MANAGEMENT OF WATER STRESS IN TOMATO (*Solanum lycopersicum* L.) THROUGH BENEFICIAL ROOT ENDOPHYTIC FUNGUS, *Piriformospora indica*

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by

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2023

DECLARATION

I, hereby declare that this thesis entitled **"MANAGEMENT OF WATER STRESS IN TOMATO (*Solanum lycopersicum* L.) THROUGH BENEFICIAL ROOT ENDOPHYTIC FUNGUS, *Piriformospora indica*"** is a bonafide record of research work done by me during the course of research and 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.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µg	Microgram
µL	Microlitre
µM	Micromolar
µm	Micromoles
^{0}C	Degree Celsius
AMF	Arbuscular mycorrhizal fungi
CD	Critical difference
CRD	Completely Randomized Design
cm	Centimeter
DAT	Days after transplanting
FC	Field capacity
Fig.	Figure
FW	Fresh weight
et al.	And other co-workers
g	Gram
g^{-1}	Per gram
i.e.,	That is
KAU	Kerala Agricultural University
Kg	Kilo gram
L	Litre
m	Meter
mg	milligram
MS medium	Murashige and Skoog medium
M	Molar
mM	Milli molar
ml	millilitre
mm	Milli meter
min.	Minute

No.	Number
NBT	Nitro blue tetrazolium
OD	Optical density
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PO	Peroxidase
PEG	Polyethylene glycol
CAT	Catalase
SOD	Super oxide dismutase
rpm	Rotations per minute
SE (m) ±	Standard error of mean
DNA	Deoxy-ribo nucleic acid
cDNA	Complementary DNA
RNA	Ribo nucleic acid
ROS	Reactive oxygen species
PCR	Polymerase chain reaction
RT-qPCR	Real-Time Quantitative Reverse Transcription PCR
RWC	Relative Water Content
CMS	Cell membrane stability
CSI	Chlorophyll stability index
ppm	Parts per million
viz.	Namely

INTRODUCTION

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a herbaceous edible fruiting plant belonging to the Solanaceae family. It is recognized as one of the most important vegetable crops worldwide, ranking second after potato in terms of consumption (FAOSTAT, 2005; Osei *et al.*, 2010). Tomato was originated and diversified in Peru and Mexico, where it was domesticated from its ancestor, *Solanum lycopersicum cerasiforme*. Today, tomato cultivation is widespread, with major production countries including China, India, USA, Italy, Turkey, and Egypt (Heuvelink *et al.*, 2020). India holds a prominent position in tomato production, ranking as the third-largest producer globally, contributing around 11.50% of the total production. With a cultivation area of 8.12 lakh hectares, India's tomato production is recorded at 205.73 lakh metric tons (NHB, 2020). However, tomato cultivation in Kerala faces challenges, resulting in sporadic production and lower productivity of 14.25 tons per hectare due to various biotic and abiotic stresses.

Tomatoes are not only widely consumed, but also recognized for their nutritional value. They are rich in health-promoting compounds, including vitamins (such as Vitamin C and E), antioxidants (like lycopene, β-carotene, lutein, and flavonoids), and minerals (Mortensen and Skibsted, 1997; Sie and Stahl, 1998). This nutritional composition contributes to the popularity of tomatoes as a versatile ingredient in various cuisines and processed forms such as paste, sauces, puree, ketchup, and canned tomatoes. Tomatoes play a crucial role in meeting the dietary needs of a growing global population. However, food production faces challenges due to climate change, including global warming and heat stress, which adversely affect crop production (IPCC, 2012).

Climate change-induced drought poses a significant challenge to food-producing regions, necessitating a 60% increase in crop productivity to meet the demands of a projected population of 9.6 billion by 2050 (Cabot *et al.*, 2014). To address this, the cultivation of drought-stressed marginal lands could be a viable option. However, drought stress affects different stages of plant growth, and commercial tomato cultivars are particularly sensitive to this stress, impacting seed germination, seedling emergence, vegetative growth, and reproduction (Zdravkovic

et al., 2013). Drought stress reduces plant tissue water content, leading to decreased water potential, photosynthesis rate, and alterations in protein synthesis, nitrogen metabolism, and cell membrane properties, ultimately hindering plant productivity (Saneoka *et al.*, 2004; Gupta *et al.*, 2014). Thus, understanding the effects of water stress on crops and implementing appropriate measures is crucial for sustainable food production under changing climatic conditions (Reynolds and Ortiz, 2010; Jefferies, 1994).

Water stress currently poses a significant threat to sustainable food production, leading to crop yield reductions up to 70 percent. Among abiotic stresses, drought is the most destructive due to its complex nature (Gosal *et al.*, 2009). To combat stresses such as drought, plants have developed diverse approaches such as stress escape, stress avoidance, and stress tolerance (Sourour *et al.*, 2017). These approaches involve intricate mechanisms, including modulation of transcriptional and expressible genes, epigenetic plasticity, metabolic reprogramming, and other phenomena that are being constantly studied and evaluated at the molecular level (Miao *et al.*, 2017). Moreover, at the cellular level, the remarkable synergistic relationship between endophytic microorganisms and their contribution to the drought tolerance capacity of agricultural crop plants has elevated the scientific understanding of microbial benefaction and host interaction to the next level (Govindasamy *et al.*, 2017).

Microorganisms that promote plant growth, such as plant growth-promoting bacteria (PGPB), rhizobia, and arbuscular mycorrhizal fungi (AMF), are classified as beneficial microbes found free-living in soils, rhizosphere/rhizoplane (e.g., rhizobacteria and ectomycorrhizal fungi), or inside plant tissues (e.g., endophytic bacteria, endomycorrhizal fungi, and AMF) (Ma *et al.*, 2011). Naturally, there exists a diverse range of endophytic microbes that play a significant role in promoting plant growth and development by forming a symbiotic relationship. These microbes induce the up-regulation of essential genes in various plant species, particularly in response to abiotic stress conditions like drought (Ngumbi and Kloepper, 2016). An exemplary organism in this regard is *Piriformospora indica*, an Agricomycetes fungus that exhibits mycorrhiza-like characteristics and is

capable of colonizing the roots of various plant species and establishing symbiotic relationships. It triggers the expression of numerous genes associated with drought stress in leaves by modulating phytohormonal signal transduction pathways (Sherameti *et al.*, 2008).

Piriformospora indica, is not specific to any host and is widely distributed. Additionally, it can be cultivated axenically and promotes plant growth, especially in nutrient-deficient soils, while also providing tolerance to various abiotic and biotic stresses, as stated by Varma *et al.* (1999). Although tomato is a warm season crop, it is susceptible to irrigation-related issues and requires frequent watering. Drought is a significant abiotic stress that can limit tomato growth and yield. However, research on the potential of utilizing *Piriformospora indica* in tomato cultivation remains limited.

A number of studies have been conducted to understand the effect of water stress in tomato and various means of water stress simulations have been adapted. The most common means to induce water stress in plants involve the gravimetric method (Earl, 2003), application of PEG (Kulkarni and Deshpande, 2007), mannitol (Raheem *et al.*,2007), ABA (Bray, 2002) and by submerging with water. Hence the study was undertaken with the following objectives,

- To investigate the ability of *Piriformospora indica*, a beneficial root-endophytic fungus, to induce tolerance to water stress in tomato.
- To study the underlying physiological and molecular mechanisms of stress tolerance in tomato colonized by *Piriformospora indica*.

2. REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum*) is a highly popular vegetable crop cultivated extensively across the globe. It holds the distinction of being the third most widely grown and second most consumed vegetable crop worldwide, trailing only behind potato and sweet potato. Additionally, when it comes to crops used for

processing purposes, tomatoes occupy the top position among all vegetables (Agrisnet, 2010). The tomato is also a widely cultivated vegetable crop in both temperate and tropical regions of India (Singh *et al.*, 2010; Nahar and Ullah, 2011). It is valued for its abundant minerals, vitamins, and organic acids content. Researchers continue to favor tomatoes due to their short growth period, ease of cultivation, high seed yield per fruit, convenience for hybridization and cytology studies, and sustained consumer demand. Tomatoes play a vital role in human diets, consisting of approximately 94% water, 2.5% total sugars, 2% total fiber, 1% proteins, and various other nutritional compounds such as acids, lipids, amino acids, and carotenoids (Koh *et al.*, 2012). The fresh fruits of tomatoes are consistently in high demand across the country throughout the year. They are considered a valuable source of essential nutrients including potassium, folate, vitamin E, soluble and insoluble dietary fibers, as well as being particularly rich in lycopene and ascorbic acid (Kaur and Kapoor, 2008).

Vegetable crops are highly susceptible to abiotic stresses, which can significantly impact their yield. Studies have shown that various abiotic stresses contribute to approximately 50% loss in crop production (Bray *et al.*, 2011). However, susceptibility of tomato to water deficit has led to extensive efforts in developing drought-resistant tomato varieties.

Plant-microorganism interactions are crucial for ecosystem functions (Cheng *et al.*, 2019). Plants host microbial communities in their root systems (Friesen *et al.*, 2011), influencing their habitat. These plant-associated microbes, particularly plant growth-promoting microorganisms (PGPM), significantly impact plant growth, nutrition, development, and health (Pascale *et al.*, 2020), benefiting agriculture (Ray *et al.*, 2020). In mutualistic symbiosis, both hosts and microbes are benefited. Arbuscular mycorrhizal fungi (AMF) are known to establish mutually beneficial relationships with about 80% of terrestrial plants (Bahadur *et al.*, 2019). This enhances plant growth, nutrient absorption, and resistance to environmental stress, with the host providing carbon (Bahadur *et al.*, 2019). *P. indica* is a cultivable endophyte that colonizes on roots of plantae (Varma *et al.*, 1999). The fungus can colonize roots of a wide range of higher plants and provide plants

multifaceted amenities such as nutrient uptake, disease resistance, stress tolerance and growth promotion involving value addition (Unnikumar *et al.,* 2013; Kord *et al.,* 2019; Mensah *et al.,* 2020). Hence, the primary aim of this study was to assess the effectiveness of *P. indica* in enhancing the growth and drought tolerance of tomato plants. The research program conducted a concise review of the work accomplished in the field of tomatoes, focusing on the objectives outlined. The review encompassed the following aspects:

2.1. Impacts of water stress on tomato

2.2. Effect of *P. indica* on plant growth and its role in enhancing drought tolerance

2.1. IMPACTS OF WATER STRESS ON TOMATO

Drought stress represents a prevalent environmental challenge with profound implications for plant growth and agricultural productivity. This phenomenon arises from an inadequate supply of water essential for the normal development and life cycles of plants. In soil, water scarcity disrupts critical physiological processes, leading to shifts in cellular water balance, reductions in plant water potential, and turgor pressure alterations (Conti *et al.,* 2019; Lisar *et al.,* 2012). This environmental stressor exerts a significant impact on species distribution, covering approximately 40% of the Earth's land surface, and affecting the livelihoods of over a billion people in these regions (Roy *et al.,* 2009). It's noteworthy that nearly 45% of the world's agricultural lands contend with drought conditions, making it a substantial constraint in the cultivation of crops like tomatoes (Bot *et al.,* 2000). The impacts of drought stress on different facets of tomato cultivation are outlined as follows:

2.1.1. Effects of water stress on vegetative and flowering characters of tomato

Drought stress is a complex combination of various stress factors, which has a multifaceted impact on the growth and development of plants (Sakya *et al.,* 2018; Zlatev and Lidon, 2012). It leads to the inhibition of processes such as cell division and enlargement, resulting in reduced vegetative and reproductive growth. Water deficiency caused by drought stress leads to a decrease in the number of flowers, subsequently reducing the number of fruits, ultimately resulting in a diminished

yield suitable for the market (Buhroy *et al.*, 2017; Losada and Rincaon, 1994; Colla *et al.*, 1999; Rahman *et al.*, 1999; Veit-Kohler *et al.*, 1999).

Crops like tomatoes are primarily grown in semi-arid regions such as the Mediterranean, where they heavily rely on irrigation. This reliance on irrigation is crucial because semi-arid areas are expected to experience more frequent drought events due to climate change (Nankishore and Farrell, 2016). Consequently, water scarcity resulting from drought periods can significantly impact tomato production, potentially causing yield reductions of up to 50% when irrigation is equivalently reduced (Cantore *et al.*, 2016). According to Doorenbos and Kassam (1979), tomato plants have their highest water demand during the flowering stage, with ripening being particularly susceptible to irregular irrigation, leading to fruit cracking (Losada and Rincaon, 1994).

Wahb-Allah *et al.* (2011) conducted an experiment at the Dirab Agricultural Research and Experimental Station of the Faculty of Food and Agriculture Sciences, King Saud University, Riyadh, to assess the impact of drought on tomato production. In this study, four commercial tomato cultivars (Imperial, Pakmore VF, Strain-B, and Tnshet Star) were examined. The results indicated that subjecting tomato plants to varying levels of drought stress significantly influenced their growth and development. Increasing water stress levels were found to progressively reduce plant height, primary branches, flower clusters per plant, fruits per cluster, the number of fruits, and total yield per plant. Additionally, individual fruit weight decreased, while the amino acid content in leaves increased with greater drought stress, and total sugar and reducing sugar content in leaves also increased.

In research conducted by Khan and colleagues in 2020, the focus was on investigating how chitosan impacted the growth and yield of tomato plants, specifically the 'Cv. Rio Grande' variety, when exposed to water stress conditions. The outcomes of their investigation shed light on the relationship between water stress duration and tomato plant height. Notably, the tallest tomato plants, with an average height of 82.69 cm, were observed in those subjected to a 6-day water stress interval. This finding was statistically comparable to the plant height, which

averaged 81.18 cm, for the group exposed to a 3-day water stress interval. In contrast, the shortest tomato plants, measuring an average height of 65.93 cm,

Rao *et al.* (2000) conducted an experiment in which four tomato varieties were subjected to three different levels of water stress. Their findings revealed a reduction in the number of branches per tomato plant as the water stress conditions intensified.

Conti *et al.* (2019) conducted an investigation aimed at quantifying the impact of prolonged drought stress on the growth of tomato stem diameter. Their study delved into the responses of six different Italian tomato varieties when subjected to an extended period of water scarcity. The experiment included three key time points: t_0 (prior to initiating the stress treatment), t_1 (midway through the stress duration), and t_2 (at the conclusion of the stress period). The findings of their research unveiled that, at t_2, all tomato varieties displayed a slight decrease in stem diameter, irrespective of whether they were well-irrigated or subjected to stress. Remarkably, there was no significant difference in stem diameter between the well-irrigated and stressed varieties.

In a separate study, Sibomana *et al.* (2013), sought to quantify the consequences of water stress on the growth and yield of tomatoes. This investigation took place at Egerton University, specifically at the Horticultural Research and Teaching Field. The selected tomato variety was "Money Maker," and the experiment involved subjecting the plants to four distinct soil moisture threshold levels: 100% PC (Potential Crop), 80% PC, 60% PC, and 40% PC. The experimental design employed a randomized complete block design with four replications. The results reported by Sibomana and coworkers indicated that plants receiving more than 60% PC exhibited elongated internodes, resulting in longer stems, with an increase of up to 36% when compared to those subjected to 40% PC moisture levels. Additionally, severe water stress, represented by the 40% PC threshold, caused a notable reduction in plant stem diameter by 18% when compared to the control group receiving 100% PC moisture levels.

The leaf serves as a crucial organ within the plant, performing essential roles such as controlling respiration and participating in the production of organic

substances that facilitate plant development and nourishment. The way tomato leaves are structured is greatly influenced by the plant's reaction to environmental factors, particularly water scarcity, as highlighted by Anjum *et al.* in 2011.Studies, such as the one by Medyouni *et al.* (2021), have documented a decrease in leaf length, leaf width and thus leaf area as a consequence of water deficit in tomato plants.

An early shift from the vegetative phase to the reproductive phase is a mechanism that enables plants to reproduce prior to the onset of severe water scarcity, which could threaten their survival. This approach, referred to as the drought escape strategy, has been researched and recorded by Ludlow (1989), Sherrard and Maherali (2006), as well as Franks (2011). Drought conditions have a notable impact on the timing of flowering in all tomato varieties. The period of flowering in tomatoes is particularly sensitive to the presence of drought stress, as highlighted in studies by Zinselmeier *et al.* (1999, 1995) and Samarah *et al.* (2009). Consequently, drought conditions lead to a significant alteration in the timing of flowering across various tomato varieties. Furthermore, the research conducted by Ram and Rao (1984) emphasized that drought stress not only significantly disrupts the flowering period but also affects nectar production, the mode of flower opening, and the maintenance of turgor in floral organs.

In another study by Sivakumar and Srividhya (2016), an experiment was undertaken to investigate the impact of drought on the flowering and yield of tomato genotypes. Their findings revealed that plants subjected to drought conditions initiated flowering earlier, with a gap of 26 days, compared to plants in control conditions that initiated flowering after 30 days. Generally, drought stress prompts early flowering, with flower initiation occurring three days earlier than in the control group. This early onset of flowering under drought conditions is likely an adaptive response to accelerate phenological development in order to complete the life cycle in an unfavorable environmental context.

Similarly, Akter *et al.* (2019) noted that the time taken for the first flowering event varied among different treatments. In their study, the plants in T2 (with 30 days of water withholding) exhibited the earliest flowering, with an average of

26.69 days, while those in T3 (with 45 days of water withholding) had a slightly later onset of flowering, at an average of 27.18 days. The control group, represented by T1, had an intermediate flowering time, occurring at around 26.89 days, which was slightly earlier than T3 but later than T2.

Buhroy *et al.* (2017) carried out a study involving thirty-two different tomato genotypes, subjecting them to drought stress conditions. Their research revealed that as the level of water deficit increased, there was a corresponding decrease in the number of clusters per plant.

According to Bhatt *et al.* (2009), susceptible tomato cultivars exhibited higher rates of flower and flower bud abscission, as well as reduced photosynthesis compared to tolerant cultivars.

Akter *et al.* (2019) reported that in their experiment, the highest number of clusters per plant, totaling 9.240 clusters per plant, was observed in the control group represented by T_1. Conversely, the lowest number of clusters per plant, totaling 7.730 clusters per plant, was recorded in T_3, where the plants experienced 45 days of drought stress. The adverse impact of drought stress on the quantity of flower clusters in tomato plants was also documented by Jangid and Dwivedi (2017). Additionally, in 2001, Sorial conducted a study involving three distinct tomato genotypes to evaluate how they performed when exposed to different degrees of water stress, specifically at levels of 100%, 50%, and 25% Field Capacity (FC.). The findings revealed that as the severity of water stress increased, there was a corresponding reduction in the number of flower clusters per plant.

Akter *et al.* (2019) evaluated the performance of 15 distinct tomato genotypes when subjected to three distinct drought treatments. Their findings indicated that the timing of fruit harvest was notably influenced by these drought treatments. In particular, tomatoes subjected to treatment T_3, which involved withholding water for 45 days, exhibited early maturation and thus were harvested sooner. Conversely, in the control group represented by T_1, fruit maturity was delayed, resulting in a longer time required for harvest. Consequently, it became evident that as the severity of drought increased, the time required for tomato plants to reach maturity decreased.

2.1.2. Impacts of water stress on tomato fruit and yield parameters

Drought stress has a detrimental impact on the productivity of agricultural crops in general. Water deficit during the flowering stage of tomato plants has been found to increase flower abortion and result in a decrease in yield due to a reduction in the number of fruits produced. Drought stress throughout the growing season commonly leads to decreased yield, primarily attributed to reductions in both fruit weight and number (Nuruddin *et al.*, 2003). The extent of yield decrease varies widely, ranging from 30% to over 80%, depending on factors such as the intensity and duration of stress exposure, growth stage of the plant, and genotype (Patanè *et al.*, 2013; Cantore *et al.*, 2016). In indeterminate tomato plants, where flowering and fruit set occur continuously, it is challenging to avoid stress during these critical stages (Pulupo *et al.*, 1996). A study observed the highest percentage of flower abortion (22%) in the most severely stressed plants (exposed to 40% of the plant's water capacity) occurring in the 3rd and 4th trusses (Sibomana *et al.*, 2013). The number of flower buds failing to develop into fruits increased with decreasing water levels, resulting in a substantial 69% reduction in yield for the most stressed plants.

Drought stress has a substantial impact on tomato plants, diminishing their vitality and overall productivity. In one study led by Sakya *et al.* (2018), seven tomato varieties were subjected to drought conditions with 8-day intervals between watering. This investigation revealed a notable reduction in tomato fruit weight, ranging from 3% to as much as 148%, when compared to normal growing conditions.

In a separate research effort conducted by Cui *et al.* (2020) in China during the 2013 and 2016 growing seasons, the effects of drought on tomatoes were explored. Different drought treatments were applied at various growth stages, resulting in a reduction in total fruit yield by 11% and 21% for the T3 and T4 treatments in 2013, and by 15% and 30% in 2016. This decline was primarily attributed to diminished fruit weight in response to drought conditions.

Giuliani *et al.* (2018) investigated the combined influence of deficit irrigation and strobilurin treatment on two tomato varieties, IT-22/025 and Ikram. While IT-22/025 exhibited higher total fruit yield under specific irrigation regimes,

both varieties experienced yield decreases. IT-22/025 saw a reduction of 43%, and Ikram exhibited a 51% decrease, primarily due to smaller fruit size when exposed to drought conditions.

Rahman *et al.* (1998) reported a decrease in fruit weight in tomatoes when subjected to increased water stress conditions. Weerasinghe *et al.* (2003) found that drought stress led to a reduction in tomato yield by decreasing the number of fruits per plant, a pattern similar to observations made by Ball *et al.* (1994) in cotton experiments.

Furthermore, Rahman *et al.* (1999) conducted experiments using drought-tolerant and drought-sensitive tomato varieties. They observed a decrease in fruit weight per plant in both varieties, with drought-sensitive ones experiencing a more substantial reduction. This decline in yield was attributed to fewer fruit clusters, diminished fruit numbers, and smaller fruit size. Similar outcomes were reported in experiments conducted by various researchers, including Thippeswami and Sreenivasa (1998), Rao *et al.* (2000), Lutfor-Rahman *et al.* (2000), and Nuruddin *et al.* (2003) in tomatoes.

Weerasinghe *et al.* (2003) carried out a study involving 45 tomato varieties in Sri Lanka, examining their performance under both regular and drought-stressed conditions. Their findings indicated that the number of fruits per tomato plant decreased when subjected to drought stress in comparison to normal conditions. Similarly, Nuruddin *et al.* (2003) conducted an experiment involving tomatoes exposed to two different levels of water deficit (65% and 80%). They observed that as the severity of water stress increased, both the quantity and size of fruits declined. Akter *et al.* (2019) noted that the highest number of fruits per cluster (3.33 per plant) was observed in T1 (control), while the lowest number of fruits per cluster (2.66 per plant) was recorded in T3 (withholding water for 45 days). In 2015, Khan *et al.* conducted an investigation and their findings indicated that when exposed to water stress conditions, the total fruit yield per plant decreased. The greatest number of fruits per plant (24.66) was observed among plants subjected to a 6-day water stress interval, while the fewest fruits per plant (15.33) were documented in plants that endured a 12-day water stress interval.

Ezin *et al.* (2010) conducted a study where they observed that subjecting tomato plants to persistent flooding over durations of two, four, and eight days led to a significant decrease in the total fruit weight when compared to a control group that wasn't subjected to flooding.

Alomari-Mheidat *et al.* (2023) noted a significant disparity in fruit dimensions between the control cohort and the water-stressed group. The fruits in the control set displayed notably larger sizes, approximately three times larger, to be precise. Similar results were obtained by Medyouni *et al.* (2021)

2.1.3. Impact of water stress on the qualitative characteristics of tomatoes

In a field experiment conducted by Nahar and Ullah (2018) on loam soil in Bangladesh, the impact of drought stress on fruit quality and osmotic adjustment in four tomato cultivars was investigated. The study revealed that, under stress conditions, the quality of the fruits improved, attributed to the synthesis of various acids such as ascorbic acid, citric acid, and malic acid. As the water deficit in the plants increased, the concentration of citric acid, malic acid, and ascorbic acid also increased. Similarly, Abdelgawad *et al.* (2019) conducted a study comparing tomato lines with different levels of ascorbate oxidase activity, including lines with reduced activity, lines with elevated activity, and a non-transgenic line (WVa106). They observed a significant correlation between the content of Vitamin C and plant growth and yield. By manipulating the ascorbate oxidase gene, the study suggested the possibility of developing cherry tomato lines that can thrive under salinity conditions, indicating the potential usefulness of this genetic manipulation approach.

In another study conducted by Hao *et al.* (2019), the effects of water stress on tomato quality and yield were investigated at three different growth stages. The researchers examined the impact of both mild and moderate water stress on different bunches of tomatoes. They discovered that while water stress played a significant role in improving fruit quality, it also resulted in a decrease in fruit yield.

Furthermore, they observed that the concentration of vitamin C was enhanced under water stress conditions compared to the control group.

Prashanth (2003) conducted a study to analyze various quality parameters of different tomato genotypes. The research revealed that the total soluble solids ranged from 3.19^{o}Brix to 5.83^{o}Brix. In a field experiment conducted by Birhanu and Tilahun (2010), the effects of moisture stress on yield and quality were examined using two tomato cultivars: Melka Shola and Melkassa Marglobe, which are commonly used for salads. The cultivars were subjected to four levels of irrigation water deficit: 0%ETc, 25%ETc, 50%ETc, and 75%ETc deficit. It was observed that as the stress level increased, the total soluble content increased, while the fruit water content decreased. Agbemafle *et al.* (2014) investigated the effects of deficit irrigation and postharvest storage on the physicochemical qualities of tomatoes. Tomato fruits (Pectomech variety) cultivated under different irrigation treatments (100% ETc, 90% ETc, 80% ETc, and 70% ETc) were analyzed for total soluble solids (TSS). The results indicated that total soluble solids increased with increasing deficit irrigation.

Hao *et al.* (2019) studied the effects of tomato quality and yield under mild and moderate water stress at different growth stages. The research revealed that total soluble solids improved during water stress compared to the control. Basit *et al.* (2020) evaluated the impact of pre-harvest foliar application of chitosan on the quality indices of tomato plants under different water stress intervals (3, 6, 9, and 12 days) after 15 days of transplantation. It was found that tomato plants treated with a 6-day water stress interval exhibited the highest total soluble solids.

Prashanth (2003) conducted a study on different tomato genotypes to analyze various quality parameters, including total soluble solids, total titratable acidity, pH, ascorbic acid, and lycopene content. The research findings showed that the total titratable acidity ranged from 0.21% to 0.70%. Amor and Amor (2007) compared the yield and fruit quality of processing tomatoes under surface and subsurface drip irrigation with 100% and 50% of crop evapotranspiration (ETc). The study revealed that the water-stressed treatment exhibited an increased pH and acidity of the fruits. Turhan *et al.* (2009) conducted a comparison of quality

characteristics among 33 tomato genotypes. The analysis included measurements of dry matter weight, sugar content, soluble solid content, titratable acids, and pH. The study found that the titratable acidity (TA) content of tomato fruit ranged from 0.22% to 0.40%. Genotypes 40443 and 62573 exhibited high values of titratable acids. Aoun *et al.* (2013) evaluated 13 traditional varieties of tomatoes collected from various locations in Tunisia. The variety IRA 9 showed a higher value in titratable acidity (9.05 g/L citric acid). Teka (2013) investigated the effect of maturity stage on post-harvest quality characteristics of tomatoes. The study indicated that the maturity stage at harvest significantly influenced the quality attributes of tomato fruit. The highest value of titratable acidity (3.98%) was recorded in fully ripe and mature green stages.

Agbemafle *et al.* (2014) examined the effects of deficit irrigation and postharvest storage on physicochemical qualities of 'Pectomech' tomatoes. The tomatoes were cultivated under different irrigation treatments (100% ETc, 90% ETc, 80% ETc, and 70% ETc). The results demonstrated that titratable acidity increased with increasing deficit irrigation. The percentage increases in titratable acidity compared to the control (100% ETc) treatment were 8.6%, 11.8%, and 14.0% for the 90% ETc, 80% ETc, and 70% ETc treatments, respectively. This indicated that tomatoes from the 70% ETc treatment had higher acid content. Basit *et al.* (2020) evaluated the effect of pre-harvest foliar application of chitosan on quality indices of tomato plants under different water stress intervals (3, 6, 9, and 12 days) after 15 days of transplantation. The study found that the maximum titratable acidity (0.496%) was observed in fruit plants treated with a 6-day water stress interval, while the minimum titratable acidity (0.415%) was recorded in fruit plants treated with a 3-day water stress interval.

Carotenoids, such as lycopene, are vital pigments present in plant photosynthetic pigment-protein complexes. They play a significant role in the vibrant colors of fruits and vegetables and serve various functions in photosynthesis. In a study conducted by Giannakoula and Ilias (2013), moderate salt stress was applied to tomato plants, resulting in enhanced lycopene levels and potentially increased concentrations of other antioxidants in the fruits. The response

of lycopene to salt stress varied between 20% and 80% in tomato fruits. Klunklin and Savage (2017) cultivated four tomato cultivars (Incas, Marmande, Scoresby Dwarf, and Window Box Red) in a greenhouse under well-watered and drought stress conditions. They observed significant differences in lycopene contents among the four cultivars when comparing well-watered conditions to drought stress. The mean lycopene levels in the drought-stressed fruits were 22.8 mg lycopene/kg DM, which was significantly higher than the well-watered tomatoes. Among the four cultivars, Window Box Red exhibited the highest lycopene content.

In a study by Randome *et al.* (2017), multiple stresses including salt, mannitol, drought, and methyl jasmonate were applied to tomato plants to assess their impact on fruit quality, specifically lycopene, beta-carotene, sucrose, and total phenolics. The researchers found that tomato plants subjected to salt stress exhibited the highest increase in lycopene content (2.8 times), while the increase for other stresses ranged from 1.1 to 1.2 times. Kareem and Karrar (2018) utilized high-performance liquid chromatography (HPLC) to measure lycopene content. Their results indicated a significant increase in lycopene production in callus subjected to drought stress compared to the content in fruits of the mother plant, demonstrating the superiority of lycopene content in drought-stressed callus. Takacs *et al.* (2020) investigated the impact of different water supply levels on both yield quantity and quality, with a focus on lycopene components. Water was supplied at 100%, 75%, and 50% of the crop evapotranspiration (ETc) levels. The results suggested that supplying water at 75% of ETc until the onset of ripening provided a balanced water supply level in terms of both yield quantity and lycopene concentration.

2.1.4. Effects water stress on physiological parameters in tomato

Relative water content (RWC) plays a crucial role in assessing water status under drought conditions. RWC is directly linked to cell volume and its relationship with transpiration and water supply to the plants (Schonfeld *et al.*, 1988). RWC is known to protect plant growth and yield characteristics from the negative effects of drought stress (Lilley and Ludlow, 1996). Garcia *et al.* (2007) subjected tomato plants to long-term, moderate, and progressive water stress, resulting in a decline

in the relative water content of the plant body due to water scarcity during drought. Hayat *et al.* (2008) imposed water stress on tomato plants by withholding water for 10 days at 20 and 30 days after sowing. They observed that water stress significantly reduced the relative water content compared to the control group.

Sibomana *et al.* (2013) investigated the impact of moisture deficit on tomato growth and yield by exposing the plants to different soil moisture levels. They found that the leaf relative water content in the most stressed plants decreased by 24.70% compared to the control group. Khan *et al.* (2015) studied the effects of drought stress on tomatoes under controlled and drought conditions. They observed a decline in the relative water content of the plant body during drought due to limited water availability. In the controlled environment, the mean relative water content was 89.28%, while under drought conditions, it was 87.73%. Zhou *et al.* (2017) conducted a study using a common greenhouse tomato cultivar 'Arvento' and two heat-tolerant tomatoes, 'LA1994' and 'LA2093'. Drought stress was induced by restricting irrigation. The study revealed that the relative water content of all cultivars significantly decreased under drought compared to the control. Hassnain *et al.* (2020) reported variations in relative water content (RWC) in tomato leaves based on different water stress intervals. The highest RWC (67.27%) was observed in plants subjected to a 6-day water stress interval, statistically different from the RWC (65.49%) observed in plants with a 3-day water stress interval. Conversely, the lowest RWC (41.50%) was recorded in tomato leaves of plants treated with a 12-day water stress interval.

A decrease in water availability can lead to an increase in proline levels in plants. This is because drought conditions stimulate the activity of Ornithine Amino-transferase (OAT), which is responsible for proline synthesis. As a result, proline accumulates in a more concentrated form compared to water. Drought also causes a reduction in plant fresh weight, further contributing to the accumulation of proline. According to Claussen (2005), the concentration of proline in tomato leaves was higher during the summer when plants experienced stress-induced differences in yield compared to the late season. The proline content in tomato leaves varied depending on nutrient concentration and total radiation, and it was closely related

to the relative water content of the leaves. The study concluded that proline can serve as a reliable indicator of environmental stress in plants, allowing for the establishment of stress thresholds for fruit yield and quality in hydroponically grown tomatoes.

In a study conducted by Khan *et al.* (2015) on the effects of drought stress on tomatoes, plants grown under controlled conditions and drought conditions in a greenhouse were compared. Proline levels were observed to increase as water quantity in the cell sap continuously decreased. In controlled conditions, the proline content was measured at 4.4 μmoles g^{-1} fresh weight, whereas plants under drought conditions had a proline content of 5.8 μmoles g^{-1} fresh weight. Sakya *et al.* (2018) investigated the relationship between physiological characteristics and tomato yield under drought stress using seven lowland tomato cultivars. Drought stress was applied through an 8-day watering interval. The proline content varied significantly among the seven cultivars under drought conditions, ranging from 5 to 16 μg g^{-1} fresh weight. The 'Ratna' cultivar showed an increase in proline content compared to the others, indicating its attempt to survive under drought conditions. In a field experiment by Kahlaoui *et al.* (2019), saline water with a conductivity of 6.57 dS m^{-1} and subsurface drip irrigation (SDI) were used on two tomato cultivars, Rio Grande and Heinz-2274, in a clay soil with high salinity. Proline was applied exogenously through foliar spray at two different concentrations. The study concluded that a low concentration of foliar-applied proline can enhance the tolerance of both tomato cultivars to salinity under field conditions.

2.1.5. Gene expression during water stress conditions

The response of plant genes to abiotic stresses such as drought, high salinity, heat, and cold plays a crucial role in stress response and tolerance. Drought stress triggers various cellular processes, including the inhibition of photosynthesis, the accumulation of reactive oxygen species that can damage cells, and the reprogramming of gene expression. Transcription factors (TFs) are key regulators of this transcriptional reprogramming, and the expression of many TF genes is influenced by drought. In a study conducted by Andrew *et al.* (2000), the researchers used LeZEP1 and LeNCED1 as probes to investigate gene expression

in leaves and roots of whole plants subjected to drought treatments, both during light/dark cycles and during dehydration of detached leaves. Their findings revealed that, under drought stress, the expression of NCED mRNA increased in both leaves and roots, while the expression of ZEP mRNA increased in roots but not in leaves.

Islam and Wang (2009) conducted a study to examine the expression pattern of dehydration-responsive element-binding protein-3 (*LeDREB3*) in tomato under various abiotic stresses. They performed organ-specific expression profiling and found that *LeDREB3* showed constitutive expression in all tested organs, with particularly strong expression in flowers. The researchers observed that the expression of *LeDREB3* was significantly induced by NaCl, drought, low temperature, and H_2O_2. Based on their findings, they proposed that the *LeDREB3* gene plays a role in the tomato plant's response to stress.

In another study by Zhang *et al.* (2011), it was discovered that over expression of miR169c can enhance plant drought tolerance. The researchers identified four potential target genes, including *SIMRP1*, which may represent a new target gene regulated by miR169. They observed that drought stress significantly down regulated the expression of *SlMRP1*. Quantitative RT-PCR analysis revealed that *SlNF-YA3* and *SlMRP1* were highly expressed in mature leaves and flowers of tomato plants. The researchers concluded that manipulating miRNAs such as miR169 to regulate genes involved in drought stress responses could potentially improve crop water-use efficiency.

Loukehaich *et al.* (2012) utilized RT-qPCR to investigate the expression profile of the drought-responsive USP gene, *SpUSP*, in a wild relative of tomato called *S. pennellii* LA716 and the cultivated tomato M82. They found that *SpUSP* exhibited high expression in leaves but low expression in roots, although its expression was detected in all tested organs. The stem of LA716 showed relatively higher expression levels of *SpUSP* compared to M82 and other tissues. Additionally, the expression of *SpUSP* peaked in the afternoon.

Gonzalez *et al.* (2013) conducted a study focusing on the epigenetic marks present in the root, an essential organ involved in sensing drought stress. Using tomato as a model plant, they specifically examined the methylated epialleles of the

Asr2 gene, which is widely found in various plant species. By performing qRT-PCR analysis under both normal and stress conditions, they observed a slight increase in *Asr2* mRNA levels as early as 10 minutes after water stress, with even higher expression at 30 minutes of stress.

In a study by Gujjar *et al.* (2014), the researchers investigated the expression of eight genes that showed significant differences when exposed to artificial drought stress in two tomato genotypes. They conducted both semi-quantitative and quantitative expression analyses of these genes under the imposed drought stress. The results confirmed that *SlPRP16, SlCYP51-17, SlMCPI19*, and *SlGDSL20* were down regulated in both genotypes, with a more pronounced down regulation in the sensitive line. *SlWRKY4* was down regulated in both lines, but with a greater fold of down regulation in the tolerant line. *SlEFH12* and *SlSNF4-15* were up regulated in the tolerant line, while *SlUSPA9* was up regulated in both lines, with a relatively higher fold of up regulation in the sensitive line.

Jiang *et al.* (2016) identified and isolated a novel transcription factor called *SlDREB1* from tomato using the yeast-one-hybrid system. They transferred the *SlDREB1* gene into Arabidopsis plants and functionally characterized it through molecular detection in vitro and drought stress experiments. Their findings revealed that the accumulation of *SlDREB1* mRNA was higher in the roots of tomato plants compared to the shoots and was strongly induced by drought, salt, or exogenous abscisic acid. The transgenic Arabidopsis plants showed significant up regulation of both *SlDREB1* and *ERD15* mRNA in response to drought stress.

In a study, researchers investigated two important transcription factors, *SlAREB1 and SlAREB2*, in cultivated tomatoes. These factors are associated with responses to abscisic acid (ABA) and various environmental stresses. The study found that both *SlAREB1* and *SlAREB2* are induced by drought and salinity in both leaves and roots, with *SlAREB1* being more responsive to stress. When *SlAREB1* was overexpressed in tomato plants, they showed increased tolerance to salt and water stress compared to non-modified plants. This enhanced tolerance was evidenced by improved physiological parameters such as relative water content and reduced damage from stress. Microarray and AFLP analyses revealed that *SlAREB1*

overexpression led to the up-regulation of genes related to oxidative stress, lipid transfer, transcription regulation, and defense against pathogens. This suggests that *SlAREB1* plays a vital role in ABA-mediated responses to abiotic stress and potentially in defense against biotic stress as well (Orellana, 2010).

Mishra *et al.* (2016) conducted an experiment to evaluate the expression profiling of tomato plants under water deficit conditions using microarray technology. Through the annotation of Affymetrix genome microarray data, they identified transcription factor (TF) genes that were differentially induced or repressed after drought stress in the CO-3 and EC-520061 genotypes, with a fold change (FC) greater than 2.0. Bai *et al.* (2018) conducted a comprehensive review focusing on the functions of *WRKY* genes in tomatoes and their homologs in other plant species, including Arabidopsis and rice. Their review particularly emphasized the roles of these genes in responding to both abiotic and biotic stresses. They found that the expression of several *SlWRKY* genes in tomato, as well as their counterparts in Arabidopsis and rice, showed significant changes under various stress conditions.

In a study by Karkute *et al.* (2018), all the *WRKY* genes in tomato were systematically classified. The researchers performed qPCR expression analysis on a selected set of 62 *WRKY* genes under drought stress conditions. The expression profiles revealed notable up-regulation of nine major *WRKY* genes in tomato. Among them, *SlWRKY58* exhibited a drastic up-regulation of 125-fold, while *SlWRKY72* showed a 36-fold up-regulation. These findings highlight *SlWRKY58* and *SlWRKY72* as potential targets for genetic manipulation to enhance drought tolerance in tomato. Thirumalaikumar *et al.* (2018) identified the NAC factor JUNGBRUNNEN1 (JUB1) as a key regulator of drought tolerance in tomato. Through their research, they observed that inhibiting SlJUB1 through virus-induced gene silencing significantly reduced drought tolerance in tomato plants. This was accompanied by increased ion leakage, elevated levels of hydrogen peroxide (H_2O_2), and decreased expression of various drought-responsive genes. These findings suggest that SlJUB1 plays a crucial role in enhancing drought tolerance in tomatoes.

2.2. EFFECTS OF *P. INDICA* ON PLANT GROWTH AND ITS ROLE IN ENHANCING DROUGHT TOLERANCE

2.2.1. Co-cultivation of tomato with beneficial root endophytic fungus, *Piriformospora indica*

2.2.1.1. ***Maintenance of P. indica culture***

Different synthetic and complex media have been employed for the cultivation and preservation of *P. indica*. Sahay and Varma (1999) successfully grew *P. indica* culture using a modified minimal agar medium with a pH of 4.8. Rhythmic growth patterns were observed when *P. indica* was cultured on modified Aspergillus minimal medium for 8 days in the absence of light at a temperature of 30°C (Pham *et al.,* 2004). The maintenance of *P. indica* culture was achieved using Aspergillus minimal medium (Druege *et al.,* 2007), and modified Kaefer medium under controlled temperature and light conditions in a growth chamber (Sun *et al.,* 2010). Optimal growth and sporulation of *P. indica* were attained by supplementing modified Kaefer medium with peptone, yeast extract, and soya bone meal at a concentration of 2.0 g/L each (Kumar *et al.,* 2011). Among the various media tested for *P. indica* cultivation, PDA (Potato Dextrose Agar) and Kaefer medium demonstrated the maximum growth (Kumar *et al.,* 2012a). Chlamydospore production of *P. indica* reached its peak when grown on a 4% (w/v) jaggery medium (Kumar *et al.,* 2012b). The maintenance of *P. indica* culture involved transferring four-week-old cultures into fresh modified Kaefer medium and incubating them in the dark at temperatures ranging from 22-24°C (Johnson *et al.,* 2013). Additionally, *P. indica* was cultured on Kaefer medium and incubated at 28°C for 10 days in other studies (Khalid *et al.,* 2020).

Tanha *et al.* (2014) successfully maintained *P. indica* culture in a complex medium (CM) and incubated it at 25°C for one month to promote sufficient sporulation. Optimal mycelial growth and chlamydospore production were observed in a four percent jaggery medium, as well as in a medium containing nitrogen, yeast extract, and peptone (Varma *et al.,* 2014).

For solid culture, *P. indica* was cultured on Hill-Kaefer medium solidified with one percent agar and incubated in the dark at 28 ± 2°C for 7 days (Kilam *et*

al., 2017). Nivedita *et al.* (2017) maintained *P. indica* culture on modified solid Aspergillus medium at 30°C in the absence of light. Narayan *et al.* (2017) cultured *P. indica* on Aspergillus modified medium standardized by Hill and Kaefer (2001) and incubated it at 30 ± 2°C for 7-10 days. The culture of *P. indica* was maintained on potato dextrose agar (PDA) medium at room temperature (28 ± 2°C) in the dark in various studies (Anith *et al.*, 2018; Cheng *et al.*, 2020).

Liquid media can be utilized for the maintenance and mass cultivation of *P. indica*. Varma *et al.* (2012) optimized the growth conditions of *P. indica* in modified liquid Hill-Kaefer medium with a pH of 6.5, a temperature of 30°C, and an agitation speed of 200 rpm. Maximum dry cell weight and spore yield were achieved five days after inoculation. *P. indica* was grown in Kaefer liquid media with an optimal pH of 6.5 and incubated at 28°C with an agitation speed of 200 rpm in other studies (Sadiqque *et al.*, 2018). Liquid culture of *P. indica* was obtained by introducing 2-3 fungal mycelial fragments into 100 ml of potato dextrose broth (PDB) medium and maintaining it at a temperature of 28°C in the dark for three days with an agitation speed of 200 rpm (Cheng *et al.*, 2020).

2.2.1.2. P. indica Co-cultivation with plants

To establish co-cultivation of *P. indica* with barley, Deshmukh *et al.* (2006) immersed two-day-old barley seedling roots in an aqueous solution of 0.05 percent Tween 20 containing 5 x 10^5 ml^{-1} *P. indica* chlamydospores and grew them in a mixture of expanded clays. Baldi *et al.* (2008) and Kumar *et al.* (2012a) conducted co-cultivation of Linum album cell suspension culture with *P. indica* in Gamborg's B5 media inoculated with different concentrations of five-day-old fungal cultures, resulting in phytopromotional effects. Kumar *et al.* (2009) established co-cultivation of *P. indica* with maize plants by growing the plants in sterile soil inoculated with one percent fungal mycelium mixed with Hoagland's solution. Achatz *et al.* (2010) performed co-cultivation of *P. indica* with barley plants by growing the plants in 300 g of sterile substrate mixed with 2 g of fungal mycelium.

For colonization in *Coleus forskohlii*, Das *et al.* (2014) filtered, washed, and placed *P. indica* mycelium from liquid Hill and Kafer medium in a potting mixture using a sandwich layer model. Satheesan *et al.* (2012) used media containing a 1:1

ratio of MS and PDA for effective co-cultivation of *P. indica* with *Centella asiatica.* Johnson *et al.* (2013) standardized co-cultivation protocols for *P. indica* with the model plant Arabidopsis thaliana. In vitro co-cultivation was established by simultaneously transferring nine to twelve-day-old Arabidopsis seedlings and four-week-old *P. indica* plugs to modify PNM medium. These seedlings were then transferred to a sterile soil-vermiculite mix for in-vivo co-cultivation. Sartipnia *et al.* (2013) immersed two-week-old tomato seedling roots in a suspension of 106 ml^{-1} of *P. indica* chlamydospores for 12 hours, transferred them into pots containing a sterilized mixture of sand and perlite substrate, and observed increased antioxidant production in tomatoes as a result of the co-cultivation.

For *in vitro* co-cultivation with *P. indica*, Banhara *et al.* (2015) germinated surface-sterilized *Lotus japonicus* and *A. thaliana* seedlings on modified solid Hoagland's medium treated with 1 ml of 0.002 percent Tween-20 suspension containing $5x10^5$ *P. indica* chlamydospores. *In vivo* co-culture was achieved by growing plants in sterile substrate inoculated with one percent fungal mycelium. Baishya *et al.* (2015) found that *Artemisia annua* callus treated with *P. indica* culture filtrate effectively increased its biomass. Roylawar *et al.* (2015) cultured *P. indica* in liquid malt extract medium incubated at 25-28°C for 15 days at 100 rpm. Co-cultivation of *P. indica* with tomatoes was achieved by transplanting ten-day-old tomato seedlings into sterile soil mixed with two percent fungal mycelium. Johnson *et al.* (2013) utilized modified Kaefer medium for the 14-day propagation of *P. indica*, and then transferred mycelium bits from this medium to PNM media for fungal growth. Vahabi *et al.* (2016) transferred four 12-day-old A. thaliana seedlings that were grown on MS media to PNM plates containing *P. indica*. Ghaffari *et al.* (2016) grew *P. indica* on a complex medium (CM) at 24°C and immersed barley seedling roots in an aqueous solution of 0.02 percent Tween-20 containing 5×10^5 spores ml^{-1}. These roots were then transferred to a substrate mixture of 2 parts sand and 1 part perlite for co-cultivation.

P. indica was introduced to the tomato plants by immersing their rootlets overnight in a solution containing 10^5 cfu/ml. Furthermore, incorporating *P. indica* mycelium at a ratio of 1/100 (weight/weight) into the substrate led to the

establishment of heavily colonized plants. Fakhro *et al.* (2010) observed that the presence of *P. indica* resulted in a significant improvement in the fresh weights of the plants and mitigated the adverse effects caused by pathogens on plant development in host tomato crops. When *in vitro* plantlets and callus were exposed to interactions with the root endophyte and its mycelial filtrate, an overall increase in plant biomass and total chlorophyll content was reported (Baishya *et al.*, 2015).

Arunkumar and Shivaprakash (2017) cultured *P. indica* in Hill-Kaefer broth medium, and 20 g of fungal mycelium was inoculated in a potting mixture using the sandwich layer model for colonization in finger millet. Nassimi and Taheri (2017) dipped two-week-old rice seedlings in a chlamydospore suspension of *P. indica* and transferred them to pots filled with a 1:1 (V:V) mixture of sterile sand and soil for co-cultivation. Rajak *et al.* (2017) conducted a co-cultivation experiment with pigeon pea by inoculating the fungal mycelium, grown in Kaefer broth medium, near the roots of two-week-old pigeon pea seedlings grown in a sterilized mixture of sand and soil in a 3:1 ratio. Su *et al.* (2017) performed *in vitro* co-cultivation of *Brassica napus* with *P. indica* by transferring two-day-old seedlings to modified PNM medium inoculated with *P. indica*. After 15 days of co-culture, the seedlings were transplanted into pots filled with a 4:2:1 mixture of sphagnum, vermiculite, and perlite.

