

The potato transcription factor StEREBP1 confers tolerance under abiotic stress conditions in transgenic potato plants

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Abstract

In an attempt to determine one of several existing plant protective mechanisms against stresses, we isolated an EREBP gene from potato (StEREBP, *Solanum tuberosum* ethylene responsive element binding protein) by reverse Northern blot analysis. The full-length sequence of StEREBP was 814 bp and contained an open reading frame encoding a putative protein of 206 amino acids, corresponding to a predicted polypeptide of 23.1 kDa. The expression of StEREBP was induced by exposure to cold, salt, ABA, drought and salicylic acid (SA). Constitutive overexpression of StEREBP in tobacco and potato plants enhanced cold, salt and pathogen tolerance. The StEREBP gene was localized to the nucleus of onion epidermis cells, and showed binding activity to both the GCC and DRE/CRT boxes. However, its capacity for DRE/CRT binding was not significant. On the basis of these results, we concluded that StEREBP plays an important role in various signal transduction pathways including ethylene signaling. Thus, to identify genes downstream of StEREBP in various signal transduction pathways, we conducted microarray analysis using TIGR 10K potato chip. StEREBP induced transcripts of many genes including PR proteins and stress inducible genes. StEREBP overexpressing potato showed increased tolerance against *Ralstonia solanacearum* and *Phytophthora infestans*(CG3134).

Result



Fig. 1. Comparison of deduced amino acid sequences of DNA binding domains of StEREBP with those of the other EREBP/AP2 related proteins.

