TO STUDY THE EFFECT OF PHYSICAL AND BIOCHEMICAL APPROACHES ON WATER USE EFFICIENCY IN TOMATO *(SOLANUM LYCOPERSICUM)*

By

MUNEEBA BANOO

(J-16-MBS-20)

Thesis submitted to Faculty of Basic Sciences

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

IN

PLANT PHYSIOLOGY

Division of Plant Physiology

Faculty of Basic Sciences

Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu

Main Campus, Chatha, Jammu 180009

2018

CERTIFICATE-I

This is to certify that the thesis entitled **"To Study the Effect of Physical and Biochemical Approaches on Water Use Efficiency in Tomato (*Solanum lycopersicum*)"** submitted in partial fulfillment of the requirement for the degree of **Master of Science in Plant Physiology** to the **Faculty of Basic Sciences,** Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is a record of bonafide research carried out by **Ms. Muneeba Banoo,** Registration No. **J-16-MBS-20** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that such help and assistance received during the course of investigation have been duly acknowledged.

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This is to certify that the thesis entitled **"To Study the Effect of Physical and Biochemical Approaches on Water Use Efficiency in Tomato *(Solanum lycopersicum)*"** submitted by **Ms. Muneeba Banoo,** Registration Number **J-16-MBS-20** to the Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, in partial fulfillment of the requirements for the degree of **Master in Plant Physiology** was examined and approved by the Advisory Committee and External Examiner(s) on 04/01/2019

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Muneebabano
Muneeba Banoo

ABSTRACT

Title of the thesis / Dissertation : **“To Study the Effect of Physical and Biochemical Approaches on Water Use Efficiency in Tomato (*Solanum lycopersicum*)”**

Name of Student : Muneeba Banoo

Registration No. : J-16-MBS-20

Major Subject : **Plant Physiology**

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Year of award of Degree : 2018

Name of University : Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

Detailed systematic studies were conducted on the effect of physical and biochemical approaches on water use efficiency in Tomato. Variety Pusa Ruby was taken as experimental material. Seeds of Pusa Ruby collected from Division of Vegetable Science and Floriculture, SKUAST-J, were sown in nursery bed at Vegetable farm and at five leaf stage it has been transplanted in plastic pots filled with soil and vermicompost in the experimental area of Division of Biochemistry. After 15 days of transplanting Paclobutrazol was introduced at different concentration (1.0ppm, 1.5ppm, 2ppm and 2.5ppm) and data was collected at three different stages of crop growth (50DAT, 100 DAT and at harvest). The present study was carried out to understand how different water-saving irrigation regimes affect the production and quality of tomato fruits (*Solanum lycopersicum* L.). In the study, Partial Root Drying (PRD) was applied as a physical technique and Paclobutrazol was used as a biochemical hormone to reduce the amount of water requirements and increase crop water use efficiency (yield/water applied) on tomatoes and to study their effect on morphological, physiological, biochemical, yield and quality parameters of tomato.

The results revealed that PRD technique and PBZ application induced the morphological, physiological and biochemical responses in relation to improving the WUE of tomato plants. In relation to morphological responses, the highest Plant height at harvest was recorded in control (88.33 cm) and PRD treated plants (80.66 cm) and lowest in plants treated with 2.5ppm PBZ (20.00 cm) and PRD+2.5ppm PBZ (26.00 cm). The highest stem thickness at harvest was found in plants treated with PBZ 2.5ppm (1.600 cm) and PRD+2.5ppm PBZ (1.733 cm) and lowest was noticed in control (0.800 cm) and PRD treated plants (0.800 cm). The highest WUE at harvest was noticed in plants treated with PBZ @ 2.5ppm (1.510 Kg L^{-1} F.W) as well as in PRD+ 2.5ppm PBZ (1.453 Kg L^{-1} F.W) and minimum was found in control (0.320 Kg L^{-1} FW) and PRD treated plants (0.507 Kg L^{-1} FW). The highest RWC at harvest was found in plants treated with 2.5ppm PBZ (82.20 %) as well as in PRD+ 2.5ppm PBZ (79.67 %) and lowest was found in control (71.23 %) and PRD treated plants (62.38 %). The highest soluble sugar in leaves at harvest was recorded in plants treated with 2.5ppm PBZ (3.903 mg g^{-1} DW) as well as in PRD+2.5ppm PBZ (4.080 mg g^{-1} DW) and therefore it was also found highest in plants treated with PRD (3.617 mg g^{-1} DW) while lowest was found in control (2.930 mg g^{-1} DW). The highest number of fruits per plant was recorded in plants treated with PBZ @ 2.5ppm (219.66) as well as in PRD+ 2.5ppm PBZ (218.33) and lowest was observed in control (128.33) and PRD treated plants (126.33). The highest lycopene content in fruits was noticed in plants treated with 2.5ppm PBZ (2.257 mg 100 g^{-1} FW) as well as in PRD+ 2.5ppm PBZ (2.203 mg 100 g^{-1}FW) and lowest was observed in control (1.677 mg 100 g^{-1}FW) and PRD treated plants (1.857 mg 100 g^{-1}FW). The highest total carotenoids in fruits was found in plants treated with PBZ @ 2.5ppm (1.407 mg 100 g^{-1} FW) as well as in PRD+ 2.5ppm PBZ (1.387 mg 100 g^{-1}FW) and lowest was observed in control (1.267 mg 100 g^{-1}FW) and PRD treated plants (1.280 mg 100 g^{-1}FW). The highest Yield per plant at harvest was recorded plants treated with 2.5ppm PBZ (2.67 Kg) as well as in PRD+ 2.5ppm PBZ(2.56 Kg) and lowest was recorded in control (1.63 Kg) and PRD treated plants (1.57 Kg).

Our results clearly indicated that, PBZ 2.5 ppm alone and in combination with PRD technique was most effective treatment to enhance morphological, physiological, biochemical parameters, yield and quality of tomato crop.

Key words: Pusa Ruby, Paclobutrazol, Partial Root Drying, Water use efficiency, Lycopene and Carotenoids.

Muneeba Banoo

Signature of Major Advisor **Signature of Student**

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PRD	Partial Root Drying	**%**	Percent
PBZ	Paclobutrazol	**m**	meter
DAT	Days after transplanting	**m^2**	meter square
ABA	Abscissic acid	**Max**	Maximum
^0C	Degree Celsius	**Min**	Minimum
RSI	Relative stress injury	**KOH**	Potassium hydroxide
WUE	Water use efficiency	**ha**	Hectare
RWC	Relative water content	**RDI**	Regulated deficit irrigation
cm	centimeter	**No.**	Number
CD	Critical difference	**CAPRI**	Controlled alternate partial root irrigation
OA	Osmotic adjustment	**SLA**	Specific leaf area
kg/L	Kilogram per litre	**RGR**	Relative growth rate
EC	Electrical conductivity	**LA**	Leaf area
g/ha	Gram per hectare	**FW**	Fresh weight
g	Gram	**OD**	Optical density
g/m^2	Gram per meter square	**ANOVA**	Analysis of variance
mg	Milligram	***et al.***	et alia = and other
RDI	Regulated deficit irrigation	**Temp.**	Temperature
TSS	Total soluble sugars	**DW**	Dry weight
ppm	Parts per million	**DI**	Deficit irrigation
HCl	Hydrochloric acid	**TEMED**	Tetramethylethylenediamine
W/V	Weight/volume	**SE (m)**	Standard error mean
SDS	Sodium dodecyl sulphate	**µg**	Micro gram
µl	Micro litre	**CRBD**	Completely randomized block design
g/lt.	Gram per liter	**kDa**	Kilodalton

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Signature of Major Advisor **Signature of Student**

CHAPTER- I

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important fruit crops grown throughout the world. In terms of human health, tomato is a major component in daily diet and nutrition globally and constitutes an important source of minerals, vitamins and antioxidants (Atkinson *et al.,* 2011). It is high in nutritional value; one medium fresh tomato (135g) provides 47% of vitamin C, 22% of vitamin A and 25% calories. Tomato is an important source of antioxidants, viz Carotenoids, Lycopene, Ascorbic acid etc. in the human diet because of their relatively high content of carotenoids. It acts as an antioxidant and scavenger of free radicals, which are often associated with carcinogenesis.

India is the second largest tomato producing country in the world producing nearly 17.5 million tonnes and the area under cultivation is 5.4 million ha with average production of 15.68 q ha^{-1}. India has world's second rank in total production and area, however it is 11th rank in productivity (Vanitha *et al.,* 2013). It is widely grown in Andhra Pradesh, Uttar Pradesh, Maharashtra, Karnataka, Bihar and Orissa. Jammu and Kashmir has 0.48% (88,080 tonnes) share in the total production. About 3.3% of total geographical area of the state is under cultivation, of which 70% is rainfed with frequent moisture stress. The tomato is a warm-season crop. It is a day neutral plant so widely it found grown in any season. The kharif crop is transplanted in July, rabi crop in October-November and zaid crop in February months.

As we know, drought is one of the most common environmental stresses that may limit agricultural production worldwide. However, this problem can be reduced by improving the water use efficiency of crop plants. Tomato is a highly water consuming crop and improving its water use efficiency implies positive economic and environmental effects (Cantero-Navarro *et al*., 2016). Water use efficiency is the ability of the crop to produce biomass per unit of water transpired. It may be defined as the ratio of biomass produced to the rate of transpiration.

WUE may be improved by partial stomatal opening, because their stomatal resistance is the major transpirational resistance and the leaves are well-adapted to atmospheric conditions. Water is essential for crop production because plants require water for growth and tissue expansion. However, well over 90% of the water required by terrestrial plants is not used in any biochemical way but lost through transpiration (Morison *et al.,* 2008). Because of the high proportion of water used for agricultural purposes and the projections that water scarcity due to unpredicted climate change will increase in the future, there is a constant need to focus on efficient use of available water resources in order to increase crop productivity per unit of used water (Mancosu *et al.,* 2015). Numerous management practices have been proposed to increase yields and improve quality attributes of tomatoes. Apart from fertilizer management, growers in recent years have attempted to develop water management strategies that maintain yields while imposing a moderate, controlled level of stress on the crops in order to improve fruit quality (Atkinson *et al.,* 2011). Efficient water use has become an important issue in recent years because the lack of available water resources in some areas is increasingly becoming a serious problem. Much effort has been spent on developing techniques such as RDI (regulated deficit irrigation) and CAPRI (controlled alternate partial root-zone irrigation or partial root-zone drying (PRD) to improve field and fruit crop water use efficiency. Simply WUE is the biomass produced for total water consumed in producing it (Heshmat *et al.,* 2013). Stomatal sensitivity for preventing high transpiration rates could be important for improving water use efficiency. Deficit irrigation techniques like conventional deficit irrigation (DI) and alternate partial root-zone drying (PRD) irrigation are among such practices which irrigate the crop with a volume of water less than the potential evapotranspiration, and the mild stress induced has a minimal effect on the yield. Accordingly, the moderate stress experienced by the plants under the DI and PRD treatments may bring about a positive effect on fruit quality (Wang and Frei, 2011). The Partial root-zone drying approaches are based on root-sourced signalling mechanism and included the following types: fixed and alternate partial root-zone drying. In fixed PRD, the one half of the root system is irrigated throughout the growing season, while the other half is exposed to soil drying during the whole growth period. In alternate PRD watering and drying parts of root zone are changed, which enables the wet side of the root to dry down and dry side to be fully irrigated (Jovanovic and Stikic, 2018).

Therefore, great emphasis is placed in the area of crop physiology and crop management for dry conditions with the aim to make plants more efficient in water use, Partial Root Drying (PRD) is applied as a physical technique and Paclobutrazol is used as a biochemical hormone to reduce the amount of water supplied and increase crop water use efficiency (yield/water applied) on tomatoes. Partial root drying (PRD) is a new irrigation and plant growing technique which improves water use efficiency without significant yield reduction. It is an irrigation technique where half of the root zone is irrigated while the other half is allowed to dry out and vice-versa. This new irrigation strategy allows the exploitation of drought-induced abscissic acid (ABA) based root to stomata signalling system to water saving. PRD could significantly increase dry matter content, firmness, and total soluble solids (TSS) concentration in tomato fruits (Davies *et al.*, 2000). PRD causes significantly stronger root-to-shoot abscissic acid (ABA) signalling, larger root systems, better leaf resistance to drought stress (Xu *et al.*, 2009), greater N and P use efficiency (Wang *et al.*, 2012), and higher water use efficiency. It has been indicated that for the successful application of PRD several factors should be taken into consideration, including: crops and variety-rootstock interaction, type and characteristics of soil, agricultural practice, specific agro-climatic conditions (Yactayo *et al.*, 2013). Partial root-zone drying strategy also includes a different approach, as “static” PRD irrigation where a reduced amount of water received by the plant was constant during the whole growth period. Another approach is “dynamic” when the amounts of irrigated water were changed according to specific crop’s phenological phase (Ahmadi *et al.*, 2014). Very recently, Adu *et al.* (2018) did not report differences in relative crop yield between PRD and RDI treated crops, but they pointed out that the effect on yield depends on crop species and soil structure. Abdelraouf (2016) conducted an experiment on potato crop and found that contrary root signals caused by PRD would make a slight reduction of the stomatal opening that would decrease the water loss substantially with only a small effect on the photosynthesis rate. He found that the opinion behind PRD is that by allowing the soil on one half of a root zone to dry, those roots will send drought signals to the shoot to reduce vegetative growth and stomatal conductance leading to reduced water use. When PRD irrigation is applied to a crop, the normal root to shoot signaling system that operates in water-deficient soils is altered, causing the drying half of the root system to release ABA thus reducing stomatal opening, whereas the fully hydrated roots preserve a favorable water status throughout the shoot parts of the plant. Partial root-zone drying (PRD) is an irrigation method that attempts to manipulate plant response to root drying in order to decrease the water demand. He found that irrigation is always needed for the production of high-yielding crops. However, the increasing global shortage of water resources requires the optimization of irrigation management in order to improve water use efficiency. Yan *et al.* (2012) investigated that to improve crop water economy, the partial root drying (PRD) technique helps to reduce the amount of water supplied and increase crop water use efficiency (yield/water applied) on tomatoes. Changes in stomatal morphological characteristics observed in PRD plants (smaller guard cells, lower stomata density) and lower conductivity affected transpiration and contributed to increase of water use efficiency, as well as enhance the photosynthetic capacity have positive impact on net. Recent comparative study indicated that alternate PRD crops have a higher yield compared to fixed PRD (Dodd *et al.,* 2015). PRD also enhances root growth and root to shoot ratio in maize plants (Wang *et al.,* 2012). The increasing efficiency of water use for fruit biomass production might be due to increasing photoassimilate allocation to the fruit of PRD plants. Compared to DI, PRD treatment was shown to induce greater ABA concentration in the xylem of tomatoes (Sun *et al.,* 2013). The hormone may be accumulated to a high level in the fruits, stimulating invertase activity and resulting in higher hexose concentration in the fruits (Ruan *et al.,* 2010).

Paclobutrazol (PBZ) is a plant growth retardant and triazole fungicide. It is a known antagonist of the plant hormone gibberellin. It acts by inhibiting gibberellin biosynthesis, reducing internodal growth to give stouter stems, increasing root growth, causing early fruitset and increasing seedset in plants such as tomato. When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate, resulting in stouter stem and short internodes with the same number of leaves (Jungklang *et al.,* 2017). Moreover, Paclobutrazol can be used as a chemical approach for reducing the risk of lodging in cereal crops (Kamran *et al.,* 2018). PBZ is used to reduce plant height for potted plant production in several species. It mainly improved resistance to drought stress, darker green leaves, higher resistance against fungi and bacteria, and enhanced development of roots. Cambial growth, as well as shoot growth, has been shown to be reduced in some tree species. Tomato yields are dependent upon several genetic, physiological and environmental factors, amongst which drought stress is known to severely hinder tomato productivity. Genetic engineering has helped in improving the drought tolerance of tomato cultivars (Peleg and Blumwald, 2011). In recent years, use of pesticides and fungicides, such as Paclobutrazol (PBZ), has shown a potential for improving crop drought tolerance (Shahrokhi *et al.,* 2011). The PBZ-induced change in leaf area under deficit irrigation may be linked to improved water use efficiency. Changes in the Root dry weight/Shoot dry weight ratio have been linked to the PBZ-induced inhibition of GA biosynthesis leading to reduced shoot growth (Bayat and Sepehri, 2012). The chlorophyll content increased in both irrigated and deficit irrigated PBZ treated plants as compared to their respective control (without PBZ). Consequently, PBZ application increased photosynthesis in tomato leaves (Nivedithadevi *et al.,* 2012). PBZ application resulted in a reduction in tomato plant height irrespective of growth

(Baninasab and Ghobadi, 2011). Paclobutrazol acts as stress protectant by maintaining relative water content, membrane stability index, photosynthetic activity, photosynthetic pigments and protects the photosynthetic machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby enhances the yield (Soumya *et al.*, 2017). Paclobutrazol (PBZ), a synthetic plant growth regulator, is a triazole-type inhibitor of gibberellin (GA) biosynthesis which affects plant growth and development. It inhibits the activity of ent-kaurene oxidase, which is an enzyme in the GA biosynthetic pathway that catalyzes the oxidation of ent-kaurene to ent-kaurenoic acid (Xia *et al.*, 2018). Practical application and promotion of this knowledge will allow farmers in water scarce areas to adapt PRD not only as a strategy for saving water, improving nutrient use and increase yield, but also for producing food with enhanced nutrition and health characteristics.

In view of above, the present study was planned with the following objectives:

1. To quantify the role of physical and biochemical approaches in improving the water use efficiency.
2. To study the effect on yield and quality parameters of fruits.

CHAPTER-II

REVIEW OF LITERATURE

Literature pertaining to physical and biochemical responses of tomato crop, especially mechanism and subsequent adaptation in growth, development, WUE, yield and quality of production has been reviewed in this chapter.

Tomato is a highly water consuming crop and improving its water use efficiency (WUE) implies positive economic and environmental effects (Cantero Navarro *et al.*, 2016). One of the indicators used to evaluate irrigation management is water use efficiency (Barideh *et al.*, 2018). Water use efficiency (WUE) is the ability of the crop to produce biomass per unit of water transpired. WUE may be improved by partial stomatal opening, because their stomatal resistance is the major transpirational resistance and the leaves are well-adapted to atmospheric conditions. Water use efficiency was considered as an important component of adaptation to water stress, where the identification of genotypes with high yield and high WUE is important. Numerous management practices have been proposed to increase yields and improve quality attributes of tomatoes (Mancosu *et al.*, 2015). Partial root drying (PRD) is a new irrigation and plants growing technique which improves water use efficiency without significant yield reduction and improves the quality of fruits. PRD causes significantly stronger root-to-shoot abscissic acid (ABA) signalling (Dodd *et al.*, 2008), larger root systems, better leaf resistance to drought stress (Xu *et al.*, 2009), greater N and P use efficiency (Wang *et al.*, 2012), and higher water use efficiency (WUE). As compared with the full irrigation, PRD could significantly increase dry matter content, firmness, and total soluble solids (TSS) concentration in tomato fruits. The moderate stress experienced by the plants under PRD treatments may bring about a positive effect on fruit quality (Wang and Frei, 2011). Paclobutrazol application reduced plant height, improved stem diameter and leaf number, altered root architecture, enhanced photosynthetic rates and WUE of tomato plants under deficit irrigation. It has been suggested that PBZ application could significantly improve tolerance in tomato plants under limited water availability through selective changes in morpho-physiology and induction of stress-related molecular processes (Pal *et al.*, 2016).

The relevant literature pertaining to the present study is reviewed under the following headings:

2.1 Water Use Efficiency:

Water use efficiency (WUE) can be defined in different ways depending on plant organization levels. The WUE for grain, straw and biological yields were calculated by dividing the yield of each by the amount of water added in each treatment (Stanhill,1987).Simply WUE is the biomass produced for total water consumed in producing it (Heshmat *et al.,* 2013). At crop level WUE is a ratio of the crop yield to total available water used by crops (Medrano *et al*., 2015). It may be defined as the ratio between marketable yield and the total water received by the crop (Giuliani *et al.,* 2017). Water use efficiency was calculated depending on total fresh biomass (WUE = kg biomass per hectare/ m^3 water consumed per hectare) (Bakr *et al.,* 2017). Water use efficiency was calculated as the ratio between total aerial plant dry matter at harvest (g $plant^{-1}$) and plant water used (L $plant^{-1}$) .Water use efficiency was also calculated as the ratio between total fruit yield and plant water used in tomatoes (WUE, g L^{-1}) (Giuliani *et al.,* 2018).

Water use efficiency is one of the indicators used to evaluate irrigation management. About two decades after the first deficit irrigation (DI), a new method known as partial root-zone irrigation (PRI) has been developed. PRI is a new method of irrigation which improves WUE without a significant decrease in the yield production. In this method, the root zone is divided into two equal parts, so that in each period of irrigation only one of those two regions is irrigated and the other side remains dry. In other words, only 50% of the root zone receives the required water and the other half of the root zone remains stressed (Barideh *et al.,* 2018). Partial root drying (PRD) is a new irrigation and plants growing technique which improves water use efficiency without significant yield reduction (Stikic *et al.,* 2003). Similarily, Paclobutrazol application enhanced photosynthetic rates and water use efficiency of tomato plants under deficit irrigation (Pal *et al.,* 2016).

2.1.1 Impact of PRD on Water Use Efficiency:

The PRD technique was developed on the basis of knowledge of root-to-shoot chemical signalling in drying soil and, therefore, understanding of this process is essential for successful application of the PRD technique. Alternating wet and dry zones of the root system are essential to trigger the continuous root-to-shoot signal. This is necessary because the root system is not able to maintain root ABA production for long periods (Loveys *et al.,* 2000). PRD has been successfully used in several crops such as tomatoes, corn, cotton, grapevines and in other vegetables (Kirda *et al*., 2004; Zegbe *et al*., 2004; Dorji *et al.,* 2005).

