Electric Gene Expression in Plant Single-cells via Ca^{2+} Dependent or Independent Pathway

Rieko HIRAI[1], Tamu KOMAZAKI[1], Yoshiko MUKAI[1], Mikako SAITO[1,2], Hideaki MATSUOKA[1,2]
[1]*Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588,Japan.*
[2]*CREST, Japan Science and Technology Agency, Kawaguchi, Saitanma 332-0012, Japan.*
bio-func@cc.tuat.ac.jp

When an electric signal was applied to rice single-cells, the Ca^{2+} concentration in many cells increased and then chitinase (RCC1) gene expression was observed. This Ca^{2+} increase was caused by Ca^{2+} influx from outside. Chitinase gene is a typical one of pathogen related (PR) proteins in rice and known to be a Ca^{2+} dependent gene. In some cells, however, no Ca^{2+} increase occurred but chitinase gene expression was observed. From these results, we suspected that the chitinase gene could be induced via Ca^{2+} dependent or Ca^{2+} independent pathway depending upon the electric signal intensity. Therefore this study is focused on the quantitative experiment about the electric signal intensity in the single-cell experiment.

Rice protoplasts were prepared from calli of *Oryza sativa* L. japonica cv. Nipponbare according to the procedure described in [1]. A plasmid composed of a cauliflower mosaic virus 35S promoter and a GFP gene (p35S-GFP) was introduced in single-cells of rice protoplast by microinjection 20 h before the electric signal application. Intracellular Ca^{2+} concentration was measured by the fluorescent dye method using Fura-2. The spatial distribution of Ca^{2+} concentration in each single-cell was analyzed with a Spectro-imaging system [2]. A pair of microelectrodes was manipulated so that the target single-cell was positioned at the center between the electrodes. The actual potential gradient between the electrodes was measured separately (Fig.1). Thus the effective cross membrane potential (V_{CMP}) for a cell with diameter of 30-50 μm was estimated as 22-36 mV under the condition of V_{ET}=20V (Fig.2).

Table 1 summarizes the result. In total, microinjection was done into 172 cells. The success of microinjection was checked by the RCC1 gene expression after electric signal application or the application of a specific eleictor at the final stage. In the case of Table 1, the success rate was 51/172=29.6%. The rate of electric gene expression via Ca^{2+} dependent pathway was 16/20=80%, while that via Ca^{2+} independent pathway was 3/14=21%. On the other hand, 6 out of 16 cells (37.5%) showed gene expression without electric signal. This should have been caused by the mechanical wound of rough microinjection.

In conclusion, the single-cell experiment has enabled the quantitative estimation of Ca^{2+} dependent and Ca^{2+} independent gene expression triggered by an electric signal.

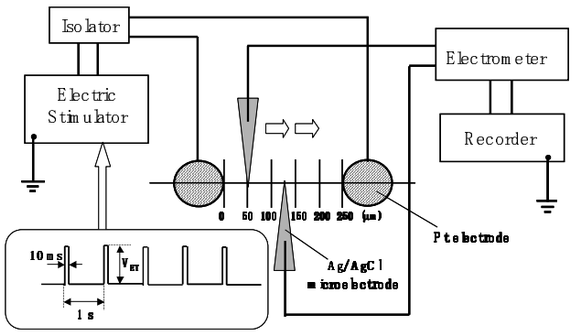

Fig.1 Electric signal application system

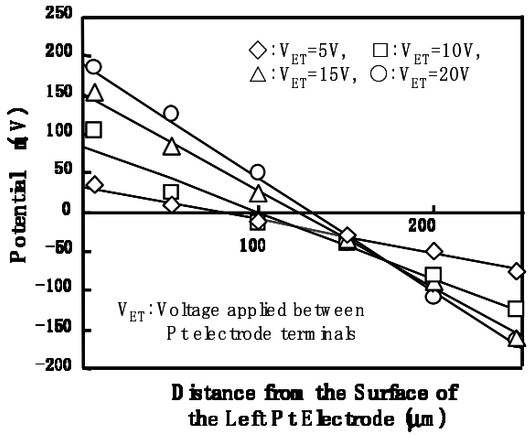

Fig.2 Potential gradients between Pt electrodes

Table 1 Chitinase gene expression induced by electric signal application

pCHI-GFP	Electric stimulus	Ca^{2+} Uptake	GFP after Electric Stimulus	GFP after elicitor addition
Microinjection 172	ON 104	+47	16	20
		-57	3	14
	OFF 68	+1	0	1
		-67	6	16
No Injection 46	ON 22	+10	0	0
		-12	0	0
	OFF 24	+0	0	0
		-24	0	0

References

[1] M.Saito et al. J. Biotechnol. 105, 41-49 (2003).
[2] H. Matsuoka et al. J. Biotechnol. 94, 299-308 (2002).
H.Matsuoka et al. Anal. Sci. 18, 1321-1324 (2002)