ELUCIDATING THE ROLE OF GROWTH PROMOTING ENDOPHYTIC FUNGUS *Piriformospora indica* FOR WATER STRESS TOLERANCE IN RICE (*Oryza sativa* L.)

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2022

DECLARATION

I, hereby declare that the thesis entitled "**Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance 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 to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Vellayani

Date: 03.10.2022

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CERTIFICATE

Certified that this thesis entitled "**Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)**" is a record of research work done independently by **Ms. LEKSHMI MOHAN S (2019-11-197)** under my guidance and supervision and this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)

by

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(2019-11-197)

THESIS

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µl	Microlitre
^{o}C	Degree Celsius
CD	Critical difference
cm	Centimeter
ml	Millilitre
M	Molar
EC	Elecrtical conductivity
ppm	Parts per million
CRD	Completely Randomized Design
rpm	Rotations per minute
et al.	and other Co workers
OD	Optical density
Fig.	Figure
g	Gram
i.e.	That is
KAU	Kerala Agricultural University
mm	Millimeter
viz.	Namely
IPCC	Inter-Governmental Panel on Climate change
IAA	Indole-3-acetic acid
SOD	Superoxide dismutase
mg	Milligram
nm	Nanometer
s	Seconds
A663	Absorbance at 663nm
A645	Absorbance at 645nm
A480	Absorbance at 480nm

A510	Absorbance at 510nm
A520	Absorbance at 520nm
A460	Absorbance at 460nm
ABA	Abscisic acid
JA	Jasmonic acid
ROS	Reactive oxygen species
DAC	Days after Cocultivation
CD	Critical Difference
CAT	Catalase
SE	Standard error

Introduction

1. INTRODUCTION

Rice is a way of life for more than half of mankind. Rice has influenced the cultures, cuisines and economies of millions of people worldwide. It has an important role in relieving poverty and hunger; and also, in ensuring food security in underdeveloped and developing countries around the globe. Domestication of rice is unquestionably one of the most significant events in history for rice provides nutritional support to around 3.5 billion people worldwide, mostly in Asia. (Gnanamanickyam, 2009).

Rice is a crucial Indian crop and millions of Indians' sustenance depends on it. India is the leading consumer as well as the second largest producer (177.65 million tons) after China (209.6 million tonnes). (FAO 2019). In many countries, including India, rice is equated with food security and is even correlated with political stability. This effectively means production should continue to increase to cater for the rise in population and the associated demand, both now and in the future (Satterthwaite *et al.*, 2010). Since the area under production in India (43.79 million hectares) (GOI, 2019) is limited, the exigency of the situation demands increase in production. This can be attained by the cultivation of diverse types of rice with required agricultural characteristics like high productivity, biotic and abiotic stress tolerance etc.

Paddy farming is some thousands of years old practice and this requires partially to fully flooded conditions. Amongst the various abiotic stresses, drought is the most devastating one which can impair the different morphological parameters and can be observed in all phenological stages of crop growth (Gaspar *et al.*, 2002, Zhang *et al.*, 2017). It is a multifaceted stress that leads to variations in the ecological, morphological, physiological, biochemical and molecular traits of plants (Bhargava and Sawant, 2013). It is estimated that half of world rice production is affected by drought (Lafitte and Bennet, 2003.) Rice crop establishment is severely hampered by soil moisture deficit during the early stages of development. It is assessed that by 2025, drought will impact 30 percent of global crop yield (Zhang, 2011).

Various measures, such as germplasm exploitation (Li *et al.*, 2016), effective management practices (Haefele *et al.*, 2016), and utilizing the significant association of rice with endophytes, can aid in improving rice grain output under drought-prone situations (Gill *et al.* 2016). Drought tolerance in rice can be improved by genetic manipulation and it has found traction in the recent years (Jena and Nissila, 2017). Water management during rice field preparation and during the whole crop development period can help in water conservation and availability during droughts. Furthermore, seed priming, foliar application of growth regulators and osmo-protectants, and adequate phosphorus (P), zinc (Zn), and silicon (Si) treatment can all help to offset drought impacts in a water-scarce environment (Taiz and Zeiger, 2006). According to Varma *et. al.* (2013) a symbiotic relationship with some endophytes can improve grain production during stress conditions. Mycorrhizal fungal hyphae can penetrate deep into the rhizosphere, transporting water and minerals to plant roots and keeping them moist even when water is scarce.

Piriformospora indica an extremely adaptable root colonising endophytic fungus, belonging to the order Sebacinales was discovered from roots of xerophytic plants in the Thar desert in Rajasthan. An increase in plant growth, early flowering, higher grain yield, changes in the plant secondary metabolites, abiotic and biotic stress adaptation are some of the positive results following the root colonization by the endophyte (Varma *et. al.,* 2012). It had positive effect on host plants growth under saline environment, water stress, temperature shocks as well as biotic stresses. *P. indica* effectively help plants to overcome abiotic stress like drought in *Arabidopsis thaliana* and Chinese cabbage (Sun *et al.*, 2010). *P. indica* colonizes root cells and enhances biomass production and water stress tolerance by increased proline accumulation and reduced malondialdehyde (MDA) during water stress in maize. Colonized plants had higher chlorophyll content and increased photosynthetic efficiency under water stress compared to the non-colonized plants (Sherameti *et al.*, 2005).

In rice, the fungal association improved biomass of seedling, uptake of functional elements like Zinc and Phosphorous, chlorophyll fluorescence, proline content, as well as the total antioxidant capacity under water stress (Saddique *e, al*., 2018). Furthermore, it also raises the leaf surface temperature, promotes the closure of stomata in addition to lowering lipid peroxidation in rice seedlings (Tsai *et. al*., 2020).

Even though the literature available on the subject of *P. indica* was aplenty, holistic studies on the fungus' capability to mitigate water stress in rice were rare. This was particularly evident regarding the rice landraces and other local varieties. The rich genetic diversity of rice landraces is important in creating an environment that facilitate agriculture in the context of a looming climate change (Gopi and Manjula, 2018) and understanding how the inoculation of growth promoting root endophyte *P. indica* to the water stress susceptible varieties as a promising strategy to enhance a plant's ability to tolerate water stress can prove to be a game changer in preserving the traditional cultivars as well as sustaining higher productivity.

In this context, the present study titled "Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)" carried out at Department of Plant Physiology, College of Agriculture, Vellayani during 2020-2021 was undertaken with the following objective.

- Elucidating the changes in morphological, physiological, biochemical and molecular mechanisms associated with water stress tolerance in *Piriformospora indica*-colonised rice.

Review of literature

2. REVIEW OF LITERATURE

Rice is a way of life for more than half of mankind. Rice has influenced the cultures, cuisines and economies of millions of people worldwide. It has an important role in relieving poverty and hunger and also in ensuring food security in underdeveloped and developing countries around the globe. Domestication of rice is unquestionably one of the most significant events in history for rice provides nutritional support to around 3.5 billion people worldwide, mostly in Asia. (Gnanamanickyam, 2009).

Rice is a crucial Indian crop and millions of Indians' sustenance depends on it. India is the leading consumer as well as the second largest producer (177.65 million tons) after China (209.6 million tonnes). (FAO, 2019). In many countries, including India, rice is equated with food security and is even correlated with political stability. This effectively means production should continue to increase to cater for the rise in population and the associated demand, both now and in the future (Satterthwaite *et al.*, 2010). Since the area under production in India (43.79 million hectares) (GOI, 2019) is limited, the exigency of the situation demands increase in production. This can be attained by the cultivation of diverse types of rice with required agricultural characteristics like high productivity, biotic and abiotic stress tolerance etc.

Rice belongs to the tribe Oryzeae of the family Poaceae and subfamily Oryzoidae. Chang (1976) reported that cultivated rice belongs to the genus Oryza, probably originated at least 130 million years ago and spread as wild grass in the supercontinent Gondwanaland which in the course of time fragmented and drifted apart to become Asia, Africa, America, Australia and Antarctica.

2.1 EFFECT OF DROUGHT ON RICE

The IPCC 2021 report has warned of an increase in drought frequency particularly in recent times, resulting in water shortages in rainfed agriculture, posing serious threats to livelihoods and food security, particularly for the world's most vulnerable people in the least developed countries (IPCC, 2021). A major barrier to achieving sustainable development is: 3.2 billion people live in agricultural regions with moderate to severe water shortages or scarcity, with 1.2 billion – nearly one-sixth of the world's population – living in highly water-constrained agricultural areas. (FAO, 2020)

Paddy farming is some thousands of years old practice and this requires partially to fully flooded conditions. Amongst the various abiotic stresses, drought is the most devastating one which can impair the different morphological parameters and can be observed in all phenological stages of crop growth. (Gaspar *et al.*, 2002, Zhang *et al.*, 2017). It is a multifaceted stress that leads to variations in the ecological, morphological, physiological, biochemical and molecular traits of plants (Bhargava and Sawant, 2013). It is estimated that half of world rice production is affected by drought. (Lafitte and Bennet, 2003.) Rice crop establishment is severely hampered by soil moisture deficit during the early stages of development. It is assessed that by 2025 drought will impact 30 percent of global crop yield (Zhang, 2011).

2.2. ADAPTATION MECHANISM TO DROUGHT TOLERANCE IN RICE

Drought resistance can be classified into drought avoidance (maintenance of tissue water potential), drought escape (flowering to complete life cycle before drought), and drought tolerance (survival mechanisms) (Levitt, 1980). Drought tolerance is the ability of plants to continue normal cellular metabolism and growth activity at low water potential despite prevailing stress condition and/or the ability to recover fast after stress. According to Basu *et al.*, (2016) a crop is considered tolerant only if it survives drought with minimal yield penalty. The adaptation mechanism of drought tolerance and the pathways regulating water stress tolerance in rice has been studied extensively.

Various measures, such as germplasm exploitation (Li *et al.*, 2015), effective management practices (Haefele *et al.*, 2016), and utilizing the significant association of rice with endophytes, can aid in improving rice grain output under drought-prone situations (Gill *et al.* 2016). Drought tolerance in rice can be improved by genetic manipulation and it has found traction in the recent years (Jena and Nissila, 2017). Water management during rice field preparation and during the whole crop development period can help in water conservation and availability during droughts. Furthermore, seed priming, foliar application of growth regulators and osmo-protectants, and adequate phosphorus (P), zinc (Zn), and silicon (Si) treatment can all help to offset drought impacts in a water-scarce environment (Taiz and Zeiger, 2006). According to Varma *et. al.* (2013) a symbiotic relationship with some endophytes can improve grain production during stress conditions. Mycorrhizal fungal hyphae can penetrate deep into the rhizosphere, transporting water and minerals to plant roots and keeping them moist even when water is scarce.

2.3. ROLE OF *P. indica* IN WATER STRESS TOLERANCE

P. indica an extremely adaptable root colonising endophytic fungus, belonging to the order Sebacinales was discovered from orchid plants in the Thar desert in Rajasthan. An increase in plant growth, early flowering, higher grain yield, changes in the plant secondary metabolites, abiotic and biotic stress adaptation are some of the positive results following the root colonization by the endophyte (Varma *et. al.,* 2012). It had positive effect on host plants growth under saline environment, water stress, temperature shocks as well as biotic stresses. *P. indica* effectively help plants to overcome abiotic stress like drought in *Arabidopsis thaliana* and Chinese cabbage (Sun *et al.,* 2010). *P. indica* colonizes root cells and enhances biomass production and water stress tolerance by increased proline accumulation and reduced malondialdehyde (MDA) during water stress in maize. Colonized plants had higher chlorophyll content and increased photosynthetic efficiency under water stress compared to the non-colonized plants (Sherameti *et al.*, 2005). In rice the fungal association improved biomass of seedling, uptake of functional elements like Zinc and Phosphorous, chlorophyll fluorescence, proline content, as well as the total antioxidant capacity under water stress (Saddique *e, al*., 2018). Furthermore, it also raises the leaf surface temperature, promotes the closure of stomata in addition to lowering lipid peroxidation in rice seedlings (Tsai *et. al*., 2020). The endophyte also has the potential to be used in biotechnological applications because of its rapid propagation, interaction with model plants and ability to possibly identify fungal factors that trigger the interaction with plants (Oelmüller *et al.*, 2009).

2.4. EFFECT OF *P. indica* ON MORPHOLOGICAL PARAMETERS UNDER WATER STRESS

2.4.1. Shoot length

Early vigour, which refers to the rapid emergence and early-stage development of plants, is a desired feature for better adaptation and crop yield since it allows for speedy plant stand and ground cover establishment (Shi *et. al*., 2020). Robust and healthy shoots and roots of rice seedlings improve drought or water stress resistance and recovery after transplanting. Vigorous shoots are required for higher energy utilization efficiency and increased photosynthates (Zhao *et. al.*, 2019). Jogawat *et. al.* (2013) in a study on the association of *P. indica* with rice reported that under stress condition, the root colonization increased the shoot length of rice seedlings. The seedlings also performed better under colonization by the fungus than uncolonized seedlings.

A study conducted by Nahar et. al. (2018) found that imposing drought diminished the growth of almost all the 21 traditional rice cultivars. The maximum relative shoot length was observed in SN03 (Bora) (93.29 percent) and minimum was recorded in (Kola Ahu) SN15 (53.89 percent). Nithya (2020) reported that water stress tolerant varieties like Ptb 29 and Ptb 30 showed higher shoot length than drought susceptible varieties like Ptb 23 and Ptb 24 under water stress conditions.

Bertolazi *et. al.* (2019) from his studies on *P. indica* colonization in wild type and transgenic rice plants inferred that morphological parameter like shoot length significantly increased in both the type of plants when colonised with the endophyte.

2.4.2. Root length

Roots absorb water and nutrients from the soil and as therefore its morphological and physiological properties have a significant impact on the shoot growth and the overall plant growth. One of the important characteristics for screening drought tolerance in rice is its root length and distribution (Gowda *et. al.*, 2011). Upland rice varieties, under water stressed conditions, develop root systems which are deep and thick so as to ameliorate the hydraulic properties of its roots (Fukai and Cooper, 1995). These attributes enhance the drought tolerance by inducing water absorption from deeper soil layers.

Rejeth (2017) on his studies on root traits associated with drought tolerance observed that when water stress was given for a period of 15 days, some improved varieties like Ptb 15, Ptb 29, and Ptb 30 showed increase in root length. Since deep rooted rice cultivars shows more tolerance to drought than shallow rooted cultivars (Chang and Loresto, 1986) these varieties can be considered as drought tolerant.

Jogawat *et. al*., (2013) found that under salt stress condition rice roots showed significant increase in length when colonised with *P. indica* and theorized that root length is one of the important parameters of salt stress analysis as the roots are in direct contact with the soil as well as absorb water from the soil.

2.4.3. Shoot dry weight

Saddique *et. al*., (2018) observed that the dry shoot weight was higher in the control rice plants of WC 297, Caawa and IR-64 inoculated with *P. indica* than the plants without *P. indica*. On comparing the drought affected plants the same trend was found with *P. indica* inoculated plants showing higher shoot dry weight. This indicates that *P. indica* has evolved ways to protect plants which is its food source.

Under severe salt stress condition, the shoot fresh and dry weight of *P. indica* colonised barley plants were 1.51 and 1.44 times higher respectively than that of non-inoculated plants (Alikhani *et. al*., 2013).

2.4.4. Root dry weight

Even though drought stress affected rice plants adversely, the colonisation by the mutualistic fungi enhanced various morphological characteristics including the root dry weight. Studies by multiple researchers has found that there was significant increase in the root dry weight in *P. indica* colonised crop plants of maize (Zhang et. al., 2018), wheat (Hosseini *et al.*, 2017), rice (Tsai *et al.*, 2020) etc. There has been extensive study on the effect of stress; especially salt stress in *P. indica* colonised and non-colonised rice plants in-lieu of the root characteristics.

In a study comparing a transgenic over-expressing the vacuolar H^+-PPase (AVP) and a wild-type (WT) rice, plants of both types showed increased dry root weight on colonisation with *P. indica* and the weight gain was more pronounced in the transgenic type (Bertolazi *et al.*, 2019).

2.4.5. Root volume

Drought affects the overall development of the root system and roots are the first responders to drought in soil by sensing water deficit and sending appropriate signals to lower stomatal conductance and leaf growth (Gollan *et al.*, 1986). Luo *et al.* (2015) reported that above-ground accumulation of biomass is decreased by reducing root volume, root mass and root length density. Rootstocks of vines were able to maintain increased gas exchange when they retained greater volume (Bartlett *et al.*, 2022).

Extensive root system growth and proliferation is one of the positive effects of *P. indica*. Waller *et al.* (2005) suggested that accelerated development of roots promoted early growth stages in *P. indica*-colonised barley seedlings under salt stress. Hosseini *et al.* (2017) found out that wheat under combined drought and mechanical stresses experienced diminished growth and on *P. indica*-colonisation, the plants recorded improved plant water status and root volume. This increased the number of absorption sites for water and nutrient and also enhances osmotic balance.

2.4.6. Root shoot ratio

Plant growth and development changes the root shoot ratio along with changing response to limiting resources above and below ground (Müller *et al.*, 2000). Chen *et al.* (2021) observed that the root shoot ratio of wheat varieties YM13 and YN19 under drought stress improved by 17.65 percent and 8.33 percent respectively. Roots absorb water from soil and primarily supports its own growth and development. Thus, is understood that the negative impact of drought on roots is lesser than that of shoots which ensures a higher root shoot ratio (Xue *et al.*, 2003). Survival of plants is dependent on the root shoot ratio as it enables to adapt to water and nutrient availability in soil. Drought stress tolerance and nutrient uptake is balanced by auxin and cytokinin to decrease shoot length and increase root length respectively and hence facilitating the survival of the plant (Kurepa and Smalle, 2022).

Zamani *et al.* (2015) reported that in *P. indica*-colonised maize grown in petroleum contaminated soil, the root shoot ratio increased which shows evidence that the endophytic fungus enhanced the growth of roots by allowing more assimilate allocation to below ground parts of the plant.

2.5. EFFECT OF *P. indica* ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS UNDER WATER STRESS

2.5.1. Relative water content (RWC)

One of the most important factors limiting potential yield and its parameters is reduced relative water content at reproductive stage (Barik *et al.*, 2018). It is also used to measure tissue water status (Sinclair and Ludlow, 1985). Dingkuhn *et al.* (1998) reported that leaf water potential is closely associated to RWC. Beena *et al.* (2012) reported that tolerant rice varieties under drought stress were able to maintain high leaf water status and there existed a significant positive correlation between biomass and relative water content. Bagheri *et al.* (2013) in studies on the effect of *P. indica* on growth and antioxidant activity found that, under salinity stress the relative water content of the plants increased. Similar results were obtained in studies by Hosseini *et al.* (2017) on wheat under drought and mechanical stress which further points to increased relative water content under *P. indica*-colonisation.

2.5.2. Specific leaf area

Specific leaf area has an important role in connecting plant carbon (C) and water cycles as it affects the distribution of leaf biomass with respect to leaf area and thereby leads to carbon accumulation relative to water loss in plant canopy (Gunn *et al.*, 1999). Wellstein *et al.* (2017) reported that under extreme drought stress, specific leaf area of grassland species of some temperate and sub-Mediterranean grass species was reduced. Lower specific leaf area is associated with improved water-use efficiency (WUE) under water stress and can be used as a strategy by stress tolerant plants. A relative decrease in surface area means that there are less ways for water to be lost. A positive correlation of SLA with leaf nitrogen content was found in trees of semi-arid region (Liu *et al.*, 2017).

2.5.3. Cell membrane stability index

Cell membrane stability index (CMS) is a direct indicator of the effect water stress have on plants. Cell membrane stability is one of the most important parameters used to study stress especially water stress or drought and subsequent selection of tolerant genotypes. PEG, a chemical desiccator, can mimic drought stress and is used to study its effect on drought stress (Premachandra and Shimada, 1987). Rejeth (2017) in his studies suggested that the CMS of water stress tolerant rice varieties were higher than that of susceptible varieties when exposed to different levels of water stress.

2.5.4. Stomatal conductance

The stomatal conductance has a direct correlation with yield as stomatal closure limits carbon absorption and assimilation leading to reduced photosynthate production and thereby potential yield loss (Liao *et al.*, 2022). Wang *et al.*, (2018) reported that gas exchange parameters like stomatal conductance decreased significantly under drought stress in young apple trees and the degree and duration plays a major factor in this. In maize seedlings, the adverse effect of salt stress in the form of NaCl was alleviated after *P. indica*-inoculation; improving stomatal conductance, lowered K^+ efflux from roots and increased K^+ content in shoots are the probable causes (Yun *et al*., 2018).

2.5.5. Transpiration rate

Drought of water stress tolerance of a plant can be determined by the transpiration. Water stress tolerant varieties retain more hydration in the plant cells by allowing less water loss through stomata. Riaz *et al.* (2013) reported that on comparing two marigold varieties under drought conditions, Super Giant had more transpiration rate than Inca F1, which may be due to the former variety's bigger and lose plant structure. Reduction in stomatal conductance in response to drought stress can cause reduction in the rate of transpiration (Hall and Schulz, 1980).

In arsenic exposed rice seedlings, a reduction in transpiration was observed and *P. indica*-inoculation enhanced the transpiration rate (Ghorbani *et al.*, 2021). *P. indica*-inoculated plants of *Trigonella foenum-graecum* on treatment with 70 mM and 150 mM NaCl concentrations, showed an increase of 54.74 percent and 76.34 percent respectively when compared to the non-colonised plants (Bisht *et al.*, 2022).

2.5.6. Photosynthetic rate

Drought inhibits carbon assimilation by damaging basic organization structure including the photosynthetic apparatus (Golldack *et al.*, 2011). Multiple studies have reported on the destructive effect of water stress damages on plant photosynthetic systems leading to reduction of photosynthesis. When the plants are exposed to severe drought condition, the photosynthetic limitation is non-stomatal while mild to moderate drought can reduce photosynthesis through stomatal limitation (Flexas and Medrano, 2002).

Tsai *et al.* (2020) reported that on *P. indica*-colonisation, water stress-induced leaf wilting and reduction in photosynthetic efficiency were diminished by promoting stomatal closure. Saddique *et al.* (2018) reported that efficiency of photosystems II and net photosynthetic are influenced by the high potassium uptake facilitated by *P. indica* and thereby maintaining optimum relative water content.

2.5.7. Total chlorophyll content

Chlorophylls, the photosynthetic pigments involved in light harvesting, plays an essential role in photosynthesis in plants. As one of the severe visible effects of drought is the degradation of chlorophyll, chlorophyll content is a parameter used in water stress studies (Ying *et al.*, 2015). Chen *et al.* (2016) reported that chlorophyll content was significantly reduced in maize seedlings as a physiological response to drought stress.

Zarea *et al.* (2012) reported that in rice and wheat, photosynthetic pigment content was elevated after *P. indica*-colonisation. Higher chlorophyll content was observed in *P. indica*-inoculated micro-propagated plantlets of *Boswellia serrata* Roxb. than un-inoculated plantlets (Suthar and Purohit, 2012). Sheramati *et al.* (2008) also reported that *P. indica*-colonised *Arabidopsis* plants showed higher chlorophyll content than non-colonised plants when exposed to drought stress.

2.5.8. Malondialdehyde content

Membrane stability decreases during water stress by reason of lipid peroxidation which leads to the loss of stability of the cell membrane (Niu and Xiang, 2018) and increased the production of MDA (Savchenko *et al.*, 2002). A higher antioxidative capacity indicates a higher abiotic stress tolerance and this was observed in a study by Tsai *et al.* (2020) where *P. indica*-colonised rice plants had lower MDA level both under water stressed and irrigated condition. Sun *et al.* (2010) in their studies on drought tolerance in Chinese cabbage reported that the endophyte slows down the accumulation of MDA which is an oxidative stress biomarker. MDA can be used as a lipid peroxidation bio-marker and is used to measure level of oxidative degradation of cell membrane in salt stress affected plants. A 1.4- and 2.2-fold higher MDA level was observed in 0.5

M and 1 M NaCl salt treatments when compared to the control sets of *P. indica*-colonised seedlings (Nivedita *et al.*, 2021)

2.5.9. Superoxide dismutase and Catalase activity

Baltruschat *et al.* (2008) suggested that antioxidant enzyme levels and ROS scavenging systems were altered in *P. indica*-colonised plants in response to salt stress. The Activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase increased during stressed condition curtailed ROS levels in cells (Zhao *et al.*, 2018). *P. indica*-colonisation confer drought tolerance to water stress affected plants in this study by significantly increasing the SOD and CAT activity and thereby reducing the ROS. This is similar to the findings that *P. indica*-conferred abiotic stress tolerance depends on enhanced antioxidant synthesis in response to the ROS (White and Torres, 2010; Zarea *et al.*, 2012). Improved Zn uptake by *P. indica* influence the antioxidant complexes (Cu/Zn-SOD) and is therefore very important in reducing cellular membrane damages by ROS scavenging (Gill and Tuteja, 2010).

2.6. EFFECT OF *P. indica* ON YIELD PARAMETERS UNDER WATER STRESS

2.6.1. Days to 50% flowering

Rice physiological traits and yield is affected by drought stress especially at flowering stage. Nithya (2020) on the morpho-physiological and plant protection traits in rice under drought condition reported that Ptb 23, Ptb 24, Ptb 29 and Ptb 30 recorded 50 percent flowering on 88th, 85.5th, 81st and 82nd day respectively.

Pan *et al.* (2017) reported that *P. indica*-colonisation conferred early flower development and promoted the siliques development in *Arabidopsis*. Gene expression of many genes including the core flowering gene *FLOWERING LOCUS T (FT),* genes controlling photoperiod – *CRYPTOCHROMES (CRY1*, *CRY2*) and *PHYTOCHROME B (PHYB),* genes related to gibberellin function (*MYB5, GA3, RGA1* and *AGL24)* were induced by the presence of *P. indica*.

2.6.2. Tillers per plant and Productive tiller per plant

During the study on the screening of wheat genotypes for drought tolerance, Mwadzingeni *et al.*, (2016) reported a reduction in tiller number under water stress condition. The highest number of productive tillers were found in genotypes LM64 and LM84 while lowest number of tillers were found in LM62 and LM95.

Su *et al.*, (2017) observed that there was a 20 percent increase in tiller numbers in *P. indica*-treated *Brassica napus* L. plants when compared to control plants. Achatz *et al.*, (2010) also recorded an increase in number of tillers in barley plants induced by *P. indica*-colonisation.

2.6.3. Panicle length

Panicle length, typically measured as one of the most important yield related traits, is one aspect of panicle architecture. Along with spikelet number and density, seed setting rate and grain plumpness, panicle length determines the number of grains per panicle and thereby directly linked to yield (Liu *et al.*, 2016). Water stress significantly affect the panicle length at all growth stages (Rahman and Yoshida, 1985). A study in millet (*Panicum milliaceum* L.) reported that the yield component like panicle length, grain yield and 1000-grain weight were significantly increased in *P. indica*-colonised seedlings under water stress (Ahmadvand and Hajinia, 2018).

2.6.4. Yield per plant

All agronomic practices in agriculture ultimately aims at increasing yield of the crop. The multitude of biotic and abiotic stresses impedes the ability of a plant to achieve the yield potential. The various crop management practices were developed to achieve this potential and the introduction of the growth promoting endophyte *P. indica* as a beneficial organism is one such promising plant stress management strategy. Growth promotion at early developmental stages by *P. indica* was reported to enhance grain yield of barley (Achatz *et al.*, 2010). In another study by Tsai *et al.* (2020) it was speculated that higher grain yield was due to promotion of panicle formation and grain filling.

Nithya (2020) on the morpho-physiological and plant protection traits in rice under drought condition reported that Ptb 23, Ptb 24, Ptb 29 and Ptb 30 recorded a yield of 8.57, 8.58, 12.18 and 12.10 g respectively. Similar results were observed by Rejeth (2017) were water stress reduced potential yield of Pattambi rice varities.

2.6.5. Spikelet fertility percentage

Drought and high temperature can cause stress-induced downregulation of genes for auxin synthesis, transport and signalling and leads to the reduction in endogenous auxin in spikelets, therby inducing spikelet sterility in rice (Sharma *et al.*, 2018). Water stress during the growth stages particularly during the flowering stage affect the spikelet fertility (Kang and Futakuchi, 2019).

2.6.6. 1000 grain weight

Water stress during grain filling stage reduces assimilate translocation and thereby decreases the grain weight and increases empty grain. 1000-grain weight is thus a measure of photosynthates produced and assimilates translocated and both factor is negatively influenced by drought (Fahad *et al.*, 2016).

Nithya (2020) on the morpho-physiological and plant protection traits in rice under drought condition reported that Ptb 23, Ptb 24, Ptb 29 and Ptb 30 had a thousand grain weight of 21.35 g, 23.35g, 25.25 g and 25.35 g respectively.

2.7. EFFECT OF *P. indica* ON MOLECULAR PARAMETERS UNDER WATER STRESS

There have been multiple studies on the molecular interaction of *P. indica* with that of the plant. Defense related genes like *ERF1, LOX2* and *PR* (Zarea *et al.*, 2012), abiotic stress response genes like *DREB2A, RD29A* and *CBL1* (Ansari *et al.*, 2013) and osmo-protectants like glycine, proline and betaine (Waller *et al.*, 2005; Trivedi *et al.*, 2013) are activated and upregulated by *P. indica*-colonisation

2.7.1. Effect of *P. indica* on expression of *OsDREB 2A* gene under water stress

Plants respond and adapt at molecular and cellular levels as well as at biochemical and physiological levels to survive under various environmental stresses. Dehydration responsive element binding (DREB) transcription factor is a group of transcription factors involved in abiotic stress tolerance (Moon *et al.* 2019). The genes in this subfamily plays an important role in abiotic stress response by recognizing dehydration responsive elements with a core motif of A/GCCGAC (Liu *et al.*, 1998). The gene products of *DREB2A* are involved in the control of gene expression in the nucleus and DREB2A interacts with the *cis*-acting dehydration-responsive element involved in drought stress-responsive gene expression (Sakuma *et al.*, 2006). Plants respond and adapt at molecular and cellular levels as well as at biochemical and physiological levels to survive under various environmental stresses. A wide array of genes is expressed or regulated by numerous transcription factors like bZIP, WRKY, AP2/ERF MYB etc., in response to these stresses (Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 2000; Golldack *et al.*, 2011).

Dehydration responsive element binding (DREB) transcription factor is one such group of transcription factors involved in abiotic stress tolerance (Moon *et al.* 2019). The genes in this subfamily plays an important role in abiotic stress response by recognizing dehydration responsive elements with a core motif of A/GCCGAC (Liu *et al.*, 1998). *OsDREB1A* to *OsDREB1G*, *OsDREB2A* and *OsDREB2B* are *DREB* genes cloned from rice (Oryza sativa), however, only *OsDREB1A*, *OsDREB1E*, *OsDREB1G*, *OsDREB2A* and *OsDREB2B* are able to specifically bind to dehydration responsive elements (Dubouzet *et al.*, 2003; Morran *et al.*, 2011).

The levels of *DREB2A* are drought inducible and is upregulated early in *P. indica* colonised drought-stressed *Arabidopsis* than in uncolonized control (Sheramati *et al.*,2008). Earlier studies in Chinese cabbage and Arabidopsis exposed to drought have demonstrated that when roots are colonised by *P. indica*, many drought-induced genes are upregulated (Sun *et al.*, 2010). The findings of Xu *et al.*, (2017) asserted that in drought stressed leaves of *P. indica*-colonised maize plants, drought-related gene *DREB2A* was upregulated.

Materials and methods

3. MATERIALS AND METHODS

The study entitled “Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)” was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani during 2019-21 with the objective to elucidate the changes in morphological, physiological, biochemical and molecular mechanisms associated with water stress tolerance in *Piriformospora indica*-colonised rice. The particulars of the materials used, methods adopted for the experiment and protocols followed during the course of experimentation are given a description in this chapter.

3.1 EXPERIMENT 1

3.1.1 Location

The experiment was conducted at Department of Plant Physiology, College of Agriculture Vellayani, situated at 8°5’ N latitude and 76°9’ E longitude and an altitude of 29 m above mean sea level.

3.1.2 Plant Material

The rice varieties used in the study consists of Ptb 23(Cheriya Aryan), Ptb 24(Chuvanna Vattan), Ptb 29(Karutha Modan) and Ptb 30(Chuvanna Modan), collected from Regional Agricultural Research Station, Pattambi, Palakkad district, Kerala.

3.1.3 Experimental details

Table1. Particulars of experiment

1.	Crop	Rice: 4 varieties Water stress tolerant rice varieties: Ptb 29 (Karutha Modan) and Ptb 30 (Chuvanna Modan) Water stress susceptible rice varieties: Ptb 23 (Cheriya Aryan), Ptb 24(Chuvanna Vattan),

2.	Design	Completely Randomized Design (CRD)
3.	Number of treatments	10 Concentration of PEG 6000: 1. PEG 6000 at 5% 2. PEG 6000 at 10% 3. PEG 6000 at 15% 4. PEG 6000 at 20% 5. Control (PEG 6000 at 0%) Colonising with *P. indica* (P) P1: *P. indica* colonised rice P2: *P. indica* non colonized rice
4.	Replication	3

3.1.4 Methodology

In this study, *P. indica* colonised and non-colonised rice seedlings were raised in plastic trays up to 14 days after sowing. Thereafter it was transferred to test tubes containing Hoagland Nutrient Solution with five levels of PEG 6000 concentration *i.e.*,5%, 10%, 15%, 20%, and 0%(control). The observations were taken seven days after treatment to identify the highest tolerating levels of water stress by *P. indica* in the rice varieties – Ptb 23, Ptb 24, Ptb 29 and Ptb 30 and also to select the best water stress tolerant variety and the most water stress susceptible variety from the aforesaid varieties.