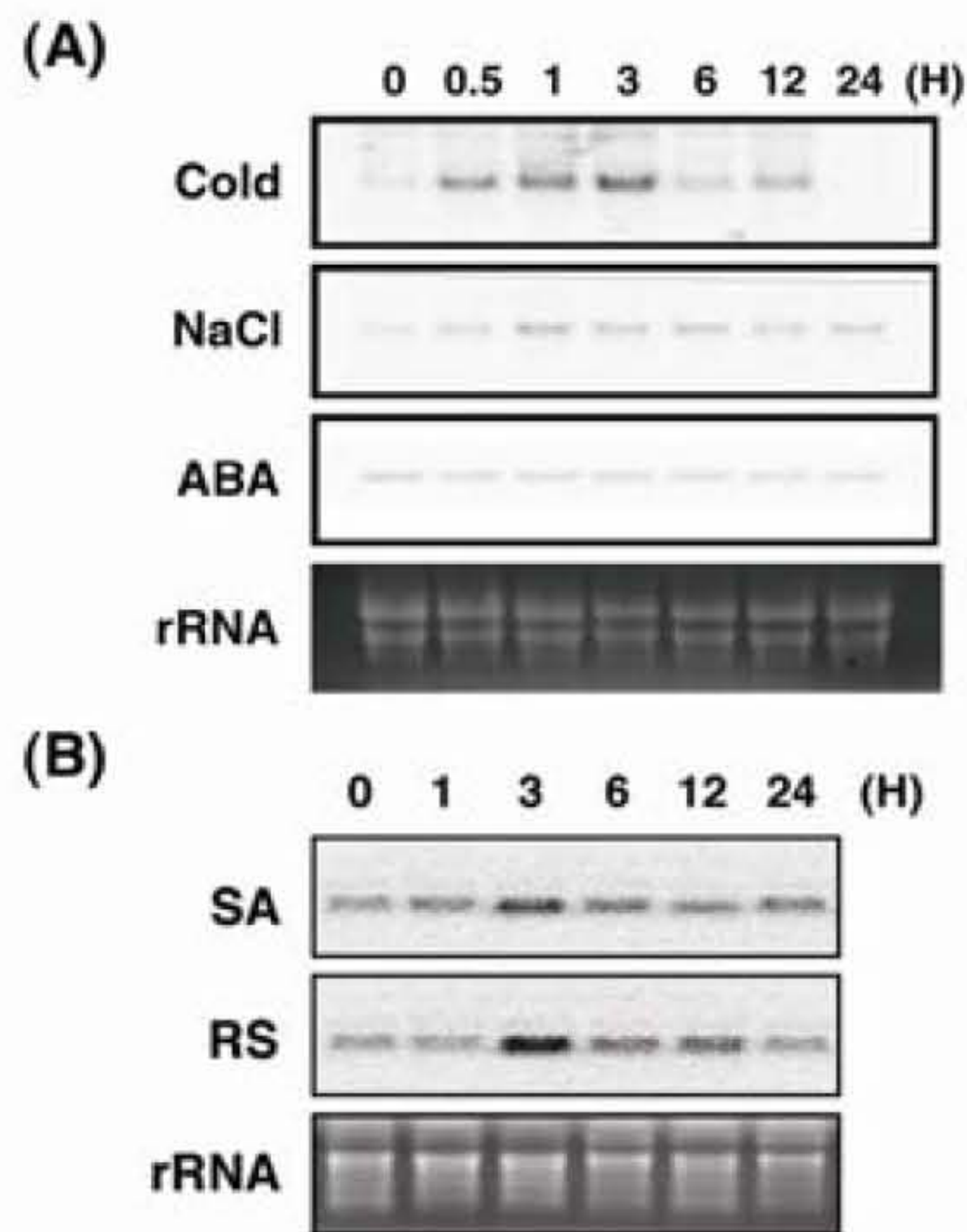


Fig. 2. Transcript level of the StEREBP was regulated under abiotic or biotic stress conditions. (A) and (B). 3-week potted potato plants were treated each stress for various times; Cold (4 °C), NaCl (100 mM), ABA (100 μM), Salicylic acid (10 μM), RS (*Ralstonia solanacearum*).



Fig. 3. Localization of StEREBP is nuclear. The smGFP-StEREBP constructs were introduced into the onion cells by particle bombardment. Expression of StEREBP-GFP fusion protein (left), Expression of GFP protein for control (right).

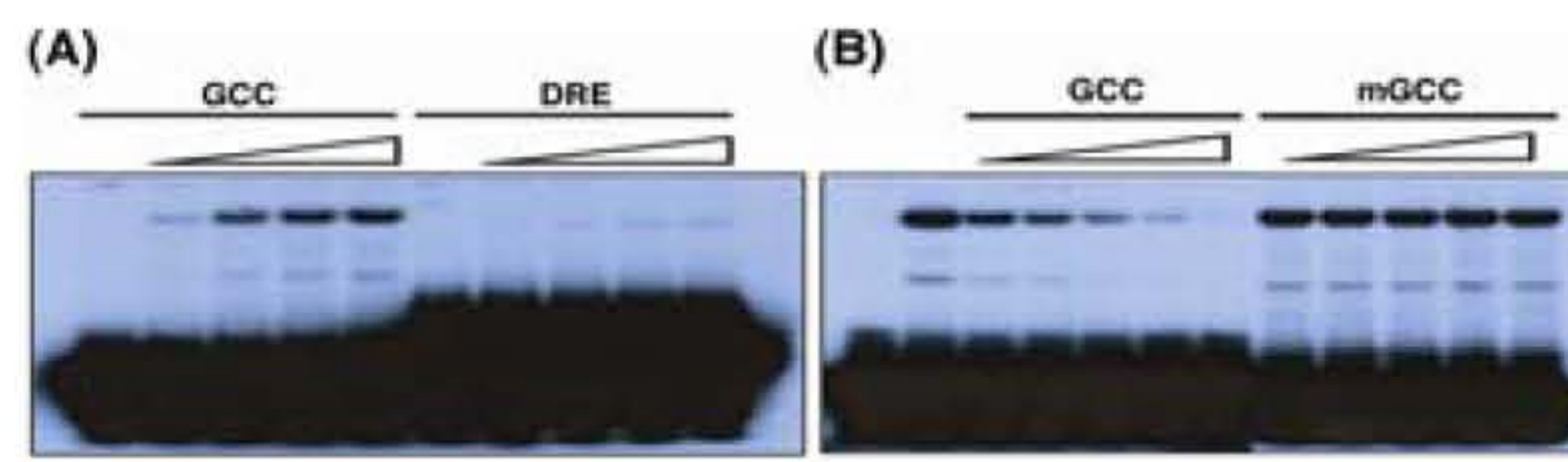


Fig. 4. Electromobility shift assay of StEREBP with GCC and DRE/CRT probe. (A) The StEREBP recombinant protein binds with GCC or DRE/CRT probe. (B) Competition assay of StEREBP with cold competition or mutated form of GCC box.

