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Short Communication

Transient Expression in Chinese Cabbage by Hydrogen Peroxide-aided Agroinfiltration

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Chinese cabbage (*Brassica campestris* ssp. napus var. pekinensis Makino) is one of the popular vegetables in oriental countries and can be eaten as fresh, fermented, and dried food. Recently, sprout vegetables grown from its seedlings have also attracted attention as well-being food. Young sprouts can be grown easily within a week using a simple home appliance. Plant transient expression system has been extensively studied owing to its several advantages such as simplicity, rapidity and low cost over the conventional plant transformation and regeneration system. Attempts have been made to commercially produce pharmaceutical recombinant proteins for therapeutic and edible vaccines using gene transformation process established through biolistic bombardment, electroporation, agroinfiltration and viral vector inoculation.[1,2] However, Chinese cabbage is not ideal for the transient expression system due to its relatively low transformation efficiency especially by *Agrobacterium* infection.[3,4,5] Therefore, to enhance the transient expression level of Chinese cabbage sprouts in this study, we applied hydrogen peroxide (HX), a chemical abrasive, prior to *Agrobacterium* infection.

We performed *Agrobacterium*-mediated transformation via vacuum-infiltration[6] (agroinfiltration) on 0-, 1-, and 2-day old for Chinese cabbage seeds Seeds were sterilized in 0.4% sodium hypochlorite solution for 1 min, washed with sterile water, and immersed in sterile water for 24 hr at 4°C. The imbibed seeds were then agroinfiltrated for 10 min for 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 in 20 ml sterilized 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. The next 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.[7] Sprouts were incubated at 37°C with X-Glc solution until blue color appeared and destained in 70% ethanol. As seen in Fig. 1, no color changes were observed in the sprouts under all experimental conditions, implying no or very slight GUS gene transformation occurred. In a comparative experiment, HX was treated to day-0-, 1-, and 2-seeds which were washed sufficiently with sterile water several times prior to agroinfiltration. GUS-positive sprouts were well observed from most of the HX-treated sprouts. HX treatment was carried by immersing seeds (day-0-seed) or young seedlings (day-1- and day-2-seed) in 5 or 10% HX solution for 2 or 4 min. These results were further evaluated in detail through the following experiment due to certain limitation in histochemical judgement on the GUS gene expression.[8]

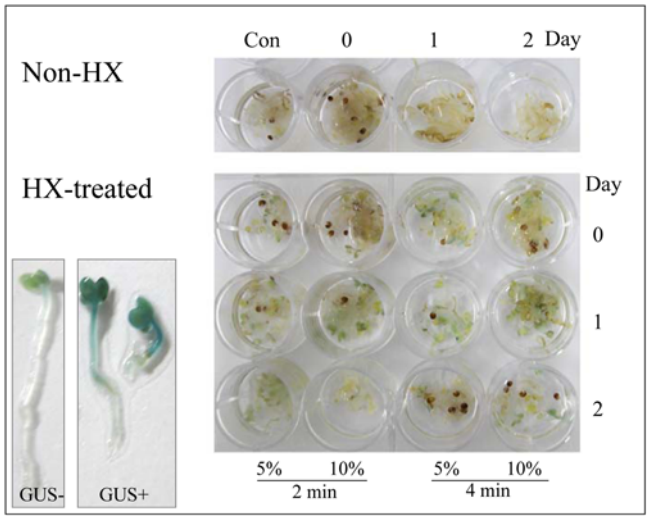

Fig. 1. GUS gene expression of Chinese cabbage sprouts. Con, non-transformed sprouts. Non-HX, no hydrogen peroxide treated to day-0-, 1-, and 2-seeds. HX, 5 or 10% hydrogen peroxide solution was treated for 2 or 4 min prior to agroinfiltration. Closed views of GUS-negative (GUS-) and GUS-positive (GUS+) sprouts are displayed in the inner box.