Solanum melongena, *Abelmoschus esculentus*, and *Capsicum annuum* seeds were surface sterilized and then transferred to a medium containing a 1:1 ratio of MS and PDB (containing *P. indica*) for co-cultivation with *P. indica*, as described by Jisha *et al.* (2019). For co-cultivation with banana, three to four-leaved banana plantlets were transferred to a rooting medium mixed with a suspension of *P. indica* chlamydospores, which was added before the medium solidified, as outlined by Li *et al.* (2019). Cheng *et al.* (2020) directly poured a *P. indica* suspension, containing approximately 60 g of mycelial mass per liter and 1×10^5 chlamydospores per milliliter, onto the soil close to the root system of one-month-old banana plantlets at a concentration of 100 ml per kilogram of soil.

2.2.1.3. Root colonization efficiency of P. indica

The colonization of *P. indica* in various plant species has been investigated, revealing interesting patterns. Deshmukh *et al.* (2006) observed that *P. indica* colonization in barley increased with root tissue maturation, with the maximum colonization occurring in the differentiation zone characterized by the presence of inter- and intracellular hyphae and intracellular chlamydospores. Similarly, in wheat, Serfling *et al.* (2007) reported the formation of intracellular hyphae in the epidermal cells after one week of inoculation, followed by the presence of chlamydospores within the epidermal and root hair cells after three weeks of inoculation. The colonization of *P. indica* in maize plants showed a gradual increase, with 20-30% colonization at the 10^{th} day and up to 70% colonization at the 20^{th} day after inoculation (Kumar *et al.*, 2009). In barley roots, Schafer *et al.* (2009) observed inter- and intracellular hyphae in the rhizodermis and cortex, with fungal sporulation initiating at 14 days after inoculation.

Other studies have also reported the colonization of *P. indica* in different plant species. Bajaj *et al.* (2014) found inter- and intracellular root colonization with intra-cellular chlamydospores in turmeric roots, ranging from 60-70% colonization. Dong *et al.* (2013) demonstrated that *P. indica* colonization in Chinese cabbage led to early root maturation, increased growth, and biomass. Aloe vera plants co-cultured with *P. indica* showed 67.5% colonization, and the inoculated plants had higher gel and aloin content (Sharma *et al.*, 2014). Das *et al.* (2014) studied the interaction of *P. indica* with *Coleus forskohlii* and reported 25.55% root colonization. Tanha *et al.* (2014) found a high degree of root colonization (90%) in globe artichoke, along with an increase in growth parameters under water stress. In *Stevia rebundiana*, *P. indica* colonization ranged from 50.0 to 53.3% in vitro-grown plantlets and 56.7 to 63.3% in greenhouse-grown plants (Kilam *et al.*, 2017). Su *et al.* (2017) investigated the effect of *P. indica* colonization in Brassica napus and observed an increase in lateral branching and root hair development. Anthurium plants showed colonization by *P. indica* at 14 days after inoculation, with fungal hyphae passing through the root epidermal cellular layers and multiplying in the cortex layers, resulting in the production of large amounts of spores (Lin *et al.*, 2019). In groundnut, Tarte *et al.* (2019) observed fully developed intracellular pear-

shaped chlamydospores arranged in single, double, or tetrad chains after 45 days of co-culture, with a root colonization ranging from 50-60%.

The presence of *P. indica* colonization in tomato plants was evident through the detection of the fungus's chlamydospores within the cortical region of the plant roots. The highest level of colonization (37.4%) was observed in plants treated with the co-cultured inoculum. Only a few adjacent cells contained spores, and these cells were either singly occupied or contained relatively small-sized spores. However, when mixed or co-cultured inoculations were employed, nearly all the cells in the cortex region of the colonized roots were filled with chlamydospores, albeit with a different distribution pattern (Anith *et al.*, 2018).

2.2.2. Role of *P. indica* in enhancing plant growth

Piriformospora indica, part of the recently established Sebacinales order, displays remarkable adaptability in forming mycorrhizal partnerships and fostering plant growth. This endophytic fungus is commonly found colonizing plant roots without causing any apparent symptoms and can establish associations with various plant groups, including bryophytes, pteridophytes, gymnosperms, and angiosperms (Varma *et al.*, 2012).

Inoculating the roots of Arabidopsis, Chinese cabbage, rice, and corn with *P. indica* has been demonstrated to stimulate root proliferation and induce alterations in biomass, as documented by Tsai *et al.* (2020). This boost in root biomass can be linked to the heightened synthesis of indole-3 acetic acid (IAA) facilitated by S. indica. Furthermore, Komis *et al.* (2015) observed that *P. indica* successfully established colonization in maize roots within a 15-day timeframe, subsequently promoting their growth by activating genes associated with microtubule-based processes (Komis *et al.*, 2015).

Chippy (2020) reported improved plant development characterized by heightened root and shoot mass, expanded leaf surface area, and increased plant stature in *P. indica* colonized plants of okra. She noted that in *P. indica*-colonized plants, there was a significant 48% increase in root fresh weight and a substantial 62% increase in shoot fresh weight compared to the control group.

In a study conducted by Saru (2021), tomato plants that had been colonized by *P. indica* exhibited significant improvements in various growth parameters compared to the control group at 60 days after colonization (DAC). These improvements included a 34% increase in shoot height, a 117% increase in the number of branches, a 96% increase in the number of leaves, an 86% increase in the number of flowers, a 45% increase in fresh shoot weight, a 66% increase in dried shoot weight, a 45% increase in fresh root weight, and a substantial 120% increase in dried root weight. Additionally, the time taken for flowering in *P. indica*-colonized plants was reduced by 12 days, and the yield per plant was significantly higher, with *P. indica*-colonized plants yielding 892.46 grams as compared to 449.51 grams in the control group.

Kaboosi *et al.* (2022) investigated how *P. indica* influenced tomato plants at various intervals (4, 8, and 12 weeks) post-inoculation. The findings consistently indicated favorable outcomes from both *P. indica* inoculation methods, resulting in augmented root and shoot dry weights. Moreover, the presence of *P. indica* substantially boosted tomato fruit yield by as much as 73%.

2.2.3. Role of *P. indica* in enhancing drought stress tolerance

Soil moisture levels, a critical factor in agriculture, often experience disruptions due to recurring water scarcity events. This disruption has a direct impact on every stage of a plant's life cycle, ranging from seed germination to seed formation. In the face of drought conditions, plants have developed various mechanisms to counteract the adverse effects of stress. These mechanisms include the development of extensive root systems, the establishment of efficient antioxidant systems, the regulation of osmotic balance, and the upregulation of stress-responsive genes, among others (Jangir *et al.*, 2021).

Although these adaptations are effective to a certain extent, more severe or prolonged drought situations can hinder plant processes and subject them to the negative consequences of drought stress. Among these consequences, oxidative stress is particularly destructive, leading to significant disruptions in plant functioning. The colonization of host plants by *P. indica* plays a crucial role in enhancing the plants' overall fitness, equipping them to better withstand various

forms of stress. Notably, during drought periods, S. indica-colonized plants exhibit more robust and improved responses to stress when compared to plants that have not been colonized by the fungus (Jangir *et al.*, 2021).

The beneficial impact of *P. indica* in mitigating the adverse effects of drought on plants can be categorized into four primary areas: (1) enhancing water and nutrient absorption (Hussin *et al.*, 2017; Swetha and Padmavathi, 2020), (2) promoting chlorophyll synthesis, (3) facilitating the accumulation of proline, and safeguarding against reactive oxygen species (ROS) through the heightened production of antioxidant enzymes (Swetha and Padmavathi, 2020).

The role of *P. indica* in stimulating the growth of host plants, improving nutrient uptake such as nitrogen (N) and phosphorus (P), and enhancing water absorption is well-documented. Researchers attribute the fungus's positive influence on enhancing plant yield under drought conditions to its origin in arid regions (Hussin *et al.*, 2017). Studies have demonstrated that drought significantly inhibits crucial enzymes involved in nitrogen metabolism, such as glutamate synthase, glutamine synthetase, and nitrate reductase (Wang *et al.*, 2016). However, this inhibitory effect was not observed in plants with colonized roots by S. indica, indicating that the fungus aids in enhancing nitrogen uptake by plants during drought conditions (Wang *et al.*, 2016).

Furthermore, Ghaffari *et al.* (2019) have pointed out that *P. indica* plays a role in optimizing the allocation of resources within the host plant and shields them from the detrimental effects of drought. It has also been documented that the colonization of plants by *P. indica* leads to the expansion of their root systems, resulting in improved uptake of water and nutrients. This, in turn, leads to increased plant biomass, regulation of leaf temperature, decreased leaf wilting, and enhanced control over stomatal closure (Tsai *et al.*, 2020).

Moreover, there is substantial evidence indicating that *P. indica* can mitigate drought-induced senescence by regulating autophagy. Ghaffari *et al.* (2019) conducted research clearly demonstrating that the presence of this endophytic fungus significantly elevates the expression of the *EXO70B1* gene, which has a pivotal role in regulating autophagy in plants. Sun *et al.* (2010) have

also identified the impact of *P. indica* on the expression of a stomatal regulator in Chinese cabbage leaves, specifically related to calcium signaling, which results in the closure of stomatal pores during drought conditions. Other advantageous effects of *P. indica* in alleviating the risks associated with drought stress encompass the prevention of thylakoid and chlorophyll protein degradation, resulting in improved photosynthetic efficiency (Saddique *et al.*, 2018; Sun *et al.*, 2010). Blanco *et al.* (2011) have documented that the colonization of plant roots by *P. indica* plays a regulatory role in photosynthesis-related proteins under drought stress conditions. This regulation encompasses various components of LHC-I and LHC-II photosystems, key enzymes in optical respiration, ferredoxin, phosphoglycolate phosphatase, and proteins within the photosystem complexes.

Recent research has revealed that the inoculation of plants with *P. indica* during drought stress leads to an increase in proline content and a reduction in malondialdehyde (MDA) accumulation (Xu *et al.*, 2017). Moreover, *P. indica*'s influence extends to the regulation of the *P5CS* gene expression, contributing to heightened proline accumulation (Abo-Doma *et al.*, 2011). This endophytic fungus also governs the expression of genes related to total antioxidant capacity (TAC), thus enhancing the plant's resilience to drought (Saddique *et al.*, 2018). Studies have further demonstrated that *P. indica* elevates the activity of essential antioxidant enzymes, namely SOD, CAT, and APX, by regulating their corresponding genes in plants (Tsai *et al.*, 2020; Zhang *et al.*, 2018).

The study conducted by Azizi *et al.* (2021) indicated that the mutualistic partnership between the plant and the fungus may assist tomato plants in enduring drought stress by means of both physiological and molecular mechanisms.

3. MATERIALS AND METHODS

The research work entitled "Management of water stress in tomato (*Solanum lycopersicum* L.) through beneficial root endophytic fungus, *Piriformospora indica*" was conducted at the Department of Vegetable Science, Department of Plant Pathology, and Department of Fruit Science, College of Agriculture, Vellayani, Thiruvananthapuram during the academic year 2019-2022 with the objectives of inducing tolerance to water stress in tomato through colonization with beneficial root-endophytic fungus, *Piriformospora indica* and to study its physiological and molecular mechanisms behind the same. The chapter details the various materials and methodologies adopted for the completion of study**.**

3.1. EXPERIMENTAL LOCATION

The pot experiments were undertaken in a temperature-controlled polyhouse situated at the Fruit Science farm of the College of Agriculture Vellayani. The farm is located at 8°5' N latitude and 76°9' E longitude, with an altitude of 29 meters above mean sea level.

3.2. EXPERIMENTAL MATERIAL

The study involved the evaluation of the effect of root endophytic fungus, *Piriformospora indica*, in conjunction with the widely recognized tomato variety, Vellayani Vijai under water stressed conditions.

3.3. EXPERIMENTAL METHODOLOGY

3.3.1. Maintenance of the fungal root endophyte *P. indica*

The root endophytic fungus, *P. indica* available at Department of Plant Pathology, College of Agriculture, Vellayani was maintained in Potato Dextrose Agar (PDA) medium (Appendix I). A fungal disc from actively growing tips of two weeks-old culture of *P. indica* was plated to the center of Petri plates containing PDA medium and incubated in dark at room temperature. The fungus was sub-cultured at two weeks interval. For maintaining broth culture, 5 mm disc of *P. indica* from a fully grown plate was transferred to a sterilized 100 ml potato dextrose broth in 250 ml conical flasks followed by incubation at room temperature with a constant shaking at 40 rpm using an orbital shaker for 15 days (Plate 1).

3.3.2. Mass multiplication of *P. indica* in potting mixture

The potting mixture was prepared using dried and finely powdered farmyard manure and coir pith amended with 2 per cent gram flour (w/w) (Jojy *et al.*, 2020). The prepared potting mixture was moistened to its field capacity and sterilized at 121°C, 15 psi for 20 min and sterilization was repeated three times in consecutive days. Fungal mycelium was filtered by passing through double layered cheese cloth and washed with sterile water two times. The autoclaved potting mixture was spread to surface sterilized plastic trays inside the Laminar Air Flow Chamber, to which 1 per cent w/w of the fungal mats was transferred and thoroughly mixed together for an even distribution of the hyphal tissues. The inoculated mixture was sprayed with sterile water for keeping it moist. Finally, the trays with potting mixture were covered using a surface sterilized cling film and kept for 7 days for getting complete fungal growth. The same procedure was repeated for the control potting mixture with the exemption of *P. indica* inoculation (Plate 1).

3.3.3. Co-cultivation of *P. indica* with tomato in potting mixture

The potting mixtures with and without *P. indica* was transferred to the surface sterilized protrays separately. The seeds of tomato var. Vellayani Vijay

were surface sterilized with 0.1 per cent mercuric chloride for 10 sec and subsequently washed twice with sterile water. The seeds were sown in the protrays and were kept in dark room for 2 days to enhance the vigour of the growing seedling. In the subsequent days, the protrays with seedlings were maintained under temperature and humidity-controlled conditions for uniform germination and growth (Plate 1).

3.3.4. Colonization of *P. indica* in tomato roots

Roots of tomato seedlings were observed at 5, 7, 10 and 15 days after co-cultivation. Roots were thoroughly washed in running water to clean all the planting medium cling to the rootlets. Roots were carefully cut into pieces of 1 cm length and were transferred to a test tube with freshly prepared 5 ml of 10 per cent KOH. The test tube with the root bits was heated in a water bath at 65°C for 5 min. Roots were taken out and washed with water and again transferred to a test tube with 1 per cent HCl for 5 min. Root bits were washed with water and placed in lactophenol trypan blue dye for 2 min to stain the fungus colonized in roots. The excess stain was removed and root bits were observed under a microscope (Leica - ICC50 HD, USA) to examine the presence of mycelia, chlamydospores and colonization in each root bit (Plate 2).

3.3.5. Pot culture studies with *P. indica* -colonized and non-colonized tomato seedlings

Pot culture experiments were conducted at Department of Vegetable Science, College of Agriculture, Vellayani in completely randomized design (CRD) to evaluate the effect of *P. indica*- colonized seedlings of tomato against different levels of water stress simulated in different ways.

3.4. EVALUATION OF TOMATO PLANTS COLONIZED WITH *P. INDICA* UNDER WATER STRESS INDUCED BY VARIOUS METHODS

3.4.1. Evaluation of tomato plants colonized with *P. indica* under drought stress

P. indica colonized and non-colonized tomato plants were given drought stress by gravimetric method. Drought stress was given for a period of 7 days starting from 45 DAS (In the gravimetric method for simulating drought stress, each

pot was filled with 4500 g of soil, while an additional 4500 g of soil was dried in an oven for 7 days to obtain the dry weight (DW). The pots with tomato plants were weighed daily and watered to reach 100%, 75%,50%, 25% of field capacity. To determine the soil water holding capacity or field capacity, an extra pot filled with 4500 g of soil was saturated with water, drained until reaching a constant weight, and recorded as the wet weight (WW). The pot weights were measured daily to maintain the relative soil water content (RSWC), with water replenished if any pot weight fell below the target weight (10g threshold). Additionally, the total plant fresh weight (WP) was estimated by measuring shoot and root weights of two extra pots. The target weight (WT) for each treatment (pot) was calculated by using equation.

$$W\,T = WD \times WP \times RSWC \times (WW - WD)$$

WD - Oven dry weight of soil

WP - total plant fresh weight

RSWC - Relative soil watercontent

WW- Wet weight (weight of water saturated soil +pot))

The plants were maintained at different levels of field capacity viz. 100%, 75%, 50% and 25%. The experimental design is given below.

a. Colonizing with *P. indica*

P1 - *P. indica*-colonized tomato plants
P2 - Non-colonized tomato plants

b. Imposing drought stress by limiting irrigation

D_1-Control (100% Field Capacity (FC)
D_2-75% FC
D_3-50% FC
D_4-25% FC

3.4.2. Evaluation of tomato plants colonized with *P. indica* under drought stress simulated by PEG

Preliminary *in vitro* studies were conducted using -1bar, -5 bar, -7bar and -10 bar concentrations of PEG and based on the result, a sub-lethal (D_2: -3bar), a higher level D_3: -7bar) and double the lethal dose (D_4: -10bar) of PEG were fixed along with the control (D_1) for *in vivo* pot culture studies. Pots were filled with 4500

g of soil, and both *P. indica* colonized and normal tomato seedlings were transplanted. The pots were organized in a completely randomized design. After 45 days of seed germination (15 DAT), the PEG treatment was administered. PEG 6000 solutions were prepared at selected concentrations based on *in vitro* studies: control (normal water), -3 bars, -7 bars, and -10 bars. The preparation procedure of PEG at different osmotic concentrations given by Michel and Kaufmann (1973) was followed. For 7 days, starting from 45 days after seed germination (DAS), the tomato seedlings were treated with PEG at different concentrations (control, -3bar, -7bar and -10bar). In the control group, water was used instead of PEG. The experimental design is given below,

a) Colonizing with *P. indica*

P_1 - *P. indica*-colonized tomato plants
P_2 - Non-colonized tomato plants

b) Application of PEG

D_1: Control (Water- -0.3 bars)
D_2: -3 bar
D_3: -7 bar
D_4: -10 bar

3.4.3. Evaluation of tomato plants colonized with *P. indica* under water stress simulated by mannitol

Preliminary *in vitro* studies were conducted using 1%, 2%, 3% and 5% concentrations of mannitol and based on the results, a sub-lethal (M_2: 3%), a higher level M_3: 5%) and double the lethal dose (M_4: 10%) were fixed along with the control (M_1) for *in vivo* pot culture studies. Pots were filled with 4500 g of soil, and both *P. indica* colonized and normal tomato seedlings were transplanted. The pots were organized in a completely randomized design. After 45 days of seed germination (15 DAT), the Mannitol treatment was administered. Mannitol at selected concentrations (i.e., 3%, 5% and 10%) were prepared and applied for 7 days. Water was used in control groups instead of mannitol. The experimental design is given below,

a) Colonizing with *P. indica*

P_1 - *P. indica*-colonized tomato plants

P_2 - Non-colonized tomato plant

b) Application of mannitol

M_1 - Control

M_2 - 3%

M_3 - 7%

M_4 - 10%

3.4.4. Evaluation of tomato plants colonized with *P. indica* under water stress simulated by ABA

Preliminary *in vitro* studies were conducted using 0.1µM, 1µM, 2µM and 4µM concentrations of ABA and based on the results, a sub-lethal (A_2: 3µM), a higher level A_3: 6µM) and double the lethal dose (A_4: 10µM) were fixed along with the control (A_1) for *in vivo* pot culture studies. Pots were filled with 4500 g of soil, and both *P. indica* colonized and normal tomato seedlings were transplanted. The pots were organized in a completely randomized design. After 45 days of seed germination (15 DAT), the ABA treatment was administered. ABA at selected concentrations based on *in vitro* studies (i.e., 3µM, 6µM, and 10µM) were prepared and applied for 7 days. Water was used in control groups instead of ABA. The experimental design is given below,

a) Colonizing with *P. indica*

P_1 - *P. indica*-colonized tomato plants

P_2 - Non-colonized tomato plants

b) Application of ABA

A_1 - Control

A_2 - 3µM

A_3 - 6µM

A_4 - 10µM

3.4.5. Evaluation of tomato plants colonized with *P. indica* under flooding

The experiment involved filling pots with 4500 g of soil, followed by the transplantation of both *P. indica*-colonized and normal tomato seedlings. The

arrangement of the pots followed a completely randomized design. After 45 days from seed germination (15 days after transplanting), the submergence treatments were initiated according to the designated durations (0, 1, 2, 3, and 5 days of submergence). Submergence was monitored by visually observing water stagnation on the surface of the pots. Periodic water addition was carried out to maintain the appropriate submergence levels specific to each treatment. The experimental design is given below,

a) Colonizing with *P. indica*

P_1 - *P. indica*-colonized tomato plants
P_2 - Non-colonized tomato plants

b) Flooding

F_1 - Control (0 days of submergence)
F_2 - 1 day of submergence
F_3 - 2 days of submergence
F_4 - 3 days of submergence
F_5 - 5 days of submergence

3.5. OBSERVATIONS

3.5.1. Vegetative and flowering characters

The following observations were common for all experiments. Five plants from each replication were selected as observational plants.

3.5.1.1. Plant height (cm)

The plant height from collar region to the tip of the plant was measured using scale in centimeters @ 30 and 60 DAT.

3.5.1.2. Primary branches per plant

Number of branches borne on the main axis of each plant was counted and expressed as number of primary branches.

3.5.1.3. Stem girth (cm)

A flexible measuring tape was used to measure the stem girth of a tomato plant. Measurement was done from the base of the stem, specifically just above the

soil level (1 cm). This ensures consistency and provides an accurate representation of the stem's circumference.

3.5.1.4. Leaf length (cm)

Ten leaves were chosen for the measurement of leaf length. To ensure consistency, middle-aged leaves were selected for the measurements.

3.5.1.5. Leaf width (cm)

The leaves selected for measuring leaf length was used for measuring leaf width also. Width was measured at the widest part of the leaf. To maintain consistency and accuracy, middle-aged leaves were specifically chosen for the measurements.

3.5.1.6. Leaf area (cm^2)

Leaf area was measured using Leaf Area Meter and expressed in cm^2.

3.5.1.7. Days to first flowering

The number of days from the date of transplanting to the occurrence of first flowering was recorded and expressed as days to first flowering.

3.5.1.8. Days to 50% flowering

The number of days from the date of transplanting to the point where 50% of the total flower buds had blossomed was recorded. This measurement was also cross-validated with the total number of flowers observed to ensure accuracy and consistency.

3.5.1.9. Flower clusters per plant

The number of flower clusters in each plant was recorded under different treatment conditions.

3.5.1.10. Flowers per cluster

The number of flowers per cluster in each plant was recorded under different treatment conditions.

3.5.2. Fruit and yield characters

The following observations were common for all the five experiments. Observations were taken from five plants from each replication.

3.5.2.1. Days to first harvest

The number of days from the date of transplanting to the day of first harvest in each treatment was recorded.

3.5.2.2. Fruits per truss

The total number of fruits in each truss was counted and represented as number of fruits per truss.

3.5.2.3. Fruits per plant

The number of fruits harvested from each observational plant in each treatment was recorded and expressed as number of fruits per plant.

3.5.2.4. Fruit set %

Fruit set was expressed as a percentage by counting both the total number of flowers and the total number of fruits per plant.

Fruit setting %= (Total no of fruits/Total no of flowers) x 100

3.5.2.5. Fruit length (cm)

At maturity, the length of the fruits was measured from the stem end to the blossom end and expressed in centimeters.

3.5.2.6. Fruit diameter (cm)

The diameter of the fruit was measured in centimeters using a vernier caliper, specifically from the center or equatorial length of the fruit.

3.5.2.7. Average fruit weight (g)

The weight of five fruits from each observational plant was recorded in electronic balance, then averaged and expressed in grams.

3.5.2.8. Fruit cracking %

The number of cracked fruits and the total number of fruits per plants were recorded and fruit cracking percentage was calculated.

Fruit cracking %= (Total no of cracked fruits/Total no of fruits) x 100

3.5.2.9. Yield per plant (g)

The weight of fully ripe fruits harvested from the observational plants in each treatment was measured using an electronic balance and expressed in grams.

3.5.3. Pest and disease incidence

3.5.3.1. Observations on infestation by pests and diseases

Pest and disease infestation in tomato plants were observed and recorded from transplanting to harvesting.

3.5.3.2. Colony Forming Units of P. indica in soil and plants

The Colony Forming Units (CFUs) of *P. indica* in both soil and plants were recorded by the serial dilution technique (Khare and Jharia, 2002).

$$\text{CFU } g^{-1} mL^{-1} = \frac{\text{Number of colonies per mL plated}}{\text{Total dilution factor}}$$

3.5.4. Quality parameters

Observations were taken from five ripe fruits from the observational plants under each replication.

3.5.4.1. Ascorbic acid

The method described by Sadasivam and Manickam (2008) was used to estimate the ascorbic acid content in plants. A working standard solution containing 100 mg/ml of ascorbic acid was pipetted into a 100 ml conical flask. To this, 4% oxalic acid was added and titrated against 2,6-dichlorophenol indophenol dye (V_1 ml). The endpoint was determined when a persistent pink color appeared for a few minutes. For the sample analysis, 0.5 g of the plant material was weighed and ground in a mortar with pestle using 15 ml of 4% oxalic acid.

The homogenate was passed through a double-layered cheesecloth to filter it. The resulting filtrate was adjusted to a known volume and then centrifuged at 10,000 rpm for 10 minutes. The supernatant obtained was collected and brought up to a volume of 25 ml using oxalic acid. A 5.0 ml aliquot of the supernatant was transferred to a conical flask, and 10 ml of 4% oxalic acid was added to it. The mixture was then titrated against a solution of dichlorophenol indophenol (DCPIP) until a pink color appeared (V_2 ml). The amount of ascorbic acid was calculated using the following method:

$$\text{Ascorbic Acid} = \frac{0.5 \text{ mg}}{V_1 \text{ ml}} \times \frac{V_2 \text{ ml}}{5 \text{ ml}} \times \frac{100}{\text{weight of sample}}$$

3.5.4.2. Lycopene

The method described by Ranganna (1976) was used to quantify the lycopene content in the fruit. A five-gram fruit sample was crushed and repeatedly extracted with acetone until the residue became colorless. The acetone extract was then transferred to a separating funnel containing 15 mL of petroleum ether and gently mixed. Next, 5 mL of a 5% sodium sulfate solution in water was added and thoroughly mixed by shaking. This step aided in separating any water present in the separating funnel and helped form a clear extract. The lower phase, which contained the carotenoid (petroleum ether extract), was transferred to another separating funnel to remove any remaining acetone. Finally, the extract was transferred to an amber-colored bottle. The extraction process involved several steps as described above.

The procedure using petroleum ether was repeated until the acetone phase became colorless. The acetone phase was then discarded, and a small amount of anhydrous sodium sulfate was added to the petroleum ether extract. The extract was transferred to a 25 mL volumetric flask and diluted to 25 mL with petroleum ether. From this, 5 mL of the diluted extract was further diluted to 25 mL with petroleum ether for color measurement. The optical density (OD) of the extract was measured at 503 nm using a UV-VIS spectrophotometer (Elico SL-160), with petroleum ether used as a blank (Sadasivam and Manikam, 1992). The lycopene content of the sample was calculated using the following formula:

$$\text{Lycopene (mg/100g)} = \frac{3.1206 \times \text{OD of sample} \times \text{volume made up} \times \text{dilution} \times 100}{\text{Weight of sample} \times 1000}$$

3.5.4.3. TSS (º Brix)

The Total Soluble Solid (TSS) of tomato fruits were measured by using hand refractometer. The tomato fruit juice was extracted with the help of needle and drop of juice was put on hand refractometer. The measured Total Soluble Solid of tomato crop was having the range of 0 to 9º B (Brix).

3.5.5. Physiological parameters

Various physiological parameters, including relative water content, cell membrane stability, chlorophyll stability index, and proline content, were recorded

in Experiments 1 to 4. Observations were taken from five observational plants under each replication.

3.5.5.1. Relative water content

The Relative Water Content (RWC) was determined by using the values of fresh weight, turgid weight, and dry weight of the leaf sample. A known amount of leaf sample was taken and cut into small pieces, and the fresh weight was measured. The turgid weight was recorded after immersing the leaf sample in water for three hours to ensure full hydration. To obtain the dry weight, the leaf samples were placed in a hot air oven at 80°C for three consecutive days.

Relative water content was calculated by using following formula and expressed as per cent.

$$\text{Relative water content (\%)} = \frac{\text{(Fresh weight -Dry weight)} \times 100}{\text{(Turgid weight-Dry weight)}}$$

3.5.5.2. Cell membrane stability

To measure electrolyte leakage, leaves were collected from plants grown under two different temperature regimes. Fresh weight of five grams of leaves was cut into small pieces (approximately 2 cm) and washed with distilled water to remove any electrolytes present on the cut edges or adhering to the surface. After drying with filter paper, the leaf pieces were placed in test tubes containing 20 mL of distilled water in two sets. Each genotype was replicated three times. One set of test tubes was placed in a water bath at 40ºC for one hour (C2), while the other set was kept at 100ºC in a boiling water bath for 15 minutes (C1). The electrical conductivities, C1 and C2, were measured using a conductivity meter (Systronics Conductivity Meter, 306). To assess membrane thermostability, the membrane stability index was calculated using the following formula:

$$\text{Membrane Stability Index} = 100 - [1 - (C1/C2) \times 100]$$

This index provides an indication of the integrity and stability of the cell membrane based on the ratio of electrical conductivities at different temperatures.

3.5.5.3. Chlorophyll stability index

For CSI (Chlorophyll Stability Index) determination, a leaf sample weighing 250 mg was homogenized using 80% acetone. The homogenized sample was then centrifuged at 3,000 rpm for 10 minutes, and the resulting supernatant was

collected and made up to a final volume of 25 ml. The absorbance of the supernatant was recorded at 652 nm to calculate the total chlorophyll content of the sample. The protocol described by Koleyoreas (1958) was followed for estimating the CSI. The CSI value was calculated using the formula:

CSI = (Total chlorophyll content in treatment / Total chlorophyll content in control) × 100

To determine the chlorophyll content, leaf discs were incubated in a mixture of acetone and dimethyl sulfoxide (DMSO) in a 1:1 ratio for 6-8 hours. After incubation, the optical density of the extract was measured at 645 nm and 663 nm using a UV-visible spectrophotometer. The total chlorophyll content was calculated based on the method described by Mafakheri *et al.* (2010) A control leaf tissue weighing 1 gram was collected and stored at room temperature, while another 1 gram of leaf tissue was boiled in a water bath at 60°C for 30 minutes. The total chlorophyll content of both samples was estimated using the DMSO-acetone method as described by Mafakheri *et al.* (2010). The CSI was then calculated using the formula described by Bajji *et al.* (2002).

3.5.5.4. Proline content

The leaf proline content of each treatment was determined using the following procedure. First, 0.5 g of leaf sample was extracted by homogenizing it in 10 ml of a 3% aqueous sulphosalicylic acid solution. The extractant was then centrifuged at 3000 rpm for 15 minutes. From the resulting filtrate, 2 ml was taken in a test tube and mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. The mixture was heated in a boiling water bath at 100°C for 1 hour. Afterward, 4 ml of toluene was added to the reaction mixture and stirred for 20-30 seconds. The intensity of the red color formed was measured at 520 nm using a spectrophotometer.

To determine the proline content in the test sample, a series of standards with pure proline were also run using the same procedure, which helped create a standard curve. The amount of proline in the test sample was then calculated from the standard curve. The calculation was performed using the formula:

Proline content in µmoles per gram tissue = (µg proline/ml x ml toluene) / (115.5 g sample)

Here, 115.5 represents the molecular weight of proline.

3.5.6. Enzymes

The activity of various enzymes, such as superoxide dismutase (SOD), catalase, and peroxidase, were determined for all experiments (Experiment 1 to 5).

3.5.6.1. Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD) was determined using the method outlined by Kakkar *et al.* (1984). Leaf samples weighing 0.5 g, obtained from the third fully opened leaves, were ground with 3.0 ml of potassium phosphate buffer. The resulting mixture was then centrifuged at 2000 rpm for 10 minutes, and the supernatants were collected for the assay. The assay mixture comprised 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.2 ml of the enzyme preparation, and water, making a total volume of 2.8 ml. The reaction was initiated by adding 0.2 ml of NADH. The mixture was incubated at 30°C for 90 seconds, and the reaction was stopped by adding 1.0 ml of glacial acetic acid. To extract the chromogen, the reaction mixture was shaken with 4.0 ml of n-butanol, allowed to stand for 10 minutes, and then centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm. One unit of enzyme activity was defined as the amount of enzyme that caused a 50% inhibition of NBT reduction in one minute.

3.5.6.2. Catalase

The catalase activity was assessed using the procedure outlined by Luck (1974). Leaf tissue weighing 1 gram was homogenized in 20 milliliters of 0.0067 M phosphate buffer (pH 7.0) (Appendix II). The homogenate was then subjected to centrifugation at 5000 revolutions per minute for 15 minutes at a temperature of 4 degrees Celsius. This extraction process was repeated twice. The resulting supernatants were combined and utilized for the enzyme assay. A volume of 40 microliters of the extract was added to a cuvette containing 3 milliliters of H_2O_2 - PO_4 buffer, while the control cuvette contained H_2O_2-free PO_4 buffer. The time

required for the absorbance to increase by 0.05 at a wavelength of 240 nanometers (Δt) was recorded to calculate the enzyme units per milliliter of the extract.

3.5.6.3. Peroxidase

The peroxidase activity in plants was determined using the procedure outlined by Reddy *et al.* (1995). A leaf sample weighing 200 mg was homogenized in 1 ml of 0.1 M phosphate buffer with a pH of 6.5 (Appendix II). The homogenate was then centrifuged at 5000 rpm for 15 minutes at 4°C. To initiate the enzyme reaction, 0.1 ml of the enzyme extract was added to 3.0 ml of a pyrogallol solution, and the mixture was adjusted to a zero reading at 430 nm. Next, 0.5 ml of a one percent hydrogen peroxide (H2O2) solution was added to the sample cuvettes, and the change in absorbance was measured every 30 seconds for a duration of 3 minutes. The peroxidase activity was quantified based on the change in absorbance per minute at 430 nm. One unit of peroxidase activity is defined as the change in absorbance per minute at the specified wavelength.

3.5.7. Molecular parameters

3.5.7.1. Gene expression by real time PCR analysis

The isolation of total RNA was performed using the total RNA isolation kit, following the instructions provided by the manufacturer (Invitrogen - Product code 10296010). The addition of TRIzol solution resulted in cell disruption and the release of RNA. Subsequent centrifugation allowed the separation of the aqueous phase, containing RNA, from the interphase and organic phase, which contained proteins. Mixing the aqueous phase with isopropanol caused RNA to precipitate as a white pellet at the side and bottom of the tube. To isolate RNA, 1 milliliter of trizol reagent was added to a 100 mg tissue sample, which was then homogenized until it formed a fine paste. The mixture was transferred to a new sterile eppendorf tube. Next, 200 microliters of chloroform were added, and vigorous shaking was carried out for 15 seconds. The tube was then incubated for 2-3 minutes at room temperature, followed by centrifugation at 14,000 revolutions per minute for 15 minutes at 4 degrees Celsius. The resulting aqueous layer was collected, and 500 microliters of 100% isopropanol were added. After incubating for 10 minutes at room temperature, the tube was centrifuged again at 14,000 revolutions per minute

for 15 minutes at 4 degrees Celsius. The supernatant was discarded, and the obtained pellet was washed with 200 microliters of 75% ethanol (Merck). The pellet was then centrifuged at 14,000 revolutions per minute for 5 minutes at 4 degrees Celsius using a cooling centrifuge (Remi CM12). Finally, the RNA pellet was dried and suspended in TE buffer (Appendix III).

3.5.7.2. cDNA Synthesis and gene expression analysis by RT-qPCR

Total RNA was extracted from the samples using Trizol (Invitrogen, USA). The purity and concentration of the extracted RNA were determined. To synthesize complementary DNA (cDNA), the cDNA preparation kit (G BIOSCIENCES, Product code 786-5019s, 786-5020) was utilized. A reaction mixture was prepared in an RNAse-free tube, consisting of 5µL of RT Easy mix, 0.5µL of oligodT, and 2 µl of RNA template (0.5µg of total RNA). Sterile distilled water was added to reach a total reaction volume of 10 µl, and the solution was gently mixed by pipetting. The cDNA synthesis was carried out in a thermal cycler (Eppendorf Master Cycler) with the following cycling conditions: 20 minutes at 42ºC and 5 minutes at 85ºC. For the Real-Time RT-qPCR analysis, SYBR Green Master Mix (G BIOSCIENCES, Product code 786-5062) was used. The analysis was performed using a Lightcycler 96 instrument (Roche). All reactions were conducted in triplicates, and the data were analyzed using the ΔΔCt method with the assistance of Light Cycler 96 SW 1.1 Software. Table1 represents the forward and reverse primers for *SlAREB1* and housekeeping gene (*GAPDH*)

Table 1. Forward and reverse primers of *SlAREB1* and *GAPDH*

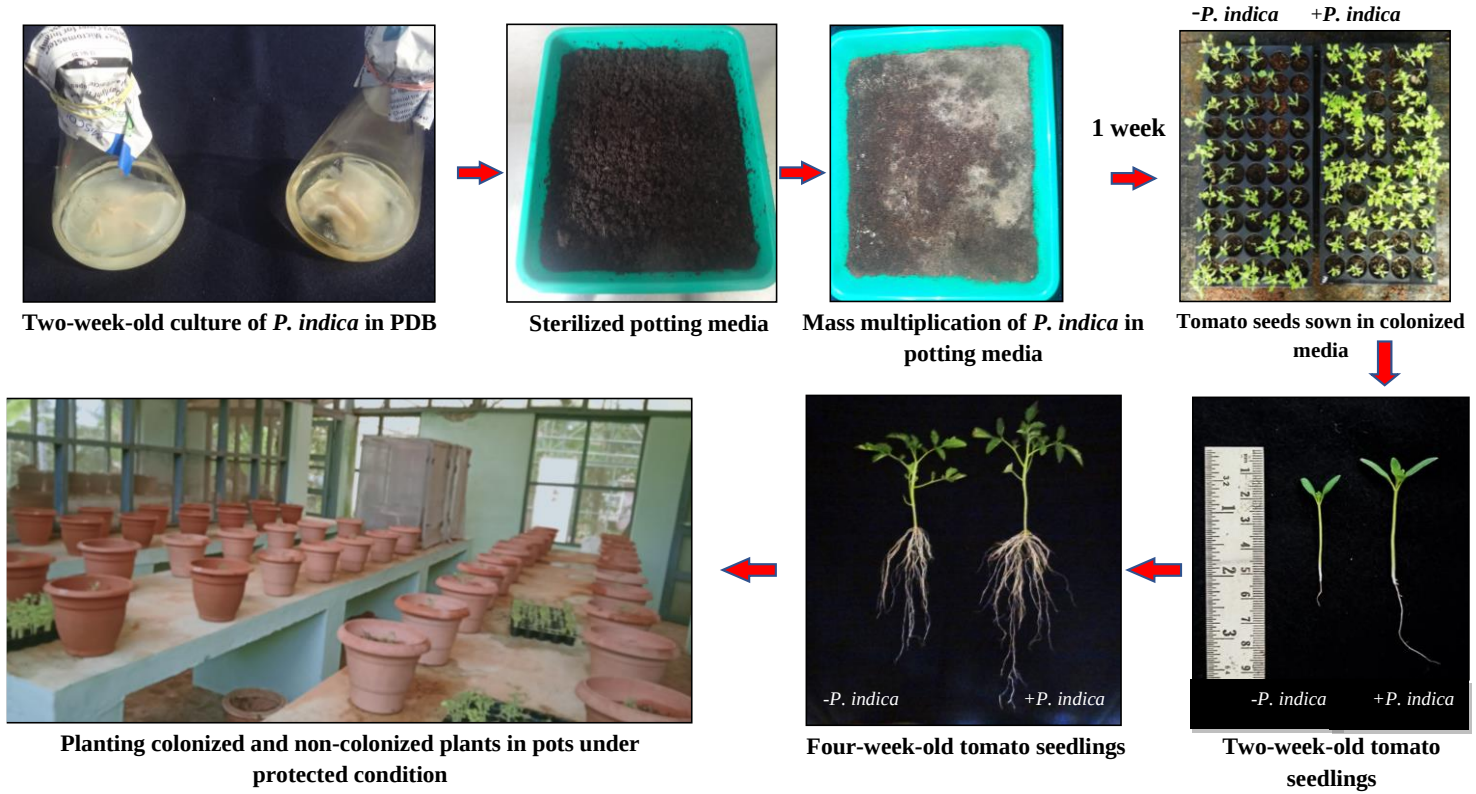
OLIGO NAME	FORWARD		REVERSE	
	SEQUENCE (5’ ->3’)	Tm	SEQUENCE (5’ ->3’)	Tm
SlAREB1	ATGGGGAGTAATTATCAT TTCAAGAAC	63.9	TTACCATGGACCAGTTTG TGTCCGTCT	72.5
GAPDH	CTG CTC TCT CAG TAG CCA ACA C	57.3	CTT CCT CCA ATA GCA GAG GTT T	54.5

3.5.7.3. Agarose gel electrophoresis

Agarose gel electrophoresis is a technique used to separate and visualize DNA fragments based on their charge and size. The fragments migrate through an agarose gel matrix when an electric field is applied. The electric field is generated by applying a potential across an electrolyte solution, also known as the buffer. To prepare the gel, a 1.5% agarose gel was made in 1x TE buffer. The agarose was melted in a hot water bath at 90°C and then allowed to cool down to 45°C. Next, 6µL of ethidium bromide at a concentration of 10 mg/mL was added to the melted agarose, which was then poured into a gel casting apparatus with the gel comb. Once the gel solidified, the comb was carefully removed. The gel tank was filled with the electrophoresis buffer, and the platform holding the gel was placed in the tank to ensure the gel was fully immersed in the buffer. The samples were loaded onto the gel, and the electrophoresis was run at 50 volts for 30 minutes. Finally, the stained gel was visualized using a gel documentation system, specifically the E gel imager from Invitrogen.

3.6. STATISTICAL ANALYSIS

Using statistical analysis platform KAU-GRAPES, the data collected for various parameters were evaluated using the analysis of variance (ANOVA) method (Gomez and Gomez, 1984) for completely random design (2 factor CRD). At a $\leq$ 5% level of significance ($p \leq 0.05$), the least significant difference test was employed to determine the interaction effects of the treatments.



Two-week-old culture of *P. indica* in PDB

Sterilized potting media

Mass multiplication of *P. indica* in potting media

Tomato seeds sown in colonized media

Two-week-old tomato seedlings

Four-week-old tomato seedlings

Planting colonized and non-colonized plants in pots under protected condition

Plate 1. Co-cultivation of tomato with *P. indica*

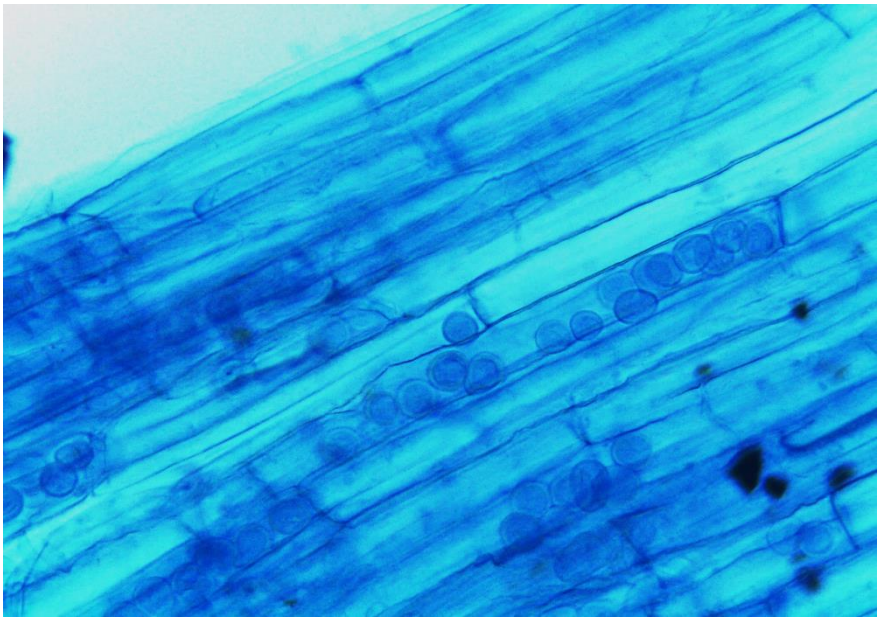


Plate 2. Root colonization of *P. indica* in tomato seedlings @15 days after co- cultivation

4. RESULT

4.1. EVALUATION OF TOMATO PLANTS COLONIZED WITH *P. indica* UNDER DROUGHT STRESS

4.1.1. Vegetative and flowering characters

Plant heights ranged from 31.10 cm to 43.80 cm at 30 days after transplanting (DAT) and from 42.26 cm to 71.00 cm at 60 DAT. Notably, at 30 DAT, *P. indica*-colonized tomato plants (P_1) measured 40.92 cm, surpassing *P. indica* non-colonized plants (P_2) with 33.76 cm. Similarly, at 60 DAT, P_1 exhibited a greater height of 59.58 cm compared to P_2 with 55.78 cm. Across different drought stress levels, the control group (D_1) consistently had the tallest plants. At 30 DAT, the control group reached a height of 40.43 cm, signifying optimal growth conditions with sufficient water. Similarly, at 60 DAT, the control group maintained a tall stature, measuring 69.70 cm, in contrast to plants subjected to water stress (Table 2). Regarding the treatment combinations, *P. indica* notably enhanced plant height under drought stress compared to non-colonized tomato plants at 30 DAT. However, at 60 DAT, the treatment combinations showed no significant differences in plant height (Plate 3).

The data in Table 2 shows the impact of *P. indica* colonization on stem girth of tomato plants under varying levels of drought through limited irrigation. At 30 DAT, P_1 had an average stem girth of 3.26 cm, while P_2 measured 2.32 cm. At 60 DAT, P_1 had an average stem girth of 3.84 cm, whereas P_2 measured 3.19 cm. Overall, *P. indica*-colonized plants (P_1) consistently exhibited significantly greater average stem girth than non-colonized plants (P_2) at both time points. Among the drought stress levels, D_1 had the highest stem girth at both 30 and 60 DAT. At 30 DAT, it measured 3.17 cm, significantly surpassing D_2, D_3, and D_4. Similarly, at 60 DAT, D_1 displayed a significant average stem girth of 3.88 cm compared to other treatments. Generally, as irrigation decreased from D_1 to D_4, stem girth decreased at both time points. Regarding treatment combinations, at 30 DAT, stem girth ranged from 1.80 cm to 3.58 cm, with P_1D_1 having the highest (3.58 cm) and P_2D_4

the lowest (1.80 cm). However, at 60 DAT, treatment combinations did not significantly differ in stem girth.

P. indica colonization significantly increased the number of primary branches in tomato plants (Table 2). Colonized tomato plants averaged 5.56 primary branches, while non-colonized ones had 2.69. Among drought stress treatments, the control (D_1) had the highest average of 4.88 primary branches, statistically comparable to D_2 (75% FC) with slightly fewer branches. Similarly, D_3 (3.63) and D_4 (3.50) showed statistically similar results in primary branch numbers. Among treatment combinations, P_1D_2 had the highest average of 6.50 primary branches per plant, signifying robust growth. P_1D_1 also had a high average, with no significant difference compared to P_1D_2 in terms of primary branches per plant.

Table 3 displays leaf length, width and area measurements at 30 DAT and 60 DAT. At 30 DAT, leaf lengths ranged from 14.32 cm to 22.60 cm, and at 60 DAT, ranged from 19.30 cm to 29.60 cm. P_1 (*P. indica*-colonized) had significantly higher average leaf length of 21.51 cm, width of 15.31 cm and area of 333.00 cm^2 at 30 DAT compared to P_2 (non-colonized). Similar trend was also observed at 60 DAT. With respect to the effect of drought stress, D_1 (control) measured highest leaf length 21.00 cm, width (15.34 cm) and area (325.93 cm^2) at 30 DAT whereas D_4 (25% FC) recorded the lowest. P_1D_1 and P_1D_2 had significantly similar leaf lengths at both time points. However, at 30 DAT, P_1D_1 had the highest (22.60 cm), and at 60 DAT also, it remained the highest (29.60 cm). P_1 with D_1 consistently yielded the highest leaf lengths, width and area, surpassing other combinations, highlighting *P. indica*-colonized plants' superior performance (Plate 4).

Colonized plants flowered at 29.91 days, significantly earlier than control (31.70 days), highlighting the effect of *P. indica* colonization in accelerating flowering. Similarly, P_1 reached 50% flowering by 56.84 days while P_2 took 57.58 days. The control group (D_1) exhibited the longest time to first flowering (34.69 days), followed by D_2 (32.83 days). With increasing drought stress (from D_1 to D_4), flowering occurred sooner, with D_4 recording the shortest time of 26.58 days. Comparing treatment combinations under the same drought stress level, *P. indica* colonization (P_1) significantly accelerated flowering compared to non-colonized

plants (P_2) under control irrigation (D_1), with P_1D_1 at 32.80 days and P_2D_1 at 37.00 days, indicating a difference in flowering onset. Comparing P_1D_1 to P_2D_1, P_1 colonized plants reached 50% flowering significantly earlier (P_1D_1: 56.84 days, P_2D_1: 64.30 days). Within the same drought stress level (e.g., P_1D_2 vs. P_2D_2), *P. indica* colonized plants consistently flowered more quickly, demonstrating significant differences in the time to reach 50% flowering. Overall, *P. indica* colonization tended to expedite flowering compared to non-colonized plants under similar irrigation conditions (Table 4).

