactive oxygen species metabolism was destroyed. The superoxide dismutase (SOD) activity increased, but ascorbate peroxidase (APX) activity decreased. In the meantime, the $O_2^{\bar{\cdot}}$ generation rate and the contents of H_2O_2 and malondialdehyde (MDA) increased. When osmotic stressed poplar seedlings were pretreated with 6-BA and AsA, the activities of SOD and APX increased, $O_2^{\bar{\cdot}}$ generation rate and H_2O_2 and MDA contents decreased, and photosynthesis photoinhibition was alleviated. The contents of reactive oxygen species and MDA in poplar leaves were negatively correlated with net photosynthetic rate and apparent quantum yield. It's indicated that the photosynthesis photoinhibition of attached leaves of poplar clone seedlings had intrinsic relations with the accumulation of reactive oxygen species under osmotic stress of root, and the alleviation effects of 6-BA and AsA on photosynthesis photoinhibition were related to their promotion effects to the scavenging system of reactive oxygen species.

Key words Poplar clone, Osmotic stress, Photosynthesis photoinhibition, Reactive oxygen species, Protecting enzymes, 6-BA, AsA.

1 引 言

强光在引起植物光抑制的同时, 还会刺激体内活性氧的产生[8,9]. 强光与其它胁迫同时发生则会加剧活性氧的生成[12]. 因此, 人们将光抑制与活性氧联系起来[21]. 活性氧的产生是光合电子传递的必然结果[1,12]. 叶绿体中分子氧不可避免地被光还原成 $O_2^{\bar{\cdot}}$ [15], $O_2^{\bar{\cdot}}$ 还能进一步转变成 H_2O_2、$^1O_2$、·OH 等活性氧[3]. 正常条件下植物能及时清除 $O_2^{\bar{\cdot}}$ 等有毒的活性氧, 使其保持在较低的水平[3]. 逆境则会加速活性氧的生成[8,10,12], 使其在体内积累, 造成氧化伤害. 细胞分裂素(CTK)能使保护酶活性增强, 防止有毒活性氧生成, 因此具有防止衰老[2,22]、抑制盐渍伤害[18]及阻止干旱时膜脂过氧化[6,7]等作用. 抗坏血酸(AsA)是植物体内有效的抗氧化剂[5,16], 水分胁迫时其含量降低[13,20,24], 外源AsA可缓解渗透胁迫引起的膜损伤[14,17]. 研究表明, 杨树光合作用光抑制与叶片中活性氧的积累有

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关[9,10].本文通过叶面施用6-BA(CTK 的一种)和AsA,研究其对根系渗透胁迫的杨树无性系幼苗光抑制和活性氧代谢的影响,进一步探讨活性氧和光抑制的关系,为阐明光抑制机理和探索缓解胁迫伤害的技术提供依据.

2 材料与方法

2.1 供试材料

试验材料为昭林杨6号(*Populus* × *xiaozhuanica* 'Zhaolin-6').其培养与渗透胁迫处理见文献[10].渗透胁迫处理前一天20:00分别用含0.04%吐温-20的25 mmol·L^{-1} AsA和0.25 mmol·L^{-1} 6-BA溶液作叶面喷施,以喷施含吐温-20的磷酸缓冲液为对照.

2.2 测定方法

渗透胁迫处理后1 h和8 h(6-BA和AsA处理后13 h和20 h)测定成熟叶片的光合参数、活性氧和丙二醛水平,以及保护酶活性等,测定方法见文献[10],每个参数均为3个叶片的3~6次测定结果的平均值.

3 结果与分析

3.1 6-BA和AsA对渗透胁迫时杨树叶片光合参数的影响

渗透胁迫时杨树无性系幼苗叶片Pn和AQY明显低于对照,6-BA和AsA处理可缓解渗透胁迫对Pn和AQY的影响.−0.5 MPa胁迫时,6-BA和AsA处理使Pn和AQY显著高于只胁迫的对照;−1.0 MPa胁迫时,6-BA和AsA处理亦使Pn和AQY升高.渗透胁迫1 h时Pn和AQY与对照差异达显著水平;胁迫8 h时Pn与对照差异不显著,AQY与对照差异显著(图1a,b).

3.2 6-BA和AsA对渗透胁迫时杨树叶片活性氧水平的影响

随胁迫强度的增大,$O_2^{\cdot-}$生成速率和H_2O_2含量升高,差异均达到显著水平.6-BA处理时$O_2^{\cdot-}$生成速率降低不显著,但H_2O_2含量显著降低,AsA处理使$O_2^{\cdot-}$生成速率和H_2O_2含量均明显降低(图1c,d).胁迫时杨树叶片活性氧水平与Pn和AQY呈显著负相关(图2).

