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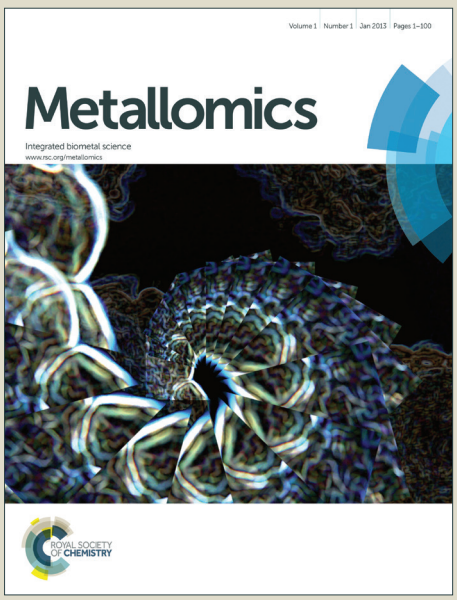

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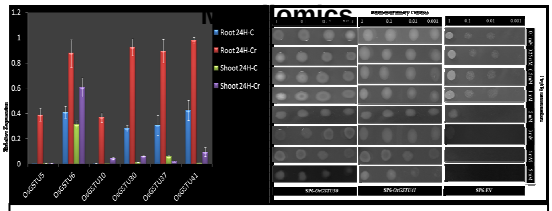

Tau class GSTs from rice up-regulated in roots upon exposure to Cr (VI) and showed resistance when expressed in *Yeast*

Transformed yeast (*Schizosaccharomyces pombe)* overexpressing rice Tau class glutathione S-transferase (*OsGSTU30* and *OsGSTU41*) show enhanced resistance to hexavalent chromium

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*Background***.** Extensive use of hexavalent chromium [Cr (VI)] in leather tanning, stainless-steel production, wood preservatives and electroplating industries have resulted in widespread environmental pollution and pose a serious threat to human health. Plant's response to Cr (VI) stress results in growth inhibition and toxicity leading to changes in components of antioxidant system. In a previous study, we observed a large number of glutathione S-transferase were up-regulated under Cr (VI) stress in rice. In this study, two rice root-specific Tau class glutathione S-transferase (GST) genes (*OsGSTU30* and *OsGSTU41*) were introduced into yeast (*Schizosaccharomyces pombe*). Transformed yeast cells overexpressing *OsGSTU30* and *OsGSTU41* were normal in growth, but had much higher levels of GST activities and showed an enhanced resistance to Cr (VI) as compared to control (transformed with empty vector). Also, higher accumulation of chromium was found in transformed yeast cells as compared to control. Manipulation of glutathione biosynthesis by exogenous application of buthionine sulfoximine (BSO) abolishes the protective effect of OsGSTs against Cr (VI) stress. These results suggest that Tau class OsGSTs plays significant role in detoxification of Cr (VI), probably by chelating and sequestrating glutathione-Cr (VI) complex into the vacuoles.

Keywords Glutathione S-transferase; Hexavalent chromium; Rice; Yeast

Introduction

Chromium (Cr) is the 21st most abundant element and seventh most abundant metal in the Earth's crust.[1] However, several industrial applications (leather tanning, stainless-steel production, wood preservatives and electroplating industries) are potentially responsible for chromium contamination in environment.[1,2] Chromium exists in several oxidation states but zero, trivalent and hexavalent states are widely present in commercial products that tremendously affects the environment.. Stable forms of chromium are the trivalent [Cr (III)] and the hexavalent [Cr (VI)] species. Among these two, Cr (VI) is the most toxic form which is usually associated with oxygen either as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxy-anions.[3] Cr (III) is mainly found as a complex with organic matter in the soil and aquatic environments and is less toxic due to its poor mobility.[4] Cr (VI) toxicity affects growth and development of plants as well as causes severe human health hazards through food chain contamination.[5] In contrast to other heavy metals like cadmium, lead, mercury and arsenic, Cr has received little attention from plant scientists. Since Cr (VI) causes toxic effects directly or indirectly so deciphering the detoxification mechanism is often desirable to counteract such issues.

Phytochelatins (PCs) are enzymatically synthesized Cys-rich peptides capable of binding heavy metal ions via thiolate coordination. Such conjugated complexes are then sequestered to the vacuoles.[6] Although PCs are known to be involved actively in heavy metal detoxification but such peptides are not involved in Cr (VI) toxicity.[3, 7] Molecular metabolism related to Cr (VI) toxicity mechanism in plants is also poorly understood. Recently, we reported detailed analysis of genome-wide transcriptome profiling in rice root following Cr-plant interaction[8] and no phytochelatin synthase transcript was detected in Cr (VI) treated rice

root. However, a large number of Glutathione S transferases were up-regulated following Cr (VI) stress in rice root[8] indicating their role in detoxification of this heavy metal.

Glutathione S-transferases (GSTs, EC 2.5.1.18) belong to a ubiquitous superfamily of multifunctional enzymes involved in cellular detoxification of a wide variety of substrates such as endobiotic and xenobiotic compounds by conjugating with glutathione.[9-11] Glutathione (GSH) is a tripeptide (γ-Glu-Cys-Gly) with a gamma peptide linkage between the amino group of cysteine and the carboxyl group of the glutamate side chain. It is a potent antioxidant which prevents cellular damage caused by reactive oxygen species such as free radicals and peroxides.[12] Several authors have observed that there was a marked decline in the GSH pool under Cr stress more severely in roots.[13] Several GSTs have been characterized in different plants with unique and even common functional specificities.[14-15] They are known to play a vital role in response to biotic and abiotic stresses.[14, 16-17] Based on sequence analysis, GSTs were classified into several classes i.e. Tau, Lambda, Phi, Dehydroascorbate reductase (DHAR), Theta, Zeta, Elongation factor 1 gamma and Tetra chloro hydroquinone dehalogenase (TCHQD).[17-18] Among these, four classes, Phi, Tau, Lambda and DHAR are specific to plants. The conserved sites of GSTs retain two set of residues required for glutathione docking and binding of co-substrates.[19] Based on differential expression profiles of genes in various tissues and developmental stages we have shown that six OsGSTs i.e. *Os09g20220* (*OsGSTU5*), *Os01g37750* (*OsGSTU6*), *Os01g27390* (*OsGSTF10*), *Os10g38600* (*OsGSTU30*), *Os01g72150* (*OsGSTU37*), and *Os01g72160* (*OsGSTU41*), were showing highest expression in root under Cr (VI) stress.[8] This data gave us a clue that these genes might play an important role in Cr (VI) detoxification in rice roots. There are very few direct evidences to prove GSTs' function in the defence against Cr (VI) stress in higher organisms. Recently we have demonstrated one member of the Lambda class of rice GSTs (*OsGSTL2*) provides tolerance for heavy metals including Cr (VI).[20] In the