Hepatitis B virus (HBV) as one of the world-threatening disease agents can be protected by vaccination using recombinant HBV surface antigen (HBsAg) protein.[9,10] 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) (data not shown) and introduced into *Agrobacterium tumefaciens* LBA 4404 for Chinese cabbage transformation. HX-treated and non-treated day-0-, 1- and 2-seeds were subjected to agroinfiltration 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 phenyl-

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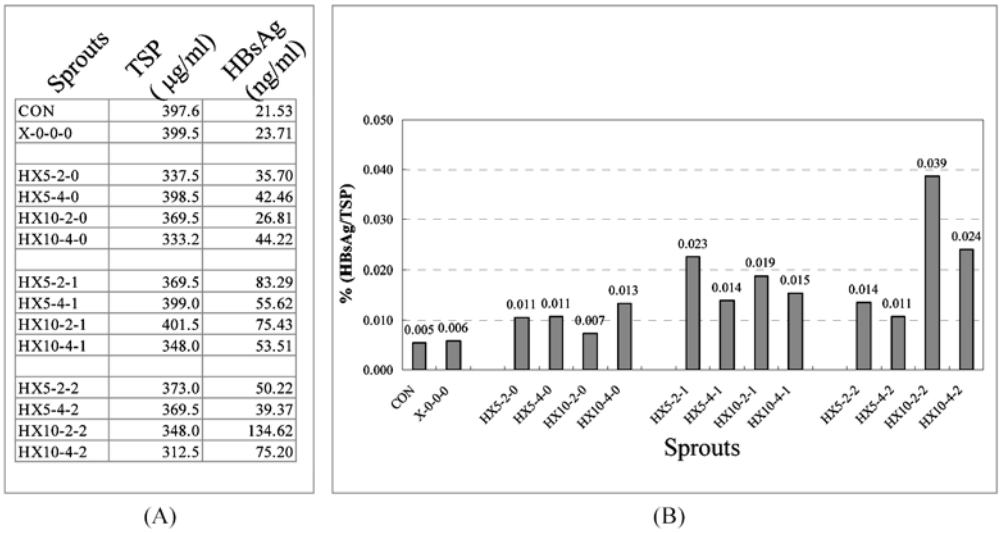

Sprouts	TSP (μg/ml)	HBsAg (ng/ml)
CON	397.6	21.53
X-0-0-0	399.5	23.71
HX5-2-0	337.5	35.70
HX5-4-0	398.5	42.46
HX10-2-0	369.5	26.81
HX10-4-0	333.2	44.22
HX5-2-1	369.5	83.29
HX5-4-1	399.0	55.62
HX10-2-1	401.5	75.43
HX10-4-1	348.0	53.51
HX5-2-2	373.0	50.22
HX5-4-2	369.5	39.37
HX10-2-2	348.0	134.62
HX10-4-2	312.5	75.20

(A) (B)

Fig. 2. Production of HBsAg protein in Chinese cabbage sprouts. Numbers attached to sprouts X (hydrogen peroxide not treated) and HX (hydrogen peroxide treated) indicate in order hydrogen peroxide concentration (0, 5 or 10%), treatment duration (0, 2 or 4 min) and age of seed (0, 1, and 2).

methylsulfonyl fluoride, 1× proteoin inhibitor (Roche).[11] 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. Figure 2-B represents percent value of HBsAg protein contained in TSP. In X-0-0-0 (sprouts of day-0-seed with no HX treatment), HBsAg protein was hardly detected as observed by its 0.006% value which is almost equal to that of non-transformed (CON) sprouts. From the results of both GUS gene expression and HBsAg synthesis, we can conclude that Chinese cabbage seeds were not responsive to *Agrobacterium* infection. In contrast, HX treatment significantly increased HBsAg production level. In particular, HX10-2-2 (sprouts of 10% HX treatment for 2 min to day-2-seed) showed 0.039% value. HX10-4-2 (10% HX, 4 min to day-2-seed) and HX5-2-1 (5% HX, 2 min to day-1-seed) also showed higher than 0.02% value. These results strongly suggest that chemical wounding caused by HX on Chinese cabbage seeds could effectively improve the transformation efficiency and, therefore, transient foreign gene expression to a significant level. Furthermore, HX treatment could also be effective for other plant species not easily transformed by *Agrobacterium* infection.

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