The results in the potato crops demonstrated that PRD has no major negative effect on the yield but improves fruit quality with a reduction of more than 50% of the consumption of water (Loveys *et al.*, 2001). Stoll *et al.*, (2000) observed that grapevine redistributed water from roots in the wet soil into root in dry soil during the night under PRD treatment. Hsiao (1990) suggested that the plant water status may equilibrate with the wettest part of the root zone which could contribute to maintenance of plant water balance. Cantore *et al.* (2000) reported reduction in fruit fresh mass and dry mass of sweet pepper when irrigation was withheld from one half of the split-root system compared to plants irrigated on both halves. In contrast, Kang *et al.* (2001) did not observe any difference in yield for hot pepper under similar treatments. Bacon (2004) in rice found that the plants growing in dry land with periodic soil drying have a higher water use efficiency and the increased WUE should be an integrated result of both short-term, as a function of atmosphere condition, and long-term, as a function of soil water availability, regulation of water loss. Improved WUE with a responsive stomatal behaviour is indeed predicted by (Cowan, 1982) from the optimization pattern of water use by plants. Jones (1992) concluded that the responsive pattern of stomatal opening would be the best pattern for both plant survival and carbohydrate production, i.e. the WUE. North and Nobel (1991) observed that the hydraulic conductivity of *Agave deserti* roots increased significantly after drying and rewetting cycles through PRD. Kang *et al.* (1999) found similar results for maize, sunflower, *Acacia confusa*, and *Leucaena glauca*. The hydraulic conductivity of apple, grape, peach, and pear tree roots also increased noticeably under a locally restricted water supply (Poni *et al.*, 1992). Such compensation may be partly attributed to the newly formed roots and also partly to the old roots which may undergo some changes when exposed to rewetting again. Many research results showed that some plants have the ability rapidly to resume water uptake after drought, and the water uptake rate would be enhanced after rewatering compared with a full water supply treatment in *Agave deserti* (North and Nobel, 1991; Huang and Nobel, 1992; Wraith *et al.*, 1995). Earlier research was also showed that hydraulic conductivity of root systems could be improved greatly when restoring wetting after drought (Kang and Zhang, 1997; Kang *et al.*, 1999). Liang *et al.* (1996) also reported that rewatering can greatly encourage the initiation and growth of secondary roots in maize. Moreover, Shi and Kang (2000) and Han and Kang (2002) reported that the ability of roots to absorb nutrients was also improved when the root zone of maize was partially watered and the partial watering was shifted alternately in a horizontal direction or along the vertical soil profile. Several research groups has shown that, using controlled alternate partial root zone irrigation (CAPRI) or PRD, the quality of fruits such as grapes in terms of sugar content can be increased (Fuller, 1997; Loveys *et al.*, 1997, 1998; Dry and Loveys, 1998, 1999, 2000 *a*, *b*; Gu *et al.*, 2000; Stoll *et al.*, 2000). They observed controlled vegetative growth of grapevine and reduced biomass production as a result of CAPRI, but the harvest index may be improved such that economic yield may not necessarily be reduced. Giuliani *et al.* (2017) revealed that in PRD experiment, the roots of tomato of the watered side of soil will keep a favourable plant status, while dehydration of the other side will promote the synthesis of abscissic acid (ABA), which will reach leaves by the transpiration stream and further reduce stomatal conductance. Loveys *et al.* (2000) conducted an experiment on tomato plant and the results showed that as a consequence of PRD treatment, the growth of whole plants was reduced with increased crop water use efficiency and sugar content. PRD also caused a significant reduction in fruit numbers but this effect was not significant for fruit biomass and fruit diameter. Further, they also observed that the sugar content and crop nutritional value increased in PRD treated plants. Davies *et al.* (2000) pointed out that reduction of carbohydrate strength in PRD treated plants resulted in a relative increase in the sink strength of tomato fruit, such that carbohydrate previously partitioned towards the side shoots is redirected towards the fruit. Alaa El-Sadek (2014) conducted an experiment on wheat and maize crops and examined that the PRD technique causes the stimulation of physiological responses which are normally associated with water stress and this result in a significant reduction in water use through the production of chemical signals in drying roots. The results confirmed an increase in irrigation water productivity using PRD comparing with conventional flood irrigation. Perez-Perez *et al.* (2012) examined that promotion of root growth and development and greater root biomass under PRD conditions increased plant hydraulic conductivity and water uptake in lemon. Several literature data also showed that PRD increase the activity of soil microorganisms and higher root nutrient uptake capacity in maize (Li *et al.*, 2010) in Potato (Sun *et al.*, 2013) in tomato (Wang *et al.*, 2013). Recently, Dodd *et al.* (2015) explained the increase of nitrogen and phosphorus uptake from different PRD treated crops such as in potatoes (Shahnazari *et al.*, 2008; Jovanovic *et al.*, 2012; Liu *et al.*, 2015; Sun *et al.*, 2015; Wang *et al.*, 2017) with so-called “Birch effect.” The effect was named on the honors of Birch, (1958) who discovered that re-wetting of previously dry soil induce an increase in N mineralization. Kudoyarova *et al.* (2015) reported that availability in water supply and mineral nutrients modified phytohormonal status (ABA and Cytokinins). Beis and Patakas (2015) also confirmed that ABA and cytokinins ratio modulated physiological and biochemical responses in PRD treated grapevine plants. In PRD plants, cytokinins controlled stomatal reaction and shoot growth, while ABA concentration play a dominate role in stomatal responses to drought in grapevines. Alternating wet and dry zones modifies phytohormonal signalling and induces changes in physical and biological processes in the soil environment with feedback on soil nutrient availability and as a result consequently improves crop nutrition. PRD frequently results in significantly higher ABA concentration in the xylem than DI, which has been considered to contribute to better stomatal control over plant water use (Dodd *et al.,* 2008; Wang *et al.,* 2010). Although there is a general consensus that tomato fruits are hydraulically isolated from the xylem stream and therefore may be inaccessible for the root-sourced ABA signalling, particularly at later stages of fruit development. Davies *et al.* (2000) demonstrated that, during the early stage of fruit growth, soil water deficit treatment or exogenous ABA application could stimulate xylem development in the tomato fruits (Davies *et al.,* 2000; Tonetto de Freitas *et al.,* 2011). Therefore, PRD is a water-saving practice and it stimulates the growth of secondary roots, which reduces the susceptibility to drought in potatoes (Abdelraouf, 2016).

2.1.2 Impact of Paclobutrazol (PBZ) on Water Use Efficiency:

Among a large number of pesticides and fungicides, Paclobutrazol has shown positive impacts on the drought-tolerance potential of tomato and other crops (Berova and Zlatev, 2003; Still and Pill, 2004; Somasundaram *et al.,* 2009). The primary action of PBZ is to inhibit ent-kaurene oxidase, which catalyzes the sequential oxidations from ent-kaurene to ent-kaurenoic acid in the early sequence of gibberellin biosynthesis (Rademacher, 1997). Among all the triazoles, PBZ performed best in terms of anthocyanin, protein, amino acid, proline, starch and sugar, contents (Gopi *et al.,* 2007). The mechanism of paclobutrazol mediated improvement in tolerance to water deficit in tomato was thoroughly investigated by (Pal *et al.,* 2016) and examined that Paclobutrazol differentially induced expression of genes and accumulation of metabolites of the tricarboxylic acid (TCA) cycle, γ-amino butyric acid (GABA-shunt pathway), glutathione ascorbate (GSH-ASC)-cycle, cell wall, sugar metabolism, abscissic acid (ABA) and expression of aquaporin (AP) protein under deficit irrigation (Pal *et al.,* 2016). They revealed that PBZ application could significantly improve tolerance in tomato plants under limited water availability through selective changes in morpho-physiology and induction of stress-related molecular processes. Integrating leaf and root observations, PBZ application led to decreased Leaf Area (LA) / Root Area (RA) ratio in irrigated plants. The PBZ-induced change in LA under deficit irrigation may be linked to improved water use efficiency. Further, Pal *et al.* (2016) reported that PBZ induced higher WUE in deficit irrigated plants affording them improved

WUE as irrigated control plants and enhanced the specific root area, especially of first order roots (i.e. root tips). The diameter of tap roots was larger, and PBZ treated tomato root system had finer side branches. Changes in the numbers of root orders and their frequency, morphology and anatomy indicate major modification of tomato root systems under PBZ treatment. As most of the roots of tomatoes were concentrated in the wet zone under drip irrigation, higher specific root area would improve the efficiency of water and nutrient uptake (Zhao and Rewald, 2012). Paclobutrazol priming of seeds facilitated basal root versus tap root system growth in tomato. It is hypothesized that basal roots, positioned in the topsoil, are most advantageous to maximize water uptake under deficit irrigation (Zhao and Rewald, 2017). Paclobutrazol (PBZ) affects the isoprenoid pathway, and alters the levels of plant hormones by inhibiting gibberellin synthesis and increasing cytokinins level. When gibberellins synthesis is inhibited, more precursors of ABA in the terpenoid pathway accumulate and that resulted to the production of abscissic acid. It acts as a stress protectant by maintaining relative water content, photosynthetic activity, photosynthetic pigments and protects the photosynthetic machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby enhances the yield (Soumya *et al.,* 2017). PBZ application has reduced plant height, improved stem diameter and leaf number, altered root architecture in tomatoes (Pal *et al.,* 2016), directly contributed to yield increase, and indirectly reduced the event of lodging (Syahputra *et al*., 2016) in rice. Xia *et al.* (2018) while working on Herbaceous peony (*Paeonia lactiflora*) found that Paclobutrazol has been associated with effects on the photosynthetic capacity of herbaceous peony plants. They found that PBZ application significantly increased photosynthetic rate (Pn), transpiration rate (Tr) and water use efficiency (WUE), PBZ increased chlorophyll content (SPAD) and the number of chloroplasts in individual cells in the foliar ultrastructure. PBZ-treated leaves had a darker green colour. The results indicated that plants treated with PBZ were superior in terms of increased photosynthetic characteristics when compared with untreated controls.

2.2 Growth parameters at different stages:

The physiological and morphological alternation of plants under partial root-zone irrigation may bring more benefits to crops than improved water use efficiency where carbon redistribution among organs is crucial to the determination of the quantity and quality of the products. Among the plant growth regulators (PGRs) used in agricultural and horticultural systems, paclobutrazol is a widely accepted regulator. Paclobutrazol is a triazole derivative that inhibits sterol and gibberellin biosynthesis (Hedden and Graebe, 1985; Lee *et al.,* 1985;

Khalil and Rahman, 1995; Khan *et al.,* 2009). This compound can markedly affect plant growth and development by altering the photosynthetic rate and modifying the phytohormone levels (Vu and Yelenosky, 1992; Wang and Lin, 1992; Huang *et al.*, 1995; Kim *et al.,* 2012). Canola plant yield could be significantly improved by paclobutrazol application (Zhou and Xi, 1993; Hua *et al.,* 2014). Aganchich *et al.* (2007) reported that alternation of wet and dry zones was vital to initiate the continuous production of root-to-shoot signal because the root system is not able to maintain root ABA production for longer periods. Lei *et al.* (2009) reported that plant height were significantly higher in control than in PRD and RDI. Leaf area per plant was affected significantly under RDI than in control, but under PRD it was affected moderately than in control. Li *et al.* (2018) observed that with elevated CO_2 concentrations condition indicated that photosynthetic rate and grain yield as well as water productivity in maize plants were higher under deficit irrigation than in full irrigation. These results open a new direction to test the efficiency of PRD strategy in specific agro-ecological conditions and under interaction of different environmental variables. Theobald *et al.* (2007) described that under PRD, leaf could have higher photosynthesis and lower transpiration, leading to more biomass and lower water usage. Stikic *et al.* (2003) showed that PRD decline 26% plant height in tomato crop as compared to well-watered plants. Leaf growth was also reduced by both decrease in the number of leaves 10% and leaf area by 22%. Davies *et al.* (2000) and Stikic *et al.* (2003) observed a significant decrease in the biomass due to decrease on shoot and root growth in tomato crop under PRD technique.

PBZ application under irrigated conditions lowered relative growth rate (RGR) by 0.7 fold at 0.8 ppm whereas this effect of PBZ was overturned during deficit irrigation revealing an increase in RGR by 0.6 fold at 1.6 ppm as compared with control plants (Pal *et al.,* 2016). Pal *et al.* (2016) reported that the application of PBZ reduced the plant height of tomatoes, improves stem diameter, altered root architecture, enhanced photosynthetic rates and WUE of tomato plants under deficit irrigation. Kamran *et al.* (2018) observed that the regulatory effects of paclobutrazol under two commonly used application methods (seed-soaking and seed-dressing) and it has been noticed that paclobutrazol improved the culm physical strength by increasing the rind penetration strength, stalk breaking strength, culm diameter, wall thickness, and dry weight per unit length of basal third internode, compared to control plants. Moreover, Paclobutrazol reduced the internode length, plant height, ear height, center of gravity height and lodging rate in both growing seasons. Similarly Pal *et al.* (2016) reported that the chlorophyll content increased in both irrigated and deficit irrigated PBZ treated tomato plants as compared to their respective control (without PBZ). In PBZ treated irrigated plants, the maximum chlorophyll content was observed at 77days after transplanting, thereafter it gradually declined, while in deficit irrigated PBZ treated plants the maximum chlorophyll content was observed at 92days after transplanting, declining thereafter and further it was concluded that, PBZ enhanced the chlorophyll content independent of water stress in both irrigated and deficit irrigated plants. Nivedithadevi *et al.* (2012) reported that PBZ application increased photosynthesis in tomato leaves. Increased rates of photosynthesis in the PBZ-treated plant could be linked with leaf structural adaptations, higher Chl (chlorophyll) index and reduced specific leaf area (SLA). Stomatal conductance increased in deficit irrigation treated with PBZ as compared to deficit irrigation control plants. Under irrigated condition, PBZ supplemented plants also showed slightly increased in stomatal conductance relative to control irrigated plants. Application of PBZ increased electron transport rate (ETR) irrespective of the growth conditions. Baninasab and Ghobadi (2011) observed reduced tomato plant height irrespective of growth under PBZ treatment and reduced size in PBZ treated plants may be linked with its inhibitory action on the GAs, which are involved in cell division. Berova and Zlatev (2003) revealed that PBZ application increased stem width and leaf number. Nevertheless the effect of PBZ on tomato roots was a reduction in root area (RA). Integrating leaf and root observations, PBZ application led to decreased leaf area and root area ratio in irrigated plants. Whereas, PBZ-induced change in leaf area under deficit irrigation may be linked to improved water use efficiency (WUE). Liang *et al.* (1996) also reported that rewatering can greatly encourage the initiation of secondary roots. Moreover, Shi and Kang (2000) reported that the ability of roots to absorb nutrients was also improved when the root zone was partially watered and the partial watering was shifted alternately in a horizontal direction or along the vertical soil profile.

Loveys (1991) was the first who applied the split-root technique (PRD) for inducing chemical signals in the root system of grapevine and the results showed that PRD reduced vine vigour and increased the quality and yield of fruit. Alternating wet and dry zones modifies phytohormonal signalling and induces changes in physical and biological processes in the soil environment with feedback on soil nutrient availability and as a result consequently improves crop nutrition. Kudoyarova *et al.* (2015) reported that availability in water supply and mineral nutrients modified by phytohormonal status (ABA and Cytokinins). In PRD treated grapevines plants, cytokinins controlled stomatal reaction and shoot growth, while ABA concentration play a dominate role in stomatal responses to drought in RDI (Beis and Patakas, 2015). Barideh *et al.* (2018) conducted an experiment on corn plants by using the alternate partial root-zone irrigation (APRI) and conventional irrigation techniques (CI) and found that the highest and lowest WUE were observed as equal to 4.88 and 3.82 g/L using the APRI and CI methods, respectively. The use of partial root irrigation method causes a reduction in water use, enhancing both the quality and quantity of the yield by creating appropriate conditions of wetness and ventilation in the roots.

2.3. Plant water status

2.3.1 Relative Water Content of leaf (RWC):

Relative water content is the ability of plant to maintain high water in the leaves under moisture stress conditions and has been used as an index to determine drought tolerance in crop plants (Basker, 2013). Relative water content is considered a measure of plant water status, it is essential because it expresses the absolute amount of water which the plant requires to reach full artificial saturation and reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC related to water uptake by the roots as well as water loss by transpiration. In addition to lowering stomatal conductance, PRD significantly increased plant water status, which may lower the hydrostatic tension in the xylem, allowing more water and calcium movement into the tomato fruit compared with DI plants (Guichard *et al.*, 2005; Tonetto de Freitas *et al.*, 2011). In accordance with this, investigation on the cause of elevated blossom end-rot incidence (BER) in tomato by osmotic or water stress has been attributed mainly to a reduction in calcium translocation into the fruit (Ho and White, 2005; Karlberg *et al.*, 2006; Magan *et al.*, 2008). Growth rates of several plants are directly proportional to the availability of water in the soil. Plant cellular water deficit occur when the rate of transpiration exceeds water uptake resulting in the reduction of the relative water content (RWC), cell volume and turgor (Lawlor and Cornic, 2002). Leaf water potential and Relative water content under PRD treatment were slightly lower whereas under RDI, they were significantly lower, resulting plants met slight water stress under PRD and strong water stress under RDI (Lei *et al.*, 2009). The reduction in evaporation under PRD relative to an RDI regime that has twice the number of emitters increases the water potential of a limited supply of water. The alternate wetting in PRD reduces drainage losses relative to a regime that always wets the same side of the plant row (Kang *et al.*, 2000). Net photosynthetic rate, transpiration rate, stomatal conductance, relative water content, leaf water potential were higher while peroxidase activity and free proline concentration were lower in the paclobutrazol-treated plants than in control (Berova and Zlatev, 2003). Plant growth in drying soil is commonly limited by a combination of chemical and hydraulic influences. Frequently, reductions in water availability result in reduced shoot turgor which can reduce shoot growth and development. Even when turgor of growing shoot cells is sustained, growth can be limited by chemical ‘signals’ generated as a result of interactions between the root and the drying soil and transmitted to the shoot via the transpiration stream. Davies *et al.* (2000) reported that water potential of PRD plants did not differ significantly from those of well-watered plants. Their results support the hypothesis that a root-sourced signal and not a leaf-sourced signal may be responsible for triggering growth reduction in PRD treated plants. Okasha *et al.* (2013) reported that PRD produced the highest values of irrigation water use efficiency in maize. When PRD irrigation is applied to a crop, the normal root to shoot signalling system that operates in water-deficient soils is altered, causing the drying half of the root system to release ABA thus reducing stomatal opening, whereas the fully hydrated roots preserve a favourable water status throughout the shoot parts of the plant. Moderate water stress induced osmotic regulation under PRD condition, leading to normal water status of the plants. Abdul Jaleel *et al.* (2007) reported that PBZ could induce leaf thickness by adding the layers of palisade and mesophyll cells. PBZ enhances the leaf thickness of *Curcuma*, which would improve the water requirements in their leaves. These may lead to an increase of RWC, a decrease of electrolyte leakage (EL) and finally the enhancement of the antioxidant system that might allow this plant to maintain its biomass and be effectively tolerant to water- deficit stress. In many plant species, relative water content in the leaves ranged from between 88% and 95% in fully turgid transpiring leaves and to about 30–40% in severely desiccated and dying leaves, depending on the species (Trouhton, 1969; Gonzalez and Gonzalez-Vilar, 2001; Schlemmer *et al.*, 2005).

2.4 Biochemical Status in Leaf

2.4.1. Proline Content:

Proline is a compatible osmolyte occurs widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Sairam and Tyagi, 2004; Verbruggen and Hermans, 2008). The proline content of tomato leaves fluctuated according to nutrient concentration and total radiation, and was closely related to the relative water content of leaves. Proline acts as a reliable indicator of the environmental stress imposed on plants, thus allowing us to establish stress thresholds for fruit yield and product quality of hydroponically grown tomato (Claussen, 2005). Recently, the proline content of tomato leaves was shown to be strongly related to the relative water content of leaves and water potential; it is thus a useful indicator of the plant's water status. Lei *et al.* (2009) conducted an experiment in tomato plants by using the technique PRD and found that the level of proline content under PRD was higher than control which means that osmotic regulation occurred. Under PRD, the leaf could have higher photosynthesis and lower transpiration, leading to more biomass and lower water usage. Proline accumulates in many plant species under a broad range of stress conditions such as water shortage, salinity, extreme temperatures, and high light intensity (Hare *et al.*, 1999). It is considered to be a compatible solute and protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source. In some plant species, proline plays a major role in osmotic adjustment such as in potato, while in others such as in tomato proline accounts for only a small fraction of the total concentration of osmotically active solutes. Therefore, its contribution to osmotic adjustment and tolerance of plants exposed to unfavourable environmental conditions is still controversial. The metabolic effects of osmolyte accumulation may, however, be equal or even more important than their role in osmotic adjustment, since stress-regulated changes in proline synthesis and degradation may also affect expression of other genes, ensuring that the genetic response to stress is appropriate to the prevailing environmental stress conditions. The accumulation of compatible solutes such as proline in response to water and salinity stress in plants is suggested to occur primarily in the cytosol to balance the osmotic potential of the vacuole where mainly non-compatible solutes are sequestered. Although proline accumulated in all vegetative organs and in fruits when plants were subjected to osmotic stress, the highest concentration was found in growing leaves. Madan *et al.* (1994) reported that the proline level and Pyrroline carboxylic acid (P5C) reductase activity were highest in young leaves and decreased linearly with increasing leaf age. It may be argued that the decrease in the proline content of tomato leaves observed later in the season was due to the increasing age of the plants. However, the investigations indicated that the proline content was only affected by the developmental stage of leaves sampled for proline analysis. An increase in proline content of tomato leaves was not detectable before 16–24 h after the beginning of stress induction conditions may either be caused by induction or activation of enzymes of proline biosynthesis or a decreased proline oxidation to glutamate, decreased utilisation of proline in protein synthesis, and enhanced protein turnover. The Relative water content was closely related to the proline accumulation in leaves. The lowering of osmotic potential by osmolyte accumulation in response to stress improves the capacity of the cell to maintain its turgor pressure at low water potential. This appears to be essential for physiological processes such as photosynthesis, enzyme activity and cell expansion (Tyree and Jarvis, 1982). Proline is well-known as an osmotic regulator that can reduce osmotic damage (Slama *et al.,* 2008; Surender-Reddy *et al.,* 2015).

Aganchich *et al*. (2007) reported that Proline content in PRD was on average by 5.88 µg/g FW higher than in control. Recently, the proline content of tomato leaves was shown to be strongly related to the relative water content of leaves and water potential; it is thus a useful indicator of the plants water status. The proline increased under PRD which means that osmotic regulation occurred. Some antioxidants such as vitamin C, vitamin E, and the activities of CAT and SOD were induced in the leaves by PBZ. Moreover, the content of vitamin C, vitamin E and CAT activity were higher in relation to water-deficit stress and PBZ treatments. This indicates that PBZ induced a number of some physiological and biochemical adaptations by maintaining growth and RWC, decreasing electrolyte leakage and proline content, increasing the vitamin C and vitamin E levels, Catalase (CAT) and Superoxide dismutase (SOD) activities that enable the plant to tolerate drought. In assessing the functional significance of accumulation of compatible solutes, it was suggested that proline synthesis may buffer cellular redox potential under heat and other environmental stresses (Wahid and Close, 2007). Accumulation of proline for osmotic regulation in drought-stressed leaves has been observed in wheat plants (Steward and Hanson, 1980 and Khan *et al.,* 2001). Martinez *et al.* (2004) reported that proline is one of the compatible solutes that accumulate in response to water stress and the accumulation of these osmolytes represents an important adaptive response to salt and water stress. Proline was considered to be the principal solute that may allow plants to overcome drought effect through osmotic adjustment, and serves as storage forms of nitrogen and carbon for future use under less stressful conditions (Sundaresan and Sudhakaran, 1995). The accumulation of Proline in plants reduces the toxic effects of ions on enzymes activity and also lowers the generation of free radicals formed by drought stress. Deepak *et al.* (1995) found that metabolic factors such as free proline contents in leaves increased significantly under severe drought stress. Jungklang *et al*. (2017) showed that water stress reduced RWC, but induced EL and proline content in the leaves. However, the leaves showed opposite results when PBZ was added to the treatments. Yang *et al.* (2009) observed a significant increase in proline amino acids commonly associated with osmotic adjustment in PBZ treated maize plants subjected to water deficit stress as compared to deficit irrigated control plants. Jungklang and Saengnil (2012) reported the

PBZ increased the tolerance of *Curcuma alismatifolia* under water-deficit stress conditions by reducing the proline content which shows that PBZ induced the water stress tolerance of the plants by maintaining fresh weight and RWC, reducing EL and Proline, enhancing the level of vitamin C and E antioxidants in the leaves. Pal *et al.* (2016) conducted an experiment on tomato and found that the proline content was reduced under water deficit stress as compared to control. The accumulation of proline, glycine and valine has been demonstrated to be intimately involved in water deficit stress response stabilizing cellular membranes and structures, scavenging free radicals and serving as a precursor for GSH. Madan *et al.* (1984) reported that the proline level were highest in young leaves and decreased linearly with increasing leaf age in tomato.