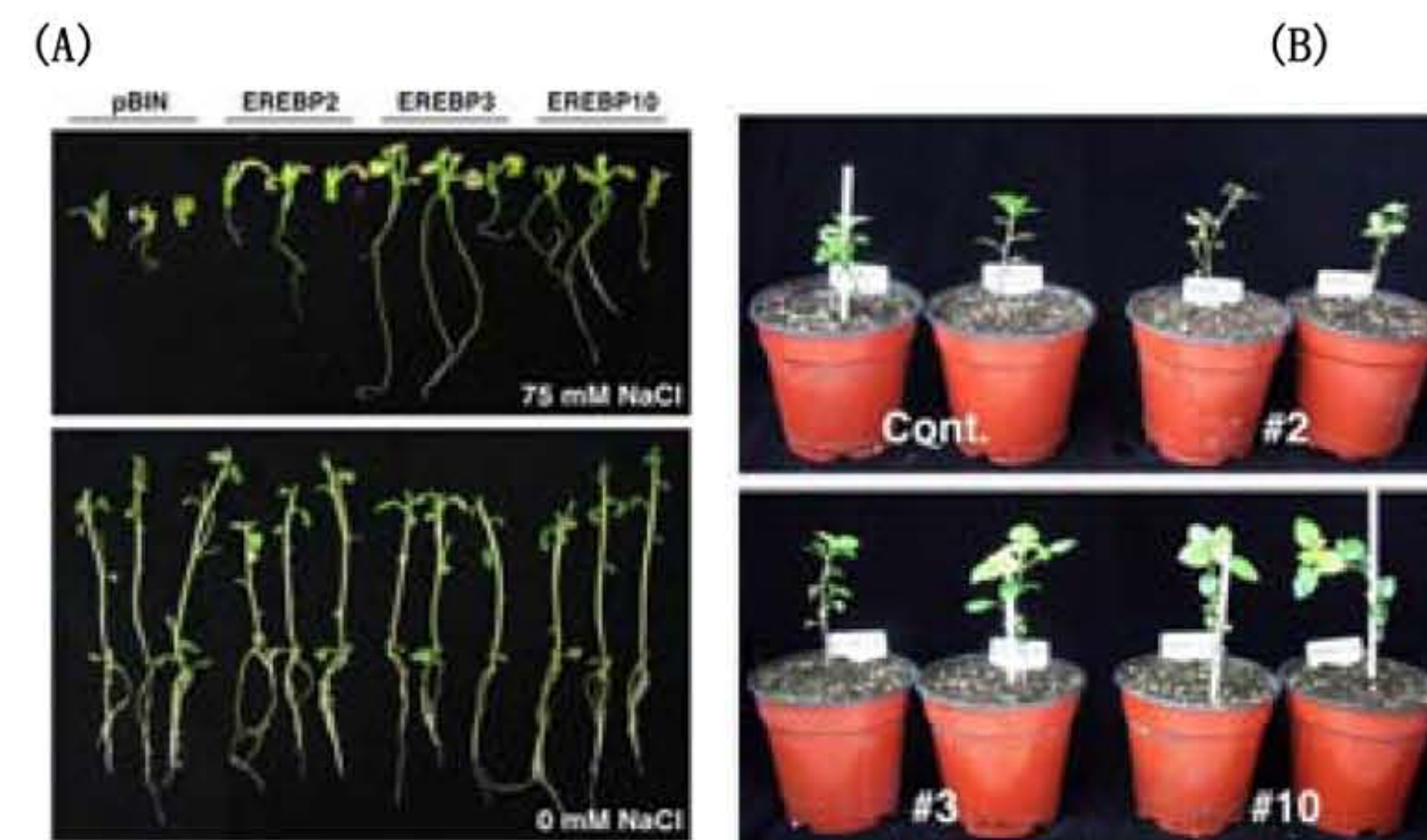


Fig. 5. Transgenic potato overexpressing StEREBP showed tolerance to salt stress and cold stress. (A). Root growth test. Potato tissues were transferred to MS medium with or without NaCl. (B) Plants were grown at low temperature condition (4-10°C) for 2 weeks.

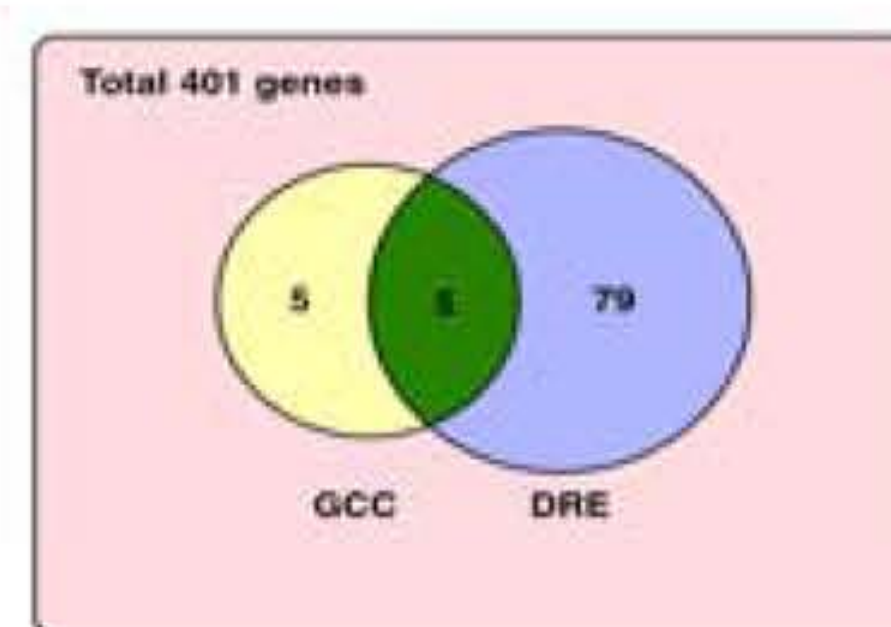


Fig. 6. Profiling of up-regulated gene transgenic potato overexpressing StEREBP using 10k DNA TIGR Chip. 89 genes are containing GCC/DRE motif in promoter among 401 genes up-regulated over 2 times.

