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ROLE OF SALICYLIC ACID AND PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) TO ENHANCE COLD TOLERANCE IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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Abstract

Better crop productivity is essential to alleviate hidden hunger but due to biotic and abiotic constraints, it is however compromised. During the last decade, revolution in off-season vegetable farming has prompted plant biologists to develop advance lines that can cope adverse biotic and abiotic stress and or probe out advance ecofriendly techniques. This study was aimed to investigate the effects of salicylic acid alone or in combination with PGPR to induce cold tolerance in tomato. Seven treatments along with one control (T0) (untreated) were applied on ten tomato genotypes. *Bacillus cereus* (ATCC 14579) and *Pseudomonas putida* (KX574857) were used as plant growth promoting rhizobacteria. Experiment was performed in open field conditions having temperature ranging between 0-10 ^{0}C. Different biological parameters were investigated like sugars, proline protein, chlorophyll a,b and carotenoids, flavonoids and ascorbic acid content, superoxide dismutase and catalase activities. Genotypes A17860 and A17876 showed 51% increase in plant height over control in T4 (Salicylic acid) and T6 (*Pseudomonas*+ Salicylic acid) during 1st year while genotype A19842 showed 59% increase in plant height, 53 % increase in protein content over control in T6 in second year. Genotypes treated with plant growth promoting rhizobacteria and salicylic acid showed better performance compared with control, although genotypic variations have also contributed to acquiring tolerance.

Key words: Plant growth-promoting rhizobacteria, *Lycopersicon esculentum*, Salicylic acid, Cold tolerance.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the second most significant vegetable crop produced in the world (Wahid *et al*., 2007). Tomato utilization has great significance because of its antioxidant compound that minimizes cancer rates (Wamache, 2005). It is enriched with minerals, nutrients, fundamental amino acids, sugars and dietary filaments also additionally, contains β-carotene, ascorbic acid and phenolics (Van Dam *et al*., 2005; Fajinmi & Fajinmi, 2010). The development and yield of any harvest is influenced by different factors, especially under unfavorable climatic conditions. In such circumstances plant growth regulator assume a significant part to modulate the growth and yield (Tiwari and Singh. 2014). As it is a short duration crop and gives a high return, it is commercially demanding and the area under development is expanding. Better crop productivity is essential to alleviate hidden hunger but due to biotic and abiotic constraints, it is however, compromised (Pardesi *et al*., 2011; Murtaza *et al*., 2022). Among these, PGPRs are ecofriendly growth promoting bacteria having a positive role in developing systemic resistance in plants against many pathogens (Ahmed & Khan, 2011a, 2011b). During the last decade, revolution in off-season vegetable farming has prompted plant biologists to develop advance lines that can cope adverse biotic and abiotic stress and or probe out advance ecofriendly techniques (Adesemoye *et al*., 2008; Ramzan *et al*., 2022). Among sustainable ecofriendly techniques, incorporation of PGPRs (Pardesi *et al*., 2011, Ahemad, 2012). Among sustainable ecofriendly techniques, incorporation of PGPRs enhanced plant growth (Pardesi *et al*., 2011, Ahemad, 2012). These root colonizing microorganisms enhance growth of plant (Bhat *et al*., 2019; Hussain *et al*., 2022 and Wang *et al*., 2019).

PGPRs plays important role in increasing yield and related traits (Piromyou *et al*., 2011). Many of the crop aspects are affected by lower temperature like cell division, water transport, growth, photosynthesis yield and finally survival. The plant enzymatic activity can also be reduced due to the lower temperature (Bukhari *et al*., 2021; Naeem *et al*., 2022). It interrupts the intake of nutrients by the plant because different enzymes are secreted by the plants to digest the materials from the soil surroundings. Subsequently, this also causes the stunted growth, which leads to death. Soil health is also adversely affected by lower temperature due to desiccation that disturb the plant water resource (Ahemad & Kibret, 2014).

Rhizobacteria promote the growth and colonize roots of monocots and dicots. PGPR stimulates the production of phytohormones and boost the adjacent root branches, enlargement of root hairs and develop the plants nutrients (Vacheron *et al*., 2013). PGPR also improve the rate of germination, root development, root and shoot weight, yield, leaf area, chlorophyll, hydraulic conductivity and nutrient mobilization (Sanghera *et al*., 2011). These PGPR are the important components of soil (Asgher *et al*, 2015). They compete for biotic stresses and maintain the soil integrity for sustainable crop productivity. They act as promoter by mobilizing nutrients in soils, producing growth regulators, controlling or minimizing pathogenic attack, improving soil fertility, modifying its structure and maintaining the improving the bioremediation of polluted soils that prevent from the toxic weight metal species (Ahmad & Khan, 2011).

Many of the genes i.e. the nitrogenase-encoding genes *nifHDK*, responsible for nitrogen fixation are activated by PGPRs activities, suggesting that PGPRs may play an impact in supporting plants to cope with cold stress. Sarma *et al.*, (2012) demonstrated that PGRs improve abiotic

stress tolerance especially cold tolerance. It regulates MAPKs (Mitogen-activated protein kinase) in controlling the interactions of defense mechanisms activated (Bruto *et al*., 2014). It is evident that PGPRs at lower temperatures can produce phytohormones that accelerate cell division and growth of plant cell, allowing them to withstand lower temperatures (Kumar *et al*., 2019).

Salicylic acid (SA) is a synthetic plant growth regulator. It is economically viable and the SA induces systemic acquired resistance (SAR) in plants, which in turn results in improved plant growth under pathogen attack (Kumar *et al*., 2019). Furthermore, foliar treatment of salicylic acid at the level of 0.5 mM on tomato plant exposed to 60 mM NaCl effectively preserved growth and the quality traits (Naeem *et al*., 2022).

The current study was aimed to determine effects of plant growth promoting rhizobacteria and salicylic acid alone and in combination in the induction of cold tolerance in genotypes of tomato.

Material and Methods

Sterilization of seeds and inoculation: Ten *Lycopersicon esculentum* Mill. genotypes' seeds were sterilized in 95 % ethanol for 3 to 4 minutes, then the seeds were soaked in 10% chlorox for 2 to 3 minutes with concomitant shaking and subsequently washed 3-4x with autoclaved distilled-water.

PGPR, *Bacillus cereus* ((ATCC 14579) and *Pseudomonas putida* (KX574857) isolated from Khewra salt range obtained from Quaid-e-Azam University, Islamabad was used during the experiment. PGPR Inoculum was prepared in the LB media by inoculating with 24 h old bacterial culture of PGPR and kept in shaking incubator for 72h at 28-30ºC at Biosciences research laboratory, Department of Biosciences University of Wah, Wah Cantt.

Sterilized seeds were placed in bacterial inocula for two to three hours; and the control sterilized seeds were placed in LB broth for the same duration. Seven treatments were made (Table 1).

Random complete block design was applied to current experiment and gross plot size in each treatment was 2.5 x 3.0 m^2. Each plot contained 20 plants maintained at distance of 50, 75 cm, respectively. Plants were grown in National Agriculture Research Centre, Islamabad. Salicylic Acid was applied at 10^5 M. Experiment was performed in open field conditions having temperature ranging between 0-10ºC.

Table 1. Treatments.

Symbol	Treatments
T0	Soaked seeds with autoclaved distilled water
T1	Soaked seeds with LB broth media without any bacterial strain
T2	Soaked seeds with bacterial strain 1 *Bacillus cereus*
T3	Soaked seeds with bacterial strain 2 *Pseudomonas putida*
T4	Control plants with foliar application of PGR, SA
T5	Soaked seeds with bacteria strain 1 + foliar application of PGR, SA
T6	Soaked seeds with bacterial strain 2 + foliar application of PGR, SA

Biochemical parameters

Protein content: Fresh leaf protein content was measured according to Lowey *et al*., *(1951).* With the aid of a mortar and pestle, fresh leaves (0.1g) at vegetative stage were crushed in phosphate buffer before being centrifuged for 15 minutes at 3000 rpm. Na-K tartrate, NaOH, $NaCO_3$, and 1 ml of $CuSO_4.5H_2O$ were all mixed with 0.1 ml solution of supernatant and vortexed for 10 mins. After mixing the 0.1 ml of folin-phenol-reagent, the mixture was incubated for 30-minutes. Folin phenol reagent (0.1 ml) was mixed, and the solution was incubated for 30 minutes. The concentration of protein was determined by the following formula:

$$\text{Protein content mg/g} = \frac{\text{K value} \times \text{Dilution factor} \times \text{Absorbance}}{\text{Weight of sample}}$$

K value = 19.6

Proline content: The Proline content was determined by the method of Bates *et al*., (1973). The leaves were homogenized for 15 min. using mortar and pestle, the extracts were centrifuged at 3000 rpm. 2ml of the supernatant was taken in the test tube, then 2ml of glacial acetic acid and 2ml of ninhydrin reagent was added. Reaction mixture was incubated at 100°C for 1 h. The reaction mixture was cooled and 4ml of the toluene was added. Brick red color appeared. After thorough mixing the toluene layer was separated. The upper layer of reaction mixture was taken, and the absorbance of supernatant was recorded at 520nm against toluene blank. The concentration of proline was determined by the following formula:

$$\text{Proline content (mg/g)} = \frac{\text{K value} \times \text{Dilution factor} \times \text{Absorbance (O.D)}}{\text{Weight of the sample}}$$

K value =19.6

Flavonoids content: Total flavonoids were determined using $AlCl_3$ method of (Zhishen *et al*., 1999). The homogenate prepared in 80% methanol were centrifuged at 3000 rpm for 10 min. $AlCl_3$ reagent was dissolved in 100ml of 80% methanol. 1ml of $AlCl_3$ reagent was added to 2ml of supernatant. After thorough mixing the absorbance was recorded at 430nm against blank. The total flavonoids content was expressed as mg quercetin equivalent per gram of extract (mg QE /g).

Chlorophyll and Carotenoids Content of Leaves: Chlorophyll and carotenoids contents were measured by using the method of Arnon, (1949). Leaves (100mg) were homogenized in 5ml of 80% acetone, incubated for 5 min at 900C in water bath. The extracts were centrifuged at 3000 rpm for 10 min. The OD of supernatant was recorded for chlorophyll a, b and carotenoids contents at 663, 645 and 480 nm against 80% acetone blank respectively.

Chlorophyll a = 12.7 x A663 -2.69 x A645
Chlorophyll b = 22.9 x A645 - 4.68 x A663
Total chlorophyll = (12.7 x A663) + (22.9 x A645)
Carotenoids = 4 × OD × Total sample vol. / Fresh weight of tomato leaves

Ascorbic acid (AsA): Fresh/preserved leaves (0.4 g) was grinded in a mortar and pestle with 4ml of 5% (v/v) trichloroacetic acid. The extract was centrifuged at 4000 × g for 10 min at 40ºC. The supernatants are ready for determination of AsA. The supernatant (0.2mL) was

treated with 1.4 ml of 75mM NaH2PO4 (pH 7.4) and 0.4 mL of 10% (v/v) TCA, 0.4 mL of 44% (v/v) H3PO4, 0.4 mL of 4% (w/v) bipyridyl (dissolved in 70% alcohol), and 0.2 mL of 3% FeCl3 (w/v). The mixture was incubated at 37ºC for 1 h, subsequently centrifuged the samples at 4800 rpm for 5 min. The OD was measured at OD525 nm and AsA concentration was determined by comparison with the standard curve.

