Effect of salt stress on medicinal plant fenugreek callus on the activity of None enzymatic antioxidants Malandialdehyde, Glutathione, and Ascorbic acid

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Abstract

The research was conducted to study the effect of salt stress on the activity of None enzymatic antioxidants Malan di aldehyde (MDA) , Glutathione (GSH), and Ascorbic acid (AsA) in the callus of the fenugreek medicinal plant. About 250 mg of seeds' induced callus was subculture in MS medium including 1.0 mg.L-1 of both BA and NAA supplemented with four concentrations of NaCl salt (6, 9, 12, and 15 ds.m-1) in addition to the control treatment without salt (2.23 dS.m-1). Cultures were incubated in the growth room for six weeks at room temperature of 25 ± 2 °C and light intensity of 1000 lux for 16 hours, followed by 8 hours of darkness per day. The results showed high levels of Malandialdehyde (MDA) , with increased activity of Glutathione (GSH), Ascorbic acid (AsA) none enzymatic antioxidant as a natural mechanism of apparent resistance salt stress and the resulting increase in free radicals.

Key Words: Fenugreek, medicinal plant ,salinity, callus culture, none enzymatic antioxidant

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1.Introduction

Fenugreek (Trigonella foenum-graecum L.) is a perennial herbaceous plant that belongs to the order Fablales and under the leguminous family Papilionace and the leguminous family Fabaceae (Flammang et ai.,2004). The whole plant, seeds and leaves were used as antioxidant (Dixit et al.,2005). In healing many diseases like antidiabetic (Raju et al.,2001). Hepatoprotective (Ulbricht et al.,2007). Anticancer (Shabbeer et al.,2009) . sexual health-modulating activities(Aswar et al.,2010) . Women's health (Doshi et al.,2012). Antihyperlipidemic (Chaturvedi et al.,2013) , Antiobesity (Gao et al.,2015) and Anti-inflammatory (Gautam et al.,2016). The health promotion and disease prevention attributes of fenugreek are due to presence of different types of phytochemicals and their varying pharmacological and biological activities (Yadav et al.,2014). Malondialdehyde (MDA) is a marker for measuring lipid peroxidation or damage to plasma lemma and organelles membranes that increases with environmental stresses (Garg and Lacsit 2010). Glutathione (GSH) it is one of the important plant defenses against damage by the reactive oxygen species (ROS) , as well as its important as metabolic components in the plant which is mostly found in its reduced form GSH in plant tissues and in all cellular parts such as cytoplasm, endoplasmic reticulum, vacuoles, mitochondria and chloroplasts (Cimini et al.,2022) . Ascorbate, plays a pivotal role in several physiological processes, including regulation of sulfate transport, signal transduction, conjugation of metabolites, detoxification of xenobiotics and the expression of stress-responsive genes (Baier and Dietz,1997). While the non-enzymatic antioxidants include water soluble (ascorbate, glutathione and flavonoids) and lipid soluble

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(ascorbate, glutathione and flavonoids) and lipid soluble (γ-tocopherol, ß- carotene) metabolites. In plant cells, specific ROS producing and scavenging systems are found in different organelles such as chloroplasts, mitochondria, and peroxisomes , ROS scavenging pathways from different cellular compartments are coordinated (Mittler, R. 2002). Salinity is one of the major problems in plant production, including medicinal plants, in many regions of the world, especially arid and semi-arid (Yasar et al.,2008). Several researches have shown that the negative impact of salinity lies in the ionic imbalance of plant cells, which leads to the occurrence of many damages such as osmotic stress, toxicity and the generation of free radicals, which in turn lead to damage to the contents of the cell such as lipids and proteins (Freeman and Beattie,2008). Under stress conditions, the plant tries to create and develop various protective and defensive mechanisms, including morphological or synthetic ones to adapt in stressful environments, in addition to possessing enzymatic and non-enzymatic systems to protect its cells from the negativity influences which resulting from stress and sometimes the formation of secondary compounds that have roles that are no less important than the main reactions (Bates et al.,1973). The aim of this research was to investigate the effects of salinity on the activity of none enzymatic antioxidant in fenugreek callus cultures.

2. Materials And Methods

2.1. Materials

The study was carried out in the laboratory of plant tissue culture/ Al-Musiab Technical College to estimate the effect of different concentrations of NaCl salt on the activity of None enzymatic antioxidants Malan di aldehyde (MDA) , Glutathione (GSH), and Ascorbic acid (AsA) in fenugreek callus cultures. About 250 mg of induced fenugreek callus was grown on the best combination of growth regulators (1.0 mg.L-1 of both BA and NAA) supplemented with four concentrations of NaCl salt (6, 9, 12, and 15 dS.m-1) in addition to the control treatment without NaCl salt (which had the value of 2.23 dS.m-1). Cultures were incubated in the growth room for six weeks at room temperature of 25 ± 2 °C and light intensity of 1000 lux for 16 hours, followed by 8 hours of darkness per day.

2.2. Methods

The Estimation of malandialdehyde (MDA) content was determined according to the method of (Heath and Packer,1968) . The estimation of Glutathione (GSH) content was estimated following the method of (Ellman.(1959) . The Determination of the total amount of Ascorbic Acid (AsA)was estimated according to the method of (Al-Ani et al.,2007). Results were analyzed using the ready-made statistical analysis system (SPSS, 2016) under the Windows computer operating system. The means were compared according to the least significant difference test (LSD) at the probability level of 0.05 to test the significant differences among the means (Al-Rawi, K. M. and Khalaf Allaha,2000).

