highly conserved and shows little variation in sequence over 12 isolates. CP is also essential for infectivity. The coat protein also determines vector specificity. The relationship with the vector is circulative and non-propagative. The virus is found in most cells of the plant except the sieve elements and can be acquired in a few seconds from infected maize by the vector. It enters the insect through the filter chamber, reaching the haemolymph, and then circulates to the salivary glands. Transmission is only effective when the insect feeds for a sustained period in the sieve elements. The success of the acquisition–transmission process is affected by factors which influence the insect–plant interactions, such as the species of host plant, the cultivar or variety and the colour of the leaves (including symptoms). The vectors and the viruses of MSV have evolved with grasses in Africa; the 2 crops most at risk in the past have been sugar-cane and maize, both introduced crops. MSV is a disease of annual grasses which is ideally suited to maize. Wheat and other small grain cereals have been affected by different strains or viruses within the streak complex but other crops such as sorghum appear resistant. *Zea perennis* and *Z diploperennis* are resistant to MSV but cultivars and hybrids show varying susceptibility. Sweetcorns are highly susceptible to MSV but many new varieties of tropical maize show high levels of tolerance, although resistance breaking strains have been reported recently. The mutation rate of the virus is unknown but perceived adaptions may the due to mixtures of genotypes within the host. Despite the presence in Europe of *Cicadulina* species, susceptible grasses and an increasing area of maize, the ecological factors required for an MSV epidemic are unlikely to be present in the foreseeable future.

Transformation of Poaceae and transgene expression. J Fütterer, I Potrykus *(Institut for Plant Sciences, ETH Zürich, Universitätstr 2, CH 8092 Zürich, Switzerland)*

Since the first generation of fertile transgenic rice in 1989, all major cereals and many grasses have been transformed. In the beginning mainly cells or structures derived from embryogenic suspension cultures were used. Establishment of such cultures was time-consuming and very genotype-dependent and plants derived from such cultures were often sterile. More recently, calli derived from immature zygotic embryos have been used, resulting in a higher percentage of fertile plants and also providing a less genotype-dependent source of regenerable material. The most commonly used methods of DNA delivery to cells are particle bombardment, electroporation, PEG-mediated DNA uptake and recently even infection by agrobacteria. Crucial for successful transformation was the establishment of tissue culture procedures and the development of the proper selection system for each species. At present, methods exist for all major species but in most cases, the overall transformation efficiencies are still very low and in many cases transformation is not really genotype independent. Consequently, very few transgenes besides the selectable marker gene and the β-glucuronidase have been expressed in transgenic Poaceae and data about expression of non-selectable genes are still quite scarce. Promoters of the maize ubiquitin I gene, the rice actin I gene, the maize adh 1 gene and of the CaMV ^{35}S RNA have all been used successfully for more or less constitutive expression of transgenes. Particularly the activity of the monocot promoters depends on the presence of a monocot intron and such an intron can also strongly enhance the activity of the CaMV ^{35}S promoter. A variety of tissue-specific promoters for green tissues, seeds, and phloem have also been described. The expression levels for a given promoter construct can vary over a wide range in different transgenic plants and even in different offspring from the same transformation event or the same primary transformant. Problems with stability of gene expression in subsequent generations have also been reported.

We will present data derived from the transformation work at the ETH in Zürich to support these general conclusions.

Some aspects of *in vitro* regeneration of bread wheat. S Bernard *(INRA, Plant Breeding Station, F-63039 Clermont-Ferrand cedex, France)*

In bread wheat, like in other cereal crops, shoot regeneration is of crucial importance in the application of *in vitro* methods for plant improvement, in particular gene transfer of agronomically useful traits. However, a number of problems remain to be solved in order to make cereal transformation as efficient as with dicotyledonous crops: different culture conditions should be devised for each step of the morphogenetic process; and (ii) various defects of the regenerants are often

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.