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The roles of Glutathione and Ascorbic Acid in Na-detoxification in terms of rooting response of Mung bean Cuttings

ادوار الكلوتاثيون والاسكوربيك أسد في ازالة سمية كلوريد الصوديوم بدلالة استجابة التجذير في عقل الماش

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Abstract:

The roles of glutathione , ascorbic acid, and their interaction in Na-detoxification removing in terms of adventitious root formation (ART) in mung bean cutting has been studied. The effect of different concentrations of the anti-oxidant GSH ranged between (10^{-3}-10^{-11}M) and ASA (100-600 ppm), the optimum concentration of GSH and ASA were (10^{-5} M and 100 ppm) respectively. The toxic concentration of NaCl was determined by using concentrations ranging from 25-300 mM. The toxic concentration was (50mM).Combination with 1: 1 ratio (V:V) for both antioxidants ASA and GSH that developed the optimal rooting response, (19.75) roots per cutting was obtained by the combination of (100: 10^{-5}) ASA: GSH. Detoxification of sodium chloride stress was occurred completely by supplying combination prior to toxic–NaCl treatment (pretreatment and after toxic NaCl treatment (post treatment) compared to their supply simultaneously with toxic NaCl.

Key wards: adventitious root formation, Ascorbic acid, glutathione, Oxidative stress, Toxicity

الخلاصة :

تمحورت الدراسة حول دور كلوتاثيون والاسكوربيك اسد وعلاقة بينهما في ازالة الاجهاد التاكسدي لسمية كلوريد الصوديوم بدلالة استجابة التجذير لعقل الماش ، قد اختبر تاثير تراكيز مختلفة من مضادي الاكسدة Glutathione (GSH) تراوحت بين (10^{-3}-10^{-11} M) و Ascorbic acid (ASA) تراوحت (100-600 ppm) ، اذ كان التركيز الأمثل من GSH و ASA هو **10^{-5} M** و100 ppm على التوالي ، وحدد التركيز السام من كلوريد الصوديوم NaCl باستعمال تراكيز تراوحت (25-300 mM) وكان التركيز السام (50) وتم مصاحبة مضادي الاكسدة بنسبة 1:1 كنسبة (V:V) لكلا مضادين الاكسدة ASAوGSH وتم الحصول على معدل (**19.75**) جذراً في العقلة الواحدة كامثل استجابة تجذير عند التوليفة (**10^{-5}M**: 100ppm) ASA :GSH وجهزت هذه التوليفة للتخفيف سمية NaCl قبل وبعد التعرض للسمية كلوريد الصوديوم وسوية وكانت المعاملة الأفضل في ازالة تاثير الاجهاد التاكسدي عند تجهيزها قبل وبعد التعرض للتركيز السام من كلوريد الصديوم حيث تمت السيطرة على الضرر التاكسدي لسمية كلوريد الصوديوم بالكامل.

Introduction:

An expanse to biotic and abiotic stresses, plants showed an expressed high levels of in reactive oxygen species(ROS), such high levels of ROS are harmful to cellular components and caused in oxidative damages which negatively affects cellular metabolism [1]. It is also known that ROS plays a central role in signal transduction that activates different responses and defense pathway for different stresses [1].The plants possess antioxidant systems which is efficient and effective, to scavense free radicals that`s accumulated under oxidative stress and the system of antioxidants is the custodian consists of enzymatic and non enzymatic antioxidants defense. The defense system works as buffers to maintain oxidative processes and reduction, including ascorbate (AsA), tocopherol, glutathione (GSH), flavonoids, alkaloids, and carotenoid in addition to the antioxidant enzyme system, which contains many antioxidant enzymes and oxidation, such as CAT, SOD, GPX and enzymes of ASA-GSH cycle such as APX, MDHAR, DHAR, GR , However, the concentrations of ASA and GSH were accumulated under some cellular parts in various stresses has been observed [2]. The ASA-GSH cycle is not only acting remove ROS toxicity but also contributes to retention of GSA and ASA pools in different cellular parts and protects the ASA-

GSH pathway from the toxic effects of ROS stressed conditions [3]. It was found that, the levels of gene expression for all ASA-GSH cycle enzymes were affected by saline stress [4]. The rooting is consists of two stage, first is initiation phase and second is Growth and Development Phase [5]. The Rooting is affected by many internal and external factors, and auxin has the primary role in the formation and organization of adventitious roots in the early stages of rooting when Initiation Phase formed. The total GSH pool was described as high GSSG compared to the final stage of rooting at the onset and elongation of the roots [6]. High oxidation of GSH during the formation of the root initiates of the tomato cuttings resulted in an increase in the ratio of the ASA-transformed in ASA-GSH cycle, and increased effectively the enzyme of the dehydroascorbate reductase at this stage of root formation and high amounts of reduced GSH can accumulate in the rooting zone [7].

