

外源抗坏血酸对臭氧胁迫下水稻 叶片膜保护系统的影响

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摘要:在田间原位条件下,运用 OTCs (open top chamber) 装置研究了外源抗坏血酸(exogenous ascorbate acid, ExAsA)对臭氧(O₃)胁迫下水稻(*Oryza Sativa* L.)叶片膜保护系统的影响。研究发现, O₃ 胁迫下的水稻叶片经过 ExAsA 处理后叶绿素 a 含量显著升高,而叶绿素 b 含量变化不明显;相对于对照,经 ExAsA 处理后的水稻叶片过氧化氢(H₂O₂)和丙二醛(MDA)含量及相对电导率(REC)均降低,超氧化物歧化酶(SOD)和抗坏血酸过氧化物酶(APX)活性明显提高,抗氧化剂类胡萝卜素(Carotene)含量升高。这表明, ExAsA 改善了 O₃ 胁迫下水稻叶片的抗氧化系统功能,减少了叶片中活性氧(activity oxygen species, AOS)的积累,抑制了脂质过氧化(lipid peroxidation, LP),延迟了 O₃ 对水稻叶片的老化作用,提高了水稻叶片对 O₃ 危害的抗性。

关键词:水稻叶片; 外源抗坏血酸; 脂质过氧化; 膜保护系统

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Effects of exogenous ascorbate acid on membrane protective system of in situ rice leaves under O₃ stress

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Abstract: The ozone is the primary gaseous pollutant with significant adverse effects on vegetation. Those effects include visible leaf injury, growth and yield reductions, accelerated senescence and altering sensitivity to biotic and abiotic stresses. Reductions in photosynthesis rate, stomata conductance, and root/shoot ratio, increase in respiration, and change in crop quality has also been observed in many crops. With fast industrial development, tropospheric ozone in the Yangtze River Delta, in eastern China, has risen since the later of the last century. The elevated ozone has become a prominent environmental and economic issue in this region.

In order to alleviate the phytotoxin of elevated level O₃ to crops, the effect of exogenous ascorbate acid (ExAsA) on membrane protective system of rice (*Oryza sativa* L.) leaves was investigated. Rice was grown in open top chambers (OTCs) under field conditions and exposed to five levels of O₃ treatments: charcoal-filtered air (CF), non-charcoal-filtered air (NF), 50 nl/L, 100 nl/L, and 200nl/L O₃. In addition, half of the plot within the OTC was treated by spraying 0.1% ExAsA solution once per week. The results showed that with spraying ExAsA, chlorophyll a content of rice leaves distinctly increased; H₂O₂ content,

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membrane permeability (indicated by the relative electrical conductivity, REC), and lipid peroxidation (indicated by the content of Malondialdehyde, MDA) of rice leaves declined; activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and carotenoid (Car) content increased. From these results, it can be speculated that ExAsA defers leaf senescence, reduces accumulation of active oxygen species (AOS) and lipid peroxidation (LP) of leaves, enhances the function of the membrane protective system, and increases the tolerance ability of rice leaves under elevated O_3 exposure.

Key words: ozone; rice leaf; exogenous ascorbate acid (ExAsA); lipid peroxidation (LP); membrane protective system

臭氧(O_3)是对陆地植被有很强毒负作用的气体污染物^[1]。它能造成农作物光合速率和气孔导度降低、呼吸增加、品质变劣^[2-5],以及老化加速、产量降低和对生物或非生物胁迫敏感性增加等变化^[6]。自 20 世纪 70 年代以来,北半球对流层 O_3 浓度每年以 1% ~ 2% 的速度增加^[7]。研究预测,如果现代人类破坏生态环境的行为得不到有效控制,这一增长速度将会继续升高^[8]。长江三角洲是我国经济发展速度较快的地区之一。近年来随着经济的发展,该地区对流层 O_3 浓度迅速升高,平均浓度为 74.6 nl/L,最高达到了 196 nl/L,高峰值主要出现在 5 月份和 9 月份^[9],造成了该地区小麦产量损失约 66.93 万 t,水稻为 59.86 万 t,直接经济损失分别达 5.39 亿元和 9.36 亿元人民币^[10],已成为当地较为突出的生态环境问题。

