

P 31

## Recent Advances in Pepper Transformation

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### Objectives

Many problems of being a failure to transform pepper shoots by co-culture experiments have been recognized for last several years at different labs in worldwide. To obtain the successful transformation system of pepper plants, we have worked on a serious of research agenda to develop a widely useful protocol for pepper transformation.

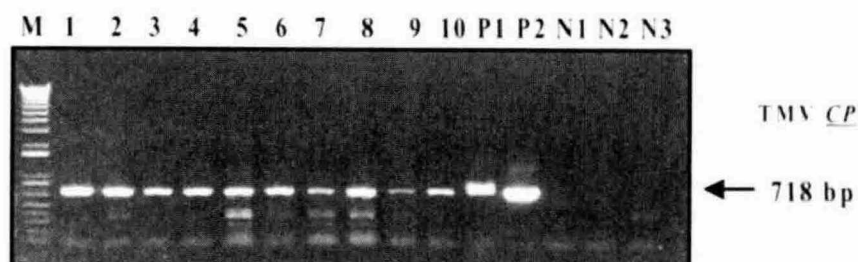
### Materials and Methods

1. Materials: 32 pepper inbred lines
2. Methods: *Agrobacterium*-mediated transformation with TMV coat protein (CP) gene; Shoot formation from callus induction with 100 mM kanamycin

### Results and Discussion

We have generated a system for shoot formation from multishoots that were induced from the edge of tissue cut of

cotyledons and hypocotyls. Most of shoots seem to grow directly from sub-epidermal cell layers near the wound surface of explants in 3-4 weeks and elongate well on the selection medium with a rate depending upon selection conditions. We have obtained around 400 plants after co-culturing with CP gene from around 40,000 explants. All of them were tested with PCR analysis and not a single shoots have contained the CP gene. However, some shoots were indirectly grown from the callus tissues that had formed around cut even after 6 weeks on the shoot induction medium. These cases were unusual and usually the callus was not easily formed from the wounding epidermis. When those shoots were transferred to elongation and rooting medium allowing to further growth, a total of twenty plants were obtained and examined by PCR analysis. Surprisingly 13 plants contained CP gene. DNA samples were isolated from the 10-20 cm tall plants and PCR data was confirmed by two different sets of primers designed from vector sequences and internal regions of CP gene. PCR Southern blot analysis provided a further evidence that we have obtained pepper plants transformed by TMV CP gene. On the poster we will present technical and basic aspects of pepper transformation as well as successfully transformed cases.



M: marker; 1-10: transformed; P1: bacterial cells harboring pTMVCP; P2: pTMVCP; N1-3: non-transformed.