# Figures

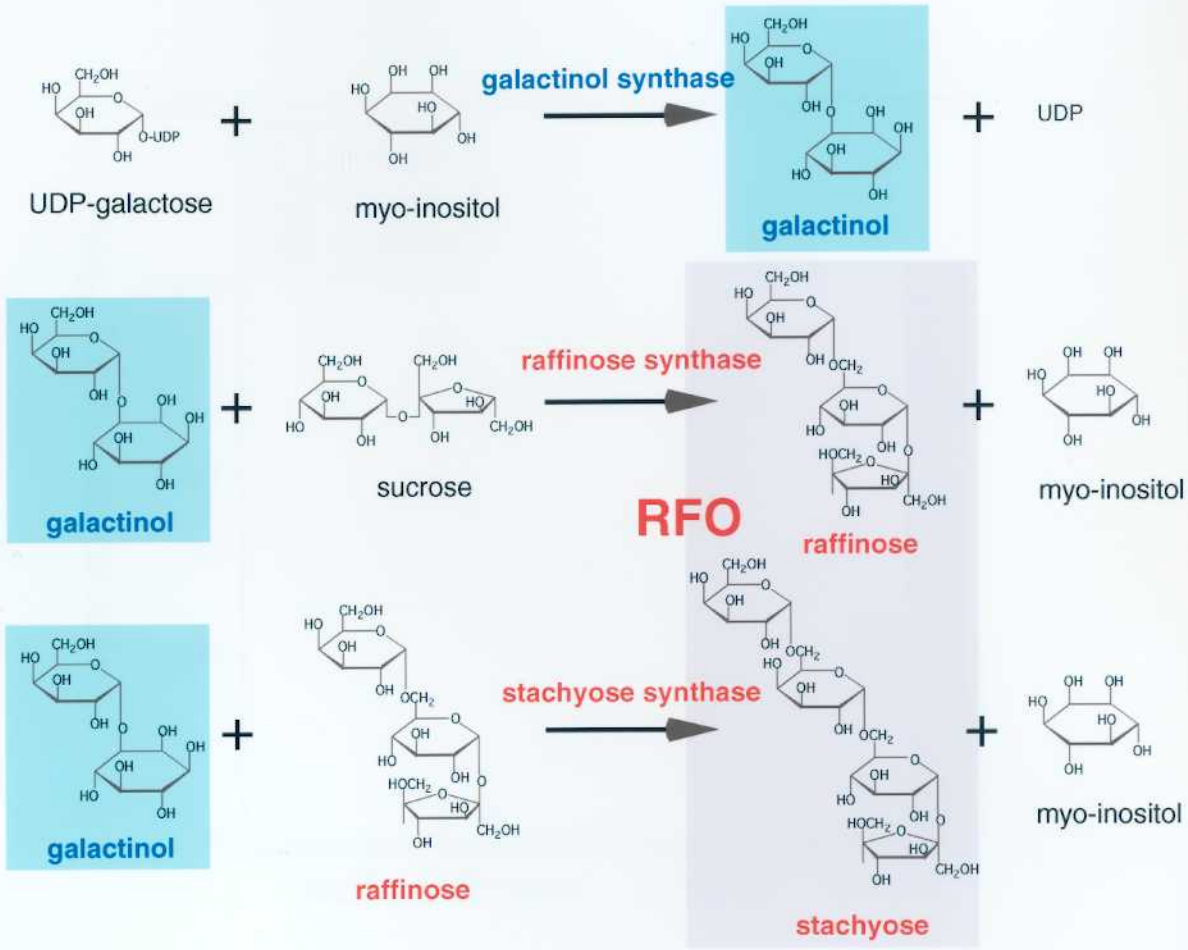


**Figure 1. Metabolic pathway of galactinol and RFO (raffinose and stachyose) in plants.**

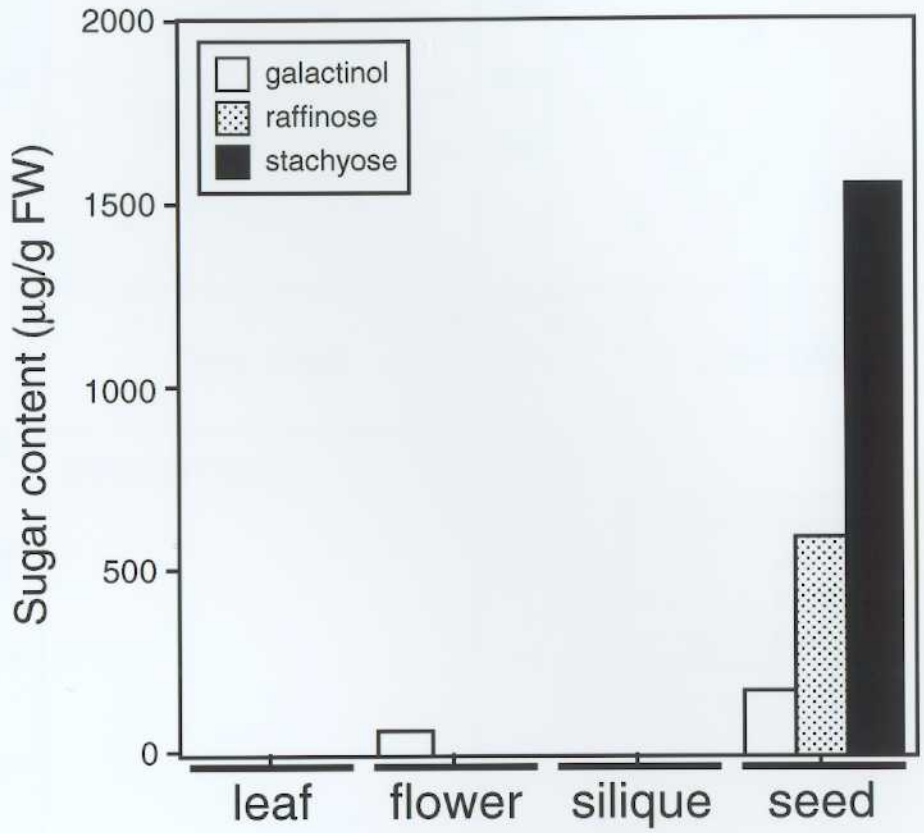


**Figure 2. RFO and galactinol contents in leaf, flower, silique and seed of *Arabidopsis*.**

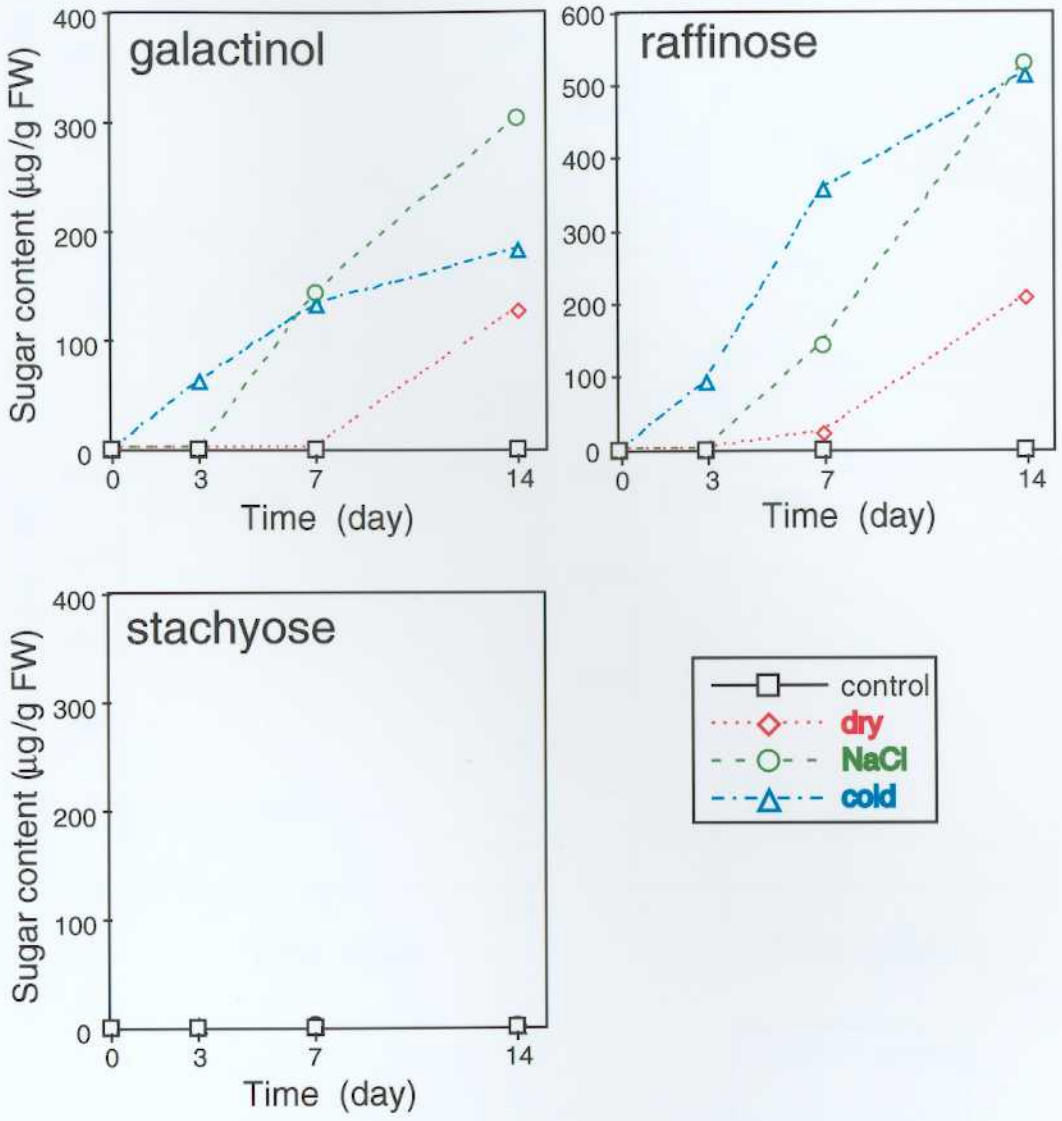


**Figure 3. Effect of drought, high salinity and cold stresses on galactinol and RFO content of vegetative tissues of *Arabidopsis*.**

Three-week-old soil-grown plants were exposed to drought (dry, ◇ ), high salinity ( 150 mMNaCl, ○ ) or cold (4°C, △ )stress, or untreated (control, □ ). Details of stress treatment are described in Materials and Methods.

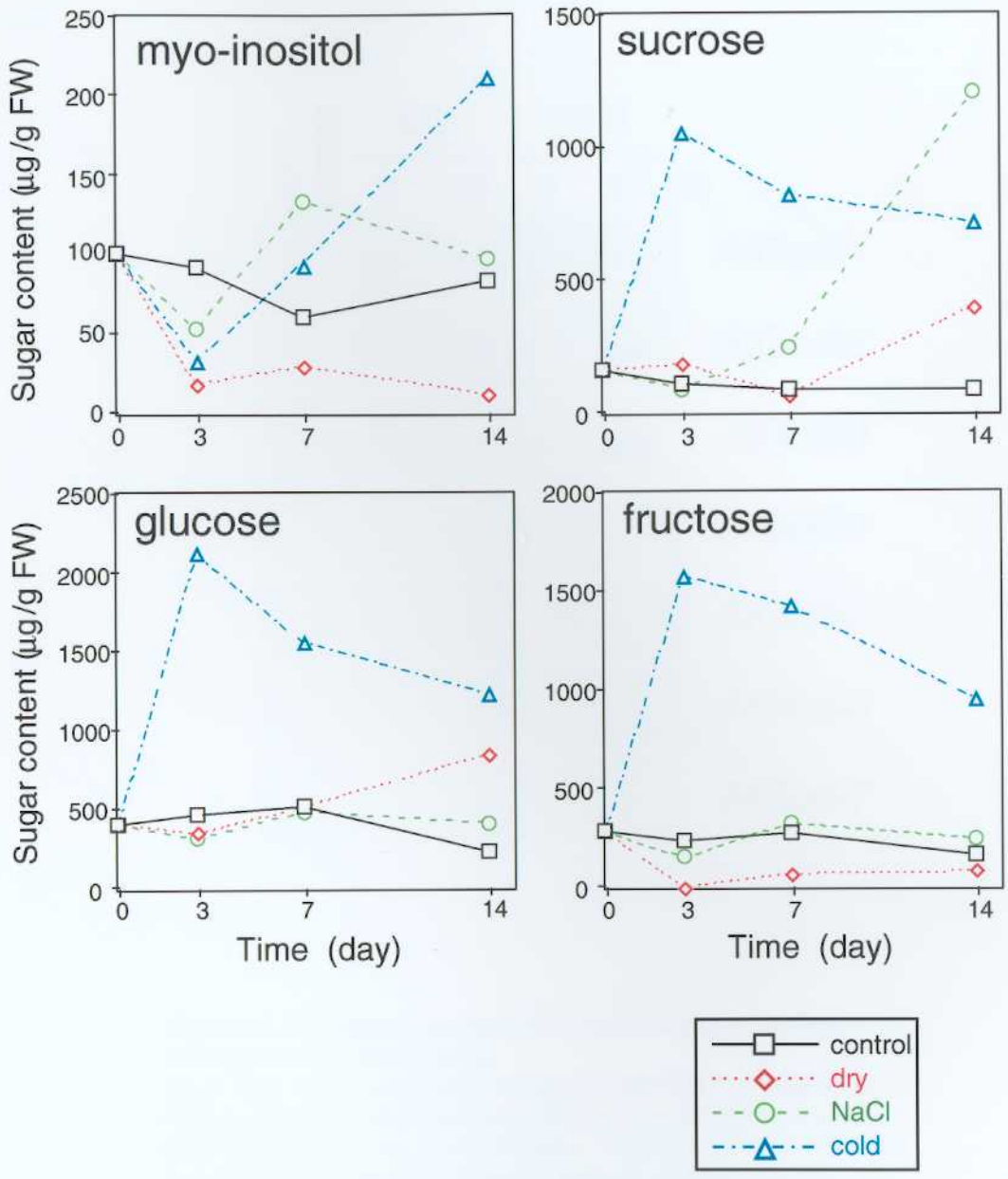