P. indica colonization significantly influenced flower clusters per plant and flowers per cluster in tomato plant (Table 4). P_1 colonized plants had more flower clusters (6.12) and flowers per cluster (7.85) compared to P_2 non-colonized plants (3.57 and 6.35 respectively). With increasing drought stress (from D_1 to D_4), the number of flower clusters and flowers per cluster gradually decreased. D_1 had the most clusters (7.17), while D_4 had the fewest (1.97). The mean number of flowers per cluster for the D_2 (75% FC) treatment was recorded as the highest (8.74) which was on par with the control (D_1) (8.45). Among treatment combinations, P_1D_1 had the highest flower (8.66), significantly surpassing all others. Comparing P_1D_1 (8.66) to P_2D_1 (5.68), *P. indica* colonization (P_1) significantly increased flower clusters per plant under both control (D_1) and 75% FC (D_2) conditions. When comparing treatment combinations, there were no significant differences in the number of flowers per cluster. However, *P. indica* colonized plants produced a greater number of flowers compared to the non-colonized counterpart across different levels of drought stress

4.1.2. Fruit and yield characters

The mean number of days to first harvest for P_1 (*P. indica* colonized plants) was recorded as 58.65 which was significantly shorter than P_2 treatment (60.22) (Table 5). The results indicated that there were significant differences in the number of days until first harvest among the different levels of drought stress also. As the severity of drought stress increased (from D_1 to D_4), the time taken for the plants to reach the first harvest stage decreased. The control group (D_1) had the longest time (65.82) to first harvest, while the plants subjected to the severe drought stress (D_4)

had the shortest time to first harvest (52.30). The results revealed a significant effect of interaction between colonization with *P. indica* and drought stress on the timing of the first harvest in tomato. The non-colonized plants under the severe drought stress (P_2D_4 combination) had the shortest time to first harvest (51.34). Under control (D_1), 75%FC (D_2), and 50%FC (D_3), *P. indica* colonized plants came to first harvest in a short time period compared to the control plants.

P. indica colonization had a significant effect on the number of fruits per truss and number of fruits per plant in tomato plants. The mean number of fruits per truss for P_1 (*P. indica*-colonized) plants was recorded as 5.02 and for P_2 (non-colonized) plants was recorded as 3.44. Likewise, number of fruits per plant for P_1 was 14.89, while for P_2 it was 11.03. The results indicated that drought stress had a significant impact on the number of fruits per truss in tomato plants. As the severity of drought stress increased, there was a gradual decrease in the average number of fruits per truss. The control group (D_1) had the highest average number of fruits per truss (6.04) and fruits per plant (19.92), while the plants subjected to the most severe drought stress (D_4) had the lowest averages. These differences were statistically significant. The results indicated that the interaction between *P. indica* colonization and drought stress had a significant impact on the number of fruits per truss in tomato plants. Comparing the treatment combinations, it can be observed that P_1D_1 (6.94) and P_1D_2 (6.94) (colonized with *P. indica*) had the highest average number of fruits per truss, with no significant difference between them. The treatment combination P_1D_1 had the highest average number of fruits per plant (20.80). P. indica colonized plants produced a greater number of fruits under control (20.80), 75% FC (18.80), 50%FC (15.20) and 25% FC (10.00) compared to the non-colonized plants (Table 5).

The results of the study showed a significant difference in fruit set percentage among the treatments (Table 5). When considering the colonization with *P. indica*, P_1 - *P. indica*-colonized tomato plants exhibited a higher fruit set percentage compared to P_2 - non-colonized tomato plants. The fruit set percentage for P_1 was 59.58%, while for P_2, it was 47.95%. As irrigation levels decreased from the control group (D_1) to D_4, the fruit set percentage gradually decreased. The

control group (D_1) had a fruit set percentage of 71.19 %, indicating optimal fruit set under normal irrigation conditions. As the irrigation level decreased, the fruit set percentage decreased as well. D_2 (75% FC) had a fruit set percentage of 65.78%, D_3 (50% FC) had 55.29%, and D_4 (25% FC) had the lowest fruit set percentage of 22.79%. The treatment combination was also found to be significant with respect to fruit set percentage and it ranged from 76.56% (P_1D_1) to 13.19% (P_2 D_4).

Regarding the fruit biometric parameters, tomato plants (P_1) had significantly longer and wider fruits compared to non-colonized ones (P_2). P_1 recorded higher fruit length (3.61 cm) and diameter (3.38 cm) whereas P_2 had only 3.04 cm and 2.91 cm respectively. In the control treatment (D_1), fruit length was 3.91 cm, slightly reduced to 3.66 cm in 75% field capacity (D_2). Further reduction to 50% field capacity (D_3) resulted in 3.31 cm fruit length, while severe drought stress (D_4) yielded the shortest, at 2.41 cm. Combinations of *P. indica* colonization (P_1) and drought stress (D_1-D_4) influenced fruit length. Similar trend was observed in fruit diameter also. P_1D_1 (colonized under control conditions) had the longest (4.14 cm) and widest (3.86 cm) fruits, significantly exceeding non-colonized control (P_2D_1). Similarly, P_1D_2 (colonized under 75% field capacity) had longer fruit (3.99 cm) and wider (3.68 cm) compared to P_2D_2 (non-colonized under 75% field capacity). As drought stress intensified, the difference in fruit length between P_1 and P_2 became more pronounced. For example, in severe drought (D_4), P_1D_4 had significantly longer fruit at 2.64 cm, while P_2D_4 measured 2.18 cm. Across all water stress levels, *P. indica* colonized plants consistently produced larger fruits compared to non-colonized ones (Table 6) (Plate 5).

P. indica colonization and drought stress from reduced irrigation significantly impacted tomato plant fruit weight (Table 6). P_1 (colonized) had higher fruit weight (23.15 g) compared to P_2 (non-colonized) at 19.19 g. As drought stress intensified, fruit weight decreased. Control (D_1) had the highest weight at 25.49 g. With irrigation reduced to 75% (D_2), 50% (D_3), and 25% (D_4) of field capacity, fruit weight progressively declined, with D_4 having the lowest at 14.25 g. Among treatment combinations, P_1D_1 (colonized under control conditions) and P_1D_2 (colonized with 75% field capacity irrigation) had the highest fruit weight,

averaging 26.64 g and 25.34 g, respectively. These were significantly higher than P_1D_3 (22.74 g) and P_1D_4 (17.90 g), indicating decreased fruit weight with increased drought stress. Similarly, P_2D_1 (non-colonized under control conditions) and P_2D_2 (non-colonized with 75% field capacity irrigation) had relatively high fruit weight, averaging 24.34 g and 23.22 g, respectively. *P. indica* colonization appeared to maintain higher fruit weight across various drought stress levels.

The colonization with *P. indica* and the different levels of drought stress caused by limiting irrigation did not have a significant impact on fruit cracking (Table 6). Among the individual treatments, P_1 (*P. indica*-colonized tomato plants) had a slightly higher fruit cracking percentage (0.77%) compared to P_2 (non-colonized tomato plants) (0.53%), but this difference was not statistically significant. Similarly, the different levels of drought stress (D_1, D_2, D_3, D_4) did not lead to significant differences in fruit cracking. Also, when considering the treatment combinations, there were no significant differences in fruit cracking. The fruit cracking percentages for all treatment combinations fell within a narrow range and did not show any consistent pattern.

The yield per plant in tomato was significantly affected by both the colonization with *P. indica* and the levels of drought stress caused by limiting irrigation (Table 6). P_1, representing *P. indica*-colonized tomato plants, had a significantly higher yield per plant (401.11g) compared to P_2, representing non-colonized tomato plants (280.03 g). Among different drought stress levels, there was a significant decrease in yield as the level of stress increased. The control group (D_1) had the highest yield per plant (582.00 g), followed by D_2 (471.82g), D_3 (252.80 g), and D_4 (55.67g). This indicates that increasing drought stress resulted in a significant reduction in the yield of tomato plants. Among the treatment combinations, P_1D_1 (*P. indica*-colonized plants under control conditions) had the highest yield per plant (621.81g), which was significantly higher compared to P_2D_1 (Non-colonized plants under control conditions) (542.19 g). *P. indica* colonized plants under 75% FC (P_1D_2) yielded significantly more (541.4 g), compared to non-colonized plants under 75%F (P_2D_2) (412.75 g). Similar trend was also observed

under 50% and 25% FC, wherein colonized plants recorded higher yield compared to non -colonized plants (359.71 g and 92.04 g respectively).

4.1.3. Quality parameters

Quality parameters such as ascorbic acid, lycopene and TSS in tomato fruits were measured under various treatments. P_1 (colonized) had significantly higher ascorbic acid content (20.62 mg g^{-1}), lycopene content (12.58 mg g^{-1}) and TSS (4.58^{o}Brix) than P_2 (non-colonized) which measured 20.08 mg g^{-1} of ascorbic acid content, 12.49 mg g^{-1} of lycopene content and TSS recording 4.46^{o}Brix. All the three parameters varied across stress levels, with the highest recorded at the most severe stress (D_4 - 25% FC). Differences among drought stress treatments were statistically significant. Among treatment combinations, the differences were found to be non-significant.

4.1.5. Physiological parameters

Relative water content, cell membrane stability, and chlorophyll stability index exhibited significant variations among treatments (Table 8). *P. indica* colonization (P_1) consistently led to higher RWC (59.32%) compared to non-colonized plants (P_2) with 54.97%. RWC decreased as drought severity increased, with control (D_1) at 70.85% and D4 at 35.76%. Similarly, CMS was significantly influenced by treatments, where *P. indica* colonization (P_1) resulted in higher CMS (68.96%) compared to non-colonized plants (P_2) with 63.38%. CMS declined with increasing drought severity, from 81.36% in the control (D_1) to 44.03% in severe drought (D_4). Chlorophyll stability, as indicated by CSI, significantly varied among treatments, with P_1 displaying a higher CSI (89.59%) compared to P_2 (83.35%). Under different drought stress conditions, CSI values also significantly differed, with the control group (D_1) having the highest CSI (106.59%). CSI values decreased with decreasing irrigation levels, with D_2 at 93.16%, D_3 at 80.14%, and the lowest recorded in D_4 (66.00%). Among treatment combinations, P_1D_1 exhibited the highest CSI (113.19%), significantly surpassing P_2D_1 (control).

Proline content was analyzed in tomato plants under various treatments, including *P. indica* colonization and differing drought stress levels (Table 9). Proline content was significantly higher in *P. indica*-colonized plants (P_1) compared

to non-colonized plants (P_2). P_1 had a proline content of 10.33 µmol g^{-1}, while P_2 had a significantly lower content of 5.97 µmol g^{-1}. Proline content increased with the severity of drought stress. Under control conditions (D_1), it was 3.23 µmol g^{-1}. At 75% field capacity (D_2), proline content was 5.01 µmol g^{-1}, rising to 9.56 µmol g^{-1} at 50% field capacity (D_3), and reaching the highest level of 14.80 µmol g^{-1} at 25% field capacity (D_4). In P_1D_1 (*P. indica*-colonized plants under control conditions), proline content was 3.28 µmol g^{-1}. As drought stress increased, proline content also rose, reaching 5.84 µmol g^{-1} in P_1D_2 (*P. indica*-colonized plants under 75% field capacity), 11.96 µmol g^{-1} in P_1D_3, and 20.24 µmol g^{-1} in P_1 D_4, highlighting the significant impact of severe drought stress on proline accumulation in *P. indica*-colonized plants. For P_2D_1 (non-colonized plants under control conditions), proline content was 3.18 µmol g^{-1}, similar to P_1D_1. However, in P_2D_2 (4.18 µmol g^{-1}), P_2D_3 (7.16 µmol g^{-1}), and P_2D_4 (9.36 µmol g^{-1}), proline content gradually increased with rising drought stress. Notably, the increase in proline content in non-colonized plants was not as pronounced as in colonized plants.

Superoxide dismutase (SOD) activity in tomato plants was assessed under various treatments, including *P. indica* colonization and different levels of drought stress (Table 9). P_1 (*P. indica*-colonized plants) exhibited higher SOD activity at 260.07 mg g^{-1} fw compared to P_2 (non-colonized plants), which had an SOD activity of 233.65 mg g^{-1} fw. In D_1 (control conditions), the SOD activity was 214.44 mg g^{-1} fw. As drought stress severity increased, SOD activity also increased. In D_2 (75% field capacity), SOD activity reached 240.47 mg g^{-1} fw, and in D_3 (50% field capacity), it further rose to 261.85 mg g^{-1}fw. The highest SOD activity was observed in D_4 (25% field capacity) at 270.68 mg g^{-1} fw, signifying the impact of severe drought stress on SOD activation in both *P. indica*-colonized and non-colonized tomato plants. The increase in SOD activity was more pronounced in *P. indica*-colonized plants compared to non-colonized plants as drought stress severity increased.

There were significant effects of *P. indica* colonization and different levels of drought stress with respect to the peroxidase (PO) activity in tomato plants (Table 9). In P_1, the *P. indica*-colonized plants, the PO activity was 40.36 min^{-1} g^{-1} fw

irrespective of drought stress which was significantly higher than the PO activity of non-colonized plants (P_2) measuring only 32.01 min^{-1} g^{-1} fw. Under drought stress conditions, the PO activity showed significant difference. Under control condition (D_1) PO activity was measured to be 28.97 min^{-1} g^{-1} fw. In D_2 (75% field capacity), the PO activity was 39.20 min^{-1} g^{-1} fw, and in D_3 (50% field capacity), it further increased to 39.20 min^{-1} g^{-1} fw. Highest PO activity was measured at 25%FC (D_4) measuring 43.70 min^{-1} g^{-1} fw. Among the treatment combinations, despite the fact that increasing drought stress led to an elevated PO activity in both colonized and non-colonized plants, the percentage increase was notably higher in the colonized plants.

Similar to PO and SOD, CAT activity was influenced by *P. indica* colonization. P_1 (colonized plants) had substantially higher CAT activity (301.21 units min^{-1} g^{-1} fw) compared to the non-colonized plants (247.07 units min^{-1} g^{-1} fw). Similarly, as the stress level increased fromD_1 to D_4, CAT activity also increased from 234.97 to 341.28 units min^{-1} g^{-1} fw. The results showed significant variations in CAT activity among the treatment combinations (Table 9). Under control conditions (D_1), P_1 exhibited a CAT activity of 252.44 units min^{-1} g^{-1} fw, whereas P_2 had a lower CAT activity of 217.51 units min^{-1} g^{-1} fw. In both P_1 and P_2, the CAT activity decreased with increasing severity of drought stress. In P_1, the CAT activity increased from 252.44 units min^{-1} g^{-1} fw in D_1 to 393.66 units min^{-1} g^{-1} fw in D_4. Similarly, in P_2, the CAT activity increased from 217.51 units min^{-1} g^{-1} fw in D_1 to 288.91 units min^{-1} g^{-1} fw in D_4. The results revealed that *P. indica* colonized plants maintained a very high CAT activity than non-colonized plants as the level of drought stress increased from 100 to 25%FC.

Table 2. Effect of *P. indica* on plant height, stem grith and primary branches per plant of tomato under drought stress by limiting irrigation

Treatments	Plant height (cm)		Stem grith (cm)		Primary branches / plant
	30 DAT	60 DAT	30 DAT	60 DAT	
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	40.92±2.70[a]	59.58±6.00[a]	3.26±0.25[a]	3.84±0.36[a]	5.56±0.96[a]
P_2 - Non-colonized tomato plants	33.76±2.60[b]	55.78±7.12[b]	2.32±0.40[b]	3.19±0.28[b]	2.69±0.79[b]
C.D (0.05)	1.21	1.14	0.10	0.10	0.45
S. E. m (±)	0.50	0.39	0.03	0.05	0.22
Drought stress by limiting irrigation					
D_1-Control	40.43±3.91[a]	69.70±2.30[a]	3.17±0.46[a]	3.88±0.36[a]	4.88±1.32[a]
D_2-75% FC	38.85±5.90[a]	61.75±1.81[b]	2.90±0.38[b]	3.68±0.39[b]	4.50±1.20[a]
D_3-50% FC	36.79±3.70[c]	55.55±3.42[c]	2.67±0.57[c]	3.39±0.40[c]	3.63±1.50[b]
D_4-25% FC	33.30±3.09[d]	43.52±2.70[d]	2.41±0.66[d]	3.11±0.32[d]	3.50±1.41[b]
C.D (0.05)	1.71	1.61	0.14	0.14	0.64
S. E. m (±)	0.59	0.56	0.05	0.07	0.31
Treatment combinations					
P_1 D_1	43.80±1.90[a]	71.00±2.50[a]	3.58±0.16[a]	4.40±0.08	6.00±0.81[a]
P_1 D_2	44.20±2.01[a]	63.00±1.00[c]	3.24±0.11[b]	3.82±0.15	6.50±0.57[a]
P_1 D_3	40.20±2.80[b]	59.14±1.00[d]	3.20±0.15[bc]	3.74±0.22	5.00±0.81[b]
P_1 D_4	35.50±0.70[cd]	44.78±2.35[f]	3.02±0.16[c]	3.38±0.19	4.75±0.50[b]
P_2 D_1	37.00±1.30[c]	68.40±1.13[b]	2.76±0.15[d]	3.54±0.05	3.75±0.50[c]
P_2 D_2	33.50±2.30[de]	60.50±2.10[d]	2.56±0.15[e]	3.32±0.08	2.50±0.57[d]
P_2 D_3	33.40±1.60[de]	51.96±0.67[e]	2.14±0.11[f]	3.04±0.11	2.25±0.50[d]
P_2 D_4	31.10±1.00[e]	42.26±0.80[g]	1.80±0.15[g]	2.84±0.11	2.25±0.50[d]
C.D (0.05)	2.42	2.27	0.19	N.S	0.90
S. E. m (±)	0.84	0.79	0.06	0.10	0.43

Table 3. Effect of *P. indica* on leaf length, leaf width and leaf area of tomato under drought stress by limiting irrigation

Treatments	Leaf length (cm)		Leaf width(cm)		Leaf area (cm^2)	
	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT
Colonizing with *P. indica*						
P_1 - *P. indica*-colonized tomato plants	21.5±1.6^{a}	27.3±2.6^{a}	15.3±1.3^{a}	22.7±1.3^{a}	333.0±10^{a}	624.2±11.0^{a}
P_2 - Non-colonized tomato plants	17.2±2.1^{b}	24.0±1.8^{b}	12.1±1.5^{b}	15.1±1.2^{b}	204.6±15^{b}	373.8±9.8^{b}
C.D (0.05)	0.72	0.92	0.39	0.74	11.89	27.37
S. E. (m)	0.35	0.45	0.19	0.36	5.81	13.37
Drought stress by limiting irrigation						
D_1-Control	21.0±2.4^{a}	29.3±1.7^{a}	15.3±1.5^{a}	22.6±1.5^{a}	325.9±19^{a}	663.7±12.0^{a}
D_2-75% FC	20.4±2.0^{a}	27.1±2.1^{b}	14.3±1.9^{b}	19.6±1.2^{b}	290.8±18^{b}	537.3±8.9^{b}
D_3-50% FC	18.8±2.3^{b}	24.6±2.4^{c}	13.2±1.1^{c}	17.6±1.4^{c}	249.4±13^{c}	443.4±14.1^{c}
D_4-25% FC	17.1±2.3^{c}	21.6±1.7^{d}	11.9±1.6^{d}	15.8±1.30^{d}	209.0±19^{d}	351.7±11.0^{d}
C.D (0.05)	1.02	1.30	0.56	1.05	16.81	38.70
S. E. (m)	0.50	0.63	0.27	0.51	8.22	18.91
Treatment combinations						
P_1 D_1	22.6±2.3^{a}	29.6±2.0^{a}	16.5±0.7^{a}	25.5±1.7^{a}	375.8±13^{a}	756.2±18^{a}
P_1 D_2	22.0±1.3^{a}	28.8±0.8^{a}	16.1±0.5^{a}	23.6±0.5^{b}	355.3±22^{b}	680.1±8.4^{b}
P_1 D_3	21.5±0.7^{a}	26.7±1.6^{b}	15.2±0.2^{b}	21.8±1.1^{c}	318.4±12^{c}	582.5±9.7^{c}
P_1 D_4	19.9±0.5^{b}	24.0±1.4^{c}	13.4±0.6^{c}	19.9±0.7^{d}	282.4±16^{d}	478.1±13.1^{d}
P_2 D_1	19.4±1.1^{b}	28.9±1.5^{a}	14.2±1.1^{c}	19.8±2.1^{d}	276.0±13^{d}	571.2±17.0^{c}
P_2 D_2	18.7±0.7^{b}	25.4±1.4^{bc}	12.4±0.3^{d}	15.5±0.4^{e}	226.3±3.7^{e}	394.5±10.4^{e}
P_2 D_3	16.2±0.2^{c}	22.5±0.8^{c}	11.2±0.2^{e}	13.5±0.4^{f}	180.3±4.2^{f}	304.4±9.7^{f}
P_2 D_4	14.3±0.6^{d}	19.3±1.1^{d}	10.4±0.4^{f}	11.7±0.6^{g}	135.6±4.1^{g}	225.3±8.5^{g}

C.D (0.05)	1.45	1.83	0.79	1.48	23.78	54.73
S. E. (m)	0.71	0.90	0.39	0.73	11.62	26.75

Table 4. Effect of *P. indica* on days to first and 50% flowering, flower clusters per plant and flowers per clusters of tomato under drought stress by limiting irrigation

Treatments	Days of first flowering	Days of 50% flowering	Flower clusters per plant	Flowers per cluster
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	29.91 ± 2.07^{b}	56.84 ± 1.79	6.12 ± 0.47^{a}	7.85 ± 2.15^{a}
P_2 - Non-colonized tomato plants	31.70 ± 2.20^{a}	57.58 ± 1.54	3.57 ± 1.85^{b}	6.35 ± 2.09^{b}
C.D (0.05)	0.73	N.S	0.36	0.29
S. E. (m)	0.25	0.27	0.12	0.10
Drought stress by limiting irrigation				
D_1-Control	34.69 ± 2.71^{a}	63.27 ± 1.38^{a}	7.17 ± 1.69^{a}	8.45 ± 0.79^{a}
D_2-75% FC	32.83 ± 2.15^{b}	59.50 ± 1.40^{b}	6.31 ± 1.72^{b}	8.74 ± 0.91^{a}
D_3-50% FC	29.14 ± 1.04^{c}	55.80 ± 1.90^{c}	3.93 ± 1.56^{bc}	7.54 ± 1.08^{b}
D_4-25% FC	26.58 ± 1.30^{d}	50.24 ± 1.70^{d}	1.97 ± 0.80^{c}	3.68 ± 0.80^{c}
C.D (0.05)	1.03	1.09	0.52	0.41
S. E. (m)	0.36	0.38	0.18	0.14
Treatment combinations				
P_1 D_1	32.38 ± 0.54^{c}	62.24 ± 1.07^{b}	8.66 ± 0.83^{a}	9.08 ± 0.39
P_1 D_2	31.04 ± 0.94^{c}	58.60 ± 1.30^{d}	7.86 ± 0.45^{b}	9.54 ± 0.45
P_1 D_3	28.90 ± 0.65^{d}	54.46 ± 1.00^{e}	6.00 ± 0.84^{c}	8.44 ± 0.62
P_1 D_4	27.35 ± 0.22^{e}	52.08 ± 1.33^{f}	5.32 ± 0.52^{c}	4.36 ± 0.40
P_2 D_1	37.00 ± 1.70^{a}	64.30 ± 0.71^{a}	5.68 ± 0.46^{d}	7.83 ± 0.55
P_2 D_2	34.62 ± 1.25^{b}	60.40 ± 0.89^{c}	4.76 ± 0.68^{d}	7.94 ± 0.26
P_2 D_3	29.38 ± 1.37^{d}	57.22 ± 1.68^{d}	2.64 ± 0.35^{c}	6.64 ± 0.47
P_2 D_4	25.82 ± 1.52^{f}	48.40 ± 1.32^{g}	1.30 ± 0.44^{d}	3.00 ± 0.35
C.D (0.05)	1.46	1.55	0.73	N.S
S. E. (m)	0.50	0.53	0.25	0.20

Table 5. Effect of *P. indica* on days to first harvest, fruit per truss, fruit per plant and fruit set % of tomato under drought stress by limiting irrigation

Treatments	Days of first harvest	Fruit per truss	Fruits per plant	Fruit set %
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	58.65±3.73^{b}	5.02±2.31^{a}	14.89±6.45^{a}	59.58±15.62^{a}
P_2 - Non-colonized tomato plants	60.22±3.60^{a}	3.44±1.94^{b}	11.03±6.97^{b}	47.95±10.35^{b}
C.D (0.05)	0.70	0.35	0.81	5.16
S. E. (m)	0.24	0.12	0.28	1.79
Drought stress by limiting irrigation				
D_1-Control	65.82±2.72^{a}	6.04±1.13^{a}	19.92±1.62^{a}	71.19±9.62^{a}
D_2-75% FC	62.06±1.49^{b}	5.79±1.28^{a}	17.15±2.05^{b}	65.78±10.33^{a}
D_3-50% FC	58.56±1.13^{c}	4.19±0.87^{b}	11.50±1.87^{c}	55.29±5.44^{b}
D_4-25% FC	52.30±1.46^{d}	0.91±0.63^{c}	3.28±1.69^{d}	22.79±13.88^{c}
C.D (0.05)	0.99	0.49	1.15	7.30
S. E. (m)	0.35	0.17	0.40	2.53
Treatment combinations				
P_1 D_1	62.50±0.93^{b}	6.94±0.75^{a}	20.80±1.64^{a}	76.56±8.90
P_1 D_2	61.00±1.09^{c}	6.94±0.26^{a}	18.80±1.44^{b}	72.95±5.74
P_1 D_3	57.85±0.82^{e}	4.78±0.82^{b}	15.20±1.03^{c}	56.40±6.32
P_1 D_4	53.26±1.02^{f}	1.42±0.34^{d}	4.76±0.80^{e}	32.40±5.93
P_2 D_1	67.14±1.54^{a}	5.14±0.54^{b}	19.04±1.13^{b}	65.82±7.56
P_2 D_2	63.12±0.99^{b}	4.64±0.59^{b}	15.50±0.79^{c}	58.62±8.80
P_2 D_3	59.28±0.97^{d}	3.60±0.42^{c}	7.80±2.01^{d}	54.19±4.70
P_2 D_4	51.34±1.21^{g}	0.41±0.19^{e}	1.80±0.50^{f}	13.19±5.72
C.D (0.05)	1.41	0.70	1.63	N.S
S. E. (m)	0.49	0.24	0.56	3.58

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 6. Effect of *P. indica* on fruit length, fruit width, fruit weight, fruit cracking % and yield per plant of tomato under drought stress by limiting irrigation

Treatments	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit cracking %	Yield per plant (g)
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	3.61±0.61^{a}	3.38±0.47^{a}	23.15±1.97^{a}	0.77±0.18	401.11±15.6^{a}
P_2 - Non-colonized tomato plants	3.04±0.58^{b}	2.91±0.62^{b}	19.19±1.34^{b}	0.53±0.27	280.03±10.6^{b}
C.D (0.05)	0.08	0.06	0.77	N.S	29.26
S. E. (m)	0.03	0.02	0.26	0.39	10.16
Drought stress by limiting irrigation					
D_1-Control	3.91±0.27^{a}	3.73±0.17^{a}	25.49±1.34^{a}	1.50±0.57	582.00±5.31^{a}
D_2-75% FC	3.66±0.37^{b}	3.48±0.22^{b}	24.28±1.10^{b}	0.00±0.00	471.82±5.30^{b}
D_3-50% FC	3.31±0.37^{c}	3.01±0.31^{c}	20.67±1.28^{c}	1.11±0.35	252.80±6.20^{c}
D_4-25% FC	2.41±0.25^{d}	2.35±0.34^{d}	14.25±1.70^{d}	0.00±0.00	55.67±4.10^{d}
C.D (0.05)	0.11	0.09	1.09	N.S	41.39
S. E. (m)	0.04	0.03	0.37	0.55	14.36
Treatment combinations					
P_1 D_1	4.14±0.17^{a}	3.86±0.09^{a}	26.64±0.46^{a}	2.00±0.37	621.81±10.4^{a}
P_1 D_2	3.99±0.14^{a}	3.68±0.08^{b}	25.34±0.60^{ab}	0.00±0.00	530.89±8.30^{b}
P_1 D_3	3.66±0.05^{b}	3.30±0.01^{c}	22.74±0.71^{d}	1.11±0.37	359.71±9.61^{c}
P_1 D_4	2.64±0.08^{e}	2.66±0.09^{d}	17.90±0.74^{e}	0.00±0.00	92.04±6.50^{d}
P_2 D_1	3.68±0.11^{b}	3.60±0.12^{b}	24.34±0.74^{bc}	1.00±0.23	542.19±7.60^{b}
P_2 D_2	3.32±0.13^{c}	3.28±0.08^{c}	23.22±0.54^{cd}	0.00±0.00	412.75±6.50^{c}
P_2 D_3	2.96±0.11^{d}	2.72±0.08^{d}	18.60±1.21^{e}	1.12±0.09	145.90±8.20^{d}
P_2 D_4	2.18±0.08^{f}	2.04±0.15^{e}	10.60±1.10^{f}	0.00±0.00	19.30±7.22^{e}
C.D (0.05)	0.15	0.13	1.20	N.S	58.53
S. E. (m)	0.05	0.04	0.59	0.79	20.32

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 7. Influence of *P. indica* on quality parameters of tomato under drought stress by limiting irrigation

Treatments	Ascorbic acid (mg g^{-1})	Lycopene (mg g^{-1})	TSS (° Brix)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	20.62 ± 0.70^{a}	12.58 ± 0.22^{a}	4.58 ± 0.25^{a}
P_2 - Non-colonized tomato plants	20.08 ± 0.73^{b}	12.49 ± 0.25^{b}	4.46 ± 0.27^{b}
C.D (0.05)	0.11	0.09	0.07
S. E. (m)	0.04	0.03	0.03
Drought stress by limiting irrigation			
D_1-Control	19.51 ± 0.22^{d}	12.24 ± 0.24^{c}	4.19 ± 0.15^{d}
D_2-75% FC	19.93 ± 0.31^{c}	12.52 ± 0.08^{b}	4.41 ± 0.06^{c}
D_3-50% FC	20.50 ± 0.43^{b}	12.61 ± 0.08^{ab}	4.67 ± 0.09^{b}
D_4-25% FC	21.45 ± 0.35^{a}	12.76 ± 0.08^{a}	4.80 ± 0.15^{a}
C.D (0.05)	0.16	0.13	0.10
S. E. (m)	0.05	0.04	0.04
Treatment combinations			
$P_1 D_1$	19.71 ± 0.13^{ef}	12.30±0.24	4.24±0.14
$P_1 D_2$	20.19 ± 0.18^{e}	12.55±0.05	4.49±0.07
$P_1 D_3$	20.86 ± 0.25^{c}	12.67±0.05	4.75±0.06
$P_1 D_4$	21.71 ± 0.21^{a}	12.78±0.06	4.84±0.07
$P_2 D_1$	19.32 ± 0.07^{f}	12.19±0.025	4.14±0.16
$P_2 D_2$	19.66 ± 0.14^{e}	12.49±0.10	4.33±0.18
$P_2 D_3$	20.15 ± 0.19^{d}	12.54±0.08	4.59±0.04
$P_2 D_4$	21.19 ± 0.16^{b}	12.74±0.09	4.77±0.04
C.D (0.05)	N.S	N.S	N.S
S. E. (m)	0.08	0.06	0.05

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 8. Impact of *P. indica* physiological parameters in tomato under drought stress by limiting irrigation

Treatments	Relative water content (%)	Cell Membrane Stability (%)	Chlorophyll Stability Index (%)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	59.32±10.82[a]	68.96±12.64[a]	89.59±18.72[a]
P_2 - Non-colonized tomato plants	54.97±12.29[b]	63.38±16.40[b]	83.35±12.80[b]
C.D (0.05)	0.77	0.96	1.76
S. E. (m)	0.25	0.33	0.61
Drought stress by limiting irrigation			
D_1-Control	70.85±1.65[a]	81.36±1.77[a]	106.59±7.20[a]
D_2-75% FC	65.80±2.83[b]	74.57±1.87[b]	93.16±4.33[b]
D_3-50% FC	56.18±3.53[c]	64.71±4.19[c]	80.14±5.66[c]
D_4-25% FC	35.76±2.58[d]	44.03±5.86[d]	66.00±4.13[d]
C.D (0.05)	1.09	1.37	2.48
S. E. (m)	0.36	0.47	0.86
Treatment combinations			
P_1 D_1	72.20±0.60[a]	81.89±1.43[a]	113.18±2.82[a]
P_1 D_2	67.86±0.55[c]	76.04±0.65[b]	96.89±2.37[b]
P_1 D_3	59.36±0.75[e]	68.56±1.52[d]	84.70±3.49[d]
P_1 D_4	37.86±0.63[g]	49.34±1.58[f]	68.40±4.63g
P_2 D_1	69.50±1.00[b]	80.84±2.08[a]	100.0±0.00[b]
P_2 D_2	63.73±0.68[d]	73.11±1.44[c]	89.44±1.40[c]
P_2 D_3	53.00±0.50[f]	60.86±0.40[e]	75.58±2.82[e]
P_2 D_4	33.66±1.75[h]	38.72±2.04[g]	63.60±1.55[f]
C.D (0.05)	1.55	1.93	3.52
S. E. (m)	0.52	0.67	1.22

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 9. Impact of *P. indica* on proline accumulation and anti-oxidant activities in tomato under drought stress by limiting irrigation

Treatments	Proline (µmol g^{-1})	Superoxide dismutase (mg g^{-1}fw)	Peroxidase (min $^{-1}$g $^{-1}$ fw)	Catalase (units min^{-1} g^{-1} fw)
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	10.33±0.79^{a}	260.07±13.27^{a}	40.36±8.07^{a}	301.21±25.81^{a}
P_2 - Non-colonized tomato plants	5.97±0.55^{b}	233.65±15.79^{b}	32.01±4.02^{b}	247.07±19.64^{b}
C.D (0.05)	0.61	4.80	1.14	7.27
S. E. (m)	0.21	1.59	0.38	2.42
Drought stress by limiting irrigation				
D_1-Control	3.23±0.38^{d}	214.44±4.61^{d}	28.97±2.44^{d}	234.97±15.91^{d}
D_2-75% FC	5.01±0.86^{c}	240.47±7.74^{c}	39.20±3.14^{c}	248.75±18.70^{c}
D_3-50% FC	9.56±0.76^{b}	261.85±8.10^{b}	32.88±5.37^{b}	271.56±21.21^{b}
D_4-25% FC	14.80±0.76^{a}	270.68±8.12^{a}	43.70±7.98^{a}	341.28±28.60^{a}
C.D (0.05)	0.86	6.78	2.29	10.28
S. E. (m)	0.30	2.24	0.76	3.43
Treatment combinations				
P_1 D_1	3.28±0.41^{f}	218.23±2.40^{e}	30.97±0.85^{d}	252.44±6.11^{e}
P_1 D_2	5.84±0.78^{e}	247.33±4.30^{c}	35.59±0.50^{c}	268.53±7.60^{d}
P_1 D_3	11.96±1.10^{b}	279.32±3.50^{b}	44.08±0.66^{b}	290.23±7.50^{b}
P_1 D_4	20.24±1.21^{a}	295.38±4.50^{a}	50.81±2.54^{a}	393.66±7.70^{a}
P_2 D_1	3.18±0.39^{f}	210.64±2.11^{e}	26.96±1.45^{e}	217.51±11.90^{f}
P_2 D_2	4.18±0.26^{f}	233.61±5.70^{d}	30.16±1.53^{d}	228.95±7.72
P_2 D_3	7.16±0.50^{d}	244.37±5.80^{c}	34.33±0.66^{c}	252.89±8.70^{e}
P_2 D_4	9.36±0.80^{c}	245.97±5.90^{c}	36.60±1.09^{c}	288.91±5.90^{c}
C.D (0.05)	1.22	9.59	2.29	14.55
S. E. (m)	0.42	3.17	0.76	4.85

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

- P. indica *+ P. indica*

100% FC

- P. indica *+ P. indica*

75% FC

- P. indica *+ P. indica*

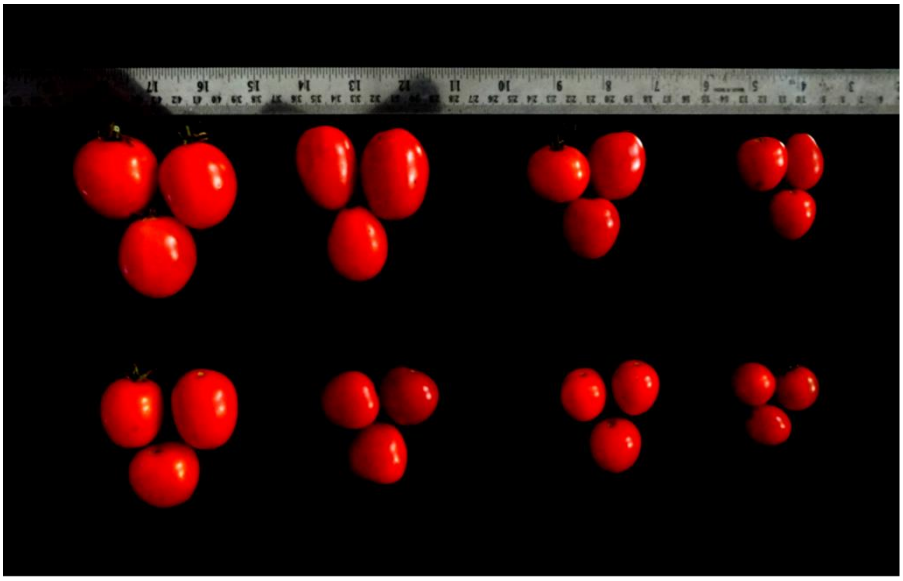
50% FC

- P. indica *+ P. indica*

25% FC

Plate 3. Tomato plants at 30 DAT under drought stress induced by limiting irrigation

Plates 4. Leaves of *P. indica* colonized and non-colonized tomato @30DAT under drought stress induced by limiting irrigation



Plates 5. Fruits harvested from *P. indica* colonized and non-colonized plants under different levels of drought stress by limiting irrigation

4.2. EVALUATION OF TOMATO PLANTS COLONIZED WITH *P. indica* UNDER DROUGHT STRESS STIMULATED BY PEG

4.2.1. Vegetative and flowering characters

The effect of *P. indica* colonization on plant height under drought stress induced by PEG treatment is summarized in Table 10. At 30 DAT, P_1 displayed significantly greater plant height at 40.48 cm compared to P_2, which had a height of 32.99 cm, regardless of drought stress levels. At 60 DAT, P_1 continued to exhibit a taller plant height of 54.39 cm compared to P_2 at 50.23 cm. Regarding the impact of drought stress, there was a consistent trend of reduced plant height as stress levels increased from D_1 to D_4. At 30 DAT, the plant height at 100% FC (D_1) was 41.00 cm, significantly taller than in the D_2 treatment (39.28 cm). The height further decreased to 36.76 cm at D_3 and 29.91 cm at D_4. Similarly, at 60 DAT, D_1 had significantly greater plant height at 67.62 cm, which progressively decreased to 31.83 cm at D_4. The combination of *P. indica* colonization and drought stress had a significant effect on plant height at both 30 and 60 DAT (Plate 6).

The effects of *P. indica* colonization and different drought stress levels significantly impact stem girth in tomato plants (Table 10). At 30 days after transplanting (DAT), P_1 exhibited a stem girth of 2.91 cm, significantly larger than P_2 with 2.16 cm. As for drought stress, increasing stress from D_1 to D_4 generally led to reduced stem girth. At 30 DAT, the control treatment (D_1) had a stem girth of 3.04 cm, significantly greater than the stem girth at D_4 (1.85 cm). Among the treatment combinations, the highest stem girth was observed in both the P_1D_1 and P_1D_2 treatment combinations, measuring 3.50 cm and 3.34 cm, respectively. These two treatments were significantly similar to each other and higher than the others. As drought stress increased from D_1 to D_4, there was a significant decrease in stem girth, regardless of *P. indica* colonization. Comparing the results, it's evident that P_1 maintained a significantly larger stem girth under control (3.50 cm), 75% FC (3.34 cm), 50% FC (2.60 cm), and 25% FC (2.20 cm) conditions compared to non-colonized control plants.

The results showed significant variations in the number of primary branches among different treatments (Table 10). *P. indica* colonization in the P_1 treatments led to a higher number of primary branches (5.38) compared to non-colonized plants (2.88). Regarding the combinations, the P_1D_1 combination (*P. indica*

colonization under control conditions) had the highest number of primary branches, averaging 7.00 branches per plant. As the drought stress level increased, both P_1 and P_2 treatments exhibited a gradual decrease in the number of primary branches. The lowest number of primary branches was observed in the P_2D_4 treatment combination, averaging 1.50 branches per plant. *P. indica* colonization resulted in a comparatively higher number of primary branches per plant at all levels of irrigation compared to non-colonized plants.

The effect of different treatments (Drought induced by PEG and *P. indica*) on leaf length, width and area in tomato plants is represented in table 11. At 30 DAT, P_1 recorded substantially higher leaf length (21.07 cm), width (14.53 cm), and area (307.64 cm^2) compared to P_2 with an average of 18.24 cm, 13.23 cm, and 202.72 cm^2 of leaf length, width and area respectively. This trend was followed at 60 DAT also. These results indicate that the colonization with *P. indica* positively influenced leaf area in tomato plants. Among all drought stress levels, at both 30 DAT and 60 DAT, D_1 exhibited larger leaves, with area measuring 302.78 cm^2 and 574.23cm^2 respectively. Increasing the stress level to D_4 (control) resulted in a drastic reduction in leaf area measuring 201.29 cm^2 and 275.28 cm^2 at 30 and 60 DAT respectively. The combination of treatments further influenced leaf area in tomato plants. Among the treatment combinations, P_1D_1 exhibited the largest leaf area at both 30 DAT (336.17 cm^2) and 60 DAT (600.10 cm^2).

P. indica-colonized tomato plants, exhibited duration of 28.81 days to first flowering compared to the P_2 treatment with non-colonized tomato plants, which took 28.33 days. This difference was not statistically significant. However, days to 50% flowering varied significantly with regard to colonization leading to only 53.95 days to reach 50% flowering in P_1, while the P_2 treatment with non-colonized tomato plants took 56.15 days. The drought stress treatments demonstrated a significant effect on the days to first flowering as well as 50% flowering. The control treatment (D_1) resulted in the delayed flowering, occurring at 33.87 days, while the plants subjected to increasing levels of drought stress by applying PEG (D_2: -3 bar, D_3: -7 bar, D_4: -10 bar) showed accelerated flowering: 29.69 days, 26.45 days, and 24.27 days, respectively. The interaction of treatments influenced the

days to first flowering and 50% flowering. Under control (D_1) *P. indica* colonized plants reached the first flowering by 32.64 days where as non-colonized plants took more days (35.10 days). As the stress level increased to -3bar and -7bar, there were no significant difference between P_1 and P_2. These findings suggest that the combined effects of *P. indica* colonization and drought stress influenced the timing of the first flowering event in tomato plants. With respect to days to 50% flowering, under conditions characterized as control (D_1), mild stress (D_2), and moderate stress (D_3), it was observed that plants that underwent colonization by *P. indica* exhibited a reduced duration for attaining 50% flowering (measuring 58.40, 57.00, and 52.80 days, respectively) (Table 12).

The results revealed that colonization with *P. indica* significantly influenced the number of flower clusters per plant and flowers per cluster in tomato plants. The P_1 treatment, consisting of *P. indica*-colonized tomato plants, exhibited an average of 5.74 flower clusters per plant and 7.34 flowers per cluster, while the P_2 treatment with non-colonized tomato plants had an average of 3.30 flower clusters per plant and 5.80 flowers per cluster. The drought stress treatments also had a significant effect on the number of flower clusters and flowers per cluster. As the water stress increased from D_1 to D_4, the number of flower clusters decreased from 6.63 to 1.82 while flowers per cluster decreased from 8.25 to 3.28. Among the treatment combinations, no distinct pattern or significant differences were observed.

4.2.2 Fruit and yield characters

The data analysis revealed a consistent and significant effect of drought stress induced by PEG application and *P. indica* colonization on the days to first harvest in tomato plants (Table 13). The colonization with *P. indica* had a significant effect on the days to first harvest in tomato plants. The P_1 treatment, which involved *P. indica*-colonized tomato plants, had an average of 55.05 days until the first harvest, whereas the P_2 treatment with non-colonized tomato plants had an average of 56.75 days. There were significant differences observed in the days to first harvest under drought stress. The control treatment (D_1) and -3 bar stress level (D_2) exhibited similar average values of 63.90 and 62.50 days, respectively. However, -7 bar stress level (D_3) resulted in a significantly shorter

duration of 54.20 days until the first harvest, and -10 bar stress level (D_4) further reduced the duration to 43.00 days. when examining the treatment combinations, there were significant variations in the days to first harvest. Among the treatment combinations, the P_2D_1 and P_2D_2 combinations had the longest durations until the first harvest, with averages of 66.60 days. On the other hand, the P_2D_4 combination had the shortest duration, with an average of 40.60 days. the results indicate that colonization with *P. indica* accelerated the days to first harvest in tomato plants under control and mild stress level (-3bar).

The results demonstrated a significant difference in the number of fruits per truss and fruits per plant between the treatments involving colonization with *P. indica and* non-colonized plants. The P_1 treatment, which comprised *P. indica*-colonized tomato plants, exhibited an average of 4.73 fruits per truss and 15.57 fruits per plant. In difference, the P_2 treatment, consisting of non-colonized tomato plants, had a lower average of 3.21 fruits per truss and 11.35 fruits per plant. Regarding the drought stress treatments, there were significant variations observed in the number of fruits. The control treatment (D_1) significantly higher average number of 6.06 fruits per truss and 21.64 fruits per plant. Analyzing the treatment combinations, there were significant differences in the number of fruits per truss and the P_1D_1 combination had the highest average with 6.90 fruits per truss, followed by P_1D_2 with 7.04 fruits per truss. On the other hand, the P_2D_1 combination had only 5.08 fruits per truss. At D_2 (-3bar) and D_3 (-7bar) also, colonized plants had significantly a greater number of fruits (6.30 and 4.58 respectively). However, at -10 bar (D_4), there was no significant difference between P_1 and P_2 with respect to number of fruits per truss (Table 13).

The data summarized in Table 13 elucidates the effect of different treatments on fruit set percentage in tomato plants. The results revealed significant variances in fruit set percentage with respect to colonization with *P. indica*. The P_1 treatment, consisting of *P. indica*-colonized tomato plants, exhibited an average fruit set percentage of 59.45%. In contrast, the P_2 treatment, comprising non-colonized tomato plants, had a lower average fruit set percentage of 45.93%.

Among the treatment combinations, the fruit set ranged from 78.27% in P_1D_1 to 16.43% in P_2D_4.

Notably, *P. indica* colonization had a positive influence on both fruit length and diameter. In the P_1 treatment, involving *P. indica*-colonized tomato plants, the average fruit length measured 3.30 cm, while in the P_2 treatment with non-colonized tomato plants, it was slightly shorter (3.11 cm). Similarly, examining fruit diameter, *P. indica* colonization exhibited a favorable outcome in tomato plants. The P_1 treatment yielded an average fruit diameter of 2.94 cm, surpassing the slightly narrower average of 2.52 cm observed in the P_2 treatment, consisting of non-colonized tomato plants. These results underscore the positive impact of *P. indica* colonization on fruit dimensions, contributing to larger fruits in colonized plants compared to their non-colonized counterparts (Plate 7).

The results demonstrated that colonization with *P. indica* positively influenced fruit weight and yield in tomato plants (Table 14). *P. indica*-colonized tomato plants, had an average fruit weight of 20.82 g and an yield of 377.94 g per plant, while the P_2 treatment, comprising non-colonized tomato plants, exhibited a substantially lower average fruit weight of 16.17 g and yield of 275.69 g per plant. In terms of the drought stress treatments, significant variations were observed. The control treatment (D_1) had an average fruit weight of 25.30 g and average yield per plant of 585.53 g, while -3 bar stress level (D_2) showed lower average of 21.30 g and yield per plant of 466.14 g. Further increases in drought stress again resulted in a significant decrease in fruit weight. At -7 bar stress level (D_3) an average fruit weight of 18.04 g and yield per plant of 237.24 g, and at -10 bar stress level (D_4) lowest average with 9.35 g and yield per plant of 18.37 g were recorded. With respect to the treatment combinations, significant differences were observed in fruit weight and yield. Among the treatment combinations, *P. indica* colonized (P_1) groups exhibited significantly higher fruit weight and yield per plant across different levels of water stress compared to the control. Highest fruit weight (26.66 g) and yield (679.50 g) were recorded in P_1D_1

The results revealed that there were no significant differences in fruit cracking between the treatments involving colonization with or without *P. indica*

and drought stress (Table 14). The P_1 treatment, consisting of *P. indica*-colonized tomato plants, exhibited an average fruit cracking rate of 0.99%, while the P_2 treatment, comprising non-colonized tomato plants, had a lower average rate of 0.22%. Variation in fruit cracking was found to be insignificant, following an irregular pattern with respect to the individual and combination effect of the different treatments.

