The role of salicylic acid in alleviating boron toxicity in mung been cuttings

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Abstract

The role of salicylic acid (SA) in alleviating boron toxicity in terms of adventitious root formation (ARF) of mung bean cuttings were studied after determination of toxic level of B and the promontory concentration of SA . Besides SA is not effective in high level of significancy in un- treated cuttings with auxin and the role of SA was tested via three kinds of applications in relation to IAA:-Post-, pre-, and simultaneous application. The results revealed the followings:

1- The toxic levels of B were (250- 600) µg/ml which reduced growth parameters in terms of rooting response to one third compared to the control , morphological symptoms represented by bleaching of primary leaves, in addition to chlorosis & necrosis .
2- The solitary and promotory concentration of SA is 10^{-4} M that enhanced rooting response by 36% over control.
3- Simultaneous application of SA & IAA at their optimum concentrations enhances rooting response by doubling the root number (110 roots) compared to IAA alone (52 root).
4- The later rooting response is better than SA pre- treated to stock plants (promotes 54.5 root / cutting) . Meanwhile, it doesn't raised to the rooting level when SA supplied to cuttings after auxin post- application) , particularly for 2-days (promote 138.2 root / cutting).
5- Complete B detoxification by treating cuttings with SA after auxin (post- application) and before exposure to the toxic level of B, was obvious Through retention of primary leaves in their normal morphological color, significantly raising the rooting responses, and verifying the synergistic effect between SA & IAA, particularly when SA supplied after 36- 40 h of cuttings excision.
6- The discussion was focused on the protective role and value of SA in preventing photosynthetic pigment damages that occurred by B, biosynthesis of protein for repairing damages , and improving the Anti-oxidant defense mechanisms.

الخلاصة

ان دور حامض السالسليك (SA) في تخفيف سمية البورون (B) بدلالة تكوين الجذور العرضية في عقل الماش قد درس بعد تحديد مستوى السمية للـ B والتركيز المحفز للـ SA. وبالنظر الى كون SA غير مؤثر بدرجة

عالية من المعنوية في العقل غير المعاملة بالاوكسين، فان الـ IAA قد جهز كمعاملة استحثاثية للتجذير، وان دور الـ SA قد فحص بثلاث طرق من خلال علاقته بالاوكسين وهي التجهيز بعد الاوكسين، قبل الاوكسين، وفي ان واحد مع الاوكسين وقد بينت النتائج مايلي :-

1-ان مستويات السمية للـ B هي (250-600) مايكروغرام/مل، حيث اختزلت مؤشرات النمو بدلالة استجابة التجذير الى الثلث(نسبة الاختزال >60%) مقارنة بعينة السيطرة، مع ظهور اعراض مورفولوجية تمثلت بابيضـاض الاوراق الاولية وتنخرها اضافة الى نقصان مستوى الكلوروفيل.

2-التركيز الوحيد والمحفز للتجذير من SA هو 10^{-4} والذي حفز استجابة التجذير باكثر من 36% فوق عينة السيطرة.

3-ان تجهيز الـSA مع IAA في ان واحد وبالتركيز الامثل لكليهما (10^{-4}M و $5x10^{-4}$M على التوالي). قد حفـز استجابة التجذير باكثر من ضعف (110 جذر/العقلة) مقارنة بالـ IAA لوحدة (52 جذر).

4-ان استجابة التجذير الاخيرة (الفقرة 3) افضل من تجهيز الـ SA قبل الاوكسين (أي للنبات الام قد حفـزت 54.5 جذر). وفي الوقت ذاته فانها لم ترتقي الى مستوى التجذير عندما يجهز الـ SA للعقل بعد الاوكسين ، خصوصاً لمدة يومين (حفزت 138.2 جذر).

5-ان ازالة سمية البورون كاملة بمعاملة العقل بالـ SA بعد الاوكسين وقبل تعرضها لمستوى السمية مـن الـ B ، تبدو واضحة عن طريق احتفاظ الاوراق الاولية بنظارتها ولونها الاعتيادي ، والزيادة المعنوية لاسـتجابة التجـذير ، وكذلك تاكيدا لصحة التاثير التآزري بين الـ SA والـ IAA وخصوصا عندما يجهز الـSA بعد 36-40 سـاعة من وقت اخذ العقل.

6-لقد تركزت المناقشة حول الدور الوقائي للـ SA في منع تحطيم صبغات البناء الضوئي الذي يحصل بتاثير الـB ، تخليق بروتين اصلاح التلف، وتحسين النظام او الميكانيكيات المضادة للاكسدة.

Introduction

Boron presents a challenge to agronomists. Management of boron in soil is made difficult by its high mobility, being easily leached under high rainfall conditions, therefore boron may accumulate to levels that become toxic to plant[1]. In plant Kingdom, there is a widespread range of sensitivity to boron depending upon concentration and time as well as method of supply [2]. Boron is one of necessary nutrients of vascular plants, but its existence with high concentrations causes toxicity. The phenomenon of boron toxicity is one of the aspects of major abiotic stress that plants are exposed to, as a result, the crop yields will be limited [3], for example : tomato, cucumber [4] & spinach [5]. In addition, the high level of boron along with salinity takes part in limiting production and growth as is in wheat [3]

The physiological effects of boron toxicity include reduced root cell division (6), decreased shoot and root growth (7,8), decrease in leaf chlorophyll, inhibition of photosynthesis (9), increased permeability membrane, peroxidation of lipids and altered activities of antioxidation pathways (10). All these effects explain the appearance of the morphological symptoms following : bleaching of primary leaves and chlorosis & necrosis as well. In addition to what is mentioned above, boron toxicity leads to oxidative stress and the production of free radical(reactive oxygen species) increases as superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical($O\dot{H}$)- that are regarded as strong oxidants to lipids, protein and nucleic

acids resulting in damage of membrane and then death of cell (11). The reduction of root growth because of boron toxicity may vary according to the plant , for example : in wheat (Triticum aestivum L), it is represented by abnormal growth of apical meristem in roots (1) and in soyabean , it is represented by formation of hypodermis and deposition of suberin in cortical cell walls (9).

