## Development of flower color mutations from the light-yellow mutant of spray-mum 'Southern Chelsea' by heavy-ion beam re-irradiation

M. Tamari,<sup>\*1</sup> G. Watanabe,<sup>\*1</sup> F. Tojima,<sup>\*1</sup> Y. Hayashi,<sup>\*2</sup> and T. Abe<sup>\*2</sup>

Heavy ion beam irradiation induces plant mutation effectively, and is used for plant breeding.<sup>1)</sup> In Kagoshima prefecture, we induced flower color mutation on a pink-colored spray-mum cultivar 'Southern Chelsea' (Registration number: 17847), using heavy ion beam or soft X-ray irradiation. As a result, a yellow flower 'Southern Chelsea Yellow' (Registration number: 26523) was developed from the pink-colored flower color. However, pure white mutants have not been obtained from previous mutagenesis experiments. We also reported that stem segments are more suitable for producing 'Southern Chelsea' mutants using Ar-ion beam irradiation.<sup>2</sup>) In this report, we describe the flower color variation that appeared due to the reirradiation of heavy ion beam on the in-vitro cultured stem of the light-yellow mutant (B25CL-16) obtained from 'Southern Chelsea.'

Cultured stem segments of 'B25CL-16' with an axillary bud were irradiated with Ar-ion beam (LET: 280 keV/  $\mu$ m) or C-ion beam (LET: 23 keV/ $\mu$ m) at doses from 0.5 to 3 Gy, and each treatment was carried out with 40 stems. After irradiation, the stems of each treatment were cultured using in vitro propagation. One month later, the elongated shoots of these tissues were subcultured with the axillary bud sections to separate the chimeric mutant sectors, and this was repeated twice. The plantlets grown from subcultured nodes were transferred to a greenhouse to investigate the flower color mutation in August flowering cultivation.

The numbers of flower-color mutants obtained by Ar-ion or C-ion beam irradiation were 89 out of 316 and 26 out of 275, respectively (Table 1, Fig. 1). In the mutation induction of 'B25CL-16,' the mutants in which the yellowish-white of flower petal were observed also included the non-irradiated group. In the cultivated chrysanthemum, the yellow pigment of the flower petal is almost carotenoid, and the white flower color is known to be obtained by the expression of the gene encoding carotenoid cleavage dioxygenase 4a (CmCCD4a).<sup>3)</sup> The yellow color mutants were thought to be decreased expression of CmCCD4a. The mutants of yellowish-white colors needs to be investigated with regard to the petal pigment in order to clarify the relationship between the gene and secondary metabolite.

The present study was supported by the Council for Science, Technology and Innovation (CSTI), Crossministerial Strategic Innovation Promotion Program

Table 1. Flower-color mutation induced by heavy-ion beam irradiation.

	Variation source				Number of Flower-color mutants			Number	Mutation
	Line class	LET (KeV/ µm)	Dose (Gy)	Number of plants <sup>1)</sup>	Yellowish -white	Yellow	Yellowish- white streak	of mutants	rate(%)
	Ar	280	0.5	95	1		13	14	14.7
			1	94	14	1	5	20	21.3
			2	89	15	19	4	38	42.7
			3	38	17			17	44.7
			Total	316	47	20	22	89	28.2
			0	36	3			3	8.3
	С	23	1	96	2		12	14	14.6
			2	95			4	4	4.2
			3	84	5		3	8	9.5
			Total	275	7		19	26	9.5
			0	36	1		3	4	11 1

1)Regenerated and flowered plants after irradiation

Fig. 1. 'B25CL-16'(A) and flower color mutation B: Yellow C: Yellowish-white steak.

(SIP), "Technologies for creating next-generation agriculture, forestry and fisheries" (funding agency: Biooriented Technology Research Advancement Institution, NARO).

References

- 1) T. Abe *et al.*, Breed. Res. **16**, 67 (2014).
- M. Tamari *et al.*, RIKEN Accel. Prog. Rep. **50**, 274 (2017).
- A. Ohmiya, Agriculture and Horticulture, 82, 11, 1153– 1160 (2007).

<sup>\*1</sup> Kagoshima Prefectural Institute for Agricultural Development

<sup>\*&</sup>lt;sup>2</sup> RIKEN Nishina Center