Biosafety Study of Transgenic Papaya DNA in Soil Environment

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Abstract

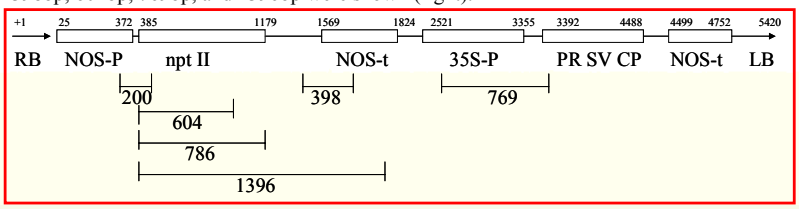
Most of the genetically modified crop use neomycin phosphotransferase gene (*nptII*) as selection marker gene, the gene that encodes the enzyme which can inactive the drug kanamycin. WHO/FAO suggested that the horizontal gene transfer (HGT) of the antibiotic resistant gene to pathogenic microorganisms and possible clinical implications must be considered. Therefore, it is important to investigate the HGT and the persistence of marker gene in the soil. Our experiments demonstrated that the HGT of nptII gene from transgenic papaya were not detected in soil. Studies on the soil persistence of transgenic papaya DNA indicated that specific transgenic sequences inserted in the genomic of transgenic papaya could be detected for five months. The concentration of nptII gene (200bp) in soil was 10^{-6} times less than the dose required for homologous gene transformation in soil[4]. Therefore, horizontal gene transfer of genomic nptII gene in soil bacteria was unlikely happened.

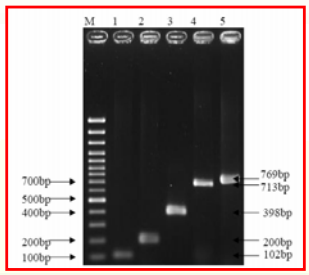
Goal

Transgenic papaya expressing ringspot virus coat protein gene (PRSV CP gene) and kanamycin resistant gene (*nptII* gene) is the first transgenic crop developed in Taiwan[1]. Our goals are : (1) build up a system to evaluate the HGT of nptII gene from transgenic papaya, and (2) develop a system to monitor the fates of transgenes in soil.

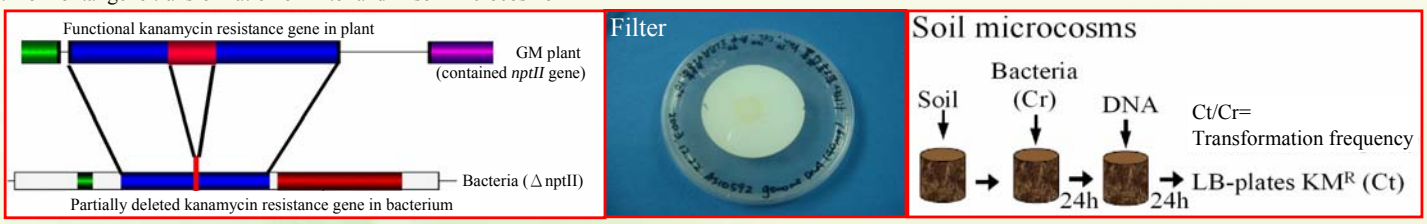
Materials and Methods

1. Genetic construct in the transgenic papaya (left). PCR amplification regions of 200bp, 398bp, 398bp, 604bp, 769bp, and 1396bp were shown (right).





2. Horizontal gene transformation on filter and in soil microcosmer[5]



3. *Acinetobacter* sp. BD413
(1) pFG4ΔnptII, 313bp[3] ; (2) pMR7ΔnptII, 10bp[2] ; (3) ATCC 33305

Table 1. Transformation of *Acinetobacter* spp. on filter and in sterile soil microcosmos

DNA	*Acinetobacter*	Filter	Sterile soil
Transgenic PCR product (1396-bp)	pFG4	10^{-6}	10^{-8}
	ATCC 33305	ND	ND
Genomic DNA	pFG4	ND	ND
	ATCC 33305	ND	ND

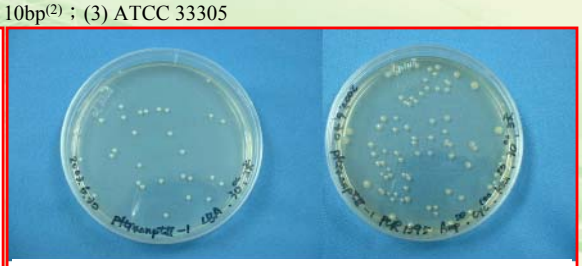


Fig. 2. *Acinetobacter* sp. BD413 PFG4ΔnptII in LBA (left), and transformed Acinetobacter sp. BD413 (kan^R) in LBA with kanamycin (50 μg/mL)(right).

4. Transgenes in soil collected form confined field at Wu-Fong

Table 2. Soil DNA extraction methods were evaluated, and CTAB/SDS/gel method was the best with 45.8% recovery.

Method	769bp (%)	398bp (%)	200bp (%)	Mean
UC-Kit	0.03	0.15	0.09	0.09
SDS/GTC				
PEG purification	3.4	11.5	2.0	5.7
Gel purification	42.8	30.2	49.3	40.8
CTAB/SDS				
PEG purification	3.7	12.3	2.4	6.1
Gel purification	57.8	32.8	46.9	45.8
Bead/SDS				
PEG purification	1.3	7.1	5.9	4.8
Gel purification	10.4	18.4	29.8	19.5

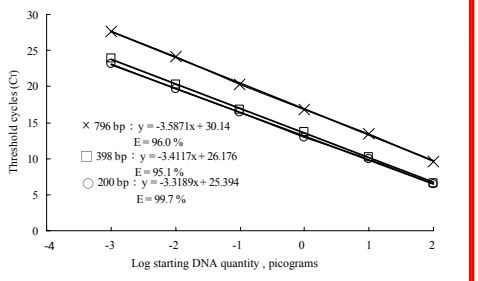


Fig 3. Standard curves of 769 bp(×), 398 bp (□), and 200 bp (○) obtained from real-time PCR. The PCR efficiencies were in the ranges of 95.1-99.7%.

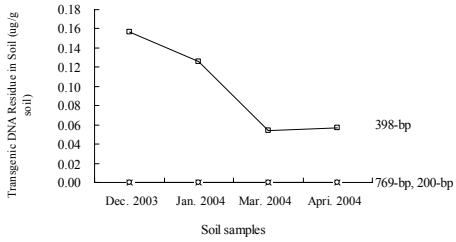


Fig. 4. Persistence of transgenic papaya DNA in soil where transgenic papaya were grown. The residues of 398-bp were less than 0.16 μg/g soil, whereas the residues of 769-bp, and 200-bp were less than 2.0×10^{-4} μg/ g soil.

Conclusion

1. No genomic nptII gene transformation was detected in strains of *Acinetobacter* sp. BD413, andBD413 (pFG4ΔnptII).
2. The persistences of 398-bp (pBI121/NOS-t) in soil samples were less than 0.16 μg/g soil, whereas the residues of 769-bp (35S-P/PRSV-CP) and 200-bp (NOS-P/nptII) were less than 2.0×10^{-4} μg / g soil.
3. The concentration of nptII gene in soil samples were 10^{-6} times less than the dose required for homologous gene transformation in soil (23.8 μg / g soil, pFG4ΔnptII bacteria). Horizontal gene transfer of genomic *nptII* gene in soil bacteria was unlikely happened.

Acknowledgments.
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References

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