EFFECT OF HEAVY-ION BEAM IRRADIATION ON *IN VITRO* CULTURED PLANT ORGANISMS

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Abstract

Growth pattern, morphological characters and molecular characters were investigated in carbon ion beam-irradiated orchid rhizomes. The ion beam irradiation significantly inhibited growth of the rhizomes as the beam dose increased. Even the frequency showed low, plants with abnormal chlorophyll containing leaves were induced at only 10Gy among the shoot induced plantlets. Some of the abnormal rhizomes were propagated and regenerated. Scorable products from 20 primers were obtained by RAPD analysis and most of the plants showed the similar DNA band patterns between control and the ion beam treated rhizomes. While the ion beam-treatment specific DNA bands were appeared in AFLP analysis of the ion beam-treated rhizomes.

INTRODUCTION

The use of physical mutagens, such as ionizing radiation and ion beam contributes basal materials for analysis of plant functions and/or for plant breeding. Since the establishment of the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture, more than 2000 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in 50 countries. In vegetatively propagated plants, many of mutants were derived from irradiating rooted stem cuttings and detached leaves [1]. Breeding of orchids has been conducted for more than 100 years, and mutation inductions by using *in vitro* cultured plant organisms have been increased [1-3]. It has known that irradiation of a new energy mutagen, heavy-ion beam has produced broad kinds of variants in the plants [3]. This study was conducted to examine an effect of ^{12}C-ion beam irradiation on morphological and molecular changes of orchid plant organisms that cultured *in vitro*.

MATERIALS AND METHODS

Two kinds of orchid rhizomes from *Cymbidium kanran* and *Cymbidium geringii* were *in vitro* cultured on modified Murashige and Skoog's medium [4], and the rhizomes plated on plastic petri-dishes were irradiated with 10Gy and 20Gy of ^{12}C-ion beam [RARF(135MeV/n) at RIKEN, Japan]. The ion beam-irradiated orchid rhizomes were subcultured on new media after 2 weeks.

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Then the rhizomes were cultured on rhizome induction media and/or shoot formation media (MS medium containing 0.1mg/L NAA and 10mg/L BAP) under the continuous fluorescence illumination (30μmole/m^2/sec) at 25±2°C. pH was adjusted to 5.8±0.1 before autoclaving at 121°C for 15 min. Arbitrary 10-mer primers (Operon Tech. Inc.) were used for the PCR based on the protocol of Willams et al. [5]. AFLP analysis was executed by the method of Mace et al. [6].

RESULTS AND DISCUSSION

Growth and morphological changes were observed in the carbon ion beam irradiated rhizomes of two orchid varieties. The carbon ion beam irradiation significantly inhibited growth of the rhizomes as the beam dose increased from 10Gy to 20Gy, when the rhizomes of two varieties were cultured on 30days after the irradiation (Fig. 1). Most of the rhizomes did not produce new rhizomes in the 20Gy of the beam treatment and did not produce new rhizomes even in one-year culture. Thus the optimal dose for root and shoot differentiation was 10Gy in the orchid cultures. The optimal dose was lower than that of the PLB (protocorm-like body) cultures of *Cybidium* [2].

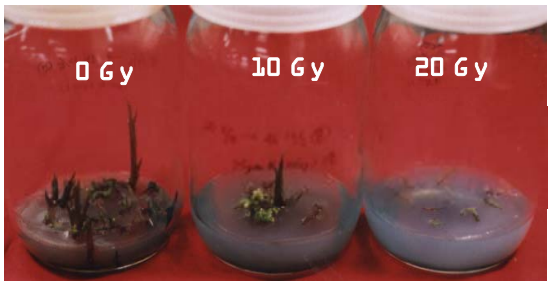

Figure 1: Shoot induction of ^{12}C ion beam irradiated orchid rhizomes in 30 days culture. *In vitro* plant organisms were subcultured on MS medium containing 10mg/l BAP and 0.1mg/l NAA.

Even the frequency showed low, plants with abnormal chlorophyll containing leaves were induced at only 10Gy among the shoot induced plantlets (Fig. 2) and albino rhizomes were induced. The abnormal spectrum of shoot leaves was more various than that of chemical-treated mutants [3, 7]. Some of the abnormal rhizomes were propagated and regenerated in order to select regular variant lines.

RAPD analysis and AFLP analysis by using the ion beam-irradiated rhizomes were carried out. Scorable products from 20 primers were obtained by RAPD analysis and most of the plants showed the similar DNA band patterns between control and the ion beam treated rhizomes. While the ion beam-treatment specific DNA bands were appeared in AFLP analysis of the ion beam-treated rhizomes (Fig. 3). To certify whether the DNA bands are ion-beam specific or not, further studies such as DNA sequencing and DNA database searching are needed.

Figure 2: Profiles of abnormal chlorophyll containing leaves induced from ^{12}C ion beam irradiated rhizomes. Plant organisms were subcultured on MS medium containing 10mg/l BAP and 0.1mg/l NAA.

CONCLUSION

An effect of ^{12}C-ion beam irradiation on morphological and molecular changes of orchid plant organisms was determined. Heavy-ion beam is a new mutagen and it gives a broad variation spectrum in abnormal chlorophyll variant induction. The variants will be used as basal materials for analyzing photosynthesis functions and plant breeding materials. From the AFLP analysis the ion beam-treatment specific DNA bands were observed**.**

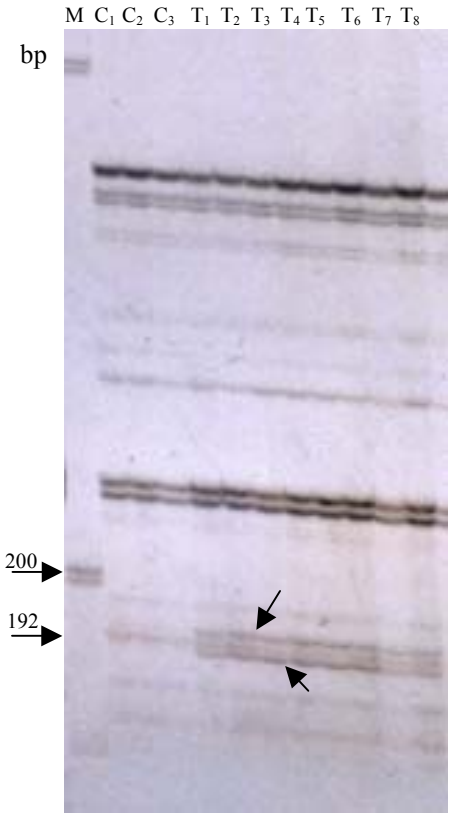

Figure 3: AFLP patterns of orchid rhizomes of control (C_1 to C_3) and ion beam treatment (T_1 to T_8) with the primers (E+ACA/M+CAG).

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