**Figure 4. Effect of drought, high salinity and cold stresses on carbohydrate content of vegetative tissues of *Arabidopsis*.**
Three-week-old soil-grown plants were exposed to drought (dry, ◇ ), high salinity (150 mM NaCl, ○ ) or cold (4°C, △ ) stress, or untreated (control, □ ). Details of stress treatment are described in Materials and Methods.

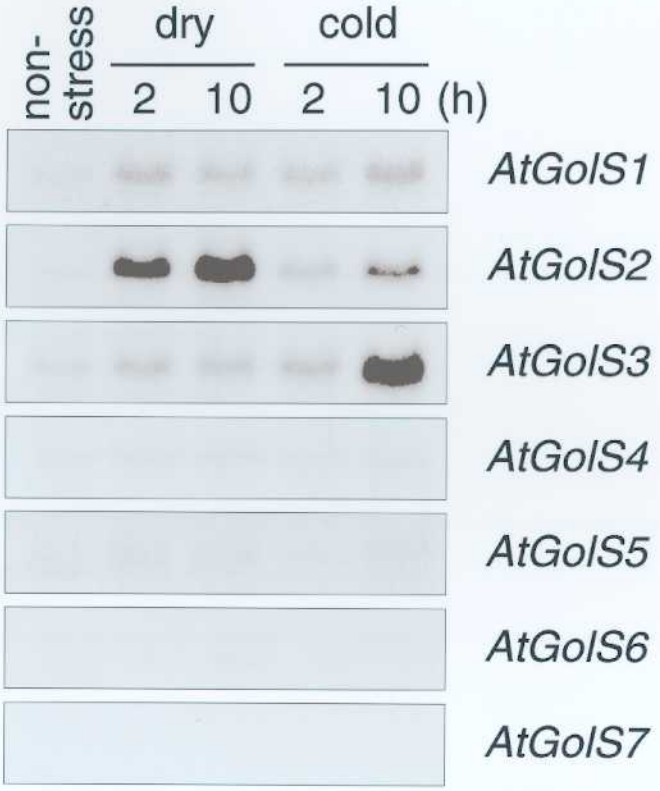


**Figure 5. Northern analysis of 7 *AtGolS* genes under drought and cold stresses.**
Each lane was loaded with 20 µg of total RNA prepared from wild-type plants that had been exposed to drought (dry) or cold (4°C) stress for 2 or 10 hours. The membranes were hybridizeded with [$^{32}$P]-labeled *AtGolS* cDNAs used as probes.

