

A Tool for Creation Artificial Symbiotic Associations of Wheat

Zilya R. Vershinina, Andrei K. Baymiev, Aleksei K. Baymiev, and Aleksei V. Chemeris

Abstract—This paper reports optimization of characteristics of bioballistic transformation of spring soft wheat (*Triticum aestivum* L. cultivar Raduga) and getting of transgenic plants, carrying pea lectin gene.

Keywords—transgenic wheat, pea lectin, rhizobia root colonization, symbiosis.

I. INTRODUCTION

CREATION of artificial symbiotic associations of cultivated plants with endophytic microorganism, which were extracted from natural symbiosis, is a new long-range direction in bioengineering of symbiotic systems. Nodule bacteria a kind of Rhizobium, which fix nitrogen in symbiosis with leguminous plants, can act as associative microsymbiont for many economically valuable nonleguminous plants, including wheat, encouraging growth of plants and protecting from phytopathogen [1], [2]. But associative processes in nature (unlike the laboratory practice) are difficult to control, and in the natural conditions useful bacteria can't stand competition and they are forced out by more aggressive, including pathogenic bacteria. The aim of researchers is to make plants to control microflora of their rhizosphere, and in case of use as useful microsymbiont of nodule bacteria, one of the tools are lectins of leguminous plant and agglutinin of nodule bacteria (secretable protein capable to recognize and selectively connect various carbohydrates).

Accumulated data in the past decades shows that lectins, at least, of graminaceous and leguminous plant, can connect with symbiotic bacteria, including associative, and help to recognize and attach microsymbiont to microsymbiont. Agglutinins of nodule bacteria also act in inter – bacteria and plant – bacteria interactions. In such a way transgenic wheat synthesizing lectin of a definite leguminous plant or agglutinin of its microsymbiont, will be mainly colonized by this microsymbiont. Using such a strategy gives a clue to formation of “artificial rhizosphere” of wheat, specifically colonized only by those bacteria which fulfill useful for plants trophic, growth initiating and/or protective functions (Fig. 1).

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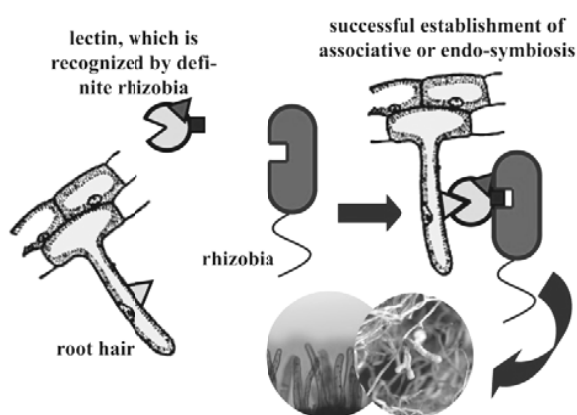


Fig. 1 Artificial colonization of non-symbiotic plants roots with the use of lectins

The aim of our work was in optimization of characteristics of bioballistic transformation of spring soft wheat (*Triticum aestivum* L. cultivar Raduga) and getting of transgenic plants, carrying pea lectin gene. This gene will let to create new associative wheat symbiosis with nodule bacteria of field pea, which has growth encouraging, fungistatic and other useful characteristics.

II. RESULTS AND DISCUSSION

Consistently the gene *psl* was cloned in the vector for transformation of plants pCambia 1301 (Fig. 2), containing GUS gene carrying a plant catalase intron, responsible for splitting β -D glucuronid and selective gene *hptII*, which gives resistance to hygromycin [3].

In order to receive transgenic plants young embryos were used as explants isolated for 12-14 days after pollination, the size of embryos was 0.5-1.5mm. Bioballistic transformation was carried out with the use of Biolistic PDS-1000/He (Bio-Rad, CIIIA). Microcarriers (1 μ gold particles) were prepared and coated with plasmid DNA according Sanford et al. [4]. The embryos were pre- and post-incubated higher-osmotic pressure medium to improve survival of wheat transformed tissue [5]. After 24h of bombardment, the embryos were transferred to the induction medium supplemented with 2mg/l 2,4-D [6] and maintained for 2 weeks in the dark, at 23°C. Then transfer any calli bearing somatic embryos to regeneration medium (enriched with 0.1mg/l of 2,4-D, 5mg/l of zeatin and 25mg/l of CuSO₄ [5]) in 9cm Petri dishes and

incubate at 22°C in the light for 2 weeks. Efficiencies of shoot regeneration with this combination of phytohormones reached 50%. The regenerating plantlets were finally transferred to half strength MS medium, enriched with 15mg/l hygromycin and 0.25mg/l NAA, and maintained for 4-6

weeks at 22°C in the light. Rooted transgenic plantlets were transferred to pots containing garden soil and grown to maturity in Binder climatic chambers operating at 21/16°C at 16/8 h light/dark cycle.

