

*Full Length Research Paper*

# Molecular analysis and phenotype characterization of the progeny of two antisense potato plants

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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 controlling the expression of unwanted proteins. The basic concept of antisense technology hinges on halting the expression of a specific mRNA by the use of a complementary sequence, which blocks the transfer of genetic information from DNA to protein (Jarald et al., 2004).

There are three ways of producing this specific inhibition 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 translation into the corresponding protein. The second credible antisense strategy is to endogenously express an antisense RNA through a recombinant expression vector harbouring antisense genes. This antisense RNA is believed 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 interfering RNAs (siRNAs) (Elbashir et al., 2001). The siRNAs

are then incorporated into a multi-protein nuclease complex, known as the RNA induced silencing complex (RISC). The complex is then guided to the target mRNA through conventional base-pairing interaction of the antisense 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 cellulose content in tuber cell walls. The transgenic potato tuber clones were obtained by transforming the potato plant with antisense constructs of the class-specific regions (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%, respectively) 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 combined effects of the two transgenes on organ and/or cellular 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 electrophoresed on 0.8% (w/v) agarose gels. Electrophoresed DNA samples were transferred onto Nylon N-membrane (Amersham). Hybridization was performed at 65°C with modified Church buffer (Church and Gilbert, 1984) containing [32P]UTP-labelled NpII 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 normalized 100 g weight.

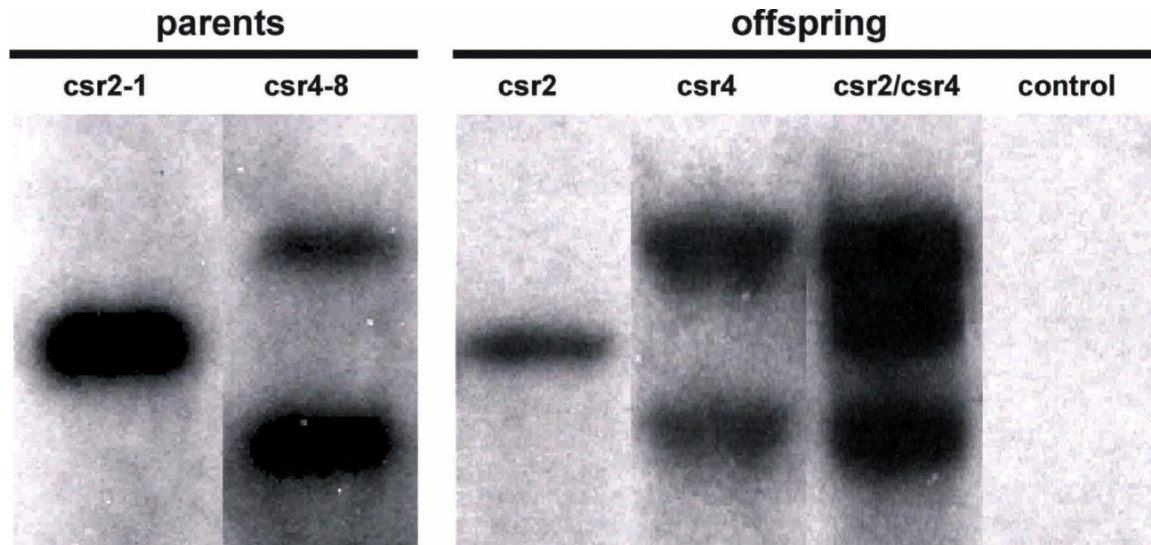
## RESULTS AND DISCUSSION

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.

