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Transforming Maize Transformation

Maize biologists have long awaited the development of an effective and efficient transformation method. Few plants share maize's importance to both agronomy and basic biology, and the ability to create transgenic maize easily and rapidly would be a tremendous advantage for both those trying to improve the agronomic characteristics of maize and those hoping to use transformation as a tool to explore fundamental questions about maize genetics and development.

Like other cereals, however, maize has not succumbed readily to attempts at genetic transformation. Whereas Agrobacterium tumefaciens provides an excellent route for introducing foreign DNA into dicots, no one has yet been able to use it to transform cereals, and researchers have scrambled to find other ways to transform these important crops. The most promising route seemed initially to be protoplast transformation. These wall-less cells take up foreign DNA more easily than their walled counterparts, and many plants, both monocots and dicots, can be regenerated from protoplasts. Protoplast transformation of cereals has been far less effective than hoped, however. For example, although fertile maize plants can be regenerated from protoplasts and it is possible to genetically transform maize protoplasts, plants regenerated from transformed protoplasts were not fertile (Rhodes et al., 1988).

A number of other possible techniques to transform maize have been pursued, including pollen transformation and bombardment of immature embryos, but until recently the only method to meet with success has been biolistic transformation of embryogenic suspension cells. By bombardering these cells with DNA containing a selectable marker and allowing them to proliferate in the presence of the selection agent, Gordon-Kamm et al. (1990), Fromm et al. (1990), and Walters et al. (1992) were able to identify calli derived from transformed suspension cells. Plants grown from these calli were fertile and transmitted the introduced gene to their progeny.

The recovery of fertile transgenic maize was a major breakthrough because it gave both applied and basic researchers the long-awaited tool that would allow them to introduce both foreign and endogenous genes into the maize genome. Unfortunately, however, the utility of this transformation method has been limited. Embryogenic suspension cells are initiated from a kind of callus, known as type II callus, that few maize lines can form. Although the ability to form type II callus can be backcrossed into agronomically important maize lines (Hodges et al., 1986), in practice this is time consuming and difficult. Moreover, even for those lines that can form type II callus, the method requires a great deal of time and labor and is, therefore, impractical for all but the most well-funded researchers.

A more universally applicable approach to maize transformation would allow rapid and efficient transformation of many different maize lines. In this issue, D'Halluin and coworkers (pages 1495–1505) describe a method to obtain fertile transgeneric maize plants that is both easier and less genotype dependent than previous methods. This new technique relies on immature zygotic embryos or type I embryogenic callus—which, unlike type II callus, forms readily from cultured zygotic embryos of most maize lines. To prepare them to take up DNA, the callus or embryos are first wounded, either enzymatically or mechanically. Foreign DNA is then transferred to them by electroporation. By assaying for transient expression of an introduced neo gene, the authors show that wounding is absolutely necessary for electroporetic DNA transfer, at least into embryos. Wounding reduces the frequency with which embryos regenerate into plants, but not significantly enough to be a problem. The electroporated embryos or callus are then cultured for several weeks on selective (i.e., kanamycin-containing) medium before resistant calli are grown into plants.

D'Halluin and coworkers show that kanamycin-resistant calli derived from immature embryos or type I callus elec- troporated with a neo gene construct gave rise at high frequency to plants that expressed neomycin phosphotransferase II (NPTII), the product of the neo gene. Although there were some signs of tissue culture-induced abnormalities, these Ro plants appeared much healthier than those derived from bombarded embryogenic suspension cells; in particular, they produced viable, functional pollen. By contrast, the Ro transformants produced by bombardment of embryogenic suspension cells are often male sterile. The relative health and fertility of the Ro transformants obtained by D'Halluin and coworkers may reflect the shorter culture times required by this method. The transformants were able to transmit the transgene, through both male and female gametes, to their progeny. In most cases, the ability to express NPTII activity was inherited as a Mendelian dominant trait, although in some instances, inheritance appeared more complex.

DNA gel blot analysis confirmed that the transformed plants contained neo gene sequences and revealed that a wide range of transformation events had occurred. Some transformants contained just one copy of the introduced gene, whereas in others, two, three, or even 20 or more copies were present. In some cases, the plasmid had clearly dimerized or trimerized before integrating into the genome, but even when many gene copies had inserted, these copies were probably closely linked. For example,
the lines with complex integration patterns, NPTII activity segregated in a Mendelian fashion (although it is also possible that in such cases, only one of the integrated genes was functional). In addition, the authors report that the NPTII-positive progeny of four different transformants, all of which had multiple plasmid insertions, had a banding pattern identical to that of their transformed parent; their NPTII-negative siblings, by contrast, contained no sequences homologous to the introduced plasmid. This result contrasts markedly with that of Spencer et al. (1992), who created bialaphos-resistant maize by biolistic transformation of cultured embryogenic cells. One such transformant contained several insertions of the transforming gene, but these copies segregated in the next generation, and just one of the copies was correlated with bialaphos resistance. It is possible that some aspect of D’Halluin and coworkers’ transformation procedure tends to promote plasmid concatemerization.

A number of questions remain about how the new transformation procedure actually works. For instance, which cells in the wounded embryo actually take up the DNA? How variable is uptake? Do slight differences in wounding protocols cause different cells to take up DNA? The authors point out that the transient expression pattern of a marker gene such as β-glucuronidase that has been electroporated into wounded embryos should reveal which cells are actually being transformed. This is a particularly important question because it bears on the issue of whether the regenerated plants arise from single cells or from multiple cells; a potential problem with transforming embryos or callus is the possibility that more than one cell will contribute to the regenerated plant. The heritability of the transgene suggests, however, that few, if any, of D’Halluin and coworkers’ regenerated plants are chimeras. It is also important to ask whether this protocol leads to successful cotransformation, what the frequency of cotransformation is, and how cotransformation can be most efficiently achieved. That is, can the cotransforming gene be carried on a different plasmid than the selectable marker gene, or should it be present on the same plasmid?

These questions will need to be answered, and many other details of the procedure worked out, before this method can be used as widely for maize as Agrobacterium-mediated transformation is for plants such as tobacco and Arabidopsis. Although embryogenic callus arises readily from electroporated immature embryos or type I callus, the ability of this callus to regenerate plants varies greatly. For example, the authors found that the competence of immature embryos to produce highly regenerable callus after transformation changes with the season. And although this method is less dependent on recipient genotype than other methods, it may not work for all genotypes, and culture conditions may need to be optimized for those lines for which it does work.

Despite these potential difficulties, however, the development of a transformation method that is easier and more efficient than previous methods has exciting implications for both applied and basic studies of maize biology. On the applied front, this method should enhance the feasibility of transforming maize plants with genes that would render them, for example, pathogen resistant, herbicide tolerant, or male sterile. On the basic front, the prospect appears closer than ever of studying gene function and regulation in stable transformants, a powerful technique that should be particularly informative for maize, many of whose physiological and developmental processes have been characterized in great genetic detail.

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REFERENCES


