Effect of Salinity Stress on expression of aquaporin (PIP 1) gene in Pokkali Vytilla V-8 (*Oryza sativa L.*): A comparative study

M. Harish[1]*, V. Salna Fathima[1], Raveendran Roshni[2], D.C. Deepthi[1] and Nair Sethulekshmy[1]
1. SCMS School of Technology and Management, Biotechnology Division- SIBB R&D, Management House, S. Kalamaserry, Cochin-33, INDIA
2. MES College, Department of Biotechnology, Marampally, North, Vazhakkulam, Aluva, Kerala 683107, INDIA
*harishmadhav20@gmail.com, harish@scmsgroup.org

Abstract
Rice is one of the most cultivated food crops worldwide, especially in various parts of Asia. A major decline in the production of rice has been reported in different parts of the continent recently. This is in turn reflected in global food security. Rice cultivation has been in threat due to leading abiotic stresses like salinity, drought, temperature etc. Therefore, stress tolerance by plants is a very important feature to be investigated. Aquaporin, a transmembrane protein in plant cells, guides water transport in various tissues and forms water channels. Aquaporin subfamilies can be found as different isoforms in plants and their selective expression depends on the type of plant and several other factors. It has been reported that Aquaporin plays a key role in providing stress tolerance to the plants. This study chooses six different varieties of Oryza sativa (Jyothy, Jaya, Harsha, Matta Triveni, Vyttila V-8 and Vaishak) cultivated under salinity stress of different concentrations (50,100 and 150-Mm NaCl).

The relative expression of the aquaporin gene in each of them was studied. The salt tolerant variety was analyzed based on the expression profiling data. This study reports that the expression of aquaporin is upregulated in a salt-tolerant varieties grown in Kerala like Pokkali Vyttila V-8. Further structure-function analysis is warranted for establishing the behaviour of AQP gene in different varieties of Oryza sativa at varying concentrations.

Keywords: *Oryza sativa L.*, aquaporin, salinity stress.

Introduction

Elevated concentrations of salt in soil can affect the osmotic balance and can result in ion toxicity. Na^+ and Cl^- ions are the primary causes of global salt toxicity, impacting as much as 50% of irrigated lands. Plant reactions to salt stress happen on various levels within the organism including cellular and molecular and have multiple effects such as balancing ion levels, regulating osmotic pressure, removing reactive oxygen species and managing nutrient levels[11]. This can influence the water absorption and efficiency of cells. Plants have the most aquaporin homologs with diverse subcellular localization patterns and solute specificities. Aquaporins are well recognized for their roles in physiological functions throughout the growth and development of plants[2]. Several aquaporin isoforms take part in water transportation and maintain osmotic equilibrium.

During salinity stress, they aid in transporting water molecules inside the cell and keep extra salts out for protection. Close to one-fifth of the earth's cultivable land and almost half of watered land are impacted by salt. Higher levels of salt can have negative effects on seed germination, seedling growth, vigor, vegetative growth, flowering and fruiting, resulting in a decrease in economic yield. They also decrease the osmotic potential of the soil solution, causing water stress in plants and harmful ion toxicity. Salt interacting with mineral nutrients can cause imbalances and deficiencies in nutrients. All these outcomes eventually result in the death of the plant, caused by both growth slowdown and damage at the molecular level. The main focus in achieving salt tolerance is to either avoid or lessen the harm, or restore balance in different stressful environments[3].

With the escalation of worldwide drought, water scarcity and soil salinization, the research focus has shifted to plant water channel proteins and their reaction to drought stress. Significant variations exist in the way water channel protein genes responding to stress and functioning in various plants, tissues and cells. Hence, researching the roles of various plant water channel proteins in plant development, growth and stress tolerance is crucial for gaining a deeper insight into the physiological functions and mechanisms of these proteins under both normal and stressful circumstances[7].

Genetic research has proved that enhancing the expression of certain aquaporin genes may increase the stress tolerance indicating its potential applications in developing more resilient crops against adverse environmental conditions. The complex regulation and varying purposes of aquaporin emphasize their crucial involvement in supporting plants to acclimatize abiotic stresses, encouraging researchers to study the role of aquaporin in achieving sustainable agricultural productivity. Identifying the limiting factors for stress tolerance helps to identify traits that can be targeted and improved through conventional breeding or other methods[8].