3.3 6-BA和AsA对渗透胁迫时杨树叶片丙二醛含量的影响

MDA含量随胁迫强度的增大而升高,其变化趋势与H_2O_2相似.6-BA和AsA处理使MDA含量明显降低(图3).胁迫时杨树叶片MDA含量与Pn和AQY呈显著负相关(图4).

3.4 6-BA和AsA对渗透胁迫时杨树叶片保护酶活性的影响

渗透胁迫时杨树无性系幼苗叶片SOD活性升高,APX活性降低;胁迫强度越大,SOD活性越高,APX活性越低.6-BA处理时SOD和APX活性显著

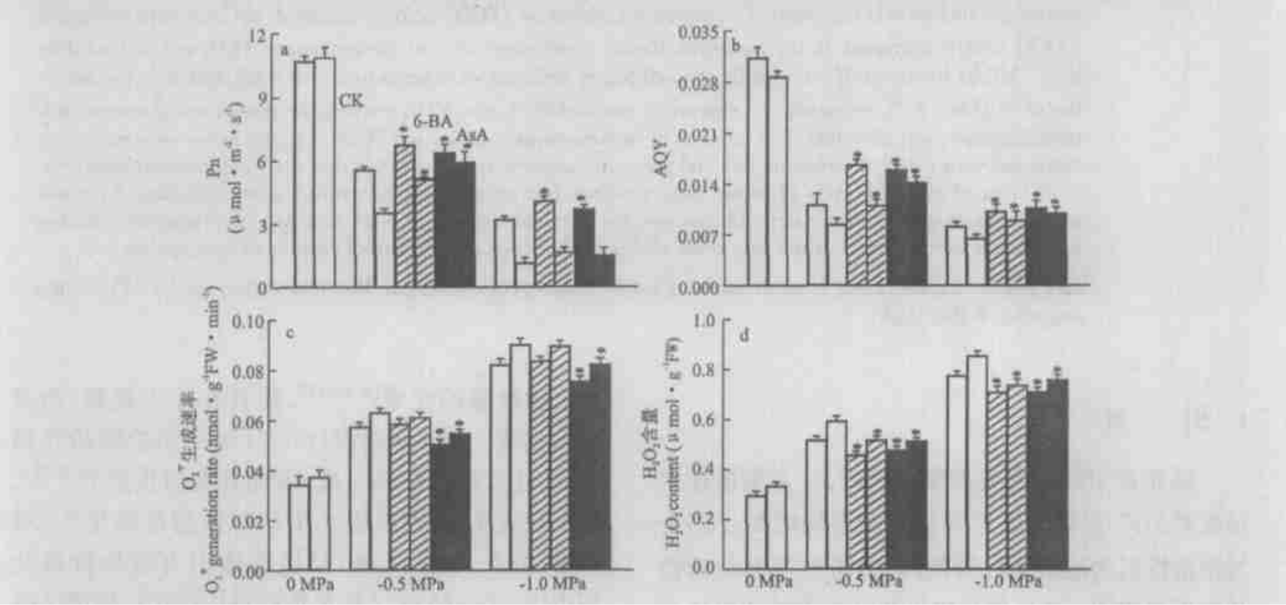

图1 6-BA和AsA对渗透胁迫时杨树叶片净光合速率(Pn)(a)、表观量子效率(AQY)(b)、$O_2^{\cdot-}$生成速率(c)和H_2O_2含量(d)的影响

Fig.1 Effects of 6-BA and AsA on net photosynthetic rate (Pn)(a), apparent quantum yield (AQY)(b), $O_2^{\cdot-}$ generation rate (c) and H_2O_2 content(d) in leaves of poplar seedlings under osmotic stress of root.

每对柱中的第一个柱为渗透胁迫1 h的测定值,第二个柱为胁迫8 h的测定值 The first bar of the twin bars indicates the value measured at 1 h after osmotic stress, the second bar indicates the value measured at 8 h after osmotic stress. * 表示6-BA或AsA处理与胁迫对照相同时间测定值间差异显著 $P<0.05$ Indicates significant different between the corresponding bars of osmotic stress and 6-BA or AsA pretreatment at $P<0.05$. 图中数据为3~6测定结果的平均值±标准误 The data are mean±SE of 3~6 replicates.