present study, we confirmed that overexpression of Tau class OsGSTs in yeast cells provides enhanced capacity of tolerance to Cr (VI). This protective effect appears to prevent cellular damage and allows yeast cells to retain high levels of metabolic activity and growth. We also showed that by inhibiting the γ-glutamylcysteine synthetase (γ-GCS) and consequently lowering the cellular glutathione (GSH) concentration abolishes the protective effect of OsGSTs against Cr (VI) stress.

Results and discussion

Selection of Cr (VI) stress responsive GSTs in rice

Genome wide rice root transcriptome analysis indicate that several members of GST gene family were up-regulated during Cr stress [8]. In this study, six genes described previously were selected based on expression analysis in rice roots upon Cr (VI) stress.[8] We validated that five selected OsGSTs, *OsGSTU5, OsGSTU6, OsGSTU30, OsGSTU37*, and *OsGSTU41* belong to 'Tau' class while *OsGSTF10* belongs to 'Phi' class gene family in rice (Table S1) based on information about classification of GST gene family in rice by Jain et al (2010).[18]

To resolve tissue-specific differences in the expression of selected genes under Cr (VI) stress, the validation was carried out through qRT-PCR. The result obtained by real time PCR agree with the trend of regulation identified by microarray analysis. As shown in Figure 1, three genes (OsGSTU30, *OsGSTU37* and *OsGSTU41*) showed comparatively higher expression under Cr (VI) stress in root. We observed that these three genes have conserved domain specific to Tau class GST gene family (Figure S2). It has already been reported that GSTs show conserved domain specificity.[18, 21]

Expression pattern analysis of selected genes in different developmental stages in Rice

To check expression level of selected genes (six), real time PCR was performed in eight different tissues and developmental stages as described in materials and methods. Three genes (*OsGSTU30*, *OsGSTU37*, and *OsGSTU41*) were showing highest expression in root indicating preferential root expression whereas *OsGSTU5* showed expression in root, mature leaf as well as anther and *OsGSTF10* showed expression in both root and young panicle. *OsGSTU6* showed highest expression in mature leaf and very less expression in root and other parts (Fig. 2). There has been increasing evidence indicating that GSTs may be implicated in many other physiological processes like plant growth, development, biotic and abiotic stresses.[22-24] Recently, Jain et al (2010)[17] reported tissue-/organ- and developmental stage-specific expression patterns of several rice GST genes. Similarly expression analysis of the selected genes during different developmental stages of rice gave us a clue about spatial expression pattern of different class of GSTs. Based on the expression pattern we selected *OsGSTU30* and *OsGSTU41* for further characterization.

Growth pattern of yeast strain following Cr (VI) stress

Two selected genes (*OsGSTU30* and *OsGSTU41*) were cloned and transformed in SP6 as described in materials and methods. Transformed SP6 growth pattern was analysed using YPD agar media with different dilutions of the strains containing different concentrations of Cr (VI). As shown in Figure 3 transformed strains showed growth in almost all dilutions up to 5 mM. However, the growth of yeast transformed with the empty vector was impaired in the presence of 0.1 mM (Fig. 3) and complete growth inhibition was observed at 3 mM Cr (VI) (Figure 3). There are several mechanisms associated with metal toxicity in plants.[10, 26-31] Role of phytochelatins in metal toxicity has already been reported but no reports are available about its role in Cr toxicity.[5, 7] However, there are several reports indicated that GST activity (enzyme) were increased during several heavy metal stresses. Halusková et al. (2009)[32] showed that barley (*Hordeum vulgare* cv. Jubilant) subjected to different heavy metal stresses

(Cd, Pb, Cu, Hg, Co, and Zn) showed a significant increase in GST activity. Cakman and Horst (1991)[33] reported that the activity of GST was also greater in the Al-tolerant lines, suggesting that this enzyme may be important not only for the detoxification of certain heavy metals but also for that of Al phytotoxicity. Kumar et al. (2013)[16] overexpressed one member of Lambda class *OsGSTL2* in *Arabidopsis* and found that transgenic plants are resistant to different heavy metals including Cr (VI). Marrs et al (1997)[34] suggested that the role of GST may be related to its possible involvement in the transport of GSH-metal complexes to the vacuole. It has also been reported that dichromate reacts with GSH at the sulfhydryl group forming an unstable glutathione– Cr complex.[13] However, the mechanism underlying the uptake and translocation of Cr in plants is not completely understood. Our data indicates that GSTs might play a role in GSH-Cr (VI) conjugation.

Growth curve analysis of recombinant yeast strain following Cr (VI) stress

We also analysed growth curve of transformed yeast at regular time interval in YPD broth at 3 mM Cr (VI) concentration. We observed that such condition favours kinetic growth pattern of yeast.[35] As shown in Figure 4, both transformed strains showed steady standard growth pattern[35] under Cr (VI) stress, although SP6-EV grew very slowly. Standard doubling time of *Schizosaccharomyces pombe* in liquid culture is almost 2-3 hrs.[36] We measured O.D. after every 4 h, based on the model where only time is considered, and not size, such as the limit cycle.[37] Our data indicates that two Tau class OsGST genes are likely to play significant role in metal detoxification by conjugating the tri- peptide (g-Glu-Cys-Gly) glutathione (GSH) to Cr (VI) and further its sequestration probably inside the vacuole.