Table 1. Up-regulated genes containing GCC or/and DRE element in transgenic plant overexpressing StEREBP.

AGI No.	Description	Category	GCC	DRE	Potato (TC)	EREBP regulation
AT4G22690	Cytochrome P450 family protein, flavonoid 3',5'-hydroxylase H1	Metabolism	1	1	TC46322	8.93
AT1G22170	Phosphoglycerate mutase	Metabolism		1	TC55957	6.50
AT4G15510	Photosystem II reaction center PsbP family protein	Metabolism	1		TC53944	5.58
AT1G27980	Pyridoxal-dependent decarboxylase family protein	Metabolism	1		TC49063	4.89
AT1G29280	WRKY 65 transcription factor	Cell rescue/defense		1	TC54604	5.11
AT1G19940	Endo-β-1,4-D-glucanase	Cell rescue/defense		1	TC54158	4.64
AT5G52040	Apoptosis regulator Bcl-2 protein	Cell rescue/defense	1		TC49742	4.21
AT5G16710	Glutathione dehydrogenase	Cell rescue/defense		1	TC41316	4.04
AT1G18150	AIMPK8	Signal transduction		1	TC49283	2.62
AT4G27200	S-locus protein kinase	Signal transduction		1	TC55477	2.73
AT2G07180	Protein kinase	Signal transduction	1		TC53776	2.90
AT4G17560	Ribosomal protein L19 family protein	Protein biosynthesis	2		TC45161	7.06
AT4G02930	Elongation factor Tu	Protein biosynthesis	1		TC45719	3.73
AT1G04520	26S proteasome regulatory subunit	Protein catabolism	1		TC42610	2.90
AT3G08690	Ubiquitin-conjugating enzyme 11	Protein catabolism	1		TC55438	2.41
AT1G50640	AREB3	Regulation of transcription	1		TC46552	2.43
AT5G52510	Transcription factor SCL5	Regulation of transcription	1		TC46670	2.84
AT1G53190	Zinc finger (C2HC4-type RING finger)	Protein binding	1		TC54792	2.75

