Functional characterisation of a soybean galactinol synthase gene under various stress conditions

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Abstract:

Galactinol synthase (GolS) has been known to play a key role in raffinose biosynthesis by catalysing the formation of galactinol. The *GolS* gene family has been recently identified in various plant species. Among them, many individual *GolS* genes have been reported to function in plant stress tolerance. In this study, we reported the construction of transgenic *Arabidopsis* overexpressing a soybean *GolS* gene, *GolS2*. There were no significant differences in the phenotypes of the transgenic and control plants during normal physiological conditions. We evaluated the performance of the transgenic plants under various stress conditions in relation to that of the control plants. The result evidenced that the overexpression of *GmGolS2* gene in *Arabidopsis* improved the plant's tolerance to salt stress but did not protect the plants against heavy metals and paraquat. Our study suggested that soybean *GolS* genes could be a potential candidate for genetic engineering to improve abiotic stress tolerance of plants.

Keywords: ***Arabidopsis thaliana,*** **galactinol synthase, overexpression, phenotypic analysis, stress tolerance.**

Classification number: **3.1**

Introduction

Plant growth and development are greatly affected by adverse environmental conditions. To respond to these stresses, regulatory compounds - including mannitol, proline, and various soluble oligosaccharides - are produced to function in cell protection and maintenance. Among them, the raffinose family of oligosaccharides is evidentially believed to perform a critical role in desiccation tolerance. As a direct precursor of raffinose, galactinol is known as a critical compound in raffinose biosynthesis. In the synthesis of galactinol, galactinol synthase (GolS) is an enzyme catalysing the formation of galactinol from UDP-D-galactose and myo-inositol. Therefore, the study on *GolS* genes may help us expand our understanding of how plants respond to stress conditions.

Up till now, *GolS* genes have been identified in many higher plant species, such as coffee (*Coffea canephora*) [1], wheat (*Triticum aestivum*) [2] and chickpea (*Cicer arietinum*) [3]. Among them, several *GolS* genes were well-established to respond to various stress conditions. For example, transgenic rice lines overexpressing *TaGolS1* and *TaGolS2* contain higher concentrations of galactinol and raffinose and exhibit enhanced cold-stress tolerance [2]. Overexpression of chickpea *CaGolS1* and *CaGolS2* in Arabidopsis conferred improved seed vigour and seed longevity to the transgenic plants [3]. More recently, *Arabidopsis thaliana AtGolS2* gene was reported to strengthen drought tolerance and increase grain yield in rice under dry field conditions [4]. In the past, overexpression of *AtGolS2* caused an increase in the galactinol and raffinose contents in leaves and exhibited improved drought tolerance of transgenic *Arabidopsis* plants [5]. The previous studies clearly indicated that genetic modification of the biosynthesis of raffinose by transformation with *GolS* genes could be an effective method for enhancing stress tolerance in plants. In this study, we generated transgenic lines of *Arabidopsis* overexpressing a soybean *GolS* gene, specifically *GolS2*. Then, transgenic plants were analysed for their abiotic stress tolerance.

Materials and methods

Materials

A. thaliana (Columbia-0 ecotype) and soybean (*Glycine max* L.) Williams 82 cultivar were used in this study.

Methods

Plant transformation: the coding sequence of *GmGolS2* (*Glyma03G38080*) from 'Williams 82' soybean genome

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was cloned into pGreen plasmid in between a cassette containing a 35S promoter and NOS terminator, which also harbours the kanamycin resistance gene. Then, this plasmid was transformed into *Agrobacterium tumefaciens* strain GV3101. Agrobacterium carrying the pGreen-35S::*GmGolS2* plasmid was used for transformation into *Arabidopsis* by following the floral dip technique [6]. Transgenic plants were selected on the kanamycin-containing medium.

Detections of GmGolS2 gene in transgenic plants: to detect the *GmGoLS2* in transgenic plants, we used PCR. The total DNA was isolated from four-week-old plants using the Exgene Plant kit (GeneAll, Korea). PCR primer sequences were aligned to 35S promoter, 5'-CCCACTATCCTTCGCAA-3' and NOS terminator, 5'-GTTGTAAAACGACGGCCAGT-3'. PCR reaction contained 0.2 µM primers, 200 µM dNTP, 1.25 U Taq DNA polymerase in 50 mM KCl, 1.5 mM $MgCl_2$ and 10 mM Tris-HCl pH 8.3. The PCR program comprised 35 amplification cycles at 95°C for 30 seconds and at 54°C and 68°C for 45 seconds each.

Morphological evaluation of transgenic Arabidopsis plants under normal condition: the sterilised *Arabidopsis* seeds were germinated in the Murashige and Skoog (MS) medium agar plates containing 30 mg/l of kanamycin. Two-week-old seedlings were transplanted into 20 cm soil-filled pots and allowed to grow at 24±2°C, relative humidity of 60-70%, under long day conditions (16-hour light/8-hour dark). The growth and development of *Arabidopsis* plants were observed and recorded at indicated times (three-, four- and five-week-old).