Salicylic acid (2-Hydroxybenzoic acid) is an organic acid derived from the amino acid phenylalanine through shikimate-phenylpropaniod pathway [12]) and is chemically similar to aspirin (acetylsalicylic acid) [13] . *salix alba* is considered as the natural source of Salicylic acid[14]. Some scientists were considered (SA) as endogenous growth regulator , having phenolic nature and contributing in many physiological processes in plants like development and growth,photosynthesis, transpiration….etc. [15] SA exhibits protective effect to plants under biotic and abiotic stress [17,16].The Plant resistance increases against salinity[17], boron toxicity related to salinity in many plants, as carrot [19], as well as protecting plant from contaminative metals [20], like alleviating toxicity of Cd [21] , Mn [22] , Hg [23] & Cu [24] . In addition , SA enhances seedlings growth under the stress of Pb_2^+ or Hg_2^+ in rice [25].

The protection role of SA is represented mainly in : regulating reactive oxygen species (ROS), antioxidants , inducing gene expression and absorbing and distributing of elements [28,27,26] . The direct physiological effect of SA is alternation of activities of antioxidant enzymes *in vivo* [29,17] . Perception and understanding of physiological principles of adventitious root formation in cuttings derived from auxin priority , and its control upon this phenomenon among all natural and synthetic chemical substances that detect of its biological activities is done in this field , and not involve any other material directly in initiation phase[30].

The dedifferentiation of cutting tissues during adventitious root formation includes alternation of cells from normal into functional developmental pathways ending with formation of root primordia and subsequent formation of new roots [31 .This requires a lot of metabolic changes as enzymes and large biomolecules during phases of induction, initiation and development of root primordia in cuttings [32,33].Besides, what have previously preceded , in spite of the little researches which refers to that SA is not effecting or may be inhibitor of adventitious roots formation as [34,35] , meanwhile in contrast to some of other researches that indicate the ability of SA to play synergetic role with auxin, that may depend on time of SA application to cuttings, that is to say before, after or simultaneously. Therefore, SA would sine a chance of knowing its expected role during phases of growth and development whenever initiation phase is submitting the control of auxin only depending upon this imagination first and considering that there is no study of the elements toxicity in terms of adventitious roots in cuttings. the aim of this research has come to explore some of the obscure chemical sides that adopt the role of SA in alleviating boron toxicity by using mung bean known of its sensitivity [36].

Materials and methods

Growth of stock plants & preparation of cuttings

Seed germination and seedling growth were carried out in growth cabinet at 25 ± 1 c º under continuous illumination supplied by warm white fluorescent tubes (1600 – 1800) lux and relative humidity of 60 – 70 % , using sterilized sawdust as

a rooting medium. Stem cuttings were prepared according to [37], from 10-day old light grwon seedlings. The cuttings had apical bud , a pair of fully expanded primary leaves, epicotyl and 3 cm of hypocotyl under cotyledonary nodes , after removal of root system.

Preparation of solutions

Indole acetic acid (IAA) was initially dissolved in a small volume of absolute ethanol (30% ethanol for salicylic acid (SA) to which d/H_2O was added to the required volume. Ethanol was presented at a final concentration of 2ml / L for both IAA and SA . These concentrations of ethanol are without effect on root formation in cuttings such as those employed here [38]. Boric acid was prepared in a wide range of concentrations (0.001-600) µg/ml to determine the toxic level of boron (B) . Thereafter, two concentrations were only used in subsequent experiments :- a) 10 µg/ml , as rooting medium for mung bean because it's necessary in formation of root promordia and its subsequent growth & development to visible roots [39]. b) 250 µg/ml , as toxic level of B that reduced growth parameters and represented by morphological symptoms by bleaching of primary leaves .

Basal treatment of cuttings

Twelve cuttings were used per treatment for rooting tests , placed 6 beakers containing 32 ml of the appropriate solution. This volume gave a solution depth of 3 cm, thereby covering the entire hypocotyl . All experiments were designed as completely randomized & the statistical analysis was done according to [40].

Results

Fig. (1) shows that cuttings treated with d/H_2O (control) for 24h developed (11) roots / cutting. Whereas, cuttings treated with low concentration (0.01,0.1 and 5) µg/ml of boric acid developed (11.2 , 12.4 , 12.3 , and 11.5) roots / cutting .These figures do not differ significantly neither from each other nor from control treatment ,so represented by block (A) . However, at concentrations (10,25,50,75,125) µg/ml roots no. per cutting were inhibited to (7.8,6.4,7.2, and 6.1) respectively . Approximately, these figures were equal to half the no. of roots compared to control (11.3) roots , which were represented by block (B). In addition, at high concentrations of boric acid (250 , 350,400,500 and 600) µg/ml, root mean no. was reduced significantly to (4.8,4.6,4.3,4.9 and 3.3) per cutting respectively, (that is, the rate of reduction became more than 60%). These cuttings were accompanied by morphological symptoms represented by bleaching of primary leaves , chlorosis , and necrosis. Thereafter, these concentrations were considered as toxic level of boron and represented by block (C) . The lower concentration 250 µg/ml was chosen and considered as the toxic level of boron for subsequent experiments.