To address water scarcity and fulfill the increasing water needs for farming, numerous Nations have started utilizing poor-quality water (such as salt water, treated water, runoff water) with elevated salinity levels. Plants will experience notable physiological and biochemical alterations such as a substantial decrease in photosynthesis rate and a significant decline in the movement of salt ions from roots to above ground under conditions of high salinity[14].

This research aims to explore the expression of aquaporin proteins in varieties of Oryza sativa. Understanding their expression patterns in different rice varieties can provide insights into mechanisms of stress tolerance, water use efficiency and overall plant physiology.

Material and Methods

Preparation of Sample: Seeds of six varieties of Oryza sativa (Jyothy, Jaya, Harsha, Matta Triveni, Vyttila V-8 and Vaishak) were collected from Regional Agricultural Research Station (RARS) Pattambi, Kerala. The seeds were soaked in distilled water for 24 hours and non-viable seeds were removed. The soaked seeds were kept for germination. The seedlings were then sown in a mix of coir pith and soil and were allowed to grow in optimum moist conditions for 10 days. The plants were then treated to salinity stress by growing them in sodium chloride (NaCl) solutions of varying concentrations (50,100 and 150-Mm) for 10 days. A control for each variety was also kept to grow without the stress. The plants were constantly checked for any changes in the physical features.

Biochemical Analysis: Proline content was determined from the leaves of all the plant varieties subjected to the treatment[5]. Purified proline was used as the standard. The leaves of each variety weighing approximately 0.2 g were taken. They were homogenized in 10 ml of 3% aqueous sulphosalicylic acid and centrifuged at 3000x g for 1 minute. 2 mL of ninhydrin and 2 mL of glacial acetic acid were added to 2 mL of the filtrate. The mixture was kept for the reaction by incubating at 100 °C for 1 hour followed by an ice bath for the termination of the reaction. The reaction mixture was separated by adding 4 mL of toluene and shaken vigorously for 15-20 seconds using a test-tube holder. The aqueous phase was collected and warmed at room temperature. Using toluene as a blank, the absorbance was measured at 520 nm. The estimation of proline concentration was done by plotting a standard curve.

Aquaporin gene amplification: RNA was isolated from the leaves of the plants after 10 days of salinity treatment using (Guanidine Thiocyanate) GTC method. cDNA was synthesized through reverse transcription from the isolated RNA. Forward and reverse primer were designed with the help of the software “E-Prime”. The polymerase chain reaction was carried out for the amplification of gene. Reaction mixture of 20 µl containing 2 µl of cDNA, 2 µl of 10x Taq buffer, 1 µl of dNTPs, 10 pm of forward and reverse primers and 0.5 µl of Taq polymerase was taken in sterile tube. The reaction takes place under the following conditions; initial denaturation at 95°C for 4 minutes, followed by 30 cycles of the following steps, denaturation at 95°C for 1 minute, annealing at 64°C for 45 seconds, extension at 72°C for 1 minute. After the extension, a final extension at 72°C for 10 minutes was also carried out and it was held at 4°C forever.

Analysis of Aquaporin Gene expression: The quantification of the expression of aquaporin gene at mRNA level was done using Real-time PCR or qPCR. The reaction was carried out using Real time machine, real plex (Eppendorf, Germany). 18S rRNA was used as an internal control for the study. Relative quantification was done where amplification of aquaporin partial gene was compared to that of 18S rRNA. The Ct value of aquaporin gene was normalized with that of 18S rRNA. The relative expression was determined in different rice varieties. Using the equation $2^{-\Delta\Delta CT}$, the expression profiling was carried out[12].

Results and Discussion

Growth of roots and leaves, proliferation, biomass, density and size are the major morphological features that respond against abiotic stress which further improves productivity under stressful conditions like salinity[4]. Significant changes in the physical features include color changes in the leaves. A visible colour change from green to yellow in the leaves was observed after the treatment. Scrolling of leaves was also noted in some plants. Changes in the texture of leaves were noted in some varieties. Table 2 shows the observations regarding the yellowing and scrolling of the leaves after the treatment.

