山菠菜胆碱单氧化物酶基因（CMO）的克隆与分析

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摘 要
甜菜碱是一类广泛存在于生物体内的渗透保护剂。高等植物中，甜菜碱的生物合成经由胆碱→甜菜碱醛→甜菜碱两步反应完成，其中第一步反应，也是甜菜碱生物合成的限速反应，由胆碱单氧化物酶（CMO）催化。本研究以耐盐植物山菠菜（Atriplex hortensis）为材料构建了盐胁迫下的GM文库，用菠菜CMO为探针从中筛选获得一个长2.42kb的CMO克隆，测序结果表明该克隆包含一个完整的开放读码框，编码一个由422个氨基酸构成的多肽，与菠菜和甜菜CMO的氨基酸序列同源性分别为23%和26%。同菠菜和甜菜中的CMO序列相比，山菠菜CMO基因（30h）也具有保守的7-8KPSF0/P/4/7簇结合区和保守的多铁原子核结合域。对盐处理条件下山菠菜CMO基因转录水平的研究表明CMO基因在盐胁迫情况下表达量增加约4倍。将CMO与4B8启动子连接后转化烟草（Nicotiana tabacum var. Xanthi）,获得了具有一定耐盐性状的转基因植株，能在1.2%NaCl的盐浓度下生长良好。

关键词 山菠菜, 甜菜碱, 胆碱单氧化物酶, 盐胁迫

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材料和方法

1 植物材料和处理方法
山菠菜（Atriplex hortensis）种植于温室内，生长到约B$W时，每天定时用#R+;盐水处理，于处理前和处理后!、#、4、AW时收取新鲜叶片，于液氮中速冻后用于提取7)N和M)N。

2 文库的构建和筛选
提取的山菠菜总7)N经$%/N?I.+GIE7)N分离出E7)N,使用

3 cDNA □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ ^
Universal Riboclone cDNA Synthesis System
Promega cDNA A M N P pExcell A M Lambda Packaging
Amersham A M N P M A M RM
1.3kb CMO cDNA 2.5 × 10^5 0.8% DMSO
CMO 65°C 18h 65°C 2 × 50°C 0.5 × SSC 0.1% SDS 20 min

2.1 CMO cDNA
BamH I EcoR I

1.3kb CMO cDNA 2.5 × 10^5 1.4 1.8 kb 0.8% DMSO
CMO 65°C 18h 65°C 2 × 50°C 0.5 × SSC 0.1% SDS 20 min

2.2 CMO cDNA

2.3 CMO cDNA

2.4 CMO cDNA

2.5 CMO cDNA

2.6 CMO cDNA

2.7 CMO cDNA

2.8 CMO cDNA

2.9 CMO cDNA

2.10 CMO cDNA
**Fig. 1** The full nucleotide and deduced amino acid sequences of *AtCMO*

* Indicates the start residue of *AtCMO* mature polypeptide |
| Shows the conserved Rieske-TyR2 Fe-2S cluster-binding region and |
| Shows the conserved mononuclear Fe-binding motif. The accession number of *AtCMO* in GenBank is AF270651

**Fig. 2** The southern blot analysis of *AtCMO*

The genomic DNA was completely digested with *BamH I* and *Sma I* and probed with a 5.8 kb fragment of the *AtCMO* cDNA.

**Fig. 3** The Northern analysis of *AtCMO* mRNA in leaves when irrigated with brine containing 2% NaCl.

Each lane contains 30 ng total RNA sample and 18S rRNA is marked as control. The numbers under lane means the days after the plant was treated.
2.4 CMO

Fig. 4 The photo of transgenic tobacco and the control. 
MS Medium in different bottles contain 0.9% A, 1.2% B, 1.5% D, 10% NaCl.

Fig. 5 The photo of four transgenic tobacco plants and the control. 
These plants are all cultured on MS medium containing 10% PEG-6000.

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Fig. 6 The Northern blot of AhCMO in transgenic tobacco. 
The lanes from left to right are control plant CK, transgenic tobacco under normal condition, and stress condition treated with 1.2% NaCl for 1 day.

Discussion

Natural ecosystems are especially challenging to plants that rely on specific metabolic pathways for survival. How can we utilize these metabolic pathways to transform important crops, improving their survival rates in response to adversity and reducing losses? This has become one of the primary goals of plant resistance gene engineering over the past decade. 

The hyperosmolyte, 1-trimethyl-2-aminoethanol, demonstrates antifreeze properties, which can accumulate in plant cells, providing protection to the membrane and proteins. It can also increase seed germination under freezing conditions. In many plants, 1-trimethyl-2-aminoethanol has been shown to play a major role in resisting osmotic stress. Therefore, the enzyme that converges the production of hyperosmolyte can be used to establish an effective osmotic stress regulation pathway in salt-sensitive crops, improving the salt tolerance of crops. 

The AhCMO gene, which was already cloned from spinach, is further cloned in this study. Our results show that AhCMO expression increases significantly under salt stress, approximately 2.0 times after 7 days. It is expected that the amount of betaine accumulated would be higher. Compared to previous studies on spinach and甜菜, our results show a much higher expression level of AhCMO, consistent with the salt-tolerant characteristics of 

gene rice to increase betaine content and improve stress resistance. Our results show that the increased betaine content is primarily attributed to the enhanced activity of the CMO enzyme in transgenic plants.

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References

Cloning and Characterization of CMO Gene from *Atriplex hortensis*

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**Abstract**  Glycine betaine is a widespread osmoregulator existed in many organisms. In higher plants, glycine betaine is synthesized via a two-step oxidation reaction: choline → betaine aldehyde → glycine betaine. The first step is also the speed-limiting step catalyzed by choline monoxygenase (CMO). Choosing halophyte *Atriplex hortensis* as material, we constructed a salt stress-induced cDNA library and isolated a 1.77-kb-length cDNA clone with spinach CMO cDNA as probe. The sequencing result showed a complete Open Reading Frame encoding a 438-amino-acid polypeptide which was 81% and 72% identified to CMO sequences of spinach and sugar beet in amino acid homology respectively. Compared with the CMO from spinach and sugar beet, the AbCMO had one conserved Rieske-Type 2Fe-2S cluster-binding region and one conserved mononuclear Fe-binding motif. The expression pattern of AbCMO under salt stress was also studied. The transcriptional level of AbCMO raised about three folds after the plant was treated with brine for 4 days. The AbCMO was then transferred into tobacco *Nicotiana tabacum* var. Xanthiic with 35S promoter and seven transgenic plants were certified by northern blot. These plants displayed some salt- and drought-stress tolerance when grew well on MS medium contained 1.2% NaCl or 10% PEG while the control was stagnated under the same condition.

**Key words**  *Atriplex hortensis*, glycine betaine, choline monoxygenase, salt stress

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