Hsp90 Inhibitor- and Heat Stress-Induced Galactinol Synthase Enhances Thermotolerance in Rice Seedlings

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Introduction

Heat stress on plants induces a set of thermotolerance-related genes. We focused on rice homologs of galactinol synthase (*OsGolS*) closely related to *Arabidopsis thaliana AtGolSs* which are induced under various stresses. Among those, galactinol synthase1 (*OsGolS1*) appeared to be highly up-regulated upon heat stress condition and geldanamycin (GDA) treatment. The up-regulation of *OsGolS1* preceding the accumulation of galactinol is a key factor that enables rice seedlings to cope with high temperature stress.

Materials and methods

Plant materials and stress treatments: 14-day-old rice seedlings (*Oryza sativa* L. cv. Hinohikari) grown in distilled water at 25°C under light (80W m^{-2}) were subjected to stress treatments as followings: 50μM GDA, heat stress at 52°C, under light condition.

RNA extraction and semiquantitative RT-PCR: Total RNA was extracted from plant tissue frozen in liquid N_2 by the SDS/phenol/LiCl method. Semi-quantitative RT-PCR was performed with ReverTra-Ace reverse transcriptase kit (TOYOBO) and GoTaq PCR Kit (Promega) with gene-specific primers.

Measurements of galactinol content: Galactinol accumulation profile under heat stress treatment was analyzed by high-performance ion-exchange chromatography using NH2P-50 4E column (Shodex), a polyamine-bonded silica-base amino column.

Results and discussion

Heat shock factor A2 (*OsHsfA2)* and *OsGolS1* were enhanced by heat stress and GDA treatments. It is supposed that heat stress treatment on plants disrupts the interaction between heat shock protein 90 (Hsp90) and heat shock factor A1 (HsfA1), which is similar to GDA treatment. That in turn HsfA1 is activated to form pre-active trimer and binds heat shock element located in promoter regions of heat inducible genes including other heat shock factors. The present study revealed that mRNA level of *OsHsfA2* increased under GDA treatments and that resultant up-regulation of OsHsfA2 protein level possibly caused induction of *OsGolS1* without heat stress condition. Recently, it has been reported that the induction of enzymes related to the biosynthesis of galactinol and raffinose family oligosaccharides (RFOs) and the resultant intracellular accumulation play pivotal roles in acquired tolerance in *Arabidopsis thaliana* under drought and temperature stresses. In the present study on rice seedlings, under heat stress condition, galactinol accumulation was observed in correlation with *OsGolS1* up-regulation. In addition, pre-treatment of the rice seedlings with GDA or raffinose enhanced their heat acclimation ability when compared to controls. The results suggested that the induction of *OsGolS1* caused the accumulation of intracellular carbohydrate content. The abundance of the sugar accelerated production of osmolytes in rice cells that can help the plants to cope with high temperature stress. (Fig. 1).

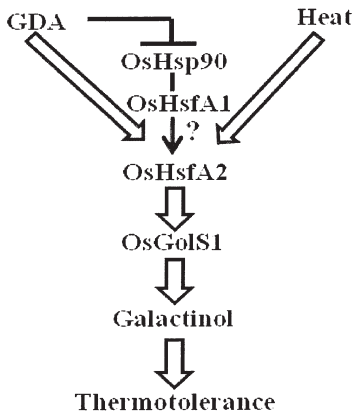

Fig. 1 A proposed model of signaling pathway involved in the regulation of OsGolS1 expression under geldanamycin (GDA) and heat stress treatments.