Sexual reproduction observed in the loss-of-apomixis mutants of guineagrass induced by heavy-ion beam irradiation

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Apomixis, an asexual mode of reproduction, provides a method for reproducing clonal plants through the seed. By using apomixis in the breeding program, we can develop a new efficient method for fixing desirable genotypes of F_1 hybrids and simplifying commercial seed production. Therefore, we aimed to isolate the gene(s) controlling apomixis from a tropical forage grass, guineagrass (*Panicum maximum* Jacq.). In a previous study, we found two mutant lines (SM-1 and SM-2) that were suggested to have lost the apomictic pathway of reproduction and to propagate using the sexual mode of reproduction.[1), 2)] In the present study, we analyzed the mode of reproduction of these loss-of-apomixis mutants with two methods: the progeny test using amplified fragment length polymorphism (AFLP) patterns and embryo sac analysis. The results showed that they lost the apomictic mode of reproduction.

The M_1 plants of SM-1 and SM-2 were generated from the dry seeds of an apomictic cultivar "Natsukaze" irradiated with $^{20}Ne^{10+}$ (63 keV/μm) ions at 200 Gy and with $^{56}Fe^{24+}$ (624 keV/μm) ions at 20 Gy, respectively.[1)] Forty M_2 plants of each line were grown in a field. DNA from the leaves of each M_2 plant was extracted and analyzed using the AFLP method. For the embryo sac analysis, inflorescences at anthesis were fixed in Farmer's fixative (100% ethanol:acetic acid = 3:1), and then dehydrated and cleared as described previously.[3)] The cleared pistils were observed with a differential interference contrast microscope (Nomarski).

The AFLP patterns of 40 M_2 plants are shown in Fig.1. In case of the apomictic cultivar "Natsukaze", the AFLP results show the same pattern among progenies. In contrast, all the AFLP patterns of M_2 plants of both SM-1 and SM-2 were different from each other. This result suggested that SM-1 and SM-2 propagated with the sexual mode of reproduction.

To confirm the sexual mode of reproduction of SM-1 and SM-2, embryo sac analysis was performed. In the case of guineagrass, an apomictic embryo sac has four nuclei (an egg, two synergids, and a polar nucleus; Fig. 2A), while a sexual embryo sac has eight nuclei (an egg, two synergids, two polar, and three antipodal cells' nuclei; Fig. 2B). The most clear differences between the sexual and apomictic embryo sacs are whether antipodal cells are formed or not, and whether the number of polar nuclei is two or one. [3)] In the embryo sac analysis of SM-1 and SM-2, we observed a few sexual embryo sacs (Fig. 2B), many aborted embryo sacs (Fig. 2C), and no apomictic embryo sac in each line. These results suggested that they lost the apomictic pathway of reproduction.

Our previous results suggested that the deleted region in the loss-of-apomixis mutants were relatively small.[2)] We are currently searching for the genes located within these deletion regions.

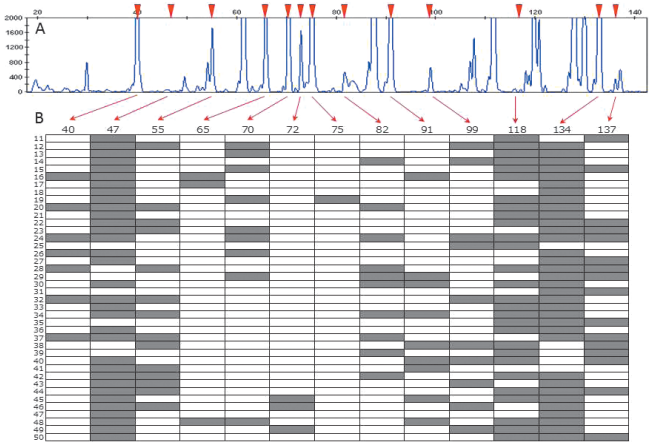

Fig. 1. AFLP patterns of M_2 plants in the sexual mutant line SM-2. (A) The AFLP pattern of "Natsukaze" detected by the ABI 3130xl capillary sequencer (as a reference). Red arrowheads indicate the loci that are polymorphic among M_2 plants. (B) Schematic presentation of AFLP patterns of M_2 plants in SM-2. White box shows the presence of the peak, and gray box shows the absence of the peak. Similar results have been obtained for SM-1 (not shown).

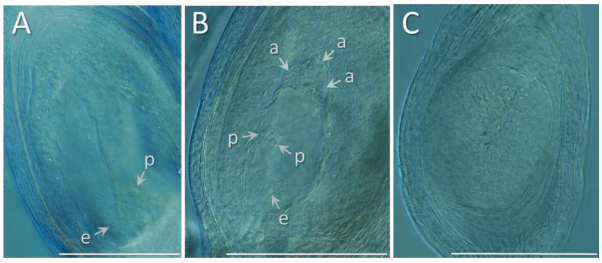

Fig. 2. Typical embryo sacs in guineagrass. (A) Apomictic embryo sac observed in "Natsukaze" (apomictic cultivar). (B and C) Sexual embryo sac (B) and aborted ovule (C) observed in the loss-of-apomixis mutants. a, antipodal cell; e, egg cell; p, polar nuclei. Synergid cells are small and difficult to observe in both types.[3)] Bar = 200 μm.

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