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Muneeba Banoo
Division of Plant Physiology, Faculty of Basic Sciences, SKUAST-J, Chatha, Jammu and Kashmir, India

Bhav Kumar Sinha
Division of Plant Physiology, Faculty of Basic Sciences, SKUAST-J, Chatha, Jammu and Kashmir, India

Gurdev Chand
Division of Plant Physiology, Faculty of Basic Sciences, SKUAST-J, Chatha, Jammu and Kashmir, India

Reena Sinha
ACRA, Dhiansar, Bari-Brahmana, SKUAST-Jammu, Bari Brahmana, Jammu and Kashmir, India

Corresponding Author:
Muneeba Banoo
Division of Plant Physiology, Faculty of Basic Sciences, SKUAST-J, Chatha, Jammu and Kashmir, India

Paclobutrazol and partial root drying induces drought tolerance in tomato (*Solanum lycopersicum* L.)

Muneeba Banoo, Bhav Kumar Sinha, Gurdev Chand and Reena Sinha

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Abstract
A study was conducted to evaluate the role of physical technique partial root drying (PRD) and plant growth retardant, paclobutrazol (PBZ) for ameliorating the effect of drought in tomato. Different concentration of PBZ (1.0, 1.5, 2.0 and 2.5 ppm) were applied in pot condition at the time of transplanting. The highest relative water content in tomato leaves was observed in PBZ @ 2.5ppm and PRD + 2.5ppm PBZ and lowest in control and PRD. In PBZ treated plants, the level of total carotenoids, lycopene and ascorbic acid in tomato fruit at harvest were higher than control. The significant reduction in total phenols, proline content and relative stress injury were recorded in plants treated with PBZ @ 2.5ppm and PRD + 2.5ppm PBZ. Changes in protein profiles were also observed in PBZ treated plants than control. The highest yield per plant was found in plants treated with PBZ @ 2.5ppm and in PRD + 2.5ppm PBZ. Paclobutrazol @ 2.5 ppm commands a great significance by controlling the growth of plants and by maintaining the relative water content, photosynthetic pigments and protects the photosynthetic machinery by enhancing the level of osmolytes, endogenous hormones and thereby enhances the yield.

Keywords: Paclobutrazol, partial root drying, photosynthetic pigments, relative water content, lycopene, protein profiles

Introduction
Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop in the world after potato. It constitutes an important source of minerals, vitamins and antioxidants (Atkinson *et al.* 2011) [4]. Tomato is an important source of antioxidants such as carotenoids, lycopene, ascorbic acid. It acts as the scavenger of free radicals which generally deals 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 area however it is 11^{th} rank in productivity (Vanitha *et al.* 2013) [27]. 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) [6]. 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. It has a significant role in improving drought tolerance by enhancing physiological response and by increasing the proline content and antioxidant activity in plants. Paclobutrazol acts as stress protectant by maintaining the membrane stability index, 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 and quality in crops (Soumya *et al.* 2017) [25].

Materials and Methods
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.

Tomato (*Solanum lycopersicum* L.) variety Pusa Ruby was used as planting material raised to study their effect on drought tolerance 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 types 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 15 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.

Leaf Relative water content (RWC)

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 Petri-dish containing distilled water and subsequently blotted gently and weighted again for Turgid Weight (TW). After taking turgid weight, the leaves were oven dried at 80 ºC for 48 h and Dry Weight (DW) was recorded separately.

The RWC was calculated using the following formula (Weatherley, 1950) [28].

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

Proline content

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

Total phenolic content

Total phenolic content of prepared extracts was determined according to the Folin- Ciocalteu method (Singleton *et al.* 1974) [24] 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.

Relative Stress Injury

The relative stress injury (RSI%) in leaves was evaluated by (Sullivan and Ross, 1979) [26]. The third fully expanded leaf from the top was collected and kept in 20 ml vials containing 10 ml de-ionised water at 25 ºC. 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.

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

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) [23]. 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. The lycopene content was calculated as lycopene mg/100g = 31.206 x absorbance/weight of sample.

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) [16]. 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 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 total carotenoids mg/100g = O.D x 13.9 x 10^4 x volume made/ weight of sample x 560 x 1000.

Ascorbic acid

The ascorbic acid content of the fruit was estimated by 2, 6-dichlorophenol Indophenol visual titration method of (AOAC 1984) [1] and expressed in mg/ 100g FW. 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. 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. 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. Ascorbic acid content of the sample was calculated as ascorbic acid mg/100 g = Titre x Dye factor x Volume made / Aliquot of extract x volume of sample x 100.

