IMPACT OF WATER STRESS ON SUCROSE METABOLISM IN RICE (*Oryza sativa L.*)

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IMPACT OF WATER STRESS ON SUCROSE METABOLISM IN RICE (*Oryza sativa L.*)

by

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DECLARATION

I hereby declare that the thesis entitled "**Impact of water stress on sucrose metabolism in rice (*Oryza sativa* L.)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Vellayani
Date: 08.12.2021

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LIST OF ABBREVATIONS

%	Percentage
A	Adenine
A_{260}	Absorbance at 260nm wavelength
A_{280}	Absorbance at 280nm wavelength
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ANOVA	Analysis of variance
At	*Arabdiopsis thaliana*
Bp	Base pair
C	Cytosine
^0C	Degree Celsius
CD	Critical difference
cDNA	Complementary DNA
CIN	Cytoplasmic invertase
CMSI	Cell membrane stability index
CRD	Completely randomized design
CWIN	Cell wall invertase
DEPC	Diethyl polycarbonate
DNA	Deoxyribonucleic acid
dNTPs	Deoxy nucleotide tri phosphates
et al	and other co-workers
EDTA	Ethylene diamine tetra acetic acid
F	Forward
FYM	Farmyard manure
G	Guanine
g	Gram

GC	Guanine-cytosine
Gm	*Glycine max*
INV	Invertase
KAU	Kerala Agricultural University
ml	Millilitre
mg	Milligram
min	Minute
mM	Millimolar
NaCl	Sodium chloride
ng	Nanogram
nm	Nanometer
OD	Optical density
Os	*Oryza sativa*
PCR	Polymerase Chain Reaction
PVP	Polyvinyl Pyrollidone
qRT-PCR	Quantitative real time PCR
R	Reverse
R/S	Root/ shoot
RARS	Regional Agricultural Research Station
RNA	Ribonucleic acid
rpm	Revolutions per minute
SE (m)	Standard error (mean)
SE (d)	Standard error (difference)
Seq	Sequence
Sp.	Species
SPS	*Sucrose phosphate synthase*
SuSy	*Sucrose synthase*
SUC2	*Sucrose transporter 2*

SWEET	*Sugar will eventually be exported transporters*
Taq	*Thermus aquaticus*
TBE	Tris-borate EDTA buffer
T_m	Melting temperature
T_a	Annealing temperature
Tris HCl	Tris (Hydroxy methyl) aminomethane hydrochloride
T	Thymine
U	Enzyme units
UDP	Uridine diphosphate
UBQ5	*Ubiquitin 5*
UV	Ultra violet
V	Volt
VIN	Vacuolar invertase
µl	Microlitre
µg	Microgram
µM	Micromolar

INTRODUCTION

1. INTRODUCTION

Rice (*Oryza sativa L.*) is the chief crop in the world as source of food for a major part of the human population. Global rice intake has increased slightly in recent years especially in Asian countries. In crop year 2018-19, around 490.27 million tonnes of rice were consumed worldwide, compared to 437.18 million tonnes in crop year 2018-19 (Statista, 2019). Therefore, due to the increasing demand for rice, it is necessary to increase the crop yield.

In rice production, water stress is considered as a major cause of yield loss. Park *et al.*, (2018) reported that due to water stress, plants are subjected to wide range of injuries, such as the inhibition of plant photosynthesis, higher oxidative stress, and variations in metabolism. Under water stress, diminished turgor pressure causes hindrance of cell expansion and impaired mitosis driven reduction in growth rate (Farooq *et al*., 2009). Water stress inhibits rice growth and biomass accumulation and alters morphological, physiological and molecular responses through stress induced genes and protein functions (Guo *et al*., 2018). Plants generate series of morpho-physiological adaptations during water stress to withstand the severe injuries from stress (Xu *et al*., 2015). Plants react to stress differently in various tissues and at various developmental stages (Ruan, 2014). In rice, reproductive stage is highly susceptible to water stress, which leads to a significant decrease in yield (Palanog *et al.,* 2014). Loss of yield in rice depends on the developmental stage, duration and severity of the water stress (Gana, 2011). Water stress significantly reduces the photosynthetic rate, and prompting consumption of the energy source and brings down the yield (Ortiz *et al*., 2008).

Sucrose, which is the predominant assimilation product produced by photosynthesis, has been transported from leaf (source organ) to the sink organ (root, seeds) in plants (Durand *et al*., 2016). Water stress improves cytoplasmic sucrose synthesis and accumulation in tolerant cultivars is a lot higher than in sensitive cultivar (Nemati *et al*., 2018). Zhu. *et al,* (2000) reported that, under drought condition plants begin the usage of its own sink for its survival, accordingly decreasing sucrose concentration. In any case, Terzi *et al.*, (2009) described that, there was no reduction in sucrose concentration

in drought tolerant plants. Du *et al.* 2020 concluded that water stress significantly increases the accumulation of sucrose in plants is seems to be an adaptation mechanism to survive during the stress condition.

Study conducted by Du *et al*. (2020) found that higher the activity level of sucrose metabolising enzymes and upregulated the expression of corresponding genes (*SPS, SuSy,* and *INV*) during water stress. Moreover, water stress up-regulated the expression levels of sucrose transporting genes (*SWEET* and *SUC*) and advanced the transport of sucrose from source to sink (Du *et al.,* 2020).

The metabolites response towards water stress shows variation among different genotypes. Therefore, comparative metabolic analysis of the responses of drought-tolerant and drought-sensitive genotypes to water stress needs to be performed to identify the mechanisms involved in adaptation to water stress. In the present study, PTB-7 was used as the drought tolerant rice variety and PTB-23 as the drought susceptible rice variety. In a previous study carried out in the Department of Plant Physiology, College of Agriculture, Vellayani, PTB-7 was identified as a variety tolerant to drought and salt stress (Beena *et al*., 2021) and Rejeth *et al*. (2020) reported that PTB-23 as a drought susceptible rice variety. Resistance towards water stress is a complex trait influenced by the function of morphological, physiological, biochemical and molecular qualities.

The present study focused on the influence of sucrose metabolism during water stress conditions by analysing the physiological, biochemical and molecular properties of selective drought tolerant and drought susceptible rice varieties.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rice is a semi aquatic annual grass (Poaceae) that belongs to the genus Oryza and *Oryza sativa and Oryza glaberrima* are the two species for cultivation and also there are 22 wild species. *Oryza sativa* can be found throughout the world, while *Oryza glaberrima* was generally developed in West Africa (Rockwood, 1992). Rice thrives in a variety of conditions, but submerged in water is the most widely used strategy. Rice is the only cereal plant that can grow in standing water for a long period of time (International Rice Commission and International Year of Rice Program, 2005). 57% of the rice is grown in irrigated land, 25% in rainfed swamps, 10% in highlands, 6% in deep waters, two or three in tidal wetlands (Chopra and Prakash, 2002).

2.1. EFFECT OF WATER STRESS ON RICE

Water stress is the most vulnerable stress for rice production and most rice varieties are susceptible to water stress (Ismail and Horie, 2017). Blum *et al*., (2011) reported that drought is insufficient soil moisture, leading to reduced plant growth and development as well as low yield. The semi-aquatic phylogenetic origin of rice makes rice more vulnerable to water stress. Water stress induces a wide range of plant responses, from cellular metabolism to changes in plant growth and yield. Kaur and Asthir, (2017) found that water stress exerts morphological effects on plant growth because cell elongation is inhibited by disruption of water flow (dehydration), yield, and membrane integrity, pigment content and photosynthesis. Stunting is one of the most important morphological effects of water stress. It reduces leaf size, increases root elongation and proliferation, impedes plant water supply and reduces water efficiency. It affects photosynthetic pigments and reduces gas exchange, leading to reduced plant yields. In addition, water stress also improves active oxygen species (Anjum *et al*., 2011).

Plants respond to water stress in different ways. One method is to escape the onset of extreme stress by completing their reproductive cycle, *i.e*., a short life cycle. Another way to avoid water loss is by maintaining optimal water levels during times of stress, mainly by reducing water loss or root growth, thereby maximizing water uptake. Second, tolerance strategies include increased root growth, rigid or small cell walls, and efficient oxygen species (ROS) collection mechanisms (Sairam and Saxena, 2000).

In rice, water stress affects different ways. Water stress is a form of dehydration, which causes the stomata to close and restricts gas exchange (Jaleel *et a*l., 2008). It reduces growth of plant by altering various morpho-physiological, biochemical and molecular processes that causes the alterations in molecular properties. Water stress is characterized by a decrease in leaf area, intercellular space and cell size (Karmer, 1969). Leaf curl disease and leaf death are considered accurate indicators to assess drought tolerance of rice (Chang *et al*., 1974). The stomata close and the rate of transpiration decreases with increasing leaf temperature, leading to leaf curl (Sobarado, 1987). Leaf rolling slows down under water pressure and the fastest recovery rate by eliminating stress is in crops considered good quality, since in rice the flagellum has a major role in filling and seed development (Evans *et al*., 1975). Drought tolerance is characterized by a deep root system (Boyer, 1996). Plants with thick and deep roots are ideal for water-stressed conditions, essential for maximum absorption of nutrients from the soil and its distribution to different areas of the plant by the xylem. Thus, water stress is positively correlated with the surface of the xylem vessels for water conduction to the upper part of the plant. It is also positively correlated with dry matter accumulation in shoots (Willumsen, 1993). Water stress can have a negative effect on root growth by reducing the rate of meristamatic activity and root elongation. Farooq *et al*. (2009) demonstrated the main negative impact of water stress on plants is reduced production of fresh and dry biomass. Important aspects of increased grain yield were large grain size, early maturation, and reduced plant height during drought-sensitive periods (Singh *et al*., 1995).

2.2. EFFECT OF WATER STRESS ON PHYSIOLOGICAL PARAMETER

Various physiological parameters are associated with drought (such as drought-induced responses) and drought tolerance. Cell membrane stability is a widely used physiological indicator to assess drought tolerance (Blum and Ebercon, 1981). Lower membrane stability reflects a greater degree of membrane lipid peroxidation, which is a consequence of a increased susceptibility to oxidative stress during various environmental stresses, including drought (Leibler *et al*., 1986). Membrane stability

index did not change much under irrigation conditions in different genotypes. However, under water stress conditions, the membrane stability index was highest in the tolerant genotype. Lower membrane strength or higher damage reflects the degree of lipid peroxidation (Dhindsa *et al*., 1981), which in turn is a consequence of higher oxidative stress due to different environmental stresses (Leibler *et al*., 1986). Premchandra *et al*. (1990) documented that cell membrane stability is a drought tolerance indicator.

Relative water content (RWC) deals with the water state of tissues and is closely related to the water potential of leaves (Sinclair and Ludlow, 1985). In response to water stress, there is a significant reduction in relative water content was observed in rice genotypes. Nithya *et al*. (2020) reported that physiological trait pathway analysis with yield components of 81 rice genotypes revealed that relative water content had a maximum positive direct effect on yield. O`Toole and Moya, (1978) suggested that the ability to maintain higher relative humidity under water stress as a possible mechanism of drought resistance in rice. Therefore, RWC showed a positive and significant correlation with biomass.

2.3. EFFECT OF WATER STRESS ON PHOTOSYNTHETIC RATE

Drought is one of the important factors regulating production efficiency in the world. Photosynthesis is an important process that controls plant metabolism. Drought stress reduces the rate of photosynthesis, leading to energy depletion and reduced yield. The reduction in crop yields is indicated by strict leaf pruning (Ephrath and Hesketh, 1991) and therefore by low rates of photosynthesis. (Chen *et al*., 1993). Stomatal closure is responsible for the reduction in leaf size (Hsiao, 1973). Plants change their behaviour in response to stress in different ways in different organs at different stages of development. Non-photosynthetic plant organs such as seeds and organic fruits account for more than 75% of global food production (Ruan *et al.,* 2010). Therefore, understanding the mechanism of their behavioural changes in the face of stress would be helpful for improving food security. Plants have the ability to convert CO_2 into organic carbon in their leaves through photosynthesis by using energy from sunlight. Sucrose is the fundamental result of photosynthesis in plants, which act as an important energy substrate and regulator of plant growth and development signals. And, in

addition, it intervenes in the reactions of various stresses. Synthesis of sucrose is occurred at the leaf, which is the source organ by the enzyme sucrose phosphate synthase (SPS). Drought can generate sucrose accumulation as a versatile tool by expanding the action of sucrose phosphate synthase. From the source organ, sucrose is transported to the sink organ, since then the enzymes such as sucrose synthase (SuSy) and invertase (INV) are responsible for hydrolysis of sucrose at the sink organ. The movement of the enzymes that break down sucrose is also related to your stress response system (Du *et al.,* 2020).

Turgor pressure variation through the osmotic effect of sucrose by the stacking of sucrose into the phloem and its dumping into the sink. This changes in the pressure causes mass flow of water, which transports sucrose as osmolyte from source to sink. This sucrose coordinate phloem movement is the significant pathway through which all segments, like nutrients, signalling particles and water, are moved to meristematic sinks, including the shoot and root apical meristems. Sucrose cleavage results hexoses fundamental to produce cellulose, starch, fructan, proteins, and antioxidant compounds (Wang *et al.,* 2000).

Sucrose synthesis and cleavage are basic to the production of food, fiber, and fuel, hence it is crucial to horticulture and energy inexhaustibility. Starches that begin from sucrose comprise ~ 90% of plant biomass, making sucrose an important yield determinant. Upon phloem unloading in sinks, sucrose is enzymatically cleaved into hexose to power and support the development of sinks like developing fruits, seeds, roots, cotton fibers and tubers (Du *et al*., 2020)

Sucrose metabolism is among the key regulatory system gives resistance to biotic and abiotic stresses. Drought is the most common stress that antagonistically affects crop development and yield. Restraint of plant photosynthesis (Ohashi *et al*., 2006), expanded oxidative stress (Porcel and Lozano, 2004), and altered metabolism (Valliyodan and Nguyen, 2006) are the explained injury manifestations caused due to drought stress in plants. Plants can create certain morphological and physiological transformations to adapt with the drought stress condition (Xu *et al*., 2015). The root/shoot (R/S) ratio is a significant boundary for estimating the drought tolerance of

plants, which demonstrates the relative distribution among root and shoot biomass (Wilson, 1988). To withstand the unfavourable natural condition like drought, plants change the location of osmolyte like sucrose from source organ to sink organ (Cuellar-Ortiz *et al*., 2008).

Now, the objective of agricultural research needs to develop procedures to improve the efficiency with limited resources (Passioura, 2012). In view of the differing environment and expanding water unavailability, effects of drought stress expected to be high. Drought condition results variety in water relations, membrane structure, physiological and biochemical cycles, and organelles. Additionally, decrease in size of leaf, stem augmentation, weakness in root expansion and low water uptake are noticed (González *et al*., 2009). During various abiotic stresses, variations in photosynthesis and carbon metabolism are normally noticed characteristics.

2.4. EFFECT OF WATER STRESS ON ROOT-SHOOT RATIO

The root-shoot ratio (R / S) and shoot-root ratio are often used to estimate the relative biomass distribution between roots and shoots (Poorter *et al.,* 2012). The distribution of carbohydrates between shoots and roots may be associated with changes in R / S. Farrar (1996) suggested that sucrose plays an important role in the distribution of biomass between shoots and roots. One of the most important determinants of plant growth is the transport of sucrose from the source to the sink (Lemoine *et al*., 2013). It is often observed that the R/S ratio increases under adverse conditions such as drought (Xu *et al*., 2015). This is due to a significant decrease in above-ground biomass as the absolute dry weight of the roots, rather than an increase in root biomass. Drought stress was no greater than under well-irrigated conditions. Mild water stress has also been reported to limit shoot growth but have a small effect on root growth (Lemoine *et al*., 2013). Mahajan and Tuteja (2005) found that leaf growth is generally more sensitive to stress than root growth. Most of the sucrose assigned to the roots is responsible for the increased R/S under drought stress (Xu *et al*., 2015).

2.5. SUCROSE METABOLISM IN PLANTS

Sucrose is the fundamental photosynthetic product in plants, and act as a significant energy substrate and signalling regulator of plant development (Du *et al*., 2020). Sucrose is synthesized in photosynthetic leaves (source) and transferred to non-photosynthetic tissue (sink). Fructose-6-phosphate and UDP-glucose were consolidated into sucrose phosphate by sucrose phosphate synthase (SPS), which is converted to sucrose by the enzyme sucrose phosphate phosphatase. Sucrose is stacked in the phloem for its movement towards the sink organ. Sucrose aggregation leads to osmosis, which creates turgor pressure in the phloem, which drives the progression of sucrose to the sink organ (Ruan, 2014).

Sucrose synthesis happens in green leaves, which act as photosynthetic source (Halford *et al*., 2010). Aldol condensation between dihydroxy acetone phosphate and glyceraldehyde-3-phosphate results the primary product fructose 1.6-bisphosphate. At that point fructose1,6-bisphosphate changed over to fructose-6-phosphate by fructose 1,6-bisphosphatase, thereafter by the activity of phosphoglucoisomerase, fructose-6-phosphate is changed over to glucose -6-phosphate, which is get changed over to glucose-1-phosphate by phosphoglucomutase. Glucose -1-phosphate consolidate with uridine triphosphate, produce uridine diphosphate (UDP) glucose with the assistance of UDP-glucose pyrophosphorylase. Sucrose phosphate synthase, which is a significant enzyme convert UDP-glucose and fructose-6- phosphate into sucrose phosphate. Sucrose phosphate eliminates the phosphate group and form sucrose by the action of sucrose phosphate phosphatase (Halford *et al*., 2010).

