

(W-IV-2) :

MATRIX ATTACHMENT REGIONS (MARs) AS A TRANSFORMATION BOOSTER IN RECALCITRANT PLANT SPECIES

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Genetic engineering technique provides a means for inserting genes that confer traits not readily available in traditional gene pool. However, practical application of such technique has been limited to only a few crop species such as cotton, potato, and tomato. Many economically important species remain recalcitrant to gene transfer manipulations. Also, many transgenes including selectable marker genes vary widely in their level of expression, and complete silencing is not uncommon. The standard agronomic practice of seedling transplantation can cause complete loss of transgene expression. According to a recent survey, nearly all of the 30 plant biotechnology companies polled experienced undesired silencing of transgenes. Moreover, silencing of selectable marker genes shortly after their incorporation into plant chromosomes may result in low recovery of transgenic tissues from selection. Therefore, management of transgene expression is important not only for maintaining transgenic phenotypes but also for enhancing survival rate of the transformed cells during selection. Matrix attachment regions (MARs) are DNA sequences that bind to the cell's proteinaceous nuclear matrix to form DNA loop domains. MARs have been shown to increase transgene expression in tobacco cells, and reduce position in mature transgenic plants. Flanking selectable marker gene(s) with MARs could therefore enhance transformation rate by increasing number of transformed cells surviving the selection. Use of MARs may help achieve the desired phenotypes in transgenic plants by increasing and stabilizing the expression of transgenes.

(W-IV-3) :

YEAST TWO HYBRID ASSAY AND ITS PRACTICAL APPLICATION

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Yeast two hybrid assay is a useful and powerful tool to investigate protein-protein interaction. It is based on the fact that many eukaryotic transcriptional regulators are composed of DNA binding domain (BD) and activation domain (AD) which are functionally independent and physically separable. When they are physically separated by recombinant DNA technology, two domains do not interact with each other and thus cannot activate the transcription of the responsive genes. However, if two domains can be brought into close proximity in the promoter region the transcriptional activation will be restored.