海草抗氧化系统及其对逆境胁迫的响应特征

卢科宇[1,2], 裴炎沼[1,2], 何　晴[1,2], 周　斌[1,2]

(1. 中国海洋大学海洋生命学院, 山东 青岛 266003; 2. 青岛海洋科学与技术试点国家实验室, 山东 青岛 266237)

摘要: 海草具有独特的进化地位和重要的生态价值, 广泛分布于潮间带和潮下带浅海海域, 易遭受多种环境因子变化的威胁, 抗氧化系统在海草抵御逆境胁迫的过程中具有非常重要的作用。本文综述了海草抗氧化系统的组成、特征及其对主要逆境胁迫的响应特征研究进展, 阐述了海草主要酶促抗氧化机制, 并将北半球代表性海草物种——鳗草抗氧化系统相关酶基因归类分析。目前对于海草逆境胁迫的研究主要集中于单一胁迫下主要抗氧化酶(如 SOD、CAT、GST)及其转录组的变化, 对于多胁迫因子协同作用和非酶抗氧化物及其他抗氧化酶的响应特征研究较少; 另外对于逆境胁迫下不同海草种类间抗氧化系统和关键基因的响应差异也有待深入研究。

关键词: 海草; 逆境胁迫; 抗氧化酶; 抗氧化系统

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海草是地球上唯一能够完全在海水中生活的被子植物, 起源于陆生高等植物, 经演化而适应海洋环境[1-3], 具有重要的进化地位[4]。海草经历了海洋-陆地-海洋的演化进程, 具有区别于陆生高等植物和大型海洋藻类的独特生理特征。鳗草(*Zostera marina*)和牟氏鳗草(*Zostera muelleri*)全基因组测序结果揭示了海草基因组具有区别于陆生高等植物的诸多特性, 如细胞壁组分基因变化、气孔基因缺失、萜类物质合成与乙烯信号转导基因缺失、紫外线防护与远红外线感受基因缺失、抗氧化系统基因收缩等, 提示海草具有不同于陆生高等植物的特殊逆境响应机制[5-6]。

植物抗氧化系统兼具响应逆境胁迫和调控活性氧信号传导的双重功能, 对其进行研究有助于全面理解植物的逆境响应过程和机制。绿色植物进行有氧代谢时产生的副产物活性氧(reactive oxygen species, ROS), 包括超氧阴离子($O_2^{\cdot-}$)、过氧化氢(H_2O_2)、单态氧($^1O_2$)和羟基自由基(·OH), 能够对植物体产生氧化损伤[7-9], 而植物也进化出了精细的抗氧化机制清除活性氧[9-10]。正常情况下, 植物体内的抗氧化系统能够及时清除过量 ROS[9-11]; 在逆境胁迫下, 植物体内 ROS 生成速度加快, 抗氧化系统清除能力相对不足, ROS 的动态平衡被打破而导致累积。累积的 ROS 在造成氧化应激和产生氧化伤害的同时, 也参与多种信息传递过程, 激活植物抗逆响应机制[9-17]。对于不同的逆境胁迫, 植物生成 ROS 的位点与种类存在差异, 而抗氧化系统本身的响应方式、程度和组分也有所区别[7, 9, 15]。

近年来, 对陆生高等植物和藻类抗氧化系统及其逆境响应已经开展了较多研究[8, 18-19], 并取得了系统性的成果[20-24]。与陆生高等植物相比, 海草由陆地到海洋的进化历程使其在适应过程中产生了独特的生理特点和抗逆机制。同时, 海草能够提供众多生态系统服务, 具有重要的生态价值[25-29]。其独特性与重要性促使人们对海草这一重要植物类群的抗氧化系统及其逆境响应机制进行深入研究。

1　海草抗氧化系统的组成及其基因分类

海草抗氧化系统包括酶促和非酶促清除系统[30], 主要包括抗氧化酶类、非酶抗氧化物以及由二者共同组成的一些循环系统如: 抗坏血酸-谷胱甘肽循

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作者简介: 卢科宇(1996—), 男, 硕士, 主要从事海洋生态学研究, E-mail: 1056016762@qq.com; 周斌(1981—), 通信作者, 从事海草生理生态学研究, E-mail: zhoubin@ouc.edu.cn

环、谷胱甘肽过氧化物酶循环、过氧化氢酶循环以及硫氧还蛋白还原酶系统和甲硫氨酸亚砜还原酶系统等[10, 30](图 1)。主要抗氧化酶有超氧化物歧化酶(superoxide dismutase, SOD)、过氧化氢酶(catalase, CAT)、抗坏血酸过氧化物酶(ascorbate peroxidase, APX)和谷胱甘肽过氧化物酶(glutathione peroxidase, GPX)等。非酶抗氧化物包括 β-胡萝卜素、维生素 A、α-生育酚(维生素 E)、抗坏血酸(维生素 C)、谷胱甘肽、黄酮类化合物, 以及某些渗透调节物质, 如脯氨酸、甘露醇等[31-32]。目前对于海草抗氧化系统的研究多集中于主要酶促反应, 对于上游转录因子和基因的研究较少。