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). 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. Thoroughly 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. Prepare a sufficient volume of separating gel mixture (30ml) by mixing Tris-HCl (pH 8.8), Ammonium per sulphate solution, 10% SDS and TEMED. 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. Prepare stacking gel (4%) by mixing the Tris-HCl (pH 6.8), Ammonium per sulphate solution, 10% SDS and TEMED (10 ml). Remove the water from the top of the gel and wash with little stacking gel solution. Pour the stacking gel mixture, place the comb in the stacking gel and allow the gel to set (30-60 min).

After the stacking gel has polymerized, 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. 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. 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. 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. The protein absorbs the Coomassie brilliant blue. 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 colourless. The proteins fractionated into band are seen coloured blue.

Number of aphids

Number of aphids per three leaves were counted at three interval stages (50 Days after transplanting, 100DAT and at harvest) and their means were calculated.

Fruit yield

Fruit yield was recorded in grams from all the three tagged plants from pots by adding up weight of fruits obtained from all the previous pickings.

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).

Results and Discussion

Plant water status (Relative water content%)

A marked increased in relative water content was observed when PBZ applied @ 2.5 ppm followed by 2.0 ppm, 1.5 ppm and 1.0 ppm as compared to control plants (Table 1). Same trend was observed in plants treated with PRD + PBZ @ 2.5 ppm, 2.0 ppm, 1.5 ppm and 1.0 ppm. When the plants were subjected to Partial Root Drying (PRD) technique, there was significant reduction observed in RWC in comparison to control. These results agreed with the findings of Soumya *et al.* (2017) [25]. They observed that application of Paclobutrazol increases the relative water content of leaves. PBZ induced several 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. Okasha *et al.* (2013) [18] found that relative water content under PRD was slightly but significantly lowered than control, which means that plants met slight water stress under PRD.

Proline content (mg/g)

A significant reduction in proline content (mg/g) was observed in PBZ @ 2.5 ppm followed by PBZ @ 2.0 ppm (Table 1). PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly reduced proline content as compared to control plants. Same trend was observed in plants treated with PRD + PBZ @ 2.5 ppm, 2.0 ppm, 1.5 ppm and 1.0 ppm. When the plants were subjected to PRD technique, there was a slight, but significant increase in proline content in comparison to control. Our results are in accordance with Pal *et al.* (2016) [20]. They observed that application of PBZ in crop decreases

the proline content of leaves as the 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. Higher accumulation of proline in PBZ-treated plants can preserve the structure of complex proteins, maintain membrane integrity under dehydration stress and reduce oxidation of lipid membranes or photo-inhibition (Hajihashemi and Ehsanpour 2013) [10]. Soumya *et al.* (2017) [25] observed that the level of proline is decreased due to the role of paclobutrazol acting as a stress-ameliorating agent in plant, as the plant does not need to accumulate the proline content in the leaves. Lei *et al.* (2009) [15] 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.

Relative stress injury (RSI%)

A significant reduction in RSI was recorded in PBZ @ 2.5 ppm followed by PBZ @ 2.0ppm (Table 1). Same trend was also observed in plants treated with PRD + PBZ @ 2.5 ppm, 2.0 ppm, 1.5 ppm and 1.0 ppm. When the plants were subjected to PRD technique, there was a slight, but significant increase in RSI at harvest in comparison to control. Our results are in agreement with Jungklang *et al.* (2017) [13]. They noticed that the PBZ application significantly decreased the electrolyte leakage because PBZ enables the plants to tolerate drought by increasing the relative water content of leaf and decreasing the proline content in leaves. PBZ treatment reduced membrane damages in stressed plants which resulted in lower level of electrolyte leakage.