In the chloroplast, leaves fix CO_2 to create triose phosphate by utilizing energy from the sunlight. Trios-phosphate act as the building unit for other metabolism, for which it is transported to cytoplasm. Inside the chloroplast, it might likewise be changed over to ADP-glucose for the synthesis of starch. At night time starch is separated into glucose or maltose and moved to cytoplasm. Sucrose biosynthesis happens in the cytoplasm. Sucrose is transport through the phloem either apoplasmically (by sucrose carriers) or symplasmically (utilizing plasmodesmata). The mass flow of sucrose through phloem towards the sink organ is done on account of the accumulation of

sucrose, which ingest water osmotically and expands the turgor pressure inside the phloem component. So the particles draw in towards the sink organ, where having low pressure contrast with phloem. (Ruan, 2014).

Unloading of sucrose into different sink organs from the phloem is either apoplasmically or symplasmically. Cell wall invertase (CWIN) changes over sucrose into glucose and fructose by apoplasmically. This transformation is occur when the sucrose is prior to being taken up by the cytoplasm. G-protein coupled receptor may be sense the apoplasmically converted glucose for signaling. Cytoplasmic invertase (CIN) and sucrose synthase (SuSy) might be cleave sucrose, that unloaded through plasmodesmata or taken up by sucrose transporters. For hydrolysis by vacuolar invertase (VIN) sucrose may go into vacuoles from the cytosol. The intracellular hexose produced in the intracellular is additionally utilized for glycolysis and synthesis of polymers, for example, fructan, cellulose and starch. Nucleus localized hexokinase or other proteins sense the hexose level to control gene

2.5.1. SUCROSE METABOLISING ENZYMES

Sucrose phosphate synthase (SPS) in the cytosol which catalysis the synthesis of sucrose, that changes the uridine diphosphate (UDP)- glucose and fructose 6-phosphate into sucrose-6-phosphate, at that point by sucrose-phosphate phosphatase (SPP) dephosphorylate the previous and yield sucrose. The rate of sucrose production is related the action of sucrose phosphate synthase (Fu *et al*., 2010).

Sucrose synthase (SuSy) and invertase are the two significant enzymes, which catalysis the cleavage of sucrose into hexose (UDP glucose and fructose). Sucrose synthase confined in the cytoplasm that reversibly degrade sucrose. Irreversibly changes sucrose into glucose and fructose by the enzyme invertase. Action of invertase is perceived as a significant controller of assimilation distribution and development of signals because of environmental changes (Hammond and White, 2011). There are different types of invertase enzyme are present in the plants. In the vacuole or cell wall contain acidic invertase (Liao *et al.,* 2020), while cytoplasm comprises of neutral or alkaline invertase. Metabolic movement at the beginning phases of plant development and the control of

sugar constituents, and in sucrose distribution at the later formative stages are significant functions of vacuolar invertase (Roitsch, 1999).

2.5.2. SUCROSE TRANSPORTERS

It is demonstrated that tonoplast and plasma membrane of parenchyma cells translocate sucrose both as input and output. In plants there are two kinds of phloem transport systems; symplastic and apoplastic pathway. Symplastic pathway is carried out by the plasmodesmata, while the apoplastic pathway requires sucrose carrier proteins such as sucrose transporter (SUC) and SWEET (Sugars Will Eventually Be Exported Transporters) gene family (Ayre, 2011). Sucrose moved from companion cells to the sieve cells through the plasmodesmata, and go into to the sink organs. AtSWEET11 and AtSWEET12 are the carriers present in *Arabidosis thaliana* which take up the sucrose into the apoplast (Chen *et al.*, 2012), at that point sucrose is conveyed by companion cell by AtSUC2 (H^+: sucrose co-carrier) (Gottwald *et al*., 2000).

2.5.3. SUCROSE AS SIGNALING MOLECULE

Sucrose act as an indirect signalling molecule, when there is low concentration of hexoses like glucose and fructose, substitute the impact of sucrose (Ehness *et al.*, 1997). In plant system, hexose detection is done by two mechanisms: the hexokinase-independent pathway and the hexokinase-dependent pathway (Gupta and Kaur, 2005). Phosphorylation of sugar is needed on account of hexokinase dependent pathway while, that isn't needed in hexokinase free pathway (Smeekens, 2000). Sucrose hydrolysing protein, for example, invertase and sucrose synthase have additionally been accounted for to have regulatory role. Studies revealed that invertases control the gene expressions in the osmotic regulation, organ development, hormonal crosstalk, cell cycle, cell division, and reproductive development (Ruan *et al.,* 2010). Then also, sucrose synthase associated with the signalling of leaf development and apical meristem of shoot (Pien *et al.,* 2001). These enzymes were discovered to be the instrument in shoot apex extension and expanding the initiation of leaf, and in cotton plants which inhibits the seed abortion (Xu *et al*., 2015).

2.6. ROLE OF SUCROSE METABOLISM IN WATER STRESS.

Sucrose metabolism is the key administrative system in plants not exclusively to the developmental processes yet additionally to the reactions to abiotic stresses. Drought stress results alteration in plant metabolism which causes osmotic irregularity. It prompts the changes in water relation, so plant cell will face dehydration. To resist cell dehydration, assimilates such as, sucrose accumulates in the plants by normalizing the osmotic irregularity. Regulation of turgor pressure and be the plant cell hydrated during drought stress condition by the assembly of solute like sucrose is called osmoregulation.

Sucrose accumulation during drought condition is a plant adaptation methodology which is broadly accepted by the whole. Sucrose assumes a role both as nutrients and signaling particles. Aggregation of sucrose for osmotic adjustment, is an adaptation method, which is significant for plants to withstand during water stress conditions. It helps in extraction of water from dry soils and in the support of cell turgor, development and exchange of gas under water stress (Chaves *et al*., 2003). The expansion in the sucrose may be a direct result of the consumption in starch content. Mohammadkhani and Heidari (2008) was reported that, in two *Zea mays* cultivars higher concentration of sucrose and lower content of starch under water stress. They proposed that upgraded sucrose content was joined by a sharp decrease in the starch level with a decline in water potential. Sucrose plays a significant part in plant metabolism as it serves as substrate in biosynthetic processes, energy production and results of hydrolytic processes and furthermore stabilize cell membrane under stress conditions. Under water stress, sucrose secures the plants in two ways. Initially, the replacement of water by the hydroxyl group of sucrose during dehydration keeps up proteins and hydrophilic interactions in membrane. Then the counteraction of protein denaturation is done by the hydrogen bond interactions of sugars with membrane and proteins. Perez *et al*., (2001) said that, fructose polymer such as fructans, soluble sugar and get from sucrose, give protection from water stress. Water stress increases the amount of sucrose (Chegah *et al.,* 2013). Praxedes *et al.,* (2006) performed a study on four clones of Robusta coffee which have different capability towards water stress showed an increment in sucrose

and hexose and a lessening in starch content. An expansion in the content of sucrose with an increment in the length of drought conditions were seen by Mohsenzade (2006). This increment in the sucrose is connected with the relative water content. They recorded that relative water content declines by 20%, and the decrease in the pace of photosynthesis and the expanding of the sucrose content. Slama *et al*. (2011) had additionally got these outcomes in alfalfa during water stress. He recorded the higher sugar accumulation.

The investigation conducted by Sperdouli and Moustakes (2012) have shown that aggregation of sucrose under drought lead to the improved adaptation by keeping an antioxidant protection. Sucrose metabolism, which contain concurrent synthesis and cleavage process during stress condition, so it is a dynamic process. These proceeded as a directing the signals and accordingly controlling the different gene expressions that is associated with development and improvement of plants (Rolland *et al*., 2006). Presence of soluble sugar in the source tissue downregulate the photosynthesis, which maintains homeostasis (Koch, 2004). Variable source-sink impacts on metabolic cycles initiated by abiotic stresses brings about various expressions of numerous proteins associated with sucrose metabolism (Wingler *et al*., 2012). Various signals enacted during drought and their reaction causes either stress tolerance or stress avoidance in plants through differential expressions of sugar transporter genes and rearrangement of sugars from source to sink (Kaur *et al.*, 2021).

Plant adapts to the stress by altering the primary metabolism like changing the activity of enzymes. During drought stress, enzymes of sucrose metabolism play a critical part. Sucrose maintains the organization of the cytoplasm and keep up osmotic regulation during water stress conditions by act as companion solutes. The other way to protect the plant cell from drought by the means of sucrose through the construction of glass. Under water lacking condition, this glass resist the harm of cell by filling up the space (Koster *et al.*, 1991). Sucrose is the primary result of photosynthesis and it is the most significant transport sugar, and sometimes as a direct or indirect regulator of gene expression (Winter and Huber, 2000). Drought stress prompts significant adjustments in sucrose metabolism. Protein phosphorylation is a significant method to figuring the

sucrose-phosphate synthase activity in response to a various environmental and endogenous signal. Diminished water potential and relative water content were noticed by Castrillo (1992) in the study of impacts of water stress on sucrose metabolism in bean plants of the Tacarigua variety developed for 25 days. Water stress impacts brought about a decrease of sucrose phosphate synthase (SPS). An expansion in sucrose synthase action expanded the exercises of both neutral and acid invertases at moderate water pressure and diminished activity at extreme water pressure. The starch/sucrose proportion declined and the proportion of total glucose/total fructose expanded. Under water stress, rice during grain filling stage sucrose synthase activation and sucrose accumulating in the stems brings about the remobilization of fructans stored in the stem and mixtures are moved to the grain (Yang and Zhang (2006). This accelerates the duration of grain filling stage. The study of Legay *et al*., (2011) with potato clones having different tolerance capacity towards water stress recommended that sucrose rearrangement is necessary under water stress conditions and observed that more sucrose was mounting up in the tolerant clone. Activity of sucrose-synthesizing and sucrose-degrading enzymes enhanced under water stress condition. The role of sucrose towards its metabolic pathways to acclimatize with the stress is by increasing its concentrations (Wang *et al*., 2000).

2.6.1. ROLE OF SUCROSE TRANSPORTER GENE UNDER WATER STRESS

Plant sucrose transporter play direct the reallocation of sucrose among source and sink under abiotic stress (Durand *et al*., 2016). Sucrose carrier genes like *AtSWEET11, AtSWEET12,* and *AtSUC2* in *A. thaliana* leaves shows upregulated articulation during the stress condition, prompts the higher sucrose transport (Durand *et al*., 2016). Du *et al*. 2019 proposed that water stress enhanced the expression levels of genes associated with sucrose transport such as *GmSWEET11/12* and *GmSUC2* in leaves and roots of soybean seedlings and increased the transport of sucrose from leaves to roots. Mathan *et al*. (2020) shows the induced expression of *OsSWEET13* and *OsSWEET15* under drought stress condition and higher sucrose content in phloem sap, then, hypothesis the role of apoplasmic transport and sucrose carriers under stress for sucrose distribution. These all are recommended that sucrose distribution and transport are the basic for the survival of plants under stress condition.

Drought stress expanded the soluble sugar and starch substance in soybean roots by managing sugar metabolism and transport. Du *et al*. (2020) concluded that this seems to be the preferred mechanism for keeping up root development and metabolism during stress condition.

2.7. STRESS RESPONSES OF REPRODUCTIVE AND VEGETATIVE TISSUES MEDIATED BY SUCROSE METABOLISM

Compared with vegetative stages, reproductive development is more susceptible to stress, especially during the seed and fruit set stage around fertilization (Kakumanu, 2012). This is outlined by the phenotypic distinction in the severity of their reactions: Abiotic stress regularly inhibits leaf extension in a reversible way however, which causes abortion of flowers, seeds, and fruitlets, and henceforth irreversible loss of yield (Muller *et al*., .2011). These are may be advantageous for plants to save limited resources during stress. During reproductive stages, drought stress seriously limits the sucrose transport from leaves to seeds. Diminished hexose-to-sucrose proportion in seeds, together results the deficiency of seed weight hence disrupts the equilibrium of sucrose metabolism and transport in leaves and seeds at reproductive stages, which appeared to be the primary reason through which drought caused seed weight to reduce (Du *et al*., 2020).

Water stress blocks sucrose import, represses invertase and sucrose synthase activity, and drains starch holds. This prompt to bringing down the concentration of glucose in reproductive organs and eventually to their early abortion. Emerging evidence, largely based on gene expression analyses, suggests that the low glucose may

(i) Inhibit directly the gene expression of cell cycle and thereby cell division (Ruan *et al*., 2012),

(ii) Decreases the hexokinase metabolic activity, which is associated with the outer membrane of mitochondria, so which results the reduction in ATP use and the ADP regeneration needed for the ATP synthesis (Kakumanu, 2012). This may cause the overproduction of reactive oxygen species by affecting electron transport chain, which leads to the oxidative damage and even programmed cell death (Foyer and Shigeoka, 2011).

MATERIALS AND METHODS

3. **MATERIALS AND METHODS**

The study entitled with “Impact of water stress on sucrose metabolism in rice (*Oryza sativa* L.*)* was conducted in the Department of Plant Biotechnology and Department of Plant Physiology, College of Agriculture, Vellayani during the year 2020-21. The objective was to study the impact of water stress on sucrose metabolism by analyzing the physiological and biochemical parameters and gene expression in selective drought tolerant and susceptible rice genotype.

POT CULTURE EXPERIMENT OF SELECTED GENOTYPE

3.1. Plant materials

Two rice genotypes (drought tolerant and drought susceptible) were used for this study.

Table 1. List of rice genotypes selected for the study

Sl. No.	Genotype
1	PTB-7 (Parambuvattan)
2	PTB-23 (Cheriya Aryan)

3.2. Location

The study was conducted in the rainout shelter of Department of Plant Physiology, College of Agriculture, Vellayani during 2020-21. The seeds of the selected genotypes for the study were collected from RARS, Pattambi.

3.3. Potting mixture preparation and transplantation.

Potting mixture containing soil, sand, FYM in the ratio 3:2:1 was filled in pots. Fifteen days old seedlings were transplanted to the pots. Cultural operations were followed as per the package of practice of Kerala Agricultural University, Thrissur.

Table 2. Particulars for the pot culture experiment

Crop	Two rice varieties
Design	CRD
No. of treatments	1. Water stress 2. Control

3.4. Methodology of imposing drought stress

In this study, plants were raised in pots in rainout shelter. Five plants per replication were maintained for control and stress treatment. Normal irrigation was given to all plants till panicle initiation. After panicle initiation, water stress was given by withdrawing the irrigation for 4 days and normal irrigation was continued for remaining plants. Samples were collected from the stressed and control plants for analysis. Irrigation was continued till grain filling period.

Plate 1. Experimental unit view with rice plants

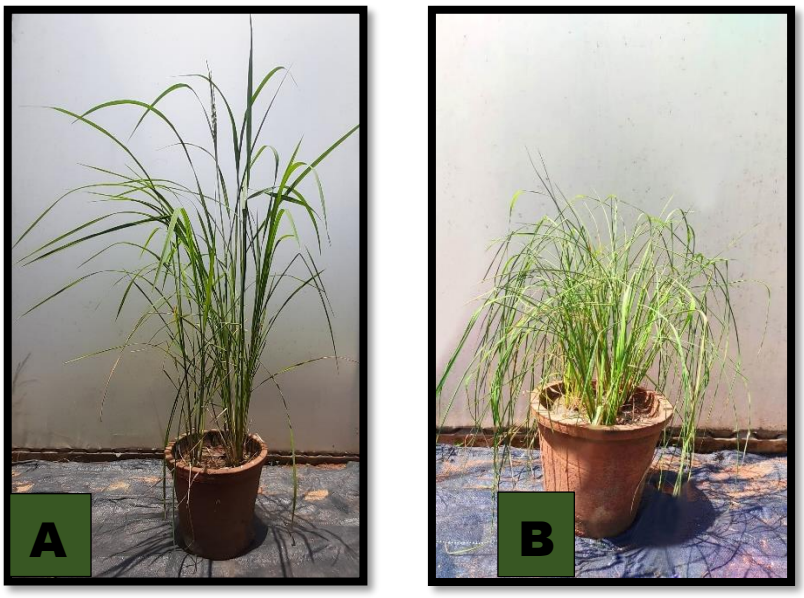


Plate 2. View of PTB 7 under water stress induced and control condition

(A - PTB 7 Control; B - PTB 7 Water stress induced)

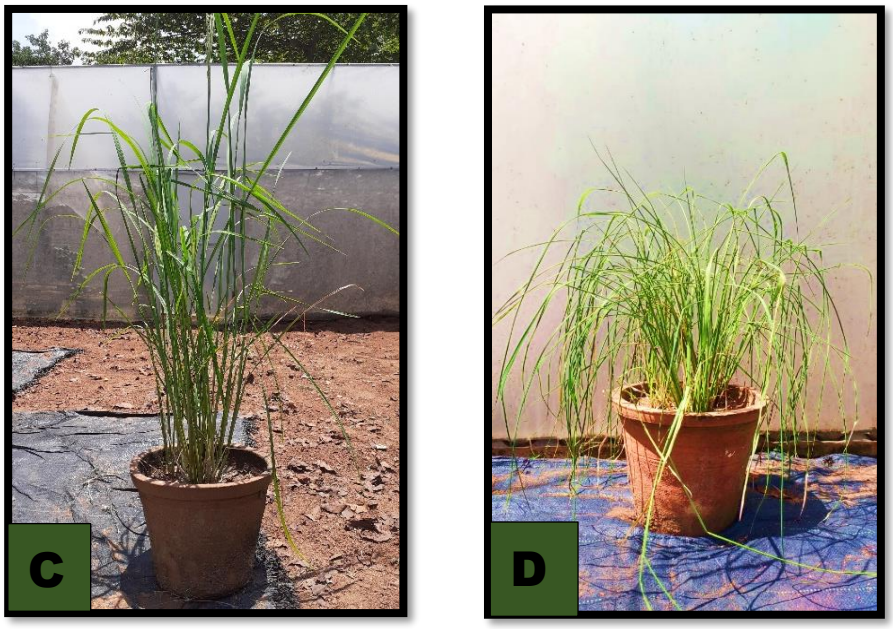


Plate 3. View of PTB 23 under water stress induced and control condition

(A - PTB 23 Control; B - PTB 23 Water stress induced)

3.5. Sampling

For physiological, biochemical and molecular analysis, root and leaf samples were collected after the induction of water stress. From both water stressed and control plants leaf samples were taken aseptically and divided into two parts. One set of plants was taken for the enzyme analysis and gene expression analysis. For that, the samples were immediately flash frozen in liquid nitrogen (-196°C) and then stored at -80°C. The other part was used to determine the relative water content, cell membrane stability index, sucrose content and reducing sugar content.

