LETTER TO THE EDITOR

On Defining T-DNA

The Agrobacterium-mediated DNA transfer system is the most widely used method for inserting genes into plant genomes; it is utilized for both basic and applied purposes. Previous studies have indicated that Agrobacterium T-DNA is present in the genomes of transformed host plants as single units or in multiple, tandemly arrayed copies (De Block et al., 1984; Spielmann and Simpson, 1986; Jorgensen et al., 1987; Deroles and Gardner, 1988a) and have emphasized that truncated T-DNA regions can frequently be observed (Holsters et al., 1983; Deroles and Gardner, 1988b). For those studies in which host plant DNA was analyzed using hybridization methods, probes specifically designed to detect DNA sequences located beyond the T-DNA border repeats in the original bacterial plasmid were usually not employed. One exceptional study described a single example of a T-DNA region that contained Ti plasmid sequences that extended beyond the usual border repeats in transformed plant tumor tissue (Ooms et al., 1982). However, the generally accepted view has been that DNA from beyond the T-DNA border direct repeats is not transferred into the host plant (Zambryski, 1992).

In the course of our development of transgenic crop plants, we have conducted a thorough analysis of the DNA inserted into our transformed plants via the Agrobacterium-mediated transfer process. Using the DNA blot hybridization technique (Southern, 1975) under standard reaction conditions (see, for example, Sanders et al., 1992), we have now examined transferred DNA in several hundred plants (representing several crop species) independently transformed using an Agrobacterium-mediated binary vector system (McBride and Summerfelt, 1990). DNA from beyond the classically defined T-DNA region has been integrated into the genomes of approximately 20 to 30% of these plants. For example, in one cocultivation experiment, 22 of 96 independently generated plants transformed with the same gene construct contained DNA sequences from beyond the border repeats. Our examination of offspring from plants transformed using another Agrobacterium-mediated binary vector system (An et al., 1985) revealed a similar frequency (20%) of "beyond the border" DNA transfer.

That stable transfer of DNA from beyond the T-DNA borders can occur in transformed plants may not come as a surprise to some plant scientists. "Read-through" of border sequences during T-strand formation in the bacterium has been reported (Stachel et al., 1987; Veluthambi et al., 1988), for example. Also, DNA sequences from far beyond the T-DNA region have been detected in petunia protoplasts during a 24-hr period after infection with Agrobacterium (Virts and Gelvin, 1985). Based on these observations in transient systems, one might predict that sequences outside the classically defined T-DNA would be available for stable incorporation into a plant genome. However, we find the high frequency at which T-DNA extensions occur in transgenic plants to be quite unexpected.

We bring these results to the attention of our colleagues for two reasons. The first is of a practical nature. Although T-DNA binary vectors are completely disarmed of the virulence and oncogenic genes normally present on Ti plasmids, assays for sequences beyond the border repeats should, and can easily, be performed when characterizing potential products. Also, plant scientists involved in projects such as cloning genes via T-DNA tags should be aware of the potential for considerably larger "tags" than had been heretofore expected, a factor that might complicate the identification of adjacent plant DNA. Second, and perhaps more importantly, we hope that this information will contribute to the design of ongoing investigations into the molecular mechanism of T-DNA transfer.

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