

A New Selection Method for Pepper Transformation: Callus-Mediated Shoot Formation

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Objectives

The objective is to develop a selection system for pepper transformation.

Materials and Methods

1. Materials: pepper inbred lines used for breeding commercial F1 hybrids.
2. Methods: *Agrobacterium*-mediated transformation with TMV coat protein (CP) gene and *PPI 1* gene; shoot formation from callus was selected by 80~100 mM kanamycin

Results and Discussion

Pepper is known as one of recalcitrant crops for transformation for many years at different labs in worldwide. To obtain a

successful transformation system of pepper plants, we have developed a system for selection by selecting shoots grown from callus that were induced from 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. However, we have never obtained a transformed pepper from the direct shoots by PCR analysis. In contrast, some shoots were indirectly grown from the callus tissues that had formed around cut of the explant on the shoot selection medium. These cases were unusual because the callus was not easily formed from the wounding epidermis. Those indirect shoots grown from the callus had a high probability to be transformed. This observation provides a strong selection means to avoid the non-transformed shoots.

Transformation rate by direct shoot formation

Gene	Explant	Shooting	Rooting	PCR (+)
TMV-CP	102,500	5,219	895	0
<i>PPI 1</i>	49,200	2,870	512	0
Total	151,700	8,089 (5.3%)	1,407 (0.9%)	0 (0.0%)

Transformation rate by indirect shoot formation

Gene	Explant	Shooting	Rooting	PCR (+)
TMV-CP	21,100	34	24	12
<i>PPI 1</i>	16,400	15	10	6
Total	37,500	39 (0.1%)	34 (0.09%)	18 (0.05%)