To measure the root to shoot (R/S) ratio and other root traits, above ground part of the rice plants were cut at the soil line and were used as the shoot biomass samples. Then the roots were taken off from the soil and rinsed thoroughly in running tap water and cleaned from all soil. These shoot and root samples were oven dried until it attained a constant weight.

3.6. MORPHOLOGICAL PARAMETERS

3.6.1. Plant height

Height of the plant was measured from the base of the tip to the primary panicle in centimeters

3.7. YIELD PARAMETER

3.7.1. Number of productive tillers

The number of tillers containing productive tillers was counted and recorded during the time of harvest.

3.8. PHYSIOLOGICAL PARAMETER

3.8.1. Root-shoot ratio

Root and shoot were separated from the whole plant by cutting at the edge of the soil line from the top of the plant and dried. After attaining a constant weight, weight of root and shoot were measured separately in grams in an electronic balance and recorded.

Root/shoot ratio = dry weight of roots/dry weight of shoot

3.8.2. Root length (cm)

Length of root (cm) was measured using centimeter scale from the tip of the longest rootlet to the cut end at the soil line

3.8.3. Root volume (cm^3)

Using water displacement method root volume in cubic centimeter was determined. Roots were removed from the soil, cleaned thoroughly and immersed in a 1000ml measuring cylinder. After that measure the displaced volume of the water, which is taken as the volume of the roots.

3.8.4. Root dry weight (g)

Roots were collected from the soil after the harvest and cleaned thoroughly for removing the remaining soil attached to the rootlets followed by drying till attaining a constant weight in a hot air oven at 80^0C. Then dry weight is measured in an electronic balance in grams.

3.8.5. Relative water content (RWC)

As per the procedure given by Barrs and Weatherley (1962) relative water content of leaves samples were measured. Immediately after excision of leaf from the plant fresh weight (FW) was recorded, without the loss of water content. After that, these leaf samples were kept floating on water for 3-4 hrs under normal room light and temperature and the turgid weight (TW) were measured. At last, the same leaf was placed at 75°C for overnight to assessing dry weight (DW). Further, the values are plugged in the following formula,

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100$$

3.8.6. Cell membrane stability index (CMSI)

Cell membrane stability index of leaf samples was determined as per the protocol describrd by Premchandra *et al.* (1990), which is modified by Sairam (1997). 100 mg leaf samples were thoroughly washed in running tap water then washed with double distilled water followed by the heating in 10 mL of double distilled water at 40°C for 30 min. Then Electrical conductivity labelled as C1 was recorded by using EC meter. After that, the samples were kept in a boiling water bath (100°C) for 10 min, and electrical conductivity (C2) was also recorded.

Cell membrane stability index = [1- (C1/C2)] x100

3.8.7. Sucrose content (mg/g)

Leaf samples were used for the estimation of sucrose content. Sucrose content of leaves was extracted and quantified by a modified method of Xu *et al.* (2015). About 100 mg of leaf sample were grounded and extracted using 80% (v/v) ethanol (Appendix I) at 80°C for 30 min, then centrifuged at 10000×g for 10 min, then collected the supernatant. The left-over was again extracted for two more times with 80% ethanol. Subsequently, supernatant of three extractions were combined and made upto the total volume of 5 ml using 80% ethanol. At 480 nm sucrose content was spectrophotometrically determined.

3.8.8. Reducing sugar (mg/g)

According to Miller (1959), estimation of reducing sugar was done by DNS method. 0.1g of leaf samples was weighed and extracted the sugars twice using hot 80% alcohol (5 ml each). Supernatant were collected and placed on water bath for evaporation. Then 10 ml of distilled water was added for dissolving the sugar. After that, 0.5 to 3 ml of extract were pipette out into each labelled test tubes, then using distilled water, all the test tubes were made up the volume to 3ml. Then added 3ml of prepared DNS reagent (Appendix II) into each test tubes.

Followed by 5 minutes heating in a boiling water bath. Then the colour developed, at that time 1 ml of 40% Rochelle salt solution (Sodium-potassium tartrate solution). After cooled down absorbance at 510 nm were measured

Using a standard graph prepared by using working standard glucose solution (Appendix III) (0 to 500 μg) the amount of reducing sugar were calculated.

3.8.9. Activity of invertase (μmol /glucose mg protein h)

- 1g plant tissue ground in pre chilled mortar pestle with 20 ml 0f 0.1M sodium citrate buffer (pH-5) (Appendix IV)
- Homogenate was passed through 2 layers of cheese cloth and centrifuged at 15000xg for 10 min at 4°C and take the supernatant.
- 0.6ml of 0.1M citrate buffer (pH-5) and 0.2 ml of 0.4 M sucrose added to 0.2ml of enzyme extract
- Incubation at 30°C for 1 hour and add 1 ml DNS and take it into boiling water bath for 5 minutes
- Cooled and diluted to 10 ml with distilled water
- Take the spectrophotometer reading at A_{560} nm. (Glucose as standard).

3.8.10. Activity of α-Amylase (mg/g h)

- 1g sample homogenized in pre-chilled mortar and pestle with 10 ml of cold distilled water at 4°C and centrifuged at 15000xg for 30 min. (enzyme extract)
- 3mM $CaCl_2$ added to 5ml of enzyme extract and heated for 5 min at 70°C to inactivate β-amylase (hot extract is used to estimate α-amylase)
- 1 mL of 1 M citrate buffer (pH-7) and 0.5 mL of 2% soluble starch (freshly prepared) added to 0.5 mL of hot enzyme extract to reach 2 mL and allowed to react for 5 minutes at 30°C.
- After 5 minutes terminate the reaction by adding 2 mL DNS reagent and heated for 5 min at 50°C.
- After cooling solution made upto 10 ml using distilled water
- Estimate the maltose content at A_{540} nm

3.8.11. Activity of ß-amylase (mg/g h)

- 1g sample homogenized in prechilled mortar and pestle with 10 ml of cold distilled water at 4° C and centrifuged at 15000xg for 30 min. (enzyme extract)
- Enzyme extract was treated with 0.1M EDTA to inactivate α-amylase
- 1 ml of citrate buffer (pH- 7) and 0.5 ml of 2% soluble starch added to the 0.5 ml of EDTA treated enzyme.
- Heated for 5 min at 30° C and cooled, terminate the reaction by 2 ml of DNS and heated for 5 minutes at 50°C
- After cooling made up to 10 ml using water
- Estimate at A_{540} nm.

3.9. MOLECULAR ANALYSIS

3.9.1. Expression analysis (Real Time-PCR)

3.9.1.1.Isolation of RNA from leaf and root samples

The isolation of RNA was done from leaf and root samples at the panicle initiation stage by using TRIzol reagent. The mortar and pestle, glassware, plastic wares (microfuge tubes, microtips), reagents were autoclaved. All the isolation process were carried out in the absence of RNase. Therefore, all the reagents used for the procedure were prepared using Diethyl Polycarbonate (DEPC) treated water (by adding 1ml of DEPC to 999ml of water (0.1%) and kept for overnight on magnetic stirrer. After overnight stirring, autoclaved twice for the complete removal of DEPC.

100 mg of leaf samples were weighed and ground to fine powders in pre-chilled mortar and pestle using liquid nitrogen. TRIzol reagent (1 ml) was added to the fine powdered samples. Then gently mixed and incubated for 5 minutes, then the mixture is transferred to pre-chilled microfuge tube and added 0.2 ml of chloroform, followed by vigorous shaking of about 15 seconds. Then incubated for 5 minutes. Then the microfuge tubes were placed in ice for 10 minutes. After the incubation, centrifuged the mixture at 12000 g for 15 minutes at 4°C. The obtained aqueous phase was safely transferred to another fresh microfuge tube. 0.5 ml ice cold

isopropanol was to the tubes and incubated for 10 minutes at room temperature. Then the mixture in the tube was mixed by slowly inverting the tubes, then centrifuged the tubes for 10 minutes at 12000 g (4°C). After the centrifugation, the supernatant was discarded and the obtained pellet was washed using 75% ethanol. Followed by spinning at 7500 g for 5 minutes at 4°C. Then removed the supernatant, pellet was air dried in the laminar air flow chamber. Dissolved the pellet in 30 µl of RNase free water (0.1% DEPC treated water). Kept for incubation for 10 minutes. At last, the isolated RNA was stored at -80°C.

3.9.1.2. Agarose gel electrophoresis

The presence of RNA was confirmed by running the samples in 2% agarose gel. It was prepared by melting 0.8g of agarose in 40ml 1X TBE buffer (APPENDIX I) (prepared in RNAase free water). Once the heat becomes ear bearable, 3µl of ethidium bromide was added to the melted gel and casted in a horizontal gel electrophoresis unit (Hoefer Power Pack, Germany).

5µl of RNA mixed with 2µl of 6X loading dye (APPENDIX V) prepared in RNAase free water was dispensed into the wells of the gel. The gel was run at $5Vcm^{-1}$ in 1X TBE buffer prepared in RNAase free water. The gel was taken out when the dye has run three-fourth of the entire distance of the gel and it was then visualized in a UV trans- illuminator system (Bio-Rad) and documented in Gel Doc system (Gel DOC ^{TM} XR+).

3.9.1.3.Quantification of RNA.

The RNA quantification was done by taking absorbance at 260 and 280 nm wavelength in UV- Visible spectrophotometer (ELICO SL 218, Double Beam, India) of 3µl sample diluted in 2997 µl of RNase free water which is taken in a glass cuvette. The concentration of RNA in the given sample was determined using the formula:

$$\text{Concentration of RNA (ng } \mu l^{-1}) = A_{260} \times 40 \times \text{Dilution factor}$$

(A_{260} – Absorbance at 260 nm)

The quality check was determined by taken the ratio of the absorbance value at 260 and 280 nm.

cDNA Synthesis

The isolated RNA was subjected to cDNA synthesis as per the manufacturer's instructions (Thermo Scientific Verso cDNA Synthesis kit). cDNA synthesis was carried out in a 20 µl reaction volume.

Table 3. Reaction mixture for the cDNA synthesis

5X cDNA synthesis buffer	4 µl
dNTP mix (5mM)	2 µl
Oligo- dT primer	0.5 µl
Random Hexamer (400 ng/µl)	0.5 µl
RT enhancer	1 µl
Verso reverse transcriptase enzyme	1 µl
RNA sample	4 µl
Nuclease free water	7 µl
Total volume	**20 µl**

The reaction mixture was run in a Thermocycler (GeneAmp PCR System 9700, Applied Biosystems) under the following conditions,

Table 4. Conditions for synthesis of cDNA:

Conditions	Temperature	Time	No. of cycles
cDNA synthesis	42°C	30 min	1 cycle
Inactivation	92°C	2 min	1 cycle

3.9.1.4.Primer designing for Real Time PCR

- Open NCBI (National Center for Biotechnology Information).
- Type the target gene on the search bar (*OsSuSy/ OsSUC2).*
- Select "Nucleotide" and click "Search".

- From the obtained result, select the *"RefSeq transcripts"* of the first variant.
- Select the "Pick Primers" on the right side of the opened window which shows the details of our gene.
- "Primer Blast" window will open and have to enter certain details such as "Range of forward primer and reverse primer, PCR product size, Primer Temperature, Exon/intron selection, Maximum target size".
- After entering all the required details click on the "Get Primers" Button.
- Select the most appropriate primer from the obtained primers.

3.9.1.5.Quality check of cDNA using UBQ5 gene

Synthesised cDNA and designed primer were confirmed by the PCR technique.

Table 5. The standard PCR mix

10X reaction buffer (1X)	2 µl
dNTP mix (100µl)	1 µl
Forward primer (10 mM)	1 µl
Reverse primer (10mM)	1 µl
Taq DNA polymerase	1 µl
Template DNA (cDNA)	1 µl
Nuclease free water	13 µl
Total volume	20 µl

Conditions were given in the thermal cycler as follows:

Initial Denaturation	94°c - 3 Minutes	
Denaturation	94°c - 30 Seconds	x 30 cycles
Annealing	50°c - 30 Seconds	
Extension	72°c - 30 Seconds	
Final Extension	72°c - 5 Minute	

Finally amplified PCR product taken and separated on agarose (1.2%) gel electrophoresis and then visualized in a UV trans- illuminator system (Bio-Rad) and documented in Gel Doc system (Gel DOC ^{TM} XR+).

3.9.1.6.Gene expression analysis using qRT PCR

Quantitative real time PCR was carried out to study the expression of sucrose metabolising genes *viz.*, *sucrose synthase* (*OsSuSy)* and *sucrose transporter (OsSUC2)* genes under water stress condition. Designed primers were used for above mentioned genes. For internal reference gene, *Ubiquitin* gene *(UBQ5)* (Park *et al.,* 2016) was taken.

Table 6. List of primers used for qRT-PCR

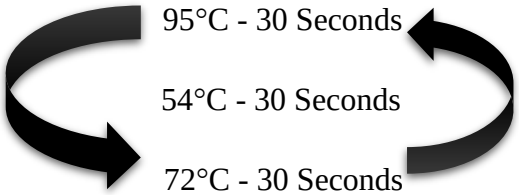
Genes	Forward primer (5'->3')	Reverse primer (5'->3')
OsSUC2	**ACCGGCATTGTCATTGCTTC**	**AATGCCCATTGCTAGACCTTG**
OsSuSy	**TGATTGCACTCTTCTCTAGGTATG**	**AGCCCGGAGAATGTCTTCAAA**
UBQ5	**GAAGTAAGGAAGGAGGAGGA**	**AAGGTGTCAGTTCCAAGG**

qRT-PCR was done in BIORAD CFXTM Real-Time Detection System and data was retrieved from BIORAD CFX Maestro 1.0 software. Reaction mix (20 µl) for qRT-PCR was prepared as follows;

Table 7. Real Time PCR Mix

SYBR Green Master mix (1X)	10 µl
cDNA Template	2 µl
Forward primer (2pM)	1 µl
Reverse primer (2pM)	1 µl
Nuclease free water	6 µl
Total Volume	20 µl

Conditions for qRT-PCR reactions performed as follows;

Initial Denaturation	95°C - 3 Minutes	
Denaturation	95°C - 30 Seconds	 X 40 cycles
Annealing	54°C - 30 Seconds	
Extension	72°C - 30 Seconds	
Final Extension	72°C - 2 Minutes	

For the normalization of real time PCR, *UBQ5* gene was used as reference gene. The obtained result was converted to fold changes *i.e.*, increase or decrease in the gene expression. ΔΔCq method was used for calculating the fold change (Rao *et al.*, 2013).

ΔCq = Cq (Query Gene) – Cq (Reference Gene)

ΔΔCq = ΔCq (Treatment) – ΔCq (Control)

FOLD CHANGE = $2^{-\Delta\Delta Cq}$

RESULTS

4. RESULTS

The experiment was conducted to study the impact of water stress on sucrose metabolism by analysing the physiological and biochemical parameters and gene expression in selected drought tolerant and drought susceptible rice genotypes, in the Department of Plant Physiology and Department of Plant Biotechnology, College of Agriculture, Vellayani during the year 2020-21. The seeds of the selected varieties were germinated and subjected to pot culture experiment. Plants were subjected to water stress by withdrawing the regular irrigation until the plant became stressed, indicated by the leaf rolling score of 9 and these plants were taken for the analysis and control plants were given normal irrigation. The physiological, biochemical and molecular parameters were recorded in both water stressed and control plants. The data obtained were analysed statistically by using ANOVA.

4.1. EFFECT OF WATER STRESS ON MORPHOLOGICAL PARAMETERS

4.1.1. Plant height

Results showed a significant difference in plant height between treatment and control. Water stress had an adverse effect on plant height. A significant reduction was observed in the height of the plant under water stress condition when compared to the control plant. Maximum plant height was observed in PTB 7 (120.07) under water stress condition. Compared to PTB 7, PTB 23 showed higher reduction by 9%, where it was 4% for PTB 7. Results observation on plant height at drought and control condition are presented in the Table 8.

Table 8. Plant height (cm) under water stress and control condition

Treatment	PTB 7	PTB 23
Control	125.33±2.03[a]	105.3±1.08[a]
Water stress	120.07±2.08[b]	96.3±1.62[b]
% increase/ decrease	-4%	-9%
SE(d)	1.700	1.054
SE(m)	1.202	0.745
CD (5%)	4.719	2.927

4.1.2. Number of productive tillers

Results showed a significant difference in plant height between treatment and control.

In this study, there was a reduction in number of productive tillers under water stress condition compared to control. Less reduction (31%) in number of productive tillers under water stress condition was found in PTB-7 (8.3), whereas PTB-23 showed higher reduction (5.7). Recorded values of number of productive tillers are represented in the Table 9.

Table 9. Number of productive tillers under water stress and control condition.