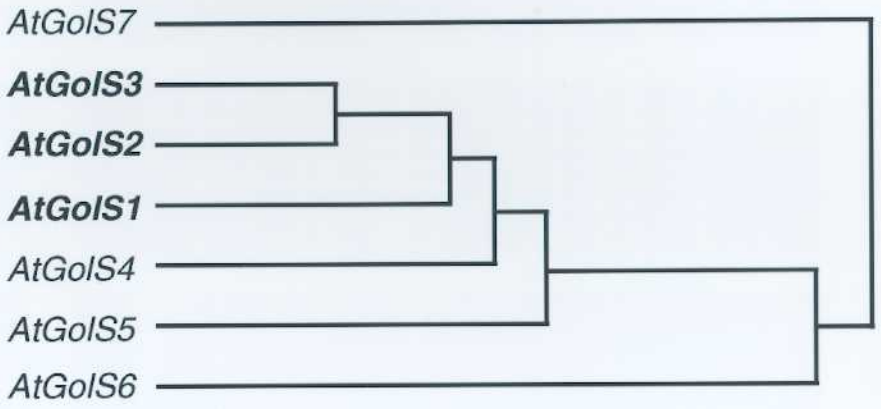


**Figure 6. The phylogenetic tree of *AtGolS* genes.**

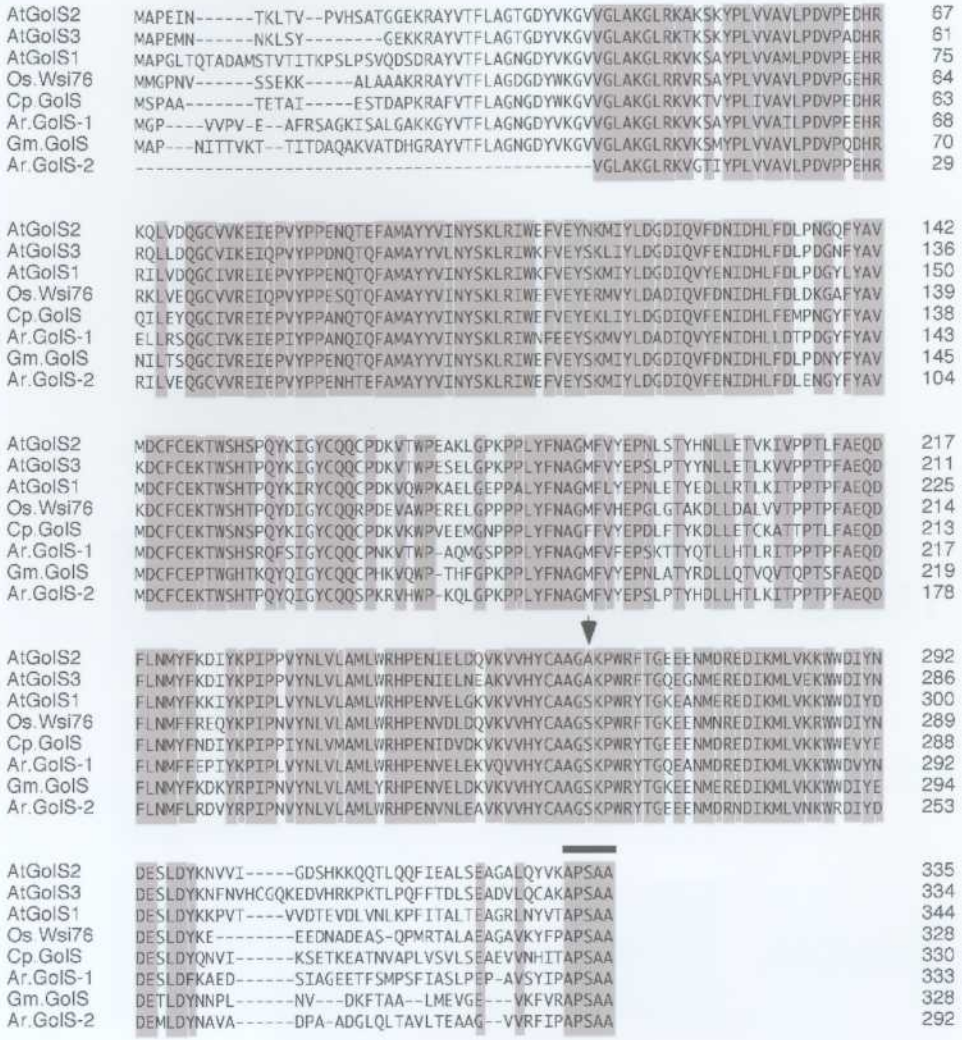


**Figure 7. Comparison of deduced amino acid sequences from cDNAs encoding galactinol synthase.**
Compared galactinol synthases are three of *Arabidopsis thaliana* (*AtGolS1, 2, 3*), *Ajuga reptans* (*Ar.GolS-1, 2*; Sprenger and Keller, 2000), *Glycine max* (*Gm.GolS*; Kerr *et al.*, 1993), *Cucurbita pepo* (*Cp.GolS*; Kerr *et al.*, 1993) and *Oryza sativa* (*Os.Wsi76*; Takahashi *et al.*, 1994). Putative serine phosphorylation site is shown as an arrow. A characteristic hydrophobic pentapeptide (APSAA) is shown as a bar.

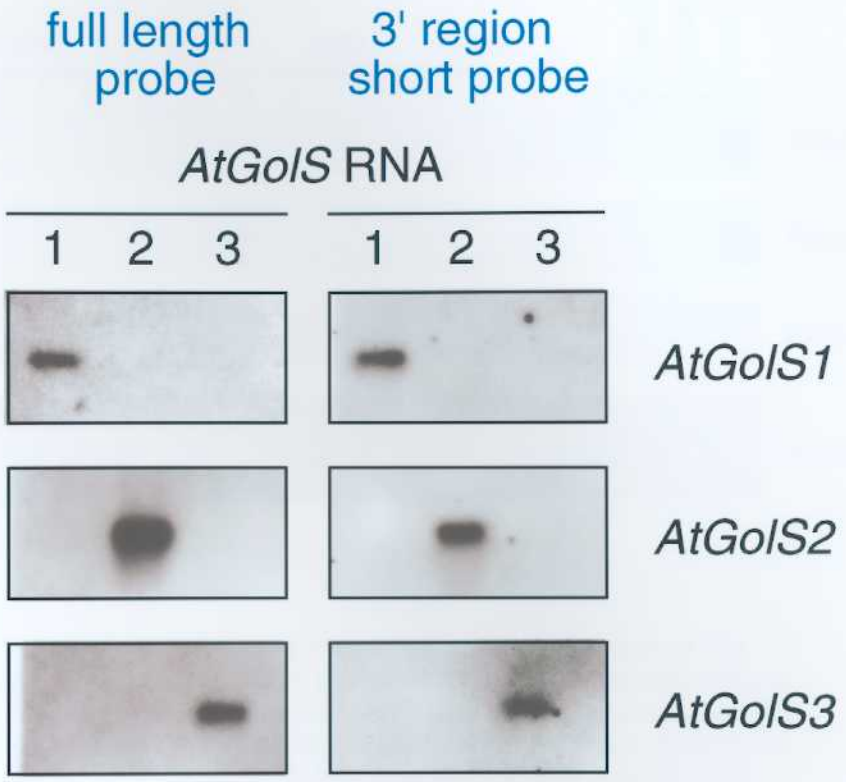


**Figure 8. Specificity of *AtGolS* probe for Northern analysis.**
Each lane was loaded with 8 pg of synthesized RNA. The RNA was fractionated on a 1% agarose gel, blotted onto a nylon membrane, and probed with digoxigenine; DIG-labeled cDNA inserts of the *AtGolS* cDNAs.