4.2.4. Quality parameters

The results showed a significant difference in the ascorbic acid content between the colonized and non-colonized plants (Table 15). The P_1 treatment, consisting of *P. indica*-colonized tomato plants, had an average ascorbic acid content of 20.68 mg g^{-1}, while the P_2 treatment of non-colonized tomato plants had an average ascorbic acid content of 20.26 mg g^{-1}. With respect to treatment combinations, no significant differences were observed in the ascorbic acid content. The ascorbic acid content ranged from 19.29 mg g^{-1} (P_2D_1 combination) to 21.83 mg g^{-1} (P_1D_4 combination).

The results also revealed a significant difference in the lycopene content and TSS between content between the fruits of colonized and non-colonized tomato plants. The P_1 treatment, consisting of *P. indica*-colonized tomato plants, exhibited a significantly higher average lycopene content of 12.35 mg g^{-1} and TSS content with an average of 4.20 °Brix while the P_2 treatment of non-colonized tomato plants had an average lycopene content of only 12.20 mg g^{-1} and TSS of 4.09 °Brix (Table 15).

4.2.5. Physiological parameters

The results revealed significant differences in the RWC, CMS and CSI between the different treatments (Table 16). P_1, comprising *P. indica*-colonized tomato plants, exhibited an average RWC of 59.03 %, CMS of 70.08%. and CSI of 88.14% while P_2, representing non-colonized tomato plants, had only an average RWC of 53.35 %, CMS of 64.62% and CSI of 79.05%. As the concentration of applied PEG increased, all the three parameters decreased for both colonized and non-colonized tomato plants. However, this decline was prominent in non-

colonized plants while *P. indica* colonized plants maintained a significantly higher RWC at all levels of drought stress induced by PEG.

The proline content in tomato plants under different treatments, including colonization with *P. indica* and drought stress induced by PEG, was analyzed (Table 17). Significant differences were observed in the proline content between the colonization treatments. P_1, representing *P. indica*-colonized tomato plants, exhibited a higher proline content of 9.17 µmol g^{-1}. In contrast, P_2, consisting of non-colonized tomato plants, had a relatively lower proline content of 5.86 µmol g^{-1}. By comparing the combinations, it is evident that proline accumulation in *P. indica* colonized plants was very high compared to the control plants as the level of water stress increased.

By referring to Table 17 significant differences were observed in anti-oxidant activities between the colonization treatments. P_1, representing *P. indica*-colonized tomato plants, exhibited a higher SOD activity of 269.20 mg g^{-1} fw. In contrast, P_2, consisting of non-colonized tomato plants, showed a relatively lower SOD activity of 236.33 mg g^{-1} fw. Similarly P_1 recorded the highest peroxidase (40.02 min^{-1} g^{-1} fw) and CAT activity (303.58units min^{-1} g^{-1} fw). As the water stress increased to D_2, D_3 and D_4, anti-oxidant enzyme activities were increased in both P_1 and P_2, but the enhancement was significantly higher in colonized plants compared to the non-colonized plants.

Table 10. Effect of *P. indica* on plant height, stem girth and number of primary branches in tomato under drought stress simulated by PEG application

Treatments	Plant height (cm)		Stem grith (cm)		Primary branches / plant
	30 DAT	60 DAT	30 DAT	60 DAT	
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	40.48±2.90[a]	54.39±3.12[a]	2.91±0.57[a]	3.62±0.55[a]	5.38±1.40[a]
P_2 - Non-colonized tomato plants	32.99±3.30[b]	50.23±3.20[b]	2.16±0.45[b]	3.04±0.49[b]	2.88±1.20[b]
C.D (0.05)	0.82	1.11	0.11	0.09	0.57
S. E. (m)	0.29	0.39	0.04	0.03	0.19
Drought stress stimulated by PEG					
D_1-Control	41.00±4.60[a]	67.62±1.80[a]	3.04±0.51[a]	3.86±0.36[a]	5.75±1.42[a]
D_2: -3 bar	39.28±4.90[b]	60.66±3.71[b]	2.89±0.50[a]	3.69±0.35[b]	4.38±1.70[b]
D_3: -7 bar	36.76±5.00[c]	49.13±3.80[c]	2.35±0.29[b]	3.12±0.39[c]	3.63±1.31[b]
D_4: -10 bar	29.91±1.91[d]	31.83±3.12[d]	1.85±0.39[c]	2.63±0.24[d]	2.75±1.50[c]
C.D (0.05)	1.16	1.57	0.16	0.13	0.81
S. E. (m)	0.41	0.55	0.05	0.05	0.28
Treatment combinations					
P_1 D_1	45.12±1.90[a]	67.92±2.41[a]	3.50±0.20[a]	4.18±0.13	7.00±0.81
P_1 D_2	43.80±2.00[a]	63.44±3.10[b]	3.34±0.20[a]	3.98±0.19	5.75±1.20
P_1 D_3	41.44±0.28[b]	52.60±1.32[d]	2.60±0.16[b]	3.46±0.11	4.75±0.50
P_1 D_4	31.58±0.79[e]	33.62±0.70[f]	2.20±0.16[c]	2.84±0.11	4.00±0.81
P_2 D_1	36.88±1.20[c]	67.32±1.30[a]	2.58±0.19[b]	3.54±0.13	4.50±0.57
P_2 D_2	34.76±0.91[d]	57.88±1.42[c]	2.44±0.16[b]	3.40±0.16	3.00±0.81
P_2 D_3	32.08±1.20[e]	45.66±0.93[e]	2.10±0.10[c]	2.78±0.19	2.50±0.57
P_2 D_4	28.24±0.78[f]	30.04±0.91[g]	1.50±0.16[d]	2.42±0.08	1.50±0.57
C.D (0.05)	1.64	2.22	0.22	N.S	N.S
S. E. (m)	0.57	0.77	0.08	0.07	0.39

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 11. Effect of *P. indica* on leaf length, leaf width and leaf area of tomato under drought stress simulated by PEG application

Treatments	Leaf length (cm)		Leaf width(cm)		Leaf area (cm^2)	
	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT
Colonizing with *P. indica*						
P_1	21.07±1.5^{a}	25.6±3.21^{a}	14.53±1.6^{a}	18.35±3.5^{a}	307.64±14.2^{a}	487.61±11^{a}
P_2	18.24±1.4^{b}	22.8±3.50^{b}	13.23±1.7^{b}	16.47±3.2^{b}	202.72±15.4^{b}	384.58±12^{b}
C.D (0.05)	0.64	0.93	0.52	0.50	11.25	13.71
S. E. (m)	0.22	0.32	0.22	0.32	3.89	4.74
Drought stress stimulated by PEG						
D_1	20.36±2.2^{a}	28.60±2.3^{a}	15.00±1.1^{a}	19.70±1.1^{b}	302.78±45^{a}	574.23±20^{a}
D_2	19.92±1.4^{a}	25.15±1.9^{b}	15.14±0.7^{a}	20.63±1.9^{a}	273.60±52^{b}	508.20±21^{b}
D_3	20.41±1.6^{a}	23.09±1.7^{c}	13.57±1.6^{b}	16.47±1.9^{c}	243.04±67^{c}	386.67±12^{c}
D_4bar	17.91±1.8^{b}	20.20±2.0^{d}	11.80±0.7^{c}	12.82±0.6^{d}	201.29±68^{d}	275.28±18^{d}
C.D (0.05)	0.90	1.31	0.74	0.71	15.90	19.39
S. E. (m)	0.31	0.45	0.31	0.45	5.50	6.70
Treatment combinations						
P_1 D_1	22.20±1.5	29.60±2.2	15.46±0.8^{a}	19.52±1.5^{bc}	336.17±34^{a}	600.10±5.2^{a}
P_1 D_2	20.70±1.0	26.80±0.8	15.52±0.5^{a}	22.40±0.6^{a}	321.16±20^{ab}	573.28±6.2^{ab}
P_1 D_3	21.80±0.8	24.58±0.4	14.94±0.9^{a}	18.18±0.5^{d}	306.92±7.4^{b}	458.62±6.1^{c}
P_1 D_4	19.56±0.9	21.60±1.1	12.20±0.8^{b}	13.28±0.5^{f}	266.30±7.4^{c}	318.46±5.1^{d}
P_2 D_1	18.52±0.5	27.60±2.0	14.54±1.3^{a}	19.88±0.7^{b}	269.40±6.2^{c}	548.36±4.4^{b}
P_2 D_2	19.14±1.2	23.50±1.0	14.76±0.8^{a}	18.86±0.6^{cd}	226.04±5.5^{d}	443.12±5.2^{c}
P_2 D_3	19.02±0.7	21.60±1.1	12.20±0.4^{b}	14.76±0.8^{e}	179.16±2.4^{e}	314.72±6.2^{d}
P_2 D_4	16.26±0.4	18.80±1.6	11.40±0.4^{b}	12.36±0.3^{f}	136.29±3.0^{f}	232.10±6.1^{e}
C.D (0.05)	N.S	N.S	1.04	1.00	22.38	27.43
S. E. (m)	0.44	0.64	0.44	0.34	7.77	9.48

Table 12. Influence of *P. indica* on flowering characters of tomato under drought stress simulated by PEG application

Treatments	Days of first flowering	Days of 50% flowering	Flower clusters per plant	Flowers per cluster
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	28.81±2.20	53.95±2.00^{b}	5.74±2.11^{a}	7.34±2.20^{a}
P_2 - Non-colonized tomato plants	28.33±3.40	56.15±1.00^{a}	3.30±1.75^{b}	5.80±2.13^{b}
C.D (0.05)	N.S	0.95	0.39	0.43
S. E. (m)	0.32	0.33	0.13	0.14
Drought stress stimulated by PEG				
D_1-Control	33.87±1.80^{a}	61.00±1.40^{a}	6.63±1.51^{a}	8.25±1.04^{a}
D_2: -3 bar	29.69±2.09^{b}	58.60±2.50^{b}	5.55±1.55^{b}	8.25±1.12^{a}
D_3: -7 bar	26.45±1.25^{c}	54.30±2.00^{c}	4.09±1.49^{c}	6.51±1.09^{b}
D_4: -10 bar	24.27±1.29^{d}	46.30±2.00^{d}	1.82±0.80^{d}	3.28±0.82^{c}
C.D (0.05)	1.28	1.28	0.55	0.60
S. E. (m)	0.45	0.44	0.19	0.21
Treatment combinations				
P_1 D_1	32.64±1.70^{b}	58.40±0.54^{bc}	7.90±0.74	9.00±0.75^{a}
P_1 D_2	30.00±0.79^{c}	57.00±1.50^{cd}	7.00±0.35	8.96±0.99^{a}
P_1 D_3	27.20±0.83^{d}	52.80±1.60^{e}	5.34±1.01	7.44±0.62^{a}
P_1 D_4	25.40±0.54^{d}	47.60±1.50^{f}	2.74±0.48	3.96±0.45^{a}
P_2 D_1	35.10±0.82^{a}	63.60±0.54^{a}	5.36±0.75	7.50±0.70^{b}
P_2 D_2	29.38±2.90^{c}	60.20±2.20^{b}	4.10±0.22	7.54±0.76^{b}
P_2 D_3	25.70±1.20^{d}	55.80±0.83^{d}	2.84±0.32	5.58±0.40^{a}
P_2 D_4	23.14±0.54^{e}	45.00±1.80^{g}	0.90±0.54	2.60±0.41^{a}
C.D (0.05)	1.81	1.90	N.S	N.S
S. E. (m)	0.63	0.66	0.27	0.29

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 13. Effect of *P. indica* on fruiting characters of tomato under drought stress simulated by PEG application

Treatments	Days of first harvest	Fruit per truss	Fruits per plant	Fruit set %
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	55.05±3.00^{b}	4.73±1.31^{a}	15.57±3.78^{a}	59.45±5.16^{a}
P_2 - Non-colonized tomato plants	56.75±2.20^{a}	3.21±1.80^{b}	11.35±4.07^{b}	45.93±6.23^{b}
C.D (0.05)	1.50	0.19	0.91	5.78
S. E. (m)	0.54	0.07	0.31	2.00
Drought stress stimulated by PEG				
D_1-Control	63.90±3.24^{a}	6.06±1.13^{a}	21.64±4.31^{a}	73.49±2.11^{a}
D_2: -3 bar	62.50±3.21^{a}	5.29±1.08^{b}	18.26±1.84^{b}	63.34±3.09^{b}
D_3: -7 bar	54.20±3.90^{b}	3.78±0.87^{c}	12.23±3.05^{c}	52.71±3.11^{c}
D_4: -10 bar	43.00±2.80^{c}	0.74±0.18^{d}	1.72±1.51^{d}	21.20±2.15^{d}
C.D (0.05)	2.22	0.27	1.29	8.18
S. E. (m)	0.77	0.09	0.45	2.84
Treatment combinations				
P_1 D_1	61.20±1.70^{b}	7.04±0.68^{a}	25.40±1.81^{a}	78.27±2.80
P_1 D_2	58.40±0.54^{b}	6.30±0.21^{b}	19.48±1.42^{b}	71.90±3.50
P_1 D_3	55.20±0.44^{c}	4.58±0.33^{cd}	15.02±1.01^{c}	61.66±3.71
P_1 D_4	45.40±1.60^{d}	1.00±0.50^{f}	2.40±1.91^{f}	25.97±2.80
P_2 D_1	66.60±1.50^{a}	5.08±0.11^{c}	17.88±1.80^{bc}	68.72±3.12
P_2 D_2	66.60±2.40^{a}	4.28±0.19^{d}	17.04±1.38^{c}	54.79±4.44
P_2 D_3	53.20±1.50^{c}	2.99±0.32^{e}	9.44±0.70^{e}	43.76±1.26
P_2 D_4	40.60±0.89^{e}	0.49±0.25^{f}	1.04±1.05^{f}	16.43±3.70
C.D (0.05)	3.14	0.53	1.82	N.S
S. E. (m)	1.09	0.18	0.63	4.01

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 14. Effect of *P. indica* on fruit biometric characteristics, fruit cracking and yield in tomato under drought stress simulated by PEG application

Treatments	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit cracking %	Yield per plant (g)
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	3.30±0.64^{a}	2.94±0.39^{a}	20.82±5.41^{a}	0.99±0.15	377.94±22.00^{a}
P_2 - Non-colonized tomato plants	3.11±0.69^{b}	2.52±0.77^{b}	16.17±6.88^{b}	0.22±0.17	275.69±19.20^{b}
C.D (0.05)	0.03	0.19	0.88	N.S	27.78
S. E. (m)	0.01	0.07	0.30	0.40	9.64
Drought stress stimulated by PEG					
D_1-Control	3.89±0.09^{a}	3.23±0.12^{a}	25.30±1.40^{a}	0.00±0.00	585.53±11.14^{a}
D_2: -3 bar	3.67±0.08^{b}	3.04±0.16^{a}	21.30±2.49^{b}	0.00±0.00	466.14±10.80^{b}
D_3: -7 bar	3.04±0.11^{c}	2.76±0.19^{b}	18.04±2.81^{c}	1.29±0.13	237.24±9.80^{c}
D_4: -10 bar	2.21±0.14^{d}	1.89±0.72^{c}	9.35±3.60^{d}	1.14±0.18	18.37±4.30^{d}
C.D (0.05)	0.05	0.28	1.24	N.S	39.29
S. E. (m)	0.02	0.10	0.43	0.57	13.64
Treatment combinations					
P_1 D_1	3.96±0.05^{a}	3.32±0.04^{a}	26.66±0.41^{a}	0.00±0.00	679.50±15.10^{a}
P_1 D_2	3.74±0.05^{c}	3.18±0.08^{ab}	23.60±0.41^{b}	0.00±0.00	499.90±9.20^{b}
P_1 D_3	3.14±0.05^{e}	2.92±0.11^{b}	20.60±0.89^{c}	2.59±0.00	305.88±13.10^{d}
P_1 D_4	2.34±0.05^{g}	2.34±0.11^{d}	12.50±0.50^{e}	1.38±0.57	26.50±12.11^{ef}
P_2 D_1	3.82±0.04^{b}	3.14±0.11^{a}	24.00±0.30^{b}	0.00±0.00	491.56±8.80^{b}
P_2 D_2	3.60±0.01^{d}	2.90±0.07^{b}	19.00±0.79^{c}	0.00±0.00	432.38±10.4^{c}
P_2 D_3	2.94±0.05^{f}	2.60±0.07^{c}	15.48±0.77^{d}	0.00±0.00	168.60±10.50^{e}
P_2 D_4	2.08±0.3^{h}	1.44±0.81^{e}	6.20±3.10^{f}	0.90±0.54	10.24±2.21^{f}
C.D (0.05)	0.06	0.39	1.76	N.S	55.57
S. E. (m)	0.02	0.13	0.61	0.81	19.29

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 15. Influence of *P. indica* on quality parameters of tomato under drought stress simulated by PEG application

Treatments	Ascorbic acid (mg g^{-1})	Lycopene (mg g^{-1})	TSS (° Brix)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	20.68±0.91[a]	12.35±0.38[a]	4.20±0.30[a]
P_2 - Non-colonized tomato plants	20.26±0.83[b]	12.20±0.13[b]	4.09±0.22[b]
C.D (0.05)	0.13	0.11	0.08
S. E. (m)	0.04	0.03	0.03
Drought stress stimulated by PEG			
D_1-Control	19.47±0.20[d]	12.15±0.12[b]	3.90±0.14[c]
D_2: -3 bar	19.92±0.16[c]	12.18±0.10[b]	3.98±0.13[c]
D_3: -7 bar	20.87±0.46[b]	12.28±0.19[b]	4.22±0.16[b]
D_4: -10 bar	21.61±0.32[a]	12.48±0.30[a]	4.47±0.15[a]
C.D (0.05)	0.18	0.15	0.12
S. E. (m)	0.06	0.05	0.04
Treatment combinations			
P_1 D_1	19.66±0.09	12.11±0.11[c]	3.91±0.11[d]
P_1 D_2	20.01±0.18	12.21±0.09[bc]	3.96±0.11[cd]
P_1 D_3	21.22±0.21	12.42±0.16[b]	4.34±0.11[b]
P_1 D_4	21.83±0.10	12.67±0.33[a]	4.58±0.11[a]
P_2 D_1	19.29±0.04	12.18±0.12[c]	3.89±0.17[d]
P_2 D_2	19.84±0.08	12.16±0.11[c]	4.00±0.16[cd]
P_2 D_3	20.53±0.37	12.15±0.11[c]	4.10±0.10[c]
P_2 D_4	21.38±0.31	12.30±0.14[bc]	4.37±0.11[b]
C.D (0.05)	N.S	0.21	0.17
S. E. (m)	0.09	0.07	0.06

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 16. Impact of *P. indica* physiological parameters in tomato under drought stress simulated by PEG application

Treatments	Relative water content (%)	Cell Membrane Stability (%)	Chlorophyll Stability Index (%)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	59.03±13.41[a]	70.08±12.66[a]	88.14±18.41[a]
P_2 - Non-colonized tomato plants	53.35±14.13[b]	64.62±15.33[b]	79.05±19.08[b]
C.D (0.05)	0.93	1.12	1.95
S. E. (m)	0.31	0.39	0.68
Drought stress stimulated by PEG			
D_1-Control	69.92±2.14[a]	81.42±1.49[a]	104.68±5.66[a]
D_2: -3 bar	64.49±3.62[b]	75.61±2.71[b]	94.54±3.36[b]
D_3: -7 bar	54.93±4.19[c]	66.65±3.23[c]	78.08±7.98[c]
D_4: -10 bar	35.43±3.27[d]	45.72±5.63[d]	57.09±5.28[d]
C.D (0.05)	1.32	1.58	2.77
S. E. (m)	0.44	0.55	0.96
Treatment combinations			
$P_1 D_1$	71.36±0.70[a]	82.58±0.26[a]	109.36±4.17[a]
$P_1 D_2$	67.76±0.75[b]	77.79±0.92[c]	97.00±1.96[b]
$P_1 D_3$	58.70±0.26[d]	69.42±1.66[e]	84.84±3.82[d]
$P_1 D_4$	38.30±1.15[f]	50.53±3.08[g]	61.38±2.25[f]
$P_2 D_1$	68.49±2.17[b]	80.26±1.25[b]	100.00±0.00[b]
$P_2 D_2$	61.21±0.02[c]	73.42±1.93[d]	92.08±2.55[c]
$P_2 D_3$	51.16±1.15[e]	63.89±1.27[f]	71.32±3.83[e]
$P_2 D_4$	32.56±0.92[g]	40.91±2.08[h]	52.80±3.43[g]
C.D (0.05)	1.87	2.24	3.91
S. E. (m)	0.62	0.78	1.36

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 17. Impact of *P. indica* on proline accumulation and anti-oxidant activities in tomato under drought stress simulated by PEG application

Treatments	Proline (µmol g^{-1})	Superoxide dismutase (mg g^{-1}fw)	Peroxidase (min $^{-1}$ g $^{-1}$ fw)	Catalase (units min^{-1} g^{-1} fw)
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	9.17±0.24^{a}	269.20±24.30^{a}	40.02±7.62^{a}	303.58±25.20^{a}
P_2 - Non-colonized tomato plants	5.86±0.58^{b}	236.33±21.75^{b}	31.89±4.34^{b}	249.67±14.20^{b}
C.D (0.05)	0.22	2.54	0.97	3.19
S. E. (m)	0.08	0.84	0.32	1.06
Drought stress stimulated by PEG				
D_1-Control	3.12±0.10^{d}	212.59±2.41^{d}	29.57±2.31^{d}	257.37±21.32^{d}
D_2: -3 bar	4.61±0.84^{c}	233.63±12.39^{c}	32.03±3.57^{c}	265.30±27.07^{c}
D_3: -7 bar	9.45±0.23^{b}	271.48±15.80^{b}	38.10±5.71^{b}	277.40±33.00^{b}
D_4: -10 bar	12.89±0.87^{a}	293.38±12.65^{a}	44.12±6.69^{a}	306.43±37.39^{a}
C.D (0.05)	0.31	3.60	1.37	4.51
S. E. (m)	0.11	1.19	0.46	1.49
Treatment combinations				
P_1 D_1	3.12±0.12^{f}	214.18±2.07^{f}	31.54±0.88^{e}	276.70±3.11^{d}
P_1 D_2	5.40±0.16^{d}	244.66±3.50^{d}	35.16±1.46^{d}	289.84±4.90^{c}
P_1 D_3	11.63±0.24^{b}	294.94±4.20^{b}	43.20±1.12^{b}	307.45±2.60^{b}
P_1 D_4	16.54±0.62^{a}	323.02±2.48^{a}	50.18±1.42^{a}	340.32±2.10^{a}
P_2 D_1	3.12±0.08^{f}	210.99±1.60^{f}	27.60±1.01^{f}	238.04±2.51^{f}
P_2 D_2	3.82±0.13^{e}	222.59±2.60^{e}	28.90±0.96^{f}	240.76±1.40^{f}
P_2 D_3	7.24±0.50^{c}	248.01±2.51^{d}	33.00±1.59^{e}	247.34±2.50^{e}
P_2 D_4	9.24±0.36^{b}	263.73±3.21^{c}	38.07±0.23^{c}	272.55±1.80^{d}
C.D (0.05)	0.43	5.09	1.95	6.38
S. E. (m)	0.15	1.68	0.65	2.11

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Plate 6. Tomato plants @ 30 DAT under drought stress simulated by application of PEG

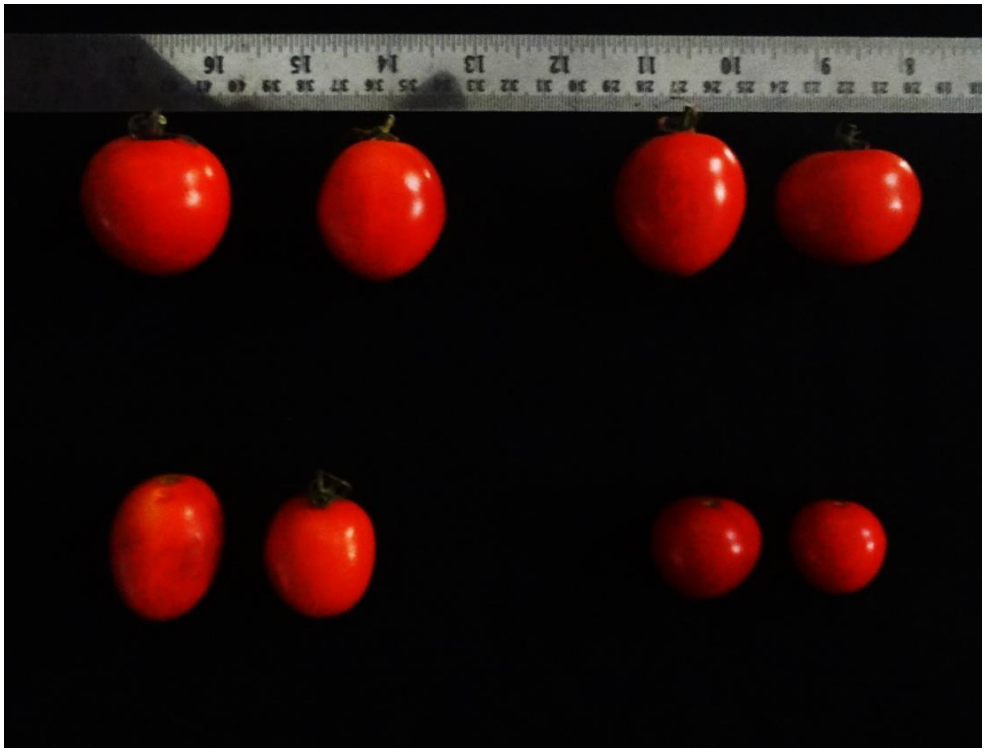


Plate 7. Fruits harvested from *P. indica* colonized and non-colonized plants under different levels of drought stress simulated by the application of PEG

4.3. EVALUATION OF TOMATO PLANTS COLONIZED WITH *P. indica* UNDER DROUGHT STRESS STIMULATED BY MANNITOL

4.3.1. Vegetative and flowering characters

The data analysis from the presented Table 18 reveals significant differences were observed in plant height between the *P. indica* colonization treatments at both 30 and 60 DAT. At 30 DAT, P_1, representing *P. indica*-colonized tomato plants, exhibited a greater plant height of 41.39cm compared to P_2, consisting of non-colonized tomato plants, which had a height of 34.72cm. Similarly, at 60 DAT, P_1 showed the highest plant height of 56.05 cm, while P_2 had a significantly lower height of 52.60 cm. These results suggest that colonization with *P. indica* promotes increased plant height in tomato plants. At 30DAT, plant height in P_1 across M_1, M_2, M_3 and M_4 were significantly higher than non-colonized plants under same conditions. However, at 60DAT, under M_1 (control) and M_2 (3%mannitol), there were no significant difference in plant height between P_1 and P_2. As the stress level increased to M_3 and M_4, plant height was maintained high in P_1 compared to P_2 (Plate 8).

The results showcased in the Table 18 indicate significant differences were observed in stem girth between the colonization treatments at both 30 and 60 DAT. At 30 DAT, P_1, representing *P. indica*-colonized tomato plants, showed a higher stem girth of 2.98cm compared to P_2, which consisted of non-colonized tomato plants, with a stem girth of 2.24cm. Similarly, at 60 DAT, P_1 exhibited the highest stem girth of 3.42cm, while P_2 had a slightly lower girth of 2.73cm. These results suggest that colonization with *P. indica* promotes increased stem girth in tomato plants. The drought stress induced by mannitol negatively affects the stem girth of tomato plants.

The number of primary branches per plant was evaluated to assess the impact of colonization with *P. indica* and drought stress induced by mannitol. P_1, representing *P. indica*-colonized tomato plants, exhibited a higher number of primary branches per plant, with an average value of 4.08. In contrast, P_2, which consisted of non-colonized tomato plants, had a lower number of primary branches, with an average value of 2.20 (Table 18).

The results depicted in the table 19 highlight the significant impact of length, width and area of tomato plant leaves measured at 30 and 60 days after

transplanting (DAT). Significant differences in leaf biometric measurements were observed between the colonization treatments at both 30 and 60 DAT. At 30 DAT, P_1, representing *P. indica*-colonized tomato plants, exhibited a higher leaf length (21.37 cm), width (14.70cm), and area (320.4cm^2) compared to P_2, which consisted of non-colonized tomato plants, with a leaf length of 17.6cm, width of 12.04 cm, and area of 215.9cm^2. Similarly, at 60 DAT, P_1 showed the consistently higher leaf length (26.2cm), width (20.10 cm) and area (530.2cm^2). Interaction effect was also found effective with respect to leaf area. Findings suggest that colonization with *P. indica* promotes an increase in leaf area in tomato plants.

The days to first flowering and 50% flowering were represented in table 20 as affected by the effects of colonization with *P. indica* and drought stress induced by mannitol. Significant differences were found in the days to first flowering between the colonization treatments. P_1, representing *P. indica*-colonized tomato plants, exhibited a shorter time to first flowering, with an average of 29.58 days. In contrast, P_2, which consisted of non-colonized tomato plants, had a delay to first flowering, with an average of 31.12days. It was observed that *P. indica* colonized plants came to first flowering stage earlier compared to the non-colonized plants at all levels of stress except M_4 (10% mannitol).

Significant variations were observed in the number of flower clusters per plant and flowers per cluster between the colonization treatments. P_1, representing *P. indica*-colonized tomato plants, exhibited a higher number of flower clusters with an average of 4.90 clusters per plant. In contrast, P_2, consisting of non-colonized tomato plants, had a lower number of flower clusters with an average of 2.95 clusters per plant. Likewise, P_1, had an average of 7.64 flowers per cluster, while P_2 had an average of only 5.81 flowers per cluster. Across the different combinations, those involving *P. indica* colonized plants exhibited significantly higher number of flower cluster compared to the control. The drought stress treatments using mannitol, also resulted in a significant difference in the number of flowers per cluster.

4.3.2. Fruit and yield characters

The colonization with *P. indica* had a significant effect on the days to first harvest. P_1, representing *P. indica*-colonized tomato plants, had an average of 59.97 days to first harvest, while P_2, comprising non-colonized tomato plants, had an average of 61.40 days to first harvest. Examining the treatment combinations, significant variations were observed in the days to first harvest. The combinations showed different average values, ranging from 53.60 (P_2M_4) to 68.00 (P_2M_1) days to first harvest. *P. indica* colonization resulted in early harvest across all levels of water stress (Table 21).

The number of fruits per truss and fruits per plant are tabulated in table 21 to assess the effects of colonization with *P. indica*, drought stress induced by mannitol, and their combinations. The statistical analysis revealed significant differences among the treatments. Colonization with *P. indica* had a significant impact on the number of fruits per truss and plant. P_1, representing *P. indica*-colonized tomato plants, had an average of 5.41 fruits per truss and 16.61 fruits per plant, while P_2, comprising non-colonized tomato plants, had only an average of 3.46 fruits per truss and 10.48 fruits per plant. Regarding the drought stress treatments using mannitol, there was a significant effect on the number of fruits per plant. The average values for M_1, M_2, M_3, and M_4 were 20.57, 17.49, 12.45, and 3.67 fruits per plant, respectively. *P. indica* colonized plants had higher number of fruits per truss compared to non-colonized plants at all levels from M_1 to M_4.

The fruit set percentage for various treatments are represented in table 21. Colonization with *P. indica* had a significant impact on the fruit set percentage. P_1, representing *P. indica*-colonized tomato plants, exhibited an average fruit set percentage of 66.44%, while P_2, comprising non-colonized tomato plants, had a lower average fruit set percentage of 52.07%.

The length and diameter of fruits were recorded to evaluate the effects of colonization with *P. indica*, drought stress induced by mannitol, and their combinations. Colonization with *P. indica* had a significant influence on fruit size. P_1, representing *P. indica*-colonized tomato plants, exhibited an average fruit length of 3.37 cm and diameter of 3.03 cm, while P_2, comprising non-colonized tomato

plants, had a substantially lower average fruit length of 2.95 cm and diameter of 2.61cm. Regarding the drought stress treatments using mannitol, a significant effect on fruit length and diameter was observed. The average lengths for M_1, M_2, M_3, and M_4 were 3.88 cm, 3.60 cm, 3.06cm, and 2.09 cm, respectively. The highest length and diameter were observed in the P_1M_1 treatment (3.98cm and 3.76cm), while the lowest were observed in the P_2 M_4 treatment (1.80cm and 1.56cm) (Table 22) (Plate 9)

Colonization with *P. indica* had a significant effect on fruit weight and yield per plant. *P. indica*-colonized tomato plants, exhibited an average fruit weight of 21.68g and average yield of 418.88g per plant, whereas P_2, comprising non-colonized tomato plants, had a lower average fruit weight of 18.16g and yield of 260.95g per plant. Drought stress induced by mannitol also significantly affected the fruit weight and yield per plant. The average yields for M_1, M_2, M_3, and M_4 were 578.03g, 475.74g, 256.12g, and 49.78g, respectively. Across the different levels of mannitol concentration, fruit weight was significantly higher in colonized plants compared to the non-colonized plants. When examining the treatment combinations, significant variations in yield per plant were observed. The average yields, ranged from 13.65g (P_2M_4) to 647.63g (P_1M_1). *P. indica* colonized plants yielded significantly higher yields at M_1 (647.63g), M_2 (565.91g), M_3 (376.09) and M_4 (85.91g) compared to the non-colonized plants (Table 22).

Colonization with *P. indica* didn't affect the fruit cracking percentage. P_1, representing *P. indica*-colonized tomato plants, exhibited an average fruit cracking percentage of 1.19%. In contrast, P_2, consisting of non-colonized tomato plants, had a lower average fruit cracking percentage of 0.21%. (Table 22).

4.3.4. Quality parameters

Colonization with *P. indica* exhibited a significant effect on the ascorbic acid, lycopene and TSS in tomato fruits. P_1, representing *P. indica*-colonized tomato plants, had an average ascorbic acid content of 20.59mg g^{-1}, while P_2, consisting of non-colonized tomato plants, had a slightly lower average content of 20.16mg g^{-1}. *P. indica*-colonized tomato plants, had an average lycopene content

of 12.53mg g^{-1} which was higher, while P_2, comprising non-colonized tomato plants, had an average content of only 12.33mg g^{-1}. Similarly, TSS content was higher for *P. indica*-colonized tomato plants (4.35^{o}Brix), compared to fruits from non-colonized tomato plants (4.21^{o}Brix) (Table 23).

4.3.5. Physiological parameters

P_1, representing *P. indica*-colonized tomato plants, exhibited an average RWC of 59.55%, while P_2, consisting of non-colonized tomato plants, had an average RWC of 54.99%. Drought stress stimulated by mannitol, there was a significant effect on the RWC of tomato plants. As the concentration of mannitol increased, the RWC decreased. The RWC values for M_1, M_2, M_3, and M_4 were 70.31%, 65.43%, 56.40%, and 36.93%, respectively. The highest CMS was observed in the combination P_1M_1, representing *P. indica*-colonized tomato plants under control conditions. Conversely, the lowest CMS was found in the combination P_2M_4, representing non-colonized tomato plants subjected to 10% mannitol-induced drought stress. At all levels, *P. indica* colonized plants had a significantly higher CMS compared to the non-colonized plants. (Table 24).

The colonization with *P. indica*, had a significant effect on CSI. P_1, representing *P. indica*-colonized tomato plants, exhibited an average CSI of 88.74%, while P_2, consisting of non-colonized tomato plants, had an average CSI of 80.31%. The difference between the two treatments was statistically significant, indicating that *P. indica* colonization positively influenced the stability of chlorophyll molecules (Table 24).

Proline content varied significantly with respect to different treatments and followed the same trend as in the experiments involving gravimetric method and PEG application (Table 24).

Regarding the colonization with *P. indica*, a significant difference in SOD, peroxidase and catalase activities were observed between the treatments. P_1, representing *P. indica*-colonized tomato plants, exhibited a significantly higher SOD (269.50mg g^{-1} fw), PO (38.72units min^{-1} g^{-1} fw) and CAT (283.24 units min^{-1} g^{-1} fw) activities of anti-oxidant enzymes compared to the non-colonized tomato plants. Even when the stress reached at its highest level, colonized plants

maintained substantially higher anti-oxidant activities compared to the control (Table 25).

- P. indica ***+ P. indica*** ***- P. indica*** ***+ P. indica***

- P. indica ***+ P. indica*** ***- P. indica*** ***+ P. indica***

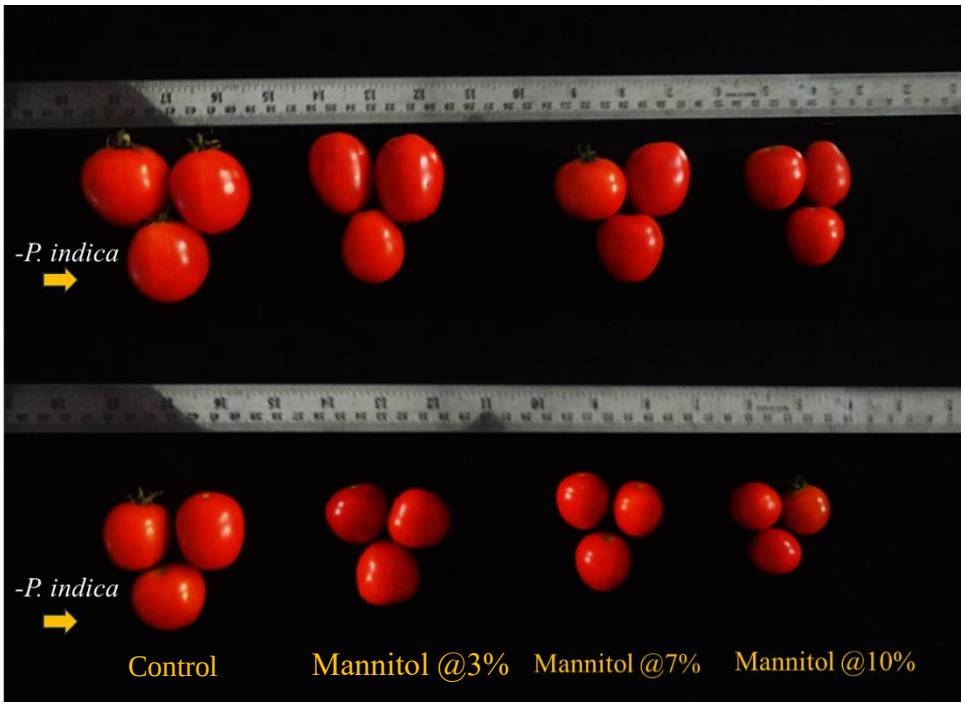


Plate 9. Fruits harvested from *P. indica* colonized and non-colonized plants under different levels of Mannitol application

Table 18. Effect of *P. indica* on plant height, stem girth and number of primary branches in tomato under drought stress simulated by mannitol application

Treatments	Plant height (cm)		Stem grith (cm)		Primary branches / plant
	30 DAT	60 DAT	30 DAT	60 DAT	
Colonizing with *P. indica*					
P_1 - *P. indica* colonized tomato plants	41.39±3.85^{a}	56.05±1.91^{a}	2.98±0.34^{a}	3.42±0.62^{a}	4.08±0.77^{a}
P_2 - Non-colonized tomato plants	34.72±5.39^{b}	52.60±4.32^{b}	2.24±0.48^{b}	2.73±0.67^{b}	2.22±1.02^{b}
C.D (0.05)	0.76	1.10	0.13	0.11	0.37
S. E. (m)	0.26	0.38	0.05	0.04	0.13
Drought stress stimulated by Mannitol					
M_1-Control	43.27±2.70^{a}	68.62±2.16^{a}	3.08±0.39^{a}	3.81±0.47^{a}	4.13±0.87^{a}
M_2- 3% Mannitol	41.10±3.30^{b}	60.98±1.42^{b}	2.72±0.31^{b}	3.42±0.35^{b}	3.41±1.15^{b}
M_3- 7% Mannitol	35.67±4.20^{c}	53.23±2.47^{c}	2.48±0.36^{c}	2.81±0.19^{c}	2.88±1.20^{c}
M_4- 10% Mannitol	32.18±4.40^{d}	34.45±4.14^{d}	2.15±0.66^{d}	2.26±0.60^{d}	2.19±1.20^{d}
C.D (0.05)	1.07	1.55	0.19	0.16	0.53
S. E. (m)	0.37	0.54	0.07	0.05	0.18
Treatment combinations					
P_1 M_1	45.52±1.48^{a}	69.38±1.80^{a}	3.40±0.21^{a}	4.24±0.11^{a}	4.75±0.64
P_1 M_2	44.06±1.06^{a}	61.60±1.30^{b}	2.98±0.14^{b}	3.72±0.19^{b}	4.25±0.95
P_1 M_3	39.60±1.02^{bc}	55.10±1.40^{c}	2.80±0.18^{b}	2.92±0.08^{de}	4.00±0.44
P_1 M_4	36.38±0.71^{d}	38.10±2.10^{e}	2.72±0.31^{bc}	2.80±0.23^{ef}	3.32±0.21
P_2 M_1	41.02±1.56^{b}	67.86±2.30^{a}	2.76±0.20^{b}	3.38±0.15^{c}	3.50±0.57
P_2 M_2	38.14±1.51^{c}	60.36±1.41^{b}	2.46±0.16^{c}	3.12±0.13^{de}	2.58±0.42
P_2 M_3	31.74±1.00^{e}	51.36±1.70^{d}	2.16±0.09^{d}	2.70±0.21^{e}	1.75±0.21
P_2 M_4	27.98±0.58^{f}	30.80±0.83^{f}	1.58±0.26^{e}	1.72±0.19^{f}	1.05±0.10
C.D (0.05)	1.51	2.19	0.27	0.22	NA
S. E. (m)	0.52	0.76	0.09	0.08	0.26

Table 19. Effect of *P. indica* on leaf length, leaf width and leaf area of tomato under drought stress simulated by mannitol application

Treatments	Leaf length (cm)		Leaf width(cm)		Leaf area (cm^2)	
	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT
Colonizing with *P. indica*						
P_1	21.37±1.7^{a}	26.22±2.9^{a}	14.67±1.3^{a}	20.06±2.2^{a}	320.39±48^{a}	530.17±14^{a}
P_2	17.62±2.3^{b}	23.01±4.6^{b}	12.04±1.7^{b}	14.60±2.9^{b}	215.93±60^{b}	345.00±37^{b}
C.D (0.05)	0.45	0.98	0.22	0.37	7.77	14.98
S. E. (m)	0.15	0.34	0.07	0.13	2.69	5.18
Drought stress stimulated by Mannitol						
M_1	22.14±1.6^{a}	29.42±2.8^{a}	15.33±1.0^{a}	20.60±2.4^{a}	340.77±46^{a}	607.47±8.3^{a}
M_2	20.36±2.0^{b}	25.87±1.8^{b}	13.93±1.4^{b}	18.62±2.7^{b}	286.26±57^{b}	484.85±9.6^{b}
M_3	18.47±2.2^{c}	23.01±2.5^{c}	12.95±1.4^{c}	16.05±3.2^{c}	245.00±57^{c}	373.07±10^{c}
M_4	17.01±2.4^{d}	20.15±2.8^{d}	11.21±1.7^{d}	14.05±3.7^{d}	200.62±62^{d}	284.97±11^{d}
C.D (0.05)	0.63	1.39	0.30	0.52	10.99	21.18
S. E. (m)	0.22	0.48	0.11	0.18	3.80	7.32
Treatment combinations						
P_1 M_1	23.48±0.3^{a}	29.84±1.5^{a}	16.32±0.2^{a}	22.84±0.3^{a}	383.17±5.2^{a}	682.60±29^{a}
P_1 M_2	22.26±0.5^{b}	27.16±1.5^{bc}	15.26±0.3^{b}	21.10±0.5^{b}	339.77±6.1^{b}	572.74±27^{b}
P_1 M_3	20.50±0.7^{c}	25.24±1.1^{cd}	14.30±0.2^{c}	19.10±0.9^{c}	299.34±9.6^{c}	476.01±18^{d}
P_1 M_4	19.24±0.7^{d}	22.64±1.4^{ef}	12.80±0.3^{d}	17.20±0.3^{d}	259.28±5.2^{d}	389.35±24^{e}
P_2 M_1	20.80±1.0^{c}	29.84±2.9^{ab}	14.34±0.3^{c}	18.36±0.2^{c}	298.36±4.2^{c}	532.35±27^{c}
P_2 M_2	18.46±0.7^{d}	27.16±0.9^{de}	12.60±0.4^{d}	16.14±0.7^{e}	232.75±4.1^{e}	396.96±28^{e}
P_2 M_3	16.44±0.6^{e}	25.24±0.8^{f}	11.60±0.4^{e}	13.00±0.3^{f}	190.66±8.4^{f}	270.12±12^{f}
P_2 M_4	14.78±0.7^{f}	22.64±0.4^{g}	9.62±0.41^{f}	10.90±0.7^{g}	141.97±3.4^{g}	180.59±5.1^{g}
C.D (0.05)	0.89	1.96	0.43	0.74	15.55	29.95
S. E. (m)	0.31	0.68	0.15	0.26	5.37	10.35

Table 20. Influence of *P. indica* on flowering characters of tomato under drought stress simulated by Mannitol application

Treatments	Days of first flowering	Days of 50% flowering	Flower clusters per plant	Flowers per cluster
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	29.58±3.07^{b}	46.75±4.6^{a}	4.90±2.28^{a}	7.64±1.82
P_2 - Non-colonized tomato plants	31.12±5.90^{a}	47.25±3.2^{b}	2.95±1.40^{b}	5.81±2.03
C.D (0.05)	0.90	N.S	0.41	0.32
S. E. (m)	0.31	0.30	0.14	0.11
Drought stress stimulated by Mannitol				
M_1-Control	35.65±3.20^{a}	54.00±2.20^{a}	6.15±2.00^{a}	8.34±0.96^{a}
M_2- 3% Mannitol	32.74±2.11^{b}	52.80±2.20^{a}	4.70±1.25^{b}	8.34±0.96^{a}
M_3- 7% Mannitol	29.10±0.87^{c}	47.20±1.20^{b}	3.25±0.89^{c}	6.32±1.30^{b}
M_4- 10% Mannitol	23.92±2.27^{d}	34.00±2.8^{c}	1.60±0.56^{d}	3.91±1.09^{c}
C.D (0.05)	1.27	1.25	0.58	0.46
S. E. (m)	0.44	0.43	0.20	0.16
Treatment combinations				
P_1 M_1	32.96±2.00^{b}	52.40±1.51^{b}	7.90±0.74^{a}	9.18±0.43^{b}
P_1 M_2	30.88±0.38^{d}	50.40±1.51^{c}	5.60±1.10^{b}	9.04±0.71^{bc}
P_1 M_3	29.25±0.50^{d}	47.80±0.83^{d}	4.00±0.35^{de}	7.44±0.62^{bc}
P_1 M_4	25.24±0.68^{f}	36.40±1.14^{e}	2.10±0.22^{f}	4.92±0.27^{bcd}
P_2 M_1	38.34±1.14^{a}	55.60±1.51^{a}	4.40±0.89^{c}	7.50±0.35^{d}
P_2 M_2	34.60±1.14^{c}	55.20±1.30^{a}	3.80±0.44^{cd}	7.64±0.61^{cd}
P_2 M_3	28.96±1.18^{e}	46.60±1.50^{d}	2.50±0.50^{e}	5.20±0.57^{bc}
P_2 M_4	22.60±2.60^{g}	31.60±1.52^{f}	1.10±0.22^{f}	2.90±0.22^{a}
C.D (0.05)	1.80	1.77	0.83	N.S
S. E. (m)	0.62	0.61	0.28	0.22

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 21. Effect of *P. indica* on fruiting characters of tomato under drought stress simulated by mannitol application

Treatments	Days of first harvest	Fruit per truss	Fruits per plant	Fruit set %
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	59.97±2.41^{b}	5.41±2.20^{a}	16.61±3.75^{a}	66.44±6.10^{a}
P_2 - Non-colonized tomato plants	61.40±2.30^{a}	3.46±1.60^{b}	10.48±3.77^{b}	52.07±7.41^{b}
C.D (0.05)	0.69	0.25	0.82	4.83
S. E. (m)	0.24	0.08	0.26	1.67
Drought stress stimulated by Mannitol				
M_1-Control	64.75±3.63^{a}	6.33±1.31^{a}	20.57±2.93^{a}	75.29±3.70^{a}
M_2- 3% Mannitol	63.60±1.90^{b}	5.56±1.39^{b}	17.49±3.12^{b}	67.53±3.20^{b}
M_3- 7% Mannitol	59.40±0.96^{c}	4.41±0.94^{c}	12.45±5.32^{c}	58.85±2.50^{c}
M_4- 10% Mannitol	55.00±1.80^{d}	1.44±1.74^{d}	3.67±1.52^{d}	35.35±3.80^{d}
C.D (0.05)	0.98	0.36	1.16	6.84
S. E. (m)	0.34	0.12	0.40	2.37
Treatment combinations				
P_1 M_1	61.50±0.61^{c}	7.52±0.39^{a}	22.84±2.11^{a}	82.07±1.84
P_1 M_2	62.00±1.22^{c}	6.84±0.47^{b}	20.30±0.67^{b}	75.81±3.61
P_1 M_3	60.00±0.70^{d}	5.24±0.48^{c}	17.40±0.82^{c}	66.51±1.00
P_1 M_4	56.40±1.14^{e}	2.04±0.36^{f}	5.90±1.08^{e}	41.38±2.50
P_2 M_1	68.00±1.73^{a}	5.14±0.41^{c}	18.30±1.40^{c}	68.52±4.47
P_2 M_2	65.20±0.83^{b}	4.28±0.21^{d}	14.68±1.32^{d}	59.25±3.37
P_2 M_3	58.80±0.84^{d}	3.58±0.23^{e}	7.50±1.36^{e}	51.20±1.78
P_2 M_4	53.60±1.14^{f}	0.84±0.25^{g}	1.44±0.86^{f}	29.33±1.80
C.D (0.05)	1.39	0.50	1.64	N.S
S. E. (m)	0.48	0.17	0.57	3.35

