

Utilization of Bowman-Birk proteinase inhibitor for prevention of cadmium influx to plant cells.

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Cadmium is a toxic heavy metal that causes serious environmental pollution in many countries. Especially, cadmium pollution is serious in Japan due to the large consumption of this heavy metal for the production of batteries and other products. The most serious cadmium pollution is seen in rice, the major food for Japanese nations. In order to establish plants that do not absorb cadmium in the body, we were screening *Coptis japonica* cDNA library for cadmium tolerant genes by use of yeast as the host organism. Among cadmium tolerant clones have found that a Bowman-Birk proteinase inhibitor gene (Fig. 1), which gave stronger tolerance to cadmium than metallothionein, a well-known heavy metal tolerant protein (Fig 2).

CjBBI	M-PFTENQS-	-KGLTEIYIV	VALE---VAA	R-----	----GSN---	28
AlfalfaBBI	MELMMNKKAM	MMKLALLVFL	LGFTSTVVDA	RFDQASFISQ	LLFNGEAANY	50
PeaBBI	M-VLMNKA-	MMKLALLVFL	LGFTATVVDA	RFDNSSFITQ	LLSNGGASN-	47
PeanutBBI	M-----	---LSQVINN	IGEASSSSDD	-----	-----	18
SoyBBI	M---V----	VLKVCLVLLF	LVGGTTSANL	RLSKLGLLMK	SDHQH-SND-	40
PhavuBBI	M---V----	VLKVCFVLLF	LLGSST-ASL	KLSELGLLMK	SGHHHESTD-	40
CjBBI	-----TSCC	NQCLCTKSIP	PQ--CHCIVD	-K-EVCHSSC	INCLCTRSIP	68
AlfalfaBBI	DVKSTTTACC	NFCPCTRSIP	PQ--CHCIDI	-G-ETCHSAC	KSCICTRSIP	96
PeaBBI	-----KACC	DSCLCTRSIP	PR--CHCNDI	-G-ETCHSAC	KICLCTRSIP	87
PeanutBBI	-----NVCC	NGCLCDRRAP	PYFECVMDIT	FDH--CPASC	NSCVCTRSNP	60
SoyBBI	D--ESSKHCC	DCCACTKSNP	PQ--CHCHDM	-RLNSCHSAC	KSCICALSYP	85
PhavuBBI	EPSESSKACC	DHCACTKSIP	PQ--CHCHDL	-RLNSCHSEC	KSCICTLSIP	87
CjBBI	PQCHCIVKL	DNCAPPSCK	MDIEQVRILS			98
AlfalfaBBI	PQCHCIDI	NRCY-PKCN	-----			113
PeaBBI	PQCHCIDI	DRCY-EKCN	-----			104
PeanutBBI	PQCHCIDI	GRCPVTECRS	-----			80
SoyBBI	AQCFCVDIT	DRCYEP-CKP	SEDDK---EN			110
PhavuBBI	AQCFCVDIT	DRCYEP-CK	-----			104

Figure 1. Multiple alignment of Bowman-Birk proteinase inhibitors

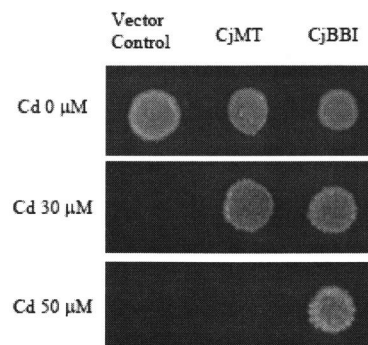


Figure 2. Cadmium tolerance of yeast transformants.
MT, metallothionein