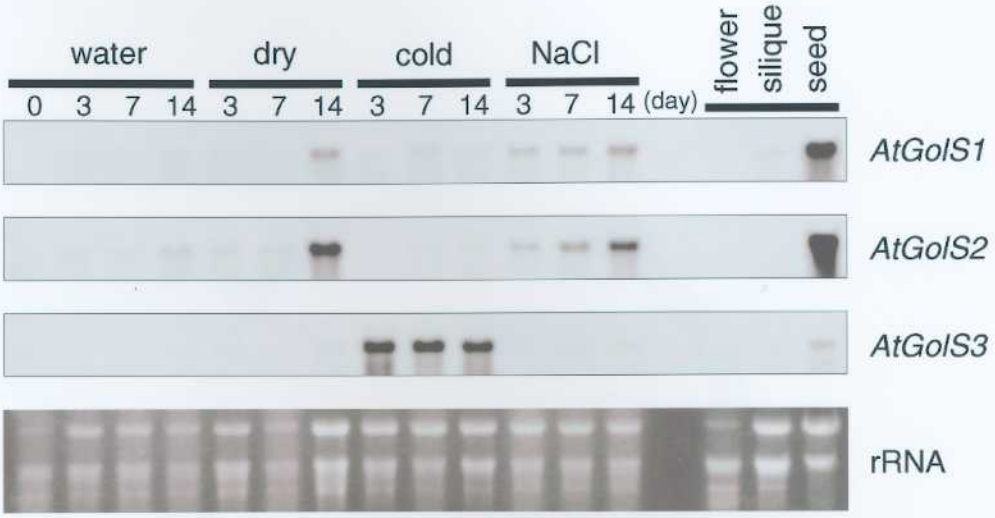


**Figure 9. Northern blot analysis of the induction of the *AtGolS* genes by water deficit stress and in various organs.** Total RNA was isolated from various *Arabidopsis* organs and whole plants which were treated in the same ways as those in Figure 1b and 1c. Each lane was loaded with 5 µg of total RNA. The RNA was fractionated on a 1% agarose gel, blotted onto a nylon membrane, and probed with digoxigenine; DIG-labeled cDNA inserts of the *AtGolS* cDNAs.

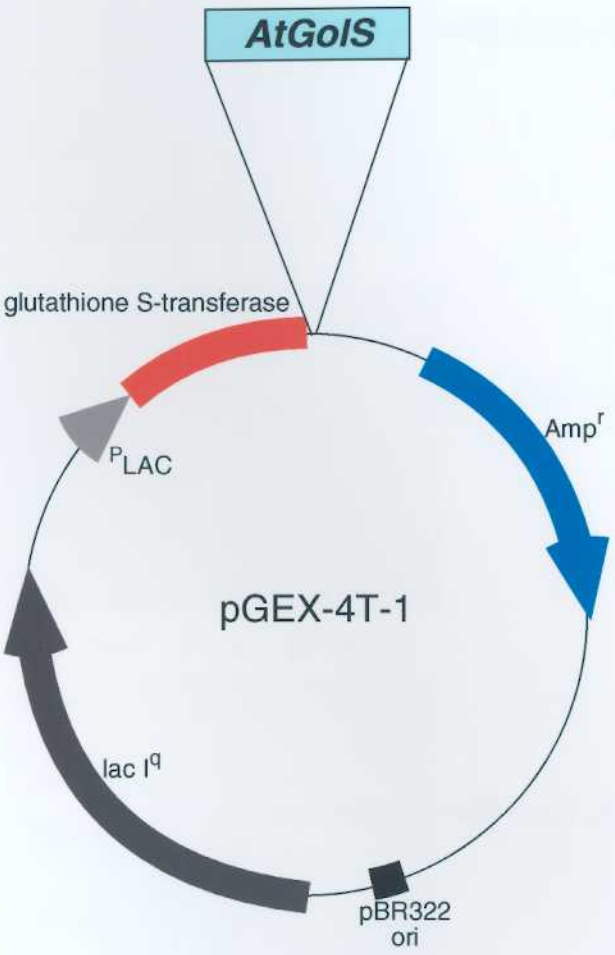


**Figure 10. Constructs of the GST-AtGolS fusion proteins.**

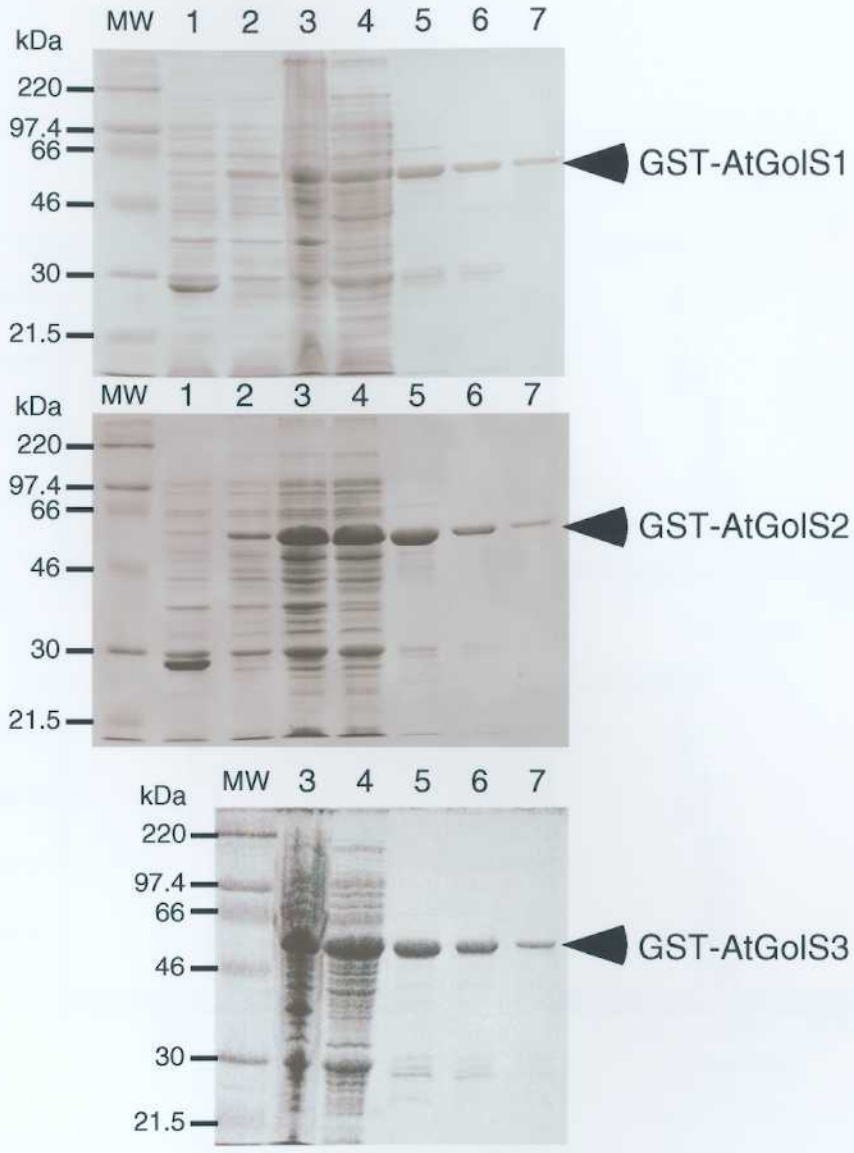


**Figure 11. Expression and purification of recombinant GST-AtGolS1, GST-AtGolS2 and GST-AtGolS3 proteins from E. coli cells.**
The insoluble pellet fraction (1, 2 and 3) and the soluble fraction (4) of crude extracts prepared from non-IPTG-treated (2) or IPTG-treated (1, 3 and 4) *E. coli* cells containing GST-AtGolS1, GST-AtGolS2 and GST-AtGolS3 were analyzed by SDS-PAGE. Fusion proteins (5 (one wash), 6 (two washes) and 7 (treee washes)) purified by use of glutathione-Sepharose beads from the soluble fraction were also analyzed by SDS-PAGE. The triangles indicate the electrophoretic positions of the recombinant proteins. The position and sizes (kDa) of molecular-weight markers (MW) are also indicated.