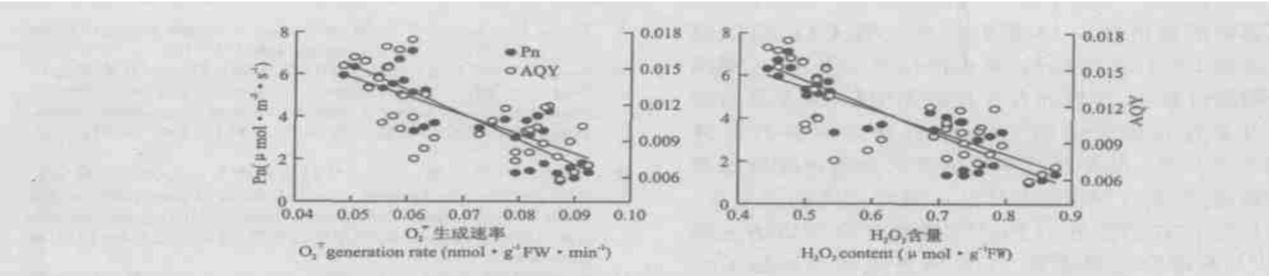

图 2　渗透胁迫时杨树叶片 $O_2^{-\cdot}$ 的生成速率和 H_2O_2 含量与 Pn 和 AQY 的关系

Fig. 2 Relationships between $O_2^{-\cdot}$ generation rate or H_2O_2 content and Pn or AQY in leaves of poplar seedlings under osmotic stress of root.

Pn= 12.2546− 116.8593× $O_2^{-\cdot}$ generation rate, r = 0.8679*; AQY= 0.0220− 0.1556× $O_2^{-\cdot}$ generation rate, r = 0.6969*; Pn= 12.0566− 12.6644 × H_2O_2 content, r= 0.8930*; AQY= 0.0223− 0.0178× H_2O_2 content, r = 0.7549*.

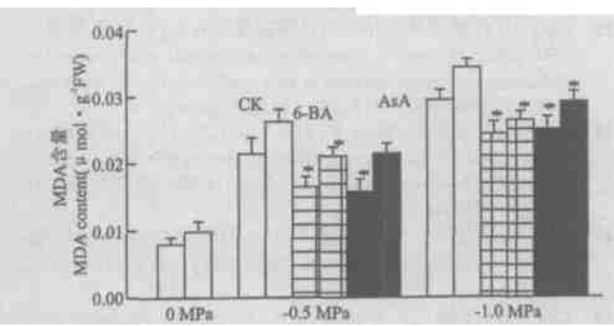

图 3　6-BA 和 AsA 对渗透胁迫时杨树叶片丙二醛(MDA)含量影响

Fig. 3 Effects of 6-BA and AsA on malondialdehyde (MDA) content in leaves of poplar seedlings under osmotic stress of root.

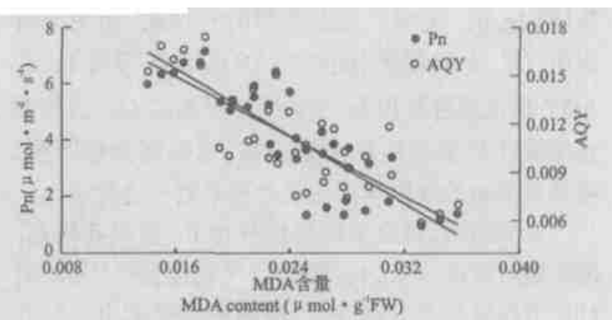

图 4　渗透胁迫时杨树叶片 MDA 含量与 Pn 和 AQY 的关系

Fig. 4 Relationships between MDA contnet and Pn or AQY in leaves of poplar seedlings under osmotic stress of root.

Pn= 11.2944− 299.0492 × MDA content, r = 0.8550*; AQY = 0.2216− 0.4586× MDA content, r = 0.7903*.

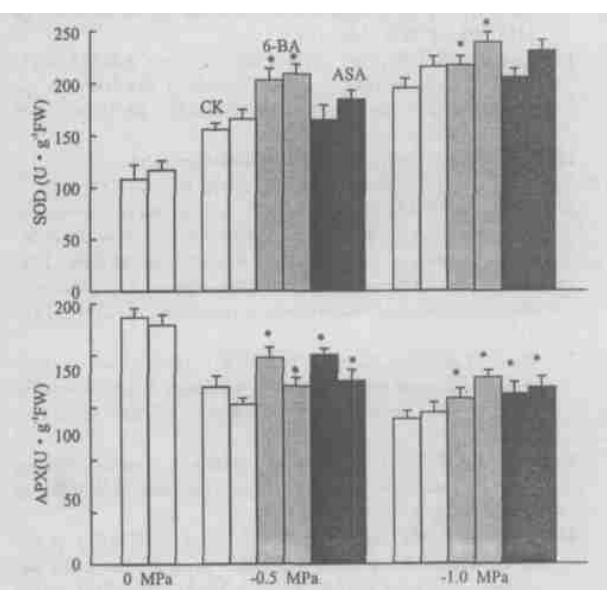

图 5　6-BA 和 AsA 对渗透胁迫时杨树叶片超氧化物歧化酶(SOD)和抗坏血酸过氧化物酶(APX)活性的影响

Fig. 5 Effects of 6-BA and AsA on the activities of superoxide dismutase (SOD) and ascorbate peroxidase(APX) in leaves of poplar seedlings under osmotic stress of root.