Electrolyte leakage: Electrolyte leakage (EL) was measured following the method of Lutts *et al*., (1995). Six randomly chosen plants per treatment (four mature leaves per plant) were taken and cut into 1 cm segments. Leaf samples were washed with distilled water to remove surface contamination and then placed in individual stopper vials containing 10 mL of distilled water. The samples were incubated at room temperature (25°C) on a shaker (100 rpm) for 24 h. The electrical conductivity of the bathing solution (EC1) was read after incubation. Subsequently, the sample was placed in an autoclave at 120°C for 20 min and a second reading of the EC (EC2) was made after cooling the solution to room temperature. The EL was calculated as EC1/EC2 and expressed as the percentage.

Enzymes assays

Superoxide dismutase (SOD): Superoxide dismutase (SOD) was determined following the method of Beauchamp & Fridovich (1971). Fresh leaves (0.2g) was ground in 4ml phosphate buffer (PH7.8), containing 1% PVP centrifuged at 15000g for 15 min at 4°C. The supernatant about 0.8ml was collected in test tube. The reaction mixture contains riboflavin (1.17×10^{-6}), methionine (0.1M), potassium cyanide (2×10^{-5}) and nitroblue tetrazolium (5.6×10^{-5}) dissolved in 3ml 0.05 M sodium phosphate buffer. The reaction mixture (3ml) was mixed with 1ml of enzyme extract. To initiate the reaction one sample was kept in light at 30ºC for 1 h and same sample was kept in dark that served as blank. The absorbance was recorded at 560nm.

Catalase (CAT): Catalase was assayed by measuring the method of Aebi. (1984). 0.5 mL of 75 mM H_2O_2 was added in 1.5 mL of 0.1 M phosphate buffer (pH 7) and 50 µL of diluted enzyme extract in 3 mL reaction mixture. The decrease in absorbance at 240 nm was observed for 1 min and enzyme activity was computed by calculating the amount of H_2O_2 decomposed.

TDS: TDS of samples were measured by standard gravimetric method by evaporating all the water and considering the known volume of water as the weight of the residue (mg) (Atekwana *et al*., 2004).

Statistical analysis

Analysis of variance was employed to analyze the data among treatments by using (SPSS18.0, SPSS Inc. IL., USA.).

Results

Physiological and biochemical characteristics are important criterion to highlight genotypes for better adaptability to adverse environmental conditions and actual insight of genotypic response to treatments.

All the genotypes showed statistically significant differences to all the treatments applied. All genotypes showed higher total dissolved solid (TDS) in T5 (*Bacillus*+ Salicylic acid) compared with all other treatments followed by T4 (Salicylic acid) and T6 (*Pseudomonas* + Salicyclic acid. Genotypes A17865 and A19842 showed least or minimum response while on the other hand, A19860 showed the maximum response. During 2nd year (2020), all the genotypes produced maximum TDS in T3 and T4 that was statistically at par with T6 (*Pseudomonas* + Salicylic acid) (Figs. 1 & 2). Results showed that in all the genotypes was significant increase over control. The genotypes A17860 and A17876 were more responsive to all treatments but response to all the genotypes was maximum in T6 (*Pseudomonas* + Salicylic acid) followed by T4 (Salicylic acid). The genotypes A17865 and A19842 showed least increase to all the treatments (Figs. 3 & 4).

Proline content significantly varied among all treatments and all the genotypes showed variable response to all treatments. All the genotypes showed maximum proline content in T3, T4 (Salicylic acid), T6 (*Pseudomonas*+ Salicylic acid) and T5 (*Bacillus* + Salicylic acid) whereas the minimum proline content was recorded in control treatment during 1st year (2019) and 2nd year (2020), where all genotypes in T6 (Pseudomonas+ Salicylic acid) showed higher proline content followed by T3 and T5 (Figs. 5 & 6). All the genotypes showed maximum flavonoid in T6 (*Pseudomonas*+ Salicylic acid) followed by T5 (*Bacillus* + Salicylic acid) whereas minimum flavonoid was recorded in T2, T3 and T4 treatment during 1st year (2019) and 2nd year (2020) (Figs. 7-8). Superoxide dismutase production was maximum in T6 (*Pseudomonas* + Salicylic acid) followed by T5 (*Bacillus* + Salicylic acid) and T4 during 1st year (2019) whereas minimum superoxide dismutase activity was recorded in control treatment while in 2nd year (2020), maximum value was shown in T5 (*Bacillus* + Salicylic acid) and T6 (*Pseudomonas* + Salicylic acid) (Figs. 9-10). All genotypes accumulated higher ACA content in T6 (Pseudomonas + Salicylic acid) compared with all other treatments followed by T4 (Salicylic acid) and T5 (Bacillus+ Salicylic acid) whereas minimum ACA value was recorded in control treatment during 2019. During 2nd year (2020), all the genotypes produced maximum ACA in T6 while T5 and T4 were statistically at par (Figs. 11 & 12). Results showed that catalase activity was higher in T6 (Pseudomonas+ Salicylic acid) compared with all other treatments followed by T4 (Salicylic acid) and T5 (Bacillus+ Salicylic acid) whereas, minimum catalase activity was recorded in control treatment during 2019. During 2nd year (2020), all the genotypes produced maximum catalase in T6 while T5 and T4 were statistically at par (Figs. 13 & 14).

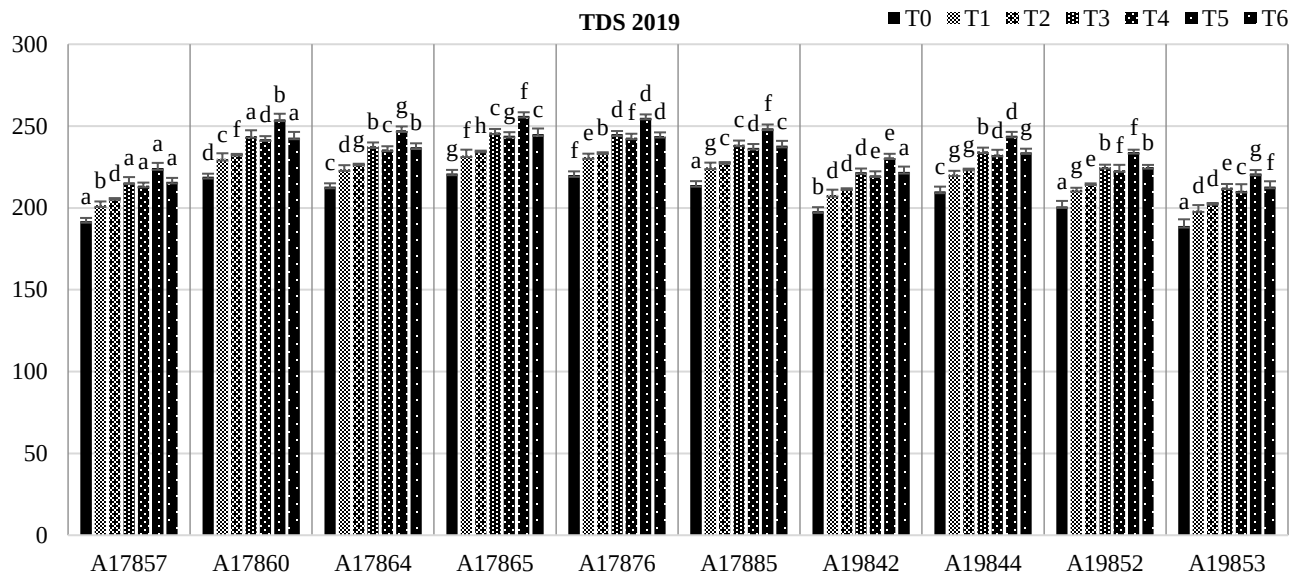

Fig. 1. TDS of 10 tomato genotypes in different treatments of PGPRs during 2019. T0 = Control, T1= Broth media, T2 = *Bacillus*, T3 = *Pseudomonas*, T4 = SA, T5 = *Bacillus* + SA and T6 = *Pseudomonas* + SA.

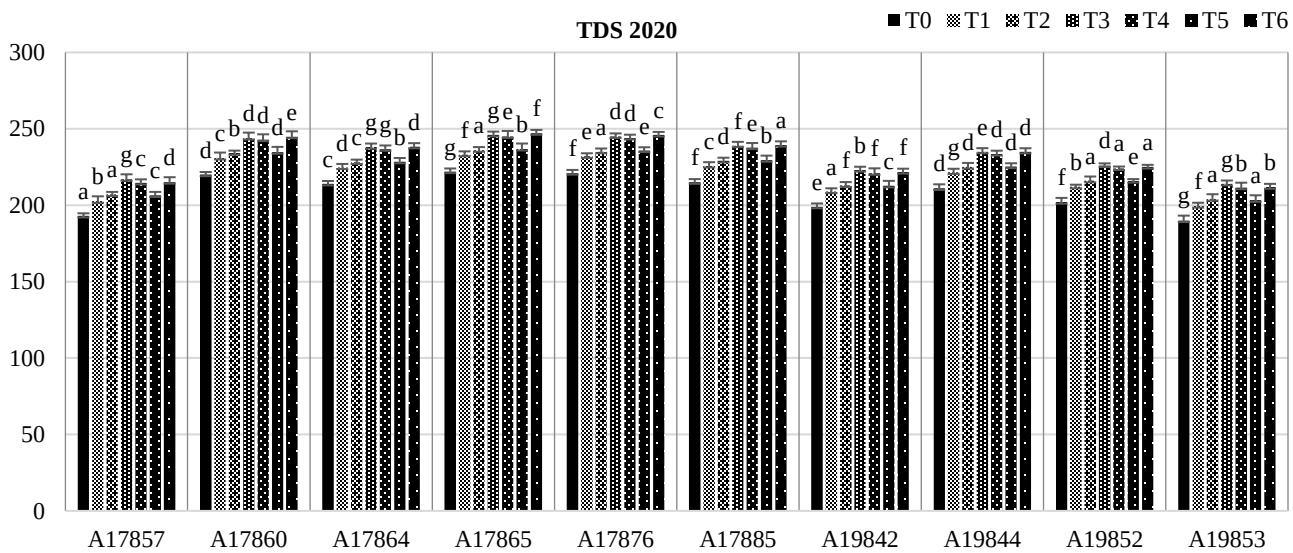

Fig. 2. TDS of 10 tomato genotypes in different treatments of PGPRs during 2020.