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 22. Effect of *P. indica* on fruit biometric characteristics, fruit cracking and yield in tomato under drought stress simulated by mannitol application

Treatments	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit cracking %	Yield per plant (g)
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	3.37±0.65[a]	3.03±0.62[a]	21.68±5.08[a]	1.19±0.04	418.88±15.1[a]
P_2 - Non-colonized tomato plants	2.95±0.77[b]	2.61±0.78[b]	18.16±5.60[b]	0.21±0.01	260.95±18.2[b]
C.D (0.05)	0.08	0.11	0.46	N.S	20.68
S. E. (m)	0.03	0.04	0.16	0.63	7.18
Drought stress stimulated by Mannitol					
M_1-Control	3.88±0.15[a]	3.64±0.14[a]	25.88±1.85[a]	0.00±0.00	578.03±7.20[a]
M_2- 3% Mannitol	3.60±0.25[b]	3.20±0.24[b]	22.40±1.81[b]	0.00±0.00	475.74±5.52[b]
M_3- 7% Mannitol	3.06±0.25[c]	2.52±0.30[c]	19.62±1.61[c]	0.84±0.05	256.12±6.20[c]
M_4- 10% Mannitol	2.09±0.33[d]	1.90±0.41[d]	11.80±2.62[d]	1.95±0.85	49.78±6.10[d]
C.D (0.05)	0.11	0.16	0.65	N.S	29.25
S. E. (m)	0.04	0.06	0.22	0.94	10.15
Treatment combinations					
P_1 M_1	3.98±0.11[a]	3.76±0.05[a]	27.56±0.68[a]	0.00±0.00	647.63±5.41[a]
P_1 M_2	3.82±0.08[b]	3.34±0.25[b]	23.94±1.13[b]	0.00±0.00	565.91±7.50[b]
P_1 M_3	3.28±0.08[c]	2.76±0.17[e]	21.04±0.62[d]	1.69±0.78	376.09±4.20[d]
P_1 M_4	2.38±0.13[e]	2.24±0.23[f]	14.20±0.67[f]	3.07±0.88	85.91±5.81[f]
P_2 M_1	3.78±0.13[b]	3.52±0.08[c]	24.20±0.44[b]	0.00±0.00	508.43±4.80[c]
P_2 M_2	3.38±0.13[c]	3.06±0.13[d]	26.26±0.90[c]	0.00±0.00	385.57±3.10[d]
P_2 M_3	2.84±0.11[d]	2.28±0.19[f]	18.20±0.67[e]	0.00±0.00	136.15±2.40[e]
P_2 M_4	1.80±0.14[f]	1.56±0.19[g]	9.40±0.82[g]	0.00±0.00	13.65±4.10[g]
C.D (0.05)	0.15	0.23	0.92	N.S	41.36
S. E. (m)	0.05	0.08	0.32	1.27	14.36

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 23. Influence of *P. indica* on quality parameters of tomato under drought stress simulated by mannitol application

Treatments	Ascorbic acid (mg g^{-1})	Lycopene (mg g^{-1})	TSS (^{o} Brix)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	20.59±0.71 [a]	12.53±0.30 [a]	4.35±0.30 [a]
P_2 - Non-colonized tomato plants	20.16±0.68 [b]	12.33±0.32 [b]	4.21±0.22 [b]
C.D (0.05)	0.15	0.15	0.05
S. E. (m)	0.05	0.05	0.02
Drought stress stimulated by Mannitol			
M_1-Control	19.62±0.27 [d]	12.19±0.26 [b]	4.04±0.14 [d]
M_2- 3% Mannitol	19.95±0.15 [c]	12.24±0.36 [b]	4.23±0.13 [c]
M_3- 7% Mannitol	20.63±0.52 [b]	12.58±0.16 [a]	4.36±0.16 [b]
M_4- 10% Mannitol	21.29±0.29 [a]	12.70±0.14 [a]	4.49±0.15 [a]
C.D (0.05)	0.22	0.21	0.07
S. E. (m)	0.07	0.07	0.02
Treatment combinations			
P_1 M_1	19.83±0.16	12.36±0.27	4.04±0.11 [e]
P_1 M_2	20.05±0.12	12.30±0.30	4.28±0.11 [d]
P_1 M_3	21.00±0.27	12.68±0.19	4.44±0.11 [b]
P_1 M_4	21.47±0.29	12.79±0.13	4.64±0.12 [a]
P_2 M_1	19.41±0.19	12.03±0.14	4.04±0.17 [e]
P_2 M_2	19.84±0.09	12.19±0.44	4.18±0.16 [d]
P_2 M_3	20.25±0.42	12.49±0.04	4.28±0.10 [c]
P_2 M_4	21.12±0.18	12.60±0.05	4.34±0.11 [c]
C.D (0.05)	N.S	N.S	0.09
S. E. (m)	0.11	0.10	0.03

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 24. Impact of *P. indica* physiological parameters in tomato under drought stress simulated by mannitol application

Treatments	Relative water content (%)	Cell Membrane Stability (%)	Chlorophyll Stability Index (%)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	59.55±13.26^{a}	71.48±12.54^{a}	88.74±16.43^{a}
P_2 - Non-colonized tomato plants	54.99±13.50^{b}	66.50±14.72^{b}	80.31±19.42^{b}
C.D (0.05)	0.99	0.99	1.89
S. E. (m)	0.33	0.34	0.65
Drought stress stimulated by Mannitol			
M_1-Control	70.31±1.43^{a}	82.47±1.63^{a}	102.82±4.49^{a}
M_2- 3% Mannitol	65.43±3.51^{b}	77.42±3.06^{b}	96.32±3.04^{b}
M_3- 7% Mannitol	56.40±3.16^{c}	68.15±2.43^{c}	81.01±6.12^{c}
M_4- 10% Mannitol	36.93±2.79^{d}	47.92±4.96^{d}	57.94±7.31^{d}
C.D (0.05)	1.41	1.40	2.67
S. E. (m)	0.47	0.48	0.92
Treatment combinations			
P_1 M_1	71.40±0.91^{a}	83.37±0.80^{a}	105.65±5.01^{a}
P_1 M_2	68.56±0.66^{b}	80.18±1.10^{b}	99.02±1.34^{b}
P_1 M_3	59.23±0.75^{d}	70.15±1.57^{d}	85.90±3.74^{d}
P_1 M_4	39.00±0.60^{f}	52.20±2.27^{f}	64.40±1.08^{f}
P_2 M_1	69.23±0.87^{b}	81.58±1.83^{ab}	100.00±0.10^{b}
P_2 M_2	62.30±1.01^{c}	74.66±0.90^{c}	93.63±0.93^{c}
P_2 M_3	53.56±0.60^{e}	66.15±0.91^{e}	76.12±3.26^{e}
P_2 M_4	34.86±2.51^{g}	43.64±2.13^{g}	51.49±3.88^{g}
C.D (0.05)	1.99	1.99	3.78
S. E. (m)	0.66	0.69	1.31

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 25. Impact of *P. indica* on proline accumulation and anti-oxidant activities in tomato under drought stress simulated by mannitol application

Treatments	Proline (µmol g^{-1})	Superoxide dismutase (mg g^{-1}fw)	Peroxidase (min $^{-1}$ g $^{-1}$ fw)	Catalase (units min^{-1} g^{-1} fw)
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	9.89±0.79^{a}	269.50±31.90^{a}	38.72±7.22^{a}	283.24±10.20^{a}
P_2 - Non-colonized tomato plants	5.75±0.55^{b}	242.04±26.80^{b}	32.10±5.01^{b}	249.79±11.23^{b}
C.D (0.05)	0.54	5.07	0.95	4.93
S. E. (m)	0.18	1.68	0.31	1.63
Drought stress stimulated by Mannitol				
M_1-Control	3.01±0.38^{d}	210.63±5.30^{d}	28.38±1.80^{d}	240.88±6.50^{d}
M_2- 3% Mannitol	4.79±1.03^{c}	241.17±11.75^{c}	32.00±3.95^{c}	255.59±7.60^{c}
M_3- 7% Mannitol	9.34±0.76^{b}	273.65±14.13^{b}	37.71±4.26^{b}	270.56±6.54^{b}
M_4- 10% Mannitol	14.15±0.86^{a}	297.63±14.14^{a}	43.54±5.15^{a}	299.03±4.23^{a}
C.D (0.05)	0.76	7.17	1.35	6.97
S. E. (m)	0.26	2.37	0.45	2.31
Treatment combinations				
P_1 M_1	3.06±0.41^{f}	212.78±6.90^{f}	29.73±1.00^{e}	252.44±6.10^{d}
P_1 M_2	5.62±0.79^{e}	250.79±1.91^{d}	35.47±0.89^{d}	269.83±5.10^{d}
P_1 M_3	11.74±1.60^{b}	294.61±8.61^{b}	41.46±1.04^{b}	290.72±1.50^{b}
P_1 M_4	19.17±1.08^{a}	319.82±6.40^{a}	48.22±0.67^{a}	319.98±5.90^{a}
P_2 M_1	2.96±0.39^{f}	208.47±2.81^{f}	27.03±1.26^{f}	229.33±4.84^{f}
P_2 M_2	3.96±0.26^{f}	231.56±8.04^{e}	28.53±1.45^{ef}	241.35±5.71^{e}
P_2 M_3	6.94±0.50^{d}	252.69±1.84^{d}	33.96±1.50^{d}	250.40±5.62^{de}
P_2 M_4	9.14±0.80^{c}	275.44±4.81^{c}	38.87±0.61^{c}	278.07±5.33^{c}
C.D (0.05)	1.08	10.13	1.90	9.86
S. E. (m)	0.37	3.35	0.63	3.26

4.4. EVALUATION OF TOMATO PLANTS COLONIZED WITH *P. indica* UNDER WATER STRESS SIMULATED BY ABA

4.4.1. Vegetative and flowering characters

At 30 DAT, the results showed that P_1, which consisted of *P. indica*-colonized tomato plants, had a significantly higher plant height of 40.74cm compared to P_2, which comprised non-colonized tomato plants with a height of 33.84cm. This indicates a positive influence of *P. indica* colonization on the plant height at an early stage of growth. At 60 DAT, similar trends were observed. The two factors and their interaction significantly affected plant height at 60 DAT. P_1 plants remained taller than P_2 plants, with heights of 60.35cm and 55.11cm, respectively (Table 26) (Plate 10).

At 30 DAT, the stem girth of *P. indica*-colonized plants (P_1) was significantly larger compared to non-colonized plants (P_2). The stem girth of P_1 plants was 3.01cm, while P_2 plants had a smaller stem girth of 2.30cm. This indicates that *P. indica* colonization positively influenced stem growth at an early stage. Similarly, at 60 DAT, P_1 plants maintained a larger stem girth of 3.44cm, while P_2 plants exhibited a smaller stem girth of 2.76cm. The difference in stem girth between the two groups remained significant, indicating a sustained positive effect of *P. indica* colonization on stem growth throughout the growth period (Table 25). Regarding water stress induced by ABA, increasing concentrations of ABA led to a reduction in stem girth both at 30 and 60 DAT. Considering the treatment combinations, variation in the stem girth depending on both *P. indica* colonization and ABA concentration was non-significant at both 30 and 60DAT.

When colonized with *P. indica* (P_1), the average number of primary branches per plant was 4.98. In comparison, non-colonized plants (P_2) had an average of 4.18 primary branches per plant. Results revealed that *P. indica* colonization contributes to a substantial increase in the number of primary branches in tomato plants. (Table 26).

At 30 DAT, P_1 had higher average leaf length of 19.86cm, which was significantly different from P_2 with a leaf length of 18.48 cm. This indicates that *P. indica* colonization had a positive effect on leaf length in tomato plants. Similar trend was noticed at 60DAT and the average leaf length for P_1 was 25.04cm, while

P_2 had an average leaf length of 22.61cm. Examining the treatment combinations, the widest leaves were observed in P_1A_1 (16.30 cm), representing *P. indica*-colonized plants under control conditions, while the narrowest leaves were found in P_2A_4 (11.46cm). At 60DAT, the widest leaves were observed in P_1 A_1 (24.20cm), representing *P. indica*-colonized plants under control conditions. Conversely, the narrowest leaves were found in P_2A_4 (13.08cm). These results indicate that *P. indica* colonization and ABA concentrations independently influence leaf width at 60 DAT. (Table 27).

At 30 DAT, the results revealed that *P. indica* colonization significantly increased leaf area in tomato plants. P_1 plants exhibited a larger leaf area (293.7 cm^2) compared to P_2 plants (209.52cm^2). Moving to 60 DAT, the positive effect of *P. indica* colonization on leaf area persisted. P_1 plants exhibited a significantly larger leaf area (482.68cm^2) compared to P_2 plants (330.06cm^2), indicating that *P. indica* colonization continues to promote increased leaf area as the plants (Table 27).

In Table 28, the impact of *P. indica* colonization and ABA-induced water stress on the time to first flowering and 50% flowering in tomato plants are presented. P_1, with *P. indica* colonization, had a significantly shorter mean flowering time of 31.13 days compared to P_2 (33.85 days). Higher ABA concentrations reduced time to flowering, with A4 (10µM ABA) at 28.14 days, while A_1 (control) was 35.05 days. Similarly, mean days to 50% flowering for *P. indica*-colonized plants (P_1) was 57.80, while non-colonized plants (P_2) took significantly longer at 59.25 days. Concerning ABA treatments, as ABA concentration increased, days to 50% flowering decreased. A_1 (control) had 60.91 days, A_2 (3µM ABA) had 60.57 days, A3 (6µM ABA) had 58.64 days, and the lowest was A_4 (10µM ABA) at 53.98 days. The interaction of *P. indica* colonization and ABA stress significantly influenced flowering time. *P. indica* colonization consistently led to earlier flowering across all ABA stress levels, indicating synergy between colonization and ABA-induced stress.

P_1 (*P. indica*-colonized) had a significantly higher mean of 7.08 clusters per plant compared to P_2 (non-colonized) at 4.13. Increasing ABA concentration led to

fewer clusters, with A_1-Control having the highest mean at 6.44, followed by A_1, A_2-3µM, A_3-6µM, and A_4-10µM at 6.26, 6.09, 5.43, and 4.66, respectively. A_1, A_2, and A_3 had similar results. This suggests that ABA-induced water stress suppressed flower cluster formation. Treatment combinations didn't yield significant effects (Table 28).

The results revealed a significant positive effect on the number of flowers per cluster with respect to colonization. P_1- *P. indica*-colonized tomato plants displayed a higher mean value of 8.06 compared to P_2- non-colonized tomato plants with a mean value of 6.45. Varying concentrations of ABA used to induce water stress had a nuanced effect on flower production. However, analyzing the treatment combinations, it is evident that the differences between the treatment combinations were not significant. (Table 28).

4.4.2. Fruit and yield characters

In the study, *P. indica*-colonized tomato plants (P_1) had a shorter time to first harvest (60.90 days) compared to non-colonized plants (P_2) at 62.88 days. ABA-induced water stress notably influenced first harvest times. The control group (A_1) had a longer duration (64.57 days) than ABA-treated groups (A_2: 62.53 days, A_3: 60.87 days, A_4: 59.60 days), indicating that drought stress accelerated the first harvest. Among combinations, P_1A_1 had the longest mean (67.38 days), while P_2A_4 had the shortest (59.36 days). Significant variations were observed across treatment combinations (Table 29).

Regarding the effect of *P. indica* colonization, the results demonstrate a significant positive effect on fruit production per truss. P_1- *P. indica*-colonized tomato plants exhibited a higher mean value of 5.88 compared to P_2 - non-colonized tomato plants with a mean value of 4.31. *P. indica* colonization, notably, positively influenced fruit production. P_1 (colonized) had a higher mean of 20.89 fruits per plant compared to P_2 (non-colonized) recording only 14.81, indicating significant enhancement in fruit yield. Statistical analysis confirmed significant differences between treatment combinations, with colonized plants consistently outperforming non-colonized ones in fruit production under all conditions (Table 29).

P. indica colonization in P_1 (colonized plants) led to higher fruit set percentages compared to P_2 (non-colonized plants). P_1 A_1 had a mean of 67.76%, significantly higher than P_1 A_2 with 61.67%. Regarding ABA-induced water stress, fruit set percentages varied with treatment concentrations. A1-Control had the highest mean at 74.79%, significantly exceeding A_2-3µM (68.23%), A_3-6µM (58.37%), and A_4-10µM (57.48%). However, no significant differences were found between treatment combinations (Table 29).

Colonizing tomato plants with *P. indica* had a significant impact on fruit length and diameter. P_1, representing *P. indica*-colonized tomato plants, exhibited a mean fruit length of 3.40cm and diameter of 3.13cm, which was significantly higher than P_2, the non-colonized tomato plants. This indicates that the presence of *P. indica* contributes to larger fruit size in tomato plants (Table 30) (Plate 11).

P. indica colonization resulted in a significantly higher mean fruit weight of 23.32g compared to P_2 (20.01g), indicating *P. indica*'s positive impact on tomato fruit weight. Regarding ABA-induced water stress, fruit weight varied notably with ABA concentrations. The control treatment (A_1-Control) had the highest mean at 25.56g, surpassing A_2-3µM (24.97g), A_3-6µM (19.97g), and A_4-10µM (16.17g), suggesting that higher ABA levels led to lighter fruits. Significant differences were found in fruit weight among treatment combinations. The highest mean fruit weight was in P_1A_1 (*P. indica*-colonized plants with A_1-Control) at 26.42g, outperforming other combinations. Conversely, the lowest mean fruit weight of 15.00g was in P_2A_4 (non-colonized plants with A_4-10µM). The differences observed in fruit cracking between *P. indica*-colonized and non-colonized plants, among the ABA treatment combinations, as well as among the treatment combinations were not statistically significant. (Table 30).

P_1 (*P. indica*-colonized) had a significantly higher average yield of 524.04g compared to P_2 (non-colonized) at 340.64g, emphasizing *P. indica*'s positive impact. Regarding ABA-induced water stress, yield varied across concentrations. A_1-Control had the highest average yield of 614.45g, significantly exceeding A_2-3µM (552.66g), A_3-6µM (373.49g), and A_4-10µM (188.79g), indicating higher ABA led to reduced yield. In treatment combinations, P_1A_1 (*P. indica*-colonized

with control) yielded the highest average at 670.97g, while P_2A4 (non-colonized with high ABA) had the lowest at 80.90g. These results highlight the combined impact of *P. indica* colonization and ABA concentration on tomato plant yield, emphasizing their importance in optimizing yield (Table 30).

4.4.4 Quality parameters

Upon examining the impact of *P. indica* colonization on the ascorbic acid content of tomato plants, it becomes apparent that P_1, consisting of *P. indica*-colonized plants, displayed a mean value of 20.62mg g^{-1}, while P_2, representing non-colonized plants, exhibited a lower mean value of 21.20mg g^{-1}. Also, mean lycopene content in P_1 was 12.42mg 100g-1, while in P_2 it was slightly lower measuring 12.33mg 100g-1. The difference between the two treatments was statistically significant. Similar trend was observed for TSS with P_1 measuring a higher value of 4.32º Brix. The findings revealed a gradual rise in ascorbic acid, lycopene and TSS levels with increasing ABA concentrations. (Table 31).

4.4.5. Physiological parameters

Regarding the influence of *P. indica* colonization, the mean RWC values for P_1 (*P. indica*-colonized tomato plants) and P_2 (non-colonized tomato plants) were 64.97% and 61.46%, respectively. The statistical analysis revealed a significant difference between these two treatments. *P. indica* colonized plants maintained a higher RWC in all conditions and this suggests that the combination of *P. indica* colonization and ABA-induced water stress has a notable influence on the relative water content in tomato plants. A significantly higher mean cell membrane stability (76.39%) and CSI (111.53%) were recorded in colonized plants compared to non-colonized plants (Table 32).

The proline content in *P. indica*-colonized tomato plants (P_1) was significantly higher compared to non-colonized plants (P_2). The mean proline content for P_1 A_1 (3.46 µmol g^{-1}) was significantly similar to P_2A_1 (2.98µmol g^{-1}). Colonizing tomato plants with *P. indica* enhances their ability to accumulate proline, which is known to play a crucial role in stress tolerance. Non-colonized tomato plants (P_2) generally showed lower proline contents across all ABA treatments compared to the corresponding P_1 treatments. (Table 33)

The results revealed significant differences in SOD activity among the different factors and treatment combinations. the analysis showed that *P. indica* colonization had a significant impact on SOD activity. The mean SOD activity for P_1 was 272.33 mg g^{-1} fw, while for P_2, it was 244.87 mg g^{-1} fw. PO activity for P_1 was 38.86 min-1 g-1 fw, while for P_2, it was 31.58 min-1 g-1 fw. The analysis of the data also revealed significant variations in CAT activity among the different factors and treatment combinations. The mean CAT activity for P_1 was 284.14units min^{-1} g^{-1} fw, while for P_2, it was 250.69units min^{-1} g^{-1} fw. These results suggest that the presence of *P. indica* positively influences the anti-oxidant activities enhancing the plant's ability to mitigate oxidative stress (Table 33). Under ABA-induced water stress conditions, the P_1 combinations (A_1 to A_4) showed higher anti-oxidant activities compared to the corresponding P_2 combinations.

Table 10. Tomato plants @ 30 DAT under water stress simulated by the application of ABA

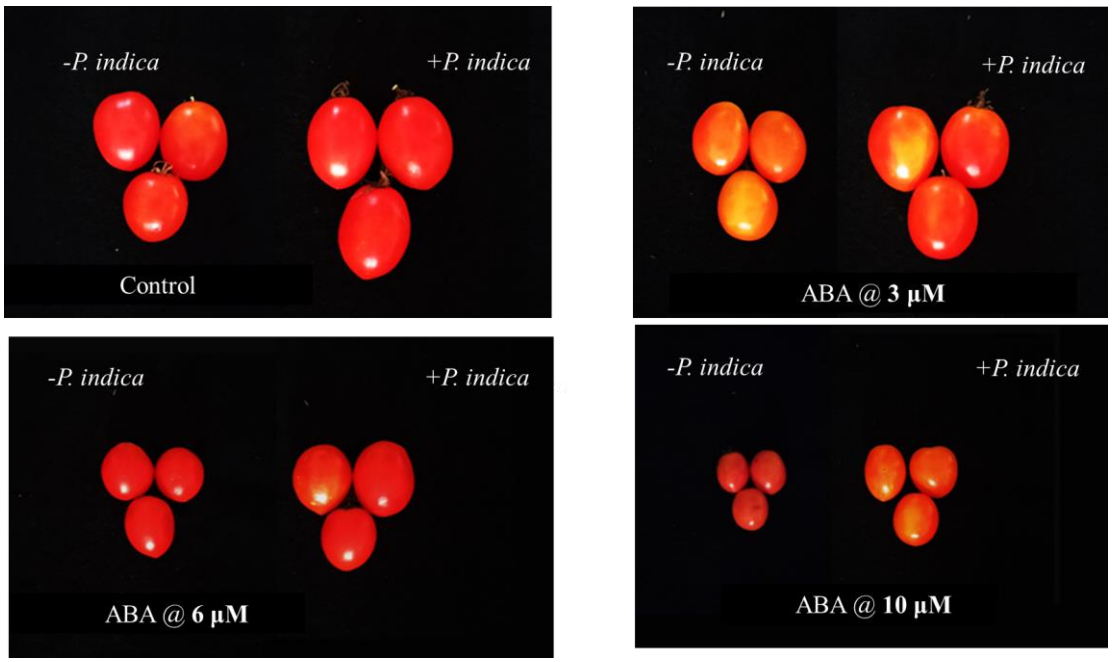


Plate 11. Fruits harvested from *P. indica* colonized and non-colonized plants under water simulated by ABA application

Table 26. Effect of *P. indica* on plant height, stem girth and number of primary branches in tomato under drought stress simulated by ABA application

Treatments	Plant height (cm)		Stem grith (cm)		Primary branches / plant
	30 DAT	60 DAT	30 DAT	60 DAT	
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	40.74±2.45[a]	60.35±2.80[a]	3.01±0.30[a]	3.44±0.45[a]	4.98±1.30[a]
P_2 - Non-colonized tomato plants	33.84±2.10[b]	55.11±3.21[b]	2.30±0.41[b]	2.76±0.56[b]	2.75±1.10[b]
C.D (0.05)	0.72	0.81	0.09	0.12	0.42
S. E. (m)	0.25	0.28	0.03	0.04	0.14
Water stress simulated by ABA					
A_1-Control	39.69±3.50[a]	62.79±1.44[a]	3.07±0.30[a]	3.79±0.35[a]	5.15±1.21[a]
A_2-3 µM	38.38±5.01[b]	60.60±2.24[b]	2.79±0.39[b]	3.23±0.43[b]	4.55±1.61[b]
A_3-6 µM	36.48±3.70[c]	55.69±1.51[c]	2.55±0.42[c]	2.85±0.38[c]	3.48±1.22[c]
A_4-10 µM	34.60±2.85[d]	51.83±1.60[d]	2.19±0.47[d]	2.53±0.44[d]	2.29±1.20[d]
C.D (0.05)	1.01	1.15	0.13	0.16	0.59
S. E. (m)	0.35	0.40	0.04	0.06	0.20
Treatment combinations					
P_1 A_1	42.84±1.41[a]	63.00±1.40[a]	3.32±0.19	4.06±0.09	6.13±0.88
P_1 A_2	43.02±0.77[a]	62.32±0.75[a]	3.16±0.05	3.59±0.18	6.03±0.58
P_1 A_3	39.78±0.54[b]	59.86±0.54[b]	2.94±0.89	3.17±0.08	4.45±0.73
P_1 A_4	37.30±0.76[c]	56.20±0.53[c]	2.60±0.14	2.94±0.09	3.33±0.52
P_2 A_1	36.54±1.10[c]	62.58±1.51[a]	2.82±0.13	3.52±0.28	4.18±0.46
P_2 A_2	33.74±1.30[d]	58.87±1.82[b]	2.42±0.08	2.86±0.22	3.08±0.29
P_2 A_3	33.18±1.81[de]	51.52±1.72[d]	2.16±0.11	2.52±22	2.50±0.36
P_2 A_4	31.90±0.22[f]	47.46±0.84[e]	1.78±0.23	2.12±0.13	1.25±0.50
C.D (0.05)	1.43	1.63	N.S	N.S	N.S
S. E. (m)	0.50	0.56	0.06	0.08	0.29

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 27. Effect of *P. indica* on leaf length, leaf width and leaf area of tomato under drought stress simulated by ABA application

Treatments	Leaf length (cm)		Leaf width(cm)		Leaf area (cm^2)	
	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT
Colonizing with *P. indica*						
P_1	19.86±2.1^{a}	25.04±3.4^{a}	14.91±1.4^{a}	20.34±3.6^{a}	293.7±6.1^{a}	482.68±10^{a}
P_2	18.48±1.8^{b}	22.61±3.3^{b}	12.90±1.1^{b}	16.46±2.2^{b}	209.52±7.1^{b}	330.06±9.6^{b}
C.D (0.05)	0.47	0.57	0.26	0.50	5.84	6.32
S. E. (m)	0.16	0.20	0.09	0.17	2.02	2.18
Water stress simulated by ABA						
A_1	21.27±1.8^{a}	26.85±2.0^{a}	15.36±1.1^{a}	20.65±1.9^{a}	306.40±4.3^{a}	505.78±9.8^{a}
A_2	20.28±0.8^{b}	26.70±1.6^{a}	14.38±1.5^{b}	20.33±1.7^{a}	285.78±3.4^{b}	481.14±9.2^{b}
A_3	18.08±0.4^{c}	22.39±0.8^{b}	13.80±1.2^{c}	18.69±1.9^{b}	224.12±3.4^{c}	381.74±8.6^{c}
A_4	17.05±0.7^{d}	19.34±1.6^{c}	12.07±0.7^{d}	13.93±1.0^{c}	190.16±4.7^{d}	256.81±5.5^{d}
C.D (0.05)	0.67	0.81	0.36	0.71	8.26	8.93
S. E. (m)	0.23	0.28	0.13	0.24	2.85	3.09
Treatment combinations						
P_1 A_1	22.58±1.3^{a}	28.44±1.2	16.30±0.3^{a}	24.20±1.7^{a}	340.47±8.2^{a}	586.79±5.6^{a}
P_1 A_2	20.83±0.8^{b}	27.78±1.6	15.72±0.4^{b}	21.86±0.9^{b}	318.05±5.9^{b}	565.88±6.1^{b}
P_1 A_3	18.39±0.2^{d}	23.04±0.4	14.92±0.2^{c}	20.52±0.2^{c}	274.46±6.1^{c}	469.46±4.5^{c}
P_1 A_4	17.62±0.5^{d}	20.88±0.1	12.68±0.2^{e}	14.78±0.6^{f}	241.83±4.7^{e}	308.60±6.1^{f}
P_2 A_1	19.96±1.1^{bc}	25.26±1.1	14.42±0.6^{c}	17.10±0.2^{e}	272.33±6.8^{c}	424.78±5.1^{d}
P_2 A_2	19.73±0.2^{c}	25.62±0.4	13.04±0.5^{d}	18.80±0.4^{d}	253.50±2.9^{d}	396.39±7.6^{e}
P_2 A_3	17.76±0.2^{d}	21.74±0.4	12.68±0.5^{e}	16.86±0.3^{e}	173.78±4.5^{f}	294.03±6.7^{g}
P_2 A_4	16.48±0.4^{e}	17.80±0.4	11.46±0.3^{f}	13.08±0.4^{g}	138.48±5.9^{g}	205.03±5.4^{h}
C.D (0.05)	0.95	N.S	0.51	1.00	11.68	12.63
S. E. (m)	0.33	0.40	0.18	0.35	4.03	4.37

Table 28. Influence of *P. indica* on flowering characters of tomato under drought stress simulated by ABA application

Treatments	Days of first flowering	Days of 50% flowering	Flower clusters per plant	Flowers per cluster
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	31.13±2.16^{b}	57.80±2.03^{b}	7.08±1.05^{a}	8.06±0.98^{a}
P_2 - Non-colonized tomato plants	33.85±3.76^{a}	59.25±2.02^{a}	4.13±0.62^{b}	6.45±0.93^{b}
C.D (0.05)	0.18	0.71	0.38	0.36
S. E. (m)	0.53	0.24	0.13	0.12
Water stress simulated by ABA				
A_1-Control	35.05±2.18^{a}	60.91±1.40^{a}	6.26±1.71^{a}	7.95±1.25^{b}
A_2-3 µM	34.79±2.08^{a}	60.57±1.74^{a}	6.09±1.73^{a}	7.81±0.92^{ab}
A_3-6 µM	32.00±2.01^{b}	58.64±1.42^{b}	5.43±1.80^{b}	7.31±0.99^{b}
A_4-10 µM	28.14±0.69^{c}	53.98±1.34^{c}	4.66±1.37^{c}	5.97±0.82^{c}
C.D (0.05)	0.76	1.00	0.54	0.50
S. E. (m)	0.26	0.35	0.19	0.17
Treatment combinations				
P_1 A_1	33.10±0.42^{b}	59.22±0.97^{b}	7.78±0.79	8.96±0.41
P_1 A_2	32.96±0.53^{b}	59.28±1.05^{b}	7.66±0.66	8.52±0.47
P_1 A_3	30.30±0.90^{c}	57.76±1.02^{c}	7.04±0.88	8.14±0.21
P_1 A_4	28.18±0.37^{d}	54.94±1.04^{d}	5.86±0.72	6.64±0.59
P_2 A_1	37.00±1.00^{a}	62.60±1.35^{a}	4.74±0.44	6.94±0.89
P_2 A_2	36.62±1.05^{a}	61.86±1.24^{a}	4.52±0.35	7.10±0.65
P_2 A_3	30.70±1.04^{b}	59.53±1.23^{b}	3.82±0.24	6.48±0.66
P_2 A_4	28.10±0.96^{d}	53.02±0.83^{e}	3.46±0.32	5.30±0.21
C.D (0.05)	1.07	1.42	N.S	N.S
S. E. (m)	0.37	0.49	0.26	0.25

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 29. Effect of *P. indica* on fruiting characters of tomato under drought stress simulated by ABA application

Treatments	Days of first harvest	Fruit per truss	Fruits per plant	Fruit set %
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	60.90±1.45^{b}	5.88±1.27^{a}	20.89±2.97^{a}	67.76±4.81^{a}
P_2 - Non-colonized tomato plants	62.88±1.30^{a}	4.31±0.98^{b}	14.81±4.61^{b}	61.67±4.70^{b}
C.D (0.05)	0.96	0.30	0.98	3000
S. E. (m)	0.34	0.10	0.34	1.03
Water stress simulated by ABA				
A_1-Control	64.57±2.19^{a}	6.11±1.20^{a}	20.92±3.04^{a}	74.79±2.61^{a}
A_2-3 µM	62.53±2.20^{b}	5.70±0.63^{a}	20.04±2.60^{a}	68.23±2.52^{b}
A_3-6 µM	60.87±0.95^{c}	5.05±1.19^{b}	18.16±3.26^{b}	58.37±2.20^{c}
A_4-10 µM	59.60±1.35^{c}	3.53±0.66^{c}	12.28±3.25^{c}	57.48±3.50^{c}
C.D (0.05)	1.36	0.42	1.39	4.24
S. E. (m)	0.47	0.14	0.48	1.47
Treatment combinations				
P_1 A_1	61.76±1.07^{bc}	7.24±0.76^{a}	23.40±1.94^{a}	78.45±2.90
P_1 A_2	61.52±2.09^{c}	6.12±0.43^{b}	22.20±1.52^{ab}	71.81±1.52
P_1 A_3	60.48±0.84^{cd}	6.10±0.58^{b}	20.86±2.20^{b}	62.03±1.84
P_1 A_4	59.85±0.85^{cd}	4.08±0.39^{d}	17.10±1.75^{cd}	58.76±1.16
P_2 A_1	67.38±1.42^{a}	4.98±0.23^{c}	18.44±1.28^{c}	71.13±2.90
P_2 A_2	63.54±2.18^{b}	5.28±0.52^{c}	15.46±0.88^{c}	64.65±2.79
P_2 A_3	61.27±0.98^{cd}	4.00±0.30^{d}	17.88±1.20^{d}	54.71±2.70
P_2 A_4	59.36±1.79^{d}	2.98±0.28^{e}	7.46±1.00^{e}	56.20±1.80
C.D (0.05)	1.93	0.60	1.97	N.S
S. E. (m)	0.67	0.21	0.68	2.08

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 30. Effect of *P. indica* on fruit biometric characteristics, fruit cracking and yield in tomato under drought stress simulated by ABA application

Treatments	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit cracking %	Yield per plant (g)
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	3.40±0.59^{a}	3.13±0.58^{a}	23.32±3.98^{a}	0.26±0.1	524.04±5.18^{a}
P_2 - Non-colonized tomato plants	3.07±0.64^{b}	2.82±0.59^{b}	20.01±4.29^{b}	0.00±0.0	340.65±7.14^{b}
C.D (0.05)	0.03	0.06	0.71	N/A	26.55
S. E. (m)	0.01	0.02	0.24	0.18	9.21
Water stress simulated by ABA					
A_1-Control	3.78±0.15^{a}	3.58±0.11^{a}	25.56±1.51^{a}	0.00±0.0	614.45±3.15^{a}
A_2-3 µM	3.73±0.16^{b}	3.33±0.15^{b}	24.97±1.84^{a}	0.00±0.0	552.66±4.24^{b}
A_3-6 µM	3.13±0.18^{c}	2.91±0.27^{c}	19.97±3.19^{b}	0.00±0.0	373.49±3.17^{c}
A_4-10 µM	2.28±0.21^{d}	2.09±0.21^{d}	16.17±1.70^{c}	0.52±0.0	188.79±4.13^{d}
C.D (0.05)	0.04	0.09	1.00	N/A	37.55
S. E. (m)	0.01	0.03	25.56±1.51a	0.00±0.0	13.03
Treatment combinations					
P_1 A_1	3.92±0.04^{a}	3.68±0.06^{a}	26.42±0.93^{a}	0.00±0.0	670.97±2.15^{a}
P_1 A_2	3.88±0.04^{b}	3.57±0.10^{ab}	26.40±1.18^{a}	0.00±0.0	652.24±7.16^{a}
P_1 A_3	3.30±0.05^{d}	3.01±0.02^{c}	22.84±0.44^{c}	0.00±0.0	476.33±6.21^{c}
P_1 A_4	2.48±0.04^{f}	2.27±0.09^{e}	17.34±1.20^{d}	0.00±0.0	296.69±5.16^{d}
P_2 A_1	3.64±0.03^{c}	3.49±0.04^{b}	24.40±0.96^{b}	0.00±0.0	557.94±6.19^{b}
P_2 A_2	3.58±0.02^{c}	3.08±0.08^{c}	23.54±1.06^{bc}	1.04±0.5	453.09±3.17^{c}
P_2 A_3	2.96±0.04^{e}	2.81±0.16^{d}	17.10±1.47^{d}	0.00±0.0	270.66±4.14^{d}
P_2 A_4	2.08±0.02^{g}	1.92±0.11^{f}	15.00±1.27^{e}	0.00±0.0	80.90±3.12^{e}
C.D (0.05)	0.06	0.12	1.42	N.S	53.10
S. E. (m)	0.02	0.04	0.49	0.33	18.43

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 31. Influence of *P. indica* on quality parameters of tomato under drought stress simulated by ABA application

Treatments	Ascorbic acid (mg g^{-1})	Lycopene ($mg100g^{-1}$)	TSS (^{o} Brix)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	20.62 ± 0.82^{b}	12.42 ± 0.13^{a}	4.32±0.23a
P_2 - Non-colonized tomato plants	20.20 ± 0.74^{a}	12.33 ± 0.19^{b}	4.25 ± 0.17^{b}
C.D (0.05)	0.10	0.05	0.05
S. E. (m)	0.03	0.02	0.02
Water stress simulated by ABA			
A_1-Control	19.49 ± 0.21^{d}	12.19 ± 0.18^{d}	4.09 ± 0.09^{c}
A_2-3 µM	19.90 ± 0.17^{c}	12.33 ± 0.06^{c}	4.12 ± 0.04^{c}
A_3-6 µM	20.81 ± 0.31^{b}	12.44 ± 0.04^{b}	4.36 ± 0.09^{b}
A_4-10 µM	21.45 ± 0.37^{a}	12.56 ± 0.05^{a}	4.56 ± 0.09^{a}
C.D (0.05)	0.14	0.08	0.07
S. E. (m)	0.05	0.03	0.02
Treatment combinations			
P_1 A_1	19.65 ± 0.17^{c}	12.29±0.12	4.09±0.12
P_1 A_2	20.05 ± 0.07^{c}	12.34±0.04	4.13±0.04
P_1 A_3	21.02 ± 0.07^{b}	12.48±0.02	4.44±0.06
P_1 A_4	21.77 ± 0.14^{a}	12.60±0.03	4.61±0.07
P_2 A_1	19.33 ± 0.11^{c}	12.08±0.17	4.08±0.07
P_2 A_2	19.75 ± 0.07^{c}	12.32±0.08	4.11±0.04
P_2 A_3	20.60 ± 0.31^{b}	12.40±0.01	4.28±0.02
P_2 A_4	21.13 ± 0.17^{a}	12.52±0.03	4.50±0.07
C.D (0.05)	N.S	N.S	N.S
S. E. (m)	0.07	0.04	0.03

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 32. Impact of *P. indica* physiological parameters in tomato under drought stress simulated by ABA application

Treatments	Relative water content (%)	Cell Membrane Stability (%)	Chlorophyll Stability Index (%)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	64.97±6.39[a]	76.39±8.20[a]	94.92±12.13[a]
P_2 - Non-colonized tomato plants	61.46±6.66[b]	72.18±10.16[b]	83.72±16.92[b]
C.D (0.05)	0.73	0.42	1.14
S. E. (m)	0.24	0.14	0.39
Water stress simulated by ABA			
A_1-Control	70.41±1.25[a]	81.70±1.53[a]	105.77±6.20[a]
A_2-3 µM	66.88±2.89[b]	79.71±1.82[b]	96.61 ±2.40[b]
A_3-6 µM	61.59±2.08[c]	76.60±2.07[c]	86.53±4.26[c]
A_4-10 µM	53.98±2.08[d]	59.13±3.92[d]	68.38±11.74[d]
C.D (0.05)	1.04	0.59	1.60
S. E. (m)	0.34	0.20	0.56
Treatment combinations			
P_1 A_1	71.36±0.70[a]	82.84±1.05a	111.53±1.81[a]
P_1 A_2	69.43±0.94[b]	81.38±0.63b	98.39±1.42[b]
P_1 A_3	63.33±0.76[c]	78.51±0.51c	90.55±0.57[d]
P_1 A_4	55.76±0.63[e]	62.84±0.32e	79.23±1.60[f]
P_2 A_1	69.46±0.85[b]	80.56±0.95b	100.00±0.01[b]
P_2 A_2	64.33±0.76[c]	78.05±0.45c	94.83±1.70[c]
P_2 A_3	59.85±1.08[d]	74.69±0.52d	82.52±0.43[e]
P_2 A_4	52.20±0.95[f]	55.43±0.41f	57.53±3.63[g]
C.D (0.05)	1.47	0.84	2.27
S. E. (m)	0.49	0.29	0.79

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 33. Impact of *P. indica* on proline accumulation and anti-oxidant activities in tomato under drought stress simulated by ABA application

Treatments	Proline (µmol g^{-1})	Superoxide dismutase (mg g^{-1}fw)	Peroxidase (min $^{-1}$ g $^{-1}$ fw)	Catalase (units min^{-1} g $^{-1}$ fw)
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	7.66±1.15^{a}	272.33±23.23^{a}	38.86±6.80^{a}	284.14±6.60^{a}
P_2 - Non-colonized tomato plants	5.38±1.42^{b}	244.87±26.21^{b}	31.58±3.65^{b}	250.69±9.20^{b}
C.D (0.05)	0.30	5.24	1.02	4.67
S. E. (m)	0.10	1.73	0.34	1.55
Water stress simulated by ABA				
A_1-Control	3.22±0.31^{d}	213.46±5.62^{d}	29.12±2.23^{d}	241.78±1.30^{d}
A_2-3 µM	3.87±0.78^{c}	244.01±11.70^{c}	32.09±3.17^{c}	256.49±6.50^{c}
A_3-6 µM	7.83±1.50^{b}	276.48±21.31^{b}	38.10±4.69^{b}	271.46±2.30^{b}
A_4-10 µM	11.16±1.6^{a}	300.46±22.42^{a}	41.58±6.53^{a}	299.93±3.50^{a}
C.D (0.05)	0.42	7.41	1.45	6.61
S. E. (m)	0.15	2.45	0.48	2.19
Treatment combinations				
P_1 A_1	3.46±0.18^{e}	215.62±7.50^{g}	30.90±1.31^{d}	253.34±4.90^{d}
P_1 A_2	4.42±0.19^{d}	253.62±2.21^{d}	34.83±1.39^{c}	270.73±6.82^{c}
P_1 A_3	9.18±0.49^{b}	297.45±9.13^{b}	42.23±1.51^{b}	291.62±2.40^{b}
P_1 A_4	13.58±0.69^{a}	322.65±6.52^{a}	47.50±0.50^{a}	320.88±6.40^{a}
P_2 A_1	2.98±0.19^{e}	211.31±3.31^{g}	27.33±1.12^{e}	230.23±6.10^{f}
P_2 A_2	3.33±0.12^{e}	234.39±7.90^{f}	29.35±0.80^{de}	242.25±5.40^{e}
P_2 A_3	6.47±0.60^{c}	255.52±1.33^{d}	33.96±1.28^{c}	251.30±4.70^{d}
P_2 A_4	8.75±0.69^{b}	278.28±5.04^{c}	35.66±1.20^{c}	278.97±4.61^{c}
C.D (0.05)	0.60	10.48	2.05	9.34
S. E. (m)	0.21	3.47	0.68	3.09

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

4.5. EVALUATION OF TOMATO PLANTS COLONIZED WITH *P. indica* UNDER FLOODING

4.5.1. Vegetative and flowering characters

After 30 days, P_1 plants were 40.06cm tall, while P_2 reached 32.88cm. Longer submergence reduced plant height, with the lowest (32.71cm) observed after 5 days (F_5). Interaction effects were significant, with P_1 taller than P_2 under the same submergence. At 60 days, P_1 reached 58.47cm, and P_2 reached 52.88cm. Increased submergence reduced height from 69.37cm (control) to 39.82cm (D_4). Plants submerged for 5 days didn't survive. Notably, P_1F_1 (70.65cm) and P_2F_1 (68.09cm) had the highest heights. Even after 3 days of submergence (P_1F_4, 52.80cm), P_1's height matched P_2F_1 (54.58cm). (Table34) (Plate 12).

Stem girth in tomato plants, influenced by *P. indica* colonization, submergence duration, and interactions, showed significant changes at 30- and 60-days post-transplantation. Colonized plants had a larger stem girth of 3.03cm and 3.58cm at 30 and 60 days, respectively, compared to non-colonized ones (Table 34).

The number of primary branches was significantly influenced by *P. indica* colonization, submergence duration, and their interactions. Colonized plants had 3.84 branches, while non-colonized had 2.20 branches, regardless of submergence. With increased submergence (up to 5 days), branches decreased notably from 4.07 to 1.46 (Table 34).

Leaf length, width and area in tomato plants were significantly influenced by colonization with *P. indica*. Colonized plants exhibited a leaf length of 20.16cm and 27.65cm at 30 and 60 days after transplanting, respectively. The interaction between *P. indica* colonization and the duration of submergence did not have a significant effect on leaf length at both 30 and 60 days after transplanting. Leaf width was highest in *P. indica* colonized plants at 30 DAT (14.19cm) and 60 DAT (21.00cm) as compared to non-colonized plants at 30 and 60 DAT (12.21cm and 17.14cm respectively) (Table 35).

P. indica colonization, duration of water submergence and their interaction had significant effect on the leaf area of tomato plants. P1 recorded a higher leaf

area at 30 DAT (305.63 cm^2**)** and 60 DAT (586.22cm^2) as against that of non-colonized plants (Table 35).

P. indica colonization significantly affected the time to first flowering and 50% flowering in tomato plants. P_1 (colonized) flowered in 28.42 days, while P_2 (non-colonized) took slightly longer at 29.92 days, highlighting the impact of colonization on flowering timing. Similarly, P_1 exhibited a mean of 49.52 days to 50% flowering, while P_2 had a higher mean of 51.94 days. The results indicated a significant difference between the treatments suggesting that the presence of *P. indica* colonization accelerated time taken to reach 50% flowering in tomato plants (Table 36).

The data also indicated that tomato plants colonized with *P. indica* (P_1) had a significantly higher number of flower clusters per plant and flowers per cluster (5.85 and 6.53, respectively) compared to non-colonized plants (P_2) (2.68 and 4.15 respectively (Table 36).

4.5.2. Fruit and yield characters

The effect of colonization of tomato plants with *Piriformospora indica*, water stress through flooding and their interactions are depicted in the table 37. From the data it is evident that tomato plants colonized with *P. indica* (P_1) took a significantly shorter time to first harvest compared to non-colonized plants (P_2) (58.30 vs. 59.59 days, respectively). Concerning the water stress imposed by flooding, there was a noticeable rush in the days to first harvest as the duration of submergence increased. Plants under 5 days to submergence did not yield any fruits and hence not included for statistical analysis

Results showed that *P. indica*-colonized tomato plants (P_1) exhibited a significantly higher fruit production (4.15 per truss) compared to non-colonized plants (P_2) (2.31 per truss). There were significant differences in the number of fruits per plant with respect to colonization, water stress and their combination. *P. indica*-colonized tomato plants (P_1) had a higher average number of fruits per plant (18.79) compared to non-colonized plants (9.25) (P_2). Additionally, water stress had a notable effect on fruit production, with the control group (F_1) showing the highest fruit yield (21.19) (Table 37).

Fruit set percentage varied significantly with respect to the colonization with *P. indica*. Tomato plants colonized with *P. indica* (55.53%) (P_1) exhibited a higher fruit set percentage compared to non-colonized plants (45.21%) (P_2) (Table 37).

Tomato plants that were colonized with *P. indica* (P_1) exhibited a significantly higher fruit length (3.32cm) and diameter (2.91cm) compared to non-colonized plants (Table 38). Also, P_1 had a mean fruit weight of 20.35 g, which was significantly higher than the non-colonized plants (P_2) with a mean weight of 17.23 g. The difference in fruit cracking between the *P. indica* colonized and non-colonized tomato was not statistically significant. Similarly, the effect of submergence and the interaction effect of colonization and water stress by flooding had no effect on fruit cracking percentage. (Table 38) (Plate 13).

The tabulated data (table 38) presents information about the yield per plant in tomato plants subjected to various treatments, including colonization with *P. indica*, water stress through flooding, and the combined effects of these factors. Examining the effects of colonization with *P. indica* on yield per plant, it can be observed that *P. indica*-colonized tomato plants (P_1) exhibited a significantly higher yield compared to non-colonized plants (P_2). The yield per plant in P_1 was 533.48 g, while P_2 had a lower yield of 191.27 g.

4.5.4. Quality parameters

The data presented in the table 39 presents the ascorbic acid, lycopene and TSS in tomato fruits under different treatments. Upon analyzing the data, it was observed that the ascorbic acid content in tomato fruits was relatively higher when colonized with *P. indica*, with values of 20.67 and measuring only19.99 mg g^{-1} for P_2 treatments, respectively. When colonized with *P. indica*, the lycopene content showed a significant increment, with values of 12.43 mg $100g^{-1}$ for P_1 and 12.35mg $100g^{-1}$ for P_2. (Table 39).

