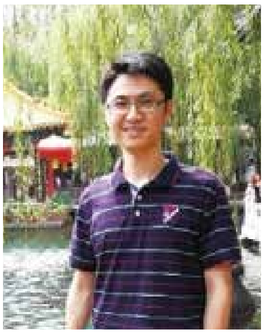
TKK Young Scientist Award in Life Sciences

Fixing Hybrid Vigor by Making Clonal Seeds

By YAN Fusheng (Staff Reporter)



Prof. WANG Kejian
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The 2020 TKK Young Scientist Award in Life Sciences went to Prof. WANG Kejian from the China National Rice Research Institute, the Chinese Academy of Agricultural Sciences (CAAS), for his achievement in clonal reproduction of hybrid rice through seeds, which thereby opens up a new route to fix hybrid vigor and one day may spare the trouble of repeatedly making the hybrid seeds each year.

Hybrid vigor, or 'heterosis', is a phenomenon in which hybrid offspring are superior to either of their parents in many aspects, such as growth rate and fitness, and has thereby been exploited by breeders to produce many elite crops.

The first success was made with corn in 1930s, and almost every corn we eat today is a hybrid. Later in the 1970s, the development of hybrid rice, known as the "three-line" and "two-line" hybrid system, greatly boosted rice yield in China.

There is, however, a big problem for these hybrid crops: You can't keep seeds from the hybrids. Because

The hybrids (left) appear the same, while their offspring (right) are quite different in many ways, such as growth rate, fitness and yield. (Credit: WANG's Lab) the seeds harvested from these elite hybrids would grow into different plants with different traits, some of them may grow slowly, some may only yield very few seeds. In other words, things would turn out differently for their offspring, which was caused by 'genetic segregation', a phenomenon shared by all sexually-reproducing organisms in spreading their genes.

Because of that, the breeders have to produce the hybrid seeds year by year, which is a very laborious job. Finding a way to bypass genetic segregation, and thereby allow the hybrids to propagate clonally through their own seeds, has therefore been strongly pursued by breeders worldwide.

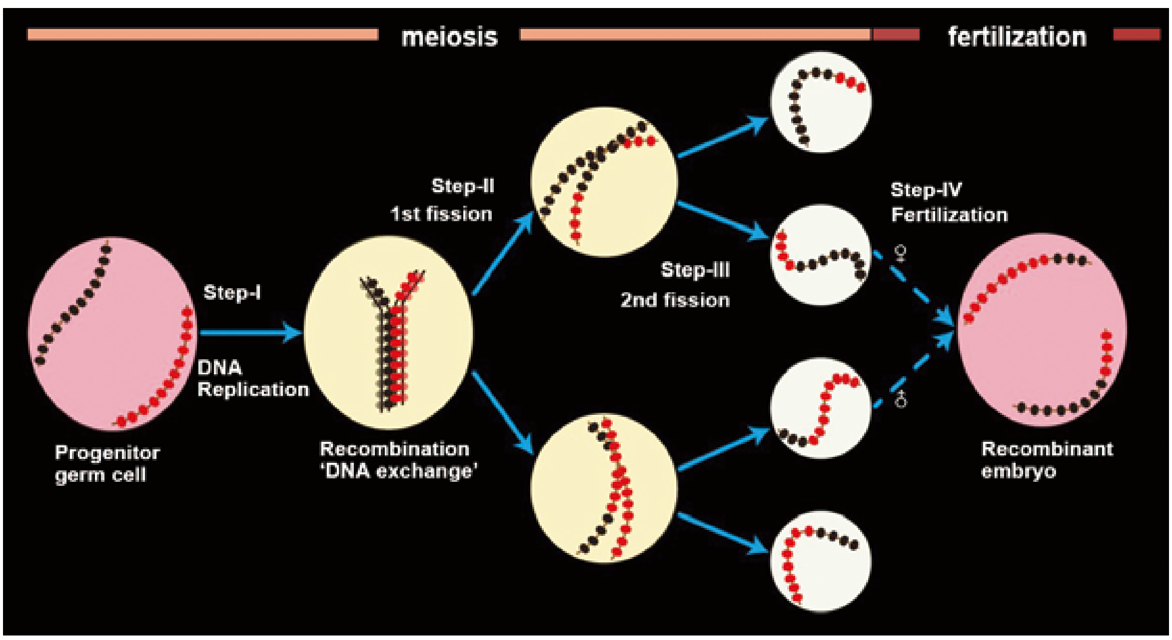
It seems nature has already shown us the way. Over 400 plant species, including many common fruits such as apple and orange, were found capable of producing 'clonal seeds' that have an identical genome to the mother, requiring no need of a father's participation. Unlike the normal seeds that tend to grow differently, clonal seeds of the same origin will grow uniformly, just like their mother. This type of asexual reproduction is also called 'apomixis'.

