The role of Trace Elements in Oxidative Hypothesis during Aging of Mung bean (*Phaseolus aureus) Cuttings via Indole acetic (IAA) level.*

Abdullah I. Shaheed
Department of Biology, College of Science, University of Babylon
Oda M. Yasser
Department of Chemistry, College of Science, University of Babylon
Abdullah O. Alwan
Department of Biology, College of Science, University of Babylon
P. O. Box (4), Hilla, Iraq

(NJC)

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Abstract

Naturally occurring auxin (IAA) was measured spectrophotometrically as indicator for oxidative processes that occurs during ageing phenomenon, in terms of rooting response of mung bean *c*uttings.

According to oxidative hypothesis fresh and aged cuttings *as well as* stock plants were supplied with nutrient solutions that are associated with anti-oxidant defense mechanisms, to investigate their effects in suppression of oxidative stress that accompanied aging phenomenon. The data revealed the followings:-

a) A decline in rooting response of aged cuttings (held in d/H_2O for 3 days) were taken from seedlings grown in Hoagland solution compared to fresh cuttings, with a percentage of decline equal to 11.8% in presence of NAA, 10^{-4} M and 30.8% in its absence. This coincided with the decline of IAA level in aged cuttings compared to fresh cuttings, and was attributed to oxidative processes that occurs during ageing.
b) A decline in rooting response of aged cuttings in d/H_2O (supplied with auxin) taken from seedlings grown in Hoagland solutions lacking for B or Fe, compared to control(complete Hoagland solution).
c) Significant increase in rooting response of cuttings aged in d/H_2O (not supplied with auxin) taken from seedlings grown in Hoagland solution lacking for Mn or B.
d) Increase in rooting response of fresh cuttings treated with low concentration of SeO_2 (0.001-10 ppm).
e) Increase in rooting response of cuttings aged in different strengths of complete, modified nutrient solution (CMNS), except the case of CMNS (SeO_2=1 ppm). This confirmed the role of selenium as anti-oxidant in defense mechanisms occurring in cuttings during ageing.
f) Increase in rootings response of cuttings aged in d/H_2O (not supplied with auxin), taken from seedlings grown in CMNS lacking for boron (In this case, B substituted by Se).
g) Decrease in rooting response of cuttings taken from seedlings grown in d/H_2O for (10) days, and aged in CMNS (Half strength) lacking for Mn or group of elements (Mn, Zn, and Se). The discussion was focused on the importance of trace elements

in their resistance to the damage that resulted by oxidative processes, occurring during aging.

Key Words: Aging , Anti-oxidant defense mechanisms, IAA biosynthesis, Oxidative hypothesis, Rooting response, Selenium, Stem cuttings, and Trace elements .

الخلاصة

تم قياس كمية الاوكسين(IAA) بطريقة Spectrophotometery في عقل الماش *Phaseolus aureus* Roxb. الطرية والمعمرة، كمؤشر لتأثير عمليات الاكسدة Oxidative Processes التي تحدث خلال ظاهرة التعمير بدلالة استجابة التجذير في العقل. وطبقاً لفرضية الاكسدة باعتبارها واحدة من الفرضيات التي تفسر ظاهرة التعمير، جهزت العقل الطرية او المعمرة او النباتات الام Stock Plants ببعض العوامل المرتبطة بميكانيكيات الدفاع المضادة للاكسدة Antioxidant defense mechanisms كالعناصر الضئيلة لمعرفة تاثيرها في اخماد او كبح عوامل الشد التاكسدي المصاحبة لظاهرة التعمير. وكانت النتائج كالاتي:

1- انخفاض استجابة التجذير في العقل المعمرة (المحفوظة لمدة ثلاثة ايام في الماء المقطر) والماخوذة من بادرات نامية في محلول Hoagland قياسا بالعقل الطرية وبنسبة 11.8% بوجود الاوكسين (NAA, 10^{-4}M) و 30.8% بعدمه. وقد تزامن ذلك مع انخفاض المحتوى الاوكسيني IAA في العقل المعمرة قياسا بالعقل الطرية ويعزا ذلك الى العمليات التاكسدية التي تحدث خلال ظاهرة التعمير.

