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DPPH (2,2-DIPHENYL-1-PICRYLHYDRAZYL) FREE RADICAL SCAVENGING ACTIVITY OF TOMATO GENOTYPES AGAINST PEG SIMULATED DROUGHT STRESS

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Abstract

Antioxidants are important substances of plants which are used to save their bodies from injuries by free reactive oxygen species. The experiment was performed to check the effect of polyethylene glycol on 26 tomato genotypes at ESMA (Extension Services and Management Academy) at Garri Dopatta, Azad Kashmir, Pakistan. The 2,2-Diphenyl-1-Picrylhydrazyl was used as an oxidant to produce the free radicals. The antioxidant behavior was found to be increased by increasing the sample concentration ranging from 25 µl to 500 µl extract concentration and decreased by increasing the Polyethylene glycol concentration. The genotype G-21-006234 showed highest antioxidant activity 53.03% at 25 µl sample extract concentration and radical scavenging activity has enhanced up to 71.54% as sample concentration was increased up to 500 µl. The activity of antioxidants was declined with an increase in polyethylene glycol concentration. Genotype G-21-00643 showed 21.189% antioxidant activity at 12.5% of polyethylene glycol. Which showed G- 31- 19289 genotypes had 52 % at 25ul sample amount at control and 39% at maximum of polyethylene glycol concentration. The results indicate that genotype G- 31- 19289 is good among the studied genotypes in antioxidant behaviour.

Key words: Tomato, Drought, Polyethylene Glycol, DPPH, Antioxidant.

Introduction

Drought is among the environmental stresses which affect the plant growth and development and are responsible to lower down the yield of the crops as well. The low soil water availability affect the internal water contents which slow down the physiological and biochemical functions of the palnt. The plants change the cellular activities by producing different defence mechanisms in response to water stress (Bohnert & Jensen, 1996).

Tomato (*Solanum lycopersicum* L.) is one of the crops in the world and important constituent food of common people (Ferrari *et al.*, 2008). The tomato is ranked higher due to its economic importance as well as its biochemical constituents like its antioxidant activity due to vitamin C and lycopene and its carotenoid contents (Abdel-Monaim *et al.*, 2012).

This crop is very sensitive to drought stress at an early growth period and the most effected stage of the tomato plant is the maturation stage (Munns, 2002). The low quantity of the water in the soil effect the rate of photosynthesis which eventually decreases the leaf size, fruit size and fruit number of tomato plant (Dodds *et al.*, 1997).

The stress tolerance is correlated with the higher amount of antioxidants which can check the cell decay (Ünyayar *et al.*, 2005). PEG is usually applied to induce the stress in crops, is harmless and unable to enter into cell and ultimately lowers the available moisture of the loam. The tomato genotypes were secreened and selected under PEG simulated drought stress by using multivariate analysis (Fakhira *et al*., 2014).

The photosynthesis and respiratory processes during the drought stress transfer high amount of electrons to the O_2 which increases the amount of reactive oxygen species (ROS) (Sánchez-Rodríguez *et al*., 2012). The inhibition of photosynthetic processes is caused by the ROS which also damage the different types of cellular structures and macromolecules (Smirnoff, 1993). Membrane lipids are directly attacked by the ROS which inactivate the metabolic enzymes, damage the nucleic acids that causes the ultimate cell death (Gill & Tuteja, 2010; Mittler, 2002). The formation and use of ROS is firmly regulated by antioxidants during normal environmental conditions while during drought stress conditions the number of ROS generally exceeds the number of antioxidants which are the source of the oxidation (Noctor & Foyer, 1998). The Vitamin C and phenols is the main free radical scavenger of tomato. The antioxidant system of the tomatoes is controlled by the genetic, environment and the maturing phase of the plant (Hallmann, 2012; Violeta *et al*., 2013). The present study was conducted to examine response of 26 genotypes of tomato to drought stress. Because, the area of Azad Jammu and Kashmir lacked the canal system for irrigation of fields and only depends upon the rain water for crops. So it is important to screen the genotypes of tomatoes which can tolerate the low water availability in the soil.