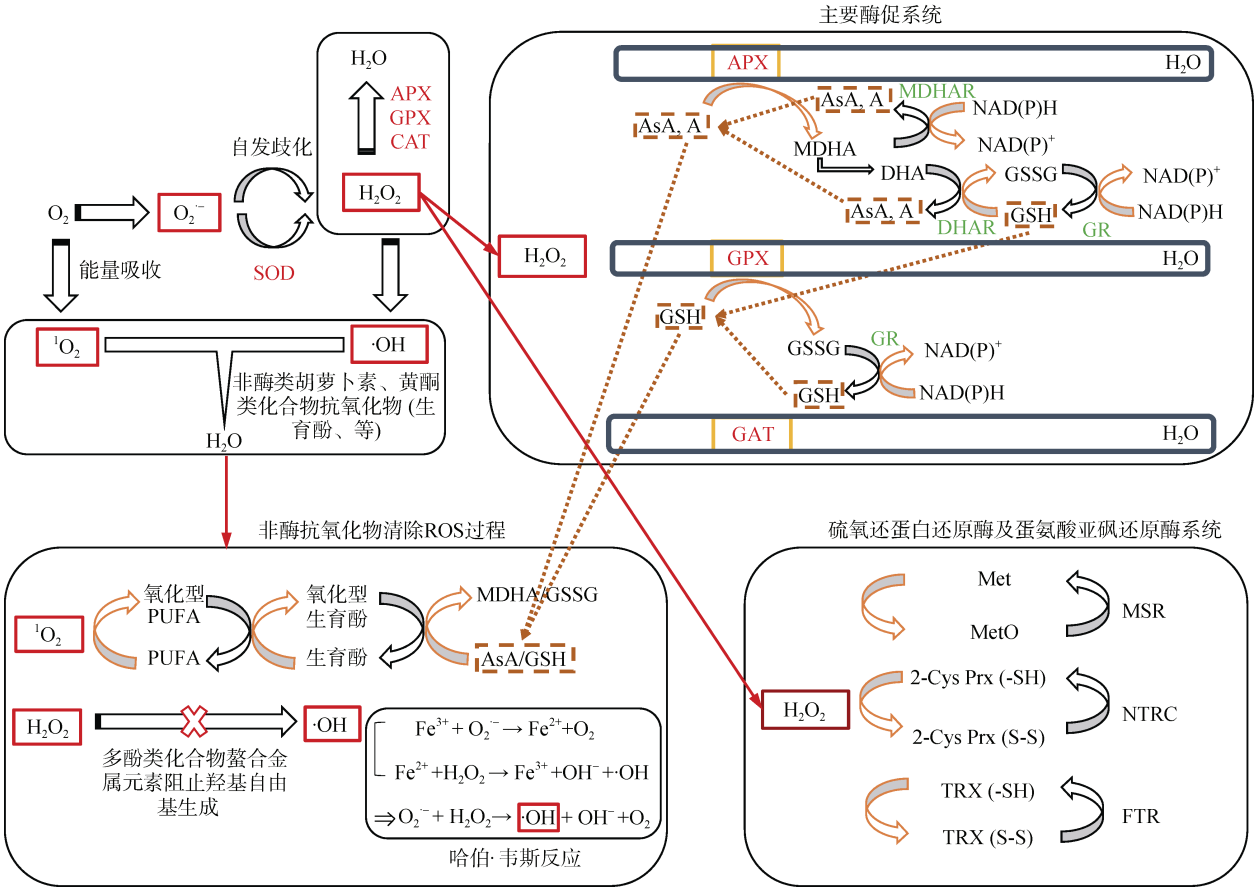

图 1　海草抗氧化系统组成图[7-10, 13, 24, 30, 33-37]

Fig. 1　Schematic of the main antioxidant system in seagrass[7-10, 13, 24, 30, 33-37]

注: $O_2^{\cdot-}$: 超氧阴离子; H_2O_2: 过氧化氢; $^1O_2$: 单态氧; ·OH: 羟基自由基; SOD: 超氧化物歧化酶; APX: 抗坏血酸过氧化物酶; GPX: 谷胱甘肽过氧化物酶; CAT: 过氧化氢酶; AsA, s: 抗坏血酸; GSH: 谷胱甘肽; (M)DHA: (单)脱氢抗坏血酸; GSSG: 氧化型谷胱甘肽; (M)DHAR: (单)脱氢抗坏血酸还原酶; GR: 谷胱甘肽还原酶; PUFA: 多不饱和脂肪酸; Met: 甲硫氨酸; MetO: 甲硫氨酸亚砜; MSR: 甲硫氨酸亚砜还原酶; 2-Cys Prx: 2-半胱氨酸过氧化物还蛋白; NTRC: NADPH 依赖的硫氧还蛋白还原酶 C; FTR: 铁氧还蛋白依赖的硫氧还蛋白还原酶。亮色箭头代表氧化过程, 相应暗色箭头代表还原过程, 红色方框表示 ROS, 红色箭头表示 ROS 的清除路径; AsA 和 GSH 是参与酶促抗氧化系统的两种重要非酶抗氧化物。