2- انخفاض استجابة تجذير عقل الماش المعمرة في الماء المقطر (المستحثة بالاوكسين المجهز) والماخوذة من بادرات نامية في محاليل Hoagland التي ينقصها البورون او الحديد وبشكل معنوي قياسا بالسيطرة (المحلول الكامل).

3- زيادة معنوية في استجابة تجذير عقل الماش المعمرة في الماء المقطر (غير المستحثة) الماخوذة من بادرات نامية في محاليل Hoagland التي ينقصها المنغنيز او البورون قياسا بالسيطرة (المحلول الكامل).

4- زيادة معنوية جدا في استجابة تجذير عقل الماش الطرية المعاملة بتراكيز واطئة من SeO_2 وبمدى (10-0.001) جزء بالمليون قياسا بالسيطرة (d/H_2O) .

5- زيادة معنوية جدا في استجابة تجذير عقل الماش المعمرة بقوى مختلفة من المحاليل المغذية المحورة، باستثناء حالة المحلول المحور الكامل (SeO_2=1 جزء بالمليون)، قياسا بالسيطرة (d/H_2O)، مما يؤكد دور السلينيوم في اليات الدفاع ضد عوامل الاكسدة التي تحدث في العقل خلال عملية التعمير.

6- زيادة معنوية في استجابة تجذير عقل الماش المعمرة في الماء المقطر (غير المستحثة) الماخوذة من بادرات نامية في المحاليل المغذية المحورة التي ينقصها البورون قياسا بعينة السيطرة (المحلول الكامل) [في هذه الحالة تم احلال B بالـ Se].

7- انخفاض معنوي في استجابة تجذير عقل الماش الماخوذة من بادرات نامية في الماء المقطر لمدة عشرة ايام والمعمرة بالمحاليل المغذية المحورة (Half Strength) التي ينقصها المنغنيز او

مجموعة العناصر(Se, Zn, Mn) وقد تم التركيز في المناقشة على اهمية العناصـــر النـــزرة فـــي مقاومة التلف الناتج عن العمليات التاكسدية التي تحدث خلال ظاهرة التعمير.

Introduction

Aging in terms of adventitious root formation (ARF), means a decline in rooting response of aged compared to fresh cuttings [1]. This decline in rooting response occurred progressively with time when inductive auxin treatment was delayed by holding cuttings in deionized H_2O particularly in Mung bean cuttings [2]. In addition, it has been proposed a free radical theory to explain the damage of plant & animal cells with progressing age[3]. The latter illustrated that lipid oxidation was correlated with plant senescence, and the anti-oxidant agents acts internally to suppress the free radicals, hence reducing the processes that occurs during aging in plants. However, free radicals and its derivatives in aged cells & organs (in Nematodes) regenerated primarily in mitochondria as undesirable products through oxidative phosphorylation[4]. Aging as a phenomenon that fundamently concerned with degenerative changes in metabolism[5]. The latter author mentioned that alteration of hormonal balances was the only molecular events leading to these changes.

On the other hand, to achieve an ideal growth, the nutrient solution must include all elements needed by plants or cuttings properly. Hoagland solution (H. S.) 0.25%, to grow leafly bean cuttings, and showed little inhibition in ARF[6]. Consequantly, some authors found a high percentage of rooting respose (with high quality) of vine cuttings taken from Fertilized plants with Zn compared to untreated plants[7]. The foregoing results may be attributed to a high level of IAA that coincided with a high level of tryptophan (auxin precursor) in plants treated with Zn. Whereas, low concentrations of Mn has no effect on ARF of Mung bean cuttings. However, Gorter was mentioned that, development of root primordia pre-requisite boron supply, while root initiation only induced by auxin[8]. The role of boron in growth and development of root primordia, by continuos cell divisions, to visible roots was demonstrated in mung bean [2]. The latter found that Mung bean cuttings taken from light grown seedlings required boron supply after 48h of inductive auxin treatment (24h). For this reason boron must be supplied during seedling growth, simultaneously during auxin treatment of cuttings, or after auxin treatment of cuttings, unless its application may extended more than 72h after cuttings were taken from seedlings.

The plant spp. That accumulates selenium (Se), fundamentally form Seleno-Amino Acids, which are non-toxic acids and not involved in toxic proteins formation. This explain, why plants accumulates (Se) are not toxified, and the importance of (Se) was resided in reduction of the toxic effects of phosphate [9]. Notwithstanding, (Se) was present in enzymes that induce oxidation-reduction reactions [10].