2.4.2 Total Soluble Carbohydrates:

Sugars constitute an important component of tomato fruit as they determine sweetness and influence the overall tomato flavour (Stevens *et al.,* 1979; Baldwin *et al.,* 1998). The major sugars in tomato fruit are glucose, fructose and sucrose (Davies and Hobson, 1981; Stevens, 1972). Sucrose is the form in which sugars are transferred from the sites of production (leaves) to fruit. Then, sucrose is broken down to glucose and fructose by the action of multiple forms of acid invertase and sucrose synthase (Yelle *et al.,* 1991). Xu *et al.* (2009) also noticed that, as compared with deficit irrigation practice, PRD leads to greater sugar and organic acid concentrations in tomato fruits. Higher accumulation of ABA in the fruits stimulates the activity of enzyme invertase and as a result the concentration of sugars hexose in the fruits is increased (Ruan *et al.,* 2010). Davies *et al.* (2000) pointed out that reduction of carbohydrate strength in PRD treated plants resulted in a relative increase in the sink strength of tomato fruit, such that carbohydrate previously partitioned towards the side shoots is redirected towards the fruit. Francaviglia *et al.* (2013) demonstrated that the improved peel colour of apple fruit under PRD was the result of changes in canopy structure and increased water use efficiency and nitrogen use efficiency, while total soluble solids accumulation (TSS) in the fruits may be due to translocation of assimilate from the leaves to fruits or metabolic changes. Metabolic changes, regulated by PRD induced phytohormones could be the result of higher conversion of starch to sugar, enhanced activities of enzymes involved in carbohydrate metabolism (starch-breaking, invertase, etc.) or *ex novo* synthesis of sucrose in the fruits. Accumulation of sugars in different parts of plants is enhanced in response to a variety of environmental stresses (Prado *et al.,* 2000). Soluble sugars seems to play an important role in osmotic regulation of cells during germination (Bolarin *et al.,* 1995). Sugars serve as signalling molecules during abiotic stress in stress-tolerant phenotypes (Rosa *et al.,* 2009). Sugar signalling pathways interact with stress pathways in a complex network to modulate the metabolic responses of plants (Gill *et al.,* 2003). Sugars that accumulate in response to stress can function as osmolytes to maintain cell turgor and have the ability to protect membranes and proteins from stress damage (Kaplan *et al.,* 2004). Starch metabolism is very sensitive to abiotic stresses generally leads to a depletion of starch content and to the accumulation of soluble sugars in leaves (Kaplan *et al.,* 2004; Basu *et al.,* 2007; Kempa *et al.,* 2008). Emery and Munger (1970) reported that indeterminate plants had more soluble solids than determinate forms of almost isogenic plants. Stikic *et al.* (2003) showed in their results that as a consequence of PRD treatment, the growth of whole plants was reduced with increased crop water use efficiency and sugar content. Fruit quality, in terms of sugars were improved at lower water application levels over the higher water application levels at final harvest. This may be due to increase in total soluble solids associated with reduced fruit water content and greater hydrolysis of starch into sugars (Subbaiah *et al.,* 2017). Several research groups have shown that, using partial root zone irrigation technique, the quality of fruits such as grapes in terms of sugar content can be increased (Fuller, 1997; Loveys *et al.,* 1997, 1998; Dry and Loveys, 1998, 1999, 2000*a*, *b*; Gu *et al.,* 2000; Stoll *et al.,* 2000). The total soluble sugar, sucrose, and starch content in the stem, leaf and bud organs were significantly increased by paclobutrazol application (Gopi *et al.,* 2007; Mobli and Baninasab, 2008; Pal *et al.,* 2016; Reddy *et al.,* 2013; Hua *et al.,* 2014) in carrot, almond, tomato, mango, canola plants respectively. The increased of total soluble sugars and sucrose content could be partially accounted for by the activating effect of paclobutrazol on enzymes related to sucrose synthesis and catalysis (Hua *et al.,* 2014).

2.4.1.2 Chlorophyll content:

Reduction of chlorophyll content has been considered as a commonly observed phenomenon in response to drought stress (Bayat *et al.,* 2009; Ebrahimiyan *et al.,* 2013) in tall fescue. Plant under water stressed conditions accumulates less chlorophyll content than the unstressed plants. Pal *et al.* (2016) conducted an experiment on tomato and found that the chlorophyll content increased in both irrigated and deficit irrigated PBZ treated tomato plants as compared to their respective control (without PBZ). In PBZ treated irrigated plants, the maximum chlorophyll content was observed at 77days, thereafter it gradually declined, while in deficit irrigated PBZ treated plants, the maximum chlorophyll content was observed at 92days and starts declining thereafter. Abdul Jaleel *et al.* (2007) reported that the average of total chlorophyll was higher on plants treated with paclobutrazol compared to control in *Catharanthus roseus*. The application of PBZ increased the total chlorophyll content as compared to control in almond (Mobli and Baninasab, 2008) and in Herbaceous peony (Xia *et al.,* 2018). This could be due to triazol applied to plants that increased the biosynthesis of chlorophyll precursor, so that plants treated with paclobutrazol usually have higher chlorophyll content compared to control.

The contrary root signals caused by PRD would make a slight reduction of the stomatal opening that would decrease the water loss substantially with only a small effect on the photosynthesis rate (Zhang and Tardieu, 1996). Reduction of vegetative vigour and canopy area allowed better exposure of grains/fruits to solar radiation (more light penetrate the canopy) and induced remobilization of assimilates from vegetative tissues to the fruits/grains that consequently could improve yield and its quality (Price *et al.,* 2013). The drought stress reduced the photosynthetic pigments such as chlorophylls and carotenoids and increased the antioxidant enzyme activities (Arivalagan and Somasundaram, 2017). Pal *et al.* 2016 demonstrates that PBZ enhanced the chlorophyll content independent of water stress in both irrigated and deficit irrigated tomato plants. At 77days, deficit irrigation treated with PBZ plants had chlorophyll content equal to irrigated plants, which suggest that PBZ compensates deficit irrigation induced reduction in chlorophyll content by maintaining higher chlorophyll content in deficit irrigated plants and thus affords them better photosynthesis similar to irrigated plants. Consequently, PBZ application increased photosynthesis in tomato leaves.

2.5. Membrane Injury in leaf

2.5.1 Relative stress injury (RSI %):

Cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants. Whitlow *et al.* (1992) demonstrated that the indicator of cell membrane integrity is electrical conductance or electrolyte leakage. Electrolyte leakage is also known as relative stress injury and it is measured by Digital Conductivity Meter which accompanies plant response to stresses. The stress induced electrolyte leakage is usually accompanied by accumulation of reactive oxygen species and often results in programmed cell death. The amount of electrolyte leakage is a function of membrane permeability. An increase in electrolyte leakage indicates an increase in membrane permeability and reduced cell tolerance to temperature change. The onset of drought stress often results in damage to plant tissue that can be quantified using a conductivity meter upon immersion in ion-free water. The cellular membranes play an important function in maintaining integrity of cell, by involving in signal transduction and ion homeostasis under environmental stresses (Kaur and Gupta, 2005; Tuteja and Sopory, 2008). Measurement of electrolyte leakage indicates the stress damage to assess the harshness of existing stress (Foyer, 1997). High membrane stability, determined in terms of changes in ion-leakage, is taken as an index of heat tolerance in several grain, forage and pasteur crops (Ismail and Hall, 1999; Blum *et al.*, 2001; Wahid and Shabbir, 2005). In principle, cell contents leak at a higher rate due to cell membrane rupture or faulty transmembrane protein pumps that regulate to and fro movement of cell fluids. Jungklang *et al.* (2017) conducted an experiment on *Curcuma alismatifolia* and found that the PBZ application significantly decreased the electrolyte leakage relative to control plants. Whitlow *et al.* (1992) showed that there were no significant difference observed in electrolyte leakage in Deficit Irrigation and Deficit irrigated with Paclobutrazol treated plant leaves. However, the roots of deficit irrigated tomato plants exhibited significantly reduced electrolyte leakage relative to control DI plants. The data suggest that PBZ has inhibitory effect on electrolyte leakage in root but no effect in leaf at this stage of plant development. The application of PBZ decreased the RSI in tomato (Pal *et al.*, 2016).

2.6. Yield parameters:

Fruit yield and quality are the important factors observed in the production of tomatoes (Muhammad and Singh, 2007). Economic yield of tomato is seriously affected by water stress. Numerous management practices have been proposed to increase yields and improve quality attributes of tomatoes. Apart from fertilizer management, growers in recent years have attempted to develop water management strategies that maintain yields while imposing a moderate, controlled level of stress on the crops in order to improve fruit quality (Atkinson *et al.*, 2011). Plants respond to drought with the activation of several signalling pathways resulting in a change of gene expression and enhancement of the biosynthesis of primary and secondary metabolites relevant for crop quality (Wang and Frei, 2011; Stagnari *et al.*, 2016). Much effort has been spent on developing techniques such as partial root-zone drying (PRD) to improve yield of crop plants. Sun *et al.* (2013) reported that alternate partial root-zone drying (PRD) irrigation treatments had no effect on tomato yield but significantly affected several organic and mineral quality attributes of the fruits. Loveys *et al.* (2001)

demonstrated that PRD has no major negative effect on the yield but improves fruit quality with a reduction of more than 50% of the consumption of water. Davies *et al.* (2000) investigated shoot-to-fruit signalling in PRD grown tomato plants and founded significant accumulation of ABA in expanded and mature leaves, but not in the fruit epidermis. Moreover, recent evidence demonstrates that exogenous ABA treatment could enhance the xylem connection between the shoot and the fruit and facilitate fruit water and calcium uptake (Tonetto de Freitas *et al*., 2011). Maintenance of turgor and total water potential in the shoot can be important for maintenance of fruit growth and development. Higher fruit yield in tomato and lower water use in PRD technique made the WUE improving (Theobald *et al.*, 2007; Abdelrouf, 2016). Davies *et al.* (2000) demonstrated that PRD reduces leaf growth of grapevines, but has no influence on fruit growth and development. Lei *et al.* (2009) conducted an experiment on tomato and found that PRD caused a significant reduction in fruit numbers but it was not significant for fruit biomass. Aganchich *et al.* (2007) reported that PRD reduced irrigation water amount by 30–50% but it had no conspicuous yield reduction and fruit quality was improved, so it meant the water use efficiency was improved. Casa and Rouphael (2014) while working in tomato with PRD technique found that marketable yields were significantly reduced, by 56% under DI and by 52% under PRD, compared to full irrigation. The comparative studies indicated that alternate PRD crops such as tomato, potato, maize, rice, wheat have a higher yield compared to control (Dodd *et al.*, 2015). Casa and Rouphael (2014) while working in tomato with PRD technique found that marketable yields were significantly reduced, by 56% under DI and by 52% under PRD, compared to full irrigation. Yield decreases were caused by reductions in fruit fresh weight (FW), rather than in the number of fruit per plant. Recently, experimental studies with elevated CO_2 concentrations condition indicated that photosynthetic rate and grain yield as well as water productivity in maize plants were higher under deficit irrigation than in full irrigation (Li *et al.*, 2018).

Plant growth retardants especially paclobutrazol, has been found beneficial in combating some of the production-related problems. Studies have shown paclobutrazol application to be promising for improving flowering and fruiting in several mango varieties (Yadava and Singh, 1998; Kulkarni, 1988; Reddy *et al.*, 2013). Canola plant yield could be significantly improved by paclobutrazol application (Zhou and Xi, 1993; Hua *et al.*, 2014). Soumya *et al.* (2017) reported that paclobutrazol acts as a stress protectant by maintaining the photosynthetic pigments machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby enhances the yield. Reddy *et al.* (2013) conducted an experiment on mango with PBZ and found that PBZ application increases the yield of mango in comparison to their control. Syahputra *et al.* (2016) found that application of PBZ increased the yield of rice and indirectly reduced the event of lodging. The application of PBZ increased the fruit yield as compared to control in wheat (Berova and Zlatev, 2003), in *Catharanthus roseus* (Abdul Jaleel *et al.,* 2007), in almond (Mobli and Baninasab, 2008) in *Camelina sativa* (Kumar *et al.,* 2012), in rice (Pan *et al.,* 2013). Zhou and Xi (1993); Hua *et al.* (2014) conducted an experiment on canola plants with paclobutrazol and found that Canola yield could be significantly improved by paclobutrazol application. Dewi *et al.* (2016); Xia *et al.* (2018) reported that application of paclobutrazol in rice plants and in herbaceous peony increased the fruit yield in comparison to their control.

2.7. Quality parameters:

Tomato is a very good source of numerous compounds with antioxidant capacity. These are mainly carotenoids (lycopene and β-carotene), flavonoid compounds as well as phenolic acids and vitamin C. High levels of ascorbic acid in tomato fruits provide health benefits for humans and also play an important role in several aspects of plant life (Matteo Di *et al.,* 2010). In general, ascorbic acid concentrations of the crop cultivars were increased as a result of water stress. Ascorbic acid in plants is involved in scavenging of oxidative stress products, such as hydrogen peroxide generated in the chloroplasts (Sairam and Tyagi, 2004). Increases in ascorbic acid concentration have been also reported previously in wheat under drought stress (Sairam *et al.,* 1998). To keep the levels of active oxygen species under control, plants have non-enzymatic and enzymatic antioxidant systems to protect cells from oxidative damage (Mittler, 2002).Non-enzymatic antioxidants including β-carotenes, ascorbic acid (AA), α-tocopherol, reduced glutathione (GSH). The increased ascorbic acid content may protect the plants from water stress (Reddy *et al.,* 2004). According to Fanciullino *et al.* (2014) water stress may influence the secondary metabolism through two interactive mechanisms: the changes of primary metabolite transport (major source in the biosynthesis of carotenoids and ascorbic acid) or oxidative stress which could affect the biosynthetic pathways of antioxidant compounds. Chen and Gallie (2004) found that ascorbic acid content of the drought stressed tomato plants significantly increased when compared to normal control plants. Tomato fruits with high total soluble solids, pH less than 4.5, high ascorbic acid, lycopene and total acidity content are preferred for processing purpose (Bose and Agarwal, 2007). Lycopene is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and tomato products. It is a carotenoid that is present in tomatoes, processed tomato products and other fruits. It is one of the most potent antioxidants among dietary carotenoids. Loveys (1991) was the first who applied the split-root technique for inducing chemical signals in the root system of grapevine grown in field conditions and reported that PRD reduced vine vigour and increased the quality of grapevine. Jovanovic and Stikic (2018) demonstrated a beneficial effect of PRD on quality of yield and its nutritional values. They found that the phenolics and carotenoids are produced more in PRD treated plants that are the important sources of bioactive components that have increased nutritional and health values. Increases in grape quality combined with reduced water use have been reported as a result of the use of PRD on a commercial field scale (Dos Santos *et al.,* 2003) and it has been found that PRD significantly increased fruit firmness, TSS, sugars, organic acids, Phosphorous (P), Potassium (K), Magnesium (Mg) concentrations in tomato juice, and Calcium and Mg concentrations in fruit dry matter, implying that PRD treatment enhanced fruit quality as compared with the deficit irrigation treatment. Casa and Rouphael (2014) reported that the PRD technique improved fruit quality in terms of total soluble solids contents (TSSC) as well as titratable acidity (TA) and juice pH, but not lycopene concentrations or fruit colour in tomato. Stikic *et al.* (2003), Jovanovic and Stikic (2018) reported that PRD has a beneficial effect on quality of yield and its nutritional or health values in terms of the secondary metabolites like phenolic compounds and antioxidants like total carotenoids including lycopene in tomatoes. Marjanovic *et al.* (2012) found that PRD tomato has some antioxidative enzymes which were upregulated during fruit expansion phase and also indicated their potential role in protection of fruits against the mild drought stress induced by PRD. Antolin *et al.* (2008) reported that under PRD changes in ABA content improved berry quality by increasing anthocyanin content and that increased mRNA induced accumulation of genes responsible for anthocyanin biosynthetic pathway (Jeong *et al.,* 2004).

Antioxidants such as vitamins C and E, and the activities of antioxidative enzymes such as catalase (CAT) and superoxide dismutase (SOD) are increased by PBZ, which in turn provides stress tolerance to plants (Somasundaram *et al.,* 2009; Srivastav *et al.,* 2010). The application of triazole (PBZ) increased the content of ascorbic acid, anthocyanin, and xanthophylls and activities of ascorbate peroxidase, superoxide dismutase, and catalase activities in Citrus lemon (Jain *et al.,* 2002), in *Vigna unguiculata* (Manivannam *et al.,* 2008), in potato (Siva Kumar *et al.,* 2010) and in mango (Reddy *et al.,* 2013). Jungklang *et al.* (2017) found that application of PBZ increased the Vitamin C of *Curcuma alismatifolia* leaves. Pal *et al.* (2016) conducted an experiment on tomato plants with paclobutrazol and found that PBZ induced higher synthesis of ascorbic acid that ensures sufficient scavenging of reactive oxygen species generated under water stress. Reddy *et al.* (2013) conducted an experiment on mango with PBZ and found that PBZ application improved quality in mango fruits in terms of lycopene as well as total carotenoids. Soumya *et al.* (2017) reported that paclobutrazol acts as stress protectant by maintaining the photosynthetic pigments machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby enhances the yield.

2.8. Molecular parameter:

PBZ differentially induced expression of genes and accumulation of metabolites of the tricarboxylic acid (TCA) cycle, γ-aminobutyric acid, glutathione ascorbate (GSH-ASC)-cycle, cell wall and sugar metabolism, abscissic acid (ABA), spermidine (Spd) content and expression of an aquaporin (AP) protein under deficit irrigation. Pal *et al.* (2016) reported that PBZ application could significantly improve tolerance in tomato plants under limited water availability through selective changes in morpho-physiology and induction of stress-related molecular processes. PBZ-treatment under deficit irrigation (having elevated ABA levels) recorded even higher protein level over the control. The increase in expression of the proteins related to cell wall, energy, and stress defense could allow PRD fruits to increase the duration of fruit growth. Upregulation of some of the antioxidative enzymes during the cell expansion phase of PRD fruits appears to be related to their role in protecting fruits against the mild stress induced by PRD (Marjanovic *et al*., 2012).

CHAPTER III

MATERIAL AND METHODS

The present study was carried out to understand how do different water-saving irrigation regimes affect the quality of tomato fruits (*Solanum lycopersicum* L.) variety Pusa Ruby. The seeds of Pusa Ruby were obtained from Division of Vegetable Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus Chatha, Jammu-180009, J&K.

3.1 Experimental site and Location

The experiment was conducted from October 2017 to April, 2018 under screen house condition as well as the laboratory work was carried out in the Division of Plant Physiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus Chatha, Jammu-180009, J&K. Geographically the experimental site is located at 320-40' N latitude and 740-58' E longitude with an altitude of 332 m above mean sea level in the Shiwalik foothills of North-Western Himalayas.

3.2 Nursery raising and transplanting

Tomato (*Solanum lycopersicum* L.) variety Pusa Ruby were raised to study their physiological, morphological, biochemical, yield and quality parameters in relation to improve their water use efficiency by implying the physical technique known as Partial Root Drying (PRD) and by the use of a plant growth regulator Paclobutrazol. Tomato seeds were germinated in commercial compost and established in a vegetable farm until the appearance of the fifth leaf. Two type of transplantation were done in soil + compost filled plastic pots i.e., one is normal transplantation and other as per the partial root drying methods. In PRD method tomato plants were transplanted, with the root system of each plant divided equally between two plastic bags in plastic pots containing the same commercial compost. Pots were watered daily for one week to allow establishment of the root systems. After five days of transplanting, as per the treatments, paclobutrazol was applied at different doses of 1.0 ppm, 1.5 ppm, 2.0 ppm and 2.5 ppm. Data were recorded at 50 days after transplanting, 100 days after transplanting and at the time of harvest.

Variety: Pusa Ruby (Indeterminate)

Date of Seed Sowing on nursery bed: 26th of October, 2017

Date of Germination: 1st of November, 2017

Date of transplanting in pots: 20th of November, 2017

Details of Treatment:

The experiment comprised of ten following treatments of tomato Variety- Pusa Ruby

Names	Notation
Control	T_1
Paclobutrazol (PBZ) -1ppm	T_2
Paclobutrazol (PBZ) -1.5ppm	T_3
Paclobutrazol (PBZ) -2ppm	T_4
Paclobutrazol (PBZ) -2.5ppm	T_5
Partial Root Drying (PRD)	T_6
PRD+PBZ -1ppm	T_7
PRD+PBZ -1.5ppm	T_8
PRD+PBZ -2ppm	T_9
PRD+PBZ -2.5ppm	T_{10}

Design: Factorial completely randomized block design (CRBD). The experiment was laid out in randomized block design with three replicates of each treatment.

Replication: Three

Sampling time :

Sampling were done at 50 days after transplanting, 100 days after transplanting and at harvest.

3.3 Observations:

The following observations were recorded on three competitive plants randomly selected and tagged from each treatment in each replication after giving paclobutrazol treatment and PRD treatment to crop at 50DAT, 100DAT and at the time of harvest.

Table 1. Meterological data during crop season (20 November 2017 to 15 April 2018)

Standard meterological week	Temperature (ºC)		Humidity (%)		Rainfall (mm)
	Max.	Min.	Morning	Evening	
47(19-25 Nov.)	23.9	6.3	94	40	0
48 (26-2 Dec.)	24.1	6.4	93	40	0
49 (3 - 9 Dec.)	23	4.6	92	41	0
50 (10-16 Dec.)	16.8	8.5	95	76	51.2
51 (17-23 Dec.)	23.1	6.1	88	46	0.0
52 (24-31 Dec.)	22.7	3.6	92	51	0
1 (1 to 7 Jan.)	17.5	3.3	94	56	0.0
2 (8 to 14 Jan.)	20.0	2.6	94	42	0.0
3 (15-21 Jan.)	21.8	3.5	91	42	0.0
4 (22-28 Jan.)	16.8	5.1	94	69	1.4
5 (29-4 Feb.)	21.4	6.1	91	49	0.0
6 (5-11 Feb.)	21.9	4.1	89	37	0.0
7 (12-18 Feb.)	20.5	7.7	92	54	6.7
8 (19-25 Feb.)	24.2	9.8	87	54	0.5
9 (26 to 4 Mar.)	24.5	12.2	84	58	0.8
10 (5-11 Mar.)	27.2	10.3	88	43	0.0
11 (12-18 Mar.)	29.2	11.6	84	38	0.0
12 (19-25 Mar.)	28.2	12.3	84	45	1.1
13 (26-1 Apr.)	32.4	13.6	84	35	0.0
14 (2-8 Apr.)	33.1	17.0	77	39	0.0
15 (9-15 Apr.)	31.7	16.3	78	36	3.8

Source: Division of Agrometerology, SKUAST- Jammu.

