

## Induction of flower color mutants by heavy-ion irradiation to leaf blades of spray-mum ‘Southern Chelsea’

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Heavy-ion beam irradiation effectively induces mutations and has been used in plant breeding<sup>1)</sup>. Ar-ion beam irradiation is expected to provide a different spectrum of mutant phenotypes from the C-ion irradiation due to their different LET values. Therefore, we irradiated Chrysanthemum tissue with an Ar-ion beam in order to obtain flower color mutants. We previously reported the induction of flower color mutants by Ar-ion irradiation to cuttings of spray-mum ‘Southern Chelsea’, which was developed by Kagoshima Prefecture<sup>2)</sup>. In addition, we reported the effects of Ar-ion beam irradiation on the regeneration of leaf blades<sup>3)</sup>. Here, we report the induction of flower color mutants by leaf blades irradiation.

Leaf blades of the spray-mum cultivar ‘Southern Chelsea’, having pink flowers, were irradiated with an Ar-ion beam (LET: 310 keV/μm) at doses of 0.1, 0.3, 0.5, 1, 2, and 3 Gy, and a C-ion beam (LET: 23 keV/μm) at doses of 1, 2, and 3 Gy. After irradiation, these tissues were cultured in vitro. Adventitiously regenerated buds were cut and grown to plantlets in vitro and they were transferred to a greenhouse to investigate the occurrence of flower color mutations.

Twenty plants among 1,716 irradiated regenerants showed flower color mutation [Table 1]. The regenerants were white, light pink, deep pink, light reddish yellow, and orange, suggesting that increase, decrease, disappearance of anthocyanin and the increase of carotenoid were induced in these mutants, respectively [Table 1].

These results were the same as those obtained by irradiation to cuttings with a C-ion beam. The mutation rates in flower color changes with Ar-ion beam irradiation were 0.5-2.6% at 0.1-1 Gy, and those with C-ion were 0.8-1.4% at 1-3 Gy [Table 1]. Irradiation to cuttings could cause simultaneous mutation in both anthocyanin and carotenoid content and yellow flower mutants were obtained<sup>2)</sup>. However, irradiation to leaf blades could not cause the simultaneous mutation in the anthocyanin and carotenoid content in this examination. Some mutants also showed flower-shape variation by Ar-ion beam irradiation [Figure 1]. Relative regeneration rate decreased to less than 10% under Ar-ion irradiation at doses of 2 Gy, and no flower color mutants were obtained at these doses. All of the regenerated plants irradiated with Ar-ion beam showed less growth than non irradiated control plants.

Ar-ion beam irradiation to leaf blades is not thought to be suitable for practical breeding in ‘Southern Chelsea’ because its regeneration rate and mutation rates are lower than that of cuttings in flower color. Currently, we are investigating the stability of flower color of the mutants derived from Ar-ion irradiation.

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

Variation source	Regeneration rate <sup>1)</sup> (%)	Number of plants <sup>2)</sup>	Number of Flower-color mutants							Number of mutants	Mutation rate (%)	
			White	Light pink	Deep pink	Deep reddish yellow	Light reddish yellow	Orange	Other			
Ar	0.1	95.6	420		2	3					5	1.2
	0.3	90.9	438								2	0.5
	0.5	65.9	264			2			1	1	4	1.5
	1	37.8	192	1						4	5	2.6
	2	8.7	6								0	0
	3	3.0	12								0	0
Total		1,332	1	4	5	0	1	5	0	16		
C	1	83.8	138			1					1	1.4
	2	64.3	114							1	1	0.9
	3	46.2	132	1						1	1	0.8
	Total		384	1	0	1	0	0	0	0	2	4
0	100	84								0	0	

1) The relative regeneration rate means the rate of regenerated shoots after the ion beam irradiation to radiationless regenerated shoots which were developed from directly adventitious shoots.

2) Regenerated and flowered plants after irradiation.

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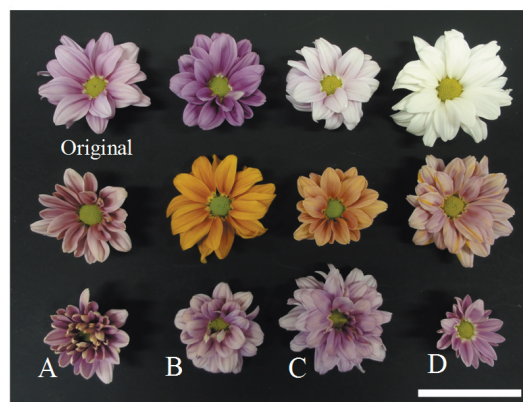


Fig.1 Spectrum of mutant phenotypes. (A,B,C,D) Mutants showing different flower shapes. Bars = 5cm.

### References

- 1) T. Abe et al., *Breed. Res.* **16**, 67 (2014).
- 2) Y. Tanokashira et al., *RIKEN Accel. Prog. Rep.* **47**, 297 (2014).
- 3) Y. Tanokashira et al., *RIKEN Accel. Prog. Rep.* **48**, 306 (2015).