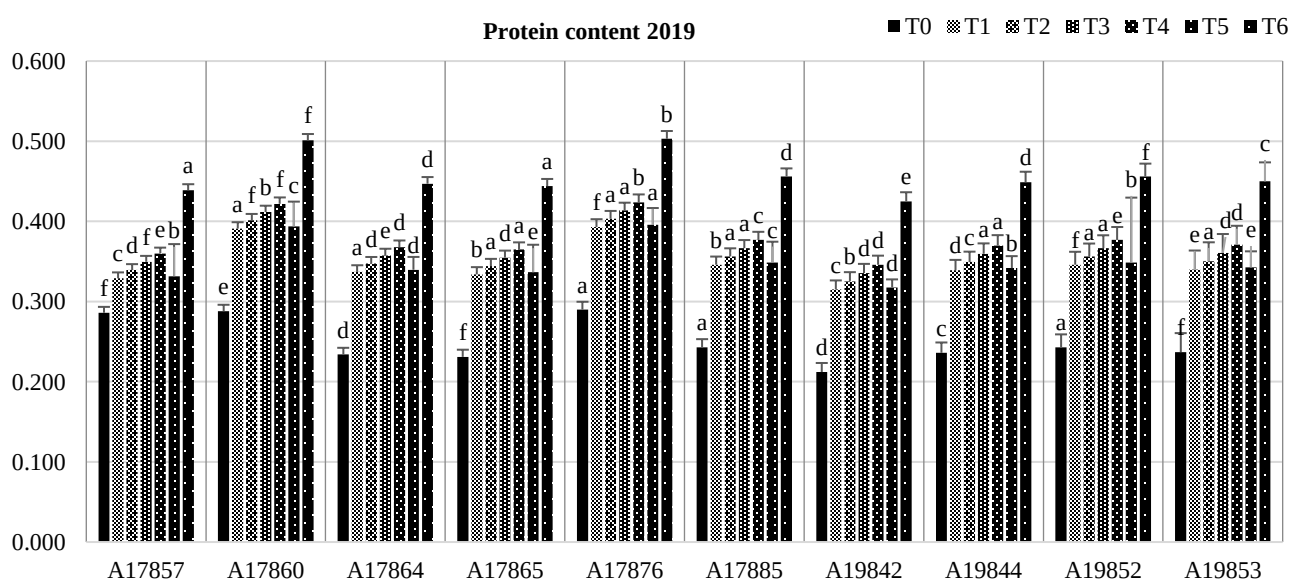

Fig. 3. Protein content of 10 tomato genotypes in different treatments of PGPRs during 2019.

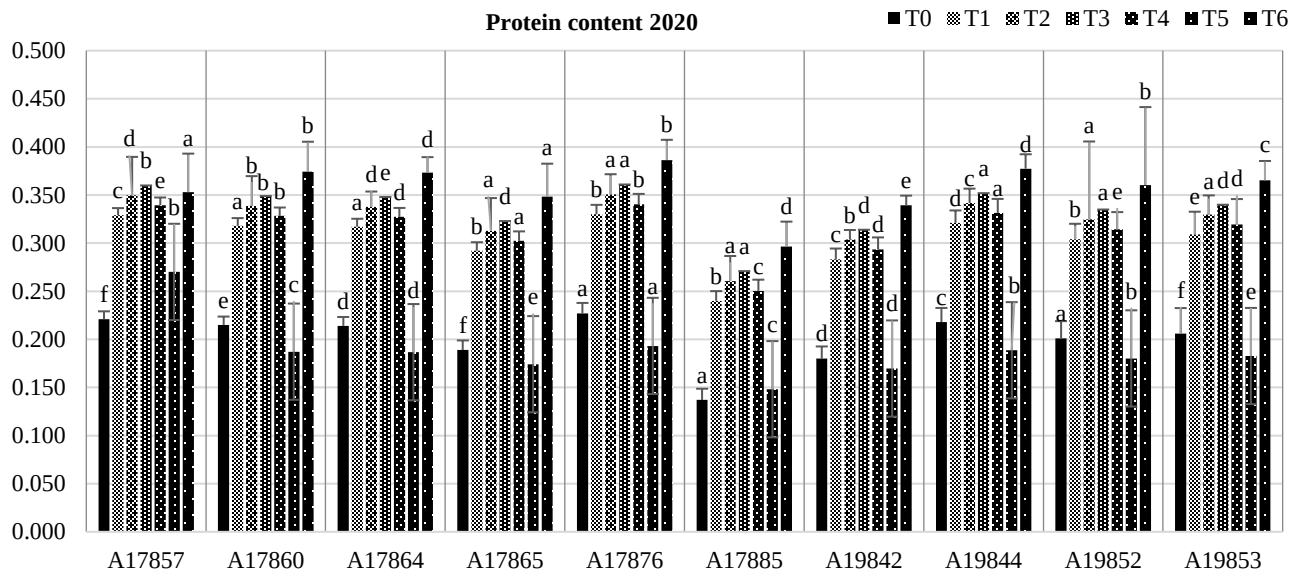

Fig. 4. Protein content of 10 tomato genotypes in different treatments of PGPRs during 2020.

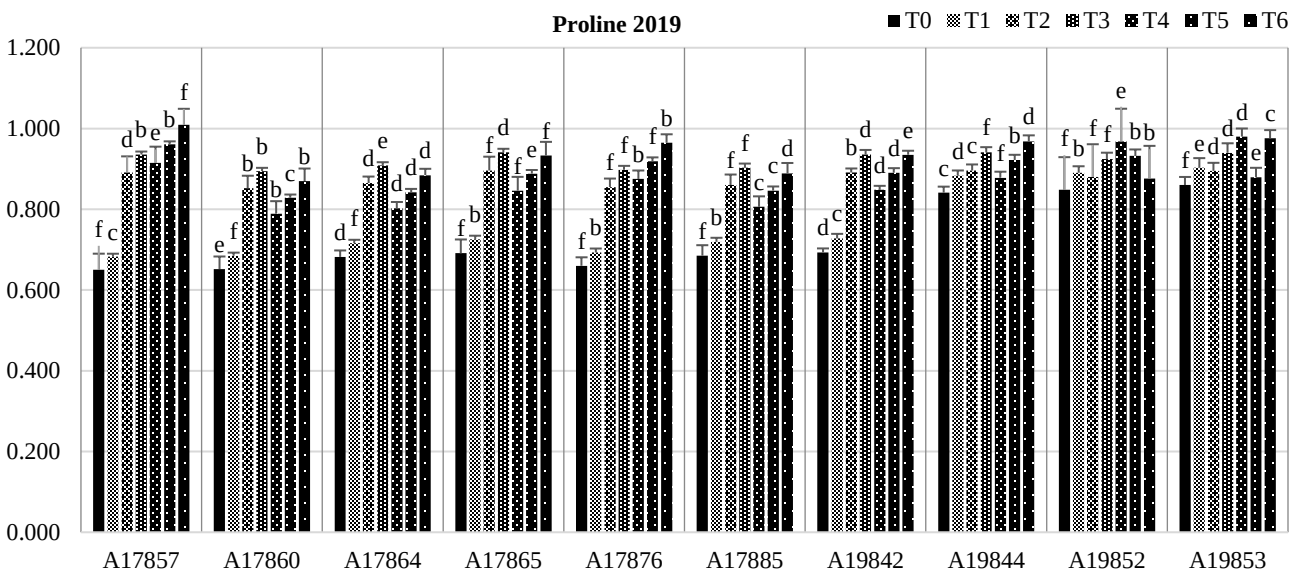

Fig. 5. Proline content of 10 tomato genotypes in different treatments of PGPRs during 2019.

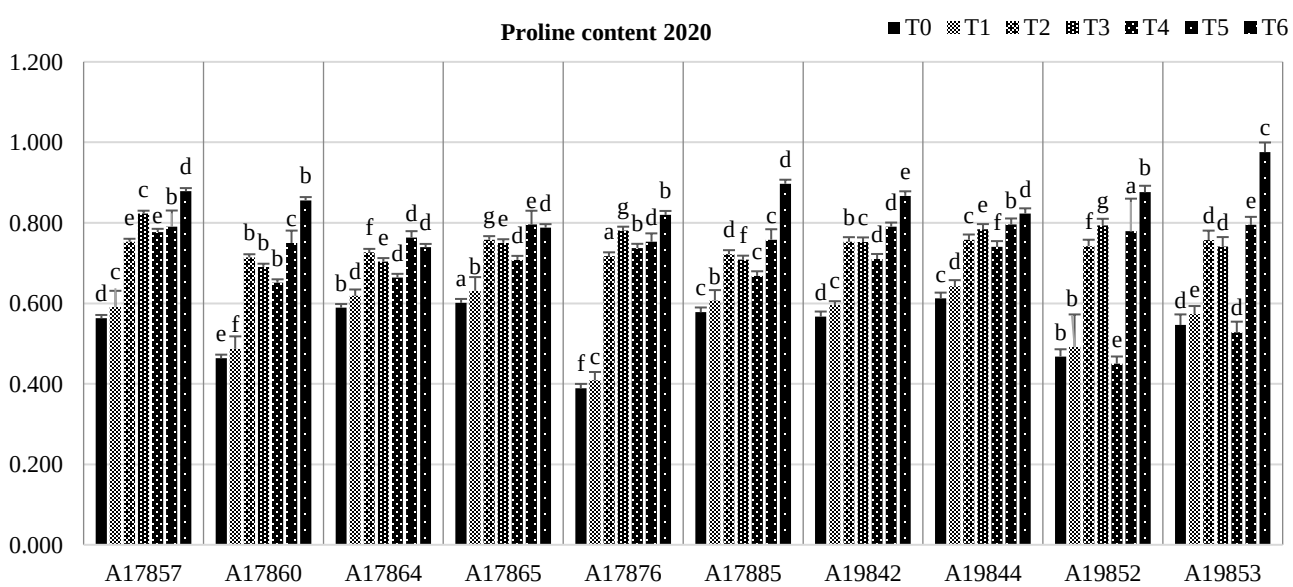

Fig. 6. Proline content of 10 tomato genotypes in different treatments of PGPRs during 2020.

