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花粉,種子與植物體耐旱機制之研究(2/3)

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摘要

萬年松（*Selaginella tamariscina*）是一種可以忍受極度缺水的復活植物。本研究以萬年松為材料欲探討植物體在忍受乾旱逆境時的可能分子機制。利用 differential display 與 5’-race 等分子工具篩選萬年松在缺水逆境的過程中受到誘導表現的基因。結果收集了約 100 個受缺水誘導表現的 cDNA clone，從其中 18 個表現較強的 clone 中得到 12 個全長 cDNA。經序列比對後，發現在其他植物皆有相似的基因存在。目前正在進行 northern blot 分析，確認這些 cDNA 的差異表現確實是受到乾旱的誘導。未來將進一步以 chromosome walking 的策略得到這些受到乾旱誘導基因的 promoter 序列，分析其 *cis*-acting element，以釐清萬年松忍受乾旱的分子機制。

Abstract

Selaginella tamariscina, a resurrection plant, can survive severe water stress. To investigate the mechanism of desiccation tolerance of resurrection plant, differential display was employed to find the dehydration induced genes in *S. tamariscina* during dehydration. About 100 dehydration induced cDNA clones were collected. Among them, the full length of 12 strongly induced cDNA clones were obtained by using 5’-race technology. These12 full-length cDNA clones were identified because high similarity to the orthologs in other plants. Northern blot analysis was also applied to conform that these cDNA clones were actually dehydration activated. In the future, chromosome walking will be executed to obtain the promoter sequence of these dehydration inducible genes, and the cis-acting element will be analyzed to elucidate the molecular mechanism of desiccation tolerance of *S. tamariscina*.

Introduction

Dehydration is one of the most common environmental stresses and is lethal to most vascular plants. Only organisms such as yeast cells, bacterial and fungal spores, nematodes, angiospermous seeds, pollen and a few resurrection plants can survive desiccated stress. Desiccation tolerance required a coordinated series of events during dehydration that are associated with preventing oxidative damage, maintaining the native structure of macromolecules and membrane, down-regulation of metabolism, partitioning of amphiphilic compounds into membranes and immobilization of the cytoplasm in a stable multi-component glassy matrix (Hoekstra et al., 2001).

The molecular basis of desiccation tolerance has been focused on a few species representing different groups: the dicotyledonous species *Craterostigma plantagineum* (Bartels et al., 1990), the monocotyledonous species *Sporobolus stapfianus* (Neale et al., 2000), and the moss *Tortula ruralis* (Oliver and Bewley,

1997).

In this study, differential display was employed to select the dehydration activated genes that may be involved in mechanism of desiccation tolerance in the desiccation tolerant tracheophytes *S. tamariscina*. Almost 100 dehydration activated cDNA bands were collected from polyacryamide gel. Eighteen dehydration strongly induced cDNA were selected for TA cloning and further sequencing. The full length of cDNA was obtained using 5'-race technique and then blast on NCBI website to get possible orthologs. Finally, 12 independent dehydration activated full-length cDNA were obtained.

Materials and Methods

Plant materials

Plants of *Selaginella tamariscina*, were purchased from flower market or obtained from wild and grown in greenhouse. For desiccation analysis, plants were dried on bench top in laboratory at room temperature for different days representing differential dehydration condition.

RNA isolation

Total RNA was isolated using the REZOL® reagent (Invitrogen). The extracted RNA was stored at –70 ^{o}C for further experiments.

Dehydration activated cDNA clone isolation and DAN sequence analysis

Differential display was carried out using RNAimage® Kit9 as described by the manufacturer. Six arbitrary primers (H-AP65, H-AP66, H-AP67, H-AP68, H-AP69 and H-AP72) paired with three kinds of polyT primers (H-T_{11}G, H-T_{11}A and H-T_{11}C) composed of 18 sets of reactions were done. After RT-PCR, the cDNA was electrophoresed in 6% denaturing polyacrylamide gel. The dehydration activated cDNA was recovered and re-amplified using the same primer pairs as previously used in RT-PCR. The re-amplified cDNA was then cloned into pGEM®-T easy vector and sequenced.