Cr (VI) estimation

As shown in Fig 5A both transformed strains showed almost three times higher Cr (VI) accumulation as compared to control indicating the role of selected genes in GSH-Cr (VI)

conjugation and its probable sequestration in vacuoles as part of its detoxification mechanism. GST expression is known to be influenced by numerous intracellular and environmental factors during different heavy metal stresses.[38] In general GSTs are responsible for conjugation of reduced glutathione (GSH) with toxic molecules and sequester them into the vacuole.[37]

GST activity analysis of transformed strain

Cr (VI) accumulation assay indicate probable role of GST in Cr (VI)-GSH conjugation. To further validate the role of GST in metal conjugation, enzymatic activity assay was performed using GST activity kit described in materials and methods. We found almost three times and six times higher GST activity in both *OsGSTU30* and *OsGSTU41* compared to SP6-EV [Cr (VI) treated] and SP6-EV respectively (Fig.5B). The above result again suggested that two Tau class OsGSTs are playing a pivotal role in GSH-Cr (VI) conjugation. Higher expression of GST specific protein in our result is an another confirmation of our hypothesis that expressed OsGSTs in transformed yeast strains utilize glutathione as a substrate and upon conjugation with Cr (VI), GSH-metal complex probably sequestered inside the vacuole.[11]

Inhibition of glutathione synthesis leads to Cr (VI) sensitivity in overexpressed yeast lines

GST utilize reduced glutathione (GSH) as sole source of substrate and transfer its thiol group to heavy metals.[11] Buthionine Sulfoximine (BSO) is a highly specific inhibitor of γ-glutamyl cysteine synthatase, first enzyme of GSH biosynthesis. To confirm specific role of GST in Cr (VI) chelation 1 mM BSO was used to inhibit GSH biosynthesis.[39] We found almost negligible growth in both control and transformed strain in minimal media containing BSO. However, minimal media devoid of BSO showed normal growth pattern (Figure 6). Our data suggests that in presence of BSO (an inhibitor of GSH biosynthesis) metal chelation was

hampered leading to metal toxicity in both transformed and control strains resulting in negligible colony growth. Various studies have confirmed that exposure of whole plants or plant cell cultures to an inhibitor of GSH biosynthesis namely, buthionine sulfoximine, conferred hypersensitivity to some metal ions. [40-42] Present analysis confirmed that selected transformed genes (OsGSTs) are specifically responsible for GSH-Cr (VI) complex formation in transformed yeast.

Conclusion

Cr (VI) is a strong oxidant and is responsible for oxidative stress in plant tissue. However, the detoxification mechanism for this heavy metal has not been studied critically. In the present study we identified two root specific Tau class GSTs (*OsGSTU30* and *OsGSTU41*) which specifically up-regulated when exposed to Cr (VI). Overexpressing these two OsGSTs in *S. pombe* resulted in an enhanced resistance to Cr (VI) and OsGSTs-expressing yeast accumulated larger amounts of Cr (VI) than the vector control. Similarly, the transgenic yeasts also showed higher GST enzyme activities. Inhibition of glutathione biosynthesis by exogenous application of buthionine sulfoximine (BSO) abolishes the protective effect of OsGSTs against Cr (VI) stress which suggests a possibility that specific Tau class of GSTs are conserved in rice for binding with Cr (VI) and its homeostasis and detoxification.

Experimental

Plant Material and growth parameters

The rice cv. Nipponbare (*Oryza sativa* subsps. *japonica*) was germinated and allowed to grow for 5 d at 26°C and then transferred to Hewitt media for growth. After 10 d of growth, seedlings of uniform size and growth were treated with 100 μM Cr (VI) under standard physiological conditions of 16 h light (115 μmol $m^{-2} s^{-1}$) and 8 h dark photoperiod at 25 ± 2°C. 100 μM concentration of Cr (VI) was prepared using $K_2Cr_2O_7$ (Cr VI - Merck). Roots

treated with 100 μM Cr (VI) after 24 h were taken to evaluate early expressed GST genes subjected to Cr stress. All the samples were ground in liquid N_2 and stored at -80°C for further analysis.

Total RNA extraction and expression analysis by Real-Time PCR

Total RNA was extracted from the treated rice roots using the QIAGEN RNeasy Plant Mini Kit (QIAGEN, MD). The yield and RNA purity were determined spectrophotometrically (NanoDrop, Wilmington, DE) and by agarose gel electrophoresis. First strand cDNA was synthesised using 5 μg purified total RNA and RevertAid First Strand cDNA synthesis Kit (Fermantas, Life Sciences, USA). Real Time PCR was performed in 20 μl for set of selected genes using Power SYBR Green PCR Master Mix (ABI, USA). List of selected genes and oligonucleotide primers (Eurofins, India) used for each gene are listed in the Additional file (Table S3). Oligonucleotide primers for rice actin gene (Table S3) were used as internal control for establishing equal amount of cDNA in all the reactions. The reactions were performed using the following cycle conditions, an initial 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and the final 5 min extension at 72°C. After obtaining ct value for each reaction, the relative expression was calculated by 2^-delta Ct method.

For development specific expression pattern analysis of six selected genes (Table S3) in eight different tissues and developmental stages real time PCR was performed. Various rice tissues/organs (seedling root, mature leaf and young leaf) and stages of reproductive development (young panicle, anther and gynaecium) were used for RNA extraction. Stages of seed development have been categorized according to days after pollination.[43] Middle and late globular embryo (S1) 5-10 dap (day after pollination) and dormancy and desiccation tolerance embryo (S2): 21-25 dap were used for expression analysis. The reactions were performed and analyzed as mentioned above.

Cloning, construct preparations and transformation

Full-length cDNA encoding *OsGSTU30* (*Os10g38600*) and *OsGSTU41* (*Os01g72160*) were amplified from cDNA library prepared from rice root through PCR using gene specific primers of both genes (Table S4) and cloned using Ins TA clone PCR Cloning Kit (Fermentas). Nucleotide sequences of cloned fragments were confirmed through sequencing of both genes. Full-length cDNA was cloned in yeast expression vector, pSP1 [44] between BamHI and HindIII restriction sites after amplification using oligonucleotide primers (Table S4) having BamHI and HindIII restriction sites in forward and reverse primers respectively. Both constructs carried T3 promoter for driving expression of *Os10g38600* and *Os01g72160* were used for transformation in yeast. Both constructs (Figure S5) were sequenced before use to confirm error free amplification and cloning.

Plasmid pSP1- *OsGSTU30*, pSP1- *OsGSTU41*and pSP1 (EV) were transformed in SP6 (*Schizosaccharomyces pombe; h⁻ leu1-32*) yeast strain.[35] Empty vector pSP1 transformed yeast cells were considered as control in each experiment. Yeast cells were grown in YPD media before transformation. Transformation of yeast cells were performed using Yeast Transformation Kit (Sigma, USA). After transformation, transformants were selected on SD (synthetic defined) -leucine media containing 2% glucose and bacteriological agar.

