ARTICLE ADDENDUM

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OsSUV3 functions in cadmium and zinc stress tolerance in rice (*Oryza sativa* L. cv IR64)

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Abbreviations: AS, antisense; Cd, cadmium; Os, *Oryza sativa; SUV3,* suppressor of Var 3; VC, vector control; WT, wild-type; Zn, Zinc

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Protein of nuclear encoded *SUV3* (suppressor of Var 3) gene is a DNA and RNA helicase, localized in mitochondria and is a subunit of the degradosome complex involved in regulation of RNA surveillance and turnover. To overcome the abiotic stress-induced loss of crop yield, a multi-stress tolerant trait is required. Beside salinity stress the heavy metals including cadmium and zinc also affect the yield and quality of food crops. Since rice is a one of the staple food therefore it is important to develop a multi-stress including salinity and metal tolerant variety. Recently we have reported the role of *OsSUV3* in salinity stress tolerance in rice; however, its role in metal stress has not been studied so far. Here we report that in response to cadmium and zinc stress the *OsSUV3* transcript level is induced in rice and its overexpression in transgenic IR64 rice plants confers the metal stress tolerance. In addition to its previously reported role in salinity stress tolerance, this study further shows the role of *OsSUV3* helicase in cadmium and zinc stress tolerance suggesting its involvement in multi-stress tolerance.

Abiotic stresses (such as high salinity, drought and flood, high and low temperatures) remain the greatest constraint to crop production. It affects growth productivity and triggers a series of, biochemical and molecular changes in plants which appears in the form of morphological and physiological changes in crops. Worldwide, it has been estimated that approximately 70% of yield reduction is the direct result of abiotic stresses.[1] Heavy metals have also become a critical environmental concern due to their potential adverse ecological effects. The regulatory limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil.[2] This threshold is continuously exceeding because of several human activities. Plants exposed to high levels of Cd causes reduction in photosynthesis, water uptake, and nutrient uptake. Soil contaminated with zinc (Zn) may cause phytotoxicity when the concentrations of Zn found in contaminated soils exceed to those required as nutrients. High levels of Zn in soil inhibit many plant metabolic functions which resulted in retarded growth of both root and shoot.[3-5] Rice is the staple food for about 50 per cent of the world's population that resides in Asia, where 90 per cent of the world's rice is grown and consumed. In Asia, India has the largest area under rice (41.66 million ha) accounting for 29.4 per cent of the global rice area. Of the total harvested area, about 46 per cent is irrigated with 28 per cent rain fed lowland, 12 per cent rain fed upland and 14 per cent flood prone. Rice is one of the major traded commodities in the world with a total quantity traded touching 16.4 million tonnes. The southeast countries account for about 40 per cent of the rice trade in the world.[6] Abiotic stress causes reduction in productivity of rice crops. Genetic modification in rice could be one of the effective way to develop stress-tolerant cultivars. Molecular techniques involve the development of genetically engineered plants by the introduction and/or overexpression of selected genes which can grow in abiotic stress conditions. In