Total phenols (mg/g)

A significant reduction in total phenols (mg/g) in tomato leaves was recorded in PBZ treated plants @ 2.5 ppm followed by PBZ @ 2ppm respectively at 50 DAT, 100 DAT and at harvest. As evident from Table 1, PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly reduced total phenols in leaves respectively at 50, 100 DAT and at harvest as compared to control. Same trend was observed in plants treated with PRD + PBZ @ 2.5 ppm, 2.0 ppm, 1.5 ppm and 1.0 ppm. When the plants were subjected to PRD technique, total phenols were significantly increased at 50 DAT, 100 DAT and at harvest in comparison to control. Similar results were also observed by Garcia-Valverde *et al.* (2013) [9]. They reported that the antioxidative effect of tomato fruits is due to the presence of polyphenols which are able to scavenge peroxyl radicals. Jovanovic and Stikic (2018) [12] also noticed that the phenolics are produced more in PRD treated plants that are the important sources of bioactive components that increased nutritional and health values. Our results are in accordance with Romero *et al.* (2016) [6]. They found that 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. Paclobutrazol maintains the relative water content of leaf, proline content and decreasing the relative stress injury and phenolic content in plants.

Table 1: Effect of physical and biochemical approaches on relative water content (%), proline content (mg/g) and relative stress injury (%) and total phenolics (mg/g) on tomato variety Pusa Ruby

Treatments	Relative water content (%)	Proline content (mg/g)	Relative stress injury (%)	Total phenols (mg/g)
T_1-Control	71.23	0.957	48.55	3.827
T_2-PBZ (1.0ppm)	77.01	0.653	42.46	3.207
T_3-PBZ (1.5ppm)	79.00	0.573	35.66	3.040
T_4-PBZ (2.0ppm)	80.72	0.490	32.12	2.867
T_5-PBZ(2.5ppm)	82.20	0.453	26.42	2.657
T_6-PRD	62.38	1.147	56.44	4.460
T_7-PRD+PBZ(1.0ppm)	75.07	0.757	47.52	3.740
T_8-PRD+PBZ(1.5ppm)	76.78	0.703	41.22	3.463
T_9-PRD+PBZ(2.0ppm)	77.60	0.663	36.21	3.203
T_{10}-PRD+PBZ(2.5ppm)	79.67	0.610	32.61	2.887
CD at 5%	4.11	0.014	4.38	0.310
±SE (m)	1.38	0.005	1.12	0.104

Effect of PBZ and PRD on antioxidants activity

Lycopene and ascorbic acid (mg/100g)

Lycopene and ascorbic acid (mg/100g) in PBZ treatment was significantly increased @ 2.5 ppm followed by 2.0, 1.5 and 1.0 ppm at harvest in comparison to control. Same trend was observed in plants treated with PRD + PBZ @ 2.5 ppm, 2.0 ppm, 1.5 ppm and 1.0 ppm. As evident from Table 2, when the plants were subjected to PRD technique, lycopene and ascorbic acid were found slightly but significantly increased as compared to control. Soumya *et al.* (2017) [25] observed 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) [21] observed 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 *et al.* (2017) [13] found that application of PBZ increased the Vitamin C of *Curcuma alismatifolia* leaves. Pal *et al.* (2016) [20] 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. Jovanovic and Stikic (2018) [12] observed 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) [17] 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. Increased the levels of antioxidant activities in plants under stress conditions are natural responses, which can help plants better tolerate the stress.

Total carotenoids (mg/100 g)

Total carotenoids (mg/100 g) in PBZ treatment were significantly increased @ 2.5 ppm followed by 2.0, 1.5 and 1.0 ppm respectively at harvest in comparison to control. Same trend was observed in plants treated with PRD + PBZ @ 2.5 ppm, 2.0 ppm, 1.5 ppm and 1.0 ppm. As evident from Table 2, when the plants were subjected to PRD technique, total carotenoids in fruits were found slightly but significantly increased as compared to control. The result was in agreement with Reddy *et al*. (2013) [21]. They conducted an experiment on mango with PBZ and found that PBZ application improved quality in mango fruits in terms of total carotenoids. Marjanovic *et al*. (2012) [17] 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.

Table 2: Effect of physical and biochemical approaches on lycopene content (mg/100g), ascorbic acid (mg/100g) and total carotenoids (mg/100g) at harvest on tomato variety Pusa Ruby

Treatments	Lycopene content (mg/100g)	Ascorbic acid (mg/100g)	Total carotenoids (mg/100g)
T_1-Control	1.677	15.20	1.267
T_2-PBZ (1.0ppm)	1.853	18.40	1.293
T_3-PBZ (1.5ppm)	1.947	21.35	1.333
T_4-PBZ (2.0ppm)	2.043	27.14	1.363
T_5-PBZ(2.5ppm)	2.257	31.65	1.407
T_6-PRD	1.857	17.53	1.280
T_7-PRD+PBZ(1.0ppm)	1.950	18.59	1.307
T_8-PRD+PBZ(1.5ppm)	2.147	21.52	1.337
T_9-PRD+PBZ(2.0ppm)	2.173	27.20	1.360
T_{10}-PRD+PBZ(2.5ppm)	2.203	32.65	1.387
CD at 5%	0.033	2.74	0.019
±SE (m)	0.011	0.92	0.006