Materials and Methods

The pot experiment was conducted in the green house of ESMA (Extension services and Management Academy) Garri Dopatta Azad Jammu & Kashmir Pakistan, using the seeds of 26 genotypes of tomato viz., G7-10593, G11-17895, G12- 17880, G26- 19293, G28-17903, G31-19289, G32-19223, G37-19895, G38-19896, G49-19889, G44-19911, G43-19907, G46-19913, G47-6231, G8-19219, G2-006233, G3-10574, G4Lo-4360, G5-017904, G6-017909, G7-88507, G11-Lo3715, G12-08527, G21-006234, G31-006234 and G45-9219 to evaluate the effects of Poly ethylene glycol (PEG 6000) induced drought on the antioxidant activities of these genotypes. The seeds of all these genotypes were

provided by the National Agriculture Research Centre (NARC) Islamabad Pakistan. The 390 earth pots of 39cm high and 20cm of diameter filled with 2 kg of soil in each. The experiment was performed in Complete Randomized Block Design (CRBD) with three replicates. The soil used for the experiment was composite. Equal amount of farm yard manure was added to the plants. Seeds were sown in pots and 30 days old seedlings were transplanted in the pots. The Polyethylene glycol (PEG) was used in five treatments which include; 0%, 5%, 7.5%, 10% and 12.5% and repeated after every seven days. The fresh leaves of the plants were collected at the flowering stage.

The antioxidant activity was tested by using the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) *In vitro* (Ferreira *et al.,* 2007). Briefly 0.25 mM solution of DPPH radical (0.5 ml) was added to the sample solution in ethanol (1 ml) at a concentration (300 µg/ml). The combination of both was shaken and put at room temperature for half an hour in the dark and absorbance was measured spectrophotometerically at 517 nm. Inhibition Percentage was calculated by comparing with the control and standard was gallic acid. The scavenging activity was calculated using the equation % = (Ao-A1)/Ao) ×100. Ao is the absorbance of the control reaction and A1 is the absorbance of the sample.

Results and Discussion

In this experiment the tomato response was evaluated at different levels of osmotic stress induced by using PEG-6000 in the medium. Several methods have applied for withdrawal of water from the environment by using chemicals like polyethylene glycol, which created low water potential in the soil. It is confirmed that addition of PEG in the soil causes the loss of water from the plant.

The antioxidant activity depends upon the ability of antioxidants to control the oxidation. The antioxidative system of the plants are highly important because they can scavange the free radicals and protect the plants from injuries (Ahmad *et al*., 2010).

P-value<0.05, indicated that treatments differ significantly from each other (Table 1). Hence the null hypothesis has rejected. Now the Duncan's multiple range test (DMRT) was applied to see which treatments differed from each other. Treatment means are presented in Table 2 in ascending order of magnitude. The comparisons of treatments are presented in Table 3. It is clear from the results that there are significant differences among all pairs of treatments except (T1, T2) and (T3, T4).

The DPPH scavenging activity of extracts of tomato leaves was concentration dependent. The scavenging percentage showed clear differences among extracts of different genotypes and all the genotypes showed the antioxidant activity.