Treatment	PTB 7	PTB 23
Control	12.0±1.00[a]	11.0±1.00[a]
Water stress	8.3±1.53[b]	5.7±0.57[b]
% increase/ decrease	-31%	-48%
SE(d)	1.054	0.667
SE(m)	0.745	0.471
CD (5%)	2.927	1.85

4.1.3. *Root traits*

In this study, root traits such as root-shoot ratio, root length, root volume and root dry weight showed a remarkable result under water stress condition. The root traits of drought tolerant variety, PTB-7 were significantly increased during stress condition when compared to the control, where drought susceptible variety, PTB-23 showed a reduction.

Plate 4. View of root extracted from stress induced and control plant of PTB-7 & PTB-23

A – PTB 7 TEST

B – PTB 7 CONTROL

C – PTB 23 CONTROL

D – PTB 23 TEST

4.1.3.1. Root-Shoot ratio

Results showed significant difference in root-shoot ratio between treatment and control varieties.

Water stress significantly increased the root-shoot ratio by suppressing the shoot growth than the root growth in tolerant variety. Maximum root-shoot ratio was found in PTB 7 (1.66) and minimum was observed in PTB 23 (0.73) under water stress. Root-shoot ration was increased by 43% in PTB 7 and decreased in PTB 23 by 35%.

Results regarding root-shoot ratio at drought and control condition are presented in the Table 10

Table 10. Root- shoot ratio under water stress and control condition

Treatment	PTB 7	PTB 23
Control	1.16 ± 0.09^{b}	1.12 ± 0.07^{a}
Water stress	1.66 ± 0.08^{a}	0.73 ± 0.05^{b}
% increase/ decrease	43%	-35%
SE(d)	0.071	0.048
SE(m)	0.051	0.034
CD (5%)	0.198	0.134

4.1.3.2. Root length (cm)

Results showed significant difference in root length between treatment and control varieties. The maximum root length was observed in PTB 7 (44.2 cm) under water stress condition, whereas the minimum root length was in PTB 23 (41.4 cm) under water stress condition. 15% increase in root length observed in PTB 7, whereas it was 9% reduction in PTB 23.

Results regarding root length at drought and control condition are presented in the Table 11.

Table 11. Root length under water stress and control condition

Treatment	PTB 7	PTB 23
Control	44.2±0.60[b]	45.7±1.63[a]
Water stress	50.9±0.77[a]	41.4±1.23[b]
% increase/ decrease	15%	-9%
SE(d)	0.567	1.177
SE(m)	0.401	0.832
CD (5%)	1.573	3.687

4.1.3.3. Root volume (cm^3)

Results showed significant difference in root volume between treatment and control varieties. Under water stress condition, PTB-7 increased the root volume by 21% compared to the control plant, whereas 15% reduction was observed in PTB-23.

Results regarding root volume at drought and control condition are presented in the Table 12.

Table 12. Root volume under water stress and control condition

Treatment	PTB 7	PTB 23
Control	32.8±1.04[b]	31.4±1.25[a]
Water stress	39.8±0.98[a]	26.7±0.60[b]
% increase/ decrease	21%	-15%
SE(d)	0.829	0.801
SE(m)	0.586	0.566
CD (5%)	2.301	2.223

4.1.3.4. Root dry weight (g)

Results showed significant difference in root dry weight between treatment and control varieties.

Under water stress condition PTB-7 showed higher root dry weight (14.69g) and lower in PTB 23 (6.92g). In PTB-7, root dry weight was increased to 37% and in PTB-23, it was decreased to 45% when compared to the control in the Table 13.

Table 13. Root- shoot ratio under water stress and control condition

Treatment	PTB 7	PTB 23
Control	10.72±0.90 [b]	12.56±0.61 [b]
Water stress	14.69±0.51 [a]	6.92±0.85 [a]
% increase/ decrease	37%	-45%
SE(d)	0.595	0.605
SE(m)	0.421	0.428
CD (5%)	1.653	2.057

4.2. EFFECT OF WATER STRESS ON PHYSIOLOGICAL PARAMETERS

4.2.1. Relative water content (RWC) (%)

Results showed that there was a significant difference for relative water content between treatment and control varieties. Relative water content of both varieties under water stress condition were reduced when compared to the control plants. In PTB-7, RWC decreased by 16% and in PTB-23, it was by 27% (Table 14).

Table 14. Relative water content under water stress and control condition

Treatment	PTB 7	PTB 23
Control	90.78±2.02 [a]	88.5±0.71 [a]
Water stress	74.63±1.20 [b]	61.7±1.58 [b]
% increase/ decrease	-16%	-27%
SE(d)	1.358	1.003
SE(m)	0.96	0.709
CD (5%)	2.102	2.785

4.2.2. Cell membrane stability Index (%)

Results showed that significant difference in cell membrane stability index between water stress induced and control plants.

Among the two varieties maximum cell membrane stability was found in PTB-7 (88.7%). Cell membrane stability index was reduced in both varieties. 10% reduction was observed in PTB-7 and 19 % was in PTB-23.

Results obtained for cell membrane stability index (%) under stress and control condition for both varieties are presented in the Table 15.

Table 15. Cell membrane stability index (%) under stress and control condition.

Treatment	PTB 7	PTB 23
Control	88.7±1.45 [a]	86.9±1.21 [a]
Water stress	79.8±1.65 [b]	70.6±1.26 [b]
% increase/ decrease	-10%	-19%
SE(d)	1.270	1.009
SE(m)	0.898	0.713
CD (5%)	3.526	2.800

4.3. EFFECT OF WATER STRESS ON BIOCHEMICAL PARAMETERS

4.3.1. Sucrose content (mg/g)

Results showed a significant difference in the sucrose content between treatment and control plants.

Water stress significantly increased the sucrose contents in leaves compared to the control plant. There was more accumulation of sucrose in the leaves of PTB 7 (6.62 mg/g) compared to PTB 23 (4.83 mg/g), that was 35% increase was found in PTB-7 and only 11% in PTB-23 (Table 16).

Table 16. Sucrose content under water stress and control condition

Treatment	PTB 7	PTB 23
Control	4.92±0.03 [b]	4.35±0.07 [b]
Water stress	6.62±0.09 [a]	4.83±0.04 [a]
% increase/ decrease	35%	11%
SE(d)	0.055	0.065
SE(m)	0.039	0.046
CD (5%)	0.150	0.181

4.3.2. Reducing sugar (mg/g)

Results shows a significant difference in the reducing sugar content between treatment and control plant.

Water stress significantly increased amount of reducing sugar in leaves compared with control. There was more accumulation of reducing sugar in the leaves of PTB 7 (37.97 mg/g) compared to PTB 23 (31.03 mg/g). In PTB-7, reducing sugar content was increased by 24% and in PTB-23, it was only by 6%.

Results regarding reducing sugar content at drought and control condition are presented in the Table 17.

Table 17. Reducing sugar content under water stress and control condition

Treatment	PTB 7	PTB 23
Control	30.53±0.75 [b]	29.40±0.56 [b]
Water stress	37.97±0.81 [a]	31.04±0.31 [a]
% increase/ decrease	24%	6%
SE(d)	0.639	0.367
SE(m)	0.452	0.259
CD (5%)	1.775	1.448

4.3.3. Activity of invertase (µmol /glucose mg protein h)

Results showed a significant difference in the activity of invertase between treatment and control plant. Activity of invertase enzyme in rice leaves were significantly higher under water stress. Notably, activity level of invertase was more enhanced in PTB 7 (4.86 µmol /glucose mg protein h) than PTB 23 (3.45 µmol /glucose mg protein h) due to water stress condition. PTB-7 showed 44% increase and PTB-23 showed 7% increase. Results regarding activity of invertase enzyme at drought and control condition are presented in the Table 18.

Table 18. Activity of invertase under water stress and control condition

Treatment	PTB 7	PTB 23
Control	3.37±0.2 [b]	3.21±0.2 [a]
Water stress	4.86±0.2 [a]	3.45±0.1 [b]
% increase/ decrease	44%	7%
SE(d)	0.17	0.12
SE(m)	0.12	0.08
CD (5%)	0.46	0.32

4.3.4. Activity of α-amylase (mg/g h)

Results showed a significant difference in the activity of α-amylase between treatment and control varieties.

Activity of α amylase enzyme in rice leaves was significantly higher under water stress. Notably, activity level of α-amylase was more increased in PTB 7 (28.5 mg/g h) than PTB 23 (18.9 mg/g h). PTB-7 showed 33% increase and PTB-23 showed 6% increase. Results regarding Activity of α amylase enzyme at drought and control condition are presented in the Table 19.

Table 19. Activity of α-amylase under water stress and control condition

Treatment	PTB 7	PTB 23
Control	21.4±0.23 [b]	17.9±0.36 [b]
Water stress	28.5±0.31 [a]	18.9±0.35 [a]
% increase/ decrease	33%	6%
SE(d)	0.221	0.291
SE(m)	0.156	0.205
CD (5%)	0.612	0.807

4.3.5. Activity of β-amylase (mg/g h)

Results showed a significant difference in the activity of β-amylase between treatment and control plant.

Activity of β-amylase enzyme in rice leaves were significantly higher under water stress. Notably, activity level of β-amylase was more increased in PTB 7 (8.7 mg/g h) than PTB 23 (6.9 mg/g h). PTB-7 showed 55% increase and PTB 23 showed 23% increase. Results regarding activity of β-amylase enzyme at drought and control condition are presented in the Table 20.

Table 20. Activity of β-amylase under water stress and control condition

Treatment	PTB 7	PTB 23
Control	5.6±0.15 [b]	4.8±0.06 [b]
Water stress	8.7±0.15 [a]	6.9±0.25 [a]
% increase/ decrease	55%	23%
SE(d)	0.124	0.149
SE(m)	0.087	0.105
CD (5%)	0.343	0.414

4.4. GENE EXPRESSION STUDY USING QUANTITATIVE REAL TIME PCR

Analysis of gene expression in leaf and root of the both varieties during panicle initiation stage of both water stress induced plant and control plant was carried out by using quantitative real time PCR. It was done. Two genes associated with the sucrose metabolism were selected for study *viz.*, sucrose synthase *(OsSuSy)* and *(OsSUC2)* with *UBQ5* (ubiquitin) as the internal reference gene.

4.4.1. Isolation of RNA from leaf and root samples

Total RNA was isolated from leaf and root of both the varieties during panicle initiation of the crop under drought stress and well-watered condition. Two bands (28S and 18S rRNA) were observed on agarose gel (2%)

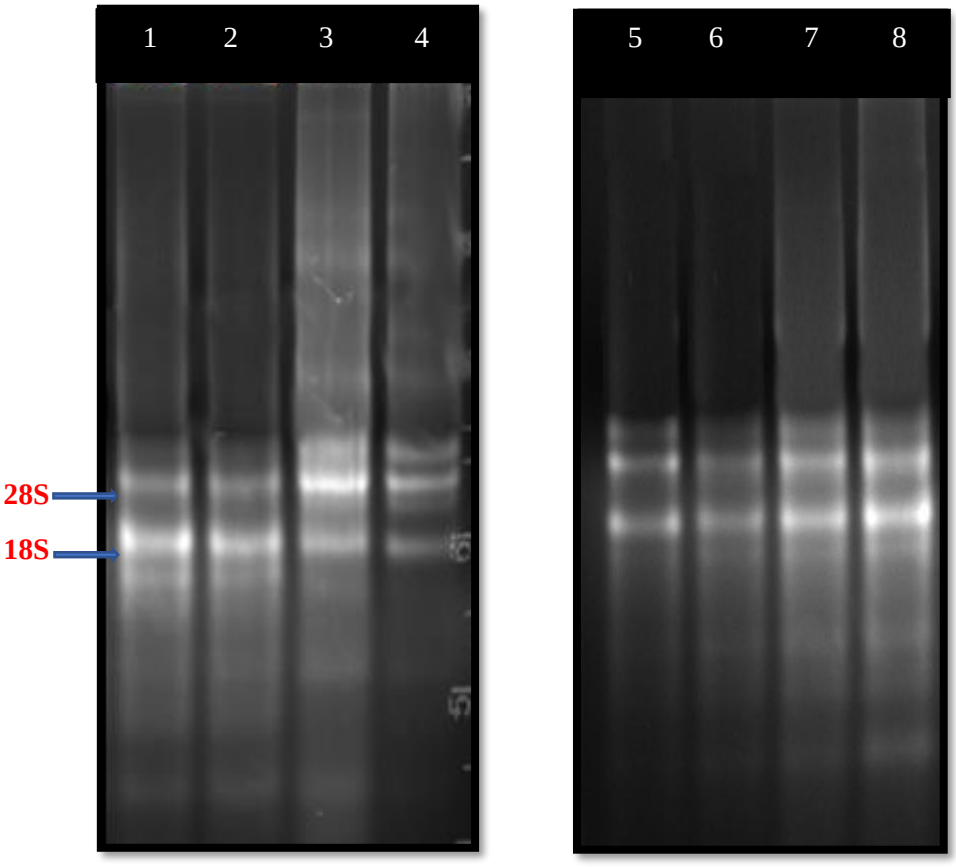


Plate 5. Gel profile with RNA bands of rice varieties.

Lane 1 – PTB 7 (root, control)

Lane 2 – PTB 7 (root, test)

Lane 3 – PTB 7 (shoot, control)

Lane 4 – PTB 7 (shoot, test)

Lane 5 – PTB 23 (root, control)

Lane 6 – PTB 23 (root, test)

Lane 7 – PTB 23 (shoot, control)

Lane 8 – PTB 23 (shoot, test)

4.4.2. Quantity and quality of isolated RNA

The quantity of the isolated RNA was determined through spectrophotometrically. Concentration of the RNA obtained was in the range of 1800-4000 ng/ µl. Good quality of RNA was obtained (*i.e.*, $A_{260/280}$ value was between 1.8 and 2). Both concentration of each RNA sample and its A_{260}/A_{280} value are represented in the Table 21.

Table 21. Concentration of and A_{260}/A_{280} value of RNA samples

Sl. No.	Variety	A_{260}/A_{280} Value	Concentration (ng/µl)
1	PTB-7 (root, control)	1.97	4011.0
2	PTB-7 (root, test)	2.12	3245.0
3	PTB-7(shoot, control)	1.86	2519.0
4	PTB-7 (shoot, test)	2.06	3054.0
5	PTB-23(root, control)	1.88	1849.0
6	PTB-23 (root, test)	1.80	3196.0
7	PTB-23(shoot, control)	2.07	2835.0
8	PTB 23 (shoot, test)	1.94	1943.0

4.4.3. Preparation and Quality check of cDNA.

In this study cDNA was synthesized using "Thermo-Scientific Verso cDNA Synthesis kit". The synthesized cDNA was confirmed using *UBQ5* gene (housekeeping gene). The expected amplicon size of the *UBQ5* gene (100 bp-200 bp) was obtained, which represented the good quality of synthesized cDNA.

4.4.4. Primer designing for Real Time PCR

Appropriate primers were designed for the genes such as *OsSuSy* and *OsSUC2* for the real time PCR by using NCBI Primer Blast.

The designed primers had the all qualities of a good primer. The length of the primer was in the range of 18-24. These primers had 40-60% GC content and ends with GC bases. Melting temperature was in the range of 50-60%.

Table 22. Details of the designed primers of *OsSuSy* and *OsSUC2* genes

	OsSUC2	*OsSuSy*
Forward primer	**ACCGGCATTGTCATTGCTTC**	**TGATTGCACTCTTCTCTAGGTATG**
Melting Temp (°C)	59.4	57.8
GC Content (%)	50.0	45.6
Length	20.0	24.0
Reverse primer	**AATGCCCATTGCTAGACCTTG**	**AGCCCGGAGAATGTCTTCAAA**
Melting Temp (°C)	58.9	59.6
GC Content (%)	49.8	47.2
Length	21.0	21.0

4.4.5. Quantitative real-time PCR analysis

Using the primers of *OsSUC2* and *OsSuSy*, the cDNA was subjected to real-time PCR with the reference gene *UBQ5*. Single peak was obtained in the melting curve of genes from the three technical replications. Single peaks in the melt curve indicates that there were no primer dimers formed during the real time PCR by using the designed primers of both genes.

