OsRuvBL1a DNA Helicase Boost Salinity and Drought Tolerance in Transgenic Indica Rice Raised by In-Planta Transformation

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Research Article

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Abstract

RuvBL, is a member of SF6 superfamily of helicases and is conserved among the various model systems. Recently rice homolog of RuvBL has been biochemically characterized for its ATPase and DNA helicase activities, however its involvement in stress is not been studied yet. This study reports the detailed functional characterization of RuvBL homolog of *Oryza sativa*, under abiotic stress through transgenic approach. An improved *Agrobacterium*-mediated *in planta* transformation method was developed in indica rice to generate the transgenic lines and study was focused on optimization of factors to achieve maximum transformation efficiency. Overexpressing *OsRuvBL1a* transgenics showed enhanced tolerance under *in vivo* salinity stress as compared to WT plants. The physiological and biochemical analysis of the *OsRuvBL1a* transgenic lines showed better performance under salinity and drought stresses. Several stress responsive interacting partners of OsRuvBL1a were identified using Y2H method. Working mechanism for boosting the stress tolerance by OsRuvBL1a has been proposed in this study. This integration of *OsRuvBL1a* gene in rice genome using *in planta* transformation method helped us to achieve the abiotic stress tolerant smart crop. This study is the first direct evidence to show the novel function of RuvBL in boosting abiotic stress tolerance in plants.

Key Message:

RuvB protein of helicase family has shown role in abiotic stress tolerance and for characterization rice transgenic plant were raised by newly developed in-vivo seed transformation protocol.

Introduction:

Global climate change and population explosion make it compulsory to utilize the full genetic potential of plants for feeding the world population (Edgerton 2009; Godfray et al. 2010; Passricha et al. 2014; Passricha et al. 2020). Abiotic stresses adversely affect the plants growth at various levels (Gao and Lan 2016; Passricha et al. 2019b). To overcome these adverse effects, stress tolerant varieties need to be developed. Genetic engineering has enlightened the path to use proteins, which are responsible for providing tolerance, and helicases such as MCM6, XPB2, PDH45, p68, OsSUV3 and OsBAT1 have been reported to impart abiotic stress tolerance (Dang et al. 2011; Liu et al. 2020; Passricha et al. 2019a; Shivakumara et al. 2017; Tuteja et al. 2014). Helicases exhibit ATPase activity and provide energy for unwinding the duplex nucleic acids (Abrahao et al. 2021; Lupas and Martin 2002) and thus are involved in various cellular functions (Nath et al. 2015; Saifi et al. 2019; Tuteja et al. 2015). Helicases are functionally coupled to the additional components of the macromolecular machines and participate in diverse biological processes. Helicases have 6 superfamilies, SF1 through SF6. Among these, SF6 comprises members of AAA+ (ATPases Associated with various cellular Activities) family, which exhibit nucleic acid unwinding activity (Abrahao et al. 2021; Saifi et al. 2018; Saifi et al. 2019; Singleton et al. 2007). Sequence analysis of AAA + superfamily shows its conservation among prokaryotes and eukaryotes. An important and highly conserved member of this AAA + superfamily is RuvB (Snider et al. 2008).
RuvB is a highly conserved protein involved in various cellular functions such as cell cycle regulation (Ahmad and Tuteja 2012), mitotic assembly (Morrison and Shen 2009; Sigala et al. 2005), transcriptional regulation of gene expression (Jonsson et al. 2001; Wood et al. 2000) and biogenesis and assembly of snoRNPs (small nucleolar ribonucleoproteins) for the generation of pre-rRNA (Zhao et al. 2008). It is a widely studied protein in other systems but is scarcely studied in plants with few reports on Arabidopsis (Holt et al. 2002; Schorova et al. 2019) and rice (Wang et al. 2011). In this study, we report the functional characterization of OsRuvBL1a, a rice homolog of RuvB1 which exhibits nucleic acid independent ATPase and nucleic acid unwinding activity (Saifi et al. 2018).

Over-expression of OsRuvBL1a encoding gene in rice plant has been used for the functional validation of the gene. Several methods have been reported for Agrobacterium-mediated transformation of the Indica rice such as tissue culture (Sahoo and Tuteja 2012), seed-piercing (Lin et al. 2009; Supartana et al. 2005), vacuum infiltration (Lin et al. 2009), floral-drip and floral-drop method (Ratanasut et al. 2017). All these methods have limitations of being laborious, having somaclonal variations, genotype-dependent, expensive and time-consuming which render indica rice transformation a tough process (Lin et al. 2009; Nishimura et al. 2005; Ratanasut et al. 2017). Some alternative fast and advantageous transformation method is needed to develop transgenics of rice (Dong et al. 2001). Callus regeneration method is the most widely used method for the rice transformation. However, the method is also beset with limitations such as, time consumption, somaclonal variations (Fukui 1983), genotype specificity and epigenetic changes (Smulders and de Klerk 2010) that reduce the rice transformation efficiency. This further reinforces the need for the development of a new transformation method (Hansen and Wright 1999).

In-planta seed transformation of rice is a new emerging and efficient method that overcomes the limitations of callus method (Bent 2000). In-planta rice transformation through seed was first attempted by Supartana et al. (2005) by piercing the pre-soaked seed embryo using Agrobacterium tumefaciens dipped needle (Supartana et al. 2005). Although this method opened the avenues for new improved way for rice transformation, however, the method need to be improvised to make it more prompt and easy to carry out. We, in the present study, have therefore attempted to improvise the in-planta transformation protocol with improved efficiency. Moreover, the improvised method is faster and easy to perform than the previously reported in-planta transformation methods (Lin et al. 2009; Ratanasut et al. 2017; Supartana et al. 2005). Further, the study involved the detailed analysis of OsRuvBL1a overexpressing transgenic lines produced using this new improved method. The recombinant OsRuvBL1a over-expressing lines seemed to be more tolerant to salinity and drought stress as surmised by reactive oxygen species (ROS) level, membrane stability and chlorophyll retention. Further, Yeast two-hybrid analysis was also carried out to predict the interacting partners of the OsRuvBL1a that may play a part in imparting the stress tolerance to rice.

**Experimental Procedure:**

*OsRuvBL1a gene selection*
Preliminary studies involving stress responsive expression level analysis was performed for OsRuvBL1a gene for the selection of candidate RuvBL gene from RuvBL gene family (Saifi et al. 2018).