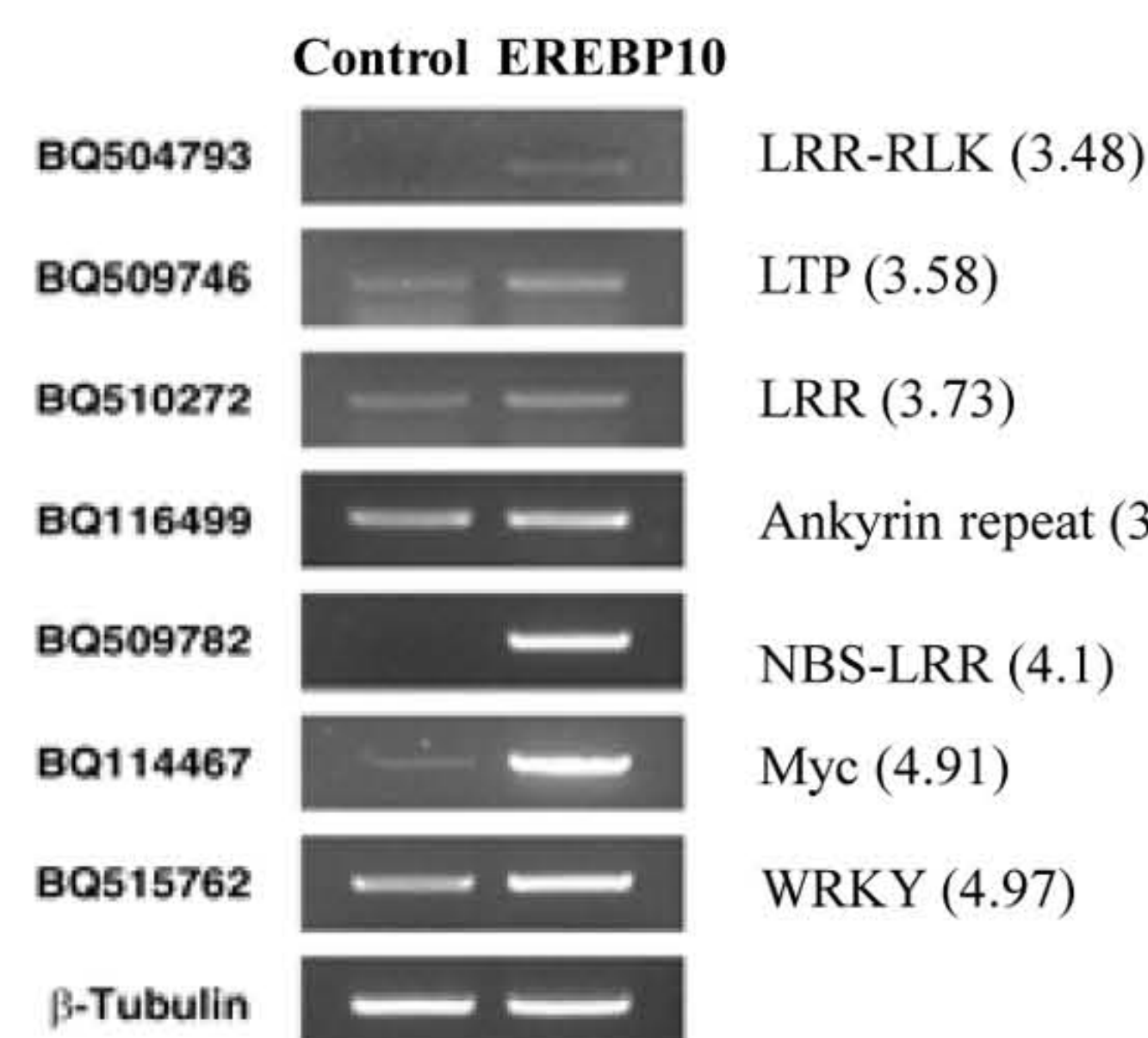


Fig. 7. Several genes containing GCC or/and DRE motif were up-regulated in transgenic potato overexpressing StEREBP. Total RNA was extracted from 3-week-old potato plants. RT-PCR analysis was performed with specific primers. Tubulin was used for control.

