Radiation hybrid technology in plants

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Radiation hybrid (RH) mapping is an extremely powerful technique developed to facilitate the construction of high-resolution physical maps of the human genome. A number of mammalian RH mapping panels are available to the scientific community for research purposes, and the transfer of this technology from mammalian systems to plant systems will greatly aid progress in the physical mapping of plant genomes.

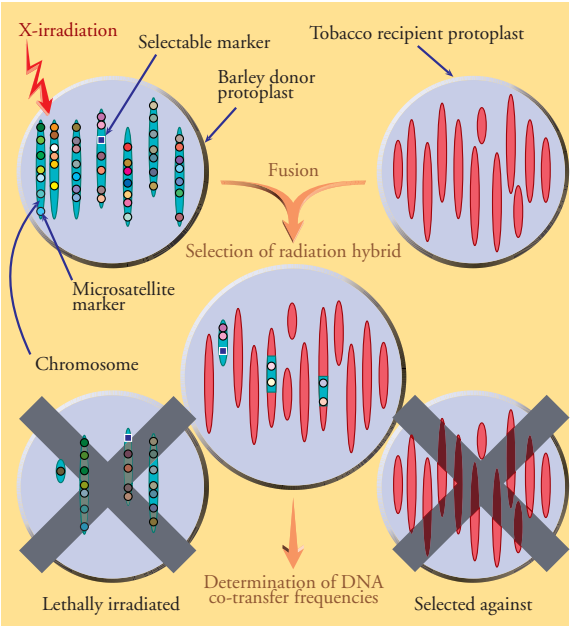

Figure 1 Irradiation and fusion gene transfer.

RH mapping is based on irradiation and fusion gene transfer (IFGT) in somatic cells (Fig. 1). Donor cells are exposed to lethal doses of irradiation which fragment the chromosomes. These fragments are then rescued by fusion with suitable recipient cells. Characterisation of resulting cell lines and subsequent mapping is achieved by assessment of co-retention frequencies for molecular marker alleles originating from the donor material and the use of statistical methods to calculate the order and distance between marker loci.

There are a number of reasons why RH panels are considered valuable tools for mapping programmes. Markers used do not rely on polymorphism to produce maps, and a simple plus/minus assay is used to assess retention or elimination of marker loci within cell lines. Problems associated with the presence of recombination hotspots during conventional mapping are avoided by utilising irradiation to induce random breakpoints within chromosomes. A further advantage of using RH mapping panels is that, by altering the irradiation dose to which donor cells are exposed, map resolution may also be manipulated. Elevated levels of irradiation treatment will induce increased chromosome fragmentation and, thus, allow the construction of high-resolution maps. Additionally, a relatively small number of lines are required to produce a mapping panel. In mammalian systems, RH panels typically number between 80 and 100 lines.

Generation of a barley whole genome radiation hybrid panel

Donor and recipient material Young, transgenic barley cell suspensions were used as a source of donor protoplasts. Incorporation of a selectable marker (herbicide resistance) within the donor genome allowed selection of putative hybrid material by manipulation of culture conditions. Tobacco, mesophyll-derived, protoplasts were utilised as recipient cells, as this species has well established protoplast fusion and culture protocols. A dicotyledon fusion partner was also chosen to ensure that molecular marker analysis of resulting putative hybrid material avoided false positive results which may occur due to homology between the donor and recipient genomes.

Irradiation and fusion gene transfer X-irradiated donor material was used in asymmetric somatic hybridisation experiments to produce a panel of radiation hybrids, exhibiting partial genome transfer, suitable for mapping procedures. X-irradiation treatment was provided by a linear accelerator and protoplasts

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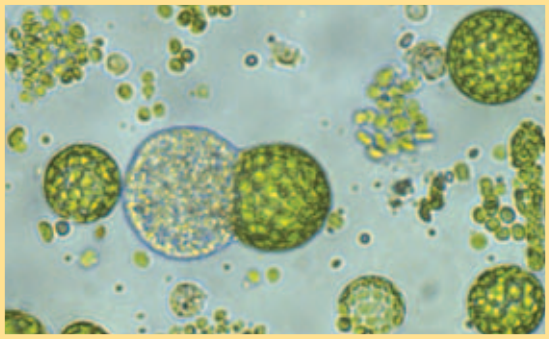

Figure 2 Barley donor (colourless) and tobacco recipient (green) protoplast fusion.

were exposed to varying doses of irradiation (0-100 Gy), prior to fusion, to induce chromosome fragmentation. Protoplasts were then mixed and aligned in an AC field where fusion was facilitated by the application of a DC pulse (Fig. 2).