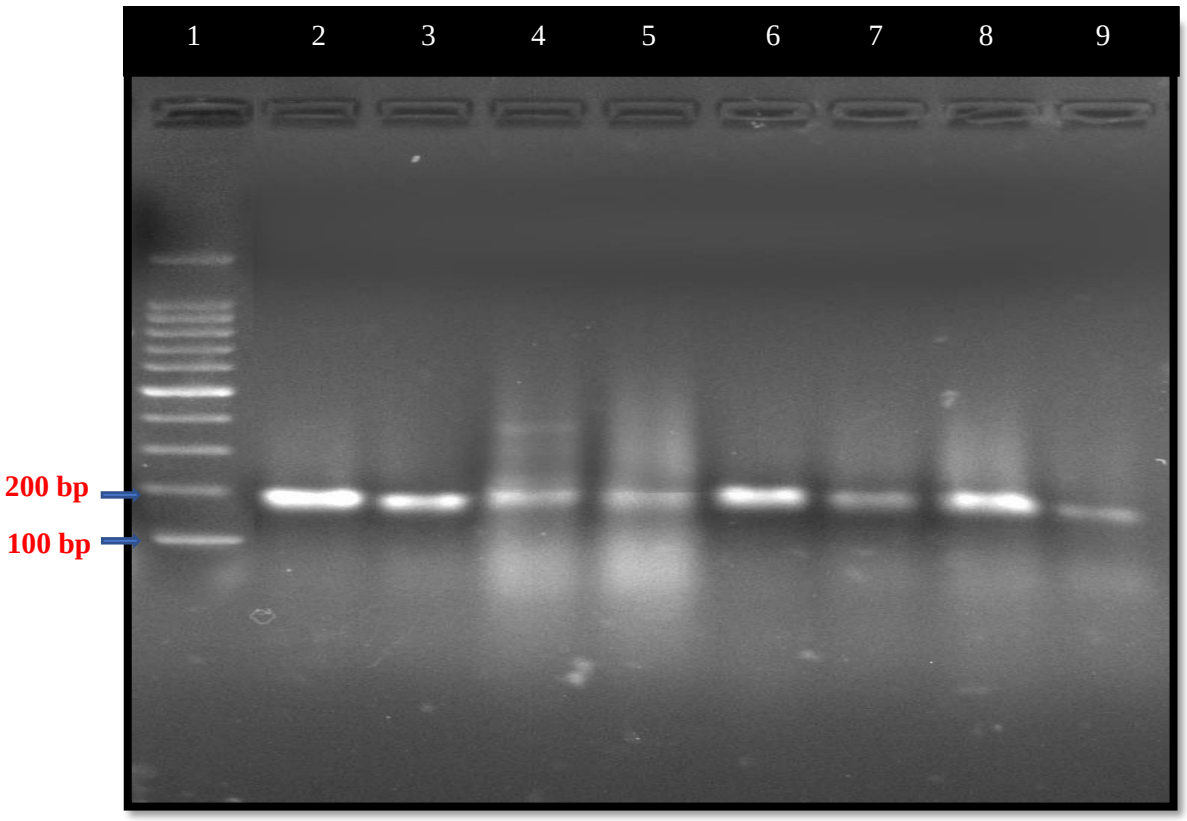


Plate 6. Amplification pattern of two rice varieties obtained by *UBQ gene.*

Lane 1 – 100 bp ladder

Lane 2 – PTB 7 (root, control)

Lane 3 – PTB 7 (root, test)

Lane 4 – PTB 7 (shoot, control)

Lane 5 – PTB 7 (shoot, test)

Lane 6 – PTB 23 (root, control)

Lane 7 – PTB 23 (root, test)

Lane 8 – PTB 23 (shoot, control)

Lane 9 – PTB-23 (shoot, test)

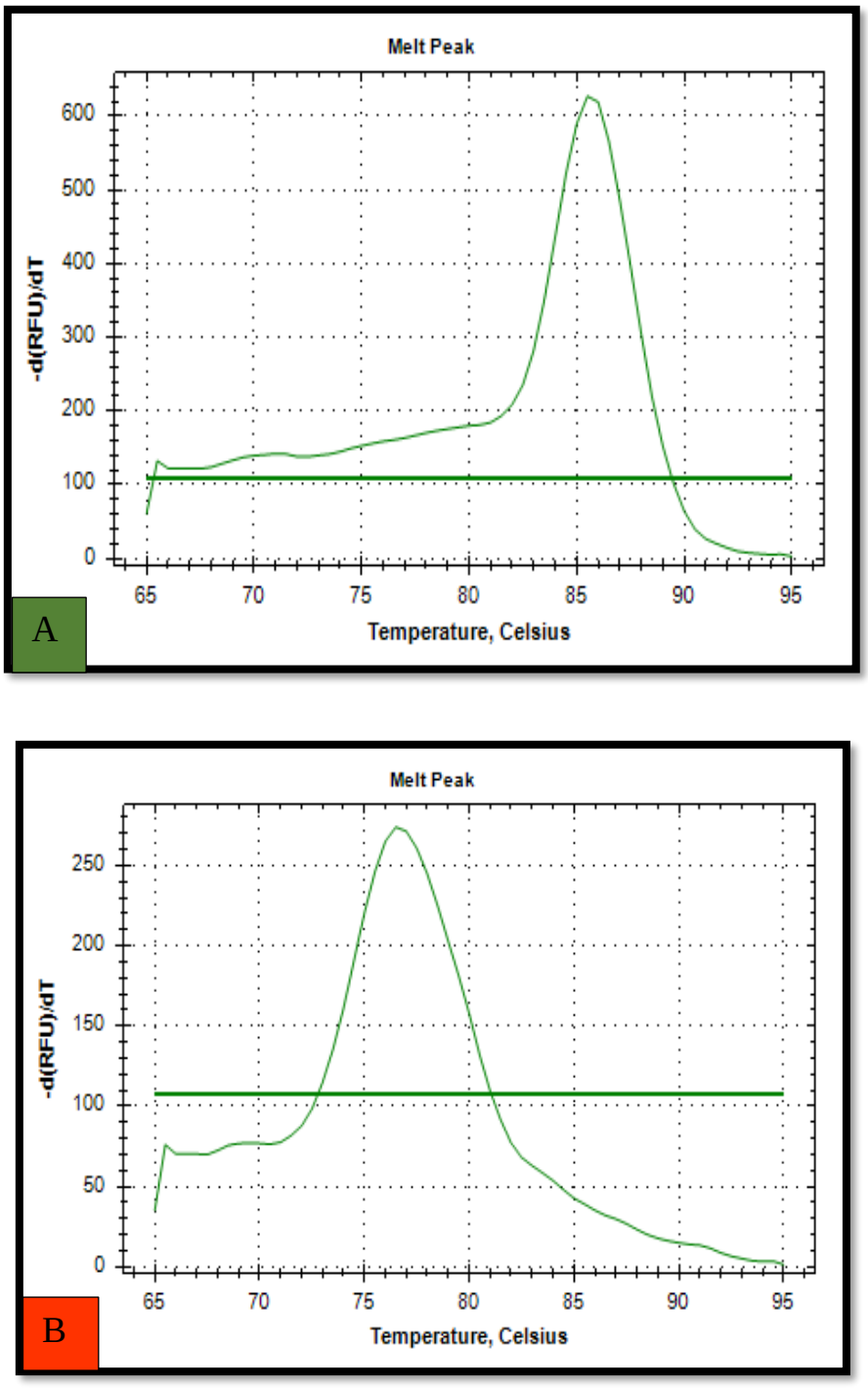


Plate 7. Melt curve analysis of genes (A- *OsSuSy* & B - *OsSUC 2*) by qRT-PCR

Differential expression of both genes in root and shoot were observed under water stress and control conditions in both varieties.

4.4.5.1. Relative expression of *OsSuSy* gene

Expression of *OsSuSy* gene was significantly higher in water stress induced plant compared to control in root and leaf of both varieties. The relative expression level of *OsSuSy* gene in root and leaf of PTB 7 was higher than PTB 23 under water stress condition.

4.4.5.1.1. Gene Expression of *OsSuSy* gene in the root

In the case of root, *OsSuSy* gene was upregulated under water stress condition. In PTB 7, an average increase of 3.78-fold expression was observed when compared to the control, but it was only 2.08-fold change in PTB 23. Results regarding the *OsSuSy* gene expression level in root at drought and control condition are presented in the Table 23.

Table 23. Gene Expression of *OsSuSy* gene in the root

Treatment	PTB 7	PTB 23
Control	1.03±0.10 [b]	1.00±0.10 [b]
Water stress	3.78±0.09 [a]	2.08±0.06 [a]
SE(d)	0.223	0.196
SE(m)	0.158	0.180
CD (5%)	0.960	0.703

4.4.5.1.2. Gene Expression of OsSuSy gene in the leaf

In leaf, the expression of *OsSuSy* gene was upregulated, but that was higher than the root. PTB 7 had higher expression of *OsSuSy* gene than PTB 23 that was 4.64-fold change. In PTB-23, it was 2.37-fold. Results regarding the *OsSuSy* gene expression level in shoot at drought and control condition are presented in the Table 24.

Table 24. Gene Expression of *OsSuSy* gene in the leaf

Treatment	PTB 7	PTB 23
Control	1.06±0.02 [b]	1.02±0.02 [b]
Water stress	4.64±0.07 [a]	2.37±0.05 [a]
SE(d)	0.052	0.013
SE(m)	0.037	0.009
CD (5%)	0.224	0.055

4.4.5.2. Relative expression of *OsSUC2* gene

Expression of *OsSUC2* gene was significantly upregulated in water stress induced plant as compared to the control plant in root and leaf of both varieties. The relative expression level of *OsSUC2* gene in root and leaf of PTB 7 was higher than PTB 23 under water stress condition.

4.4.5.2.1. Gene Expression of *OsSUC2* gene in the leaf

In root, *OsSUC2* gene showed a higher expression in stressed plant than control plant. PTB 7 had higher expression of *OsSUC2* gene than PTB 23 that was 5.62-fold change. In PTB 23, it was 2.01-fold. Results regarding the *OsSUC2* gene expression level in root at drought and control condition are presented in the Table 25.

Table 25. Gene Expression of *OsSUC2* gene in the leaf

Treatment	PTB 7	PTB 23
Control	1.00±0.01 [b]	1.00±0.04 [b]
Water stress	5.62±0.09 [a]	2.01±0.11 [a]
SE(d)	0.06	0.081
SE(m)	0.043	0.057
CD (5%)	0.259	0.348

4.4.5.2.2. Gene Express ion of *OsSUC2* gene in the shoot

In the case of shoot, the *OsSUC2* gene was upregulated, but not much higher than the root, PTB 7 had 3.9-fold expression as compared to the control, while PTB 23 had 2.03-fold expression. Results regarding the *OsSUC2* gene expression level in shoot at drought and control condition are presented in the Table 26.

Table 26. Gene Expression of *OsSUC2* gene in the shoot

Treatment	PTB 7	PTB 23
Control	1.00±0.11 [b]	1.00±0.05 [b]
Water stress	3.9±0.11 [a]	2.03±0.13 [a]
SE(d)	0.077	0.080
SE(m)	0.025	0.027
CD (5%)	0.076	0.074

DISCUSSION

5. DISCUSSION

Rice gets adversely affected by several abiotic and biotic stresses, leading to serious yield losses. One of the major abiotic stresses is the water stress, which reduces the crop production. Tolerance to stress is an important character with different phenotypes in rice at different developmental stages. The capacity of tolerance or susceptibility to the water stress is related to the action of various genes associated with the various pathways which co-ordinated with the stress.

Sucrose is the primary product of photosynthesis that not only act as the carbon source of the plant metabolism but also a signalling component for the plant growth and development. Water stress reduces the photosynthetic rate (Cuellar-Ortiz *et al*., 2008). However, it increases the activity of sucrose synthesising enzyme (sucrose phosphate synthase) in the source organ and results in the sucrose accumulation (Yang *et al.,* 2001) during water stress condition. In source as well as sink organ, enzymes such as sucrose synthase and invertase are responsible for the sucrose hydrolysis (Ruan *et al.,* 2010). These also show an increased activity under water stress, may causes the accumulation of hexose (Praxedes *et al.,* 2006).

Water is the main factor in agricultural production and the drought stress that has an antagonistic effect on crop production and leads to a decrease in yield. Environmental changes have serious effects on water resources and the frequency of droughts and floods is likely to increase in the future. Drought is an abiotic stress that, on the contrary, affects the development and improvement of plants at the molecular and metabolic level and, consequently, reduces productivity.

5.1. EFFECT OF WATER STRESS ON PHYSIOLOGICAL PARAMETERS

Plant height of both genotypes was negatively affected by water stress. Under water stress condition, 4% decrease in plant height was observed in PTB 7 and 9% in PTB 23. Previous studies reported that, during drought stress the rice cultivars at vegetative stage undergo a significant reduction in plant height (Manickavelu *et al*., 2006). The study conducted by Beena *et al.* (2012) reported that 10.4% reduction in plant height was observed in rice during drought stress. These results may be due

to the impairments in the cell division and cell expansion, which are responsible for the loss of cell turgor under water stress condition that is leads to the reduction in plant height (Allahmoradi *et al*., 2011). Razmjoo *et al.* (2008) reported that during drought stress conditions, plants limit their nutrient uptake because of the less soil moisture, which causes the reduction in length of the stem. Effect of water stress on plant height in both genotypes are expressed in the Fig. 1.

Water stress adversely affect the number of productive tillers, thereby causes the yield loss. In this study, there was a significant reduction in number of productive tillers under the application of water stress on both varieties, 31% decrease in PTB 7 and 48% reduction in PTB 23. This is because of the less transport of assimilates during water stress (Bhutta, 2006). The study conducted by Beena *et al. (*2012) reported that 25.7% reduction in number of productive tillers was observed in rice. The previous studies also supporting this observation by showed the result same as obtained (Castillo *et al.,* 2007). Effect of water stress on number of productive tillers in two rice genotypes (drought stress tolerant and susceptible) is expressed in Fig. 2.

Previous study of Du *et al*., (2020) found that, in soybean plants drought stress enhanced R/S ratio and promoted the drought resistance. The present study also showed that water stress induced an increase of the R/S ratio in stress tolerant rice variety (PTB 7), which was due to the restriction of shoot biomass accumulation compared to roots. In the case of susceptible variety (PTB 23) under water stress condition, there was 35% reduction in R/S ratio. R/S ratio can be considered as an important parameter in determining drought tolerance in rice (Mathan *et al.,* 2020). Genotypes having higher root characters were found to tolerate drought. Effect of water stress on root-shoot ratio in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig 3.

In the present study, under water stress, there was an increment in the root length. PTB 7 showed increase (15%) and PTB 23 showed the reduction (9%) under water stress condition. Better increment in root length indicates the improved root penetration ability (Yu *et al.,* 1995), which is a survival mechanism under

unfavourable condition. Effect of water stress on root length in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 4.

Root volume under water stress condition showed an increase by 21% in PTB 7 (tolerant) but in the case of susceptible variety (PTB 23), showed a decrease in root volume by 15%. This may be due to the decrease in moisture availability under water stress. Similar results were reported by Rejeth *et al. (*2020) and Nag (2008). Effect of water stress on root volume in the two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 5.

Root dry weight showed an increase of 37% in tolerant rice genotypes (PTB 7) due to water stress compared to control condition. Cruz *et al*. 1986, concluded that the increase in biomass of root under water stress condition is a survival mechanism to tolerate the crop under unfavourable stress condition. However, susceptible genotype, PTB 23 showed a decrease in root dry weight under stress condition compared to control condition. Effect of water stress on root volume in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig 6.

The results showed a significant decrease in RWC when exposed to water conditions when compared to control conditions. It was observed in PTB 7 was 74.63% and 61.7% in PTB 23 with percent decrease of 16% and 27% respectively. This may due to the adverse effect of water stress on water balance, and thus the reduced water potential of the leaves. Chowdhury *et al*. (2017) reported that drought-sensitive varieties show a significantly lower water potential than drought-resistant varieties. Baroowa and Gogoi (2016), discovered that different varieties have different varieties of ability of absorbption and water loss by transpiration through their stomata. Therefore, RWC can be used as an important indicator of water status under drought stress (Parvin *et al.,* 2015; Shanazari *et al.,* 2018). Effect of water stress on relative water content in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 7.

In this study, the result showed a significant decrease in the cell membrane stability index in both varieties when exposed to water stress condition when compared to

control condition. This is may be due to the overproduction of reactive oxygen species, which destroys cell membranes by changing the composition of phospholipids and fatty acids under drought stress conditions (Ratnasekera and Subhashi, 2015). In this study, the cell membrane stability index observed for PTB 7 under water stress was 79.8% and for PTB 23 was 70.6%, with reduction rates of 10% and 19%, respectively. Tyagi *et al*. (1999) reported high membrane stability index in resistant genotypes under water stress. The cell membrane stability index is considered to be the first defence mechanism in plants under stress conditions. Therefore, maintaining membrane stability and integrity is the plant's ability to withstand drought resistance (Ahmadizadeh *et al.*, 2011). Therefore, the cell membrane stability index can be used as an important indicator of water status under drought stress (Shanazari *et al*., 2018). Effect of water stress on cell membrane stability index in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 8.

5.2. EFFECT OF WATER STRESS ON BIOCHEMICAL PARAMETERS.

In this study, sucrose content was increased in both genotypes under water stress condition. A 35% increase was observed in PTB-7 and 11% in PTB-23. Previous study of Van den Ende and Valluru (2008) has shown that the greater sucrose accumulation was associated with better plant resistance to abiotic stress. Before reducing the photosynthesis, growth of the plant gets inhibited by the onset of water stress (Wang *et al.*, 2016), which could lead to the sucrose accumulation in source organ and disrupts the balance of sucrose metabolism. The sucrose accumulation detected in rice leaves in this study is consistent with data from an earlier study in which drought caused an increase in sucrose content in the source organs of the plant (Cuellar Ortiz *et al*., 2008)., where drought induced an increase in sucrose content in plant source organs (Cuellar-Ortiz *et al*., 2008). Effect of water stress on sucrose content in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 9.

In the current study, result showed that with the imposition of water stress, content of reducing sugar was increased in both varieties. The reducing sugar contents was

significantly increased in PTB 7 and PTB 23 about 24% and 6%, respectively. It was previously reported that, there was reduction in photosynthesis which affected the accumulation, mobilization, and distribution of sugars (Zayed and Zeid, 1998). Similar to our results, he had described that dried chickpea *(V. radiata)* seedlings increased hydrolytic enzymes such as α-amylase, which is also a reason of increased reducing sugar content. The accumulation of these organic solutes made it possible to improve cytoplasmic osmoregulation and thus increased plant's tolerance to the stress condition (Stancato, 2001). Effect of water stress on reducing sugar content in the two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 10.