*Generation of OsRuvBL1a overexpressing rice transgenics with in-planta transformation method*

(i) Plant material and seed sterilization

Healthy and mature seeds of IR64 variety of *Oryza sativa* were collected from IARI, New Delhi, India and dehusked seeds were sterilized as described by Sahoo and Tuteja (2012).

(ii) Preculture of seeds

Surface-sterilized seeds were precultured in 100 mL of ½ MS liquid medium with 0.5 mg L\(^{-1}\) of GA\(_3\) in a 250mL Erlenmeyer flask incubated in an orbital shaker (Kuhner, Switzerland) set at a constant temperature of 28°C for different time periods i.e., 0, 3, 12, 18, 24, and 48 hrs. Optimized concentration as surmised from the optical density of *Agrobacterium* at 600 nm (OD = 0.4) and co-cultivation duration (up to 24 hrs) is given in the supplementary tables, S2 and S3.

(iii) *Agrobacterium* strains and binary vector

Two strains of *Agrobacterium tumifaciens* (LBA4404 and EHA105) with gene cassette were used for rice seed transformation.

(iv) Preparation of *Agrobacterium* inoculum

*Agrobacterium* cells showing absorbance of 0.6-0.8 at 600 nm were harvested at 3500 rpm for 20 min at 4°C. The harvested bacterial cells were resuspended in co-cultivation medium (Table S7).

(v) Seed transformation

Precultured seeds were dipped in *Agrobacterium* inoculum for different durations (3, 12, 18 and 24 hrs) and kept on shaker (180rpm) at two different temperatures (23°C and 28°C) with different concentrations of acetosyringone (0, 40, 100, 200 and 400 mg/L).

(vi) Effect of hygromycin concentration on germination percentage

Germination percentage was calculated as the average number of seeds germinated over 5 to 10 days’ period. The influence of hygromycin was observed on seed germination (Figure S1).

(vii) Seed germination, selection and acclimatization

Agro-infected seeds were inoculated on ½ MS Plates, and incubated in the dark at 27°C. After 2 weeks, the germinated embryos with small shoots were transferred to jam bottles containing ½ MS media supplemented with hygromycin for selection and then transferred to vermiculite for hardening. Root
morphology was closely observed in jam bottles. Putative positive plants which survived on hygromycin were transferred to vermiculite.

**(viii) Screening of putative transformants**

(a) PCR analysis: The PCR reactions were carried out by using two primer combinations such as *hptII* specific primers (F’- CAACCTTTTATGAAAAAGCCTGAACCTCACCGC and R’- CCACCTAGTCTCGAGTCTATTTTTGCCTCGGGACGAG) and CaMV35S promoter forward and gene specific reverse (ACGGATCCCTCGAGATGAGGATCGAGGAGGAGGTGCAGTCGG).

(b) Histochemical analysis of *Gus* gene expression: Histochemical analysis of *Gus* gene expression was performed on leaf sheath from transgenic and WT leaf using the procedure described by Vitha *et al.* (1995).

**(ix) Transformation efficiency**

Transformation efficiency was calculated based on the presence of the transgene/s in transgenic plants as described by Vitha *et al.* (1995).

**(x) Optimization of co-cultivation media**

To attain the higher transformation efficiency, we studied the effect of different *Agrobacterium* concentrations (corresponding to the Optical density 0.3, 0.4, 0.5 and 0.6), pH (5.0-6.0) of media, surfactant (Tween 20 and Triton X-100) and growth regulator (GA$_3$). The influence of *Agrobacterium* strains (LBA4404 and EHA105) on the *in-planta* transformation was also studied.

*Copy number analysis*

Copy number analysis was performed by using two different approaches of qPCR assay and then its confirmation with Southern hybridization analysis as reported by Passricha *et al.* (2016).

*Analysis of transgenic plants*

(i) Expression analysis of *OsRuvBL1a* gene in transgenic plants

Transcript level of *OsRuvBL1a* gene in rice overexpressing transgenic lines was performed by qPCR method with WT plant as control and *Actin* as reference gene as described by Tuteja *et al.* (2013).

(ii) Transgene inheritance and homozygous line selection

Transgene inheritance in *OsRuvBL1a* overexpressing transgenic lines was analyzed by PCR and Gus method. Positive plants were analyzed for homozygous lines by using qPCR approach as described by Passricha *et al.* (2016) in T$_1$ generation (Passricha et al. 2016). List of primers used in this experiment is given in Table S8.
Physiological analysis of OsRuvBL1a transgenic plants

Different physiological analysis such as germination assay, root/shoot length, chlorophyll retention, dry weight and test grain weight were performed as described by (Garg et al. 2012) and Tuteja et al. (2013).

Biochemical analysis for salinity tolerance of transgenic plants

Biochemical analysis for transgenic lines and WT plants was performed as described by Garg et al. (2012) for salinity (200 mM NaCl) and drought (150 mM mannitol) stress.

In vivo stress tolerance

In vivo stress tolerance in OsRuvBL1a overexpression transgenic was analyzed as described by Tuteja et al. (2013) for 200 mM NaCl.

Qualitative and quantitative measurement of ions

Rice OsRuvBL1a overexpressing transgenic lines with WT control were grown for 15 days in normal tap water. The seedlings were then subjected to salinity stress by addition of 200 mM NaCl for 12 hrs duration. Crown roots of these plants (stressed and non-stressed) were used for the confocal imaging for Na\(^+\) and Ca\(^{2+}\) accumulation using the method described by Nath et al., (2016). The mean fluorescence pixel intensity (MFPI) was calculated using five independent measurements of the respective fluorescent confocal images using ImageJ program (Collins 2007).

Cell Viability

15 days old seedlings of WT and OsRuvBL1a transgenics were subjected to salinity (200 mM NaCl) and drought (150 mM mannitol) stress for 12 hrs duration. Crown roots of these seedlings were analyzed for cell viability by staining of roots using propidium iodide (3 µg/mL; Hi Media) for 1 min as described by Nath et al. (2016).