3.1.5 Preparation of media for mass multiplication of *P. indica*

The beneficial fungal root endophyte, *P. indica* from Department of Plant Pathology, College of Agriculture, Vellayani was maintained in potato dextrose agar (PDA) medium. Fungal disc from actively growing margin of two weeks-old culture of *P. indica* was transferred to 250

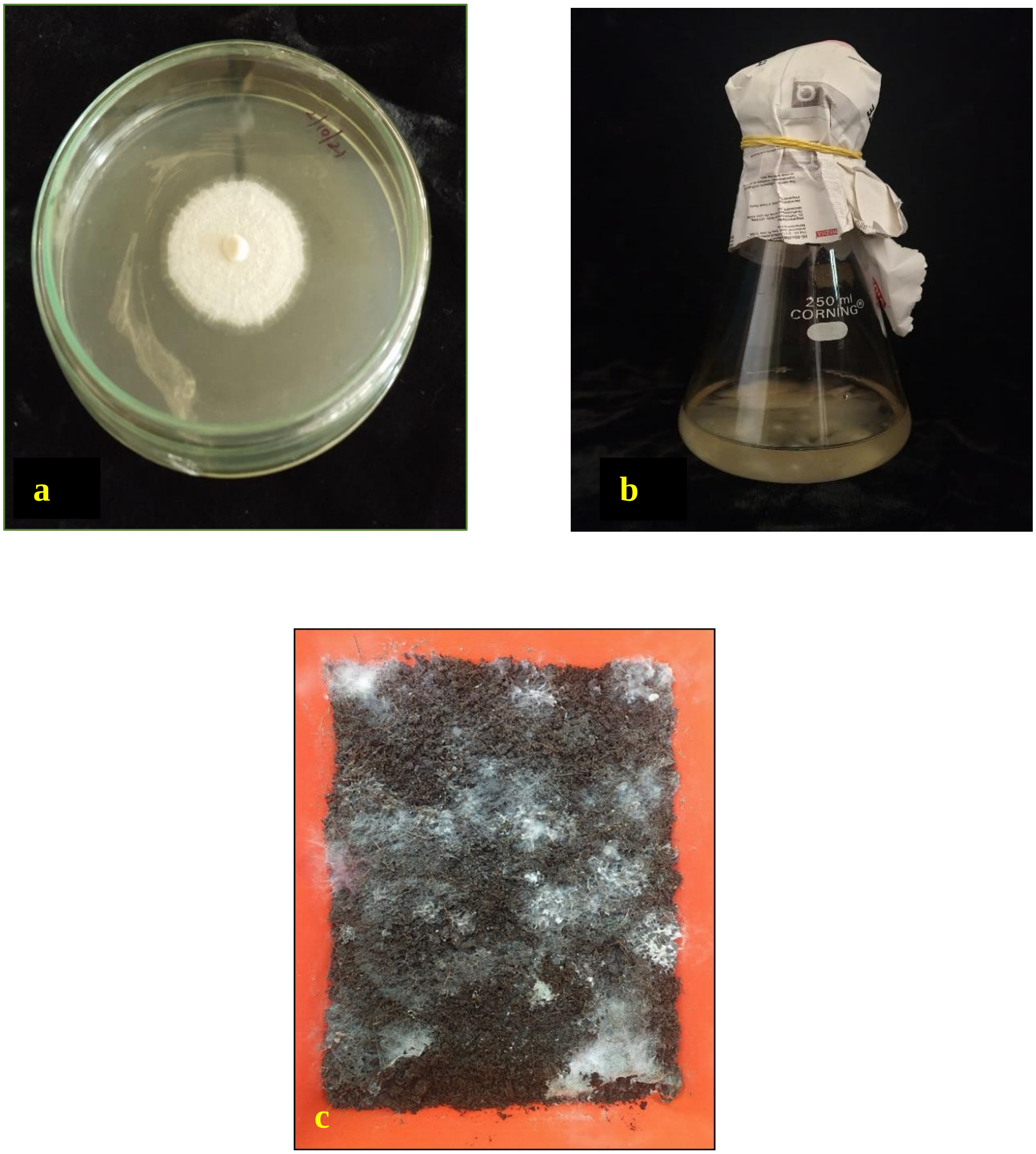

Plate. 1. Mass multiplication of P. indica (a) *P. indica* grown in Potato Dextrose Agar (PDA) (b) Mycelial mat of P. indica formed in Potato Dextrose Broth (c) mass multiplied in coir pith–cow dung media.

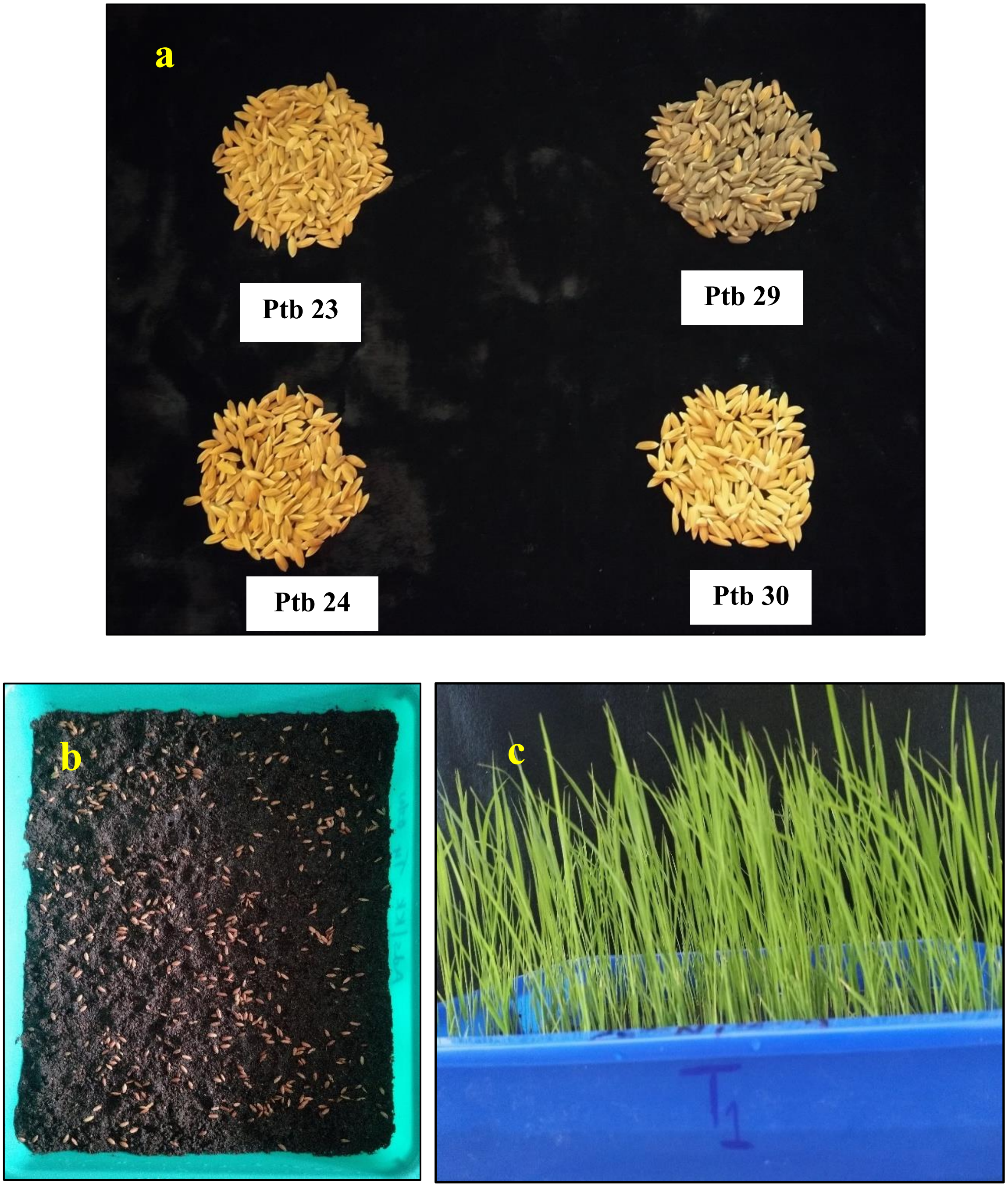

Plate. 2. Raising of *P. indica*-colonised seedlings of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 rice varieties. (a) Seeds of rice varieties Ptb 23, Ptb 24, Ptb 29 and Ptb 30 obtained from RARS, Pattambi (b) Rice seeds sown in *P. indica* mass multiplied medium (c) 7 days old rice seedlings grown in P. indica mass multiplied media.

ml conical flask containing 100 ml Potato Dextrose Broth (PDB) and incubated in dark at room temperature for 2 weeks. For colonization of *P. indica*, the incubated PDB containing fungal mycelium was mixed with sterilized composted coir pith- cow dung (1:1) mixture medium filled in plastic trays. It was kept covered for promoting fungal growth. After 3 weeks surface sterilized rice seeds were placed in those trays and maintained in temperature and humidity -controlled conditions for uniform germination.

3.1.6 Root colonization of rice by *P. indica*

Roots of rice seedlings were collected at 5, 15, and 25 days after co-cultivation. Roots were washed thoroughly with running water to make it free from planting medium. Those bits were cut into small pieces of 1 cm length and transferred into test tube containing 5 ml of freshly prepared 10 per cent KOH. It was placed in water bath at 65°C for 5 min. The test tube was taken out and contents were washed once with water and treated with 1 per cent HCl for 5 min. The treated root bits were again washed with water and transferred into lactophenol trypan blue for 2 min to stain the fungus in roots. The root bits were observed under compound microscope (M/s. Leica, USA) for the presence of chlamydospores and colonization in each root bit.

3.1.7 Preparation of PEG-6000 solution for inducing water stress.

The following concentrations of PEG-6000 were prepared in a modified Hoagland nutrient media.

Table 2. Particulars of PEG-6000 solution

Sl. no.	Concentration of PEG-6000 solution (%)	PEG-6000 required per liter of final solution (g)	Electrical conductivity (EC) of final solution (dSm^{-1})	Water potential of final solution (MPa)
1.	0	0	2.6	-
2.	5	50	2.3	-0.499
3.	10	100	2.0	-1.480

4.	15	150	1.8	-2.950
5.	20	200	1.5	-4.906

3.1.8 Observations

3.1.8.1 Biometric parameters.

3.1.8.1.1 Shoot length

The shoot length was measured using a ruler from stem base to shoot tip of both control and treatment plants and were expressed in cm.

3.1.8.1.2 Root length

Root length was measured from the root-shoot junction to the tip of longest root-let and expressed in cm.

3.1.8.1.3 Shoot dry weight

The shoot weight was recorded separately after drying the shoot portion in hot air oven at 80^{o}C for 48 hours till reaching constant weight. Then dry weight is measured in an electronic balance and expressed in g.

3.1.8.1.4 Root dry weight

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^{o}C. Then dry weight was measured in an electronic balance in grams.

3.1.8.1.5 Seedling vigour index – 1

Seedling vigor index (SVI) 1 was calculated according to formula proposed by Abdul-Baki and Anderson (1973). It was determined by multiplying germination percentage with seedling length.

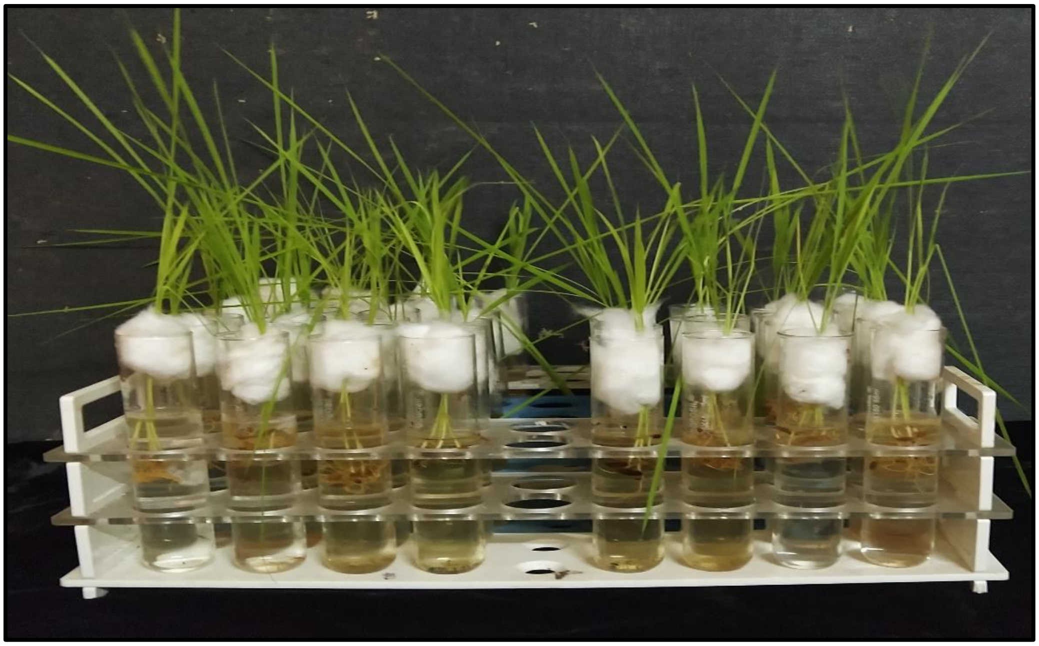

Plate. 3. Experimental setup for inducing water stress in *P. indica*-colonised and non-colonised seedlings of rice varieties Ptb 23, Ptb 24, Ptb 29 and Ptb 30 in test tubes containing different concentrations (0%, 5%, 10%, 15%, 20%) of PEG 6000 in modified Hoagland nutrient solution.

Seedling vigor index (SVI) 1 = Germination percentage x Seedling length (Root + Shoot) where Germination percentage = (number of germinated seed/total number of seeds) x 100

3.1.8.1.6 Seedling vigour index – 2

Seedling vigor index (SVI) 2 was computed by multiplying germination percentage by seedling dry weight.

Seedling vigor index (SVI) 2 = Germination percentage x Seedling dry weight where Germination percentage = (number of germinated seed/total number of seeds) x 100

3.1.8.1.7 Root shoot ratio

The root shoot ratio was calculated as follows

Root shoot ratio = Root dry weight / Shoot dry weight

3.1.8.1.8 Number of root branches

The number of branches of roots arising from the root-shoot junction of the plants are counted and recorded.

3.2 EXPERIMENT 2

3.2.1 Location

The experiment was conducted at Department of Plant Physiology, College of Agriculture Vellayani, situated at 8°5’ N latitude and 76°9’ E longitude and an altitude of 29 m above mean sea level.

3.2.2 Plant Material

The rice varieties used in the study consists of Ptb 23 (Cheriya Aryan), and Ptb 29 (Karutha Modan) collected from Regional Agricultural Research Station, Pattambi, Palakkad district, Kerala. This was selected based on observations of experiment 1.

3.2.3 Experimental details

Table 3. Particulars of experiment 2

1.	Crop	Rice: Two varieties Water stress tolerant: Ptb 29 (Karutha Modan) Water stress susceptible: Ptb 23 (Cheriya Aryan)
2.	Design	Completely Randomized Design (CRD)
3.	Number of treatments	8 Level of stress: 1. Water stress 2. Control (Irrigated) Colonising with *P. indica* (P) 1. P1: *P. indica* colonised rice 2. P2: *P. indica* non-colonised rice Varieties 1. Best water stress tolerant variety 2. Most water stress susceptible variety
4.	Replication	Three

3.2.4 Methodology

In this study, *P. indica* colonised and non-colonised rice seedlings of the selected rice varieties Ptb 23 and Ptb 29, from experiment 1 were raised in pots of 30 cm height and 25 cm width in rainout shelter. A set of three replication were maintained for each variety under water stressed and irrigated conditions. Water stress was induced by stopping irrigation for 5 days during maximum tillering, panicle initiation and flowering stages and normal irrigation resumed thereafter. Physiological parameters were observed at maximum tillering, panicle initiation and flowering stages, while the morphological and yield parameters were observed at harvest stage. The biochemical and molecular parameters were analysed during flowering stage.

3.2.5 Preparation of potting mixture and transplanting

Pots were filled with potting mixture of soil, sand and FYM in the ratio 3:2:1. 20 days old seedlings of *P. indica* colonised and non-colonised Ptb 23 and Ptb 29 rice varieties raised in experiment 1 was transplanted to the pots at the rate of two seedlings per pot.

3.2.6. Observations

3.2.6.1 Morphological parameters

3.2.6.1.1 Shoot length

The shoot length was measured with a ruler from stem base to shoot tip after harvest of both control and treatment plants and were expressed in cm.

3.2.6.1.2 Root length

Length of root was measured with centimeter scale from the tip of the longest rootlet to the cut end at the soil line and expressed in cm

3.2.6.1.3 Shoot dry weight

The shoot weight was recorded separately after drying the shoot portion in hot air oven at 80^0C for 48 hours till reaching constant weight. Then dry weight was measured in an electronic balance in mg.

3.2.6.1.4 Root dry weight

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 mg.

3.2.6.1.5 Root volume

Using water displacement method root volume in cubic centimeter was determined. Roots were removed from the soil and cleaned thoroughly then immersed in a 1000ml measuring cylinder. After that the displaced volume of water was measured, which was taken as the volume of roots and expressed in cm^3

3.2.6.1.6 Root shoot ratio

The root shoot ratio was calculated as follows

Root shoot ratio = Root dry weight / Shoot dry weight

3.2.6.2 Physiological and biochemical parameters

3.2.6.2.1 Relative water content (RWC)

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

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

3.2.6.2.2 Specific leaf area

Specific leaf area was calculated by selecting a fully expanded leaf. The area of leaf was recorded with the help of graphical method. Leaf sample was oven dried at 70°C for about 24 hours till constant weight was obtained. Specific leaf area was calculated from the following equation as.

Specific leaf area = (Leaf area / Dry weight)

The calculated value is expressed in $cm\ g^{-1}$

3.2.6.2.3 Cell membrane stability index

Cell membrane stability index (CMSI) was determined in accordance to the procedure proposed by Sairam *et al.* (1997). 100 mg of leaf samples after through washing, were taken in two sets, containing 10 ml of double distilled water. One set was heated at 40ºC for 30 minutes, while other set was boiled at 100ºC for 10 minutes. Then electrical conductivity of both sets was measured as C_1 and C_2 respectively.

$$CMSI\ \% = [1- (C_1/C_2)] \times 100$$

3.2.6.2.4 Stomatal conductance

Stomatal conductance was estimated between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and were expressed in m H_2O moles m^{-2} s^{-1}.

3.2.6.2.5 Transpiration rate

Transpiration rate was quantified between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and expressed in m H_2O moles m^{-2} s^{-1}

3.2.6.2.6 Photosynthetic rate

Photosynthetic rate was quantified between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and was expressed in µ CO_2 moles m^{-2} s^{-1}.

3.2.6.2.7 Total chlorophyll content

Chlorophyll content of leaves were quantified in accordance to the procedure proposed by Arnon (1949). Fresh leaf samples (0.5 g) were cut into small bits and put into test tubes containing10 ml DMSO: 80% acetone mixture (1:1 v/v) and incubated overnight at room temperature. The coloured solution obtained was then transferred to a measuring cylinder and volume made up to 25 ml with the DMSO-acetone mix. The absorbance was taken at 663 and 645 nm spectrophotometricaly.

The total chlorophyll content was quantified with the help of the given formulae.

Total Chlorophyll = [20.2 (O.D. value at 645) + 8.02 (O.D. value at 663)] x V/1000 x W

V = Final volume of the acetone extract

W = Fresh weight in gram

3.2.6.2.8 Malondialdehyde content

Malondialdehyde content was appraised to measure the extent of lipid peroxidation proposed by Heath and Packer (1968) using thiobarbituric acid (TBA). Fresh leaf (0.5 g) samples were homogenized in 3 ml of 5 % (w/v) trichloroacetic acid (TCA). After centrifugation at 12000g (12 minutes), 1 ml of supernatant was collected and mixed with four ml of 20 percent TCA containing 0.5 % TBA, heated it at 95ºC for 30 min and cooled immediately on ice. This solution was again centrifuged at 12000g for 10 minutes. The difference in absorbance at 532 and 600nm was used to calculate the MDA content (extinction coefficient - 155 mM^{-1} cm^{-1}).

3.2.6.2.9 Superoxide dismutase activity

Superoxide dismutase activity was measured using the according to the method proposed by Kono (1978). Enzyme extract solution was prepared by grinding 0.5 g of fresh leaf samples in 50 mM potassium phosphate buffer (pH 6.5) and was centrifuged at 10,000g for 12 minutes. The supernatant collected was used as enzyme extract. 1.3 ml of 50 mM potassium phosphate buffer, 500 µl nitroblue tetrazolium (NBT), 100 µl Triton-X 100 were taken in a test tube. The reaction for the superoxide radical production was initiated by adding 100 µl hydroxylamine hydrochloride to the aforementioned mixture. After 2 mins, 70 µl of the enzyme extract was added and absorbance was calculated at 540 nm, an increase in absorbance was noted at time interval of 1 minute. The SOD activity was expressed as units (amount of enzyme required to inhibit NBT reduction by 50 %) min^{-1} mg^{-1} protein, which was then converted to U mg^{-1} FW.

3.2.6.2.10 Catalase activity

Catalase (CAT) activity was enumerated according to the method proposed by Aebi (1983). Enzyme extract solution was prepared by grinding 0.5 g of fresh leaf samples in 50 mM potassium phosphate buffer (pH 6.5) and was centrifuged at 10,000g for 12 mins. The supernatant thus collected was used as enzyme extract. Enzym extract (100 µl) was diluted 50 mM potassium phosphate buffer (1 ml), The reaction was initiated by adding 100 µl of 100mM H_2O_2. Change in absorbance was calculated at 240 nm, at time interval of 15 sec. for 2 minutes. The CAT activity was expressed as units^{-1} min^{-1} mg^{-1} protein, which was then converted to U mg^{-1} FW.

3.2.6.2.11 Invertase activity

Enzyme extract solution was prepared by grinding 1g plant tissue in pre chilled mortar pestle with 20 ml 0f 0.1M sodium citrate buffer (pH-5). Homogenat passed through 2 layers of cheese cloth and centrifuged at 15000xg for 10 minutes at 4^0 C and the supernatant was used as enzyme extract. 0.6ml of 0.1M citrate buffer (pH-5) and 0.2 ml of 0.4 M sucrose was added to 0.2ml of the enzyme extract. It was then incubated at 30^0 C for 1 hour and 1 ml DNS was added and kept in boiling water bath for 5 minutes**.** After that it was cooled and diluted to 10 ml with distilled water**.** The spectrophotometer reading at A_{560} nm was taken with glucose as standard. Invertase activity was expressed in µmol mg^{-1} pro. h^{-1}

3.2.6.3 Yield parameters

3.2.6.3.1 Days to 50% flowering

The days required for exertion of 50% of the panicles in each replication was observed and recorded.

3.2.6.3.2 Tillers per plant

In each replication, total number of tillers at the time of harvest was counted and recorded.

3.2.6.3.3 Productive tiller per plant

In each replication, the number of tillers bearing panicle was counted at harvest stage by manual counting and recorded.

3.2.6.3.4 Panicle length

The length of the primary panicle from each plant was measured from the neck node to the tip of the apical grain using a centimeter scale and expressed in cm.

3.2.6.3.5 Yield per plant

The grains collected per plant was weighed after harvesting and expressed in g.

3.2.6.3.6 Spikelet fertility percentage

Spikelet fertility was deliberated after harvest by manually counting the total number of spikelets, and number of complete and partially filled spikelets.
Spikelet fertility percentage = (Filled spikelets/total number of spikelets) x 100.

3.2.6.3.7 1000 grain weight

Fully filled, bold one thousand grains were collected from the harvested grains, weighed and expressed in grams.

3.2.6.4 Molecular analysis

3.2.6.4.1 Expression analysis of *OsDREB 2A* gene

3.2.6.4.1 Sample collection

RNA was isolated from flag leaf of the rice plants. 100 mg of leaf samples were used for RNA isolation, three biological replicates were maintained for each treatment. Leaf samples collected were immediately covered in aluminium foil and labeled then transferred to liquid nitrogen. Leaf samples were collected from rice plants that were grown under water stressed and irrigated conditions.

3.2.6.4.2 RNA isolation

RNase is ubiquitous in nature, which degrades RNA molecules. Hence during RNA isolation, special care has to be taken to avoid RNA degradation by RNase. The activity of endogenous RNases inside the plant cells can be inhibited by action of inhibitors present in extraction buffer (phenol, guanidine isothiocyanate). In order to prevent the degradation of RNA by RNases from external environment we had to wear gloves and treat the glasswares and solutions with Diethylpyrocarbonate (DEPC), a strong RNase inhibitor. 0.1 % DEPC prepared in distilled water was used to treat labwares to remove the traces of RNases. Labwares were dipped in 0.1 % DEPC for 24 hours at dark followed by baking at 180 °C for five hours in a hot air oven or autoclaving, after wrapping in aluminum foil in order to remove remaining traces of DEPC. The used DEPC water was double autoclaved before disposal. All the reagents (like buffers) used RNA isolation were also prepared using double autoclaved DEPC treated water.

RNA isolation was performed using modified TRIzol reagent method (Yin *et al.*, 2016). 100 mg of flag leaves were collected and wrapped in aluminium foil prior to its transfer to liquid nitrogen. Leaf samples were homogenized into fine powder in a pre-chilled mortar and pestle using liquid nitrogen. One ml of TRIzol reagent was added to it and the components transferred into a 1.5 ml RNase-free tube. It was mixed immediately and shaken vigorously for 20 seconds. The mixture was then incubated for 5 minutes in room temperature. 0.2 ml chloroform (for each 1 ml of TRIzol reagent) was added and shaken well for 20 seconds. It was again incubated for 5 minutes. The tubes were centrifuged at 12000g for 15 min at 4°C. From the three phases obtained, the RNA was found in the aqueous phase. The aqueous phase was transferred into a fresh 1.5 ml RNase-free tube. An equal volume of isopropyl alcohol was added to the tube and was mixed by inversion. The mixture was then incubated at room temperature for 10 minutes followed by centrifugation at 12000g for 10 minutes at 4°C. The supernatant was discarded. The RNA pellet washed with one ml of 75% RNase-free ethanol by gentle inversion and centrifuged at 7500g for five minutes at 4°C. The supernatant was discarded and the RNA pellet was air dried for five minutes. 80 µL of RNase-free water was added to the air-dried pellets to dissolve the RNA and was stored at -80 °C.

3.2.6.4.3 Agarose gel electrophoresis

Agarose gel electrophoresis was performed to check the quality and integrity of the isolated RNA by loading 3 µl sample on a 1.2 % agarose gel at 70 V using 1 X TAE buffer. After half an hour, the gel was analyzed using Gel Doc XR+ Gel Documentation system (Bio-Rad).

3.2.6.4.4 Quantification of RNA

The concentration and purity (A_{260}/A_{280}) of isolated RNA was determined spectrophotometricaly. 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. (The good quality RNA shows 1.8 – 2).

3.2.6.4.5 cDNA synthesis

cDNA was synthesized using Thermo scientific Verso cDNA Synthesis kit. The reaction was done by incubating the reaction mixture in a thermal cycler in accordance with the manufacturer's instruction. In order to minimize the RNases attack, all the steps were carried out in RNase free condition. The synthesis of cDNA was carried out in a 20 µl reaction volume which contained;

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 thermal profile used for cDNA synthesis was as follows:

Step	**Temperature (ºC)**	**Time (min)**
Priming	25	5
Reverse transcription	46	20
RT inactivation	95ºC	1
Optional Step	Hold at 4ºC	-

3.2.6.4.6 Primer selection

The primers used for expression analysis of OsDREB 2A (Zhang *et.al.,* 2013) are as follows

Forward primer: 5′-GGATCCATGCTGTTTCGATTTGTG-3′

Reverse primer: 5′-GGTACCCTAATAGGAGAAAAGGCT-3′

3.2.6.4.7 Quality check of cDNA

Synthesised cDNA and designed primer were confirmed by the PCR technique. The standard reaction mix contains:

2X AB HS SYBR Green qPCR Mix (2 X)	10 µl
Forward primer (5 µM)	0.5 µl
Reverse primer (5 µM)	0.5 µl
cDNA	5 µl
DNase free water	4 µl
Total volume	20 µl

Finally amplified PCR product was 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 ™ XR+).

3.2.6.4.8 Real-Time PCR analysis

The expression analysis of water stress tolerance conferring OsDREB 2A genes was done using qRT-PCR. Real Time PCR analysis was done using 5 µl of cDNA as template. Gene expression was normalized using β-actin gene (reference gene). Each reaction was performed in triplicates with a volume of 20 µL. In addition, an NTC was also set for each primer by replacing the cDNA with 5 µl DNase free water.

Initial denaturation was carried out at 95 °C for 3 mints. Followed by 40 cycles (95 °C for 10 s, 60 °C for 30 s). Directly after the amplification step, melt curve analysis was performed to monitor primer-template specificity. Experiments were performed in CFX Mastero™ Real-Time PCR system (Bio-Rad). After amplification, the relative fold change in gene expression was analyzed using Comparative Ct method (Livak and Schmittgen, 2001). Data analysis was done using MS Excel software 2010.

The Reaction mix used for qPCR reaction contains:

2X AB HS SYBR Green qPCR Mix (2 X)	10 µl
Forward primer (5 µM)	0.5 µl
Reverse primer (5 µM)	0.5 µl
cDNA	5 µl
DNase free water	4 µl
Total volume	20 µl

Results

4. RESULTS

The results of the present study titled "Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)" carried out at Department of Plant Physiology, College of Agriculture, Vellayani during 2019-2021 with the objective to elucidate the changes in morphological, physiological, biochemical and molecular mechanisms associated with water stress tolerance in *Piriformospora indica*-colonised rice are presented in this chapter. The initial study aimed at identifying the highest tolerating level of water stress in seedling stage by virtue of root colonisation by *P. indica* in two water stress tolerant varieties (Ptb 29 and Ptb 30) and two water stress susceptible varieties (Ptb 23 and Ptb 24) of rice by treatment with Poly Ethylene Glycol (PEG) 6000 at concentrations of 0, 5,10, 15, and 20 per cent. Based on the observation on different parameters the best water stress tolerant variety and the variety most susceptible to water stress were selected. The selected varieties were the evaluated for water stress tolerance during the different growth stages by studying the morphological, physiological, biochemical, yield parameters and molecular aspects. The data collected throughout the study was statistically analysed, and the results are discussed in this chapter.

4.1. EXPERIMENT 1

The preliminary screening of *P. indica* colonised and non-colonised Ptb 23, Ptb 24 (water stress susceptible) and Ptb 29, Ptb 30 (water stress tolerant) varieties for water stress tolerance was done by treating with 0, 5, 10, 15 and 20 per cent of Poly Ethylene Glycol (PEG 6000) in modified Hoagland nutrient solution. All the varieties on root colonisation with *P. indica* showed higher water stress tolerance while the water stress tolerant varieties Ptb 29 and Ptb 30 showed tolerance to water stress even in the absence of colonisation with *P. indica*.

4.1.1. Root colonization of rice by *P. indica*

The colonisation by root endophytic fungus *Piriformaspora indica* was observed and confirmed seven days after cocultivation in all the rice varieties through the presence of double walled chlamydospores.

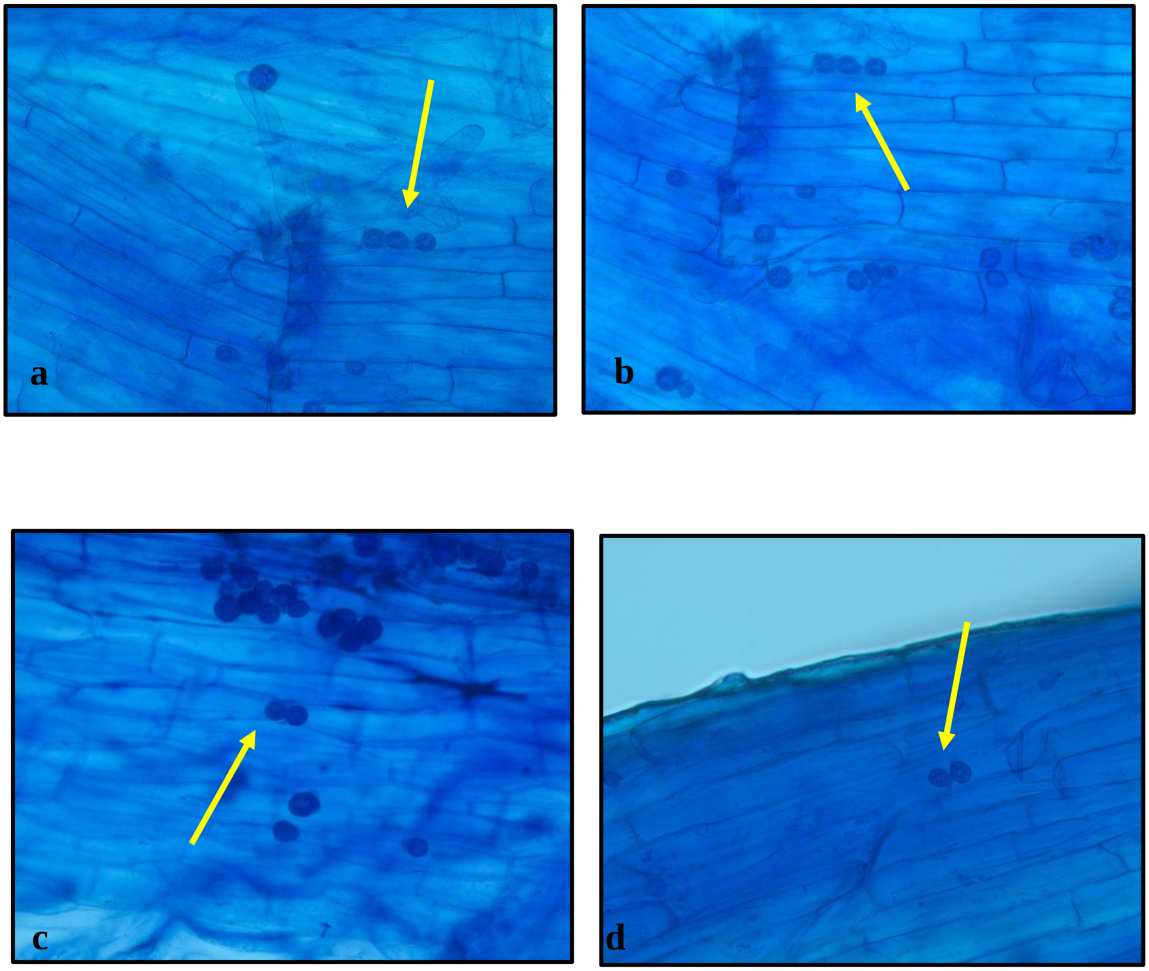

Plate. 4. In-vitro root colonisation of *P. indica* in the seedlings of a) Ptb 23, b) Ptb 24, c) Ptb 29 and d) Ptb 30 rice varieties at 7 DAC (DAC- Days after co-cultivation) (presence of double walled chlamydospores noted)

4.1.2 Shoot length

P. indica colonised seedlings of all the varieties showed increase in shoot length compared to non-colonised seedlings when subjected to water stress. There was an increase in shoot length when the varieties were treated with PEG 6000 up to a concentration of 10per cent. However, in higher concentrations of PEG 6000 *i.e.,* in 15 per cent and 20 per cent PEG 6000 concentrations the seedling did not exhibit any significant increase in shoot length. *P. indica* colonised seedlings maintained green colour in higher concentrations of PEG 6000 (15 and 20 per cent) while the non-colonised seedlings of Ptb 23 showed symptoms of shoot curling and drying. Among the two water stress susceptible varieties, Ptb 24 showed better tolerance when exposed to varying concentrations of PEG 6000 by showing higher seedling length. Ptb 29 appeared better equipped to deal with water stress than Ptb 30 by maintaining shoot length and greenness which is evident from the findings depicted in table 4.

