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Effect of polyethylene glycol and trehalose sugar on some biochemical parameters of rice plant (*Oryza sativa* L.) cultivar Anbar 33

Ameer A.Abbood [*1] , Hussein K. Al-Kaaby [*2] , Luma H. Adul-Qadir [*3]

Department of Biology, College of Education for pure sciences
University of Basrah

ameeradnan223@yahoo.com

Abstract

A laboratory experiment was conducted using the anvils to study the effect of water-tightening induced by polyethylene glycol (PEG) and the effect of interference between PEG and trehalose sugar biochemical parameters of Iraqi rice plant Anbar 33. Rice seeds were planted in plastic anvils 12 cm in diameter and weighing 700 g of a mixture of 1: 2 with dates and sand, respectively, were added to the anvils with watering water PEG substance with three (5, 10 and 15) g / l and trehalose sugar at three concentrations (0, 5 and 10) mmol / L and the experiment continued for 10 weeks, after which the concentrations of each of Proline and sucrose sugar amino acid, also estimated the efficacy of the catalase and Peroxidase enzymes, the results showed a significant increase in the concentration of both proline and sucrose and the activity of the catalyzes (CAT) enzyme as well as the decrease in the effectiveness of peroxidase (POD) while improved trehalose added externally on the leaves the effectiveness of an enzyme (POD) as well as a reduction of both sucrose and proline and the effectiveness of the enzyme (CAT) Especially at the 5 and 10 mmol / L concentrations of trehalose sugar. The study aims to determine the role of trehalose sugar when interacting with polyethylene glycol in improving rice plant tolerance to water stress.

keywords: water stress, trehalose sugar, antioxidants, *Oryza sativa*

Introduction

Rice is a staple food for more than 3.5 billion people globally, including all eastern, southeastern, and western Asia, which provides for Iraq (CGIAR, 2016). Rice is superior to the rest of the crops in offering food to the world's population. It provides nearly 20% of the food energy supply in Wheat and maize provide 5.19%, respectively (Alexandratos and Jelle, 2012). People consume about 95% of the rice produced (FAO, 2017).

Water stress is one of the types of non-vital environmental stresses that restrict the productivity of crop plants, as it affects plant growth and development and reduces grain yield, and thus leads to an aggravation of the food shortage and to reduce losses due

to drought and achieve stability in the yield of annual crops, it is necessary to conduct studies that enable increasing Tolerance to drought in crops, especially rice, as it is considered a highly sensitive crop (Labell and Field, 2007) .

Through our review of the sources, it was noted that many chemical compounds have been used to induce water stress, such as mannitol, sorbitol, and polyethylene glycol (PEG) (Wani *et al*., 2010) which was used in the current study, and it is a non-ionic, non-toxic, water-soluble polymer due to its high molecular weight ranging between 8000 1500 g / mol can not be depleted into the plant cell, meaning that it does not affect its metabolic processes and as a result creates conditions of water stress similar to what the plant undergoes in (Verslue *et al*., 1998)

Trehalose is a non-reducing natural sugar that consists of two molecules of glucose sugar found in the vast majority of living organisms such as bacteria, yeasts, and invertebrates, as well as in plants. That characterizes a significant role as a carbon source in primitive organisms (Iordachescu and Imai 2008). Despite its presence in plants in a small amount, it is noted that its levels rise when exposed to abiotic stresses. It is believed that Trehalose plays a protective role against various abiotic stresses such as temperatures, Highness, salinity, and drought (Kosar *et al*., 2019).

Since rice is one of the important economic plants in Iraq and the world, it is sensitive to drought and salinity. Therefore, the current study aimed to demonstrate the effect of water tightening using PEG and its interference with trehalose sugar in the concentration of proline and sucrose and in the effectiveness of catalase and peroxidase enzymes.