The antioxidant activity of different studied genotypes showed that higher 53.05% antioxidant activity was produced by the genotype G-21-006234 at control (no PEG solution) with 25 µl aqueous leaf extract. Higher the amount of extract concentration higher was the antioxidant activity. At the concentration of 500 µl leaf extract the genotype G-21-00623 showed the maximum antioxidant activity 71.54%. The results recorded during the experiment clearly indicated that the free radical scavenger behavior was increased with increase of extract while in other case the antioxidant behavior was low with high amount of PEG. The 53.06% at control was shown by G-21-006234 and 21.19% was at highest PEG concentration. Among all the genotypes studied the least antioxidation was 23.924% shown by G7-10593 at control and 18.755% at 12.5% PEG (Fig. 1). The genotype G 31-19289 showed the good response for antioxidant activity (Fig. 2). The radical scavenging activity of this genotype was 52.42% at 25 µl leaf extract with control and 39.21% at the lowest PEG concentration and this amount of the radical scavenging was the highest among the other studied genotypes. The cluster analysis of the data of the antioxidant activity of tomato genotypes grouped into 4 main clusters. The cluster 1 consisted of 8 genotypes which had average antioxidant activity of 57.47 and cluster 2 comprised of 2 genotypes which showed same data. The cluster 3 consisted of 2 genotypes and cluster 4 grouped 14 genotypes.

Table 1. One-way analysis of variance.

Source	DF	SS	MS	F-ratio	P-value
Treatment	4	2291	572.7	13.94	0.00
Error	125	5136.2	41.1		
Total	**129**	**7427.1**			

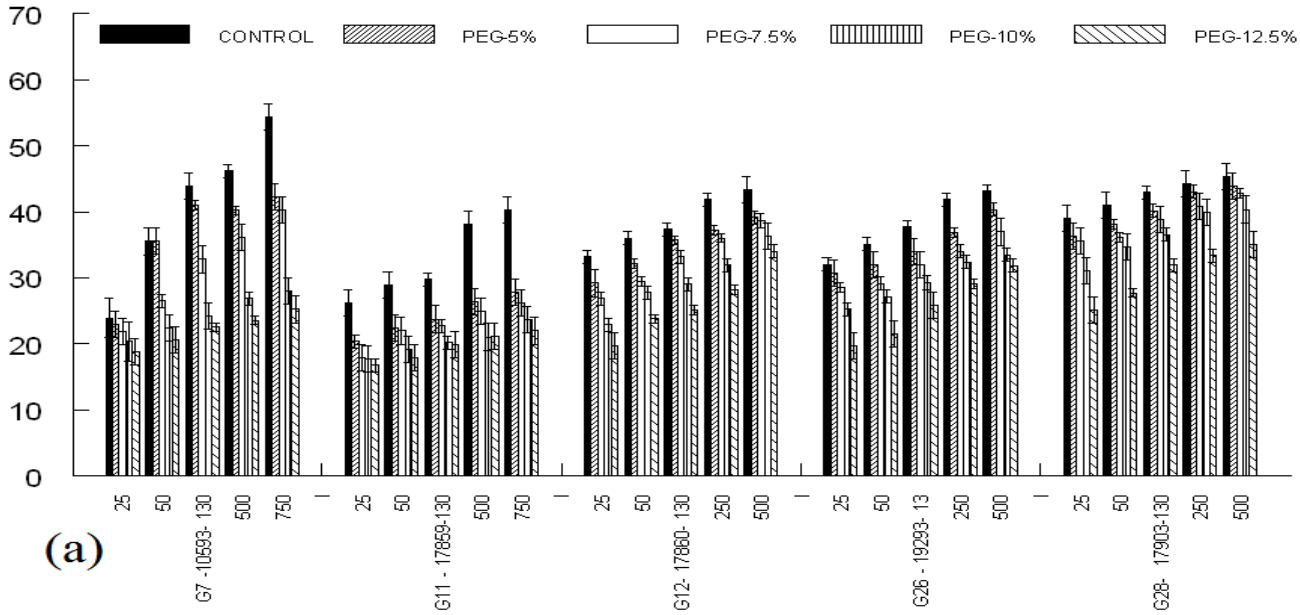
Table 2. Treatments means of DPPH on 26 genotypes of tomatoes.

