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Marker- Free Transgenic Development in Plants

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SUMMARY

Selectable marker genes are widely used for the efficient transformation of crop plants. In most cases, selection isbased on antibiotic or herbicide resistance. Due mainly to consumer concerns, a suite of strategies (site-specific recombination, homologous recombination, transposition and co-transformation) have been developed to eliminatethe marker gene from the nuclear or chloroplast genome after selection. Current efforts concentrate onsystems where marker genes are eliminated efficiently soon after transformation. Alternatively, transgenic plantsare produced by the use of marker genes that do not rely on antibiotic or herbicide resistance but instead promoteregeneration after transformation. Here, the merits and shortcomings of different approaches and possibledirections for their future development are discussed.

INTRODUCTION

A Transgenic organism is a genetically engineered organism developed through transformation. Selection of transformed cell from the non- transformed cell is one of the most important steps of genetic modification event. To distinguish transformed cell from non-transformed cells, Selectable Marker Genes (SMGs) are used in plant transformation protocol. Antibiotics, herbicides or other drugs resistant genes are generally used as SMGs. Once transgenic events have been selected SMGs are generally of no use. But the presence of such genes in transgenic plants raised the concern of development of herbicide resistant weed and antibiotic resistant microorganism through horizontal gene transfer which may have adverse consequences for the environment and human health. So the development of marker -free transgenic plants is essential for their commercial development and from the biosafety and consumer viewpoints.

Strategies to Develop Marker Free Transgenic Plants:

Alternatives to Herbicide or antibiotic- resistant genes:

Some marker genes provide metabolic advantages to transformed cells over non- transformed ones. So transformed cells experience better growth than non- transformed cells. The selection of transgenic events based on differential growth of transformed cells instead of killing non-transformed cells using toxic substances (antibiotics, herbicides). Such a system is based on non-toxic selective chemicals. Example of such markers are *pmi* (mannose), xylose isomerase (xylose), *daol* (D =-amino acid), atlD(arabiotol), AtTPSI(glucose), TSBI (tryptophan), ipt, rolA, B, C, genes etc. Besides these green fluorescent protein (GFP) from jellyfish, luciferase from firefly also act as a visible marker to recognize transformed cells visually.

Co- Transformation:

It is based on the principle of integration of the gene of interest (transgene) and transformation marker (SMG) in different locations of plant genome followed by segregation in the progenies. After segregation progenies with only transgenes are selected. These two genes can be delivered by –(i) two different Agrobacterium strains each containing binary vector, (ii) single strain containing two binary vectors or (iii) single strains having two T DNAs within the same vectors. As it relies on sexual reproduction (segregation), this method cannot be applied to vegetatively reproducing plants and sterile plants. It does not suit for species with long life cycle as this process is time- consuming.

Homologous Recombination:

This method is based on the principle of double – strand DNA break repair mechanism. Double strand break can be repaired by Homologous Recombination (HR) or Non-Homologous End Joining (NHEJ). Frequency of HR is higher when homologous sequence near the break is available. Genes in between homologous repeat

sequence can be deleted during homologous recombination. Recovery of a high frequency of kanamycin resistant gene free tobacco plants was reported when kanamycin gene was inserted between two direct repeat *attP* regions. Induction of double- strand break for higher homologous recombination can be achieved by transient expression of *I-Scel*restriction enzyme in- vivo. Major disadvantage of this system is uncontrolled recombination, excision of non-target genes and low efficiency.

Transposition:

Some elements (DNA sequence), able to change their position within a genome, can also be used to generate marker- free transgenic crops. This strategy is based on placing either gene of interest or marker gene on a mobile element (transposableelement) so that they separate away from each other after transposition. Enzyme transposase excises the transposable elements (TE) and repositions it into another position of the genome. Ac/Ds transposable element system-based marker free transgenic plants have been demonstrated in tobacco and tomato. The recovery frequency of marker – free event is very less as due to the tendency of TE reinsert in linked position and transposition is a rare event.

Site Specific Recombination:

Some microbes originated site specific recombination enzyme acts as molecular scissors which cut the DNA at specific site and also ligate it in another specific region. Site specific recombination has been successfully used in the production of marker -free plants. Recombinase enzyme encoding gene is introduced along with marker and transgene in the target genome. Genes to be removed is flanked by specific palindromic sequence. Recombinase identifies this sequence and cuts it out. This removed portion get degenerated in-vivo. Some example of site-specific recombination system is Cre/lox from bacteriophage. Lysine rich transgenic maize variety was developed successfully using Cre/lox system.

CONCLUSION

In the modern era genetic manipulation is important for early release of improved crop variety. biosafety issue pertaining to the release of transgenic plants have restricted a number of promising GM crops to be commercialized. Several novel marker elimination techniques like homologous recombination, gene targeting use of Zinc finger nucleases have been reported with the increased concern of genetic contamination. There is no evidence that supports the concerns raised by different consumers organization and environmentalists that foreign gene may escape and persist in the environment and food chain leads to unavoidable hazards.

REFERENCES

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