Materials and Methods

Germination Percentage Test - 1 The seeds of class Anbar 33 were preserved with laboratory temperature and tested for vitality, by taking a hundred and distributing them over four petri dishes (10 cm diameter) with adding (5) cm 3 of distilled water to a filter paper inside each dish. Then, the percentage of seed germination was calculated to reach (98 ± 2)% after the passage of (48) hours It was calculated from the following formula.

$$\text{Germination percentage} = \frac{\text{Number of vegetable seeds}}{\text{The total number of seeds}} \times 100\ \%$$

Seeds Surface Sterilization 2-

The seeds were placed in ethyl alcohol (70%) for five minutes with gentle stirring, washed with distilled and sterile water, and then sterilized superficially with mercury chloride $HgCl_2$.

As a solution of 1% mercury chloride and the seeds were sterilized with this substance for a period of (6-8) minutes with constant stirring, they were washed with sterile distilled water three times to get rid of the effects of sterile material. (Ahmed *et al*., 2016)

Prepare a seed growing medium 3-

Sterile seeds were previously planted in 12 cm plastic bins with a weight of 700 g container on a mixture of sand and peat with a ratio of 1: 2 and at the rate of 5 seeds for each anvil and watered with a full-force Hockland solution based on the field capacity calculated by the gravimetric method until the formation of seedlings. After the seedlings were formed and reached 14 days of age, they were treated with the above nutrient solution containing PEG of molecular weight 1500 in concentrations (5, 10, 15) g / L only 1 in

addition to the treatment of interference between the previous PEG concentrations with trehalose (Trh) sugar 5 and 10 mmol/l. In addition to control treatment with a nutrient solution only, at three replications per treatment. The transactions are as follows as shown in Table 1.

Table (1). Experience transactions

Transaction code	Transactions	Two-week-old seedlings resulting from germination of seeds of the rice plant, cultivar Anbar 33
T1 (control)	0 g / L PEG + 0 mmol/l Trh	
T2	5 g / L PEG	
T3	5g / L PEG + 5 mmol/l Trh	
T4	5g / L PEG + 10 mmol/l Trh	
T5	10 g / L PEG only	
T6	10 g / L PEG + 5 mmol/l Trh	
T7	10 g / L PEG + 10 mmol/l Trh	
T8	15 g / L PEG only	
T9	15 g / L PEG + 5 mmol/l Trh	
T10	15 g / L PEG + 10 mmol/l Trh	

The activity of Catalase was estimated according to the method described by(Hadwan and kadhum, 2018) The effectiveness of the peroxidase enzyme was estimated according to the method described by (Pitotti *et al*., 1995 in addition to estimating proline according to the method (Bates *et al*., 1973) and a concentration was estimated. Sucrose according to instructions provided by the company Medical & Biological Laboratories.

statistical analysis

In statistical analysis, I use Complete Random Design (CRD) to experiment with two factors, the first is the PEG concentrations (0, 5, 10, 15) g/L and the second factor is trehalose sugar (0, 5, 10) mmol/l and three replicates, and I use The SPSS 24 statistical program for analyzing the results, and after obtaining the table of variance analysis, the averages were compared using the Revised Least Significant Difference Test (RLSD) at a probability level of 0.05 (Kettebekov *et al*., 2002).

Results and discussion

The results show in (Figure 1) that the increase in PEG concentrations when treating the plant with it resulted in a significant increase in the concentration of proline, as different concentrations of PEG 5), 10 and 15) g/l high levels of proline were recorded (41.33 and 47.33

and 53) mg/gm respectively compared to a control treatment recorded (34.66) mg/g.

The results also indicated in the same figure the role of the trehalose interference with PEG in a significant decrease in the proline concentration, as the interference of 5 PEG + 10 Trh (T4) recorded the lowest rate of proline accumulation as it reached (36.33) mg/g.