T_5	T_4	T_3	T_2	T_1
40.930	44.377	45.729	49.436	52.807

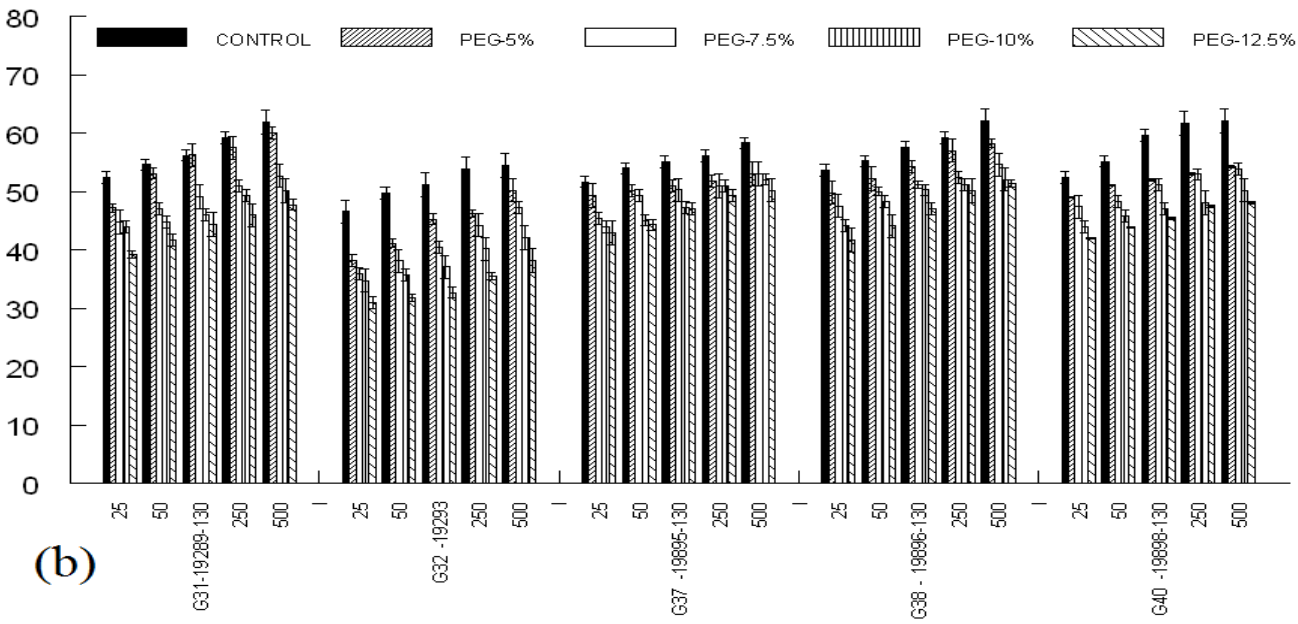
Table 3. Comparison of treatments means.

T_1-T_5	52.807-40.930	11.877>3.42
T_1-T_4	52.807-44.377	8.43>3.42
T_1-T_3	52.807-45.729	7.078>3.42
T_1-T_2	**52.807-49.436**	**3.371<3.42***
T_2-T_5	49.436-40.930	8.506>3.42
T_2-T_4	49.436-44.377	5.059>3.42
T_2-T_3	49.436-45.729	3.707>3.42
T_3-T_5	45.729-40.930	4.799>3.42
T_3-T_4	**45.729-44.377**	**1.352<3.42***
T_4-T_5	44.377-40.930	3.447>3.42

Antioxidant components of Plants are vital constituents that safeguard the bodies from the damages results due to free radicals formed in plants during drought stress (Ahmad *et al*., 2010). Antioxidants neutralize ROS and their undesirable effects and work at different phases like avoidance, capture and repair. Different mechanisms are used by the antioxidants like reduction by addition of hydrogen, reducing singlet oxygen and capturing of free radicals (Agarwal *et al.*, 2008). The study indicated that antioxidant activity depended upon the concentration of sample which has increased by increasing the sample amount and vice versa. Our results were in accordance with the results recorded by the screening of drought tolerant genotypes of sugarcane through biochemical markers against polyethylene glycol (Abbas *et al.*, 2014). The ROS and lipid peroxidation produced during abiotic environmental stresses cause oxidative damage to lipid metabolism which interrupt the plant lipid metabolism (Elkahoui *et al.,* 2005).