The aim of this study is to investigate the influence of oxidative processes that proposed to increase during aging in terms of IAA biosynthesis (Level). In addition, to elevate that effect by anti-oxidant defense mechanisms, represented by trace elements to improve rooting response of cuttings aged in nutrient solutions, particularly those supplied with Se.

Materials & Methods

Cultivation of Stock Plants: Seeds of Mung bean (*Phaseolus aureus* Roxb. Var. local) were soaked overnight, sown in moistened (with distilled H_2O or tested solutions) sterilized sawdust in plastic trays. Seedlings raised in growth chamber provided with a continous light (light intensity 3000-3500 Lux), temperature $25 \pm 1^\circ C$ and relative humidity 60-70%.

Preparation of Cuttings: Cuttings were prepared from 10-day-old light grown seedlings according to[11]. These cuttings described by having small terminal bud, pair of fully expanded primary leaves, a whole epicotyl and hypocotyl (3-cm length) under cotyledonary nodes, after removal of root system.

Basal Treatment of Cuttings: Dipping of the whole hypocofyl (3-cm depth) in glass vials required 15ml of tested solutions. Fresh cuttings were treated for 24h with d/H_2O, auxin (NAA, 10^{-4}M), or tested solutions then transferred to boric acid ($10\,\mu g/ml$) for 6 days [12], before counting the root numbers (Twelve cuttings/ treatment).

Aging Treatments: Cuttings were held immediately after taken from 10-day-old seedlings in d/H_2O for 3-days or alternatively in tested solution for the above period, if the purpose is controlling of aging phenomenon. Physiologically, aged cuttings treated with NAA, 10^{-4}M for 24h, then transferred to boric acid ($10\ \mu g/ml$) for further 6 days before counting the root number per cutting. Completely randomized design (CRD) with 3 replicates was conducted in all experiments for statistical analysis according to [13].

Preparation of Solutions:

a) **Synthetic Auxin Solution:** Naphthalene Acetic Acid (NAA) was intialy dissolved in small amount of absolute alcohol (2%)according to(Middleton *et al*,1978a) to prepare (10^{-4} M). The latter concentration was already considered as the optimal conc. with the same kind of cuttings according to [15].

b) **Boric Acid Solution:** Prepared at (10 μg/ml) and employed as rooting medium [12].

c) **Hoagland Solution (H.S.)**: Prepared as proposed by [16] for solution cultures [17] to grow Mung bean seedlings & for cuttings treatment.

d) **Hoagland Solutions Lacking for some Nutrients**: Few nutrient solutions lacking for some trace elements were prepared (Table-2), to show the deficiency effects of these elements on growth of seedlings & rooting response of cuttings.

e) **Selenium Oxide (SeO_2) Solution:** Prepared by dissolving 0.02g of SeO_2 in 200ml of d/H_2O to achieve 100ppm as stock solution, then diluted to the required concentrations.

f) **Modified Hoagland Solutions (MHS):** Selenium was added to Hoagland solution to produce modified Hoagland solution (M.H.S). The latter was achieved according to a series of preliminary experiments (composition of H.S., strength of H.S., presence/absence of Se in H. S., Optimal concentration of Se added to H.S. and both modified, complete solutions & rooting response of cuttings.

Quantitative Determination of IAA: Naturally occurring auxin (IAA) was measured spectrophotometrically in primary leaves and terminal bud, epicotyls and hypocotyls, according to [18,19]. The above procedure (was modified) include the reaction of IAA with Acetic Anhydride to form 2-

Methyl-Indole-α Pyrone. Synthetic IAA was used for standard curve.

Results

A) Physiological aspects

Rooting response of fresh & aged cuttings were taken from seedlings grown in complete Hoagland solutions or in solutions lacking for certain other trace elements, both full strength for (10) days, was shown in Table (1-A). These results revealed that fresh, untreated cuttings (general control treatment) and transferred to boric acid for further 6 days were developed 22.41 roots/cutting. Such rooting response was attributed to endogenous auxin (IAA). Meanwhile, fresh cuttings were induced by inductive auxin treatment (NAA, 10^{-4} M) for 24h, developed 66.16 roots/cutting with increased percentage estimated as 195.2% over control.