Exogenous application of GSH into the rooting media, increased the number of roots that develops on tomato cuttings while exogenous application of GSSG did not affect rooting of the cuttings. ASA level was found raised in the third day during from the conversion the root initiation to the development phase. It was proposed that ASA is essential to reduce content of H_2O_2 in the rooting region through an increasing the efficiency of H_2O_2 scavenging enzymes that stimulate the presence of ASA[6].

Little informations of considering ASA-GSH cycle in plant defenses against abiotic stresses , therefore this study was carried out to understand the relationship between antioxidants ASA and GSH and their role in reducing the damage that caused by salt stress in terms of rooting response of Mung bean cuttings as an experimental system.

Materials and methods

A local seeds of the *Vigna ratiata* (L.) Wilczek were employed. A uniform seeds were selected for cultivation. Seeds were soaked with current water for 12 hours and implanted in parallel lines on sterile sawdust as a medium for seed planting using perforated plastic basins from the bottom where seeds are placed in larger, non-perforated basins.

Water was added to large basins and placed in a controlled growth cabinet, which characterized by continuous light at intensity ranged 1600- 1800 Lux, 25 ± 1 temperature and relative humidity 70-60% and water was added as needed for up to 10 days.

Mung bean cuttings preparated from similar 10 day-old light grown seedlings according to Hess [8], which has a small terminal bed , pair of fully expanded primary leaves, an epicotyl , and hypocotyl of 3 cm in length under the site of cotyledonary nodes.

Solutions of different concentrations were Prepared from GSH (10^{-3}-10^{-11} Moler), ASA (100-600 ppm), sodium chloride solution (25-300mM). In addition to boric acid solution that used as medium for rooting in concentration of 5 µg / ml (9).

The basal parts of the cuttings were supplied with test solutions by placing 4 cuttings per glass vials. Each treatment included three vials, as (12) cuttings per treatment. The hypocotyl of cuttings with length of 3 cm was soaked in 15 ml of test solution. The hypocotyl was treated with distilled water, ascorbic acid , glutathione and sodium chloride solutions for 24 hours, then transferred into rooting medium (5 µg/ ml boric acid) for six days after which the number of roots was couned in each cutting.

Completely Randomized Design was used, the data were statistically analyzed using the computer and the L.S.D values were reliable to compare the mean of the coefficients at a probability level (0.05) in all experiments [9].

Results

1. Determination of the toxic concentration of sodium chloride in terms of rooting response

Figure 1 shows the effect of different concentrations of Nacl salt in response of Mung bean cuttings. The results showed that low concentrations of Nacl (25 mM/ml) caused a significant increase of rooting response(14.33 roots) when statistically compared with control which developed 11.25 root / cutting. Concentration of 50 mM / ml of was significantly inhibited the number of roots to 6.50 which is the threshold in which the rate of rooting was reduced approximately by half

(54.54% reduction of the growth rate in terms of adventitious rooting), and on this bases was considered as a toxic concentration for Mung bean cuttings and was reliable in subsequent experiments. High concentrations 100 mM / ml and beyond inhibit the rooting response completely (0.0%).

