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- 단 보 -

Sodium Hypochlorite Solution As a Chemical Wounding Agent for Improving *Agrobacterium*-mediated Chinese Cabbage Seed Transformation

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Chinese cabbage (*Brassica campestris* ssp. napus var. pekinensis Makino) seeds/seedlings were transformed via vacuum-infiltration with recombinant *Agrobacterium tumefaciens* LBA4404 cells. The agroinfiltration method was determined to be unsuccessful for Chinese cabbage transformation during the analysis of hepatitis B surface antigen expression by ELISA. However, treatment of sodium hypochlorite solution, prior to agroinfiltration, to pregerminated or germinating 1 day- or 2 days-old seeds was proven effectively to enhance transformation efficiency, suggesting that chemical wounding caused by sodium hypochlorite reaction might facilitate *Agrobacterium* infection and, therefore, transient gene expression in Chinese cabbage sprouts.

Key words – sodium hypochlorite, agroinfiltration, transient expression

Transient expression system using plant has been widely investigated due to several promising advantages including feasibility and low cost of performance. It sometimes purposes even commercial production of many of high value recombinant proteins. To do this, gene transformation step must be processed and, therefore, rehearsed using gene delivery skills such as biolistic bombardment, electroporation, agroinfiltration, and viral vector inoculation[5,6]. Chinese cabbage (*Brassica campestris* ssp. napus var. pekinensis Makino), a well-recognized fresh vegetable, has been attracted because of its sprouts as well-being food. We can enjoy the young sprouts easily even at home using a simple device. Chinese cabbage, however, is probably not suitable for the transient expression system because of its low efficiency for transformation via *Agrobacterium* infection[2-4]. In this study, we examined how sodium hypochlorite (SHC), a chemical abrasive, affected *Agrobacterium*-mediated Chinese cabbage seed transformation by means of histochemistry and ELISA techniques.

We performed *Agrobacterium*-mediated transformation via vacuum-infiltration[1] (agroinfiltration) for Chinese cabbage seeds on 0-, 1-, and 2-day. Seeds were sterilized in 0.2% sodium hypochlorite solution for 1 min. They were then washed with sterile water and placed for imbibition for 24~48 hr at 4°C. The imbibed seeds were agroinfiltrated for 10 min for β-glucuronidase (GUS) reporter gene transformation (day-0-seed agroinfiltration) and planted. The *Agrobacterium* cell cultures (100 μl of 16 hr-grown at 27°C) harboring pBI121 GUS expression vector were added to the seeds soaked in 20 ml of sterile distilled water. Vacuum-infiltration continued for 10 min. Transformed and non-transformed seeds were placed on a pre-wet paper towel at 27°C in the dark for growth. At the following days, days 1 and 2 non-treated germinating seeds (day-1-seed and day-2-seed) were also independently agroinfiltrated and grown under the same conditions mentioned above. At 6th day, sprouts were harvested for histochemical GUS gene analysis[8]. Sprouts were incubated at 37°C with X-Glc solution until blue color appeared and destained in 70% ethanol. In Fig. 1, Con and X-0 in the upper part represent non-transformed sprouts and sprouts from agroinfiltrated day-0-seed, respectively. Unexpectedly, Con sprouts displayed stronger GUS-stain than X-0. In any way, both of the results were presumed quite unlikely, probably indicating certain level of intrinsic GUS enzyme activity in chinese cabbage sprouts or remnants of *Agrobacterium* activity. In repeated experiments, results were similarly monitored. Meanwhile, GUS activity from sprouts of SHC-treated and agroinfiltrated day-0-, 1-, and 2-seed was disappointingly low or negligible. At this point, we raised a question whether this observation quite true or false. So, we decided to estimate the level of transformed gene expression by ELISA[9].

HBsAg, a major hepatitis B virus (HBV) envelop

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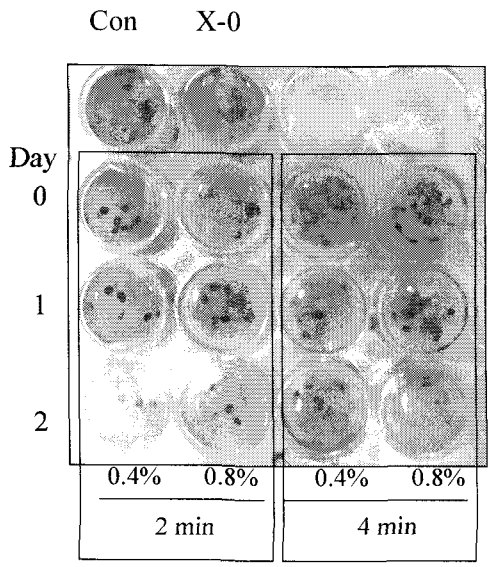

Fig. 1. Detection of GUS gene expression in Chinese cabbage sprouts by means of histochemistry. Con, non-transformed sprouts. X-0, sprouts from agroinfiltrated day-0-seed. Day-0-, 1-, and 2-seeds were treated with 0.4% and 0.8% SHC solution for 2 and 4 min prior to agroinfiltration.