The full-length cDNA clones were obtained by BD SMART^{TM} RACE cDNA Amplification Kit as described as the manufacturer.

The nucleotide sequence of each full-length cDNA was deduced into peptide sequence and blasted on NCBI website.

Result

Leaves of *S. tamariscina* curved into round shape when the water content was

lower than 10% of fresh weight. After re-watering and in saturated moisture content for 7 hours, *S. tamariscina* regained normal features as control plant. This result indicated that *S. tamariscina* was a member of resurrection plants.

Based on differential display strategy, about 100 dehydration activated bands on denature polyacrylamide gel were collected. Potion of the result was presented in figure 2. Only 18 of the strongly dehydration induced cDNAs were selected for further analysis. 5'-race was employed to obtain the full-length cDNA sequences of the selected ones (Table1). The resulting cDNA sequences were translated into amino acid sequences and blasted on the NCBI website to find possible orthologs in other organisms.

Future work will focus on obtaining the promoter sequence of these dehydration inducible genes, and the cis-acting element will be analyzed to elucidate the molecular mechanism of desiccation tolerance of *S. tamariscina*.

Reference

Bartels D, Schneider K, Terstappen G, Piatkowski D, Salamini F (1990) Molecular cloning of abscisic acid-modulated genes which are induced during desiccation of the resurrection plant *Craterostigma plantaginum*. Planta 181: 27-34

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. TRENDS in Plant Science 6: 431-438

Neale AD, Blomstedt CK, Bronson P, Le T-N, Gutteridge K, Evans J, Gaff DF, Hamill JD (2000) The isolation of genes from the resurrection grass *Sporogolus stapfianus*, which are induced during severe drought stress. Plant, Cell and Environment 23: 265-277

Oliver MJ, Bewley JD (1997) Desiccation-tolerance of plant tissue: A mechanistic overview. Horticultural Reviews 18: 171-213

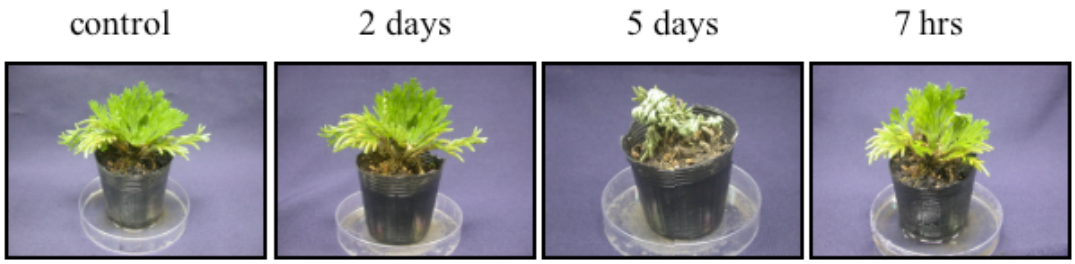

Fig.1 Resurrection of *S. tamariscina* during dehydrating and re-watering cycle. Leaves of watered control plant open well. After 2 days dehydration, the feature of plant was not change. When dehydrated for 5 days, the leaves of plant curved into global which the water content was less than 10% in fresh weight. When rehydrating under saturation condition, the plant recovered to the feature the same as control plant.