SOD、CAT、APX 和 GPX 等关键酶是海草体内应对逆境胁迫的重要防卫系统(图 1)。SOD 作为抗氧化系统中的第一道防线, 将难以直接清除的 $O_2^{\cdot-}$歧化为 H_2O_2, CAT、APX 和 GPX 则将 H_2O_2 转化为 H_2O(图 1)[7, 9, 33-37]。其中 APX 通过催化抗坏血酸(ascorbic acid, AsA)与 H_2O_2 的反应参与到抗坏血酸-谷胱甘肽循环中, 其氧化产物单脱氢抗坏血酸(monodehydroascorbic acid, MDHA)与脱氢抗坏血酸(dehydroascorbic acid, DHA)能够通过相应的单脱氢抗坏血酸还原酶(MDHAR)和脱氢抗坏血酸还原酶(DHAR)还原为 AsA, 从而维持这一重要非酶抗氧化物的浓度[7, 9, 13, 35]。而另一种重要的非酶抗氧化物谷胱甘肽(GSH)作为还原剂参加了 DHA 的还原反应。类似地, GPX 催化 GSH 清除 H_2O_2 产生的

氧化态谷胱甘肽(GSSG)也能通过谷胱甘肽还原酶(GR)再生 GSH。这两种主要非酶抗氧化物除了通过酶促反应清除 H_2O_2 外，还能够参与清除其他三种 ROS[7-10, 24, 38-39]。重要的抗氧化酶还包括谷胱甘肽 S-转移酶(glutathione S-transferase, GST)和过氧化物酶(peroxidase, POD)等[34, 37, 40-42]。

另外，硫氧还蛋白/硫氧还蛋白还原酶系统和甲硫氨酸亚砜/甲硫氨酸亚砜还原酶系统也是植物体内应对氧化胁迫的重要途径(图 1)[43]。在逆境胁迫下产生的过量 ROS 除了对肽主链造成一般的氧化损伤外，一些含硫氨基酸侧链的功能和结构也可能会在氧化应激过程中被特定的修饰所改变。如甲硫氨酸(Met)和半胱氨酸(Cys)其侧链中含有一个硫原子，是最容易被氧化的氨基酸[44]。Met 和 Cys 的氧化损伤可分别由甲硫氨酸亚砜还原酶(methionine sulfoxide reductase, MSR)系统和硫氧还蛋白还原酶(thioredoxin reductase, TrxR)系统修复，ROS 则在蛋白质修复的过程中被清除。其中 Met 被过量 ROS 和 H_2O_2 氧化形成的甲硫氨酸亚砜(MetO)可以通过 MSR 还原为 Met[45-47]。因此 MetO 的形成是氧化损伤的标志之一，该损伤可由 MSRs 控制或逆转[44]；而 TrxR 系统是由一系列蛋白质组成：硫氧还蛋白(Trx)、过氧化物氧还蛋白(Prx)和硫氧还蛋白还原酶(TrxR)。这些蛋白与还原性烟酰胺腺嘌呤二核苷酸磷酸(NADPH)相互作用，作为辅因子清除 H_2O_2 或其他 ROS[48-49]。

单态氧和羟基自由基的清除主要依靠非酶抗氧化物(图 1)。除 GSH 和 AsA 外，还包括类胡萝卜素、生育酚、黄酮类化合物和脯氨酸等。这些非酶抗氧化物都能够清除单态氧，多酚类化合物和脯氨酸还能够参与清除羟基自由基[7-10, 14, 50-51]。其中生育酚还能参与清除超氧化物，并通过 GSH 和 AsA 的作用再生，表明抗氧化物之间存在协作关系[7-8, 10, 13, 24, 52]。由于抗氧化系统的各个组分能够清除的活性氧种类存在差异，而且存在的位点也有区别(例如生育酚、类胡萝卜素和 CAT 的主要存在位点分别在细胞膜、叶绿体和过氧化物酶体)，所以除了浓度以外，各组分之间比例的变化也将影响活性氧的清除效果以及海草整体上对逆境胁迫的响应[9, 14, 53]。总体而言，抗氧化物基因的过表达能够提高抗逆性，且多种抗氧化酶基因同时过表达能够起到相互协同的作用[10]。

控制海草抗氧化系统关键酶和非酶抗氧化物基因的上游核转录因子调控过程也是氧化应激的重要控制环节，影响抗氧化系统相关基因的转录。在正常生理状态下这些转录因子在胞质内与各自的抑制蛋白结合为复合体而呈现非活性状态。当受到胁迫时，复合体会被氧化剂等物质激活而解离，转录因子被转移入核内诱导含有特异启动子(如抗氧化元件 antioxidant response element, ARE)的基因转录，调控氧化应激。这些信号通路主要有 Nrf2-Keap1 信号通路、NF-κB 信号通路等，但在海草等水生高等植物中研究较少[54-61]。

