THE CROP JOURNAL XX (2017) XXX–XXX

Available online at www.sciencedirect.com

ScienceDirect

Soybean hairy roots produced *in vitro* by *Agrobacterium rhizogenes*-mediated transformation☆

Li Chen[a,b,1], Yupeng Cai[a,b,1], Xiujie Liu[a,b], Chen Guo[a,b], Shi Sun[b], Cunxiang Wu[b], Bingjun Jiang[b], Tianfu Han[b], Wensheng Hou[a,b,*]

[a]National Center for Transgenic Research in Plants, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[b]Ministry of Agriculture Key Laboratory of Soybean Biology (Beijing), Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China

ARTICLE INFO

Article history:
Received 31 May 2017
Received in revised form 24 August 2017
Accepted 10 September 2017
Available online xxxx

Keywords:
Agrobacterium rhizogenes
Hairy root
Protocol
Soybean
Transformation

ABSTRACT

Soybean is one of the world's most important oil and protein crops. Efficient transformation is a key factor for the improvement of soybean by genetic modification. We describe an optimized protocol for the *Agrobacterium rhizogenes*-mediated transformation of soybean and the induction of hairy root development *in vitro*. Cotyledons with 0.5-cm hypocotyls were cut from 5-day-old seedlings and used as explants. After infection and co-cultivation, hairy roots were produced in induction culture medium after 10–12 days. Using this method, 90%–99% of the infected explants of five different cultivars produced hairy roots within one month. Observations using reporter constructs showed that 30%–60% of the hairy roots induced were transformed. Based on high transformation efficiency and short transformation period, this method represents an efficient and rapid platform for study of soybean gene function.

1. Introduction

Agrobacterium rhizogenes contains a root-inducing (Ri) plasmid that contains root locus (*rol*) genes in the T-DNA region, including *rolA*, *rolB*, *rolC*, and *rolD*, and is able to induce hairy roots from the wounded surface of explants after infection [1]. The hairy roots can be maintained in culture or hosted in composite plants with untransformed aerial tissue. *Agrobacterium rhizogenes*-mediated transformation has been widely used in many plants, such as *Glycine max* [2], *Capsicum annuum* [3], *Lotus corniculatus* [4], *Prunus* [5], *Pisum sativum* [6], and *Catharanthus roseus* [7].

Soybean (*Glycine max* (L.) Merr.) is one of the most important crops and has high oil and protein contents. With the development of biotechnology, advances in breeding, functional research and targeted genetic modifications have become essential for studying soybean. Thus, efficient transformation systems are required to advance soybean research. At present, *Agrobacterium tumefaciens*-mediated transformation and biolistic methods are the methods most

☆ Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

* *Corresponding author at: National Center for Transgenic Research in Plants, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China.*
E-mail address: houwensheng@caas.cn (W. Hou)

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cj.2017.08.006

Please cite this article as: L. Chen, et al., Soybean hairy roots produced *in vitro* by *Agrobacterium rhizogenes*-mediated transformation..., The Crop Journal (2017), https://doi.org/10.1016/j.cj.2017.08.006

frequently used for soybean transformation [8,9]. However, these techniques are too inefficient and labor-intensive to meet the increased demands of research [2].

Several successful soybean studies have been performed using *Agrobacterium rhizogenes* and taking advantage of the hairy root system. For gene function studies, overexpression of *GmACSL2* (long-chain acyl-CoA synthetase 2) in soybean hairy roots was observed to reduce lipid and fatty acid content, suggesting that *GmACSL2* is an important enzyme that catalyzes the five fatty acids (C16:0, C18:0, C18:1, C18:2, and C18:3 fatty acids) to form acyl-coenzymes [10]. Overexpression of *TaNHX2* (Na^+/H^+ antiporter 2) in hairy roots improves the salinity tolerance of transgenic roots. Under salt stress, a general growth inhibition in hairy roots was observed, but hairy roots transformed with the control vector without *TaNHX2* showed much less growth (on a dry-weight basis) than transgenic hairy roots overexpressing *TaNHX2* [2]. For promoter studies, the specificity of the soybean root promoter could be used to detect expression in hairy roots. Using this method, activities, enhancers, repressors, and the core region of the promoter could be easily observed in the hairy root system [11,12]. The soybean hairy root system was also used to test the expression efficiency of an RNAi vector and was successfully applied to the CRISPR/Cas9 system [13–15].

Compared with *A. tumefaciens*-mediated transformation of soybean, the *A. rhizogenes*-mediated hairy root transformation system has a high transformation efficiency and short transformation period. The procedure can be completed within one month. Hairy roots are usually non-chimeric, because they are derived from single cells and each hairy root consists of uniformly transformed cells [16]. Furthermore, hairy roots can grow without exogenous hormones [17].

In this report, the process for producing soybean hairy roots is described and illustrated. Using this method, 90%–99% of the infected explants of five different cultivars produced hairy roots within one month, and 30%–60% of the hairy roots induced were transformed. In addition, the formation of calluses from hairy roots can be successfully induced. An efficient *in vitro* hairy root system was established, it would be an efficient and rapid platform for study of soybean gene function.