In the absence of water stress, the shoot length of of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 15.07, 15.21, 16.00 and 14.43 cm respectively while the same for non-colonised seedlings were 14.07, 14.40, 14.73 and 14.13 cm respectively. Meanwhile under the extreme stress of 20 per cent PEG 6000 solution the average shoot length of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 9.43, 10.00, 9.33 and 9.00 cm respectively while the same for non-colonised seedlings were 8.97, 9.17, 9.30 and 9.13 cm respectively.

4.1.3 Root length (cm)

The results of root length of *P. indica* colonised and non-colonised rice varieties at seedling stage under different concentrations of PEG 6000 are presented in table 5.

P. indica colonised seedlings showed lower root growth than non-colonised seedlings in all the varieties. Among the water stress susceptible varieties, non-colonised seedlings of Ptb 23 showed higher root length with the highest under control conditions (4.67 cm) followed by non-colonised seedlings under five percent PEG 6000 concentration, while the lowest was recorded in non-colonised seedlings of Ptb 23 under 20 percent PEG 6000 concentration (2.03 cm). On comparing the two water stress tolerant varieties, higher root length was observed in non-colonised seedlings of Ptb 29 when PEG 6000 was not applied. The lowest root length is shown in *P. indica* colonised Ptb 29 under 20 percent PEG 6000 concentrations (2.10 cm)

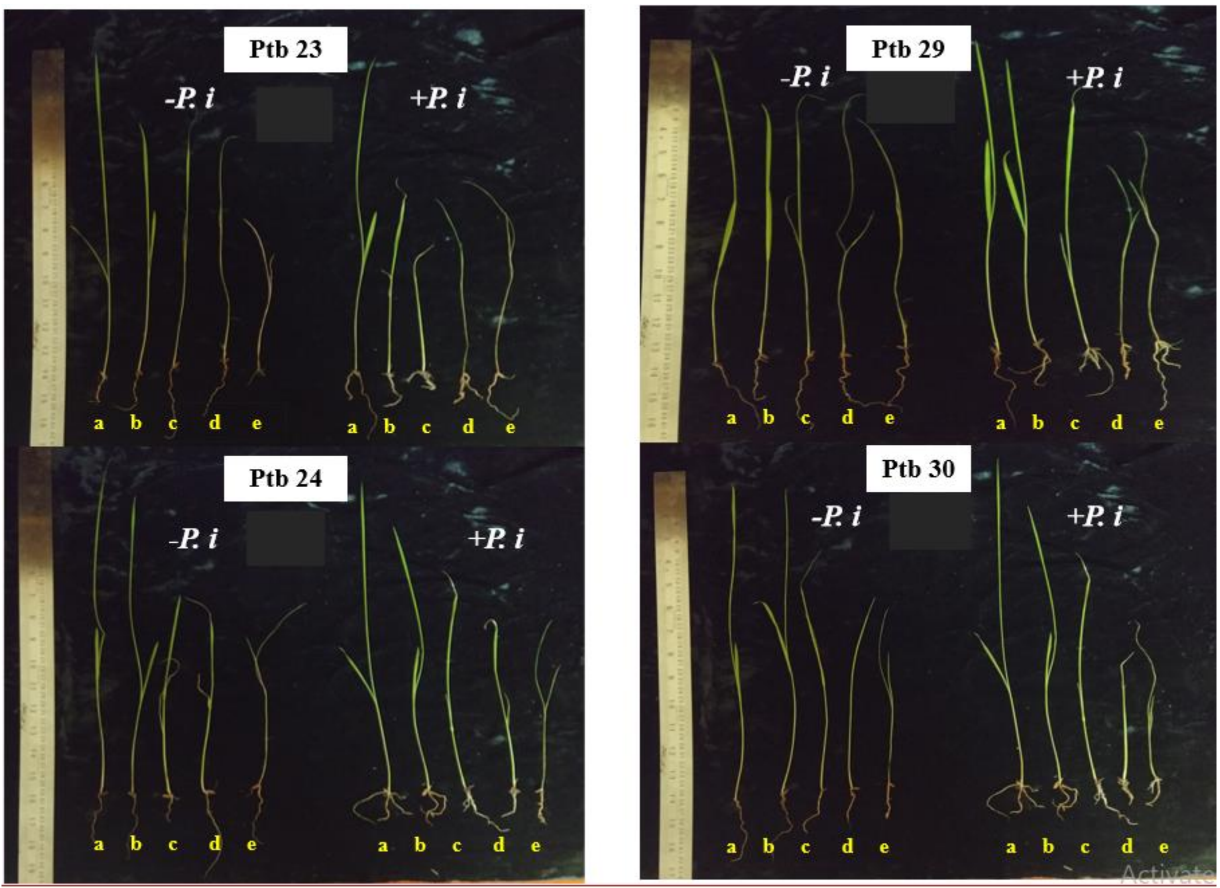

Plate. 5. Effect of P. indica-colonisation on seedling length of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment (different levels of PEG 6000 conc: a - 0%, b - 5%, c - 10%, d - 15% and e - 20%) (-*P. i* – without *P. indica*, +*P. i* – with *P. indica*)

In the absence of water stress, the root length of of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 3.30, 3.10, 3.10 and 3.30 cm respectively while the same for non-colonised seedlings were 467, 4.53, 4.73 and 4.47 cm respectively. Meanwhile under the extreme stress of 20 percent PEG 6000 solution the average root length of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 2.03, 2.53, 2.10 and 2.57 cm respectively while the same for non-colonised seedlings were 2.26, 2.30, 2.20 and 2.37 cm respectively.

4.1.4 Shoot dry weight

The results of shoot dry weight of *P. indica* colonised and non-colonised rice varieties at seedling stage under different concentrations of PEG 6000 are presented in table 6.

The rice varieties on interaction with *P. indica* showed higher shoot dry weight than the non-colonised seedlings in most treatment combinations. In the absence of water stress, the average shoot dry weight of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 3.40, 3.44, 3.38 and 3.38 g respectively while the same for non-colonised seedlings were 3.28, 3.38, 3.34 and 3.26 g respectively. As the concentration of PEG 6000 increased a decrease in shoot dry weight was observed across the two colonisation statuses of all four varieties. Under the stress of 20 percent PEG 6000 solution the average shoot dry weight of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 1.12, 1.54, 1.56 and 1.44 g respectively while the same for non-colonised seedlings were 1.16, 1.20, 1.22 and 1.26 g respectively.

The highest shoot dry weight was observed in Ptb 24 (3.44 mg) a water stress susceptible variety under control conditions while the lowest shoot dry weight was noticed in another water stress susceptible variety Ptb 23 (1.16 mg) under extreme water stress condition of 20 percent PEG 6000 concentration. When considering the tolerant varieties, the highest shoot dry weight was seen in *P. indica* colonised-control seedlings (3.38 mg) of Ptb 29 and the lowest shoot dry weight was observed in non-colonised rice seedlings of Ptb 29 under stress level of 20 percent PEG 6000 concentration (1.22 mg).

Table 4. Effect of *P. indica*-colonisation on seedling shoot length (cm) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change
1.	Control (0)	14.07	15.07	+7.11	14.40	15.21	+5.56	14.73	16.00	+8.62	14.13	14.43	+2.12
2.	5	11.13	12.97	+16.56	12.27	13.37	+11.90	13.27	14.40	+8.51	12.00	12.53	+4.41
3.	10	10.70	11.43	+6.88	11.27	12.73	+12.95	12.33	12.47	+1.13	11.07	11.37	+2.71
4.	15	9.53	10.53	+10.49	10.40	10.47	+0.67	10.10	10.97	+8.61	9.30	10.33	+12.90
5.	20	8.97	9.43	+5.12	9.17	10.00	+9.05	9.30	9.33	+0.32	9.13	9.00	-1.42

SEm (±)	0.582	SEm PV (±)	0.260	SEm PS (±)	0.291	SEm VS (±)	0.411
CD (0.05)	NS	CD (0.05) PV	NS	CD (0.05) PS	0.819	CD (0.05) VS	NS

Values are mean of three replications

Table 5. Effect of *P. indica*-colonisation on seedling root length (cm) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change
1.	Control (0)	4.67	3.30	-29.33	4.53	3.10	-31.57	4.73	3.10	-34.46	4.47	3.30	-26.17
2.	5	4.10	2.60	-36.59	4.47	3.10	-30.65	4.37	2.80	-35.93	4.20	2.90	-30.95
3.	10	3.73	2.70	-27.61	3.43	2.80	-14.22	3.47	2.77	-20.17	3.70	2.73	-26.21
4.	15	2.27	2.13	-6.17	2.30	2.63	+14.35	3.00	2.37	-21.00	2.37	2.40	+1.27
5.	20	2.26	2.03	-10.57	2.30	2.53	+10.00	2.20	2.10	-4.55	2.37	2.57	+12.66

SEm (±)	0.179	SEm PV (±)	0.08	SEm PS (±)	0.09	SEm VS (±)	0.127
CD (0.05)	0.505	CD (0.05) PV	0.226	CD (0.05) PS	0.252	CD (0.05) VS	0.357

Values are mean of three replications

4.1.5 Root dry weight

The results of root dry weight of *P. indica* colonised and non-colonised rice varieties at seedling stage under different concentrations of PEG 6000 are presented in table 7.

The rice varieties on interaction with *P. indica* showed higher root dry weight than the non-colonised seedlings in most treatment combinations. In the absence of water stress, the average root dry weight of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 1.14, 1.26, 1.12 and 1.14 mg respectively while the same for non-colonised seedlings were 1.06, 1.14, 1.08 and 1.06 mg respectively. As the concentration of PEG 6000 increased a decrease in root dry weight was observed across the two colonisation statuses of all four varieties. Under the stress of 20 percent PEG 6000 solution the average root dry weight of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 0.42, 0.39, 0.44 and 0.42 mg respectively while the same for non-colonised seedlings were 0.38, 0.36, 0.39 and 0.40 g respectively.

The highest root dry weight was observed in *P. indica* colonised Ptb 24 (1.26 mg) a water stress susceptible variety under control conditions while the lowest root dry weight was noticed in another water stress susceptible variety Ptb 23 (0.38 mg) under extreme water stress condition of 20 percent PEG 6000 concentration. When considering the tolerant varieties, the highest root dry weight was seen in *P. indica* colonised-control seedlings (1.14 mg) of Ptb 30 and the lowest root dry weight was observed in non-colonised rice seedlings of same variety under stress level of 20 percent PEG 6000 concentration (0.40 mg).

4.1.6 Seedling vigour index – 1

The results of seedling vigour index- 1 of *P. indica* colonised and non-colonised rice varieties at seedling stage under different concentrations of PEG 6000 are presented in table 8.

The rice varieties on colonisation with *P. indica* showed higher SVI-1 than the non-colonised seedlings in most treatment combinations. In the absence of water stress, the average SVI-1 of *P.indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 434.067, 422.400, 440.733 and 416.133 respectively while the same for non-colonised seedlings were 312.067, 294.200295.000 and 311.433 respectively. As the concentration of PEG 6000 increased a decrease

Table 6. Effect of *P. indica*-colonisation on seedling shoot dry weight (mg) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change
1.	**0 (Control)**	3.28	3.40	+3.61	3.38	3.44	+1.77	3.34	3.38	+1.19	3.26	3.38	+3.68
2.	**5**	2.8	3.1	+9.15	2.88	3.18	+10.42	2.90	3.14	+8.28	2.88	3.10	+7.64
3.	**10**	2.70	2.72	+0.74	2.80	2.86	+2.14	2.84	2.90	+2.11	2.68	2.70	+0.75
4.	**15**	2.30	2.60	+13.04	2.40	2.28	-5.00	2.40	2.44	+1.67	2.36	2.26	-4.2
5.	**20**	1.16	1.12	-3.44	1.20	1.54	+28.8	1.22	1.56	+27.87	1.26	1.44	+14.28

SEm (±)	0.078	SEm PV (±)	0.035	SEm PS (±)	0.039	SEm VS (±)	0.055
CD (0.05)	NS	CD (0.05) PV	NS	CD (0.05) PS	0.11	CD (0.05) VS	NS

Values are mean of three replications

Table 7. Effect of *P. indica*-colonisation on seedling root dry weight (mg) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change
1.	**Control (0)**	1.06	1.14	+7.55	1.14	1.26	+7.01	1.08	1.12	+3.70	1.06	1.14	+7.5
2.	**5**	0.94	1.02	+8.5	0.90	1.08	+20.00	0.92	1.06	+15.22	0.94	1.08	+14.89
3.	**10**	0.84	0.90	+7.1	0.77	0.84	9.09	0.84	0.88	+4.76	0.80	0.84	+5.00
4.	**15**	0.78	0.84	+7.69	0.74	0.78	+5.41	0.82	0.74	-9.76	0.74	0.76	+2.70
5.	**20**	0.38	0.42	+9.52	0.36	0.39	+8.33	0.39	0.44	+5.00	0.4	0.42	+5.00

SEm (±)	**4.066**	SEm PV (±)	**1.818**	SEm PS (±)	**2.033**	SEm VS (±)	**2.875**
CD (0.05)	**NS**	CD (0.05) PV	**NS**	CD (0.05) PS	**NS**	CD (0.05) VS	**NS**

Values are mean of three replications

in SVI-1 was observed across the two colonisation statuses of all four varieties. Under the stress of 20 percent PEG 6000 solution the average SVI - 1 of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 212.967, 248.167, 207.300 and 222.133 respectively while the same for non-colonised seedlings were 192.433, 247.000, 198.333 and 240.000 respectively

The highest SVI-1 was observed in *P. indica* colonised control seedlings of Ptb 29 (440.733), a water stress susceptible variety while the lowest SVI-1 was noticed in seedlings of water stress susceptible variety Ptb 23 (192.433) under extreme water stress condition of 20 per cent PEG 6000 concentration. When considering the tolerant varieties, the highest SVI-1 was seen in *P. indica* colonised-control seedlings of Ptb 30 and the lowest was observed in non-colonised rice seedlings of same variety under stress level of 20 percent PEG 6000 concentration.

4.1.7 Seedling vigour index – 2

The results of seedling vigour index-2 of *P. indica* colonised and non-colonised rice varieties at seedling stage under different concentrations of PEG 6000 are presented in table 9.

The rice varieties on interaction with *P. indica* showed higher SVI-2 than the non-colonised seedlings in most treatment combinations. In the absence of water stress, the average SVI-2 of *P.indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 409.2, 411.6, 406.2 and 407.4 respectively while the same for non-colonised seedlings were 393.6, 400.2, 397.7 and 389.4 respectively. As the concentration of PEG 6000 increased a decrease in SVI-2 was observed across the two colonisation statuses of all four varieties. Under the stress of 20 percent PEG 6000 solution the average SVI - 2 of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 151.8, 171.6, 183.6 and 166.8 respectively while the same for non-colonised seedlings were 145.8, 145.8, 136.2 and 145.2 respectively.

The highest seedling vigour index- 2 was observed in *P. indica* colonised control seedlings of Ptb 24 (411.4), a water stress susceptible variety while the lowest SVI-2 was noticed in seedlings of tolerant variety Ptb 29 (136.2) under extreme water stress condition of 20 percent PEG 6000 concentration.

Table 8. Effect of *P. indica*-colonisation on seedling vigour index-1 of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change
1.	Control (0)	312.067	434.067	+39.09	294.200	422.400	+43.58	295.000	440.733	+49.40	311.433	416.133	+33.62
2.	5	245.133	381.967	+55.82	291.267	415.733	+42.73	265.400	406.267	+53.08	272.967	390.533	+43.07
3.	10	253.700	346.833	+36.71	263.167	321.733	+22.25	261.233	324.467	+24.21	257.067	344.300	+33.93
4.	15	202.533	213.533	+5.43	247.400	217.467	-12.10	217.267	340.900	+56.90	226.333	219.868	-2.86
5.	20	192.433	212.967	+10.67	247.000	248.167	+0.47	198.333	207.300	+4.5	240.000	222.133	-6.21

SEm (±)	**7.738**	SEm PV (±)	**3.461**	SEm PS (±)	**3.869**	SEm VS (±)	**5.472**
CD (0.05)	**NS**	CD (0.05) PV	**NS**	CD (0.05) PS	**10.889**	CD (0.05) VS	**NS**

Values are mean of three replications.

4.1.8 Root shoot ratio

The results of root shoot ratio of *P. indica* colonised and non-colonised rice varieties at seedling stage under different concentrations of PEG 6000 are presented in table 10.

The rice varieties on interaction with *P. indica* showed higher root shoot ratio than the non-colonised seedlings in most treatment combinations. In the absence of water stress, the average root shoot ratio of *P.indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 0.348, 0.331, 0.331, and 0.337 respectively while the same for non-colonised seedlings were 0.311, 0314, 0.323 and 0.325 respectively. As the concentration of PEG 6000 increased a decrease in root shoot ratio was observed about the two colonisation conditions of all four varieties. Under the stress of 20 percent PEG 6000 solution the average root shoot ratio of *P. indica* colonised Ptb 23, Ptb 24, Ptb 29 and Ptb 30 were 0.273, 0.285, 0.293 and 0.292 respectively while the same for non-colonised seedlings were 0.290, 0.224, 0.311 and 0.317 respectively.

4.1.9 Number of root branches

The results of number of root branches of *P. indica* colonised and non-colonised rice varieties at seedling stage under different concentrations of PEG 6000 are presented in table 11.

The number of root branches were higher in *P. indica* colonised seedlings of all the four varieties and across all the stress conditions. The highest number of root branches was observed in *P. indica* colonised control seedlings of Ptb 29 (2.67), a water stress susceptible variety and Ptb 24 (2.67). However, the all the four varieties in the absence of root colonisation by *P. indica* did not produce branches and the average number of roots remained 1.

Table 9. Effect of *P. indica*-colonisation on seedling vigour index-2 of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change	-*P. indica*	+*P. indica*	% change
1.	Control (0)	393.6	409.2	+3.96	400.2	411.6	+2.85	397.7	406.2	+2.13	389.4	407.4	+4.62
2.	5	340.2	376.2	+10.58	341.4	382.8	+12.13	344.3	377.4	+9.61	341.4	376.2	+10.31
3.	10	319.2	324.6	+1.56	325.2	333.6	+2.58	327.6	345.6	+5.05	313.8	291.6	-0.71
4.	15	276.6	307.2	+11.06	282.6	292.2	+3.39	282.2	292.8	+3.76	280.2	272.4	-2.78
5.	20	145.8	151.8	+4.11	145.8	171.6	+17.69	136.2	183.6	+34.80	145.2	166.8	+14.87

SEm (±)	**16.002**	SEm PV (±)	**7.156**	SEm PS (±)	**8.001**	SEm VS (±)	**11.315**
CD (0.05)	**45.034**	CD (0.05) PV	**20.14**	CD (0.05) PS	**22.517**	CD (0.05) VS	**31.844**

Values are mean of three replications.

Table 10. Effect of *P. indica*-colonisation on seedling root shoot ratio of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change
1.	Control (0)	0.311	0.348	+11.89	0.314	0.331	+4.51	0.323	0.331	+2.47	0.325	0.337	+3.69
2.	5	0.303	0.359	+18.48	0.281	0.375	+33.45	0.317	0.378	+19.10	0.336	0.348	+3.57
3.	10	0.309	0.315	+1.94	0.287	0.300	+4.53	0.296	0.303	+2.33	0.296	0.311	+5.07
4.	15	0.300	0.353	+19.67	0.259	0.325	+25.48	0.336	0.304	-9.52	0.314	0.336	+7.00
5.	20	0.290	0.273	-5.86	0.234	0.285	+21.79	0.311	0.293	-5.79	0.317	0.292	-7.88

SEm (±)	**0.035**	SEm PV (±)	**0.016**	SEm PS (±)	**0.018**	SEm VS (±)	**0.025**
CD (0.05)	**NS**	CD (0.05) PV	**NS**	CD (0.05) PS	**0.05**	CD (0.05) VS	**NS**

Values are mean of three replications.

Table 11. Effect of *P. indica*-colonisation on number of root branches of seedlings of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment

Sl. No.	PEG 6000 conc. (%)	Varieties											
		Ptb 23			Ptb 24			Ptb 29			Ptb 30		
		+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change	+*P. indica*	-*P. indica*	% change
1.	Control (0)	1.00	2.67	+167	1.00	2.67	+167	1.00	2.67	+167	1.00	2.67	+167
2.	5	1.00	2.33	+133	1.00	2.33	+133	1.00	2.33	+133	1.00	1.67	+67
3.	10	1.00	2.00	+100	1.00	2.00	+100	1.00	2.00	+100	1.00	1.33	+33
4.	15	1.00	1.67	+67	1.00	1.67	+67	1.00	1.67	+67	1.00	1.67	+67
5.	20	1.00	1.33	+33	1.00	1.67	+67	1.00	2.00	+100	1.00	1.33	+33

SEm (±)	0.279	SEm PV (±)	0.125	SEm PS (±)	0.139	SEm VS (±)	0.197
CD (0.05)	NS	CD (0.05) PV	NS	CD (0.05) PS	0.392	CD (0.05) VS	NS

Values are mean of three replications.

4.2 EXPERIMENT 2

In this study, *P. indica* colonised and non-colonised rice seedlings of the selected water stress susceptible variety (Ptb 23) and water stress tolerant variety (Ptb 29), from experiment 1 were raised in rainout shelter. Water stress was induced by stopping irrigation for 5 days during maximum tillering, panicle initiation and flowering stages and normal irrigation resumed thereafter. Physiological parameters were observed at maximum tillering, panicle initiation and flowering stages, while the morphological and yield parameters were observed at harvest stage. The biochemical and molecular parameters were analysed during flowering stage. The data collected was statistically analysed, and the results are tabulated and explained in this chapter.

4.2.1 Morphological parameters

4.2.1.1 Shoot length

The shoot length of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 under irrigated and water stress conditions was taken at harvest stage and recorded in table 12.

The result showed that the shoot length of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23 under control condition. It also indicates that rice plants colonised by *P. indica* recorded higher shoot length than non-colonised plants of both varieties. The shoot length of *P. indica* colonised Ptb 29 under water stress condition was 73.07 cm and under irrigated condition was 89.10 cm, while that of non-colonised plants were 69.30 and 84.17 cm respectively. A similar trend was observed in Ptb 23 with *P. indica*-colonised plants under water stress condition showing an average shoot length of 61.83 cm and irrigated plants with shoot length of 78.70 cm while non-colonised plants with a shoot length of 48.00 cm and 74.60 cm respectively.

4.2.1.1 Root length

The root length recorded under both irrigated and water stress condition at harvest stage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 were presented in table 13. There is a significant variation in root length among the various treatment combinations. The result shows that the root length of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23. Ptb 29 also showed higher root length under water

Table 12. Effect of *P. indica*-colonisation on shoot length (cm) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	**+*P. indica***	**% change**	**- *P. indica***	**-+*P. indica***	**% change**
1.	**Water stress**	48.00	61.83	+28.81	69.30	73.07	+5.44
2.	**Irrigated**	74.60	78.70	+5.49	84.17	89.10	+5.88
SEm (±)	1.963	SEm PV (±)	1.388	SEm PS (±)	1.388	SEm VS (±)	1.388
CD (0.05)	5.884	CD(0.05) PV	4.161	CD(0.05) PS	NS	CD(0.05) VS	4.161

Table 13. Effect of *P. indica*-colonisation on root length (cm) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	**+*P. indica***	**% change**	**- *P. indica***	**+*P. indica***	**% change**
1.	**Water stress**	45.10	47.53	+5.39	52.93	63.43	+19.83
2.	**Irrigated**	48.47	52.73	+8.79	42.77	46.40	+8.49
SEm (±)	1.164	SEm PV (±)	0.823	SEm PS (±)	0.823	SEm VS (±)	0.823
CD (0.05)	3.491	CD(0.05) PV	2.468	CD(0.05) PS	NS	CD(0.05) VS	2.468

Values are mean of three replications.

stressed condition when compared to irrigated condition. The root length of *P. indica* colonised Ptb 29 under water stress condition (63.43 cm) was higher than that of non-colonised plants (52.93 cm). When irrigated the root length of *P. indica*-colonised Ptb 29 was 46.40 cm and that of non-colonised plants was 42.77 cm. *P. indica*-colonised Ptb 23 under water stressed condition showed an average root length of 47.53 cm while that of non-colonised plants was 45.10. On irrigation the root length of Ptb 23 with *P. indica* (52.73 cm) was on par with that of Ptb 23 plants without *P. indica* (48.47 cm).

4.2.1.1 Shoot dry weight

The shoot dry weight of *P. indica*-colonised and non-colonised plants of Ptb 23 and ptb 29 under irrigated and water stress conditions was taken at harvest stage and recorded in table 14.

The result showed that the shoot dry weight of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23 under water stressed condition. It also indicated that rice plants colonised by *P. indica* recorded higher shoot dry weight than non-colonised plants of both varieties. The shoot dry weight of *P. indica* colonised Ptb 29 under water stress and irrigated condition was 23.97 and 39.61 g respectively, while that of non-colonised plants was 13.58 and 34.87 g respectively. In *P. indica*-colonised plants of Ptb 23 under water stress condition, the shoot dry weight was 24.59 g which was lower than that of irrigated condition (32.24 g). Ptb 23 rice plants without *P. indica* under water stressed and irrigated condition recorded shoot dry weights of 18.70 and 31.62 g respectively.

4.2.1.1 Root dry weight

The root dry weight recorded under both irrigated and water stress condition at harvest stage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 were presented in table 15.

There was a significant variation in root dry weight among the various treatment combinations. The result showed that the root dry weight of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23. Ptb 29 showed higher root dry weight under water stressed condition when compared to irrigated condition. The root dry weight of *P. indica* colonised Ptb 29 under water stress condition (16.60 g) was significantly higher than that of non-colonised plants (8.87 g). When irrigated the root dry weight of *P. indica* colonised

Table 14. Effect of *P. indica*-colonisation on shoot dry weight (g) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	*+P. indica*	% change	*- P. indica*	*-+P. indica*	% change
1.	Water stress	18.70	24.59	+31.49	13.58	23.97	+76.51
2.	Irrigated	31.62	35.24	+11.45	34.87	39.61	+13.59
SEm (±)	0.97	SEm PV (±)	0.686	SEm PS (±)	0.686	SEm VS (±)	0.686
CD (0.05)	NS	CD(0.05) PV	2.056	CD(0.05) PS	2.056	CD(0.05) VS	NS

Table 15. Effect of *P. indica*-colonisation on root dry weight (g) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	*+P. indica*	% change	*- P. indica*	*-+P. indica*	% change
1.	Water stress	6.27	10.47	+66.98	8.87	16.60	+87.15
2.	Irrigated	10.97	14.43	+31.54	21.73	23.50	+8.15
SEm (±)	0.656	SEm PV (±)	0.464	SEm PS (±)	0.464	SEm VS (±)	0.464
CD (0.05)	1.967	CD(0.05) PV	1.391	CD(0.05) PS	1.391	CD(0.05) VS	NS

Values are mean of three replications.

Ptb 29 was 23.50 g and that of non-colonised plants was 21.73 g. *P. indica* colonised Ptb 23 under water stressed condition showed an average root dry weight of 10.47 g while that of non-colonised plants was 6.27 g. On irrigation the root dry weight of Ptb 23 with *P. indica* (14.43 g) was higher than that of Ptb 23 plants without *P. indica* (10.97 g).

4.2.1.1 Root shoot ratio

The root shoot ratio observed under both irrigated and water stress condition at harvest stage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 were presented in table 16.

The result showed that the root shoot ratio of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23. Ptb 29 showed higher root shoot ratio under water stressed condition when compared to irrigated condition. The root shoot ratrio of *P. indica* colonised Ptb 29 under water stress condition (0.70) was higher than that of non-colonised plants (0.66). When irrigated the root shoot ratio of *P. indica* colonised Ptb 29 was 0.62 and that of non-colonised plants was 0.59. *P. indica* colonised Ptb 23 under water stressed condition showed an average root shoot ratio of 0.42 while that of non-colonised plants was 0.33. On irrigation Ptb 23 with *P. indica* showed higher root shoot ratio (0.41) than Ptb 23 plants without *P. indica* (0.35).

4.2.1.1 Root volume

The root volume of *P. indica* colonised and non-colonised plants of Ptb 23 and ptb 29 under irrigated and water stress conditions was observed at harvest stage and recorded in table 17.

The root volume of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23 under both water stressed and irrigated conditions. It also revealed that rice plants colonised by *P. indica* recorded higher root volume than non-colonised plants of both varieties. The root volume of *P. indica* colonised Ptb 29 under water stress and irrigated condition was 40.53 and 50.63 cm^3 respectively, while those of non-colonised plants were 34.30 and 45.27 cm^3 respectively. In *P. indica*-colonised plants of Ptb 23 under water stress condition, the root volume was 23.60 cm which was lower than that of irrigated condition (29.70 cm^3). Ptb 23 rice plants without *P. indica* under water stressed and irrigated condition recorded root volume of 16.53 and 27.67 cm respectively.

Table 16. Effect of *P. indica*-colonisation on root shoot ratio of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	***+P. indica***	**% change**	***- P. indica***	***-+P. indica***	**% change**
1.	**Water stress**	0.33	0.42	+27.27	0.66	0.70	+6.06
2.	**Irrigated**	0.35	0.41	+17.14	0.59	0.62	+5.08
SEm (±)	0.031	SEm PV (±)	0.022	SEm PS (±)	0.022	SEm VS (±)	0.022
CD (0.05)	NS	CD (0.05) PV	NS	CD (0.05) PS	NS	CD (0.05) VS	NS

Table 17. Effect of *P. indica*-colonisation on root volume (cm^3) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	***+P. indica***	**% change**	***- P. indica***	***-+P. indica***	**% change**
1.	**Water stress**	16.53	23.60	+42.77	34.30	40.53	+18.16
2.	**Irrigated**	27.67	29.70	+7.34	45.27	50.63	+11.84
SEm (±)	0.648	SEm PV (±)	0.458	SEm PS (±)	0.458	SEm VS (±)	0.458
CD (0.05)	1.942	CD (0.05) PV	1.373	CD (0.05) PS	NS	CD (0.05) VS	NS

Values are mean of three replications.