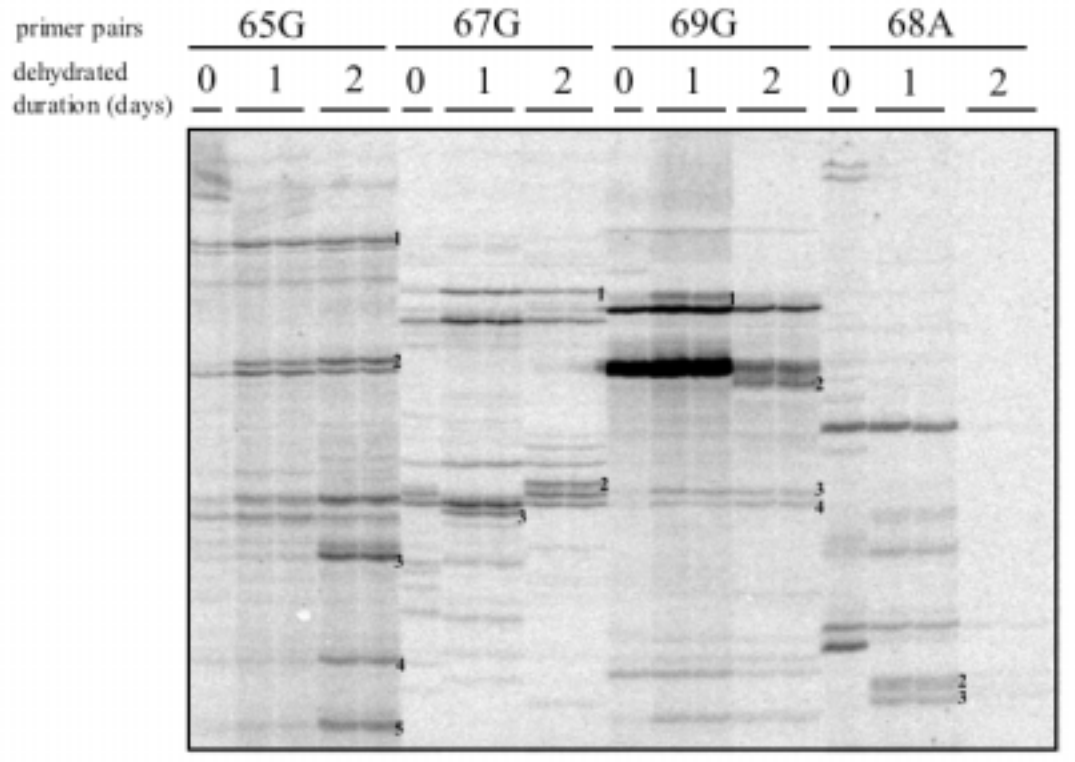

Fig. 2 cDNA expression pattern of *S. tamariscina* before and after dehydration. Primer pairs 65G represented H-AP65 paired with H-T_{11}G, and so on. *S. tamariscina* dehydrated for 0, 1, and 2 days are examined. The dehydrated activated cDNA bands (numbered) were interested and collected for further 5'-race examination.

Table 1 The full-length cDNA clones and their similar orthlogs in other plants.
The full-length cDNA sequence was deduced into amino sequence and blasted on NCBI website. The orthlogs with highest identity were list.

clone	ORF length	Similar protein	identity	species
5-68A1	672	Lil3 protein	51%	*Oryza sativa*
5-68A2	423	putative 16kDa membrane protein	57%	*Nicotiana tabacum*
5-72A8	1254	UDP-glucoronosyl/UDP-glucosyl transferase family protein	35%	*Arabidopsis thaliana*
65A11-1	1713	putative Ca2+-dependent lipid-binding protein	37%	*Oryza sativa*
65A4-1	744	hydrolase, alpha/beta fold family protein	36%	*Arabidopsis thaliana*
65A81-2	708	DNA polymerase-related	36%	*Arabidopsis thaliana*
67C21-4	426	germin-like protein (GLP6)	42%	*Arabidopsis thaliana*
72A61-3	477	disease resistance-like protein	55%	*Oryza sativa*
65C3-3-2	831	thylakoid lumen 18.3 kDa protein	63%	*Arabidopsis thaliana*
65C716-2	810	thylakoid lumen 18.3 kDa protein	62%	*Arabidopsis thaliana*
65C721-1	639	polyneuridine aldehyde esterase	36%	*Rauvolfia serpentina*
65C723-4	510	expressed protein	27%	*Arabidopsis thaliana*