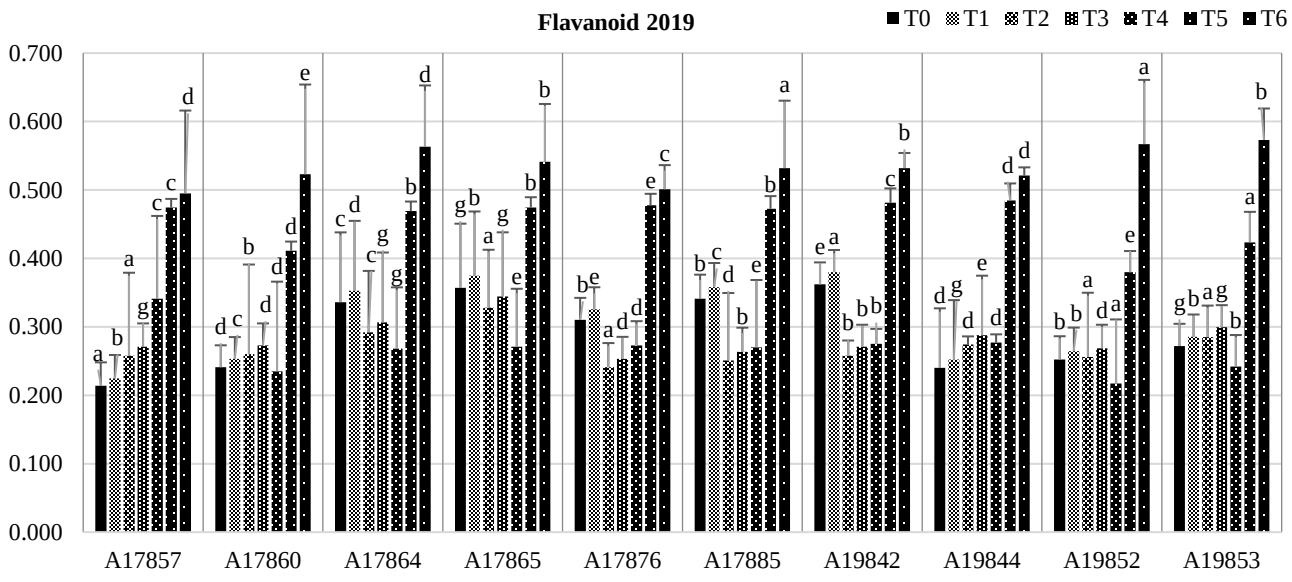

Fig. 7. Flavonoid of 10 tomato genotypes in different treatments of PGPRs during 2019.

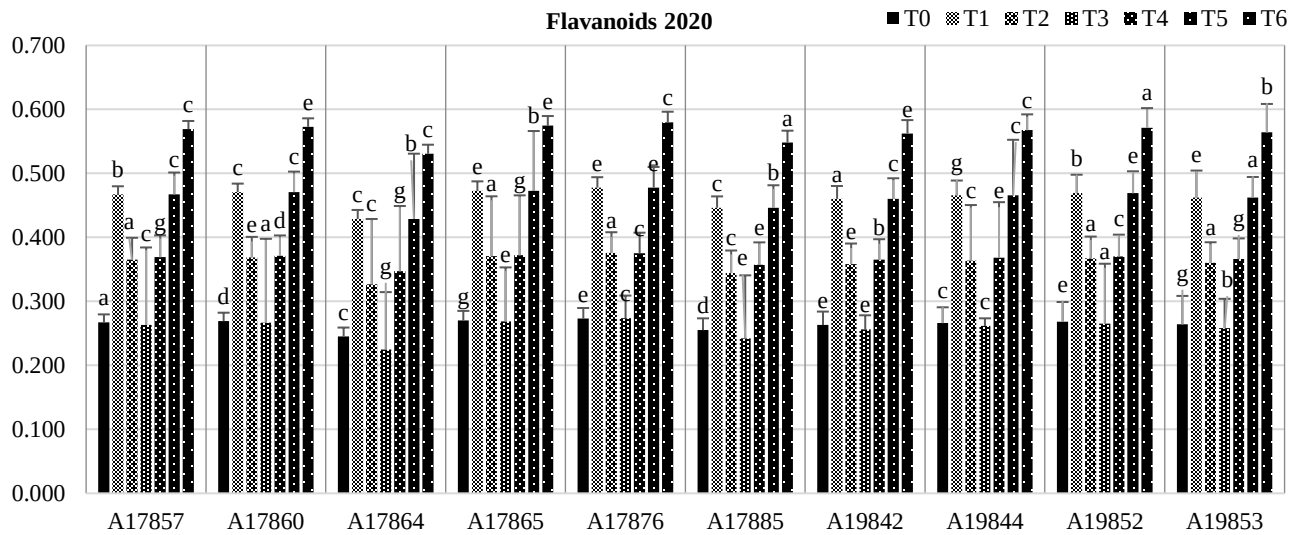

Fig. 8. Flavonoid of 10 tomato genotypes in different treatments of PGPRs during 2020

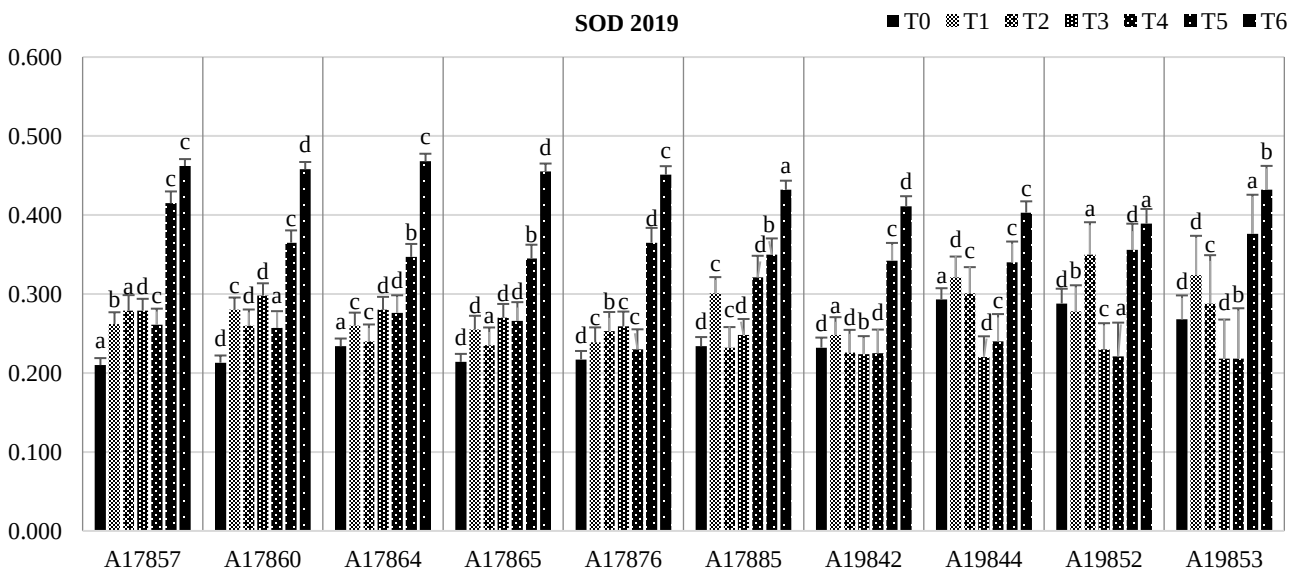

Fig. 9. SOD of 10 tomato genotypes in different treatments of PGPRs during 2019.

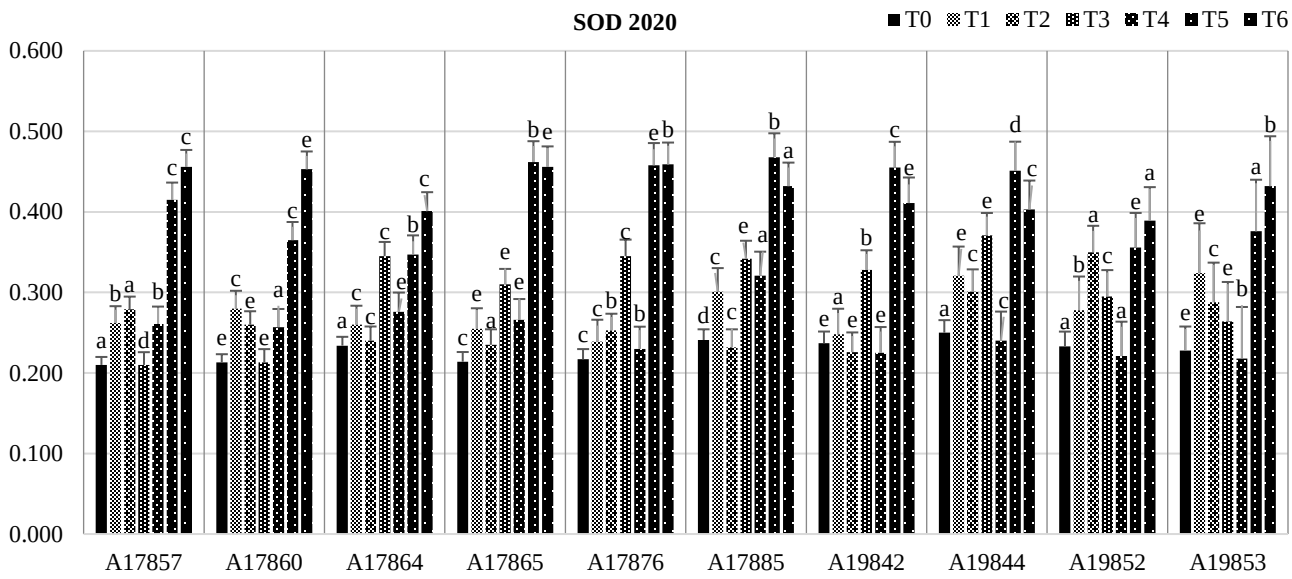

Fig. 10. SOD of 10 tomato genotypes in different treatments of PGPRs during 2020.

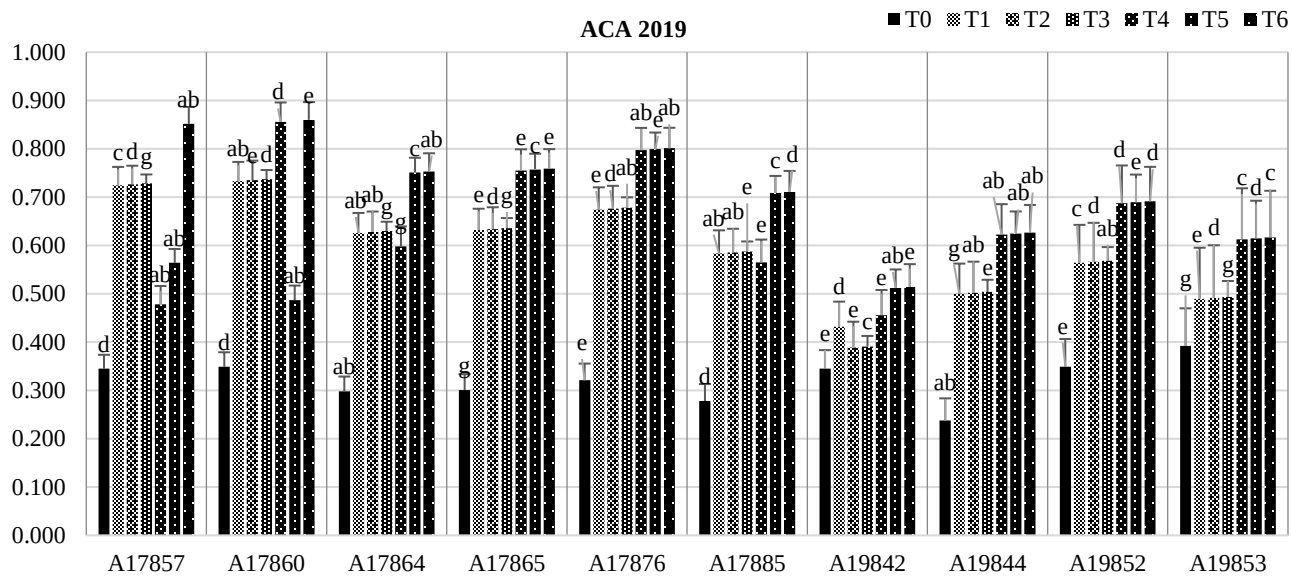

Fig. 11. ACA of 10 tomato genotypes in different treatments of PGPRs during 2019.