Identification of interacting partners

Interacting partners of OsRuvBL1a were identified by yeast two-hybrid (Y2H) assay. The ORF of OsRuvBL1a and another member of this family, OsRuvBL2a were cloned in frame in both the pGBK7 and pGAD7T vectors at Nde I and EcoR I restriction sites. Rice cDNA library was cloned in pGAD7T vector. The yeast two-hybrid assay was performed as described by Fields and Song (1989). Few interacting partners from Y2H results were validated with Bimolecular fluorescence complementation (BiFC) assay for one-to-one interaction as well as for authenticity of Y2H results. Selected interacting partners were cloned in pSPYNE vector and OsRuvBL1a was cloned in pSPYCE vector for BiFC. Abaxial surface of Nicotiana benthamiana leaves were co-transformed with Agrobacterium cells containing pSPYCE and pSPYNE constructs having OsRuvBL1a and selected interacting partners along with p19 RNAi suppressor gene construct as described by Passricha et al. 2019. Transformed plants incubated at 22°C for 2 days
and then screened with fluorescence microscopy for interactions. pSPYCE and pSPYNE empty vectors were used as negative control.

Agrobacterium cells containing each of the OsRuvBL1a-pSPYCE construct & LecRLK-SPYNE construct were inoculated in 5mL LB containing rifampicin, Kanamycin and Chloramphenicol. Agrobacterium containing p19 RNAi suppressor gene construct were similarly inoculated in 5mL LB media containing rifampicin, gentamycin, and kanamycin using a 2mL syringe, the Agrobacterium cocktail was injected into the abaxial surface of a leaf of Nicotiana benthamiana. Plants were then kept at 22°C and screened after 2 days using a fluorescence microscope (ZEISS AXIO Imager.Z2 microsystems from Germany).

**Statistical analysis**

Average 50 seeds, in three replications were used for each combination and data represented in Table S2 and S4 is the mean±SE calculated by using MSTAT computer program. Least significant difference (LSD) among the means at P>0.05 level of probability was considered as significant. For confocal study, data are presented as relative units of pixel intensities and the average fluorescence intensity was calculated via three independent measurements of confocal images using ImageJ software (NIH) in arbitrary units. In addition, respective back-ground pixel intensity (unstained area) of the confocal image was also considered to calculate the final mean fluorescence pixel intensity using ImageJ (NIH). For all graphs, data were analyzed by ANOVA (Analysis of Variance). Dunnett's multiple comparison test was used to test the significance between WT and transgenic plants by using GraphPad Prism software version 6.0. The statistical significance was represented as “*” and “**” for p<0.05 and p<0.01 respectively.

**Locus ID of OsRuvBL1a**: LOC_Os01g62040

**Results:**

**Gene Selection and characterization**

OsRuvBL1a gene has been selected based on our preliminary studies in which it was found to be an abiotic stress-responsive gene (Saifi et al. 2018) and thus, make it a potential candidate gene mediating abiotic stress tolerance in rice. OsRuvBL1a encodes for a 55 kDa protein (Fig. 1 a & b). Recently, it has been reported that purified recombinant protein showed nucleic acid independent ATPase (Fig. 1 c & d) and ATP dependent DNA unwinding activity (Fig. 1e) (Saifi et al. 2018).

**Development of in-planta transformation method for rice with mature seeds**

OsRuvBL1a overexpressing rice transgenics were produced by using improved *in-planta* transformation method, which is less labour-intensive, easy and fast, using the mature rice seeds as explant. This report briefly describes the OsRuvBL1a overexpression transgenic of rice by using this improved *in-planta* method and its optimization for various factors.
(i) Effect of *in-planta* transformation and hygromycin concentration on germination percentage

The hygromycin concentration of 20 mg/L was observed as lethal (Minimum Inhibitory Concentration, MIC) as more than 50% of seeds failed to germinate (Table S1). Later same concentration (20 mg/L) was used for selecting the putative transformed seedlings (Fig. 2a). Wild type control and non-transgenic seedlings died on hygromycin selection (Fig. 2ai and 2aiii) whereas putative transgenic plants survived and were selected (Fig. 2aii and 2aiv).

(ii) Optimization of acetosyringone concentration, co-cultivation duration and effect of different strains on *in-planta* transformation efficiency

Different concentrations of acetosyringone (0, 40, 100, 200 and 400 mg/L) were supplemented with *Agrobacterium* inoculum to study the effect of acetosyringone concentration on transformation efficiency of this method. Among the different concentrations and co-cultivation duration, *Agrobacterium* strain LBA4404 with 200 mg/L of acetosyringone incubated for 24 hrs showed maximum transformation of 32.7±0.9% after PCR with *hptII* gene specific primers and CaMV35S forward and gene specific reverse primers, respectively (Fig. 2bi and 2bii) and Gus screening (Fig. 2c) (Table S2). Similarly, for *Agrobacterium* strain EHA105 mediated transformation, the same concentration of acetosyringone (200 mg/L) resulted in maximum transformation of 36.0±2.0% when incubated for 24 hrs (Table S3).

(iii) Influence of pre-culture duration on *in-planta* transformation efficiency

The transformation efficiency was observed for different durations of pre-culture (0, 3, 12, 18, 24 and 48 hrs). A pre-culture period of 24 hrs was found to be the most suitable for T-DNA delivery, resulting in a significant increase in the transformation frequency from 32.7±0.9% to 41.33±3.5% for LBA4404 strain (Table S4).

(iv) Optimization of co-cultivation media for *in-planta* transformation method

1. *Agrobacterium* inoculum concentration: Highest percentage of transformed plants was observed when optical density (O.D.) of *Agrobacterium* inoculum was kept 0.4 at 600nm. Further increase in O.D. resulted in a decrease in transformation efficiency.
2. Optimization of pH: pH of co-cultivation medium is the authoritative divisor for the expression of *vir* genes and T-DNA transfer. In this study we obtained the highest efficiency at pH 5.8.
3. Surfactants: Best standard conditions (24 hrs incubation with 200 mg/L acetosyringone concentration) were chosen to test the effect of different surfactants on transformation efficiency. Two surfactants (Tween 20 and Triton X-100) were applied with three different concentrations (0.05, 0.1 and 0.2 %). The application of surfactants at higher concentration showed continuous decline in the transformation efficiency.
4. Growth Regulator: A growth regulator, GA$_3$ in co-cultivation medium, did not show any significant change in transformation efficiency.
This *in planta* method is strain-independent ([Table S5](#)) because both the strains of *Agrobacterium* (LBA4404 and EHA105) showed maximum transformation efficiency by 24 hrs co-cultivation. We have optimized that rice seeds pre-cultured for 24 hrs in MS medium with pH-5.8 and co-cultivated with *Agrobacterium* cells (at O.D. = 0.4) for 24 hrs in the presence of 200 mg/L acetosyringone showed maximum transformation efficiency. The transformed seeds were selected on 20 mg/L hygromycin supplemented MS medium to narrow down the selection process.