Cr (VI) resistance in YPD-agar

Transformed yeast cells were grown in 5 ml YPD broth (Sigma) to an OD_{600}nm 1 using a shaker under controlled condition (Temp-30°C, 220 rpm).YPD agar plates treated with different concentrations of Cr (VI) (0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 5 mM) were prepared using $K_2Cr_2O_7$ (Cr VI Merck). Different dilutions (1, 0.1, 0.01, and 0.001) of previously grown culture were prepared using YPD broth for dilution and 5 µl of each were spotted onto YPD agar plates accordingly. Inoculated plates were placed in incubator at 30°C temperature and plates were analysed after 48 hrs of incubation.

Growth curve analysis

Yeast cells were grown on 5 ml YPD broth as mentioned in previous section to obtain primary culture. 100 ml YPD broth containing 3 mM Cr (VI) was prepared and inoculated with 100 µl of primary culture. YPD-broth was taken as control. Initial absorbance was taken using spectrophotometer at 600 nm. Subsequent absorbance was measured after every 4 h up to 24 h. Growth curve was prepared based on absorbance data.

Cr (VI) estimation

Transformed yeast cells were grown onto YPD media containing 3 mM Cr (VI) as described previously for quantification of total metal accumulation. Cells were harvested by centrifugation at 5000 rpm for 10 minute at 4°C and dried in an oven at 55°C for 2 days. For estimation of Cr in yeast cells, 0.1g oven dried yeast cell cultures were taken and digested in 3ml of HNO_3 at 120°C for 5 days and filtered in 10 ml of Milli Q water. Cr estimation of filtrates was carried out with the help of Atomic Absorption Spectrometer (AAS, Perkin Elmer, AAnalyst 400). The standard reference materials of metal (E-Merck, Germany) were used for the calibration and quality assurance for each analytical batch. The detection limit was 0.1 ppm.

GST activity assay

Yeast cells were grown in 100 ml of YPD containing 3 mM Cr (VI) at condition mentioned in previous section. Yeast cultures were centrifuged at 5,000×g for 10 min, and each harvested pellet was suspended in an extraction buffer solution consisting of 25 mM Tris–HCl (pH 8.0), 400 mM NaCl, 10 % glycerol, 1 mM 2-mercaptoethanol, and 0.1 % Tween-20. The suspended yeast cells were sonicated for 30 cycles (15 sec pulse-15 sec break) and centrifuged at 13,000×g for 10 min at 4 °C. The supernatant obtained was used for determination of GST activity assay. The total cell protein of the supernatant was estimated

by using the standard protocol of Bradford (1976).[45] GST activity in recombinant yeast against control was determined by using Glutathione-S-Transferase assay kit (Sigma) following user's manual which utilizes 1-Chloro-2, 4-dinitrobenzene (CDNB) as substrate, suitable for the broadest range of GST isozymes. The GSTs in the sample catalyses the conjugation of CDNB to glutathione producing S-(2, 4-dinitrophenyl) glutathione, and enzyme activity was monitored spectrophotometrically at 600 nm.

Inhibition of glutathione synthesis and Cr (VI) sensitivity in overexpressed yeast lines

In vitro enzyme specificity of *OsGSTU30* (Os10g38600) and *OsGSTU41* (Os01g72160) expressing GST in recombinant yeast was determined by using buthionine sulfoximine (BSO, Sigma)) an inhibitor of γ-Glutamyl cysteine synthatase, first enzyme of GSH biosynthesis. Synthetic dropout (Sigma) agar plates containing essential amino acids, 0.5mM Cr ($K_2Cr_2O_{7)}$ and 1 mM BSO were prepared. Different dilutions (10^{-1}, 10^{-2}, and 10^{-3}) of yeast primary culture grown in SD media (Sigma) were prepared using SD broth for dilution and 5 µl was spotted on each SD plates containing amino acids, Cr (VI) and BSO agar plates accordingly. SP6-EV strain was taken as control. Inoculated plates were placed in incubator at 30°C temperature and plates were analysed after 60 hrs of incubation.

Abbreviations

Cr	Chromium
GST	Glutathione S-transferase
BSO	Buthionine sulfoximine
GSH	Reduced glutathione
CrO_4^{2-}	Chromate
$Cr_2O_7^{2-}$	Dichromate
PCs	Phytochelatins
DHAR	Dehydroascorbate reductase
TCHQD	Tetra chloro hydroquinone dehalogenase

$K_2Cr_2O_7$	Pottasium dichromate
N_2	Nitrogen
Dap	Day after pollination
SD	Synthetic defined
YPD	Yeast pottato dextrose
AAS	Atomic absorption spectrometer
PPM	Parts per million
NaCl	Sodium chloride

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Legends of Figures:

Figure 1: Quantitative real time PCR analysis of six GSTs (*OsGSTU5*, *OsGSTU6*, *OsGSTF10*, *OsGSTU30*, *OsGSTU37*, and *OsGSTU41*) in rice root and shoot following 0.1 mM Cr (VI) stress. Relative expression in control and Cr (VI) treated sample in both root and shoot were calculated using 2 ^-delta Ct. Bars show the mean of triplicate cultures and error bars represent the SD (Standard Deviation). Rice seeds were germinated hydroponically for 10 days under normal condition followed by 24 h exposure to 0.1mM Cr (VI). Root and Shoot samples were harvested after 24 h exposure to Cr (VI).

Figure 2: Quantitative real time PCR analysis of six GSTs in 8 different developmental stages of rice showing highest expression of selected genes (SP6-*OsGSTU30* and SP6-*OsGSTU41*) in root. Relative expression for all genes, in all tissues were calculated using 2 ^-delta Ct. Bars show the mean of triplicate cultures and error bars represent the SD (Standard Deviation).Young leaves and roots were harvested from rice plant grown hydroponically while remaining stages were harvested from rice plant grown in field under controlled conditions. Stage 1 and Stage 2 represent 5-10 dap embryo and 21-25 dap embryo respectively. (dap: days after pollination)

Figure 3: Growth pattern of transformed yeast expressing *OsGSTU30* and *OsGSTU41* following Cr (VI) stress. 5µl from different dilutions of primary culture (1, .1, 0.01 and 0.001) were spotted onto YPD agar media plates containing different concentrations of Cr (VI) [0.1mM, 0.25mM, 0.5mM, 1mM, 2mM, 3mM, 4mM and 5mM]. An empty vector transformant (pSP1) was used as control (SP6-EV). Yeast pre-cultures having OD600-1.0 were serially diluted and incubated at 30 °C for 3 days.

