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ScienceDirect
Gene 409 (2008) 11–19
GENE
www.elsevier.com/locate/gene

# Germline transformation of Shepherd's purse (*Capsella bursa-pastoris*) by the 'floral dip' method as a tool for evolutionary and developmental biology

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Received 10 July 2007; received in revised form 29 October 2007; accepted 30 October 2007
Available online 17 November 2007
Received by W. Martin

**Abstract**

*Capsella bursa-pastoris* is an attractive model system for evolutionary and developmental biology. To facilitate future studies on gene function, the 'floral dip' method was adapted to achieve germline transformation of *C. bursa-pastoris*. The *GFP* and *BASTA-resistance* (*BAR* $^r$) genes were used as markers for screening or selecting, respectively, putative transgenic *C. bursa-pastoris* plants and the β-glucuronidase (*GUS*) gene as well as the *GFP* gene for monitoring transgene expression level. We tested two *Agrobacterium* strains, LBA4404 and GV3101, for their ability to transform *C. bursa-pastoris*. In contrast to *Arabidopsis thaliana*, for which both strains were able to transform different ecotypes, only GV3101 gave satisfactory transformation rates with *C. bursa-pastoris*. Furthermore, we evaluated the effects of different concentrations of sucrose and the surfactant Silwet L-77 on the efficiency to generate transgenic *C. bursa-pastoris* plants and identified an efficient medium containing 10% (w/v) sucrose and 0.02–0.05% (v/v) Silwet L-77. Using Southern hybridisation, we confirmed the integration of the marker gene in the plant genome and the stable heredity of the introduced genes in the next generation.
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*Keywords:* *Agrobacterium*-mediated transformation; Floral-dipping; *GUS*-expression; Herbicide resistance; *Capsella*; Transgenic Shepherd's purse

## 1. Introduction

Shepherd's purse, *Capsella bursa-pastoris* (L.) Medik., is a close relative of the genetic model plant *Arabidopsis thaliana* (Koch and Kiefer, 2005) and belongs to the five most wide-spread flowering plants on our planet (Hurka et al., 2003). It is a member of a small genus within the mustard family (Brassicaceae) which may only comprise three species; these, however, show remarkable differences in ploidy level, breeding systems and habitat range (Zunk et al., 1999; Hurka et al., 2005). Two of the *Capsella* species, *C. grandiflora* and *C. rubella*, are diploid, while the third one, *C. bursa-pastoris*, is tetraploid. *C. bursa-pastoris* is predominantly selfing, and is distributed mainly in disturbed, 'man-made' habitats, almost all over the world (Hurka and Neuffer, 1997). Concerning reproductive biology and ploidy level, comparison with outgroup species strongly suggests that *C. grandiflora* represents the most ancestral and *C. bursa-pastoris* the most derived character states (Hurka et al., 2005).

Within *Capsella*, *C. bursa-pastoris* is of special interest. It is a classical model plant for investigations on the early development of eudicot embryos (Schulz and Jensen, 1968). Moreover, *C. bursa-pastoris* shows some interesting and potentially

*Abbreviations:* BAP, Benzylaminopurine; BAR$^r$, Basta-resistance gene; CaMV, Cauliflower Mosaic Virus; *Cbp*AG, *Capsella bursa-pastoris* Agamous; CHSA, Chalcone synthase; DIG, Digoxigenin; GFP, Green fluorescent protein; GUS, β-glucuronidase; MAS, Mannopine synthase; MS, Murashige & Skoog; NPTII, Neomycin phosphotransferase II; NOS, Nopaline synthase; OD, Optical density; pAG7, Agropine synthase polyadenylation signal; SI, Self-incompatibility.

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doi:10.1016/j.gene.2007.10.033

important morphogenetic phenomena that are, however, quite rare and hence cannot be easily studied elsewhere. In the flowers of the homeotic mutant *Stamenoid petals* (*Spe*), for example, stamens develop in the second floral whorl, where usually petals would occur, while all other floral organs are unaffected (Nutt et al., 2006; Hintz et al., 2006). The *Spe* mutant is interesting from both a developmental and an evolutionary point of view (Nutt et al., 2006; Hintz et al., 2006). Several lines of evidence suggest that homeotic changes played a considerable role during the evolution of flowers, but the relevance of homeotic transformations during the origin of morphological novelties has remained a very controversial topic (reviewed by Theiβen, 2006). Thus *C. bursa-pastoris* is an attractive experimental system for evolutionary and developmental biology.