Though apomixis has not been found in major crops, this gave crop breeders a hope that there may exist a secret passage that could ferry sexual reproduction to apomixis and thereby allow the hybrids to clonally propagate through their own seeds. Finding this secret passage thereby became the 'holy grail' in plant biotechnology and agriculture.

Since then, many breeders attempted to understand how apomixis works in nature and discovered many apomixis-related genes. Their attempts in applying these genes for crop apomixis were impressive but ultimately futile. Then they became discouraged and most of them discontinued their pursuits.

To tackle this problem directly, WANG's Lab sought to use gene-editing technology to cripple meiosis – a cell division process in which a progenitor germ cell undergoes two fissions of the nucleus and giving rise to four gametes, or sex cells (pollens, ova and sperms), each possessing half the number of chromosomes of the original cell – to see whether meiosis can be degenerated to mitosis, another form of cell division that produces a copy or 'clone' of the original cell.

In early experiments, they crippled the first step of meiosis, in which chromosomes replicate and get close in

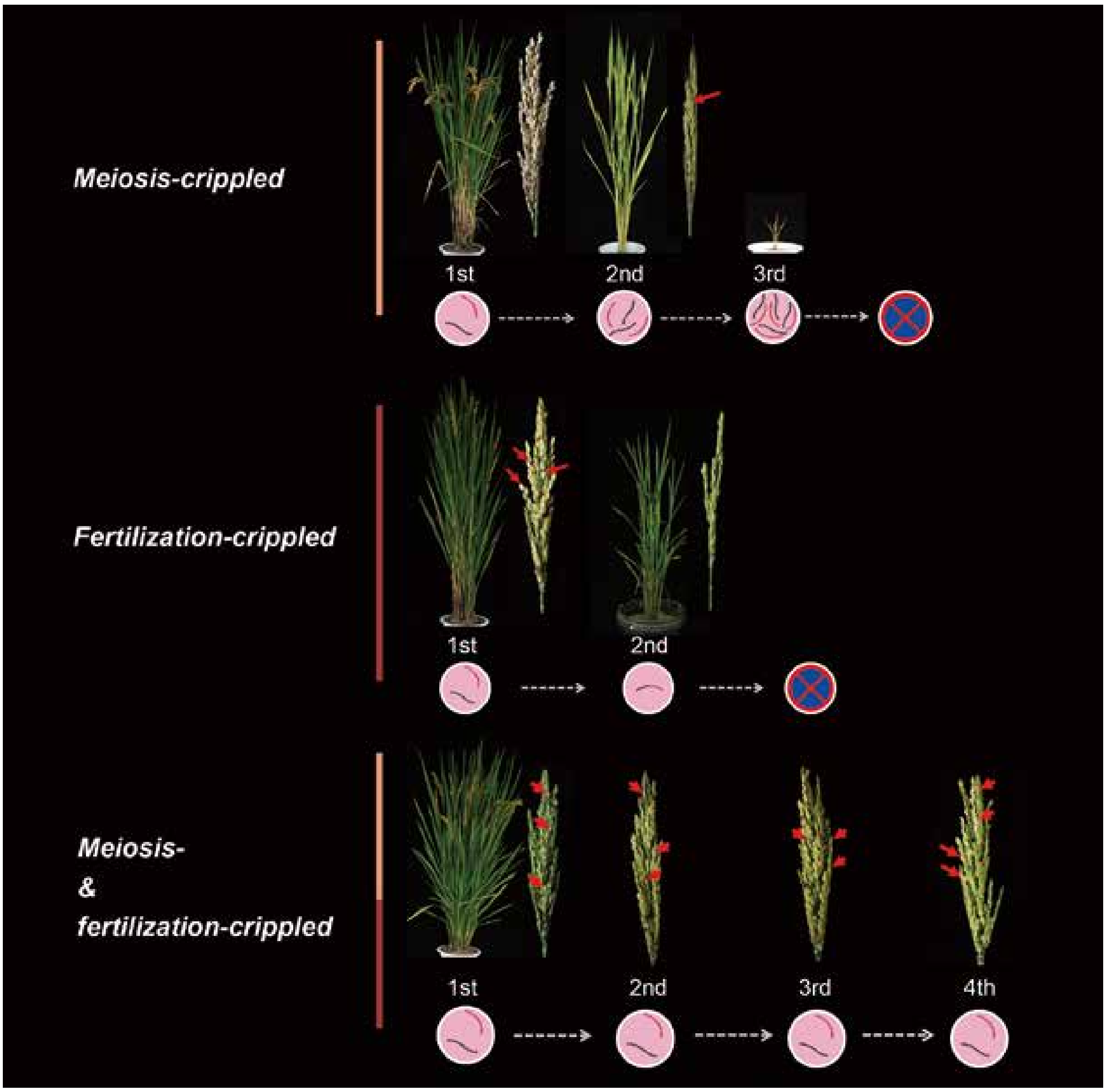


Schematic illustration of meiosis and fertilization, which together form the cycle of sexual reproduction. The male or female progenitor germ cell, likes any body cell having two sets of paired chromosomes as indicated by black and red chains of beads (diploid), is designed to form gametes, cells of fungal spores, plant's pollen, or animal's sperms and eggs, which have a single set of unpaired chromosomes (haploid). Meiosis causes DNA exchanges between the paired chromosomes and gives rise to male or female gametes of different genetic backgrounds, which is the cause for genetic segregation. The fusion of male and female gametes recovers the ploidy and thereby forms genetically different embryos. (Image by WANG's Lab) pairs to exchange DNA, and found that the gene-edited hybrid rice grew normally but failed to yield seeds. When they crippled the first two steps at the same time, they still got no seeds.

"These results were expectable, because the processes of meiosis are essentially quite the same for all sexually-reproducing organisms, no matter as small as fly and mosquito, or as big as dog and cattle, or even we humans. Messing with meiosis is very likely to end up with infertility," stated Prof. WANG.

Then, something unexpected happened when they disabled all the three major steps of meiosis. The gene-edited hybrids grew normally and their ears of paddy were ripe and golden, just like the ones from the normal hybrid rice.

"We were very delighted, even couldn't believe it was truly happening. We double checked and planted the seeds, and found that they can sprout and grow to full term just like the seeds harvested from normal hybrid rice. The yield of these second-generation plants was, however, greatly reduced, with only very few seeds. When we planted them, we can easily tell, there were



Crippling of meiosis and fertilization, the two foundations for sexual reproduction. The seeds (indicated by red arrow) were planted for the next generations. (Image by WANG's Lab) something wrong with the third-generation seedlings, they all died young," recalled Prof. WANG in a public talk, "we then ran some genetic tests and found that the plant's genome doubles in each generation, from two to four, then eight and finally the plant, like a car without brake, overdrives and clashed."