However, cuttings were aged for 3 days in d/H_2O then transferred to boric acid with out auxin treatment (Table 1-B) was developed 15.5 roots/cutting compared to fresh untreated cuttings with auxin (22.41). In other words a decline in rooting response was estimated as 30.5% and attributed to the processes that occurs during aging phenomenon. Meanwhile, cuttings aged in d/H_2O and induced by auxin (Table 1-A) were developed 58.33 roots/cutting, with a decline percentage (11.8%) compared to fresh induced cuttings with auxin (66.16 roots/cutting). This percentage of decline in rooting response is statistically not significant, because the stock plants were grown in complete Hoagland solution. In addition, for controlling the processes that occurs during aging & to know the effective nutrients, this study involved cuttings taken from seedlings grown in complete Hoagland solutions or in solutions lacking for certain other trace elements, both full strength.

The morphological products represented by mean roots number per cutting taken from seedlings grown in nutrient solutions lacking for Zn, Mn, B, Cu, Fe (pH=5.3, 5.5, 5.4, 5.4, and 5.3) respectively (Table 1-A) was (51.41, 53.25, 46.25, 49.83, and 41.08) roots respectively. The parentage of decline in roots number compared to control (58.33 roots & pH=5.6) if considered 100% was 11.9%, 8.7%, 20.7%, 14.6%, and 29.6%) respectively. Statistically, these figures are not significant except treatments that lacking for B or Fe having significant, negative differences on 5% level of probability for the former & 1% level for the second respectively.

Table (1-B) revealed that the roots number of cuttings were taken from seedlings grown in Hoagland solutions lacking for Zn, Mn, B, Cu, and Fe (pH=5.3, 5.5, 5.4, 5.4, and 5.3 respectively) was 17.8, 18.5, 19.0, 17.3, and 15.2 roots respectively. However, the rooting response of treatments lacking for Zn, Mn, B, and Cu was increased as percentage (15%, 19.4%, 22.6%, and 11.3% respectivily) whereas in treatment lacking for Fe was decreased as percentage (2.2%), compared to control (15.5 roots). Statistically, all the above figures are not significantly different, except treatments lacking for Mn & B. These treatments recognized by a positive significant difference on 5% level of probability compared to control.

Effect of SeO_2 on rooting response:

The effect of SeO_2 on rooting response of fresh cuttings, taken from seedlings grown in d/H_2O for 10 days was shows in Table (2). Treatment of cuttings with different concentrations of SeO_2 revealed the followings: Low concentrations (0.001, 0.01, 0.1, 1, 10) ppm, at pH=5, developed a number of roots equal 22.2, 23, 24, 19.9, and 28.5 roots respectively. In other words, the percentage of increment in roots no. was 170.2%, 180.5%, 192.7%, 142.8%, and 247.6% respectively, compared to control (8.2 roots/cutting) if considered 100%. Statistically, all the above treatments having a significant differences on 0.01 level compare to control. However, cuttings treated with 30 ppm (pH=4.5) developed 13.2 roots with no significant differences. Meanwhile, high conc.s (50 & 100)ppm (pH=3.6 & 3.4 respectively) of SeO2 were completely inhibited rooting response.

Depending on (a) conclusions of [20,21,22,23and 9], that deals with the ability of legumes to accumulates selenium in high concentrations, and (b) rootings response of mung bean cuttings to different conc.s of selenium (Table-2), primerly selenium at 10 ppm was involved in the constitution of Hoagland solution. There after, mung bean seedlings were grown in modified Hoagland solution containing 10 ppm of Se for 10 days under controlled conditions. The result of such preliminary experiment was revealed a short seedlings to prove that Se had inhibiting effect on seedlings growth compared to that grown in Hoagland solution lacking for selenium, or tap H_2O.

Rooting response of fresh & aged cuttings treated with Hoagland solutions & modified solutions (different strengths).