2. Materials and methods

2.1. Plant materials

Five soybean cultivars (Williams 82, Jack, Zigongdongdou, Heihe 27, and Zhonghuang 30) were used for *Agrobacterium rhizogenes*-mediated transformation.

2.2. Plasmid construction

In addition to the desired transgenic construct, plasmids for the transformation of soybean require a linked reporter gene marker. We designed a plasmid with three reporter genes (*GUS*, *GFP*, and *DsRed2*) under the constitutive promoter CaMV 35S for rapidly checking for positive hairy roots.

2.3. Strain

The plasmid vector with the three reporter genes (*GUS*, *GFP*, and *DsRed2*) was mobilized into *Agrobacterium rhizogenes* K599 *via* electroporation for use in later soybean infection.

2.4. Preparation of culture medium

The media used in this protocol were (1) solidified YEP medium, composed of 10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 5 g L^{-1} NaCl, and 15 g L^{-1} agar (pH 7.0); (2) liquid YEP medium, composed of 10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, and 5 g L^{-1} NaCl (pH 7.0); (3) MS liquid medium, composed of 4.33 g L^{-1} MURASHIGE & SKOOG BASAL SALT MIXTURE (PhytoTechnology, M524), 30 g L^{-1} sucrose (pH 5.8) and 1 mL L^{-1} of MURASHIGE & SKOOG VITAMIN SOLUTION (PhytoTechnology, M533); (4) germination culture medium (GCM), composed of 3.1 g L^{-1} GAMBORGS BASAL SALT MIXTURE (PhytoTechnology, G768), 20 g L^{-1} sucrose, 7 g L^{-1} agar (pH 5.8), and 1 mL L^{-1} of GAMBORGS VITAMIN SOLUTION (PhytoTechnology, G249); (5) co-cultivation culture medium (CCM), composed of 0.433 g L^{-1} MURASHIGE & SKOOG BASAL SALT MIXTURE, 30 g L^{-1} sucrose, 3.9 g L^{-1} MES (Sigma, M3671), 7 g L^{-1} agar (pH 5.4), 1 mL L^{-1} of MURASHIGE & SKOOG VITAMIN SOLUTION, 150 mg L^{-1} DTT (Sigma, D5545), and 0.02 g L^{-1} AS (Sigma, D134406); (6) washing culture medium (WCM), composed of 2.165 g L^{-1} MURASHIGE & SKOOG BASAL SALT MIXTURE, 30 g L^{-1} sucrose (pH 5.8), 1 mL L^{-1} of MURASHIGE & SKOOG VITAMIN SOLUTION, 250 mg L^{-1} cefotaxime sodium salt (Sigma, C7039), and 250 mg L^{-1} carbenicillin disodium salt (INALCO, 1758–9317); (7) induction culture medium (ICM), composed of 2.165 g L^{-1} MURASHIGE & SKOOG BASAL SALT MIXTURE, 30 g L^{-1} sucrose, 0.6 g L^{-1} MES, 7 g L^{-1} agar (pH 5.8), 1 mL L^{-1} of MURASHIGE & SKOOG VITAMIN SOLUTION, 250 mg L^{-1} cefotaxime sodium salt, and 250 mg L^{-1} carbenicillin disodium salt; and (8) callus induction culture medium (CICM), composed of 2.165 g L^{-1} MURASHIGE & SKOOG BASAL SALT MIXTURE, 30 g L^{-1} sucrose, 0.6 g L^{-1} MES, 7 g L^{-1} agar (pH 5.8), 1 mL L^{-1} of MURASHIGE & SKOOG VITAMIN SOLUTION, 1 mg L^{-1} 2,4-D (Sigma, D7299), 0.2 mg L^{-1} 6-BA (Sigma, B3408), 250 mg L^{-1} cefotaxime sodium salt, and 250 mg L^{-1} carbenicillin disodium salt. All media were autoclaved at 121 °C for 15 min.

2.5. Detection of transgenic hairy roots

According to reporter gene in the designed vector, any reporter gene can be used to detect transgenic hairy roots. GUS can be assessed by GUS staining, whereas GFP and DsRed2 can be assessed by fluorescence. Hairy roots were directly screened using a dissecting fluorescence microscope (Nikon SMZ1500), and transgenic hairy roots showed GFP or DsRed2 fluorescence labeling. Histochemical GUS assays were performed following Jefferson [18]. The hairy roots were placed in GUS staining solution (50 mmol L^{-1} sodium phosphate, pH 7.0, 0.5 mmol L^{-1} potassium ferrocyanide, 0.5 mmol L^{-1} potassium ferricyanide, 0.5 mg mL^{-1} 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc), 0.1%

Please cite this article as: L. Chen, et al., Soybean hairy roots produced *in vitro* by *Agrobacterium rhizogenes*-mediated transformation..., The Crop Journal (2017), https://doi.org/10.1016/j.cj.2017.08.006