## Flower color mutants of chrysanthemum obtained using C-ion beam irradiation

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Chrysanthemum is a major agricultural product in Hyogo Prefecture. We tried to use ion beams to induce flower color variation in chrysanthemum. We irradiated 53 cultivars of chrysanthemum with carbon beams. We have previously reported their mutant frequency and flower color mutants.<sup>1)</sup> We tried to increase variation in flower color by using a single origin. We irradiated flower color mutants from 'Benitsubaki' with C-ion beams and obtained more mutants.

'Benitsubaki' is a purplish red small mum that blooms at the end of November (Fig. 1). In 2009, we irradiated cuttings with C-ion beams (energy, 135MeV/nucleon; LET, 23 keV/ $\mu$ m) at doses of 4 and 6 Gy. After irradiation, herbaceous cutting was performed, and four weeks later, fix planting was carried out in a glass house. Cultivation conditions were no pinching and no picking the bud. Other conditions for cultivation were standard. At the time of full bloom, we observed the mutation rate with respect to flower shapes. Stability of the mutation was confirmed in 2010. We irradiated the cuttings of VT4Pi and VT6RB mutants with C-ion beams at doses of 4 and 6 Gy in 2011. Other conditions were the same in 2009. Relative DNA contents of somatic nuclei in 'Benitsubaki' and mutants derived after re-irradiation were compared using flow cytometry.

The mutation frequencies of 'Benitsubaki' were 6.3% at 4 Gy and 4.3% at 6 Gy. We obtained two flower color mutants, deep pink (VT4Pi) and red (VT6RB), that bloomed at the end of November. These mutations were stable in 2010. Since the flower color of VT4Pi was not different from that of the original 'Benitsubaki', we tried re-irradiation to obtain more varied mutants.

The mutation frequencies of VT4Pi were 7.8% at 4 Gy and 3.9% at 6 Gy. We obtained 4 flower color mutants from VT4Pi (Fig. 1). One of them was double-colored, purplish red outside and white inside (VT4Pi6WT-Wh), that bloomed at the middle of December. Two mutants were also double-colored flowers that bloomed at the end of December and were deep pink outside and white inside (VT4Pi6Pi-Wh) and light pink outside and white inside (VT4PiLtPi-Wh). The fourth mutant was also double-colored, orange and outside yellow inside (VT4Pi6Or-Yr) and bloomed at the middle of December. The difference among three mutants, VT4Pi6WT-Wh, VT4Pi6Pi-Wh and VT4PiLtPi-Wh, was the strength of the color. The mutation frequencies of VT6RB were 7.3% at 4 Gy and 14.3% at 6 Gy. The yellow mutant (VT6RB6Yr·

RB) bloomed in the middle of December (Fig. 1).

Although this mutant had a reddish bud, the flowers were bright yellow with green core.

Flower color changes under the influence of cultivation temperature. We are currently carrying out cultivation experiments to reveal the effect of temperature on the blooming period and flower color of these mutants. In terms blooming period, all the obtained mutants bloomed late. The mutation frequency by re-irradiation was higher than that after a single irradiation. The relative DNA contents in the mutants obtained after re-irradiation did not decrease to that in 'Benitsubaki'. These results indicate that ion-beam re-irradiation of mutants is effective in increasing the variety of mutants.



Fig. 1 'Benitsubaki' and its flower color mutants

References

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<sup>1)</sup> K. Tamaki et al.: RIKEN Accel. Prog. Rep. 44 p.276-277 (2010).