Irradiation of seeds with C-ion and Ar-ion beams for *Streptocarpus* mutagenesis to understand leaf meristem genetics

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Streptocarpus Lindl. (Gesneriaceae) attract developmental biologists owing to their variable shoot/leaf forms, which include caulescents with ordinary shoot apical meristem (SAM) and acaulescent (unifoliates and rosulates) lacking SAMs (*e.g.*, Figs. 1a–c). Anisocotyly (Fig. 1b), the unequal cotyledon development producing a macrocotyledon also marks this genus/family.[1)] *Streptocarpus rexii* Lindl. is rosulate and has been studied as a model to understand their unique cotyledon and leaf development. Previous results have shown relocated expression of SAM genes in their leaf meristems.[1)] Thus, the genetic and functional shift of meristems from the shoot apex laterally to the leaf might result in their unique acaulescent forms. This work is important to understand plant meristems and their effects on plant form. To date, a genetic map, transcriptome, and genome have been generated for *S. rexii*,[2)] which are important resources to isolate the underlying genetic loci. Here, we use forward genetics utilizing heavy-ion mutagenesis to facilitate this aim.[3)]

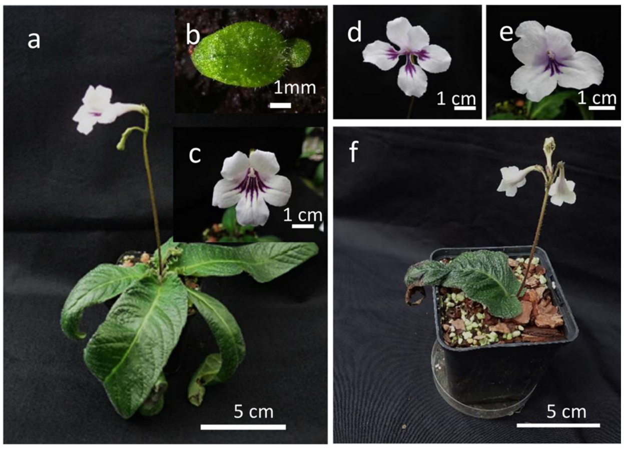

Fig. 1. Images of *Streptocarpus rexii* wild type and heavy-ion irradiated mutants. a-b) *S. rexii* wild type. b) Anisocotyly. c) Wild type flower. d-e) C200 flower mutations. f) C200 qualifier "JW" M_1 plant showing delayed leaf initiation phenotype.

Seeds of *S. rexii* (RBGE 20170766*A) cultivated in the glasshouses at Royal Botanic Garden Edinburgh (RBGE) were harvested in October 2018, certified at Science & Advice for Scottish Agriculture (SASA; Phytosanitary Certificate No. SG/SASA/2018/033), and transported to RIKEN (Wako, Japan). C-ion ($^{12}C^{6+}$) and Ar-ion ($^{40}Ar^{17+}$) irradiations were conducted at RIBF. The high linear energy transfer (LET) value of C-ion was set to 30 keV μm^{-1}, and that of Ar-ion to 184 keV μm^{-1}. For C-ion, doses of 50, 100, 150, 200, 300 and 400 Gy, and for Ar-ion, 10, 20 and 50 Gy were used, labelled C50, C100, C150, C200, C300, C400, Ar10, Ar20, and Ar50, respectively. The lethal effects of the treatments were assessed as the germination rate and seedling growth. The seeds were sown in Petri dishes on filter paper soaked with tap water and examined 2 and 3 weeks later. The growth of seedlings (the cotyledon size) was measured 7 weeks after sowing. The flower phenotype was also observed for mutations in the M_1 generation from 20–26 plants per treatment. To obtain larger M_2 populations for screening, M_1 seeds of selected treatments were sown and cultivated. The vegetative phenotype was observed while focusing on mutations in leaf initiation. Germination was observed in all treatments; however, it was delayed in C400 and Ar50. The final germination rate was low only for Ar50 (Table 1). A strong suppression of seedling growth was observed in C300, C400, and Ar50, and all seedlings eventually died. Over 20% of M_1 plants of C150, C200, Ar10 and Ar20 showed flower mutations, such as split corollas and shift in symmetry (Figs. 1c–e).

Table 1. Effects of heavy-ion irradiation on Streptocarpus rexii.

	Germination% 2 weeks	Germination% 3 weeks	Macrocotyledon size (μm^2)	Num. of flower mutants per total plants observed
	average ± SE, *N*=10, biological triplicates or duplicate*			
Wild type	78 ± 7	78 ± 7	2724 ± 88	0/20
C50	62 ± 2	72 ± 2	6380 ± 1564	2/23
C100	81 ± 3	83 ± 2	4453 ± 925	1/23
C150	67 ± 1	75 ± 5	3689 ± 861	6/26
C200	70 ± 11	76 ± 6	1984 ± 38	6/26
C300	66 ± 10	71*	965 ± 63	lethal
C400	45 ± 6	73 ± 8	703 ± 36	lethal
Ar10	71 ± 13	76 ± 11	4148 ± 1064	5/24
Ar20	76 ± 4	85 ± 4	5332 ± 877	7/26
Ar50	46 ± 18	53 ± 17	964 ± 36	lethal

Considering all results together, C150, C200, and Ar20 were just below the lethal dosage, and showed clear flower mutations. Thus, they were selected for large scale mutant screening. More than 1000 M_1 plants of C150, C200, and Ar20 were cultivated to obtain M_2 seeds. The estimated fertility rates (in May

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2021) were 62, 47, and 40% for C150, C200, and Ar20, respectively. Five of C200 M_1 plants, and 25 of Ar20 showed a delayed leaf initiation phenotype (Table 1; Fig. 1f). M_2 seeds of these were harvested from four C200 and 12 Ar20 plants. Now, these mutant phenotypes were observed for mutant stability in following generations.

References
1) K. Nishii *et al.*, Dev. Genes Evol. **220**, 25 (2010).
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