Photo plate 1: Experimental view of Tomato crop under screen house

Photo plate 2: Seedling of tomato variety (Pusa Ruby)

Photo plate 3: Left - Implementation of Partial Root Drying (PRD) technique during transplantation

Right – Establishment of PRD technique with one sided partial root irrigation

Photo plate 4: Effect of Paclobutrazol (PBZ) treatment on Plant height in Tomato

Left: Control , Right: PBZ (1.0, 1.5, 2.0 and 2.5ppm)

3.3.1 Growth characters

Plant height (cm): Plant height of the tagged plants was measured in centimetres as the distance from ground level to the tip of the plant at three different stages 50 DAT, 100 DAT and at harvest.

Stem thickness (cm): Stem thickness was measured with the help of Vernier Caliper in centimetre at all the three stages.

Number of leaves: Number of leaves was recorded from selected plants from each treatment.

Root dry weight (g): Total root dry weight in grams was recorded from selected plants from each treatments.

Shoot dry weight (g): Total shoot dry weight in grams was recorded from selected plants from each treatments.

Leaf Area (cm^2): The leaf surface area one side was calculated manually and recorded throughout the period of crop development at 50DAT, 100DAT and at harvest (Blanco and Folegatti, 2003).

Partitioning coefficient (%): The partitioning coefficient of root and shoot was measured at harvest. It was calculated by using following formula

$$\text{Partitioning coefficient } (\%) = \frac{\text{Dry weight of root and shoot}}{\text{Total dry weight of plant}} \text{x100}$$

Specific leaf area: The specific leaf area was calculated as the one sided leaf surface area divided by its dry oven mass at harvest. It was expressed in $cm^2 \ g^{-1}$ (Cornelissen *et al.*, 2003)

Relative growth rate: The RGR was measured at harvest. It was expressed in $g \ d^{-1}$. It was calculated by using following formula (Hunt, 1982)

$$RGR = W_2 - W_1 / T_2 - T_1$$

Where, W_2 and W_1 is the final and initial plant dry weight at time T_1 and T_2.

3.3.2 Maturity traits

3.3.2.1 Harvesting: The first picking was done after 90 days of transplanting. The subsequent 15 pickings were done at an interval of 7 days.

3.3.3 Fruit characters, yield and yield attributing traits

Number of flowers per cluster: Number of flowers per cluster was noted from three plants and means were worked out of each treatment and control.

Number of fruits per cluster: Number of fruits per cluster was taken from three plants and means were worked out of each treatment and control.

Number of fruits per plant: Number of fruits per plant was recorded from three plants and means were worked out of each treatment and control.

Fruit yield per plant (Kg): Fruit yield was recorded from all the three tagged plants by adding up weight of fruits obtained from all the previous pickings.

Number of locules in fruit: Tomato fruits of each treatment were cut transversely and locules were counted.

Fruit diameter (cm): Diameter of fruit was measured in (cm) with the help of a Vernier caliper at the center (equatorial length) of the fruit.

Fruit volume (cm^3): Fruit volume was measured by water displacement method. In water displacement method, harvested tomato fruit was poured in partially water filled measuring cylinder. Initial and final level of water was noticed. Fruit volume was calculated by the following formula (Calbo and Nery, 1995).

Calculation:

$$\text{Fruit volume } (\text{cm}^3) = \frac{\text{Final water level} - \text{ Initial water level}}{\text{Density of water}}$$

Whereas,

Density of water = 0.998 g/cm^3

Fruit density (g/cm^3): The fruit density was measured by the fresh weight of fruit divided by the volume of fruit which was calculated by the following formula.

Calculation :

$$\text{Fruit density } (\text{g per cm}^3) = \frac{\text{Weight}}{\text{Volume}}$$

Fresh weight of fruit (g): Fresh weight of fruit was taken at harvest from three plants and mean were worked out of each treatment and control. It was expressed in grams.

Dry weight of fruit (g): Dry weight of fruit was taken at harvest from three plants and mean were worked out of each treatment and control. It was expressed in grams.

Water use efficiency: Water use efficiency was calculated at harvest in fruits from three tagged plants and their mean were worked out. It was expressed as Kg L^{-1} FW. It was calculated by the total fruit yield produced per plant divided by the total water used by the plants in each treatment (Giuliani *et al.,* 2018).

$$\text{WUE (Kg per litre FW)} = \frac{\text{Total fruit yield produced by the plants}}{\text{Total water consumed by the plants}}$$

3.4.4 Quality parameters

3.4.4.1 Lycopene content :

The lycopene content of tomato fruit was measured by using a spectrophotometer and expressed in mg 100 g^{-1} FW (Sadasivam and Manickam, 1996)

Reagents :

1. Acetone (AR grade)

2. Petroleum ether (AR)

3. Anhydrous sodium sulphate

4. 5% sodium sulphate

Procedure:

Tomato fruits (3-4) were taken and pulped well to a smooth consistency in a waring blender. 5-10 g of this pulp was weighed and extracted repeatedly with acetone using pestle mortar or waring blender until the residue was colourless. The acetone extract was pooled and transferred to a separating funnel containing about 20ml petroleum ether and mixed gently. To this, 20 ml of 5% sodium sulphate solution was added and the separating funnel was shaken gently. As the volume of petroleum ether reduces during these processes because of its evaporation, 20 ml more of petroleum ether was added to the separating funnel for clear separation of two layers. Most of the colour was noticed in the upper petroleum ether layers. The two phases were separated and the lower aqueous phase was re-extracted with additional 20 ml petroleum ether until the aqueous phase became colourless. The petroleum ether extract was cooled and washed once with a little distilled water. The washed petroleum ether extract containing carotenoids was poured into a brown bottle containing about 10 g anhydrous sodium sulphate and was kept aside for 30 minutes or longer. The petroleum ether extract was decanted into a 100 ml volumetric flask through a funnel containing cotton wool. Then, sodium sulphate was washed with petroleum ether until it became colourless and the washings were then transferred to the volumetric flask. Finally, the volume was made up to 100ml and the absorbance was measured in a spectrophotometer at 503 nm using petroleum ether as blank.

Calculation:

Absorbance (1 unit) = 3.1206 µg lycopene / ml

$$\text{Lycopene (mg /100 g F W)} = \frac{31.206 \text{ x absorbance}}{\text{Weight of sample(g)}}$$

3.4.4.2 Total carotenoids :

Total carotenoids content of tomato fruit was measured by using a spectrophotometer and expressed in mg/100 g F.W. (Mahadevan and Sridhar, 1986).

Procedure:

A known fresh weight of sample (1gm) was extracted with acetone and add a few drops of sodium sulphate. The extractions were repeated and the extract was collected in a beaker and to it added 10% KOH. The extract was heated on a water bath for 30 minutes and then transferred to a separating funnel. To this 50 ml of petroleum ether was added. The separating funnel was shaken and allowed to stand for at least 10 minutes till the layers got separated. The lower layer was drained and the upper layer of petroleum ether containing pigment was collected in a volumetric flask and the volume was made up to 50 ml with petroleum ether and O.D was recorded as 452 nm against petroleum ether as blank. The total carotenoids were calculated as per the formula:

$$\text{Total carotenoids (mg / 100 g F.W)} = \frac{\text{O.D x } 13.9 \text{ x } 10^4 \text{ x Volume made}}{\text{Weight of sample x 560 x 1000}}$$

3.4.4.3 Ascorbic acid :

The ascorbic acid content of the fruit was estimated by 2, 6-dichlorophenol Indophenol visual titration method of (A.O.A.C, 1984) and expressed in mg/ 100g FW.

Reagents

3% Meta phosphoric acid (HPO_3): 30 g of HPO_3 were dissolved in distilled water and volume made up to 1000 ml.

Ascorbic acid standard

100 mg of 1-ascorbic acid were dissolved in 3% HPO_3 and volume made upto 100 ml. 10 ml from this solution were further diluted to 100 ml with 3% HPO_3.

Dye solution

50 mg of the sodium salt of 2, 6-dichlorophenol indo-phenol were dissolved in 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate. It was cooled and diluted with glass-distilled water to 200 ml and filtered.

Procedure:

Standardization of dye

5 ml of 3% HPO_3 were added to 5 ml of standard ascorbic acid and titrated with the dye solution to a pink colour which persisted for 15 seconds. The dye factor i.e. mg of ascorbic acid per ml of dye was determined using the formula:

Dye factor = 0.5/titre

Preparation of sample

20 ml of fruit juice extracted from 100 g of fruit was taken and dissolved in 3% HPO_3 to make up the volume 100 ml. It was then filtered through filter paper.

Determination of ascorbic acid

An aliquot of 5 ml of the HPO_3 extract of the sample was titrated with the standard dye to a pink end point. The titration was repeated thrice and average volume of dye used was calculated.

Calculation

Ascorbic acid content of the sample was calculated by using the formula:

$$\text{Ascorbic acid (mg per 100 g)} = \frac{\text{Titre x Dye factor x Volume made}}{\text{Aliquot of extract x volume of sample x 100}}$$

Where,

Volume of sample taken for estimation = 20 ml

Volume made up = 100 ml

Aliquot of extract taken for estimation = 5 ml

3.4.5 Plant water status:

3.4.5.1 Relative water content (RWC%) of leaf:

For RWC, the second or third fully expanded leaf from the top was brought from the field in polyethylene bags and stored in an ice box. Immediately, twenty leaf discs were weighted on electronic balance (Citizen Scale, CY510, Poland) and Fresh Weight (FW) was determined. The weighted leaf discs were floated overnight in a petridish containing distilled water and subsequently blotted gently and weighted again for Turgid Weight (TW). After taking turgid weight, the leaves were oven dried at 80º C for 48 h and Dry Weight (DW) was recorded separately. The RWC was calculated using the following formula (Weatherley, 1950).

$$\text{RWC}\,(\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \text{ X } 100$$

3.4.6 Biochemical estimation :

3.4.6.1 Proline content :

Proline content was estimated by using the method of (Bates *et al.*, 1973).

Reagents

1. 3% aqueous sulphosalicylic acid (w/v)
2. Acid ninhydrin (prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6.0 M o-phosphoric acid until dissolved)
3. Toluene

Extraction

Three hundred mg of leaves were separately homogenised in 5 ml of 3 % sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes and supernatant was taken.

Procedure

Two ml of supernatant was taken in a test tube and 2.0 ml reagent acid ninhydrin was added. This mixture was then kept in boiling water bath for 1 h at 100º C and thereafter reaction was terminated by keeping tubes in ice bath. Then 4.0 ml of toluene was added.

After vigorous shaking, the upper coloured organic phase was taken after attainment of room temperature and absorbance was recorded at 520 nm by using toluene as blank. Standard curve was prepared by using graded concentration of proline in 3% sulphosalicylic acid. The proline content was expressed as mg g^{-1} FW.

3.4. 6.2 Total soluble carbohydrates (TSC) :

Total soluble carbohydrates was determined with the method of Yemm and Willis (1954) using anthrone reagent. Two hundred mg dry samples of leaf were homogenised separately in 80% ethanol using acid washed sand as an abrasive. The homogenate was refluxed thrice with 80% ethanol. The supernatant from different extraction was pooled and volume made to 5ml with 80% ethanol. The extract so obtained was used for estimation of TSC.

Reagents

Anthrone reagent:

Anthrone reagent was prepared by dissolving 0.4g anthrone in 100ml concentrated H_2SO_4.

Procedure

An aliquot from the above extract measuring 0.2 ml was evaporated to dryness in a test tube in a boiling water bath. On cooling the residue left in the test tubes was dissolved in one ml of distilled water and mixed with 4.0 ml of the anthrone reagent. The mixture was heated in a water bath for 10 minutes. After cooling, absorbance was recorded at 620 nm using computer aided spectrophotometer (Systronic India Spectrophotometer 117).

3.4.6.3 Estimation of chlorophyll in leaf:

Estimation of chlorophyll was done by acetone method (Arnon, 1949) and expressed in mg g^{-1} FW.

Chemical required:

Acetone

Preparation of reagent:

80% acetone: 80 ml in 20 ml of Distilled water

Extraction

0.2 g of fresh plant leaf material was placed in a test tube and grind with 10 ml of 80% acetone in mortar using pestle.

Procedure

Chlorophyll extracted into acetone solution was collected from test tubes and centrifuge the extract at 5000 rpm for 15 minutes. Collect the supernatant from centrifuge tubes and transfer into another test tubes and discard the residue. Make the final volume of the filtrate up to 25 ml with 80% acetone. Concentration of chlorophylls a, b and total chlorophyll were quantified in samples by reading the optical density at 663 and 645 nm. Calculate the chlorophylls a, b and total chlorophyll using the formula.

Calculation:

The amount of chlorophyll was calculated in mg chlorophyll per g F.W tissue using the following equations:

$$\text{mg chlorophyll a / g tissue} = 12.7\ (A663) - 2.69\ (A645) \times V / 1000 \times W$$

$$\text{mg chlorophyll b /g tissue} = 22.9\ (A645) - 4.68\ (A663) \times V / 1000 \times W$$

$$\text{mg total chlorophyll /g tissue} = 20.2\ (A645) + 8.02\ (A663) \times V / 1000 \times W$$

$$\text{mg total carotenoids/ g tissue} = 0.114 + 480\ (A663) - 0.638\ (A645) \times V / 1000 \times W$$

where,

A = absorbance at specific wavelengths

V = final volume of chlorophyll extract

W = fresh weigh of tissue extracted

3.4.6.3 Total phenols :

Total phenolic content of prepared extracts was determined according to the Folin-Ciocalteu method (Singleton *et al.*, 1974) and expressed in mg g^{-1} DW. The quantitative determination of total phenols was done by spectrophotometer. Briefly, 100 µl of the sample extract was taken and the volume was raised to 1000 µl by adding 900 µl distilled water. 1ml of 1 N Folin- Ciocalteu reagent was then added and the reaction mixture was kept at room temperature for 5 minutes.

After 5 minutes, 3 ml of 20% Na_2CO_3 was added. After 10 minutes of incubation at room temperature, the absorbance of the reaction mixture was taken at 760 nm using double beam UV-VIS spectrophotometer. The results were expressed as milligram gallic acid equivalents (mg GAEs) per gram of the extracts.

3.4.7 Membrane Injury

3.4.7.1 Relative Stress Injury :

The relative stress injury (RSI%) in leaves was evaluated by (Sullivan and Ross, 1979).

Procedure

The third fully expanded leaf from the top was collected and kept in 20 ml vials containing 10 ml de-ionised water at 25º C. After 4 h, the electrical conductivity (EC) of the solution was measured by the Water Analysis Kit (Naina, India Ltd., NDC 732) and designated as $EC_{a.}$ Then the samples were kept in boiling water bath for 50 minutes to achieve total killing of tissue. After cooling, the EC of the solution was again measured and designated as $EC_{b.}$ The relative stress injury (RSI) was calculated as follows:

$$\text{RSI}\ (\%) = 1 - \frac{EC_a}{EC_b}\ X\ \ 100$$

3.4.8 Protein separation by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The protein profiling through SDS-PAGE in tomato leaves was determined by (Laemmli, 1970).

Principle: SDS is an anionic detergent which binds strongly to, and denatures, proteins. The number of SDS molecules bound to a polypeptide chain is approximately half the number of amino acid residues in that chain. The Protein-SDS complex carries net negative charges, hence move towards the anode and the separation is based on the size of the protein.

Material and Reagents

Stock Acrylamide Solution (30%)

Acrylamide	:	29.2g
Bisacrylamide	:	0.8g
Double distilled water (ddH_2O)	:	100ml

Separation Gel Buffer (pH 8.8)

0.6M Tris-HCl : 7.26g

ddH$_2$O : 100ml

Tris base dissolve in 50 ml ddH$_2$O and adjust the pH8.8 with HCl. Then make up the volume 100ml.

Stacking Gel Buffer (pH 6.8)

0.6M Tris-HCl : 7.26g

ddH$_2$O : 100ml

Tris base dissolve in 50 ml ddH$_2$O and adjust the pH6.8 with HCl. Then make up the volume 100ml.

Polymerizing Agents

1. Ammonium persulphate 5% : 0.5 g/ 10 ml, prepare freshly before use.
2. TEMED fresh from the refrigerator.

Electrode Buffer (pH 8.2-8.4)

0.05M Tris : 12g

0.192 M Glycine : 28.8g

0.1% SDS : 2g

ddH$_2$O : 2 L

No adjustment required. Electrode buffer may be used 2-3 times

Sample Buffer (5X concentration)

Tris- HCl buffer pH 6.8 : 5 ml

SDS : 0.5g

Sucrose : 5g

Mercaptoethanol : 0.25ml

Bromophenol Blue(0.5% W/V) : 1ml

ddH$_2$O : 10ml

Store frozen in small aliquots. Dilute to 1x concentration and use.

Sodium dodecyl sulphate 10% solution : store at room temperature.

Standard Protein Marker

Protein stain solution

Comassie brilliant blue R 250 : 0.1g
Methanol : 40ml
Acetic acid : 10ml
ddH_2O : 50ml

First, dissolve the dye in methanol and proceed. Use fresh preparation every time.

Destainer

Methanol : 40ml
Acetic acid : 10ml
ddH_2O : 50ml

Procedure

1. Throughly clean and dry the glass plates and spacers, then assemble them properly. White petroleum jelly or 2% agar (melted in a boiling water bath) is then applied around the edges of the spacers to hold them in place and seal the chamber between the glass plates.
2. Prepare a sufficient volume of separating gel mixture (30ml) by mixing the following.

	For 15% gel	For 10% gel
Stock acrylamide solution	20ml	13.3ml
Tris-HCl (pH 8.8)	8ml	8ml
Water	11.4ml	18.1ml
Ammonium persulphate solution	0.2ml	0.2ml
10% SDS	0.4ml	0.4ml
TEMED	20µl	20µl

3. Mix gently and carefully, pour the gel solution in the chamber between the glass plates. Layers distilled water on top of the gel leave to set for 30-60min.

4. Prepare stacking gel (4%) by mixing the following solutions (total volume 10 ml)

	4% gel
Stock acrylamide solution	13.5ml
Tris-HCl (pH 6.8)	1ml
Water	7.5ml
Ammonium persulphate solution	50 µl
10% SDS	0.1ml
TEMED	10µl

5. Remove the water from the top of the gel and wash with little stacking gel solution.
6. Pour the stacking gel mixture, place the comb in the stacking gel and allow the gel to set (30-60 min).
7. After the stacking gel has polymerised, remove the comb without distorting the shapes well. Carefully install the gel after removing the clips, agar etc. in the electrophoresis apparatus. Fill it with electrode buffer and remove any trapped air bubbles at the bottom of the gel. Connect the cathode at the top and turn on the direct current power briefly to check the electrical circuit. The electrode buffer and the plates can be kept cooled using a suitable facility so that heat generated during the run is dissipated and does not affect the gel and resolution.
8. Prepare samples for electrophoresis, following suitable extraction procedure. Adjust the protein concentration in each sample using the 5-strength sample buffer and water in such a way that the same amount of protein (50-200µg) in a volume (25-50µl) not greater than the size of the sample well. As general practice, heat sample solution in boiling water for 2-3 min to ensure complete interaction between the protein and SDS.
9. Cool the sample solution and take up the required volume in a microsyringe and carefully inject it into the sample well through the electrode buffer. Making the position of well on the glass plate with a marker pen and the presence of bromophenol blue in the sample buffer facilitate easy loading of the sample buffer.
10. Turn on the current to 10-15mA for initial 10-15 min until the samples travel through the stacking gel. The stacking gel helps concentration of the samples. Then continue the run at 30 mA until the bromophenol blue reaches the bottom of the gel (3h). However, the gel may be run at a high current (960-70mA) for short period (1h) with proper cooling.

11. After the run is complete, carefully remove the gel from between the plates and immerse in staining solution for at least 3h or overnight with uniform shaking. The protein absorb the Comassie brilliant blue.
12. Transfer the gel to a suitable container with at least 200-300ml destaining solution and shake gently continuously. Change the destainer frequently, particularly during initial periods, until the background of the gel is colorless. The proteins fractionated into band are seen colored blue. As the proteins of minute qualities are stained faintly, destaining process should be stopped at appropriate stage to visualise as many bands as possible. The gel can be photographed or stored in polythene bags or dried in *vacuo* for a permanent record.

3.4.9 Statistical analysis

Data were analysed using Completely Randomized Block Design (CRBD). Treatments were compared using critical difference (CD) at 5 % level of significance. Data were subjected to analysis of variance (ANOVA) using Online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar 125004, Haryana, India).

CHAPTER-IV

RESULTS

The present study was carried out on tomato plant (*Solanum lycopersicum* L.) variety (Pusa Ruby) to study their physiological, morphological, biochemical, yield and quality parameters in relation to improve their water use efficiency by implying the physical technique known as Partial Root Drying (PRD) and by the use of a biochemical hormone known as Paclobutrazol. Tomato seeds were germinated in commercial compost and established in a vegetable farm until the appearance of the fifth leaf. The plants were transplanted in plastic pots under glass house in plastic pots containing the same commercial compost. After transplanting, Paclobutrazol was applied at different doses (1.0 ppm, 1.5 ppm, 2.0 ppm, 2.5 ppm). Data were recorded at 50 days after transplanting, 100 days after transplanting and at the time of harvest. The results obtained were described below:

4.1 Growth Parameters:

4.1.1 Plant height (cm):

A significant reduction in plant height was recorded in Paclobutrazol (PBZ) treated plants @ 2.5 ppm (12.00, 18.00 and 20.00 cm) followed by PBZ @ 2ppm (14.33, 19.66 and 23.66) respectively at 50, 100 DAT and at harvest. As evident from Table 2, PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly reduced plant height (17.66, 22.33 and 26.00) and (20.00, 25.00 and 30.66) respectively at 50, 100 DAT and at harvest as compared to control plants (37.33, 79.00 and 88.33) at 50, 100 DAT and at harvest respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a slight, but significant reduction in plant height at 50, 100 DAT and at harvest (29.00, 66.66 and 80.66) in comparison to control (37.33, 79.00 and 88.33) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant reduction in plant height was recorded in PRD+PBZ treated plants @ 2.5 ppm (14.00, 19.33 and 26.00) at 50, 100 DAT and at harvest respectively followed by PRD+PBZ @ 2ppm (15.33, 22.33 and 27.66), PRD+PBZ @ 1.5 ppm (19.66, 25.33 and 30.66) and 1.0 ppm (24.66, 28.66 and 33.66) at 50, 100 DAT and at harvest respectively in comparison to control (37.33, 79.00 and 88.33) and PRD treated plants (29.00, 66.66 and 80.66).

