【別紙２】

論文名：THE EFFECT OF ^{12}C-ION BEAM IRRADIATION AND OVEREXPRESSION OF THE GIBBERELLIN BIOSYNTHETIC AND METABOLIC GENES ON MORPHOLOGY AND ARTEMISININ CONTENT OF *ARTEMISIA ANNUA* L.

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Artemisia annua L. is a commercial source of artemisinin production. Nevertheless, artemisinin content is relatively low and varies depending on the genotype and cultivation environment. In order to broaden the genetic variability and develop novel strains with high artemisinin contents, mutation induction by ^{12}C-ion beam irradiation and production of transgenic plants overexpressing the gibberellin biosynthetic and metabolic genes were examined in *A. annua*.

Nodal segments of *A. annua* were irradiated with various doses of ^{12}C-ion beam. Irradiation at 2.5 Gy had a slight lethal effect to nodal segments while a noticeable lethal effect was observed at 5 and 10 Gy. Furthermore, at higher doses (20 and 50 Gy), a severe lethal effect was observed. Mutations at the DNA level of axillary bud-derived shoots were performed by RAPD. The mutation frequency at 10 Gy was about 1.7 and 2.1 times higher than that at 2.5 and 5 Gy, respectively. When artemisinin production was investigated in 72 irradiated mutants, about 14 and 7% of them showed higher artemisinin contents and higher artemisinin yields compared to the control, wild type plants, respectively. The highest artemisinin content in mutants was 1.43% DW, which was 3.2-fold higher than the control. On the other hand, the highest artemisinin yield in mutants was 3.68 mg/plant, which was 1.4-fold higher than the control. In addition, extracts from irradiated mutants exhibited an antibacterial activity against *Staphylococcus aureus*, but those from the control did not. Clear differences in the metabolic fingerprint were observed between the control and irradiated mutants by GC-MS analysis.

In order to modify only specific traits, gibberellin biosynthetic (*TfGA3ox* and *TfGA20ox2*) and metabolic (*TfGA2ox2*) genes from *Torenia fournieri* were transferred to *A. annua* by *Agrobacterium*-mediated transformation. After selection and confirmation, only *TfGA20ox2*-overexpressing transgenic plants were successfully obtained. Compared with the control, non-transgenic plants, all the transgenic plants showed higher plant heights and higher artemisinin contents. The highest artemisinin content in *TfGA20ox2*-overexpressing transgenic plants was 0.72% DW, which was two-folds higher than the control. In addition, the highest artemisinin yield in the transgenic plants was 1.16 mg/plant, which was 1.9-fold higher than the control.

The present study showed the validity of heavy-ion beam irradiation and *Agrobacterium*-mediated transformation with the gibberellin biosynthetic gene for improving artemisinin contents in *A. annua*. The mutants and transgenic plants obtained in the present study may be useful as valuable materials for both functional genetic studies and breeding program of *A. annua*.