Proline plays a crucial role as an amino acid for basic plant metabolism. The build-up of proline in plant cells when exposed to salt is a frequent occurrence. It is believed that proline safeguards plant tissues from stress by functioning as an osmo-protectant, scavenging ROS and storing nitrogen and carbon source[1].

Table 1
Observations regarding yellowing and scrolling of leaves

Name of the variety	Yellowing of leaves found and the concentration of NaCl (Mm)	Scrolling of leaves Found and the concentration of NaCl (mM)
Jaya	Yes 50	Yes 100,150
Jyothy	No	No
Harsha	Yes 50	No
Matta Triveni	Yes 100,150	No
Vyttila V-8	No	No
Vaishakh	Yes 150	Yes 50,100

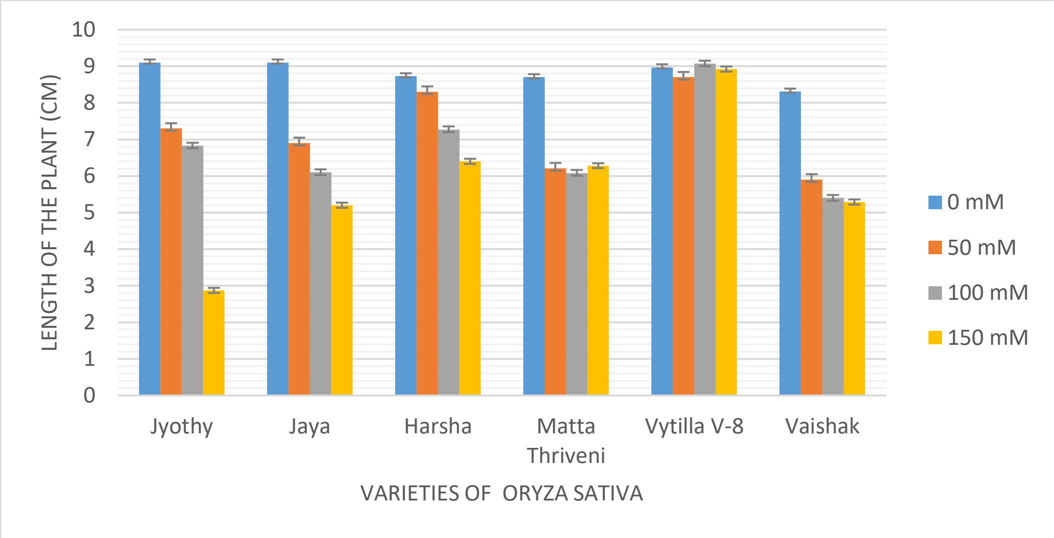

Figure 1: Graph representing the lengths of leaves of rice varieties grown in 0, 50, 100 and 150- Mm concentrations of NaCl