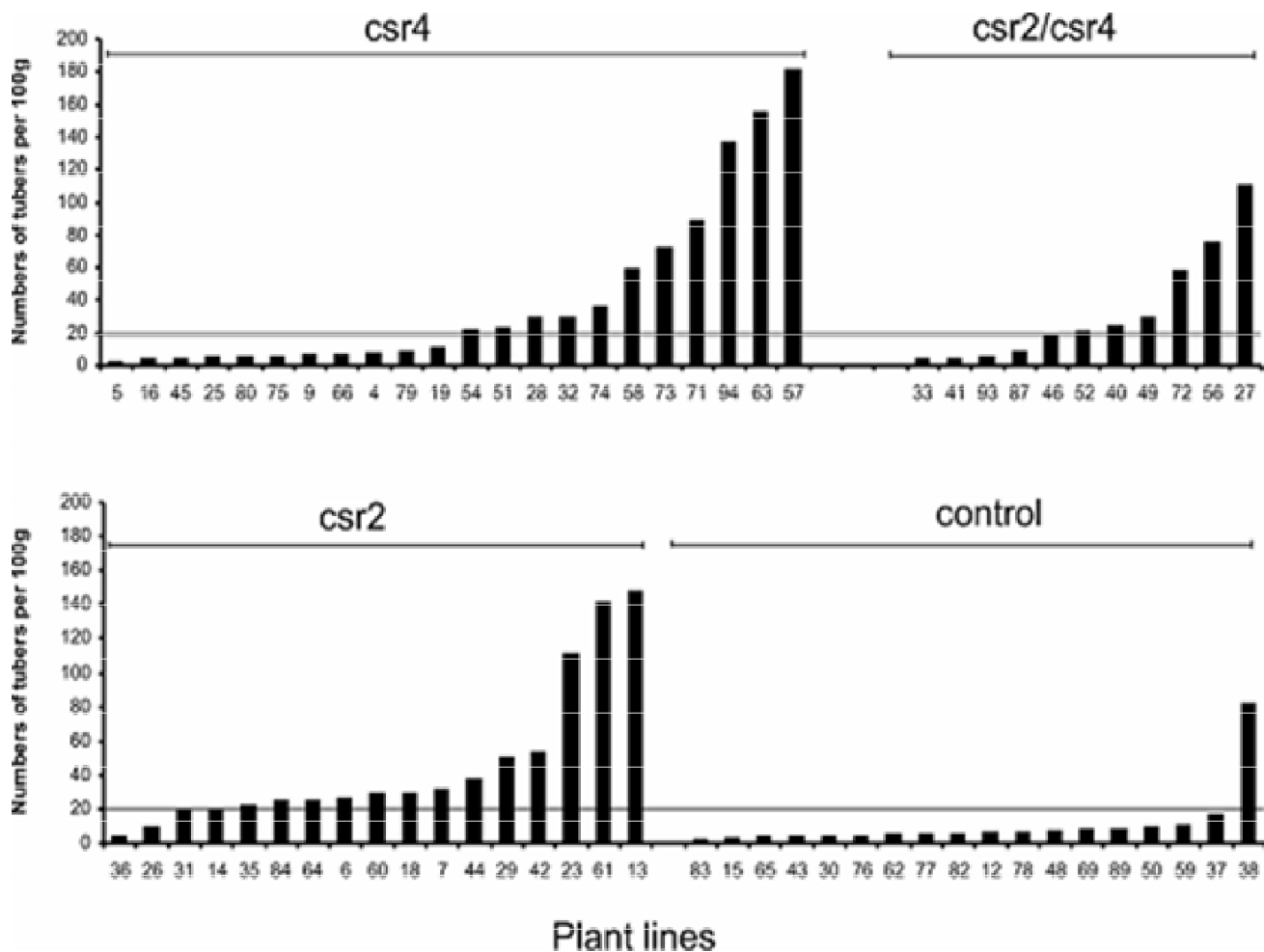
This analysis revealed an expected co-segregation of both the single copy and the tandem repeat of *csr4* transgenes, which implies that they were integrated in the same locus, thus leading to an expected segregation ratio of 1:1:1:1 of the four classes ( $\chi^2_3 = 3.3$ ;  $P < 0.05$ ). The observed 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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## REFERENCES

- Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409:363-366.
- Church GM, Gilbert W (1984). Genomic sequencing. *Proc. Natl. Acad. Sci. USA.* 81: 1991-1995.
- Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411:494-498.
- Hammond SM, Bernstein E, Beach D, Hannon GJ (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 404:293-296.
- Jarald E, Edwin S, Dubey P, Tiwari AA, Thakre V (2004). Nucleic acid drugs: a novel approach. *Afr. J. Biotechnol.* 3: 662-666.
- Oomen R, Tzitzikas EN, Bakx EJ, Straatman-Engelen I, Bush MS, McCann MC, Schols HA, Visser RGF, Vincken JP (2004). Modulation of the cellulose content of tuber cell walls by antisense expression of different potato (*Solanum tuberosum* L.) *CesA* clones. *Phytochemistry* 65: 535-546.