CONTROL
PEG-5%
PEG-7.5%
PEG-10%
PEG-12.5%
70
60
50
40
30
20
10
0
25
50
G7 -10593- 130
500
750
G11 - 17859-130
G12- 17860- 130
G26 - 19293- 13
G28- 17903-130
250

(a)


CONTROL
PEG-5%
PEG-7.5%
PEG-10%
PEG-12.5%
80
70
60
50
40
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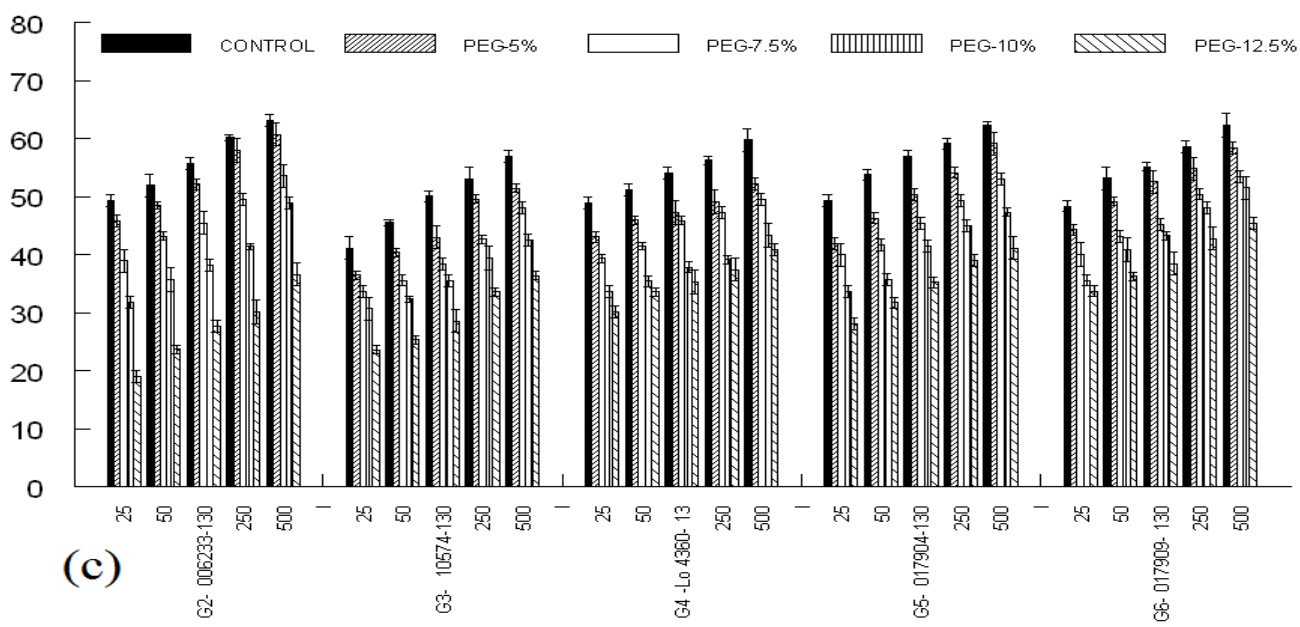
20
10
0
25
50
G31-19289-130
250
500
G32 -19293
G37 -19895-130
G38 - 19896-130
G40 -19898-130

(b)


CONTROL
PEG-5%
PEG-7.5%
PEG-10%
PEG-12.5%
80
70
60
50
40
30
20
10
0
25
50
G2- 006233-130
250
500
G3- 10574-130
G4 -Lo 4360- 13
G5- 017904-130
G6- 017909- 130

(c)