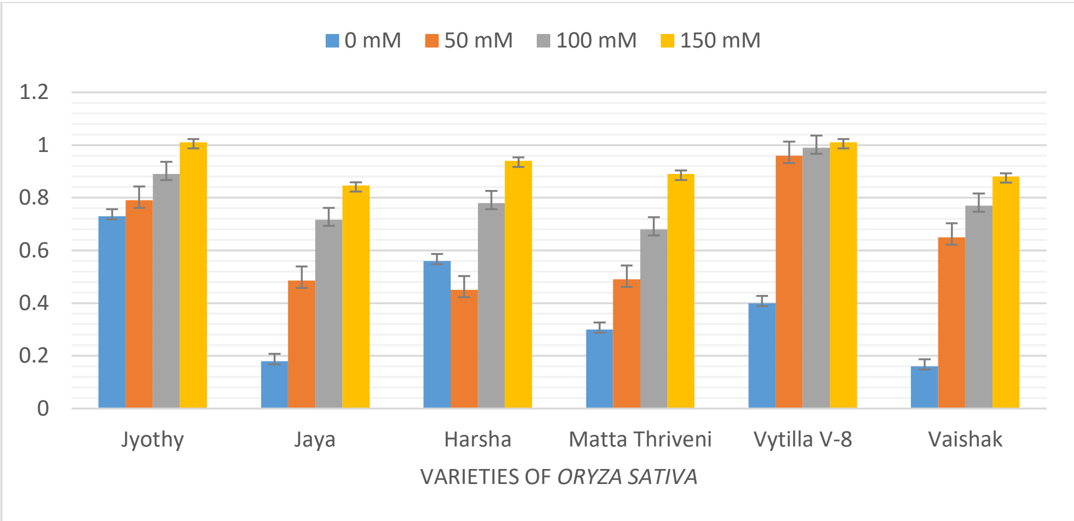

Figure 2: Estimation of proline content (in µM) in varieties of *Oryza sativa*

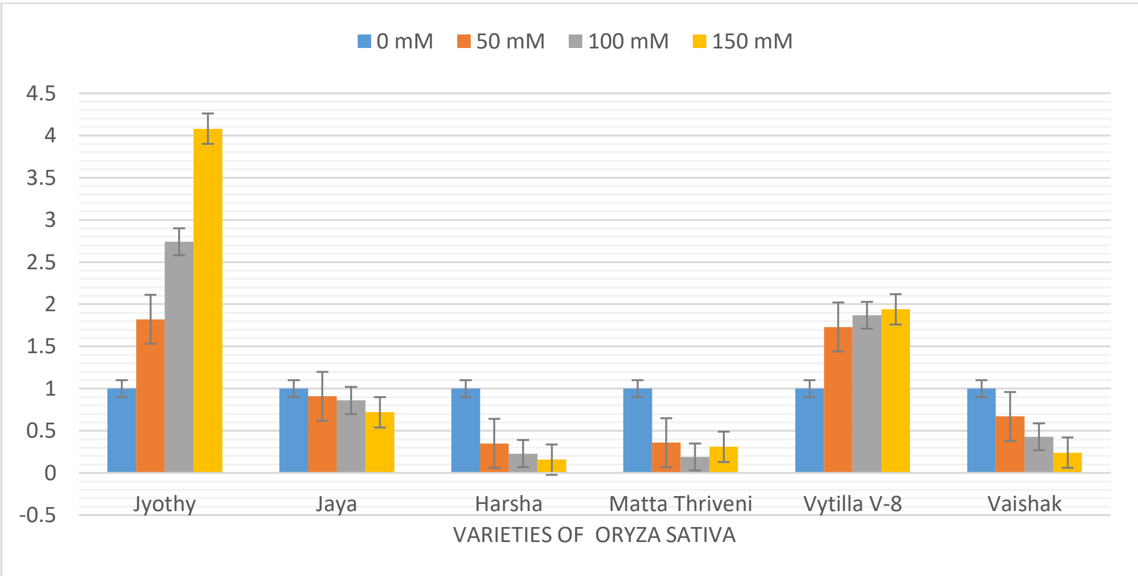

Figure 3: Graphical representation of relative expression level of aquaporin in varieties of Oryza sativa grown in varying concentrations of NaCl

During times of stress, plants undergo an adaptive response by producing and storing free amino acids, with a focus on proline accumulation. Thus, proline may serve as an indicator of metabolic stress. The ability of plants to withstand unfavorable conditions, particularly lack of water, has been linked to the build-up of proline, a non-protein amino acid, produced in the leaves of plants experiencing water stress[15]. Figure 2 shows the graphical representation of proline content in varieties of Oryza sativa grown in 0, 50, 100 and 150-mM NaCl.

It is clear from the graph that the six varieties contain different concentrations of proline. If proline can be used as a stress related metabolic marker as stated by Saha et al[15], Vyttila-8 can be confirmed as a saline tolerant variety of *Oryza sativa*. A gradual increase in the proline content can be observed in Vyttila-8 at varying concentrations compared to other varieties.

