Transforming monocots using agrobacterium

Agrobacterium tumefaciens is said to infect dicots naturally. What are the potential obstacles in Agrobacterium-mediated transformation of monocots? Discuss how did the breakthrough (success in transforming monocots using Agrobacterium) come about? (60 marks)

Gene transfer using Agrobacterium is a method of transferring genes by using a carrier to insert the gene of interest into the recipient host plant cells. This technology is based on the discovery of infection tumor in the dicotyledone plants caused by a bacterium, named Agrobactertum tumerfaciens. The species Agrobacterium is a soil bacterium which is capable to infect and caused plant wound and then developed into crown galls, normally formed at the trunk of many types of dicot plants. This Agrobactereium spp. has a special DNA, which has a small ring inside the cytoplasm called Ti plasmid (tumour inducing plasmid). On the Ti plasmid, there is a DNA fragment called T-DNA (transfer DNA) which contains the gene causing crown galls development. Plant cells have genes to code for the production of auxin and cytokinin, the two plant hormones which are used as energy sources by Agrobacterium. The use of Ti plasmid in gene transfer into plants is done by replacing the gene related to plant hormone production and the gene producing opine substance with the desirable trait gene on the T-DNA and then using the Agrobacterium to transfer the gene to the plant chromosomes.

Transformation of dicotyledenous plants using Agrobacterium tumefaciens has been well established and widely used but not so in the case of monocotyledonous plants. The potential obstacle in Agrobacterium-mediated transformation of monocot plants includes:

Agrobacterium is responsive to phenolic compounds such as acetosyringone which are produced when the plant was wounded. The released phenolic compound from the wounded plant cells will stimulate the performance of vir gene on the Ti plasmid, leading to the transferring T-DNA to the plant chromosome. Most of the dicot plants produced this phenolic compound. On the other hand, most monocot plants did not produce the compounds or produced it in a smaller quantity, therefore resulted in the low efficiency of the Agrobacterium attachment. Furthermore, the wounded cells in the monocot plants multiplied less than in dicot plants.

Tissue browning and necrosis following Agrobacterium infection is still a major obstacles especially in cereals. For example in case of wheat, following Agrobacterium infection, wheat embryo and root cells may produce hydrogen peroxide, which altered cell wall decomposition and resulted in a higher level of cellular necrosis and subsequently caused cell death. However the improvement method to resolve the cell death and to improve the transformation efficiency has been demonstrated in cereals (Frame et al., 2002)

Apart from necrosis, physical characteristic and genotype, other factors affected transformation efficiency are strains of Agrobacterium used, binary vector, selectable marker gene and promoter, inoculation and co-culture conditions, inoculation and co-culture medium, osmotic treatment, desiccation, Agrobacterium density and surfactants, tissue culture and regeneration medium (Cheng et al., 2004).

The Agrobacterium has specificity in attaching monocot plants. Most of monocot plants with important economic value are not hosts of the Agrobacterium, therefore the transformation efficiency involving them is low (Lippincott, 1978).

Explants type, quality and source also affect the transformation efficiency foe example embryogenic callus derived from mature seed of rice was reported to be the best explant for Agrobacterium-mediated transformation of rice due to its active cell division (Hiei et al., 1994).

The breakthrough on the transformation of monocot plants using Agrobacterium started when Hiei et al. (1994), done a research on Japonica rice. They reported a stable transformation of Japonica rice by using Agrobacterium. They reported results of evaluations using molecular and genetic analysis on the R0, R1 and R2 progenies. The LBA 4404, the super-binary vector of Agrobacterium strain was demonstrated as the most effective vector for the transformation of three Japonica cultivars tested. Their success has open up the possibility of using Agrobacterium for transforming monocot plants such as maize, barley and wheat.

In 1996, Ishida et al., has done a transformation research on maize by using a similar approach as developed by Hiei et al (1994). Their transformation efficiency was further improved by the addition of silver nitrate in the culture medium. Other factors that may influence transformation efficiency were also investigated that included incubation time and co-cultivation period.

Zhao et al. (2002) optimized the transformation conditions based on Ishida’s protocol and it was demonstrated that maize can be transformed with high

efficiency by using Agrobacterium method. The gene transfer was done by using a combination of standard binary vector with the addition of antioxidant cysteine in the co-culture medium. In the same year, other researchers included had demonstrated that elite maize cultivars could also be transformed by using Agrobacterium-medated transformation method.

Soon after maize, the successful Agrobacterium-mediated transformation of wheat and barley was reported (Jones H. D, 2005, Tingay et al., 1997). Compared with rice and maize, progress with wheat and barley has been slower. Various factors that influence the transformation efficiency have been further investigated. It was reported that the use of surfactant such as Silwett L-77 and desiccation treatment during co-cultivation increased the transformation efficiency of wheat.

In the case of barley, since the success of Tingay et al., (1997) in transforming barley by using Agrobacterium, a number of other researchers around the world have reported the successful production of transgenic barley plants. However majority of the successful reports of Agrobacterium-mediated transformation of barley are restricted with model genotype ‘ golden promise’ and ‘ igri’. Therefore, optimizations of parameters are required to extend the Agrobacterium-mediated transformation in other elite barley cultivars.

The transformation of sorghum is the least successfully manipulated. Zhao et al. (2000) developed an efficient Agrobacterium-mediated transformation system for sorghum and from the research it showed that the embryos from the field had higher transformation frequency than those from the

greenhouse. Other transformation of monocotyledon plant reported such as Agrobacterium-mediated transformation of turfgrasses, such as creeping bentgrass (Yu et al., 2000), Italian ryegrass (Bettany et al., 2003), and tall fescue (Wang and Ge, 2005) were also reported.

Although the delivery of foreign gene into several monocot species via Agrobacterium tumefaciencs has now become a routine technique, there are still serious limitations on the used of this technology on other major monocots. In order to achieve better success in transforming monocot using Agrobacterium, many factors and conditions were being investigated, such as selection of which target tissues which are highly responsive, adjustment of gene transfer conditions to increase the possibility of Agrobacterium attachment into the cell by adding phenolic substances such acetosyringone during co-cultivation period or in co-cultivation medium, that are similar to the substance released by plant cells when they are naturally wounded, using efficient promoter gene to stimulate the expression of the gene in monocot plants and the used of super-virulent of Agrobacterium strains to increase the transformation efficiency (Cheng et al., 2004).