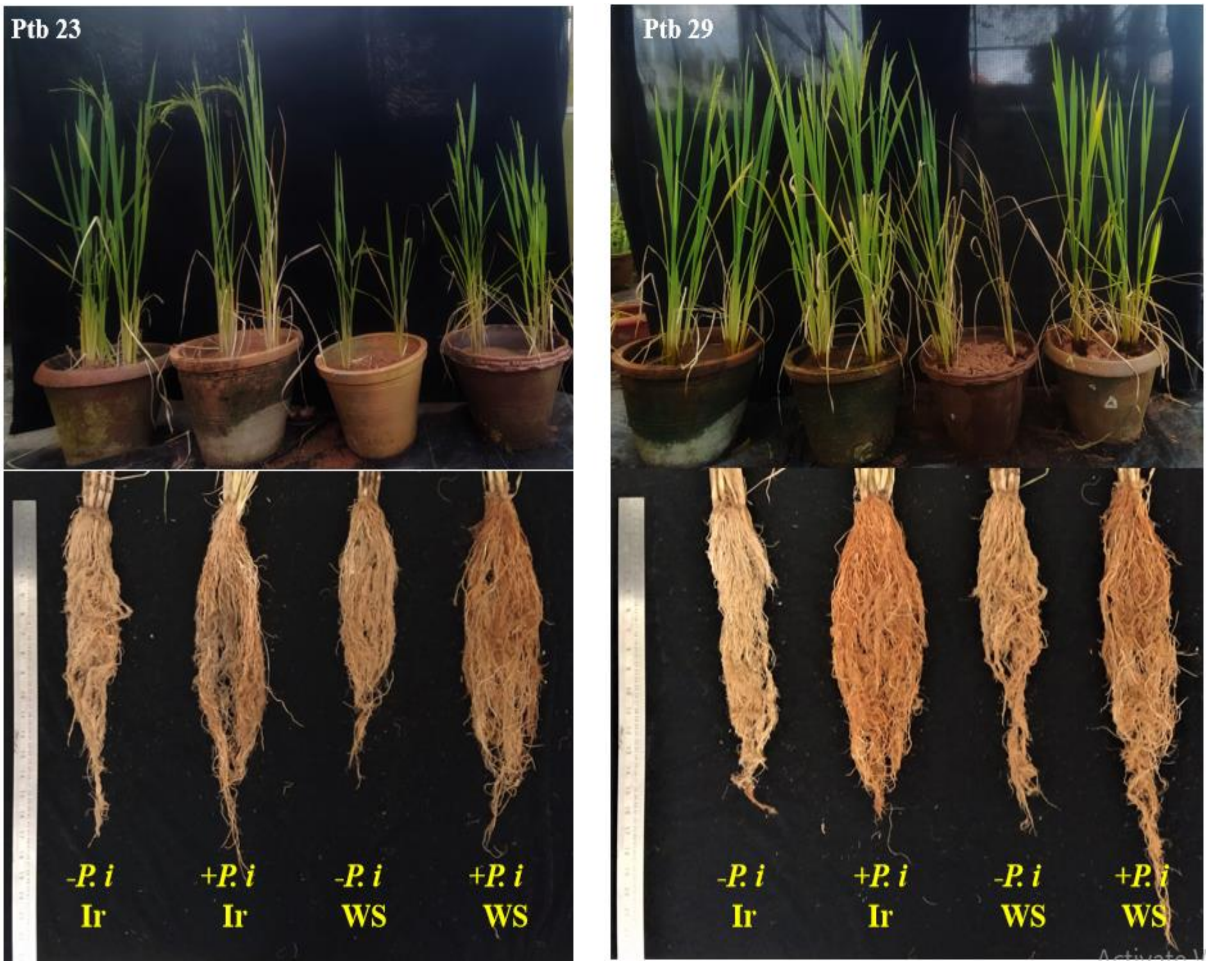

Plate. 6. Effect of *P. indica*-colonisation on shoot and root length of Ptb 23 and Ptb 29 rice under irrigated (Ir) and water stress (WS) conditions (-*P. i* – without *P. indica*, +*P. i* – with *P. indica*)

4.2.2 Physiological and biochemical parameters

4.2.2.1 Relative water content (RWC) (%)

The relative water content of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 under irrigated and water stress conditions were observed at maximum tillering, panicle initiation and flowering stages and recorded in table 18.

Ptb 29 variety showed higher RWC than Ptb 23 plants at all three stages of observation. At maximum tillering stage, water stressed plants of *P. indica* colonised Ptb 29 recorded an RWC of 78.83 per cent while for non-colonised plants it was 74.73 per cent. Irrigated rice plants of Ptb 29 with and without *P. indica* colonisation showed RWC of 88.20 and 86.03 per cent respectively. In the absence of *P. indica* colonisation, the water stressed Ptb 23 plants showed the lowest RWC of 69.67 per cent while for irrigated plants it was 80.93 per cent. The results indicated that *P. indica* colonisation improved RWC in both water-stressed (76.20 per cent) and irrigated (82.27 per cent) plants of Ptb 23.

During the panicle initiation stage, the RWC of Ptb 29 rice plants with and without *P. indica* under water stress were 78.33 and 73.40 per cent respectively while that of plants under irrigated conditions were 87.17 and 86.83 per cent respectively. Ptb 23 rice plants with the root colonising endophyte under water limited condition showed an RWC of 75.30 per cent while non-colonised plants recorded a lower value of 67.97 per cent. On irrigation the values increased to 80.77 and 78.30 per cent respectively.

At flowering stage, the presence of *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the RWC of water stressed rice plants (73.43 and 84.70 per cent respectively) on comparison with that of irrigated plants (79.17 and 87.73 per cent respectively). However, in the absence of *P. indica* the RWC of Ptb 23 and Ptb 29 under water limited situations were 67.77 and 76.87 per cent respectively while that of under normal irrigation schedule were 76.93 and 85.87 per cent respectively.

Table 18. Effect of *P. indica*-colonisation on relative water content (%) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions at a) maximum tillering, b) panicle initiation and c) flowering stages.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	**+ *P. indica***	**% change**	**- *P. indica***	**+ *P. indica***	**% change**
a) Maximum tillering stage							
1.	**Water stress**	69.67	76.2	+9.37	74.73	78.83	+5.49
2.	**Irrigated**	80.93	82.27	+1.62	86.03	88.20	+2.52
SEm (±)	0.714	SEm PV (±)	0.505	SEm PS (±)	0.505	SEm VS (±)	0.505
CD (0.05)	2.141	CD(0.05) PV	NS	CD (0.05) PS	1.514	CD(0.05) VS	NS
b) Panicle initiation stage							
1.	**Water stress**	67.97	75.3	+10.78	73.4	78.33	+6.72
2.	**Irrigated**	78.3	80.77	+3.15	86.83	87.17	+0.39
SEm (±)	1.247	SEm (±)	0.882	SEm (±)	0.882	SEm (±)	0.882
CD (0.05)	3.737	CD(0.05) PV	NS	CD (0.05) PS	2.643	CD(0.05) VS	2.643
c) Flowering stage							
1.	**Water stress**	67.77	73.43	+8.35	76.87	84.7	+10.19
2.	**Irrigated**	76.93	79.17	+2.91	85.87	87.73	+2.17
SEm (±)	1.596	SEm (±)	1.129	SEm (±)	1.129	SEm (±)	1.129
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	NS	CD(0.05) VS	3.384

Values are mean of three replications.

4.2.2.1 Specific leaf area (cm g^{-1})

The specific leaf area of *P. indica* colonised and non-colonised plants of Ptb 23 and ptb 29 under irrigated and water stress conditions were observed at maximum tillering, panicle initiation and flowering stages and recorded in table 19.

Ptb 29 variety showed higher specific leaf area than Ptb 23 plants at all three stages of observation. At maximum tillering stage, water stressed plants of *P. indica* colonised Ptb 29 recorded an SLA of 316.53 cm g^{-1} while for non-colonised plants it was 247.30 cm g^{-1}. Irrigated rice plants of Ptb 29 with and without *P. indica* colonisation showed specific leaf area of 347.47 and 325.29 cm g^{-1} respectively. In the absence of *P. indica* colonisation, the water stressed Ptb 23 plants showed the lowest specific leaf area of 195.30 (cm g^{-1}) while for irrigated plants it was 258.40 (cm g^{-1}). The results indicated that *P. indica* colonisation improved specific leaf area in both water-stressed (235.31 cm g^{-1}) and irrigated (301.75 cm g^{-1}) plants of Ptb 23.

During the panicle initiation stage, the specific leaf area of Ptb 29 rice plants with and without *P. indica* under water stress were 336.47 and 261.52 cm g^{-1} respectively while that of plants under irrigated conditions were 351.61 and 322.40 cm g^{-1} respectively. Ptb 23 rice plants with the root colonising endophyte under water limited condition showed a specific leaf area of 225.85 cm g^{-1} while non-colonised plants recorded a lower value of 196.79 cm g^{-1} and under irrigated condition the observed values are 292.24 and 257.65 cm g^{-1} respectively.

At flowering stage, *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the specific leaf area of water stressed rice plants (315.63 and 256.93 cm g^{-1} respectively) on comparison with that of irrigated plants (355.57 and 303.53 cm g^{-1} respectively). However, in the absence of *P. indica* the specific leaf area of Ptb 23 and Ptb 29 under water limited situations were 201.70 and 284.31 cm g^{-1} respectively while that of under normal irrigation schedule were 282.64 and 326.90 cm g^{-1} respectively.

4.2.2.1 Cell membrane stability index (%)

The cell membrane stability index of *P. indica* colonised and non-colonised plants of Ptb 23 and ptb 29 under irrigated and water stress conditions were observed at maximum tillering, panicle initiation and flowering stages and recorded in table 20.

Table 19. Effect of *P. indica*-colonisation on specific leaf area (cm g^{-1}) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions at a) maximum tillering stage, b) panicle initiation and c) flowering stages.

Sl. No.	Treatments	Varieties: Ptb 23 (water stress susceptible) - *P. indica*	+ *P. indica*	% change	Ptb 29 (water stress tolerant) - *P. indica*	+ *P. indica*	% change
a) Maximum tillering stage							
1.	**Water stress**	195.30	235.31	+29.70	247.31	316.53	+27.99
2.	**Irrigated**	258.40	301.75	+16.78	325.29	347.47	+6.82
SEm (±)	6.006	SEm PV (±)	4.247	SEm PS (±)	4.247	SEm VS (±)	4.247
CD (0.05)	18.005	CD(0.05) PV	NS	CD (0.05) PS	12.731	CD(0.05) VS	NS
b) Panicle initiation stage							
1.	**Water stress**	196.79	225.85	+14.77	261.52	336.47	+28.66
2.	**Irrigated**	257.65	292.24	+13.43	322.40	351.61	+9.06
SEm (±)	10.463	SEm (±)	7.398	SEm (±)	7.398	SEm (±)	7.398
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	NS	CD(0.05) VS	NS
c) Flowering stage							
1.	**Water stress**	201.70	256.93	+27.38	284.31	315.63	+11.02
2.	**Irrigated**	282.64	303.53	+7.39	326.90	355.57	+8.77
SEm (±)	7.032	SEm (±)	4.972	SEm (±)	4.972	SEm (±)	4.972
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	NS	CD(0.05) VS	14.907

Values are mean of three replications.

Table 20. Effect of *P. indica*-colonisation on cell membrane stability index (%) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions at a) maximum tillering, b) panicle initiation and c) flowering stages.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	**+ *P. indica***	**% change**	**- *P. indica***	**+ *P. indica***	**% change**
a) Maximum tillering stage							
1.	**Water stress**	77.9	82.4	+5.77	90.5	95.9	+5.96
2.	**Irrigated**	83.9	85.2	+1.55	96.5	98.9	+2.49
SEm (±)	0.892	SEm PV (±)	0.631	SEm PS (±)	0.631	SEm VS (±)	0.631
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	1.842	CD(0.05) VS	NS
b) Panicle initiation stage							
1.	**Water stress**	79.93	84.6	+5.84	92.27	97.27	+5.41
2.	**Irrigated**	84.8	85.47	+0.79	97.87	98.33	+0.47
SEm (±)	0.824	SEm (±)	0.583	SEm (±)	0.583	SEm (±)	0.583
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	NS	CD(0.05) VS	NS
c) Flowering stage							
1.	**Water stress**	81.6	85.73	+5.06	95.43	97.83	+2.51
2.	**Irrigated**	84.9	84.87	-0.03	97.57	98.2	+0.65
SEm (±)	0.824	SEm (±)	0.583	SEm (±)	0.583	SEm (±)	0.583
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	NS	CD(0.05) VS	NS

Values are mean of three replications.

Ptb 29 variety showed higher cell membrane stability index than Ptb 23 plants at all three stages of observation. At maximum tillering stage, water stressed plants of *P. indica* colonised Ptb 29 recorded a cell membrane stability index of 95.90 per cent while for non-colonised plants it was 90.50 per cent. Irrigated rice plants of Ptb 29 with and without *P. indica* colonisation showed cell membrane stability index of 98.90 and 96.50 percent respectively. In the absence of *P. indica* colonisation, the water stressed Ptb 23 plants showed the lowest cell membrane stability index of 77.90 per cent while for irrigated plants it was 83.90 per cent. The results indicated that *P. indica* colonisation improved cell membrane stability index in both water-stressed (82.40 per cent) and irrigated (85.20 per cent) plants of Ptb 23.

During the panicle initiation stage, the cell membrane stability index of Ptb 29 rice plants with and without *P. indica* under water stress were 97.27 and 92.27 per cent respectively while that of plants under irrigated conditions were 98.33 and 97.87 per cent respectively. Ptb 23 rice plants with the root colonising endophyte under water limited condition showed a cell membrane stability index of 84.60 per cent while non-colonised plants recorded a lower value of 79.93 per cent and under irrigated condition the observed values were 85.47 and 84.80 per cent respectively.

At flowering stage, the presence of *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the cell membrane stability index of water stressed rice plants (85.73 and 97.83 per cent respectively) on comparison with that of irrigated plants (84.87 and 98.20 per cent respectively). However, in the absence of *P. indica* the cell membrane stability index of Ptb 23 and Ptb 29 under water limited situations were 81.60 and 95.43 per cent respectively while that of under normal irrigation schedule were 84.90 and 97.57 per cent respectively.

4.2.2.1 Stomatal conductance (m H_2O moles m^{-2} s^{-1})

Ptb 29 variety showed higher stomatal conductance than Ptb 23 plants at all three stages of observation (Table 21). At maximum tillering stage, water stressed plants of *P. indica* colonised Ptb 29 recorded a stomatal conductance of 344.33 m H_2O moles m^{-2} s^{-1} while for non-colonised plants it was 230.33 m H_2O moles m^{-2} s^{-1}. Irrigated rice plants of Ptb 29 with and without *P. indica* colonisation showed stomatal conductance of 392.00 and 371.67 m H_2O moles m^{-2} s^{-1} respectively. In the absence of *P. indica* colonisation, the water stressed Ptb 23 plants showed the

Table 21. Effect of *P. indica*-colonisation on stomatal conductance (m H_2O moles m^{-2} s^{-1}) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions at a) maximum tillering stage, b) panicle initiation and c) flowering stages.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	**+ *P. indica***	**% change**	**- *P. indica***	**+ *P. indica***	**% change**
a) Maximum tillering stage							
1.	**Water stress**	146.00	247.67	+69.64	230.33	344.33	+49.49
2.	**Irrigated**	284.33	295.67	+3.99	371.67	392.00	+5.47
SEm (±)	6.318	SEm PV (±)	4.467	SEm PS (±)	4.467	SEm VS (±)	4.467
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	13.393	CD(0.05) VS	NS
b) Panicle initiation stage							
1.	**Water stress**	169.00	204.33	+20.57	300.33	342.00	+13.87
2.	**Irrigated**	262.33	277.33	+5.72	465.33	484.00	+3.37
SEm (±)	2.804	SEm (±)	1.983	SEm (±)	1.983	SEm (±)	1.983
CD (0.05)	8.406	CD(0.05) PV	5.944	CD (0.05) PS	5.944	CD(0.05) VS	5.944
c) Flowering stage							
1.	**Water stress**	171.33	254.33	+48.44	290.97	354.67	+21.89
2.	**Irrigated**	268.03	282.67	+5.46	347.67	376.67	+8.34
SEm (±)	3.958	SEm (±)	2.798	SEm (±)	2.798	SEm (±)	2.798
CD (0.05)	11.865	CD(0.05) PV	NS	CD (0.05) PS	8.39	CD(0.05) VS	8.39

Values are mean of three replications.

lowest stomatal conductance of 146.00 m H_2O moles m^{-2} s^{-1} while for irrigated plants it was 284.33 H_2O moles m^{-2} s^{-1}. The results indicated that *P. indica* colonisation improved stomatal conductance in both water-stressed (247.67 m H_2O moles m^{-2} s^{-1}) and irrigated (295.67 m H_2O moles m^{-2} s^{-1}) plants of Ptb 23.

During the panicle initiation stage, the stomatal conductance of Ptb 29 rice plants with and without *P. indica* under water stress were 342.00 and 342.00 m H_2O moles m^{-2} s^{-1} respectively while that of plants under irrigated conditions were 484.00 and 465.33 m H_2O moles m^{-2} s^{-1} respectively. Ptb 23 rice plants with the root colonising endophyte under water limited condition showed a stomatal conductance of 204.33 m H_2O moles m^{-2} s^{-1} while non-colonised plants recorded a lower value of 169.00 m H_2O moles m^{-2} s^{-1} and under irrigated condition the observed values were 277.33 and 262.33 m H_2O moles m^{-2} s^{-1} respectively.

At flowering stage, the presence of *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the stomatal conductance of water stressed rice plants (254.33 and 354.67 m H_2O moles m^{-2} s^{-1} respectively) on comparison with that of irrigated plants (282.67 and 376.67 m H_2O moles m^{-2} s^{-1} respectively). However, in the absence of *P. indica* the stomatal conductance of Ptb 23 and Ptb 29 under water limited situations were 171.33 and 290.97 m H_2O moles m^{-2} s^{-1} respectively while that of under normal irrigated condition were 268.03 and 347.67 m H_2O moles m^{-2} s^{-1} respectively.

4.2.2.1 Transpiration rate (m H_2O moles m^{-2} s^{-1})

Ptb 29 variety showed higher transpiration rate than Ptb 23 plants at all three stages of observation (Table 22). At maximum tillering stage, water stressed plants of *P. indica* colonised Ptb 29 recorded a transpiration rate of 1.37 m H_2O moles m^{-2} s^{-1} while for non-colonised plants it was 0.81 m H_2O moles m^{-2} s^{-1}. Irrigated rice plants of Ptb 29 with and without *P. indica* colonisation showed transpiration rate of 1.54 and 1.36 m H_2O moles m^{-2} s^{-1} respectively. In the absence of *P. indica* colonisation, the water stressed Ptb 23 plants showed the lowest transpiration rate of 0.48 m H_2O moles m^{-2} s^{-1} while for irrigated plants it was 1.15 m H_2O moles m^{-2} s^{-1}. The results indicated that *P. indica* colonisation significantly improved transpiration rate in both water-stressed (0.94 m H_2O moles m^{-2} s^{-1}) and irrigated (1.26 m H_2O moles m^{-2} s^{-1}) plants of Ptb 23.

Table 22. Effect of *P. indica*-colonisation on transpiration rate (m H_2O moles m^{-2} s^{-1}) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions at a) maximum tillering stage, b) panicle initiation and c) flowering stages.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+ *P. indica*	% change	- *P. indica*	+ *P. indica*	% change
a) Maximum tillering stage							
1.	**Water stress**	0.48	0.94	+95.83	0.81	1.37	+69.14
2.	**Irrigated**	1.15	1.26	+9.56	1.36	1.54	+13.23
SEm (±)	0.041	SEm PV (±)	0.029	SEm PS (±)	0.029	SEm VS (±)	0.029
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	0.088	CD(0.05) VS	0.088
b) Panicle initiation stage							
1.	**Water stress**	0.35	0.92	+162.86	0.61	1.30	+113.11
2.	**Irrigated**	1.17	1.27	+8.54	1.25	1.37	+9.6
SEm (±)	0.118	SEm (±)	0.083	SEm (±)	0.083	SEm (±)	0.083
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	0.25	CD(0.05) VS	NS
c) Flowering stage							
1.	**Water stress**	0.48	1.05	+118.75	0.70	1.20	+71.42
2.	**Irrigated**	1.21	1.36	+12.50	1.31	1.38	+5.34
SEm (±)	0.031	SEm (±)	0.022	SEm (±)	0.022	SEm (±)	0.022
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	0.065	CD(0.05) VS	0.065

Values are mean of three replications

During the panicle initiation stage, the transpiration rate of Ptb 29 rice plants with and without *P. indica* under water stress were 1.30 and 0.61 m H_2O moles m^{-2} s^{-1} respectively while that of plants under irrigated conditions were 1.37 and 1.25 m H_2O moles m^{-2} s^{-1} respectively. Ptb 23 rice plants with the root colonising endophyte under water limited condition showed a transpiration rate of 0.92 m H_2O moles m^{-2} s^{-1} while non-colonised plants recorded a lower value of 0.35 m H_2O moles m^{-2} s^{-1} and under irrigated condition the observed values were 1.27 and 1.17 m H_2O moles m^{-2} s^{-1} respectively.

At flowering stage, the presence of *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the transpiration rate of water stressed rice plants (1.05 and 1.20 m H_2O moles m^{-2} s^{-1} respectively) on comparison with that of irrigated plants (1.36 and 1.38 m H_2O moles m^{-2} s^{-1} respectively). However, in the absence of *P. indica* the transpiration rate of Ptb 23 and Ptb 29 under water limited situations were 0.48 and 0.70 m H_2O moles m^{-2} s^{-1} respectively while that of under normal irrigation schedule were 1.21 and 1.31 m H_2O moles m^{-2} s^{-1} respectively.

4.2.2.1 Photosynthetic rate (µ CO_2 moles m^{-2} s^{-1})

Ptb 29 variety showed higher photosynthetic rate than Ptb 23 plants at all three stages of observation (Table 23). At maximum tillering stage, water stressed plants of *P. indica* colonised Ptb 29 recorded a photosynthetic rate of 20.67 µ CO_2 moles m^{-2} s^{-1} while for non-colonised plants it was 14.13 µ CO_2 moles m^{-2} s^{-1}. Irrigated rice plants of Ptb 29 with and without *P. indica* colonisation showed photosynthetic rate of 25.57 and 23.57 µ CO_2 moles m^{-2} s^{-1} respectively. In the absence of *P. indica* colonisation, the water stressed Ptb 23 plants showed the lowest photosynthetic rate of 4.27 µ CO_2 moles m^{-2} s^{-1} while for irrigated plants it was 11.80 µ CO_2 moles m^{-2} s^{-1}. The results indicated that *P. indica* colonisation increased photosynthetic rate in both water-stressed (5.70 µ CO_2 moles m^{-2} s^{-1}) and irrigated (14.20 µ CO_2 moles m^{-2} s^{-1}) plants of Ptb 23.

During the panicle initiation stage, the photosynthetic rate of Ptb 29 rice plants with and without *P. indica* under water stress were 21.47 and 14.20 µ CO_2 moles m^{-2} s^{-1} respectively while that of plants under irrigated conditions were 27.93 and 25.67 µ CO_2 moles m^{-2} s^{-1} respectively. Ptb 23 rice plants with the root colonising endophyte under water limited condition showed a

Table 23. Effect of *P. indica*-colonisation on photosynthetic rate (μ CO_2 moles m^{-2} s^{-1}) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions at a) maximum tillering stage, b) panicle initiation and c) flowering stages.

Sl. No.	**Treatments**	**Varieties**					
		Ptb 23 (water stress susceptible)			**Ptb 29 (water stress tolerant)**		
		- *P. indica*	**+ *P. indica***	**% change**	**- *P. indica***	**+ *P. indica***	**% change**
a) Maximum tillering stage							
1.	**Water stress**	4.27	5.7	+36.71	14.13	20.67	+46.28
2.	**Irrigated**	11.8	14.2	+20.33	23.57	25.93	+10.01
SEm (±)	0.331	SEm PV (±)	0.234	SEm PS (±)	0.234	SEm VS (±)	0.234
CD (0.05)	0.992	CD(0.05) PV	0.701	CD (0.05) PS	0.701	CD(0.05) VS	NS
b) Panicle initiation stage							
1.	**Water stress**	4.9	7.87	+60.61	14.20	21.47	+51.19
2.	**Irrigated**	12.73	14.8	+16.26	25.67	27.93	+8.80
SEm (±)	0.345	SEm (±)	0.244	SEm (±)	0.244	SEm (±)	0.244
CD (0.05)	1.034	CD(0.05) PV	0.731	CD (0.05) PS	0.731	CD(0.05) VS	0.731
c) Flowering stage							
1.	**Water stress**	4.23	8.50	+100.94	15.3	21.4	+39.87
2.	**Irrigated**	13.63	14.40	+5.64	24.6	26.97	+9.63
SEm (±)	0.513	SEm (±)	0.363	SEm (±)	0.363	SEm (±)	0.363
CD (0.05)	NS	CD(0.05) PV	1.088	CD (0.05) PS	1.088	CD(0.05) VS	NS

Values are mean of three replications.

photosynthetic rate of 7.87 µ CO_2 moles m^{-2} s^{-1} while non-colonised plants recorded a lower value of 4.90 µ CO_2 moles m^{-2} s^{-1} and under irrigated condition the observed values were 14.80 and 12.73 µ CO_2 moles m^{-2} s^{-1} respectively. At flowering stage, the presence of *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the photosynthetic rate of water stressed rice plants (85.73 and 97.83 µ CO_2 moles m^{-2} s^{-1} respectively). At flowering stage, the presence of *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the photosynthetic rate of water stressed rice plants (8.50 and 21.40 µ CO_2 moles m^{-2} s^{-1} respectively) on comparison with that of irrigated plants (14.40 and 26.97 µ CO_2 moles m^{-2} s^{-1} respectively). However, in the absence of *P. indica* the photosynthetic rate of Ptb 23 and Ptb 29 under water limited situations were 4.30 and 15.30 µ CO_2 moles m^{-2} s^{-1} respectively while that of under irrigated conditions were 13.63 and 24.60 µ CO_2 moles m^{-2} s^{-1} respectively.

4.2.2.1 Total chlorophyll content (mg g^{-1})

Ptb 29 variety showed higher total chlorophyll content than Ptb 23 plants at all three stages of observation (24). At maximum tillering stage, water stressed plants of *P. indica* colonised Ptb 29 recorded a total chlorophyll content of 1.71 mg g^{-1} while for non-colonised plants it was 1.39 mg g^{-1}. Irrigated rice plants of Ptb 29 with and without *P. indica* colonisation showed total chlorophyll content of 1.95 and 1.82 mg g^{-1} respectively. In the absence of *P. indica* colonisation, the water stressed Ptb 23 plants showed the lowest total chlorophyll content of 1.06 mg g^{-1} while for irrigated plants it was 1.21 mg g^{-1}. The results indicated that *P. indica* colonisation improved total chlorophyll content in both water-stressed (1.28 mg g^{-1}) and irrigated (1.25 mg g^{-1}) plants of Ptb 23.

During the panicle initiation stage, the total chlorophyll content of Ptb 29 rice plants with and without *P. indica* under water stress were 2.26 and 1.85 mg g^{-1} respectively while that of plants under irrigated conditions were 2.52 and 2.33 mg g^{-1} respectively. Ptb 23 rice plants with the root colonising endophyte under water limited condition showed a total chlorophyll content of 1.13 mg g^{-1} while non-colonised plants recorded a lower value of 1.08 mg g^{-1} and under irrigated condition the observed values were 1.23 and 1.09 mg g^{-1} respectively. At flowering stage, the presence of *P. indica* colonisation on both Ptb 23 and Ptb 29 improved the total chlorophyll content of water stressed rice plants (1.23 and 2.26 mg g^{-1} respectively) on comparison with that of irrigated plants (1.22 and 2.42 mg g^{-1} respectively). However, in the absence of *P. indica* the total chlorophyll

Table 24. Effect of *P. indica*-colonisation on total chlorophyll content (mg g^{-1}) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions at a) maximum tillering stage, b) panicle initiation and c) flowering stages.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+ *P. indica*	% change	- *P. indica*	+ *P. indica*	% change
a) Maximum tillering stage							
1.	**Water stress**	1.06	1.28	+20.75	1.39	1.71	+23.02
2.	**Irrigated**	1.21	1.25	+3.30	1.82	1.95	+7.14
SEm (±)	0.02	SEm PV (±)	0.014	SEm PS (±)	0.014	SEm VS (±)	0.014
CD (0.05)	NS	CD(0.05) PV	0.043	CD (0.05) PS	0.043	CD(0.05) VS	0.043
b) Panicle initiation stage							
1.	**Water stress**	1.08	1.13	+4.62	1.85	2.26	+22.16
2.	**Irrigated**	1.09	1.23	+12.84	2.33	2.52	+8.15
SEm (±)	0.028	SEm (±)	0.02	SEm (±)	0.02	SEm (±)	0.02
CD (0.05)	0.084	CD(0.05) PV	0.06	CD (0.05) PS	NS	CD(0.05) VS	0.06
c) Flowering stage							
1.	**Water stress**	0.98	1.23	+26.03	2.01	2.26	+12.44
2.	**Irrigated**	1.10	1.22	+10.91	2.28	2.42	+6.14
SEm (±)	0.04	SEm (±)	0.029	SEm (±)	0.029	SEm (±)	0.029
CD (0.05)	NS	CD(0.05) PV	NS	CD (0.05) PS	0.088	CD(0.05) VS	0.088

Values are mean of three replications.

content of Ptb 23 and Ptb 29 under water limited situations were 0.98 and 2.01 mg g^{-1} respectively while that of under normal irrigation schedule were 1.10 and 2.28 mg g^{-1} respectively.

4.2.2.1 Malondialdehyde (MD) content (nmol g^{-1} FW)

The malondialdehyde content of *P. indica* colonised and non-colonised plants of Ptb 23 and ptb 29 under irrigated and water stress conditions was taken at harvest stage and recorded in table 25.

The result showed that the MDA of water stress tolerant variety Ptb 29 was lower than that of water stress susceptible variety Ptb 23 under water stressed condition. It also indicated that rice plants colonised by *P. indica* recorded lower MDA content than non-colonised plants of both varieties. The MDA content of *P. indica* colonised Ptb 29 under water stress and irrigated condition was 4.63 and 3.07 nmol g^{-1} FW respectively, while that of non-colonised plants were 9.13 and 15.13 nmol g^{-1} FW respectively. In *P. indica*-colonised plants of Ptb 23 under water stress condition, the MDA content was 11.5 nmol g^{-1} FW which was higher than that of irrigated condition (6.47 nmol g^{-1} FW). Ptb 23 rice plants without *P. indica* under water stressed and irrigated condition recorded MDA content of 18.6 and 7.63 nmol g^{-1} FW respectively.

4.2.2.1 Superoxide dismutase (SOD) activity (U mg^{-1} FW)

The SOD activity was recorded under both irrigated and water stress condition at harvest stage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 were presented in table 26.

The result showed that the SOD activity in water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23. Ptb 29 showed higher SOD activity under water stressed condition when compared to irrigated condition. The SOD of *P. indica* colonised Ptb 29 under water stress condition (23.40 U mg^{-1} FW) was higher than that of non-colonised plants (13.40 U mg^{-1} FW). When irrigated the SOD of *P. indica* colonised Ptb 29 was 16.20 U mg^{-1} FW and that of non-colonised plants was 14.97 U mg^{-1} FW. *P. indica* colonised Ptb 23 under water stressed condition showed an average SOD of 12.57 U mg^{-1} FW while that of non-colonised plants was 7.30 U mg^{-1} FW. Under irrigated conditions the SOD activity of Ptb 23 with *P. indica* (11.90 U mg^{-1} FW) was higher than that of Ptb 23 plants without *P. indica* (10.30 U mg^{-1} FW).

Table 25. Effect of *P. indica*-colonisation on malondialdehyde (MDA) (nmol g^{-1} FW) content of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+*P. indica*	% change	- *P. indica*	-+*P. indica*	% change
1.	Water stress	18.6	11.5	-38.17	9.13	4.63	-49.29
2.	Irrigated	7.63	6.47	-15.20	15.13	3.07	-79.71
SEm (±)	0.396	SEm PV (±)	0.28	SEm PS (±)	0.28	SEm VS (±)	0.28
CD (0.05)	1.188	CD(0.05) PV	NS	CD(0.05) PS	0.84	CD(0.05) VS	0.84

Table 26. Effect of *P. indica*-colonisation on superoxide dismutase activity (U mg^{-1} FW) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+*P. indica*	% change	- *P. indica*	-+*P. indica*	% change
1.	Water stress	7.30	12.57	+72.19	13.40	23.40	+74.62
2.	Irrigated	10.30	11.90	+15.53	14.97	16.20	+8.21
SEm (±)	0.47	SEm PV (±)	0.332	SEm PS (±)	0.332	SEm VS (±)	0.332
CD (0.05)	NS	CD(0.05) PV	NS	CD(0.05) PS	0.997	CD(0.05) VS	0.997

Values are mean of three replications.

4.2.2.1 Catalase activity (U mg^{-1} FW)

The catalase activity of *P. indica* colonised and non-colonised plants of Ptb 23 and ptb 29 under irrigated and water stress conditions was taken at harvest stage and recorded in table 27.

The result showed that the catalase activity of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23 under water stressed condition. It also indicated that rice plants colonised by *P. indica* recorded lower catalase than non-colonised plants of both varieties.

The catalase activity of *P. indica* colonised Ptb 29 under water stress and irrigated condition was 16.77 and 12.33 U mg^{-1} FW respectively, while that of non-colonised plants were 8.23 and 10.70 U mg^{-1} FW respectively. In *P. indica*-colonised plants of Ptb 23 under water stress condition, the Catalase activity was 7.53 U mg^{-1} FW which was higher than that of irrigated condition (4.83 U mg^{-1} FW). Ptb 23 rice plants without *P. indica* under water stressed and irrigated condition recorded catalase activity of 4.83 and 7.30 U mg^{-1} FW respectively.

4.2.2.1 Invertase activity

The invertase activity was recorded under both irrigated and water stress condition at harvest stage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 were presented in table 28.

The result indicated that the invertase activity in water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23. Ptb 29 showed higher invertase activity under water stressed condition when compared to irrigated condition.

The invertase activity of *P. indica* colonised Ptb 29 under water stress condition (4.95 U mg^{-1} FW) was higher than that of non-colonised plants (5.17 U mg^{-1} FW). When irrigated the invertase of *P. indica* colonised Ptb 29 was 4.04 U mg^{-1} FW and that of non-colonised plants was 3.51 U mg^{-1} FW. *P. indica* colonised Ptb 23 under water stressed condition showed an activity of 3.41 U mg^{-1} FW while that of non-colonised plants was 4.58 U mg^{-1} FW. Under irrigated conditions the invertase activity of Ptb 23 with *P. indica* (3.10 U mg^{-1} FW) was lower than that of Ptb 23 plants without *P. indica* (3.32 U mg^{-1} FW).

Table 27. Effect of *P. indica*-colonisation on catalase (unit mg^{-1} FW) activity of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	*+P. indica*	% change	*- P. indica*	*-+P. indica*	% change
1.	Water stress	4.83	7.53	+55.90	8.23	16.77	+103.77
2.	Irrigated	7.30	6.97	-4.52	10.7	12.33	+15.23
SEm (±)	0.306	SEm PV (±)	0.216	SEm PS (±)	0.216	SEm VS (±)	0.216
CD (0.05)	0.916	CD(0.05) PV	0.648	CD(0.05) PS	0.648	CD(0.05) VS	0.648

Table 28. Effect of *P. indica*-colonisation on invertase activity (U mg^{-1} FW) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	*+P. indica*	% change	*- P. indica*	*-+P. indica*	% change
1.	Water stress	4.58	3.41	-25.54	5.17	4.95	-4.25
2.	Irrigated	3.32	3.1	-3.92	3.51	4.04	+15.09
SEm (±)	0.038	SEm PV (±)	0.027	SEm PS (±)	0.027	SEm VS (±)	0.027
CD (0.05)	0.113	CD(0.05) PV	0.08	CD(0.05) PS	0.08	CD(0.05) VS	0.08

Values are mean of three replications.

