Production of flower color mutants of spray-mum 'Southern Chelsea' by Ar-ion beam irradiation

M. Tamari,[*1] Y. Tanokashira,[*1] Y. Hayashi ,[*2] T. Hirano,[*2,*3] and T. Abe[*2]

Heavy-ion beam irradiation effectively induces mutations of plants and has been used in plant breeding.[1] Ar-ion beam irradiation is expected to expand the spectrum of mutant phenotypes and mutation rate owing to its higher LET compared to a C-ion beam. We previously reported the induction of flower-color mutants of spray-mum cultivar 'Southern Chelsea' cuttings using Ar-ion beam irradiation.[2] In addition, we reported the effects of Ar-ion beam irradiation on the regeneration from leaf blades[3] and speculated that Ar-ion beam irradiation on leaf blades was not practical because of the low relative regeneration fequency of less than 10% at doses of 2 Gy Ar-ion beam irradiation.[4] Here, we report the production of flower-color mutants by the irradiation of stem segments of 'Southern Chelsea.'

Eighty and forty stem segments of 'Southern Chelsea,' were irradiated with Ar-ion beams (LET: 280 keV/μm) at doses of 0.5, 1, 2, and 3 Gy and C-ion beams (LET: 23 keV/μm) at doses of 1, 2, and 3 Gy, respectively. After irradiation, these tissues were cultured in vitro, and 3 nodes that had axillary buds cut out from an extended shoot were subcultured to separate mutation sites. This subculture step was repeated when shoots sprouted from axillary buds were extended sufficiently. Plantlets grown from re-subcultred nodes were transferred to a greenhouse to investigate flower- color mutation.

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

Variation source		Number of plants[1] (%)	Number of flower-color mutants						Number of mutants	Mutation rate(%)
Line class	Dose (Gy)		White	Yellow	Orange	Yellowish pink	Light pink	Other		
Ar	0.5	171							0	0
	1	165						1	1	0.6
	2	151	2	1	2	8		1	14	9.3
	3	74	1		4		1		6	8.1
	Total	561	3	1	6	8	1	2	21	3.7
	0	12							0	0
C	1	111							0	0
	2	102							0	0
	3	101			1				1	1.0
	Total	314	0	0	1	0	0	0	1	0.3
	0	46						1	1	2.2

1) Regenerated and flowered plants after irradiation.

The numbers of flower-color mutants obatined by Ar-ion beam irradiation at 1, 2, and 3 Gy were 1, 14, and 6, respectively (Table 1). On the other hand, only one flower-color mutant was obtained by C-ion beam irradiation at all doses (Table 1).

The flower colors of mutants obtained by Ar-ion beam irradiation were white, yellow, orange, yellowish pink, and light pink (Table 1). This fact suggests that the direction of flower-color mutation by Ar-ion beam irradiation is the decrease or disappearance of anthocyanin and increase of carotenoid. Especially, one yellow-flower mutant, which was thought to be difficult to obtain from 'Southern Chelsea,' having pink flowers, appeared after Ar-ion beam irradiation at 2 Gy (Table 1). It is thought that this yellow-flower mutant was caused by the disappearance of anthocyanin and simultaneous increase of carotenoid. Some mutants also showed the morphological variation of flower shape because of Ar-ion beam irradiation (data not shown). The growth of many mutants from stem segments with Ar-ion beam irradiation was equal to that of control plants (data not shown). Because the growth of almost all mutants from leaf blades by the same irradiation condition was lower than that of control plants,[4] this result suggests that stem segments will be more suitable for producing mutants of spray-mum using Ar-ion beam irradiation.

From the results presented above, it has become possible to produce many flower-color mutants of spray-mum by Ar-ion beam irradiation to stem segments. On the basis of these results, it is supposed that the low rate of regeneration and mutation by Ar-ion beam irradiation in several spray-mum cultivars will be improved by using stem segments as irradiation targets.

The present study was supported by the Council for Science, Technology and Innovation (CSTI), Cross-ministerial Strategic Innovation Promotion Program (SIP), "Technologies for creating next-generation agriculture, forestry and fisheries" (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).

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).
4) Y. Tanokashira et al.: RIKEN Accel. Prog. Rep. **49**, 261(2016)

*1 Kagoshima Prefectural Institute for Agricultural Development
*2 RIKEN Nishina Center
*3 Faculty of Agriculture, University of Miyazaki