

observed, such as albinism, chromosomal or somaclonal variation, spike sterility.

Various types of source explants have been evaluated and can be classified according to their degree of organisation or their level of ploidy.

At the diploid level, somatic embryogenesis has been attempted on actively growing tissues, such as leaf bases, scutellums of immature embryos, or very young inflorescences which constitute meristem mosaics. In this process, the callus and embryogenic steps are relatively easy to achieve in a large range of genotypes, whereas short-term and even more long-term regeneration are much more genotype-dependent.

Suspension cultures of isolated callus-derived cells or protoplast should represent interesting targets for transformation studies. However the regeneration of high numbers of fertile healthy plants remains far from being routinely achieved in wheat.

At the haploid level, the development of a transformation system using microspores or microspore-derived embryos has great potential for the genetic modification of wheat: homozygous transformants can be obtained after one generation after chromosome doubling. Significant efforts have been made to develop this androgenetic process in wheat and triticale at Clermont-Ferrand. Many environmental factors have been optimized in a first step, using a large range of genetic material. We have also studied the genetic control of the process using aneuploid material and segregating pools of DH lines, together with chromosomal and RFLP markers, and identified chromosomes associated to each step of *in vitro* androgenesis.

The above-mentioned studies have led to sustained progress leading to yields of 20–70 green plants per spike in elite materials, thereby enabling the haplo-diploidisation technique to be integrated in breeding strategies, cultivar development and molecular genome mapping. Selected doubled-haploid lines with high embryogenic and regeneration potential are currently being used in transformation experiments using biolistics.

**Soil-borne wheat mosaic virus RNA stability and molecular pathology.** J Chen, SA MacFarlane, TMA Wilson (*Scottish Crop Research Institute, Dundee DD2 5DA, UK*)

Repeated mechanical passaging of soil-borne wheat mosaic virus (SBWMV) or growth at high

temperatures (25–30°C) results in rapid deletion of part of RNA2. During early passages, plants contain a mixed population of deletion mutants which are always located in the coat protein-readthrough (CP-RT) gene. In later passages, 1 deletion mutant became dominant. This stable mutant is deleted between nts 1 420 and 2 180, resulting in the loss of 759 nts (253 aa) from the CP-RT gene. Smaller deletions in earlier passages are not intermediates in the larger deletion process. Only full-length SBWMV RNA2 is transmitted to wheat roots by the viruliferous fungal vector, *Polymyxa graminis* from field soil and there is no intraplant barrier to the movement of deleted forms of RNA2 between roots and leaves. The spontaneous stable deletion mutant is associated with increased symptom severity following further mechanical inoculation to young healthy test plants.

**Barley mild mosaic bymovirus: existence of 2 subgroups.** M Meyer, JT Dessens (*INRA, unité de pathologie végétale, route de Saint-Cyr, F-78026 Versailles cedex, France*)

A *Polymyxa*-transmitted isolate of barley mild mosaic virus (BaMMV-P) was collected from a virus-infected barley field near Reims. It was propagated in mechanically inoculated Magie cultivar. Comparison of the RNA content shows that BaMMV-M contains smaller RNA-2 than BaMMV-P. The nucleotide sequence of the smaller RNA-2 indicate that this RNA lacks approximately 1 000 nucleotides of its C terminal protein gene. As a consequence, this RNA-2 encodes an N terminal protein of 25 kDa and a C terminal protein of 34 kDa instead of 73 kDa for the wild type RNA-2.

The coat protein gene and the 3'-untranslated regions of RNA-1 and RNA-2 of BaMMV-M and BaMMV-P have been cloned and sequenced. Comparison of the nucleotide and amino acid sequences indicates that the 2 isolates contain distinct RNA-1 and RNA-2 molecules, and hence contain distinct BaMMV strains. One strain is present mainly in BaMMV-P and belongs to a subgroup including 2 German (from Braunschweig and Ascherleben) and a Japanese Ka1 isolate. The other strain is present mainly in BaMMV-M and belongs to a subgroup including a UK (Streatley) isolate. These results also indicate that the mechanical transmission causes a shift in the virus population in favor of the strain with smaller RNA-2.