高于对照. AsA 处理对 SOD 活性无显著影响, 但可使不同胁迫强度下叶片 APX 活性显著升高(图 5).

4　讨　　论

渗透胁迫使杨树无性系幼苗叶片 AQY 降低(图 1b), 表明发生了光合作用的光抑制[9,19]. 胁迫时 Pn 下降, 使碳同化利用的光能减少、过剩光能增加. 这些过剩光能若不能及时有效地耗散掉, 就会通过多种途径在植物体内产生有毒的活性氧[8,12,15]. 渗透胁迫时杨树叶片 $O_2^{-\cdot}$ 生成速率加快, SOD 活性升高, 歧化反应生成的 H_2O_2 量增多(图 1c, d 和图 5). 胁迫时 APX 活性降低(图 5), H_2O_2 的清除减少, 因此, 使其在植物体内积累(图 1d), 或通过 Fenton 反应或 Haber-Weiss 反应产生毒性更大的·OH, 导致膜脂过氧化, MDA 含量升高(图 3), 膜透性增大, 启动氧化伤害. 活性氧代谢失衡进一步影响光合功能[10,14,17]. 水分胁迫时玉米、小麦叶片保护酶活性降低, 导致膜脂过氧化[6,7,24], 盐渍胁迫时亦有类似的报道[18]. 胁迫对细胞保护酶活性的影响没有固定的模式, 酶活升与降和植物材料、胁迫种类、胁迫强度及速度等有关. Zhang 等[25]、Davis 等[4]的研究结果证明了这一点.

6-BA 预处理使胁迫时杨树叶片 SOD 和 APX 活性升高(图 4), H_2O_2 和 MDA 含量降低, Pn 和 AQY 升高(图 1a), 缓解了光合作用的光抑制. 有关 CTK 刺激细胞保护酶活性的报道较多[2,22], 胁迫时 CTK 对 Pn 的影响亦有报道[6,7,17], 但 CTK 对光抑制影响的报道较少. 本研究结果表明, CTK 可以通

过激活细胞保护酶活性, 降低活性氧水平, 防止或减缓膜脂过氧化, 缓解光合作用的光抑制. AsA 是植物体内有效的抗氧化剂[5,16], 水分胁迫时其含量降低[13,14,20,24]. AsA 预处理可延缓水分胁迫时叶绿素的降解, 提高叶绿素含量[14,17], 维持较高的光系统, 尤其是 PSII 的活性与 Pn[17], 缓解光合作用的光抑制[9]. 本研究结果表明, AsA 预处理使渗透胁迫时杨树叶片 $O_2^{\cdot}$ 生成速率降低(图 1c), APX 活性升高(图 5), 导致 H_2O_2 含量下降(图 1d), 膜脂过氧化受到抑制, MDA 含量降低(图 3). 同时, Pn 和 AQY 升高(图 1a, b), 缓解了光合作用的光抑制. 相关分析表明, $O_2^{\cdot}$ 生成速率、H_2O 和 MDA 含量均与 Pn 和 AQY 呈极显著负相关. 保护酶抑制剂或 $O_2^{\cdot}$ 诱导剂处理使叶片中活性氧水平升高, 光抑制加剧[9,10]. 可见光抑制的发生与活性氧的积累有一定关系.

研究表明, 胁迫早期杨树叶片 Pn 受气孔限制, 随胁迫时间的延长, Pn 转受非气孔限制[9,11], 同时叶片活性氧水平升高[9,10]. 推测胁迫早期由于气孔关闭光合作用利用的光能减少, Mehler 反应加快, 活性氧生成增多, 随胁迫时间的延长, 较高水平的活性氧进一步引起 Pn 降低, 导致光合作用光抑制. 保护酶激活剂 CTK 和抗氧化剂 AsA 处理使杨树叶片活性氧水平降低, 缓解了光合作用光抑制, 表明活性氧积累是渗透胁迫时杨树光抑制的主要原因.

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