Figure 4: Growth curve of both transformed strain, SP6-*OsGSTU30* and SP6-*OsGSTU41* following Cr (VI) stress showing rapid growth as compared to SP6-EV. Such growth pattern favours kinetic growth as difference in growth can be observed during regular time intervals.

Figure 5: A. Cr (VI) estimation in yeast. Both SP6-*OsGSTU30* and SP6-*OsGSTU41* strain showed almost six times higher accumulation than SP6-EV. Bars show the mean of triplicate cultures and error bars represent the SD. Values marked with similar letters are not significantly (Duncan's test: $p < 0.05$) different. B. GST activity in yeast grown on YPD broth containing 3 mM Cr (VI) showing almost three times and six times higher GST activity in both *OsGSTU30* and *OsGSTU41* as compared to SP6-EV. Bars show the mean of triplicate cultures and error bars represent the SD. Values marked with similar letters are not significantly (Duncan's test: $p < 0.05$) different. Each experiment was carried out at least three times.

Figure 6: Growth pattern of SP6-*OsGSTU30* and SP6-*OsGSTU41* following BSO treatment. 5 µl from different dilutions of primary culture were spotted on SC agar media containing 0.5 mM Cr (VI) and 1 mM BSO. All plates were grown on 30°C in dark and observed after 48 hrs.

Legends of Supplementary Figures

Table S1: Gene family of selected GST genes in rice.

Figure S2: Multiple sequence alignment of partial nucleotide sequence showing conserved domain specific to GST.

Table S3: List of primers used for RT-PCR analysis of Cr (VI) stress responsive genes in rice roots.

Table S4: List of primers of both genes (*OsGSTU30*, *OsGSTU41*) used for cloning and transformation. Underline sequences showing sequence of restriction site.

Figure S5: Construct map of cloning vector used for transformation of *OsGSTU30* (A) and *OsGSTU41* (B) genes of Rice into *Schizosacharomyces pombe* (SP6) strain of yeast. pSP1 vector was used for transformation. 'P' denotes promoter site whereas 'T' denotes terminator site.