component, has been extensively investigated and characterized due to its importance as a vaccine antigen against HBV[10,11]. To estimate efficiency of Chinese cabbage seed transformation, we carried out ELISA for HBsAg antigen instead of fluorometric assay for GUS enzyme using 4-methyllumbelliferyl β-D glucuronide because of feasibility under our lab circumstances. Plant expression vector containing 0.7 kb DNA fragment encoding HBsAg (pBIHBsAg) was constructed. PCR-amplified HBsAg DNA from pAM6 (ATCC 40101) was cloned into GUS DNA-deleted pBI121 (pBI121ΔGUS) and introduced into *Agrobacterium tumefaciens* LBA 4404 for Chinese cabbage transformation (data not shown). Agroinfiltration was processed for day-0-, 1- and 2-seeds following SHC treatment as described earlier. Sprouts were homogenized in the extraction buffer containing 20 mM sodium phosphate, pH 7.0, 0.15 M NaCl, 20 mM sodium ascorbate, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 1x protein inhibitor (Roche)[12]. Centrifugation (12,000×g, 15 min) was performed twice to collect clear homogenate to be analyzed for HBsAg protein content by ELISA using Abbott IMx detector system. Total soluble protein (TSP) was determined according to Bio-Rad Protein Assay system. The results are shown in Fig. 2-A. Fig. 2-B represents percent value of HBsAg protein contained in TSP. Con sprouts and X-0-0-0 sprouts(sprouts of day-0-seed with no SHC treatment) were shown to contain 0.006 and 0.007% HBsAg protein in TSP, respectively. The 0.007% value presumably meant little HBsAg protein synthesis in agroinfiltrated sprouts because Con sprouts were observed by its 0.006% value which is almost equal to that of X-0-0-0 sprouts. These findings were quite different from GUS detection analysis and led to a conclusion that the GUS-positivity was, in fact, false-positive. In contrast, SHC treatment resulted in significant increase in HBsAg production. Especially, SC.4-4-1(sprouts of 0.4% SHC treatment for 4 min to day-1-seed) showed 0.027% value. Also, SC.4-2-0(0.4% SHC, 2 min, day-0-seed), SC.4-2-1(0.4% SHC, 2 min, day-1-seed), SC.8-2-1(0.8% SHC, 2 min, day-1-seed) and SC.8-4-1(0.8% SHC, 4 min, day-1-seed) showed HBsAg expression higher than 0.015%. These results strongly suggested that chemical wounding

A.

Sprouts	TSP (μg/ml)	HBsAg (ng/ml)
CON	383.5	22.88
X-0-0-0	387.8	26.88
SC.4-2-0	394.5	80.98
SC.4-4-0	366.0	48.41
SC.8-2-0	353.5	28.82
SC.8-4-0	354.5	30.49
SC.4-2-1	350.8	56.48
SC.4-4-1	354.4	95.32
SC.8-2-1	339.5	52.20
SC.8-4-1	373.5	88.92
SC.4-4-2	399.0	21.10
SC.4-2-2	380.5	24.70
SC.8-2-2	346.6	28.30
SC.8-4-2	345.1	30.40

B.

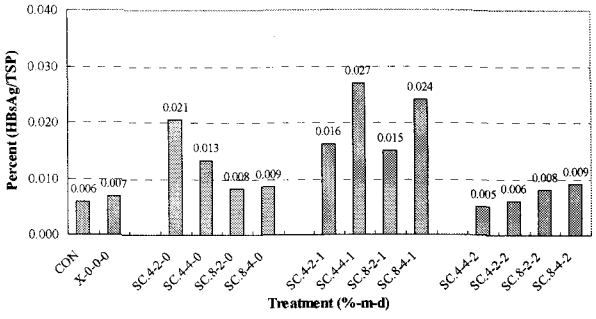

Fig. 2. HBsAg protein content in Chinese cabbage sprouts. Numbers attached to sprouts X(no SHC treated) and SC(SHC treated) indicate in order SHC concentration (0, 0.4 and 0.8%), treatment duration (0, 2 and 4 min) and age of seed (0, 1, and 2) for agroinfiltration.

caused by SHC on Chinese cabbage seeds could effectively improve the transformation efficiency and, therefore, transient heterologous gene expression to a significant level. More endeavor will be of great help to employ Chinese cabbage sprouts as one of the promising transient expression systems in the future[7]. In addition, SHC solution could be applied as an effective reagent to improve the efficiency of *Agrobacterium*-mediated transformation in other plant species.

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초록 : Sodium hypochlorite처리에 따른 배추종자의 *Agrobacterium*이용 형질전환 증대

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배추 종자 및 유묘에 대하여 GUS발현 또는 hepatitis B surface antigen (HBsAg)발현 벡터를 지니는 *Agrobacterium tumefaciens* LBA4404 세포를 이용하여 진공침윤(agroinfiltration)에 의한 형질전환을 시도하였다. 특히 ELISA를 이용한 HBsAg발현의 정량적 분석에서 agroinfiltration 방법은 형질전환효율이 매우 저조하게 나타났다. 그러나 차아염소산나트륨 용액을 발아 전 또는 발아 중인 배추종자에 처리한 후 agroinfiltration을 실시한 경우 형질전환 효율이 2~5배 증가하였다. 따라서 차아염소산나트륨 등의 화학연마제에 의한 종자의 상처발생이 *Agrobacterium*의 감염을 용이하게 함으로써 배추유묘에서의 일시유전자발현을 증대시키는 것으로 제안되고 있다.