Performance of the transgenic plants under various stress treatments: the seeds of transgenic plants overexpressing *GmGolS2* were surface sterilised, placed in the dark at 4°C for two days, and then sown on selective half-strength MS medium agar plates. The seedlings were transferred onto half-strength MS medium supplemented with various concentrations of NaCl (for high salinity condition) and $CdCl_2$ (for heavy metal condition). The survival rates were visually observed and recorded after two days of treatments. For paraquat leaf disc assay, the procedures described in the previous study were followed [7].

Results and discussion

Development of transgenic plants overexpressing GmGolS2 gene

To examine the function of *GmGolS2* gene in plants, we transformed *A. thaliana* plants with *Agrobacterium* carrying the plasmid 35S::*GmGolS2*. The individual kanamycin-resistant plants were finally selected.

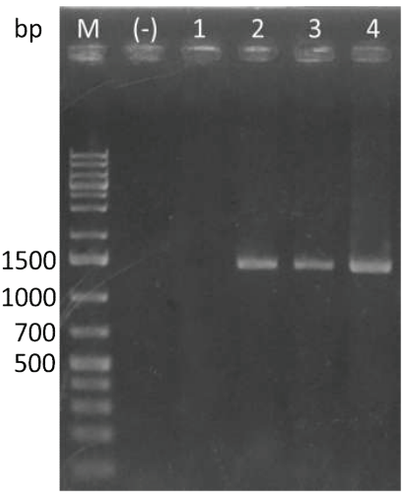

Fig. 1. Verification of the presence of *GmGolS2* gene in *Arabidopsis* transgenic lines. M: 1 kb DNA ladder; lane (-): negative control; lane 1: wild-type control; lane 2-4: transgenic lines.

The transgenic lines were confirmed by PCR. The total DNA extracted from young leaves of each transgenic lines was used as templates. Then, PCR products were visualised on 1.3% agarose gel with 1 kb DNA markers. As shown in Fig. 1, no band was found in the wild-type plant. The presences of a target band (~ 1.5 kb) in lane 2, 3 and 4 clearly confirmed the insertion of *GmGolS2* gene in 3 transgenic lines. In this work, one transgenic line was selected for further studies.

Phenotype evaluation of transgenic Arabidopsis overexpressing GmGolS2

Evaluation of the growth and development of the transgenic plants under normal condition is an important step to functionally characterise these plants in various stress conditions. Sterilised homozygous transgenic *Arabidopsis* seeds were germinated in selective MS medium agar plates, two-week-old seedlings were transplanted into pots. The growth conditions in the greenhouse included a 16h photoperiod, a day/night thermo period of 24±2°C, and a day/night relative humidity of 60-70%. The observations were recorded after 3 weeks.

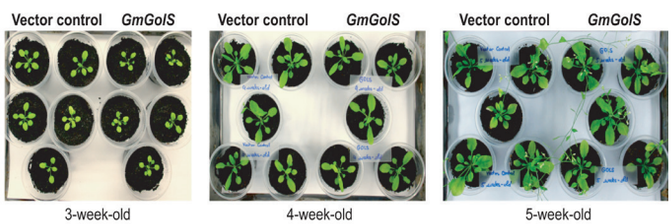

Fig. 2. The evaluation of the morphology of the 35S::GmGolS2 transgenic plants.

As shown in Fig. 2, no significant difference in morphology was visible between the transgenic lines and the control. This observation confirmed that the overexpression of *GmGolS2* gene in *Arabidopsis* did not affect the growth and development of transgenic plants under normal conditions.

Performance of 35S::GmGolS2 Arabidopsis under various stress treatments

In the past, the *GolS* gene family was identified in many plant species [1-3], and most *GolS* genes were reported to be highly expressed under various abiotic stress treatments. For instance, it has been reported that the overexpression of *AtGolS2* caused high accumulation of galactinol and raffinose in leaves and exhibited enhanced drought tolerance of transgenic *Arabidopsis* plants [5]. The previous authors clearly demonstrated that the overexpression of *GolS* genes increased the galactinol and raffinose contents with enhanced abiotic stress tolerance in transgenic plants. Thus, to test whether 35S::*GmGolS2* plants altered their responses to abiotic stress, the transgenic plants were treated under high-salinity, heavy metal, or paraquat conditions.

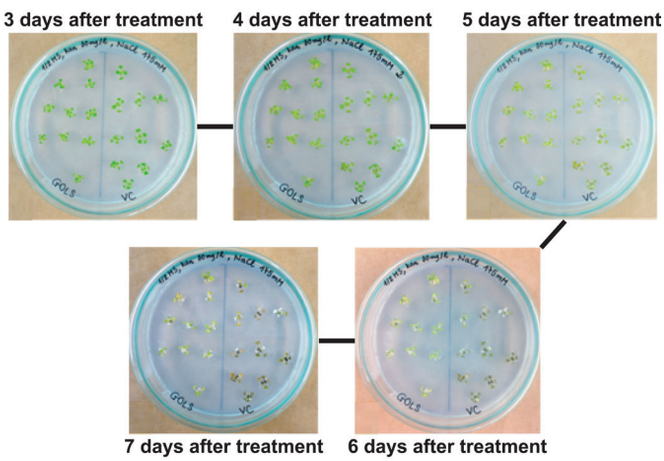

Fig. 3. Survival rates of transgenic plant under high salinity condition.