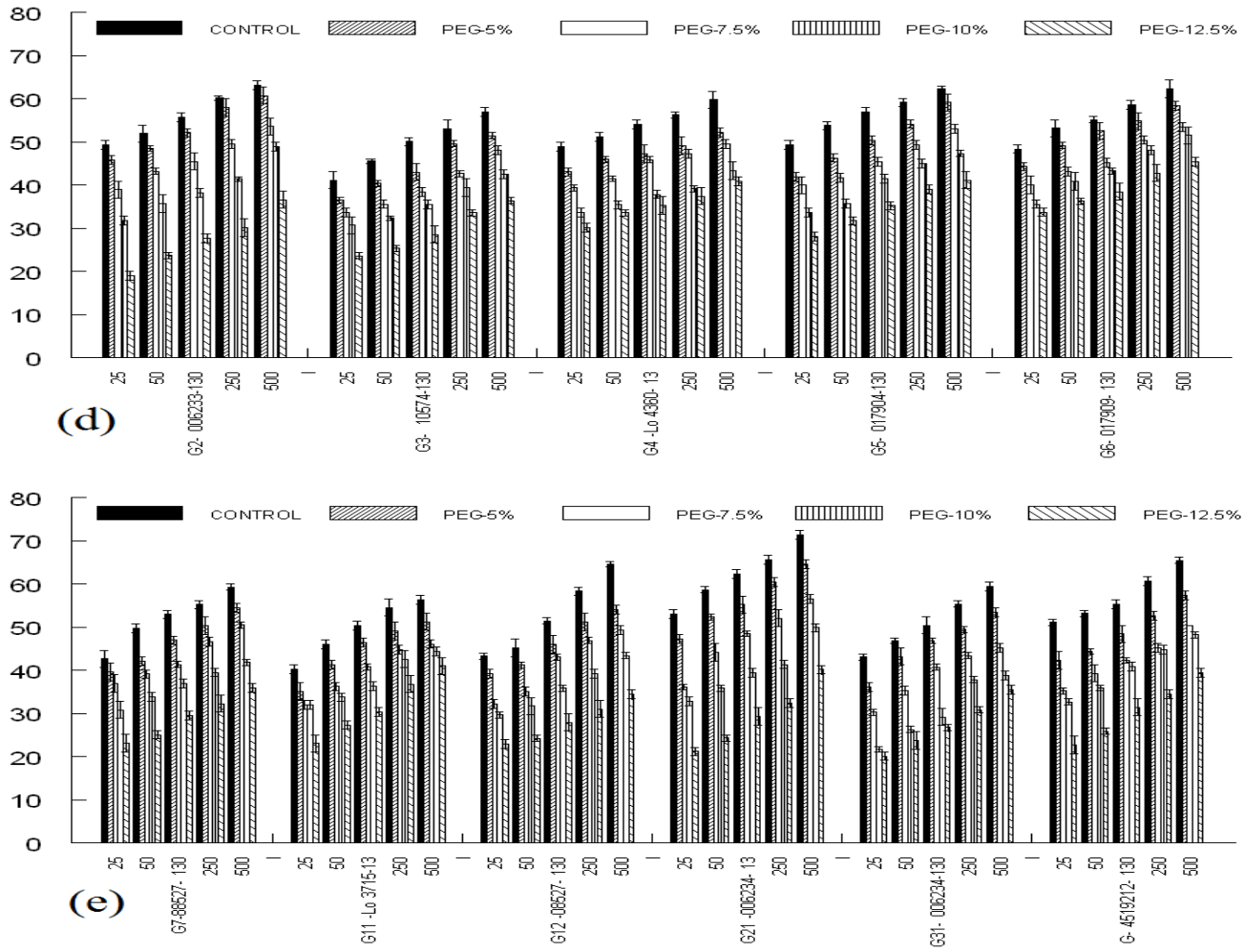


Fig. 1. Antioxident activity of aqueous extract of leaves of genotypes (a) (G7-10593, G11-17895, G26-19293, G28-17903), (b) G31-19289, G32-19223, G-37-19895, G38-19896, G49-19889, (c) G44-19911, G43-19907, G16-19913, G47-6231, G8-19219, (d) G2-006233, G3-10574, G4LO-4360, G5-017904, G6-017909, (e) G7-88507, G11-LO3715, G12-08527, G21-006234, G31-006234 and G45-9219). DPPH radical scavenging activity of different genotypes of tomatoes against (PEG). Values are means ± SD (n=3).