To establish the ideal modified Hoagland solution, it depend on achieving the optimum concentration of selenium in order to involve it in Hoagland solution and hence, to know its effect on aging phenomenon. So, cuttings were taken from seedlings grown in d/H_2O for 10 days, then treated with different strengths of Hoagland solutions & modified solution (pH=6.5) by adding SeO_2 at different concentrations. The goal of this experiment is to know the rooting response in both fresh & aged cuttings as shown in Tables (3 & 4) with reiterated experiment depending on results of Table (3) that deals with rooting response of mung bean cuttings (fresh) to Hoagland solutions & modified solutions at different strengths. Selenium was supplied as SeO_2 at 0.5 ppm to Hoagland solution & modified solutions both at half strength to grow mung bean seedlings for 10 day under controlled conditions. A negative result was obtained by growing seedlings that avoiding the uniformity and with small etiolated leaves, compared to solutions lacking for Se or tap H_2O. A final attempt, SeO_2 was supplied at 0.01 ppm to Hoagland & modified solutions both at half strength, to grow vigorous seedlings & highly uniform. A positive result was obtained considered the concentration of 0.01 ppm selenium is the optimum to achieve the ideal modified Hoagland solution that would used in the subsequent experiments. It is noteworthy, that concentrations of selenium (0.001-10) ppm were revealed a high rooting responses characterized by a highly significant differences on 1% level of probability compared to control (Table 2).

Rooting responses of fresh & aged cutting, were taken from seedlings grown in complete, modified nutrient solutions & solutions lacking for certain other trace elements (Half strength) for 10-days was shown in Table (5-A & B). The results of Table (5-A) revealed that, cuttings aged in d/H_2O and induced with auxin after aging period (3-days) developed 14.3 roots/cutting. In other words, rooting response in aged cutting was declined about 51.3% compared to fresh induced cuttings (29.3 roots). This decline was attributed to processes that occurs during aging and leads to diminish rooting response in aged cuttings. However, to control these processes & to know the effect of trace elements, cuttings were taken from seedlings grown in complete, modified nutrient solution & solutions lacking for certain trace elements. The average root numbers of cuttings in treatments lacking for Se, Zn, Mn, B, Cu, Fe, group of (Se, Zn, and, Mn), and group of (B, Cu, and Fe) was 11.8, 11.3, 13.9, 17.1, 17.4, 13.4, 18.2, and 11.1 roots respectively (Table 5A). The rooting response was declined in treatments that lacking for Se, Zn, Mn, Fe, group of (B, Cu, and Fe) compared to control treatment (complete modified solution). Whereas, the rooting response was increased in treatments that lacking for B & Cu, and group of (Se, Zn, and Mn). But statistically all the above figures in table (5-A) having no significant differences compared to control.

On the other hand, cuttings were not induced with auxin (Table 5-B), showed that the average no. of roots in treatments lacking for Se, Zn, Mn, B, Cu, Fe, group of (Mn, Se, and Zn), and group of (B, Cu, and Fe) was 6.4, 7.8, 9, 10.2, 9.2, 9.5, 8.8, and 7.5 respectively. However, rooting response was increased in treatments lacking for Zn, Mn, B, Cu, B, group of (Se, Zn, and Mn), and group of (B, Cu, and Fe) as percentage 9.2%, 25.5%, 41.8%, 27.9%, 32.5%, 23.2%, and 4.6% respectively. Whereas, the decrease was only noticed in treatments lacking for Se (10.5%) compared to control (7.2 roots) if considered as 100%. Statistically, all the above figures in table (5B) are not significantly different compared to control except the treatment lacking for boron.

The effects of complete, modified nutrient solutions having SeO_2, 0.01 ppm) and solutions lacking for some other trace elements (Half Strength) on rooting response of mung bean cuttings which aged in these solutions & already taken from seedlings grown in d/H_2O (Table 6). The roots no. of control treatment (complete modified solution) was 18.1 with increment estimated as percentage was (77.8%), compared to general control treatment (d/H_2O) was 10.2. However, cuttings were aged in solutions lacking for Se, Zn, Mn, B, Cu, Fe, group of (Mn, Zn, and Se), and group of (Fe, Cu, and B) developed 15.2, 19.8, 12.5, 14.8, 14.6, 13.7, 12.3, and 15 respectively. Statistically, all the above figures having no significant differences except the treatment lacking for Mn and group of (Mn, Zn, and Se) recognized with a negative significant differences on 5% level. In addition to the general control treatment (d/H_2O) that recognized with a negative highly significant differences on 1% level of probability compared to control.