By analyzing the data, it can be concluded that all factors, including the colonization with *P. indica*, water stress induced by flooding and their interaction are non-significant in relation to the Total Soluble Solids (TSS) levels in tomato fruits. For the factor of colonization with *P. indica*, the TSS levels showed minimal

variation between P_1 (*P. indica*-colonized) and P_2 (non-colonized) treatments, with values of 3.86 and 3.88 ^{o} Brix, respectively. *P. indica* colonization did not have a significant impact on the TSS levels (Table 39).

4.5.5. Physiological parameters

The results revealed that colonizing tomato plants with *P. indica* significantly influenced the activity of anti-oxidant enzymes. The SOD activity in P_1 plants was 280.69mg g^{-1} fw, while in P_2 plants, it was only 255.36mg g^{-1} fw. The highest SOD activity was observed in P_1F_4 (3 days to submergence, +*P. indica*) with 321.62mg g^{-1} fw, followed closely by P_1 F_5 (5 days to submergence, + *P. indica*) with 318.22mg g^{-1} fw. Peroxidase activity for P_1 plants, colonized with *P. indica*, was notably higher (46.30min $^{-1}$ g $^{-1}$ fw), in comparison to the non-colonized P_2 plants. These results indicate a favorable influence of *P. indica* colonization on peroxidase activity. The interaction between *P. indica* colonization and the duration of submergence showed a significant impact on the PO activity in tomato plants

The results also demonstrated a significant difference between the colonized P_1 plants and the non-colonized P_2 plants with respect to catalase activity. P_1 plants exhibited a substantially higher CAT value of 316.62units min^{-1} g $^{-1}$ fw, in contrast to the CAT value of 258.46units min^{-1} g $^{-1}$ fw observed in P_2 plants (Table 40).

4.6. MOLECULAR PARAMETERS

4.6.1. Expression profile of drought stress related transcription factor-*SlAREB1*

Tissue-specific (leaf) expression analysis of *SlAREB1* was performed using real-time PCR in *P. indica* colonized plants and non-colonized tomato plants under normal and drought stress conditions. The fold change in the expression of *SlAREB1* recorded was 0.367 in *P. indica* colonized plants under normally irrigated conditions, 0.752 in non-colonized plants under drought stress conditions, and 1.765 in *P. indica* colonized plants under drought stress conditions (Table 41 to 43).

4.7. COLONY FORMING UNITS OF *P. indica* IN SOIL AND PLANTS

Colony Forming Units (CFU) of *P. indica* were quantified in both the soil (rhizosphere) and tomato roots, with observations taken from moderate-level stress

treatments across all five experimental trials. The spore count in soil and plant roots are represented in the table 44.

4.8. INCIDENCE OF PEST AND DISEASES

Bacterial wilt was noticed during the early vegetative stage. Minor incidence of spotted wilt was also noticed at mature stages (Plate 14).

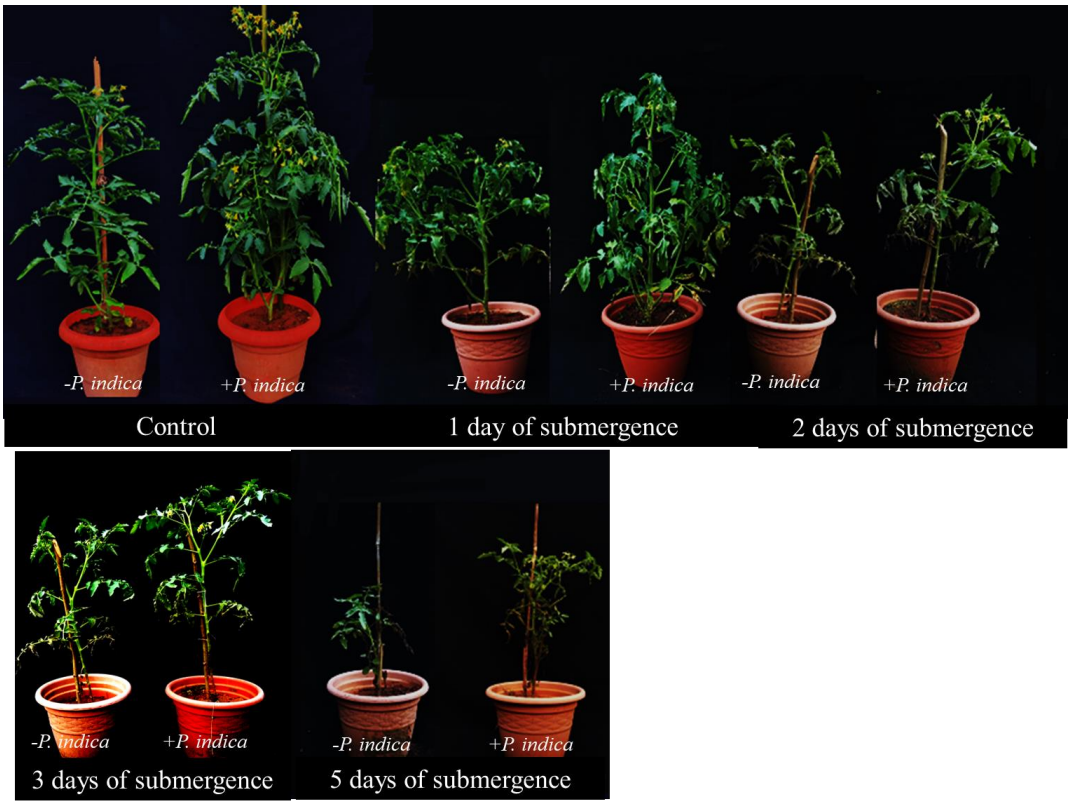


Plate 12. Tomato plants @30 days after transplantation under stress simulated by flooding

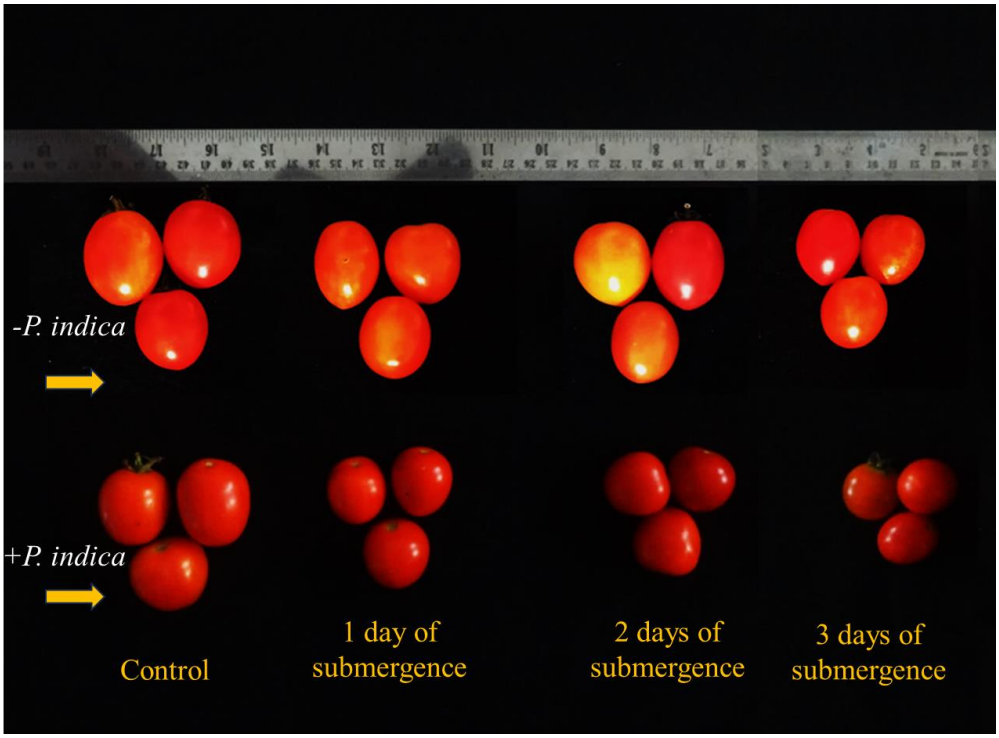


Plate 13. Fruits harvested from *P. indica* colonized and non-colonized plants under different durations of flooding

Table 34. Effect of *P. indica* on plant height, stem girth and number of primary branches in tomato under water stress by flooding

Treatments	Plant height (cm)		Stem grith (cm)		Primary branches / plant
	30 DAT	60 DAT	30 DAT	60 DAT	
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	40.05±3.81^{a}	58.47±2.02^{a}	3.03±0.24^{a}	3.58±0.40^{a}	3.84±0.31^{a}
P_2 - Non-colonized tomato plants	32.87±2.0^{b}	52.88±2.38^{b}	2.24±0.43^{b}	2.94±0.24^{b}	2.20±0.41^{b}
C.D (0.05)	0.98	1.23	0.06	0.07	0.37
S. E. (m)	0.48	0.43	0.02	0.03	0.13
Water stress by flooding					
F_1-Control	39.17±3.20^{a}	69.37±2.30^{a}	2.98±0.35^{a}	3.65±0.30^{a}	4.07±0.43^{a}
F_2-1 day of submergence	38.85±3.90^{a}	58.63±2.36^{b}	2.91±0.31^{a}	3.36±0.28^{b}	3.850±0.50^{a}
F_3-2 days of submergence	36.39±3.35^{b}	54.89±2.90^{c}	2.71±0.39^{b}	3.14±0.18^{c}	3.02±0.45^{b}
F_4-3 days of submergence	35.21±2.90^{b}	39.82±2.70^{d}	2.48±0.45^{c}	2.90±0.32^{d}	2.70±0.34^{b}
F_5-5 days of submergence	32.71±2.90^{c}	-	2.12±0.60^{d}	-	1.46±0.23^{c}
C.D (0.05)	1.56	1.74	0.10	0.11	0.59
S. E. (m)	0.54	0.60	0.03	0.04	0.20
Treatment combinations					
P_1 F_1	43.80±1.99^{a}	70.65±2.55^{a}	3.30±0.08^{a}	4.20±0.15^{a}	4.50±0.30^{ab}
P_1 F_2	44.20±2.00^{a}	62.69±0.95^{c}	3.18±0.11^{ab}	3.62±0.08^{b}	5.30±0.37^{a}
P_1 F_3	39.38±0.67^{b}	58.04±1.21^{d}	3.08±0.08^{bc}	3.30±0.10^{c}	4.00±0.31^{bc}
P_1 F_4	37.70±1.20^{b}	42.52±1.05^{g}	2.90±0.12^{c}	3.22±0.13^{cd}	3.55±0.30^{c}
P_1 F_5	35.20±1.20^{c}	-	2.70±0.12^{d}	-	1.85±0.30^{de}
P_2 F_1	34.54±1.88^{cd}	68.09±1.17^{b}	2.66±0.08^{d}	3.10±0.16^{de}	3.65±0.15^{c}
P_2 F_2	33.50±2.29^{cd}	54.58±0.89^{e}	2.64±0.11^{d}	3.10±0.10^{de}	2.40±0.27^{d}
P_2 F_3	33.40±1.67^{cd}	51.74±2.96^{f}	2.34±0.05^{e}	2.98±0.04^{e}	2.05±0.20^{d}
P_2 F_4	32.72±1.79^{d}	37.12±1.13^{h}	2.06±0.13^{f}	2.58±0.13^{f}	1.85±0.20^{de}
P_2 F_5	30.22±1.88^{e}	-	1.54±0.11^{g}	-	1.07±0.18^{e}
C.D (0.05)	2.20	2.46	0.14	0.15	0.83

S. E. (m)	0.77	0.85	0.05	0.05	0.28

Table 35. Effect of *P. indica* on leaf length, leaf width and leaf area in tomato under water stress by flooding

Treatments	Leaf length (cm)		Leaf width(cm)		Leaf area (cm^2)	
	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT
Colonizing with *P. indica*						
P_1	20.16±1.7[a]	27.6±2.12[a]	14.19±1.7[a]	21.00±2.3[a]	305.6±2.5[a]	586.2±2.0[a]
P_2	17.48±1.8[b]	24.4±2.18[b]	12.21±1.4[b]	17.14±2.3[b]	225.5±2.3[b]	420.5±1.7[b]
C.D (0.05)	0.38	0.84	0.31	1.12	10.03	39.21
S. E. (m)	0.13	0.41	0.11	0.39	3.46	13.61
Water stress by flooding						
F_1	20.96±1.4[a]	27.8±2.04[a]	15.34±1.5[a]	20.80±2.4[a]	319.7±1.3[a]	583.0±0.9[a]
F_2	19.93±1.4[b]	27.2±1.84[a b]	13.96±1.1[b]	20.19±1.4[a]	275.8±1.2[b]	547.8±1.8[a]
F_3	19.22±1.6[c]	26.6±1.85[b]	13.53±1.7[b]	20.57±2.5[a]	259.0±2.1[c]	547.9±1.2[a]
F_4	17.67±1.5[d]	22.5±2.12[c]	11.91±0.7[c]	14.73±2.0[b]	207.8±2.5[d]	334.8±2.0[b]
F_5	16.3±1.3[e]	-	11.26±0.8[d]	-	179.3±1.1[e]	-
C.D (0.05)	0.61	1.19	0.49	1.59	14.18	55.45
S. E. (m)	0.21	0.41	0.17	0.55	4.90	19.25
Treatment combinations						
$P_1 F_1$	22.24±0.7	29.6±2.07	16.48±0.7[a]	24.20±2.7[a]	364.7±1.2[a]	717.0±1.4[a]
$P_1 F_2$	21.18±0.8	28.8±0.83	14.96±0.5[b]	21.10±1.4[b c]	314.7±3.1[b]	605.5±0.8[b]
$P_1 F_3$	20.56±1.2	28.2±0.83	15.10±0.2[b]	22.94±0.8[a b]	308.4±1.6[b]	645.1±0.9[a b]
$P_1 F_4$	19.02±0.6	24.0±1.41	12.46±0.3[d]	15.76±2.3[e f]	284.5±1.3[d]	377.3±1.3[d]
$P_1 F_5$	17.82±0.6	-	11.96±0.3[e]	-	212.7±2.0[e]	-
$P_2 F_1$	19.68±0.3	26.1±1.02	14.20±1.1[c]	17.40±0.8[d e]	274.7±1.36[c]	449.0±2.3[c d]

P_2 F_2	18.68±0.6	25.7±0.97	12.96±0.2^{d}	19.28±0.5^{c} $_{d}$	236.8±1.15^{d}	490.1±1.6^{c}
P_2 F_3	17.88±0.3	25.0±1.22	11.96±0.3^{e}	18.20±0.4^{d}	209.6±2.6^{e}	450.7±2.0^{c} $_{d}$
P_2 F_4	16.32±0.4	21.0±1.58	11.36±0.4^{e}	13.70±1.2^{f}	180.9±2.2^{f}	292.3±1.8^{e}
P_2 F_5	14.82±0.4	-	10.56±0.4^{f}	-	145.9±1.4^{g}	-
C.D (0.05)	N.S	N.S	0.69	2.24	18.14	78.42
S. E. (m)	0.30	0.60	0.24	0.78	6.32	27.22

Table 36. Effect of *P. indica* on flowering characters of tomato under water stress by flooding

Treatments	Days of first flowering	Days of 50% flowering	Flower clusters per plant	Flowers per cluster
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	28.48±2.29^{b}	49.42±1.67^{b}	5.85±0.47^{a}	6.53±1.35^{a}
P_2 - Non-colonized tomato plants	29.92±3.90^{a}	51.94±1.72^{a}	2.68±0.69^{b}	4.15±1.13^{b}
C.D (0.05)	0.52	0.49	0.22	0.72
S. E. (m)	0.18	0.17	0.08	0.25
Water stress by flooding				
F_1-Control	34.26±2.39^{a}	56.06±1.96^{a}	6.32±0.71^{a}	7.70±1.53^{a}
F_2-1 day of submergence	30.76±2.00^{b}	53.18±2.11^{b}	5.60±0.76^{b}	6.98±1.48^{a}
F_3-2 days of submergence	27.75±0.48^{c}	50.86±1.85^{c}	4.35±1.00^{c}	5.30±1.54^{b}
F_4-3 days of submergence	27.00±0.66^{cd}	48.52±1.29^{d}	3.07±0.47^{d}	4.31±1.70^{c}
F_5-5 days of submergence	26.27±0.62^{d}	44.79±1.32^{e}	1.98±0.45^{e}	2.41±1.45^{d}
C.D (0.05)	0.82	0.78	0.35	1.13
S. E. (m)	0.29	0.27	0.12	0.40
Treatment combinations				
P_1 F_1	32.10±0.73^{b}	53.42±0.87^{c}	7.74±0.59^{a}	8.77±1.09
P_1 F_2	29.54±0.95^{c}	51.30±0.83^{d}	7.20±0.45^{b}	8.07±0.85
P_1 F_3	27.60±0.54^{de}	49.20±0.67^{e}	6.02±0.14^{c}	6.80±1.50

P_1 F_4	27.30±0.83^{de}	47.44±0.71^{f}	4.96±0.11^{d}	5.50±1.80
P_1 F_5	25.90±0.54^{f}	45.75±0.82^{g}	3.32±0.21^{f}	3.50±1.70
P_2 F_1	36.42±0.85^{a}	58.70±1.48^{a}	4.90±0.63^{d}	6.63±1.12
P_2 F_2	31.98±2.10^{b}	55.06±0.71^{b}	4.00±0.18^{e}	5.90±1.14
P_2 F_3	27.90±0.41^{d}	52.52±0.63^{c}	2.68±0.17^{g}	3.80±1.30
P_2 F_4	26.70±0.27^{ef}	49.60±0.57^{e}	1.18±0.24^{h}	3.12±0.11
P_2 F_5	26.64±0.47^{ef}	43.84±1.00^{h}	0.64	1.32±0.39
C.D (0.05)	1.17	1.10	0.50	N.S
S. E. (m)	0.41	0.38	0.17	0.56

Table 37. Influence of *P. indica* on fruiting characters in tomato under water stress by flooding

Treatments	Days of first harvest	Fruit per truss	Fruits per plant	Fruit set %
Colonizing with *P. indica*				
P_1 - *P. indica*-colonized tomato plants	58.30±1.89^{b}	4.15±0.72^{a}	18.79±1.70^{a}	55.53±5.59^{a}
P_2 - Non-colonized tomato plants	59.59±1.36^{a}	2.31±0.40^{b}	9.25±1.30^{b}	45.21±6.66^{b}
C.D (0.05)	0.59	0.08	1.11	5.50
S. E. (m)	0.20	0.03	0.39	1.90
Water stress by flooding				
F_1-Control	62.06±1.64^{a}	5.23±0.91^{a}	21.19±1.1^{a}	68.52±6.58^{a}
F_2-1 day of submergence	60.27±1.74^{b}	3.87±1.24^{b}	18.67±1.21^{b}	54.82±5.20^{b}
F_3-2 days of submergence	57.81±0.83^{c}	2.35±1.20^{c}	10.34±0.90^{c}	44.34±5.59^{c}
F_4-3 days of submergence	55.64±0.73^{d}	1.45±0.30^{d}	5.88±0.70^{d}	33.79±4.50^{d}
C.D (0.05)	0.84	0.25	1.57	7.78
S. E. (m)	0.29	0.09	0.54	2.69
Treatment combinations				
P_1 F_1	60.62±0.69^{b}	6.22±0.40^{a}	23.12±1.45^{a}	71.81±5.05
P_1 F_2	59.10±0.85^{c}	5.10±0.61^{b}	24.70±1.77^{a}	63.93±5.95
P_1 F_3	57.54±0.53^{d}	3.38±1.00^{d}	16.70±1.14^{c}	51.51±7.84

P_1 F_4	55.94±0.56[e]	1.88±0.50[f]	10.62±0.94[d]	34.88±5.72
P_2 F_1	63.50±0.79[a]	4.24±0.60[c]	19.25±0.82[b]	65.24±6.89
P_2 F_2	61.44±1.63[b]	2.64±0.31[e]	12.64±1.12[d]	45.71±6.87
P_2 F_3	58.08±1.04[cd]	1.32±0.22[g]	3.98±0.71[e]	37.17±3.43
P_2 F_4	55.34±0.82[e]	1.02±0.30[g]	1.14±0.50[f]	32.71±5.30
C.D (0.05)	1.19	0.36	2.23	N.S
S. E. (m)	0.41	0.12	0.77	3.80

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 38. Effect of *P. indica* on fruit biometric characteristics, fruit cracking and yield in tomato under water stress by flooding

Treatments	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit cracking %	Yield per plant (g)
Colonizing with *P. indica*					
P_1 - *P. indica*-colonized tomato plants	3.32±0.25[a]	2.91±0.24[a]	20.35±5.18[a]	0.00±0.0	533.48±66.8[a]
P_2 - Non-colonized tomato plants	2.94±0.18[b]	2.67±0.18[b]	17.23±4.74[b]	0.30±0.7	191.27±53.9[b]
C.D (0.05)	0.11	0.08	0.58	N.S	27.37
S. E. (m)	0.04	0.03	0.20	0.15	9.46
Water stress by flooding					
F_1-Control	3.76±0.19[a]	3.21±0.15[a]	25.02±1.81[a]	0.00±0.0	533.48±22.9[a]
F_2-1 day of submergence	3.61±0.25[a]	3.11±0.12[a]	20.96±2.14[b]	0.25±1.3	402.20±20.0[b]
F_3-2 days of submergence	3.02±0.25[b]	2.74±0.18[b]	17.05±1.93[c]	0.00±0.0	186.73±22.3[c]
F_4-3 days of submergence	2.12±0.20[c]	2.10±0.24[c]	12.14±1.50[d]	0.35±0.3	76.95±12.3[d]
C.D (0.05)	0.15	0.12	0.83	N.S	38.71
S. E. (m)	0.05	0.04	0.28	0.21	13.38
Treatment combinations					
P_1 F_1	3.90±0.10	3.28±0.14	26.62±0.70	0.00±0.0	616.81±40.9[a]

$P_1 F_2$	3.80±0.29	3.22±0.04	22.88±0.87	0.00±0.0	563.57±29.5[a]
$P_1 F_3$	3.22±0.08	2.86±0.25	18.62±1.24	0.00±0.0	311.91±13.1[c]
$P_1 F_4$	2.34±0.15	2.28±0.13	13.28±1.19	0.00±0.0	141.31±15.5[e]
$P_2 F_1$	3.62±0.14	3.14±0.13	23.43±0.75	0.00±0.0	450.16±28.1[b]
$P_2 F_2$	3.42±0.08	3.00±-.07	19.04±0.57	0.50±1.2	240.85±12.6[d]
$P_2 F_3$	2.82±0.20	2.62±0.13	15.48±0.87	0.00±0.0	61.56±9.0f
$P_2 F_4$	1.90±0.14	1.92±-0.17	10.99±0.85	0.70±1.1	12.51±2.50[f]
C.D (0.05)	N.S	N.S	N.S	N.S	54.39
S. E. (m)	0.07	0.06	0.41	0.30	18.88

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 39. Influence of *P. indica* on quality parameters in tomato under water stress by flooding

Treatments	Ascorbic acid (mg g^{-1})	Lycopene (mg g^{-1})	TSS (º Brix)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	20.67 ± 0.86^{a}	12.43 ± 0.19^{a}	3.86±0.13
P_2 - Non-colonized tomato plants	19.99 ± 0.66^{b}	12.35 ± 0.15^{b}	3.88±0.14
C.D (0.05)	0.08	0.06	N.S
S. E. (m)	0.03	0.02	0.03
Water stress by flooding			
F_1-Control	19.52 ± 0.21^{d}	12.18 ± 0.11^{c}	3.90±0,13
F_2-1 day of submergence	19.81 ± 0.29^{c}	12.34 ± 0.09^{b}	3.86±0.14
F_3-2 days of submergence	20.60 ± 0.57^{b}	12.48 ± 0.06^{a}	3.80±0.13
F_4-3 days of submergence	21.39 ± 0.43^{a}	12.55 ± 0.13^{a}	3.91±0.13
C.D (0.05)	0.11	0.09	N.S
S. E. (m)	0.04	0.03	0.04
Treatment combinations			
P_1 F_1	19.70 ± 0.10^{d}	12.18±0.13	3.92±0.11
P_1 F_2	20.06 ± 0.16^{c}	12.41±0.04	3.88±0.17
P_1 F_3	21.13 ± 0.16^{b}	12.49±0.07	3.80±0.12
P_1 F_4	21.79 ± 0.09^{a}	12.62±0.14	3.84±0.11
P_2 F_1	19.35 ± 0.11^{e}	12.18±0.11	3.88±0.16
P_2 F_2	19.56 ± 0.11^{d}	12.27±0.09	3.84±0.11
P_2 F_3	20.07 ± 0.09^{c}	12.47±0.05	3.80±0.15
P_2 F_4	21.00 ± 0.14^{b}	12.47±0.08	3.98±0.13
C.D (0.05)	0.16	N.S	N.S
S. E. (m)	0.05	0.04	0.06

(DAT- Days after Transplanting; Different letters within the same column represent significant differences; Values are mean of 5 observations)

Table 40. Influence of *P. indica* on anti-oxidant activities in tomato under water stress by flooding

Treatments	Superoxide dismutase (mg g^{-1}fw)	Peroxidase (min^{-1} g^{-1} fw)	Catalase (units min^{-1} g^{-1} fw)
Colonizing with *P. indica*			
P_1 - *P. indica*-colonized tomato plants	280.69±26.78^{a}	46.30±5.75^{a}	316.62±16.26^{a}
P_2 - Non-colonized tomato plants	255.36±27.86^{b}	35.47±4.34^{b}	258.46±15.69^{b}
C.D (0.05)	7.65	0.83	3.00
S. E. (m)	2.57	0.28	1.01
Water stress by flooding			
F_1-Control	212.50±5.49^{c}	30.19±1.18^{e}	258.46±16.31^{e}
F_2-1 day of submergence	242.50±16.92^{b}	35.36±3.17^{d}	266.39±16.06^{d}
F_3-2 days of submergence	291.03±15.12^{a}	41.97±7.05^{c}	278.49±14.00^{c}
F_4-3 days of submergence	299.08±16.74^{a}	47.79±8.38^{b}	307.52±16.38^{b}
F_5-5 days of submergence	295.03±11.73^{a}	49.11±8.46^{a}	326.82±15.41^{a}
C.D (0.05)	12.05	1.32	4.74
S. E. (m)	4.07	0.44	1.60
Treatment combinations			
P_1 F_1	215.03±6.90^{g}	31.09±0.88^{fg}	277.79±2.95^{e}
P_1 F_2	252.29±1.92^{e}	38.05±1.17^{d}	290.93±4.70^{d}
P_1 F_3	296.31±4.69^{b}	48.35±1.35^{b}	308.54±2.66^{c}
P_1 F_4	321.62±5.42^{a}	56.31±1.57^{a}	341.41±2.17^{b}
P_1 F_5	318.22±4.47^{a}	57.71±1.57^{a}	364.41±2.27^{a}
P_2 F_1	209.97±2.85^{g}	29.30±0.55^{gf}	239.13±2.37^{g}
P_2 F_2	232.71±4.03^{f}	32.67±1.49^{f}	241.85±1.38^{f}
P_2 F_3	285.75±5.73^{bc}	35.58±0.49^{e}	248.43±2.53^{f}
P_2 F_4	276.54±4.89^{cb}	39.27±0.23^{c}	273.64±4.70^{e}
P_2 F_5	271.84±4.80^{d}	40.52±0.23^{c}	289.24±4.70^{d}
C.D (0.05)	17.10	1.87	6.71
S. E. (m)	5.76	0.63	2.26

Table 41. Expression of *SlAREB1* in *P. indica* colonized tomato plants under normal condition

	Experimental Well 1	Experimental Well 2	Experimental Well 3	Control Well 1	Control Well 2	Control Well 3	Average Experimental Ct Value	Average Experimental Ct Value	Average Control Ct Value	Average Control Ct Value	ΔCt Value (Experimental)	ΔCt Value (Control)	Delta Delta Ct Value	Expression Fold Change
	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	TE	HE	TC	HC	ΔCTE	ΔCTC	ΔΔCt	2^-ΔΔCt
Housekeeping Gene	26.23	25.96	25.96	35.37	35.37	35.37	-	26.05	-	35.37	2.78	1.34	1.44	0.367716716
Gene being Tested	33.50	26.50	26.50	36.71	36.71	36.71	28.83	-	36.71	-				

Table 42. Expression of *SlAREB1* in non-colonized tomato plants under drought stress condition

	Experimental Well 1	Experimental Well 2	Experimental Well 3	Control Well 1	Control Well 2	Control Well 3	Average Experimental Ct Value	Average Experimental Ct Value	Average Control Ct Value	Average Control Ct Value	ΔCt Value (Experimental)	ΔCt Value (Control)	Delta Delta Ct Value	Expression Fold Change
	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	TE	HE	TC	HC	ΔCTE	ΔCTC	ΔΔCt	2^-ΔΔCt
Housekeeping Gene	26.00	26.00	26.05	35.37	35.37	35.37	-	26.02	-	35.37	2.75	2.34	0.41	0.752623374
Gene being Tested	29.30	28.50	28.50	37.71	37.71	37.71	28.77	-	37.71	-				

Table 43. Expression of *SlAREB1* in *P. indica* colonized tomato plants under drought stress condition

	Experimental Well 1	Experimental Well 2	Experimental Well 3	Control Well 1	Control Well 2	Control Well 3	Average Experimental Ct Value	Average Experimental Ct Value	Average Control Ct Value	Average Control Ct Value	ΔCt Value (Experimental)	ΔCt Value (Control)	Delta Delta Ct Value	Expression Fold Change
	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	Raw Ct Value	TE	HE	TC	HC	ΔCTE	ΔCTC	ΔΔCt	2^-ΔΔCt
Housekeeping Gene	28.28	28.28	28.28	35.37	35.37	35.37	-	28.28	-	35.37	1.52	2.34	-0.82	1.765405993
Gene being Tested	29.80	29.80	29.80	37.71	37.71	37.71	29.80	-	37.71	-				

Table 44. Colony forming units of *P. indica* in soil and plant roots

Sl. No.	Experiments	Spore population in soil and plant roots ($CFUg^{-1}ml^{-1}$)	
		Soil	Plant root
1	Evaluation of tomato plants colonized with *P. indica* under drought stress (50% FC)	1.00×10^6	2.25×10^6
2	Evaluation of tomato plants colonized with *P. indica* under drought stress simulated by PEG (-7 bar)	0.50×10^6	2.50×10^6
3	Evaluation of tomato plants colonized with *P. indica* under water stress simulated by mannitol (7% mannitol)	0.50×10^6	2.00×10^6
4	Evaluation of tomato plants colonized with *P. indica* under water stress simulated by ABA (6 µM)	0.25×10^6	1.50×10^6
5	Evaluation of tomato plants colonized with *P. indica* under flooding (3 days of submergence)	0.75×10^6	2.00×10^6

5. DISCUSSION

Tomato (*Solanum lycopersicum* L.) is a widely recognized herbaceous edible fruiting plant belonging to the Solanaceae family and is considered one of the most essential vegetable crops globally. Tomatoes are not only a staple in our diets but also offer a wealth of essential nutrients and health-promoting compounds, making them a crucial component of global food security. However, challenges such as climate change-induced drought threaten tomato production, especially in regions like Kerala, India, where sporadic production and lower productivity persist due to various biotic and abiotic stresses. Addressing these challenges is essential to ensure a stable supply of this vital crop.

To combat the adverse effects of water stress on tomato cultivation, the current study entitled "Management of water stress in tomato (*Solanum lycopersicum* L.) through beneficial root endophytic fungus, *Piriformospora indica*" was conducted in the Department of Vegetable Science, College of Agriculture, Vellayani during 2019-22 which focused on the potential of *Piriformospora indica*, a beneficial root-endophytic fungus, to induce tolerance to water stress in tomato plants. The study employed various methods to induce water stress in tomato plants, including gravimetric method, application of PEG, mannitol, and ABA, and submergence, to comprehensively assess the effects of water stress on tomato plants and the potential benefits of *Piriformospora indica* colonization. The results obtained are discussed in this chapter.

5.1. EVALUATION OF TOMATO PLANTS COLONIZED WITH *P. indica* UNDER WATER STRESS INDUCED BY VARIOUS METHODS

5.1.1. Plant height

The height of the plants was significantly influenced by the varying levels of field capacities, which ranged from 100% to 25%. Plants that were colonized by *P. indica* showed notable increase in height, with 21% and 6% more height compared to non-colonized plants at 30 and 60 days after transplanting, respectively irrespective of the levels of drought stress (Fig. 1). On the other hand, when the irrigation was reduced to 25% of the field capacity, there was a considerable reduction in plant height, with decreases of 17% and 24% at 30 and 60 days after

transplanting, respectively. Sibomana *et al.* (2013) conducted a similar study to examine how different soil moisture threshold levels affected the growth and yield of tomatoes under water stress conditions. The research revealed that severe water deficit resulted in a 24% decrease in plant height compared to the control group. Plants under water stressed condition shows stunted shoot growth to prevent the loss of more water through transpiration (Etesami *et al.*, 2015; Barnawal *et al.*, 2019). Notably, the colonization by *P. indica* had a positive impact on tomato plant height across all field capacities. It led to a maintenance of 18%, 31%, 20%, and 14% more plant height than non-colonized plants at 100%, 75%, 50%, and 25% field capacities, respectively.

Under the stress simulated by PEG-6000, tomato plants showed a reduced shoot growth of 18% and 52% (at 30 and 60DAT respectively) when the concentration reached -10bar. Reduced plant height by PEG induced stress was also reported in tomato (6 Kumar *et al.*, 2021), maize (Robin *et al.*, 2021), and sugarcane (Reyes *et al.*, 2023). *P. indica* colonization led to a 22% increase in plant height compared to control plants without any PEG treatment, 30 days after transplantating. However, at 60 days after transplanting (DAT), the heights of colonized and non-colonized plants were statistically similar. With increasing concentrations of applied PEG from -3bar to -10bar, colonized plants exhibited 26%, 29%, and 11% more height compared to non-colonized plants treated with -3bar, -7bar, and -10bar concentrations, respectively.

When mannitol was used for inducing stress, plant height reduced by 25% and 49% at 30 and 60 DAT, respectively as the concentration of mannitol reached 10%. Decrease in shoot length under different concentrations of mannitol was also reported in maize (Kaya *et al.*, 2013; Mozdzen *et al.*, 2015) and chilly (Garg *et al.*, 2019). Under mannitol induced water stress also, *P. indica* colonization could maintain 10% more plant height than control at 30 DAT. As the concentration of applied mannitol increased to 10%, plant height reduced in both colonized and non-colonized plants, but colonized plants had 30% more plant height than that of non-colonized plants.

ABA application reduced plant height from 39.69cm to 34.60cm at 30 DAT and 62.79cm to 51.83cm at 60 DAT respectively. The correlation between the elevated accumulation of ABA in plants exposed to stress conditions and the resulting stunted growth has been supported by experimental evidence. Additionally, when ABA was externally applied at higher concentrations, it inhibited the growth of non-stressed plants that were adequately watered (Zhang and Davies, 1990). *P. indica* colonization consistently maintained higher plant heights at both 30 and 60DAT across different concentrations of ABA applied.

When tomato plants were submerged for different durations *viz*.1, 2, 3 and 5 days, plant height was reduced by 16% at 30 DAT irrespective of *P. indica* colonization. The reduced availability of nitrate and nitrogen caused by flooding and the subsequent denitrification process can have detrimental effects on plant growth. Nitrate is a vital source of nitrogen for plants, and its depletion can lead to nutrient deficiencies, negatively impacting various aspects of plant growth and development (Blom, 1999; Vos *et al.*, 2005; Hamonts *et al.*, 2013; Mu and Chen, 2021). Under submerged conditions, colonized plants had maintained significantly higher plant heights compared to the non-colonized plants.

In all the five experiments, under stressed condition *P. indica* colonized plants maintained higher plant height across different levels of water stress induced by different methods. Colonized plants experience improved growth during drought stress due to the advantageous effects of *P. indica* (Fig. 2). This fungus plays a vital role in enhancing the plant's ability to absorb essential nutrients, including nitrogen (N) and phosphorus (P). Furthermore, *P. indica* positively influenced the plant's water uptake efficiency (Boorboori and Zhang, 2022). Waller *et al.* (2005) observed that *P. indica*-colonized plants exhibited improved and robust secondary and tertiary roots. As a consequence of this enhanced root development, the overall biometric parameters of the plants increased. Increased plant height in *P. indica* colonized plants under water stressed condition has been reported in Chinese cabbage (Sun *et al.*, 2010), tomato (Fakhro *et al.*, 2010), finger millet (Tyagi *et al.*, 2017) and rice (Saddique *et al.*, 2018). *P. indica* promotes shoot growth in terms of length, fresh weight (FW), and dry weight (DW). The fungus regulates plants'

cytokinin-responsive genes differently (Vadassery *et al.*, 2008) and produces cytokinin, which may be a reason for the increased shoot length.

5.1.2. Stem girth

Stem girth was reduced gradually as the level of water stress increased to 25%FC at both 30 and 60 DAT. Similarly reduced stem diameter under drought stress was reported in tomato by Kirnak *et al.* (2001); and Sibomana *et al.* (2013). When PEG-induced stress was applied, a substantial reduction of 39% and 31% in stem girth was observed at 30 and 60 days after transplanting (DAT), respectively, as the concentration increased to -10bar. Similarly, mannitol-induced stress resulted in significant reductions of 30% and 40% at 30 and 60 DAT, respectively, with a concentration of 10%. Moreover, the application of ABA also had a notable impact on stem girth. At a concentration of 10 μM, there were reductions of 28% and 33% in stem girth at 30 and 60 DAT, respectively. These findings highlight the notable impact of these stress-inducing agents on the growth of the plants during the specific time points. Reduced stem diameter under PEG induced stress was reported by Sirkeci *et al.* (2021) and Reyes *et al.* (2023). Zhu *et al* (2020) reported reduction in plant height as well as diameter under drought stress induced by application of PEG and mannitol. Restriction of shoot growth by exogenous application of ABA has been reported earlier by Leskovar and Cantliffe (1992). Increasing concentration of ABA decreased the stem diameter in tomato as reported earlier by Vu *et al.* (2015). Water logged condition affecting overall plant growth and having a negative impact on stem diameter has been reported (Tian *et al.*, 2020, Rahman *et al.*, 2021). Similar findings were obtained in the preset study also.

P. indica, on the other hand, counters the negative effects of water stress by increasing the production of antioxidants, both enzymatic and nonenzymatic, including secondary metabolites that scavenge harmful ROS. It also helps in higher water uptake and maintains efficient photosynthesis, resulting in reduced ROS generation. The fungus additionally boosts the production of osmolytes, which aid in osmotic adjustment within the cells. Furthermore, *P. indica* improves water and nutrient uptake and enhances photosynthetic efficiency. All these mechanisms lead to enhanced growth of colonized plants under stressed conditions (Jangir *et al.*,

2021). In the present study, *P. indica* colonized tomato plants maintained higher stem diameter compared to non-colonized plants across different ranges of water stress induced by various factors. Aliloo *et al.* (2016) and Su *et al.* (2017) reported a higher stem diameter in plants colonized by *P. indica* under stressed condition compared to control plants.

5.1.3. Primary branches per plant

The growth and development of branches in tomato plants can be considered as indicators to improve their productivity (Ohta *et al.*, 2017). Under increased drought stress, where the stress level reached 25%, there was a significant decline of 64% in the number of primary branches in tomatoes. Under drought stress, plant physiological processes are disrupted, leading to reduced water absorption and transmission to various plant parts (Lisar *et al.*, 2012; Conti *et al.*, 2019). Consequently, the number of branches per plant decreases under drought conditions. This finding is consistent with the results reported by Salama *et al.* (2017).

When PEG, mannitol, and ABA were applied to induce stress, reductions of 12%, 50%, and 42% were observed in the number of primary branches per plant at their highest concentrations of -10 bar, 10%, and 10 μM, respectively. Under waterlogged conditions, the number of branches per plant exhibited a decline when compared to the control plants (Sathi *et al.*, 2022). In the present study also 64% reduction was reported when tomato plants were submerged for 5 days.

In the study conducted by Saru (2021), fungal colonization in tomato plants led to an impressive 117% increase in the number of branches. Moreover, in the present research, it was observed that the colonized plants produced significantly a greater number of primary branches compared to the non-colonized plants when subjected to the same intensity of stress treatment.

5.1.4. Leaf length

The leaf is a vital organ with significant functions, including regulating respiration and contributing to the synthesis of organic matter, which supports plant growth and nutrition. The plant's response to environmental conditions, particularly water deficit, has a significant impact on the structure of tomato leaves (Anjum *et*

al., 2011). Increasing drought stress to 25% FC reduced the leaf length at both 30 and 60 DAT by 18% and 26% respectively. Reduction in leaf length due to water depreciation has been reported in tomato (Medyouni *et al.*, 2021). Consistently same results were obtained when PEG, mannitol and ABA were applied to induce water stress. Increasing concentration of PEG resulted in reduction in leaf length as reported by Ayaz *et al.* (2015) in tomato. Application of ABA had same effect as that of water stress and resulted in reduction of leaf length Quarrie and Jones (1977). The decrease in leaf size is typically due to the suppression of both cell division and cell expansion, similar to what has been documented in studies on water stress (Slatyeb, 1967).

Plants colonized by *P. indica* displayed significantly higher leaf length under a range of stress levels induced by limited irrigation, PEG, mannitol, ABA, and waterlogging. Such enhancement in leaf size was noticed in *P. indica* colonized plants as reported by Xu *et al.* (2017).

5.1.5. Leaf width

According to Medyouni *et al.* (2021), water deficit was found to decrease leaf width in tomatoes. In the present current study, drought stress at 25% FC resulted in a significant reduction of 22% and 30% in leaf width at 30 and 60 days after transplanting (DAT), respectively. Moreover, the application of PEG (-10bar), mannitol (10%), ABA (10µmol), and waterlogging for 3 days also led to a notable decrease in leaf width, ranging from 20% to 30% in tomato plants. Furthermore, it was observed that higher concentrations of ABA inhibited plant growth, as documented by Humplík *et al.* (2017) and 46 Yoshida *et al.* (2019). However, the colonization of *P. indica* had a remarkable effect on the plants, aiding them in maintaining significantly higher leaf width compared to normal tomato plants under various stress levels induced by different methods. This highlights the potential beneficial impact of *P. indica* colonization in mitigating the adverse effects of stress on tomato plants.

5.1.6. Leaf area

Changes in leaf area have occurred as a consequence of the inhibition of leaf growth under restricted water supply (Petrović *et al.*, 2021). A decrease by 35% and

47% in leaf area was noted at 30 and 60 DAT when drought stress was increased to 25% FC. Decreased leaf area due to insufficient water was reported earlier in tomato (Medyouni *et al.*, 2021). The decrease in leaf size may be linked to a decline in the mitotic activity of epidermal cells, resulting in a reduced total number of leaf cells (Farooq *et al.*, 2009). Similar observations were made in other plant species such as *Pennisetum glaucum* L (Kusaka *et al.*, 2005), *Phaseolus vulgaris*, *Sesbania aculeata* (Ashraf and Iram, 2005), and sesame (Hassanzadeh *et al.*, 2009).

Similar trends were observed when stress was induced by applying PEG, mannitol, and ABA. At the highest applied concentration, a notable decrease in leaf area ranging from 33% to 53% was recorded. Around 28% reduction in leaf area was observed in wheat genotypes under drought stress induced by the application of 15% PEG (Sharma *et al.*, 2022). The decrease in cell growth and cell generation are recognized factors that lead to a decline in leaf size (Alves and Setter, 2004). According to Vu *et al.* (2015), higher ABA concentrations led to a reduction in leaf area in tomato seedlings. This decrease in leaf size may have consequences for the plant's overall photosynthetic capacity and growth.

The presence of *P. indica* has a significant impact on leaf area and various agronomic attributes related to crop improvement. *P. indica* exhibits a strong potential for enhancing both vegetative and reproductive growth (Su *et al.*, 2017). Under different ranges of stress induced in the current study, *P. indica* colonized tomato plants possessed significantly higher leaf area compared to those plants which were not inoculated. Enhanced leaf area was recorded in maize colonized with *P. indica* under PEG induced stress (Xu *et al.*, 2017; Hosseini *et al.*, 2018). Increased leaf area was also observed in *P. indica* colonized plants of maize (Xu *et al.*, 2017), proso millet (Ahmadvand and Hajinia, 2018) and in egg plant (Swetha and Padmavathi, 2020) under water stress induced by limiting irrigation.

P. indica exerts multiple positive effects on plant growth. It stimulates shoot growth, resulting in increased shoot length, fresh weight (FW), and dry weight (DW) of the shoots. This effect is achieved through the differential regulation of plants' cytokinin-responsive genes by *P. indica* (Vadassery *et al.*, 2008). Additionally, *P. indica* itself produces cytokinin, which likely plays a significant

role in the observed increase in shoot length. Furthermore, *P. indica* enhances the translocation process through the xylem and phloem, assisting in osmotic adjustment at the cellular level during drought. This process helps to maintain cell turgor, ultimately promoting the growth of plant cells (Ahmadvand and Hajinia, 2018; Nagarajan and Nagarajan, 2010). Moreover, *P. indica* positively influences leaf characteristics, leading to enhanced leaf area and an increased number of leaves per plant across various host plants (Hosseini *et al.*, 2018; Xu *et al.*, 2017; Zhang *et al.*, 2018; Tyagi *et al.*, 2017; Ahmadvand and Hajinia, 2018; Swetha *et al.*, 2020).

The analysis of gene expression in Chinese cabbage triggered by *P. indica* demonstrates its significant role in activating genes related to carbohydrate transportation, hormone signalling, cell wall metabolism, and root development (Lee *et al.*, 2011). Additionally, the growth improvement in Chinese cabbage due to *P. indica* involves the induction of auxin, evident from increased auxin levels attributed to the activation of genes responsible for cell wall acidification and auxin transportation. Likewise, *P. indica* can engage with sugar beet Hs1 PRO-1 2 to influence the early growth of tobacco seedlings. The phosphorus transporter genes in *P. indica* facilitate plant phosphorus uptake, as these genes are induced in the external mycelium (Yadav *et al.*, 2010). In summary, *P. indica* strongly enhances plant growth through various mechanisms involving nutrients, auxin, miRNA models, specific gene expressions, phytoremediation, immunomodulation, and acting as a bio-herbicide (106 Khalid *et al.*, 2019).

5.1.7. Days to first flowering

According to the findings of Ram and Rao (1984), drought stress has a notable impact on various aspects of flowering, including the flowering duration, nectar production, flower opening mechanism, and the maintenance of turgor in floral organs. Two common strategies employed by plants to adapt to drought conditions are altering their growth rate and adjusting their flowering time (Schmalenbach *et al.*, 2014). An early transition from vegetative to reproductive development allows plants to reproduce before the advent of severe water deficit jeopardizes their survival. This strategy, known as the drought escape strategy, has been studied and documented by Ludlow (1989), Sherrard, and Maherali (2006),

and Franks (2011). In the present study, when water stress was intensified to 25% FC (field capacity), tomato plants exhibited a significantly accelerated flowering response, reaching the flowering stage by 27 days after transplanting. In contrast, under normal conditions, it took 35 days for the plants to initiate flowering. In a similar manner, as the concentration of PEG was raised from -1 bar to -10 bars, there was a significant 28% reduction in the time taken for the first flowering event. Similarly, increasing the mannitol concentration from 1% to 10% resulted in a notable 32% reduction in the days to first flowering. Furthermore, elevating the ABA concentration from 1 µmol to 10 µmol led to a distinct 19% reduction in the time to first flowering. Sivakumar and Srividhya (2016) observed a significant reduction in the time taken for flowering to occur in tomatoes when they subjected the plants to increased drought stress, transitioning from 100% field capacity (FC) to 50% FC. Akter *et al.* (2019) found that the time taken for first flowering was accelerated in T2 (30 days of water withholding) with an average of 26.69 days, whereas it was delayed in T3 (45 days of water withholding) with an average of 27.18 days. Comparatively, T1 (control) exhibited a slightly earlier flowering at an average of 26.89 days. This accelerated flowering response during drought conditions could be attributed to the plants' rapid phenological development, as they attempt to complete their life cycle within an unfavourable environmental setting.

The flowering period in plants colonized by *P. indica* was shortened by 12 days as reported by Saru (2021). Here, in all the five experiments *P. indica* colonized tomato plants took significantly shorter period to reach flowering compared to the non-colonized plants kept under well irrigated condition. The presence of *P. indica* has a substantial impact on crucial genes in the gibberellic acid (GA) pathway, which plays a vital role in controlling the timing of flowering. This finding is consistent with the understanding that early flower induction is influenced by GA (Kim *et al.*, 2017). Furthermore, *P. indica* colonization results in elevated levels of gibberellins in the roots of Chinese cabbage (Lee *et al.*, 2011) and barley seedlings (Schäfer *et al.*, 2009). In barley plants, the gene responsible for deactivating active GA (GA2ox) is down-regulated upon *P. indica* inoculation, indicating that the fungus enhances GA biosynthesis. Furthermore, the study

conducted by Kim *et al.* (2017) indicated that *P. indica* colonization led to the up-regulation of key genes involved in gibberellin biosynthesis, specifically Gibberellin 20-Oxidase2, Gibberellin 3-Oxidase1, and Gibberellin requiring 1, in plants. This up-regulation resulted in an increase in GA4 content. As a result, *P. indica* facilitated early flowering in *Arabidopsis* by enhancing the gibberellin levels. This suggests that the GA pathway plays a significant role in contributing to the early-flowering phenotype induced by *P. indica* inoculation.