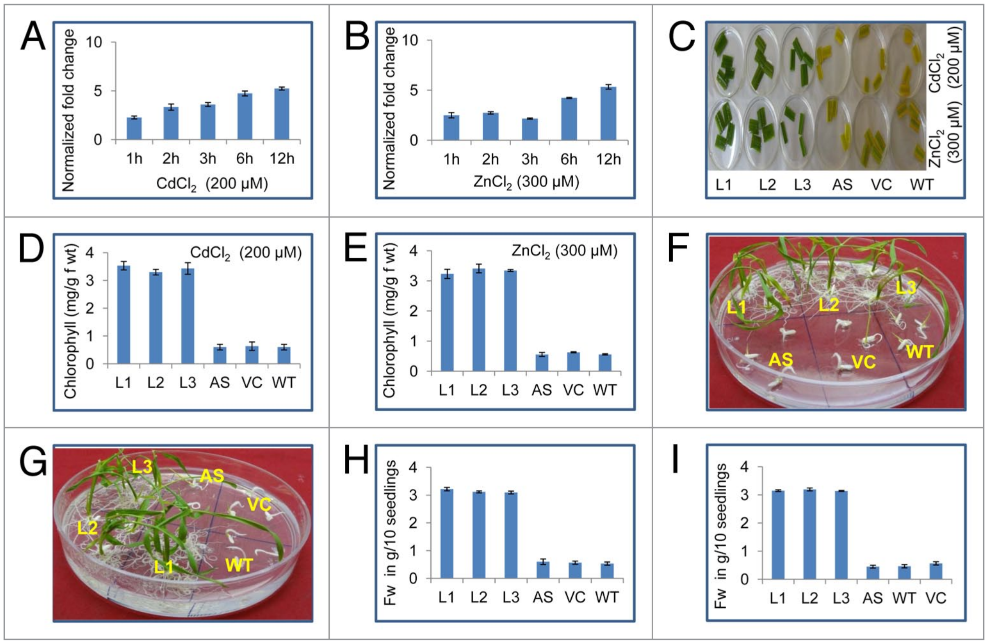

Figure 1. Response of *OsSUV3* transgenic plants to heavy metal stress. Quantitative real-time PCR analysis of Os*SUV3* under different abiotic stress conditions (**A**) 200 μM $CdCl_2$ (**B**) 300 μM $ZnCl_2$. Total RNA isolation was done from leaf samples collected at different time intervals (viz. One h, 2 h, 3 h, 6 h, 12 h). Bars indicate the standard error (± SE) calculated from three independent experiments. (**C**) Leaf disk senescence assay of *OsSUV3* transgenic rice plants along with antisense (AS), vector control (VC) and wild-type (WT) in presence of 200 μM $CdCl_2$ and 300 μM $ZnCl_2$. (**D**) Estimation of total chlorophyll content of *OsSUV3* transgenic plants along with AS, VC and WT plants after $CdCl_2$ stress. (**E**) Estimation of total chlorophyll content of *OsSUV3* transgenic plants after $ZnCl_2$ stress. (**F**) Germination test of T2 *SUV3* transgenic AS, VC and WT seeds in MS plate supplemented with 200 μM $CdCl_2$. (**G**) Germination of T2 *SUV3* transgenic seeds after 300 μM $ZnCl_2$. (**H**) Fresh weight per 10 seedlings from MS plate supplemented with 200 μM $CdCl_2$. (**I**) Fresh weight per 10 seedlings from MS plate supplemented with 300 μM $ZnCl_2$.

our previous study[7] we have isolated and characterized *OsSUV3* gene and reported that the encoded protein contains DNA and RNA helicase activity and functions in providing salinity stress tolerance by maintaining antioxidant machinery and enhancing photosynthesis in rice (*Oryza sativa* L. cv IR64). SUV3 helicase was initially reported in yeast (*Saccharomyces cerevisiae*) as a dominant suppressor allele. It plays an important role in the RNA surveillance system, regulates the stability of mature mRNAs, the removal of aberrantly formed mRNAs and the rapid degradation of non coding processing intermediates. In this study we report OsSUV3 provides first direct evidence of its function in imparting metal stress tolerance without reduction of biomass.

The 1.74 kb rice *SUV3* (*OsSUV3*) gene has been cloned (accession number: GQ982584) and IR64 rice transgenic plants overexpressing this gene in sense and antisense orientations have been raised as described earlier.[7] As an ideal control the rice transgenic plants with empty vector (pCAMBIA1301) were also raised as the method described earlier[7] and named as vector control (VC) plant. For isolation of total RNA for quantitative real-time PCR (qRT-PCR) the wild-type (WT) rice (*Oryza sativa* cv IR 64) plants were grown in vermiculite in standard green house condition for 21 d with regular supplementation of Hoagland solution. The plants were allowed to grow in metal stress like $CdCl_2$ (200 μM) and $ZnCl_2$ (300 μM). Leaf samples were harvested at different time (1h, 2h, 3h, 6h and 12h) intervals. Isolation of total RNA and the expression analysis of *OsSUV3* gene was performed by qRT-PCR according to method described earlier[8] using primers (forward 5′-CAG TTG AGA TGG CCG ACA-3′ and reverse 5′-CAG CTG GGT CAC CAC AAA-3′) and was normalized with α-tubulin primers (forward 5′-GGT GGA GGT GAT GAT GCT TT-3′ and reverse 5′-ACC ACG GGC AAA GTT GTT AG-3′). The qRT-PCR was repeated for three times independently for each time point. Relative gene expression was calculated using the 2-ΔΔCT values following Livak's method.[9] For metal stress tolerance the leaf disk (~1cm × 1cm) from T1 *SUV3* overexpressing transgenic rice (sense and antisense) and control plants (empty vector transformed [VC] and WT) were float in metal solutions (200 μM $CdCl_2$ and 300 μM $ZnCl_2$) for 72 h and total chlorophyll content were measured according to method described earlier.[10] For checking the post-germination growth in presence of metal stress the transgenic rice (*OsSUV3*) T2 seeds were grown on MS media containing predetermined concentration of $CdCl_2$ (200 μM) and $ZnCl_2$ (300 μM) in green house at 28 °C under 16 h light for 2 wk and their growths such as fresh biomass were observed.