These early results greatly enhance the morale of the members in WANG's Lab who took part in this study. By crippling all three major steps, they successfully turned meiosis into a 'mitosis-like' cell division, termed *MiMe* (mitosis instead of meiosis). Instead of giving rise to sperms or eggs with a single set of chromosomes (haploid), *MiMe* produces clonal sperms or eggs that have two sets of chromosomes (diploid), just like any other cells of the plant. However, they were facing another obstacle: the progeny's genome doubles to become tetraploid when the *MiMe*-derived clonal sperms and eggs, as diploid, participate in normal self-fertilization.

Then they attempted to stop 'genome-doubling' by crippling candidate genes linked with fertilization process, so that they can set up a brake system for this 'overdriven car'. Messing with most of the candidate genes caused infertility. Luckily, one plant was found to produce a few seeds. They planted these seeds and found that the seedings were dwarf and completely barren. Genetic analysis revealed that the plant's genome was reduced by half, which was a good sign.

"It seemed that we had found the brake we were looking for. So, the next step was to put the accelerator and the brake together, hoping the car would then drive smoothly and safely," recalled Prof. WANG, "With great unsettling excitement, we crippled all the four major steps of sexual reproduction at the same time, and things worked out as we wished. There were seeds, though not so many. We carefully planted those seeds, took care of them, and we found that the plants were able to continue to produce seeds in the second generation, the third, the fourth and now the sixth generation. Our tests showed that the plants maintained two sets of genomes, neither increased nor decreased. Our study demonstrates that these gene-edited hybrids are capable of long-term self-seeding. More importantly, we have proved that the secret passage does exist."

This critical breakthrough made by WANG's Lab was published in *Nature Biotechnology* in 2019, entitled "Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes."

"This work proves the feasibility of apomictic reproduction in hybrid rice, and is a major breakthrough in the field of apomictic reproduction," commented YUAN Longping, a globally renowned Chinese agricultural scientist known as the "father of hybrid rice," as soon as he was informed about this breakthrough.

"Due to the problem that we can't keep seeds from the hybrid crops or other economical plants, there is a huge potential of plant hybrid vigor remains to be explored. We hope one day we can extend to other crops and economical plants," stated Prof. WANG, "Of course, our current work only proves that the secret passage between sexual and apomictic reproduction exists, and there are still problems to be tackled with. In particular, nine of ten seeds would unfortunately fall off, and only one can make it through. In future, we will continue to explore and optimize this passage, hoping to widen this passage so that all seeds can safely make to the other side."