Previous studies have shown that water stress controls the sucrose metabolic balance in leaves through changes in the activity of sucrose-metabolic enzyme (Pinheiro *et al*., 2001; Xu *et al*., 2015). The study conducted by Pinheiro *et al.* (2001) stated that, in *Lupinus albus* leaves water stress increases acid invertase enzyme activity. Study conducted by Chandra *et al., (*2012) were also supported the result of the present study, which also shows the higher activity of invertase enzyme was one of the major causes of sucrose accumulation in sucarcane. Du *et al*. (2020) described the activity levels of enzymes involved in sucrose metabolism, he found that, enzymes in the sucrose metabolism significantly enhanced under drought stress, indicating that soybean leaves consistently adjust their metabolic capacity to adapt with water stress. Also he found that activity of sucrose metabolising enzymes was higher in tolerant variety (PTB 7) than susceptible variety (PTB 23), which is also similar to the result of the present study. Effect of water stress on activity of invertase in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 11.

In higher plants, starch is an important temporary energy store that can be rapidly mobilized as a readily available source of sugar for the growth and development of sink organs in response to unfavourable conditions (Stitt and Zeeman, 2012). Water stress induces starch recycling in roots by increasing the activity of the enzymes such as α- and β-amylase (Kalpan *et al.*, 2006). In this study also α- and β-amylase

activities were significantly higher under drought stress, this leads to the increased starch content and promoted sucrose accumulation in roots. Previous studies have suggested that starch accumulation in the root system due to abiotic stress can improve starch status and root gravitation, increase nutrient uptake and root juice (Thitisaksakul *et al*., 2017). Starch can also be used as a stored energy for subsequent recycling to support root growth when favourable conditions are restored (Luquet *et al*., 2008). Osmotic stress promotes starch breakdown (Thalmann *et al.,* 2016). Du *et al.* (2020) concluded from their study that the increased starch degradation in soybean leaves promoted the conversion of starch to sucrose, which was beneficial for sucrose accumulation. Effect of water stress on α- and β-amylase content in two rice genotypes (drought stress tolerant and susceptible) is expressed in the Fig. 12 and 13 respectively.

5.3. EXPRESSION ANALYSIS OF GENES INVOLVED IN SUCROSE METABOLISM UNDER WATER STRESS

In this study, water stress significantly upregulated the expression level of *OsSuSy* and *OsSUC2* in the shoot and root of the rice genotypes. This results in accordance with earlier studies, when it was reported higher expression of *GmSuSy* and *GmSUC2* in soybean leaves and roots (Du *et al.,* 2020).

Sugar metabolism is also important for root development under drought stress (Xu *et al*., 2015). In this study, there was an upregulation of sucrose synthase gene in rice (*OsSuSy)* in shoot and root. Du *et al.* (2020) also found that drought stress increased the activity of SuSy and positively regulated the expression of *GmSuSy* in soybean leaves and roots. In this study and previous studies reported that sucrose synthase (*SuSy)* gene expression is higher in leaves than roots. This may lead to the accumulation of sucrose in leaves. Wang *et al. (*2016) previously concluded that drought stress reduced the growth of plant before inhibiting the photosynthesis, which causes the sucrose accumulation in leaves. In the study of Van den Ende and Valluru, (2008) described that the higher accumulation of sucrose (soluble sugars) was a natural tolerance of plants to the abiotic abiotic stress. Among the two varieties selected for this study PTB 7 shows higher tolerance to the water stress

condition. Expression analysis of *OsSuSy* gene under water stress in root and shoot expressed in Fig. 14 and 15 respectively.

Previous study reported that sucrose transporter (*SUCs)* expression level was related with sucrose transport capacity (Durand *et al.,* 2016), which was drought stress upregulated *AtSUC2* gene expression level in *Arabidopsis thaliana* and enhances the flow of carbon from leaves to roots. These changes improved the utilization efficiency of sucrose and starch. In addition, drought promoted the transport of sucrose from leaves to roots. Since the carbon source necessary for root growth and metabolism is transported from the leaves, improving the transport of sucrose from the leaves to the roots helps maintain root growth under drought stress. This seems to be the preferred mechanism for maintaining root growth and metabolism in response to drought stress (Mathan *et al.,* 2020). The present study determined that, *OsSUC2* gene expression level in root under water stress was higher when compared to the same in shoot. Also, the expression of the gene was high in PTB 7 than PTB 23. These results suggest that water stress increased the metabolic cycle of sucrose, resulting in increased accumulation of sucrose in the roots, which is a stress tolerant mechanism of rice to survive under stress condition. By this, we can also be concluded that PTB 7 is the tolerant rice variety. Expression analysis of *OsSUC2* gene under water stress in shoot and root expressed in Fig. 16 and 17 respectively.

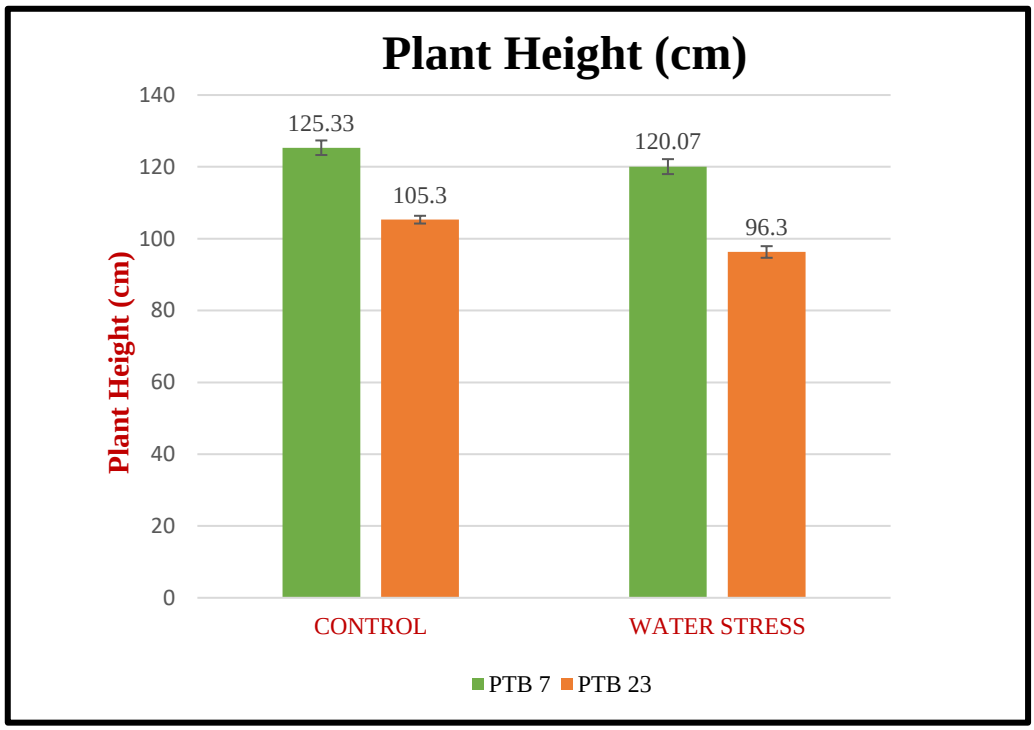


Fig 1. Effect of water stress on plant height in both rice genotype

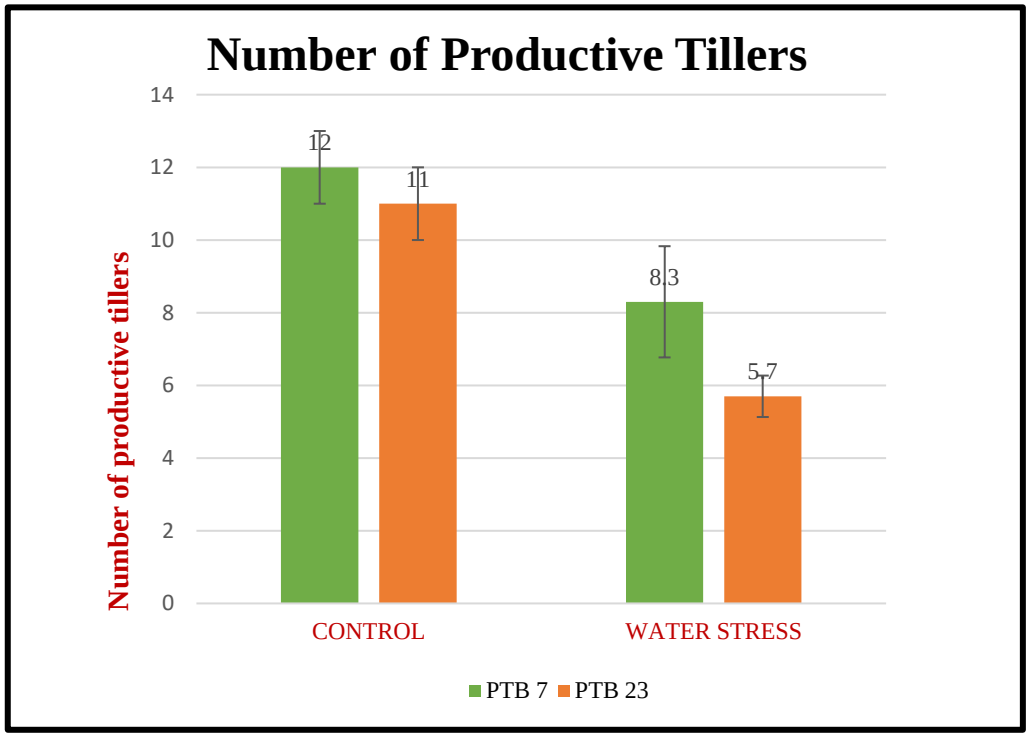


Fig 2. Effect of water stress on number of productive tillers in two rice genotypes

