DNA marker analysis revealed that the deletion is relatively small in loss-of-apomixis mutants

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Apomixis produces seed progeny that are genetically identical to the mother plant. This process is widely observed among wild plant species but is almost completely absent in major crop species. Apomixis will have a great impact on agriculture through clonal seed production. As the first step of the agricultural use of apomixis, we aimed to isolate the gene(s) controlling apomixis for application in major crops. Guinea grass (*Panicum maximum* Jacq.), a major tropical forage grass, has some characteristics suitable for the study of apomixis. However, recent studies have suggested that recombination is suppressed at the apomixis-controlling locus in guinea grass. To narrow down the apomixis-controlling genomic region, we developed deletion mutants for this region by using irradiation with heavy-ion beams.[1), 2), 3)] In a previous study, we found that two mutant lines (SM-1 and SM-2) showed different AFLP patterns between M_2 progenies within each line.[3)] This result suggested that they lost the apomicitic pathway of reproduction and propagated using the sexual mode of reproduction. In the present study, we analyzed the deletion size of these loss-of-apomixis mutants with apomixis-specific sequence-tagged site (STS) markers.

The M_1 plants of SM-1 and SM-2 were generated from dry seeds (an apomictic cultivar 'Natsukaze') irradiated with $^{20}Ne^{10+}$ (63 keV/μm) ions at 200 Gy and $^{56}Fe^{24+}$ (624 keV/μm) ions at 20 Gy, respectively.[2)] Approximately ten M_2 plants of each line were grown in a field. DNA from five M_2 plants of each line were extracted from leaves and analyzed using polymerase chain reaction (PCR) with 83 apomixis-specific STS markers. The SM-1-1, SM-1-4, and SM-1-6 plants were blighted before analysis.

Fig. 1 shows that SM-1 lost four markers (CA-A14-252, CI-A1-296, CI-T1-217, and CK-T2-374) among the 83 analyzed markers (4.8%). In case of the SM-2 line, two markers (CA-A3-354 and CA-A11-355) among the 83 analyzed markers (2.4%) were lost in only three M_2 plants (SM-2-3–5). Fig. 1 also shows that SM-2-1 possessed all markers, suggesting that it had an intact apomixis chromosome. In contrast, SM-2-2 lost all markers, suggesting that it lost the entire apomixis chromosome. These results can be explained by the exchange of chromosomes during the process of sexual reproduction in SM-2. SM-2-3–5 were suggested to have a partially deleted apomixis chromosome.

These results suggested that the size of the deletion in SM-1 and SM-2 was relatively small, probably several percent of the apomixis-controlling genomic region. Previously, we obtained 22 other mutants in which the apomixis-controlling genomic region was partially deleted, and these mutants lost 2–12 STS markers out of a total of 48 markers (4.2–25%).[3)] Compared to these mutants, the size of the deletion in SM-1 and SM-2 were suggested to be relatively small. However, we could not estimate the exact physical size of these deletions because the whole DNA sequence of this region has not been obtained. Sequencing and scaffolding of BAC clones of this region is currently in progress. This result will be useful in estimating the exact size of the deletions and in searching for the genes within these deletion regions.

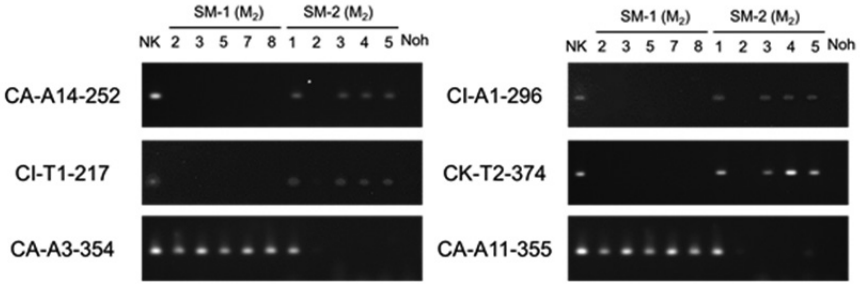

Fig. 1 PCR analysis using STS markers lost in SM-1 or SM-2 mutant line. Five M2 individuals were analyzed in each mutant line. An apomictic cultivar 'Natsukaze' (NK) and a sexual line 'Noh PL1' (Noh) were used as the positive and negative controls, respectively.

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