Less can be more, for plant breeders too

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Imagine you are a rice breeder and one day within a large field you discover a plant that has just the characteristics you have been looking for. You happily take your special plant to the laboratory where you find out that the spontaneous, beneficial event was due to inactivation of a single gene. This is a great observation; however, there are many different strains grown in different parts of the world, well adapted to the particular region they grow in.

How can you now transfer the inactivated gene to other strains of rice? Conventionally, you would have to go through years and years of breeding, until you have successfully transferred that single gene, without affecting all the other genes that are responsible for the target strains being so well adapted to their local environment. Would it not be great, if one could do this faster?

Using inactivated genes for rice breeding might sound far-fetched, but is not unusual. For example, the main change enabling the green revolution in rice resulted from loss of a gene that normally makes rice grow tall (and hence prone to toppling over if a plant makes many heavy rice grains). Thus, transferring inactivated genes is something rice breeders are indeed very much interested in.

Researchers at the Max Planck Institute (MPI) for Developmental Biology in Tübingen, Germany in collaboration with the International Rice Research Institute in the Philippines, have now generated a tool that should greatly speed up this particular aspect of rice breeding: According to a study published in *PLoS ONE* this week, a team led by Norman

Warthmann (MPI) successfully demonstrated highly specific gene silencing using so-called artificial miRNAs in rice (Oryza sativa).

MicroRNAs are 20-22 bp long RNA molecules. In animals as well as in plants they have important functions in regulating gene activity. In plants, they cause highly specific degradation of sequence-matched messenger RNAs, which encode enzymes, regulatory factors or other proteins. The end effect is that the corresponding gene is silenced. With artificial miRNAs (amiRNAs), this natural silencing pathway can be harnessed to inactivate genes of interest to the breeder, with unprecedented specificity.

Detlef Weigel's research group at the Max Planck Institute in Tübingen had initially pioneered this technique in the model plant Arabidopsis thaliana. The plethora of potential applications in agriculture now motivated them to try the method in rice. One of the rice genes they targeted is called Eui1.

When Eui1 is inactive, the uppermost part of the rice plant and parts of the flowers grow taller and the plants can more easily fertilize neighboring plants; breeders use this genetic trick for hybrid seed production. Originally identified as a spontaneous mutant in a japonica rice variety, the eui1 mutation was introduced into indica varieties by several years of breeding.

With an artificial miRNA targeting the Eui1 messenger RNA, the researchers at the International Rice Research Institute obtained within weeks plants with the desired property in two different rice varieties, including the agronomically important indica variety IR64, the most commonly grown strain in South-East Asia. Similarly, the researchers also report successful silencing of two other genes, Pds and SPl11.

Besides allowing the quick transfer of reduced gene function between different varieties, artificial miRNAs also accelerate the initial identification of important genes and the discovery of functions of genes that have not been studied before. Potential applications in rice breeding are manifold and they don't stop at rice genes. By targeting pathogen-derived genes, for example, it should be possible to enhance virus and insect resistance. In addition, because they act dominantly, they are also perfectly suited for hybrid breeding.

MiRNAs have been found in all plant species examined so far. It should hence be possible to adapt the technique of gene silencing by artificial miRNAs to other crops and it may provide an important new avenue to enhance agronomic performance and nutritional value. Computer software to design the required oligonucleotide sequences and detailed protocols to produce amiRNAs are provided free of charge on the authors' web site, at wmd2.weigelworld.org . Similarly, the artificial miRNA vector is provided free of charge to colleagues.

Citation: Warthmann N, Chen H, Ossowski S, Weigel D, Hervé P (2008) Highly Specific Gene Silencing by Artificial miRNAs in Rice. PLoS ONE 3(3): e1829. doi:10.1371/journal.pone.0001829

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