Hybrid sterility between Asian and African rice can be mitigated by tetraploidization

Almost all rice varieties cultivated worldwide belong to the Asian rice (*Oryza sativa*), and modern rice varieties with high yielding capacity and good eating quality are developed through intraspecific crosses within *O. sativa*. In contrast, the African rice (*O. glaberrima*), cultivated only in limited regions of West Africa, exhibits distinct traits such as resistance to certain pests and diseases and adaptability to low-fertility soils. Hybridizing these two species may enable the development of varieties that can be cultivated in environments where *O. sativa* cultivation has been difficult. However, interspecific F_1 hybrids between *O. sativa* and *O. glaberrima* exhibit severe sterility of pollen grains (hybrid sterility), preventing seed production; thus, it is essential to develop fertile interspecific hybrids to overcome this sterility.

In this work, we investigated the relationship between hybrid sterility and ploidy level of the hybrid and found that tetraploid F_1 hybrids can restore pollen fertility and set seeds by self-pollination.

We examined the pollen fertility of F_1 hybrids between an *O. glaberrima* variety (Og) and three *O. sativa* varieties (Os1, Os2, and Os3 belonging to the *temperate japonica*, *indica*, and *aus* subspecies, respectively), and found that while diploid F_1 hybrids showed 0% pollen fertility (complete sterility), tetraploid F_1 hybrids exhibited pollen fertility ranging from 5.7% to 28.1%, enabling self-pollination (Fig. 1).

Genetic analysis of the fertile microspores in the tetraploid hybrids provided insight into the fertility restoration mechanism (Fig. 2). For example, in the *S2* locus, which is one of the loci inducing pollen grain sterility in the hybrid, all microspores carrying the *S2* allele from *O. glaberrima* would be sterile in diploid hybrids. However, they could maintain fertility in the tetraploid situation by carrying the *S2* allele from *O. sativa* simultaneously.

These results suggest that employing tetraploid hybrids can facilitate genetic exchange between the two species, allowing for the development of diverse hybrids with valuable traits, which was difficult in the diploid situation. Furthermore, diploid hybrids between the two species could be developed successfully by rescuing the fertile microspores in the tetraploid hybrid through anther culture methods. Further investigation on pollen fertility and agronomic traits of these diploid hybrids will be required to estimate their usability as hybrids with intermediate genomic compositions between the two species.

Authors: Kuniyoshi, D. [JIRCAS], Ishihara M., Yamamori K., Koide Y., Kishima Y. [Hokkaido Univ]

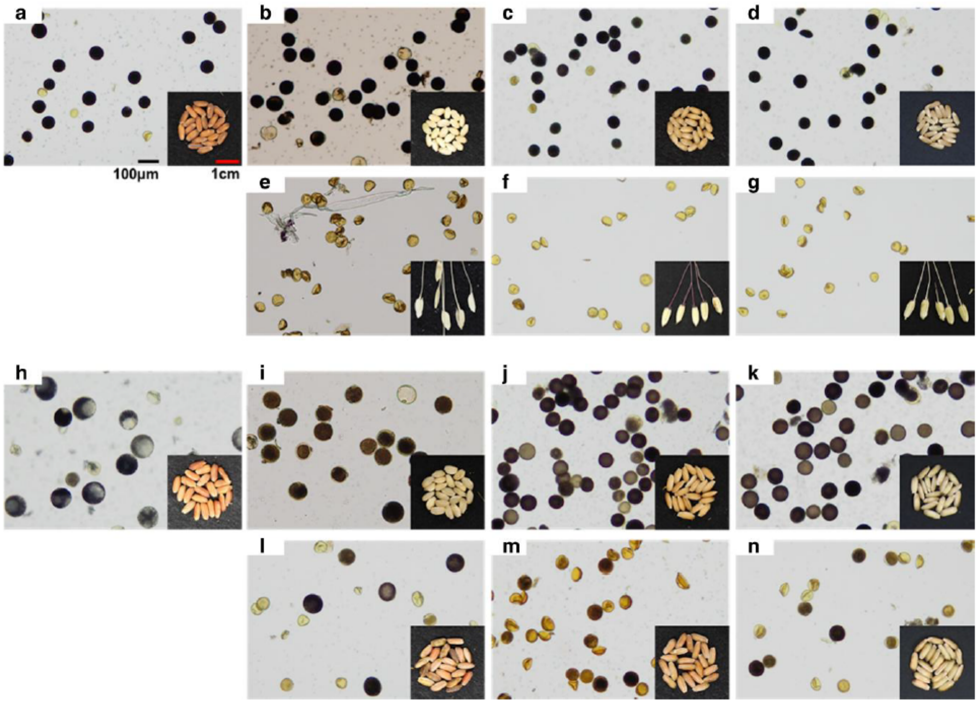

Fig. 1. Observation of fertility of pollen and self-pollinated seeds

Pollen samples were stained with I_2-KI staining then observed. Fertile pollen: black color. Sterile pollen: yellow to brown color.

a–g: Pollen and seeds in the diploid materials.

a: *O. glaberrima* variety (Og); b–d: *O. sativa* varieties (Os1, Os2, Os3). Os1, Os2, and Os3 belong to *temperate japonica*, *indica*, and *aus* subspecies, respectively.

e–g: Og/Os1, Og/Os2, Og/Os3 F_1 hybrids. Diploid hybrids were pollen-sterile, resulting in no seed set.

h–n: Pollen and seeds in the tetraploid materials.

h: Og; i–k: Os1, Os2, and Os3.

l–n: Og/Os1, Og/Os2, and Og/Os3 F_1 hybrids in the tetraploid situation. Pollen fertility was restored in the tetraploid situation; thus, self-pollinated seeds were obtained for all three combinations.

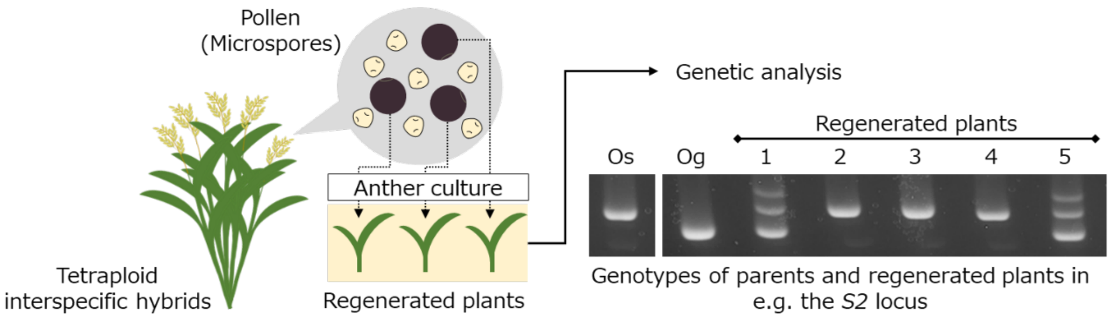

Fig. 2. Genetic analysis of hybrid sterility loci

Diploid plants were produced from fertile microspores of the tetraploid hybrids and analyzed. The hybrid sterility loci (e.g. the *S2* locus) which induce sterility of pollen were targeted for this analysis. The image of gel electrophoresis represents the result of analysis of the *S2* locus: among the plants induced from the fertile micropores, three plants (2, 3, and 4) carried the *S2-Os* allele while two (1 and 5) carried both *S2-Os* and *S2-Og*. Basically, microspores carrying the *S2-Og* allele would be sterile, but this result confirmed that they could maintain fertility in the tetraploid hybrid by carrying it with the *S2-Os* allele.