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Rapeseed Breeding in the Biotechnology Age

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Gene technology and conventional breeding

Plant breeding today is a science in rapid change and development. New tools and techniques are being added complementing the existing conventional methods.

In the forties polyploidy was introduced as in important new technique. In the sixties mutation breeding was the fancy tool. In the eighties somatic hybridization was considered as an important new method to enlarge the genetical variation for the plant breeders. Today gene technology or gene transfer is considered as a new very powerful tool. Since plant breeding on a scientific base started about 100 years ago this new technology will probably have the greatest impact of all breeding innovations. There are several reasons for this :

1) Genes from quite distant species, or even artificially constructed, can be isolated and transferred to agricultural crop plants.
2) New traits which are difficult to improve with conventional breeding like insect tolerance or drought tolerance can be improved.
3) These genes are easy to introduce into breeding programs : Normally single dominant genes.
4) In the gene isolation and transformation process these genes are well investigated and described. E.g. PCR primers are designed for rapid and effective marker assisted selection in breeding programmes.

Today you often hear the question : "Will this new technique replace the conventional breeding". The answer is no. First, it is very wrong to put gene technology against conventional breeding : Gene technology is a new powerful tool which has been added to the other tools used in plant breeding. Second, transformation will be used for transfer of specific genes conferring specific traits. If we have introduced a gene for insect tolerance of course we do not neglect all other characters that can be improved via conventional breeding. Third, transfer of a gene conferring a certain trait is normally done only in one background and only one transformation event (=elite event) is selected for further breeding. This event is then introduced in the breeding programme via back-crossing. New transformations involving the same gene are not done.

The reasons why only one elite event is used and why transformation is not repeated with the same gene in a species are the following:

1) Back-crossing is faster: A transformation results in a number of primary transformation events. The best of those is selected to become an elite event. This selection includes e.g. copy number, expression level, stability in different environments and different genetic backgrounds. It is obvious that such an elite event selection takes several years and both laboratory and field resources.
2) A new event requires new regulatory files.
3) It is easier and more effective to use only one event in a breeding programme.
4) The receptor line that has to be used for transformations is not always a good agronomic performer. E.g. a disease resistance gene is normally transformed into a susceptible line to facilitate elite event selection.

SeedLink™ hybridization technology

Plant Genetic Systems (PGS) has developed a new hybridization system that has been applied to a number of crops a.o. oilseed rape (*Brassica napus*). Both the male sterility (Ms) and restoration of fertility (Rf) genes have been introduced in oilseed rape using an Agrobacterium based transformation technology.

Male sterility was introduced in oilseed rape by tapetum cell specific expression of a ribonuclease gene, *barnase*. This gene inhibits anthers from producing pollen. Male fertility in the F_1-hybrid is restored by a tapetum cell specific gene encoding the *barnase* inhibitor, *barstar*.

In the transformation vectors both the Ms and Rf genes have been linked to a herbicide tolerance marker gene, i.e. tolerance to glufosinate ammonium.

The Ms and Rf genes are both single dominant genes. The

tight linkage to herbicide tolerance gives two great advantages:

1) Plant breeders using SeedLink™ genes can select directly plants carrying Ms or Rf genes in segregating populations. This avoids steps like testcrosses to confirm presence of restorer genes.
2) Since male sterility is conferred via a single dominant gene which is always present in a hemizygous stage, the female needs to be rogued for the 50% segregating fertile plants. Chemical roguing with the herbicide can be done very effectively. This ensures very pure F1 hybrid seed lots.

SeedLink™ is very stable : Both the male sterility and the restoration are stable and complete in different environments and also in different genetic backgrounds.

Finally the SeedLink™ system does not give any biological penalty, thereby ensuring the full exploitation of the potential heterosis.

PGS hybrid winter oilseed rape breeding programme

The main breeding goals in the winter oilseed rape breeding programme in Europe are the following :

- high seed yield
- good winterhardiness
- good straw stiffness
- good shattering resistance
- drought tolerance
- disease resistance
  - Black leg (*Phoma*)
  - Light leafspot (*Cylindrosporium*)
  - Stem rot (*Sclertinia*)
- good seed quality
  - high oil content
  - high protein content
  - low glucosinolate cotent
  - improved fatty acid profiles

To achieve these goals hybrids offer several advantages. The heterosis or hybrid vigour gives not only improved seed yield but can also have a poositive influence on traits like winterhardiness and straw stiffness.

The different steps in PGS' winter oilseed rape hybrid breeding program involves:

- parent development
- general combining ability testing of the parents
- specific F1-hybrid testing for commercial products.

Parent development

For good heterosis genetical distance between the two parents is important. To develop the two parents in a hybrid with a great genetical distance tools like AFLP, RFLP and RAPD are used routinely in the breeding programme.

AFLP is also used to introduce e.g. Ms genes in new backgrounds via accelerated back-crossing. With this technique a line conversion can be completed at BC_2 or BC_3 instead of crossing up to BC_6 with the recurrent parent.

At PGS the parental lines are developed via the double haploid technique (DH). On the male side the DH s are produced with Rf genes already in place. The DH s are normally tested 2 years for per se performance of agronomic and quality characters. 8000 DH s are produced every year. After per se performance evaluation about 900 are selected for combining ability tests.

General combining ability testing of the parents

The parental lines are tested for general combining ability with a few testers. An advantage with the SeedLink™ male sterility is that also parents without restorer gen can be tested wite a Ms female. In such a case 50% of the F1 plants will be fertile and 50% sterile. For combining ability tests however it is sufficient if half of the plants contain pollen. The best combiners of the tested parents can then be converted to an Ms or Rf line via marker-assisted accelerated backcrossing.

The normal situation is however that the male parents are developed within the system, containing the Rf gene in homozygous stage. The general combining ability testing of the parental lines are done on three locations in UK, France and Germany. Of the 900 tested parental lines about 100 are selected for further testing in specific combinations.

Specific F1 hybrid testing

At this stage. the best 100 parent lines are tested as F1 hybrids in specific combinations for commercial products. These F1 hybrids are trialled in 9 locations in the most important rapeseed growing countries in Europe. The tests continue over three more years with increasing number of locations and decreasing number of F1 hybrids. Parallel with the last two test years the hybrids are also entered into official registration trials in the different countries. If a hybrid performs better than the check varieties it normally gets registration after the two years of official testing. Certified seed production, marketing and sales can then start.