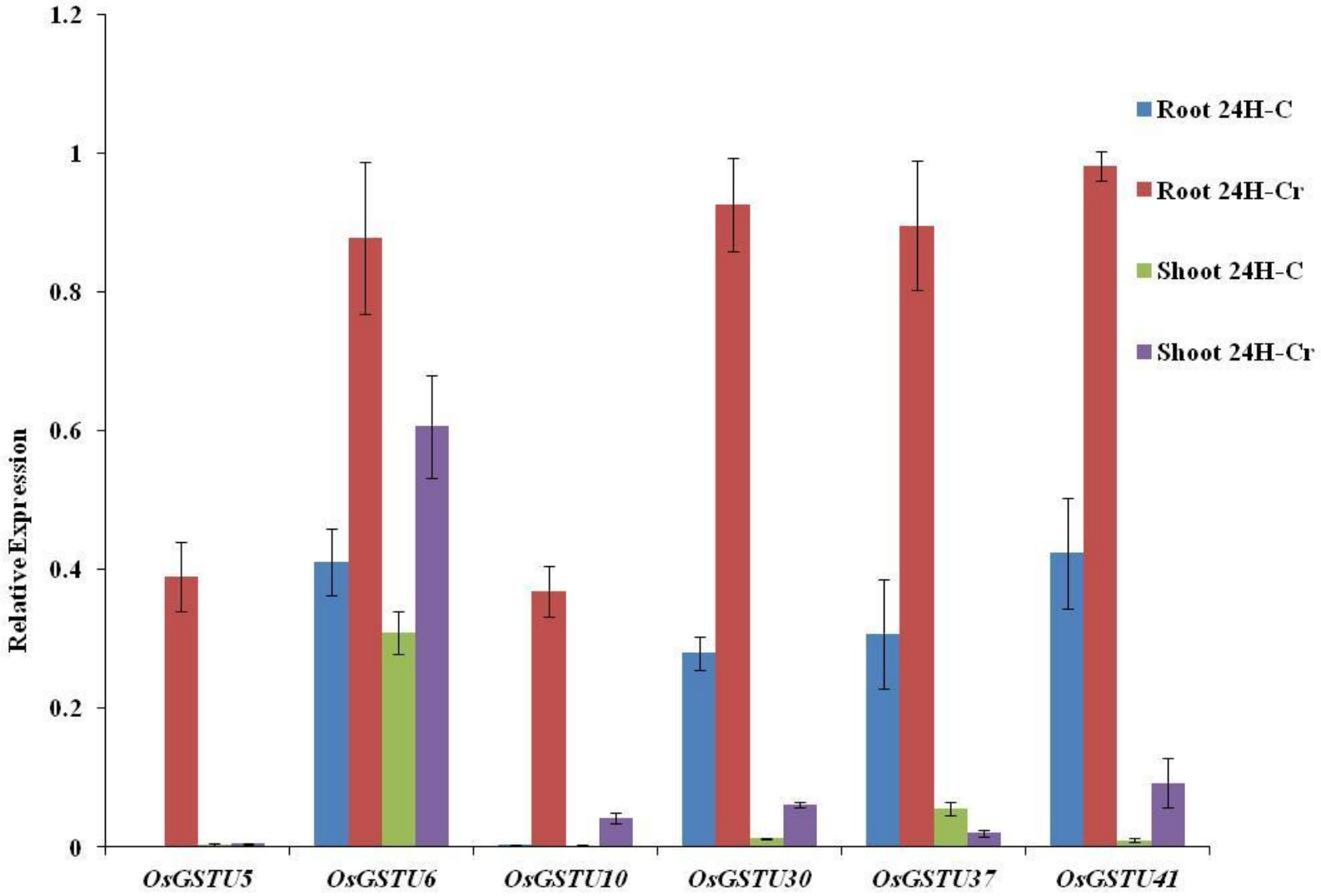

Figure 1

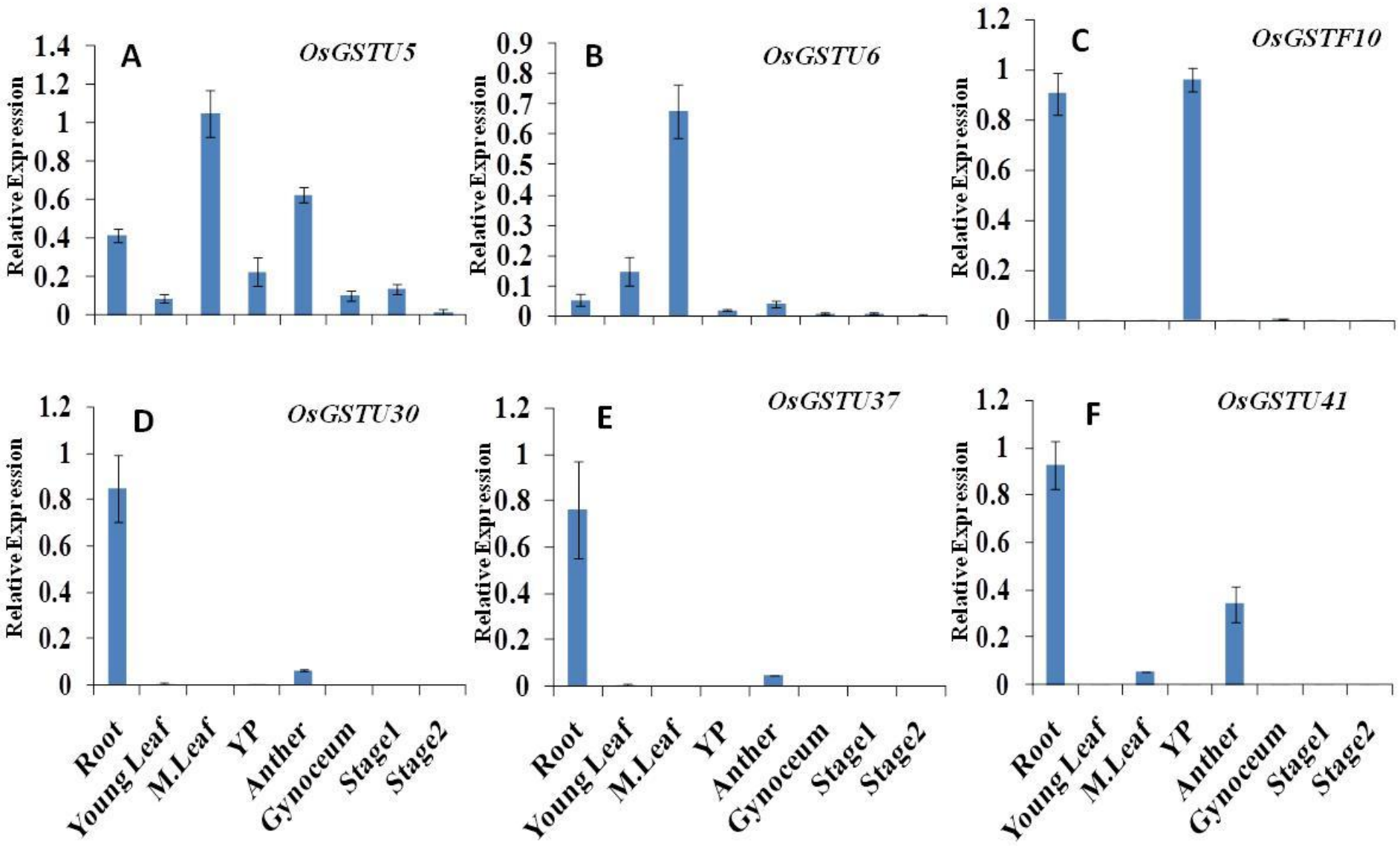

Figure 2