针对 O_3 浓度增加和 O_3 植物伤害,自 20 世纪 70 年代开始,欧美发达国家的学者进行了大量 O_3 植物伤害的防治与治理研究,并发现了一些对 O_3 植物毒性具有抑制作用的物质^[11-13]。这些物质不仅能改善植物体生理机能^[14],增强植物体抗逆能力,还对植物生长有促进作用^[15]。抗坏血酸(ascorbate acid, AsA)就是具有这种功能的物质。内源抗坏血酸(endogenous ascorbate acid, EnAsA)直接参与植物体内活性氧(activity oxygen species, AOS)的清除代谢,是植物体内重要的抗氧化剂^[16-18],而外源抗坏血酸(exogenous ascorbate acid, ExAsA)则能使受胁迫的植物组织丙二醛(Malondialdehyde, MDA)含量下降,脂质过氧化(lipid peroxidation, LP)得到抑制^[19]。本文以田间原位条件下水稻为材料,研究 ExAsA 对 O_3 胁迫下水稻叶片膜保护系统的影响,旨在探寻防治 O_3 对农作物伤害的可能方法,为预防和治理大气污染提供科学依据。

1 材料与方法

1.1 实验设计

实验设于浙江省嘉兴市双桥农场。OTC-1 (open top chamber) 型开顶式气室用钢筋和聚乙烯塑料膜构建,主体为正八面柱体,主要构成部分包括过滤系统、通风系统、布气系统及框架等^[20]。 O_3 由浙江省余姚市圣莱特电器有限公司生产的 O_3 发生器产生,用美国 MONITOR 公司生产的 ML9810BO₃ 分析仪对 OTC 内 O_3 浓度进行即时监测。实验有 2 m × 2 m 的小区 15 个,纵横间隔 3 m。每小区又被均匀分成两个小区(如图 1),一侧喷施 ExAsA 作为处理,另一侧不喷 ExAsA 作为对照。于 2004 年 6 月 28 日对各小区土地进行翻耕,7 月 2 日在各小区按行距 × 穴距为 0.25 m × 0.20 m 移栽水稻(*Oryza sativa* L.)秧苗。待秧苗返青后,把构建好的 OTC-1 型开顶式气室安放于小区上,并适应性熏气 3 d,正式熏气于 2004 年 7 月 21 日始,止于当年 10 月 10 日。每天熏气的时间为 9:00 ~ 17:00,下雨和星期天停止熏气,共熏气 65 d。0.1% (M/V) ExAsA 每星期喷施 1 次,喷施量为 250 ml/m²。熏气设 5 个处理:空气(no-filter NF, 20 ~ 50 nl/L)、过滤(charcoal-filter CF, 5 ~ 15 nl/L)、50 nl/L (45 ~ 55 nl/L)、100 nl/L (95 ~ 105 nl/L) 和 200 nl/L (190 ~ 210 nl/L),每个处理 3 个重复。在整个水稻生长期所有田间管理方式与当地保持一致,使水肥和病虫害等不成为限制因子。

1.2 生理生化指标的测定

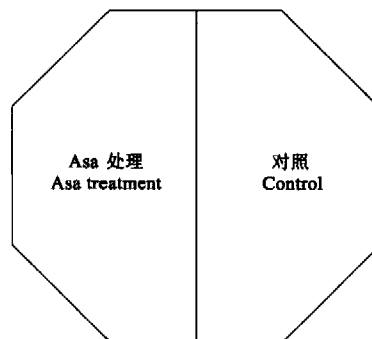


图 1 OTC 内分区示意图

Fig. 1 Sketch of division in OTC

在水稻的灌浆盛期,对各处理分别剪取剑叶 30 片,剔除中脉并剪切成碎片混匀后测定以下指标(各 5 次重复):