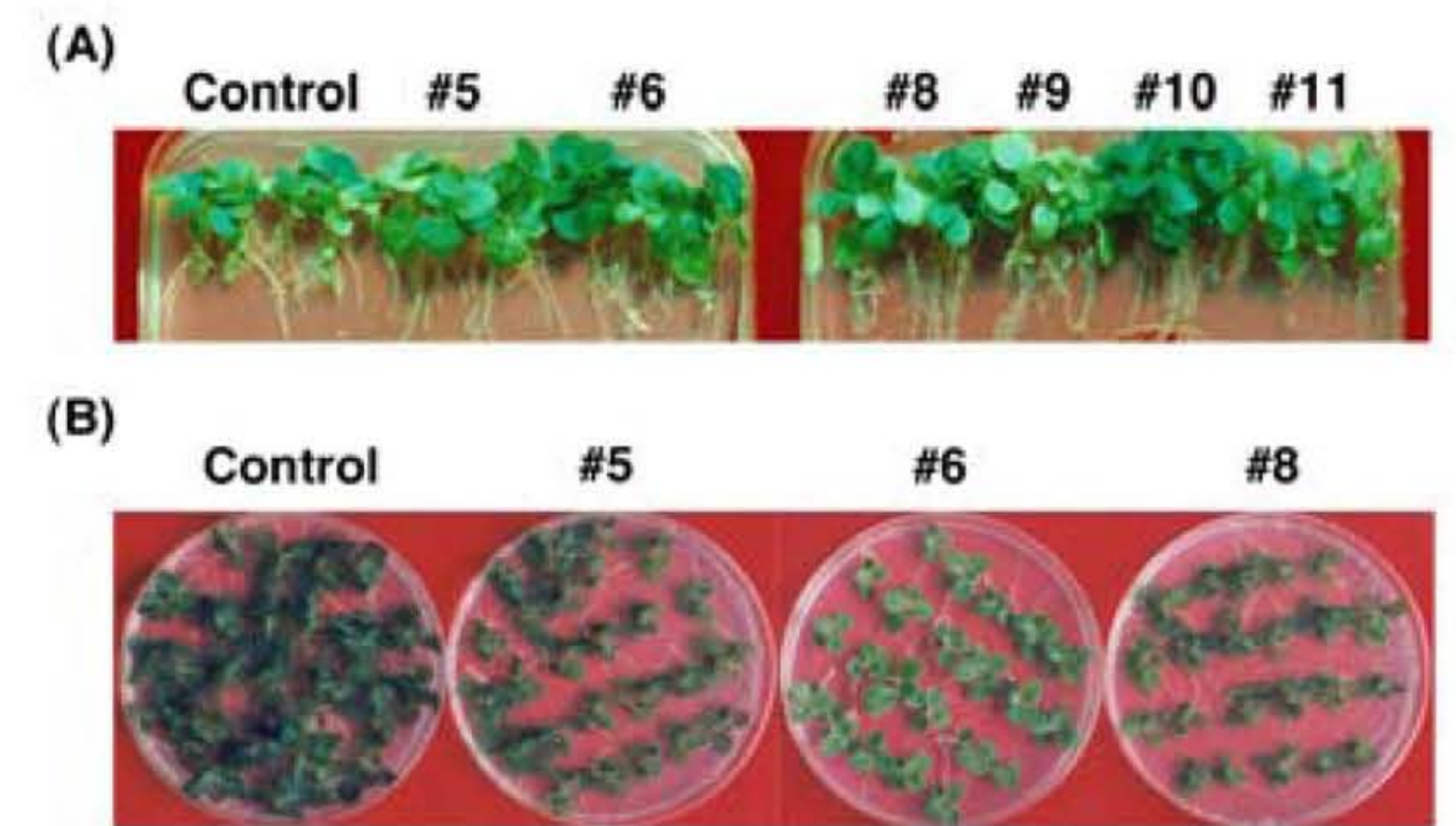


Fig. 8. Transgenic tobacco plants overexpressing StEREBP enhanced tolerance against abiotic stress. (A) Salt tolerance of StEREBP. One-week-old seedling were transferred to MS medium containing 300 mM NaCl. (B) Freezing tolerance of StEREBP. Two-week-old seedling were treated at -6°C for 24 hour in the dark and transferred to normal growth condition.

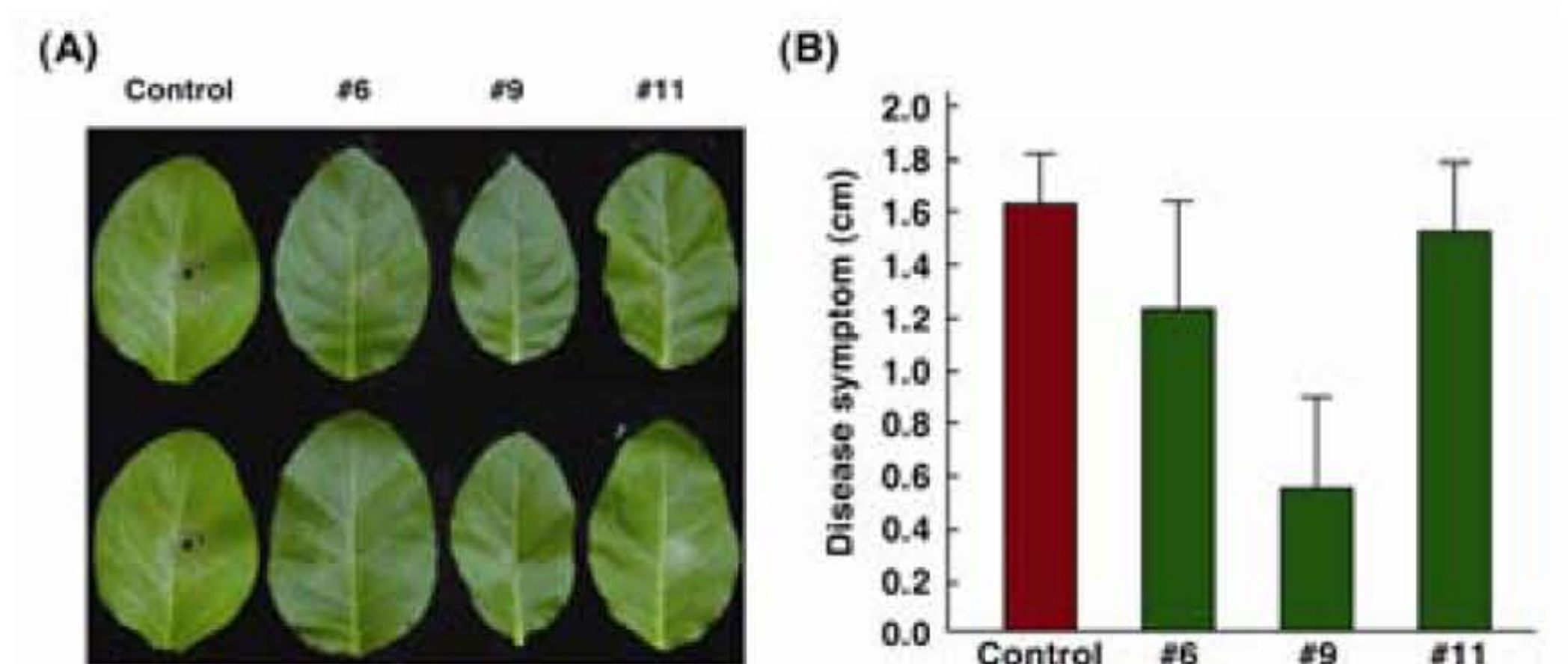


Fig. 9. Transgenic tobacco overexpressing StEREBP showed resistance to fungal pathogen *Phytophthora nicotianae*. (A) *P. nicotianae* was treated transgenic tobacco leaves by pin infiltration method. Photograph was taken after at 28 °C for 3 days. (B) Disease symptom was measured after 5 days at 28°C.