其中核因子 E2 相关因子 2(Nrf2)是抗氧化系统的关键调节因子。Nrf2-Keap1 信号通路主要由转录因子 NF-E2 相关因子 2(Nrf2)及其伴侣分子 Keap1 组成。Nrf2/Keap1 系统通过与抗氧化反应元件(ARE)相互作用，调控一系列解毒酶和抗氧化酶基因的表达来维持机体氧化还原的平衡状态[54]。例如 Nrf2 控制谷胱甘肽生物合成的限速步骤也控制谷胱甘肽过氧化物酶 2 和还原酶 1 的表达，从而影响抗坏血酸-谷胱甘肽循环[62]；Nrf2 也能影响控制胞质硫氧还蛋白 1(TXN1)[63]、硫氧还蛋白还原酶 1(TXNRD1)[64-67]和硫氧还蛋白 1(SRXN1)[68]的表达，并且调控体内产生 NADPH 的各个途径，从而对抗氧化系统进行调控[69-72]。在非应激条件下，Keap1 是 E3 泛素连接酶复合物的氧化还原调控底物接头，其结合 Nrf2 形成 Nrf2-Keap1 复合物，从而抑制 Nrf2 的活性并持续引导 NRF2 降解以确保 Nrf2 以较低浓度存在，并将 Nrf2 限制在细胞质中[55-57]。当细胞暴露于氧化胁迫时，Keap1 上的高活性半胱氨酸基团被过量 ROS 氧化使其构象产生变化，Keap1 被亲电子分子修饰后，阻止其靶向蛋白 Nrf2 的降解，复合体释放 Nrf2 并将其转运到细胞核中，从而使 Nrf2 在氧化胁迫下在细胞核中快速积累，诱导含有抗氧化元件 ARE 的基因转录[58-60]。拥有 ARE 的基因编码形成合作酶网络，Nrf2 可通过转录网络调控抗氧化系统以应对外界胁迫[61]。

目前在基因水平对海草抗氧化系统的研究相对较少。由于仅有鳗草和牟氏鳗草完成了全基因组测序，因此本文以北半球代表性海草鳗草为代表，根据鳗草全基因组测序结果等研究成果，对鳗草抗氧化酶基因进行了分类注释。根据抗氧化酶参与 ROS 清除的不同作用，将相关酶分为 3 类进行注释，分别为：直接清除 ROS 的抗氧化酶(表 1)、参与清除 ROS 的蛋白和酶(表 2)、催化抗氧化剂再生的酶(表 3)[6]。

表 1　鳗草直接清除 ROS 的抗氧化酶基因[6]

Tab. 1　*Zostera marina* directly scavenges ROS with antioxidant enzymes

相关酶及涉及的反应过程	基因名称	基因代码
Superoxide dismutase(SOD)	*ZMFeSOD*	Zosma106g00140
$O_2^-+ O_2^-+2H^+ \rightarrow H_2O_2+O_2$	*ZMFeSOD*	Zosma1306g00070
	ZMCu/ZnSOD	Zosma72g00490
	ZMCu/ZnSOD	Zosma5g01030
	ZMCu/ZnSOD	Zosma16g01180
	ZMMnSOD	Zosma270g00070
	ZMSOD	Zosma6583g00010
	ZMSOD	Zosma12595g00010
	ZMSOD	Zosma12509g00010
	ZMSOD	Zosma10604g00020
Ascorbate peroxidase(APX)	*ZMAPX*	Zosma21g01150
$2Asc+ H_2O_2 \rightarrow 2MDA+2H_2O$	*ZMAPX*	Zosma230g00360
	ZMAPX1	Zosma123g00790
	ZML-APX3	Zosma1g02970
	ZML-APX6	Zosma4g01780
	ZMStromal-APX	Zosma182g00510
Catalase(CAT)	*ZMCAT*	Zosma11924g00010
$2H_2O_2 \rightarrow 2H_2O+O_2$	*ZMCAT*	Zosma9250g00010
	ZMCAT	Zosma228g00030
Glutathione peroxidase(GPx)	*ZMGPx1*	Zosma180g00060
$H_2O_2+2GSH \rightarrow 2\ H_2O+GSSG$	*ZMGPx5*	Zosma44g01480
	ZMGPx	Zosma533g00130
	ZMGPx	Zosma269g00080
	ZMGPx	Zosma41g01010
Peroxiredoxin(PrxR)	*ZMPrxR*	Zosma12201g00010
$2P\text{-}SH+H_2O_2 \rightarrow P\text{-}S\text{-}S\text{-}P+2H_2O$	*ZMPrxR*	Zosma12323g00010
	ZMPrxR	Zosma12946g00010
	ZMPrxR	Zosma13231g00010
	ZMPrxR	Zosma161g00470
	ZMPrxR	Zosma1861g00010
	ZMPrxR	Zosma2227g00010
	ZMPrxR	Zosma6632g00010
	ZMPrxR-2F	Zosma16g01570
	ZMPrxR Q	Zosma176g00240
	ZM1-Cys PrxR	Zosma44g00080
	ZM1-Cys PrxR	Zosma44g00090
	ZM2-Cys PrxR	Zosma2g03310

表 2　鳗草参与清除 ROS 的蛋白和酶基因[6]

Tab. 2　*Zostera marina* participates in scavenges elimination of ROS via the genes of enzymes