(1)叶绿素和类胡萝卜素(Car)含量的测定,采用 Arnon 法^[21]。

(2)质膜相对透性的测定,按李锦树^[22]方法。

(3)丙二醛(MDA)含量,采用硫代巴比妥酸(TBA)法^[23]。

(4)SOD 活性测定,称取剑叶碎 0.5g,按 1:5(W/V)加入 50mmol/L 含 1% 聚乙烯吡咯烷酮(PVP)和 10mmol/L 巯基乙醇的磷酸缓冲液(pH7.8)冰浴研磨,于 $15000 \times g$ (4℃)离心 20min,上清液为粗酶液。活性测定按 Del longo^[24] 等的方法,稍有改进。在 3ml 反应体系其中包括 0.1mmol/L EDTA (pH8.0), 13mmol/L 的蛋氨酸, 75×10^{-6} mol/L NBT, 2×10^{-6} mol/L 核黄素。酶活性采用抑制氮蓝四唑(NBT)光化学还原 50% 的酶量为一个酶活性单位。

(5)APX 活性测定 酶液同上。采用 Nakane 和 Aasda 方法^[25]测定,略有改进。3ml 反应体系中含有 0.1mmol/L K_2HPO_4 - KH_2PO_4 缓冲液(pH7.5), 0.5 mmol/L AsA, 0.4 mM H_2O_2 和少量酶液。在 290nm 下测定 4min 内吸光值的变化。

(6)POD 活性测定 酶液同上,采用 Kar 和 Choudhuri 方法^[26]测定,略有改进。 ΔA_{470} 上升 0.01/min 为一个酶活力单位。

1.3 数据分析

对所有测定数据进行方差分析(ANOVA)。

2 结果与分析

2.1 对叶绿素的影响

叶绿素(Chl a 和 Chl b)是植物进行光合作用的主要色素。图 2a、b 表明水稻叶片中 Chl a 和 Chl b 含量均随 O_3 浓度升高显著降低($p < 0.05$),且呈显著的负相关($r_{a, O_3} = -0.9274$, $r_{a, ExAsA + O_3} = -0.9771$, $r_{b, O_3} = -0.9274$, $r_{b, ExAsA + O_3} = -0.9771$);而经 ExAsA 处理的水稻叶片中 Chl a 含量显著增高,50、100nl/L 和 200 nl/L 处理分别比对照高 16.8%、44.7%和 11.6%。Chl b 含量则没有明显变化。