4.1.2 Number of leaves:

A marked decrease in number of leaves was noticed when the plants treated with Paclobutrazol (PBZ) @ 2.5 ppm (28.00, 36.00 and 46.66) followed by PBZ @ 2ppm (30.66, 39.00 and 50.66) at 50, 100 DAT and at harvest respectively. As evident from Table 3, PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly reduced number of leaves (32.66, 42.00 and 54.00) and (34.66, 44.33 and 56.66) respectively at 50, 100 DAT and at harvest as compared to control plants (55.66, 105.00 and 118.66) at 50, 100 DAT and at harvest respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a slight, but significant reduction in number of leaves at 50, 100 DAT and at harvest (48.33, 88.66 and 101.30) in comparison to control (55.66, 105.00 and 118.66) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant reduction in number of leaves was recorded in PRD+PBZ treated plants @ 2.5 ppm (26.33, 34.33 and 49.66) at 50, 100 DAT and at harvest respectively followed by PRD+PBZ @ 2ppm (29.33, 38.66 and 52.33), PRD+PBZ @ 1.5 ppm (32.33, 42.66 and 55.33) and 1.0 ppm (35.66, 45.00 and 58.00) at 50, 100 DAT and at harvest respectively in comparison to control (55.66, 105.00 and 118.66) and PRD treated plants (48.33, 88.66 and 101.30).

4.1.3 Leaf Area (cm^2):

Data presented in Table 4 showed a significant reduction in leaf area in Paclobutrazol (PBZ) treated plants @ 2.5 ppm (45.44, 75.35 and 139.55 cm^2) followed by PBZ @ 2ppm (65.33, 102.33 and 175.00) at 50, 100 DAT and at harvest respectively. PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly reduced leaf area (80.44, 124.11 and 220.66) and (94.87, 143.22 and 247.33) respectively at 50, 100 DAT and at harvest as compared to control plants (365.80, 759.53 and 991.60) at 50, 100 DAT and at harvest respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a slight, but significant reduction in leaf area at 50, 100 DAT and at harvest (247.26, 510.88 and 755.53) in comparison to control (365.80, 759.53 and 991.60) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant reduction in leaf area was recorded in PRD+PBZ treated plants @ 2.5 ppm (55.88, 99.72 and 199.33) at 50, 100 DAT and at harvest respectively followed by PRD+PBZ @ 2ppm (82.94, 130.05 and 262.22), PRD+PBZ @ 1.5 ppm (104.60, 157.07 and 306.44) and 1.0 ppm (125.20, 179.10 and 353.05) at 50, 100 DAT and at harvest respectively in comparison to control (365.80, 759.53 and 991.60) and PRD treated plants (247.26, 510.88 and 755.53).

Table 2: Effect of physical and biochemical approaches on plant height (cm) at various growth stages in tomato (Pusa Ruby)

Plant height (cm)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1-Control	37.33	79.00	88.33
T2-PBZ (1ppm)	20.00	25.00	30.66
T3-PBZ (1.5ppm)	17.66	22.33	26.00
T4-PBZ (2ppm)	14.33	19.66	23.66
T5-PBZ (2.5 ppm)	12.00	18.00	20.00
T6-PRD	29.00	66.66	80.66
T7-PRD+PBZ(1ppm)	24.66	28.66	33.66
T8-PRD+PBZ(1.5ppm)	19.66	25.33	30.66
T9-PRD+PBZ(2ppm)	15.33	22.33	27.66
T10-PRD+PBZ(2.5ppm)	14.00	19.33	26.00
CD at 5%	*5.34*	*5.09*	*3.97*
SE (m)	*1.79*	*1.71*	*1.33*

Table 3: Effect of physical and biochemical approaches on Number of leaves at various growth stages in tomato (Pusa Ruby)

Number of leaves			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1-Control	55.66	105.00	118.66
T2-PBZ (1ppm)	34.66	44.33	56.66
T3-PBZ (1.5ppm)	32.66	42.00	54.00
T4-PBZ (2ppm)	30.66	39.00	50.66
T5-PBZ (2.5ppm)	28.00	36.00	46.66
T6-PRD	48.33	88.66	101.30
T7-PRD+PBZ(1ppm)	35.66	45.00	58.00
T8-PRD+PBZ(1.5ppm)	32.33	42.66	55.33
T9-PRD+PBZ(2ppm)	29.33	38.66	52.33
T10-PRD+PBZ(2.5ppm)	26.33	34.33	49.66
CD at 5 %	*4.66*	*6.41*	*9.32*
SE (m)	*1.57*	*2.15*	*3.13*

Table 4: Effect of physical and biochemical approaches on Leaf Area (cm^2) at various growth stages in tomato (Pusa Ruby)

Leaf Area (cm^2)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1-Control	365.80	759.53	991.60
T2-PBZ (1ppm)	94.87	143.22	247.33
T3-PBZ (1.5ppm)	80.44	124.11	220.66
T4-PBZ (2ppm)	65.33	102.33	175.00
T5-PBZ (2.5ppm)	45.44	75.35	139.55
T6-PRD	247.26	510.88	755.53
T7-PRD+PBZ (1ppm)	125.20	179.10	353.05
T8-PRD+PBZ (1.5ppm)	104.60	157.07	306.44
T9-PRD+PBZ (2ppm)	82.94	130.05	262.22
T10-PRD+PBZ (2.5ppm)	55.88	99.72	199.33
CD at 5%	*46.25*	*46.62*	*59.05*
SE (m)	*15.56*	*15.69*	*19.87*

Photo plate 4: Effect of Paclobutrazol (PBZ) treatment on Plant height in Tomato

Left: Control , Right: PBZ (1.0, 1.5, 2.0 and 2.5ppm)

Photo plate 5: Effect of PRD technique and PRD+(1.5, 2.0 and 2.5ppm) PBZ on plant height in Tomato

Left: Control, Right: PRD and PRD+ (1.5, 2.0 and 2.5ppm) PBZ

Photo plate 6: Visual comparison of leaf morphology of tomato plant leaf treated with PBZ and the untreated control

4.1.4 Stem thickness (cm):

Stem thickness was significantly increased in plants treated with Paclobutrazol (PBZ) treated plants @ 2.5 ppm (1.200, 1.533 and 1.600 cm) followed by PBZ @ 2ppm (1.067, 1.467 and 1.533) at 50, 100 DAT and at harvest respectively (Table 5). PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly increased stem thickness (0.867, 1.133 and 1.433) and (0.733, 0.900 and 1.267) respectively at 50, 100 DAT and at harvest as compared to control plants (0.467, 0.667 and 0.800) at 50, 100 DAT and at harvest respectively. When the plants were subjected to Partial Root Drying (PRD) technique, stem thickness was slightly but significantly increased at 50, 100 DAT and at harvest (0.433, 0.633 and 0.800) in comparison to control (0.467, 0.667 and 0.800) respectively. In various combination of physical and biochemical technique viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, stem thickness was significantly increased in PRD+PBZ treated plants @ 2.5 ppm (1.267, 1.467 and 1.733) at 50, 100 DAT and at harvest respectively followed by PRD+PBZ @ 2ppm (1.133, 1.267 and 1.700), PRD+PBZ @ 1.5 ppm (0.867, 0.967 and 1.400) and 1.0 ppm (0.733, 0.933 and 1.267) at 50, 100 DAT and at harvest respectively in comparison to control (0.467, 0.667 and 0.800) and PRD treated plants (0.433, 0.633 and 0.800).

4.1.5 Relative water content (RWC %):

The Relative water content (RWC) was markedly increased in plants treated with paclobutrazol @ 2.5 ppm and 2.0 ppm (86.62 and 84.93 %) whereas non significantly increased @ 1.0 ppm and 1.5 ppm (81.12 and 83.20) respectively at 50 DAT in comparison to control (80.50). As evident from Table 6, PBZ when applied @ 1.0 ppm, 1.5 ppm, 2.0 ppm and 2.5 ppm, the RWC was significantly increased (79.32, 80.79, 82.88 and 84.07) and (77.01, 79.00, 80.72 and 82.20) respectively at 100 DAT and at harvest in comparison to control (72.58 and 71.23) respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a slight, but significant reduction in RWC at 50, 100 DAT and at harvest (64.40, 63.20 and 62.38) in comparison to control (80.50, 72.58 and 71.23) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, the RWC was significantly increased (79.51, 81.58, 82.54 and 84.18) at 50 DAT, (76.72, 78.10, 78.99 and 81.31) at 100 DAT and (75.07, 76.78, 77.60 and 79.67) at harvest respectively in comparison to PRD alone (64.40, 63.20 and 62.38), respectively. In comparison to control, RWC was slightly but non significantly increased in PRD+PBZ @ 1.0, 1.5, 2.0 and 2.5 ppm at 50 DAT but significantly increased at 100 DAT and at harvest.

4.1.6 Total phenols (mg g^{-1} DW):

A significant reduction in total phenols in tomato leaves was recorded in Paclobutrazol (PBZ) treated plants @ 2.5 ppm (2.130, 2.310 and 2.657 mg g^{-1} dry wt.) followed by PBZ @ 2ppm (2.277, 2.573 and 2.867) respectively at 50, 100 DAT and at harvest. As evident from Table 7, PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly reduced total phenols in leaves (2.343, 2.747 and 3.040) and (2.487, 2.977 and 3.207) respectively at 50, 100 DAT and at harvest as compared to control plants (3.007, 3.547 and 3.827) at 50, 100 DAT and at harvest, respectively. When the plants were subjected to Partial Root Drying (PRD) technique, total phenols was significantly increased at 50, 100 DAT and at harvest (3.990, 4.247 and 4.460) in comparison to control (3.007, 3.547 and 3.827) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant reduction in total phenols was recorded in PRD+PBZ treated plants @ 2.5 ppm (2.143, 2.767 and 2.887) at 50, 100 DAT and at harvest respectively followed by PRD+PBZ @ 2ppm (2.377, 3.003 and 3.203), PRD+PBZ @ 1.5 ppm (2.683, 3.263 and 3.463) and non significant reduction was found in PRD+PBZ @ 1.0 ppm (2.817, 3.477 and 3.740) at 50, 100 DAT and at harvest respectively in comparison to control (3.007, 3.547 and 3.827) but a significant reduction was found in comparison to PRD treated plants (3.990, 4.247 and 4.460).

4.1.7 Soluble sugar (mg g^{-1} DW):

A marked increased in soluble sugar was observed when PBZ applied @ 2.5 ppm (3.773, 3.847 and 3.903 mg g^{-1} dry wt.) followed by PBZ @ 2ppm (3.720, 3.813 and 3.853) respectively at 50, 100 DAT and at harvest. As evident from Table 8, PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly increased soluble sugar (3.657, 3.763 and 3.823) and (3.507, 3.707 and 3.767) respectively at 50, 100 DAT and at harvest as compared to control plants (2.337, 2.900 and 2.930) at 50, 100 DAT and at harvest respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a significant increased in soluble sugar at 50, 100 DAT and at harvest (3.230, 3.560 and 3.617) in comparison to control (2.337, 2.900 and 2.930) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant increased in soluble sugar was recorded in PRD+PBZ treated plants @ 2.5 ppm (3.850, 3.943 and 4.080) at 50, 100 DAT and at harvest respectively followed by PRD+PBZ @ 2ppm (3.790, 3.920 and 3.960), PRD+PBZ @ 1.5 ppm (3.757, 3.863 and 3.923) and 1.0 ppm (3.690, 3.847 and 3.883) at 50, 100 DAT and at harvest respectively in comparison to control (2.337, 2.900 and 2.930) and PRD treated plants (3.230, 3.560 and 3.617).

Table 5: Effect of physical and biochemical approaches on Stem thickness (cm) at various growth stages in tomato (Pusa Ruby)

Stem thickness (cm)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1- Control	0.467	0.667	0.800
T2-PBZ (1ppm)	0.733	0.900	1.267
T3-PBZ (1.5ppm)	0.867	1.133	1.433
T4-PBZ (2ppm)	1.067	1.467	1.533
T5-PBZ (2.5ppm)	1.200	1.533	1.600
T6-PRD	0.433	0.633	0.800
T7-PRD+PBZ (1ppm)	0.733	0.933	1.267
T8-PRD+PBZ (1.5ppm)	0.867	0.967	1.400
T9-PRD+PBZ (2ppm)	1.133	1.267	1.700
T10-PRD+PBZ (2.5ppm)	1.267	1.467	1.733
CD at 5 %	*0.180*	*0.188*	*0.295*
SE (m)	*0.061*	*0.063*	*0.099*

Table 6: Effect of physical and biochemical approaches on Relative water content (%) at various growth stages in tomato (Pusa Ruby)

Relative water content (%)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1- Control	80.50	72.58	71.23
T2-PBZ (1ppm)	81.12	79.32	77.01
T3-PBZ (1.5ppm)	83.20	80.79	79.00
T4-PBZ (2ppm)	84.93	82.88	80.72
T5-PBZ (2.5ppm)	86.62	84.07	82.20
T6- PRD	64.40	63.20	62.38
T7-PRD+PBZ (1ppm)	79.51	76.72	75.07
T8-PRD+PBZ (1.5ppm)	81.58	78.10	76.78
T9-PRD+PBZ (2ppm)	82.54	78.99	77.60
T10-PRD+PBZ (2.5ppm)	84.18	81.31	79.67
CD at 5 %	*4.34*	*3.90*	*4.11*
SE (m)	*1.46*	*1.31*	*1.38*

Table 7: Effect of physical and biochemical approaches on total phenols (mg g^{-1} DW) at various growth stages in tomato (Pusa Ruby)

Total phenols (mg g^{-1} DW)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1-Control	3.007	3.547	3.827
T2-PBZ (1ppm)	2.487	2.977	3.207
T3-PBZ (1.5ppm)	2.343	2.747	3.040
T4-PBZ (2ppm)	2.277	2.573	2.867
T5-PBZ (2.5ppm)	2.130	2.310	2.657
T6-PRD	3.990	4.247	4.460
T7-PRD+PBZ (1ppm)	2.817	3.477	3.740
T8-PRD+PBZ (1.5ppm)	2.683	3.263	3.463
T9-PRD+PBZ (2ppm)	2.377	3.003	3.203
T10-PRD+PBZ (2.5ppm)	2.143	2.767	2.887
CD at 5%	*0.310*	*0.204*	*0.310*
SE (m)	*0.105*	*0.069*	*0.104*

Table 8: Effect of physical and biochemical approaches on total soluble sugar (mg g^{-1} DW) at various growth stages in tomato (Pusa Ruby)

Total soluble sugar (mg g^{-1} DW)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1- Control	2.337	2.900	2.930
T2-PBZ (1ppm)	3.507	3.707	3.767
T3-PBZ (1.5ppm)	3.657	3.763	3.823
T4-PBZ (2ppm)	3.720	3.813	3.853
T5-PBZ (2.5ppm)	3.773	3.847	3.903
T6-PRD	3.230	3.560	3.617
T7-PRD+PBZ (1ppm)	3.690	3.847	3.883
T8-PRD+PBZ (1.5ppm)	3.757	3.863	3.923
T9-PRD+PBZ (2ppm)	3.790	3.920	3.960
T10-PRD+PBZ (2.5ppm)	3.840	3.943	4.080
CD at 5%	*0.193*	*0.108*	*0.111*
SE (m)	*0.065*	*0.036*	*0.037*

4.1.8 Chlorophyll *a* (mg g^{-1} FW):

A marked increased in chlorophyll *a* was observed when PBZ applied @ 1.0, 1.5, 2.0 and 2.5 ppm (2.700, 2.887, 3.040 and 3.430 mg g^{-1}) respectively at 50 DAT (Table 9). PBZ when applied @ 1.0, 1.5, 2.0 and 2.5 ppm alone, also greatly increased chlorophyll *a* (2.433, 2.513, 2.587 and 2.647) and (2.293, 2.373, 2.490 and 2.563) respectively at 100 DAT and at harvest as compared to control plants (2.287, 2.213 and 2.177) at 50, 100 DAT and at harvest respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a slight but non significant increase in chlorophyll *a* at 50, 100 DAT and at harvest (2.323, 2.180 and 2.127) in comparison to control (2.287, 2.213 and 2.177), respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant increased in chlorophyll *a* (2.713, 2.833, 3.010 and 3.112) respectively at 50 DAT. In PRD+PBZ @ 1.0 and 1.5 ppm, there was a non significant increase was observed in chlorophyll *a* (2.147 and 2.230) and (2.145 and 2.228), respectively at 100 DAT and at harvest in comparison to control (2.213 and 2.177) and PRD treated plants (2.180 and 2.127), respectively. However, in PRD+PBZ @ 2.0 and 2.5 ppm, the chlorophyll *a* was significantly increased (2.290 and 2.360) and (2.283 and 2.327) respectively at 100 DAT and at harvest in comparison to control (2.213 and 2.177) and PRD treated plants (2.180 and 2.127).

4.1.9 Chlorophyll *b* (mg g^{-1} FW):

Chlorophyll *b* was significantly increased in Paclobutrazol (PBZ) treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm (1.437, 1.480, 1.550 and 1.637 mg g^{-1}) respectively at 50 DAT. As evident from Table 10, PBZ when applied @ 1.0, 1.5, 2.0 and 2.5 ppm alone, also greatly increased Chlorophyll *b* (1.277, 1.363, 1.413 and 1.460) and (1.147, 1.257, 1.283 and 1.327) respectively at 100 DAT and at harvest as compared to control plants (0.713, 0.543 and 0.503) at 50, 100 DAT and at harvest respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a slight but non significant increase was found at 50, 100 DAT and at harvest (0.687, 0.557 and 0.510) in comparison to control (0.713, 0.543 and 0.503) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant increase in Chlorophyll *b* was recorded (1.427, 1.477, 1.510 and 1.543) at 50 DAT, (1.280, 1.317, 1.347 and 1.377) at 100 DAT and (1.183, 1.210, 1.257 and 1.290) at harvest respectively in comparison to control (0.713, 0.543 and 0.503) and PRD treated plants (0.687, 0.557 and 0.510)

4.1.10 Total chlorophyll (SPAD value):

Total chlorophyll content was significantly increased when PBZ applied @ 1.0, 1.5, 2.0 and 2.5 ppm (43.10, 45.40, 46.96 and 49.50) respectively at 50 DAT in comparison to control (29.60). As evident from Table 11, PBZ when applied @ 1.0, 1.5, 2.0 and 2.5 ppm alone, the total chlorophyll in leaf was non significant at 100 DAT and at harvest (29.96, 34.03, 37.20 and 40.90) and (27.36, 29.50, 30.63 and 31.86), respectively in comparison to control (26.86 and 22.16), respectively. When the plants were subjected to Partial Root Drying (PRD) technique, there was a non significant increased was found at 50, 100 DAT and at harvest (30.46, 28.73 and 23.23) in comparison to control (29.60, 26.86 and 22.16) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, the total chlorophyll was significantly increased (45.70, 47.30, 48.16 and 49.03) respectively at 50 DAT. Total chlorophyll was found non significant @ 1.0, 1.5, 2.0 and 2.5 ppm (30.63, 33.90, 34.50 and 36.46) and (26.10, 27.63, 29.23 and 30.36) respectively at 100 DAT and at harvest in comparison to control (29.60, 26.86 and 22.16) and PRD treated plants (30.46, 28.73 and 23.23).

4.1.11 Total carotenoids (mg g^{-1} FW):

Total carotenoids was significantly increased in Paclobutrazol (PBZ) treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm (0.543, 0.567, 0.587 and 0.623 mg g^{-1}) respectively at 50 DAT (Table 12). PBZ when applied @ 1.0, 1.5, 2.0 and 2.5 ppm alone, also greatly increased total carotenoids (0.517, 0.530, 0.553 and 0.577) and (0.360, 0.390, 0.427 and 0.460) respectively at 100 DAT and at harvest as compared to control plants (0.247, 0.210 and 0.183) respectively at 50, 100 DAT and at harvest. When the plants were subjected to Partial Root Drying (PRD) technique, there was a non significant increased was found at 50, 100 DAT and at harvest (0.270, 0.247 and 0.217) in comparison to control (0.247, 0.210 and 0.183) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant increased in total carotenoids was recorded (0.587, 0.617, 0.633 and 0.667) at 50 DAT, (0.513, 0.533, 0.563 and 0.590) at 100 DAT and (0.367, 0.383, 0.403 and 0.433) at harvest respectively in comparison to control (0.247, 0.210 and 0.183) and PRD treated plants (0.270, 0.247 and 0.217).