The relative expression of aquaporin gene was determined. It is obvious from the resulting data that aquaporin plays a key role in response of plants to abiotic stresses like salinity. The six varieties of Oryza sativa; Jaya, Jyothy, Harsha, Matta Triveni, Vyttila-8 and Vaishakh have shown different levels of aquaporin expression under salinity stress (0, 50,100 and150-Mm NaCl). It has been reported in many studies that the expression of aquaporin in plants depends on several parameters including the experimental set-up and not just the level of stress. Also, aquaporin genes and their function follow a complex mechanism of regulation at multiple[17]. Figure 3 represents the graphical representation of aquaporin gene in five different varieties of Oryza sativa grown in varying concentrations of NaCl solution i.e. in Jyothy and Vyttila-8, the expression of aquaporin increased with rising concentration of NaCl.

In Jyothy variety, the expression levelled up three-fold from 0 to 150-mM concentrations. In Vyttila V-8, there was a gradual increase in the expression. In the remaining varieties like Jaya, Harsha, Matta, Triveni and Vaishak, the aquaporin gene expression showed a decline in the level as the concentration of NaCl increased. This can be indicative of the fact that Jyothy and Pokkali Vyttila-8 are two of the saline tolerant rice varieties grown in Kerala and the expression of aquaporin is upregulated in them.

Otherwise, we can say that the expression of aquaporin is upregulated in salt-tolerant varieties like Jyothy and Vyttila-8. There is no debate to the fact that aquaporins play significant roles in water transport and thus in the stress tolerance. Aquaporin gene expression is adjusted at RNA and protein levels through numerous mechanisms, enabling plants to quickly change their membrane water permeability in accordance with variations in leaf water potential and environmental conditions[9].

Aquaporins play a crucial role in regulating leaf water content and transpiration rates. It is crucial to comprehend the signals that control aquaporin's impact on water transportation, to investigate how leaf rolling affects the expression and function of aquaporin genes[6]. Studies have detected the expression of aquaporin genes in young seedlings of certain plant species, indicating their potential involvement in leaf movements. The functional analysis of aquaporins in the context of leaf movements and water channel activity provides insights into the potential interplay between leaf scrolling and aquaporin gene expression[15].

The expression of aquaporin is not always solely related to the stress tolerance as shown in many studies and it is dependent on several factors. Additionally, the regulation of aquaporin gene in plants under stress follows complex mechanisms at multiple levels. A comprehensive structural analysis should be carried out for establishing the abnormal behavior of AQP gene in different varieties of *Oryza sativa* at varying concentrations.

Conclusion

The six varieties of Oryza sativa chosen for the study (Jyothy, Jaya, Harsha, Matta Triveni, Vyttila-8, Vaishak) have shown different levels of expression of aquaporin gene under saline stress conditions of different concentrations (50, 100, 150, 200 and 250 mM). The expression of aquaporin is upregulated in salt-tolerant varieties. Therefore, Jyothy and Pokkali Vyttila V-8 being salt-tolerant varieties of *Oryza sativa*, are ideal for growing in saline conditions and show upregulation of aquaporin. The study was all about the relative expression level of aquaporin in leaves of the six varieties.

The stress tolerance is not solely related to expression of aquaporin as shown in many studies and it is dependent based on various spatial, temporal and environmental factors. The regulation of aquaporin gene in plants under stress follows complex mechanisms at multiple levels and in case of salinity stress, it can be increased to enhance the plant's tolerance. The fact that aquaporins play significant roles in water transport and thus in the stress tolerance, cannot be denied. The yield advantage of salt-tolerant rice is partly due to its higher biomass accumulation, faster growth rate, stronger antioxidant capacity and stronger osmotic regulation ability under salt stress[17].

An in-depth examination of the gene's structure is necessary to determine how the aquaporin gene's expression levels vary across different types of rice in reaction to varying levels of salt stress. Several research projects have examined how genes are expressed when exposed to environmental signals along with the quantities, distribution and water transportation function of aquaporins. These researches indicate that individual aquaporins have distinct functions in maintaining plant water balance when exposed to different environmental pressures[10].

Salt stress significantly impacts the growth and yield of rice. Unraveling the mechanism of salt stress requires intricate research methods. Therefore, to understand its molecular mechanism, to improve the yield and development of salt-tolerant varieties and to achieve significant results in the future, it is necessary to combine the use of high-throughput cutting-edge technology with research foundations[13].

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