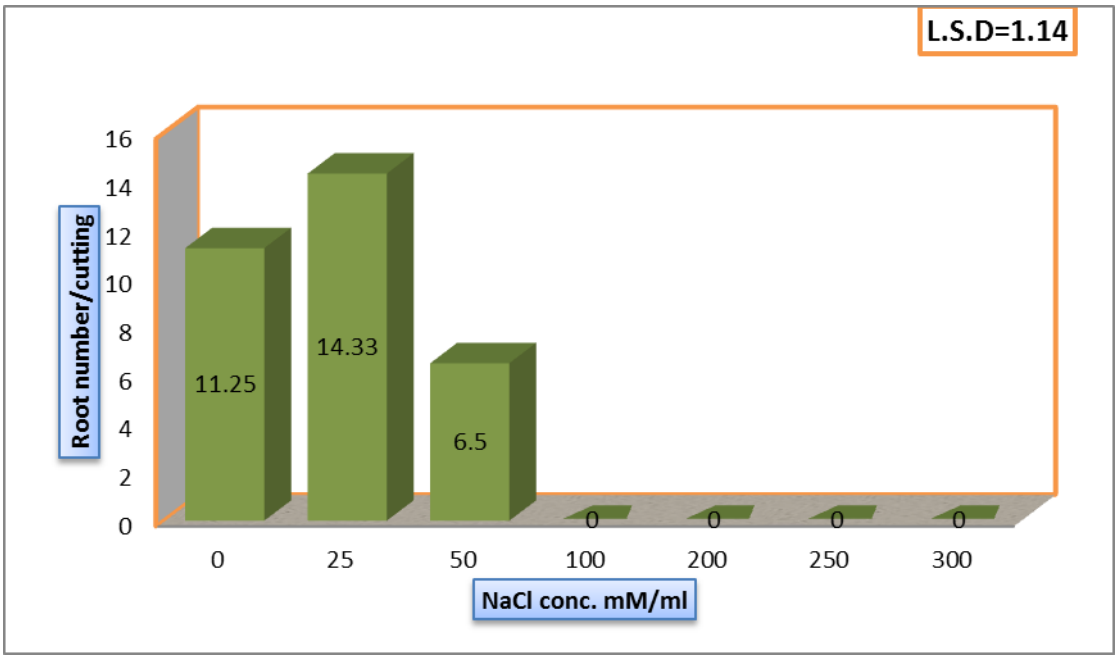


Figure (1):The effect of NaCl concentration on rooting response of Mung bean cuttings

cuttings were treated directly with distilled water or different concentrations of Nacl (25-300 mM/ml) for 24 hours. The Mung was then transferred to boric acid at a concentration of 5 µg / ml for 6 days.

2-Determination of the optimal concentration of glutathione in rooting response

Table (1) shows the effect of different concentrations of glutathione on rooting response of fresh Mung bean cuttings were treated with distilled water developed 9.5 roots. Increasing the concentration of glutathione from 10^{-11} to 10^{-5}M correlated with increased rooting response and developed up to 23.45 root per cutting (an increase of 165.78% at 10^{-5} in comparison to control treatment. Concentration of 10^{-5} has reliable in subsequent experiments as the optimal concentration of glutathione in rooting response.

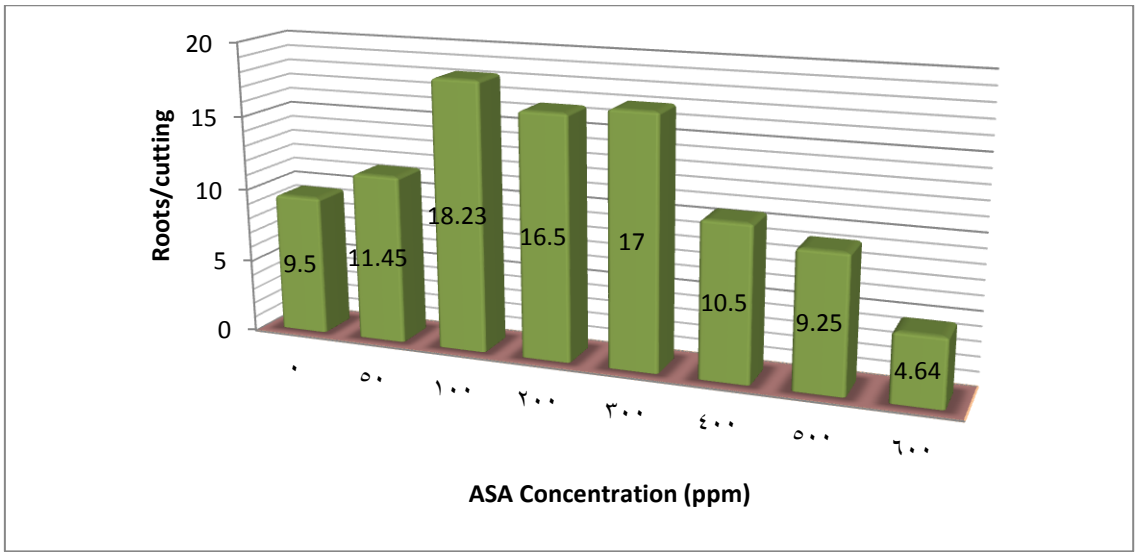
Table (1): The effect of GSH concentration on root response of Mung bean cutting