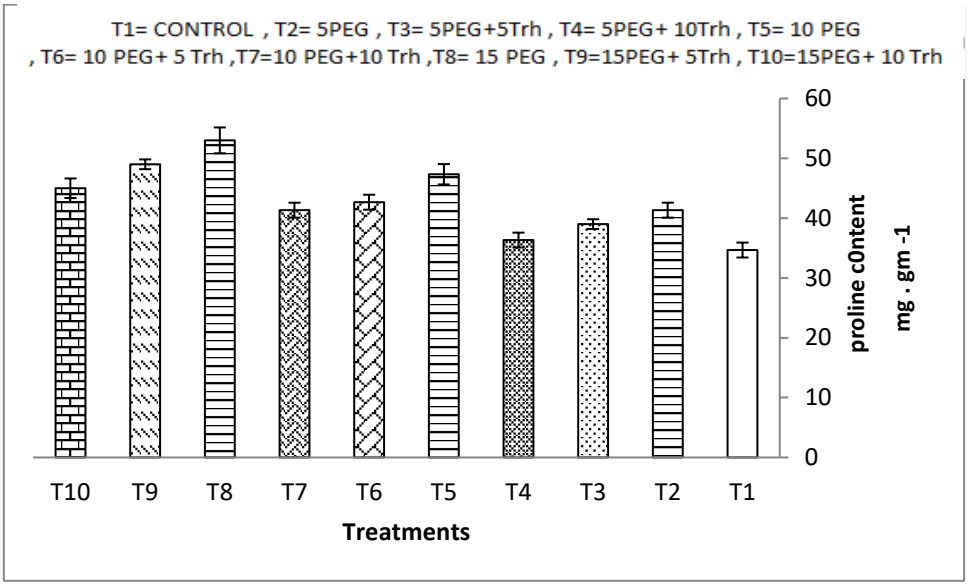

Fig. (1) Effect of PEG and its Interaction with Trehalose Sugar on Proline Content (mg/gm) fresh Weight) in rice plant cultivar Anbar 33

The increase in the accumulation of proline by increasing the severity of water stress is attributed to the conversion of some amino acids (such as Glutamic acid) that were formed as a result of the breakdown of routines into proline under the influence of drought (Pirjo, 1999; Mitysik *et al*. 2002) Proline released from hydrolyzed protein may form under the influence of water stress to participate in the process of osmotic regulation, and it is mentioned that the accumulation of proline is adaptive in cases of water stress because it is a method of osmotic regulation and these results are consistent with (Rontein *et al*., 2002) on the rice plant and wheat plant (Ali *et al*.,2007), as both studies observed proline accumulation under the influence of water stress.

A significant decrease in the proline content of the leaves of the rice plant was observed when treating the plants with a solution containing trehalose sugar in combination with PEG, and this may be because trehalose is a compatible solvent and because its action identical to proline was accumulated instead of it.

It is noted from the results of (Figure 2) a significant increase in the plant content of sucrose by increasing PEG concentrations, as stress concentrations (5, 10 and 15) g \l were recorded rates reached (0.72, 0.92 and 1.25 (ɱg/gm, respectively, compared to the control treatment that recorded an average of (0.54) ɱg/g .

The same figure also indicated the role of the trehalose interference with PEG in a significant decrease in the concentration of sucrose, as(T7) (10 PEG + 10 Trh) interference recorded

the lowest rate g/ gm compared to the rest of the interventions.
.M (0.57)