相关酶及涉及的反应过程	基因名称	基因代码
Ferritin	*ZMFerritin*	Zosma105g00360
Fe+P→P-Fe	*ZMFerritin*	Zosma105g00430
	ZMFerritin	Zosma175g00320
Blue Copper Protein	*ZM Blue copper protein*	Zosma75g00400
Cu+P→P-Cu		
Alternative Oxidase(AOX)	*ZMAOX1*	Zosma120g00200
$2e^- + H^+ + O_2 \rightarrow H_2O$		
Thioredoxin(Trx)	*ZMTrx*	Zosma10352g00020
P-S-S-P→P-SH	*ZMTrx*	Zosma110g00370
	ZMTrx	Zosma125g00410
	ZMTrx	Zosma194g00220
	ZMTrx	Zosma42g00310
	ZMTrx family	Zosma161g00120
	ZMTrx h1	Zosma4g00450
	ZMTrx H-type	Zosma74g00380
	ZMTrx H-type 1	Zosma632g00030
	ZTrx M3	Zosma41g01280
	ZMTrx O1	Zosma189g00190
	ZMTrx Y	Zosma373g00110
	ZMTrx-like HCF164	Zosma49g00620
	ZMTrx-like 1-1	Zosma53g00120
	ZMTrx-like 3	Zosma330g00090
	ZMTrx-like 4B	Zosma125g00410
	ZMTrx-like AAED1	Zosma207g00240
	ZMm-typeTrx1	Zosma15g01700

表 3　鳗草催化抗氧化剂再生酶基因[6]

Tab. 3　Genes of antioxidant regenerating enzymes in *Zostera marina*

相关酶及涉及的反应过程	基因名称	基因代码
Monodehydroascorbate Reductase(MDHAR)	*ZMMDHAR*	Zosma1159g00010
$MDA+NAD(P)H+H^+ \rightarrow ASC+NAD(P)^-$	*ZMMDHAR*	Zosma153g00080
Dehydroascorbate Reductase(DHAR)	*ZMDHAR*	Zosma64g00760
DHA+2GSH→ASC+GSSG	*ZMDHAR*	Zosma243g00090
	ZMDHAR	Zosma3g01440
Glutathione Reductase (GR)	*ZMGR*	Zosma206g00070
GSSG+NAD(P)H→2GSH+NAD(P)	*ZMGR1*	Zosma82g00060
Glutaredoxin (GLR)	*ZMGLR*	Zosma175g00390
DHASc+2GSH→ASC+GSSG	*ZMGLR*	Zosma1861g00010
	ZMGLR	Zosma6177g00030
	ZMGLR	Zosma8720g00010
	ZMGLR family	Zosma8g00210
	ZMGLR family	Zosma27g00500
	ZMGLR family	Zosma315g00080
	ZMGLR family	Zosma400g00060
	ZMGLR family	Zosma400g00070
	ZMGLR family	Zosma400g00080
	ZMGLR family	Zosma64g00630

2 海草抗氧化系统对逆境胁迫的响应特征

2.1 对高温胁迫的响应

全球变暖导致的高温胁迫是海草面临的主要环境问题之一。高温胁迫能够影响海草的光合作用与呼吸进程, 影响其生长和代谢平衡, 并导致海草体内 ROS 过量生成和累积而引起氧化损伤[73-78]。具体表现为在高温胁迫下海草的光合酶系统受到损伤, 最终呼吸作用会大于光合作用, 导致消耗的 O_2 增加以及 ROS 的生成增加, 并且海水温度升高可以直接抑制海草体内抗氧化酶(如 GST)的活性, 从而削弱抗氧化系统清除 ROS 的能力。因此, 过量的 ROS 在植物体内累积, 能够影响正常的生理过程, 造成氧化损伤[79-80]。鳗草、大洋波喜荡草(*Posidonia oceanica*)、小丝粉草(*Cymodocea nodosa*)、泰来草(*Thalassia hemprichii*)等均通过增加抗氧化酶的含量或提高抗氧化酶的活性来应对高温胁迫, 但参与响应的抗氧化酶种类、响应程度和速度等受到海草种类、生境、胁迫强度与持续时间等诸多因素的影响。大多数情况下, SOD 作为抗氧化系统的第一道防线在清除 ROS 过程中具有重要作用, 比如高温胁迫能够诱导大洋波喜荡草、小丝粉草、泰来草 *SOD* 基因表达量显著上调以应对 ROS 的累积, 避免氧化损伤[34, 74, 80]; 鳗草的 *SOD* 基因会在非极端高温诱导时上调表达, 并在一段时间内保持其过表达状态, 同时保持较高的光合能力[81-84]。一定范围内的高温胁迫能够通过激活 SOD 活性而不是提高基因表达程度来提高活性氧清除能力, 而在极端高温下(≥25 ℃)SOD 酶活性急剧下降, 使抗氧化系统的高温胁迫响应能力急剧下降[77, 83]。相对于其他抗氧化酶组分活性受到抑制而逐渐降低、自由基清除能力变弱, SOD 的重要性还体现在能够在连续多次的高温胁迫过程中始终保持有效响应[34, 77]。其他种类的抗氧化酶也在海草响应高温胁迫中发挥重要作用, 比如高温能够诱导泰来草和诺氏鳗草 *CAT* 和 *GST* 基因表达量增高[80, 85], 鳗草中这两种酶的活性也有显著升高[78, 84]。海草抗氧化系统的其他组分对高温胁迫的响应相对于上述几种主要抗氧化酶具有较高的可变性[34], 这种可变性与物种和生境的相关性较高, 总体而言高纬度物种和深水生态型的适应程度低, 其抗氧化系统响应速度慢、强度低, 除主要抗氧化酶外需要更多的抗氧化系统组分参与响应, 且高温胁迫后的恢复较慢[34, 81, 86-87]。可见, 包括温度升高在内的气候变化能够影响海草抗氧化能力等生理过程, 进而改变海草的分布与种群结构[76]; 而基于气候驯化的生态适应对于海草应对高温胁迫具有重要意义。