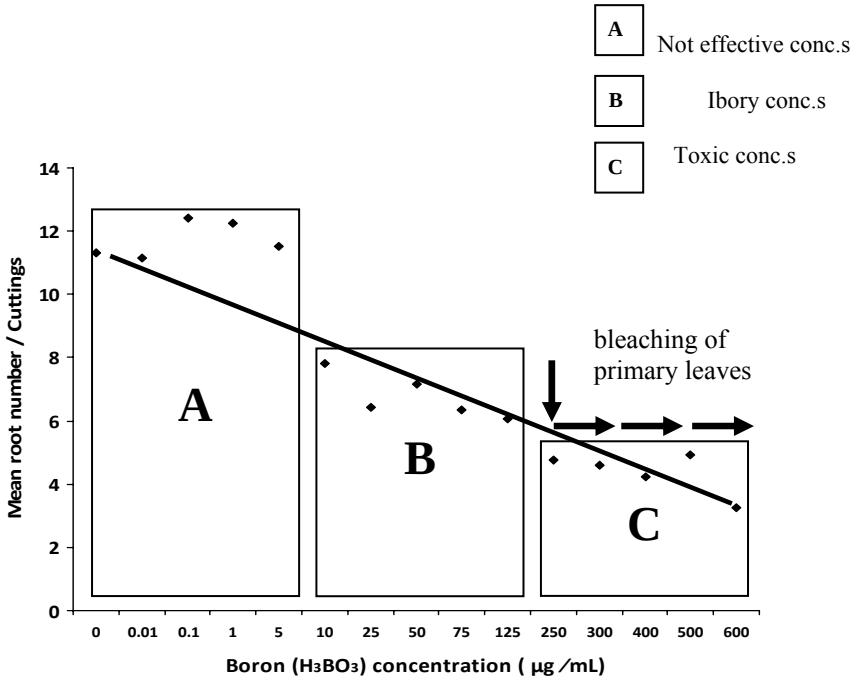


Figure 1 : Rooting response of mung been cuttings supplied with different concentration of boron (H_3BO_3) / to determine the toxic level . Cuttings were taken from 10 – day – old light grown seedling , treated for 24h with borate except for control . Root number were determinate 6 day after transfer to d/H_2O .L.S.D at (0.05) = 0.690.

The influence of different concentrations of salicylic acid (SA) on rooting response of mung bean cuttings was observed through Table (1). Cuttings of control (d/H_2O) developed (10.5 roots/cutting), cuttings treated with SA for 24h at (10^{-12} -10^{-5})M developed number of roots which are not significantly different from control , particularly at $10^{-10}$and 10^{-5})M or less at the rest of above concentrations . However , increasing the concentration to (10^{-4} M) raised the no. of roots to 14 roots/cutting which differ significantly from other concentrations & control treatment . While the highest concentration (10^{-3})M inhibited the roots number to less than (72%) in comparison to control (2.9 root) . The foregoing results confirm that SA might act as rooting promoter at the optimum concentration (10^{-4}M) for mung bean cutting and was employed for the subsequent experiments.

Table (1): influence of salicylic acid on rooting response of mung bean cuttings

Treatment for 24h with	Mean roots no./cutting
d/H_2O	**10.50**
SA 10^{-3} M	*** 2 . 9**
SA 10^{-4} M	**14 . 33***
SA 10^{-5} M	**9 . 5**
SA 10^{-6} M	**7. 09**
SA 10^{-7} M	**7. 08**
SA 10^{-8} M	**7 . 25**
SA 10^{-10} M	**9 . 25**
SA 10^{-12} M	**7. 25**

L.S.D at (0.05) =1.27

Table (2) shows that IAA supplied exogenously at (5×10^{-4}) M promoted roots to 58.8 ,which approximately equal six folds compared to control (10.5 roots) . Whereas , SA at optimum concentration (10^{-4})M and Boron at toxic level (250) µg/ml developed individually 8.8 and 4.7 roots /cuttings respectively. However , post – application of SA at optimum concentration (10^{-4})M for cuttings pre-treated with IAA (5×10^{-4} M) for the 1st 24h, developed 77.2 roots /cuttings, that represents (31.3%) over IAA alone . In addition, the influence of B at the toxic level (that reduced the root no. to 4.7 roots/cuttings) was able to be detoxified by supplying SA for 24h to cuttings which were already pre-treated with IAA before B supplied at the toxic level (250 µg/ml). Notwithstanding, cuttings were maintaining the freshness appearance of their leaves by keeping their chlorophyll content and developing a no. of roots equal to 70.9 which do not significantly differ from cuttings that are not supplied with toxic level of B (77.2), or approximately doubling the no. of the roots (138.2) compared to cuttings that were supplied with SA and already pre-treated with IAA (particularly when SA supplied for 48h). As a conclusion , SA was detoxifying B toxicity completely by avoiding the appearance of morphological bleaching of primary leaves, in addition to doubling the root no. when cuttings pre-treated with IAA, thereafter, confirming the synergistic role of SA with auxin (IAA).

Table (2) : The influence of salicylic acid in B-detoxification when supplied after auxin (post-application) for mung bean cuttings.