*C. bursa-pastoris* is quite easy to cultivate and propagate. Its life cycle is fairly short and allows the growth of two to three generations per year (depending on the variety). And despite being tetraploid, *C. bursa-pastoris* switched already to disomic inheritance during evolution (Hurka and Düring, 1994), which makes crossing experiments easier to interpret. The order, orientation and sequence of genes is very similar in *Arabidopsis* and *Capsella*, with more than 90% sequence identity within exons (Acarkan et al., 2000; Koch and Kiefer, 2005). This allows the identification of genes within *Capsella* with the help of the *Arabidopsis* genome (*Arabidopsis* Genome Initiative, 2000). Due to its close relationship to the model plant *A. thaliana* numerous experimental tools are available to study the genus *Capsella*, and more are being developed. Experimentation along these lines will be further facilitated by the sequencing of the genome of *Capsella rubella* (Joint Genome Institute, United States Department of Energy).

To investigate traits from an ecological, developmental or evolutionary point of view transgenic technology is often extremely helpful. For example, the evolution of the self-incompatibility system in *Arabidopsis* was studied by transfer of two S locus genes from the self-incompatible *A. lyrata* into the self-compatible *A. thaliana* (Nasrallah et al., 2002), and similar experiments may help to investigate the evolution of the SI (self-incompatibility) system that is active in *C. grandiflora* (Paetsch et al., 2006) as well. But so far successful transformation of *Capsella* has not been reported.

In *Capsella*'s close relative *A. thaliana*, genetic transformation became a very simple procedure by development of the 'floral dip' method (Clough and Bent, 1998). 'Floral dip' is an *in planta* method, because genes are delivered into intact plants. Unlike previous methods (reviewed by Bent, 2000), the 'floral dip' procedure requires neither tissue culture nor vacuum infiltration and is hence very labour-efficient. The 'floral dip' method could also be applied to *Arabidopsis lasiocarpa*, radish (*Raphanus sativus ssp. longipinnatus*) and *Medicago truncatula* with good success (Tague, 2001; Curtis and Nam, 2001; Trieu et al., 2000), but similar attempts with *C. bursa-pastoris* failed (Tague, 2001).

The previous studies in *Arabidopsis* and *Raphanus* revealed that the presence of the tri-siloxane 'Silwet L-77' and of sucrose in the inoculation medium as well as the developmental stage of the dipped plants significantly influence the efficiency of transgenic plant production. Here we report a protocol for successfully generating genetically modified *C. bursa-pastoris* plants. Besides the parameters mentioned above we also found that choice of the right strain of *Agrobacterium* is essential for efficient transformation.

## 2. Materials and methods

### 2.1. Plant material

*C. bursa-pastoris* (L.) Medik. selfed offspring was used from individuals of the line 'wt 6/1+2' with normally developed floral whorls, and individuals from line '*Spe* 9/9', a variety where petals in the second floral whorl are replaced by stamens; both lines are from population '1947', growing in Gau-Odernheim (Rheinhessen, Germany). Additional selfed offspring used was from line '*Spe* 2/4', of the population '1948', growing near Warburg (Westfalen, Germany) (Nutt et al., 2006). On the following plant line names will be abbreviated by '1947-*Spe*', '1947-wt' and '1948-*Spe*', respectively. *Capsella* population and plant line numbers refer to the Brassicaceae Germ Plasm Collection of the Department of Systematic Botany, University of Osnabrück, Germany. Generation times differ between the plant lines. From sowing to the start of bolting, line 1947-wt needs 6 weeks, line 1947-*Spe* needs 8 to 12 weeks, and line 1948-*Spe* needs 5 to 6 weeks under our standard growing conditions. All plant lines need 4 weeks from start of flowering to senescence of the inflorescence and ripening of fruits and seeds.

### 2.2. Plant growth conditions

Plants were cultivated in a mixture of soil-based seedling substrate (Klasmann, Germany), Vermiculite (Klasmann, Germany) and sand (8:1:1 by vol) supplemented with 1 g/l of each Osmocote mini (Scotts, The Netherlands) and Triabon (Compo, Germany) as long-term fertilizer. Prior to germination seeds were stratified for 4 days at 4 °C on top of humid soil surface. Growing conditions were set to a 16-h-photoperiod with 250–300 μmol photons/m$^2$s at 22 °C and 8h at 18 °C without light, and relative humidity levels of about 50% (day) to 60% (night).

### 2.3. Bacterial strains and plasmids

The *Agrobacterium tumefaciens* strains GV3101/pMP90 (Koncz and Schell, 1986) and LBA4404 (Hoekema et al., 1983) were used in this study. Each strain carried either the binary vector mGFP5-ER (Haseloff et al., 1997), pGPTV-bar (www.biotech.unl.edu) or pFGC5941 (www.chromdb.org). The T-DNA cassette of mGFP5-ER-vector contains a modified *GFP* gene driven by the CaMV (Cauliflower Mosaic Virus) 35S promoter (Fig. 1a). The additional kanamycin resistance gene *NPTII* of the vector mGFP5-ER has not been used in this study. The vector pGPTV-bar contains the *BASTA-resistance* gene (*BAR* $^r$) and a *GUS* gene as a second selectable marker, both driven by NOS promoters (Fig. 1b). The vector pFGC5941, which was designed for RNAi experiments, carries the *BAR* $^r$

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