**Copy number**

Results of both the qPCR and Southern blot hybridization methods for copy number analysis of putative transgenic lines (L-1 to L-5) showed that L-1, L-3, L-4 and L-5 have single copy insertion whereas L-2 showed two copies of target gene in rice genome ([Fig. 2d and 2e](#)). Based on these results single copy lines L-1, L-3, L-4 and L-5 were used for further analysis.

**Expression analysis of OsRuvBL1a in transgenic plant**

Four transgenic plants (L-1, L-3, L-4 and L-5) overexpressing *OsRuvBL1a* gene were screened to identify the transgenic lines which showed higher expression for *OsRuvBL1a* gene by qPCR method. Out of 4 transgenic lines, transcript level quantification showed that line L-1, L-4 and L-5 have the highest expression for the transgene with around 13, 23 and 15 folds’ increase, respectively ([Fig. 2f](#)).

**Transgene inheritance**

The segregation pattern for T-DNA was tested as a single dominant Mendelian gene segregation method. About 45 seeds were taken from each line (L-1, L-4 and L-5) and the results were validated using the classical chi-square ($\chi^2$) test taking 1 as degree of freedom. According to $\chi^2$ test value T$_1$ population did not follow the Mendelian segregation pattern because average chi square value was much higher than table value (20.90) ([Table S6](#)). Gus histochemical assay was also performed in T$_1$ seeds and seedlings for the positive transgenics and it showed the formation of blue coloration in transgenic seeds and seedlings ([Fig. 2g](#)).

**Homozygous transgenic line**

The identification of homozygous lines was performed with qPCR in T$_1$ transgenic plants for the lines L-1, L-4 and L-5 for 14 plants. Out of 14 plants in the line L-1, four plants (namely, L1.1, L1.2, L1.6 and L1.10) possessed 2 copies ([Fig. 2h](#)). In the line L-4, three plants (namely, L4.5, L4.7 and L4.10) possessed 2 copies of *OsRuvBL1a* ([Fig. 2i](#)). Similarly, in the line L-5, 3 three plants (L5.2, L5.6 and L5.9) showed 2 copies for the transformed gene and hence considered as homozygous ([Fig. 2j](#)).

**Physiological analysis of OsRuvBL1a transgenic plants**

To examine the role of overexpression of *OsRuvBL1a* gene in conferring the abiotic stress tolerance in rice, comparative analysis of several physiological tests was performed in WT type and *OsRuvBL1a*
overexpressing transgenic lines (L1, L4 and L5) under salinity and drought stress conditions. Germination assay revealed that under stress-free control conditions there was no significant difference in germination pattern in WT as compared to OsRuvBL1a transgenic lines (Fig. 3a-d), however, when grown under salinity (200 mM NaCl) and drought (150 mM mannitol) stress the transgenic lines were germinated earlier than the corresponding WT. The seeds exposed to salinity stress had the germination percentage of ~60%, 75% and 55% (Fig. 3d); whereas, under drought stress the germination rate was 68%, 80% and 62% for lines L-1, L-4 and L-5, respectively (Fig. 3d). The WT seeds exhibited only ~15% germination under both the stress conditions (Fig. 3d). High root/shoot ratio and strong root architecture helps plant to overcome the salinity (Fig. 3e) and drought stress conditions (Fig. 3f). Transgenic lines (L-1, L-4 and L-5) showed root/shoot ratio of 1.2, 1.3 and 1.5, respectively under salinity stress (Fig. 3g) and 0.7, 0.8 and 0.8, respectively under drought stress (Fig. 3g). Whereas WT plants showed root/shoot ratio of 0.2 and 0.6 under salinity and drought stress (Fig. 3g). Higher dry weight of plant indicates lower water retention capacity. Lines L-1, L-4 and L-5 showed 1.7, 1.8 and 1.5-fold lesser dry weight, respectively than WT plants under the salinity stress conditions (Fig 3h). Under drought stress, all the three transgenic lines showed 1.8-fold lesser dry weight as compared to WT plants (Fig. 3h), whereas under the normal control conditions, transgenic lines and WT plants did not have significant difference in their respective dry weight (Fig. 3h). Stress conditions affected the photosynthetic machinery of plants and caused chlorophyll degradation (Fig. 3I). Total chlorophyll content measured in WT and OsRuvBL1a transgenic lines showed about 5-fold high chlorophyll content in all the three transgenic lines (L-1, L-4 and L-5) as compared to WT plants during salinity stress (Fig. 3j). Under drought stress, lines L-1 and L-5 showed about 2.5-fold higher retention of chlorophyll content as compared to WT plants, whereas L-4 line showed 1.5-fold higher chlorophyll retention (Fig. 3l).

**Biochemical analysis of OsRuvBL1a transgenic plants**

Abiotic stresses affect ROS machinery in plants and it was detected and quantified by some biochemical tests such as malondialdehyde (MDA) accumulation, proline, H$_2$O$_2$ content and cell membrane stability in T$_2$ generation (Fig. 4a-l). Generation of H$_2$O$_2$ and MDA in a cell is the marker of negative effects of stress since these molecules are result of cell degradation. This study showed significantly less H$_2$O$_2$ and MDA accumulation in transgenic line L-4 under both the salinity (Fig. 4a & b) as well as the drought stress (Fig. 4 g & h) as compared to the corresponding WT plants. To overcome the water deficit condition imposed by the salinity and drought stress, plants are known to accumulate osmolytes such as proline. OsRuvBL1a transgenic lines showed higher level of proline content as compared to WT with line L-4 showing maximum accumulation of proline under both salinity (Fig. 4c) and drought stresses (Fig. 4i). All transgenic lines showed higher electrolyte retention as compared to the WT plants with line L-4 showing maximum electrolyte retention under salinity stress (Fig. 4d) and line L-1 showed maximum electrolyte retention under drought stress (Fig. 4j). Overall these observations suggest the reduced effect of stress and damage to cell membrane in transgenic lines and confirm the role of OsRuvBL1a in maintenance of the cell membrane stability under the abiotic stresses. The activity of ROS scavenging enzymes-ascorbate peroxidase (APX) and catalase were also estimated. Both the enzymes APX and catalase use
H$_2$O$_2$ as substrate to reduce it into water. Under salinity stress condition transgenic line L-4 showed higher APX and catalase activities as compared to WT plants (Fig. 4 e & f). Under drought stress the transgenic line L-4 showed maximum activity of APX and catalase (Fig. 4 k & l). These biochemical analyses showed improved ROS scavenging machinery and maintenance of cell integrity in transgenic lines as compared to WT plant under stress conditions.