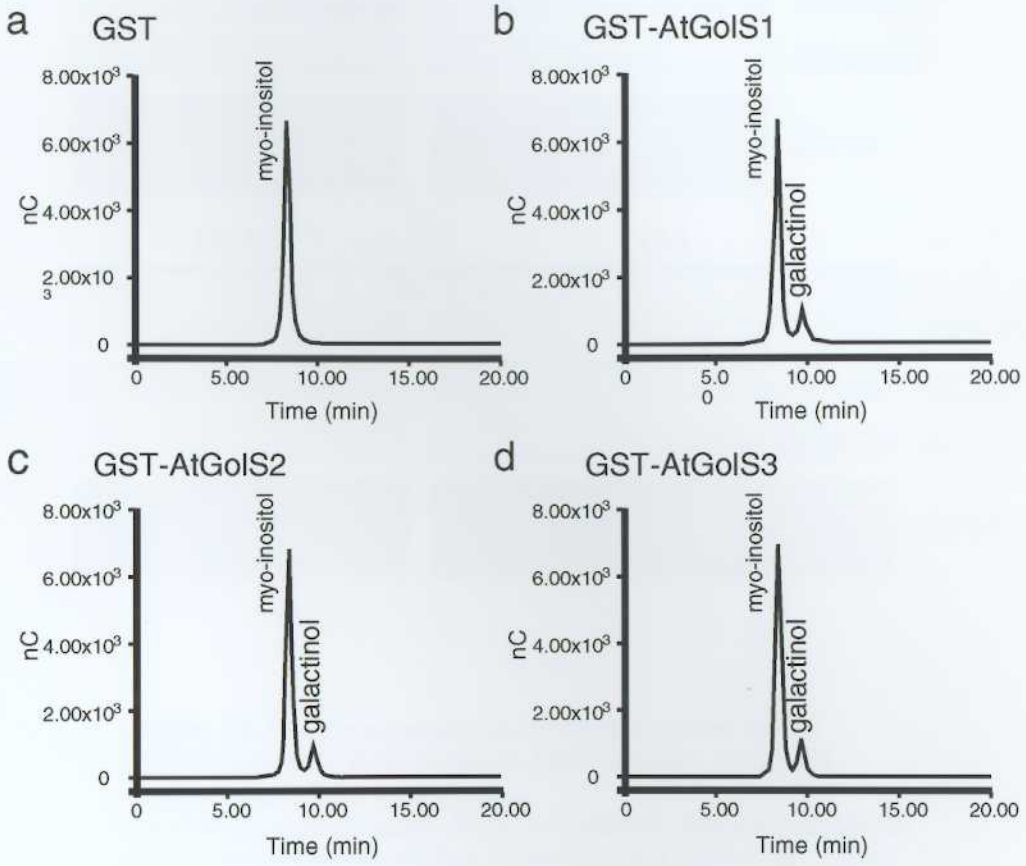


**Figure 12. HPLC profiles of carbohydrate metabolites of GST (a), GST-AtGolS1 (b), GST-AtGolS2 (c), GST-AtGolS3 (d) recombinant proteins.** The reaction mixture contained myo-inositol as substrate.

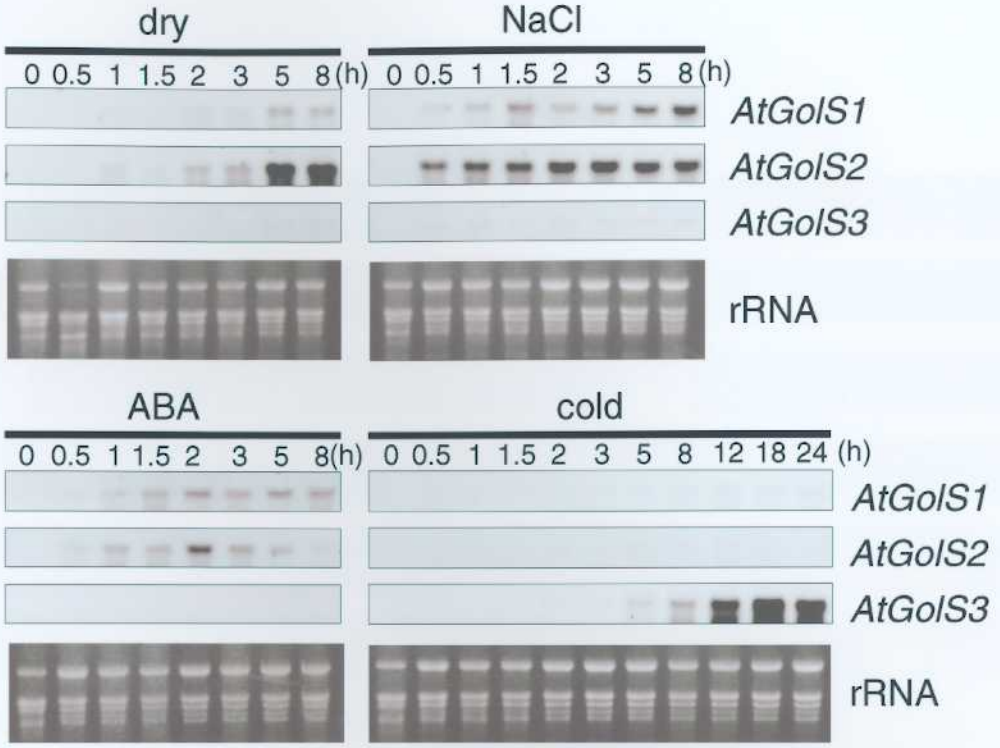


**Figure 13. Northern analysis of the induction of the *AtGolS* genes by dehydration, high salinity, and cold stresses, and ABA treatment.**
Total RNA was prepared from 3-week-old *Arabidopsis* plants grown on GM plates with or without stress treatment. Stress treatment was given as follows; transfer plants from agar plates on Whatman 3MM filter paper (dry), transfer plants from agar plates to hydroponic culture with 250 mM NaCl (NaCl), transfer plants from agar plates to hydroponic culture with 100 μM ABA (ABA), transfer plants on agar plates to growth chamber at 4°C (cold). Each lane was loaded with 5 μg of total RNA. The RNA was fractionated on a 1% agarose gel, blotted onto a nylon membrane, and hybridized with digoxigenine; DIG-labeled cDNA inserts of the *AtGolS* cDNAs as probes.

**Figure 14. Comparison of deduced amino acid sequences from cDNAs encoding raffinose synthase.**
Compared raffinose synthases are *Arabidopsis thaliana* (*AtRafS1*) and *Cucumis sativus* (*CsRafS1*; accession No. AC007138).