4.1.12 Proline content (mg g^{-1} FW):

A significant reduction in Proline content was recorded in Paclobutrazol (PBZ) treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm (0.593, 0.573, 0.560 and 0.537 mg g^{-1}) respectively at 50 DAT. As evident from Table 13, PBZ when applied @ 1.0, 1.5, 2.0 and 2.5 ppm alone, also greatly decreased Proline content (0.580, 0.527, 0.483 and 0.473) and (0.653, 0.573, 0.490 and 0.453) respectively at 100 DAT and at harvest as compared to control plants (0.890, 0.973 and 0.957) respectively at 50, 100 DAT and at harvest. When the plants were subjected to Partial Root Drying (PRD) technique, there was a significant decreased was

Table 9: Effect of physical and biochemical approaches on Chlorophyll *a* (mg g^{-1} FW) at various growth stages in tomato (Pusa Ruby)

Chlorophyll *a* (mg g^{-1} FW)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1- Control	2.287	2.213	2.177
T2-PBZ (1ppm)	2.700	2.433	2.293
T3-PBZ (1.5ppm)	2.887	2.513	2.373
T4-PBZ (2ppm)	3.040	2.587	2.490
T5-PBZ (2.5ppm)	3.430	2.647	2.563
T6-PRD	2.323	2.180	2.127
T7-PRD+PBZ (1ppm)	2.713	2.147	2.145
T8-PRD+PBZ (1.5ppm)	2.833	2.230	2.228
T9-PRD+PBZ (2ppm)	3.010	2.290	2.283
T10-PRD+PBZ (2.5ppm)	3.112	2.360	2.327
CD at 5%	*0.199*	*0.088*	*0.109*
SE (m)	*0.067*	*0.029*	*0.037*

Table 10: Effect of physical and biochemical approaches on Chlorophyll *b* (mg g^{-1} FW) at various growth stages in tomato (Pusa Ruby)

Chlorophyll *b* (mg g^{-1} FW)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1- Control	0.713	0.543	0.503
T2-PBZ (1ppm)	1.437	1.277	1.147
T3-PBZ (1.5ppm)	1.480	1.363	1.257
T4-PBZ (2ppm)	1.550	1.413	1.283
T5-PBZ (2.5ppm)	1.637	1.460	1.327
T6-PRD	0.687	0.557	0.510
T7-PRD+PBZ (1ppm)	1.427	1.280	1.183
T8-PRD+PBZ (1.5ppm)	1.477	1.317	1.210
T9-PRD+PBZ (2ppm)	1.510	1.347	1.257
T10-PRD+PBZ (2.5ppm)	1.543	1.377	1.290
CD at 5%	*0.079*	*0.089*	*0.066*
SE (m)	*0.027*	*0.030*	*0.022*

Table 11: Effect of physical and biochemical approaches on Total chlorophyll (SPAD value) at various growth stages in tomato (Pusa Ruby)

Total chlorophyll (SPAD value)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1-Control	29.60	26.86	22.16
T2-PBZ (1ppm)	43.10	29.96	27.36
T3-PBZ (1.5ppm)	45.40	34.03	29.50
T4-PBZ (2ppm)	46.96	37.20	30.63
T5-PBZ (2.5ppm)	49.50	40.90	31.86
T6-PRD	30.46	28.73	23.23
T7-PRD+PBZ(1ppm)	45.70	30.63	26.10
T8-PRD+PBZ(1.5ppm)	47.30	33.90	27.63
T9-PRD+PBZ(2ppm)	48.16	34.50	29.23
T10-PRD+PBZ(2.5ppm)	49.03	36.46	30.36
CD at 5%	*4.65*	*N.S*	*N.S*
SE (m)	*1.56*	*3.02*	*2.38*

Table 12: Effect of physical and biochemical approaches on total carotenoids (mg g^{-1} FW) at various growth stages in tomato (Pusa Ruby)

Total carotenoids (mg g^{-1} FW)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1-Control	0.247	0.210	0.183
T2-PBZ (1ppm)	0.543	0.517	0.360
T3-PBZ (1.5ppm)	0.567	0.530	0.390
T4-PBZ (2ppm)	0.587	0.553	0.427
T5-PBZ (2.5ppm)	0.623	0.577	0.460
T6-PRD	0.270	0.247	0.217
T7-PRD+PBZ(1ppm)	0.587	0.513	0.367
T8-PRD+PBZ(1.5ppm)	0.617	0.533	0.383
T9-PRD+PBZ(2ppm)	0.633	0.563	0.403
T10-PRD+PBZ(2.5ppm)	0.667	0.590	0.433
CD at 5%	*0.044*	*0.044*	*0.057*
SE (m)	*0.015*	*0.015*	*0.019*

Table 13: Effect of physical and biochemical approaches on Proline content (mg g^{-1} FW) at various growth stages in tomato (Pusa Ruby)

Proline content (mg g^{-1} FW)			
Days after transplanting			
Treatments	50DAT	100DAT	At harvest
T1-Control	0.890	0.973	0.957
T2-PBZ (1ppm)	0.593	0.580	0.653
T3-PBZ (1.5ppm)	0.573	0.527	0.573
T4-PBZ (2ppm)	0.560	0.483	0.490
T5-PBZ (2.5ppm)	0.537	0.473	0.453
T6-PRD	1.503	1.107	1.147
T7-PRD+PBZ(1ppm)	0.627	0.690	0.757
T8-PRD+PBZ(1.5ppm)	0.577	0.593	0.703
T9-PRD+PBZ(2ppm)	0.560	0.510	0.663
T10-PRD+PBZ(2.5ppm)	0.527	0.493	0.610
CD at 5%	*0.065*	*0.010*	*0.014*
SE (m)	*0.022*	*0.003*	*0.005*

found at 50, 100 DAT and at harvest (1.503, 1.107 and 1.147) in comparison to control (0.890, 0.973 and 0.957) respectively. In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, there was a significant decrease in proline content was recorded (0.627, 0.577, 0.560 and 0.527) at 50 DAT, (0.690, 0.593, 0.510 and 0.493) at 100 DAT and (0.757, 0.703, 0.663 and 0.610) at harvest, respectively in comparison to control (0.890, 0.973 and 0.957) and PRD treated plants (1.503, 1.107 and 1.147).

4.1.13 Fresh weight of root (g) :

Fresh weight of root was significantly decreased when PBZ applied @ 1.0, 1.5, 2.0 and 2.5 ppm (5.31, 5.26, 5.21 and 5.13 g) respectively at harvest in comparison to control (6.37) (Table 14). When the plants were subjected to Partial Root Drying (PRD) technique, the fresh weight of root was found non significant (6.45) as compared to control (6.37). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, the fresh weight of root was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (5.34, 5.29, 5.25 and 5.20) respectively at harvest in comparison to control (6.37) and PRD treated plants (6.45).

4.1.14 Dry wt. of root (g) :

Dry weight of root in Paclobutrazol (PBZ) treatment was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (3.84, 3.77, 3.73 and 3.68 g) respectively at harvest in comparison to control (4.06). As evident from Table 14, when plants were subjected to Partial Root Drying (PRD) technique, dry weight of root was noticed non significant (4.10) as compared to control (4.06). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, dry weight of root was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (3.87, 3.81, 3.74 and 3.68) respectively at harvest in comparison to control (4.06) and PRD treated plants (4.10).

4.1.15 Fresh wt. of shoot (g) :

Similarly, fresh weight of shoot in Paclobutrazol (PBZ) treatment was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (43.74, 43.55, 43.40 and 43.21 g) respectively at harvest in comparison to control (52.91). As evident from Table 14, plants were subjected to Partial Root Drying (PRD) technique, fresh weight of shoot was found non significant (52.87) as compared to control (52.91). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, fresh weight of root was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (43.72, 43.52, 43.38 and 43.18) respectively at harvest in comparison to control (52.91) and PRD treated plants (52.87).

4.1.16 Dry wt. of shoot (g) :

Dry weight of shoot (Table 14) was significantly decreased when plants treated with PBZ @ 1.0, 1.5, 2.0 and 2.5 ppm (22.91, 22.89, 22.86 and 22.84 g) respectively at harvest in comparison to control (28.97). In Partial Root Drying (PRD) technique, fresh weight of root was found non significant (28.92) as compared to control (28.97). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, fresh weight of root was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (22.89, 22.85, 22.82 and 22.78) respectively at harvest in comparison to control (28.97) and PRD treated plants (28.92).

4.1.17 Pericarp thickness (cm) :

Pericarp thickness of tomato fruit was slightly increased in PBZ treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm (0.63, 0.66, 0.70 and 0.73 cm) respectively at harvest in comparison to control (0.56) but they are statistically non significant (Table 15). When the plants were subjected to Partial Root Drying (PRD) technique, pericarp thickness was found non significant (0.56) as compared to control (0.56). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, pericarp thickness was slightly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (0.63, 0.70, 0.73 and 0.76) respectively at harvest in comparison to control (0.56) and PRD treated plants (0.56) and they are statistically non significant.

4.1.18 Fruit diameter (cm) :

Fruit diameter (Table 15) was significantly decreased in PBZ treated plants @ 2.5 ppm (3.82 cm) in comparison to control (4.43). PBZ when applied @ 1.0, 1.5 and 2.0 ppm alone, fruit diameter was found non significant. When the plants were subjected to Partial Root Drying (PRD) technique, fruit diameter was found non significant (4.73) as compared to control (4.43). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, fruit diameter was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (3.86, 3.83, 3.76 and 3.60) respectively at harvest in comparison to control (4.43) and PRD treated plants (4.73).

4.1.19 Yield per plant (Kg) :

Tomato yield per plant was significantly increased in Paclobutrazol (PBZ) treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm (2.44, 2.51, 2.62 and 2.67 Kg) respectively at harvest in comparison to control (1.63). As evident from Table 15, When the plants were subjected to Partial Root Drying (PRD) technique, the yield per plant was found non significant (1.57) as compared to control (1.63). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, the yield per plant was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (2.33, 2.48, 2.54 and 2.56) respectively at harvest in comparison to control (1.63) and PRD treated plants (1.57).

4.1.20 Water Use Efficiency (Kg L^{-1}) :

A marked increase in water use efficiency was noticed when plants were subjected to PBZ @ 1.0, 1.5, 2.0 and 2.5 ppm (1.380, 1.423, 1.480 and 1.510 Kg L^{-1}) respectively at

Table 14: Effect of physical and biochemical approaches on fresh weight of root (g), fresh wt. of shoot (g), dry wt. of root (g) and dry wt. of shoot (g) at harvest on tomato (Pusa Ruby)

Treatments	**Fresh wt. of root(g)**	**Dry wt. of root(g)**	**Fresh wt. of shoot (g)**	**Dry wt. of shoot(g)**
T1-Control	6.37	4.06	52.91	28.97
T2-PBZ (1ppm)	5.31	3.84	43.74	22.91
T3-PBZ (1.5ppm)	5.26	3.77	43.55	22.89
T4-PBZ (2ppm)	5.21	3.73	43.40	22.86
T5-PBZ (2.5ppm)	5.13	3.68	43.21	22.84
T6-PRD	6.45	4.10	52.87	28.92
T7-PRD+PBZ(1ppm)	5.34	3.87	43.72	22.89
T8-PRD+PBZ(1.5ppm)	5.29	3.81	43.52	22.85
T9-PRD+PBZ(2ppm)	5.25	3.74	43.38	22.82
T10-PRD+PBZ(2.5ppm)	5.20	3.68	43.18	22.78
CD at 5%	*0.11*	*0.11*	*0.31*	*0.32*
SE (m)	*0.04*	*0.03*	*0.10*	*0.10*

Table 15: Effect of physical and biochemical approaches on Pericarp thickness (cm), Fruit diameter (cm), Yield/plant (Kg) and WUE (Kg L^{-1}) at harvest on tomato variety (Pusa Ruby)

Treatments	**Pericarp thickness (cm)**	**Fruit diameter (cm)**	**Yield/plant (Kg)**	**Water use efficiency (WUE: kg L^{-1})**
T1-Control	0.56	4.43	1.63	0.320
T2-PBZ (1ppm)	0.63	4.16	2.44	1.380
T3-PBZ (1.5ppm)	0.66	4.13	2.51	1.423
T4-PBZ (2ppm)	0.70	3.93	2.62	1.480
T5-PBZ (2.5ppm)	0.73	3.82	2.67	1.510
T6-PRD	0.56	4.73	1.57	0.507
T7-PRD+PBZ (1ppm)	0.63	3.86	2.33	1.313
T8-PRD+PBZ (1.5ppm)	0.70	3.83	2.48	1.410
T9-PRD+PBZ (2ppm)	0.73	3.76	2.54	1.440
T10-PRD+PBZ (2.5ppm)	0.76	3.60	2.56	1.453
CD at 5%	*NS*	*0.56*	*0.07*	*0.039*
SE(m)	*0.05*	*0.18*	*0.02*	*0.013*

Photo plate 7: Effect of PBZ treatment on fruiting in Tomato

Left: Control , Right: PBZ (1.0, 1.5, 2.0 and 2.5ppm)

Photo plate 8: Effect of PRD technique and PRD+PBZ on fruiting in Tomato

Left: Control, Right: PRD and PRD+ (1.0, 1.5, 2.0 and 2.5ppm) PBZ

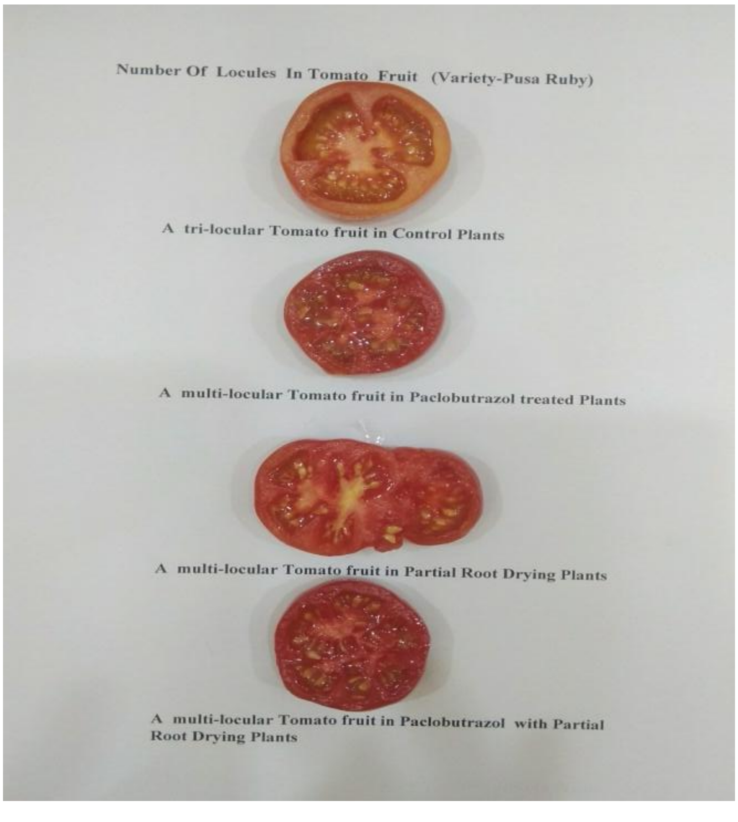


Photo plate 9: Effect of PBZ, PRD and PRD + PBZ on quality of fruits and their locules in tomato harvest in comparison to control (0.320). Whereas in Partial Root Drying (PRD) technique, the water use efficiency was significantly increased (0.507) as compared to control (0.320) (Table 15). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, the water use efficiency was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (1.313, 1.410, 1.440 and 1.453) respectively at harvest in comparison to control (0.320) and PRD treated plants (0.507).

4.1.21 Fruit volume (cm^{-3}) :

Fruit volume of tomato was significantly increased in Paclobutrazol (PBZ) treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm at harvest (16.39, 16.77, 17.22 and 17.82 cm^{-3}) respectively in comparison to control (14.36). As evident from Table 16, when the plants were subjected to Partial Root Drying (PRD) technique, fruit volume was found non significant (14.28) as compared to control (14.36). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, fruit volume was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (15.36, 16.03, 16.87 and 17.47) respectively at harvest in comparison to control (14.36) and PRD treated plants (14.28).

4.1.22 Fruit density (g cm^{-3}) :

A marked increase in fruit density of tomato fruit was noticed in PBZ treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm (1.190, 1.223, 1.263 and 1.300 g cm^{-3}) respectively at harvest in comparison to control (1.020) (Table 16). Whereas in Partial Root Drying (PRD) technique, fruit density was found non significant (0.987) as compared to control (1.020). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, fruit density was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (1.157, 1.190, 1.203 and 1.230) respectively at harvest in comparison to control (1.020) and PRD treated plants (0.987).

4.1.23 Fresh wt. of fruit (g) :

Fresh wt. of tomato fruit (Table 16) was significantly increased in PBZ treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm (18.93, 19.03, 19.21 and 19.26 g) respectively at harvest in comparison to control (17.04). When the plants were subjected to Partial Root Drying (PRD) technique, fresh wt. of fruit was found non significant (17.02) as compared to control (17.04). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, fresh wt. of fruit was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (18.96, 19.11, 19.21 and 19.32) respectively at harvest in comparison to control (17.04) and PRD treated plants (17.02).

4.1.24 Dry wt. of fruit (g) :

Dry wt. of tomato fruit in PBZ treatment was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (7.95, 8.13, 8.24 and 8.33 g) respectively at harvest in comparison to control

(7.24). As evident from Table 16, when the plants were subjected to Partial Root Drying (PRD) technique, dry wt. of fruit was found non significant (7.22) as compared to control (7.24). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, dry wt. of fruit was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (7.98, 8.13, 8.19 and 8.24) respectively at harvest in comparison to control (7.24) and PRD treated plants (7.22).

4.1.25 Partitioning coefficient of root (%) :

Partitioning coefficient of root in Paclobutrazol (PBZ) treatment was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (11.76, 11.47, 11.24 and 11.09 %) respectively at harvest in comparison to control (12.57). As evident from Table 17, when the plants were subjected to Partial Root Drying (PRD) technique, the partitioning coefficient of root was found slightly but significantly increased (12.76) as compared to control (12.57). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, the partitioning coefficient of root was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (11.82, 11.59, 11.38 and 11.22) respectively at harvest in comparison to control (12.57) and PRD treated plants (12.76).

4.1.26 Partitioning coefficient of shoot (%):

Partitioning coefficient of shoot (Table 17) in Paclobutrazol (PBZ) treated plants was significantly decreased (85.86, 85.12, 84.22 and 83.92 %) when PBZ applied @ 1.0, 1.5, 2.0 and 2.5 ppm respectively at harvest in comparison to control (87.43). Under Partial Root Drying (PRD) technique, the partitioning coefficient of shoot was found slightly but significantly increased (87.52) as compared to control (87.43). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, the partitioning coefficient of shoot was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (85.42, 84.56, 84.22 and 83.42) respectively at harvest in comparison to control (87.43) and PRD treated plants (87.57).

4.1.27 Lycopene content (mg 100g^{-1} F.W)

Lycopene content of fresh tomato fruit was significantly increased (1.853, 1.947, 2.043 and 2.257 mg 100g^{-1}) in PBZ treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm respectively at harvest in comparison to control (1.677). As evident from Table 17, when the plants were subjected to Partial Root Drying (PRD) technique, lycopene content was found slightly but significantly increased (1.857) as compared to control (1.677). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, lycopene content was significantly increased (1.950, 2.147, 2.173 and 2.203) @ 1.0, 1.5, 2.0 and 2.5 ppm respectively at harvest in comparison to control (1.677) and PRD treated plants (1.857).

4.1.28 Ascorbic acid (mg $100g^{-1}$ F.W)

As evident from Table 17, ascorbic acid content was significantly increased (18.40, 21.35, 27.14 and 31.65 mg $100g^{-1}$) in tomato fruits treated with Paclobutrazol @ 1.0, 1.5, 2.0 and 2.5 ppm respectively at harvest in comparison to control (15.20). When the plants were subjected to Partial Root Drying (PRD) technique, ascorbic acid was found slightly but non significantly increased (17.53) as compared to control (15.20). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, ascorbic acid was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (18.59, 21.52, 27.20 and 32.65) respectively at harvest in comparison to control (15.20). PRD+PBZ at 1.5, 2.0 and 2.5 ppm, ascorbic acid was significantly increased @ 1.5, 2.0 and 2.5 ppm (21.52, 27.20 and 32.65) respectively in comparison to PRD treated plants but in PRD+PBZ @1.0 ppm, the ascorbic acid was slightly but non significantly increased in comparison to PRD treated plants (17.53).

4.1.29 Total number of fruits per plant

Total number of fruits per plant was significantly increased (206.00, 210.66, 215.33 and 219.33) in PBZ treated plants @ 1.0, 1.5, 2.0 and 2.5 ppm respectively at harvest in comparison to control (128.33) (Table 18). When the plants were subjected to Partial Root Drying (PRD) technique, total number of fruits per plant was found non significant (126.33) as compared to control (128.33). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, total number of fruits per plant was significantly increased (205.33, 209.66, 214.66 and 218.33) respectively at harvest in comparison to control (128.33) and PRD treated plants (126.33).

4.1.30 Relative stress injury (%)

Relative stress injury was significantly decreased in Paclobutrazol (PBZ) treatment @ 1.0, 1.5, 2.0 and 2.5 ppm (42.46, 35.66, 32.12 and 26.42 %) respectively at harvest in comparison to control (48.55) (Table 18). When the plants were subjected to Partial Root Drying (PRD) technique, relative stress injury was found significantly increased (56.44) as compared to control (48.55). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, relative stress injury was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (47.52, 41.22, 36.21 and 32.61) respectively at harvest in comparison to control (48.55) and PRD treated plants (56.44).

4.1.31 Specific leaf area (cm^2 g^{-1})

Specific leaf area in Paclobutrazol (PBZ) treatment was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (43.53, 39.90, 33.19 and 32.86 cm^2 g^{-1}) respectively at harvest in comparison to control (157.70). As evident from Table 18, when the plants were subjected to

Partial Root Drying (PRD) technique, specific leaf area was found significantly decreased (124.70) as compared to control (157.70). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, specific leaf area was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (60.16, 56.48, 54.81 and 52.73) respectively at harvest in comparison to control (157.70) and PRD treated plants (124.70).

4.1.32 Relative growth rate (g day^{-1})

Relative growth rate was significantly decreased in Paclobutrazol (PBZ) treatment @ 1.0, 1.5, 2.0 and 2.5 ppm (0.154, 0.150, 0.144 and 0.138 g day^{-1}) respectively at harvest in comparison to control (0.173) (Table 18). When the plants were subjected to Partial Root Drying (PRD) technique, relative growth rate was found non significant (0.167) as compared to control (0.173). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, relative growth rate was significantly decreased @ 1.0, 1.5, 2.0 and 2.5 ppm (0.149, 0.142, 0.136 and 0.131) respectively at harvest in comparison to control (0.173) and PRD treated plants (0.167).

4.1.33 Total carotenoids (mg 100g^{-1} F.W fruit)

Total carotenoids in Paclobutrazol (PBZ) treatment was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (1.293, 1.333, 1.363 and 1.407 mg 100g^{-1}) respectively at harvest in comparison to control (1.267). As evident from Table 19, when the plants were subjected to Partial Root Drying (PRD) technique, total carotenoids was found slightly but significantly increased (1.280) as compared to control (1.267). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, total carotenoids was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (1.307, 1.337, 1.360 and 1.387) respectively at harvest in comparison to control (1.267) and PRD treated plants (1.280).

4.1.34 Number of locules in fruit

Number of locules in Paclobutrazol (PBZ) treatment was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (5.33, 5.33, 6.00 and 6.00) respectively at harvest in comparison to control (3.00) (Table 19). When the plants were subjected to Partial Root Drying (PRD) technique, number of locules in tomato fruit was found significantly increased (6.00) as compared to control (3.00). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, number of locules was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (5.33, 6.00, 6.00 and 6.00) respectively at harvest in comparison to control (3.00) and they are also found significantly increased in PRD+PBZ @ 1.0 ppm (5.33) in comparison to PRD alone but non significant in PRD+PBZ @ 1.5, 2.0 and 2.5 ppm in comparison to PRD treated plants (6.00).

4.1.35 Number of flower trusses per plant

As evident from Table 19, number of flower trusses per plant was significantly increased in Paclobutrazol (PBZ) treatment @ 1.0, 1.5, 2.0 and 2.5 ppm (19.33, 21.00, 22.00 and 24.00) respectively at harvest in comparison to control (11.33). When the plants were subjected to Partial Root Drying (PRD) technique, number of flower trusses per plant was found slightly but non significantly increased (11.66) as compared to control (11.33). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, number of flower trusses per plant was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (19.33, 20.33, 22.33 and 24.00) respectively at harvest in comparison to control (11.33) and PRD treated plants (11.66).

4.1.36 Number of flowers per cluster

Number of flowers per cluster was significantly increased in Paclobutrazol (PBZ) treatment @ 1.0, 1.5, 2.0 and 2.5 ppm (11.66, 12.33, 13.00 and 15.00) respectively at harvest in comparison to control (8.00) (Table 19). When the plants were subjected to Partial Root Drying (PRD) technique, number of flowers per cluster was found non significant (6.66) as compared to control (8.00). In various combination viz, PRD+PBZ at 1.0, 1.5, 2.0 and 2.5 ppm, number of flowers per cluster was significantly increased @ 1.0, 1.5, 2.0 and 2.5 ppm (10.33, 12.00, 13.66 and 14.33) respectively at harvest in comparison to control (8.00) and PRD treated plants (6.66).