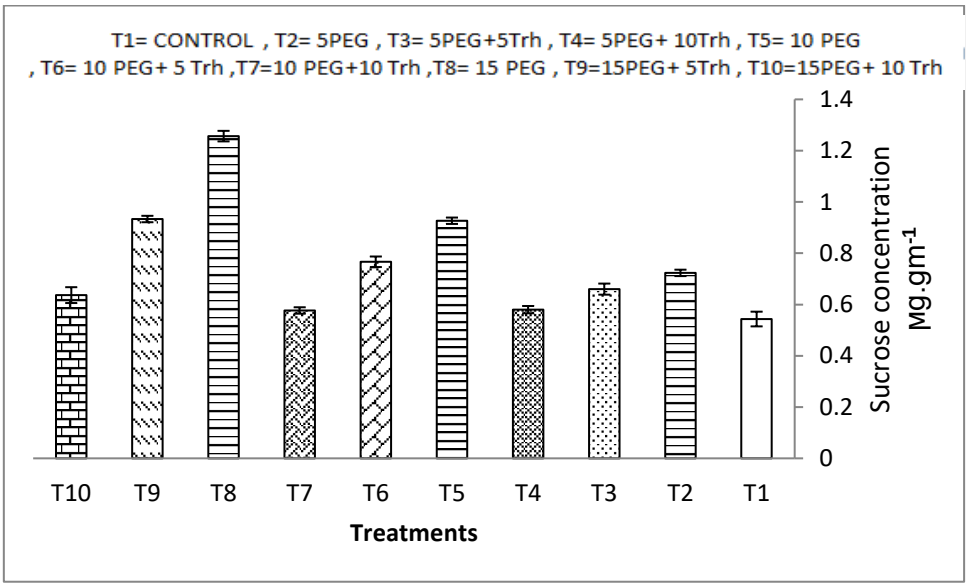

Figure (2) Effect of PEG and its Interaction with Trehalose Sugar on Sucrose Concentration (Mg.gm^{-1} fresh Weight) rice plant cultivar Anbar 33

The reason for the significant increase in the concentration of sucrose by increasing concentrations of water stress is because sucrose may participate in the tolerance of dehydration by protecting large particles through Preferential hydration during water loss(Buitink *et al*., 2002) or that the cause of the accumulation of sucrose may also be caused by a decrease in the mobilization of sucrose by the enzyme invertase Providing glucose as a source of energy for metabolism is the latter decreasing with water loss et al., 2002 (Walters *et al*.,2002) It also indicated Bianchi *et al* .,(1991) and Müller *et al*., (1995) the accumulation of sucrose in the leaves of Resurrection plants as the result agreed above with(Kameli *et al*., 1993) and (Bohnert *et al*., 1995).

In contrast to the above results, a decrease in the accumulation of sucrose was observed when plants were treated with trehalose sugar under the effect of water stress. Perhaps the reason is that trehalose sugar is one of the sugars that accumulates under the influence of water stress to protect large biological molecules and may act as a Compatible soluteinstead of the accumulation of other sugars as the severity of stress Aqueous is not required to accumulate more than a compatible solute This Müller, (1997) has observed a decrease in the concentration of sucrose in soybean roots when absorbing a quantity of trehalose sugar by microorganisms as the results agreed with (Herold *et al*., 1981) when he observed Low level Sucrose addition of trehalose to spinach.

The results shown in (Figure 3) indicate a significant increase in the levels of catalase enzyme by increasing the PEG concentrations, as they gave different concentrations of PEG (5, 10 and 15) g/ l the highest rates reached (0.68, 0.75 and 0.86) min/g, respectively, while the control

treatment gave a rate of(0.34) minute.g^{-1}. The results also showed in the same figure the role of trehalose interference with PEG concentrations in a significant decrease in the levels of catalase enzyme, where a treatment interaction of 5 PEG + 10 Trh (T4) was recorded. The lowest rate was(0.46) minute.g$^{-1.}$