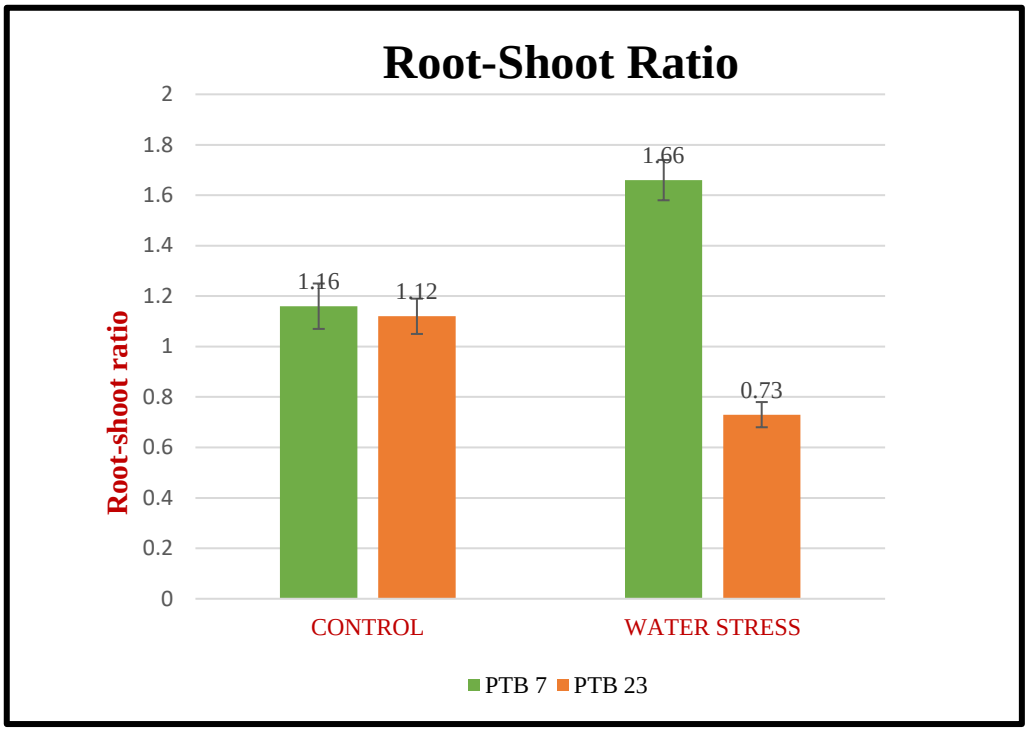


Fig 3. Effect of water stress on root-shoot ratio in two rice genotypes

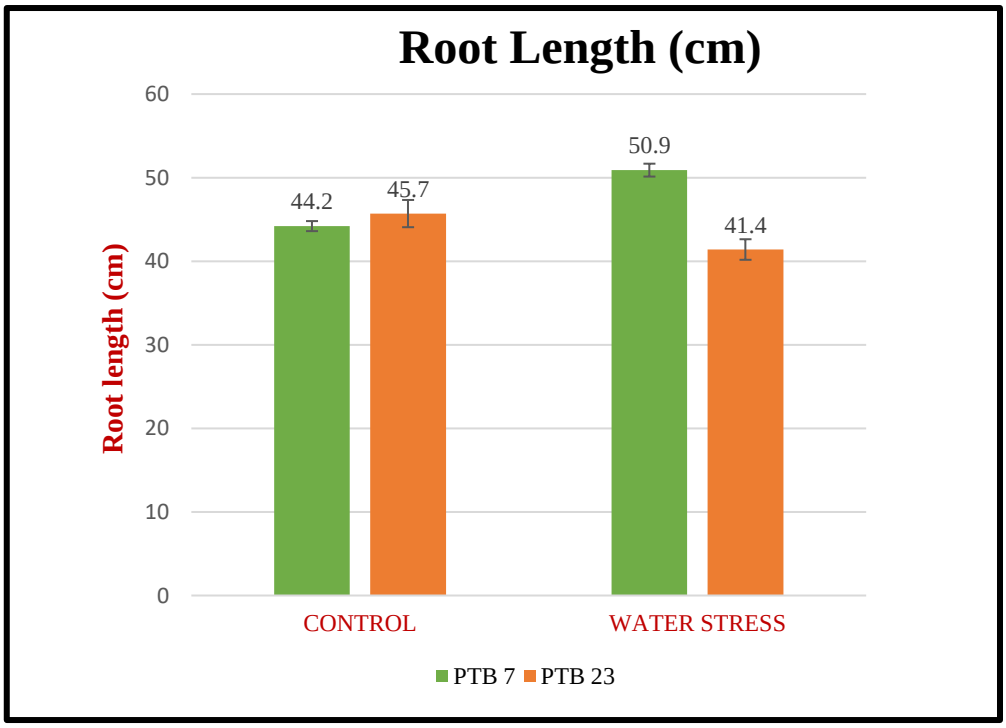


Fig 4. Effect of water stress on root length in two rice genotypes

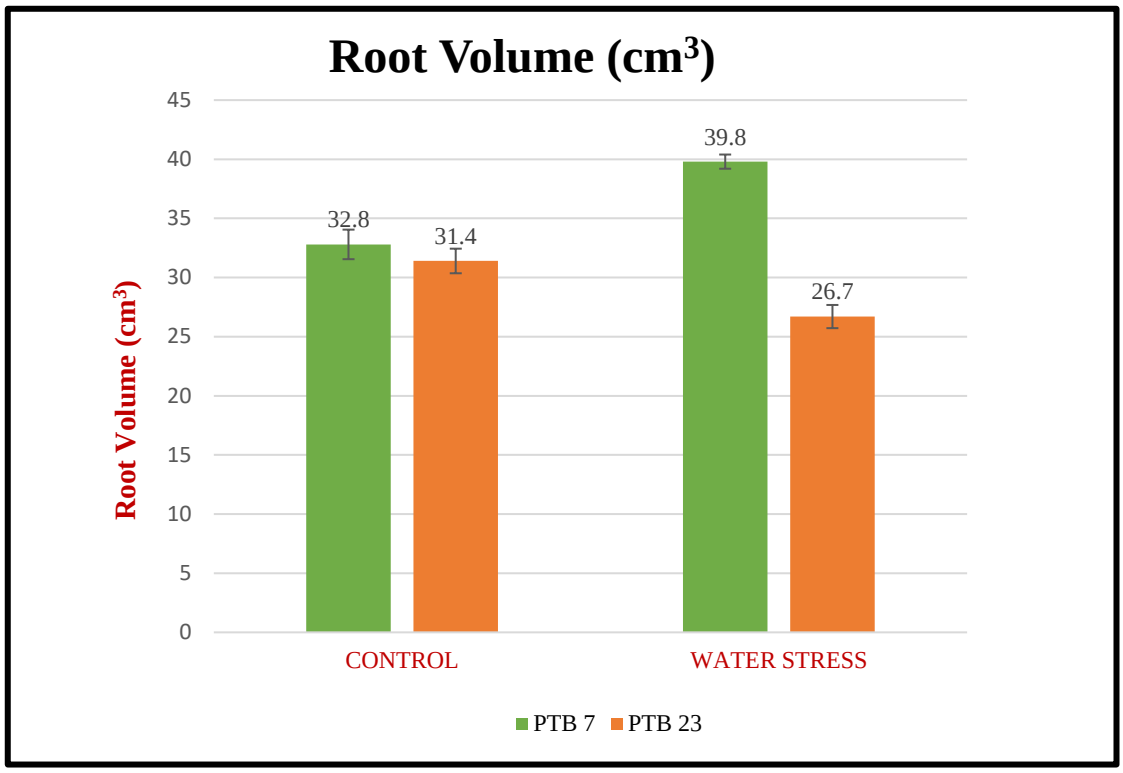


Fig 5. Effect of water stress on root volume in two rice genotypes

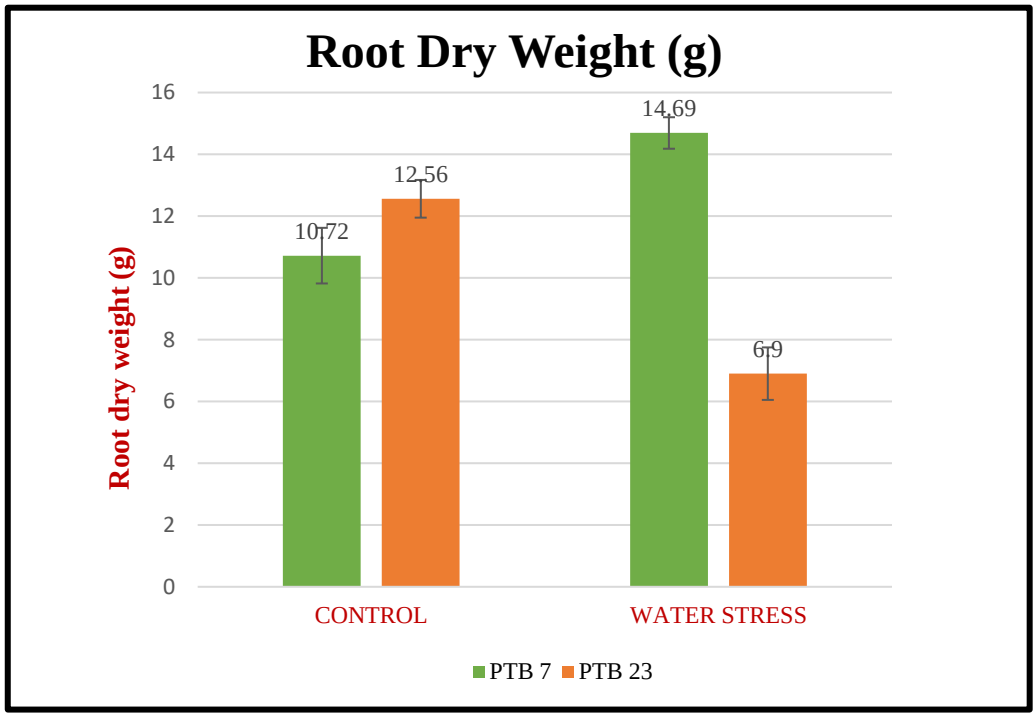


Fig 6. Effect of water stress on root dry weight (g) in two rice genotypes

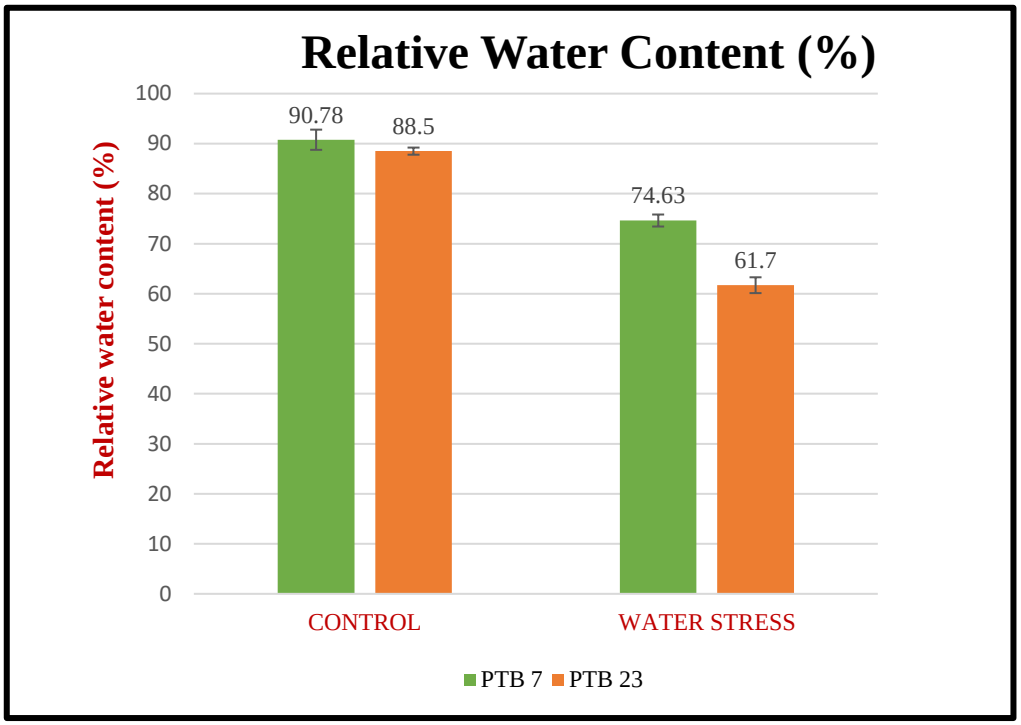


Fig 7. Effect of water stress on relative water content (%) in two rice genotypes

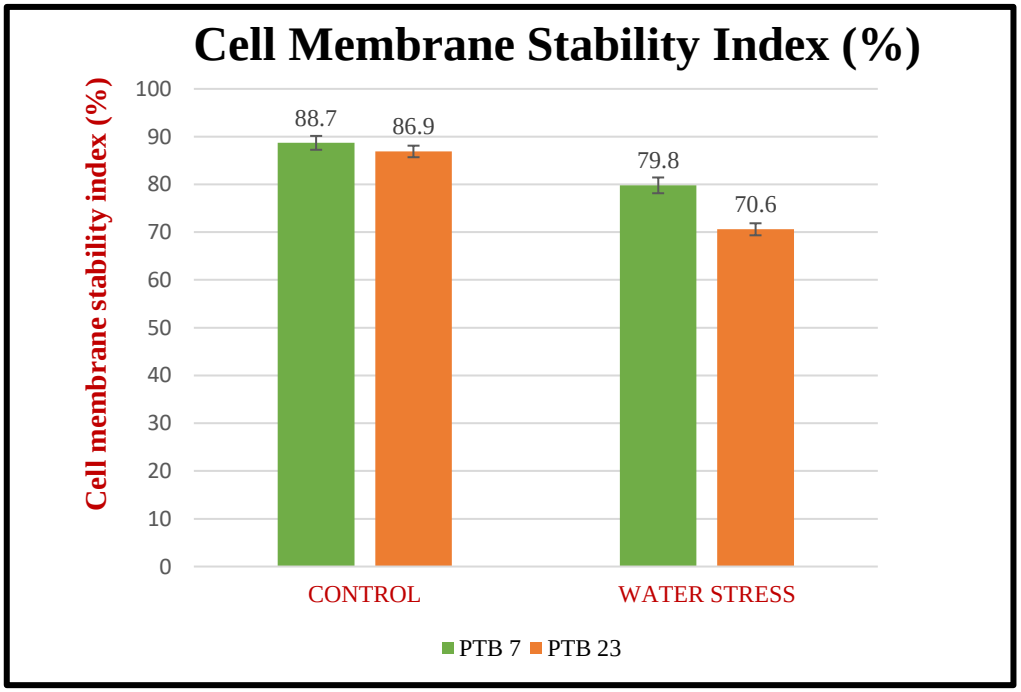


Fig 8. Effect of water stress on cell membrane stability index (%) in two rice genotypes

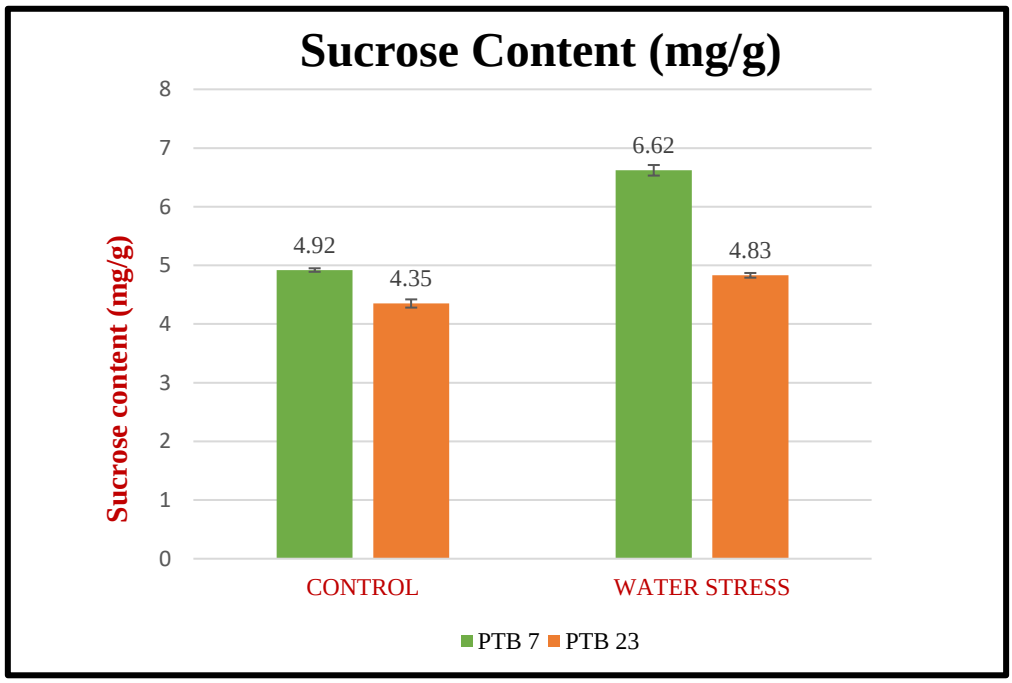


Fig 9. Effect of water stress on sucrose content (mg/g) in two rice genotypes

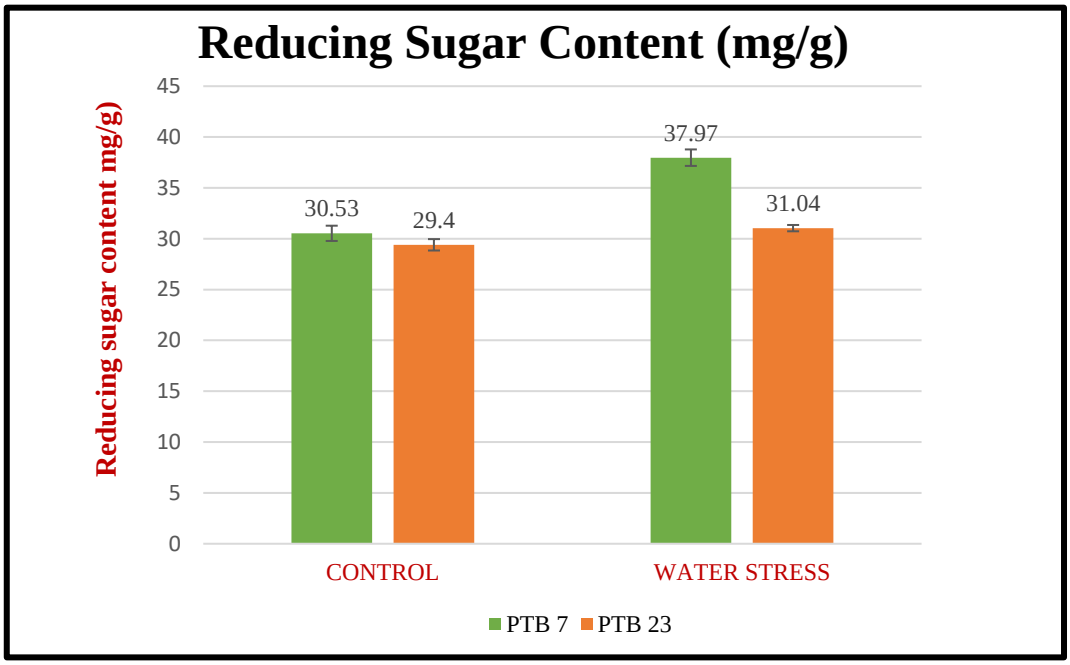


Fig 10 . Effect of water stress on reducing sugar (mg/g) in two rice genotypes

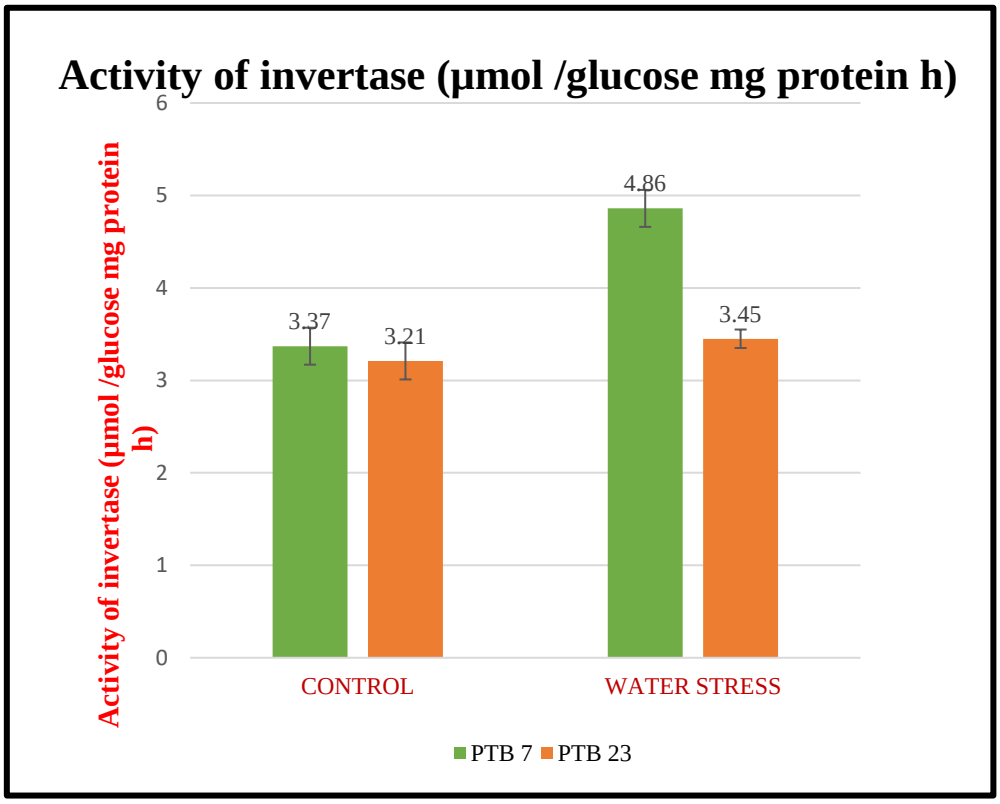


Fig 11 . Effect of water stress on invertase activity (µmol /glucose mg protein h)) in two rice genotypes

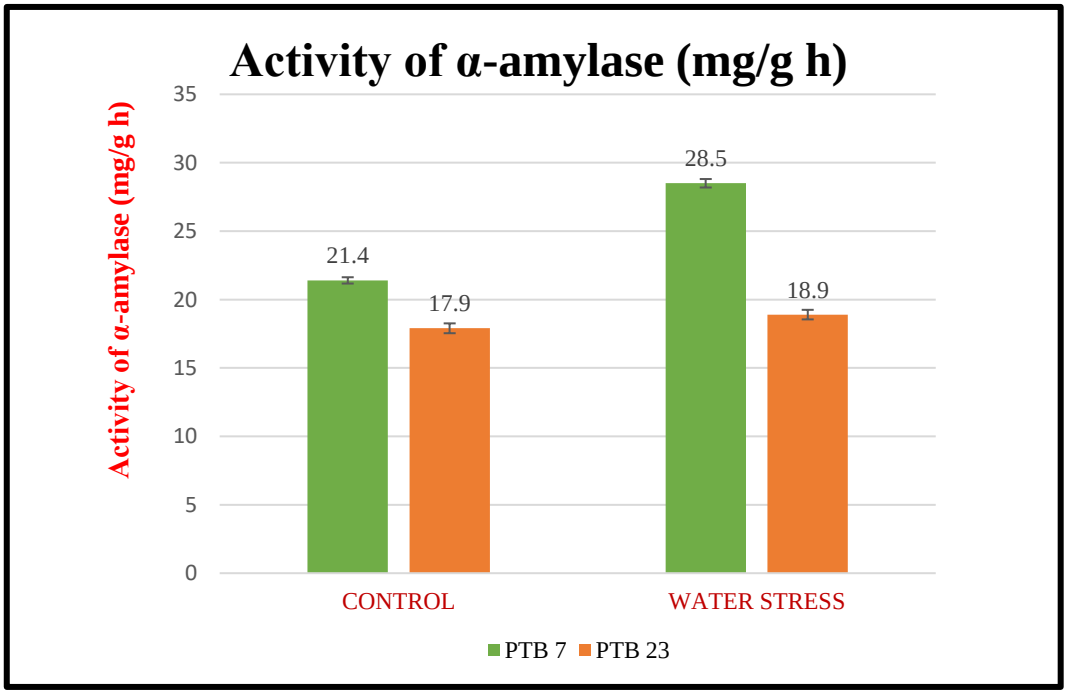


Fig 12 . Effect of water stress on α-amylase activity (mg/g h) in two rice genotypes

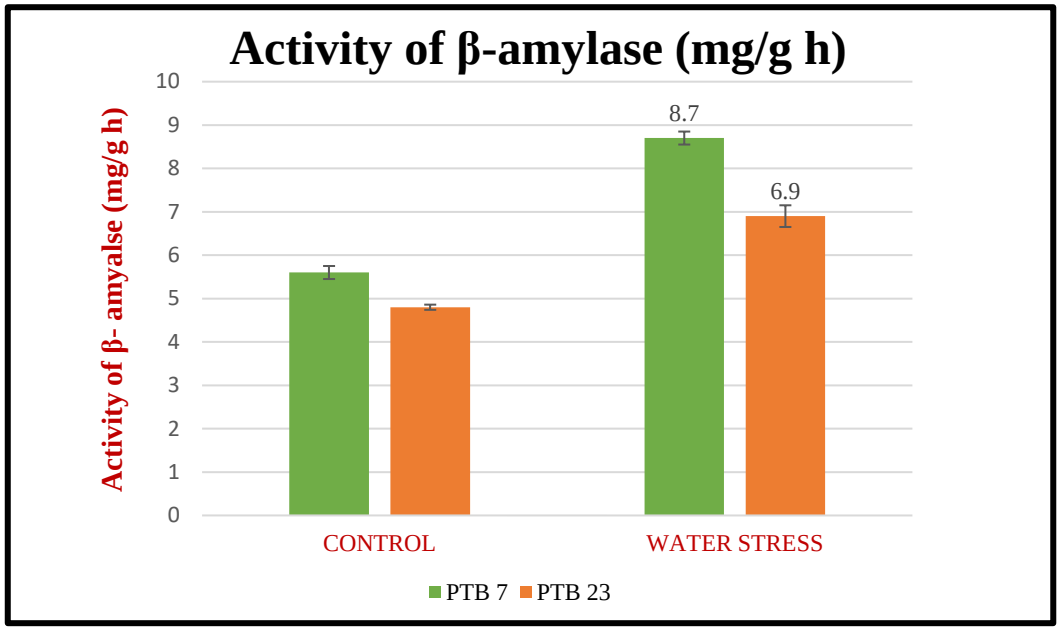


Fig 13 . Effect of water stress on β-amylase activity (mg/g h) in two rice genotypes