4.1.37 Protein profiling of tomato leaves:

Changes in protein profile were studied by electrophoresis on SDS-PAGE to identify the differences in protein band pattern in tomato leaves at harvest under different treatments of Paclobutrazol and Partial Root Drying technique with respect to their control conditions. Photo Plate 10 shows the banding pattern in tomato leaves variety Pusa Ruby.

The protein bands of different molecular weight (MW) with high and low intensities were present. The tomato leaves raised under normal conditions showed protein bands with MW of 75.83 kDa in control and PRD in combination with PBZ @ 2.0 and 2.5 ppm whereas it was found absent in PRD and PBZ alone. In Paclobutrazol treated plants, the new protein band of MW 12.06 kDa, 16.33 kDa and 23.92 kDa were identified. A new protein band of MW 63.13 kDa were observed in PRD treated plants alone and also in combination with PBZ

The protein bands with MW of 55.16 kDa and 47.38 kDa were found almost in all treatments but they were observed more thick and intensed in PBZ alone @ 1.0, 1.5, 2.0 and 2.5 ppm and also in combination with PRD @ 1.0 ppm in comparison to control and

PRD alone. In control plants with water, the protein bands were noticed very few and thin whereas these were appeared more thick and condensed in PBZ treatment.

Table 16: Effect of physical and biochemical approaches on Fruit volume (cm^3), Fruit density (**g cm**$^{-3}$), Fresh wt. of fruit (g) and Dry wt. of fruit (g) at harvest on tomato variety (Pusa Ruby)

Treatments	**Fruit volume(cm^3)**	**Fruit density (g cm^{-3})**	**Fresh wt. of fruit (g)**	**Dry wt. of fruit(g)**
T1-Control	14.36	1.020	17.04	7.24
T2-PBZ (1ppm)	16.39	1.190	18.93	7.95
T3-PBZ (1.5ppm)	16.77	1.223	19.03	8.13
T4-PBZ (2ppm)	17.22	1.263	19.21	8.24
T5-PBZ (2.5ppm)	17.82	1.300	19.26	8.33
T6-PRD	14.28	0.987	17.02	7.22
T7-PRD+PBZ(1ppm)	15.36	1.157	18.96	7.98
T8-PRD+PBZ(1.5ppm)	16.03	1.190	19.11	8.13
T9-PRD+PBZ(2ppm)	16.87	1.203	19.21	8.19
T10-PRD+PBZ(2.5ppm)	17.47	1.230	19.32	8.24
CD at 5%	*1.23*	*0.175*	*0.88*	*0.30*
SE(m)	*0.41*	*0.059*	*0.29*	*0.10*

Table 17: Effect of physical and biochemical approaches on partitioning coefficient (P.C %) of root and shoot, lycopene content (**mg 100g^{-1} FW**) and ascorbic acid (**mg 100g^{-1} FW**) at harvest on tomato variety (Pusa Ruby)

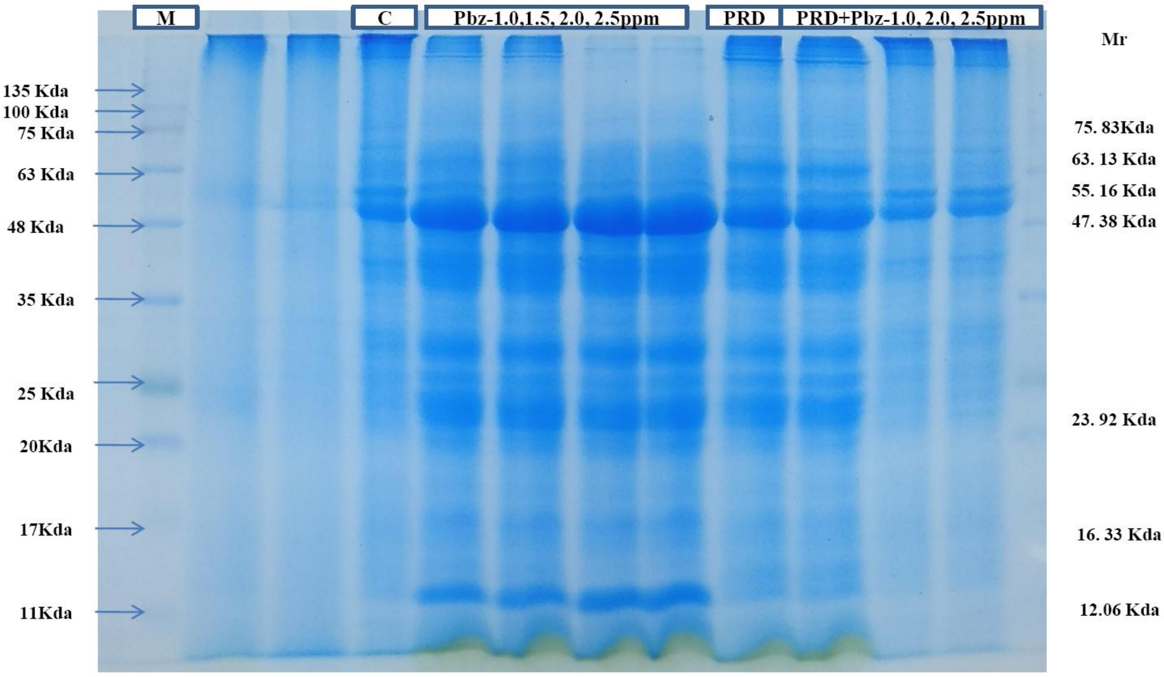
Treatments	**P.C. of root (%)**	**P.C. of shoot (%)**	**Lycopene content (mg 100g^{-1} FW)**	**Ascorbic acid (mg 100g-^{1} FW)**
T1-Control	12.57	87.43	1.677	15.20
T2-PBZ (1ppm)	11.76	85.86	1.853	18.40
T3-PBZ (1.5ppm)	11.47	85.12	1.947	21.35
T4-PBZ (2ppm)	11.24	84.22	2.043	27.14
T5-PBZ (2.5ppm)	11.09	83.92	2.257	31.65
T6-PRD	12.76	87.52	1.857	17.53
T7-PRD+PBZ(1ppm)	11.82	85.42	1.950	18.59
T8-PRD+PBZ(1.5ppm)	11.59	84.56	2.147	21.52
T9-PRD+PBZ(2ppm)	11.38	84.22	2.173	27.20
T10-PRD+PBZ(2.5ppm)	11.22	83.42	2.203	32.65
CD at 5%	*0.09*	*0.07*	*0.033*	*2.74*
SE (m)	*0.03*	*0.02*	*0.012*	*0.92*

Table 18: Effect of physical and biochemical approaches on total number of fruits per plant, relative stress injury (%), specific leaf area **(cm² g-^{1})** and relative growth rate (g day^{-1}**)** at harvest on tomato variety (Pusa Ruby)

Treatments	**Total number of fruits per plant**	**Relative stress injury (%)**	**Specific leaf area(cm² g-^{1})**	**Relative growth rate (g day^{-1})**
T1-Control	128.33	48.55	157.70	0.173
T2-PBZ (1ppm)	206.00	42.46	43.53	0.154
T3-PBZ (1.5ppm)	210.66	35.66	39.90	0.150
T4-PBZ (2ppm)	215.33	32.12	33.19	0.144
T5-PBZ (2.5ppm)	219.66	26.42	32.86	0.138
T6-PRD	126.33	56.44	124.70	0.167
T7-PRD+PBZ(1ppm)	205.33	47.52	60.16	0.149
T8-PRD+PBZ(1.5ppm)	209.66	41.22	56.48	0.142
T9-PRD+PBZ(2ppm)	214.66	36.21	54.81	0.136
T10-PRD+PBZ(2.5ppm)	218.33	32.61	52.73	0.131
CD at 5%	*16.28*	*4.38*	*17.39*	*0.007*
SE(m)	*5.48*	*1.12*	*5.85*	*0.002*

Table 19: Effect of physical and biochemical approaches on total carotenoids (mg $100g^{-1}$ FW), number of locules in fruit, number of flower trusses per plant and number of flower per cluster in tomato variety (Pusa Ruby)

Treatments	Total carotenoids (mg $100g^{-1}$ FW fruit)	Number of locules in fruit	**Number of flower trusses per plant**	**Number of flowers per cluster**
T1-Control	1.267	3.00	11.33	8.00
T2-PBZ (1ppm)	1.293	5.33	19.33	11.66
T3-PBZ (1.5ppm)	1.333	5.33	21.00	12.33
T4-PBZ (2ppm)	1.363	6.00	22.00	13.00
T5-PBZ (2.5ppm)	1.407	6.00	24.00	15.00
T6-PRD	1.280	6.00	11.66	6.66
T7-PRD+PBZ(1ppm)	1.307	5.33	19.33	10.33
T8-PRD+PBZ(1.5ppm)	1.337	6.00	20.33	12.00
T9-PRD+PBZ(2ppm)	1.360	6.00	22.33	13.66
T10-PRD+PBZ(2.5ppm)	1.387	6.00	24.00	14.33
CD at 5%	*0.019*	*0.62*	*4.05*	*2.23*
SE(m)	*0.006*	*0.21*	*1.36*	*0.75*


M
C
Pbz-1.0,1.5, 2.0, 2.5ppm
PRD
PRD+Pbz-1.0, 2.0, 2.5ppm
Mr
135 Kda
100 Kda
75 Kda
63 Kda
48 Kda
35 Kda
25 Kda
20Kda
17Kda
11Kda
75. 83Kda
63. 13 Kda
55. 16 Kda
47. 38 Kda
23. 92 Kda
16. 33 Kda
12.06 Kda

CHAPTER-V

DISCUSSION

Physiological, biochemical and molecular processes in plants are governed by interaction of genetic constitution and the environment in which it is growing, which have either direct or indirect impact on growth, development, metabolism and finally the survival and yield potentiality of the plants. Modern approaches in agricultural research, in the present era have been mainly focused on sustainable agriculture under changing climatic condition throughout the world. The studies on genotypes and environment interaction are of prime importance particularly in country like India, where striking variations in agro-climatic conditions exist. In such a country, it may be possible that same crop and even the same variety may respond differently under similar kind of treatment in different part of the country. Water availability is a limiting factor for growing crop worldwide. Water scarcity impedes plant growth via direct affects on cell division and expansion and perturbs ion balance and induces senescence. Therefore this problem can be reduced by improving the water use efficiency of crop plants.

Water saving techniques have a great importance in the area of crop physiology and crop management for dry conditions with the aim to take more crop per drop of water. However, these techniques may also exert secondary, often unpredicted influences on the growth and yield of the crop plants by affecting changes in growth pattern, plant morphology, anatomy as well as biochemical composition and finally the yield and quality of the crop.

Tomato (*Solanum lycopersicum* L.) was chosen as a biological material in the present investigation, as this crop is one of the most important vegetable crops grown in different parts of the country. In the present investigation, the important physio-morphological, biochemical and molecular aspects of this crop in relation to the physical and biochemical water saving techniques were investigated. The pertinent findings of the present study are being discussed in this chapter.

The data recorded for all the parameters are in itself very explanatory with reference to objectives. However, the previous works carried out in this direction have been duly consulted for a very sound interpretation of the findings in the light of proposed objectives. As far as physiological, biochemical and molecular studies in relation to physical and biochemical approaches for maximising the water use efficiency and yield in this particular crop are concerned, the experimental findings are being discussed in order to elucidate their relevance in the following pages of this chapter.

It is apparent that the growth and development of tomato plants were favourably influenced by the application of Paclobutrazol and Partial Root Drying technique. It is reasonable because Paclobutrazol is a plant growth retardant and PRD is water saving irrigation technique therefore, its application at appropriate concentrations and time help in proper growth, development and yield of plants. Discussions for all the characters are given below:

5.1 Growth Parameters:

5.1.1. Plant height:

As far as the growth characters are concerned, first of all, the plant height was significantly decreased in plants treated with Paclobutrazol (PBZ) @ 2.5 ppm and in PRD+PBZ @ 2.5 ppm as compared to control and PRD alone (Table 2). Paclobutrazol effectively reduced the plant height because; it is a triazole-type inhibitor of gibberellins biosynthesis which affects plant growth and development. It inhibits the activity of ent-kaurene oxidase, which is an enzyme in the GA biosynthetic pathway that catalyzes the oxidation of ent-kaurene to ent-kaurenoic acid (Xia *et al.,* 2018).Our observations are in agreement with other researchers (Mobli and Baninasab, 2008) in Almond, (Baninasab and Ghobadi, 2011) in Cucumber, (Hua *et al.,* 2014) in Canola (Dewi *et al.,* 2016) in rice, (Pal *et al.,* 2016) in tomato, and (Xia *et al.,* 2018) in herbaceous peony. They observed that the height of plants treated with paclobutrazol decreased in accordance with the increase of paclobutrazol concentration. Lei *et al.* (2009) also found that, PRD had weaker vegetative growth as compared with control and same results was observed on tomato by (Kirda *et al.,* 2004).

5.1.2. Number of leaves and leaf area:

In present investigation, the number of leaves (Table 3) and leaf area (Table 4) was significantly reduced by PBZ treatment alone and also in combination with PRD (PRD+PBZ) @ 2.5 ppm. Maximum number of leaves and leaf area was observed in control and PRD alone. The PBZ induced reduction in leaf area may be linked to inhibition of GA biosynthesis whereas small leaves helps in minimum loss of water by the leaf surface and it also helps in improvement of water use efficiency. Our findings are in accordance with Pal *et al.,* (2016) in which they noticed that PBZ application led to decreased leaf area and root area ratio in tomato plants. PBZ application led to decreased leaf area ratio in irrigated plants (Berova and Zlatev, 2003). Paclobutrazol treatment induced reduction in leaf area under deficit irrigation and also in both irrigated and deficit irrigated conditions in tomato plants (Mobli and Baninasab, 2008; Parent *et al.,* 2009; Mahdieh *et al.,* 2009). Lei *et al.* (2009) noticed that, leaf area under PRD was significantly decreased in comparison to control. Leaf growth reduction was the result of both a decrease in the number of leaves and leaf area (Stikic *et al.,* 2003).

5.1.3. Stem thickness :

The stem thickness was significantly increased in PBZ alone and PRD+PBZ treatment @ 2.5 ppm as compared to control and PRD alone (Table 5). Increased stem thickness in PBZ treated plants due to reduced GA endogenous level and hence increased stem width because cell division still occurs, but the new cells do not elongate, resulting in stouter stem and short internodes. Our observations are in vicinity to other researchers, who observed that the plants treated with PBZ exhibit stunted growth due to reduced GA endogenous level (Berova and Zlatev, 2003; Mobli and Baninasab, 2008; Upreti *et al.,* 2013). PBZ application has reduced plant height and therefore improved stem diameter (Pal *et al.,* 2016). Jungklang *et al.* (2017) also reported that gibberellins production is inhibited in PBZ treated turmeric plants whereas cell division continue, but the new cells do not elongate, resulting in stouter stem and short internodes with the same number of leaves.

5.1.4. Biomass :

The fresh weight as well as dry weight of roots and shoots of tomato was significantly decreased in PBZ alone and PRD+PBZ treated plants @ 2.5 ppm in comparison to control and PRD alone (Table 14). Changes in the root fresh weight/dry weight and shoot fresh weight/dry weight have been linked to the PBZ induced inhibition of GA biosynthesis leading to reduced shoot/root growth. GA induced growth and activity of the enzyme xyloglucan transglycosylase (XET) in plant tissues. This enzyme hydrolyses xyloglucans of the cell walls internally and causes molecular rearrangement in the cell wall matrix which could promote extension of cell wall. Berova and Zlatev (2003); Gopi *et al.* (2007); Bayat *et al.* 2012 supporting our findings. They noticed that fresh weight and dry weight of roots and shoots was significantly decreased in wheat, carrot and maize plants treated with Paclobutrazol. Pal *et al.* (2016) also observed that the application of PBZ at different concentration significantly reduced shoot dry weight and root dry weight considerably in tomato plants. In PRD treatment, there was found no significant difference in total dry biomass as compared to control (Lei *et al.*, 2009 and Perez-Perez *et al.*, 2012).

5.1.5. Specific leaf area :

In present investigation, specific leaf area was significantly decreased in PBZ alone and in PRD+PBZ @ 2.5 ppm in comparison to control and PRD alone (Table 18). Reduction in specific leaf area in PBZ treated plant is due to the leaf structural adaptation and also helping in higher chlorophyll index and higher rate of photosynthesis. Our observations are in agreement with Pal *et al.* (2016) in tomato and Abdul Jaleel *et al*. (2007) in *Catharanthus roseus*. They found a significant reduction in specific leaf area in PBZ treated tomato and china rose plant.

5.1.6. Relative growth rate :

Relative growth rate was significantly decreased in PBZ alone and in PRD+PBZ @ 2.5 ppm in comparison to control and PRD alone (Table 18). Reduction in RGR may be due to the reduction in plant height under PBZ treated plants. Our observations are in agreement with other researchers study results (Pal *et al.*, 2016). They noticed that PBZ application under irrigated conditions lowered relative growth rate (RGR) in tomato plants.

5.2 Plant water relations

5.2.1. Relative water content:

In the present investigation, the relative water content (RWC) of leaves in tomato was significantly increased in PBZ alone and PRD+PBZ treated plants @ 2.5 ppm in comparison to control and PRD treatment (Table 6). Our results are in agreement with other researchers (Berova and Zlatev 2003; Jungklang *et al.* 2017; Soumya *et al.* 2017) findings and they noticed that the application of Paclobutrazol increases the relative water content of leaves. Jungklang and Saengnil (2012) also found that application of PBZ increases the RWC of *Curcuma alismatifolia* leaves. PBZ induced a number of some physiological and biochemical adaptations by maintaining growth and relative water content of leaf, decreasing relative stress injury and proline content that enables the plant to tolerate drought. In PRD alone, the RWC was significantly decreased as compared to control. This was also proved by Lawlor and Cornic (2002); Lei *et al.* (2009) which shows that as compared to control, relative water content under PRD was slightly but significantly lowered than control, which means that plants met slight water stress under PRD. The findings of other researchers, De Souza *et* *al.* (2005) in grapevine, Aganchich *et al.* (2007) in olive and Lei *et al.* (2009) in tomato revealed that the relative water content was significantly decreased under PRD which means that plants met slight water stress under PRD. Relative water content is the ability of plant to maintain high water in the leaves under moisture stress conditions and has been used as an index to determine drought tolerance in crop plants (Basker, 2013). Relative water content is considered a measure of plant water status, it is essential because it expresses the absolute amount of water which the plant requires to reach full artificial saturation and reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC related to water uptake by the roots as well as water loss by transpiration. When PRD irrigation is applied to a crop, the normal root to shoot signalling system that operates in water-deficient soils is altered, causing the drying half of the root system to release ABA thus reducing stomatal opening, whereas the fully hydrated roots preserve a favourable water status throughout the shoot parts of the plant (Okasha *et al.,* 2013). The Relative water content was closely related to the proline accumulation in leaves. The lowering of osmotic potential by osmolyte accumulation in response to stress improves the capacity of the cell to maintain its turgor pressure at low water potential. This appears to be essential for physiological processes such as photosynthesis, enzyme activity and cell expansion (Tyree *et al.,* 1982).

5.2.2. Proline content :

Proline is one of the compatible solutes that accumulate in response to water stress and the accumulation of these osmolytes represents an important adaptive response to salt and water stress (Martinez *et al.,* 2004). It has been well known, that the metabolites like proline, sugars were accumulate to a high level in plants when they are under water stress (Ashraf and Foolad, 2007; Kovacik *et al*., 2009; Parween *et al*., 2012; Afzal *et al*., 2014). Proline is a compatible osmolyte occurs widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Sairam and Tyagi, 2004; Verbruggen and Hermans, 2008). In some plant species, proline plays a major role in osmotic adjustment such as in potato, while in others such as in tomato proline accounts for only a small fraction of the total concentration of osmotically active solutes (Perez-Alfocea *et al.,* 1993). In the present investigation, the highest proline content was found in PRD treatment alone than control. Our results are in agreement with other researchers study results (Lei *et al.,* 2009). They conducted an experiment on tomato by using the PRD technique and found that as compared to control, the level of proline was higher in PRD treatment. It can be indicated that osmotic regulation was induced by water stress. Claussen (2005) during their research in tomato crop by applying the PRD technique found that the proline content of tomato leaves was shown to be strongly related to the relative water content of leaves; it is thus a useful indicator of the plant's water status and the proline content of tomato leaves fluctuated according to nutrient concentration and total radiation, and was closely related to the relative water content of leaves. Madan *et al.* (1994) reported that the proline level were highest in young leaves and decreased linearly with increasing leaf age in tomato. Aganchich *et al*. (2007) contributed a lot of research on PRD technique and conducted that Proline content in PRD was higher than in Control. Accumulation of proline for osmotic regulation in drought-stressed leaves has been observed in plants (Steward *et al.,* 1980 and Khan *et al.,* 2001). In our research findings, it was concluded that the proline content of fresh tomato leaves was significantly decreased in PBZ alone and in combination with PRD (PRD+PBZ) @ 2.5 ppm (Table 13). Our results are in agreement with other researchers (Berova and Zlatev 2003; Gopi *et al.* 2007; Yang *et al.* 2009; Pal *et al.* 2016) study results which shows that application of PBZ in crop decreases the proline content of leaves because PBZ acts as a drought tolerant in crop species. Jungklang *et al*. (2017) in *Curcuma alismatifolia* also showed that water stress reduced RWC, but induced electrolyte leakage and proline content in the leaves. However, the leaves showed opposite results when Paclobutrazol (PBZ) was added to the treatments that means the PBZ application increases the RWC of leaf but decreases the Proline content of leaf that enables the plants to tolerate drought.