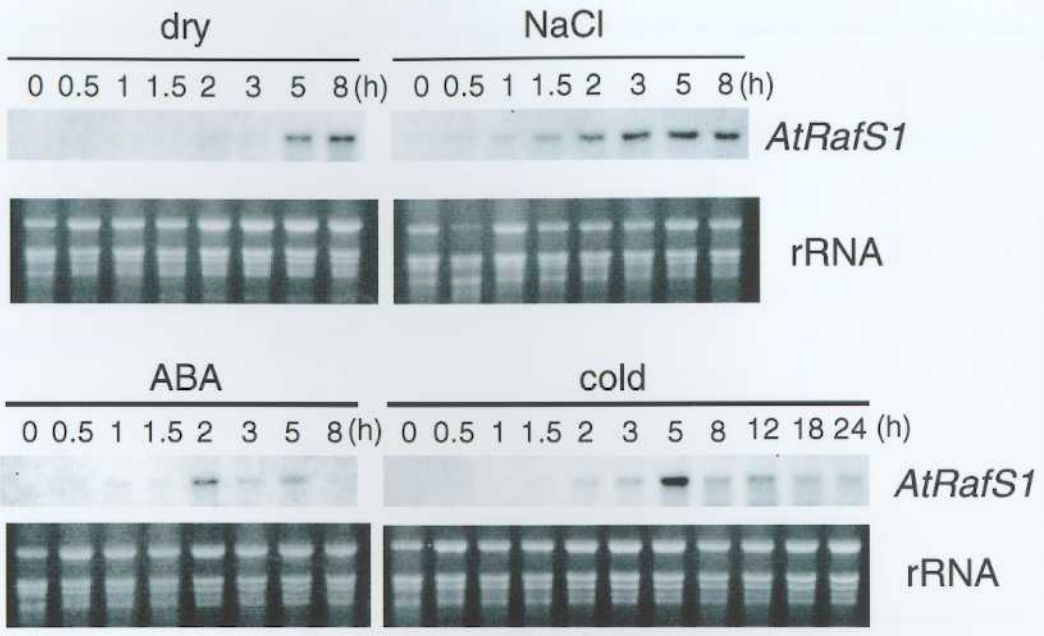


**Figure 15. Northern analysis of the induction of the *AtRafS1* genes by dehydration, high salinity, and cold stresses, and ABA treatment.**
Total RNA was prepared from 3-week-old *Arabidopsis* plants grown on GM plates with or without stress treatment. Stress treatment was given as follows; transfer plants from agar plates on Whatman 3MM filter paper (dry), transfer plants from agar plates to hydroponic culture with 250 mM NaCl (NaCl), transfer plants from agar plates to hydroponic culture with 100 μM ABA (ABA), transfer plants on agar plates to growth chamber at 4°C (cold). Each lane was loaded with 5 μg of total RNA. The RNA was fractionated on a 1% agarose gel, blotted onto a nylon membrane, and hybridized with digoxigenine; DIG-labeled cDNA inserts of the *AtRafS1* cDNAs as probes.

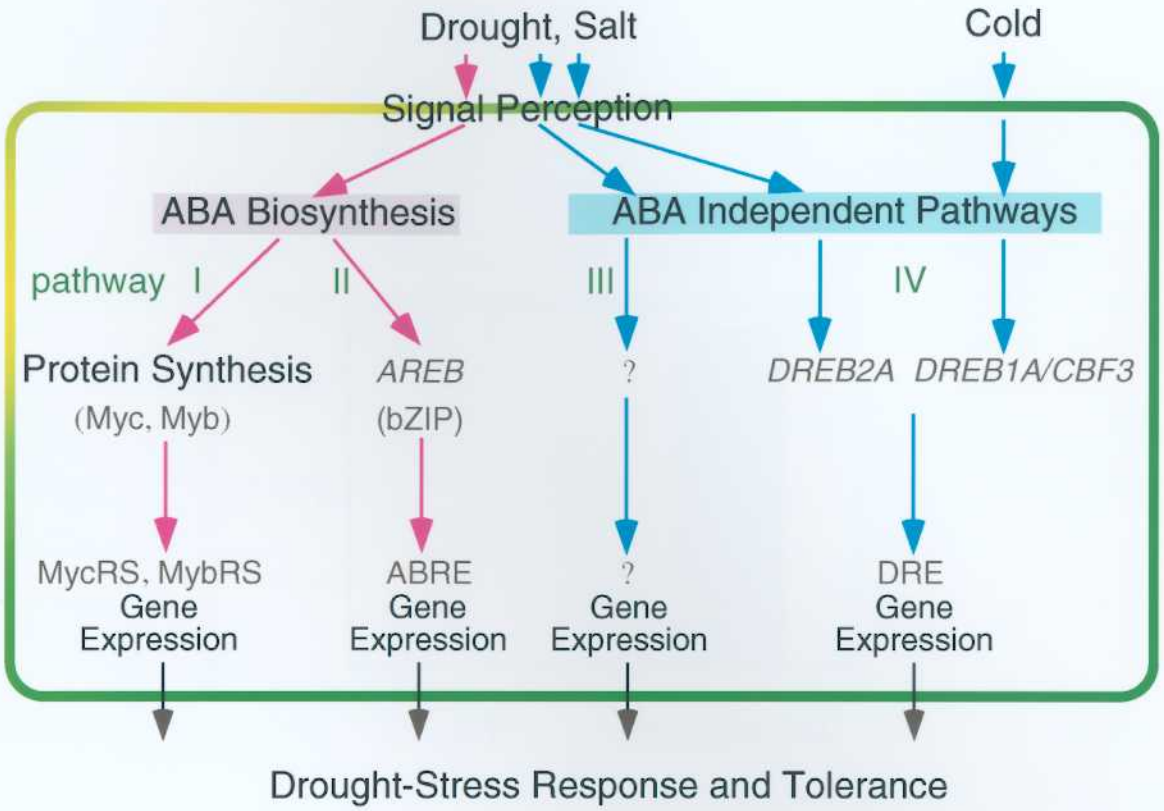


**Figure 16. A hypothetical model of signal transduction cascades in molecular response to water deficit stresses in *Arabidopsis*.**
There are at least four independent signal transduction pathways between the perception of water deficit stress signal and gene expression. Two of them are ABA-dependent (Pathways I and II) and two are ABA-independent (Pathways III and IV). One of the ABA-dependent pathways requires protein biosynthesis (Pathway I). In another ABA-dependent pathway, ABRE functions as an ABA-responsive element and does not require protein biosynthesis (Pathway II). In one of the ABA-independent pathways, DRE is involved in the regulation of genes not only by drought and salt but also by cold stress (Pathway IV). Another ABA-independent pathway is controlled by drought and salt, but not by cold (Pathway III).

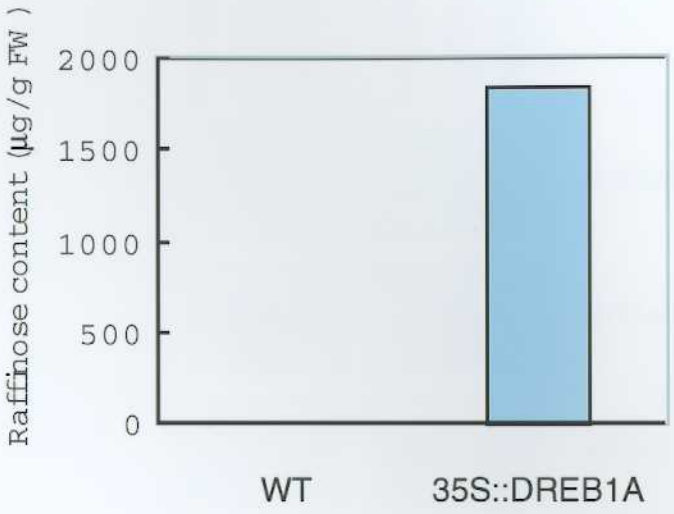


**Figure 17. Raffinose contents of wild type plants and 35S::DREB1A plants.**

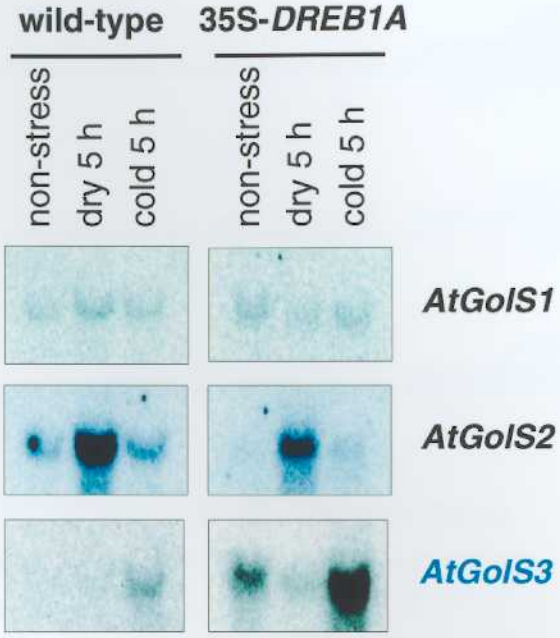


**Figure 18. Expression of *AtGolS* genes in the transgenic *Arabidopsis* plants that overexpress *DREB1A* cDNA.**
Total RNA was prepared from 3-week-old *Arabidopsis* wild-type plants and 35S-*DREB1A* plants grown on GM plates that had been treated with drought (dry) or low temperature at 4°C (cold) or untreated (non stress) at indicated times.
Each lane was loaded with 10 μg of total RNA. The RNA was fractionated on a 1% agarose gel, blotted onto a nylon membrane, and hybridized with [$^{32}$P]-labeled *AtGolS1, 2* and *3* cDNAs as probes.