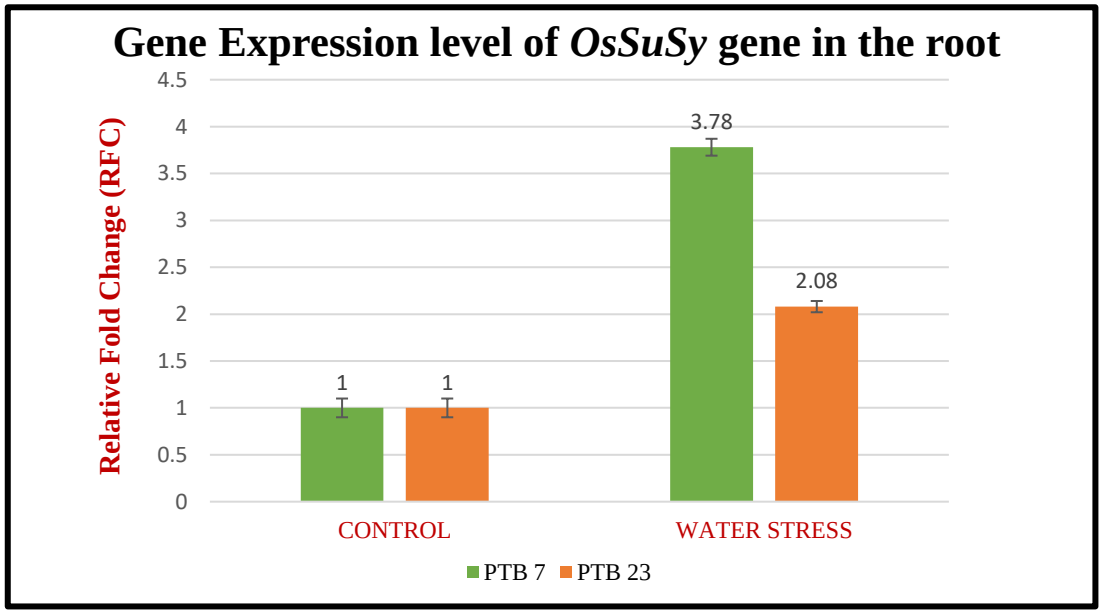


Fig 14 . Relative expression of *OsSuSy* in the root under water stress.

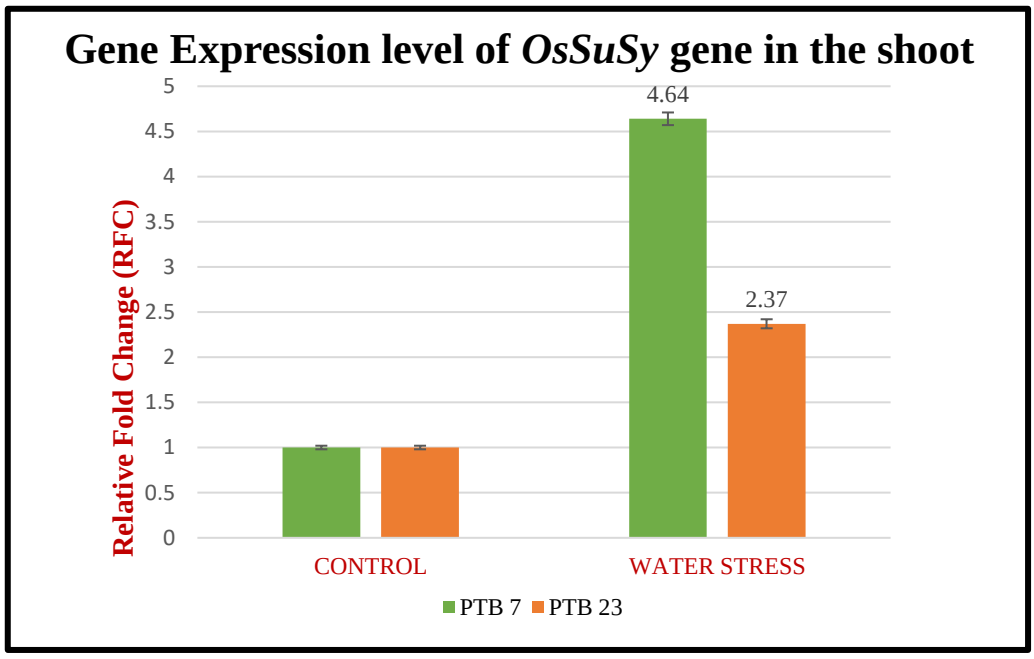


Fig 15 . Relative expression of *OsSuSy* in the shoot under water stress.

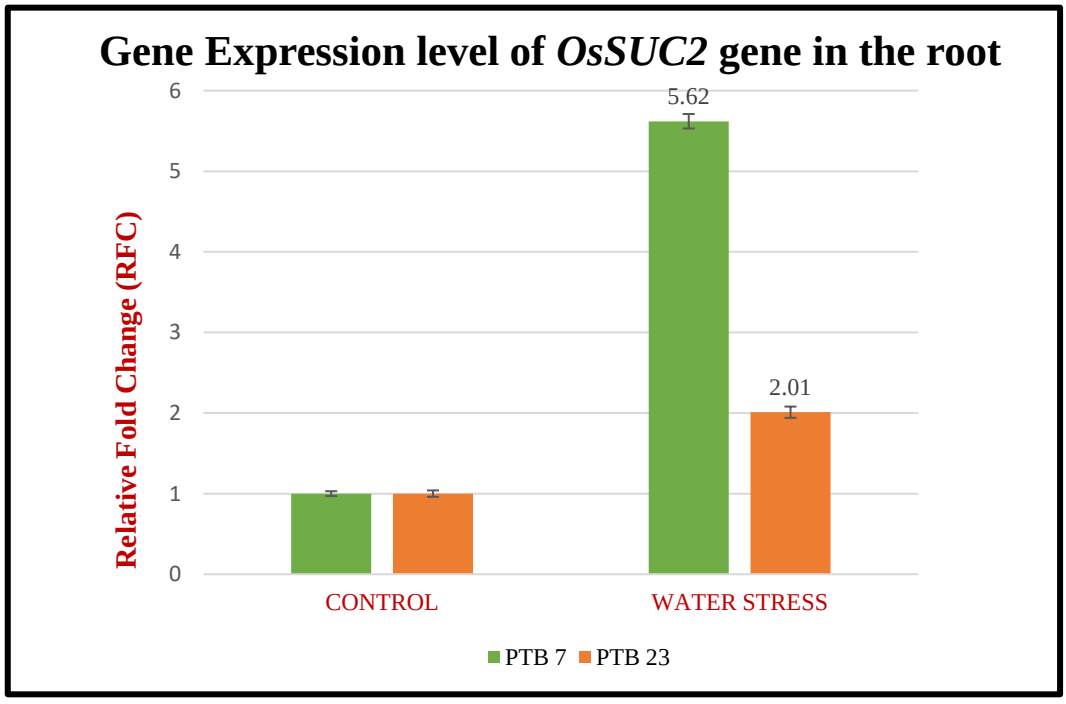


Fig 16. Relative expression of *OsSUC2* in the root under water stress.

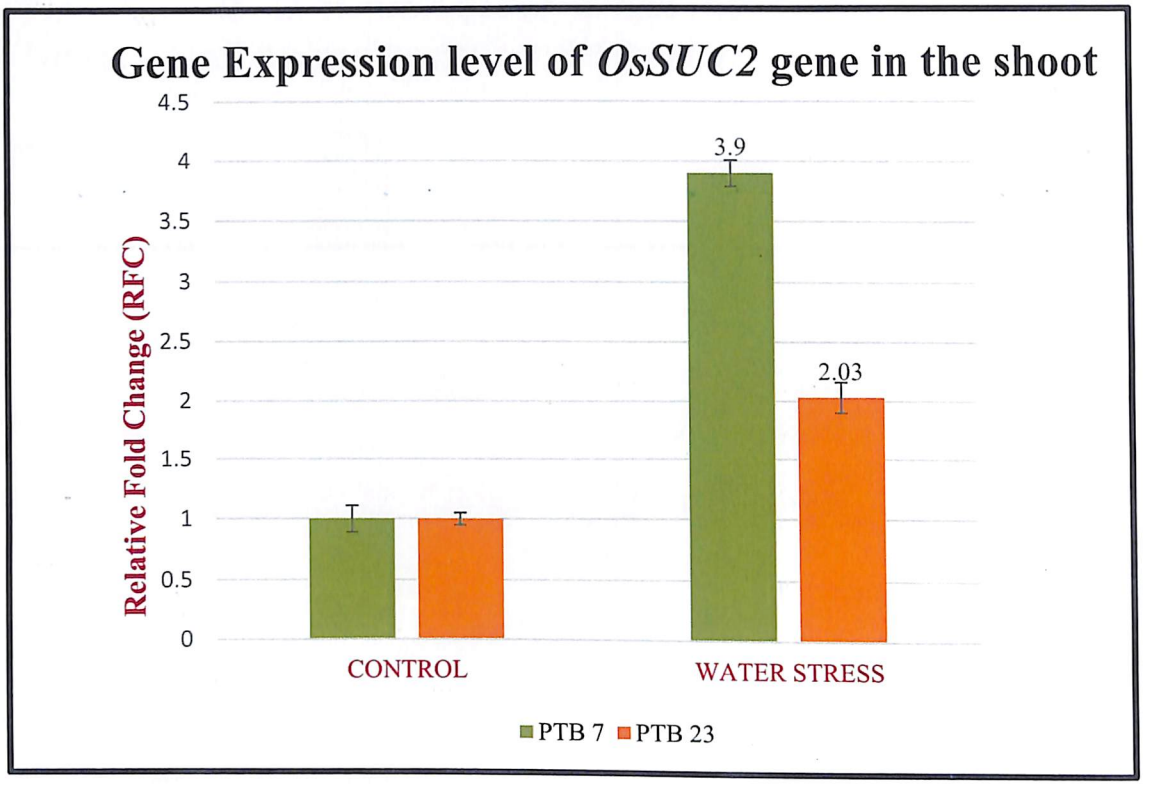


Fig 17. Relative expression of *OsSUC2* in the shoot under water stress.

SUMMARY

6. SUMMARY

The study on “Impact of water stress on sucrose metabolism in rice (*Oryza sativa* L.)” was conducted at Department of Plant Physiology and Department of Plant Biotechnology of College of Agriculture, Vellayani during 2020-21, with the objective of studying the impact of water stress on sucrose metabolism by analyzing the physiological and biochemical parameters and gene expression in selective drought tolerant and susceptible rice genotypes.

Rice is the staple food of approximate 90% population around the world and it is cultivated almost in all the countries. Being a semi aquatic annual grass, it is very much vulnerable to the water stress conditions, which may lead to its less productivity. In order to adapt these kinds of unfavorable situations, plants generally have certain mechanisms. One of the major methods is the enhanced accumulation of osmolytes or osmoprotectants like sucrose in the plant. This study primarily concentrated on changes of sucrose metabolism during water stress condition by analysing the action of enzymes involved in sucrose metabolism such as invertase, α- and β-amylase. Also the expression level of genes associated with the sucrose metabolism and transport include sucrose synthase (*OsSuSy*) and sucrose transporter (*OsSUC2*) gene.

In order to study the changes of sucrose metabolism during water stress condition, two rice genotypes were selected. One was drought tolerant PTB 7 and other was drought susceptible PTB 23. They were subjected to water stress by the withdrawal of irrigation for 5 days at the panicle initiation stage. Then the morphological, physiological, biochemical and molecular parameters were studied from the stress induced plants and control (unstressed) plants.

Plant height, number of productive tillers, root traits such as root-shoot ratio, root length, root volume, root dry weight were recorded for assessing the stress tolerance level of the plants and observed that PTB 7 had more tolerance to water stress than PTB 23, and also PTB 7 showed enhanced sucrose metabolism.

For assessing the sucrose metabolism, the activity of the sucrose metabolising enzymes were estimated. It was found that, the activity of enzymes were increased under stress condition for mitigating the stress situation in plants. Then expression level of genes such as *OsSuSy* and *OsSUC2* from root and leaves of both varieties were quantified using quantitative real time PCR. For the conducting qRT-PCR, total RNA was isolated and then quality and quantity of the isolated RNA were checked spectrophotometrically, after RNAs with good quality and quantity were taken for the cDNA synthesis.

Primers for the two genes were designed by using NCBI Primer BLAST. Then primers were checked for their specificity and quality in a software called Primer Express. After checking all the parameters essential for the good primer, most appropriate two primers were selected for the study. The designed primers were standardized for the annealing temperature through gradient PCR that was determined at 59^{o}C. Expression pattern of selected genes were studied by Real time PCR. *UBQ5* (ubiquitin) gene was used as the internal reference gene. For calculating the relative fold change in gene expression, the Ct values obtained from the analysis of Real time PCR were used. Comparative ΔΔCq method was used for calculating the relative fold change.

Both genes were highly expressed during the water stress condition in root and leaf of both varieties. However, PTB 7 showed comparatively higher expression of two genes (*OsSuSy* and *OsSUC2*) than PTB 23, which indicates that enhanced sucrose metabolism is a characteristics of water stress tolerance.

This study showed that water stress induced various morpho-physiological, biochemical as well as molecular changes in rice plants for their survival. Water stress enhanced the activity of enzymes associated with the sucrose metabolism and upregulated the gene expression of *OsSuSy* and *OsSUC2* in root and leaf of rice. So, sucrose metabolism seems to be the preferred method in rice to survive in response to water stress condition

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APPENDIX

APPENDIX I

80% ethanol: 50 ml

- Ethanol 40 ml
- Distilled water 10 ml

APPENDIX II

DNS Reagent

Dinitrosalicylic acid (1g), crystalline phenol (200 mg) and sodium sulphite (50 mg) were dissolved simultaneously in 1% NaOH solution (100 ml).

APPENDIX III

Standard Glucose solution (stock)

100 mg of glucose in 100 ml distilled water. Working standard: Dilute 10 ml of stock solution to 100 ml with distilled water (100 μg/ml)

APPENDIX IV

Sodium citrate buffer

- EDTA- 0.5mM
- DTT-3mM
- Sodium diethyldithiocarbamate-3mM
- Bovine serum albumin-1%
- PVP-2%

APPENDIX V

Gel loading dye

- Bromophenol blue 25mg
- Xylene cyanol FF 25mg
- Glycerol 3.3ml
- Distilled water 6.7ml

APPENDIX VI

1X TBE (Tris Borate EDTA) :1000 ml

- Tris base 27g
- Boric acid 13.75g
- 0.5M EDTA (pH 8.0) 10ml
- Final volume made up to 500ml with autoclaved distilled water. From 5X TBE, 1X was prepared before use.

TBE Buffer (1X)

- TBE Buffer (5X) 200ml
- Distilled water 800ml

ABSTRACT

IMPACT OF WATER STRESS ON SUCROSE METABOLISM IN RICE (*Oryza sativa L.*)

by

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ABSTRACT

The study entitled “Impact of water stress on sucrose metabolism in rice (*Oryza sativa* L.)” conducted at Department of Plant Biotechnology and Department of Plant Physiology, College of Agriculture, Vellayani during 2020-21. The objective was to study the impact of water stress on sucrose metabolism by analyzing the physiological and biochemical parameters and gene expression in selective drought tolerant and susceptible rice genotypes.

In this study, two rice varieties, drought tolerant variety, PTB 7 (Parambuvattan) and drought susceptible, PTB 23 (Cheriya Aryan) were grown in pot culture and after the panicle initiation stage, crops were subjected to water stress by withdrawing irrigation until the plants experienced the symptoms of stress (leaf rolling). Then the various physiological parameters were studied five days after the induction of water stress. Extraction and estimation of sucrose metabolizing enzymes such as invertase, α- and β-amylase were done five days after induction of stress spectrophotometrically. Expression levels of sucrose synthase *(SuSy)* and sucrose transporter gene (*SUC2)* were analyzed from both root and leaf seven days after induction of water stress.

Under water stress, physiological parameters such as cell membrane stability index, relative water content and yield trait like number of productive tillers were significantly reduced, activity of enzymes such as invertase, α- and β-amylase were increased and gene expression level of sucrose synthase (*SuSy*) and sucrose transporter (*SUC-2),* which are associated with the sucrose metabolism were upregulated. Water stress enhanced the sucrose content and reducing sugar content in rice plant. There was significant increase in root traits in PTB 7 but they were reduced in PTB 23. Also, there was a reduction in shoot biomass than the root biomass in tolerant rice variety, which leads to an increase in root to shoot (R/S) ratio.

In this study, sucrose metabolism and transport were increased in both drought tolerant and susceptible variety under water stress condition. However, PTB 7 (drought tolerant) showed an improved sucrose metabolism than PTB 23 (drought susceptible) during water stress condition.