Treatment for 24h with	Subsequent treatment	Mean roots no./cutting
d/H_2O	B / 10 µg/ml for 6 days .	10.25
IAA / 5×10^{-4}M	B / 10 µg/ml for 6 days .	58 .75
Salicylic acid 10^{-4} M	B / 10 µg/ml for 6 days .	8 . 83
H_3BO_3 250 µg/ml	B / 10 µg/ml for 6 days .	4 . 66
IAA / 5×10^{-4}M	SA 10^{-4}Mfor 24h / d/H_2O for 6 days.	77.166
IAA / 5×10^{-4}M	SA 10^{-4}M for 24h / B 250 µg/ml for 24h / d/H_2O for 6 days.	70 . 91
IAA / 5×10^{-4}M	SA 10^{-4}M for 48h / B 250 µg/ml for 24h / d/H_2O for 6 days.	138.166 *
IAA / 5×10^{-4}M	B 250 µg/ml for 24h / d/H_2O for 6 days.	32.75

Stem cuttings was treated with d/H_2O, IAA (5×10^{-4} M), SA (10^{-4}M), and Boric acid (250 µg/ml) for 24h. There after , transferred to boron rooting medium (10 µg/ml)for 6 day or SA for 24h or 48h before supplying H_3BO_3 at toxic level , then to d/H_2O for 6 day. L.S.D at (0.05) = 17.04

The influence of salicylic acid (SA) when supplied to the stock plant (pre-treatment), before derived cuttings treated with IAA (5×10^{-4} M), then B at the toxic level was observed in Fig (2). Cuttings derived from seedlings supplied with SA (10^{-4}M) developed an average of adventitious roots, and the latter was increased proportionally with raising the duration time of SA application to the stock seedlings from (4-7) days. Significantly, root no. increased particularly at seventh day, in cuttings that were supplied with toxic level of B, which confirmed B-detoxification by supplying stock plants (pre-application).

It is noteworthy that the above relation was inversed after (eight –ten) days that accompanied with the time of cotyledons shrivels & drop-off spontaneously from mung bean seedlings, whereas, rooting response was increased in cuttings supplied with normal concentration of Boron in rooting medium (10 µg/ml). According to the foregoing results, it is reasonable to recommend, that application of SA to stock plant for 7 days in mung bean before, treating the derived cuttings with IAA then the toxic level of B, in order to overcome B toxicity in a ratio equals to 3 folds compared to control (cuttings were supplied with normal level of B as a rooting medium).

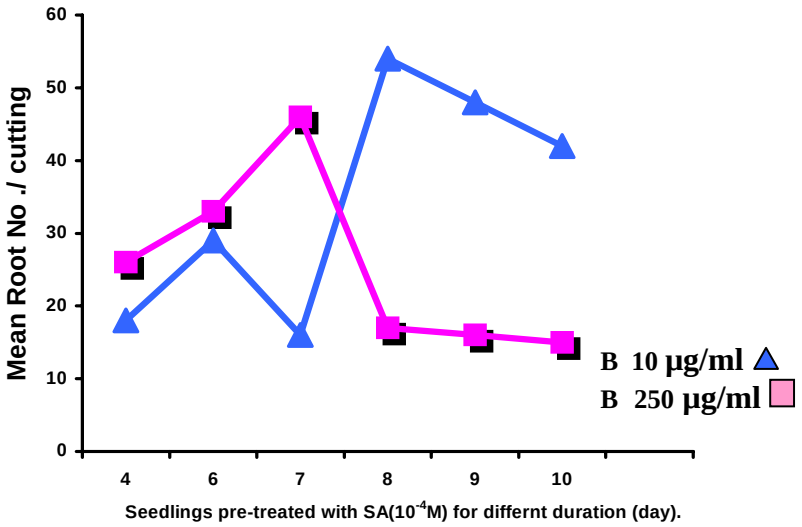


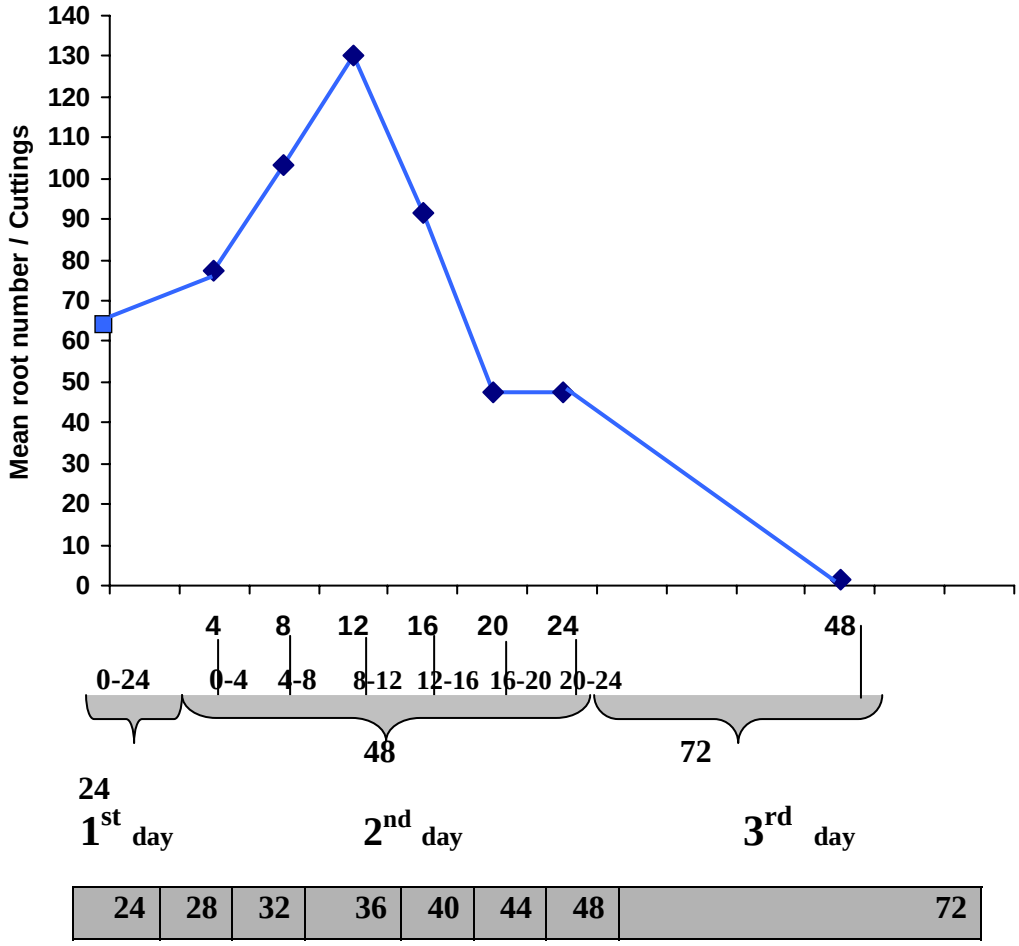
Figure 2 : Effect of pre-treatment of SA to the stock plants before treatment of the derived cuttings with optimum concentration of IAA (5×10^{-4}M) then ,toxic level of B (250 µg/ml) , on rooting response of mung bean cuttings. L.S.D at (0.05) = 21.96