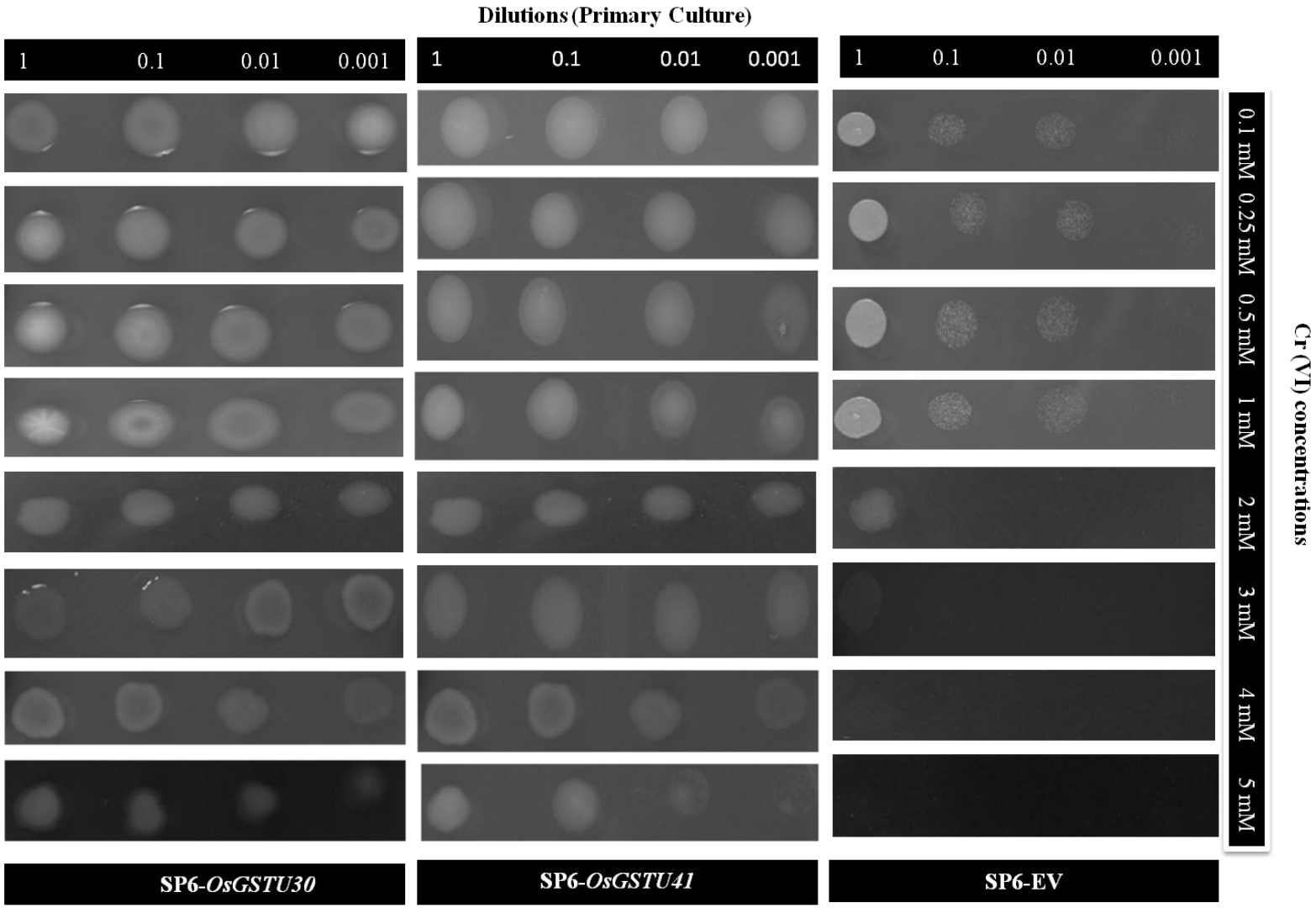

Figure 3

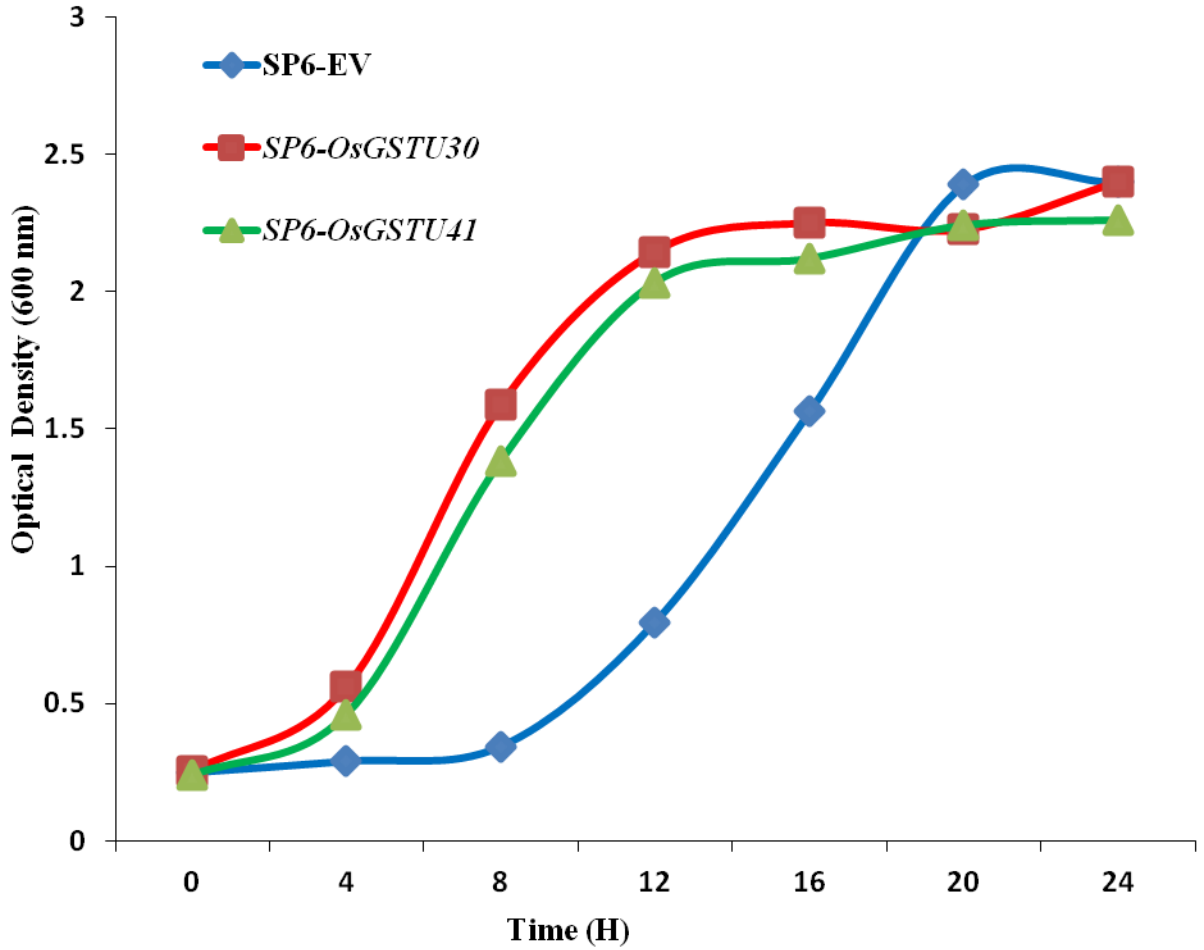

Figure 4