2.2 对重金属胁迫的响应

重金属是影响海草的主要胁迫因素之一[88-92]。重金属的过量累积能够对海草产生严重的生理伤害, 包括抑制生长、降低光合速率等, 同时也能诱导海草体内 ROS 的过量生成而产生氧化损伤[93-100]。抗氧化系统对于重金属的敏感性可能是由于重金属离子能通过与抗氧化酶的巯基蛋白结合来破坏其结构[94, 98-99], 或是在高浓度下造成严重的细胞损伤, 使与抗氧化系统相关的代谢循环受损而导致整个抗氧化系统的崩溃[94]。海草抗氧化系统对重金属胁迫的响应特征与重金属的种类和浓度有关, 如抗氧化酶基因的表达程度与重金属浓度在一定限度内呈正相关[37, 40, 42]。海草对 Cu^{2+}胁迫非常敏感, 在短时间、低浓度的 Cu^{2+}胁迫下, 海草能够上调抗氧化酶(GPX、CAT、SOD、GR、APX 和 GST)和抗氧化剂(GSH)的基因表达来清除额外生成的 ROS; 而在长时间或高浓度的 Cu^{2+}胁迫下, 抗氧化系统组分的基因表达受到抑制, ROS 得不到及时清除而导致氧化损伤[37, 41, 93-94, 100-101]。但是对牟氏鳗草的研究表明, 在低浓度 Cu^{2+}胁迫下并不响应的 *POD* 与 *GST* 基因随着 Cu^{2+}浓度的升高而出现过表达[98]。

对于其他重金属(Fe、Mn、Zn、Cd、Cr、Pb、Hg、Ni)而言, 即使在较高浓度下, 海草抗氧化系统的相关组分也能通过基因上调表达来提高 ROS 清除能力[41, 46]。其中, 海草对 Cd^{2+}的耐受能力最强, 例如大洋波喜荡草只有受到高浓度 Cd^{2+}胁迫时才需要提高 GST 的活性[102]; 鳗草在 Cu^{2+}胁迫下会增加 GSH 的生成量来协助清除 ROS, 而即使暴露于高浓度的 Cd^{2+}胁迫下, *GSH* 基因的表达也没有显著升高[93]。Greco 等认为 Cu^{2+}与 Cd^{2+}在海草体内的累积是竞争性的, 因此低浓度 Cd^{2+}的存在甚至可能会减轻 Cu^{2+}对海草的氧化损伤[93]。此外, 乙酰胆碱酯酶(AchE)对重金属也具有高敏感性, 因而被认为通过基因上调表达参与了对重金属胁迫的响应, 并与 SOD 共同组成第一道防线[42]。

对于纳米金属颗粒胁迫的响应研究主要集中于纳米银微粒, 实验证明其在极低含量下也能导致海草体内 ROS 过量生成, 而不同种类的海草对其响应

程度存在差异。小丝粉草能够通过提高 SOD 和 APX 等主要抗氧化酶的活性, 有效防止氧化损伤[103]; 而长萼喜盐草(*Halophila stipulacea*)只能通过增加 SOD 的生成来应对氧化胁迫且 APX 的活性受到抑制, 因此并不能避免脂质过氧化[104-105]。与之相似的, 氧化锌微粒也能导致氧化应激, 引起海草抗氧化系统的响应变化, 但其作用机制尚不明确[106]。

在一定的重金属浓度范围内, 抗氧化系统的活性随着重金属浓度升高而提升, 但这并不一定代表其能够有效地清除额外生成的 ROS, 保护植物组织免受氧化损伤。例如, 大洋波喜荡草随着 Hg^{2+}胁迫强度的提高不断增加 GST 和 CAT 的生成量, 而代表脂质过氧化程度的丙二醛(MDA)含量也在增加, 其在高浓度 Hg^{2+}胁迫下通过植物螯合物才能有效降低氧化损伤的程度[44]。不同抗氧化酶对不同重金属的响应程度也不同, 例如日本鳗草 SOD 的含量及活性在 Cd^{2+}胁迫下不发生明显变化, CAT 则对 Pb^{2+}胁迫无显著响应[37]; 泰来草在受到 Zn^{2+}、Cu^{2+}、Cd^{2+}胁迫时, *SOD* 基因的表达受到显著抑制, 而 POD 则一直保持过表达状态[100]。迄今为止, 重金属对海草抗氧化系统的影响研究仍限于单一重金属对单一海草物种的胁迫和响应, 对多种重金属协同效应的研究较少, 且普遍缺少胁迫的分子机制研究。