Fig (3) shows the influence of SA during growth and development phase in rooting response in mung bean cuttings pre-treated with IAA (5×10^{-4} M) during the first day (24h). Cuttings of control treatment (cuttings were treated with IAA for the first 24h, then transfer to boric acid/rooting medium) developed 65.9 roots/cuttings. Whereas, cuttings supplied with SA during the 2nd (24-28h), which already pre-treated with IAA during the 1st day (24h), developed 81.5 roots/cuttings (with increment =12.4 %). In addition, when cuttings were supplied with SA during the 3rd day (48-72h) which were already pre-treated with IAA during the 1st day (24h) & d/H_2O during the 2nd day, the rooting response was inhibited completely (1.4 roots/cuttings).

The foregoing results indicate, the promotory role of SA when supplied only during the 2nd day, and the inhibitory role of SA when supplied only during the 3rd day, with the pre-treatment of IAA during the 1st day in both cases. In addition, the above results also revealed the need to investigate the accurate time for SA influence during the 2nd day.

For this reason, SA was supplied successively for cuttings for each 4h during the 2nd day (e.s. after 24-28, 28-32, 32-36, 36-40, 40-44 and 44-48h) after supplying IAA for the 1st 24h. However, rooting in terms of root no./cutting was

(77.1, 103.3, 130.3.91.7, 47.9 and 49.1) respectively. In other words, the no. of roots increased proportionally after supplying SA till 36-40h from time of cuttings excision, then declined beyond that time to a level less than the control treatment.



SA supplied successively for each 4h during the 2nd day or the whole 3rd day.

Figure 3 : Effect of the salicylic acid through growth & development phase in the Rooting response of mung been cuttings pre-treated with Auxin(5×10^{-4}M) through the first 24 h . L.S.D at (0.05) = 12.31.

The interaction between SA & IAA when supplied simultaneously during the 1st 24h was shown in table (3). Cuttings were treated with IAA or SA individually developed 58.2 or 13.7 roots/cutting respectively. Whereas, cuttings treated within a combination from the optimum concentration or both IAA (5×10^{-4} M) and

SA (10^{-4}M) developed 110 roots/cuttings. However, application of IAA at the optimum concentration + SA at concentration higher than optimum (10^{-3}M) or lower than the optimum such as (10^{-5} or 10^{-8}M), the rooting response was reduced to the 96.7, 63.5, or 68.6 roots/cuttings at the three cases above. Finally, as a conclusion, the application of SA with IAA simultaneously promotes a rooting response twice than that which belongs to IAA or SA alone. In addition, it is considered better than supplying SA before auxin (pre-treatment) as in fig (3), but it does not rise to the effect of SA when supplied after auxin (post-treatment) as in Table (2).

Table (3): The interaction between IAA& SA when supplied simultaneously in rooting response of mung bean cuttings

Treatment for 24h with	Mean roots no./cutting
IAA 5×10^{-4} M	**58.16**
SA 10^{-4} M	**13.66**
IAA 5×10^{-4} M+ SA 10^{-3} M	**96.66**
IAA 5×10^{-4} M+ SA 10^{-4} M	**109.66**
IAA 5×10^{-4} M+ SA 10^{-5} M	**63.50**
IAA 5×10^{-4} M+ SA 10^{-8} M	**68.66**

L.S.D at (0.05) =31.