5.2.3. Total soluble sugars:

Sugars constitute an important component of tomato fruit as they determine sweetness and influence the overall tomato flavour (Stevens *et al.,* 1979; Baldwin *et al.*, 1998). Accumulation of sugars in different parts of plants is enhanced in response to a variety of environmental stresses (Prado *et al.,* 2000). When the plants were subjected to Partial Root Drying (PRD) technique, there was a significant increased in soluble sugar. Our results are in agreement with other researchers (Davies *et al.,* 2000; Loveys *et al.,* 2000; Stikic *et al.,* 2003; Xu *et al.,* 2009) in tomato and (Francaviglia *et al.,* 2013) in apple. They found that as compared with deficit irrigation practice, PRD leads to greater sugar and organic acid concentrations in tomato fruits. Ruan *et al.* (2010) also found that higher accumulation of ABA due to mild stress in PRD stimulates the activity of enzyme invertase in the fruits and as a result the concentration of sugars hexose in the fruits is increased. Increases in grape quality combined with reduced water use have been reported as a result of the use of PRD on a commercial field scale that PRD significantly increased fruit firmness, TSS, sugars, organic acids, P, K, Mg concentrations in tomato juice, and Ca and Mg concentrations in fruit dry matter, implying that PRD treatment enhanced fruit quality as compared with the deficit irrigation treatment (De Souza *et al.,* 2003; Dos Santos *et al.,* 2003). Starch metabolism is very sensitive to abiotic stresses generally leads to a depletion of starch content and to the accumulation of soluble sugars in leaves (Kaplan *et al.,* 2004; Basu *et al.,* 2007; Kempa *et al.,* 2008). Emery and Munger (1970) reported that indeterminate plants had more soluble solids than determinate forms of almost isogenic plants. Similarly, Subbaiah *et al.* (2017) conducted an experiment on mango and they found that fruit quality, in terms of sugars were improved at lower water application levels over the higher water application levels. This may be due to increase in total soluble solids associated with reduced fruit water content and greater hydrolysis of starch into sugars. Several research groups have shown that, using partial root zone irrigation technique, the quality of fruits such as grapes in terms of sugar content can be increased (Fuller, 1997; Loveys *et al.*, 1997, 1998; Dry and Loveys, 1998, 1999, 2000*a*, *b*; Gu *et al.*, 2000; Stoll *et al.*, 2000). In the present investigation, the total soluble sugar was significantly increased in PBZ alone and PRD+PBZ @ 2.5 ppm in comparison to control plants (Table 8). Our results was proved by the other researchers (Gopi *et al.*, 2007, Mobli and Baninasab, 2008; Pal *et al.*, 2012; Reddy *et al.*, 2013; Hua *et al.*, 2014). They found that the total soluble sugar, sucrose, and starch content in the stem, leaf and bud organs were significantly increased by paclobutrazol application in carrot, almond, tomato, mango, canola plants respectively. The increased of total soluble sugars and sucrose content could be partially accounted by the activating effect of paclobutrazol on enzymes related to sucrose synthesis and catalysis (Hua *et al.*, 2014).

5.2.4. Relative stress injury:

The indicator of cell membrane integrity is electrical conductance or electrolyte leakage also known as relative stress injury (Whitlow *et al.*, 1992). The stress induced electrolyte leakage is usually accompanied by accumulation of reactive oxygen species and often results in programmed cell death. An increase in electrolyte leakage indicates an increase in membrane permeability and reduced cell tolerance to temperature change. Measurement of electrolyte leakage indicates the stress damage to assess the harshness of existing stress (Foyer *et al.*, 1997). When the plants were subjected to Partial Root Drying (PRD) technique, the relative stress injury was found significantly increased as compared to control. In PRD, the RSI was increased due to mild water stress to plants. In PBZ treatment alone and in combination with PRD, PRD+PBZ @ 2.5 ppm, the relative stress injury was significantly decreased (Table 18). Our results are in agreement with other researchers (Whitlow *et al.,* 1992; Pal *et al.,* 2012; Soumya *et al.,* 2017; Jungklang *et al.,* 2017). They noticed that the PBZ application significantly decreased the electrolyte leakage relative to control plants. Jungklang and Saengnil (2012) found that application of PBZ decreased the electrolyte leakage of *Curcuma alismatifolia* leaves because PBZ enables the plants to tolerate drought by increasing the RWC of leaf and decreasing the proline content in leaves.

5.2.5. Total phenols:

Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress (Bhattacharya *et al.,* 2010). Total phenols was significantly increased in PRD treated plants in comparison to control as these are mainly produced in response to water stress. Garcia-Valverde *et al.* (2013) reported that the antioxidative effect of tomato fruits is due to the presence of polyphenols which are able to scavenge peroxyl radicals. Moreover, polyphenols extracted from peel, pulp and seeds of selected fruits, including tomatoes, have an antiproliferative effect in several cancer cell lines (Li *et al.,* 2013). Wang and Frei (2011); Stagnari *et al.* (2016) investigates that the plants respond to drought with the activation of several signalling pathways resulting in a change of gene expression and enhancement of the biosynthesis of primary and secondary metabolites relevant for crop quality. Results of Jovanovic and Stikic (2018) demonstrated a beneficial effect of PRD on quality of yield and its nutritional values. They found that the phenolics and carotenoids are produced more in PRD treated plants that are the important sources of bioactive components that have increased nutritional and health values. According to Romero *et al.* (2016) the reduced vegetative growth and increased light penetration into the canopy in PRD vines together with the increased ABA content and salicylic acid in berries might have an increasing effect on production of phenolic compounds. In PBZ treatment alone and in PRD+PBZ @ 2.5 ppm, total phenols were significantly decreased as compared to control and PRD treatment (Table 7). Paclobutrazol maintains the RWC of leaf, proline content and decreasing the relative stress injury, and enhancing the antioxidant activities so, there is no need to produce the secondary metabolites like phenols in higher amount.

5.2.6. Chlorophyll content:

In the present research investigation, the maximum chlorophyll content was found in PBZ alone and in combination with PRD (PRD+PBZ) @ 2.5 ppm and minimum in control and PRD treated plants (Table 9, 10 and 11). This result are in agreement with other researchers (Abdul Jaleel *et al.,* 2007; Mobli and Baninasab, 2008; Kumar *et al.,* 2012; Xia *et al.,* 2018). They found that that the average of total chlorophyll was higher on plants treated with paclobutrazol as compared to control. This could be due to triazol applied to plants that increased the biosynthesis of chlorophyll precursor, so that plants treated with paclobutrazol usually have higher chlorophyll content compared to control. Pal *et al.* (2016) conducted an experiment on tomato regarding PBZ application found that the chlorophyll content increased in both irrigated and deficit irrigated PBZ treated plants as compared to their respective control (without PBZ), thereafter it gradually declined. In PRD treatment, the chlorophyll content was decreased than PBZ treated plants due to mild water stress to PRD plants. Drought not only causes dramatic loss of pigments but also leads to disorganization of thylakoid membranes, therefore reduction in chlorophyll contents is expected (Ladjal *et al.,* 2000). Paclik *et al.* (1996) observed that water stress reduced chlorophyll *a* and *b* contents by 38% compared with the adequately watered plants. Similarly, Arivalagan and Somasundaram (2017) while conducting an experiment on tomato under water stress conditions found that the water stress reduced the photosynthetic pigments such as chlorophylls and increased the antioxidant contents and antioxidant enzymes activities. Reduction of chlorophyll content has been considered as a commonly observed phenomenon in response to water stress (Bayat *et al.,* 2009; Ebrahimiyan *et al.,* 2012). Plant under water stressed conditions accumulates less chlorophyll content than the unstressed plants.

5.3 Water Use Efficiency:

In the present investigation, the WUE was significantly increased in PBZ and PRD+PBZ treatment @ 2.5 ppm as compared to control and PRD alone (Table 15). Our results are in agreement with other researchers (Pal *et al.,* 2016; Soumya *et al.,* 2017; Xia *et al.,* 2018; Giuliani *et al.,* 2018). They found that Paclobutrazol application enhanced photosynthetic rates, chlorophyll content, high fruit yield with minimum usage of water, thus improving water use efficiency in plants. Water use efficiency is one of the indicators used to evaluate irrigation management. Water use efficiency (WUE) is a measure of a crop's capacity to convert water into plant biomass or yield. Water use efficiency was also calculated as the ratio between total fruit yield and plant water used in tomatoes (g L^{-1})

(Giuliani *et al.,* 2018). When the plants were subjected to Partial Root Drying (PRD) technique, the water use efficiency was significantly increased as compared to control. Therefore in comparison to control the WUE of PRD treated plants were highest and this was proved by other researchers (Stikic *et al.,* 2003; Okasha *et al.,* 2013; Abdelrouf, 2016; Barideh *et al.,* 2018; Jovanovic *et al.,* 2018). Due to partial root irrigation, there is minimum use of water and high fruit yield under PRD in comparison to control helps in improving the WUE of crop plants.

5.4 Molecular Parameter:

PBZ differentially induced expression of genes and accumulation of metabolites of the tricarboxylic acid (TCA) cycle, γ-aminobutyric acid, glutathione ascorbate (GSH-ASC)-cycle, cell wall and sugar metabolism, abscissic acid (ABA), spermidine (Spd) content and expression of an aquaporin (AP) protein under deficit irrigation. The high molecular weight protein band was observed in PBZ alone and in combination with PRD, which was very thick and intensed in comparison to control and PRD alone. Pal *et al.* (2016) reported that PBZ application could significantly improve tolerance in tomato plants under limited water availability through selective changes in morpho-physiology and induction of stress-related molecular processes. PBZ-treatment under deficit irrigation (having elevated ABA levels) recorded even higher protein level over the control. The increase in expression of the proteins related to cell wall, energy, and stress defense could allow PRD fruits to increase the duration of fruit growth. Upregulation of some of the antioxidative enzymes during the cell expansion phase of PRD fruits appears to be related to their role in protecting fruits against the mild stress induced by PRD (Marjanovic *et al.,* 2012).

5.5 Yield and Quality Parameters

5.5.1. Fruit yield:

Fruit yield and quality are the important factors observed in the production of tomatoes (Muhammad and Singh, 2007). In the present investigation, the fruit yield (Kg) per plant was significantly increased in PBZ alone and PRD+PBZ treatment @ 2.5 ppm in comparison to control and PRD treated plants (Table 15). Our results are in agreement with other researchers (Berova and Zlatev, 2003; Abdul *et al.,* 2007; Kumar *et al.,* 2012; Pan *et al.,* 2013; Pal *et al.,* 2016; Dewi *et al.,* 2016; Xia *et al.,* 2018). They noticed that application of paclobutrazol in crop plants would increased the fruit yield. Zhou and Xi, (1993); Hua *et* *al.,* (2014) conducted an experiment on canola plants with paclobutrazol and found that canola yield could be significantly improved by paclobutrazol application. Paclobutrazol application increased the chlorophyll content which led to greater rate in photosynthesis and higher yield. Paclobutrazol also increased the activity of oxidative enzymes and enhanced plant resistance to water lodging. The PBZ treated leaves experienced late senescence. This could be due to an increase in the activity of oxidative enzymes which prevented cell maturation. Slow senescence in leaves can prolong the phase of seed development and maturation. As a consequence, the yield can be increased. When the plants were subjected to Partial Root Drying (PRD) technique, the yield per plant was found non significant as compared to control. Our results was agree with other researchers (Lei *et al.,* 2009; Theobald *et al.,* 2007; Abdelrouf, 2016). They suggested that higher fruit yield and lower water use in PRD technique made the WUE improving. Sun *et al.* (2013) investigated that PRD is a water saving irrigation technique and they executed an experiment on tomato crop which reported that the irrigation treatments had no effect on tomato yield but significantly affected several organic and mineral quality attributes of the fruits. Loveys (1991) was the first who applied the split-root technique for inducing chemical signals in the root system of grapevine grown in field conditions and the results showed that PRD reduced vine vigour and increased the quality and yield of fruit.

5.5.2. Fruit biomass:

Fresh weight of fruit, dry weight of fruit, fruit volume, fruit density and total number of fruits per plant was significantly increased in PBZ and PRD+PBZ treatment @ 2.5 ppm as compared to control and PRD treated plants (Table 16). The highest number of fruits, fruit biomass, fruit volume and fruit density was found in PBZ treated plants and also in PRD+PBZ @ 2.5 ppm as compared to control and PRD alone. This was proved by other researchers (Reddy *et al*., 2013). They reported that application of PBZ increases the fruit biomass, PBZ enhanced the photosynthetic rate and thereby enhances the fruit yield which may be linked with the highest number of fruits and fruit biomass in PBZ treated plants. In PRD treatment, our results showed that PRD caused a slight but non significant reduction in fruit numbers and fruit biomass as compared to control. Davies *et al.* (2000) demonstrated that PRD reduces leaf growth of grapevines, but has no influence on fruit growth and development. Our results are in agreement with other researchers (Stikic *et al.,* 2003; Lei *et al.,* 2009).

5.5.3. Ascorbic acid and total carotenoids in fruit:

Tomato fruits contain a high level of antioxidants such as vitamin C, polyphenols, and carotenoids. High levels of ascorbic acid in tomato fruits provide health benefits for humans and also play an important role in several aspects of plant life (Matteo Di *et al.*, 2010). To keep the levels of active oxygen species under control, plants have non-enzymatic and enzymatic antioxidant systems to protect cells from oxidative damage (Mittler, 2002). Non-enzymatic antioxidants including β-carotenes, ascorbic acid, α-tocopherol, reduced glutathione (GSH). According to Fanciullino *et al.* (2014) water stress may influence the secondary metabolism through two interactive mechanisms: the changes of primary metabolite transport (major source in the biosynthesis of carotenoids and ascorbic acid) or oxidative stress which could affect the biosynthetic pathways of antioxidant compounds. In the present investigation, ascorbic acid content and total carotenoids of fresh tomato fruits in Paclobutrazol treatment was significantly increased @ 2.5 ppm at harvest in comparison to control and PRD alone (Table 17). Our results are in agreement with other researchers (Jain *et al.*, 2002) in Citrus lemon (Manivannam *et al.*, 2008) in *Vigna unguiculata* (Siva Kumar *et al.*, 2010) in potato (Reddy *et al.*, 2013) in mango. They showed that application of triazole (PBZ) increased the content of ascorbic acid, anthocyanin, and xanthophylls and activities of ascorbate peroxidase, superoxide dismutase, and catalase activities. Jungklang and Saengnil (2012) found that application of PBZ increased the Vitamin C of *Curcuma alismatifolia* leaves. Pal *et al.* (2016) conducted an experiment on tomato plants with paclobutrazol and found that PBZ induced higher synthesis of ascorbic acid that ensures sufficient scavenging of reactive oxygen species generated under water stress. Some antioxidants such as vitamin C, vitamin E, and the activities of CAT and SOD were induced in the leaves by PBZ. Moreover, the content of vitamin C, vitamin E and CAT activity were higher in relation to water-deficit stress and PBZ treatments. This indicates that PBZ induced a number of some physiological and biochemical adaptations (maintaining growth and RWC, decreasing EL and proline content, increasing the vitamin C and vitamin E levels, and CAT and SOD activities) that enable the plant to tolerate drought. When the plants were subjected to Partial Root Drying (PRD) technique, the ascorbic acid was found slightly but non significantly increased as compared to control. This result was proved by Chen and Gallie (2004) they noticed that ascorbic acid content of the drought stressed plants significantly increased when compared to control tomato plants. The increased ascorbic acid content may protect the plants from stress (Reddy, 2004). In general, ascorbic acid concentrations of the crop cultivars were increased as a result of water stress. Ascorbic acid in plants is involved in scavenging of oxidative stress products, such as hydrogen peroxide generated in the chloroplasts (Sairam *et al.,* 2000). Increases in ascorbic acid concentration have been also reported previously in wheat under drought stress (Sairam *et al.,* 1998). Tomato fruits with high total soluble solids, pH less than 4.5, high ascorbic acid, lycopene and total acidity content are preferred for processing purpose (Bose and Agarwal, 2007). Antioxidants such as vitamins C and E, and the activities of antioxidative enzymes such as catalase (CAT) and superoxide dismutase (SOD), are increased by PBZ, which in turn provides stress tolerance to plants (Somasundaram *et al.,* 2009; Srivastav *et al.,* 2010).

5.5.4. Lycopene content:

Lycopene is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and tomato products. It is a carotenoid that is present in tomatoes, processed tomato products and other fruits. It is one of the most potent antioxidants among dietary carotenoids. Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases, such as cancer and cardiovascular disease. Tomato is a very good source of numerous compounds with antioxidant capacity. These are mainly carotenoids (lycopene and β-carotene), flavonoid compounds as well as phenolic acids and vitamin C. In Paclobutrazol treatment and PRD+PBZ @ 2.5 ppm, the lycopene content was significantly increased at harvest in comparison to control and PRD alone (Table 17). Our results are in agreement with other researchers (Soumya *et al.,* 2017). They found that paclobutrazol acts as stress protectant by maintaining the photosynthetic pigments machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby enhances the yield. Reddy *et al.* (2013) conducted an experiment on mango with PBZ and found that PBZ application improved quality in mango fruits in terms of lycopene as well as total carotenoids. When the plants were subjected to Partial Root Drying (PRD) technique, the lycopene content was found slightly but significantly increased as compared to control. This result was proved by other researchers Casa and Rouphael, (2014); Stikic *et al.,* (2003); Jovanovic and Stikic, (2018) study results. They found that PRD has a beneficial effect on quality of yield and its nutritional or health values in terms of the secondary metabolites like phenolic compounds and antioxidants like lycopene content in tomato fruits. Marjanovic *et al.* (2012) found that PRD tomato has some antioxidative enzymes which were upregulated during fruit expansion phase and also indicated their potential role in protection of fruits against the mild drought stress induced by PRD.

CHAPTER VI

SUMMARY AND CONCLUSION

Present investigation on physical and biochemical water saving techniques and their roles in plant growth and sustainable development have given a new dimension to modern agriculture. Partial Root Drying (PRD) and Paclobutrazol (PBZ) at definite proportion play vital roles in the life processes of plants. PBZ is a plant growth retardant which is required in minute quantities for plant processes and it also effective in combination with physical technique PRD. This technique is helpful for maintaining water relations, photosynthesis, regulating phytohormone synthesis, level of soluble sugar, proteins, pollen grain formation and active ion absorption. With these considerations and in view of the economic importance of tomato (*Solanum lycopersicum* L.) as an important vegetable crop of India as well as of the world, Present investigation entitled " To study the effect of physical and biochemical approaches on water use efficiency in tomato (*Solanum lycopersicum*)" was undertaken which can be summarized as follows:

This experiment was carried out in pots, having soils with equal amount of Farm Yard Manure at the experimental house of the Division of Plant Physiology, SKUAST-J. Growth retardant PBZ was therefore, applied at different levels, i.e., 1.0, 1.5, 2.0 and 2.5 ppm and PRD in combination with PBZ at four different level i.e., PRD + (PBZ 1.0, 1.5, 2.0 and 2.5 ppm). A number of observations pertaining to morphological, physical, biochemical and molecular changes in plants were made, e.g., plant height, number of leaves, leaf area, stem thickness, Relative Water Contents (RWC), Proline content, chlorophyll a and b, total carotenoids, total chlorophyll and total soluble sugars at 50 DAT, 100 DAT and at harvest. When plants attained their maturity level, the observations on yield and quality, e.g., number of flower trusses, number of flower per cluster, total number of fruits, fruit yield, water use efficiency, specific leaf area, relative stress injury, ascorbic acid, lycopene content, fruit volume, fruit density, number of locules, fruit diameter and total carotenoids in fruit were done at harvest and the following conclusion were drawn.

As regard the plant height, number of leaves, leaf area and specific leaf area of tomato were significantly decreased in PBZ alone and in PRD+PBZ @ 2.5 ppm followed by 2.0 ppm, 1.5 ppm and 1.0 ppm in comparison to control and PRD alone at 50 DAT, 100 DAT and at harvest. When the plants were subjected to PRD technique, there was a slight, but significant reduction in plant height at 50, 100 DAT and at harvest in comparison to control.

Contrarily stem thickness was significantly increased in PBZ alone and in plants treated with PRD+PBZ @ 2.5 ppm at all the growth stages. Effect of PBZ was more pronounced than PRD technique and combination of PRD+PBZ respectively.

Fresh and dry weight of root and shoot was significantly decreased in PBZ alone @ 2.5 ppm and also in combination with PRD + PBZ @ 2.5 ppm in comparison to control and PRD alone. Effects of various treatments were at par in all the stages.

Relative Growth Rate (RGR) and Relative Stress Injury (RSI) were significantly decreased in plants treated with PBZ @ 2.5 ppm alone and also in combination with PRD + PBZ @ 2.5 ppm in comparison to control and PRD alone treated plants, while RGR and RSI did not vary significantly at different stages.

Application of PBZ alone and in combination with PRD increased the percent RWC of leaves. Maximum RWC was noticed in plants treated with PBZ @ 2.5 ppm and PRD+PBZ @ 2.5 ppm in comparison to control and PRD treated plants at harvest. Stem thickness also following the same trend.

As a result of increased total chlorophyll (SPAD value), chlorophyll ‘a’, chlorophyll ‘b’, the photosynthetic ability was higher in plants treated with PBZ @ 2.5 ppm and also in combination of PRD+PBZ @ 2.5 ppm than other treatment in comparison to control and PRD treated plants at 50 DAT while not vary significantly at other two growth stages. Contrarily proline contents increased in plants treated with PRD alone and in control at all the growth stages, while proline concentration decreased in plants treated with PBZ alone and in combination of PBZ + PRD at all the growth stages.

A pronounced increase in total souble sugar was noticed while total phenols decreased in response to various treatments of PBZ alone @ 2.5 ppm and in combination with PRD+. PBZ @ 2.5 ppm at all the growth stages, while increase in total soluble sugar was maximum at 50 DAT, whereas total phenols were decreased maximum at harvest.

Treatment of PBZ alone and in combination of PRD markedly effect the yield and its various attributes i. e. number of flower trusses per plant, number of flower per cluster, number of fruits per plant, fruit yield per plant. Pronounced effect of 2.5ppm PBZ greatly was observed in all parameters related to yield and its attributes in comparison to PRD and in combination of PRD. Effective treatment of PBZ increased the fruit yield per plant with minimum use of water hence, increased the water use efficiency (WUE) of crop plants.

Therefore, maximum WUE was also recorded in plants treated with 2.5ppm PBZ and PRD+PBZ 2.5 ppm in comparison to control and PRD alone.

Fruit quality parameters i. e. fruit volume, fruit density, pericarp thickness, number of locules in fruit, fresh and dry weight of fruit was increased in plants treated with 2.5 ppm PBZ and also in combination PRD + 2.5 ppm PBZ as compared to control and PRD treated plants.

A pronounced increase in the concentration of lycopene, ascorbic acid and total carotenoids in fruits was noticed in plants treated with 2.5 ppm PBZ and in combination with PRD + 2.5ppm PBZ as compared to control and PRD alone.

Application of 2.5 ppm PBZ by soil drenching method may be beneficial for better yield and quality production of tomato crop. This dose of PBZ could not be recommended at this stage to farmers prior to its confirmation at the field scale. Nevertheless, the positive results with reference to PBZ in tomato crop is undoubtedly encouraging for making elaborate studies at field scale with adequate concentrations of PBZ. Moderate water stress induced osmotic regulation under PRD condition, leading to normal water status of the plants, higher activity of antioxidant enzymes, higher Photosynthetic rate and lower transpiration, the same level of biomass and lower water use thus providing some part of mechanism to higher WUE under PRD condition. PRD also gives better performance when applied in combination with PBZ.

Therefore, it may suggested that application of 2.5 ppm PBZ commands a great significance in maintaining a number of physiological, biochemical and molecular adaptations by maintaining growth and RWC, decreasing evaporational loss of water and proline content, increasing the vitamin C levels, that enable the plant to grow under water limiting environments.

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CERTIFICATE- IV

Certified that all the necessary corrections as suggested by the external examiner and the Advisory Committee have been duly incorporated in the thesis entitled **"To Study the Effect of Physical and Biochemical Approaches on Water Use Efficiency in Tomato** *(Solanum lycopersicum)*" submitted by **Ms. Muneeba Banoo**, Registration No. **J-16-MBS-20**.

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