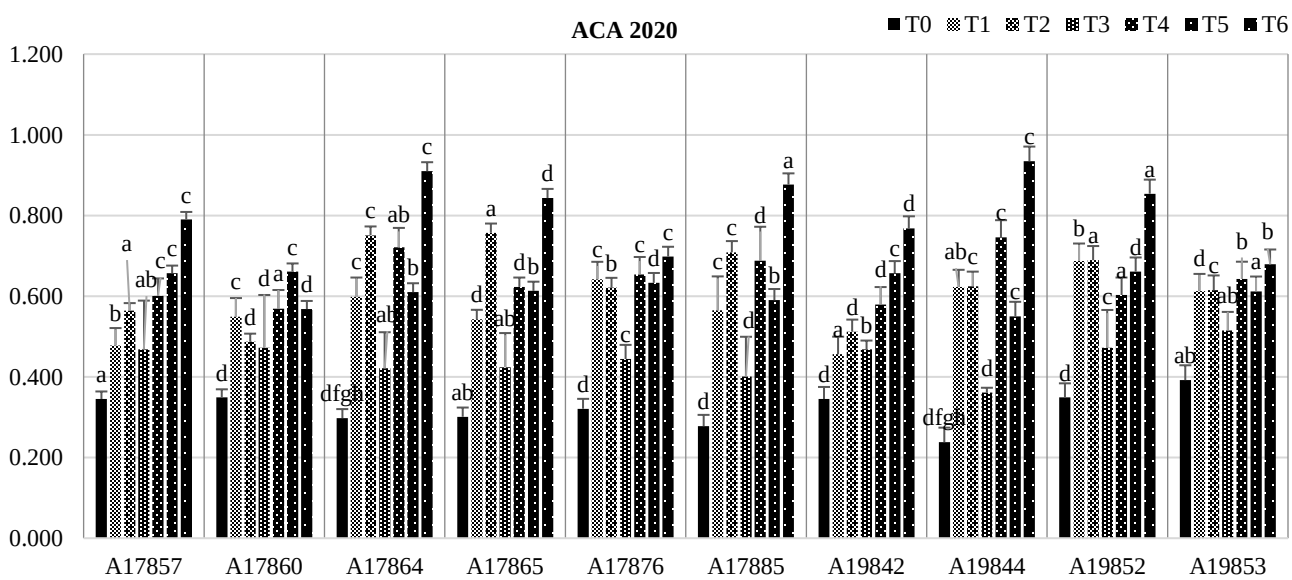

Fig. 12. ACA of 10 tomato genotypes in different treatments of PGPRs during 2020.

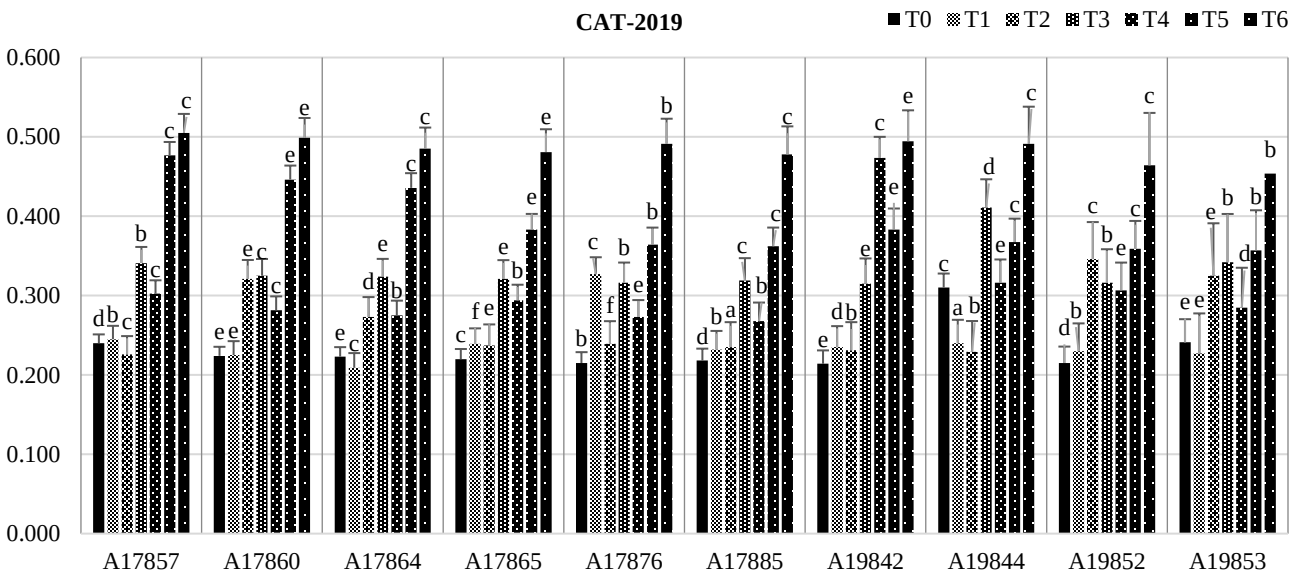

Fig. 13. CAT of 10 tomato genotypes in different treatments of PGPRs during 2019.

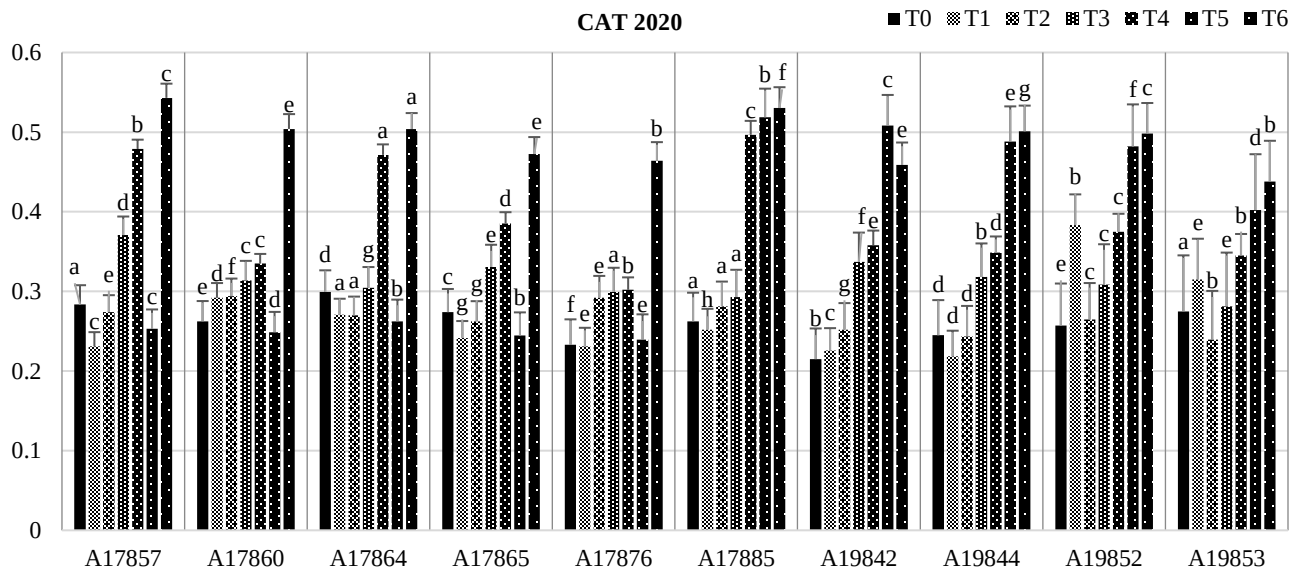

Fig. 14. CAT of 10 tomato genotypes in different treatments of PGPRs during 2020.

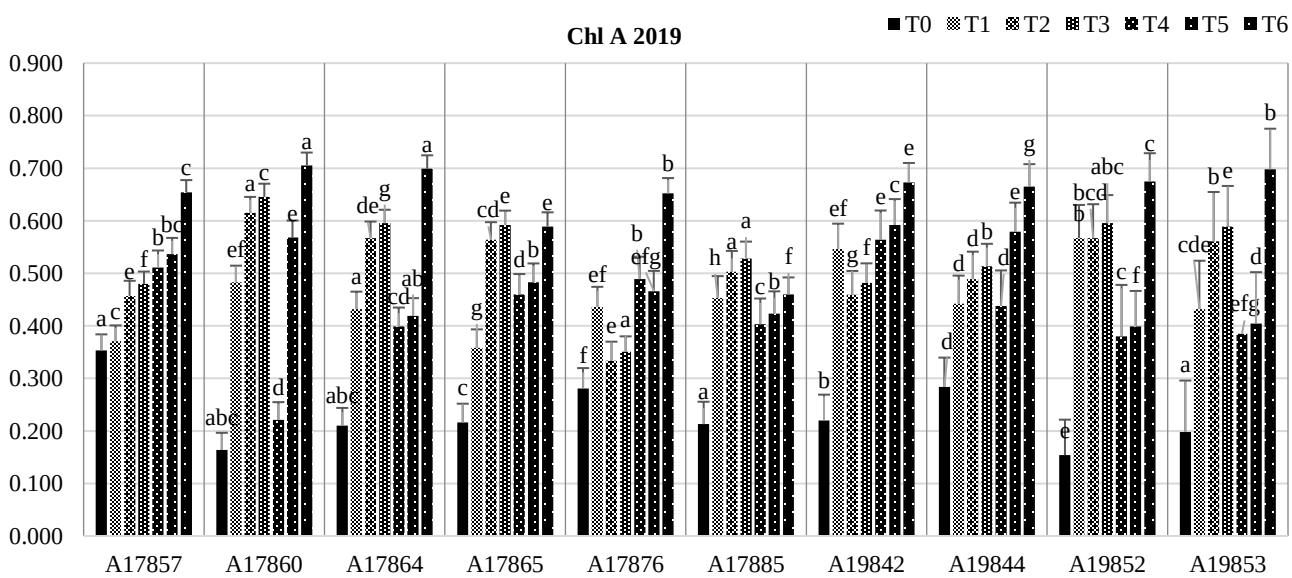

Fig. 15. Chl-a of 10 tomato genotypes in different treatments of PGPRs during 2019.