**Determination of ion content and cell death in response to abiotic stresses**

The quantification of ions accumulation and cell death in plant tissue is another method to observe the effect of stress conditions on plants. In this study, we measured the effect of salinity stress and drought stress on rice seedlings. Sodium ion (Na$^+$) accumulation in root tissue during salinity stress was studied as a method for quantification of Na$^+$ ion imbalance in plants during stress conditions. CoroNa green dye was used for the non-destructive monitoring of relative accumulation of Na$^+$ ion in roots of WT and OsRuvBL1a overexpressing transgenic lines by using the confocal microscopy. The transgenic line L-5 showed the least fluorescence and lines L-1 and L-4 also showed lesser fluorescence as compared to WT roots under salinity stress (Fig. 5 a & b). These results suggest that more Na$^+$ ion accumulation occurred inside WT roots during salinity stress as compared to the transgenic lines. For the study of calcium ion (Ca$^{2+}$) accumulation an esterified form of Fluo-4, Fluo4-AM was used. Fluo4-AM is a Ca$^{2+}$ sensitive fluorescent probe indicator which shows increase in fluorescence upon binding with cytosolic Ca$^{2+}$. Confocal microscopy of WT and transgenic roots under salinity stress was used to observe the cytosolic Ca$^{2+}$ accumulation. Higher fluorescence in WT roots as compared to transgenic line L-1 suggested an accumulation of more cytosolic Ca$^{2+}$ in WT roots under stress conditions (Fig. 5 c & d). Stress conditions also cause ionic imbalance and oxidative stress that make the cells inviable. Cell viability can be studied in plant root in non-destructive manner by using propidium iodide (PI) dye. PI is a membrane impermeant dye and hence viable cells show less or no fluorescence whereas it penetrates in dead cells and intercalates in double stranded DNA and provides florescence on excitation. The cell viability study of rice root tissue under salinity and drought conditions showed higher fluorescence in roots of WT plants as compared to transgenic lines under salinity stress (Fig. 5 e & f) and drought stress (Fig. 5 g & h). These results showed that stress conditions caused higher cell death in WT plants as compared to overexpressing transgenic lines. These studies suggest the role of OsRuvBL1a in providing stress tolerance under stress conditions at ionic and cell viability level.

**Salinity and drought stress tolerance under in vivo conditions**

WT and the transgenic plants (L-1, L-4 and L-5) overexpressing OsRuvBL1a gene showed significantly different behaviors under salinity and drought stress (Fig. 6). At day-1, all the plants of same age were exposed to salinity stress (200 mM NaCl) as well as drought stress (non-availability of water) as shown in Fig. 6 a & c. On day 20 salinity stress, the WT plant could not survive salinity stress till 20 days, on the other hand, the OsRuvBL1a transgenic plants withstood the stressed condition (Fig. 6b). Among the
transgenics, the L-4 line was found to be the most tolerant line. For drought stress, WT plant died after 15 days while OsRuvBL1a-overexpressing transgenic plants were thriving (Fig. 6d).

**Isolation and identification of interacting partners of OsRuvBL1a through yeast two-hybrid method**

Physiological and biochemical analysis of overexpressing transgenic lines showed better performance as compared to WT under salinity and drought stress. To understand the working mechanism of OsRuvBL1a, its interacting partners were identified by using yeast two-hybrid method. Sequential selection of transformed yeast on two drop outs (2-DO) (-Leu, -Trp), 3-DO (-His, -Leu, -Trp) and 4-DO (-Ade, -His, -Leu, -Trp) media plates followed by filter lift assay showed positive interacting clones. The colonies found positive on X-gal assay were sequenced and analyzed using rice genome to find the interacting partners of OsRuvBL1a enlisted as Table 1. Y2H (one-to-one interaction) as well as BiFC studies showed that OsRuvBL1a does not self-interact whereas, it exhibits strong interaction with another member of its family, OsRuvBL2a. The OsRuvBL1a-OsRuvBL2a interaction led to the formation of a hetero-oligomeric structure by these proteins. BiFC based one-to-one interaction study of OsRuvBL1a with few selected partners showed positive interactions and validate the Y2H results (Figure S2). These validated interacting partners may have some direct or indirect role in conferring the stress tolerance to the plants.
| Class                  | Locus ID            | Gene Name                                      | Putative Function                                      |
|-----------------------|---------------------|------------------------------------------------|--------------------------------------------------------|
| **Hormonal Signaling**| LOC_Os08g44510      | UDP-N-acetylglucosamine–peptide N-acetylglucos-aminyltransferase | Negative regulator of GA signaling                      |
|                       | LOC_Os10g31770      | START domain containing protein                | ABA signaling, Biotic and Abiotic stress tolerance       |
|                       | LOC_Os01g13030      | OsIAA3 - Auxin-responsive Aux/IAA gene family member | Auxin signaling                                         |
|                       | LOC_Os07g28480      | glutathione S-transferase                      | Auxin and oxidative stress-signaling                    |
|                       | LOC_Os03g20790      | ethylene-insensitive 3                         | Me-JA and SA signaling                                  |
|                       | LOC_Os12g43600      | RNA recognition motif containing protein        | Response to stress and RNA silencing and splicing       |
| **Gene Regulation**   | LOC_Os03g60080      | NAC domain-containing protein 67               | Stress-responsive transcription factor                   |
|                       | LOC_Os06g39700      | DNA-directed RNA polymerase subunit alpha       | Transcription                                           |
|                       | LOC_Os01g63980      | ZOS1-17 - C2H2 zinc finger protein              | Transcription                                           |
|                       | LOC_Os01g54100      | CK1_CaseinKinase_1a.2                          | Transcription elongation                                |
|                       | LOC_Os05g41110      | Ribosomal protein L7Ae                         | Translation                                             |
|                       | LOC_Os01g24690      | 60S ribosomal protein L23A                      | Translation                                             |
|                       | LOC_Os07g40580      | eukaryotic translation initiation factor 5A     | Translation                                             |
|                       | LOC_Os06g43210      | zinc finger, C3HC4 type domain containing protein | Ubiquitination pathway                                 |
|                       | LOC_Os01g62230      | Core histone H2A/H2B/H3/H4 domain containing protein | Chromatin remodeling                                   |
|                       | LOC_Os06g45390      | Expressed protein                              | RNA binding, post transcriptional gene regulation and translation |
| Class                          | Locus ID          | Gene Name                          | Putative Function                                                                 |
|-------------------------------|-------------------|------------------------------------|----------------------------------------------------------------------------------|
| LOC_Os01g50100                | ABC transporter, ATP-binding protein | Multiple drug resistance, Telomerase reverse transcriptase activity, Activation of double strand break machinery |
| LOC_Os05g50370                | DnaJ domain containing protein | Chaperon and protein transportation by vesicle coat proteins                      |
| LOC_Os06g12580                | Pro-resilin precursor | Chaperon                           |
| LOC_Os11g47970                | AAA-type ATPase family protein | Rubisco Activase, A chaperon for Rubisco under Heat stress                       |
| LOC_Os07g05810                | Glycine-rich protein | Peroxisome biogenesis, transport mechanism                                      |
| LOC_Os12g19381                | Ribulose bisphosphate carboxylase small chain | Involved in CO₂ metabolism         |
| LOC_Os08g15170                | ATP synthase epsilon chain | ATP synthesis                       |
| LOC_Os08g03290                | glyceraldehyde-3-phosphate dehydrogenase | glycolytic pathway, response to ER stress, heat, H₂O₂, oxidative, salt stress, sucrose and temperature stimuli |
| LOC_Os08g19980                | NBS-LRR disease resistance protein | R-gene involved in biotic stress regulation                                       |
| LOC_Os03g28940                | ZIM domain containing protein | Jasmonate Signalling                |
| LOC_Os07g48500                | Stress-responsive protein | Stress-responsive and ROS scavenging                                              |