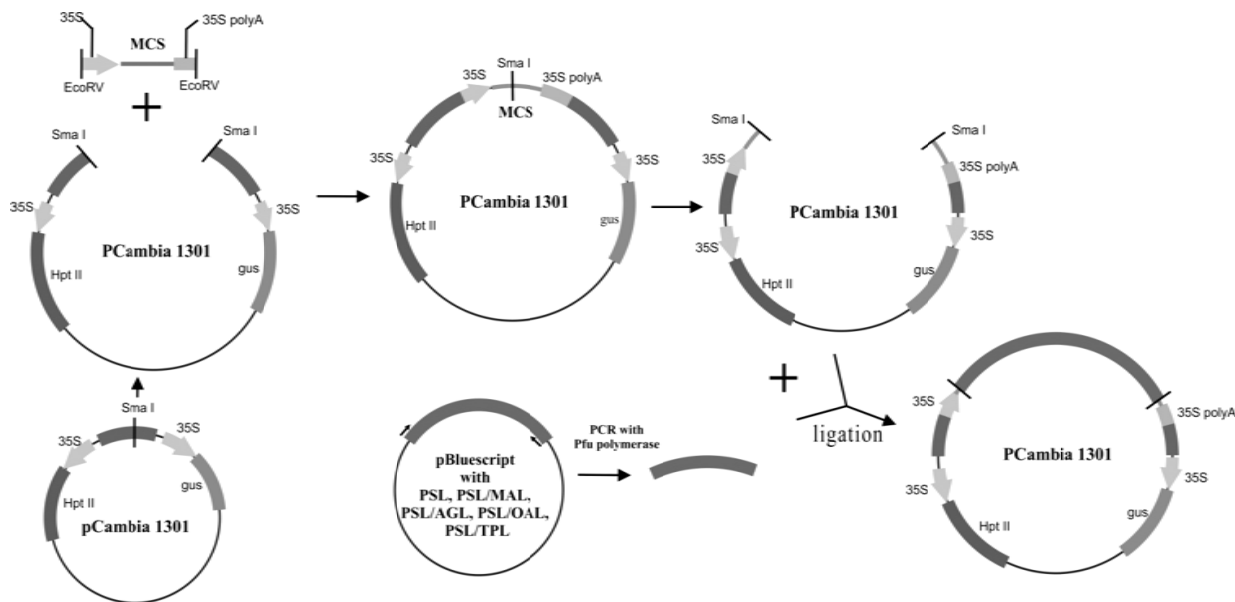


Fig. 2 Cloning strategy of the *psf* into pCambia 1301

Efficacy of transformation was tested by checking the activity of transfer gene products (*hpt II*, *gus* and *psf*) in transformed wheat tissue built by pCambia 1301-*psf*. The presence of *psf* sequence was confirmed by PCR analysis of genomic DNA obtained from individual shoots. Total DNA was extracted as described by Graham [7]. For *psf* gene, two oligonucleotide primers (5'-ATAATGGCTT-CTCAAACCC-3' and 5'-GCAAAAAA ACTATGCATCT-GCA-3') were designed according to Gatehouse et al. [8] and gave rise to an 840-bp fragment. Amplification was carried out by Taq polymerase in a temperature program of 94°C for 40 s, 53°C for 1min 30s and 72°C for 1min 30s with 30 cycles (Fig. 3). Histological assays for GUS expression were performed with histochemical substrate X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide) and protocol described in Jefferson et al [9]. Plant materials under test were incubated in the dark at 37 °C in a GUS assay mixture (Fig. 4).

Result of this work was to optimize parameter biobalistic transformation of wheat cultivar Raduga: helium pressure of 6500kPa and the target distance of 6 cm. The final stage of the study was achieved transformation efficiency of 3%.

This work was supported by Russian Foundation for Basic Research (12-04-31277, 12-04-31284).

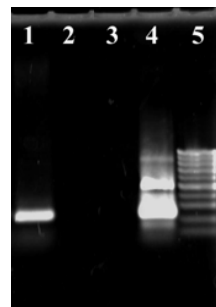


Fig. 3 PCR-analysis of some transformed clones

1, 2 – clones
3 – neg. control
4 – pos. control
5 – 100bp DNA marker

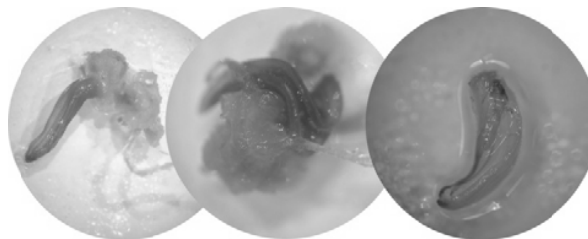


Fig. 4 GUS-histochemical staining of the regenerating wheat plantlets

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