Number of aphids/three leaves

Significant reduction in number of aphids was in paclobutrazol (PBZ) @ 2.5 ppm (9.00) followed by PBZ @ 2ppm (11.00) respectively at 50, 100 DAT and at harvest (Table 3) in comparison to control (17.75). PBZ when applied @ 1.5 ppm and 1.0 ppm alone, also greatly reduced aphid number at 50, 100 DAT and at harvest. When the plants were subjected to PRD technique, there was a slight, but significant reduction in number of aphids at 50, 100 DAT and at harvest in comparison to control. Same trend was observed in plants treated with PRD + PBZ @1.0, 1.5, 2.0 and 2.5 ppm. Similar pattern has been observed in other studies (Chorbadjian *et al.*, 2011) [7]. They found that PBZ can enhance the resistance of plants to insect pest and effectively reduced the number of aphids because; it is a triazole-type inhibitor of gibberellins biosynthesis with multi-stress ameliorant properties (Soumya *et al.*, 2017) [25].

Yield/plant (kg)

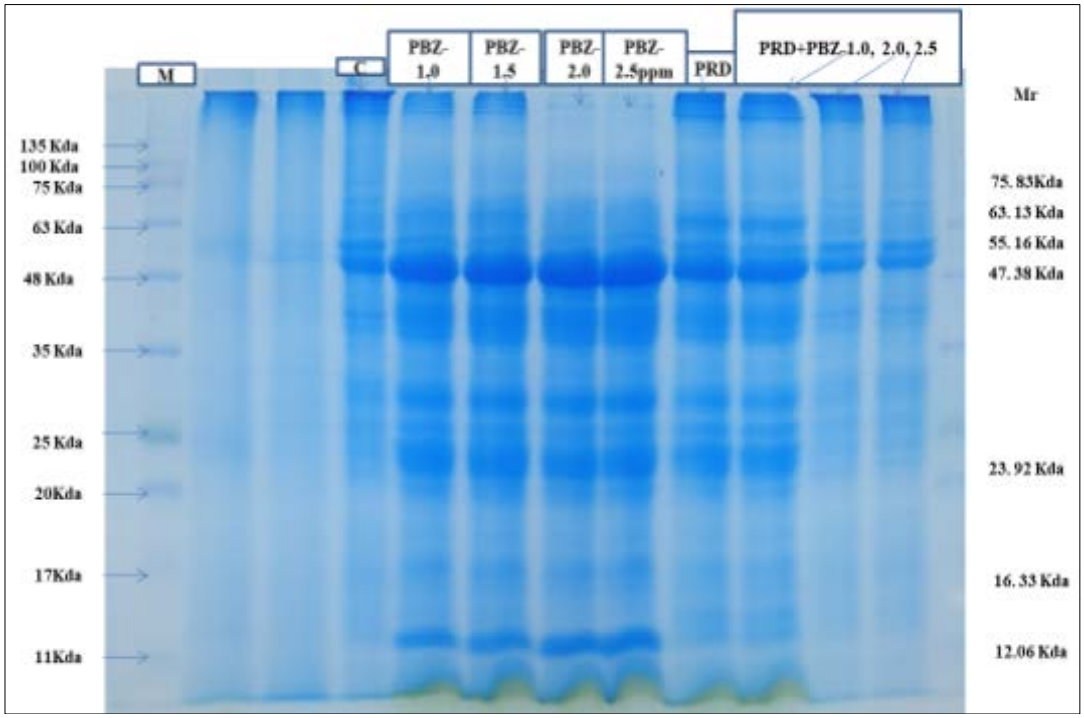
Tomato yield per plant was significantly increased in paclobutrazol (PBZ) treated plants in comparison to control. When the plants were subjected to PRD technique, the yield per plant was non-significant as compared to control. In various combinations PRD+PBZ at 1.0 ppm, the yield increased significantly at harvest in comparison to control and PRD treated plants (Table 3). Similar pattern of high yield in PBZ treated plants has been observed in other studies (Xia *et al.*, 2018) [29]. They noticed that application of paclobutrazol in crop plants would increase the fruit yield. Paclobutrazol is effective in enhancing the yield of several horticultural crops as it inhibits gibberellic acid (GA) biosynthesis which changes the sink-source relationship by reallocating carbohydrates to other organs (Ashraf and Ashraf, 2020) [3]. The yield of canola plant could be significantly improved by paclobutrazol application (Hua *et al.*, 2014) [11]. Ashraf and Ashraf, (2020) [3] reported that the application of PBZ at 1.0 g enhances yield and quality in mango. Paclobutrazol application increased the chlorophyll content which led to greater rate in photosynthesis thereby enhances the yield. When the plants were subjected to PRD technique, the yield per plant was found non-significant as compared to control. Our results were in agreement with Lei *et al.*, (2009) [15]. They suggested that PRD technique improves water use efficiency of crop plants without significant effect on yield.