4.2.3 Yield parameters

4.2.3.1 Days to 50% flowering

The days to 50 per cent flowering of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 under irrigated and water stress conditions were observed at harvest stage and recorded in table 29.

The result showed that the water stressed plants of both the varieties achieved 50 per cent flowering before that of irrigated plants. It also indicated that root colonisation by *P. indica* resulted in early flowering under both water stressed and irrigated conditions in the two rice varieties studied.

In Ptb 29, the 50 per cent flowering of *P. indica* colonised water stressed plants was on 73.67th day while that of irrigated plants was on 76.67th day. Non-colonised plants completed 50 per cent flowering on 76.33rd (water stressed) and 77.33rd (irrigated) day. The water stress susceptible variety Ptb 23 on colonisation by the root endophyte exhibited 50 per cent flowering on 75.67th (water stressed) and 83.33rd (irrigated) day while non-colonised plants manifested the same on 77.33rd and 85.67th day respectively.

4.2.3.1 Tillers per plant

The tillers per plant observed under both irrigated and water stress condition at harvest stage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 were presented in table 30.

The plants of water stress tolerant variety Ptb 29 produce more tillers per plant compared to the susceptible variety Ptb 23. The results also revealed that *P. indica* colonised plants had more tillers than non-colonised plants.

Ptb 29 on colonistion with the root endophyte formed 8.33 (water stressed) and 10.00 (irrigated) tillers which is considerably more compared to non-colonised plants (6.67 and 9.67 respectively). Whereas Ptb 23 on colonisation with *P. indica* had 6.00 tillers per plant under water stressed condition while on irrigation the number increased to 8.67. Normal plants of Ptb 23 under water stressed condition showed poor number of tillers (4.00) than control plants (7.00).

Table 29. Effect of *P. indica*-colonisation on days to 50% flowering of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	*+P. indica*	% change	*- P. indica*	*-+P. indica*	% change
1.	Water stress	77.33	75.67	-2.15	76.33	73.67	-3.48
2.	Irrigated	85.67	83.33	-2.73	77.33	76.67	-0.85
SEm (±)	0.833	SEm PV (±)	0.589	SEm PS (±)	0.589	SEm VS (±)	0.589
CD (0.05)	2.498	CD(0.05) PV	1.767	CD(0.05) PS	1.767	CD(0.05) VS	NS

Table 30. Effect of *P. indica*-colonisation on number of tillers per plant of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- P. indica	*+P. indica*	% change	*- P. indica*	*-+P. indica*	% change
1.	Water stress	4.00	6.0	+33.33	6.67	8.33	+24.89
2.	Irrigated	7.00	8.67	+23.85	9.67	10.00	+3.41
SEm (±)	0.471	SEm PV (±)	0.333	SEm PS (±)	0.333	SEm VS (±)	0.333
CD (0.05)	NS	CD(0.05) PV	NS	CD(0.05) PS	0.999	CD(0.05) VS	NS

Values are mean of three replications.

4.2.3.1 Productive tillers per plant

The number of productive tillers per plant of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 under irrigated and water stress conditions were observed at harvest stage and recorded in table 31.

The water stress tolerant variety Ptb 29 produced higher number of productive tillers per plant compared to the susceptible variety Ptb 23 under water stressed condition. The results also indicated that *P. indica* colonised plants had more productive tillers than non-colonised plants.

Ptb 29 on colonistion with the root endophyte formed 5.33 (water stressed) and 6.33 (irrigated) productive tillers which was higher when compared to non-colonised plants (3.67 and 4.33 respectively). Whereas Ptb 23 on root colonisation with *P. indica* had on an average 3.67 productive tillers per plant under water stressed condition while on irrigation the number increased to 4.33. Normal plants of Ptb 23 under water stressed condition showed poor number of tillers (1.33) than control plants (4.00).

4.2.3.1 Panicle length (cm)

The panicle length of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 under irrigated and water stress conditions was taken at harvest stage and recorded in table 32.

The result showed that the panicle length of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23 under corresponding water stressed and control conditions. It also indicated that rice plants colonised by *P. indica* recorded higher panicle length than non-colonised plants of both varieties.

The panicle length of *P. indica* colonised Ptb 29 under water stress condition was 23.17 cm and under irrigated condition was 26.17 cm, while that of non-colonised plants were 69.30 and 84.17 cm respectively. A similar trend was observed in Ptb 23 with *P. indica*-colonised plants under water stress condition showing an average panicle length of 22.73 cm and irrigated plants with panicle length of 25.73 cm while non-colonised plants with a panicle length of 18.23 cm and 22.11 cm respectively.

Table 31. Effect of *P. indica*-colonisation on number of productive tillers per plant of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+*P. indica*	% change	- *P. indica*	-+*P. indica*	% change
1.	Water stress	1.33	3.67	+175.94	3.67	5.33	+45.23
2.	Irrigated	4.00	4.33	+8.25	4.33	6.33	+46.18
SEm (±)	0.312	SEm PV (±)	0.22	SEm PS (±)	0.22	SEm VS (±)	0.22
CD (0.05)	NS	CD(0.05) PV	NS	CD(0.05) PS	0.661	CD(0.05) VS	0.661

Table 32. Effect of *P. indica*-colonisation on panicle length (cm) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+*P. indica*	% change	- *P. indica*	-+*P. indica*	% change
1.	Water stress	18.73	22.73	+21.36	21. 63	23.17	+7.11
2.	Irrigated	22.11	25.73	+16.37	19.50	26.17	+34.20
SEm (±)	0.548	SEm PV (±)	0.388	SEm PS (±)	0.388	SEm VS (±)	0.388
CD (0.05)	NS	CD(0.05) PV	1.163	CD(0.05) PS	1.163	CD(0.05) VS	1.163

Values are mean of three replications.

4.2.3.1 Yield per plant (g)

The yield per plant of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 under irrigated and water stress conditions were observed at harvest stage and recorded in table 33.

The yield per plant of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23 under water stressed condition. It also showed that rice plants colonised by *P. indica* recorded higher shoot dry weight than non-colonised plants of both varieties.The yield of *P. indica* colonised Ptb 29 under water stress and irrigated condition were 22.63 and 26.67 g respectively, while that of non-colonised plants were 20.10 and 25.04 g respectively. In *P. indica*-colonised plants of Ptb 23 under water stress condition, the yield per plant was 20.10 g which was lower than that of irrigated condition (25.04 g). Ptb 23 rice plants without *P. indica* under water stressed and irrigated condition recorded a yield of 11.83 and 23.33 g respectively.

4.2.3.1 Spikelet fertility percentage (%)

The spikelet fertility percentage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 under irrigated and water stress conditions was taken at harvest stage and recorded in table 33.

The spikelet fertility percentage of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23 under water stressed and irrigated conditions. The result also showed that rice plants colonised by *P. indica* recorded higher spikelet fertility percentage when compared with non-colonised plants of both varieties. The spikelet fertility percentage of *P. indica* colonised Ptb 29 under water stress and irrigated condition was 75.13 and 87.20 per cent respectively, while those of non-colonised plants were 68.80 and 84.73 per cent respectively. In *P. indica*-colonised plants of Ptb 23 under water stress condition, the spikelet fertility percentage was 62.87 per cent which was lower than that of irrigated condition (54.10 per cent). Ptb 23 rice plants without *P. indica* under water stressed and irrigated condition recorded spikelet fertility percentages of 54.10 and 79.20 per cent respectively.

Table 33. Effect of *P. indica*-colonisation on yield (g) per plant of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+*P. indica*	% change	- *P. indica*	-+*P. indica*	% change
1.	Water stress	11.83	20.10	+69.91	14.4	22.63	+57.15
2.	Irrigated	23.33	25.04	+7.16	25.93	26.67	+2.85
SEm (±)	0.384	SEm PV (±)	0.271	SEm PS (±)	0.271	SEm VS (±)	0.271
CD (0.05)	NS	CD(0.05) PV	NS	CD(0.05) PS	NS	CD(0.05) VS	0.814

Table 34. Effect of *P. indica*-colonisation on spikelet fertility percentage (%) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions.

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+*P. indica*	% change	- *P. indica*	-+*P. indica*	% change
1.	Water stress	54.10	62.87	+16.21	68.80	75.13	+9.20
2.	Irrigated	79.20	83.4	+5.30	84.73	87.20	+2.91
SEm (±)	0.776	SEm PV (±)	0.549	SEm PS (±)	0.549	SEm VS (±)	0.549
CD (0.05)	2.328	CD(0.05) PV	NS	CD(0.05) PS	1.646	CD(0.05) VS	1.646

Values are mean of three replications.

4.2.3.1 1000 grain weight

The 1000 grain weight recorded under both irrigated and water stress condition at harvest stage of *P. indica* colonised and non-colonised plants of Ptb 23 and Ptb 29 were presented in table 35.

The result showed that the 1000 grain weight of water stress tolerant variety Ptb 29 was higher than that of water stress susceptible variety Ptb 23. Ptb 29 showed higher 1000 grain weight under water stressed condition when compared to irrigated condition.

The 1000 grain weight of *P. indica* colonised Ptb 29 under water stress condition (23.30 g) was significantly higher than that of non-colonised plants (19.33 g). When irrigated the 1000 grain weight of *P. indica* colonised Ptb 29 was 27.13 g and that of non-colonised plants was 25.76 g. *P. indica* colonised Ptb 23 under water stressed condition showed 1000 grain weight of 21.71 g while that of non-colonised plants was 17.73 g. Irrigated plants of Ptb 23 with *P. indica* showed 1000 grain weight of 21.93 g while that of Ptb 23 plants without *P. indica* was 20.07 g.

Table 35. Effect of *P. indica*-colonisation on 1000 grain weight (g) of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Sl. No.	Treatments	Varieties					
		Ptb 23 (water stress susceptible)			Ptb 29 (water stress tolerant)		
		- *P. indica*	+*P. indica*	% change	- *P. indica*	-+*P. indica*	% change
1.	Water stress	17.73	21.17	+19.40	19.33	23.03	+19.14
2.	Irrigated	20.07	21.93	+9.27	25.76	27.13	+5.32
SEm (±)	0.43	SEm PV (±)	0.304	SEm PS (±)	0.304	SEm VS (±)	0.304
CD (0.05)	NS	CD(0.05) PV	NS	CD(0.05) PS	0.912	CD(0.05) VS	0.912

Values are mean of three replications.

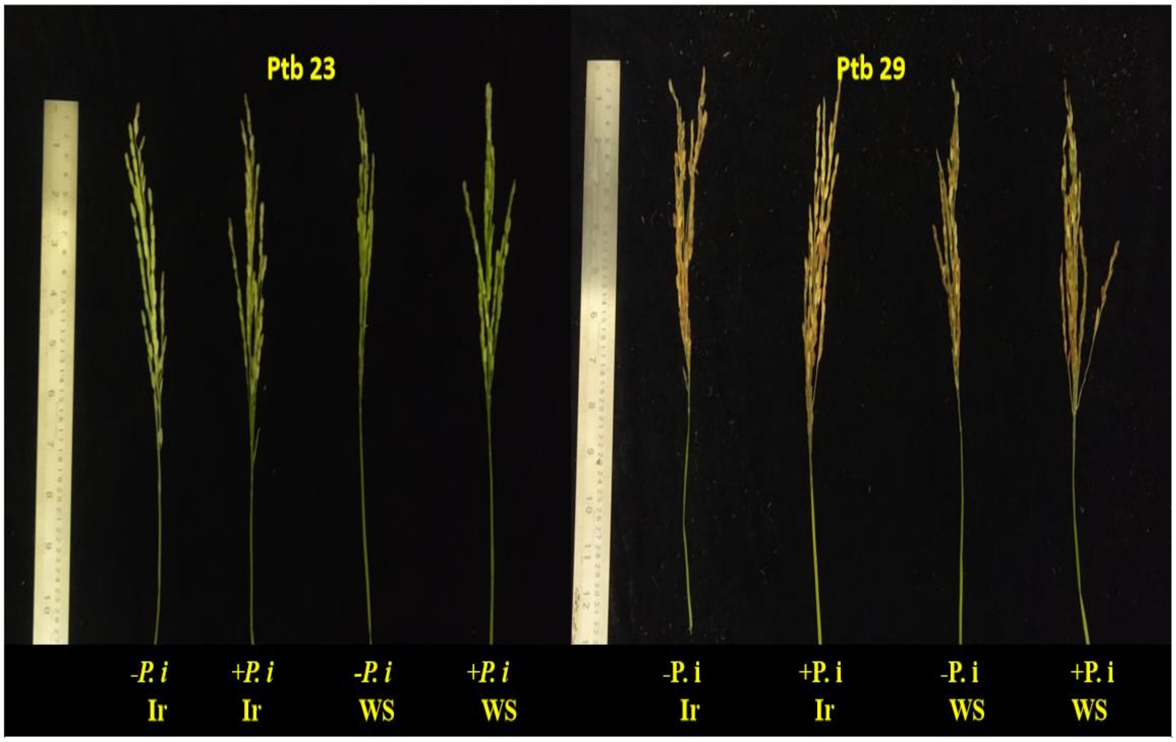

Plate. 7. Effect of *P. indica*-colonisation on panicle length (cm) of Ptb 23 and Ptb 29 rice under irrigated and water stress conditions

4.2.4 *Molecular analysis*

Expression analysis of OsDREB 2A gene using Real-time PCR.

4.2.4.1 RNA isolation

Total RNA was isolated from fresh flag leaf tissues of Ptb 23 and Ptb 29 rice using the TRIzol reagent method. RNA samples isolated were stored at -80°C.

4.2.4.1 Agarose gel electrophoresis

The RNA was observed as two bands (28 S and 18 S) in 1.2 % AGE. The purity of the RNA was assessed by measuring absorbance at 260nm and 280nm and the values are shown in Table 36.

4.2.4.1 *Quantification of RNA*

Concentration as well as purity values of RNA isolated were represented in Table 36.

Table 36. Yield and purity of isolated RNA.

Treatments	**Ptb 23**		**Ptb 29**	
	Concentration (ng/µl)	**A_{260}/A_{280}**	**Concentration (ng/µl)**	**A_{260}/A_{280}**
Water stress - *P. indica*	838.68	2.01	799.38	2.13
Water stress + *P. indica*	876.2	2.05	898.57	2.15
Irrigated - *P. indica*	969.6	2.02	902.1	2.03
Irrigated + *P. indica*	876.2	2.05	898.57	2.15

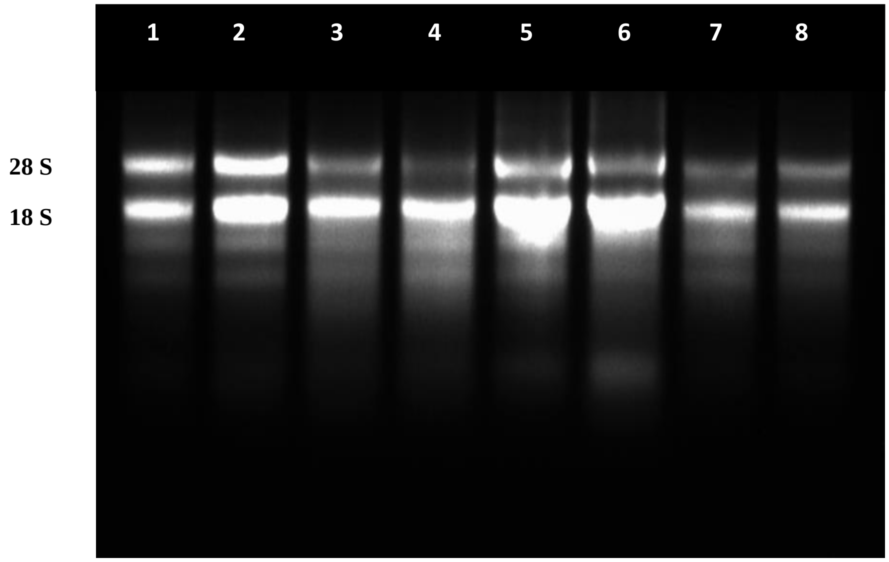

Plate. 8. Gel image of Total RNA isolated from rice flag leaf tissues . Lane 1,2,3,4 represents the RNA isolated from leaf tissues of Irrigated - *P. indica*, Irrigated + *P. indica*, Water stress - *P. indica* and Water stress + *P. indica* treated Ptb 23 respectively and Lane 5,6,7,8 represents RNA isolated from leaf tissues of Irrigated - *P. indica*, Irrigated + *P. indica*, Water stress - *P. indica* and Water stress + *P. indica* treated Ptb 29 respectively

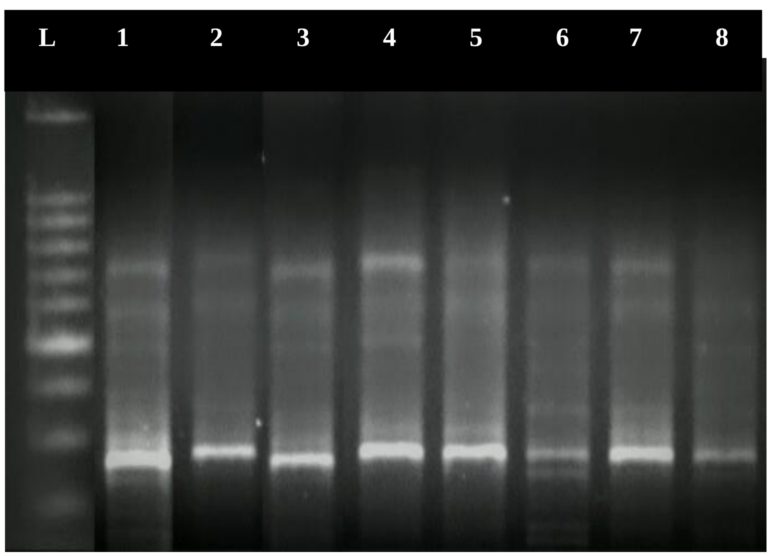

Plate. 9. Amplification pattern of two rice varieties obtained by *OsActin gene*. Lane L- 50bp ladde; Lane 1,2,3,4 represents the RNA isolated from leaf tissues of Irrigated - *P. indica*, Irrigated + *P. indica*, Water stress - *P. indica* and Water stress + *P. indica* treated Ptb 23 and Lane 5,6,7,8 represents RNA isolated from leaf tissues of Irrigated - *P. indica*, Irrigated + *P. indica*, Water stress - *P. indica* and Water stress + *P. indica* treated Ptb 29

4.2.4.1. cDNA synthesis

The isolated RNA samples of both rice varieties were diluted to a concentration of 150 ng/µl. Then converted to cDNA using iScript cDNA synthesis Kit (Bio-rad). For each 20µl reaction 6 µl RNA sample was used. The cDNA thus produced was stored at (-20ºC). The synthesized cDNA was confirmed using *OsActin* (amplicon size 93 bp)

4.2.4.1. Annealing temperature standardization

The optimum annealing temperature was selected as 60°C after performing thermal gradient qPCR at 57°C, 59°C and 51°C, since the reaction performed at 60°C shows lowest threshold cycle (Ct) values.

Table 37. Standardization of annealing temperature of Real-Time primers.

Temperature	**Threshold cycle (Ct) values**
	OsDREB 2a
57°C	39.75
59°C	33.38
61°C	36.33

4.2.4.1. Expression analysis of OsDREB 2a gene.

Expression analysis of *OsDREB 2a* gene was studied using *OsActin* as reference gene. The threshold cycle (Ct) values were used to find relative expression of *OsDREB 2a* in *P. indica* colonised plants of Ptb 23 and Ptb 29 rice varieties under water stressed and irrigated conditions. using Comparative Ct method. It was observed that the root colonisation by endophytic fungus *P. indica* increased the expression of *OsDREB 2a* gene in water stressed rice plants in both the varieties studied in the experiment. (Table 38).

The expression of *OsDREB 2a* increased during water stress in non-colonised Ptb 23 by 1.5 and Ptb 29 by 1.8 folds. In *P. indica* colonised plants, the induction of water stress significantly increased the expression of *OsDREB 2a* by 2.2 (Ptb 23) and 5.1 (Ptb 29) folds as compared to the non-colonised irrigated (control) plants.

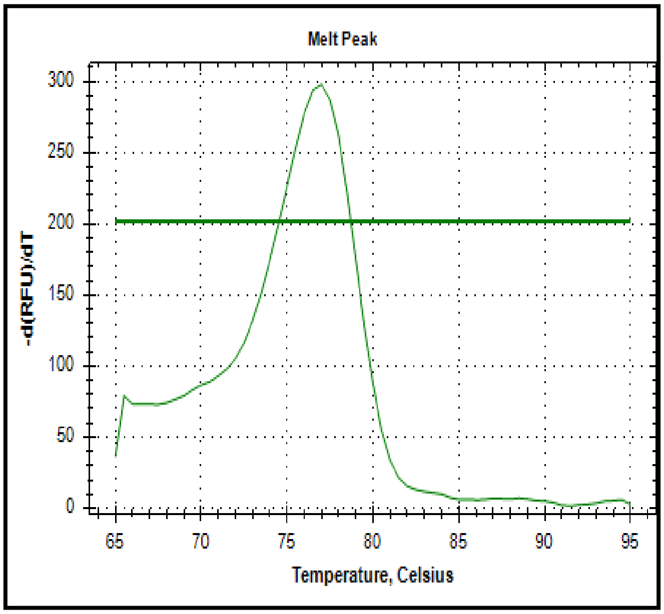

Fig.1. Melt curve analysis of *OsDREB 2A*

Table 38. Effect of *P. indica*-colonisation on expression of *OsDREB 2a* of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions

Treatments	Relative fold change (RFC)	
	Ptb 23	**Ptb 29**
Water stress - *P. indica*	1.5±0.2	1.8±0.4
Water stress + *P. indica*	2.2±0.3	5.1±0.2
Irrigated – *P. indica*	1.0±0.0	1.0±0.1
Irrigated + *P. indica*	1.1±0.1	0.9±0.2
SE(d)	0.11	0.25
SE(m)	0.08	0.18
CD (0.5%)	0.48	1.07

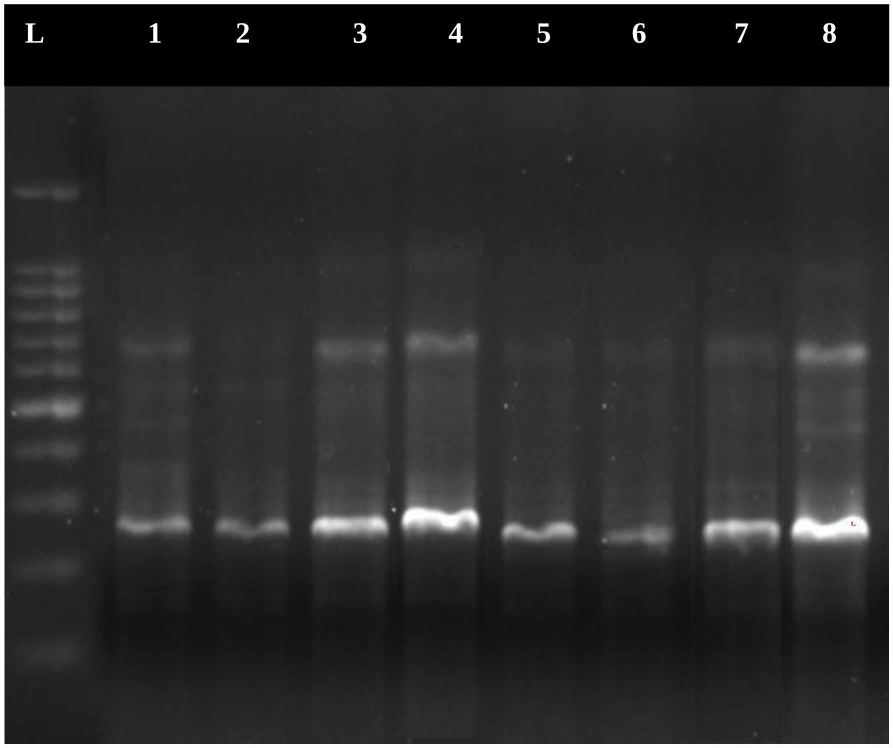

Plate. 10. Effect of *P. indica*-colonisation on expression of *OsDREB 2a* of drought sensitive and tolerant varieties of rice under water stress and irrigated conditions. *Lane L-50bp ladder; Lane 1,2,3,4 represents the RNA isolated from leaf tissues of Irrigated - P. indica, Irrigated + P. indica, Water stress - P. indica and Water stress + P. indica treated Ptb 23 and Lane 5,6,7,8 represents RNA isolated from leaf tissues of Irrigated - P. indica, Irrigated + P. indica, Water stress - P. indica and Water stress + P. indica treated Ptb 29*

Discussion

5. DISCUSSION

Water is one such commodity that we cannot afford to overlook and mismanage. This fact is further reinforced by the IPCC 2021 report that warned of an increase in drought frequency in recent times and the near future, resulting in water shortages in rainfed agriculture, posing serious threats to livelihoods and food security, particularly for the world's most vulnerable people in the least developed countries. (IPCC, 2021). When there is not enough water to meet all demands that is needed for an ecosystem to function effectively, the demise of some or all organisms is an eventuality.

A wide range of adverse environmental conditions affect the growth and development of crop plants. Amongst the various stresses that curtail the crop growth and productivity, water deficit or water stress is one that affect the overall production of crop (Foley *et al.,* 2011). Rice, one of the most important food crops is also one of the most drought sensitive crops owing to its thin cuticular wax, swift stomatal closure and shallow or small root system (Serraj *et al.,* 2011). Rice being the staple food crop for millions of people worldwide (Kush, 2005), not only accounts for their daily caloric needs but also ensures food security and political stabilities of many developing and underdeveloped countries.

The particular genotype, species, age, developmental phase of plants and the severity of stress imposed will factor on the varied responses of plants to drought stress (Waseem *et al.,* 2011). To adapt to the drought condition, plants have the ability to manifest itself with many survival mechanisms, namely drought avoidance (maintenance of tissue water potential), drought escape (flowering to complete life cycle before drought), and drought tolerance (survival mechanisms) (Levitt, 1980). Fukai and Cooper (1995) further emphasized that plants adjust to external pressures on the of their molecular responses which leads to the various morpho-physiological adaptations.

Various measures, such as germplasm exploitation (Li *et al.,* 2016), effective management practices (Haefele *et al.,* 2016), and utilizing the significant association of rice with endophytes, can aid in improving rice grain output under drought-prone situations (Gill *et al.* 2016). A novel approach to manage water stress is the use of a fungal root endophyte, *P. indica* that establish a symbiotic relationship with plants and positively reprogrammes the plant transcriptomes, proteomes and metabolomes (Zuccaro *et al.,* 2011; Bakshi *et al.,* 2017; Bajaj *et al.,* 2018; Nivedita *et al.,* 2021) which includes phytohormone synthesis and signaling that affect growth, nutrient uptake, flowering, seed production; and protection against biotic and abiotic stresses (Schäfer *et al.,* 2009; Varma *et al.,* 2012; Xu *et al.*, 2018; Liu *et al.,* 2019).

In the present study, four rice genotypes collected from RARS Pattambi were used to evaluate the changes in morphological, physiological, biochemical and molecular mechanisms associated with water stress tolerance in *P. indica*-colonised rice.

5.1. ROOT COLONIZATION OF RICE BY *P. INDICA*

P. indica was cultured in Potato Dextrose Agar (PDA) media by periodical subculturing maintained at room temperature (28±2ºC) in dark. It was then grown in Potato Dextrose Broth in dark conditions at room temperature for two weeks in order to facilitate fungal growth as thick mucous like mycelial formation above the liquid layer. (Sharma *et al.*, 2014; Lakshmipriya, 2017; Chippy, 2021).

For colonization of *P. indica*, the incubated PDB containing fungal mycelium was mixed with sterilized composted coir pith- cow dung (1:1) mixture medium filled in plastic trays. It was kept covered for promoting fungal growth. White cotton-like mycelial formation above the mixture indicated the presence of *P. indica* and was confirmed under microscope. This biodynamic preparation of composted coir pith- cow dung (1:1) mixture infused with *P. indica* has the potential to be evolved into a formulation compatible for field application of nutrients in farming. The efficacy of the aforementioned mixture enriched with the growth promoting endophytic fungi *P. indica* with biotic and abiotic stress tolerance ability (Waller *et al.,* 2005; Gill *et al.,* 2016; Yun *et al.,* 2018; Mensah *et al.,* 2021) as an organic potting media can be capitalized as an effective methodology to minimize the use of agrochemicals.

Surface sterilized seeds of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 rice varieties of were placed in those trays after 3 weeks of fungal growth. A similar set of trays containing the same composted coir pith- cow dung (1:1) mixture but without mixing the *P. indica* culture were used to germinate the seeds of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 rice varieties as control plants. These trays were maintained in temperature and humidity-controlled conditions for uniform germination.

The seeds germinated after three days of sowing in both set of trays. The roots of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 varieties' seedlings were appraised microscopically for root colonisation. Isolated or chains of double walled pear shaped chlamydospores were observed in the root sections of seedlings after seven days of cocultivation. Similar studies involving rice seedlings inoculated with living chlamydospores of *P. indica*, colonisation was observed in sixty percent of the entire root at fifteen days post inoculation(dpi) and at twenty days dpi the percentage of colonisation increased to seventy (Jogawat *et al.,* 2013). In other cereal crops the duration required for colonisation and chlamydospore formation varied slightly. In wheat plants chlamydospores were observed six days after inoculation (Rabiey *et al.,* 2015) while in barley the colonisation was observed fourteen days after inoculation (Alikhani *et al.*, 2013). It is understood that a compatible interaction between the root endophyte and the plant is facilitated through down-regulation of plant's innate immunity by manipulating multiple phytohormone signalling pathways (Schäfer *et al.,* 2009; Jacobs *et al.,* 2011).

5.2. PRELIMINARY SCREENING OF VARIETIES FOR WATER STRESS TOLERANCE

The effect of *P. indica* on water stress in the seedling stages of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 rice varieties was assessed in this study by treating with 0, 5, 10, 15 and 20 per cent of Poly Ethylene Glycol (PEG 6000) in modified Hoagland nutrient solution. All the varieties pertaining to this study on root colonisation with *P. indica* showed higher water stress tolerance while the water stress tolerant varieties Ptb 29 and Ptb 30 showed tolerance to water stress even in the absence of colonisation with *P. indica*.

Growth promotion of rice seedlings was assessed seven days after treatment. The water stress susceptible varieties of Ptb 23 and Ptb 24 both showed increased shoot length on *P. indica* colonisation. However, in Ptb 23 the increase was more pronounced. For instance, there was a sixteen percent increase in shoot length of *P. indica* colonised Ptb 23 seedlings when exposed to five percent PEG 6000 concentration solution. In the water stress tolerant varieties, the shoot length was found to be higher in colonised seedlings. In a similar study by Saddique *et al.* (2018), the negative effect of oxidative stress in plants subjected to 15 percent PEG-6000 solution experienced by non-colonised plants were absent in *P. indica*-colonised rice seedlings. Many prominent works on the field of fungal-plant interaction have reported on *P. indica*-colonisation and the production of phytohormones like indole-3-acetic-acid (IAA), cytokinin (CK) and Gibberellins (GA), that gives growth and yield benefits, and abscisic acid (ABA), ethylene (ET), salicylic acid (SA) and jasmonates, which enhances stress tolerance through systemic resistance response (Varma *et al.*, 2012; Gill *et al.*, 2016). Sirrenberg *et al.*, (2007) has reported that *P. indica* was able to produce IAA in liquid culture. Since auxins are involved in cell division, cell elongation and organ development and stimulating shoot growth, the *P. indica*-induced auxin production or signaling in roots may have caused the colonised plants to exhibit increased shoot length in this study (Vadassery *et al.*, 2008; Lee *et al.*, 2011).

The root length observed in the experiment however showed contrasting results. The colonised seedlings showed lower root length than the non-colonised set. Drought affected maize seedlings showed significantly reduced root length compared to the control set, while the *P. indica* colonised seedlings recorded enhanced root length under stressed condition (Zhang *et al.*, 2018). In a similar study, inoculation with *P. indica* conferred a 48.18 percentage increase in root length in *Brassica napus* L (Su *et al.*, 2017). The trend also indicated that as the level of stress increased, the length of roots progressively decreased. The seedlings of Ptb 29 and Ptb 30 proved to be better under the higher concentration of PEG 6000 solutions. Deshmukh *et al.* (2006) suggested that on *P. indica*-colonisation, the endophyte either actively kills the cells or detect cells undergoing programmed cell death (PCD) and the fungal hyphae builds a meshwork surrounding the plasmolysed protoplasts following penetration of the cells. This may be one of the causes of reduced root length in the initial days of seedling growth. The increased auxin response as a result of *P. indica*-colonisation may be another reason for reduced root length in this study. It is possible that the initial growth was negatively affected by inhibition of primary root growth by auxin as the observations were taken only for 7 days after treatment in the experiment. The effect of auxin may have been able to compensate in the later stages of plant growth by the induction of lateral root formation (Laskowski *et al.*, 1995) as auxin-induced root branching may be one of the beneficial effects of *P. indica* (Sirrenberg *et al.*, 2007). The effect of auxin in inhibiting primary root elongation is most probably mediated by crosstalk with the gaseous hormone ethylene (Swarup *et al.*, 2002; Qin and Huang, 2018). The number of root branches, however, exhibited an opposite effect to that of root length. The presence of *P. indica* significantly augmented the number of roots or root branches. The percentage increase in number of root branches of seedlings decreased progressively as the water stress level increases. The results did not reveal any significant deviation in root branch numbers between the four varieties of rice on the treatment with the different levels of stress.

