CONSTRUCTION OF TRANSFORMATION VECTORS FOR EFFICIENT DOWN-REGULATION OF GENES IN TRANSGENIC PLANTS

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Down-regulating genes is one way to generate transgenic plants in genetic engineering. Knocking out a gene by antisense technology has been applied to modify the oil palm fatty acid biosynthesis pathway, but it is not the most efficient approach. Recently, the discovery in plants of RNA interference (RNAi) mediated by sequence-specific degradation of double stranded RNA (dsRNA) has led to the development of highly efficient methods for silencing plant genes. Specifically designed genetic constructs containing introns flanked by inverted repeats (self-complementary 'harpin'), or intron-harpin RNA (ihpRNA), have been confirmed as a useful strategy for silencing gene function. Hence, MPOB has developed a series transformation vectors to facilitate gene silencing through ihpRNA technology.

ihpRNA VERSUS ANTISENSE TECHNOLOGIES

The ihpRNA is accomplished through sequences of inverted repeats flanked with an intron, where a normal sequence in the 5'-3' direction is followed by the same sequence in the 3'-5' direction. This formation will result in the folding back of two complementary sequences from a single-stranded RNA molecule to form a 'mimic' dsRNA required for starting the RNAi pathway in plants, which can silence the gene at 90%-100% efficiency as compared to only 15%-30% using antisense RNA technology (*Figure 1*).

APPLICATION OF ihpRNA TECHNOLOGY IN OIL PALM GENETIC ENGINEERING

The ihpRNA backbone vectors were constructed for transformation into oil palm (*Figure 2*). The vector contained two specific restriction enzyme sites, *Bsi*WI-*Aat*II and *Asc*I-*Avr*II, separated by an intron. The cloning of the target gene into the vectors requires amplification of the PCR fragment using a pair of specific primers with two restriction enzyme sites at the 5' end of each primer (*Figure 3*). The inner enzymes were used to first clone the gene fragment into the vector in a sense orientation. Then the fragment digested with the external enzymes was inserted into the vector

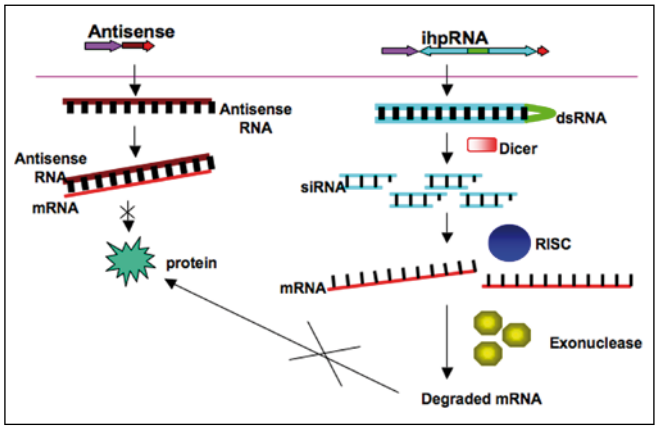

Figure 1. Antisense and ihpRNA technologies.

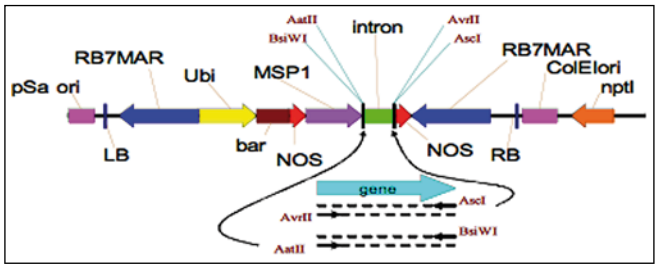

Figure 2. The ihpRNA backbone vector for oil palm genetic engineering.

Forward primer

*Aat*II *Avr*II

GC **GACGTC** **CCTAGG** GACGGCGAAAG

Reverse primer

*Bsi*WI *Asc*I

GC **CGTACG** **GGCGCGCC** TCAGAACTTG

Figure 3. Primers used for cloning of target gene.

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in an antisense orientation to produce ihpRNA transformation vectors (*Figure 4*). Restriction enzyme and PCR analyses were performed to confirm that all the genes were inserted in the expected orientation (*Figure 5*).

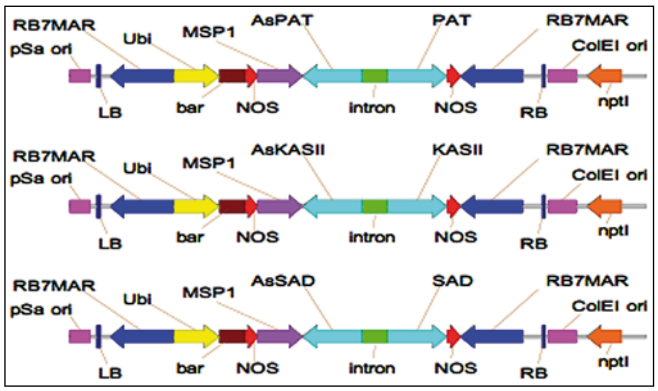

Figure 4. A list of ihpRNA transformation vectors.

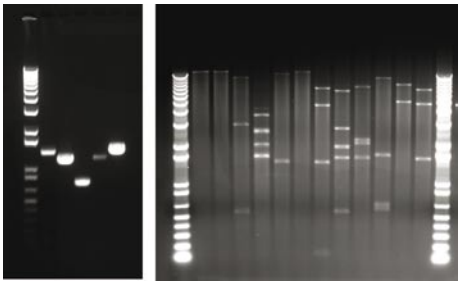

Figure 5. The PCR and restriction enzymes analyses of ihpRNA transformation vectors.

ECONOMIC AND SERVICE COST

The need (and difficulty) to individually construct the ihpRNA vectors greatly limits application of this technology in plant genetic engineering. To overcome this, several versions of ihpRNA vectors, pHannibal and Gateway, have been developed by biotechnology companies such as CSIRO and INVITROGEN. However, their use is limited by their costs and licensing requirements instead. This service by MPOB is to construct the specific ihpRNA vectors required at minimal cost – RM 5000 to RM 10 000 (depending on the complexity and nature of the gene) per construct for any gene for any plant.

WHO SHOULD BENEFIT

Members of the oil palm industry interested to genetically modify oil palm to produce novel products can use this technology. Those who want to genetically modify any other plant for any purpose should also benefit.

For more information kindly contact:

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