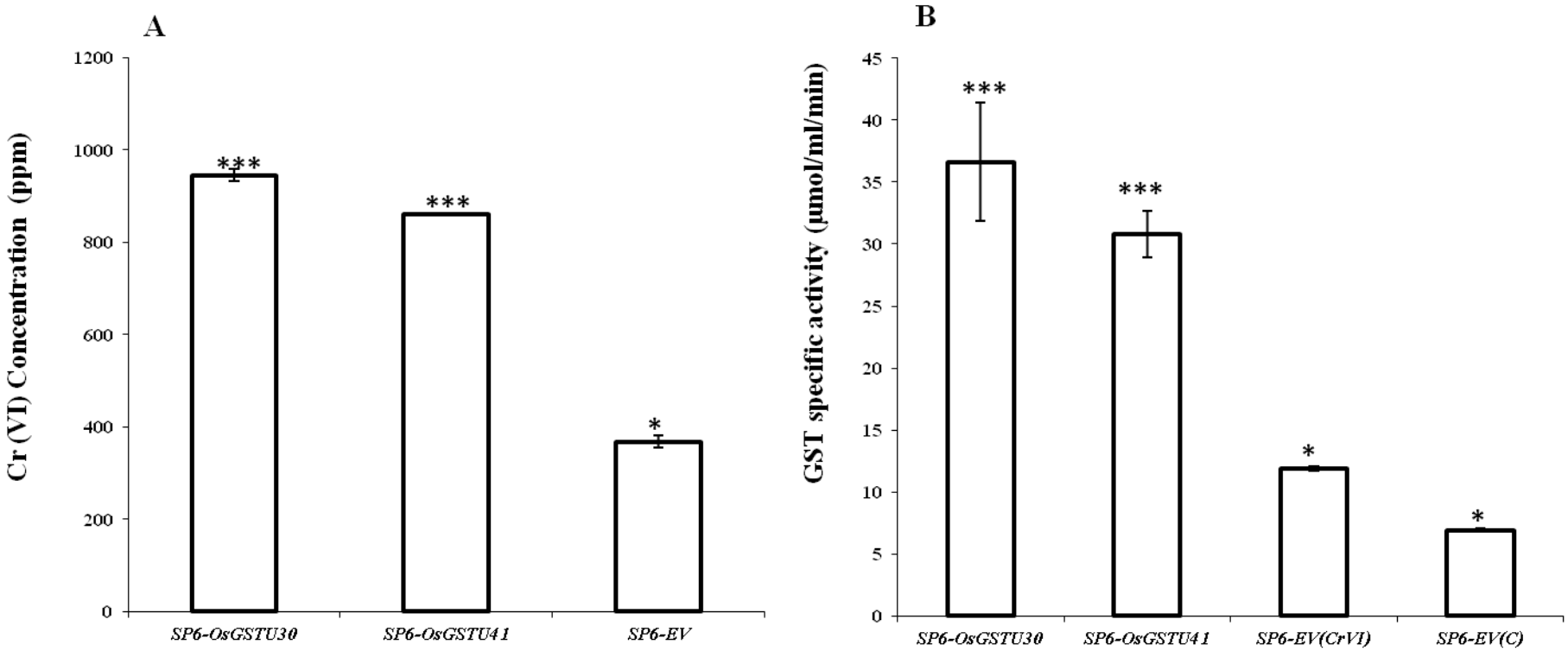

Figure 5

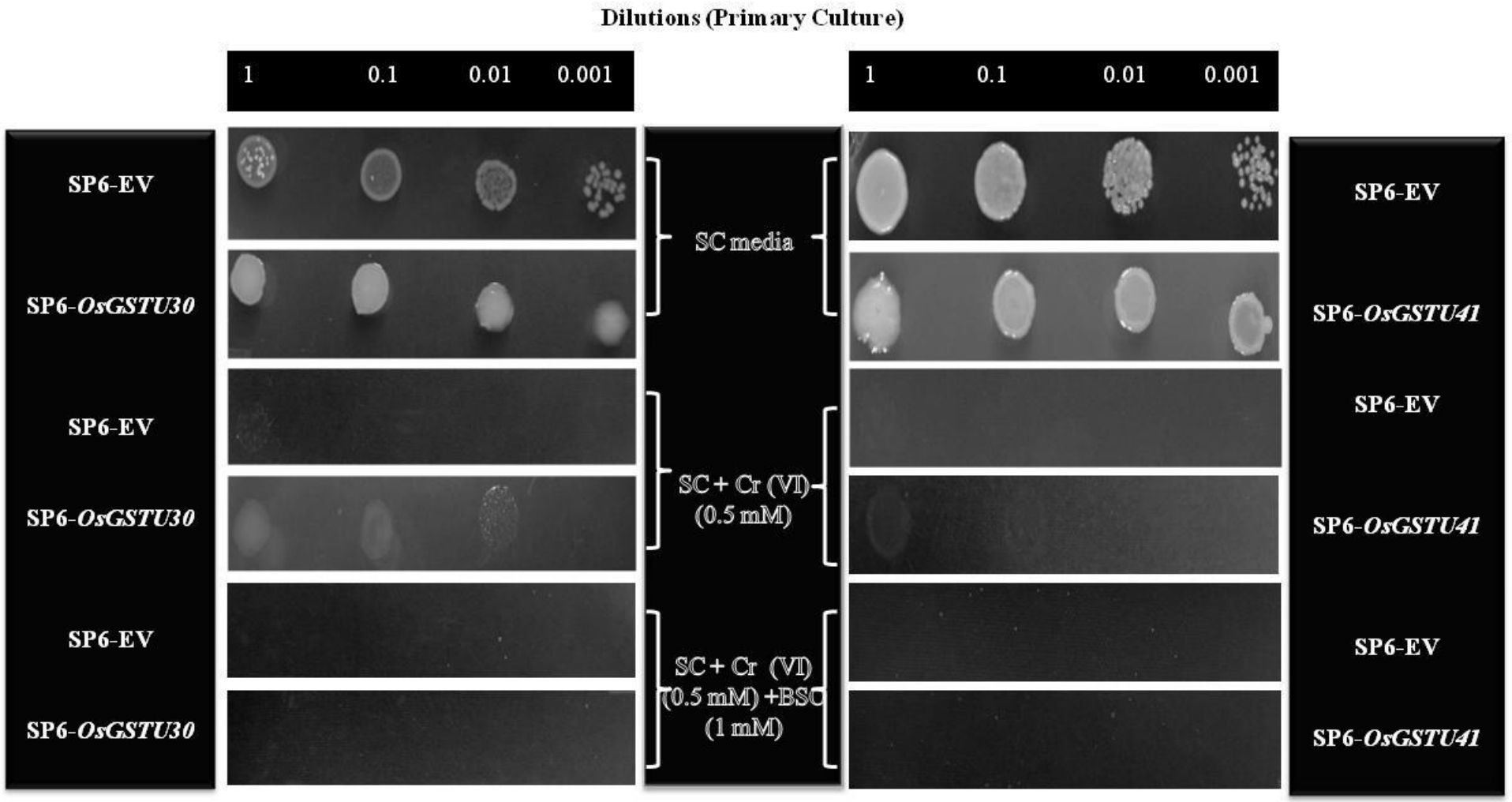

Figure 6