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Production of marker-free transgenic tomato plants with the super sweet protein gene under the control of cis-regulatory elements using pMF vector system

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Generation of transgenic plants without any foreign genetic material relieves public concerns and facilitates future commercialization of GM crops. In our investigation we used the pMF vector containing of the recombinase R and a CodA-nptIIbi-functional selectable gene for produce tomato plants carrying the super sweet thaumatin II gene under the control of tomato fruit-specific ELIP or E8 gene promoter and tomato RuBisCo terminator. In the early selection approach, a total of 155 Km-resistant calluses were treated to induce recombination. Eighty three shoots from 116 was non-transgenic escapes, 32 contained nptII gene fragment and only one marker-free line with correctly excised DNA that confirmed by PCR and Southern was appear. In the alternative delayed strategy we have obtained a total of 170 transgenic lines. About half of them contained a partial sequence of the T-DNA but the majority of checked had two or more inserts. For the second round of shoot regeneration we choose 35 transgenic lines. One hundred twenty one resistant plants were obtained from 18 original lines. Most of them lost resistance to kanamycin in spite of the sequence of nptII gene were not detected only in one marker-free line. We suppose that an incomplete excision and chromosomal rearrangements due to the presence of multiple and aberrant or partial T-DNA insertions occur in other cases. The thaumatin II gene expression has been confirmed by RT-PCR, Western blotting and organoleptic analyses. Two completely marker-free transgenic tomato plants were obtained by two different selection strategies altogether. This result demonstrates that the pMF vector system is an acceptable for production marker-free transgenic tomato but probably objective physiological and molecular biological features do not allow achieving the high efficiency for *Solanum* species.

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Microbial load of operating theatre at Ayder Referral Hospital, northern Ethiopia

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Microbial contamination of the operating theatre (OT) is a major cause of nosocomial infections (NI). The study assessed the level of microbial contamination and to determine the antimicrobial resistance of the bacterial isolates. Settle plate's method was used for air sample collection while swab method was used to collect samples from surfaces and other articles in the major OT. Collected samples were transported and microbiologically processed using standard procedures. One hundred twenty air, 36 article and 12 surface samples were taken for microbiological evaluation. The highest level of microbial contamination was detected in the OT air before proper cleaning-fumigation as compared to after the intervention. Moreover, microbial growth was found on surfaces and semi-critical articles. On the other hand articles which were sterilized by autoclave showed no microbial growth. The five types of bacteria isolated were coagulase negative *Staphyllococci* (68; 53.4%), *Staphyllococcus aureus* (42; 33.1%), *P. aeruginosa* (13; 10.2%), *E. coli* (2; 1.6%) and *Bacillus* spp. (2; 1.6%). Methicillin resistance *S. aureus* (MRSA) account for 7.7% of the *S. aureus* isolates. The highest resistance was found against penicillin G and ampicillin with a resistance rate of 52.7% and 44.5% respectively. Multi-drug resistance was observed among 23(36.5%) of the bacterial isolates. In conclusion, there was high level of microbial contamination in the OT particularly in air and semi-critical articles. However, it has been dramatically reduced through proper cleaning-fumigation of the OT. Therefore, efforts should be made to ensure strict infection control practices in the OT.

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