**Discussion:**

In a recent study we have reported that OsRuvBL1a exhibits nucleic acid independent ATPase and DNA unwinding activity (Saifi et al. 2018). ATPase activity in other systems has been reported in the presence of ssDNA and dsDNA (Ahmad and Tuteja 2012; Gribun et al. 2008; Kanemaki et al. 1997) but nucleic acid independent activity has not been reported yet. To functionally validate the OsRuvBL1a gene under stress conditions, overexpressing transgenic lines were generated with the help of a newly developed Agrobacterium-mediated in-planta transformation method.

Many in-planta transformation methods are already in use for rice (Ratanasut et al. 2017; Supartana et al. 2005), brinjal (Subramanyam et al. 2013), maize (Hiei et al. 2006) and field bean (Keshamma et al. 2011). The in-planta rice transformation protocol used in present study was improvised to reduce labor
and time of the transformation protocol that too with an improved transformation efficiency. The optimization of transformation efficiency is primary requirement for a transformation protocol of choice. The transformation efficiency is a factor of media composition, incubation period, acetosyringone concentration and seed treatments (Mayavan et al. 2013). Our method is based on the principle that transformation of meristematic cells at an early stage makes the inheritance stable from the parent generation to the next generation (Sajib et al. 2008). In this method, rice seeds were allowed to germinate in MS liquid medium with Agrobacterium to facilitate the integration of transgene during germination, as meristematic cells are rapidly dividing and highly receptive at this stage (Gordon-Kamm et al. 2002; Villemont et al. 1997). Similar increase in transformation efficiency has also been reported in several crops including wheat, tomato and barley (Rai et al. 2011).

A number of factors such as medium characteristics (like pH, sugar concentration, salt concentration), bacterial suspension, acetosyringone amount and growth hormones influence the transformation efficiency of an in planta transformation method. The negligible effect of different strains of A. tumefaciens strain (LBA4404 and EHA105) on germination percentage of IR64 variety seeds also supports the hypothesis. MIC of hygromycin provides add-on tools for selecting the transgenic plants (Tuteja et al. 2012). The pH of the medium is an important determinant for efficient growth of Agrobacterium cells and transformation of SAM cells. Media set at pH 5.8 worked best for the protocol used in the study. Fierer and Jackson (2006) also reported that acidic pH is optimum for bacterial growth. Agrobacterium cell concentration is another critical component to be optimized for maximum transformation efficiency. An O.D. of 0.4 at 600 nm was found to be the optimum concentration for Agrobacterium growth, since it contained highly active cells in their log phase of development (McCormac et al. 1998; Zhou et al. 2003). Another major component of co-cultivation media is acetosyringone that provides chemotaxis to the Agrobacterium and activates the vir genes present on the helper plasmid (Kumlehn et al. 2006; Winans et al. 1988). In this study, concentration of acetosyringone was optimized by performing the in planta seed transformation with various concentrations of acetosyringone and 200 mg/L acetosyringone was found to be the optimum concentration after 24 hrs of co-cultivation. High concentration of acetosyringone showed a reduction in total seed germination percentage which ultimately reduced the transformation efficiency probably because of the supra-optimal concentration of non-polar solvents (alcohol/DMSO) (Antony et al. 2015). Few reports claimed the influence of growth regulators such as gibberellic acid on the transformation efficiency by breaking the dormancy of rice seeds (Vieira et al. 2002), although in this study the concentration of GA3 used did not make any significant change. The addition of surfactant in the inoculation media increases the transformation efficiency by reducing the surface tension of water and facilitated the attachment of Agrobacterium cells to the surface of explant as well as increased the time of attachment by eliminating the inhibitory substances (Huang and Wei 2005). However, we observed lower transformation efficiency in the presence of surfactants. Similar results for surfactant have been reported by Hosein et al. (2012) (Hosein et al. 2012). Pre-culture medium increases the number of transformation event by promoting cell division, because actively dividing cells are amenable to T-DNA delivery and integration (Sangwan et al. 1991). In addition, pre-culture also prevents the negative effect of Agrobacteria and selection agent in the seed
transformation. Similarly, previous reports also claimed an improvement of transformation efficiency due to pre-culturing (Mariashibu et al. 2013). After optimization of several factors, we conclude that this newly developed *in-planta* transformation method is better than previous methods because it is less labor-intensive, less time-consuming, strain-independent and cost-effective as well.

Salinity and drought inhibit seed germination and cause retarded root/shoot growth due to the osmotic imbalance in plant cells (Khan and Hemalatha 2016; Munns 2002; Passricha et al. 2019b; Ra et al. 2016). The transgenic plants overexpressing *OsRuvBL1a* showed higher seed germination, longer primary roots and high root/shoot ratio that overcame the osmotic imbalance. The transgenic lines showed higher chlorophyll content, cell membrane stability and higher water retention capacity as compared to the WT plants under stress condition. These improved features assure the survival of plants under stressed condition as reduction in chlorophyll degradation (Mao et al. 2012), maintenance of membrane integrity and electrolyte balance in cell is a necessity to withstand the stress conditions.