Previously, transgenic *Arabidopsis* plants overexpressing *TsGolS2* were treated with 0, 50, 100, 150, and 200 mM NaCl. Among them, with 200 mM NaCl, the germination rates of transgenic lines were recorded to be significantly higher than the control plants [8]. Here, we reported the survival rates of our transgenic plants under 175 mM NaCl. Seven days after cultivation on half-strength MS medium with 175 mM NaCl, the transgenic plants still maintained growth, whereas the vector control plants exhibited growth inhibition or died; even the high salt medium inhibited the growth of both transgenic and control plants (Fig. 3). These observations revealed that the overexpression of *GmGolS2* gene conferred salt resistance to transgenic *Arabidopsis* during their growth on the MS plates. Thus, our results indicate that the *GmGolS2* gene functions on improving salt stress tolerance in plants.

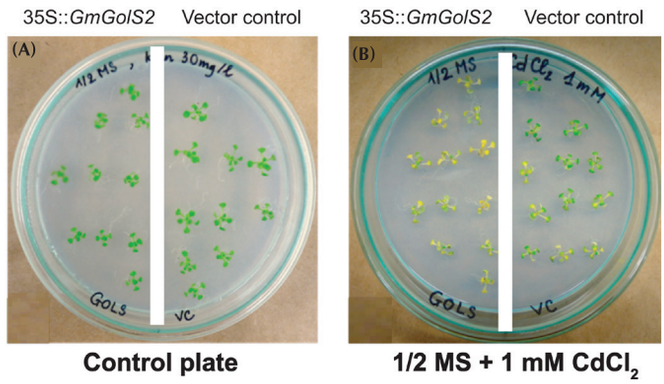

Fig. 4. Survival rates of 12-day-old transgenic plants under (A) normal condition and (B) heavy metal treatment.

Next, to examine the function of *GmGolS2* in heavy metal resistance, transgenic seeds were germinated, grown on selective half-strength MS agar plates and then transferred onto half-strength MS containing 1 mM $CdCl_2$. The result, as shown in Fig. 4, indicates that most transgenic seedlings were yellowing, but a majority of control plants were still green. It seemed that the over-expression of *GmGolS2* did not have a protective role in the plants against heavy metal (Cd) stress.

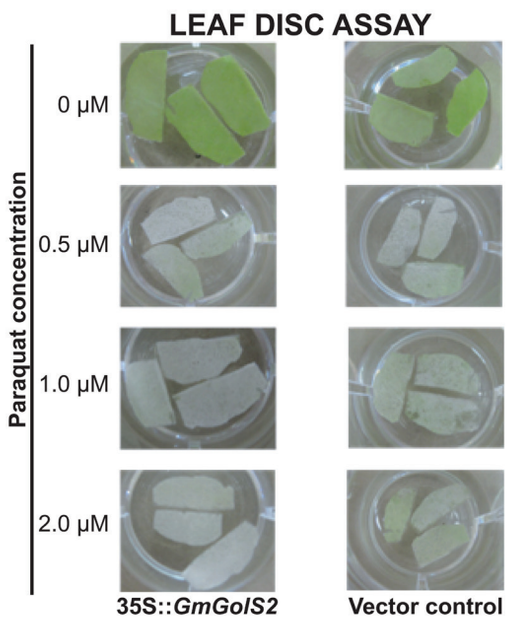

Fig. 5. Paraquat leaf disc assay of transgenic plants.

Finally, we also examined the sensitivity of transgenic plants to paraquat by using leaf disc assay. Paraquat is a recognised compound that generates reactive oxygen species (ROS) in the cell, causing cell injury and cell death [9]. As shown in Fig. 5, paraquat caused loss of the regular green

coloration of transgenic leaves; the leaf discs of the control plants lost their green colour a little bit slower under the same treatment, suggesting that *GmGolS2* did not provide protection against paraquat-induced ROS.

Conclusions

The transgenic *Arabidopsis* plants overexpressing the *GmGolS2* gene have been successfully created by the floral dip method. The presence of *GmGolS2* gene was verified by the PCR test with designed primers.

During normal growth conditions, no morphological differences were observed between the transgenic lines and the control plants. We found that the overexpression of *GmGolS2* gene in *Arabidopsis* did not affect the growth and development of transgenic plants.

The overexpression of *GmGolS2* gene improved tolerance to salt stress but not to heavy metal and paraquat stress in the Arabidopsis plants. This study suggested that soybean *GolS2* gene could be a potential candidate for molecular breeding and genetic engineering to improve abiotic stress tolerance of plants.

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