Cloning plants from seeds

December 15 2009, by Albert Sikkema

(PhysOrg.com) -- Wageningen geneticists (The Netherlands) are developing a method to replicate the parents of a chosen plant. Known as 'reverse breeding', this will have a big impact for the breeding industry.

Just as humans have no idea which characteristics a child will inherit during child-bearing, plants which propagate with pollen and pistil produce a wide variety of offsprings. Breeders want to keep this complex variation within limits. 'We are going to turn the process around this time', says geneticist Hans de Jong. 'We are going to reproduce the parents of the ideal plant.'

De Jong's is carrying out his work on reverse breeding together with PhD student Erik Wijnker and a research team from breeding firm Rijk Zwaan. This company's R&D-director Rob Dirks came up with this new idea, which has been patented in the meantime. The Laboratory of Genetics is now investigating if this approach is feasible and has published an article this month in *Plant Biotechnology Journal.*

'We want to delimit the processes which determine the enormous genetic variation in plants', says De Jong. 'As such, we need to understand the process of genetic recombination and bring it under control.' The genome of plants and animals consists of many chromosome pairs. During fertilization, the seed and ovule fuse to create a unique offspring, which has one chromosome out of a pair from each parent. A parent does not actually transfer one of its two chromosomes to its offsprings. The chromosome which has been given away is a combination of two parental chromosomes, because pieces of chromosomes are exchanged

via so-called cross-overs. It is these cross-overs which result in new combinations of father and mother characteristics and big variations in the offsprings. 'We are now able to hinder these cross-overs', says Wijnker. 'In so doing, we limit the recombination and are able to do breeding on the chromosome level.'

This opens up new vistas for the breeding industry. De Jong says: 'Currently, breeders make use of carefully selected parental lines in which both chromosome copies are exactly the same.' Breeders cross the parental lines to develop the ideal plant with the desired characteristics: the hybrid.

For example, we have the hybrid for big tasty tomatoes. But when this is crossed with other tomatoes, the combination of desired characteristics will be lost in subsequent generations. The tomato grower therefore needs to keep buying new hybrid seeds. That is how breeders earn their keep. The ideal hybrid can only be reproduced if you have its parents. Breeding firms therefore guard the parental lines closely and keep these away in secret fields.

De Jong and Wijnker want to replicate these hybrids in their lab using reverse breeding. The underlying scientific question is: how can you retrieve the parents of the ideal tomato plant? If this were possible, we would sow a big sack of seeds of many varieties all over the place and look out for the plant which grows best under existing environmental circumstances, says De Jong. 'We would then reproduce the parents of that plant.' The secret fields of the breeding firms would become a thing of the past.

To do this, the researchers first set out to engineer the meiosis process, when gender cells are formed in which the cross-overs occur. They have succeeded for the model plant Arabidopsis and for several crops. The good thing about Arabidopsis is that it only has five pairs of

chromosomes. The cross-overs during meiosis will result in a maximum of two-to-the-power-of-five, which makes 32 different viable gender cells.

Afterwards, these gender cells, in which only one copy of the chromosome resides, need to be developed into an adult plant with two copies, just like the original parent. Here again, the duo have succeeded in getting pollen from Arabidopsis to germinate into a seedling, by letting the few chromosomes duplicate themselves. 'A month ago, this wasn't possible with Arabidopsis. Then, we had help from an American colleague who made a fantastic discovery in the lab, and we're back on track', De Jong says. The next step is to cross the seedlings with one another. They will then use marker technology to find out which of the 32 possible parents are responsible for the ideal plant. They will supply evidence which shows that reverse breeding is possible in a new publication soon.

The breeding industry, however, looks the other way. Arabidopsis has very few chromosomes and can result in only 32 possible gender cells. 'The chance of finding the parents is therefore very big', says Wijnker. Rice and tomato have twelve chromosome pairs. 'With such a sharp rise in complexity, the chances of finding the parents will become less', De Jong says. A crop such as bread wheat (21 chromosome pairs) is too complex. 'We don't think that we can use this technique in wheat.' But reverse breeding is within reach for agricultural crops such as spinach (six chromosome pairs), rye and cucumber (seven), beetroot (nine) and maize (ten).

Provided by Wageningen University