Glutathione concentration(mole)	Mean roots number /cutting
0	9.50
10^{-11}	14.50
10^{-9}	18.75
10^{-7}	16.00
10^{-5}	23.45
10^{-3}	20.25
L.S.D at 0.05	3.18

3 - Determination of optimal concentration of ascorbic acid in rooting response

Figure (2) indicates the effect of different concentrations of Ascorbic acid on rooting response of Mung bean cuttings. Cuttings were treated with distilled water developed 9.50 roots per cutting. However, cuttings were treated with different concentrations of Ascorbic acid for 24 h. stimulates rooting response at 100-300 ppm particularly, and highest response 18.2 roots at concentration of 100 ppm although it doesn't differ statistically among them, while concentrations higher than 400

ppm was inhibitory for the rooting response and linearly decreased with increasing the concentration up 600 ppm. Finally, concentration of 100 ppm was considered as the optimum concentration that concerned with Na- detoxification in subsequent experiments.



Figure(2):The effect of ASA concentration on rooting response of Mung bean cuttings

4 - Effect of combinations between GSH and ASA in rooting response of Mung bean cuttings

In Table 2, the treatment of the Mung bean cuttings with distilled water developed 7.8 roots per cutting and combination between GSH at optimum conc. 10^{-5} mM (threshold) and different Conc. of AS A (threshold) , 100 ppm , less than threshold , 50 ppm of more than threshold , 400 ppm) developed 18.5 , 15.6 and 14.2 roots/ cutting respectively . On the other hand, combination between AsA of optimum. Conc., l00M (Threshold) and different Conc. of GSH (Threshold) 10^{-5} less then Threshold 10^{-7}M and more then threshold 10^{-3}M were developed 19.35 ,17.5 and 21.25 roots / cutting respectively.

Obviously, the higher roots response (21.25 roots / cutting) represented by the combination of 100 ppm of ASA (threshold) plus 10^{-3}M GSH (more than Threshold). Note with standing, the above combination is not differ statistically from the combination of 100 ppm of ASA (Threshold) plus 10^{-5} M of GSH (Threshold too), that developed 19.75 roots /cutting. Consequently, The foregoing results particularly the last case (the threshold of ASA : Threshold of GSH) 100 ppm: 10^{-5} M that developed 19.75 roots /cutting was considered the optimum combination for Na-detoxification in subsequent experiments.

Table (2): The interaction effect GSH and ASA on the rooting response of Mung bean cuttings

Ratio GSH: ASA	Mean root number/cutting
D.W.	7.8
10^{-5} :100	18.5
10^{-5} :50	15.6
10^{-5} :400	14.25
100 :10^{-5}	19.75
100 :10^{-7}	17.5
100 :10^{-3}	21.25
L.S.D at 0.05	2.13

5- Effect of Glutathione and ascorbic acid on the removal of Nacl toxicity in Mung bean cuttings

Table 3 refers to the effect of combination between GSH 10^{-5}M and ASA100ppm combination , as exogenously supplied before(Pre–treatment), after (post–treatment) and simultaneously with toxic NaCl on rooting response of Mung bean cuttings. However, distilled water treatment developed 14.50 root per cutting. Moreover, the combination of GSH 10^{-5}M: ASA100 ppm within 24 hours were developed 25.66 roots per cutting and, while sodium chloride alone had an inhibitory effect (8.83), both of which were statistically significant. Exogenous application of the combination (within the first 24 hours) prior to NaCl (within of
NaCl pre-treatment was developed (19.00) roots. On the other hand, (post–treatment of combination after the toxic level of NaCl was developed 22.33 roots. While application the combination simultaneously with toxic level developed 12.08roots. Seemingly, supplying the combination pre or post. Is better than simultaneous supply by toxic NaCl, and post- application is better siqanficantly. It should be recon cerervded that application of the combination between (ASA, 100 ppm + GSH, 10^{-5}M) having the capability to remove Completely the toxic effect oxidation stress of Na.