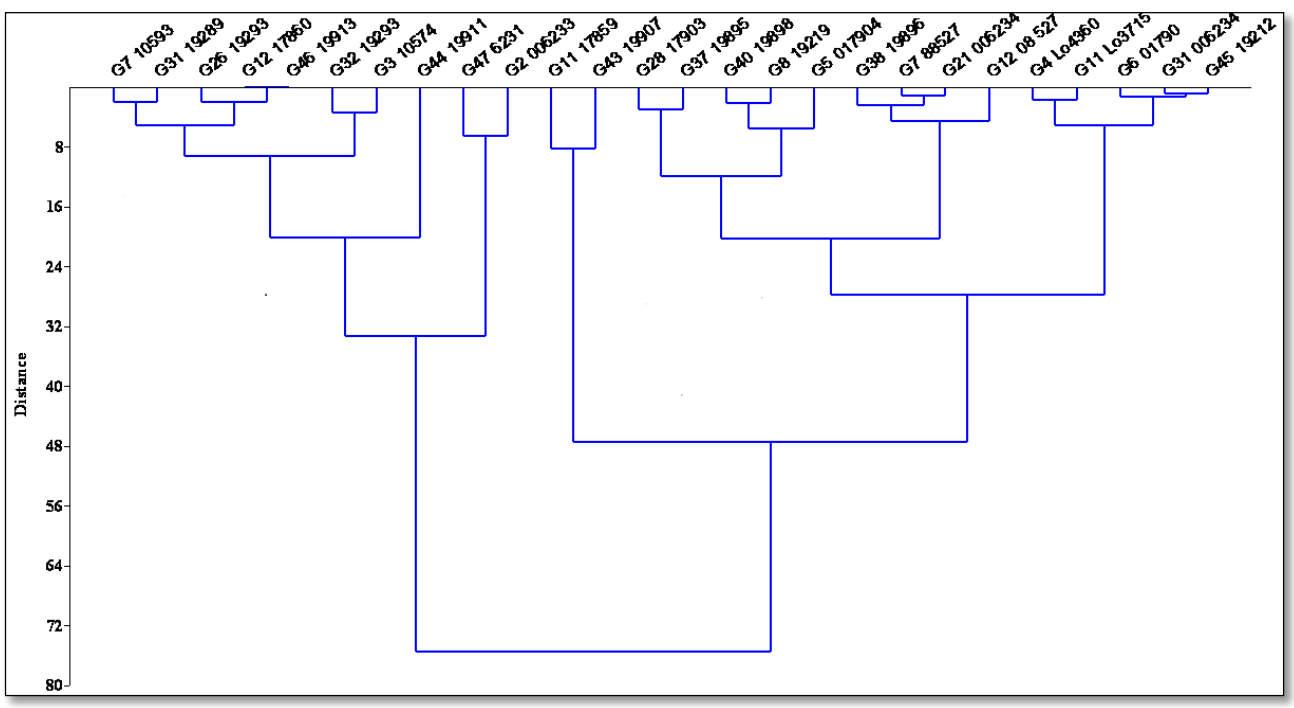


Fig. 2. Dendrogram of 26 genotypes of tomato based on the antioxidant activity.

It is concluded from the results that antioxidant activity of tomato genotypes grown under PEG simulated drought stress was increased by increasing the sample concentration and decreased by increasing the PEG concentration. The genotypes showed better results could further be explored and used in drought areas of Pakistan.

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