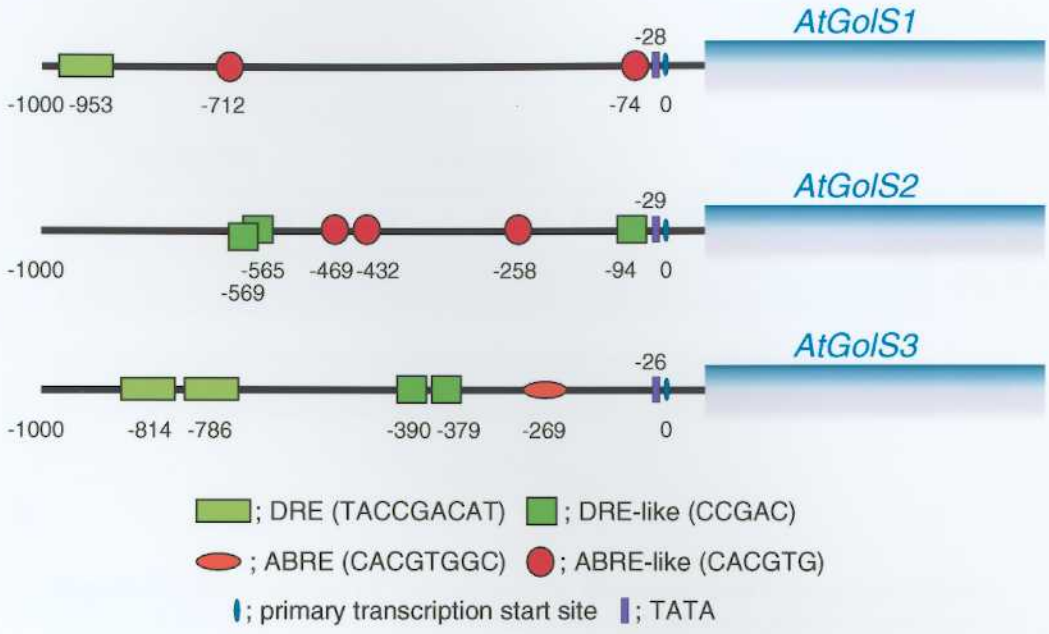


**Figure 19. Comparison of the promoter region of the *AtGolS* genes.**
DRE (TACCGACAT), DRE-like (CCGAC), ABRE (CACGTGGC) and ABRE-like (CACGTG, G-box sequence) motifs are shown in the 1000-bp upstream regions of 5' termini of the full-length cDNA clones. Numbers with minus signs indicate nucleotides upstream of the 5' terminus of the putative transcription start sites of *AtGolS* genes.

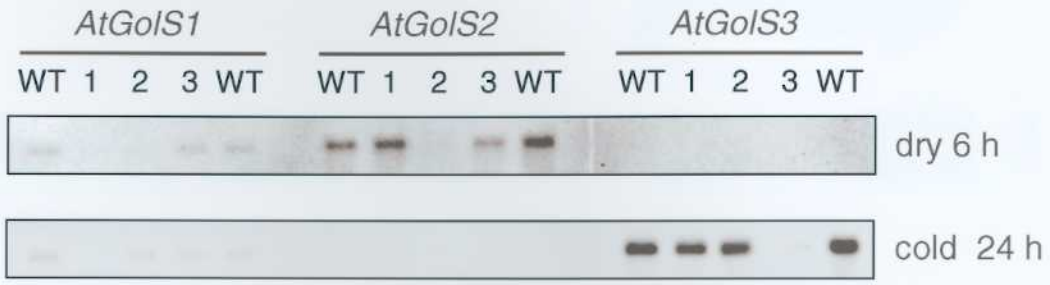


**Figure 20. Expression of transgenes in the antisense transgenic plants.**
Total RNA was prepared from 3-week-old *Arabidopsis* plants grown on GM plates with stress treatment. Stress treatment was given as follows; transfer plants from agar plates on Whatman 3MM filter paper (dry) and transfer plants on agar plates to growth chamber at 4°C (cold). Each lane was loaded with 5 µg of total RNA. The RNA was fractionated on a 1% agarose gel, blotted onto a nylon membrane, and hybridized with digoxigenine; DIG-labeled cDNA inserts of the *AtGolS* cDNAs as probes.

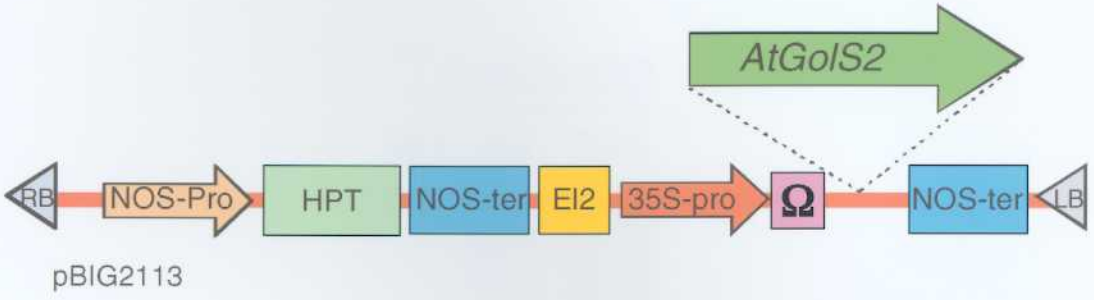


**Figure 21. Constructs of the CaMV 35S-promoter::*AtGolS2* cDNA fusion genes.**
*AtGolS2* cDNA was subcloned into *Bam* HI site of pBIG2113 vector in the sense orientation. NOS-Pro, nopaline synthase promoter region; HPT, hygromycin phosphotransferase; NOS-ter, nopaline synthase terminator region; E12, 5'-up-stream sequence of CaMV 35S promoter (-419 to -90) X 2; 35S-pro, CaMV 35S promoter; Ω, 5'-upstream sequence of TMV, RB, T-DNA right border; LB, T-DNA left border.


**Figure 22. Expression of *AtGolS2* in the *AtGolS2*-overexpressing transgenic plants.**

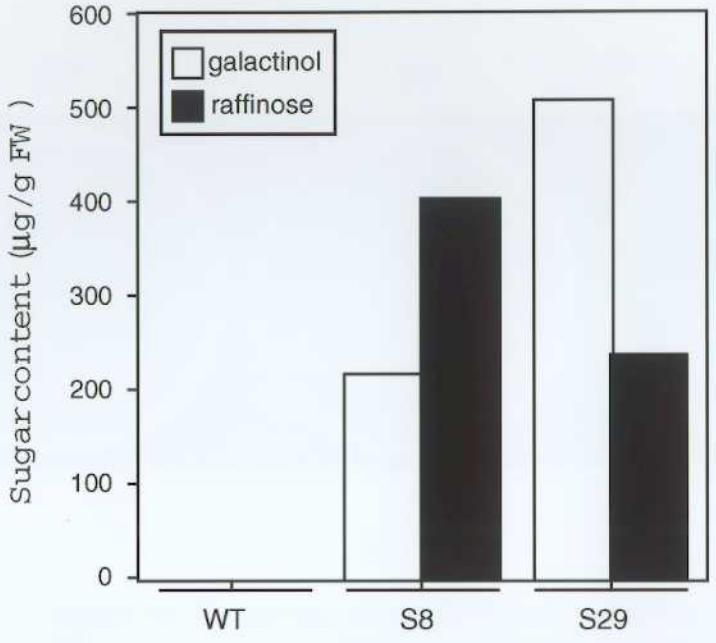


**Figure 23. Galactinol and raffinose content of the *AtGolS2* transgenic plants.**
Galactinol and raffinose were measured in 3-week-old plants. Control transgenic plants with the vector pBIG2113N (wild type); transgenic plants with 35S-*AtGolS2*-sense (S8, S29).