B) Biochemical aspects:

IAA content (m mol/g fresh plant tissue) in hypocotyls of fresh cuttings were taken from seedlings grown in complete modified nutrient solutions & solutions lacking for some other trace elements (Half strength) for 10-days, was shown in Fig (1a). The results revealed that IAA content in hypocotyls of cuttings taken from seedlings grown in complete, modified solutions (control treatment) was 19.076 m Mole. However, IAA content in samples lacking for Se, Mn, B, Cu, Fe, group of (Mn, Zn, and Se), and groups of (Fe, Cu, and B) was (18.08, 17.854, 17.312, 18.171, 18.42, 17.719, and 17.176) m Mole respectively. Statistically, a highly significant differences between all treatments on 1% level of probability, except the treatment that lacking for Fe on 5% level. On the other hand, treatment lacking Zn was recognized by raising the level of IAA content to 19.257 m Mole but not significantly different.

Fig. (1b) showed that IAA content in hypocotyl of cuttings were taken from seedlings grown in complete, modified solutions and aged for 3 days in d/H_2O (control) was 13.873 m Mole. However, IAA content in treatment lacking for some other trace elements was declined in all treatments but not significantly except treatment lacking for group of elements (Mn, Zn, and Se) was decreased significantly on 5% level of probability compared to control. On the other hand, treatment lacking Se & Zn were recognized by raising IAA content to 14.234 & 14.37 m Mole respectively, but statistically not significant.

The effect of aging on IAA content in hypocotyls of cuttings were taken from seedlings grown in d/H_2O for 10 days & then aged for 3 days in complete, modified nutrient solution & solutions lacking for some other trace elements (Half strength) was shown in Fig (1c). IAA content in hypocotyls of cuttings aged in complete, modified solutions (Control) was 13.307 m Mole. Meanwhile, IAA content in all other treatments was recognized statistically with no significant difference except treatments lacking for Se & Mn was positively significant different on 5% level of probability whereas, treatment of d/H_2O (general control) was negatively significant different on 1% level of probability.

The initial amount of IAA in different parts of fresh & aged cuttings, both taken from seedlings grown in d/H_2O for 10 days, was shown in Fig. (1d). IAA content in primary leaves, epicotyl, and hypocotyl was 16.995, 14.777, and 11.022 m Mole respectively in fresh cuttings whereas, 14.778, 9.936, and 11.067 m Moles respectively in cuttings were aged for 3 days in d/H_2O .

Discussion

The quantitative determination of IAA in fresh & aged cuttings of mung bean were taken from seedlings grown in d/H_2O (see fig. 1d) denoted total IAA of fresh cuttings (42.794) m Mole declined in aged cuttings to (35.781) m Moles. However, IAA content in hypocotyl of aged cuttings was declined to (13.873) m Moles compared to (19.076) m Mole of fresh cuttings, were both taken from seedlings grown in modified nutrient solutions/ half strength (Fig. 1a & b) respectively.

The foregoing results are in agreement with the physiological results that associated with decline of rooting response in aged compared to fresh cuttings (Table 5 & other tables) from one side & confirm one of multihypotheses that explain aging causes (decline of endogenous IAA) from other side. The above hypothsis was verified by Shaheed & Al-Alwani (2001) using the same kind of cuttings & auxin bioassay technique. It is noteworthy, that quantitative determination of auxin in this study was done by using spectrophotometric method, which is more accurate.

The decline of IAA content in aged cuttings (during aging period) perhaps attributed to:

A) Decrease in auxin biosynthesis in primary leaves of aged cuttings [25], which considered as a central source of IAA biosynthesis. In addition, wilkins (1975) was mentioned that, a decline or stopping of IAA biosynthesis in fully expanded leaves[26]. This was confirmed by our results (Fig. 1d) which revealed that IAA content in primary leaves of aged cuttings (14.778) m Moles was declined significantly compared to fresh cutting (16.995) m Moles.

B) Decline in basipetal transport of IAA, in aged cuttings of mung bean compared to fresh cuttings after 24h treatment of c^{14}-IAA as foliar application was confirmed by [2] .

C) Conversion of free IAA to conjugated IAA in aged cuttings [27].

D) Oxidative processes that occurs at high level in aged cuttings (during aging) which should be discussed among our results, that obtained from this study.

The increase of rooting response (195.2 %) in fresh cuttings (induced with NAA, 10^{-4}M) were taken from seedlings grown in Hoagland solutions (Table 1) compared to fresh cuttings taken from seedlings grown in d/H_2O, was attributed to the effect of nutritional elements rather than hormonal factors in adventitious root formation (ARF). This was confirmed by Shaheed & Salim (2002a) using the same kind of cuttings [28].

