Molecular markers in sunflower

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Abstract
We present sunflower as a crop that is ready for exploitation with molecular markers. AFLP[tm] analysis is currently the method of choice for saturation linkage mapping, bulk segregant analysis, and backcross breeding.

Keywords
AFLP, RFLP, cytoplasm, hybrid, yield, backcross

Introduction
Sunflower is the world's fourth largest oil crop. In 1990, the world production was 22 million tonnes (compared with 103 for soya, 33.6 for cotton and 25.3 for rapeseed), and this accounted for 11% of the world's need for oilseed (1). Improvements in the harvest of sunflower have increased dramatically from 1960 (when the world production was 7 million tonnes) , due to the use of cytoplasmic male sterile systems to produce hybrid sunflower. As can be seen in figure 1, sunflower has kept pace with the production of oil crops worldwide.

Part of the increase in demand for this oil crop has been stimulated by the recognition of the advantages of sunflower oil for the human diet. The oil is particularly low in polyunsaturates, and sunflower margarine is cheaper than butter, resulting in a shift in consumer preferences away from animal based oils. A typical sunflower contains the saturated acids stearic acid (C18, 3.7%), palmitic acid (C16, 6.4%); and the unsaturated fatty acids oleic acid (C18:1, 23.8%), linoleic acid (C18:2, 65%) and 0.2% linolenic acid (C18:3, 0.2%). This spectrum is most similar to soya, but with reduced palmitic and linolenic acids, and more linoleic. The oil forms 48-55% of the dry weight. Speciality genotypes have also been developed , for instance the high oleic types, for use in particular activities.

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Apart from its use in food products, sunflower oil has been used in biodiesel fuel, polymers, paints, varnishes in Europe, South Africa and Australia.

In the European Union, Spain is the largest cultivator of sunflower (1.2million hectares) followed closely by France (with just over 1 million hectares). These two countries account for nearly 90% of the production within the European Union.

Breeding targets

Sunflower is susceptible to several diseases, of which the most serious is sclerotinia, followed by mildew, rusts, phomopsis, botrytis, albugo and phoma. Resistance genes are available to most of the common diseases, but too much dependence on single major resistance genes can lead to selection of virulent pathogens. A sensible strategy is to use molecular markers to enable the selection of genotypes with several resistance genes to the same pathogen in the same variety. Resistance to sclerotinia is multigenic, and markers can be of great assistance in assembling resistant genotypes.

Sunflower meal has relatively high fibre and ash content, which reduces the metabolisable energy, and is also low in lysine. However, it is high in sulphur amino acids, and seems to lack antinutritional factors. These positive factors need to be maintained whilst selecting against the negative characters.

The most important factor is yield, with lodging and seed set being key factors in determination of harvested yield. Most of the area of cultivation is subject to periodic drought conditions, and genotypes must be drought tolerant.

As noted above, a key factor in the improvement of the yield of modern cultivars has been the use of the Leclerq cms cytoplasm, to enable hybrids to be made easily. Many other cytoplasms are known, though the Leclerq cytoplasm is used in about 98% of current hybrids. The backcross method can be used to transfer the cms cytoplasm into a new genotype. Molecular markers are useful in this context to select for those individuals in the backcross population that contain the maximum amount of the recurrent parent genome. Similarly, markers can also be used to maintain genes from a donor genotype in backcross individuals, such as restorer genes, doing so avoids the need for testcrossing.

In addition to the use of a cms cytoplasm, it is also necessary to choose genotypes for hybrid production that maximise the heterosis between the parents. Molecular markers are useful in this context to maximise the genetic distance between the parents of a hybrid combination, and also to

examine the genetic pool to find groups of genotypes (heterotic groups) that are particularly well suited to the production of high yielding hybrids.

Available molecular markers

Many RFLPs are available, but have been developed by particular companies, and hence are not freely available. These markers tend to be slow in routine use, and require DNA of high quality and large quantity. An alternative to the routine use of RFLPs is to convert RFLPs known to be linked to particular genes into another, PCR-based format, eg. an Allele Specific Oligonucleotide assay (ASO) or a cleaved amplified polymorphic sequence (CAPS) assay. RFLPs find one particular niche application in the fingerprinting of cytoplasms, for identification of novel sources of cms. **AGROGENE** is fully equipped to offer all these services to its customers.

AFLP^{tm} is a technology invented in the Netherlands by **KeyGene**, who have filed for patent protection world wide on the use of this technology. **AGROGENE** is licensed by **KeyGene** to provide a commercial service using this new technology. Some of the advantages of this new technology are that a large number of molecular markers can be revealed at the same time in the same reaction, the system uses the polymerase chain reaction (and so is relatively quick), and requires only small quantities of DNA. The number of AFLPs available is, to all practical extents, unlimited, and so the ability to saturate the genetic map of a species is feasible, it is also an ideal tool to perform bulk segregant analysis to find markers closely linked to genes controlling simple traits.

A combination of the use of molecular markers for selection, and embryo culture to speed up the generation time for sunflowers has been proposed by Pelletier in 1992. The proposed scheme results within 8 generations in a fixed line, already selected by markers for agronomic performance, and tested for combining ability. A similar combination of molecular marker analysis and embryo rescue can be used to speed up backcross breeding - as the use of markers will typically shorten the number of backcross generations required in a programme by 2 or 3 cycles.

fig. 1 Isaac, Bonjean and Liu