Discussion

In this study, investigation was carried out about the role of SA in mitigation (amelioration) of plant resistance to one environmental stress factors, boron toxicity. Previously, it was mentioned, about the protective effect of SA which involved anti-stress programs and acceleration of natural growth processes after removal of stress factors [41]. The result of this study revealed many important points :

First :- determination of B – toxic level in mung bean cutting was done depending on the appearance of morphological symptoms that were represented by bleaching of primary leaves, chlorosis, and necrosis. However, reduction of growth parameters was represented by rooting response in terms of the no. of adventitious roots per cutting. Meanwhile, the toxic level of B was (250 µg/ml) and so on Fig (1). Additionally, [42] mentioned that the appearance of bleaching and necrosis at high level of B was limiting the capability

of leaves for photosynthetic supplying, while on the root growth level, it decreased the absorption of water & minerals. It is noteworthy that B-toxicity was represented by the above concentration (250-600) µg/ml, which are higher than the international standard that was mentioned by [37]. The latter considered that mung bean is sensitive plant for B within a limit of (0.5-1.0) µg/ml. In this study, we are depending on the morphological symptoms to determine the toxic levels rather than the quantitative analysis for boron in plant tissues

Second :- the promotory role of SA in rooting response at 10^{-4}M was {promotion (36%) more than control}, however, higher concentration (10^{-3}M) was inhibitory whereas, lower concentration was in general not effective and some other concentrations are inhibitory in little percent (Table-1). Recently, this case was confirmed by many researchers, they pointed out that the effect of SA in physiological process was different. It was promotory for some and inhibitory for other, depending on SA concentration, plant species, developmental stage, and environmental conditions [43,44]. Accordingly , SA is ubiquitous phenolic compound in plants , and considered by some an endogenous growth regulator and participator in regulation of plant physiological processes [45,46,15]. However, exogenous application of SA may affect uptake and transport of ions [47], inhibition of ethylene biosynthesis , transpiration and stress tolerance [48,49], and promote adventitious root formation in many plants in particular mung bean[50]. In addition , it has been mentioned that SA revealed different degrees of promotion depending on properties that deal with species(*populus* cuttings) rather than concentration, meanwhile, SA was not effective in *Tillia* cuttings [35] whereas, the inhibitory role of SA resides in improvement of IAA oxidation during auxin sensitive phase (24-96h)[51].

Third :- the interaction between SA & IAA , represented by supplying SA simultaneously with IAA in the best combination involved the optimum concentration of both IAA (5×10^{-4} M) and SA (10^{-4})M. This combination promotes rooting response more than 2 folds (109.6 roots /cutting) compared to cuttings treated with IAA alone (52 roots /cutting) as in Table (3). The above response is better than supplying SA to the stock plants. In other words , before auxin treatment (pre-treatment) as in Fig (3) which developed (54.5) roots, but at the same time it does not rise to the level of rooting response

when SA supplied to cuttings after auxin treatment (post-application) as in Table (2), particularly when supplied for 2 days (developed 138.2 roots /cutting). The foregoing results denoted the application of SA for mung bean cuttings after routine treatment of IAA for 24h (post-application) is the best not only for rooting response induction but for maintaining the synergetic effect between each other, in addition to B-detoxification.[52] mentioned that, SA has synergetic effect with IAA in mung bean cuttings, but not in *Acer* cuttings. However, such synergism seems to be related to different plant species and time of cutting excision more than SA concentration and method of application [53], but SA was described to have favorable influence with IAA in rooting of mung bean cuttings, as the influence of SA with NAA on *populus* cuttings & aspirin (acetylsalicylic acid) with *phaseoulus* cuttings [54].

The pre-application of SA was less effective in rooting response, that enabled Van Der Vrieken [55] and his colleagues (1997) to denote its inhibitory effects when supplied before auxin in rooting of apple stem slices. The explanation of this effect was attributed to the IAA-oxidation during auxin-sensitive phase by SA that promotes IAA-oxidation[51].

Fourth :- B-detoxification was completely occurred via treatment of cutting by SA after auxin (post-application) and before exposure to toxic level of B, and that was obvious through morphological maintenance of freshness of primary leaves & raising the average of roots in cutting, that is confirming synergist effect between SA and IAA unchanged. In addition to enhancement of SA in B-detoxification that increased with the increasing the duration of time from 24-48h (Table-2), which confirms the need of cutting for SA more than 24h after auxin treatment (during growth and development phase of adventitious roots).

Obviously, the results of Fig (3) show the promotory role of SA when supplied only during the 2nd day after pre-treatment of cuttings with IAA during the 1st day.

The above results imposed the need to investigate the precise and effective time of SA application during the 2nd day. For this reason, SA was supplied successively every 4h during the 2nd day, that made the roots no. increase proportionally until 36-40h after cutting excision, then decline to a level less than the control treatment. The foregoing

results coincided with a high precision with : a) Rapid mitosis b) Accumulation of Nucleic acids c) Root initial formation, which become evident within 36-40h in mung bean cuttings [56,57] d) Increasing the activity of IAA-oxidase, promotes root primordia formation and its subsequent development [58]. On the other hand, it was coincided with increasing the endogenous level of H_2O_2, that was detected after 36h from cutting preparation of mung bean via NADPH oxidase pathway during ARF, this was considered as one of the constituents which are necessary for induction of adventitious roots [59]. The latter mentioned that H_2O_2 may act as signal transduction molecule in auxin response during ARF of mung bean cutting. It is noteworthy, that SA promotes increasing H_2O_2 content in plants via inhibition of catalase and ascorbate peroxidase [60,61].

Fifth :- the promotory effect of SA when supplied to the stock plant (pre-application) before treating the derived cuttings with IAA then B at toxic level (250 µg/ml), is detoxifying B particularly when SA supplied until seven - day-old of seedlings old. This agreed with previous results [62] that involved the proportional relationships between the no. of developed roots and increasing seedling age till seven or eight day whether cuttings (the same variety of cuttings as in this study) were treated or untreated with auxin. However, the roots no. were increased significantly, particularly at the seventh day in cutting supplied with toxic level of B that confirms B-detoxification by supplying SA during seedling growth(keeping in mind that ,this effect is less compared to its application to IAA simultaneously or after IAA). It is noteworthy, that this relationship was reversed (rooting response was declined proportionally)after 8-10 days of seedling age. This was coincided with the cotyledons shrivel and drop-off spontaneously in mung been seedling (with nutritional & hormonal substance) that have been previously determined between 8-10 days [63].In addition ,the progressive decline of carbohydrate content in aqueous extracts of mung been cotyledons excised from seedling at different ages (4,5,6,and 7 day), reflected the progressive (loss) in rooting response of mung been cuttings, that were prepared from seedling in fully expanded primary leaves stage, (after cotyledons dropping off) [63].