The shoot dry weight and root dry weight of the seedlings was able to convey the established assumption that as the level of water stress increases, the biomass also decreases. This reduction in biomass owing to its reduction in shoot and root weight was more pronounced in Ptb 23 and Ptb 24 than in Ptb 29 and Ptb 30. These water stress susceptible varieties (Nithya, 2020) showed symptoms of mild to complete wilting as the stress levels progressed. The presence of the endophytic fungus proved to be a factor in the improvement of both shoot and root dry weight. Studies conducted in plant species from varied families like maize (Varma *et al.,* 1999), wheat (Serfling *et al.,* 2007), pepper (Anith *et al.,* 2011), tomato (Fakhro *et al.,* 2010) etc., have found evidence of enhanced vegetative growth as a result of *P. indica*-colonisation. As biomass production is depended on photosynthetic activity of plant, it can be speculated that *P. indica* enhances the photosynthetic activity and transport of photoassimilates. The increase in root dry weight in contrast to the decrease in root length can be attributed to the increase in the number of branches as well as the thickness of roots. The root shoot ratio, which is the measure of root biomass in proportion to the shoot biomass was also positively influenced by the presence of *P. indica*. The root shoot ratio showed an inverse relation with the concentration of PEG 6000. However, the values did not show any significant variation in the water stress applied and its effect on the different varieties.

The seedling vigour indices 1 and 2 were both dependent on the germination percent of the seeds. The germination rate was found to be slightly higher in the trays with *P. indica* in the current study. This factor along with the higher seedling length (shoot length + root length) and seedling dry weight (shoot dry weight + root dry weight) may have contributed to the increased SVI 1 and SVI 2 respectively in seedlings with *P. indica*. As the water stress levels increased, both SVI 1 and SVI 2 decreased. Ptb 29 fared slightly better than the other varieties in these indices under the different levels of PEG 6000 concentration. Drought stress at the seedling stage invariably affects the seedling vigour, growth and development (Mishra *et al.,* 2019). The *P. indica*-varieties interaction was observed to be non-significant which suggest that the rate of germination of all the set of seeds in trays with *P. indica* was uniform. Some research in sunflower and common bean plants suggests that *P. indica*-colonisation enhanced seed germination (Varma *et al.*,2014), however there was no evidence of that in the present study.

5.3. EVALUATION OF THE EFFECT OF *P. indica* ON WATER STRESS TOLERANCE IN RICE

The *P. indica*-colonised and non-colonised rice seedlings of the selected water stress susceptible variety (Ptb 23) and water stress tolerant variety (Ptb 29), from the screening experiment were raised in rainout shelter. Water stress was induced by stopping irrigation for five days during maximum tillering, panicle initiation and flowering stages and normal irrigation was resumed thereafter.

5.3.1. Effect of *P. indica* on morphological parameters under water stress

The beneficial effects of *P. indica* as a growth promoting endophytic fungus on various growth parameters has been widely reported in multiple crops. Many studies have found different plausible causes to understand these effects and many more researches to identify the exact reasons, mechanisms and pathways behind these effects are still underway.

The shoot and root length both increased significantly in the plants inoculated with the growth endophyte in both the varieties. The effect of the water stress on shoot and root length was more pronounced in Ptb 23. Ptb 29 had longer roots which establishes the variety's water stress tolerance capacity (Nithya, 2020). The shoot and root dry weight also increased in the *P. indica*-colonised plants. The shoot and root dry weight together contributes to the biomass of the plant. In this study the *P. indica*-colonised plants produced more biomass than non-inoculated plants. Water stress affected the biomass production negatively in both the varieties. The colonising effect of the fungus was able to create a level playing field between water stress susceptible Ptb 23 and water stress susceptible Ptb 29 in terms of biomass. The root shoot ratio which is dependent on the biomass can throw light upon the partitioning of photosynthates is also influenced by external stimuli (Rogers *et al.*, 1995), most important being the availability of water. A plant with higher root shoot ratio may possibly be able to absorb more nutrient from the soil and help in increasing above-ground biomass and also increases resistance to the stresses including drought (Blàha., 2019). When there is a deficiency of water for the plants, root growth is favoured over shoot growth. Rice plants with deep roots are more tolerant to water stress as they maintain productivity in such situations (Uga et al, 2013). When water stress which corresponds to a lower water potential occurs, osmotic adjustments in the root system helps maintain some level of turgidity which leads to water uptake by re-establishment of water potential gradient. These adjustments facilitate root growth under water limited conditions (Hsiao and Xu, 2000). The root volume was also found to be higher in *P. indica*-colonised plants in both Ptb 23 and Ptb 29 however, the effect was more conspicuous in Ptb 23 with a 42.77 percent increase in colonised plants over non-colonised plants. Ptb 29, true to its water stress tolerant characteristic had significantly higher root volume. Hosseini *et al.* (2017) found out that, wheat under combined drought and mechanical stresses experienced diminished growth and on *P. indica*-colonisation, the plants recorded improved plant water status and root volume. This increased the number of absorption sites for water and nutrient and also enhances osmotic balance.

The enhanced plant shoot-root growth and biomass production as well as other changes in plant morphology after *P. indica*-colonisation can be related to the plant response to increase auxin level in roots either produced by the fungus (Sirrenberg *et al.*, 2007; Vadassery *et al.,* 2008) or by the plant due to stimulation by the fungus (Hua *et al.,* 2017). A possible chemical crosswalk pathway between the plant and the fungus was opened in *P. indica*-colonised Chinese cabbage roots – upregulate the expression of AUX1 and downregulate PIN3 expression (Lee *et al.,* 2011; Dong *et al.,* 2013); AUX1 and PIN3 are auxin-transport related and signaling proteins. Nutrition absorption capacity was bolstered by the endophyte-colonisation, which lead to the stimulation of root hair development with more lateral branching (Su *et al.,* 2017). A well-developed root system improves the absorption of water and mineral nutrients (Dolatabadi *et al.,* 2011). It is also possible that the growth promotion by *P. indica*-induction be related to the improved nutrient uptake by roots from soil (Nautiyal *et al.,* 2010; Gosal *et al.,* 2011). The enhanced biomass production may also be due to the phytohormone homeostasis or other metabolic factors as discussed in the further pages.

5.3.2. Effect of *P. indica* on physiological and biochemical parameters under water stress

Relative water content (RWC) is one of the parameters that indicate plant water status. The present study showed a significant interaction between the rice varieties and water stress with plants of Ptb 29 being able to sustain higher water content in their canopy. A positive effect of the endophyte was observed in both the varieties, however, in the susceptible variety the results were more pronounced. *P. indica*-colonised plants of Ptb 23 under water stressed conditions recorded 9.37, 10.38 and 8.35 percentage increase in RWC during panicle initiation, maximum tillering and flowering stages respectively. This may be possible due to the extensive root system developed in the plant due to *P. indica*-colonisation as discussed in the previous section. The plants may have able to retain more water even in stressed situation through stomata behaviour regulation and reducing oxidative stress by ROS scavenging systems (Tsai *et al.*, 2020).

The water stressed plants recorded lower specific leaf area (SLA) than irrigated plants in both varieties during the three growth stages as this enables plants to conserve water as much as possible. There was significant increase in the specific leaf area in *P. indica*-colonised plants of both varieties than non-colonised plants. This indicates that *P. indica*-colonised plants were not as affected as non-colonised plants by the water stress. In water stress affected plants of both the varieties, SLA indicate the plants' response to minimize water loss. Ptb 23 was more affected by the water limited condition during all three growth stages but the *P. indica*-colonised plants were able to recover faster. Lower SLA is associated with improved water-use efficiency (WUE) under water stress and can be used as a strategy by stress tolerant plants (Wellstein *et al.*, 2017).

Cell membrane stability index (CMS) is a direct indicator of the effect water stress have on plants. In the current study, rice plants of Ptb 29 under water stressed condition had higher CMS than Ptb 23. This is comparable to the studies of Rejeth (2017) where the CMS of water stress tolerant rice varieties were higher than that of susceptible varieties when exposed to different levels of water stress. Incidentally, *P. indica* colonisation improved the membrane stability of cells in stressed plants and the percentage increase was comparable in both the varieties. It decreases during water stress because of lipid peroxidation which leads to the stability of the cell membrane loss (Niu and Xiang, 2018) and increased the production of malondialdehyde (Savchenko *et al.*, 2002). This was validated in this study too, as the malondialdehyde content was increased in the water stress affected plants. However, plants in the presence of *P. indica* showed significantly reduced MDA content which indicates that *P. indica* lowers the adverse consequences of drought. Stress affected Ptb 29 plants had lower MDA content than Ptb 23, which revealed that the former variety is more tolerant to water stress. A higher antioxidative capacity indicates a higher abiotic stress tolerance and this was observed in a study by Tsai *et al.* (2020) where *P. indica*-colonised rice plants had lower MDA level both under water stressed and irrigated condition.

Gas exchange parameters like stomatal conductance, transpiration rate and photosynthetic rate showed increases in the *P. indica*-colonised plants than in non-colonsed plants. Similar findings were reported by Bertolazi *et al.* (2019) in their study on the efficiency of wild-type and transgenic rice in over-expressing the Vacuolar H^+-PPase. Furthermore, the percentage increase in the values were higher and more significant in the water stress tolerant variety Ptb 23. Plants exposed to water limited conditions had lower stomatal conductance, transpiration rate and photosynthetic rate owing to lower relative water content. Plants try to minimize the water loss by regulating the stomatal closure. Regulation of stomatal closure is facilitated by ABA, hydrogen peroxide, nitric oxide and it also involves Ca^{2+} signalling. In leaves of *P. indica*-colonised Chinese cabbage, the expression of a Ca^{2+} sensing regulator was up-regulated (Sun *et al.*, 2010) which suggest that *P. indica* is able to provide water stress tolerance by minimizing water loss from the leaves through stomatal regulation. Johnson *et al.*, (2018) suggested the presence of cellotriose, an elicitor-active cell wall moiety released by *P. indica*, which induces mild defense-like response including changes in membrane potential and production of reactive oxygen species and is involved in cytoplasmic calcium elevation. The stomatal conductance has a direct correlation with yield as stomatal closure limits carbon absorption and assimilation leading to reduced photosynthate production and thereby potential yield loss (Liao *et al.*, 2022)

Total chlorophyll content reduced in plants under water stress while water stressed *P. indica*-colonised plants had higher chlorophyll content than their counterparts. Retention of chlorophyll was significantly higher in water stressed plants of Ptb 29 than Ptb 23. Studies in various crops like rice (Jogawat *et al.*, 2013), banana (Li *et al.*, 2021), strawberries (Liu *et al.*, 2022) and sweet potatoes (Li *et al.*, 2021) have found that *P. indica*-colonisation preserved photosynthetic pigments and as a result higher chlorophyll content was recorded. Higher photosynthetic pigments also imply a higher photosynthetic capacity which further validates the results of this study.

Water stress causes the pile-up of ROS (reactive oxygen species) which damages various cellular components. Most common ROS includes superoxide anions, peroxide and hydroxyl ions (Bahuguna *et al.,* 2015). H_2O_2 produced during stressed condition also promote ROS accumulation. Consequently, plants adopt various adaptive mechanisms like reduction in ROS accumulation (Sailaja *et al.,* 2015). The Activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidases increased during stressed condition reduced ROS levels in cells (Zhao *et al.*, 2018). *P. indica*-colonisation confer drought tolerance to water stress affected plants in this study by significantly increasing the SOD and CAT activity and thereby reducing the ROS. This is similar to the findings that *P. indica*-conferred abiotic stress tolerance depends on enhanced antioxidant synthesis in response to the ROS (White and Torres, 2010; Zarea *et al.*, 2012). Water stress conditions induced a vacuolar invertase activity in mature leaf, leaf sheath and primary roots of maize plants and was accompanied by an increase in vacuolar invertase protein and *Ivr2* transcripts (Kim *et al.*, 2000). *P. indica*-colonised plants showed a reduced invertase activity than non-colonised plants. The effect of the endophyte was easily noticed and significant in water stressed Ptb 23 plants colonised with *P. indica*.

5.3.3. Effect of *P. indica* on yield parameters under water stress

In the present study, water stress hastened the flowering process in both the varieties with the more significant ramification on the stress susceptible variety Ptb 23. However, in plants under moisture stress and inoculated with *P. indica*, the days taken to 50 percent flowering was closer to that of well irrigated plants indicating that the endophyte enable the plants to tide over the distressing effects of water stress by reprogramming the biochemical and molecular mechanisms.

The tillers per plant and productive tillers per plant are indicative of the yield of the plant. Drought stress at the growth stages particularly at the flowering stage has a strong influence on the rice physiological influences and yield (Yang *et al.*, 2019). The varieties that show strong recovery capability that contributes to maintaining relatively high grain production can be characterized as stress tolerant as was evident regarding the Ptb 29 in this study. *P. indica*-colonised plants under stress showed significantly a greater number of tillers and productive tillers than non-colonised plants with the values more pronounced in Ptb 23. Ptb 29 under water stressed condition produced more tillers and productive tillers than Ptb 23. Panicle length, typically measured as one of the most important yield related traits, is one aspect of panicle architecture. Along with spikelet number and density, seed setting rate and grain plumpness, panicle length determines the grain number per panicle and thereby directly linked to yield (Liu *et al.*, 2016). Water stress significantly affect the panicle length at all growth stages (Rahman and Yoshida, 1985). Panicle length was found to be longest in plants inoculated with *P. indica* in both the varieties. Ptb 29 had longer panicle than Ptb 23 under water stress situation which further emphasize on the former variety's water stress tolerant capacity. A similar study in millet (*Panicum milliaceum* L.) reported that the yield component like panicle length, grain yield and 1000-grain weight were significantly increased in *P. indica*-colonised seedlings under water stress (Ahmadvand and Hajinia, 2018).

Drought and high temperature can cause stress-induced downregulation of genes for auxin synthesis, transport and signalling and leads to the reduction in endogenous auxin in spikelets, therby inducing spikelet sterility in rice (Sharma *et al.*, 2018). Water stress during the growth stages particularly during the flowering stage affect the spikelet fertility (Kang and Futakuchi, 2019). Ptb 23 under water stress conditions were most affected and *P. indica*-colonisation was able to offset the stress effect by a 16.21 percentage increase over non-colonised plants; auxin production is enhanced in *P. indica*-colonised plants (Vadassery *et al.*, 2008), which may have led to the increased spikelet fertility.

Water stress during grain filling stage reduces assimilate translocation and thereby decreases the grain weight and increases empty grain. 1000-grain weight is thus a measure of photosynthates produced and assimilates translocated and both factor is negatively influenced by drought (Fahad *et al.*, 2016). In this study, it is evident that water stress influenced the size of grain and it varied in both the variety. The grain from water stress affected plants weighed considerably lower than well irrigated control plants. *P. indica*-colonisation contributed to a hike in the grain weight and this considerably important in Ptb 23.

All agronomic practices in agriculture ultimately aims at increasing yield of the crop. The multitude of biotic and abiotic stresses impedes the ability of a plant to achieve the yield potential. The various crop management practices were developed to achieve this potential and the introduction of the growth promoting endophyte *P. indica* as a beneficial organism is one such promising plant stress management strategy. The culmination of negative effect of water stress on the morphological, physiological, biochemical and other yield parameters in this study eventually resulted in reduced yield and this was apparent in the case of water stressed plants of Ptb 23 free of *P. indica*. The role of *P. indica* as a growth promoter and aiding in water stress tolerance (Varma *et al.,* 1999) is also strengthened from the yield value of *P. indica*-colonised plants of Ptb 23 showing a 69.91 percentage increase over non-colonised plants under water stress. Ptb 29 proved to be superior to Ptb 23 in terms of most of the parameters studied here and thus able to produce more yield. Growth promotion at early developmental stages by *P. indica* was reported to enhance grain yield of barley (Achatz *et al.,* 2010). In another study by Tsai *et al.* (2020) it was speculated that higher grain yield was due to promotion of panicle formation and grain filling.

5.3.4. Effect of *P. indica* on expression of *OsDREB 2A* gene under water stress

Plants respond and adapt at molecular and cellular levels as well as at biochemical and physiological levels to survive under various environmental stresses. Dehydration responsive element binding (DREB) transcription factor is a group of transcription factors involved in abiotic stress tolerance (Moon *et al.* 2019). The genes in this subfamily plays an important role in abiotic stress response by recognizing dehydration responsive elements with a core motif of A/GCCGAC (Liu *et al.,* 1998). The gene products of *DREB2A* are involved in the control of gene expression in the nucleus and DREB2A interacts with the *cis*-acting dehydration-responsive element involved in drought stress-responsive gene expression (Sakuma *et al.,* 2006). The levels of *DREB2A* are drought inducible and is upregulated early in *P. indica* colonised drought-stressed *Arabidopsis* than in non-colonised control (Sheramati *et al.,*2008). Earlier studies in Chinese cabbage and Arabidopsis exposed to drought have demonstrated that when roots are colonised by *P. indica*, many drought-induced genes are upregulated (Sun *et al.,* 2010). In the current study, presence of *P. indica* in the roots enhanced the expression of *OsDREB2A* gene. In *P. indica* colonised plants, the induction of water stress significantly increased the expression of *OsDREB2A* by 2.2 (Ptb 23) and 5.1 (Ptb 29) folds as compared to the non-colonised irrigated (control) plants. Similarly, the findings of Xu *et al.,* (2017) asserted that in drought stressed leaves of *P. indica*-colonised maize plants, drought-related gene *DREB2A* was upregulated.

During the course of the current study, it was inferred that rice plants of Ptb 23 and Ptb 29 were able to cope with water stress and utilize resources for growth promotion in the presence of *P. indica* by regulating some phytohormone signalling pathways, activating ROS scavenging mechanisms through antioxidants and inducing the expression of various stress-responsive genes like *DREB2A*. This interference by *P. indica* influence the morphological, physiological biochemical and molecular parameters and enable the plants to tolerate abiotic and biotic stresses including water stress. However, the exact mechanism by which *P. indica* confers benefit to the host plant is not fully understood. Further studies should be conducted to understand the exact molecular mechanisms and underlying signalling pathways.

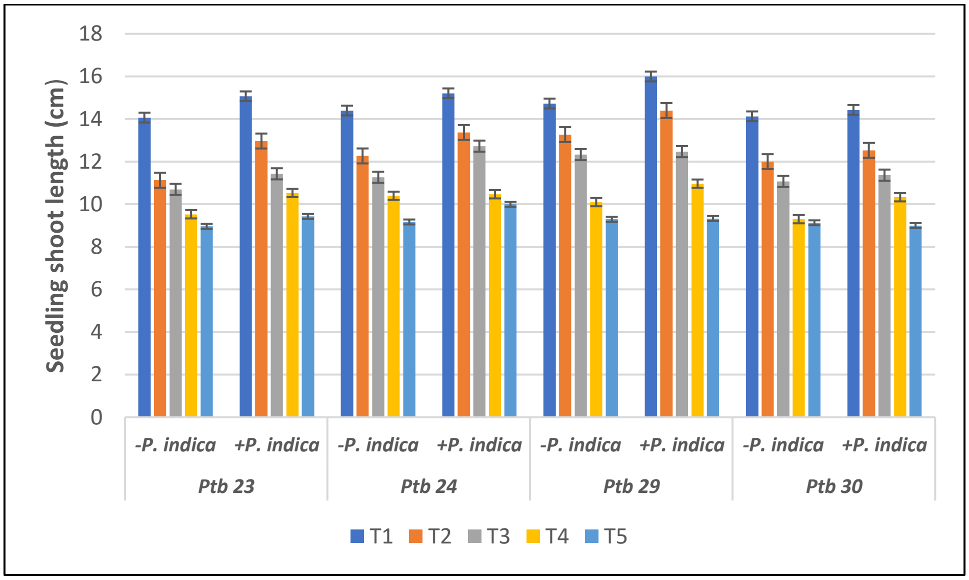

Fig.2. Effect of *P. indica*-colonisation on seedling shoot length (cm) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment. (T1 - 0%, T2 - 5%, T3 - 10%, T4 - 15% and T5 - 20% PEG 6000 concentrations)

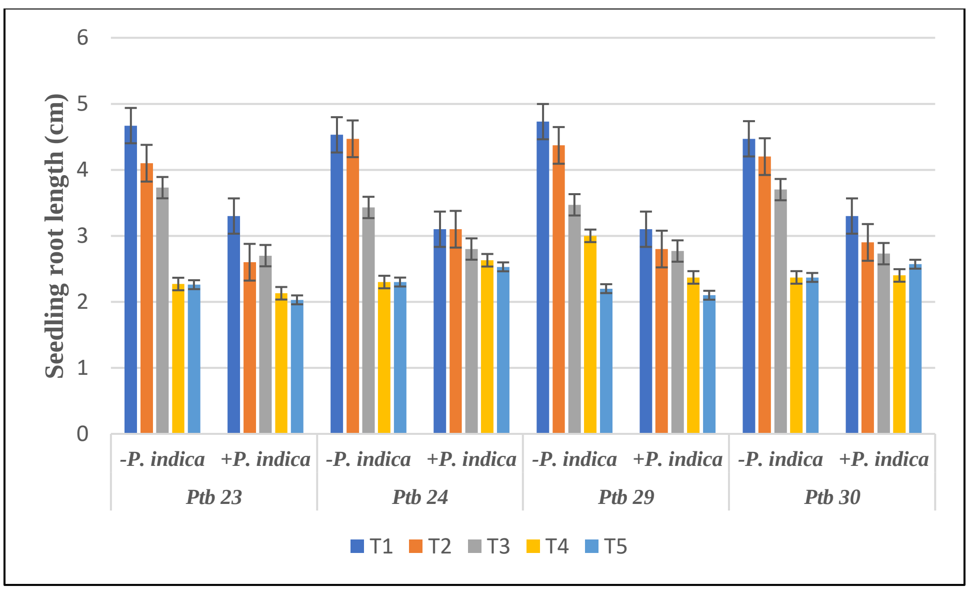

Fig.3. Effect of *P. indica*-colonisation on seedling root length (cm) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment. (T1 - 0%, T2 - 5%, T3 - 10%, T4 - 15% and T5 - 20% PEG 6000 concentrations)

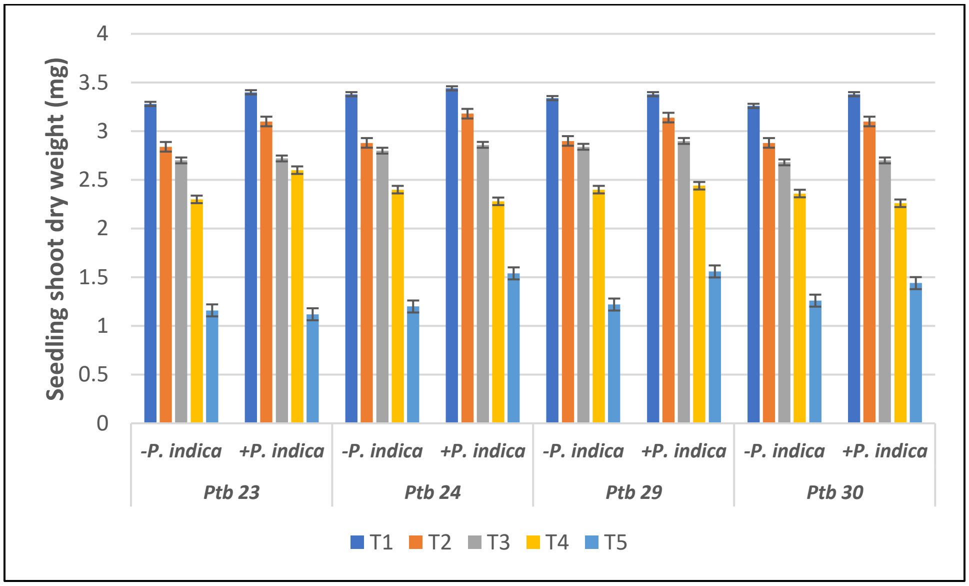

Fig.4. Effect of *P. indica*-colonisation on seedling shoot dry weight (mg) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment. (T1 - 0%, T2 - 5%, T3 - 10%, T4 - 15% and T5 - 20% PEG 6000 concentrations)

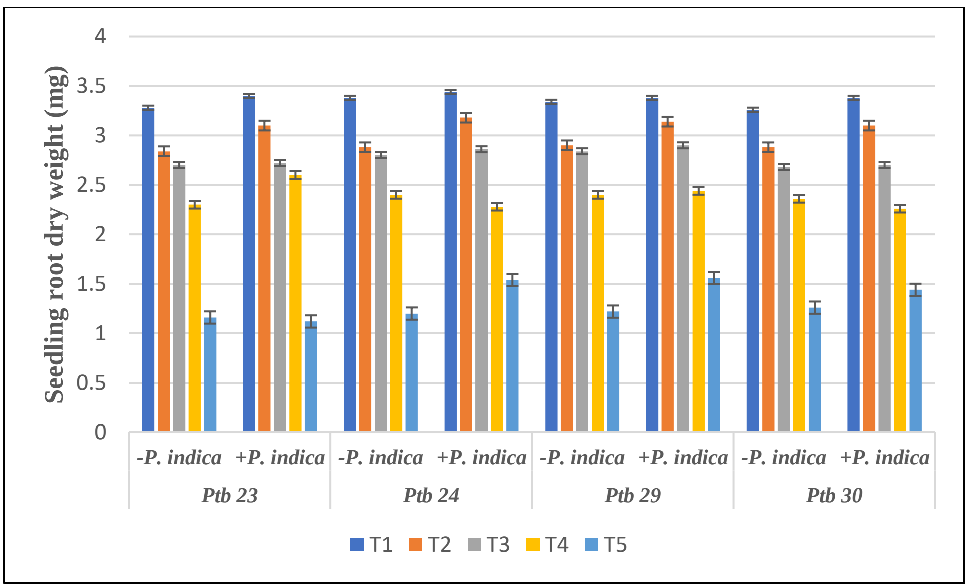

Fig.5. Effect of *P. indica*-colonisation on seedling root dry weight (mg) of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment. (T1 - 0%, T2 - 5%, T3 - 10%, T4 - 15% and T5 - 20% PEG 6000 concentrations)

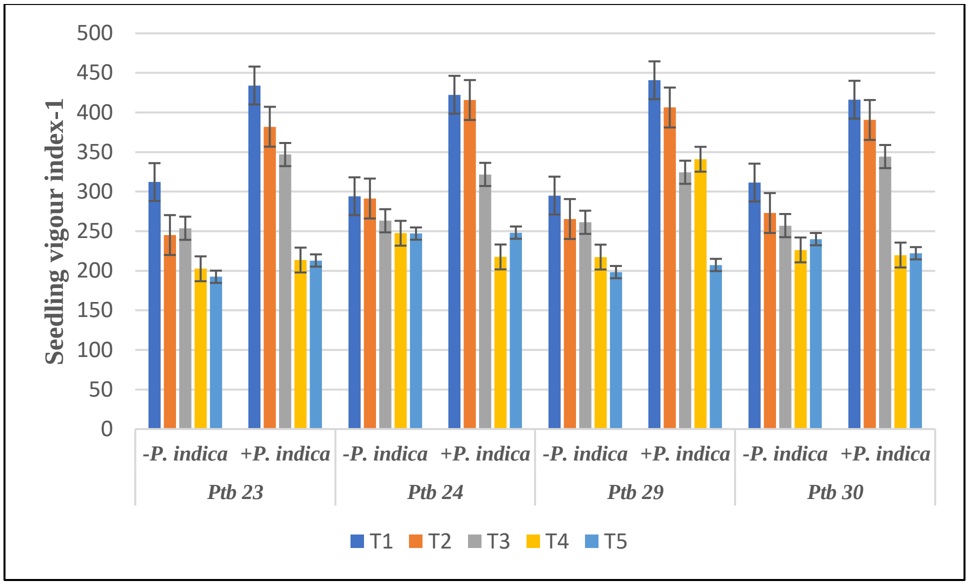

Fig.6. Effect of *P. indica*-colonisation on seedling vigour index-1 of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment. (T1 - 0%, T2 - 5%, T3 - 10%, T4 - 15% and T5 - 20% PEG 6000 concentrations)

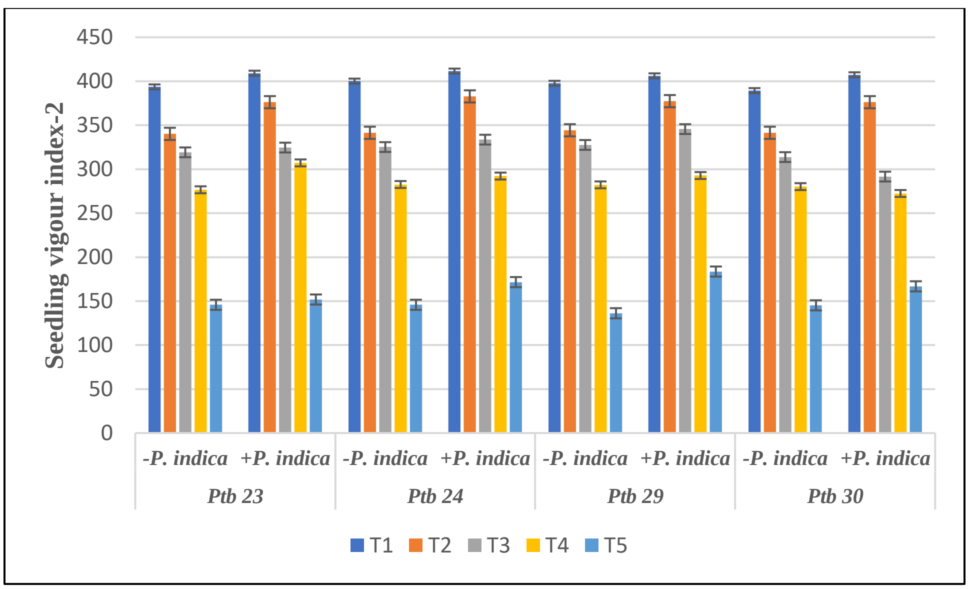

Fig.7. Effect of *P. indica*-colonisation on seedling vigour index of drought sensitive and tolerant varieties of rice under different concentrations of PEG 6000 at 7 days after treatment. (T1 - 0%, T2 - 5%, T3 - 10%, T4 - 15% and T5 - 20% PEG 6000 concentrations)

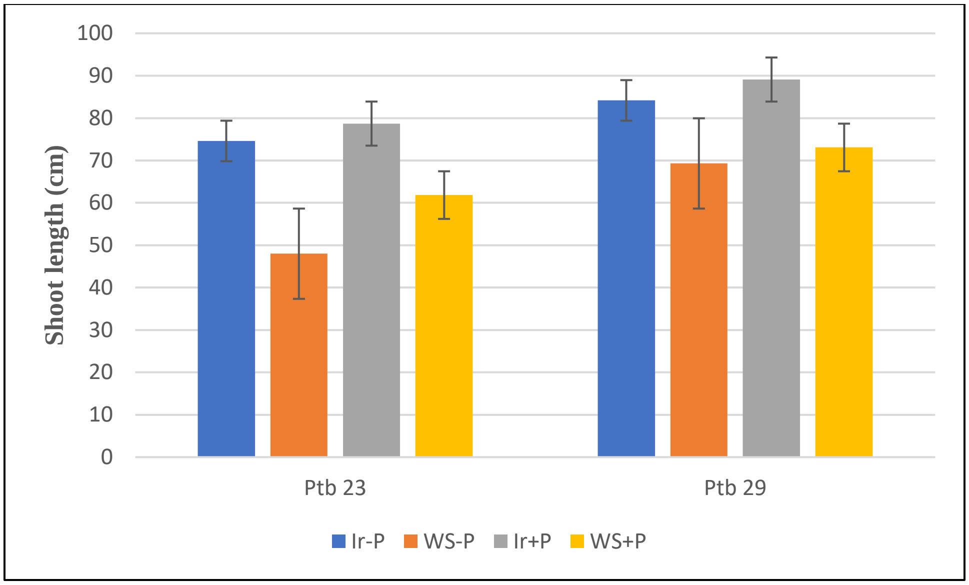

Fig.8. Effect of *P. indica*-colonisation on shoot length (cm) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated (Ir) conditions. (-P - without *P. indica*, +P – with *P. indica*)

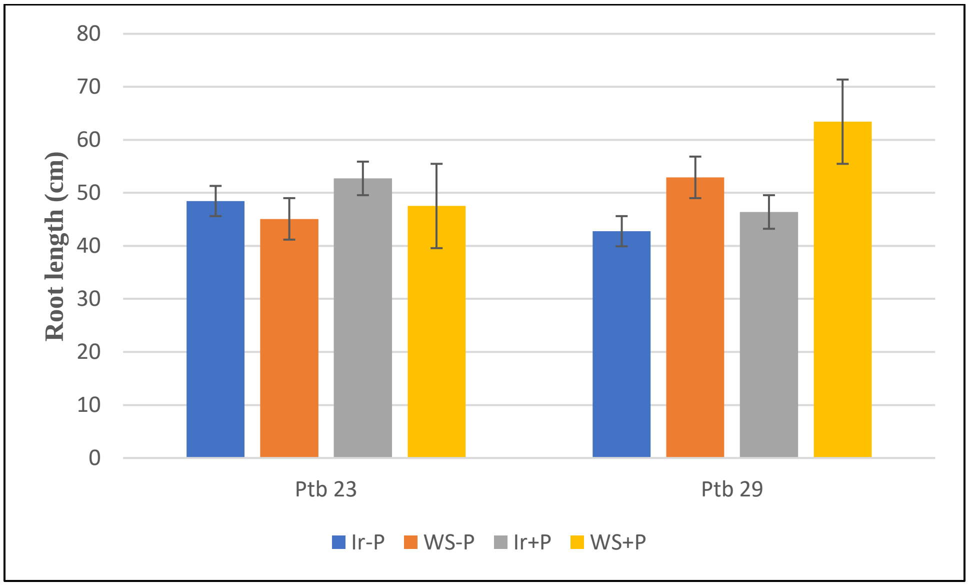

Fig.9. Effect of *P. indica*-colonisation on root length (cm) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

