JUST AGRICULTURE
multidisciplinary e-Newsletter

Genetically Modified Plant: A Vector for Antibiotic Resistome?

Sagar, S. P.[1], Sanam Tulja[2] and Prathima, M. N.[3]
[1]Ph. D. Scholar, Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi
[2]Ph.D. Scholar, Department of Agricultural Sciences, UAS, Bangalore
[3]Ph. D. Scholar, Department of Agricultural Microbiology, UAS, Dharwad

ARTICLE ID: 66

Introduction

Antibiotics, the result of competition among microbial species over a period of evolution and in the same manner they responded with varied resistance mechanisms to combat the effect of antibiotics. The issue of clinically emerged antibiotic resistance is due to the exchangeable genetic elements through either vertical or horizontal transfer of multiple genes for antibiotic resistance (Sande *et al*., 1990). Furthermore, antibiotic resistance attributes act through chemical degradation of antibiotics like β-lactamase enzyme or inactivate antibiotics through addition of chemical groups through *npt-II*.

As selection of transformed plant cells isn't an easy approach among larger number of cells to be screened and there the selection based on phenotype isn't convenient. Antibiotic resistance markers (ARM) play a vital role at this point. They are often used in plant biotechnology by construction of vectors to study plant transformation, like genetically modified (GM) *Escherichia coli* or *Agrobacterium tumefaciens*, which directly transfer plant cells and at some instances, genetically engineered plants retain ARM as it follows a bacterial promoter sequence (Malik and Saroha, 1999). Though studies presented as ARM's are lost in the GM plants either through selection or segregation between the gene of interest and the ARM, and instances where ARM get either inactivated through mutation or lost as there won't be any function of adequate requirement to plant, there are no much evidence for an exact mechanism that prevail in removal of such ARM's.

Commonly used AMR's include neomycin phosphotransferase II (*npt-II*) (Malik and Saroha, 1999; European Federation of Biotechnology, 2001) that inactivates kanamycin and neomycin, a group of aminoglycosides. Others like ampicillin resistance gene (beta-lactamase, *bla*) and aminoglycoside resistance gene (aminoglycoside adenyl transferase, *aad*)

JUST AGRICULTURE
multidisciplinary e-Newsletter

that degrade ampicillin and streptomycin, respectively. There are also instances of such genes being incorporated into the plant products (Malik and Saroha, 1999) and if such is the case, it may assist in dissemination of such antibiotic resistome in bacteria via endophytes and the food web. Hence, it's a matter of concern from the point of current scenario of deceases caused due to antibiotic resistance in pathogens.

Genes governing as ARM in plant biotechnology

- ***bla*, ampicillin resistance:** Usage of ampicillin was wide during 1960's due to its vast range of activity against community acquired urinary tract and respiratory pathogens. Later it was replaced by amoxicillin as it causes less gastrointestinal side effects. Organisms resistant to amoxicillin were known during 1970's through a presence of *bla* genome in a plasmid. The product of this gene β-lactamase hydrolyses these antibiotics and restricts it's usage and hence, the third generation antibiotics like carbapenems and cephalosporins were developed (Malik and Saroha, 1999).
- ***npt-II*, kanamycin and neomycin resistance:** Including the mentioned antibiotics it also provides resistance against number of analogues of aminoglycosides such as butirosin and paromomycin. Further, it won't inactivate the clinically used gentamicin as it lacks 3' hydroxyl necessary for the action *npt-II*. But, will inactivate other non-clinicallyused gentamicin (Azucena and Mobashery, 2001; Smith and Baker, 2002).
- ***hph* [APH(4)], hygromycin resistance:** Though it's laboratory usage continues, no significant evidence of the ARM is noticed in commercialized plants (Miki and McHugh, 2004). Furthermore, it's utility in human and veterinary treatment is not that significant. Only the *Escherichia coli*-derived APH(4)-Ia, also referred to as hygromycin phosphotransferase (hph) has been utilized in plant transformation (Day, 2003). Hygromycin resistance is conferred by APH(4)-Ib (derived from *Pseudomonas pseudomallei*), aminoglycoside phosphotransferase (APH) 4-Ia (derived from *E. coli*) and APH (7") (derived from *Streptomyces hygroscopicus*) (Wright and Thompson, 1999).

Alternative to ARM

Selective technologies

Herbicide tolerance could be used as a selectable marker. Examples include EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), *bar* (phosphinothricin acetyl transferase)

JUST AGRICULTURE
multidisciplinary e-Newsletter

and *Bxn* (bromoxinil nitrilase) resistant to glyphosate, glufosinate and bromoxinil, respectively (Day, 2003), but may arise the issue of providing a source of herbicide resistance to sexually compatible weed species or even limit the control option for re-emergence of plant species in following crop rotations.

Furthermore, growth of plant cells in unusual growth medium like glucouronides, mannose, xylose and others could be employed in either positive or negative selection of transformed plant cells (Haldrup *et al*., 1998; Joersbo *et al*., 1998). But, it may lead to different metabolic pathways and hence, the complete analysis of such effect should be monitored from the point of ecological dissemination of new metabolic pathways through genes (European Federation of Biotechnology, 2001).

Co-transformation

Here marker gene and the desired gene are placed on different DNA segments or genomic regions, so that the marker gene can be removed out after obtaining the transformed cells through transitional breeding (Miki and McHugh, 2004). Two Agrobacterium strains with separate plasmids each with single T-DNA (Daley *et al*., 1998) will suit here. But one has to produce comparatively larger number of plants to select genetically transformed plants (Miki and McHugh, 2004).