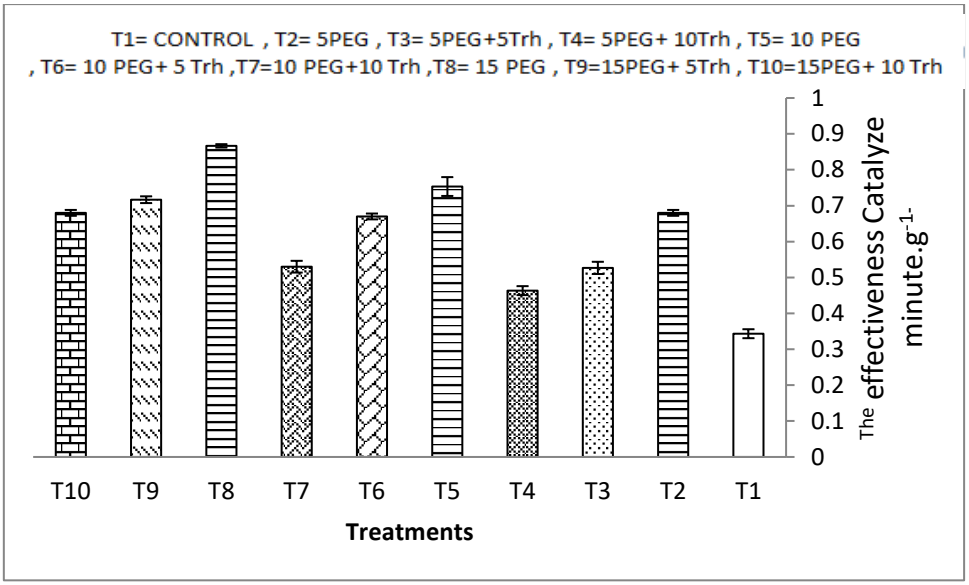

Figure (3) Effect of PEG Substance and its Interaction with Trehalose Sugar on the Effect of Catalase Enzyme (minute.g^{-1})In) rice plant cultivar Anbar 33

Perhaps the reason for increasing the effectiveness of catalase enzyme is its ability to break down free radicals, which gives the plant a greater opportunity to grow and develop. The reason for increasing the effectiveness of catalase enzyme is that it is one of the means to resist water stress, which leads to the development of oxidative stress represented by increasing the types of active oxygen (ROS). (Harmful to the plant because of its role in its disposal, which is the removal of hydrogen peroxide **H_2O_2** (Gara *et al*., 2003) Moloudi *et al*. (2013) indicated that it is an enzyme found in all eukaryotes, as the breakdown of H_2O_2 generated in the peroxysomes to H_2O and O_2 as an important role to maintain the equilibrium of X During stress oxidation, if the results are identical to (Nazarli and Faraji, 2011)

A decrease in the effectiveness of the catalase enzyme when treating plants with a solution that contains trehalose sugar and perhaps the reason for that is the role of trehalose sugar by preserving the amount of water inside plant cells and reducing the severity of stress, thus reducing the effectiveness of some enzymatic antioxidants. The catalase enzyme may be one of them as indicated Nounjan and Theerakulpisut.,(2012). When studied on rice plant (*Oryza sativa* L.), the effectiveness of catalase enzyme decreased when treating with trehalose sugar under the influence of saline stress.

The results shown in (Figure 4) indicate a significant decrease in the levels of peroxidase enzyme by increasing PEG concentrations, as different PEG concentrations were given (5, 10 and

15) g/ 1 the lowest rates were (2.54, 2.33 and 1.73) minute. g^{-1}, respectively, while the control treatment gave an average of (3.04) minute.g^{-1}. The results showed in the same figure the role of trehalose interference with PEG concentrations in a significant increase in the levels of peroxidase enzyme, as a 10 PEG + 10 Trh (T7) interference treatment recorded the lowest rate reached 2.63) minutes.$g^{-1.}$