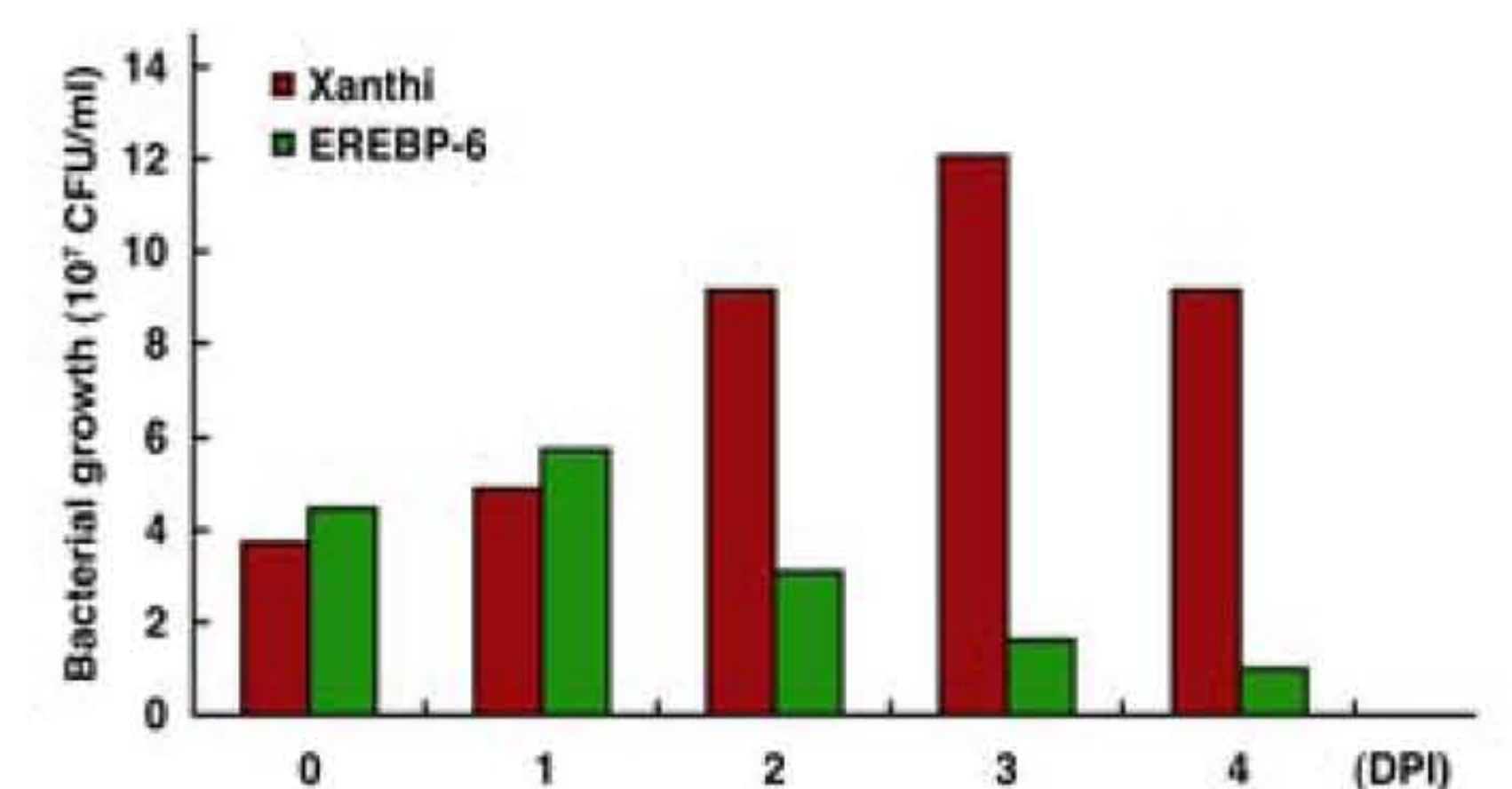


Fig. 10. Transgenic tobacco overexpressing StEREBP showed resistance to bacterial pathogen *Pseudomonas syringae* pv. *tabaci*.