Table 3: Effect of physical and biochemical approaches on number of aphids per three leaves and yield/plant (kg) in tomato (Pusa Ruby)

Treatments	No. of aphids (per three leaves)			Yield/plant (kg)
	At 50 DAT	At 100 DAT	At harvest	At harvest
Control	17.75	13.50	9.25	1.63
PBZ (1.0 ppm)	14.25	10.75	5.50	2.44
PBZ (1.5 ppm)	12.25	8.75	4.00	2.51
PBZ (2.0 ppm)	11.00	6.00	3.00	2.62
PBZ (2.5 ppm)	9.00	4.75	2.50	2.67
PRD	20.75	15.75	10.75	1.57
PRD + PBZ (1.0 ppm)	18.25	11.75	7.00	2.33
PRD + PBZ (1.5 ppm)	15.50	9.75	5.50	2.48
PRD + PBZ (2.0 ppm)	12.00	5.25	4.00	2.54
PRD + PBZ (2.5 ppm)	8.00	3.00	1.25	2.56
C.D. at 5%	*(0.38)*	*(0.38)*	*(0.31)*	*(0.07)*
± S.E.(m)	*(0.13)*	*(0.13)*	*(0.10)*	*(0.02)*

Protein profiling of all the treatments

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. Fig.3 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 kilo Daltons (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 thicker and intense 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 thicker and more condensed in PBZ treatment. Pal *et al*. (2016) [20] 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 defence 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) [17]. Protein measurement showed that drought stress had no effect on protein content while PBZ treatment significantly increased it. In this study, SDS-PAGE of extracted proteins revealed that PBZ treatments increased some proteins and reduced some other. PBZ treatment increased the accumulation of low molecular weight of proteins, e.g. 12.06 kDa. (Amini *et al*. 2007) [2]. Dani *et al*. (2005) [8] reported that salt and osmotic stresses can increase the expression of stress proteins. This protein has a critical function in plant development under biotic and abiotic stresses (Amini *et al*. 2007) [2].



Lane M for marker, C for Control, PRD for Partial Root Drying, PBZ for Paclobutrazol (1.0, 1.5, 2.0 and 2.5ppm) and PRD+ (1.0, 2.0 and 2.5ppm) PBZ and Mr for relative molecular weight

Fig 3: Effect of PBZ (1.0, 1.5, 2.0 and 2.5ppm), PRD and PRD + (1.0, 2.0 and 2.5ppm) PBZ on SDS-PAGE protein profiling of tomato leaves

Conclusion

Water availability is a limiting factor for growing crop worldwide. Water scarcity impedes plant growth via direct effects on cell division and expansion and perturbs ion balance and induces senescence. Crop plants mitigate adverse effects of drought stress to some extent by the exogenous application of PBZ. PBZ induce several physiological and biochemical alterations that generally lead to morphological modifications with consequent effect on yield in tomato. Hence, it is suggested that the use of PBZ would effectively improve the water use efficiency of crop plants and increase the biomass under water stress conditions in tomato. The effective concentrations of 2.5 ppm of PBZ were found to be the most suitable for the purpose. It helps to maintain relative water content, membrane stability index, and protects the photosynthetic machinery by enhancing the levels of photosynthetic pigments, antioxidant activities and thereby enhances the yield. The study, therefore recommends the use of PBZ to mitigate stressful environment under water stress conditions. Moderate water stress induced osmotic regulation under PRD condition, leading to normal water status of the plants. PRD also gives better performance when applied in combination with PBZ.

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