Similarly, overexpressing *OsRuvBL1a* transgenic plants showed lesser accumulation of ROS as compared to WT plants under salinity and drought stress, suggesting the role of *OsRuvBL1a* in regulation of ROS scavenging pathway. H$_2$O$_2$ is one of the major products of ROS and its higher accumulation in plants showed the negative effects of stress on plants (Gill and Tuteja 2010; Huda et al. 2013; Yadav et al. 2018). *OsRuvBL1a* transgenic plants showed lower accumulation of H$_2$O$_2$ in stress conditions and it is in agreement with the previously reported studies that states lesser accumulation of ROS helps the plants in stress tolerance (Huda et al. 2013; Tuteja et al. 2013). In addition, the transgenic plants showed higher activity of enzymes such as catalase and ascorbate peroxidase which are key players in regulation of ROS level in cell (Gill and Tuteja 2010) and their high activity in transgenic plants supports the low level of H$_2$O$_2$ of these plants even under stress conditions. This study also showed a low level of MDA formation due to the less lipid peroxidation which provides membrane stability (Huda et al. 2013; Wong-Ekkabut et al. 2007) and high level of proline accumulation. Proline is an osmolyte that helps the cells to increase its water retention capacity under physical and physiological stresses (Garg et al. 2012; Tu et al. 2014).

Salinity stress creates ionic imbalance in the cells, thereby, leading to the cell death. The measurements of intracellular ion concentration in transgenic plants revealed that the *OsRuvBL1a* encoding gene is involved in lowering of the accumulation of sodium ions inside cells as compared with the corresponding wild types grown in identical conditions. Lower sodium ion accumulation in the salinity tolerant plants as compared to susceptible plants has also been reported earlier studies also (Kavitha et al. 2012; Nath et al. 2016; Passricha et al. 2020; Zhang et al. 2011). As a stress tolerance mechanism the plants are known to sequester excess Na$^+$ ions inside the intracellular compartments to protect the essential organelles and molecules from their deleterious effects (Blumwald 2000; Flowers and Colmer 2008).

Ca$^{2+}$ ion is another important ion that plays an important role in regulating cell physiology as a messenger of numerous cell signalling pathways. Our results revealed a higher concentration of Ca$^{2+}$ ions in WT roots than the transgenic lines grown in identical conditions. These results can be correlated
with higher rate of cell deaths in WT plants grown under stress conditions. These results further reinforce that OsRuvBL1a is involved in regulation of many cellular activities since the Ca^{2+} ion flux has been reported to initiate onset of various cellular pathways, which initially protect cells from stress but eventually lead to cell death under continuous stress conditions (Lin et al. 2008).

Hybridization studies revealed that OsRuvBL1a interacts with OsRuvBL2a, which is another member of the RuvB family, however no evidence of inter-molecular interaction is found between two OsRuvBL1a molecules. RuvB1 and RuvB2 interactions are also been reported earlier that results in the formation of heterohexameric ring structure (Abrahao et al. 2021; Cheung et al. 2010; Niewiarowski et al. 2010; Schorova et al. 2019). Formation of a complex structure as a result of OsRuvBL1a and OsRuvBL2a interaction needs to be looked upon by structural studies.

The interacting partners of OsRuvBL1a that might play some direct or indirect role in the stress tolerance have been listed in Table 1. Based upon the results of the dihybrid analysis, a hypothetical model has been proposed (Fig. 7), which highlights the possible importance of OsRuvBL1a in imparting the stress tolerance to the rice crop. Reactive oxygen species (ROS) and lipid peroxidase are efficient markers of stress (Sewelam et al. 2016). The genes involved in the ROS scavenging pathway are known to be controlled by OsRuvBL1a via hormonal regulation as surmised by the interaction of the OsRuvBL1a with SPINDLY (Furstenberg-Hagg et al. 2013; Shimada et al. 2006), START (Hubbard et al. 2010; Klingler et al. 2010), eIF5a (Wang et al. 2012) OsI/A3 and ZIM domain containing proteins (Ishiga et al. 2013; Wang et al. 2017).

OsRuvBL1a directly interacts with stress responsive transcription factor such as NAC domain containing protein (Huang et al. 2016; Rahman et al. 2016) and EIN3 is involved in the expression of stress-responsive genes to cope with the abiotic as well as biotic stress through MeJA-induced leaf senescence (Cho and Yoo 2014; Zhang et al. 2016). OsRuvBL1a has also been shown to interact with core histone domain containing protein H2A/H2B/H3/H4 which are part of the of nucleosome core. In addition, the OsRuvBL1a also interacts with the C3HC4 zinc finger protein, which is known to be involved in chromatin remodeling by monoubiquitination of histone H2B (Liu et al. 2007), for accessibility of DNA-to-DNA damage repair machinery for repair of any damage caused due to ROS and lipid peroxidation. Earlier studies in other systems such as yeast and human have already shown RuvB1 as a component of different chromatin remodelling complexes (Gorynia et al. 2011; Jeganathan et al. 2015; Jin et al. 2005). However, no such study has been carried out in the plant system, and our study may provide a lead to further explore the role of OsRuvBL1a as a part of some chromatin remodelling complex and thereby effectuating the stress tolerance in rice.

OsRuvBL1a directly interacts with R-gene NBS-LRR in NBS-LRR-Avr complex to provide biotic stress tolerance by facilitating the expression of R-gene responsive genes involved in hypersensitive response (DeYoung and Innes 2006; Marone et al. 2013; McHale et al. 2006; Song et al. 2017). OsRuvBL1a directly interacts with stress-responsive proteins such as GST (Glutathione-S-Transferase) involved in xenobiotic and peroxide detoxification (Dixon et al. 2009; Raza 2011), glyceraldehyde-3-phosphate dehydrogenase
response to various abiotic stimuli like ER stress, temperature, H$_2$O$_2$, oxidative stress, redox state, salt stress and sucrose stimulus (Kappachery et al. 2014), glycine-rich protein (Ortega-Amaro et al. 2014; Yao et al. 2016) and chaperon proteins such as dnaJ (Wang et al. 2015; Zhao et al. 2010) and pro/resilin precursor protein (Vitamvas et al. 2012; Yang et al. 2016). OsRuvBL1a also helps in the improvement of photosynthetic efficiency by interacting with Rubisco activase that is involved in the removal of inhibitory sugars from Rubisco under normal cellular conditions and also acts as a chaperon during heat stress (Chen et al. 2015; Keown et al. 2013; Kumar et al. 2016).