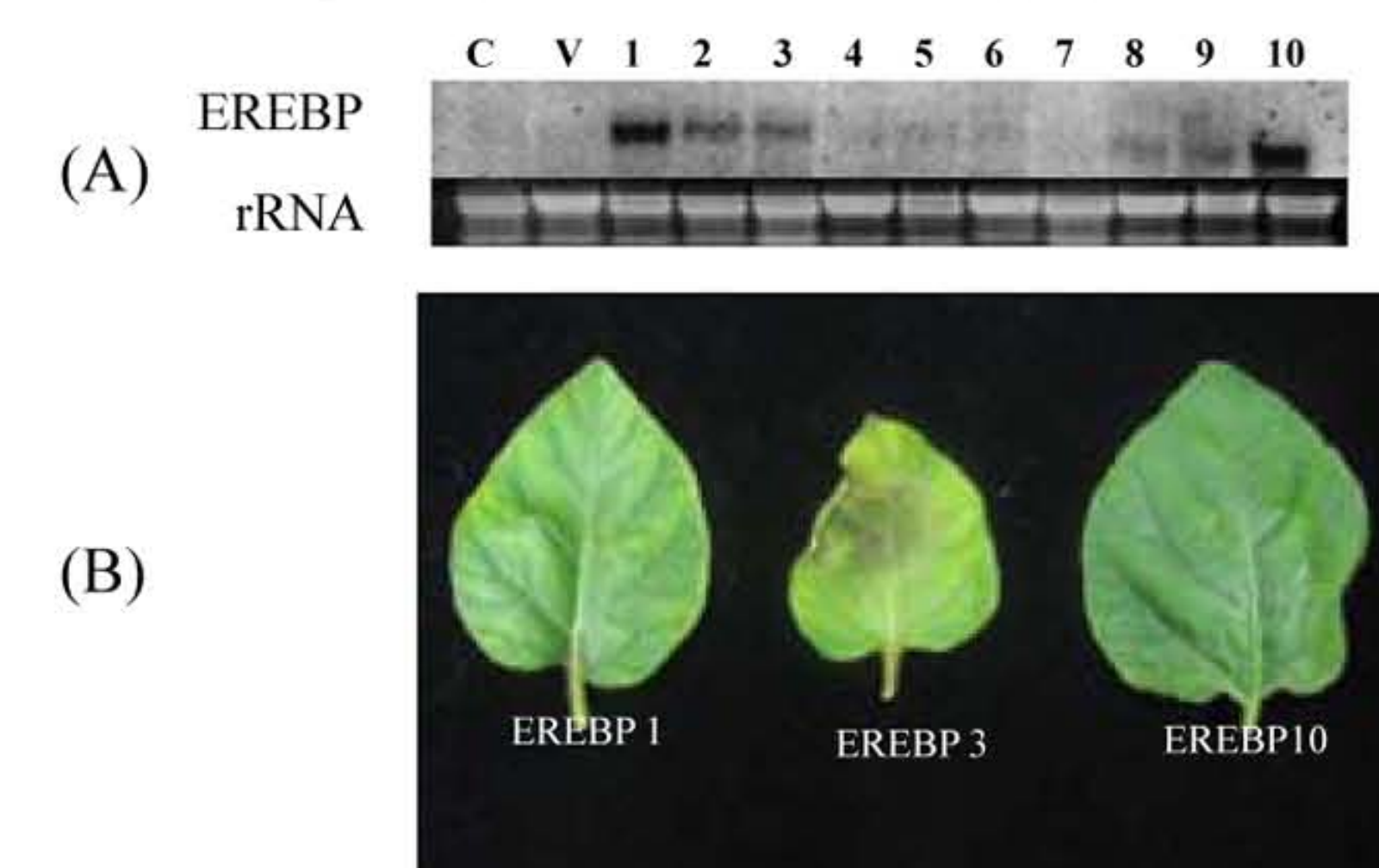


Fig. 11. Transgenic potato overexpressing StEREBP showed resistance to Oomycetes *Phytophthora infestans* (KACC 40718). A, mRNA accumulation of EREBP; B, Response of transgenic potato on 5 days after infiltration of sporangium

Conclusion

1. The transcript level of StEREBP was induced by biotic or abiotic stresses.
2. StEREBP bound to the GCC box (an element responsive to ethylene) and to the dehydration responsive element.
3. Transgenic plants overexpressing StEREBP showed tolerance to cold or salt stress and resistance to fungal or bacterial pathogens.
4. Transgenic plants overexpressing StEREBP up-regulated several genes containing GCC box related to variety stress such as low temperature or pathogen attack.
5. Therefore, we suggest that StEREBP give the tolerance or resistance to abiotic or biotic stress through StEREBP regulate various genes.