Sixth :- cuttings were maintaining their color and freshness of primary leaves when supplied with SA during its exposure to the toxic level of B, that may be

attributed to the role of SA in increasing chlorophyll content of leaves [64,65], thereafter increasing photosynthetic rate [65]. [66] describes that SA application reduced the no. & size of necrotic lesions produced by an infection, so the application of SA must be carried out before infection or exposing to a biotic stress. However, SA acts on initiation of some metabolic changes such as , pathogenesis related proteins and phytoalxins [67], or through improvement of anti-oxidant system in leaves [68].

In addition, SA detoxified B that is related to the salinity of carrot (*Daucus carota* L. cv. Nantes) [19] by increasing the accessory pigments of photosynthesis, such as carotenoids & anthocianin in storage roots [69]. These pigments were decreased under stress conditions caused by B [19], and increase of their contents was related to suppling SA & their protective value in mung bean [70].

Reference

1. Reid, R.J., Hayes, J.E., Post, A., Stangoulis, J.C.R & Graham, R.D . ***Plant Cell Envir*** , 2004, **25**, 1405.
2. Schon, M.K., Novacky, A & Blevins, D.G . ***Plant Physiol***. ,1990 , **93**, 566.
3. Grieve, C.M., & Poss, J.A ., ***J. Plant Nutr*** ., 2000 , **23**, 1217.
4. Alpaslan, M & Gunes, ***A . Plant Soil*** . ,2001, **236**, 123.
5. Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E & Nuray Cicek ., ***Plant Physiol***., 2007, **164**, 728.
6. Liu, D., Jiang, W., Zhang, L & Li, L***. Isr. J. Plant Sci***., 2000, **48**, 47.
7. Nable R.O., Cartwright B., and Lance R.C.,. In: Bassam N.E., Dambroth M., Loughman B.C. (Eds), Genetic Aspects of Plant Mineral Nutrition.(1990). Kluwer Academic Publishers, Dortrecht: 243.
8. Lovatt, C. J & Bates, L. M., ***J. Exp. Bot***., 1984, **35**, 297.
9. Ghanati, F., Morita, A & Yokota, H. ***Plant Sci***., 2005 , **168**, 397.
10. Karabal, E., Yu cel, M & ktem, H.A .***Plant Sci***., 2003, **164**, 925.
11. Del Rio L. A.., Corpas J., Sandalio L. M., Palma J. M & Barroso J. B. ***International Union of Biochemistry and Molecular Biology (IUBMB) Life***., 2003 , **55**, 71.
12. Tachet L, Baltz R, Chong J, Kauffmann S, Fritig B & Saindrenan P . ***FEBS Lett.***, 1998, **437**, 319.
13. http://www.inchem.org/documents/icsc/icsc/eics0563.htm , (2008).
14. Stone E . ***Philosophical Transactions***., 1763 , **53**, 195.
15. Hayat, S & Ahmad, A. (Eds.)(2007) , Springer , Netherlands .
16. Molina, A., Bueno, P., Mar´ın, M.C., Rodrıguez-Rosales, M.P., Belver, A., Venema, K & Donaire, ***J.P. New Phytol***. 2002 , **156**, 409.
17. He, Y., Liu, Y., Cao, W., Hua, M., Xu, B & Huang, B. ***Crop Sci***., 2005, **45**, 988.
18. Shakirova, F.M., Bezrukova, M.V & Izv, Ross. Akad. Nauk, ***Ser : Biol***., 1997, **2**, 149.
19. Eraslan, F., Inal, A., Gunes, A & Alpaslan, M. ***Scientia Horticulturae*** . 2007, **113**, 120.
20. Mishra, A. & Choudhuri, M.A., ***Biol. Plant***., 19994 , **2**, 409.
21. Zawoznik, MS., Groppa, MD., Tomaro, ML & Benavides, MP. ***Plant Sci*** ., 2007, **173**, 190.
22. Shi, Q & Zhu, Z ., ***Envir & Exper Bot*** ., 2008 , **63**, 317.