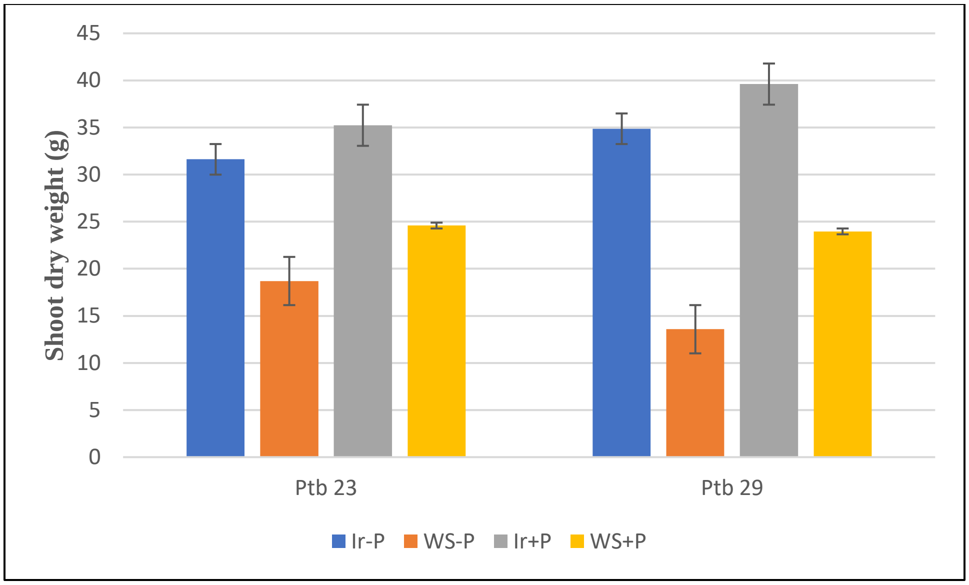

Fig.10. Effect of *P. indica*-colonisation on shoot dry weight (g) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

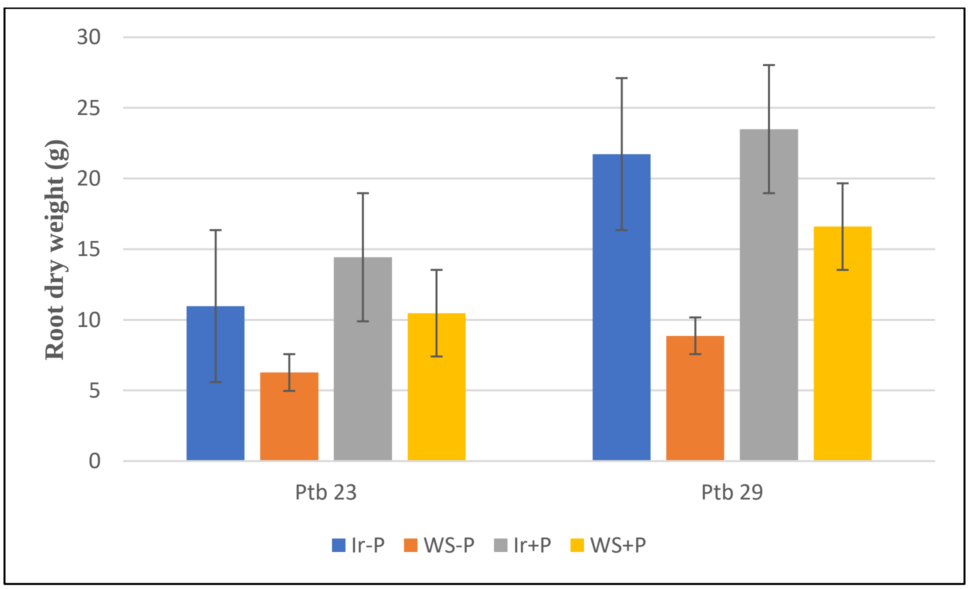

Fig.11. Effect of *P. indica*-colonisation on root dry weight (g) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

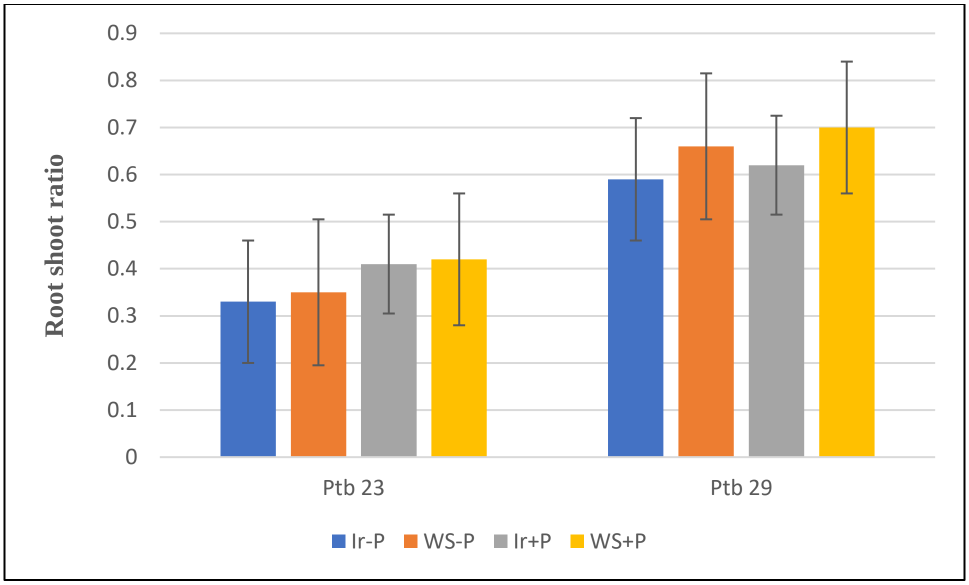

Fig.12. Effect of *P. indica*-colonisation on root shoot ratio of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

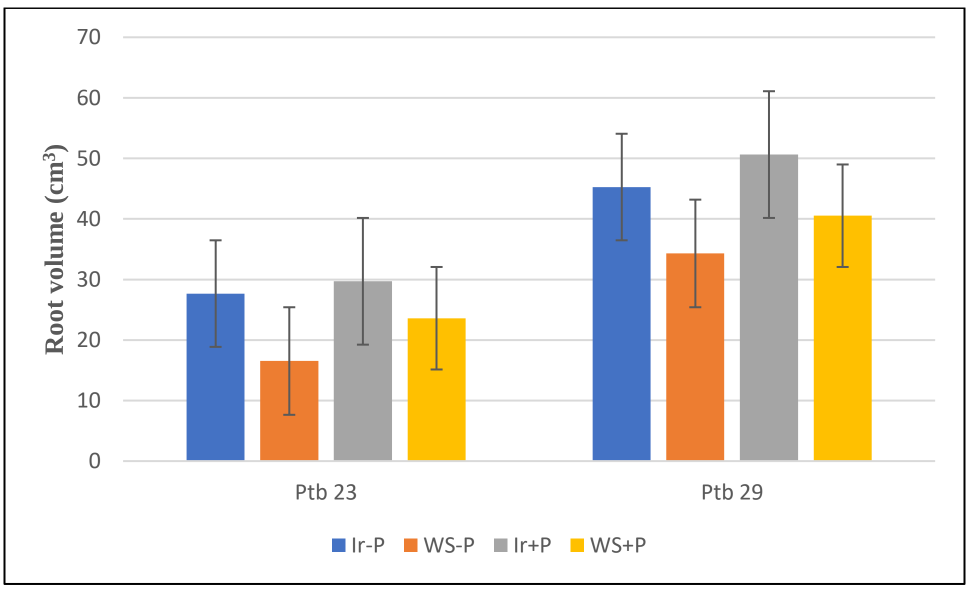

Fig.13. Effect of *P. indica*-colonisation on root volume (cm^3) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

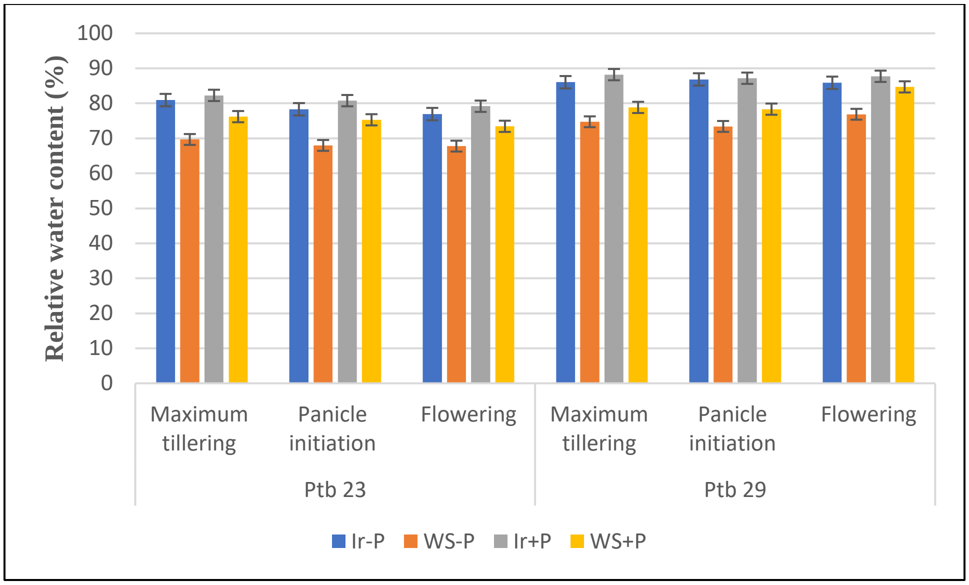

Fig.14. Effect of *P. indica*-colonisation on relative water content (%) Ptb 23 and Ptb29 varieties of rice under water stress (WS) and irrigated conditions at maximum tillering, panicle initiation and flowering stages. (-P - without *P. indica*, +P – with *P. indica*)

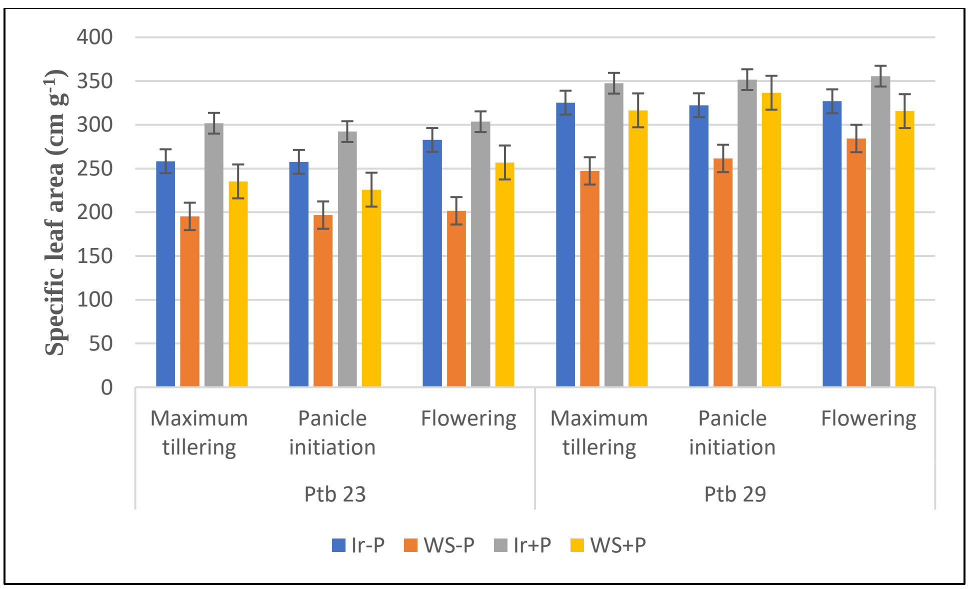

Fig.15. Effect of *P. indica*-colonisation on specific leaf area (cm g^{-1}) of Ptb 23 and Ptb29 varieties of rice under water stress (WS) and irrigated conditions at maximum tillering, panicle initiation and flowering stages. (-P - without *P. indica*, +P – with *P. indica*)

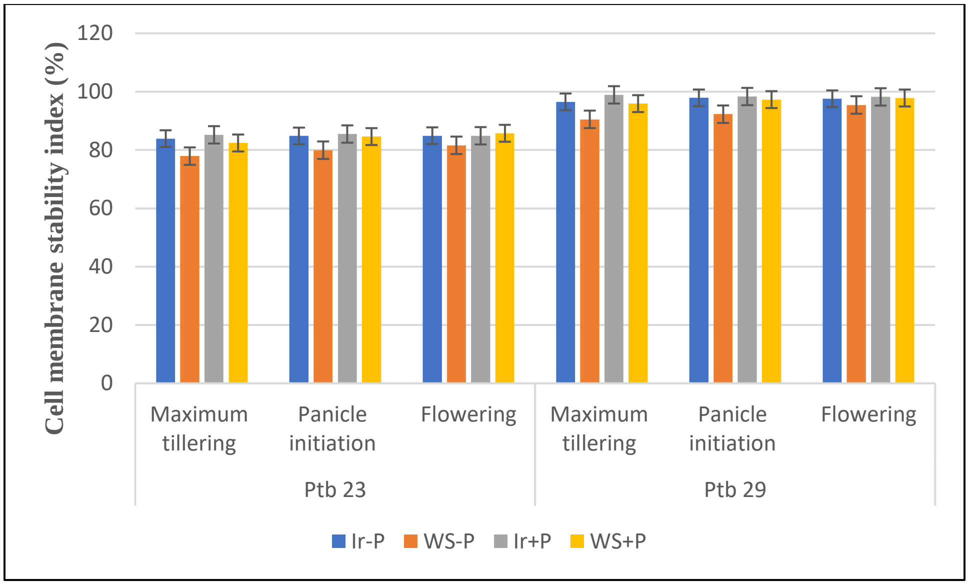

Fig.16. Effect of *P. indica*-colonisation on cell membrane stability index (%) of drought sensitive and tolerant varieties of rice under water stress (WS) and irrigated conditions at maximum tillering, panicle initiation and flowering stages. (-P - without *P. indica*, +P – with *P. indica*)

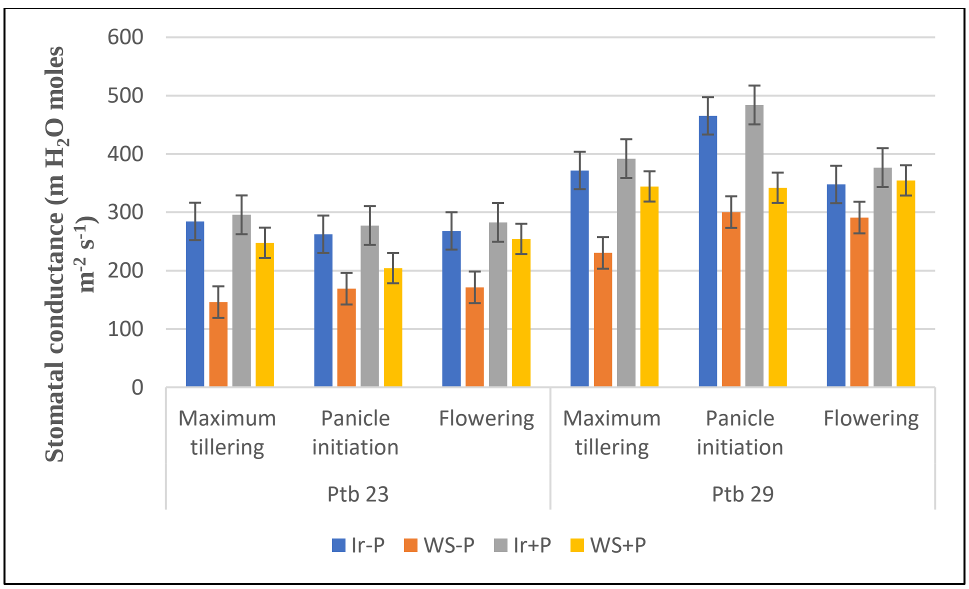

Fig.17. Effect of *P. indica*-colonisation on stomatal conductance (m H_2O moles m^{-2} s^{-1}) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions at maximum tillering, panicle initiation and flowering stages. (-P - without *P. indica*, +P – with *P. indica*)

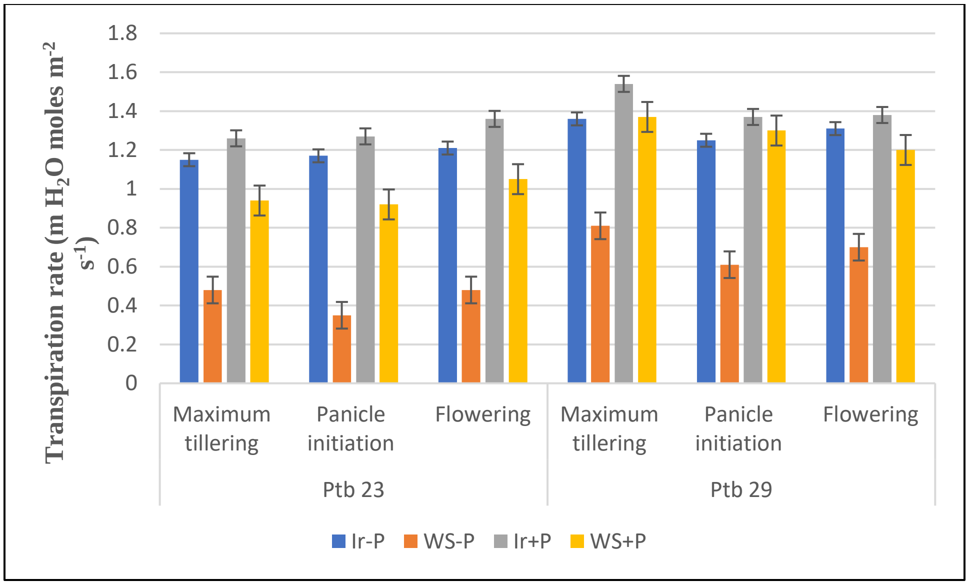

Fig.18. Effect of *P. indica*-colonisation on transpiration rate (m H_2O moles m^{-2} s^{-1}) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions at maximum tillering, panicle initiation and flowering stages. (-P - without *P. indica*, +P – with *P. indica*)

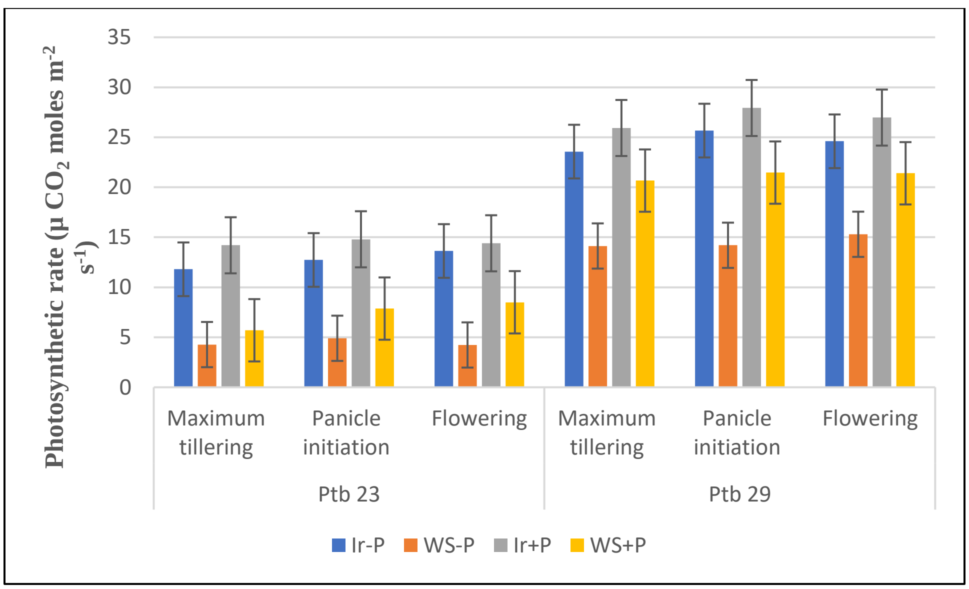

Fig.19. Effect of *P. indica*-colonisation on stomatal conductance (m CO_2 moles m^{-2} s^{-1}) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions at maximum tillering, panicle initiation and flowering stages. (-P - without *P. indica*, +P – with *P. indica*)

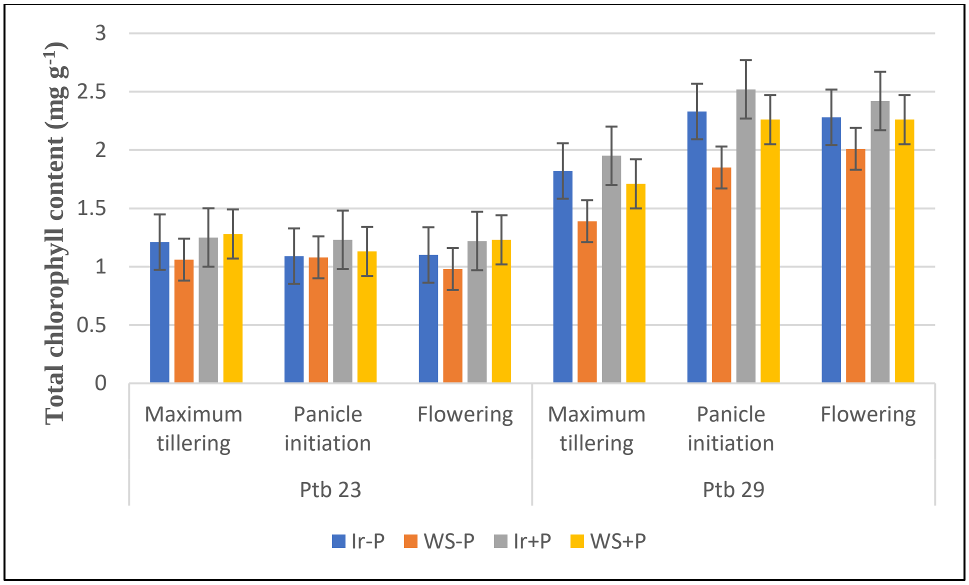

Fig.20. Effect of *P. indica*-colonisation on total chlorophyll content (mg g^{-1}) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions at maximum tillering, panicle initiation and flowering stages. (-P - without *P. indica*, +P – with *P. indica*)

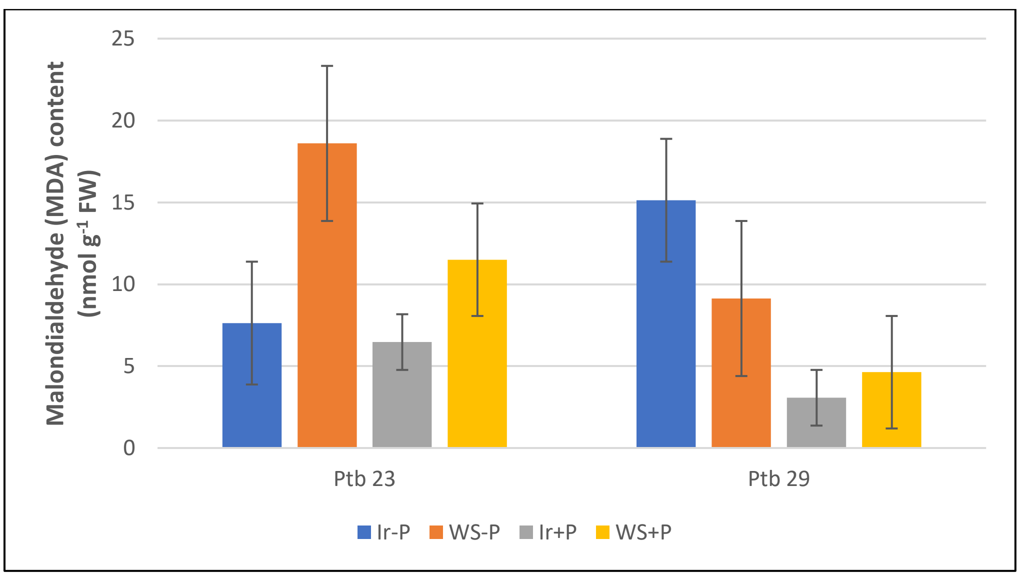

Fig. 21. Effect of *P. indica*-colonisation on malondialdehyde (MDA) (nmol g^{-1} FW) content of Ptb 23 and Ptb29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

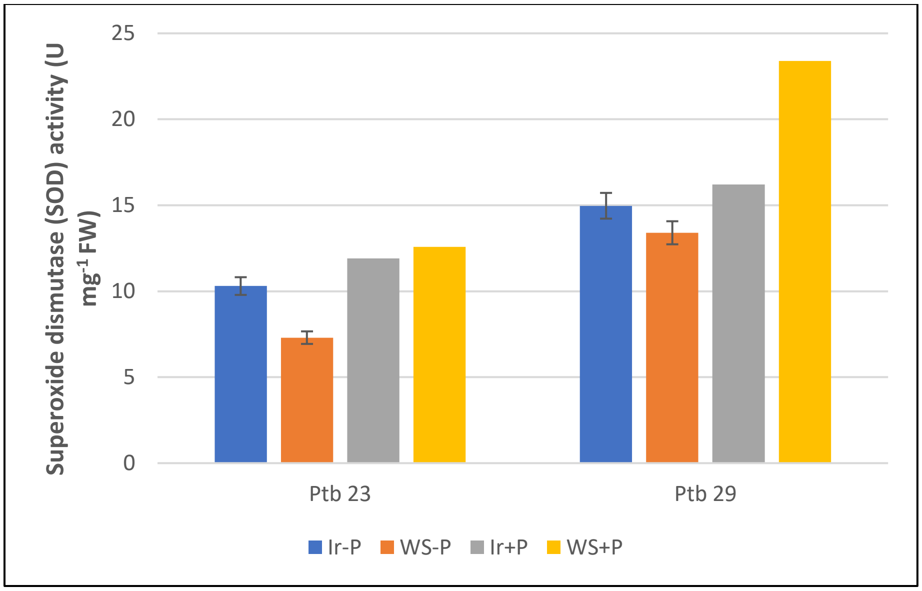

Fig. 22. Effect of *P. indica*-colonisation on superoxide dismutase activity (U mg^{-1} FW) content of Ptb 23 and Ptb29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

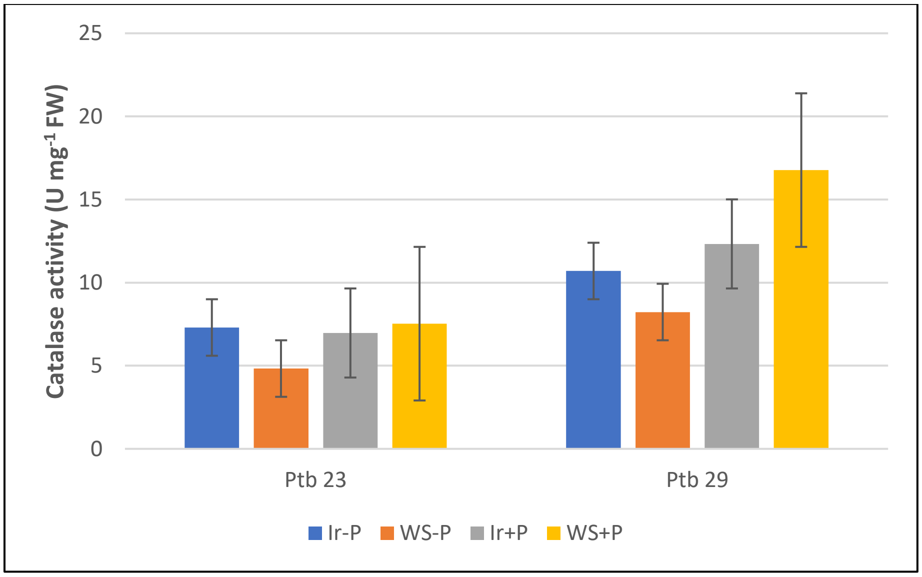

Fig. 23. Effect of *P. indica*-colonisation on catalase (unit mg^{-1} FW) Ptb 23 and Ptb29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

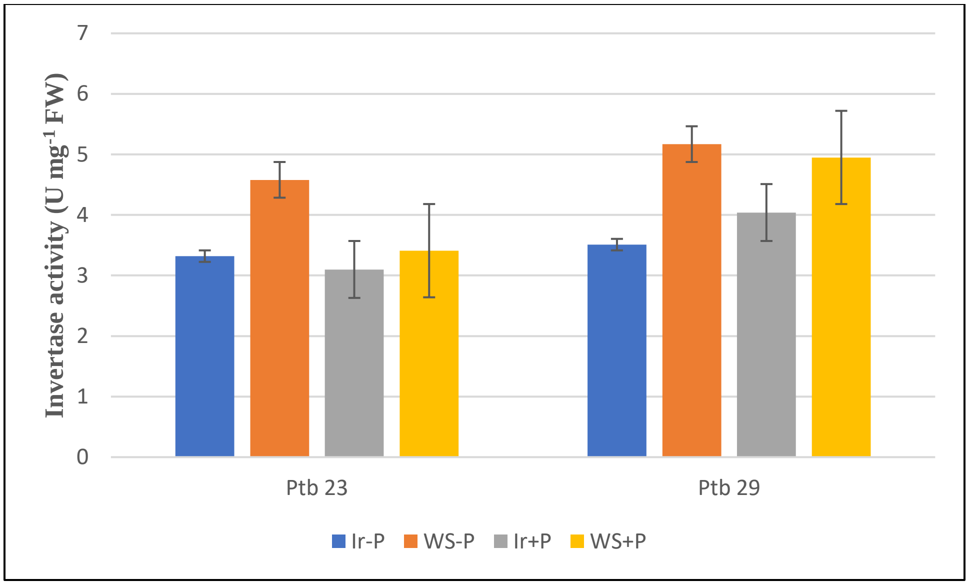

Fig. 24. Effect of *P. indica*-colonisation on invertase activity (U mg^{-1} FW) Ptb 23 and Ptb29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

Fig. 25. Effect of *P. indica*-colonisation on days to 50% flowering of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

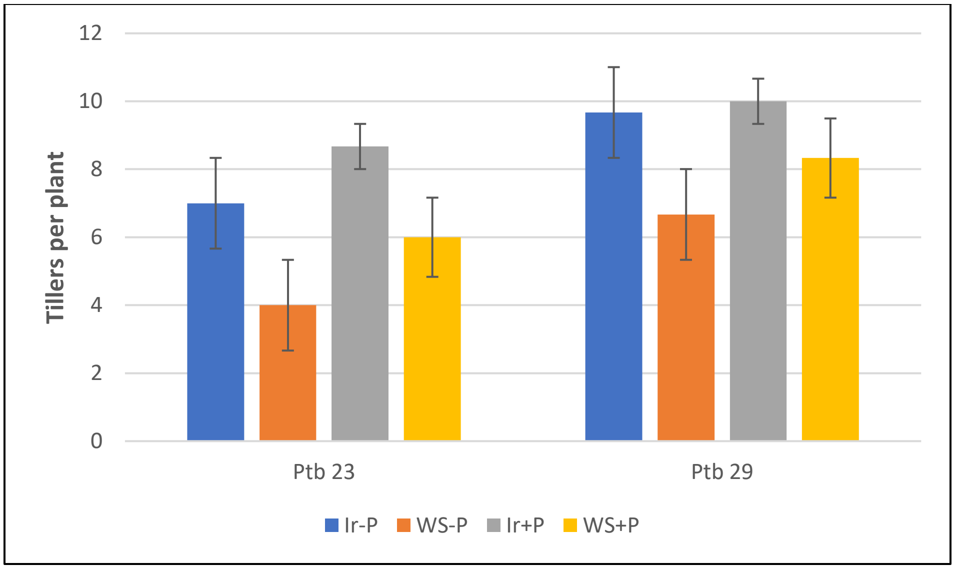

Fig. 26. Effect of *P. indica*-colonisation on tillers per plant of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

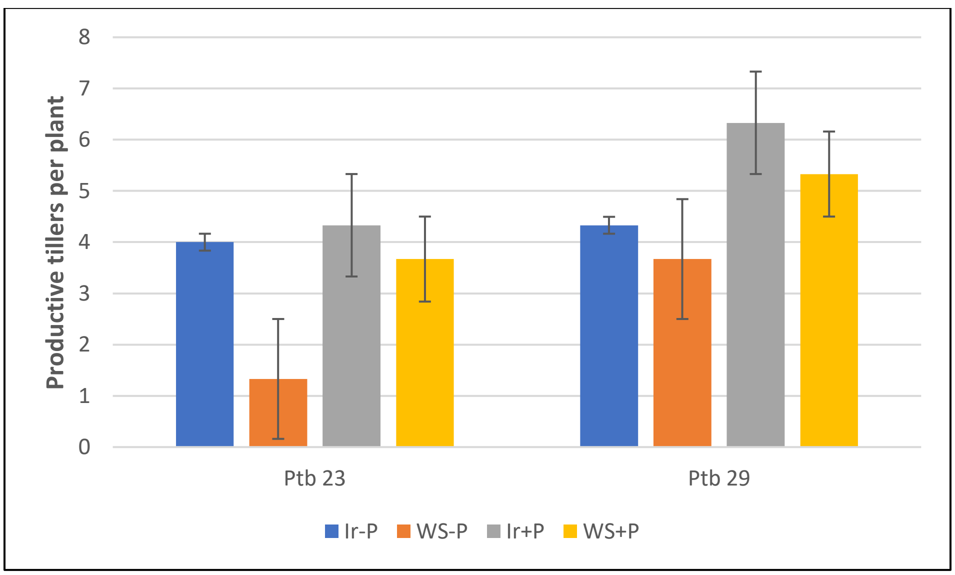

Fig. 27. Effect of *P. indica*-colonisation on productive tillers per plant of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

