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Full Length Research Paper

Genetic Profiling and Phenotypic Assessment of Progeny from Antisense Potato Lines

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Two transgenic potato lines csr2-1 and csr4-8, containing two different antisense constructs, csr2 and csr4, respectively, were crossed to investigate the possibility of achieving double transformants with combined effects of the two antisense transgenes on plant phenotypes and cellulose deposition. Molecular analysis revealed an expected segregation ratio of 1:1:1:1 of the four classes. Phenotype characterization revealed that offspring containing either one or both transgenes produced more tubers than the control plants but individual tubers were mostly smaller and had lesser weight than the control tubers.

Key words: Antisense technology, double transformants, genetic crossing, potato, segregation analysis.

INTRODUCTION

Antisense technology has increasingly huge potentials in cellular and tissue engineering, in addition to its wide-spread use in studying gene function as well as control-ing the expression of unwanted proteins. The basic con-cept of antisense technology hinges on halting the expression of a specific mRNA by the use of a comple-mentary sequence, which blocks the transfer of genetic information from DNA to protein (Jarald et al., 2004).

There are three ways of producing this specific inhibit-tion of gene expression. These include the use of single-stranded oligonucleotides that bind to a specific mRNA, forming a DNA-RNA duplex, which in turn inhibits tran-slation into the corresponding protein. The second credi-ble antisense strategy is to endogenously express an antisense RNA through a recombinant expression vector harbouring antisense genes. This antisense RNA is be-lieved to form a duplex with the complementary mRNA sequence and blocks translation by the ribosome. In the third strategy, which is RNA interference (Hammond et al., 2000), long double-stranded RNA (dsRNA) molecules are cleaved by a dsRNA-specific nuclease named Dicer (Bernstein et al., 2001) into 21–23 base pairs small inter-fering RNAs (siRNAs) (Elbashir et al., 2001). The siRNAs are then incorporated into a multi-protein nuclease com-plex, known as the RNA induced silencing complex (RISC). The complex is then guided to the target mRNA through conventional base- paring interaction of the anti-sense strand of the siRNA and eventually degrades the target mRNAs homologous to the single stranded siRNA (Hammond et al., 2000).

(Oomen et al., 2004) employed the antisense RNA expression vector strategy to generate antisense potato plants with varying degrees of down-regulation of cellu-lose content in tuber cell walls. The transgenic potato tuber clones were obtained by transforming the potato plant with antisense constructs of the class-specific re-gions (CSR) of four corresponding potato cellulose synthase genes (CSR1, 2, 3 and 4). Two transgenic lines csr2-1 and csr4-8, which showed considerable cellulose reduction (40 and 60%, respect-ively) in their tuber cell walls were identified by the anthrone colorimetric assay (Oomen et al., 2004). Hence our interest in exploring the possibility of achieving double transformants with com-bined effects of the two transgenes on organ and/or cel-lular phenotype, and ultimately on cellulose synthesis and deposition.

In this study, we have crossed the transgenic potato lines csr2-1 and csr4- 8, whose tuber cell walls exhibit low levels of cellulose as compared to the control. We report the segregation analysis of the transgenes as well as whole plant phenotype characterization, which indicate

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Figure 1. The 819 base pairs promoter sequence.

combined effects of the two transgenes on tuber production in the progeny.

MATERIALS AND METHODS

Plant material and growth conditions

Potato (Solanum tuberosum) plants used for the cross carried, in antisense orientation, csr2 sequence of the potato CesA2 gene (accession number AY221089) or the csr4 sequence of the CesA4 gene (accession number AY221088) (Oomen et al., 2004)). Expression of the antisense constructs was targeted to the tuber by using a granule bound starch synthase (gbss) promoter to drive its expression. The 819 base pairs promoter sequence is presented in Figure 1.

Pollen of the plant line csr4-8 was used to fertilize plant line csr2-1 to produce berries. A total of 488 seeds were removed from the berries, dried and prepared for germination. 100 seedlings, representing the different genotypes, were grown in soil in the greenhouse under 3,000 lux and in a light/dark period of 16/8 h.

Molecular analysis

Copy number of transgene integration of the parents and segregation pattern in the offspring was determined by southern analysis. A total of 84 F1 plants were used for segregation analysis. Genomic DNA was isolated from young leaves by using GenElute™ Plant Genomic DNA Miniprep Kit (Sigma) . 8 µg of the prepared DNA was digested with restriction enzyme HindIII and electropho-resed on 0.8% (w/v) agarose gels. Electrophoresed DNA samples were transferred onto Nylon N-membrane (Amersham). Hybri-dization was performed at 65°C with modified Church buffer (Church and Gilbert, 1984) containing [32P]UTP-labelled NptII probe.