23. Zhou, Z.S., Guo, K., Elbaz, A.A & Zhi Min Yang ., ***Envir . & Exper. Bot*** ., 2009, **65**, 27.
24. El-Tayeb, M. A., El-Enany, A. E & Ahmed, N. L)., ***Plant Growth Regul*** ., 2006 , **50**,191.
25. Mishra A, & Choudhuri M.A., ***Seed Sci .Technol***., 1997, **25**, 263.
26. Chen, W & Singh, K.B., ***Plant J.***, 1999 , **19**, 667.
27. Metwally, A., Finkemeier, I., Georgi, M & Dietz, K., ***J. Plant Physiol*** ., 2003, **132**, 272.
28. Wang, Y.S., Wang, J., Yang, Z.M., Wang, Q.Y., Li, B., Li, S.Q., Lu, Y.P., Wang, S.H & Sun, X., ***Acta Bot. Sin***., 2004 , **46**, 819.
29. Wang, L.J & Li, SH., ***Plant Growth Regul*** ., 2006, **48**, 137.
30. Blakesley, D., Weston GD & Elliott MC., ***Plant Growth Regul***, 1991, **10**, 1-12
31. Ballester, A., San-Jose, M.C., Vidal, N., Fernandez-Lorenzo, J.L & Vieitez, A.M., , ***Ann. Bot***., 1999, **83**, 619.
32. Tartoura, K., da Rocha, A & Youssef, S. ***Plant Growth Regul*** . , 2004 , **42**, 253.
33. Batish DR., Singh HP., Kaur S., Kohli RK & Yadav S.S. ***J. Plant Physiol***. 2008 , **165**, 297.
34. Morsink, W.A.G. & V.G. Smith . ***Can. J. Forest Resear*** ., 1975 , **5**, 500.
35. Isfendiyarouglu, M & Ozeker, E. ***J. Bot*** ., 2008 , **40(3)**, 1135.
36. Huber, G & Knafla, P.ENG. A. Equilibrium Environmental Inc.(2009).
37. Hess, C. E. ***Plant Physiol*** . , 1961, **36** , suppl . 21
38. Shaheed, A. I.(1987). Ph. D. Thesis, University of Sheffield, U. K .
39. Middleton,W., Jarvis,B.C. and Booth , A. (1978 a). New Phytol., 81:279.
40. Al-Kinani, Aied Kareem. (2009). Book of Statistics, University of Babylon, Iraq.
41. Sakhabutdinova, A . R., Fatkhutdinova, D. R., Bezrukova, M. V & Shakirova, F. M. ***Bulg.***, ***J. plant physiol, special issue***, 2003, 314-319.
42. Nable, R.O & Moody D.B., ***Plant & Soil*** , 1992, **140**, 311–314.
43. El-Mergawi, R.A. & Abdel-Wahed, ***M.S.A. Egyptian J. Agron.***, 2003, **26**, 49.
44. Iqbal, M., M. Ashraf, A. Jamil & U.R.M. Shafiq, ***J. of integrative plant Biol***., 2006 , **48(2)**, 181.
45. Raskin, I. In: Davies PJ (ed) . 1995, Kluwer Academic Publishers, Dordrecht, p188.
46. Rajasekaran, LR & Blake, TJ ., ***J. Plant Growth Regul***., 1999, **18**, 175.
47. Khadiga, A. Bebars. Egypt., ***J. Appl. Sci***., 1993, **8(3)**, 259.
48. Waseem M., H.U.R. Alhar & M. Ashraf., ***J. Bot***., 2006, **38(4)**, 1127.
49. Khan, W., Prithiviraj, B & Smith, D.L., ***J. plant Physiol.***, 2003, **160**, 485.
50. De Klerk, G. J., Marinova, S., Rouf S. & Ter Brugge., ***J. Acta Horti.***, 1997, **447**, 247.
51. Kling, G.J. & Meyer, M.M., ***Hort. Sci*** ., 1983 , **18(3)**, 352.
52. Bojarczuk, T. & L. S.Jankiewicz., ***Acta Agrobotanica ,*** 1975, **28**, 121.
53. Singh, S. P. ***Adv. Hort. For***., 1993, **3**, 207.
54. Van Der Krieken, W.M., J. Kodde, M.H.M. Visser, D. Tsardakas, A. Blakmeer, K. De Groot &L. Leegstra. 1997. In: *Biology of Root Formation and Development*, (Ed.): A. Altman and Y. Waisel, Plenum Pres, New York, 95-104 p.
55. Chandra, G. R., Gregory, L. E. & Worley, J. F., ***Plant & Cell Physio***., 1971, **12**, 317.
56. Blazich,F.A. & Heuser.C.W., ***Amer.J.Soc.Hort.Sci***., 1979 ,**104**, 63.
57. Chibbar, R. N. , Gurumurti, K., & Nanda, N. N. ***Experintia*** ., 1979, **15**, 202.
58. Li, S.W., Xuea, L., Xub, S., Fengb, H & Anb, L., ***Envir .& Exp. Bot***., 2009 , **65**, 63.
59. Luo, J. P., Jiang, S. T., & Pan, L. ,***J. Plant Sci***., 2001 , **161**, 125.
60. Kawano, T & Muto, S. ***J Exp. Bot*** . , 2000 , **51**, 685.
61. Shaheed, A.I. ***Iraqi J. Sci*** .1995 , **36 (2)**.

62. Abdulhassan, N.A (2006).M.S.C.Thesis , Babylon univ . Iraq.
63. Leslie CA, Romani RJ, ***Plant Physiol*** , 1988, **88**, 833.
64. Zhao, H.J., Lin, X.W., Shi, H.Z & Chang, S.M . ***Acta agron. Sin*** ., 1995, **21**, 351.
65. Enyedi, A. J., Yalpani, N., Silverman, P & Raskin, I . ***Proc. Natl. Acad. Sci. USA***., 1992 , **89**, 2480.
66. Klessig, D. F & Malamy, ***J. Plant Mol. Biol*** ., 1994 , **26**, 1439.
67. Senaratna, T., McKay, C.E., McKersie, B.D & Fletcher, R.A. ***J. Plant Physiol***., 1988, **33**, 56.
68. Brosche, M & Strid, A. ***Physiol. Plant***., 2003 , **117**, 1.
69. Moharekar, S.T., Lokhand SD., Hara T., Tanaka R., Tanaka A & Chavan P.D. ***Photosynthetica*** ., 2003 , **41**, 315.