2.3 对光胁迫的响应

光是海草生产力、分布和丰度的重要决定因素。海草的光合速率低于陆生植物, 具有较低的光补偿点与光饱和点, 可以保证在水下弱光环境中正常生长, 是海草对弱光环境的适应[107-109]。但在近岸海域中, 由于悬浮颗粒增加、附生植物或浮游藻类过度生长而导致的光散射和/或光衰减增加, 可能会进一步削弱海草的可利用光照, 导致海草光合产能不足, 从而影响海草生长繁育[110]。弱光胁迫对海草的影响可能涉及更多的光适应基因, 对抗氧化系统影响较小。长期弱光胁迫使大洋波喜荡草光合作用碳反应关键调节酶 Rubisco 的基因表达下调, 参与碳水化合物裂解的酶和蛋白水解酶表达上调, 而抗氧化系统 CAT、SOD 和 APX 三种抗氧化酶的合成减少, 但未呈现受胁迫状态[110-113]。

相比于光照不足, 海草对强光胁迫更加敏感, 而抗氧化系统作为光保护机制的重要部分, 对海草响应强光胁迫至关重要。生长在潮间带和浅水环境中的海草经常在一天中的部分时间暴露在过饱和光强下, 甚至直接暴露于阳光直晒之下, 这可能会导致强光胁迫。由于海草的光饱和点较低不能充分利用光能, 过剩的光能会导致 ROS 产生, 破坏海草体内的代谢平衡, 产生光抑制[114]。在强光胁迫下, 海草的光适应相关基因(Rubisco 酶、铁氧还蛋白、叶绿素结合蛋白)和光保护相关基因(抗氧化酶、叶黄素循环相关基因、生育酚的生物合成)上调, 表明了抗氧化系统作为防御机制被激活[115]。持续强光胁迫能够激活牟氏鳗草和大洋波喜荡草 AsA-GSH 循环, 表现为 APX、GPX、MDHAR 等抗氧化酶和相关基因上调表达, 并显著提高 AsA 和 GSH 的合成相关的酶与蛋白的表达量; 同时 CAT、POX、GST、生育酚和类黄酮化合物的合成量增加[110, 115]。大洋波喜荡草在强光胁迫下并没有产生光损伤或氧化损伤, 说明抗氧化系统能够起到有效的保护作用[110]。与之不同的是, 卵叶喜盐草(*Halophila ovalis*)与泰来草作为典型的热带海草, 在强光胁迫下一般不会显著增加 ROS 生成量或抗氧化系统的活跃程度, 体现了其对热带强光环境的适应性[116]。深水生态型与浅水生态型大洋波喜荡草之间存在的抗逆基因差异, 也说明其对于光胁迫具备足够的适应能力[110]。在不同纬度及深度环境下, 不同生态型海草的抗氧化系统均对光胁迫表现出适应特征, 表明了海草抗氧化系统能够应对光强变化产生的胁迫。

2.4 对其他环境因子胁迫的响应

除上述的主要逆境胁迫外, 海草抗氧化系统还会响应其他环境胁迫, 包括附生生物、高盐度、海洋酸化、缺氧及硫化物等。

1) 对附生生物胁迫的响应。有关海草抗氧化系统对附生生物响应的研究主要局限于大洋波喜荡草。附生生物一般附着在海草叶片表面上, 能够导致光照衰减, 降低光合作用, 但是不会导致氧化胁迫。附生生物一般通过直接创伤导致海草体内产生过量 ROS, 引起海草体内 APX、GPX、CAT、SOD、DHAR 和 GSH 等多种抗氧化组分活性或含量的增加, 抗氧化系统总体清除能力显著提升; 但现有研究认为这种清除能力的提升不足以应对 ROS 的快速产生和过量累积, 导致氧化胁迫产生, 表现为 MDA 含量显著增加[115, 117]。不同海草物种对附生生物的响应可能存在差异, 因此需要更多相关研究来完善认识。

2) 对盐胁迫的响应。海草在高或低盐度胁迫下均能激活其抗氧化系统的大部分组分, 包括非酶抗氧

化物和与 AsA-GSH 循环相关的抗氧化酶, 然而由于 SOD 和 CAT 这两种主要抗氧化酶的活性受到抑制, 海草还是会受到一定程度的氧化损伤[118-119]。在陆生植物中, 高盐胁迫诱导的氧化应激会导致植物卡尔文循环消耗的 NADPH 和光合作用固定的 CO_2 减少而影响 ASA-GSH 循环, 电子可能从 PSI 转移至 O_2 而形成 O^{2-}, 并引发链式反应[120-122]。对海草而言, 在高盐胁迫下其线粒体和叶绿体的相对面积随盐度的增加而增加, 总光合受到抑制, 净光合作用减少[123-124], 表明高盐度胁迫导致了海草光合器官损伤。在光、盐度与营养盐联合胁迫下, 光胁迫成为主导因素。强光胁迫能够促进 SOD 和 POD 等抗氧化酶活性提高以应对氧化胁迫[124]。我们推测海草与陆生植物的抗氧化系统具有相同的盐胁迫响应机制, 如黄秋葵(*Abelmoschus esculentus*)、水稻(*Oryza sativa*)、大豆植株在盐胁迫下能够诱导 CAT、POD 等主要抗氧化酶活性增强[121, 125-126]。此外, 有研究表明植物可以通过一种非典型的双特异性蛋白酪氨酸磷酸酶 ATPFA-DSP3(DSP3)调节蛋白质磷酸化介导植物抗氧化系统对盐胁迫的响应[127]。