This study focused on the detailed characterization of rice homolog of RuvBL1 (OsRuvBL1a). OsRuvBL1a exhibits ATPase and helicase activities that could be essential to perform cellular functions. Transgenic Rice plants over-expressing OsRuvBL1a encoding gene were developed for its functional validation through an efficient _Agrobacterium_-mediated _in-planta_ transformation method. This study expounds a highly efficient method for rice transformation. OsRuvBL1a interacts with different proteins which directly or indirectly are involved in providing stress tolerance to the plant as shown in working mechanism (Fig. 7). Multiple physiological and biochemical analysis showed the better performance of OsRuvBL1a overexpressing transgenic lines under salinity and drought stress as compared to WT plants. This is the first report that details the role of RuvBL1 in plants and the information of its characteristics, highlighted through the present study, lays the foundation for the potential use of this enzyme in crop improvement. Furthermore, its role in mitigating the negative effects of abiotic stress in plants indicate its potential use in engineering plants for overcoming abiotic stress.

**Declarations:**

**Conflict of interest**

We do not have any conflict of interest to declare.

**Author Contribution Statement**

SKS and NP conducted the experiment, NT and MN designed the study and SSG help in writing the manuscript. RT and NT thoroughly reviewed the manuscript.

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Figures
Figure 1

Biochemical characterization of OsRuvBL1 (a) SDS-PAGE of purified OsRuvBL1a. (b) Western blot analysis of purified OsRuvBL1a (Lane 1-purified protein and M-protein marker). (c) ATPase assay of OsRuvBL1a in absence of nucleic acid (C-control). (d) ATPase assay of OsRuvBL1a in presence of ssDNA (C-control). (e) Helicase activity of OsRuvBL1a (Lanes C-negative control without protein, B-boiled substrate).
Figure 2

In-planta rice transformation for overexpression of OsRuvBL1a (a) Putative transgenic plants showed survival on hygromycin supplemented media and WT plant showed death (i and iii) and transgenic lines survived (ii and iv) under hygromycin selection. (b) PCR selection of transgenic plants (i) with hygromycin F’ and R’ primers showing 1 kb amplicon (ii) with CaMV35S F’ and OsRuvBL1a R’ primers showing ~1.4 kb amplicon. (c) GUS analysis of transgenic plants at T0 stage (i) WT plant as control and (ii-iv) transgenic plants showed positive GUS assay. (d) Copy number analysis with real-time method showed lines L-1, 3, 4 and 5 with single site insertion and L-2 with double insertion site. (e) Southern blot hybridization showed lines L-1, 3, 4 and 5 with single site insertion. (f) qPCR based transcript level analysis in OsRuvBL1a overexpressing transgenic lines for lines L-1, 3, 4 and 5. (g) Color formation in GUS analysis of T1 generation at different stages of seed germination. (h-j) Homozygous plants in lines L-1, 4 and 5.
Figure 3

Physiological analysis of OsRuvBL1a overexpression transgenic lines under salinity and drought stress conditions (a-c) Germination assay of OsRuvBL1a overexpression transgenic lines L-1, L-4 and L-5 with respect to WT under salinity and drought stress conditions (a) on ½ MS (control) (b) on 200 mM NaCl and (c) on 150 mM mannitol (d) Germination percentage analysis of WT and transgenic lines under salinity and drought stress. (e-g) Root-shoot length and ratio analysis of WT and transgenic lines under salinity (200 mM NaCl) and drought (150 mM mannitol) stress. (h) Dry weight analysis of WT and transgenic lines under control, salinity and drought conditions. (i-j) Chlorophyll estimation of WT and OsRuvBL1a transgenic lines under salinity conditions (200, 300 and 350 mM NaCl). (k-l) Chlorophyll estimation of WT and OsRuvBL1a overexpressing transgenic lines under drought conditions (10%, 20% and 30% PEG). The significant difference between the mean values (n = 3) of WT and OsRuvBL1a overexpressing transgenic lines (L1, 4 and 5) were determined by analysis of variance (ANOVA) using Graphpad PRISM. The statistical significance was represented as “*” and “**” for p<0.05 and p<0.01 respectively.
Figure 4

Biochemical analysis of OsRuvBL1a overexpressing transgenic lines for different time durations (1, 6, 12 and 24 hrs) (a-f) Under 200 mM NaCl concentration and (g-l) Under 150 mM mannitol concentration (drought) showed stress tolerance in OsRuvBL1a transgenic lines for WT, L-1, L-4 and L-5 (a, g) Hydrogen peroxide content, (b, h) Lipid peroxidation in terms of malondialdehyde (c, i) Proline content (d, j) Cell membrane stability in terms of percentage electrolyte retention (e, k) Enzymatic activity of Ascorbate peroxidase and (f, l) Catalase. The significant difference between the mean values (n = 3) of WT and OsRuvBL1a overexpressing transgenic lines (L1, 4 and 5) were determined by two way analysis of variance (ANOVA) using Graphpad PRISM. The statistical significance was represented as “*” and “**” for p<0.05 and p<0.01 respectively.
Figure 5

Confocal microscopy based analysis of roots (A, B) Na\(^+\) ion localization under 200 mM NaCl for 12 hrs. (C, D) Ca\(^{2+}\) ion localization under 200 mM NaCl for 12 hrs. (E, F) Cell viability analysis under 200 mM NaCl for 12 hrs. (G, H) Cell viability analysis under 150 mM mannitol for 12 hrs. The significant difference between the mean values (n = 3) of WT and OsRuvBL1a overexpressing transgenic lines (L1, 4 and 5) were determined by two way analysis of variance (ANOVA) using Graphpad PRISM. The statistical significance was represented as “*” and “**” for p<0.05 and p<0.01 respectively.
Figure 6

In vivo stress tolerance study (a,b) Salinity and (c,d) drought stress tolerance under in vivo conditions for same age plants (a,c) Plants after salinity and drought stress Day 1 with WT and transgenic lines (L-1, 4 and 5) where all plants are healthy. (b,d) Plants on 20th and 15th day after giving salinity and drought stress showing death of WT plant whereas OsRuvBL1a overexpressing transgenic line showed green tissue and survived the stress.
Figure 7

Hypothetical model for the working mechanism of OsRuvBL1a under stress condition.

Supplementary Files

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