On the other hand, the decline in rooting response of cuttings aged in d/H_2O whether induced with NAA, 10^{-4}M (Table- 1A) or not (Table- 1B), was attributed to the oxidative processes that occurs during aging which leads to diminish rooting response in aged cuttings, as is the case with Table (5). The foregoing results was confirmed by[3,4] as mentioned above. In addition, it has been mentioned that gradual decrease in biological activities in aged cuttings, such as deterioration of ribosome & r RNA & destruction of chlorophyll, proteins, and nucleic acid in mature leaves [29]. Alternatively, a decline in IAA content with increasing leaves age [25] or increasing cuttings age [24] or due to decline in nutritional status of aged cuttings associated with proteins, carbohydrates, and mineral nutrients[28]. Or permeability perturbation due to changes in constitution & properties of plasma-membrane due to decline in proteins & phospholipids [30]. or decline in transpiration rate of aged cuttings, that might be associated with uptake of supplied auxin by hypocotyl & its subsequent acropetal transport to the leaves [2]. The latter author subsequently, found a decline in basipetal transport of C^{14}-IAA when supplied to the leaves of aged mung bean cuttings as foliar application.

Statistically, Table 1A revealed a significant decrease in rooting response (46.25 roots/cutting) of induced mung bean cuttings with auxin (aged in d/H_2O) were taken from seedlings grown in Hoagland solution lacking for Boron, Compared to control or complete Hogland solution (58.33 roots/cutting). Whereas rooting response was coincided with significant increase in cuttings not induced but having the same above treatments (Table 1B). To elucidate this contradiction in rooting, som authors [31] where pointed out to the boron requirment, that increase with the increment of supplied auxin concentrations in case of induced cuttings with auxin (NAA) & vice versa. In other words boron requirements decreased depending on endogenous IAA in cuttings not induced with auxin.

The delecate hormonal balance between endogenous IAA & supplied auxin from one side & the interaction between boron & auxin from the other side may help to explain the significant increase in rooting response of aged cuttings (not induced with auxin) lacking for boron (Table 1B). However, rooting response was increased to 10.17 roots compared to control treatment or complete, modified Hoagland solution (7.17 roots/cutting). The foregoing results was coincided with the decline of IAA content (12.877 m Mole) compared to control (13.873 m Moles) as in Fig (1b). Meanwhile keeping in mind, the presence of selenium as anti-oxidant in concentration (0.01 ppm) that ionized into Se^{+2} & Se^{+4} through its association with enzymes that promotes oxidation-reduction reactions.

The results of Table 1A showed a significant decline in rooting response of aged (induced with auxin) cuttings were taken from seedlings grown in Hoagland solution lacking for Fe (41.08 roots) compared to complete Hoagland solution (58.33 roots). It was attributed to Fe ions which acts as anti-oxidants & oxidants simultaneously. In other words, acting as anti-oxidants when bounded to some enzymes & acting as oxidants and anti-oxidants simultaneously when it is free, due to having an orbital (d) & the possibility of this orbital to gain or loss electrons. For this reason Fe ionized into Fe^{+2} & Fe^{+3} and its importance in metabolism appeare through its involvement directly in cytochromes, which are essential for electron flow in Mitochondria and chloroplasts [(32)]. However, the defficiency of Fe causes decrease in chlorophyll & deterioration of chloroplast structure [(20)]. The latter changes reflect its effects on nutritional status particularly in aged cuttings [(28)].

The results of Table 1B revealed a significant increase in rooting response of aged (not induced) cuttings were taken from seedlings grown in Hoagland solution lacking for Mn (18.5 roots) compared to complete Hoagland solution (15.5 roots). It might be attributed to the role of Mn as oxidant agent (ionized into Mn^{+2} & Mn^{+4}). Mn has a recognizable role in degradation of IAA via activation of IAA-oxidase in cutting base. In other words the defficiency of Mn promotes the accumulation of IAA in cuttings & then enhance rooting response [(33)]. Among the studies of[(34)] on rooting response of mung bean cuttings Mn at low concentrations has no effects whereas, high roots response associated with high concentrations may be due to the possibility of replacement of Mn by some other cations (Bivalence) paticularly in respiration reactions such as Mg^{+2}, Co^{+2}, Zn^{+2}, and Fe^{+2} [(35)]. The latter author mentioned, that Mn can be replaced partially by cobelt in Oxalo-Succinic Decarboxylase, an enzyme involved in krebs cycle.