3) 对缺氧及硫化物胁迫的响应。全球变暖及富营养化引起的缺氧现象正成为海洋生态系统的重要威胁[128-130]。缺氧胁迫会显著降低海草的光合作用, 并影响海草的碳/氮代谢, 而海草抗氧化系统在缺氧胁迫下也会被激活以应对 ROS 产生的危害, 如鳗草在缺氧胁迫下显著上调编码 CAT、SOD、POD、GST、MDHAR 等抗氧化系统相关酶的基因[128]。缺氧不仅对海草有直接影响, 还会引起硫化物对海草的入侵。海草一般生长在高度还原性的沉积物中, 沉积物中的有机质经过厌氧代谢产生的硫化物在低氧环境下会侵入海草内部, 进而破坏海草的分生组织并抑制海草的光合作用[131]。而目前海草对硫化物胁迫响应的分子机制研究较少, 关于海草抗氧化系统如何应对硫化物胁迫尚不清晰。

4) 对海洋酸化的响应。对海草抗氧化系统而言, 海水中 CO_2 含量(pCO_2)升高导致的海洋酸化并非逆境胁迫, 反而能够在一定程度上缓解海草的氧化压力。高 pCO_2 驯化(一般需要持续数月)能够使海草稳态下抗氧化酶和抗氧化物(SOD、CAT、APX、GR 和 GSH)活性降低, 表明抗氧化系统在相对不活跃的情况下也能有效地阻止氧化损伤, 表明了海草的高 pCO_2 偏好; 而未驯化的海草在低 pCO_2 下抗氧化酶和抗氧化物表达量短期内提高, 在高 pCO_2 下表达下降, 但下降趋势不明显[132]。相关研究一般利用海洋火山口这一自然实验室, 但是其导致的温度变化和排放的其他气体带来了大量的干扰, 例如 Lauritano 等用同种同源的海草在不同火山口进行培养实验却得出了截然不同的结果[133]; 而 Ravaglioli 等在证明火山口高不确定性的同时, 还表明营养盐的浓度会显著影响海草抗氧化系统对 pCO_2 变化的响应[134]。由于 pH 的改变还会影响海草附生生物群落[135], 因此在气候变化及海洋酸化的背景下, 揭示海草抗氧化系统的响应情况需要更多深入全面的研究。

3 展望

1) 海草抗氧化系统组分在逆境胁迫下的协同变化和多层次关联分析: 目前对海草抗氧化系统响应逆境胁迫的研究关注于主要抗氧化酶的变化, 往往忽视非酶抗氧化物和参与 AsA-GSH 循环的其他抗氧化酶。抗氧化系统是复杂的多酶多剂系统, 不同组分之间存在响应差异, 某一种或某几种组分的变化不能全面反映抗氧化系统的总体状态。应当结合基因组、转录组、蛋白质组等多个研究层次的成果, 将抗氧化酶活性变化与基因表达变化关联分析, 综合分析逆境胁迫下抗氧化系统的响应特征及机制, 这是全面开展海草抗逆生理机制研究的重要基础。

2) 不同海草物种抗氧化系统对逆境胁迫的响应差异: 海草抗氧化系统对逆境胁迫的响应存在显著的种间差异, 需要将海草种间差异响应特征与关键差异基因相关联, 进而基于差异基因的功能阐明抗氧化系统响应存在种间差异的原因, 这是揭示海草逆境胁迫耐受性种间差异机制的重要前提。

3) 多个胁迫因子联合作用下海草抗氧化系统的响应特征与机制: 海草抗氧化系统的逆境胁迫响应研究仍局限于少数胁迫因子, 与海草面对的复杂实际环境相距甚远, 需要开展多个胁迫因子联合作用下海草抗氧化系统的响应特征与机制研究, 这是全面分析复杂环境条件下海草逆境响应机制的基础。

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Research progress on the antioxidant system of seagrass and its response to stress

LU Ke-yu[1, 2], PEI Yan-zhao[1, 2], HE Qing[1, 2], ZHOU Bin[1, 2]

(1. College of Marine Life Science, Ocean University of China, Qingdao 266003, China; 2. Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao 266237, China)

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Abstract: Seagrass has a unique evolutionary status and important ecological value. It is widely distributed in the intertidal and infralittoral zones of littoral waters. Seagrass is often threatened by a variety of environmental stressors. The seagrass antioxidant system plays a very important role in the tolerance to abiotic stress. This study reviews the composition and characteristics of the antioxidant system of seagrass and its response to stress. Moreover, the major enzymatic antioxidation mechanisms of *Zostera marina*, a representative seaweed species in the Northern Hemisphere, were elaborated and genes of antioxidation enzymes were classified and analyzed. Current research on stress in seagrass has concentrated on changes in major antioxidases (e.g., superoxide dismutase, catalase, and glutathione S-transferase) and the relevant transcriptomes under a single stressor. However, few studies have considered the response to multiple stressors and non-enzymatic antioxidants as well as the responses of other antioxidant enzymes. Besides, there is a great research gap in antioxidant systems and key genes among different seagrass species under adversity stress.

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