A significant increase in the range of 2.4-fold to 6.3-fold was observed till 12 h in the expression level of *OsSUV3* under the exposure to 200 μM $CdCl_2$ treatment (**Fig. 1A**). In the presence of 300 μM $ZnCl_2$, the expression level was increased from 2.7-fold to 4.9-fold till 12 h. A slight decrease at 3 h was also observed (**Fig. 1B**). Leaf disks from T1 sense transgenic lines were found more tolerant to both the metals (200 μM $CdCl_2$ and 300 μM $ZnCl_2$) as compared with WT, VC and AS plants after 72h (**Fig. 1C**). Loss of chlorophyll was lesser in sense transgenic lines under the stress. Total chlorophyll content was significantly higher in transgenic (80%) as compared with WT, AS and VC plants after 200 μM $CdCl_2$ and 300 μM $ZnCl_2$ (**Fig. 1D and E**). Seeds of T2 sense transgenic plants (L1-L3) showed good post-germination growth under 200 μM $CdCl_2$ and 300 μM $ZnCl_2$ stress while WT, AS and VC seeds showed much lesser germination under same conditions (**Fig. 1F and G**). At 200 μM

$CdCl_2$ and 300 μM $ZnCl_2$ a statistically significant growth difference was observed in between the WT, antisense, VC and transgenic lines. Under exposure to 200 μM $CdCl_2$ and 300 μM $ZnCl_2$ the fresh weight of transgenic lines were significantly increased (more than 70%) as compared with WT, AS and VC plants (**Fig. 1H and I**).

The expression of *OsSUV3* transcript was upregulated in the presence of cadmium and zinc in rice seedlings. These observations suggest that *OsSUV3* is involved in maintaining the homeostasis of these ions. Earlier it has been shown that in response to NaCl the transcript level of *OsSUV3* gene was induced several folds.[7] Enhanced expression of *OsSUV3* with respect to the metals stress suggests that it might be regulated through Cd^{2+} and Zn^{2+} dependent signal transduction pathways. The physiological parameters like fresh biomass of the plant are frequently used as a parameter to monitor the effects of heavy metal stress. Reduction of total fresh weight arises due to altered physiological phenomena in the presence of toxic levels of cadmium and zinc.[11] Reduction of chlorophyll in response to cadmium and zinc exposure indicates growth retardation and weakening of pigment biosynthetic pathway. It has been reported that Cd^{2+} induces depletion of chlorophyll content in a variety of plants.[12,13] However, in this study transgenic rice plants overexpressing *OsSUV3* possess significantly higher chlorophyll under Cd^{2+} stress. Hence, it appears that the transgenic rice plants are in a better state with respect to these pigments. This might be due to the possibility of *OsSUV3* mediated Cd^{2+} ion efflux thereby maintaining the ion homeostasis for normal growth of exposed cells. Overall, this study indicates the novel role of *OsSUV3* in metal (cadmium and zinc) stress tolerance. Previously its role in salinity stress tolerance has been shown[7] and now in metal stress tolerance (this study), suggesting future potential for using *OsSUV3* in genetic approaches to improve plant performance and multi-stress tolerance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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