The Integration of biotechnology and conventional breeding in Brassica oilseed improvement

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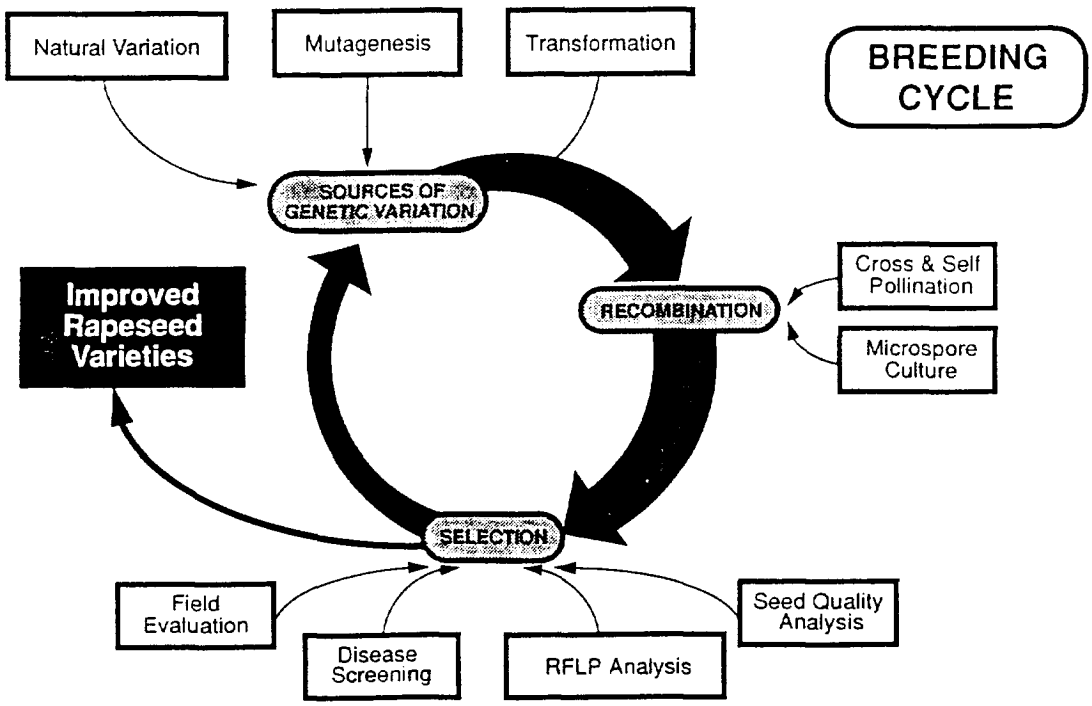
Targets for Oilseed Rape Improvement at Agrigenetics

The major targets for oilseed rape (*Brassica napus*) improvement at Agrigenetics are:

1. High stability Canola oils (under contract with the SVO Specialty Products Division of the Lubrizol Corporation)
2. High oil Canola (in collaboration with Maribo Seed of Denmark)
3. Improved meal protein using Phaseolin seed storage protein genes
4. Resistance to the broad spectrum herbicide Ignite (Basta)
5. Insect resistance using Bt toxin genes

To expedite the achievement of these targets, biotechnologies such as transformation, microspore culture and RFLP analysis are used in conjunction with conventional breeding techniques. These technologies are viewed as part of the breeding cycle (Figure 1) in which improved rapeseed varieties are developed. The components of this cycle are discussed along with actual examples where appropriate.

Figure 1 : Breeding Cycle



Sources of Genetic Variation

These are the inputs to the breeding cycle and can be of several types.

Natural variation is the preferred source of variation if it is available. Agronomic traits, resistance to diseases such as blackleg, seed characteristics and other traits where adequate natural variation is available within the species or in closely related species are incorporated into the breeding cycle through conventional breeding techniques. Sometimes microspore culture is employed to fix the variation prior to incorporation into the breeding cycle (eg disease resistance, fatty acid composition).

If natural variation cannot be found, the next approach employed is that of **mutagenesis**. This technology is particularly effective where the intention is to block a pathway. For example, mutagenesis has been employed to block the fatty acid desaturation pathway in both *B. napus* and *Brassica rapa* resulting in mutants with significantly elevated levels of oleic acid (Auld et al, 1992).

To incorporate novel traits which do not exist in the species and which cannot be created through mutagenesis, **transformation** technology is employed. This involves isolation of the gene coding for the trait (eg Phaseolin seed storage protein gene from *Phaseolus vulgaris*, insecticidal protein genes from *Bacillus thuringiensis*), construction of a cassette containing the gene with an appropriate promoter and a selectable marker (eg PAT gene from Hoechst) and incorporation of this cassette into oilseed rape using Agrobacterium mediated transformation.

Recombination

Once the variation has been identified or created, it is incorporated into the breeding cycle and recombined with elite germplasm. To accomplish this, conventional **cross and self pollination** techniques are employed. **Microspore culture** is employed where the number of genes being recombined is large and at the end of a backcrossing program to produce true breeding varieties. A new technique for doubling of chromosome number is now being employed whereby the microspores are treated with the herbicide trifluralin.

