

P35

## SSH(suppression subtractive hybridization) 방법을 이용한 야생벼(*Oryza grandiglumis*) 유래 유용 유전자 탐색

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## Isolation of wild rice(*Oryza grandiglumis*) specific gene using PCR-base suppression subtractive hybridization

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### 실험목적

CCDD genome의 야생 벼 *Oryza grandiglumis*에서 wounding 및 병충해 침입에 대한 반응기작으로 차별 발현되는 유전자들을 탐색하여 유전자원화하고 기능을 확인함

### 재료 및 방법

- 공시재료: 야생 벼 (*Oryza grandiglumis*)
- 실험방법
  - 1) 야생 벼(*Oryza grandiglumis*) wounding 및 fungal elicitor 처리
  - 2) Suppression Subtractive Hybridization (SSH)
  - 3) 유전자들의 염기서열 분석 및 발현 검정

### 실험결과

- wounding 및 fungal elicitor 처리와 비처리 재료간의 SSH를 통해 776개의 clone 확보

- Primary screen(dot blot) 결과 115개의 유발 유전자 확인
- 염기서열 분석 및 유용한 유전자 확인(Table 1)
- 현재 유용 유전자들에 대한 individual northern 및 Race를 통한 전체 유전자 확보 실험 진행중

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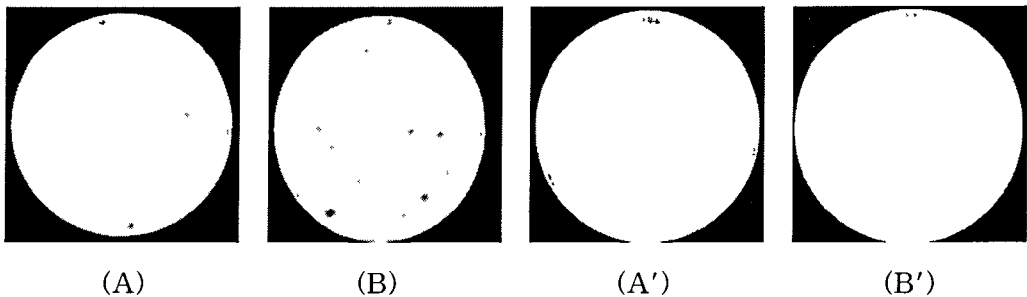


Fig. 1. RNA dot blot screen of arrayed subtractive clones with forward-subtracted cDNA probes (A & B) and reverse-subtracted cDNA probes(A' & B'). Membrane A and A' arrayed same clones from # 406 to # 540 , and membrane B and B' arrayed # 541 to # 675.

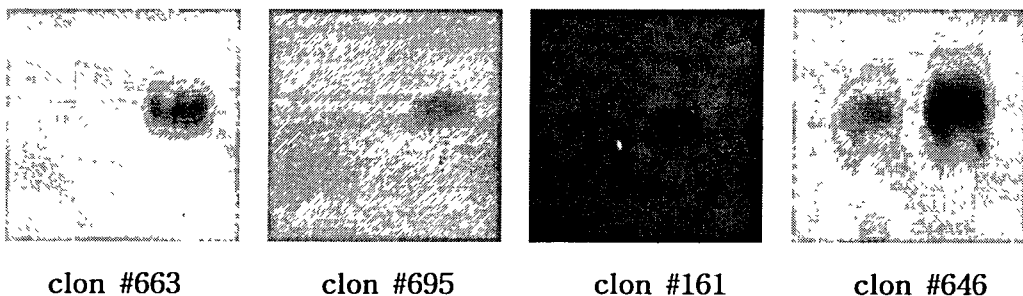


Fig 2. Confirmation of primary screened subtractive clones by individual northern blot.

Clon #663: putative integral membrane protein

Clon #695: putative endo-1,4,-beta-glucanase

Clon #161: putative  $\text{Na}^+$ -D-glucose cotransport regulator gene

Clon #646: putative pseudo-response regulator 5

Table 1. Differentially expressed wild rice (*O. grandiglumis*) cDNA clones induced upon wounding and fungal elicitor

clon #	best homology to	clon #	best homology to
8	ARE1-like protein ( <i>A. thaliana</i> )	78	carbonate dehydratase ( <i>A. thaliana</i> )
23	Pol polyprotein ( <i>A. thaliana</i> )	86	unknown
31	<i>A. thaliana</i> BAC T12C245	92	Bowman-Birk proteinase inhibitors ( <i>O. sativa</i> )
37	rubisco activase	94	metallothionein-like protein ( <i>O. sativa</i> )
39	Actin ( <i>Nicotiana tabacum</i> )	95	ribose 5-phosphate isomerase ( <i>A. thaliana</i> )
44	ABA and salt stress-responsive rice cDNA	99	photosystem I protein
48	transcription factor E2F ( <i>Xenopus laevis</i> )	605	phosphoesterase ( <i>Pyrococcus horikoshii</i> )
49	ABC transporter ( <i>A. thaliana</i> )	611	gal-pol polyprotein ( <i>O. sativa</i> )
55	transposase ( <i>O. sativa</i> )	646	pseudo-response regulator 5 ( <i>A. thaliana</i> )
71	integral membrane protein ( <i>A. thaliana</i> )	660	3-phosphoshikimate 1-vinyltransferase
74	unknown	663	integral membrane protein ( <i>A. thaliana</i> )
76	hypothetical transmembrane protein	686	forkhead homolog 4 ( <i>Drosophila</i> )
77	phosphonbuloknase ( <i>Triticum aestivum</i> )	695	endo-1,4-beta-glucanase ( <i>P. horikoshii</i> )