5.1.8. Days to fifty percentage flowering

Days to fifty percent flowering followed the same trend as in days taken to reach first flowering stage. Increasing drought stress from100 to 25% FC decreased the days taken to fifty percent flowering by 20%. With the elevation of PEG concentration to -10 bars, mannitol concentration to 10%, and ABA concentration to 10 µmol, there was a substantial reduction in the time taken for fifty percent flowering, specifically by 24%, 37%, and 11%, respectively. Such findings were also reported in wheat (Kadam *et al.*, 2017), tomato (Namitha, 2017) and bean (Nuñez Barrios *et al.*, 2005) under water stress. When tomato plants were submerged for 5 days, the days to 50% flowering reduced by 20%.

P. indica colonized tomato plants exhibited a significant advancement in reaching fifty percent flowering compared to non-colonized plants. This consistent trend was observed under various conditions of water stress induced by limiting irrigation, as well as through the application of PEG, mannitol, ABA, and even during submergence. In previous studies, it was observed that *P. indica* had a positive impact on inoculated plants. This positive impact manifested through various mechanisms, such as widespread growth and increased proliferation of the root system. Additionally, the inoculated plants exhibited an advancement in their flowering, occurring earlier than in non-inoculated plants (Johnson *et al.*, 2014; Shrivastava and Varma, 2014; 73 Das *et al.*, 2012).

5.1.9. Flower clusters per plant

In the study, the number of flower clusters per plant decreased by 12%, 45%, and 72% as the level of drought stress increased to 75%, 50%, and 25% of the field capacity (FC) respectively. Flower production and maintenance require

significant energy in the form of carbon, nutrients, and water (Galen 2000; Teixido and Valladares 2014; Roddy *et al.*, 2019; Gallagher and Campbell 2017). As a result, plants experiencing limited water availability often produce flowers that are smaller, fewer in number, or have shorter lifespans (Carroll *et al.* 2001; Burkle and Runyon 2016; Teixido *et al.* 2016; Gallagher and Campbell 2017). Harmful effect of drought stress in number of flower clusters in tomato was also reported by Jangid and Dwivedi (2017). In 2001, Sorial carried out a study using three different tomato genotypes to assess their performance when subjected to varying levels of water stress (100%, 50%, and 25% F.C.). The results indicated that as the water stress level increased, the number of flower clusters per plant decreased.

In the study involving PEG-induced water stress, the number of flower clusters per plant decreased significantly with increasing stress levels. Specifically, when subjected to concentrations of -1 bar, -7 bar, and -10 bar, the reduction in flower clusters per plant was 16%, 38%, and 72%, respectively. When mannitol was utilized to induce water stress, a consistent and gradual decline in the number of flower clusters was observed as the concentration of mannitol was increased. These findings highlight the sensitivity of flower production to water stress, and the results could be valuable in understanding the response of plants to different stress levels. Su *et al.* (2013) demonstrated a notable reduction in the quantity of flowers in *Arabidopsis thaliana* when subjected to drought stress, as compared to the control group. Under ABA-induced stress, there was no statistically significant difference observed at a concentration of 1µmol. However, at a concentration of 10µmol, a notable decrease of 25% in flower clusters was observed. Similarly, Hernandez-Aarmenta (1985) conducted a study with bell pepper plants under water stress conditions. Their findings revealed that reduced soil moisture negatively affected both vegetative and reproductive growth, resulting in a lower production of flower clusters per plant. Nawata and Sakuratani (1999) conducted an experiment comparing drought-tolerant and drought-sensitive tomato varieties under drought conditions. They found that both types of varieties showed a decrease in the number of flower clusters per plant, but the reduction was more significant in the drought-sensitive varieties compared to the drought-tolerant ones.

Keeping colonized and non-colonized tomato plants under submerged condition also had a negative impact on number of flower cluster per plant. Number of flower clusters reduced gradually as the duration of submergence increased. When the plants were submerged for 5 days, 68% reduction was recorded. Similar findings were reported in tomato by Ezin at al. (2010).

Regardless of the water stress levels induced by different methods, tomato plants colonized by *P. indica* exhibited a higher number of flower clusters compared to non-colonized plants. A study conducted by Pham *et al.* (2004) reported a greater number of inflorescences in *P. indica*-inoculated plants compared to non-inoculated plants. Similarly, Saru's (2021) research on tomatoes also documented increased flower production in *P. indica* colonized plants. These findings suggest that the presence of *P. indica* positively influences flower production in tomato plants, regardless of the water stress conditions.

5.1.10. Flowers per cluster

The variation in the number of flowers per cluster followed a similar trend to that observed in the case of flower clusters. With an increase in drought stress to 25% FC, there was a notable decrease of approximately 56% in the number of flowers per cluster. This reduction is consistent with previous findings in tomato studies reported by Ganeva *et al.* (2019) and Subramanian *et al.* (2006), where a significant decrease in the number of flowers per cluster was also observed.

In a similar manner, the application of PEG to induce water stress resulted in a substantial 60% reduction in the number of flowers per cluster at a concentration of -10 bar. Likewise, using mannitol at a concentration of 10% led to a significant decrease of 53% in the number of flowers per cluster. When exposed to ABA at lower concentrations of 3µmol, there was no noticeable effect on the number of flowers. However, at a higher concentration of 10µmol, there was a significant drop of 24% in the number of flowers per cluster.

Water stress induced by submerging the plants resulted in a significant reduction in the number of flowers per cluster. Increasing the duration of submergence from 1 day to 5 days led to a substantial decrease of 65% in the

number of flowers per cluster. This indicates that prolonged submergence severely impacts flower production in the plants.

P. indica colonized plants consistently produced higher number of flowers compared to non-colonized plants across different levels of water stress. The presence of the fungus had a positive effect on flower production. Compared to the uninoculated controls, plants inoculated with the fungus showed a significant increase in the number of flowers (Pham *et al.*, 2004, Das *et al.*, 2012, Fakhro *et al.*, 2010, Rai *et al.*, 2001). The increase in flower production in plants can be attributed to two main factors: improved nutrient uptake, especially potassium (K+), and possible hormonal effects (Perner *et al.*, 2007). Higher levels of K+ in the plant improve nutrient absorption, which contributes to more flowers being produced (Abdelaziz *et al.*, 2019). Additionally, hormones like gibberellins, responsible for bud production, may be transported faster with higher K+ levels. Furthermore, studies have emphasized the importance of phosphorus as a nutrient in promoting bud formation and development, affecting the number of flowers, size of pollen grains, and seed formation. For instance, in tomatoes, phosphorus has been shown to promote flower formation, increase fruit mass, seed count, pollen count, and average pollen production in individual flowers (Poulton *et al.*, 2002).

5.1.11. Days to first harvest

Days to harvest were affected by different water stress methods. When the drought stress was increased to 25% of the field capacity, there was a 20% decrease in the number of days it took to reach the first harvest stage. Additionally, in cowpea, researchers (Fatokun *et al.*, 2012) observed early flowering and fruiting under water-stressed conditions.

Under PEG-induced water stress, a concentration of -10 bar resulted in a 20-day reduction in the time required for harvesting. Conversely, increasing the mannitol concentration by 10% led to a 10-day decrease in the number of days needed for harvest. Furthermore, the application of ABA at a concentration of 10 µmol reduced the time to harvest by an additional 5 days.

Upon subjecting both colonized and non-colonized plants to a 5-day submergence, the flowers were unable to develop into fruit, leading to their

premature dropping. Furthermore, submergence lasting 3 days led to a notable reduction of 6 days in the time required to reach the first harvest.

Plants colonized by *P. indica* exhibited an earlier arrival at the initial harvest stage, even when subjected to varying levels of water stress induced through different methods. The phenomenon of early flowering and fruiting has been documented in plants colonized by *P. indica*, as highlighted by both Mensah *et al.* (2020) and Dass *et al.* (2012).

5.1.12. Fruits per truss

Ramping up water stress to 25% of field capacity had an adverse effect on the number of fruits per truss. A significant 84% reduction in the count of fruits per truss was documented at the 25% FC stress level. In a similar vein, Geneve *et al.* (2019) observed a substantial decrease of 76-28% in the formation of fruits per cluster upon reducing water regimes by 50%. Coinciding with these findings, Sato *et al.* (2001)'s research confirmed comparable patterns of heightened flower abortion and restricted fruit development resulting from the interplay of drought and elevated temperatures. According to Wu *et al.* (2022), tomato plants cultivated with optimal irrigation displayed a greater fruit yield compared to those subjected to limited irrigation.).

A comparable pattern emerged when stress was induced using PEG, mannitol, and ABA. As the applied stress concentration intensified to -10 bars, a substantial 87% decrease in the quantity of fruits per truss was observed in tomato plants. Meanwhile, with mannitol at a concentration of 10%, the reduction stood at approximately 77%, and elevating ABA concentration to 10 µmols led to a reduction of 42%. Earlier research asserted that when exposed to water scarcity, tomato plants exhibited a diminished capacity to efficiently uptake and process nutrients, potentially resulting in a decline in fruit production (Wang *et al.*, 2019)

When tomato plants were submerged for 5 days no flowers and fruits were formed. Under 3 days of submergence, a reduction number of fruits per truss by 72% was noticed. The reduction in number of fruits could be due to the inhibition of photosynthesis and adverse effect of flooding (Ezin *et al.*, 2010).

P. indica colonization resulted in an enhancement in fruits formed per truss irrespective of the level of water stress by 31%, 32%, 36%, and 26% under water stress by limiting irrigation, application of PEG, mannitol and ABA respectively. Under flooding induced water stress also, *P. indica* enhanced the number of fruits per truss by 44% irrespective of number of days under submergence. Furthermore, it has been documented that *P. indica* can mitigate the harmful consequences of stress by promoting the absorption of water and minerals (Abadi and Sepehri, 2016). The enhanced root size resulting from the colonization of *P. indica* could potentially enhance and optimize the uptake of nutrients from the rhizosphere (Sun *et al.*, 2010; Das *et al.*, 2014), and could be the reason for enhanced number of fruits (Abdelaziz *et al.*, 2019).

5.1.13. Fruits per plant

The decline in the count of fruits per plant became apparent, paralleling the decrease in the number of fruits formed per truss, as elaborated earlier. When considering drought induced by restricted irrigation, the number of fruits decreased by a significant 83% at a 25% field capacity (FC). Under stress induced by PEG, the fruit count diminished by 92% at a concentration of -10 bars. Weerasinghe *et al.* (2003) discovered that the presence of drought stress leads to a decrease in tomato yield by lowering the number of fruits produced per plant. Similarly, a comparable pattern emerged with mannitol-induced water stress, where the number of fruits reduced by 82% at the highest concentration of 10%. At an ABA concentration of 10 µmol, there was a reduction of 42% in the number of fruits per plant. Water scarcity results in a notable decrease in the count of blossoms, subsequently leading to a diminished production of fruits and ultimately resulting in a lower yield with reduced market value (Buhroy *et al.*, 2017; Losada and Rincaon, 1994). Comparable findings were also documented by Hassnain *et al.* (2020). In the current study, it was observed that tomato plants did not produce any fruits when submerged for a period of 5 days, regardless of whether they were colonized by *P. indica*. However, when subjected to submersion for a duration of 3 days, there was a significant decrease of 72% in the number of fruits per plant. Similar findings were reported by Kuo and Chen (1980) and Kuo *et al.* (1982).

Colonization by *P. indica* in tomato led to a rise in fruit count by 20% and 45% compared to plants that were not colonized, both in conditions with no stress and under water stress (Abdeldaym and Sabra,2018). In the present study also, tomato plants colonized by *P. indica* consistently exhibited a greater number of fruits per plant across all five experiments involving diverse stress induction methods (Fig. 3). Colonized tomato plants exhibited a two-fold increase in fruit production, as documented by Fakhro *et al.* (2010). *P. indica* demonstrated its multifunctional capabilities by engaging in various essential roles. These encompassed efficient nutrient uptakes, enhancing the plant's ability to resist diseases, improving its tolerance to stressful conditions, and actively promoting overall growth. This was elucidated by Unnikumar *et al.* (2013) in their research.

5.1.13. Fruit set percentage

In the context of a fruit-producing vegetable crop like the tomato, the fruit set emerges as the predominant attribute in appraising its capacity to withstand drought conditions. Fruit set percentage was negatively affected by increasing drought stress to 25% field capacity. There was a reduction in fruit set by 68% at 25%FC. The phases of reproduction in tomatoes, such as flower and fruit development, are particularly vulnerable to the impact of drought stress (Salter, 1954). The presence of drought stress led to a decline in the percentage of successful flower and fruit formation in chickpeas (Fang *et al.* 2010). In the current investigation, the imposition of water stress through the use of PEG at a concentration of -10 bars led to a significant 71% decrease in fruit set. Comparable patterns were noted in cases of water stress induced by applying mannitol (10%) and ABA (10µmol), where reductions of 53% and 23% in fruit set percentage were observed, respectively. Following a period of submersion lasting 3 days, a significant reduction of 50% in fruit set was observed. Nevertheless, when the submersion duration was extended to 5 days, there was a complete absence of fruit set.

As explained by Horchani *et al.* (2008), there exist two potential explanations for the shedding of tomato flowers and fruits in response to drought stress. One is the accumulation of stress-induced ethylene in the above-ground

structures. The second is the possible limitation of carbohydrate supply to flowers and fruits due to restricted photosynthetic activity.

Consistently, tomato plants colonized by *P. indica* exhibited elevated fruit set percentages in comparison to non-colonized plants, both in regular and stress-induced conditions. Similar findings were reported by Das *et al.* (2012) and Abdelaziz *et al.* (2019). In a study conducted by Kaboosi *et al.* (2022) in tomato, the presence of *P. indica* positively influenced the transformation of flowers into fruits, leading to a significant 44% augmentation of this particular characteristic in the colonized plants.

5.1.14. Fruit length

The length of the fruit exhibited a gradual reduction as the intensity of water stress heightened, achieved through methods such as controlled irrigation restriction, the application of substances like PEG, mannitol, ABA, and even submergence. As water stress intensified to reach 25% of the field capacity, there was a resultant reduction of 38% in the fruit length. As outlined by Prudent *et al.* (2010), in tomatoes, the application of water deficiency negatively regulated cell division and the development of fruit tissue. Application of PEG (-10bar) caused a reduction in fruit length by 43%. Alomari-Mheidat *et al.* (2023) reported a noteworthy contrast in fruit size between the control group and the water-stressed group. The fruits in the control group were considerably larger, boasting a size that was roughly three times greater. Raising the levels of mannitol and ABA to 10% and 10µmol, respectively, also triggered a decrease in fruit length by 46% and 39%, independently of whether the plants were colonized by *P. indica*. Additionally, prolonging the submergence period yielded a similar outcome, causing a 43% reduction in fruit length. The study conducted by Parkash *et al.* (2021) also demonstrated a reduction in fruit length in cucumbers through the deliberate intensification of water stress.

Under both controlled and stressed conditions, plants colonized by *P. indica* exhibited notably greater fruit lengths compared to non-colonized plants. Similar reports were also documented by Wang *et al.* (2015) and Abdelaziz *et al.* (2019) in tomato

5.1.15. Fruit diameter

In this study, subjecting plants to an intensified level of water stress, specifically at 25% of the field capacity (FC), yielded a significant and remarkable 37% reduction in fruit diameter. This outcome aligns with the findings of Medyouni *et al.* (2021), who observed a decrease in tomato fruit diameter as a result of water deprivation, further supporting the notion of diminished fruit diameter in response to water deficit. The mechanism behind this limitation in fruit growth during water deficit revolves around the diminished flow of both xylem and phloem within the plant's vascular system. This intricate interplay could be linked to changes in the plant's water status, alterations in the hydraulic characteristics of xylem, and fluctuations in carbohydrate synthesis occurring within the leaves, as proposed by Li *et al.* (2023).

The study further demonstrated that the induction of water stress through the application of PEG, mannitol, and ABA resulted in a significant decrease in fruit diameter. Moreover, subjecting the plants to a 3-day submersion period yielded a noteworthy 34% reduction in fruit diameter. In an investigation carried out by Hossain (2003), three varieties of mungbean were subjected to three levels of water stress (30%, 50%, 70% field capacity). The study revealed that as the degree of water stress rose, the size of pods diminished. A comparable outcome was noted by Taub (2003) in the case of chickpeas.

Consistently, the plants that were colonized by *P. indica* displayed significantly larger fruit diameters in contrast to their non-colonized counterparts. This observation aligns with a previous study conducted by Kaboosi *et al.* (2022), which reported a substantial 34% decrease in fruit diameter of non-inoculated tomato plants as compared to the inoculated ones. Additionally, a study by Yan *et al.* (2021) documented an increase in fruit size among *P. indica* colonized *Passiflora edulis* plants.

5.1.16. Fruit weight

Subjecting the tomato plants to controlled irrigation reduction to induce drought resulted in a significant 44% decline in the weight of their fruits, irrespective of whether they were colonized or not. This aligns with the prior findings of Medyouni *et al.* (2021), who reported a 43% reduction in tomato fruit weight due to restricted water availability. The majority of research findings indicate that the scarcity of water caused by drought stress disrupts the usual operations of tomato plants by significantly impacting their strength and overall productivity (Techawongstein *et al.*, 1992). A significant 63% decrease in fruit weight was noted as the concentration of PEG was elevated to -10 bar. These results align with those of a recent investigation conducted by Flores-Saavedra *et al.* (2023). Increasing the concentration of mannitol and ABA also resulted in the reduction of individual fruit weight. Under submerged conditions for 3days, the fruit weight was reduced by 51%. Ezin *et al.* (2010) found that subjecting tomato plants to continuous flooding for two, four, and eight days resulted in a notable reduction in the overall weight of fruits when compared to the control group

Inoculation with *P. indica* influenced the fruit weight of tomato positively, enhancing the fruit weight under control as well as water stress condition (Fig. 4). When tomato plants were treated with *Talaromyces omanensis*, an endophytic fungus, in a study conducted by Flores-Saavedra *et al.* (2023), a significant increase in both the weight and width of the fruits was observed, setting it apart from the impact of drought treatment. Similarly, the utilization of arbuscular mycorrhizal fungi in the presence of drought stress resulted in an improvement in the overall weight and physical size of *Capsicum annuum* fruit, as highlighted in the findings of Mena-Violante *et al.*'s study (2006). Moreover, the endophytic fungus *Piriformospora indica* demonstrated its capacity to enhance the biomass of tomato fruits, specifically by raising their fresh weight and enhancing the content of dry matter, as established through the investigation conducted by Fakhro *et al.* (2010).

5.1.17. Fruit cracking percentage

The percentage of fruit cracking remained largely unaffected by variations in water stress levels and the presence of *P. indica* colonization across all conducted

experiments. Fruit cracking transpires when there is a simultaneous occurrence of swift intake of water and solutes into the fruit, coupled with a weakening of the tomato skin's resilience and flexibility due to ripening or other influencing factors. In outdoor settings, the primary trigger for fruit cracking often arises from abrupt reductions in soil moisture tension brought about by irrigation or rainfall (Peet, 1992). In the current investigation, water stress was introduced prior to the stage of fruit formation, which might account for the limited fluctuations observed in fruit cracking percentage concerning the degree of water stress intensity. Additionally, it was observed that the colonization of *P. indica* did not exert a significant influence on the aforementioned outcome.

5.1.18. Yield per plant

Numerous studies have confirmed that drought has a negative impact on crop yield. For instance, Arshad *et al.* (2008) showed significant reductions in tomato yield due to drought treatment. Decreases in yield and changes in flowering patterns were observed under drought stress, likely because of damage to leaf gas exchange, which restricted tissue growth. Additionally, processes like phloem loading, moving nutrients, and distributing dry matter were also constrained by drought stress (Anjum *et al.*, 2011; Farooq *et al.*, 2009). In the context of the present study, as the level of drought stress intensified to 25%FC, the yield experienced a significant decline of almost 90%. Increase in PEG concentration to -10bar caused a reduction by 96% in yield of tomato. Similarly yield was reduced by 91% and 69% as the mannitol concentration and ABA concentration escalated to 10% and 10µmol respectively. Water stress created through flooding the plants for 3days resulted in 70% reduction in yield. The reduction in yield during periods of drought stress is probably a consequence of factors like premature fruit shedding before harvest, constrained carbohydrate supply due to a decline in photosynthesis rate, and direct hindrance of fruit expansion due to reduced cell turgor caused by water stress (Powell, 1974; Bradford and Hsiao, 1982; Kramer and Boyer, 1995; Lopez *et al.*, 2012;).

Nonetheless, the utilization of microbial treatments resulted in the enhancement of yield attributes. This improvement was ascribed to a range of

mechanisms, including heightened auxin production (Khalid *et al.,* 2004), nitrogen fixation (Luna *et al.,* 2012), and the process of phosphate solubilization (Oteino *et al.,* 2015). Together, these mechanisms acted in concert to foster augmented plant growth and increased productivity. The colonization of *P. indica* led to a significant 43% increase in yield, regardless of the drought conditions induced through reduced irrigation in tomato plants. Furthermore, when comparing the yield of plants that were colonized with those that were not, there was a noticeable enhancement in yield by 14%, 28%, 146%, and 376% under the conditions of normal irrigation, 75%FC, 50%FC, and 25%FC, respectively. This result was consistent in experiments wherein water stress was induced by the application of PEG, mannitol, ABA and submergence.

Studies have indicated that during periods of drought, crucial enzymes involved in nitrogen metabolism, such as glutamate synthase, glutamine synthetase, and nitrate reductase, are significantly inhibited (Wang *et al.*, 2016). Nonetheless, this inhibitory impact was absent in plants that had their roots inhabited by *P. indica*. This suggests that the fungus aids in enhancing the plants' ability to absorb nitrogen, even when faced with drought circumstances (Wang *et al.*, 2016). According to Ghaffari *et al.* (2016), *P. indica* enhances the reallocation of resources within the host plant and shields it from the adverse consequences of drought. *P. indica* brings about positive changes in the growth of host plants during their early growth phase itself (Nautiyal *et al.*, 2010; 138. Anith *et al.*, 2011). This fungus has a significant impact on the root system of many plants. For example, when faced with drought conditions, it increases the length, fresh weight, and dry weight of roots in various cereals and eggplants (Saddique *et al.*, 2018; Tsai *et al.*, 2020; Zhang *et al.*, 2018; Xu *et al.*, 2017; and Swetha and Padmavathi, 2020, Hosseini *et al.*, 2018). This improvement in root traits helps plants absorb more nutrients and water, which in turn supports healthier growth even under stressful conditions. This ultimately increases the chances of plants surviving stress.

The augmentation in growth, including the expansion of lateral roots, is believed to stem from *P. indica*'s function in enhancing both the synthesis and movement of auxin. Additionally, it triggers the activation of genes linked to auxin

signalling within the host plants, as noted in references (Tanha *et al.*, 2014; Lee *et al.*, 2011; Xu *et al.*, 2018). Moreover, *P. indica* exerts distinct regulatory control over plant genes that respond to cytokinins (Vadassery *et al.*, 2008). The fungus itself produces cytokinins, which likely play a role in the observed enhancement of shoot length. Furthermore, *P. indica* facilitates the efficient transport of substances through both xylem and phloem. This process becomes particularly significant during periods of drought stress as it supports osmotic adjustments at the cellular level. Ultimately, this mechanism upholds cellular turgor, thereby fostering the growth of plant cells. *P. indica*'s influence leads to increased nutrient uptake and improved photosynthetic pigments in host plants during drought stress, which also contributes to better agronomic traits (Ahmadvand and Hajinia, 2018; Nagarajan and Nagarajan, 2010).

The collective impact of *P. indica* on root development, shoot growth, flowering, and agronomic traits culminates in a substantial improvement in crop yield (Jangir *et al.*, 2021). By enhancing plants' ability to withstand drought stress and efficiently utilize available resources, *P. indica* contributes to a more productive and resilient agricultural system (Boorboori and Zhang, 2022). Its potential to mitigate the negative effects of drought stress on yield makes it a valuable tool for sustainable crop management, particularly in regions prone to water scarcity.

5.1.19. Ascorbic acid

In response to oxidative pressure, the presence of ascorbate in tomato fruits rises during periods of water stress (Murshed *et al.*, 2013). In the present study, increasing the drought stress to 25% FC resulted in an increase in ascorbic acid content by 9%. This result was also observed when water stress was induced by application of PEG, mannitol, ABA and submergence. Increased accumulation of ascorbic acid in tomato fruits was also reported when grown under water stressed condition (Bogale *et al.*, 2016; Nahar and Ullah, 2018). This discovery is firmly corroborated by Tambussi *et al.* (2000), who also documented that the elevation in ascorbic acid levels could serve as a viable approach to safeguarding membranes against oxidative harm during water scarcity.

Colonization by *P. indica* has revealed its potential to amplify ascorbic acid content in fruits by a substantial range of 1.6% to 2.6% across varying water stress conditions. This increase in ascorbic acid levels is closely associated with *P. indica*'s influence on nutrient accessibility and absorption within plants. The colonization with *P. indica* has been demonstrated to notably enhance nutrient uptake, with specific emphasis on phosphorus (P) and potassium (K), as supported by findings from Nautiyal *et al.* (2010) and Abdelaziz *et al.* (2019). This augmentation in nutrient uptake, particularly the elevated presence of potassium, assumes a pivotal role in fostering the synthesis and accumulation of ascorbic acid within plant tissues, as underscored by earlier research (Duchêne *et al.*, 2020). Subramanian *et al.* (2016) demonstrated that the quality of tomato fruits was enhanced through mycorrhizal association, resulting in increased levels of ascorbic acid and a reduction in acidity. The study showed that approximately 65% of the phosphorus absorbed by mature plants was directed towards the fruits, serving as a significant phosphorus sink. This led to the translocation of considerable amounts of monocalcium phosphate to the fruits by mycorrhizal plants, which contributed to the neutralization of fruit acidity. Additionally, the study found that mycorrhizal associations helped mitigate the adverse effects of drought on fruit quality.

5.1.20. Lycopene

The variation in lycopene content displayed significant sensitivity to both colonization and drought stress factors. As the intensity of drought stress increased, there was a gradual rise in lycopene content, with a notable enhancement of 4% achieved when the stress intensity reached 25% of field capacity (FC). Similarly, the application of water stress through PEG, mannitol, and ABA led to enhancements of 2%, 4%, and 3%, respectively, at their highest applied concentrations. Additionally, when subjected to submergence conditions, extending the duration of submergence to 3 days resulted in a marginal increase of 3% in lycopene content. These findings collectively underscore the intricate relationship between lycopene accumulation and various stress factors, highlighting how subtle variations in stress intensity and duration can influence lycopene content in plants.

In the exploration of the relationship between colonization and water stress, it became evident that their combined effect did not result in a significant alteration in the lycopene content of fruits. However, what's particularly noteworthy is that the solitary impact of *P. indica* colonization consistently displayed a positive influence on lycopene content throughout various experimental settings. This positive influence can be attributed to *P. indica*'s capacity to enhance the uptake of potassium in plants, an aspect emphasized by Abdelaziz *et al.* (2019). This increase in potassium (K) levels, in turn, holds a pivotal role in influencing lycopene production by activating specific enzymes involved in the biosynthetic pathway, as pointed out by Tavallali *et al.* in 2018. Relevant to this context, Varma *et al.* (2012) highlighted that the stability of lycopene levels in tomato fruits from plants treated with *P. indica* remains consistent across a range of diverse growth conditions.

5.1.21. TSS

In the series of conducted experiments, excluding water stress induction through submergence, both the colonization of *P. indica* and the introduction of water stress exhibited noteworthy effects on total soluble solids (TSS) levels (Fig. 5). The escalation in the intensity of water stress yielded a modest increase in the TSS content of the fruits. This trend of higher TSS levels with intensified water stress intensity aligns with the findings from various studies focused on tomatoes (Subramanian *et al.*, 2016; Kazemi *et al.*, 2021; Sivakumar and Srividhya, 2016). Nahar and Ullah (2011) provided insight into the potential enhancement of fruit quality in tomatoes during water scarcity. Their findings indicated that the synthesis of ascorbic, citric, and malic acids might contribute to this effect.

Documenting mycorrhizal association in tomatoes, Subramanian *et al.* (2016) observed elevated TSS in comparison to the control group, under both normal and drought-stressed conditions. Similarly, our own research replicated these results, demonstrating that plants colonized by *P. indica* produced fruits with significantly higher TSS levels, unaffected by the imposition of drought stress. In line with these findings, Abdelaziz *et al.* (2019) also noted an increase in TSS in tomato fruits from *P. indica* colonized plants under stressed conditions. This

increase in TSS, combined with enhanced firmness, has been linked to an extended shelf life, as reported by Wang *et al.* (2015).

5.1.22. Relative water content (RWC)

Various important characteristics such as relative water content (RWC), leaf water potential, stomatal resistance, transpiration rate, leaf temperature, and canopy temperature play a pivotal role in influencing the water relations within plants. Relative water content is a crucial indicator of plant water status, serving as a reflection of the metabolic vitality within plant tissues. It holds particular significance as an indicator of dehydration tolerance (Anjum *et al.*, 2011).

In the course of the present investigation, the induction of water stress was achieved by methodically reducing irrigation to 25% of the field capacity. This deliberate approach resulted in a substantial decrease of 49% in the relative water content (RWC). A parallel trend emerged during the subsequent experimental phase, where an elevation of PEG concentration to -10 bars produced an analogous reduction in RWC. Furthermore, the introduction of mannitol at a concentration of 10% led to a noteworthy decline of 47% in RWC. Similarly, the application of ABA at a concentration of 10µmols brought about a noticeable reduction of 23% in RWC. In a separate investigation conducted by Khan *et al.* (2015), involving tomato plants within a controlled environment, the average relative water content was determined to be 89%. In contrast, under conditions of drought stress, the recorded relative water content experienced a decrease, reaching a value of 87%.

It's worth noting that the findings of the present study align with a common trend observed in various plant species when subjected to drought stress, as well-documented by Nayyar and Gupta (2006). Under drought conditions, leaves tend to experience substantial declines in RWC and water potential. This, in turn, leads to noticeable reduction in transpiration rate, while causing an increase in leaf temperature, as noted in the study conducted by Siddique *et al.* (2000).

The introduction of *P. indica* colonization had a pronounced positive impact on the relative water content (RWC) in tomato plants, demonstrating its efficacy in both regular and stress-induced conditions. Focusing on the specific contribution of *P. indica*, its colonization consistently yielded a remarkable 5-10% increase in

RWC among tomato plants (Fig. 6). This effect remained consistent regardless of the extent of water stress, irrespective of the method used to induce it, whether through restricted irrigation, the application of PEG, mannitol, or ABA. Furthermore, this influence extended across a spectrum of water stress levels applied, showcasing that tomato plants colonized by *P. indica* consistently maintained significantly higher RWC levels compared to control plants subject to equivalent levels of water stress (Fig. 7). As documented earlier, plants serve as a carbon source for *P. indica,* and in return, *P. indica* enhances the host plant's ability to absorb nutrients (Parniske, 2008). Specifically, *P. indica* plays a significant role in boosting the uptake of phosphorus (P) by the host plant (Yadav *et al.*, 2010). This improved phosphorus uptake, resulting from the colonization of plant roots by *P. indica*, leads to several positive effects. These include reducing malondialdehyde content, increasing the levels of osmolytes and nitrogen compounds, maintaining optimal relative water content in leaves, and enhancing the efficiency of photosystem II and the rate of net photosynthesis (Tariq *et al.*, 2017; Wu *et al.*, 2018; Azizi *et al.*, 2021). Enhanced RWC under normal and water stressed condition by *P. indica* colonization has also been reported in wheat (Hosseini *et al.*, 2017), *Eleusine coracana* (Tyagi *et al.*, 2017), and rice (Bagheri *et al.*, 2013).

5.1.23. Cell Membrane Stability

It has been widely recognized that various abiotic stresses primarily target cellular membranes. When exposed to a combination of drought and heat stress, the stability of cell membranes rapidly diminished (Wang and Huang, 2004). Elevating drought stress to 25% of field capacity (FC) resulted in a 45% reduction in Cell Membrane Stability (CMS). This reduction was consistent when high concentrations of PEG and mannitol were applied (at -10 bar and 10%, respectively). ABA-induced water stress at the highest concentration (10 µmol) led to a 27% decrease in CMS. These findings were substantiated by Hayat *et al.* (2008), who also documented a decrease in membrane stability in tomatoes exposed to water stress. Moreover, the observations made by Mohawesh (2016) affirmed the

connection between a gradual reduction in water levels and a continuous decline in the membrane stability index (MSI). This trend became particularly pronounced when evaluating deficit irrigation approaches at levels of 40% and 20% of the field capacity.

Drought triggered oxidative stress in plants by generating reactive oxygen species (ROS), as highlighted in studies like Farooq *et al.* (2009). These ROS, which encompassed radicals such as O_2^-, H_2O_2, and OH, had the capability to directly target membrane lipids, thereby resulting in an elevation of lipid peroxidation, a phenomenon detailed by Mittler (2002). This heightened ROS production due to drought stimulated a surge in malondialdehyde (MDA) content. MDA content was widely recognized as an indicator of oxidative damage, as noted by Moller *et al.* (2007). It aptly served as a marker for assessing the extent of membrane lipid peroxidation. The decrease in membrane stability reflected the degree of lipid peroxidation initiated by ROS.

The endophytic interaction plays a pivotal role in bolstering the resilience of cell wall structures amid drought conditions. This symbiotic relationship potentially enhances the osmotic potential of cells, enabling them not only to absorb water efficiently but also to retain it more effectively than their non-colonized counterparts (Dupont *et al.*, 2015). In the current investigation, the colonization of *P. indica* played a pivotal role in enabling plants to uphold a considerably elevated level of CMS across a spectrum of water stress intensities in comparison to the control group. Specifically, when subjected to moderate water stress conditions (50%FC, -7bar PEG, 7% mannitol, and 6µmol ABA), the presence of *P. indica* led to an augmentation of CMS by a range of 5-11%. Moreover, under conditions of heightened and severe stress (25%FC, -10bar PEG, 10% mannitol, and 10 µmol ABA), plants colonized by *P. indica* exhibited an even more pronounced elevation in CMS, surpassing non-colonized plants by 11-21%. Increased membrane stability by *P. indica* inoculation has been also reported by Dehghanpour-Farashah *et al.* (2019) and Yaghoubian *et al.* (2022). Through a series of intricate mechanisms, endophytes appear to shield cell walls from the detrimental effects of dehydration-induced stress. This defense mechanism involves the activation of genes

responsible for orchestrating the synthesis of essential cell wall polysaccharides such as cellulose, hemicellulose, and pectin, in addition to the modulation of cell wall modifiers and specialized cell wall proteins (Vardharajula *et al.*, 2011). The endophytic partnership also triggers the activation of genetic processes responsible for preserving the integrity of the cell wall during periods of drought-induced stress. These microorganisms stimulate the host's genes involved in producing essential components like cellulose, hemicellulose, and pectin, as well as genes related to cell wall modification and cell wall proteins, all of which contribute to enhancing the robustness of the host's cell wall structure (Vardharajula *et al.*, 2011) (Fig. 8 & 9).

5.1.24. Chlorophyll Stability Index

Under the influence of drought stress, various changes occur in the structures and functions of photosynthetic mechanisms. These alterations encompass modifications in photosynthetic pigments like chlorophyll a and b. Additionally, the CO_2 uptake process is hampered due to stomatal closure, and there's a deficiency in assimilating photosynthates because the activity of chloroplasts is inhibited (Liu *et al.*, 2016).

In conditions of water scarcity, the production of chlorophyll is impeded. Here, Chlorophyll Stability Index was decreased by 38% as the intensity of water stress reaches 25% FC. Similarly, under water stress induced by PEG, mannitol and ABA application resulted in gradual substantial reduction in CSI. The quantity of chlorophyll present in leaves serves as an indicator of the photosynthetic capability of plant tissues. In cases of drought, various crops have exhibited reduced or unaltered levels of chlorophyll content, signifying water scarcity. This phenomenon has been reported in several studies focused on drought conditions (Barutcular *et al.*, 2016).

Sustaining chlorophyll levels is crucial for facilitating photosynthesis during periods of water stress. *P. indica* colonization maintained a higher CSI compared to the control plants across varying intensities of drought stress. The presence of *P. indica* colonization consistently upheld an elevated Chlorophyll Stability Index (CSI) in comparison to the control plants, even when subjected to

different levels of drought stress. The fungus mitigates the adverse impacts of water scarcity on chlorophyll content in diverse plants, as evidenced by numerous investigations involving induced drought using PEG or real-world field drought experiments (Jogawat *et al.*, 2013; Khalid *et al.*, 2017; Tyagi *et al.*, 2017; Swetha and Padmavath, 2020; Sun *et al.*, 2020; Hosseini *et al.*, 2018). Furthermore, research indicates that the fungus enhances chlorophyll content irrespective of water availability (Tyagi *et al.*, 2017). Treatment with *P. indica* triggers an increase in chlorophyll synthesis, as plants colonized by the fungus exhibit an enhanced water absorption capacity even under conditions of low soil water potential (Swetha and Padmavath, 2020).

5.1.25. Proline content

Plants that exhibit heightened proline concentrations have shown an enhanced ability to withstand drought stress, leading to improved growth in conditions with environmental limitations (Giorio *et al.*, 2018). When subjected to drought stress, there was a notable surge of over 300% in proline accumulation, especially evident when the intensity reached 25% of field capacity (FC). Similar patterns emerged when the concentrations of PEG and mannitol reached -10 bars and 10%, respectively. Additionally, elevating the concentration of ABA to 10 µmol led to a remarkable 250% augmentation in proline accumulation. Within plant cells, the levels of intracellular proline have been observed to undergo an increase of more than a hundredfold under stress conditions, as documented by Handa *et al.* (1983) and Verbruggen and Hermans (2008). Proline played a role in mitigating the impacts of drought by assisting in maintaining negative water potential and turgor within plant cells. This, in turn, supported the extraction of water from the soil, allowing for the maintenance of open stomata even in challenging conditions. Furthermore, there had been suggestions that proline served not solely as an osmolyte, but also as an efficient scavenger of reactive oxygen species (ROS). This dual capability contributed both to the adjustment of cellular osmotic conditions and to the reinforcement of non-enzymatic antioxidant defense mechanisms, as indicated in earlier research (Reddy *et al.*, 2004; Sánchez-Rodríguez *et al.*, 2010; Doupis *et al.*, 2011; Rejeb *et al.*, 2014).

Tomato plants colonized by *P. indica* exhibited significantly elevated proline accumulation when compared to the control plants under conditions of water stress. During instances of moderate stress, characterized by 50% field capacity (FC), -7 bar PEG, 7% mannitol, and 6µmol ABA, proline accumulation in the colonized tomato plants was notably enhanced by a range of 41-69%. Furthermore, when subjected to more severe stress conditions, such as 25% FC, -10 bar PEG, 10% mannitol, and 10µmol ABA, the proline accumulation witnessed an even more substantial increase, ranging from 55 to 116%. These findings are in line with the reports by Ghorbani *et al.* (2019), who found that when tomato roots are colonized by *P. indica*, there is an augmentation in the buildup of betaine, glycine, and proline within the roots, along with an increase in the concentration of photosynthetic pigments. In recent research, it has been demonstrated that introducing *P. indica* to plants during drought conditions leads to an elevation in proline levels and a reduction in the accumulation of malondialdehyde (MDA) (Tsai *et al.*, 2020; Xu *et al.*, 2017) Furthermore, *P. indica* exerts control over the expression of the P5CS gene (Abo-Doma *et al.*, 2011), resulting in an augmentation of proline accumulation which in turn increases the plant's resistance to drought (Fig. 10 & 11).

5.1.26. Superoxide dismutase

Superoxide dismutase (SOD) functions as a metalloenzyme and plays a crucial role in the plant cell's defense system against the harmful effects of reactive oxygen species (ROS). It facilitates the transformation of superoxide radicals (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), making it a highly efficient component of the cell's antioxidant protection mechanism (Mishra *et al.*, 2003). As the level of drought stress escalated to 25%FC, we observed a notable 26% increase in SOD activity. When subjected to PEG and mannitol stress at -10 bar and 10%, respectively, SOD activity exhibited even more substantial enhancements, with increments of 38% and 41%, respectively. A similar outcome was achieved when ABA was employed to induce water stress. In the case of submergence, extending the duration to 5 days resulted in a remarkable 38% boost in SOD activity. Notably, previous studies have also reported an augmentation in

SOD content due to reduced irrigation in various crops such as tomato (Rady *et al.*, 2020), faba bean (Rady *et al.*, 2021), and maize (Desoky *et al.*, 2021). SODs play a pivotal role as the foremost defense mechanism against abiotic stress, serving as a critical component in the plant's protective system. The impact of all SODs on the immediate or secondary modification of various reactive oxygen species (ROS) has been substantiated by Mittler (2006).

The colonization of *P. indica* consistently amplified SOD activity in response to escalating water stress levels. During standard conditions, the presence of *P. indica* did not induce any alteration in SOD activity when compared to non-colonized tomato plants. However, as the intensity of stress heightened, across all the conducted experiments, the colonized plants consistently exhibited significantly elevated SOD activity in comparison to the non-colonized plants subjected to equivalent levels of water stress. Likewise, in chickpea plants, the total SOD activity displayed an increase among those that were colonized by *P. indica* in comparison to the control plants, when subjected to biotic stress (Narayan *et al.*, 2017). Under conditions of salt stress, plants colonized by *P. indica* exhibited notably elevated levels of SOD and CAT enzyme activities compared to plants that were not colonized. These findings imply that the enhancement of enzymes responsible for scavenging reactive oxygen species (ROS) likely played a role in the enhanced stress tolerance observed due to *P. indica* colonization in tomato (Hosseini *et al.*, 2017) (Fig. 12 & 13).

5.1.27. Catalase

Catalases are key enzymatic agents responsible for directly transforming H_2O_2, playing a crucial role in the detoxification of reactive oxygen species (ROS) during stress conditions (Amor *et al.*, 2005). This phenomenon is closely connected to the observation that peroxisomes experience proliferation under stress, potentially assisting in the removal of diffused H_2O_2 from the cytosol (Amor *et al.*, 2005). Raising drought stress to 25% FC led to a CAT activity decrease of 45% in tomato plants, regardless of whether they were colonized by *P. indica*. Correspondingly, elevating the concentrations of PEG, mannitol, and ABA to their maximum levels brought about a CAT activity reduction ranging between 19% and

24%. Extending the submergence duration to 5 days resulted in a notable 26% rise in CAT activity. Additionally, a study by Çelik *et al.* (2017) demonstrated an elevation in Catalase (CAT) activity in response to drought stress in two different industrial tomato varieties. It's believed that the heightened catalase activity is an adaptive trait that could be instrumental in mitigating potential tissue damage caused by moderating levels of H_2O_2.

In all five conducted experiments, plants colonized by *P. indica* consistently upheld notably elevated catalase activity levels both during standard conditions and under water stress conditions. Across diverse stress environments induced through various methods, fungal colonization exhibited the ability to sustain approximately 13-22% higher catalase activity in tomato plants that were colonized. Increased catalase activity in colonized tomato under stress was also reported by Abdelaziz *et al.* (2019) and in rice by Bagheri *et al.* (2013). Enhanced antioxidant enzyme activity reduces the likelihood of an oxidative burst, which involves excessive production of reactive oxygen species (ROS). This suggests that *P. indica* could potentially evade the host plant's oxidative defense mechanisms while being colonized (Kumar *et al.*, 2009) (Fig. 14 & 15).

5.1.28. Peroxidase

Peroxidase activity, much like catalase and SOD, responded to water stress conditions. In tomato plants, peroxidase activity displayed a significant enhancement ranging between 40-60%, with the increase correlating with escalating water stress levels. This pattern was consistent across all five experiments, irrespective of whether the plants were colonized by *P. indica*. Numerous researchers have documented the augmentation of peroxidase activity in plants under water stress conditions (Farooq *et al.*, 2009; Chai *et al.*, 2016; Anjum *et al.*, 2011; Murshed *et al.*, 2013).

Also, the colonization with *P. indica* resulted in enhancement of around 18-31% in peroxidase activity irrespective of water stress levels in all the experiments. In the study conducted by Li *et al.* (2017), it was shown that under conditions of salt stress, *Medicago truncatula* plants that had been primed with *P. indica* exhibited a notable increase of 44% in peroxidase activity and a 38% increase in

catalase activity. Additionally, when *P. indica*-primed cowpea plants were examined, they displayed elevated levels of defensive enzymes such as peroxidase and polyphenol oxidase. This priming also offered protection against the *black eye cowpea mosaic virus*, as observed in studies by Alex (2017) and Chandran (2019). It appeared that *P. indica* had the potential to indirectly promote the production of antioxidant enzymes by aiding in the accessibility of diverse macro and micronutrients (Kaboosi *et al.*, 2022) (Fig 16 & 17).

5.2. MOLECULAR PARAMETERS

5.2.1. Expression profile of drought and water stress related genes -*SlAREB1*

Moisture stress triggers a variety of cellular mechanisms, including the suppression of photosynthesis, accumulation of harmful reactive oxygen species within cells, and the restructuring of gene expression. Among these processes, transcription factors (TFs) play a pivotal role in orchestrating the reconfiguration of gene expression. Furthermore, the impact of moisture stress extends to influencing the expression of numerous TF genes.

In the landscape of the ABA signalling pathway, a notable cohort of transcription factors belongs to the *AREB/ABFs*, constituting a prominent class of TFs encoding basic-domain leucine zipper (bZIP) TFs. These factors are categorized within the group-A subfamily and possess an affinity for binding to ABA-responsive elements (ABREs) situated within the promoter region of target genes (Kang *et al.*, 2002; Fujita *et al.*, 2013; Banerjee and Roychoudhury, 2017). Prior investigations conducted through the yeast one-hybrid screening approach in Arabidopsis unveiled the existence of four AREB/ABF proteins (ABF1, AREB1/ABF2, ABF3, AREB2/ABF4) (Choi *et al.*, 2000; Uno *et al.*, 2000). Importantly, among this group, the AREB1/ABF2, ABF3, and AREB2/ABF4 genes emerge as entities that are activated in response to both elevated salinity and drought conditions. Their pivotal roles as regulators within the ABA signalling pathway in reaction to stressors have been validated through thorough genetic transformation analysis (Fujita *et al.*, 2005; Abdeen *et al.*, 2010; Yoshida *et al.*, 2010).

In the present investigation, a comprehensive analysis of gene expression patterns within two distinct categories of tomato plants: those colonized by *P. indica* and those remained non-colonized. These analyses encompassed two distinct environmental conditions, encompassing both maintenance at 50% field capacity (FC) and standard conditions. Our approach involved the utilization of quantitative reverse transcription polymerase chain reaction (RT-qPCR) to assess gene expression levels.

The outcomes of our investigation unveiled a striking and significant upregulation in the expression of the *SlAREB1* gene particularly in tomato plants colonized by *P. indica*. The magnitude of this enhancement was notable, with a fold increase of approximately 1.36 compared to the control group. In the context of drought stress at 50% FC, it was observed that both colonized and non-colonized tomato plants exhibited an elevation in gene expression. However, an intriguing distinction emerged: while non-inoculated plants exhibited a relative expression level that was merely 1.75 times higher than that of the control, the *P. indica* colonized plants displayed a notably heightened relative expression level of 2.76 times that of the control. This distinction underscores the potentially synergistic effect of *P. indica* colonization in augmenting the gene expression response under conditions of drought stress. Xu *et al.* (2022) conducted a study that identified four distinctive ABF/AREB transcription factors in the tomato plant. Their investigation revealed that among these factors, *SlAREB1* exhibited a robust and significant response to both abscisic acid (ABA) and saline-alkaline stress conditions (Fig. 18). In a study conducted by Orellana *et al.* (2010) found that both *SlAREB1* and *SlAREB2* are induced by drought and salinity in both leaves and roots, with *SlAREB1* being more responsive to stress. When *SlAREB1* was overexpressed in tomato plants, they showed increased tolerance to salt and water stress compared to non-modified plants. This enhanced tolerance was evidenced by improved physiological parameters such as relative water content and reduced damage from stress.