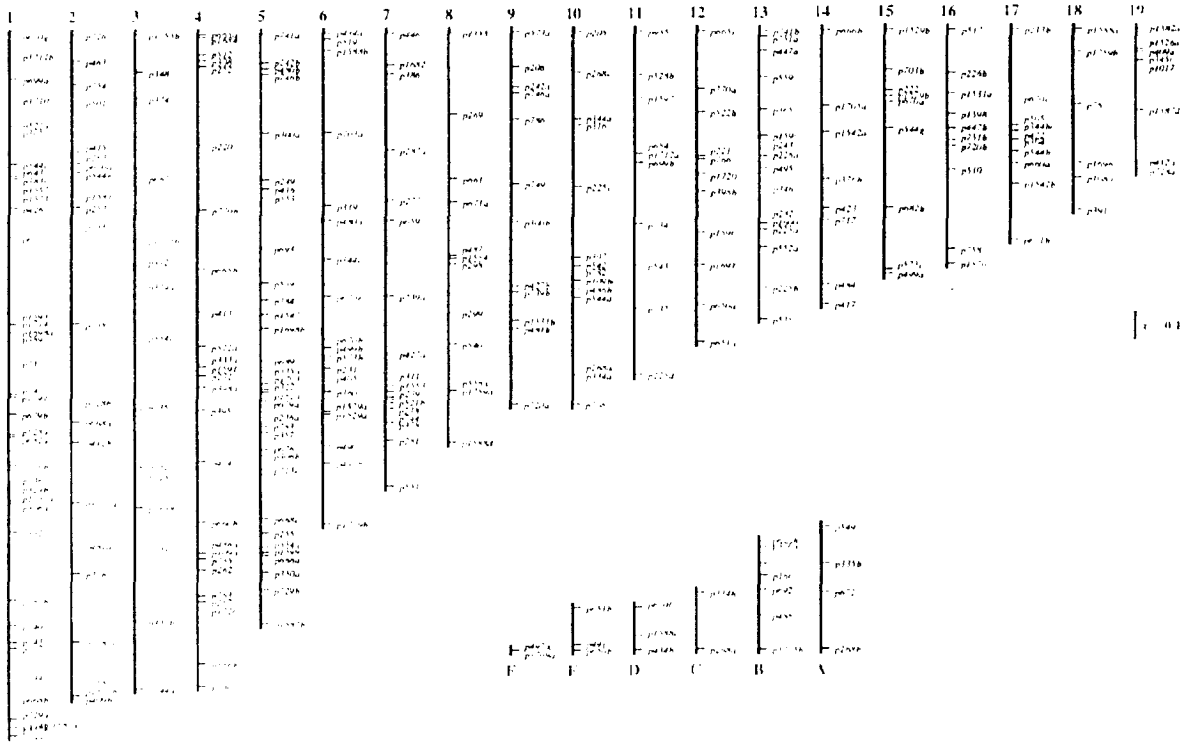
Selection

After recombination of traits has been achieved using cross and self pollination and microspore culture, the recombinants are screened in order to identify the desired individuals.

Seed quality analysis is employed to screen for oil content, fatty acid composition of the seed oil and glucosinolate content. Oil content is done using NMR technology, fatty acid composition is analyzed using gas chromatography of the fatty acid methyl esters using half-seed, half-embryo (from microspore culture) and bulk seed samples. Glucosinolate content is determined using glucose TesTape and HPLC techniques.

RFLP maps for both *B. napus* (Figure 2) and *Brassica rapa* have been developed at Agrigenetics. **RFLP analysis** using this map/probe set is employed for selection in a number of ways. Firstly, this technology is employed to assist in the recovery of the recurrent parent genotype in a backcrossing program (Chyi et al, 1991). Using RFLP assisted selection, only 2 backcrosses are required to achieve recurrent parent recovery comparable to 4 backcrosses without selection. Another application of RFLP analysis is to identify probes which have close linkages to genes which are difficult to score phenotypically, particularly in the heterozygous state (eg disease resistance). Selection for these traits is accomplished indirectly by selecting for the flanking RFLP markers. A third application of RFLP analysis is to develop a riflotype (molecular fingerprint) for a variety or parent of a hybrid for proprietary protection purposes.

Figure 2 : RFLP Map for *Brassica napus*



Disease screening for blackleg (*Leptosphaeria maculans*) is accomplished using growth chamber and field assays. The focus is on natural resistance available in *B. napus* and not that from *B. juncea*.

Field evaluation is a critical component of the breeding cycle. Strains arising from the breeding cycle are first evaluated in non-replicated nurseries at 2 locations in the target production region. The best strains from these nurseries are advanced to replicated trials at 5-6 locations and the best strains from the replicated trials are advanced to registration trials (Canola varieties) or used for contract production (specialty varieties).

Status of Oilseed Rape Improvement at Agrigenetics

Using the technologies discussed above, the achievement of the five major targeted improvements is underway. The status of these projects is summarized below.

1. High stability Canola oils have been developed at Agrigenetics under contract with the SVO Specialty Products Division of the Lubrizol Corporation. Pilot scale production of these oils has been completed and small scale commercial production is underway.

2. High oil Canola is being developed in collaboration with Maribo Seed of Denmark. Agrigenetics is testing high oil strains for Maribo in North and South America and we expect to have the first high oil variety registered in Canada in 1994. These strains represent a major improvement in oil content, up to 3% higher than current varieties.

3. Improvements in meal protein using Phaseolin seed storage protein genes from *Phaseolus vulgaris* is underway using transformation technology. A transgenic field trial is being conducted in western Canada in 1993 to evaluate the effects of these transgenes on protein content and amino acid composition of the spring Canola variety Profit.

4. Transformants of oilseed rape with resistance to the broad spectrum herbicide Ignite (Basta) have been produced and the resistance has been introduced into the breeding cycle for backcrossing into elite Canola and specialty oil strains. The first field trials with this material will be in 1994.

5. The development of oilseed rape which has resistance to Lepidopteran pests such as cabbage loopers and diamond back moth larvae is underway using Bt toxin genes. In 1991, Agrigenetics conducted the first US transgenic trial evaluating Bt in oilseed rape. Transformants with new Bt toxin genes will be available for field evaluation in 1994-95.

References

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