Agrobacterium-mediated transformation in potato using different explants and co-cultivation media and histochemical detection of pathogenesis-related promoters

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Abstract: The aim of the study was to compare different co-cultivation media (solid and liquid) and explants (leaf and microtuber) on *Agrobacterium*-mediated transformation in potato and to detect the β-glucuronidase (GUS) expression using plant-originated pathogenesis-related (PR) promoters. Leaf and microtuber discs were inoculated with GV 2260 *Agrobacterium* p35S GUS-INT, AoPR1-GUS-INT and PR1a-GUS and co-cultivated on either liquid or agar-solidified medium. Liquid co-cultivation medium increased transformation frequency of potato in use of all *A. tumefaciens* plasmids and explant types. Mean transformation frequency was 15% in liquid medium compared to 4.1% on solid medium for leaf explants. Similarly, liquid medium (3.12%) gave higher transformation frequency than solid medium (1.25%) in microtuber discs. Moreover, low GUS expression in all organs was observed for PR promoters (AoPR1 or PR1a) compared to CaMV35S promoter. Both salicylic acid and wounding treatments enhanced AoPR1 and PR1a promoter gene activity in leaf, stolon, tuber and microtuber.

Key words: potato, leaf discs, microtuber discs, co-cultivation media, pathogenesis-related promoters.

Abbreviations: GUS, β-glucuronidase; PR, pathogenesis-related; SA, salicylic acid.

Introduction

The most common method used for the transformation in plants is the use of *Agrobacterium tumefaciens*. *Agrobacterium*-mediated transformation is influenced by a large number of variables including bacteria strain, plant genotype, pH, co-cultivation media, temperature, co-cultivation time and explants type (URANBEY et al., 2005). Genetic transformation in potato was achieved using leaf, stem, tuber and microtuber discs (SHEERMAN & BEVAN, 1988; ELISEU et al., 1994; ANNIE et al., 1995; KUMAR et al., 1995; MORAVCIKOVA et al., 2003). It was emphasized that microtubers were better source of explant material resulting in more uniform response during genetic transformations (KUMAR et al., 1995). Furthermore, the solid co-cultivation medium has routinely been used in most reported *Agrobacterium*-mediated transformation protocols (DONG & MCHUGHEN 1991; HOLFORD et al., 1992; MUTHUKUMAR et al., 1996; CERVERA et al., 1998), whereas liquid co-cultivation medium has only been preferred in a few studies (HERMAN et al., 1989, PAULA et al., 1993). The development of transgenic plants includes the development of uniform and high-frequency adventitious shoot regeneration with efficient transformation techniques for the introduction of genes into the crop plants. Since microtubers are acquired under controlled conditions, a more uniform response during transformation may be obtained in potato as well. Moreover combination of microtubers and liquid co-cultivation medium for transformation study in potato has not been reported up to date.

Pathogenesis-related (PR) proteins are a heterogeneous group of proteins induced in plants by the presence of exogenous chemicals besides pathogen infection. PR promoters and their relation with salicylic acid (SA) have previously been reported (OHSHIMA et al., 1990; PARK & KLOEPPER, 2000). Analysis of PR promoters provided an insight into the area of plant response (LINTHORST, 1991). These plant-originated promoters are used as alternatives to the virus-originated CAMV 35S promoters. The PR-1a promoter may be used to induce the expression of *Bacillus thuringiensis* δ-endotoxin in transgenic plants. Use of PR pro-

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