Protoplast fusion assessments have revealed that an X-irradiation dose of 50 Gy resulted in the most efficient throughput of material. Experiments, where protoplasts were exposed to irradiation levels exceeding 50 Gy, were less efficient, generating resistant cell lines at a frequency too low to be suitable for the construction of mapping panels.

Characterisation of radiation hybrid cell lines

PCR screen for selectable marker All cell lines, which survived culture upon herbicide selection medium, were assessed by PCR for the presence or absence of the selectable marker. A screen of five putative hybrid cell lines revealed four herbicide resistant lines which are PCR positive for the selectable marker (Fig. 3).

Co-retention of microsatellite marker loci within cell lines PCR-positive RH cell lines were further characterised using 37 barley-specific microsatellite markers offering genome-wide cover. Cell lines which retain the marker, as in RH 23 and RH 33 (Fig. 4), are scored 1 and those where the marker loci has been eliminated, as in RH 61 (Fig. 4), score 0. This allows

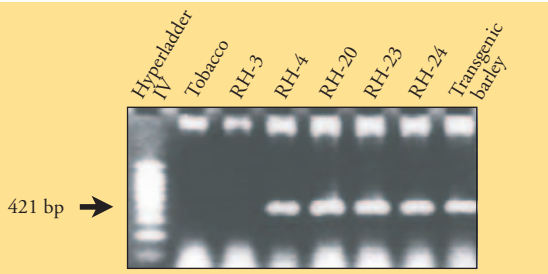

Figure 3 PCR-based validation of radiation hybrid cell lines.

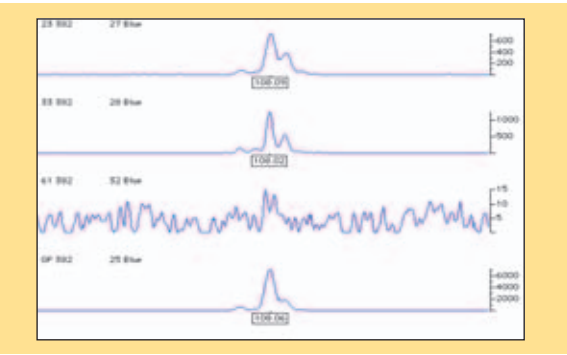

Figure 4 Microsatellite marker characterisation of radiation hybrid cell lines (RH 23, 33, 61 and control barley from top to bottom).

us to build up a picture of which parts of the genome have been retained in individual cell lines. Retention frequency varies among cell lines but marker data collected reveals that barley DNA is retained within the tobacco genome at a frequency similar to that of currently available human RH panels. Assessment of the microsatellites chosen for characterisation of the RH lines has also revealed that certain genomic regions are more frequently retained than others but that, across the panel, it is possible to secure retention of most of the genome for whole genome mapping purposes.

Conclusions

This research has established a protocol for the generation of the first whole genome radiation hybrid panel in barley and this panel is currently being exploited to physically map a group of barley-specific microsatellite markers.

Ongoing work on this project includes further validating the panel using previously mapped markers. Two approaches are being undertaken to achieve this goal. Firstly, PCR screens are being carried out for closely linked pairs of markers that have been physically mapped using BACs. Both markers should always be either present or absent in RH lines. Secondly, work carried out at JIC on physical mapping has revealed an RFLP marker mapping closely to the transgene. This marker should be retained at a frequency similar to the selectable marker (100%) and will also further validate the panel.

Exploitation of this technology is focusing on further production of RH lines to be used concomitantly with the current 50 Gy RH panel for high density mapping of single chromosomes using ESTs produced at SCRI. Future work will also focus on studying cell lines using fluorescence *in situ* hybridisation to visualise barley chromosome fragments retained within the recipient genome.