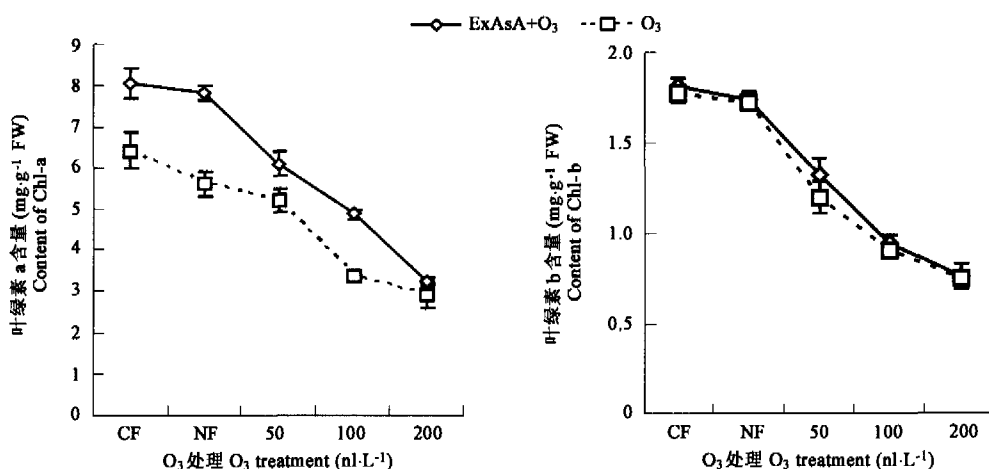


图 2 ExAsA 对 O_3 胁迫下水稻叶片叶绿素含量的影响

Fig.2 Effect of ExAsA on chlorophyll content of rice leaves under O_3 stress

2.2 对水稻叶片脂质过氧化的影响

2.2.1 对 H_2O_2 含量的影响 H_2O_2 是生物体内重要的一种活性氧(active oxygen species AOS),其浓度大小是生物受胁迫程度的一种反映。图 3 表明,无论是否经过 ExAsA 处理, H_2O_2 含量均随 O_3 浓度的增加而显著升高($p < 0.05$),且呈显著正相关($r_{ExAsA + O_3} = 0.97808$, $r_{O_3} = 0.97401$),但 ExAsA 处理后的水稻叶片 H_2O_2 含量都比

相应对照低,50、100nl/L 和 200nl/L 处理分别比对照低 30.9%、35.0%、9.8%。

2.2.2 对 MDA 含量的影响 MDA 是植物器官衰老或受逆境胁迫时膜脂发生过氧化作用形成的产物之一,其含量大小反映质脂过氧化程度。图 4a 表明,处理和对照水稻叶片 MDA 含量均随 O_3 浓度的升高而显著增加 ($p < 0.01$);但经 ExAsA 处理后,MDA 含量低于对照,50、100nl/L 和 200 nl/L 处理分别降低 7.9%、3.4%、10.1%。

2.2.3 对质膜相对透性的影响 相对电导率(relative electron conduction, REC)是表示质膜透性的重要指标之一。图 4b 表明,水稻叶片 REC 随 O_3 浓度增加显著性增加 ($p < 0.05$),且呈显著正相关($r_{O_3} = 0.8548$, $r_{ExAsA + O_3} = 0.8607$);而经 ExAsA 处理后,水稻叶片 REC 低于对照,50、100nl/L 和 200nl/L 处理分别降低 7.1%、5.1%、7.3%。