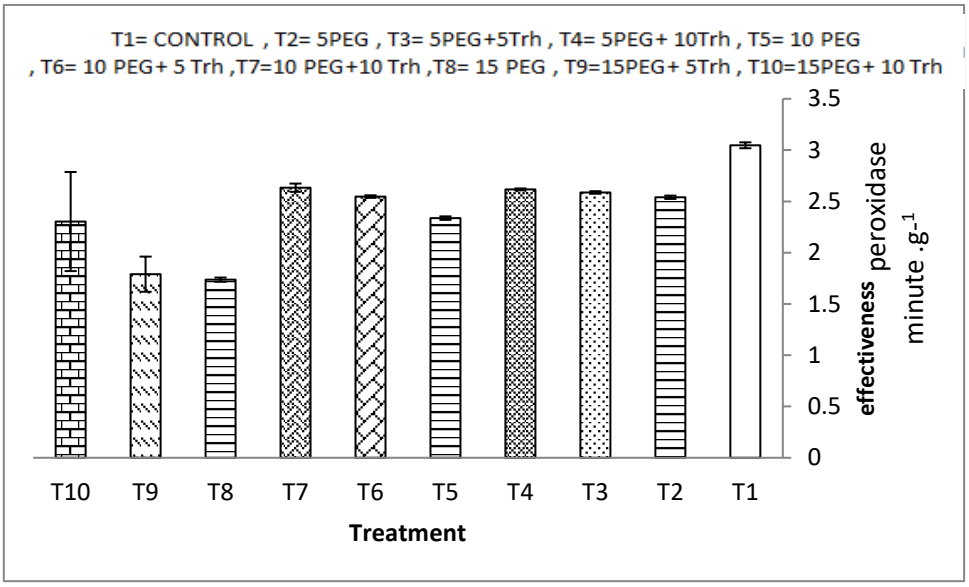

Figure. (4) Effect of PEG and its Interaction with Trehalose Sugar on the Effect of the Peroxidase Enzyme (minutes.g^{-1}) in rice plant cultivar Anbar 33

The reason for the decrease in the effectiveness of the peroxidase enzyme by increasing the water stress is due to the increase in free radicals in large quantities, which in turn leads to a decrease in some antioxidant enzymes. The peroxidase enzyme may be including Lee *et al*.,(2012). These results are consistent with Asadi (2014) when studying on date palm while observing Sharifi *et al*. (2012), when looking on wheat, increased the effectiveness of peroxidase (POD) by increasing levels of water stress, as the enzyme works to remove the toxicity of effective oxygen types (ROS) by acting as a complementary group that accelerates proton oxidation, giving compounds bound with H_2O_2 and thus leading to breakdown H_2O_2 and so on Toxic toxicity as it urges and accelerates the conversion of H_2O_2 into water and oxygen in addition to its role in increasing the stability of the cell membrane and chlorophyll. In contrast, a significant increase in the activity of an enzyme was observed when treating rice plants with trehalose sugar under the effect of water stress. It works to increase the efficiency and effectiveness of antioxidants, including the enzyme peroxidase, as indicated Ibrahim and Abdellatif, (2016) to increase the potency of POD In their studies on wheat plant under the influence of water stress.

Conclusion

It was noted through the results that the treatment of plants with concentrations of polyethylene glycol 5, 10 and 15 g / l

significantly reduced plant growth. At the same time, it was observed that the use of Trehalose sugar, at concentrations of 5 and 10 mmol / l, combined with concentrations of polyethylene glycol led to improved tolerance. Rice plant for water stress, by the aforementioned biochemical measurements.

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تأثير الكلايكول متعدد الأثلين وسكر التريهالوز في بعض المعايير البايوكيميائية لنبات الرز *Oryza sativa* (L.) صنف عنبر 33

أمير عدنان عبود حسين خلف زاير لمى حسين عبد القادر

قسم علوم الحياة - كلية التربية للعلوم الصرفة - جامعة البصرة

المستخلص

أجريت تجربة مختبرية بأستعمال السنادين لدراسة تاثير الشد المائي المستحث بمادة الكلايكول متعدد الأثيلين (PEG)، وتأثير التداخل بين PEG وسكر التريهالوز، في بعض المعايير البايوكيمائية لنبات الرز العراقي صنف عنبر 33. زرعت بذور الرز في سنادين بلاستيكية قطر12 سم وبوزن 700 غم من خليط من 1:2 بتموس ورمل على التوالي أضيف إلى السنادين مع ماء السقي مادة PEG بتراكيز (5 و10 و15) غم / لتر و سكر التريهالوز بثلاثة تراكيز (0 و 5 و 10) مليمول / لتر واستمرت التجربة لمدة 10 اسابيع تم بعدها قياس تراكيز كل من الحامض الأميني البرولين وسكر السكروز، كما قدرت فعالية إنزيمي الكاتليز والبيروكسيديز المضادان للأكسدة. أوضحت النتائج زيادة معنوية في تركيز كل من البرولين والسكروز وفعالية انزيم الكاتاليز (CAT) فضلاً عن انخفاض فعالية البيروكسيديز (POD). في حين حسن التريهالوز المضاف خارجياً على الأوراق فعالية انزيم (POD) فضلاً عن انخفاض كل من السكروز والبرولين وفعالية انزيم (CAT) لاسيما عند التركيزين 5 و 10 مليمول/ لتر من سكر التريهالوز.