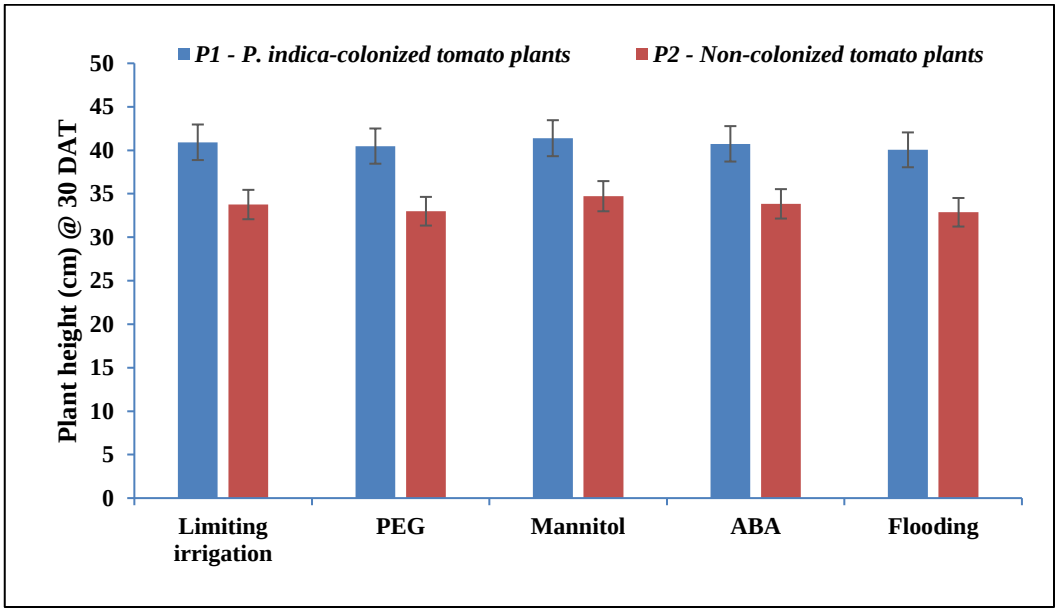
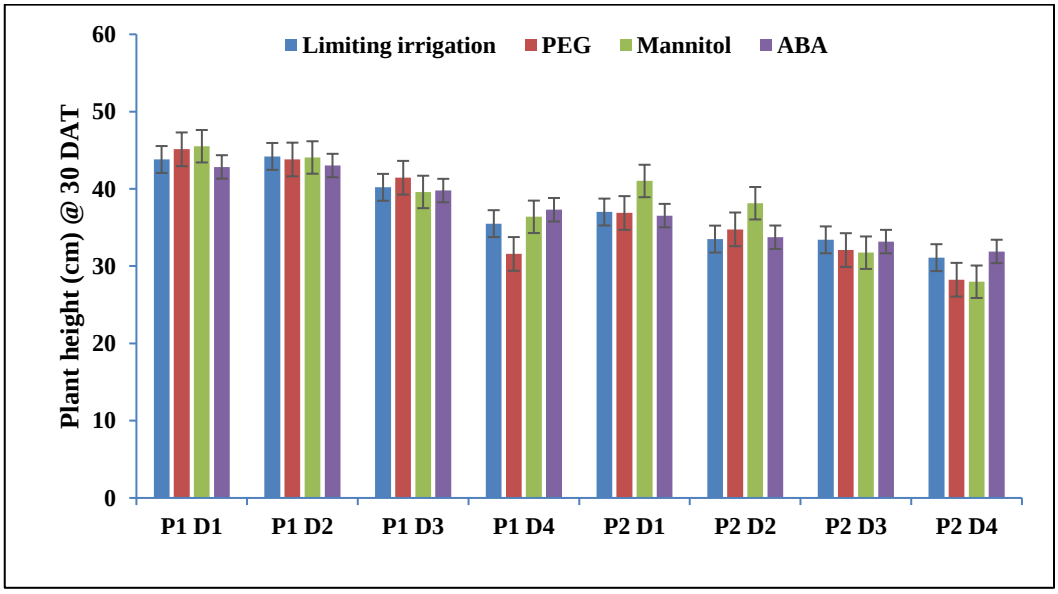


Fig. 1. Influence of *P. indica* on plant height @30 DAT in tomato plants under simulated water stress conditions



P_1-*P. indica*-colonized tomato
P_2- Non-colonized tomato

D_1-Control
D_2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA
D_3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA
D_4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA

Fig. 2. Variation in plant height @30 DAT in tomato plants under different levels of drought

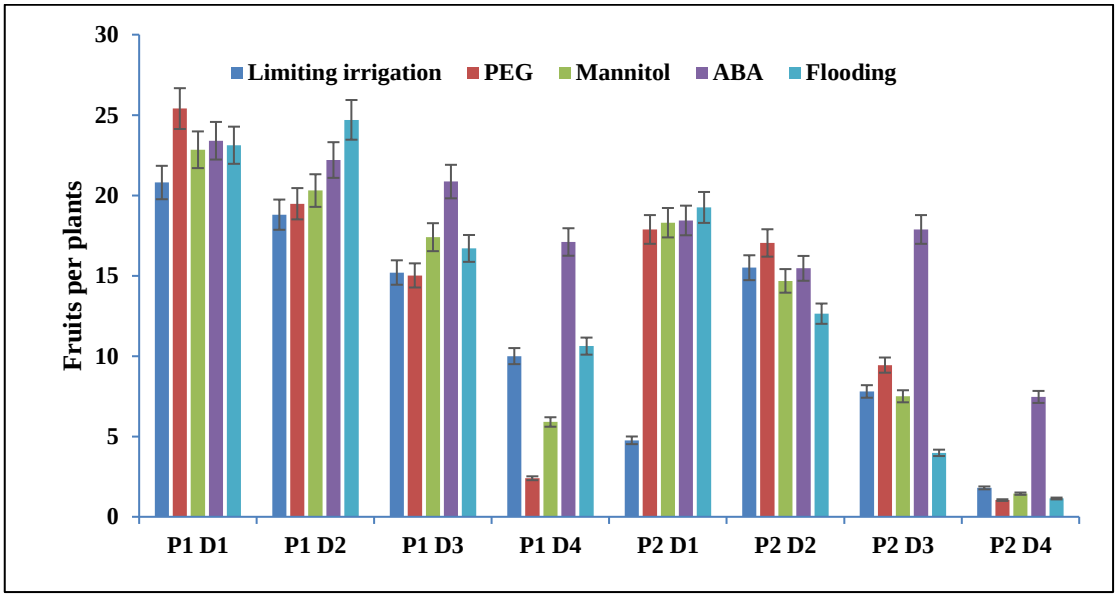


Fig. 3. Changes in number of fruits per plants in tomato under different levels of water stress

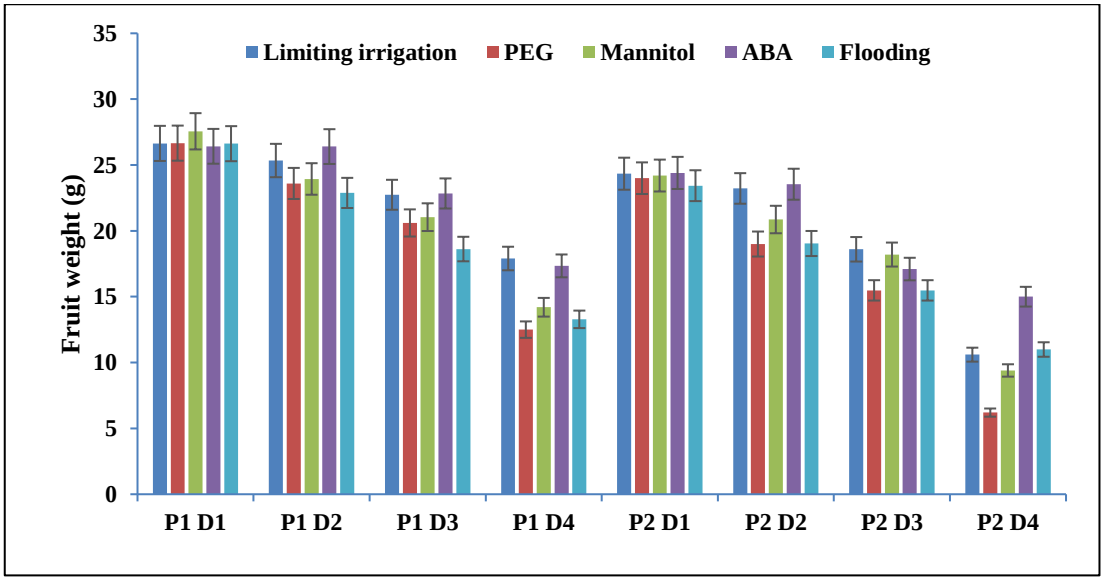


Fig.4. Variations in fruit weight of tomato under different levels of water stress

P_1-*P. indica*-colonized tomato
P_2- Non-colonized tomato

D_1-Control
D_2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA/ 1 day submergence
D_3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA/ 2 days of submergence
D_4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA/ 2 days of submergence

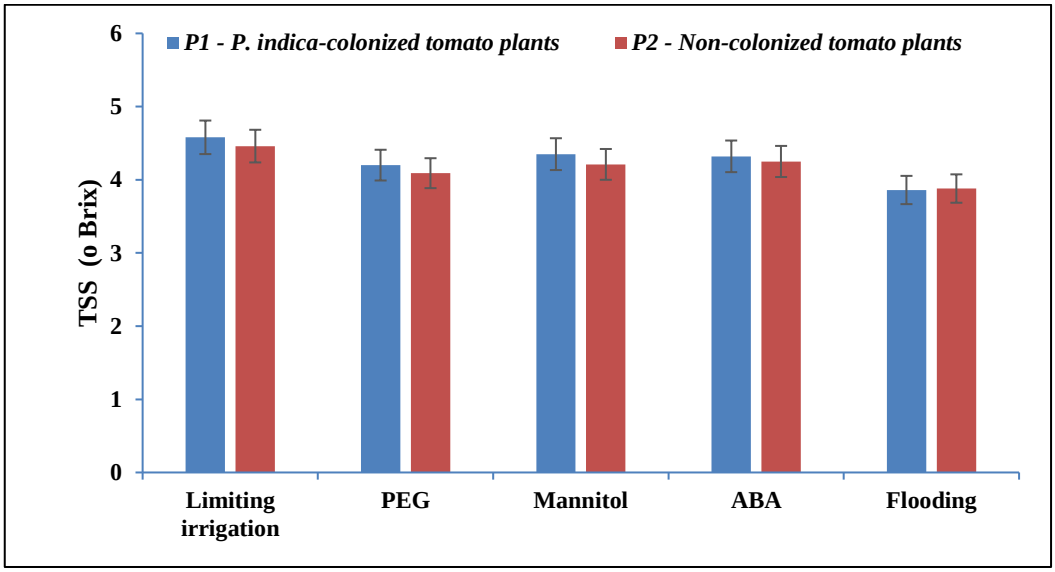


Fig.5. Influence of *P. indica* colonization on TSS of tomato fruit under simulated water stress conditions

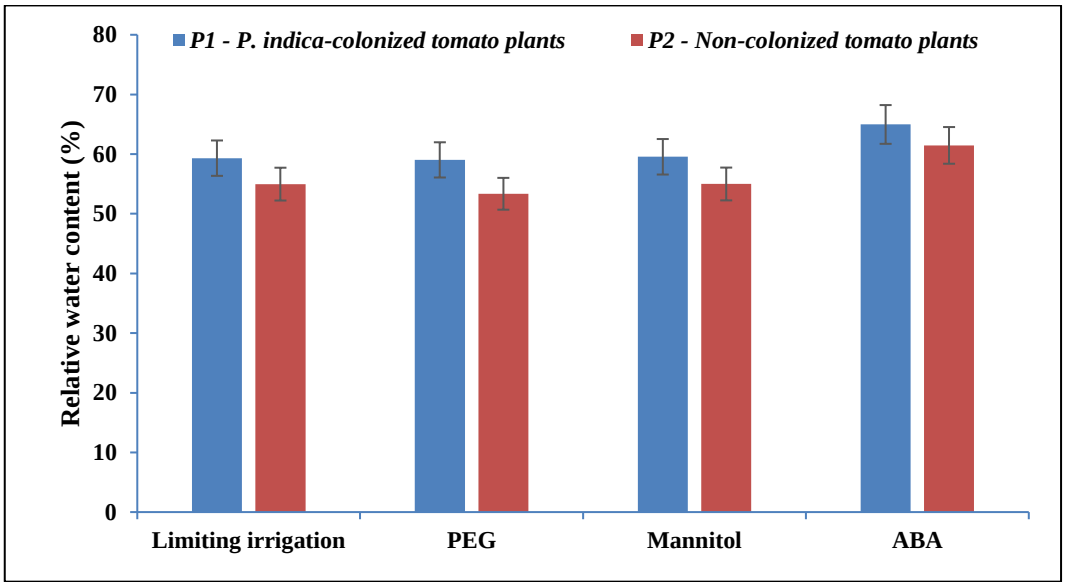
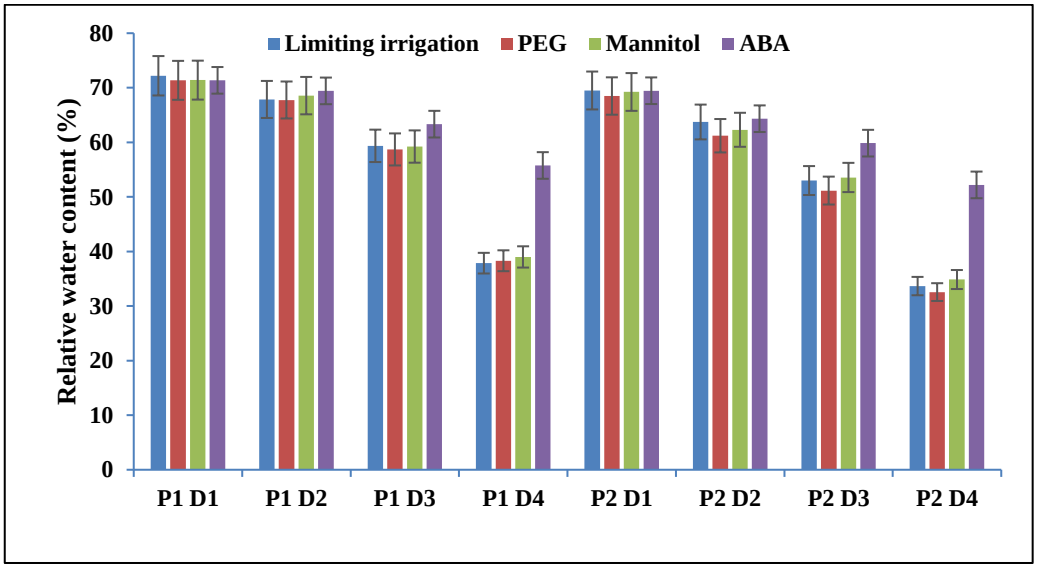


Fig.6. Effect of *P. indica* colonization on RWC of tomato plants under simulated drought conditions



P_1-*P. indica*-colonized tomato
P_2- Non-colonized tomato

D_1-Control
D_2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA
D_3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA
D_4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA

Fig.7. Changes in RWC of tomato plants under different levels of drought stress

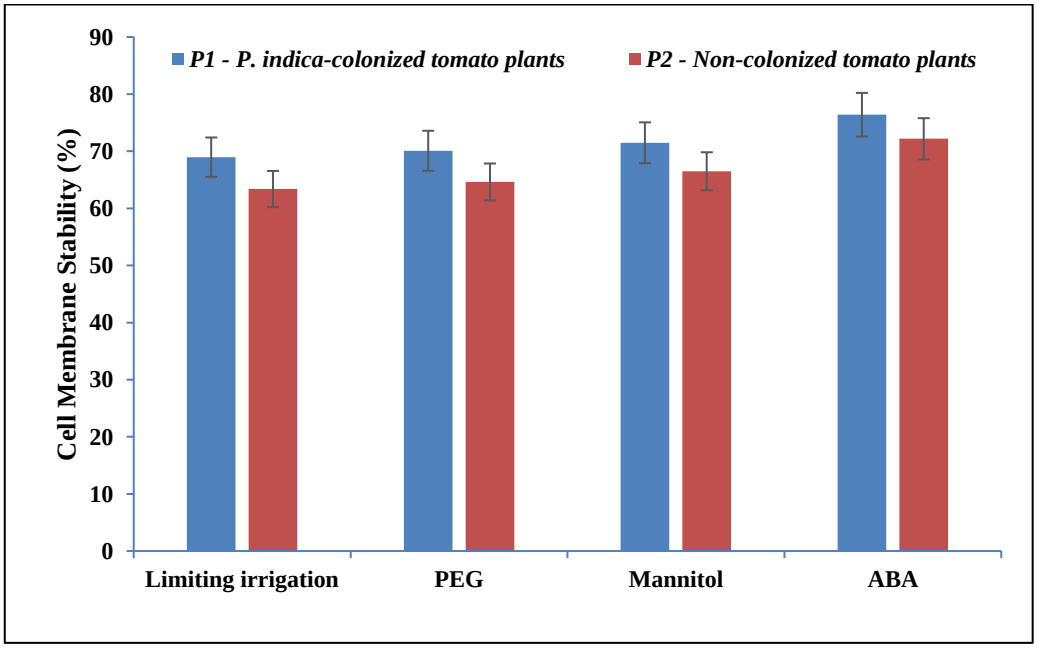
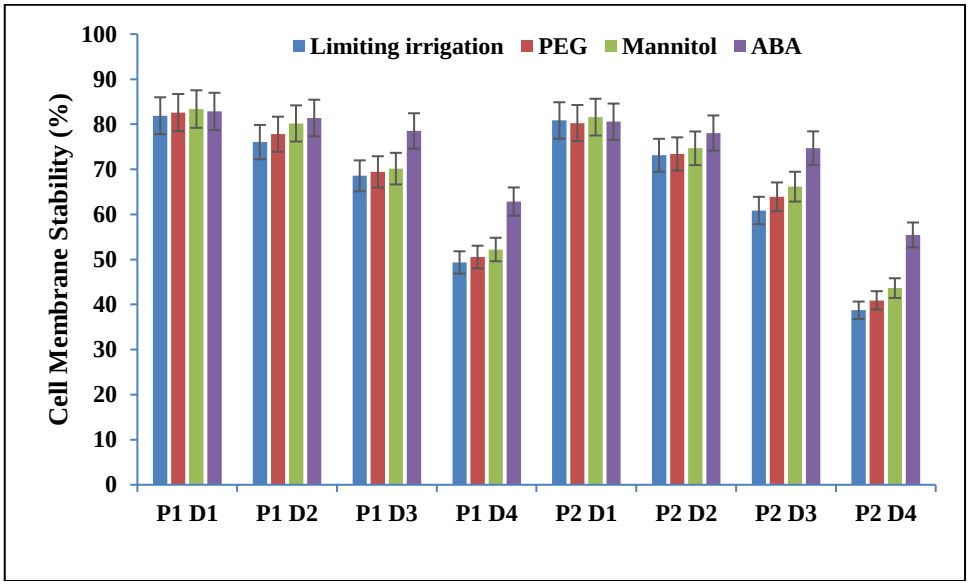


Fig.8. Influence of *P. indica* colonization on CMS of tomato plants under simulated drought conditions



P_1-*P. indica*-colonized tomato
P_2- Non-colonized tomato

D_1-Control
D_2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA
D_3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA
D_4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA

Fig.9. Variations in CMS of tomato plants under different levels of drought stress

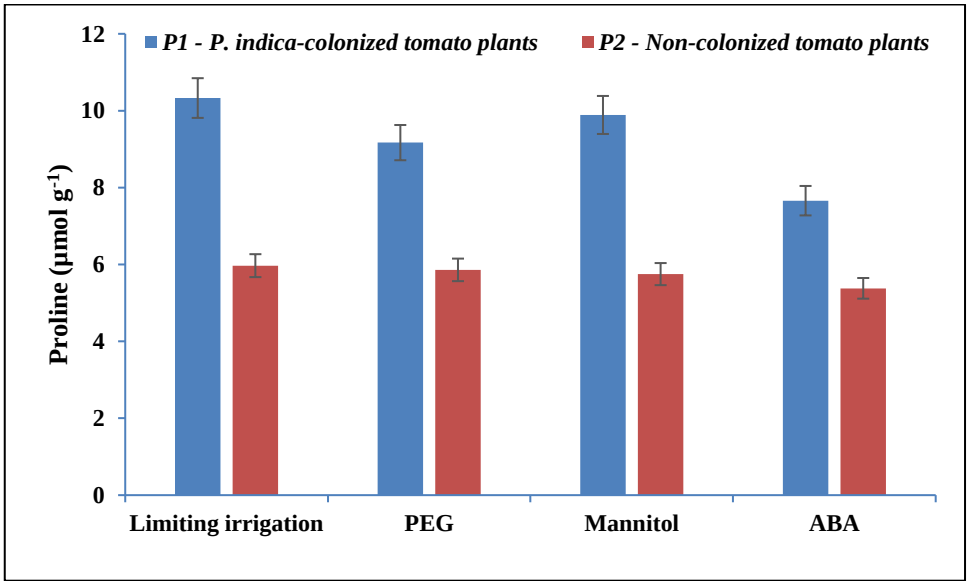
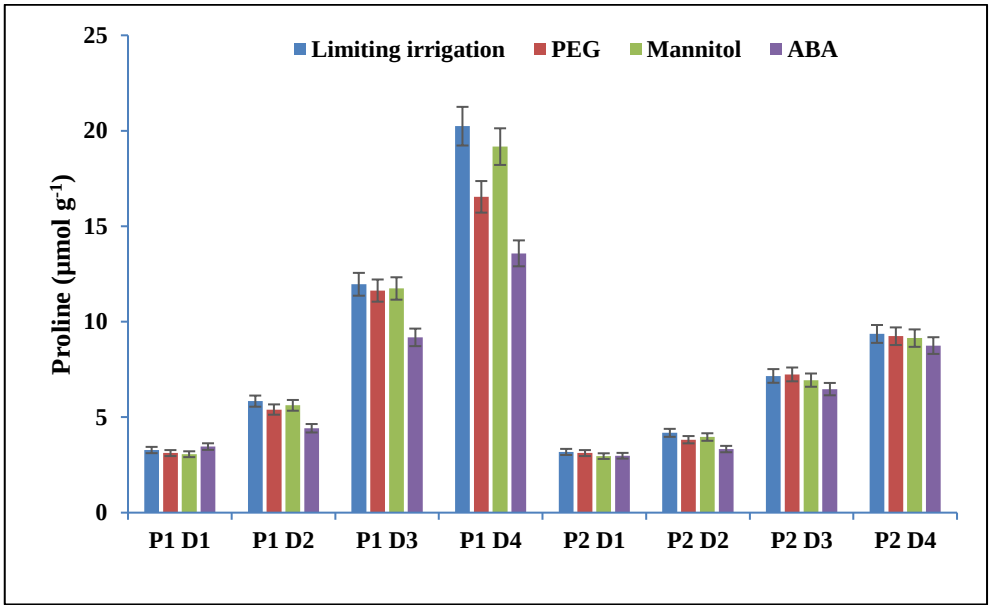


Fig.10. Effect of *P. indica* colonization on proline content in tomato plants under simulated drought conditions



P1-*P. indica*-colonized tomato
P2- Non-colonized tomato

D1-Control
D2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA
D3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA
D4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA

Fig.11. Changes in proline content in tomato plants under different levels of drought stress

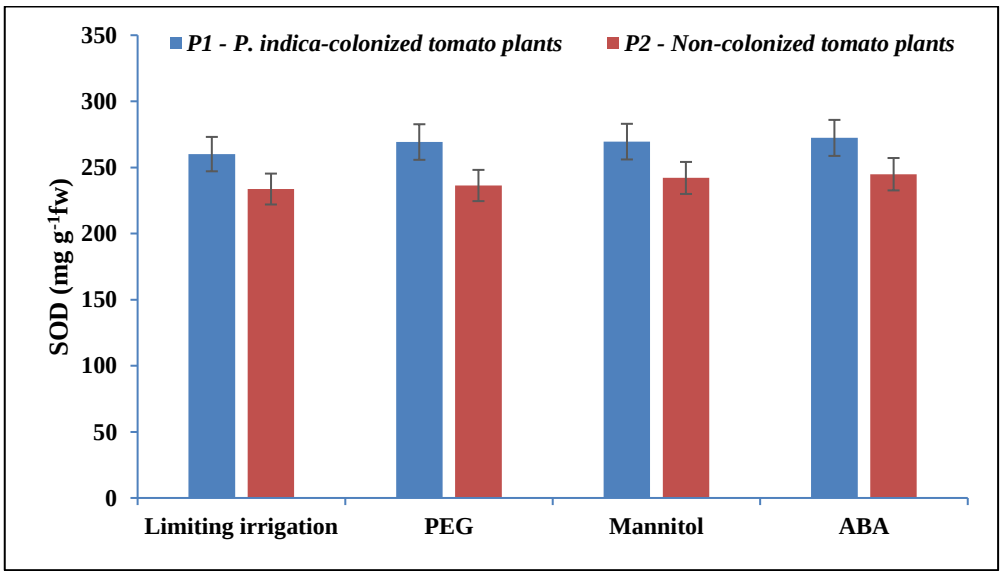
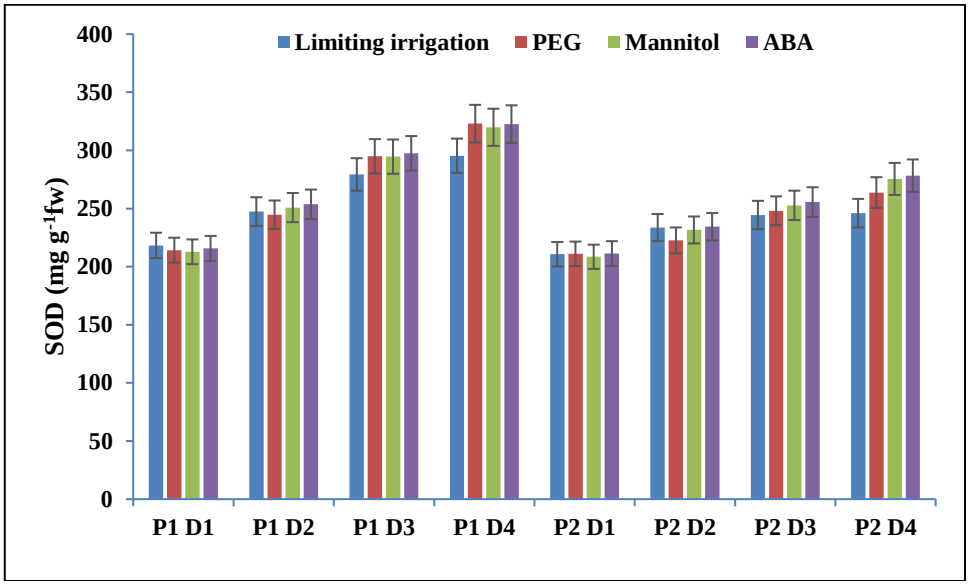


Fig.12. Effect of *P. indica* colonization on SOD activity in tomato plants under simulated drought stress conditions



P1-*P. indica*-colonized tomato
P2- Non-colonized tomato

D1-Control
D2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA
D3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA
D4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA

Fig.13. Changes in SOD activity in tomato plants under different levels of drought stress

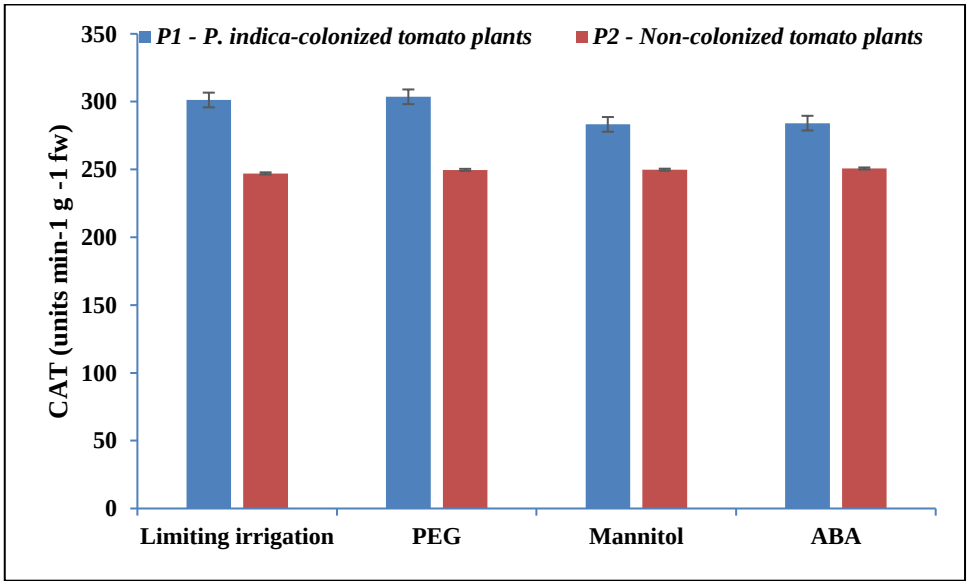
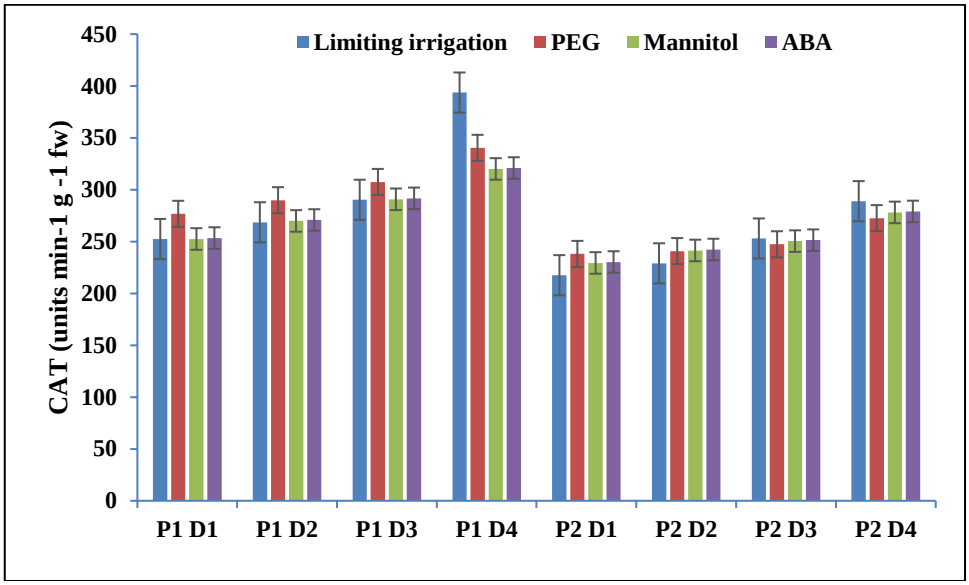


Fig.14. Influence of *P. indica* colonization on CAT activity in tomato plants under simulated drought conditions



P_1-*P. indica*-colonized tomato
P_2- Non-colonized tomato

D_1-Control
D_2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA
D_3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA
D_4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA

Fig.15. Variations in CAT activity in tomato plants under different levels of drought stress

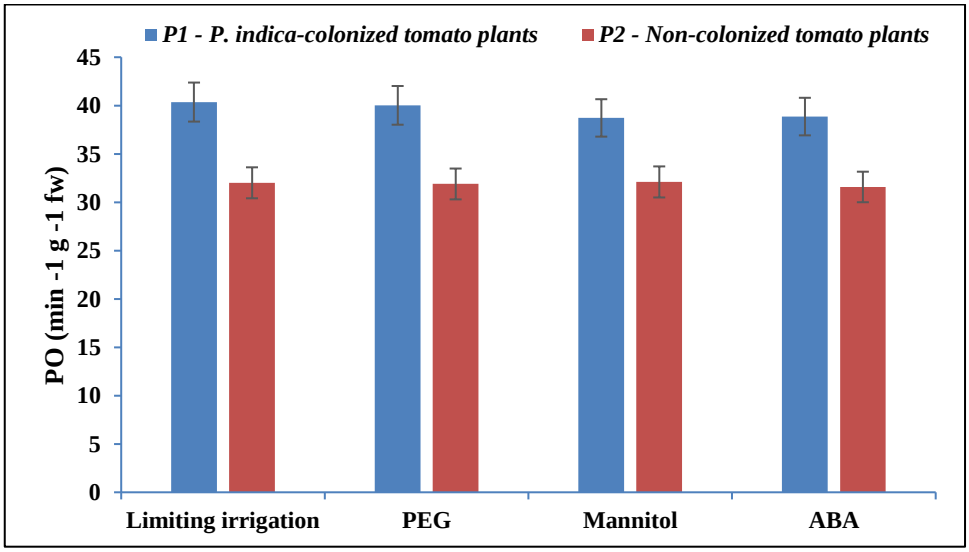
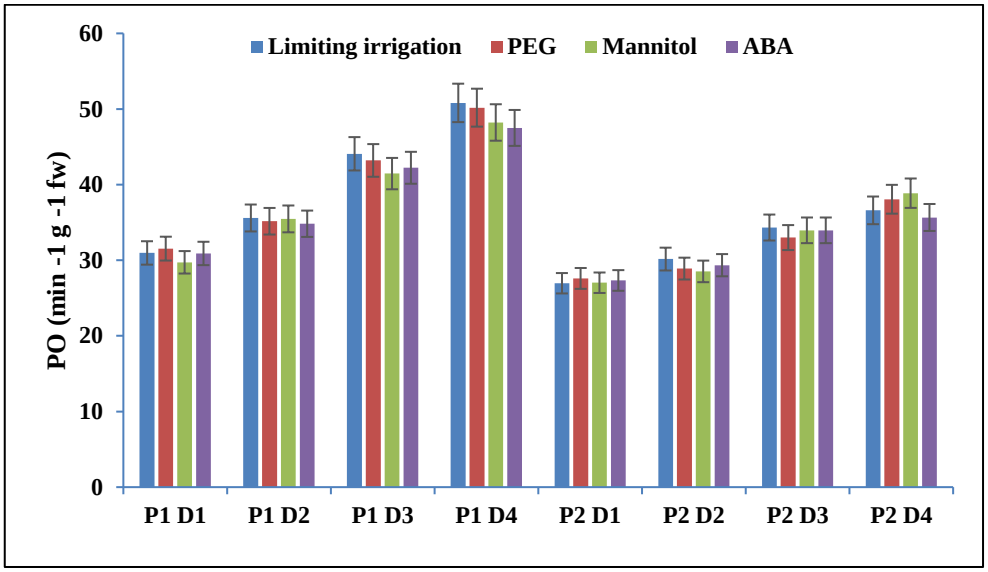


Fig.16. Influence of *P. indica* colonization on peroxidase activity in tomato plants under simulated drought stress conditions



P_1-*P. indica*-colonized tomato
P_2- Non-colonized tomato

D_1-Control
D_2-75% FC/-3bar PEG/3% mannitol/ 3µM ABA
D_3-50% FC/-7bar PEG/7% mannitol/ 6µM ABA
D_4-25% FC/-10bar PEG/10% mannitol/ 10µM ABA

Fig.17. Variations in peroxidase activity in tomato plants under different levels of water stress

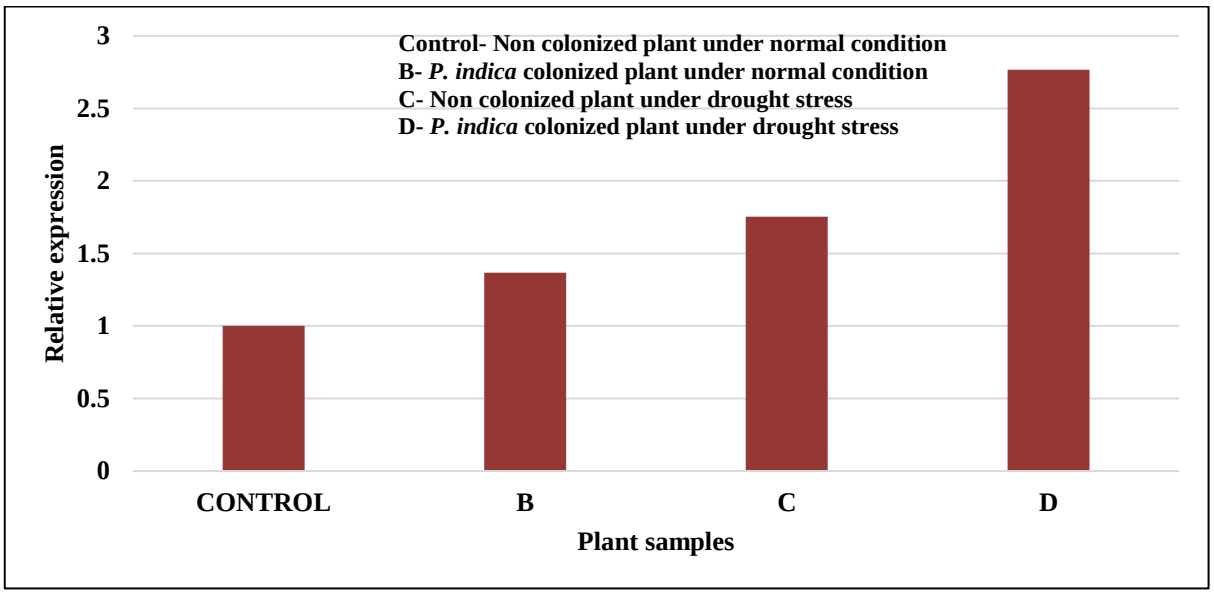


Fig.18. Relative expression of *SlAREB1* in *P. indica* colonized and non-colonized tomato plants

6. SUMMARY

The study entitled "Management of water stress in tomato (*Solanum lycopersicum* L.) through beneficial root endophytic fungus, *Piriformospora indica*" was conducted in the Department of Vegetable Science, College of Agriculture, Vellayani during 2019-2022. The study was conducted with the objectives of inducing tolerance to water stress in tomato through colonization with beneficial root-endophytic fungus, *Piriformospora indica* and to study its physiological and molecular mechanisms behind the same. The salient findings of research work are summarized below.

1. The evaluation of tomato plants colonized with *P. indica* under various simulated water stress conditions revealed a consistently positive impact on multiple aspects of plant growth, including vegetative and flowering characteristics, as well as fruit yield-related traits. Across all five experiments conducted, P_1 (colonized plants) consistently displayed greater values for plant height, stem girth, primary branches per plant, leaf length, leaf width, and leaf area when compared to P_2 (non-colonized plants).
2. As the level of stress increased, there was a noticeable decline in vegetative characters. However, *P. indica* colonization played a pivotal role in helping the plants maintain comparatively better growth parameters even under severe stress conditions. For instance, at 30 days after transplanting (DAT) and under 25% field capacity (FC), colonized plants achieved a height of 35.50 cm, significantly surpassing the height of non-colonized plants receiving the same level of irrigation, which measured 31.10 cm.
3. The presence of *P. indica* colonization consistently expedited both flowering and fruiting in tomato plants across all experiments under normal and stressed conditions. In normal conditions, where there was no water stress, colonized plants initiated flowering 3-6 days ahead of their non-colonized counterparts. Likewise, colonized plants reached their first harvest 3-7 days earlier than the non-colonized plants.
4. The number of flower clusters per plant and the number of flowers per cluster exhibited significant increases in colonized plants across all experiments. In

conditions of drought stress, simulated by controlled irrigation reduction, P_1 consistently displayed notable outcomes, recording an average of 6.12 flower clusters and 7.85 flowers per cluster. These findings were consistently observed in other experiments as well.

5. As stress levels heightened, both the number of fruits per truss and the number of fruits per plant exhibited reductions. Nevertheless, plants colonized by *P. indica* consistently maintained higher counts, even under severe stress conditions. In experiments where stress was induced using PEG, mannitol, and ABA, *P. indica* colonization led to a substantial increase in the number of fruits per plant, with figures reaching 15.57, 16.61, and 20.89, respectively.
6. Water stress had a detrimental effect on fruit set percentage, with reductions of up to 68% observed under severe stress conditions. However, regardless of the level of water stress, *P. indica* colonization consistently maintained approximately 6-14% higher fruit set percentages.
7. Colonized plants consistently yielded larger tomatoes, as evidenced by their greater fruit length and diameter, even across varying stress levels, when compared to non-colonized plants. Additionally, the fruit weight of colonized plants consistently exceeded that of non-colonized plants in all experiments, with weights ranging from 20.35g to 23.32g.
8. In the initial experiment, where drought stress was induced through controlled irrigation reduction, *P. indica* colonized plants demonstrated significantly higher yields, producing nearly double the yield obtained from non-colonized plants (401.11g vs. 280.03g). Similarly, in experiments involving the application of PEG, mannitol, and ABA, P_1 (colonized plants) consistently achieved superior yields, measuring 377.94g, 418.88g, and 524.04g, respectively. In the experiment involving varying submersion durations, colonized plants yielded significantly more, with a harvest of 533.48g.
9. Across all experiments, the qualitative attributes of tomato fruits, including ascorbic acid, lycopene, and TSS, displayed an upward trend with rising stress levels. Importantly, *P. indica* colonization consistently led to improved fruit

quality, manifesting as higher levels of ascorbic acid, lycopene, and TSS in all experiments, regardless of whether the plants were subjected to stress or not.

10. In all experiments, physiological parameters such as RWC, CMS, and CSI exhibited a notable decline as water stress levels increased, with reductions of up to 50% observed in RWC under severe stress conditions. However, the presence of *P. indica* colonization consistently mitigated this decline, enhancing RWC by approximately 5-10% compared to non-colonized plants across various water stress levels.
11. When subjected to moderate water stress conditions (50% FC, -7bar PEG, 7% mannitol, and 6 µmol ABA), the presence of *P. indica* led to an increase in CMS ranging from 5-11%. Furthermore, under conditions of heightened and severe stress (25% FC, -10bar PEG, 10% mannitol, and 10 µmol ABA), *P. indica*-colonized plants exhibited even more substantial CMS improvement, surpassing non-colonized plants by 11-21%. Similar trends were observed for CSI as well.
12. Tomato plants colonized by *P. indica* exhibited significantly elevated proline accumulation when compared to the control plants under conditions of water stress. During instances of moderate stress, characterized by 50% field capacity (FC), -7 bar PEG, 7% mannitol, and 6µmol ABA, proline accumulation in the colonized tomato plants was notably enhanced by a range of 41-69%. Furthermore, when subjected to more severe stress conditions, such as 25% FC, -10 bar PEG, 10% mannitol, and 10µmol ABA, the proline accumulation witnessed an even more substantial increase, ranging from 55 to 116%.
13. The colonization of *P. indica* consistently amplified anti-oxidant activities (SOD, catalase and peroxidase) in response to escalating water stress levels. As the intensity of stress heightened, across all the conducted experiments, the colonized plants consistently exhibited significantly elevated anti-oxidant activities in comparison to the non-colonized plants subjected to equivalent levels of water stress. The increased anti-oxidant activities in *P. indica*-colonized plants suggests an enhanced ability to scavenge superoxide radicals and protect against oxidative stress caused by drought

14. *P. indica* colonization in tomato plants significantly enhanced the expression of the stress responsive transcription factor-*SlAREB1*, particularly under drought stress conditions. When both colonized and non-colonized tomato plants were subjected to drought stress (50% field capacity), non-colonized plants showed only 1.75 times increase in gene expression compared to the control, whereas *P. indica*-colonized plants displayed a much higher 2.76 times increase. This highlights the potential synergy between *P. indica* colonization and the gene's response to drought stress.

In conclusion, the presence of *P. indica* colonization had a consistently positive impact on various aspects of tomato plant growth and development under different water stress conditions. It resulted in improved vegetative and flowering characteristics, increased fruit yield, enhanced fruit quality, and heightened stress tolerance. Colonized plants consistently outperformed their non-colonized counterparts across all experiments, highlighting the potential of *P. indica* as a valuable ally in sustainable agriculture, particularly in mitigating the adverse effects of water stress on tomato crops.

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Appendix – I

Potato Dextrose Agar (PDA) medium

Potato: 200g

Dextrose: 20g

Agar: 20g

Distilled water: 1 litre

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 3.522g KH_2PO4 and 7.298g $Na_2HPO4.2H20$ in distilled water and make up to 1000 ml.

Appendix – III

Buffers for PCR products and Gel electrophoresis

10 X TE buffer

0.1 M Tris-Cl 0.01 M EDTA

Prepare 800 ml of distilled water in a suitable container

Add 15.759 g of Tris-Cl (desired pH) to the solution

Add 2.92 g of EDTA (pH 8.0) to the solution

Make up the volume to 1L using di

MANAGEMENT OF WATER STRESS IN TOMATO (*Solanum lycopersicum* L.) THROUGH BENEFICIAL ROOT ENDOPHYTIC FUNGUS, *Piriformospora indica*

by

ARUNA S.
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ABSTRACT
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ABSTRACT

Management of water stress in tomato (S*olanum lycopersicum* L.) through beneficial root endophytic fungus, *Piriformospora indica*

The study entitled 'Management of water stress in tomato (*Solanum lycopersicum* L.) through beneficial root endophytic fungus, *Piriformospora indica*' was conducted at College of Agriculture, Vellayani during 2019- 2022 with the objective to to enhance water stress tolerance in tomato plants through colonization with the beneficial root-endophytic fungus, *Piriformospora indica*, and to explore the underlying physiological and molecular mechanisms driving this tolerance.

The comprehensive study comprised five distinct experiments wherein *P. indica*-colonized and non-colonized tomato plants of the variety Vellayani Vijai were meticulously evaluated under varying water stress conditions. The co-cultivation of tomato seedlings with the fungus was carried out according to the established standard protocol (Johnson *et al*., 2011). Following the co-cultivation phase, both colonized and non-colonized tomato seedlings were transplanted into pots under protected conditions at 30 days after sowing (DAS). Subsequently, water stress was induced by adapting different methods to simulate water stress (limiting irrigation, applying polyethylene glycol (PEG), mannitol, abscisic acid (ABA) and flooding) at 45 DAS, persisting for a duration of seven days.

In all the experiments, the results consistently demonstrated the positive impact of *P. indica* colonization on various vegetative traits, such as plant height, stem girth, primary branches per plant, leaf length, leaf width, and leaf area. Conversely, with the escalation of water stress levels, these vital vegetative characteristics exhibited noticeable declines. Moreover, *P. indica* colonized tomato plants consistently exhibited significant advancements in achieving first and fifty percent flowering compared to their non-colonized counterparts. This consistent

trend held true across diverse water stress conditions, whether induced by restricted irrigation, the application of PEG, mannitol, ABA, or even submergence.

The impact of *P. indica* colonization was evident in various aspects of flowering and fruiting. The number of flower clusters per plant, flowers per cluster, fruit set percentage, fruits per truss, and fruits per plant were all significantly enhanced. *P. indica* colonization consistently resulted in approximately 6-14% higher fruit set. Additionally, colonized plants showed an impressive increase up to 36% in the number of fruits formed per cluster. For instance, under mannitol-induced stress, colonized plants demonstrated a remarkable 58% increase in the number of fruits per plant.

Colonized plants consistently yielded larger tomatoes, as evidenced by their greater fruit length and diameter, even across varying stress levels, when compared to non-colonized plants. The fruit weight in colonized plants consistently exceeded that of non-colonized plants in all experiments, with a pronounced 16-28% enhancement. Colonized plants demonstrated significantly higher yields, producing up to double the yield obtained from non-colonized plants (401.11g vs. 280.03g as observed in first experiment). Similarly, in experiments involving the application of PEG, mannitol, ABA, and submergence, colonized plants consistently achieved superior yields. As the stress levels increased the effect of *P. indica* became more evident. For instance, when comparing the yield of plants that were colonized with those that were not, there was a noticeable enhancement in yield by 14%, 28%, 146%, and 376% under the conditions of normal irrigation, 75% field capacity (FC), 50% FC, and 25% FC, respectively.

Across all experiments, the qualitative attributes of tomato fruits, including ascorbic acid, lycopene, and TSS, displayed an upward trend with rising stress levels. Importantly, *P. indica* colonization consistently led to improved fruit quality, manifesting as higher levels of ascorbic acid, lycopene, and TSS in all experiments, regardless of whether the plants were subjected to stress or not.

In all experiments, physiological parameters such as relative water content (RWC), cell membrane stability (CMS), and chlorophyll stability index (CSI)

exhibited a notable decline as water stress levels increased, with reductions of up to 50% observed in RWC under severe stress conditions. However, the presence of *P. indica* colonization consistently mitigated this decline, enhancing RWC by 6-12%, CMS by 13-27% and CSI by 16-35% compared to non-colonized plants under severe stress conditions in all conducted experiments.

Tomato plants colonized by *P. indica* exhibited significantly elevated proline accumulation when compared to the control plants under conditions of water stress. During instances of moderate stress, characterized by 50% field capacity (FC), -7 bar PEG, 7% mannitol, and 6µmol ABA, proline accumulation in the colonized tomato plants was notably enhanced by a range of 41-69%. Furthermore, when subjected to more severe stress conditions, such as 25% FC, -10 bar PEG, 10% mannitol, and 10µmol ABA, the proline accumulation witnessed an even more substantial increase, ranging from 55 to 116%.

The colonization of *P. indica* consistently amplified anti-oxidant activities (superoxide dismutase, catalase and peroxidase) in response to escalating water stress levels. As the intensity of stress heightened, in all the conducted experiments, the colonized plants consistently exhibited significantly higher anti-oxidant activities in comparison to the non-colonized plants subjected to equivalent levels of water stress. The increased anti-oxidant activities in *P. indica*-colonized plants suggests an enhanced ability to scavenge superoxide radicals and protect against oxidative stress caused by drought.

P. indica colonization in tomato plants significantly enhanced the expression of the stress responsive transcription factor-*SlAREB1*, particularly under drought stress conditions. When both colonized and non-colonized tomato plants were subjected to drought stress (50% FC), non-colonized plants exhibited only 1.75-fold increase in gene expression compared to the control (normally irrigated), whereas *P. indica*-colonized plants displayed a significantly higher 2.76-fold increase. This highlights the potential synergy between *P. indica* colonization and gene expression in response to drought stress.

In conclusion, colonization with *P. indica* had a consistently positive impact on multiple facets of tomato plant growth and development, even under varying water stress conditions. Across all experimental conditions, the colonized tomato plants consistently outperformed their non-colonized counterparts, highlighting the potential of *P. indica* as a valuable ally in sustainable agriculture, particularly in mitigating the adverse effects of water stress on tomato crops. This symbiotic relationship provides a promising avenue for optimizing water usage in tomato cultivation. Further studies should be conducted to elucidate the role of other antioxidants, and molecular mechanisms involved in this host-endophyte interactions. Field and multi-locational studies should also be conducted for confirmation of the results.