Strategies for variety-independent genetic transformation of important cereals, legumes and woody species utilizing particle bombardment

Paul Christou
John Innes Centre, Norwich Research Park, Norwich NR4 7UH, U.K.

Key words: gene transfer, crop species, particle bombardment, transgenic plants, cereals, legumes, woody plants

Summary

The limiting component in the creation of transgenic crops has been the lack of effective means to introduce foreign genes into elite germplasm. However, the development of novel direct DNA transfer methodology, by-passing limitations imposed by Agrobacterium-host specificity and cell culture constraints, has allowed the engineering of almost all major crops, including formerly recalcitrant cereals, legumes and woody species. The creation of transgenic rice, wheat, maize, barley, oat, soybean, phaseolus, peanut, poplar, spruce, cotton and others, in an efficient and in some cases, variety-independent fashion, is a significant step towards the routine application of recombinant DNA methodology to the improvement of most important agronomic crops. In this review we will focus on key elements and advantages of particle bombardment technology in order to evaluate its impact on the accelerated commercialization of products based on agricultural biotechnology and its utility in studying basic plant developmental processes and function through transgenesis. Fundamental differences between conventional gene transfer methods, utilizing Agrobacterium vectors or protoplast/suspension cultures, and particle bombardment will be discussed in depth.

Introduction

Agricultural biotechnology promises to improve crop productivity, complementing traditional breeding by decreasing our dependence on harmful chemicals, pesticides, fertilizers and antibiotics, etc. Contemporary social and environmental trends emphasize improved safety and quality of agricultural products. Genetic engineering is now available to breeders and can supplement conventional practices in improving crop performance. It is important for the breeder to identify goals and objectives for the genetic modification of crops, which are both scientifically feasible and economically viable. In the past, agriculture has been an energy- and labour-intensive industry. Biotechnology offers the opportunity to reduce both these costs. In order to achieve this objective it is important to identify the genetic need for crop improvement as defined by plant breeders. Factors including basic physiology and genetics of pest resistance, the many years and locations needed to evaluate and identify stress tolerance, and the length of time (in generations) required to break up undesirable genetic linkages or to assemble desirable traits, need to be examined very carefully (Cullis, 1987). Even in cases involving successful separation of the introduced gene from linked deleterious genes, the gene’s inheritance and expression may be altered unpredictably in the new genetic background.

In order to apply genetic engineering successfully to crop improvement, one should be able to identify and isolate agronomically-useful genes, modify them according to strict specifications, and transfer them between species; ultimately, of course, these genes need to be recovered in mature plants and used in a breeding programme. Currently-available methods are designed only to transfer single or few genes. Many traits connected with final plant productivity are the result of multigene families that are only recognized in the context of their ability to be manipulated in a breeding programme, but with little or no knowledge of the biochemical basis of their action (Goodman et al., 1987). In contrast, success in plant genetic engineering
will rely, to a great degree, on a thorough understanding
of the molecular, genetic and metabolic characteristics
and properties of traits to be transferred (Barton & Brill,
1983). In the years ahead, applications of new
biotechnology techniques to agriculture are likely to
enable the high growth rate of crop productivity to
continue by following three general strategies: increase
yields by using more productive plant strains, cut losses
from pests and disease, and improve storage.

The available gene pool with traditional breeding
methods is restricted by the sexual incompatibility of
many interspecific and intergeneric crosses (Nisbet &
Webb, 1990). Genetic manipulation and in vitro culture
provide a means for the gene pool to be substantially
broadened by allowing transfer of specific genes con­trolling well-defined traits from one organism to anoth­er, thus improving crops in a less haphazard way. By
avoiding back-crossing programmes, which may take
years in some cases, considerable time and financial
resources can be conserved.

With conventional transformation methods, appli­cation of biotechnology to crop improvement has been
limited to species amenable to such techniques. Too
much emphasis has been bestowed on model plant sys­tems that were shown to be easily amenable to genetic
and cellular manipulation. As a result, we developed a
good understanding of the molecular biology of
tobacco, potato, tomato and petunia with very little
effort directed towards the important agronomic and
industrial crops. In any biotechnology programme
it is important to work directly with elite cultivars.
As Norman Borlaug observed, variety development is
dynamic and subject to constant change due to the way
pathogens mutate. Agronomic advances also dictate
the constant evolution of improved cultivars. Working
with model systems amenable to laboratory technolo­gies, may appear to be attractive in the early stages of
any research programme. However, short-term gains
will be offset by the need to transfer all the technology
from the model system to elite cultivars, a proposition
that may not always be feasible (Borlaug, 1984).

Elaboration of methods for gene transfer into a wide
range of plant species is a direct result of the develop­ment of in vitro techniques for the culture and propa­gation of cells and tissues. Genes can be accessed from
many diverse sources, e.g. plants, animals, bacteria,
fungi and even viruses. It is theoretically possible to
design a gene to be expressed in a tissue/organ-specific
manner at particular developmental stages in the plant’s
life cycle.

Targets for genetic transformation include: resis­tance to broad-spectrum, environmentally-safer herbi­cides which cannot normally be used with suscepti­ble crops; isolation, characterization and cloning of
disease- and pest-resistance genes from other plant
species or bacteria such as Bacillus thuringiensis; elim­ination of bloating in animals grazing on forage crops
such as clover by introducing tannin genes; improv­ing protein content of grain and forage legumes for
increased nutritional quality; oil quality improvements;
understanding the mechanism of nitrogen fixation by
Rhizobium-legume symbiotic relationships in order to
improve nitrogen fixation in legumes and extend it to
non-leguminous crops.

Comparative evaluation of gene transfer methods
and criteria for stable transformation

Recognition of the ability of the soil bacterium
Agrobacterium tumefaciens to transfer a portion of its
DNA to plants was perhaps one of the most impor­tant milestones in plant biotechnology (Barton et al.,
1983; Caplan et al., 1983; Herrera-Estrella et al.,
1983): Major advances contributing to the popularity of
Agrobacterium-based transformation systems include
the development of disarmed strains where the onco­genes, which result in tumorigenesis and thus diffi­culties in the regeneration capabilities of transformed
tissues, are deleted from the plasmid. The development
of binary vectors, in which the T-DNA borders are
located on a small but wide-host range plasmid and the
virulence genes of the Ti-plasmid are located on an
independent plasmid and act in trans to effect the exci­sion of the T-DNA from the vector plasmid, was also
crucial in expanding the use of Agrobacterium-based
vectors in plant transformation. Any DNA fragment
can be transferred from the Ti plasmid of Agrobacteri­um tumefaciens (or Ri plasmid from A. rhizogenes) as
long as it is located in between the T-DNA borders
flanking the wild type Ti/Ri plasmid T-DNA. These
small plasmids can be easily manipulated in E. coli
and Agrobacterium (Binns, 1988). Chimeric genes
containing selectable resistance gene markers, such as
the antibiotics kanamycin (Becker et al., 1982) or
hygromycin (van den Elzen et al., 1985), methotrex­ate (Pua et al., 1987), herbicides such as Basta (De
Block et al., 1987) and glyphosate (Amrhein et al.,
1983), or screenable markers such as Lux (Ow et al.,
1986) or GUS (Jefferson et al., 1987), can be intro­duced between the T-DNA borders. These genes are