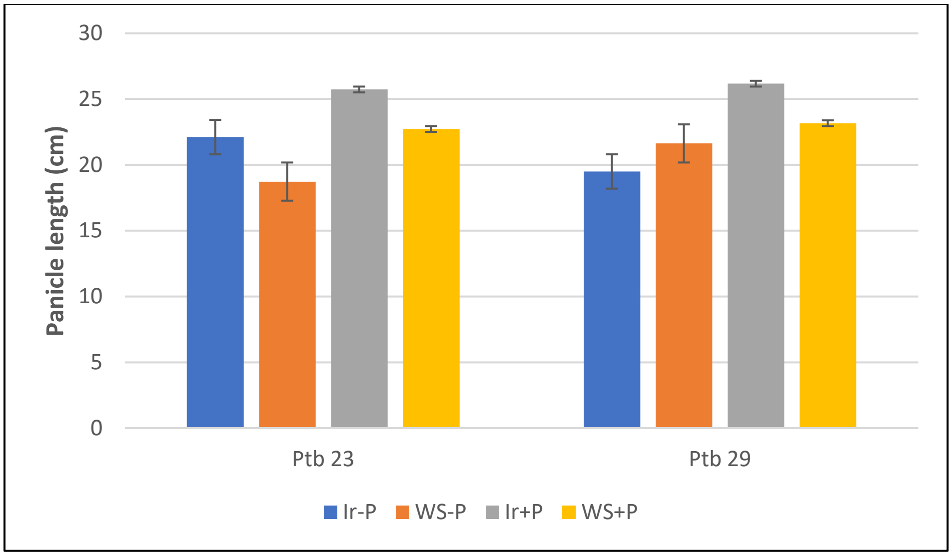

Fig. 28. Effect of *P. indica*-colonisation on panicle length (cm) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

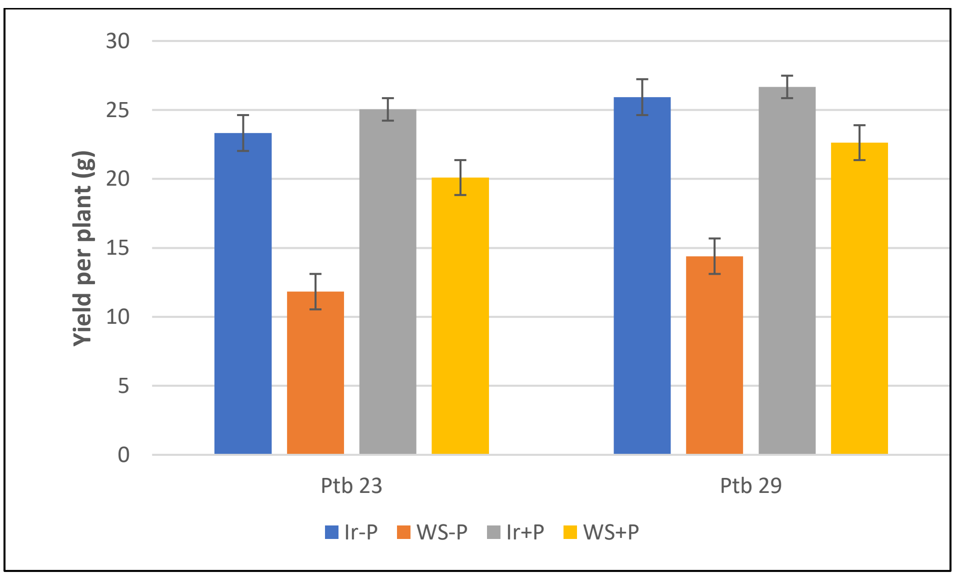

Fig. 29. Effect of *P. indica*-colonisation on yield per plant (g) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

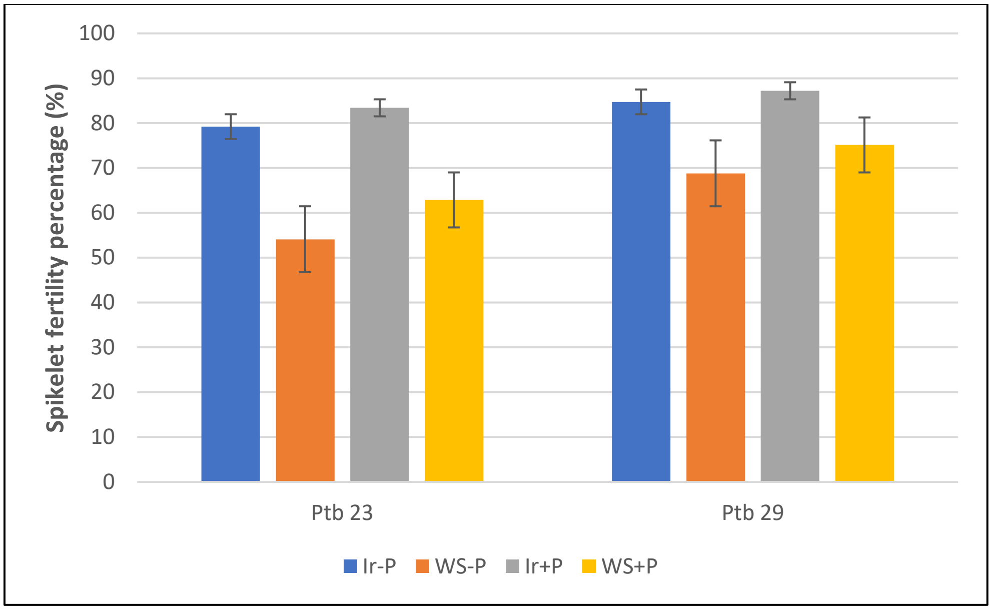

Fig. 30. Effect of *P. indica*-colonisation on spikelet fertility percentage (%) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

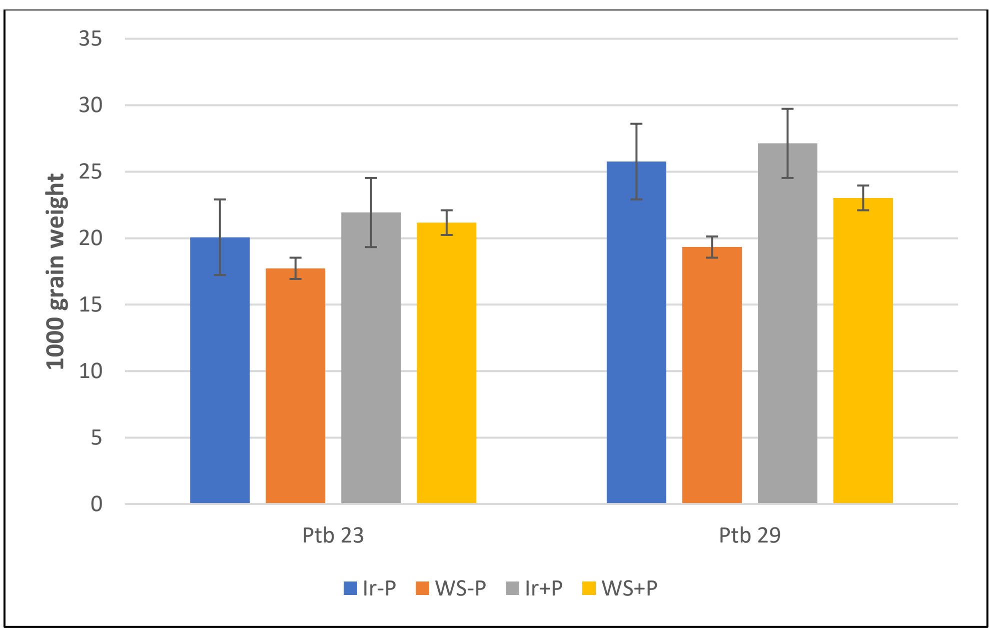

Fig. 31. Effect of *P. indica*-colonisation on 1000 grain weight (g) of Ptb 23 and Ptb 29 varieties of rice under water stress (WS) and irrigated conditions. (-P - without *P. indica*, +P – with *P. indica*)

Summary

6. SUMMARY

The research work titled "Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)" carried out at Department of Plant Physiology, College of Agriculture, Vellayani during 2020-2021 with the objective to elucidate the changes in morphological, physiological, biochemical and molecular mechanisms associated with water stress tolerance in *Piriformospora indica*-colonised rice.

The initial study aimed at identifying the highest tolerating level of water stress in seedling stage by virtue of root colonisation by *P. indica* in two water stress tolerant varieties (Ptb 29 and Ptb 30) and two water stress susceptible varieties (Ptb 23 and Ptb 24) of rice by treatment with Poly Ethylene Glycol (PEG) 6000 at concentrations of 0, 5,10, 15, and 20 per cent.

Surface sterilized seeds of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 rice varieties of were placed in trays containing the *P. indica*-infused composted coir pith- cow dung (1:1) mixture after 3 weeks of fungal growth. A similar set of trays but without mixing the *P. indica* culture were used to germinate the seeds of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 rice varieties as control plants. These trays were maintained in temperature and humidity-controlled conditions for uniform germination. The seeds germinated after three days of sowing in both set of trays. The roots of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 varieties' seedlings were appraised microscopically for root colonisation. Isolated or chains of double walled pear shaped chlamydospores were observed in the root sections of seedlings in trays with *P. indica* after seven days of cocultivation.

In the preliminary screening of varieties for water stress tolerance, growth promotion of rice seedlings was assessed seven days after treatment. All the varieties pertaining to this study on root colonisation with *P. indica* showed higher water stress tolerance while the water stress tolerant varieties Ptb 29 and Ptb 30 showed tolerance to water stress even in the absence of colonisation with *P. indica*. Shoot length, shoot and root dry weight, number of root branches, SVI 1 and SVI 2 increased in *P. indica*-colonised plants than non-colonised plants under water stressed condition, while the root length decreased. *P. indica*-induced auxin production in roots may have caused the colonised plants to exhibit increased shoot length and biomass and slower root growth in this study. Based on the observations on different parameters, Ptb 29 was considered as the best water stress tolerant variety and as Ptb 23 performed better than Ptb 24 under water stress condition, the former was selected as the best water-stress susceptible variety.

The *P. indica*-colonised and non-colonised rice seedlings of the selected water stress susceptible variety (Ptb 23) and water stress tolerant variety (Ptb 29), from the screening experiment were raised in rainout shelter and were the evaluated for water stress tolerance during the different growth stages by studying the morphological, physiological, biochemical, yield parameters and molecular aspects. Water stress was induced by stopping irrigation for five days during maximum tillering, panicle initiation and flowering stages and normal irrigation was resumed thereafter.

The effect of *P. indica*-colonisation on morphological parameters were studied and it was found that *P. indica*-colonised plants under water stress exhibited higher shoot and root length, shoot and root dry weight, root-shoot ratio and root volume, however, it was more conspicuous in the water stress susceptible variety. The enhanced plant shoot-root growth and biomass production as well as other changes in plant morphology after *P. indica*-colonisation can be related to the plant response to increase auxin level in roots either produced by the fungus or by the plant due to stimulation by the endophyte.

The physiological and biochemical parameters were observed during maximum tillering, panicle initiation and flowering stages. Relative water content was found to be higher in water stressed *P. indica*-colonised plants in both Ptb 23 and Ptb 29, although the effect of colonisation was more visible in Ptb 23. There was significant increase in the specific leaf area in *P. indica*-colonised plants of both varieties than non-colonised plants. In the current study, rice plants of Ptb 29 under water stressed condition had higher cell membrane stability index than Ptb 23. Incidentally, *P. indica*-colonisation improved the membrane stability of cells in stressed plants and the percentage increase was comparable in both the varieties. It decreases during water stress because of lipid peroxidation which leads to loss of cell membrane stability and increased production of malondialdehyde. However, plants in the presence of *P. indica* showed significantly reduced MDA content which indicates that *P. indica* lowers the adverse consequences of drought. Ptb 29 had lower MDA content than Ptb 23, which further established that Ptb 29 is more tolerant to water stress. Gas exchange parameters like stomatal conductance, transpiration rate and photosynthetic rate showed increase in *P. indica*-colonised plants than in non-colonsed plants. Plants try to minimize the water loss by regulating the stomatal closure which is facilitated by ABA, hydrogen peroxide, nitric oxide and Ca^{2+} signalling. *P. indica*-colonisation is presumed to up-regulate the expression of some Ca^{2+} sensing regulators thereby influencing stomatal movement. Total chlorophyll content was lower in plants under water stress while water stressed *P. indica*-colonised plants had higher chlorophyll content than non-colonised plants. Retention of chlorophyll was significantly higher in water stressed plants of Ptb 29 than Ptb 23.

The increased activity of antioxidant enzymes such as superoxide dismutase and catalase in *P. indica*-colonised plants during stressed condition reduced ROS levels in cells and thereby minimizing or preventing damage to cellular components. *P. indica*-colonised plants showed a reduced invertase activity than non-colonised plants. The effect of the endophyte was easily noticed and significant in water stressed Ptb 23 plants colonised with *P. indica*.

All agronomic practices in agriculture ultimately aims at increasing yield of the crop. The multitude of biotic and abiotic stresses impedes the ability of a plant to achieve the yield potential. The various crop management practices were developed to achieve this potential and the introduction of the growth promoting endophyte *P. indica* as a beneficial organism is one such promising plant stress management strategy. *P. indica*-colonisation improved the number of tillers and productive tillers as well the panicle length in plants under water stressed condition. The spikelet sterility was higher in water stressed plants while *P. indica*-colonisation was able to improve spikelet fertility. The grain from water stress affected plants weighed considerably lower than well irrigated control plants. *P. indica*-colonisation contributed to a hike in the grain weight and this considerably important in Ptb 23. All these factors contributed to the final yield of the plant and unsurprisingly, *P. indica*-colonised plant sets produced more yield than non-colonised plant sets under water stressed conditions. Ptb 29 proved to be superior to Ptb 23 in terms of most of the parameters studied here and thus able to able to produce more yield. However, the yield of *P. indica*-colonised plants of Ptb 23 showed a 69.91 percentage increase over non-colonised plants under water stress and this suggests that *P. indica*-colonisation was more noticeable and effective in water stress susceptible variety- Ptb 23.

The effect of *P. indica* on expression of *OsDREB2A,* a member of the DREBP subfamily of AP2/ERF transcription factor in rice and is involved in abiotic stress response namely water stress, was studied here. It was found to be upregulated in *P. indica*-colonised plants when exposed to water stress.

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Appendices

Appendix - I

Potato Dextrose Agar (PDA) medium

Potato: 200 g

Dextrose: 20 g

Agar: 20 g

Distilled water: 1 L

Appendix - II

Buffers for Enzyme analysis

1. 0.1 M Sodium phosphate buffer (pH 6.5)

Stock solutions

A: 0.2 M solutions of monobasic sodium phosphate (27.8 g in 1litre)

B: 0.2 M solutions of dibasic sodium phosphate (53.65 g in 1 litre)

68.5 ml of A mixed with 31.5 ml of B diluted to a total of 200 ml

2. 0.067 M Phosphate buffer (pH 7)

Dissolve 3.522 g KH_2PO_4 and 7.298 g $Na_2HPO_4.2H_2O$ in distilled water and make up to 1000 ml.

Appendix - III

Components of modified Hoagland nutrient solution

Compound	Molecularweight	Concentration of stock solution	Volume of stock soln per L of final solution	Final Conc. of element	
	g mol^{-1}	mM	ml	µM	ppm
Macronutrient					
KNO_3	101.10	1,000	6.0	16,000	224
$Ca(NO_3). 4H_2O$	236.16	1,000	4.0	6,000	235
$NH_4H_2PO_4$	115.08	1,000	2.0	4,000	160
$MgSO_4. 7H_2O$	246.48	1,000	1.0	2,000	62
				1,000	32
				1,000	24
Micronutrient					
KCl	74.55	25.0		50.0	1.77
H_3BO_3	61.83	12.5		25.0	0.27
$MnSO_4. H_2O$	169.01	1.0		2.0	0.11
$ZnSO_4. 7H_2O$	287.54	1.0	2.0	2.0	0.13
$CuSO_4.5H_2O$	249.68	0.25		0.5	0.03
H_2MoO_4 (85% MoO_3)	161.97	0.25		0.5	0.05
NaFeEDTA (10% Fe)	558.50	53.7	0.3-1.0	16.1-53-7	1.00-3.00

Adopted from Taiz and Zeiger (2002)

Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)”

by

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ABSTRACT

Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)

The research work titled "Elucidating the role of growth promoting endophytic fungus *Piriformospora indica* for water stress tolerance in rice (*Oryza sativa* L.)" carried out at Department of Plant Physiology, College of Agriculture, Vellayani during 2020-2021 with the objective to elucidate the changes in morphological, physiological, biochemical and molecular mechanisms associated with water stress tolerance in *Piriformospora indica*-colonised rice.

The roots of Ptb 23, Ptb 24, Ptb 29 and Ptb 30 varieties' seedlings were appraised microscopically for root colonisation after germinating in trays containing *P. indica*-infused composted coir pith- cow dung (1:1) mixture maintained in temperature and humidity-controlled conditions. Isolated or chains of double walled pear shaped chlamydospores were observed in the root sections of seedlings in trays with *P. indica* after seven days of cocultivation.

On treatment with Poly Ethylene Glycol (PEG) 6000 at concentrations of 0, 5,10, 15, and 20 per cent, the varieties on root colonisation with *P. indica* showed higher water stress tolerance while the water stress tolerant varieties Ptb 29 and Ptb 30 showed tolerance to water stress even in the absence of *P. indica*. Shoot length, shoot and root dry weight, number of root branches, SVI 1 and SVI 2 increased in *P. indica*-colonised plants than non-colonised plants under water stressed condition, while the root length decreased. Based on the observations on different parameters, Ptb 29 was considered as the best water stress tolerant variety and as Ptb 23 performed better than Ptb 24 under water stress condition, the former was selected as the best water-stress susceptible variety.

The *P. indica*-colonised and non-colonised rice seedlings of Ptb 23 and Ptb 29 were the evaluated for water stress tolerance during the different growth stages by studying the morphological, physiological, biochemical, yield parameters and molecular aspects. *P. indica*-colonised plants under water stress exhibited higher shoot and root length, shoot and root dry weight, root-shoot ratio and root volume, however, it was more conspicuous in the water stress

susceptible variety. The enhanced plant shoot-root growth and biomass production as well as other changes in plant morphology after *P. indica*-colonisation can be related to the plant response to increase auxin level in roots either produced by the fungus or by the plant due to stimulation by the endophyte.

Relative water content was found to be higher in water stressed *P. indica*-colonised plants in both Ptb 23 and Ptb 29, although the effect of colonisation was more visible in Ptb 23. There was significant increase in the specific leaf area in *P. indica*-colonised plants of both varieties than non-colonised plants. In the current study, rice plants of Ptb 29 under water stressed condition had higher cell membrane stability index than Ptb 23. Plants in the presence of *P. indica* showed significantly reduced MDA content which indicates that *P. indica* lowers the adverse consequences of drought. Ptb 29 had lower MDA content than Ptb 23, which further established that Ptb 29 is more tolerant to water stress. Gas exchange parameters like stomatal conductance, transpiration rate and photosynthetic rate showed increase in *P. indica*-colonised plants than in non-colonsed plants. Retention of chlorophyll was significantly higher in water stressed plants of Ptb 29 than Ptb 23. The increased activity of antioxidant enzymes such as superoxide dismutase and catalase in *P. indica*-colonised plants during stressed condition reduced ROS levels in cells and thereby minimizing or preventing damage to cellular components. *P. indica*-colonised plants showed a reduced invertase activity than non-colonised plants.

P. indica-colonisation improved the number of tillers, productive tillers as well the panicle length in plants under water stressed condition. The spikelet sterility was higher in non-colonised and water stressed plants. *P. indica*-colonisation contributed to a considerable increase in grain weight in Ptb 23. All these factors contributed to the final yield of the plant and unsurprisingly, *P. indica*-colonised plant sets produced more yield than non-colonised plant sets under water stressed conditions. Even though Ptb 29 produced more yield., *P. indica*-colonisation was more noticeable and effective in the water stress susceptible variety- Ptb 23.

Keeping in view of our results, it can be emphasised that *P. indica* can mitigate the ill effects of water stress. The findings obtained from this study can be used as a foundation for future lines of research related to rational improvement of rice plants against water stress using endophytes.

സംഗ്രഹം

"നെല്ലിലെ ജലസമ്മർദ സഹിഷ്ണുതക്കായി പിരിഫോർമോസ്പോറ ഇൻഡിക്ക എന്ന വളർച്ചയെ പ്രോത്സാഹിപ്പിക്കുന്ന വേരിൽ അന്തർവ്യാപന ശേഷിയുള്ള മിത്രകുമിളിനുള്ള പങ്ക്"

"നെല്ലിലെ ജലസമ്മർദ സഹിഷ്ണുതക്കായി പിരിഫോർമോസ്പോറ ഇൻഡിക്ക എന്ന വളർച്ചയെ പ്രോത്സാഹിപ്പിക്കുന്ന വേരിൽ അന്തർവ്യാപന ശേഷിയുള്ള മിത്രകുമിളിനുള്ള പങ്ക്" എന്ന തലക്കെട്ടിലുള്ള ഗവേഷണം വെള്ളായണി കാർഷിക കോളേജിലെ സസ്യ ധർമ്മശാസ്ത്ര വിഭാഗത്തിൽ 2019 -2021 കാലയളവിൽ നടത്തി. പി. ഇൻഡിക്ക കോളനിവൽക്കരിച്ച നെല്ലിലെ ജലസമ്മർദ സഹിഷ്ണുതയുമായി ബന്ധപ്പെട്ട ബാഹ്യരൂപഘടന, ഫിസിയോളോജിക്കൽ, ബിയോകെമിക്കൽ മോളിക്യൂലർ മെക്കാനിസങ്ങളിലെ മാറ്റങ്ങൾ വ്യാഖ്യാനിക്കുക എന്നതായിരുന്നു പഠനത്തിന്റെ ലക്ഷ്യം.

പി. ഇൻഡിക്ക കലർന്ന കമ്പോസ്റ്റ്-ചകിരിച്ചോറ്-ചാണകപ്പൊടി (1:1) മിശ്രിതം അടങ്ങിയ ട്രേകളിൽ താപനിലയും ഈർപ്പവും നിയന്ത്രിത അവസ്ഥയിൽ നിലനിർത്തി മുളപ്പിച്ചതിനുശേഷം Ptb 23, Ptb 24, Ptb 29, Ptb 30 എന്നീ നെല്ലിനങ്ങളുടെ തൈ-വേരുകളിൽ പി. ഇൻഡിക്കയുടെ കോളനിവത്കരണം സൂക്ഷ്മതലത്തിൽ വിലയിരുത്തിയപ്പോൾ ഏഴു ദിവസം പ്രായമായ പി.ഇൻഡിക്കയോടുകൂടിയ ട്രേകളിൽ മുളച്ച തൈകളുടെ വേരുഭാഗങ്ങളിൽ ഇരട്ട ഭിത്തികളുള്ള പിയർ ആകൃതിയിലുള്ള ഒറ്റപ്പെട്ടതോ ചങ്ങലയോ ആയി ക്ലാമിഡോസ്പോറുകളുടെ സാന്നിധ്യം കണ്ടെത്തി.

0, 5,10, 15, 20 ശതമാനം സാന്ദ്രതയിൽ പോളി എഥിലീൻ ഗ്ലൈക്കോൾ (PEG) 6000 ഉപയോഗിച്ചുള്ള ജലസമ്മർദ്ദ പഠനത്തിൽ, മേല്പറഞ്ഞ നെല്ലിനങ്ങൾ എല്ലാം പി. ഇൻഡിക്ക-കോളനിവൽക്കരണത്താൽ ഉയർന്ന ജലസമ്മർദ്ദ സഹിഷ്ണുത കാണിച്ചു, അതേസമയം സഹജമായ ജലസമ്മർദ്ദ സഹിഷ്ണുത പുലർത്തുന്ന ഇനങ്ങളായ Ptb 29, Ptb 30 എന്നിവ പി.

ഇൻഡിക്കയുടെ അഭാവത്തിൽ പോലും ജല സമ്മർദ്ദത്തോട് സഹിഷ്ണുത കാണിച്ചു. ജലസമ്മർദ്ദമുള്ള അവസ്ഥയിൽ കോളനിവൽക്കരിക്കാത്ത സസ്യങ്ങളെ അപേക്ഷിച്ച് പി. ഇൻഡിക്ക കോളനിവത്കരിച്ച തൈകളുടെ തണ്ടിന്റെ നീളം, തണ്ടിന്റെയും വേരിന്റെയും ഉണങ്ങിയ ഭാരം, ശാഖാവേരുകളുടെ എണ്ണം, SVI 1, SVI 2, എന്നിവ വർധിക്കുകയും വേരിന്റെ നീളം കുറയുകയും ചെയ്തു. വിവിധ പാരാമീറ്ററുകളിലെ നിരീക്ഷണങ്ങളുടെ അടിസ്ഥാനത്തിൽ, Ptb 29 മികച്ച ജലസമ്മർദ്ദം സഹിഷ്ണുതയുള്ള ഇനമായി തിരഞ്ഞെടുത്തു. കൂടാതെ ജലസമ്മർദ്ദാവസ്ഥയിൽ Ptb 24 നേക്കാൾ മികച്ച പ്രകടനം കാഴ്ചവെച്ചതിനാൽ, Ptb 23 ജലസമ്മർദം അധികം ബാധിക്കാത്ത ഇനമായി കണക്കാക്കുകയും ചെയ്തു.

പി. ഇൻഡിക്ക കോളനിവത്കരിച്ചതും അല്ലാത്തതുമായ Ptb 23, Ptb 29, നെല്ലിനങ്ങളിൽ വിവിധ വളർച്ചാ ഘട്ടങ്ങളിലെ ജലസമ്മർദ്ദസഹിഷ്ണുത വിലയിരുത്തുന്നതിനായി സസ്യങ്ങളുടെ ബാഹ്യരൂപഘടന, ഫിസിയോളജിക്കൽ, ബയോകെമിക്കൽ, വിളവ് പാരാമീറ്ററുകൾ, മോളിക്യൂലർ വശങ്ങൾ എന്നിവ നിരീക്ഷിച്ചു. ജലസമ്മർദ്ദവസ്ഥയിൽ പി. ഇൻഡിക്ക കോളനിവത്കരിച്ച സസ്യങ്ങളിൽ തണ്ടിന്റെയും വേരിന്റെയും നീളം ഉണങ്ങിയഭാരം, റൂട്ട്-ഷൂട്ട് അനുപാതം, വേരിന്റെ വ്യാപ്തം എന്നിവ ഉയർന്നു. എന്നിരുന്നാലും, ജലസമ്മർദ്ദത്തിന് വിധേയമാകുന്ന ഇനങ്ങളിലായിരുന്നു ഇത് കൂടുതൽ പ്രകടമായത്. പി. ഇൻഡിക്ക കോളനിവത്കരണത്താലുള്ള തണ്ടുകളുടെയും വേരുകളുടേയും അധികവളർച്ച, അധിക ജൈവാംശ ഉത്പാദനം, ബാഹ്യരൂപഘടനയിലെ മാറ്റങ്ങൾ എന്നിവ ഫംഗസ് മൂലമോ ചെടിയുടെ ഉത്തേജനം മൂലമോ വേരുകളിൽ ഉത്പാദിപ്പിക്കുന്ന ഓക്സിന്റെ അളവിലുള്ള വർദ്ധനവ് കാരണം ഉണ്ടാകുന്ന സസ്യ പ്രതികരണവുമായി ബന്ധപ്പെട്ടിരിക്കുന്നു.

ജലസമ്മർദ്ദത്തിനു വിധേയമായ പി. ഇൻഡിക്ക കോളനിവത്കരിച്ച Ptb 23, Ptb 29 സസ്യങ്ങളിൽ ഉയർന്ന ആപേക്ഷിക ജലാംശം

കണ്ടെത്തിയെങ്കിലും കോളനിവൽക്കരണത്തിന്റെ സ്വാധീനം കൂടുതൽ പ്രകടമായത് Ptb 23-ൽ ആയിരുന്നു. കോളനിവൽക്കരിക്കാത്ത സസ്യങ്ങളെ അപേക്ഷിച്ച് രണ്ട് ഇനങ്ങളുടെയും പി. ഇൻഡിക്ക കോളനിവൽക്കരിച്ച സസ്യങ്ങളിൽ പ്രത്യേക ഇലകളുടെ വിസ്തൃതിയിൽ ഗണ്യമായ വർദ്ധനവുണ്ടായി. നിലവിലെ പഠനത്തിൽ, Ptb 29-ന്റെ നെൽച്ചെടികൾക്ക് ജല സമ്മർദ്ദമുള്ള അവസ്ഥയിൽ Ptb 23-നേക്കാൾ ഉയർന്ന സെൽ മെംബ്രൺ സ്ഥിരത സൂചിക ഉണ്ടായിരുന്നു. പി. ഇൻഡിക്കയുടെ സാന്നിധ്യമുള്ള ചെടികളിൽ മലോൻഡിഎൽഡിഹൈഡിന്റെ അളവ് ഗണ്യമായി കുറഞ്ഞതായി കണ്ടെത്തി. ഇത് പി. ഇൻഡിക്ക വരൾച്ചയുടെ പ്രതികൂല പ്രത്യാഘാതങ്ങൾ കുറയ്ക്കുന്നു എന്ന് സൂചിപ്പിക്കുന്നു. Ptb 29-ലുള്ള Ptb 23-നേക്കാൾ കുറഞ്ഞ MDA യുടെ അളവ്, Ptb 29 സഹജ ജലസമ്മർദ്ദ സഹിഷ്ണുത ചൂണ്ടിക്കാട്ടുന്നു.

സ്റ്റോമാറ്റൽ കണ്ടക്ടൻസ്, ട്രാൻസ്പിറേഷൻ റേറ്റ്, ഫോട്ടോസിന്തറ്റിക് നിരക്ക് എന്നിവ പോലുള്ള ഗ്യാസ് എക്സ്ചേഞ്ച് പാരാമീറ്ററുകൾ പി. ഇൻഡിക്ക സാന്നിധ്യമില്ലാത്ത സസ്യങ്ങളെ അപേക്ഷിച്ച് കുമിൾ സാന്നിധ്യമുള്ള സസ്യങ്ങളിൽ വർധിച്ചു. Ptb 29-ന്റെ ജല സമ്മർദ്ദമുള്ള ചെടികളിൽ ക്ലോറോഫിൽ നിലനിർത്തുന്നത് Ptb 23-നേക്കാൾ വളരെ കൂടുതലായി കാണപ്പെട്ടു. പി. ഇൻഡിക്ക കോളനിവൽക്കരിച്ച ചെടികളിലെ സൂപ്പർഓക്സൈഡ് ഡിസ്മ്യൂട്ടേസ്, കാറ്റലേസ് തുടങ്ങിയ ആന്റിഓക്സിഡന്റ് എൻസൈമുകളുടെ വർദ്ധിച്ച പ്രവർത്തനം കോശങ്ങളിലെ ROS അളവ് കുറയ്ക്കുകയും അതുവഴി സെല്ലുലാർ ഘടകങ്ങളുടെ കേടുപാടുകൾ കുറയ്ക്കുകയോ തടയുകയോ ചെയ്യുന്നു. പി. ഇൻഡിക്ക കോളനിവത്കരിച്ച സസ്യങ്ങളിൽ കോളനിവൽക്കരിക്കാത്ത സസ്യങ്ങളെ അപേക്ഷിച്ച് ഇൻവെർട്ടസ്ന്റെ പ്രവർത്തനം കുറഞ്ഞു.

പി. ഇൻഡിക്ക കോളനിവൽക്കരണം ജലസമ്മർദ്ദമുള്ള ചെടികളിലെ ചിനപ്പുകളുടെ എണ്ണം ഉൽപാദനക്ഷമമായ ചിനപ്പുകളുടെ എണ്ണം

കതിരുകളുടെ നീളം എന്നിവ മെച്ചപ്പെടുത്തി. ഫങ്കസ് സാനിധ്യമില്ലാത്തതും ജലസമ്മർദ്ദമുള്ളതുമായ ചെടികളിൽ സ്പൈക്ക്ലെറ്റ് സ്റ്റെറിലൈറ്റി കൂടുതലായിരുന്നു. പി. ഇൻഡിക്ക കോളനിവത്കരണത്തെത്തുടർന്ന് Ptb 23-ൽ ധാന്യത്തിന്റെ തൂക്കത്തിൽ ഗണ്യമായ വർദ്ധനവുണ്ടായി. പി. ഇൻഡിക്ക-കോളനിവൽകൃത സസ്യങ്ങൾ ജലസമ്മർദ്ദമുള്ള സാഹചര്യങ്ങളിൽ കോളനിവൽക്കരിക്കാത്ത സസ്യ സെറ്റുകളേക്കാൾ കൂടുതൽ വിളവ് ഉത്പാദിപ്പിച്ചു. Ptb 29 കൂടുതൽ വിളവ് ഉത്പാദിപ്പിച്ചെങ്കിലും, പി. ഇൻഡിക്കയുടെ ഗുണം കൂടുതൽ ശ്രദ്ധേയവും ഫലപ്രദവുമായത് Ptb 23-ലാണ്. ഈ പഠനത്തിന്റെ ഫലങ്ങൾ കണക്കിലെടുക്കുമ്പോൾ, ജലസമ്മർദ്ദത്തിന്റെ ദൂഷ്യഫലങ്ങൾ ലഘൂകരിക്കാൻ പി. ഇൻഡിക്കയ്ക്ക് കഴിയുമെന്ന് ഊന്നിപ്പറയാം. എൻഡോഫൈറ്റുകൾ ഉപയോഗിച്ച് ജലസമ്മർദ്ദത്തിനെതിരെ നെൽച്ചെടികളുടെ യുക്തിസഹമായ മെച്ചപ്പെടുത്തലുമായി ബന്ധപ്പെട്ട ഭാവിയിലെ ഗവേഷണങ്ങൾക്ക് ഈ പഠനത്തിൽ നിന്ന് ലഭിച്ച കണ്ടെത്തലുകൾ അടിത്തറയായി ഉപയോഗിക്കാം.