Phenotype characterization

Following molecular characterization of the 84 plants and at maturity, the tubers were harvested and grouped accordingly. The tubers for each F1 plant were then weighed to generate data on number of tubers from a particular offspring plant that correspond to norma-lized 100 g weight.

RESULTS AND DISCUSSION

Southern analysis of the parent plants (Figure 2) revealed that parent csr2-1 contained 2 copies of the transgene in tandem while parent csr4-8 contained 3 copies (1 single insertion and 1 tandem repeat) . Based on the transgene insertion number in the parent, we determined the segregation pattern of the offspring, using Southern analysis.

This analysis revealed an expected co-segregation of both the single copy and the tandem repeat of csr4 transgenes, which implies that they were integrated in the same locus, thus leading to an expected segregation ratio of 1:1:1:1 of the four classes (^{2}3 = 3.3; P<<0.05). The observed segregation pattern of a total of 84 plants analyzed was: twenty five plants contained the csr4 construct, twenty four the csr2 construct, fifteen both the csr2 and csr4 construct, and 18 plants contained none of the constructs. Although, the number of plants containing the double constructs or no construct was lower than that of plants containing either of the two transgenes, statistically it is well within the expected segregation ratio.

There were differences in tuber production whereas there was no visible difference in plant morphology, phyllotaxis, growth and development of the offspring. It was observed that 100% and 90% of the control and csr4 plants produced tubers respectively, whereas for the csr2 and csr2/csr4 plants, only 70% of them produced tubers.

It was clear that offspring plants containing either one or both antisense transgenes produced more tubers than the control plants, but individual tubers were mostly smaller and had lesser weight than the control tubers. By deduction from Figure 2, 88% of the csr2 plants and 64% of the csr2/csr4 plants produced tubers with an average weight of equal or less than 5 g, whereas 50% of the csr4 plants and 6% of the control plants produced tubers with an average weight of equal or less than 5 g.

On the whole, our results present molecular evidence of Mendelian inheritance of the two antisense transgenes

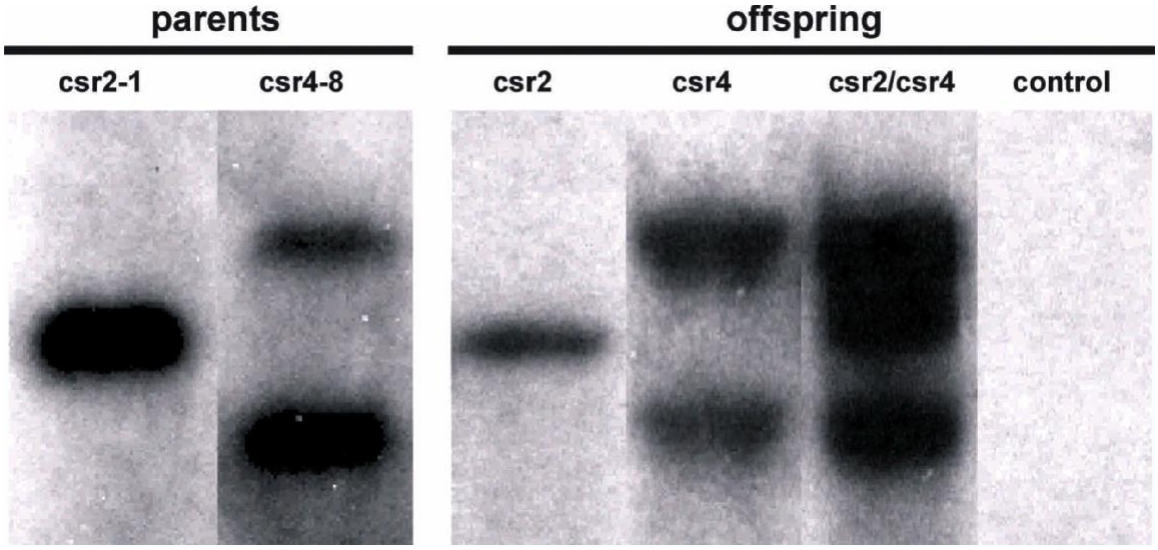

Figure 2. Southern blots showing transgene integration in the parents and the observed segregation pattern in the offspring. Double insertions of the csr2 construct in the csr2-1 parent and triple insertions of the csr4 construct in the csr4-8 parent.