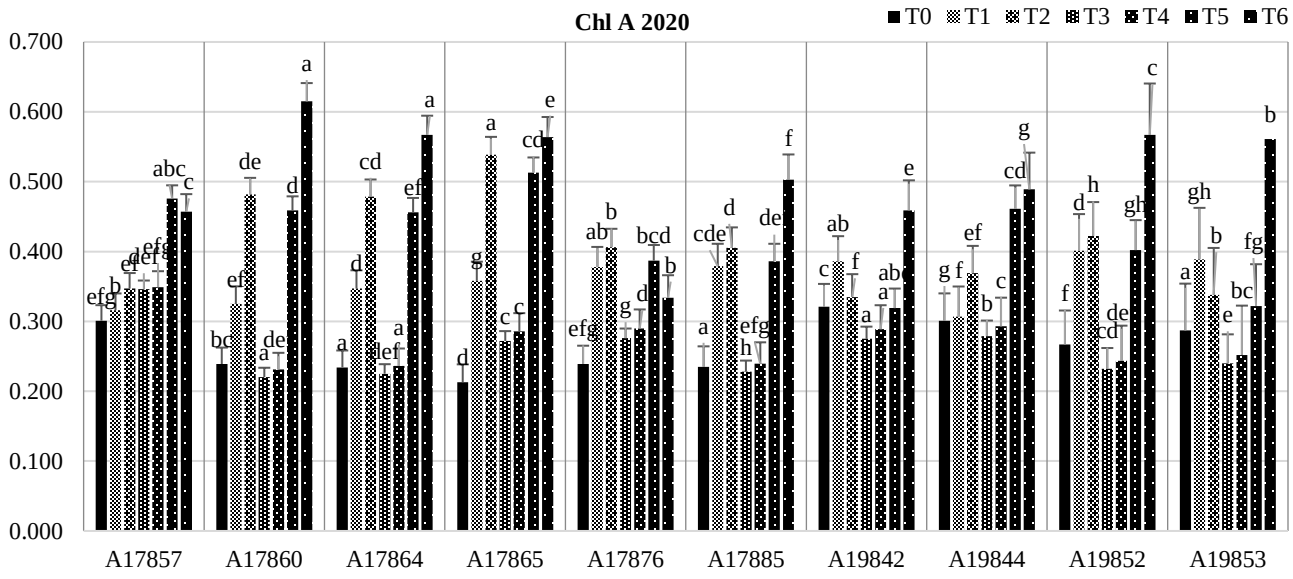

Fig. 16. Chl-a of 10 tomato genotypes in different treatments of PGPRs during 2020.

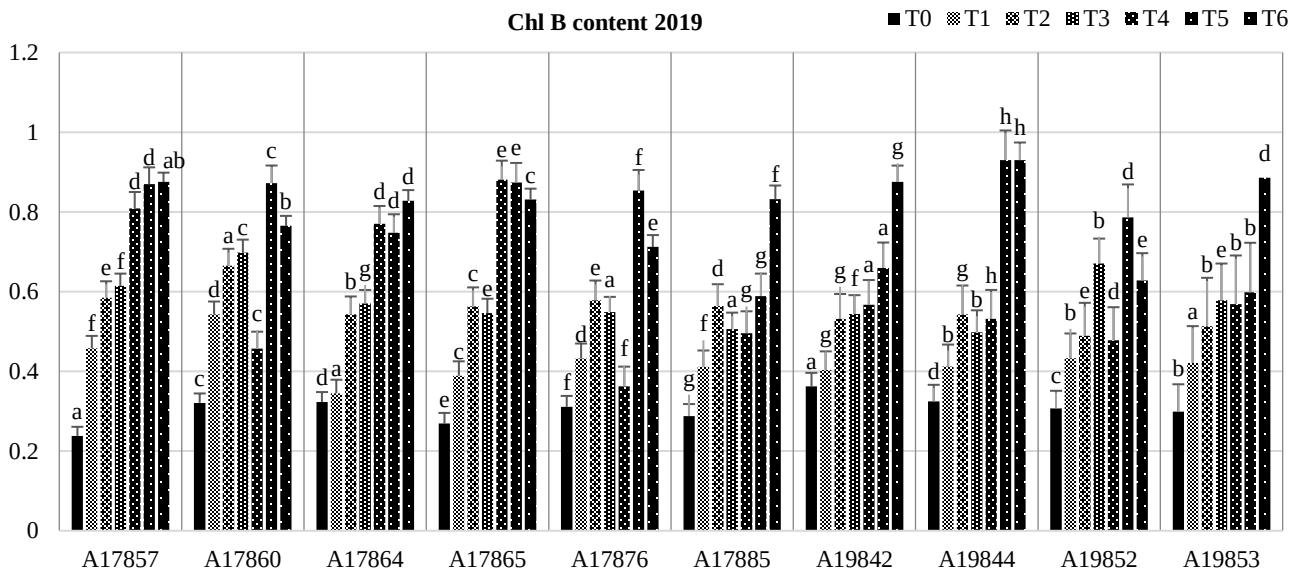

Fig. 17. Chl-b of 10 tomato genotypes in different treatments of PGPRs during 2019.

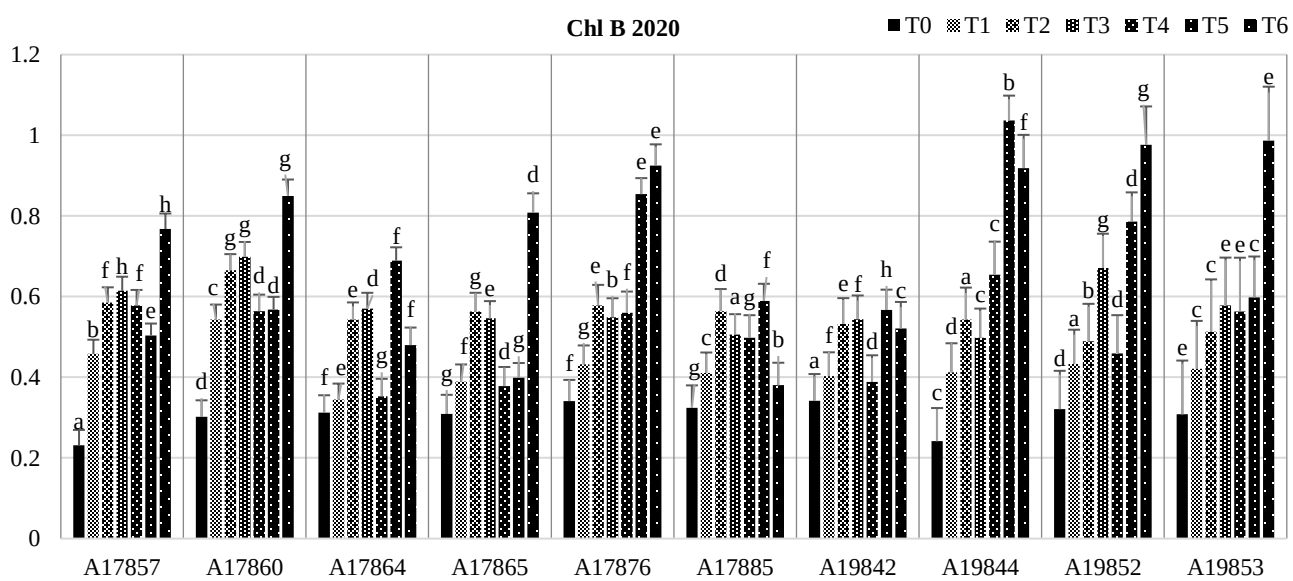

Fig. 18. Chl-b of 10 tomato genotypes in different treatments of PGPRs during 2020.

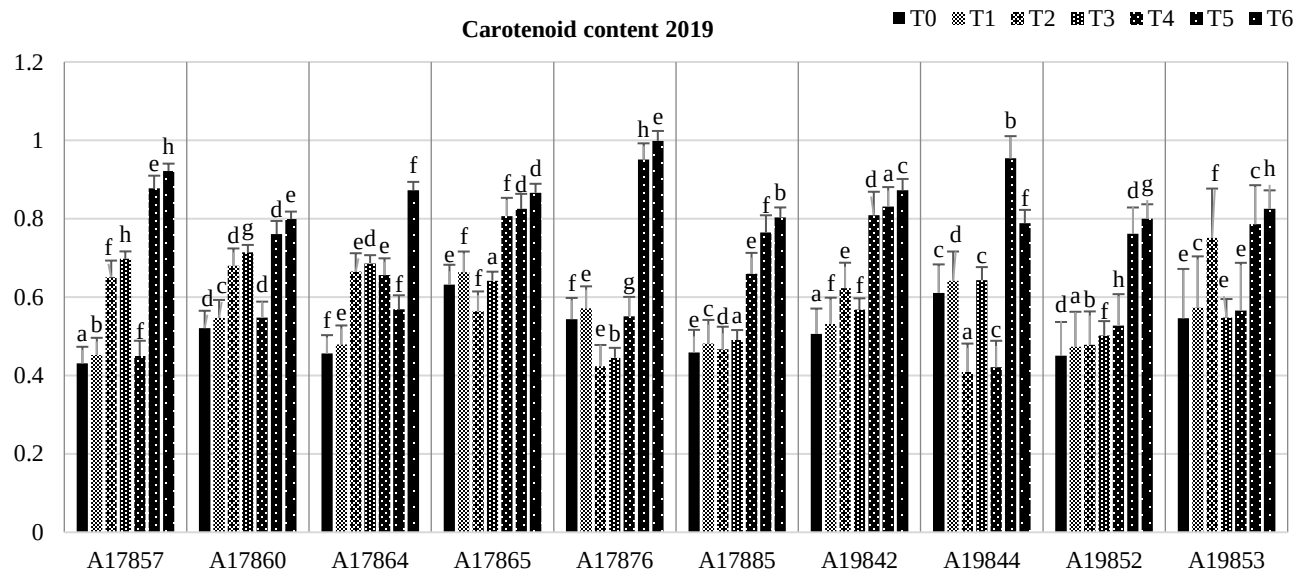

Fig. 19. Carotenoid content of 10 tomato genotypes in different treatments of PGPRs during 2019.

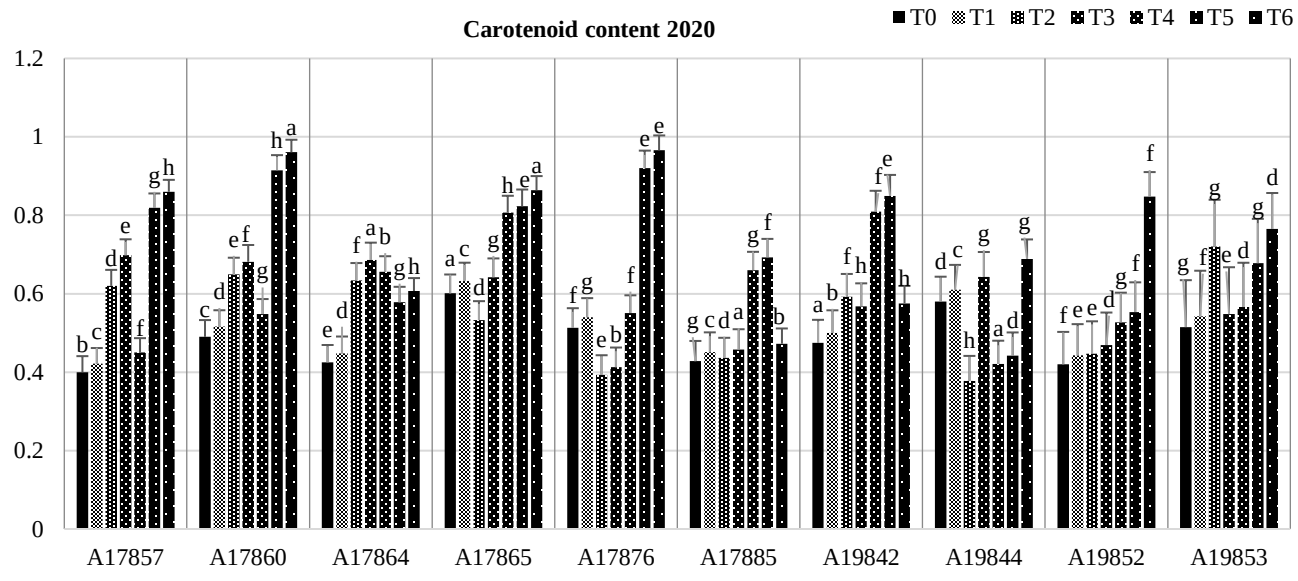

Fig. 20. Carotenoid content of 10 tomato genotypes in different treatments of PGPRs during 2020.