Table(3) : The effect of GSH and ASA on removing the toxicity of NaCl of Mung bean cuttings

Treatment with :	Mean Root number
d.w for 24h	14.50
GSH 10^{-5}: ASA100 for 24h	25.66
Nacl 50 M/ml for 24h	8.83
GSH 10^{-5} :ASA100 for 24h → Nacl 50 M/ml for24h	19.00
Nacl 50 M/ml for 24h → GSH 10^{-5} :ASA100 24h	22.33
GSH 10^{-5} :ASA100 + Nacl 50 M/ml for 24h	12.08
L.S.D at (0.05)	2.26

Discussion :

The role of the antioxidants glutathione and ascorbic acid was investigated in order to improve the tolerance of Mung bean (sensitive) to saline toxicity in terms of rooting response. The level of the toxic sodium was determined in the Mung bean cuttings based on the reduction of growth indicator in terms of the roots number development in per cutting by 50% , and the appearance of morphological symptoms associated with toxicity.

The results of the current study showed that, the number of roots was reduced by about half (6.5) in cuttings the toxic concentration of NaCl at 50mM compared to control cuttings (11.25) and in reduction is equall 54.54% Figure (1).Howover, [10] referred to decrease the rooting response of Mung bean cuttings when exposed to Cadmium toxicity. The decrease of rooting response is attributed to the decline in basic rooting requirements such as auxin (IAA), carbohydrates and proteins [11].

On the other hand, exogenous supply of cuttings with glutathione for 24 hours led to increase roots number, and the catalytic effect of GSH was its significantly affected the rooting response particular at high concentrations 10^{-5}-10^{-3}. It was found by [6] that, the high concentrations of GSH that were more than 5mM had a lethal effect on the cells of rooting zone results of the study were compatible with the results of [12] which indicated tha the reduced GSH encouraged the adventitious root formation (ARF) in the Medicago cuttings.

In addition, ASA at a concentration of 100 ppm stimulated the rooting response and this could be attributed to that was mentioned by [13] that ascorbate controls the hydroxyproline synthesis process, which incurporated many proteins necessary for cell growth and G1, G2 of cell cycle. In addition , ASA is one of the important factors for ellivating H_2O_2 toxicity and its high concentrations helps the cells to maintain the chemical stress of oxidation and reduction. It was

confirmed by [14] that, ASA at a concentration of 350 mg / L alone or associated with the IBA hormone promotes the implantation of the cuttings of the Punica medical plant. Supplying both antioxidants at different concentrations, based on the principle of fixing one of the antioxidants in the optimal concentration (eg 100 ppm for ASA) and the addition of the other anti-oxidants to work of combinations with the second anti-oxidants with the optimum concentration of GSH 10^{-5} , Secondly below the optimum concentration of 10^{-7} and thirdly higher than the optimal concentration 10^{-3} and in the second case of the GSH is proved in the optimum concentration 10^{-3} and the addition of ASA in the same method as the optimum concentration of 100, the lower is 50 and the higher is 400.

Based on the results, it appears that the best response of the rooting response was the result of the interaction between ASA and GSH, which developed 22.33 root per cutting when the optimal ASA (100 ppm) in terms of the rooting response with GSH when prepared with a higher concentration GSH concentrations($100:10^{-3}$) This may be due to the role of non enzymatic antioxidants GSH and ASA and their efficacy in removing the toxicity of NaCl and their interaction in the continuity of the ASA-GSH cycle and increasing the efficiency of the enzymes of this cycle and its role in suppressing the toxic effects of free radical that prodrced in cutting under NaCl stress.

For controlling Nacl toxicity, the antioxidants ASA and GSH were supplied at the optimal concentration of rooting (100 ppm ASA and 10^{-5} M GSH). such combination that supplied exogenous before , after or simultanous with toxic concentration of NaCl. Table (3) referred to the combination that had catalytic effect relative to control (treated with distilled water) and that the toxic concentration of salt had a similar inhibitory effect as compared to control and combination doesnt affects the removal of NaCl toxicity when it was supplied simultaneously (together) but is able to remove NaCl toxicity when it it was supplied before and after exposure to NaCl (i.e. protective and therapeutic role). Inaddition, an induction of increased rooting response at 31.03% and 54% respectively. The combination removal NaCl toxicity when supplied as Pre-treatment NaCl and Post-treatment NaCl. High production of H_2O_2 during oxidative stress inhibits the formation of roots in Mung bean cuttings [16].

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