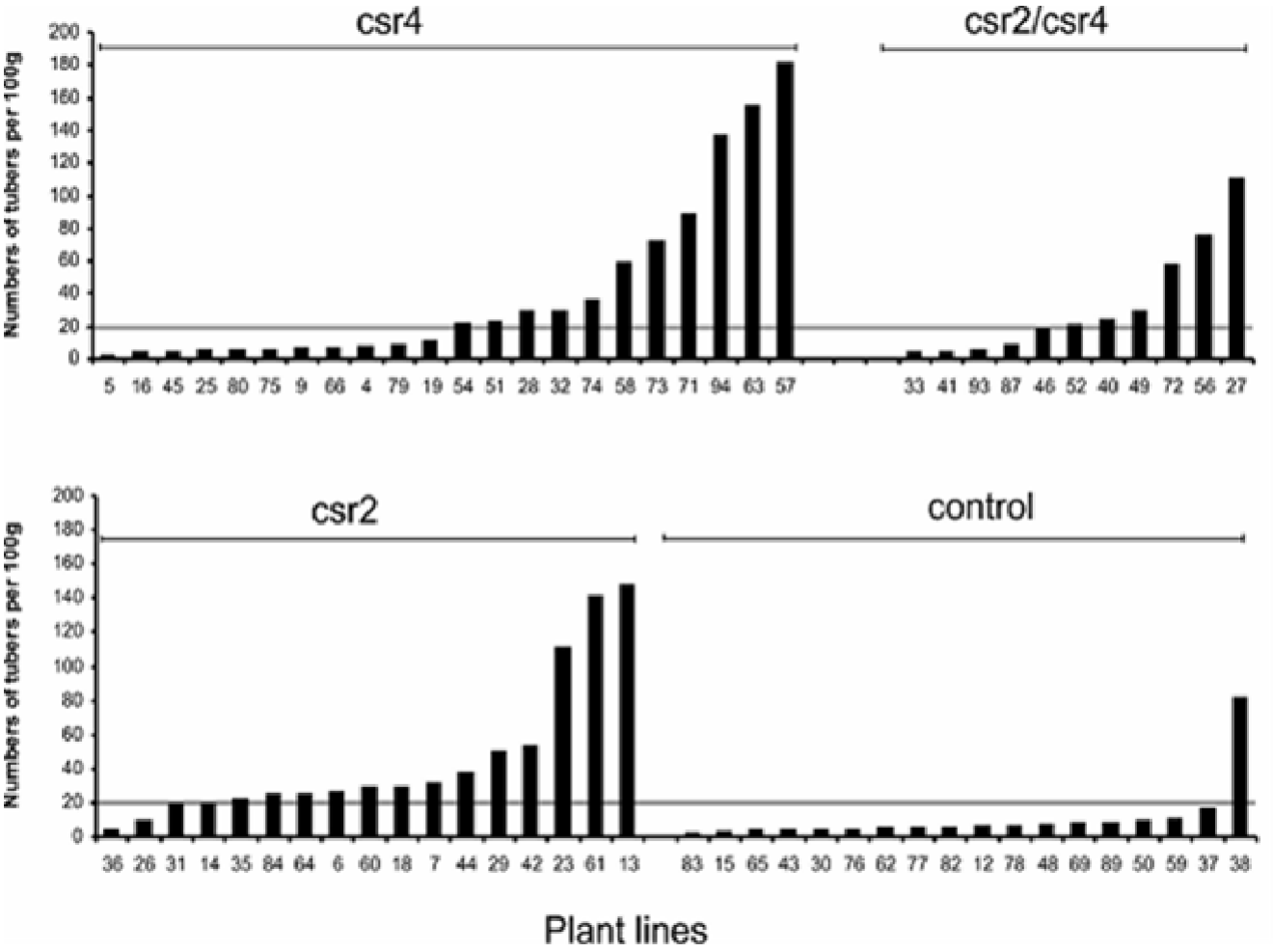

Figure 3. Number of tubers of offspring plants corresponding to normalized 100 g weight. Average weight of tuber for a particular F1 plant is deduced by dividing the normalized 100 g weight by the corresponding number of tubers.

genes in the progeny. These plants did not exhibit striking additive effects of the transgenes in the whole plant morphology. We are currently analyzing the tubers further for cellular phenotypes since the antisense genes were targeted to the potato tuber.

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