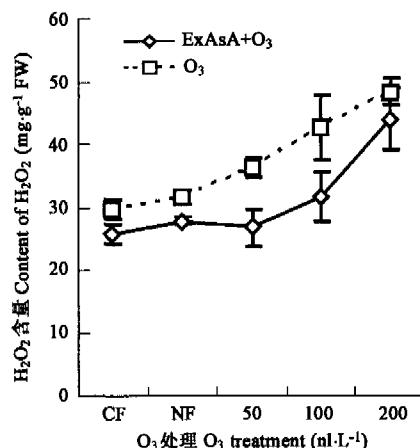


图 3 ExAsA 对 O_3 胁迫下水稻叶片 H_2O_2 含量的影响

Fig.3 Effect of ExAsA on H_2O_2 content of rice leaves under O_3 stress

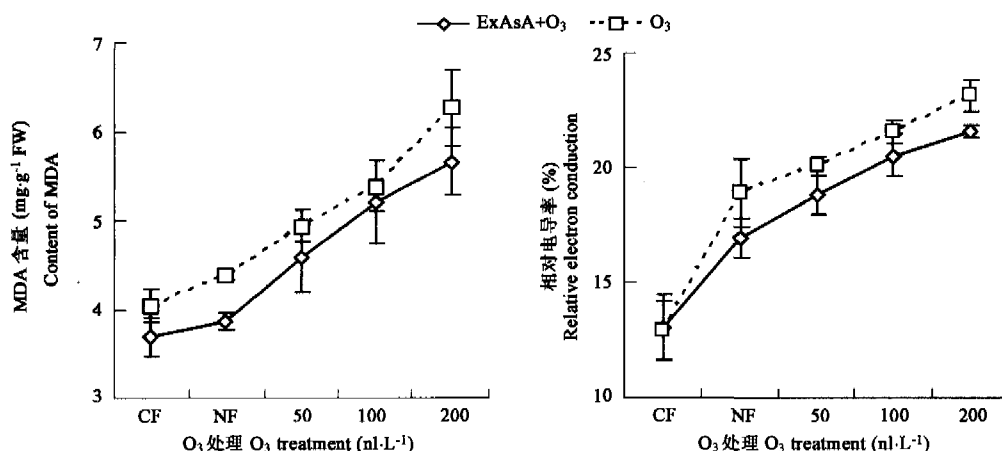


图 4 ExAsA 对 O_3 胁迫下水稻叶片 MDA 含量及 REC 的影响

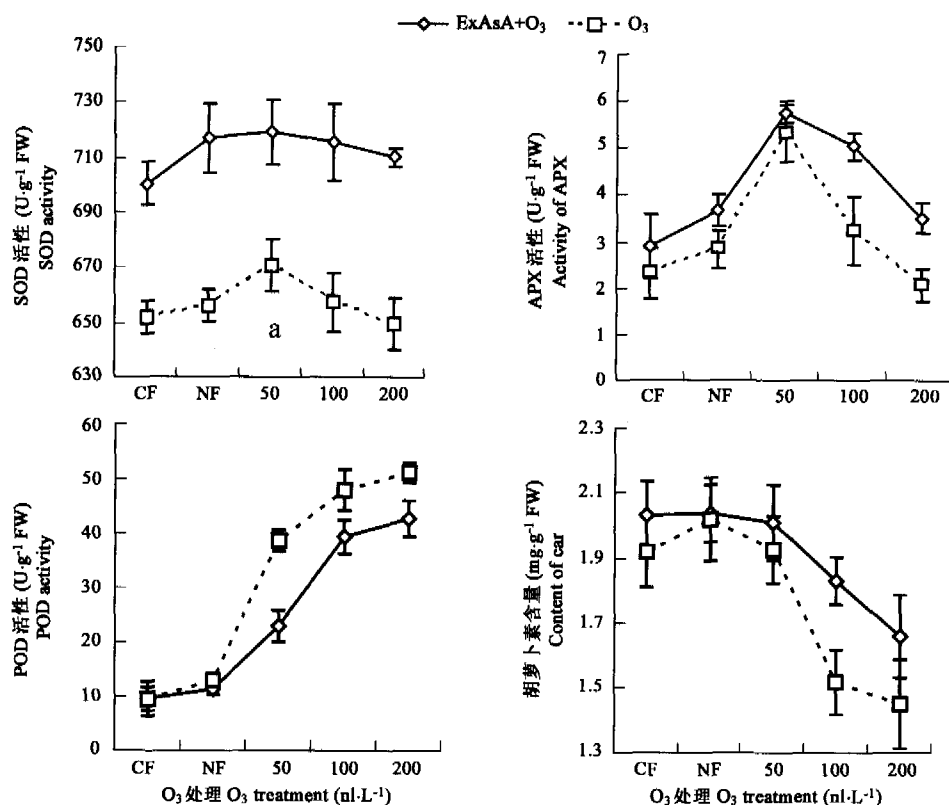
Fig.4 Effect of ExAsA on MDA content and REC of rice leaves under O_3 stress

2.3 对水稻叶片抗氧化体系的影响

图 5a、b 表明,喷施 ExAsA 后水稻叶片的 SOD 和 APX 活性均高于相应对照,且和对照有相似的酶活性变化趋势。50、100nl/L 和 200 nl/L O_3 处理下,喷施 ExAsA 后 APX 活性分别提高 8.3%、55.5%和 69.2%,SOD 分别提高 6.9、8.8 和 9.3%。图 5c 表明, O_3 胁迫下水稻叶片中 POD 活性随 O_3 浓度升高而升高,但喷施 ExAsA 后水稻叶片中 POD 活性显著低于相应对照,50、100nl/L 和 200nl/L 处理下分别降低 40.5%、17.8%和 16.5%。Car 除吸收光能参与植物的光合作用外,还是植物体内重要的抗氧化剂^[27]。图 5d 表明, O_3 胁迫下水稻叶片 Car 含量随 O_3 浓度的升高而降低,且呈显著的负相关($r_{O_3} = -0.8908$, $r_{ExAsA + O_3} = -0.9841$),但喷施 ExAsA 后水稻叶片 Car 含量显著高于相应对照,50nl/L、100nl/L 和 200 nl/L 处理下分别提高 4.3%、20.5%和 14.4%。