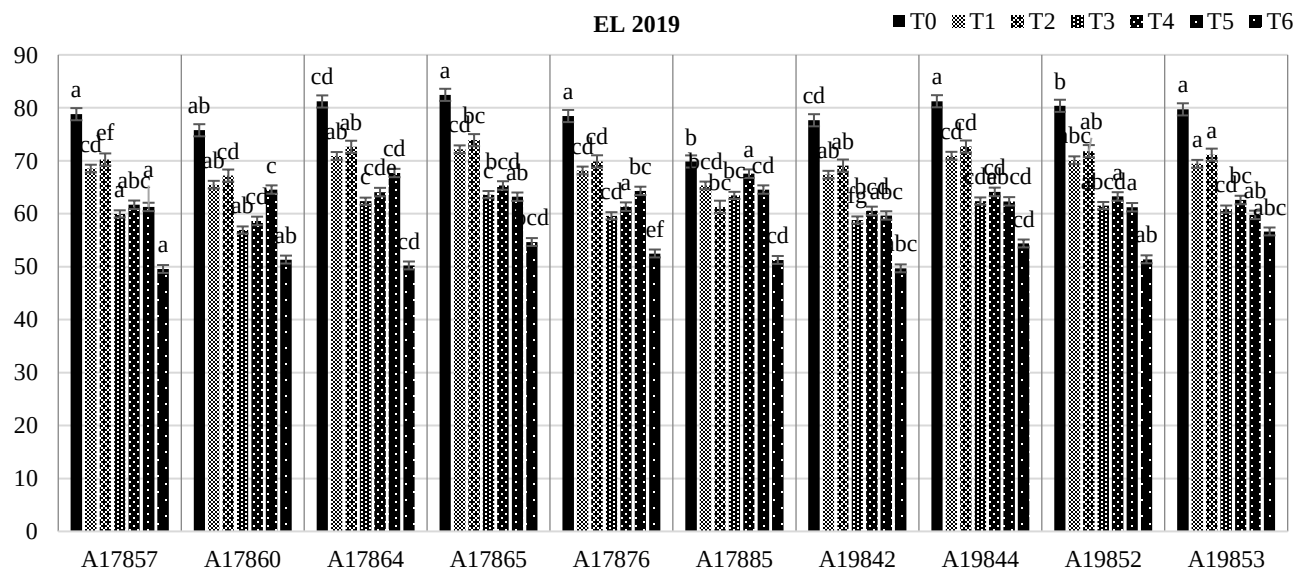

Fig. 21. Electrolyte leakage of 10 tomato genotypes in different treatments of PGPRs during 2019.

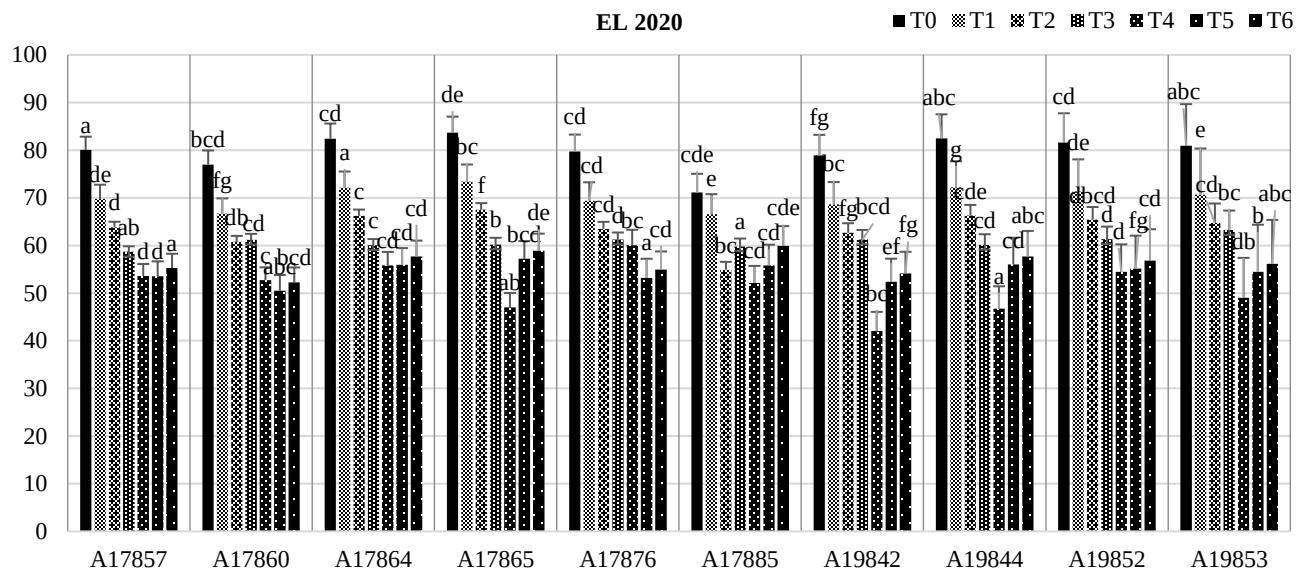

Fig. 22. Electrolyte leakage of 10 tomato genotypes in different treatments of PGPRs during 2020.

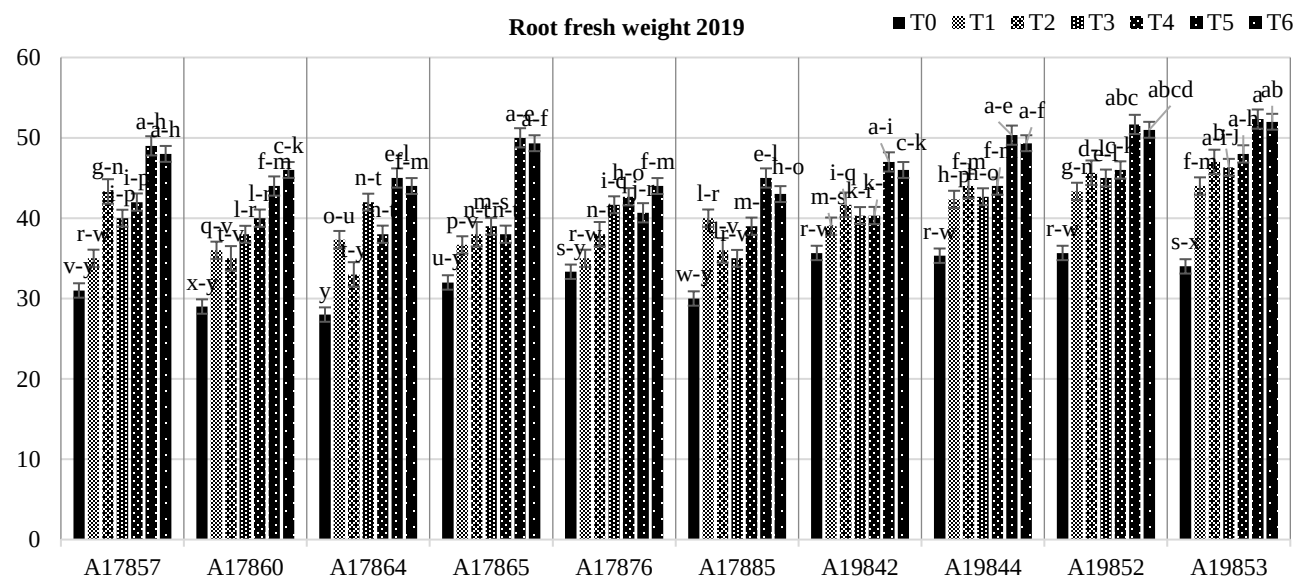

Fig. 23. Root fresh weight of 10 tomato genotypes in different treatments of PGPRs during 2019.

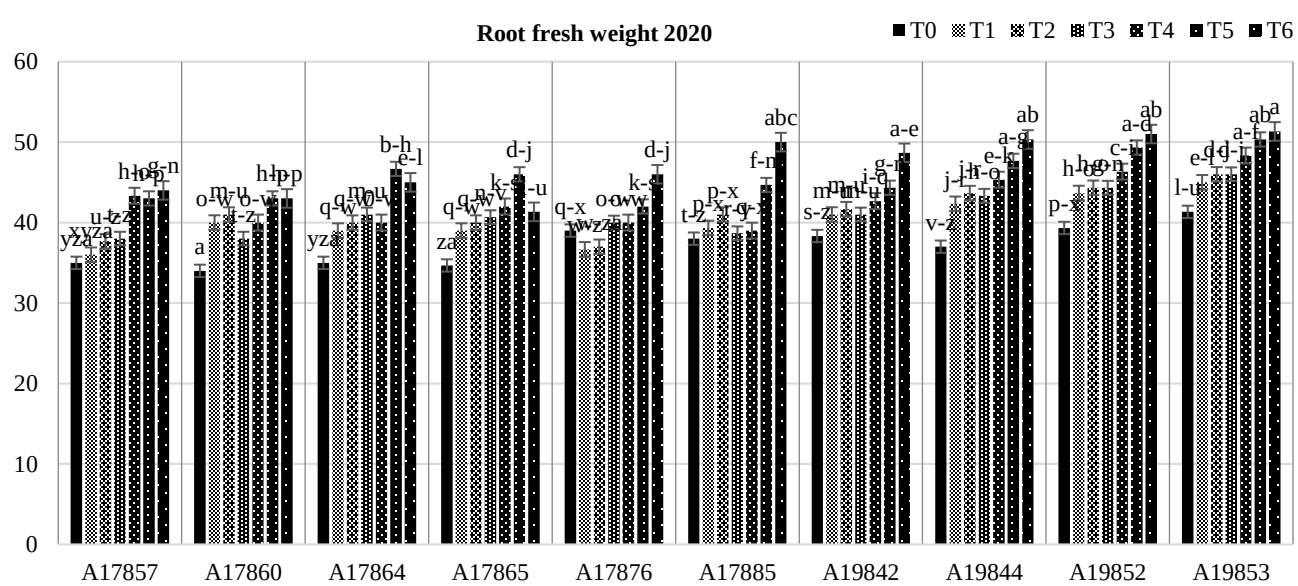

Fig. 24. Root fresh weight of 10 tomato genotypes in different treatments of PGPRs during 2020.