3. Results And Discussion

3.1. Results

3.1.1. Malandialdehyde (Mda) Estimation

Table (1) , showed the effect of NaCl on Malan di aldehyde (MDA) content of fenugreek callus . Levels of MDA were increased significantly in callus that treated with NaCl Ec (6, 9, 12 , and 15 dS.m-1) to give 2.722 , 3.840 , 3.521 and 4.863 µmole.g-1 respectively , compared to the control treatment Ec (2.23 dS.m-1) that gave 1.266 µmole.g-1.

Table 1. Effect of salt stress caused by the addition of NaCl salt on the MDA content of fenugreek callus after 6 weeks of cultivation.

NaCl Ec. (dS.m-1)	MDA (µmole.g-1)
2.23	1.266
6	2.722
9	3.840
12	3.521
15	4.863
L.S.D(0.05) =0.126	

3.1.2. Glutathione Estimation

There is a significant increase in GSH content (26.627 , 32.107 , and 37.308 µmole.g-1) (the table 2) in the concentrations of 6, 9, and 12 dS.m-1 of NaCl respectively as compared to control treatment (16.088 µmole.g-1), whereas, NaCl at 15 dS.m-1 caused significant decrease in GSH content (22.024 µmole.g-1) because it led to decrease the in metabolic processes in callus.

Table 2: Effect of salt stress caused by the addition of NaCl salt on the Glutathione content of fenugreek callus after 6 weeks of cultivation.

NaCl (dS.m-1)	Glutathione (µmole.g-1)
2.23	16.088
6	26.627
9	32.107
12	37.308
15	22.024
L.S.D(0.05) =4.653	

3.1.3. Ascorbic acid (AsA) Estimation

results of the table (3) there is a significant increase in Ascorbic acid (AsA) content (29.165, 32.949, and 39.626 µmole.g-1) in the treatments of 6, 9, and 12 dS.m-1 of NaCl respectively, whereas, the treatment of 15 dS.m-1 of salt caused a significant decrease in Ascorbic acid content (29.213 µmole.g-1) due to decrease in the metabolic processes in callus. The control treatment concentration (2.23 dS.m-1) gave 27.082 µmole.g-1.

Table 3: Effect of salt stress caused by the addition of NaCl salt on the Ascorbic acid (AsA) CONTENT of fenugreek callus after 6 weeks of cultivation.

NaCl (dS.m-1)	Ascorbic acid (µmole.g-1)
2.23	27.082
6	29.165
9	32.949
12	39.626
15	29.213
L.S.D(0.05) = 3.591	

3.2. Discussion

The main objective of the current research was Investigation of the effects of salt stress on the activity of None enzymatic antioxidants Malandialdehyde (MDA) , Glutathione (GSH), and Ascorbic acid (AsA) in vitro. Medicinal fenugreek seeds have been germinated in vitro on MS medium containing the best combination of growth regulators (1 NAA + 1 BA mg.L-1) to obtain the highest fresh weight of the callus after 45 days, then the resulting callus was treated with different salt concentrations Ec (6 , 9 , 12 ,and 15 dS.m-1) of NaCl , in addition to the salt-free control treatment Ec (2.23 dS.m-1) . The content of MDA , GSH , and AsA were estimated in the callus. Where it was found that the treatment with a saline concentration of Ec (15 dS.m-1) was significantly superior in produced the highest value of MDA content compared to the rest of the treatments and the control treatment, where the MDA content reached (4.863 µmole.g-1), These results were in agreement with (Garg and Lacsit,2010). The results indicated that the increasing in enzymatic and non-enzymatic antioxidants successfully scavenge reactive oxygen species (ROS), prevent oxidative injury to membranes and enhance tolerance to oxidative stress in plants (Ehsan et al.,2021). The level of MDA indicates the degree of salt tolerance as described in nodal explant Limnophila aromatica in vitro under NaCl treatment, the increase in MDA could be associated with ion accumulation and active ROS production under salt stress (Dogan,2020).

While the results of GSH were an agreement with (Amerjani ,2015) on C.roseus plant. The increase in GSH might be due to the main oxidation under salinity and it is necessary for increasing the stress protect antioxidant enzyme glutathione reductase (GR). (Shehab et al.,2010) reported that ascorbate and glutathione contents were increased under PEG-induced drought stress in rice callus culture . Concentration of GSH increased progressively during drought stress, concomitant with high level of GR activity, indicating that GSH plays an important role in scavenging the ROS by ascorbate-glutathione cycle. GSH is an ideal biochemical to protect plants against stress including oxidative stress, and takes part in the control of H2O2 levels (Aazami et al.,2021). On the other hand, and from the results of the table (3) for the AsA content , the results are consistent with (Misra et al. ,2014) on C.roseus plant , and these results was associated with the stress protecting mechanisms of the plants under salinity (Aazami et al.,2021) .

4. Conclusions

From the above- mentioned results it could be conclude that salt stress elicitation is effective method In the synthesis of non-enzymatic system of antioxidants. Therefore, in order strengthen antioxidant metabolites in medicinal plant fenugreek , uses of abiotic elector can be suggested

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