3 讨论

EnAsA 是植物体内一类很强的抗氧化剂^[16-18, 28],对 O_3 和 AOS 有较高的解毒能力^[29-31],自身则被氧化成单脱氢抗坏血酸(MDHA)和脱氢抗坏血酸(DHA)。研究发现在植物细胞非原生质内,EnAsA 的浓度可达毫摩尔(millimolar mM)数量级^[32, 33],因此 EnAsA 被认为是植物抵御 O_3 和其它氧化胁迫的第一道防线^[34]。chl_a、chl_b

图 5 ExAsA 对 O₃ 胁迫下水稻叶片抗氧化体系的影响Fig. 5 Effect of ExAsA on antioxidation system of rice leaves under O₃ stress

是植物色素中最基本的光合色素,其含量的下降是叶片衰老最明显特征^[35]。实验发现,经 ExAsA 处理的水稻叶片 Chla 含量显著高于相应对照。说明 ExAsA 被水稻叶片吸收后可能起到 EnAsA 的作用,直接参与了对 O₃ 和 AOS 的解毒,抑制了 O₃ 和 AOS 对 chl a 的破坏,从而使 chl 得到保护,水稻叶片 chl 总量增加,缓解 O₃ 对水稻的老化作用。实际上 chl 的降解除与叶片衰老有关外,还与 LP^[36] 和 POD 分解 H₂O₂^[37] 密切相关。本实验中水稻叶片 Chla 含量与 MDA 含量和 POD 活性之间呈显著的负相关($r_{\text{chl}a-\text{MDA}, \text{ExAsA}+\text{O}_3} = -0.9944$, $r_{\text{chl}a-\text{MDA}, \text{O}_3} = -0.9564$; $r_{\text{chl}a-\text{POD}, \text{ExAsA}+\text{O}_3} = -0.9757$, $r_{\text{chl}a-\text{POD}, \text{O}_3} = -0.9186$), 也证明了这一结论。

O₃ 经气孔进入植物体后使 AOS(如 O₂⁻ 和 H₂O₂)大量产生并富集。在长期进化过程中植物体本身也形成了酶类(如 SOD, APX)和非酶类(如 Car)两大 AOS 清除系统, SOD 催化 O₂⁻ 生成 H₂O₂ 和 O₂^[38], APX 使 H₂O₂ 转化成 H₂O^[39, 40], 而 Car 则通过清除单线态氧和猝灭三线态叶绿素来保护光合膜^[41]。实验表明, ExAsA 处理后水稻叶片中 SOD 和 APX 活性以及 Car 含量都高于相应 O₃ 处理下的水稻叶片,而叶片中 AOS(如 H₂O₂)浓度、质膜过氧化产物 MDA 和质膜透性 REC 低于相应 O₃ 处理。这说明 ExAsA 改善了水稻叶片抗氧化系统的功能,使 LP 得到抑制^①,从而使水稻叶片内 H₂O₂ 和 MDA 含量及 REC 降低,增强了水稻对 O₃ 的耐受能力。实验还发现 CF 和 NF 处理下的水稻叶片经 ExAsA 处理后,和相应的对照相比, Chla 含量升高(图 2 a)、H₂O₂(图 3)和 MDA 含量降低(图 4 a)、REC 值降低(图 4 b)以及抗氧化系统能力增强(图 4)也证明了上述结论。实验还发现 POD 活性随 O₃ 浓度升高而增强(图 5 c),这可能与 POD 具有生长素(IAA)氧化酶特性^[42] 有关,所以经 ExAsA 处理后 POD 的 IAA 氧化酶功能受抑制, POD 活性降低。

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