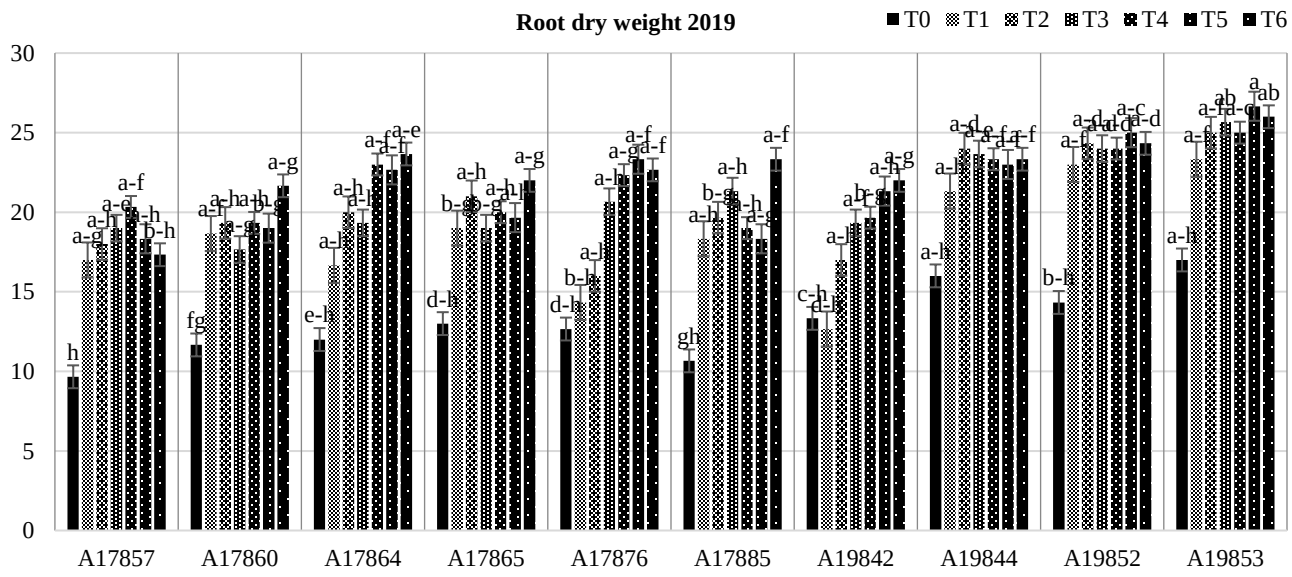

Fig. 25. Root dry weight of 10 tomato genotypes in different treatments of PGPRs during 2019.

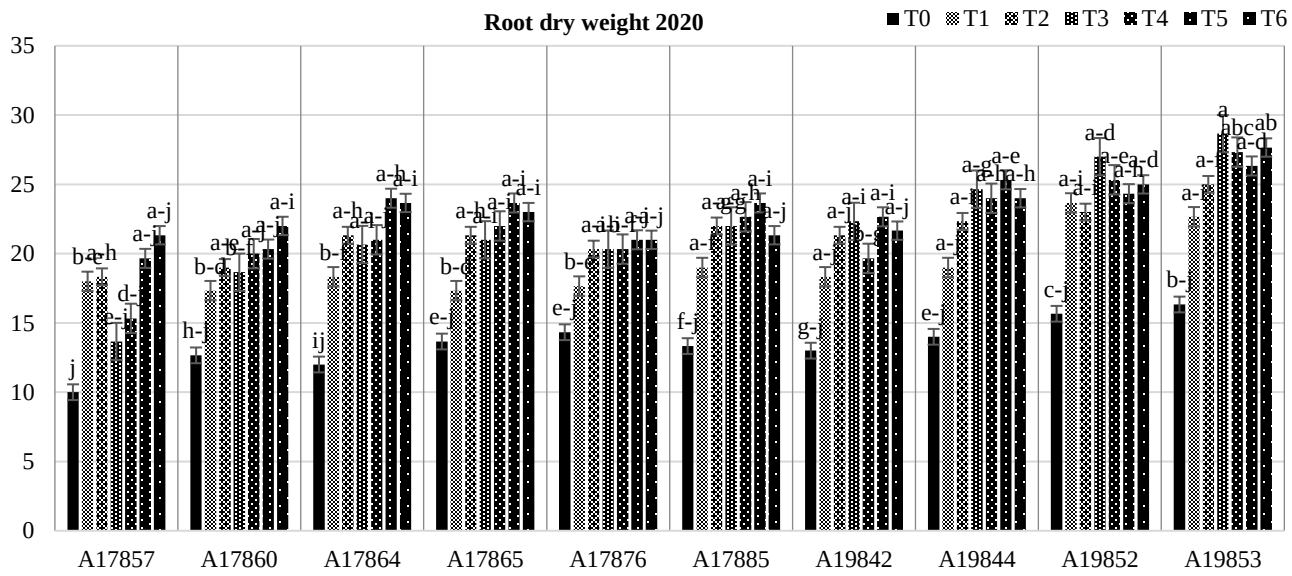

Fig. 26. Root dry weight of 10 tomato genotypes in different treatments of PGPRs during 2020.

Results showed that the content of chlorophyll-a and b were improved in all treatments compared with control. All genotypes produced higher chlorophyll-a & b in T6 (Pseudomonas+ Salicylic acid) followed by T5 (*Bacillus*+ Salicylic acid) and T4 (Salicylic acid) whereas minimum chlorophyll-a &b was recorded in control treatment during 2019 and 2020 (Figs. 15, 16, 17 & 18). Results showed that carotenoid content was improved in all treatments compared with control. All genotypes produced higher carotenoid content in T6 (*Pseudomonas*+ Salicylic acid) followed by T5 (*Bacillus*+ Salicylic acid) and T4 (Salicylic acid) whereas, minimum carotenoid content was recorded in control treatment during 2019 and 2020 (Figs. 19 & 20). Results regarding electrolyte leakage show that maximum electrolyte leakage was observed in T0 (control) while lowest electrolyte leakage was shown by all genotypes in T6 (*Pseudomonas*+ Salicylic acid) followed by T5 (*Bacillus*+ Salicylic acid) and T4 (Salicylic acid) during 2019 and 2020 (Figs. 21 & 22). In 2020, the maximum root fresh weight was recorded in T6 (*Pseudomonas*+ Salicylic acid) followed by T5 (*Bacillus*+ Salicylic acid) (Figs. 23 and 24). Similarly, the maximum root dry weight during 2019 was observed in T5 (*Bacillus*+ Salicylic acid) followed by T6 (*Pseudomonas*+ Salicylic acid) while the minimum root dry weight was recorded in control T0 treatment (Figs. 25 and 26).

Discussion

All the genotypes showed variable response to all the treatments. Findings enclosed both PGR and PGPRs enhanced the quality of fruit by enabling plants to cope chilling stress. The current study demonstrated that T6 (*Pseudomonas*+ Salicylic acid) was proved better among all treatments followed by T5 (*Bacillus*+ Salicylic acid) and T4 (Salicylic acid) in all the tested genotypes. Genotypes A17865 and A19842 showed least or minimum response while on the other hand, A19860 showed the

maximum response following the treatments T4 (Salicylic acid), T5 (*Bacillus*+ Salicylic acid). Compared with all other treatments followed by T6 (*Pseudomonas*+ Salicylic acid. Use of rhizobacteria have potential of producing Indole acetic acid (IAA) (Ul Hassan & Bano, 2015). Applications of the SA affected all growth process in the tomatoes varieties as in the previous studies (Wani & Khan, 2010). SA stimulated the growth of the tomatoes varieties and enhanced their resistance in harsh environments (Dong *et al*., 2011; Saleem *et al*., 2020). It also promoted the growth of the PGPR in the soils and promoted the activities of the useful microorganisms (Pastor *et al*., 2013).

PGRs control stem elongation and produce compact plants. Triazoles, control plant height and produce compact plants (Schluttenhofer *et al*., 2011). Isah *et al*., (2019) demonstrated that the strength and concentration of plant growth regulators (PGRs), genetic makeup of any variety and growing conditions affect floral initiation and development. Results of present study was correlated with Raskin *et al*., (1990) who claimed that plant initiating early flowering showed higher concentrations of SA.

SA also induces the immunity in the different crops. It is also concluded the abiotic stresses are involved in the cellular signaling of the many plant varieties as in tomatoes (Tucuch *et al*., 2017). SA stimulated the growth parameters of the tomatoes varieties and enhanced the resistance in harsh environments (Dong *et al*., 2014; Saleem *et al*., 2020). It's promoting the growth of the PGPR in the soils, also increases the biological process and promotes the activities of the useful microorganisms (Khamar *et al*., 2016; Kosovo *et al*., 2012; Wu *et al*., 2019).

Increase in the chlorophyll and carotenoids contents within the leaves of PGPR- and SA treatments may be attributed to greater availability of nutrients and improved organic substances in the rhizosphere (Esitken *et al*., 2006; Nadeem *et al*., 2007). Numerous reviews demonstrated that PGPR booster speed up the photosynthesis in flora under stress condition (Kohler *et al*., 2009; Bhattacharyya & Jha, 2012; Heidari & Golpayegani, 2012). Useful outcomes of foliar application of SA on growth of plant, chlorophyll content and accumulation of mineral nutrients under saline environment have been reported previously (Yildirim *et al*., 2008).

Findings of this study showed that stress exposed plants showed increased electrolyte leakage. Tolerant plants showed lesser electrolyte leakage compared with sensitive plants. This shows membrane integrity of plants under stress.

Finding showed that PGR and PGPRs treated plants exhibited an increase in root fresh and dry weight due to *Pseudomonas*+ Salicyclic acid and *Bacillus*+ Salicyclic acid while decrease weight was observed in Control plot with PGR and PGRs. Increased root weight due to PGRs was also reported by Jilani *et al*., (2012). Cato *et al*., (2013) conducted a novel study to investigate the effects of different chemicals on the tomatoes. They found that roots were also affected due to SA. Chauhan *et al*., (2017) also investigated the effects of different chemicals on the tomatoes. Achard *et al*., (2009) found that many of the growth parameters were affected due to chemical treatment of the soil applications of the SA to the tomato crops.

Conclusion

It is inferred from the present investigation that both the PGPR as well as SA favorably affected the growth and biochemical parameters of tomato exposed to low temperature stress. But it is evident that the combined treatment of PGPR and SA were more effective particularly SA + *Pseudomonas* treatment significantly improved the TDS, ASA and also the osmoregulant, proline production. The PGPR and SA treatment imparted low temperature tolerance to tomato via reducing electrolyte leakage increasing the flavonoids contents and enhancement of the activities of antioxidant enzymes. The combined treatment of SA with PGPR is an ecofriendly cost effective method recommended for inducing cold tolerance to plants.

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