Recombinase-mediated excision

It is a site-specific recombinase mediated excision system. Here, the selectable marker gene will be flanked by sequence of specific recombination target and introduce the recombinase protein subsequently. The bacteriophage P1 *Cre/ lox* system, the *Saccharomyces cereviseae* 2 mm circle

FLP/FRT system, the *pSR1* system of *Zygosaccharomyces rouxii* and the Gin recombinase system of phage Mu (Gilbertson, 2003; Miki and McHugh, 2004) are the four such systems well known and of which *Cre/ lox* system is well studied and utilized in methodologies.

Homologous recombination

The excision of DNA sequence between two repeated DNA sequences can be achieved when they're adjacent in a chromosome through homologous recombination (Lichtenstein *et al*., 1994). The frequency of homologous recombination generally occurs at a low frequency in plant species and that vary with the plant species too. While it is noted that

JUST AGRICULTURE
multidisciplinary e-Newsletter

the recombination in maize of closely linked repeated sequence occur at a frequency of 0.5 % (Sudupak *et al*., 1993). Current research is oriented towards conditions that increase such magnitude of recombination, thereby provide a practical way of inserting desired gene rather than achieving selection.

Conclusion

Afterall very basic questions arise, viz., effect of these ARM's is through the gene itself? or it's protein product? or will they transfer ARM into bacteria? or will they cause deleterious effects in human cell? Out of 54 g/ day of total DNA consumed just 54 µg of transgene was intaken along with the total and that's almost one-millionth of total ingested DNA (Aumaitre *et al*., 2002). Even such ARM genes are digested and processed just like other genes from different products, thereby, no prominent deleterious effect could be identified. Furthermore, in human or animal gut ARM proteins are frequently produced and been exposed to those since history, hence, antibiotic resistant bacteria are selected among gut microbiome. Well known mechanisms like vertical and horizontal gene transfer aid in transfer of such ARM among bacterial population and will lead ultimately in dissemination of antibiotic resistome through rhizosphere microflora or endophytes. So, it's an important task for researcher to overcome such adverse effects specifically with clinically administered antibiotics. Even the GM plants are to be dealt from the point of ethical issues in a way towards ecologically balanced days ahead.

References

Aumaitre, A., Aulrich, K., Chesson, A., Flachowsky, G., & Piva, G. (2002). New feeds from genetically modified plants: substantial equivalence, nutritional equivalence, digestibility, and safety for animals and the food chain. *Livestock production science, 74*, 223-238.

Azucena, E., & Mobashery, S. (2001). Aminoglycoside-modifying enzymes: mechanisms of catalytic processes and inhibition. *Drug Resistance Updates, 4*, 106-117.

Daley, M., Knauf, V. C., Summerfelt, K. R., & Turner, J. C. (1998). Co-transformation with one *Agrobacterium tumefaciens* strain containing two binary plasmids as a method for producing marker-free transgenic plants. *Plant cell reports, 17*, 489-496.

Day, A. (2003). Antibiotic resistance genes in transgenic plants: their origins, undesirability and technologies for their elimination from genetically modified crops. In: *Transgenic*

Plants: Current Innovations and Future Trends ed. Stewart, N. C. pp. 111-156. Wymondham, UK: Horizon Scientific Press.

European Federation of Biotechnology. (2001). Task group on public perceptions of biotechnology. *Antibiotic resistance markers in genetically modified (GM) crops*. Briefing paper 10. Delft, the Netherlands: European Federation of Biotechnology.

Gilbertson, L. (2003). Cre-lox recombination: creative tools for plant biotechnology. *Trends in Biotechnology, 21*, 550-555.

Haldrup, A., Petersen, S. G., & Okkels, F. T. (1998). The xylose isomerase gene from *Thermoanaerobacterium thermosulfurogenes* allows effective selection of transgenic plant cells using D-xylose as the selection agent. *Plant Molecular Biology. 37*, 287-296.

Joersbo, M., Donaldson, I., Kreiberg, J., ... & Petersen, S. (1998). Analysis of mannose selection used for transformation of sugar beet. *Molecular Breeding, 4*, 111-117.

Lichtenstein, C., Paszkowski, J., & Hohn, B. (1994). Intrachromosomal recombination between genomic repeats. In: *Homologous Recombination and Gene Silencing in Plants* ed. Paszkowski, J. pp. 95-122. Dordrecht, the Netherlands: Kluwer.

Malik, V. S., & Saroha, M. K. (1999). Marker gene controversy in transgenic plants. *Journal of Plant Biochemistry and Biotechnology, 8*, 1-13.

Miki, B., & McHugh, S. (2004). Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *Journal of Biotechnology, 107*, 193-232.

Sande, M. A., Kapusnik-Uner, J. E., & Mandell, G. L. (1990). Antimicrobial agents-general considerations. In: *The Pharmacological Basis of Therapeutics* ed. Gillman, A. G., Rall, T. W., Nies, A. S., & Taylor, P. pp. 1018-1046. New York: Pergammon.

Smith, C. A., & Baker, E. N. (2002). Aminoglycoside antibiotic resistance by enzymatic deactivation. *Current Drug Targets. Infectious Disorders, 2*, 143-160.

Sudupak, M. A., Bennetzen, J. L., & Hulbert, S. H. (1993). Unequal exchange and meiotic instability of disease-resistance genes in the Rp1 region of maize. *Genetics, 133*, 119-125.

Wright, G. D., & Thompson, P. R. (1999). Aminoglycoside phosphotransferases: proteins, structure, and mechanism. *Frontiers in Bioscience, 4*, d9-d21.