**Figure 24. Phenotype of plants exposed to drought stress.** Three-week-old plants were exposed to drought stress. Drought stress was performed by withholding water for 14 days. After the drought stress, the plants were rehydrated for 5 days. Plants used for the analysis were transgenics with the vector pBIG2113N (wild type) as a control, 35S-*AtGolS2*-sense-8 (S8) and 35S-*AtGolS2*-sense-29 (S29)

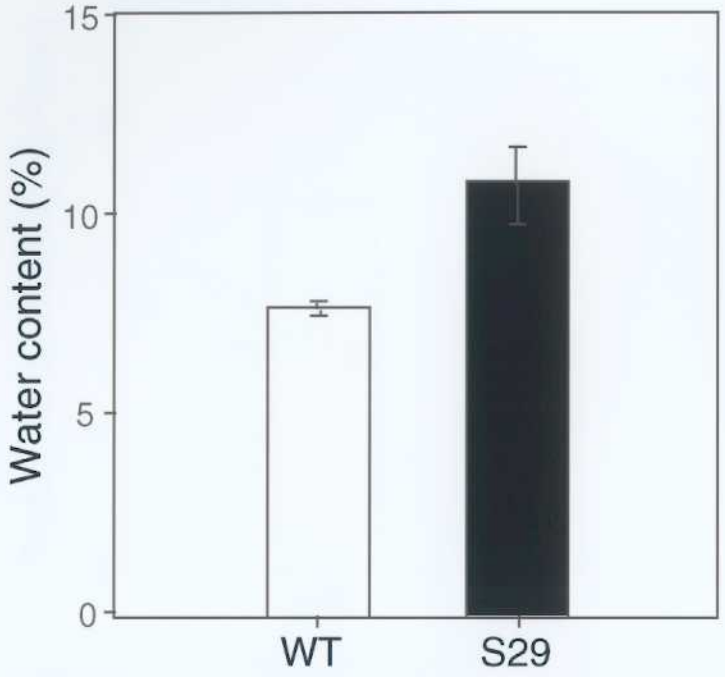


**Figure 25. Water content of the soil for the growth of plants after drought stress.**
The 3-week-old plants were exposed to drought stress. Drought stress was performed by withholding water for 14 days. Plants used for the analysis were transgenic with the vector pBIG2113N (wild-type) and the 35S-sense-*AtGolS2*-29 (*AtGolS2*-S-29) fusion genes.

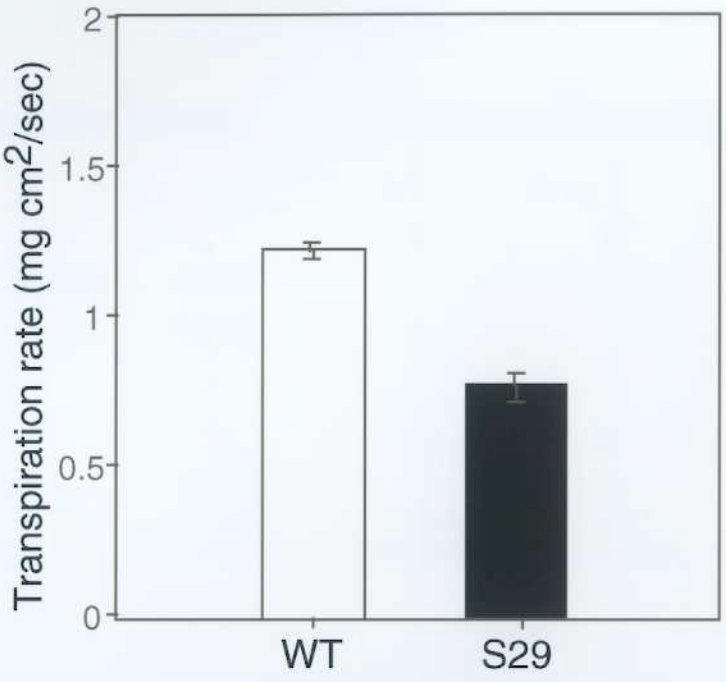


**Figure 26. Transpiration rates of wild type plants and 5-week-old *AtGolS2* transgenic plants (S29) under normal growth conditions.**
Transpiration rates of *Arabidopsis* plants were measured in fully expanded leaves with a portable photosynthesis system (model LI-6400, Li-Cor, Lincoln, NE, USA) under the followimg conditions: 100 μmol/m$^2$/s, 350 ppm $CO_2$, 22°C, and 70% relative humidity.

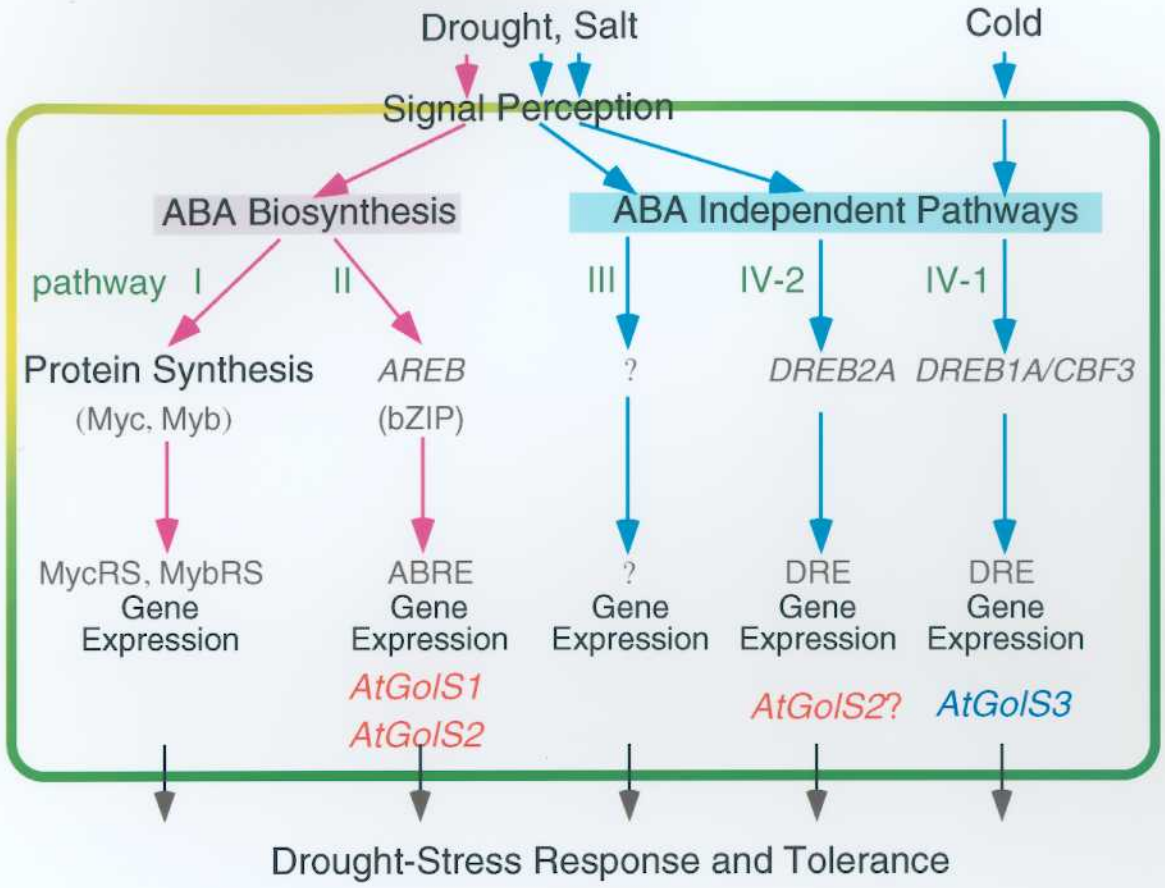


**Figure 27. A hypothetical model of signal transduction cascades in molecular response to water deficit stresses in *Arabidopsis*.**
Northern analysis and promoter analysis indicated that *AtGolS1* and 2 existed in ABA-dependent pathway (Pathway II) and suggested that *AtGolS2* also existed in ABA independent pathway through *DREB2A* (Pathway IV-1). Furthermore,from the Northern analysis in the 35S::*DREB1A* plants, it was indicated that *AtGolS3* existed in ABA independent pathway through *DREB1A* (Pathway IV-2).