A significant decline of rooting response (Table-6) in cuttings were taken from seedlings grown in d/H_2O & aged in modified solutions, half strength, lacking for Mn or group of elements (Mn, Zn, and Se) compared to complete solution. However, the same treatments revealed no significant increase in rooting response compared to d/H_2O treatment. The reason may be attributed to (a) The role of Mn, Zn, and Se as anti-Oxidants & oxidants simulaneously (b) A decline in nutritional status of aged cuttings (cuttings were taken from seedlings grown in d/H_2O for 10-days) rather than hormonal factors & its effects. (c) Changing of pH value (6.5) compared to its value (5.5) in table (1). The role of Mn in degradation or oxidation of IAA via its promotion of IAA-oxidase activity was confirmed by quantitative estimation of IAA (Fig. 1) in cuttings aged in modified, nutrient solutions (half strength) lacking for Mn, that developed (15.004) m Moles, which is the highest value compared to control.

Table 2 raises two main points: firstly, the inhibitory effects of high concentrations of Se (30-100 ppm) on rooting response which leads to wilting

& death of cuttings due to the salt stress which represented by the role of Se by acting as reducing agent that converted from Se^{+4} to Se^{+2}, and then scavenge electrons. Meanwhile contribute as electron's doner to cell walls causing its oxidation, hence converted again to Se^{+4}. The foregoing results was confirmed by [36]. The latter authors mentioned, that salt stress may be promot the formation of reactive oxygen species that was able to damage the fundamental lipid of membrane, proteins & nucleic acids. These changes leads to accumulation of lipid peroxidation products in root, stem, leaf tissues, which causes increase in leakage of fundamental electrolytes of plasmamembrane that already promoted by stress [36]. This may help to give acceptable interpretations for the decline in rooting response of mung bean cuttings aged in Hoagland solutions (Full Strength) compared to d/H_2O treatment (see Table 4).

Secondly, highly significant increase in rooting response with low concentrations of Se (0.001-10 ppm) (Table 2). This response was attributed to:- (a) The role of Se ions as anti-oxidants via its association with enzymes that promotes (oxidation-reduction) reactions. In addition, Se acts as Co-enzyme in Glutathione oxidase that convert reduced Glutathione (GSH) to oxidized Glutathione (GSSG) as a result of oxidation processes. This conversion was occurred through the role of ascorbic acid as anti-oxidant in regeneration of Glutathione-cycle, and protection of vitamin E. To confirm the above information [10] pointed out to some spp. of bacteria & animals that required Se, contains few essential proteins mostly enzymes. These enzymes promotes (oxidation-reduction) reactions & the presence of Se was necessary for the activation of such enzymes. (b) Some accumulator species such as legumes tolerates high conc.s of some mineral elements like Se. These plants incorporates Se with the amino acid that contain S (e. g. Cysteine, Methionine) or organic acids (e. g. Acetate, Malate or Citrate) [37]. (c) Many studies giving reason for increased plant growth when supplied with nutrient solution having high conc.s of Se to selenate that reduces the toxic effects of phosphate [9]. (d) De-toxification of plants that accumulates Se in high conc.s, due to the formation of Seleno-Amino Acids (SAA) [9]. The latter author, mentioned, that such acids are not toxic & not incorporated in the formation of toxic proteins. The foregoing informations explain the role of Se in raising the rooting response of mung bean cuttings (both fresh & aged) treated with different strengths of modified solutions (Tables 3 & 4) compared to cuttings treated with Hoagland solutions. However, Table (3) developed a highly significant rooting response in fresh cuttings treated with modified solution (Half & Full strength) & significant rooting response with quarter strength compared to control. In addition, Table (4) developed a highly significant rooting response when aged cuttings treated with modified solutions at strengths (Full, half, and quarter) in case-C & at strengths (half & quarter) in case-B compared to control. These obtained result, denoted the role of Se in stopping of aging phonomenon completely. It is noteworthy, that cuttings aged in nutrient modified solutions were respond in terms of ARF, as was the case in fresh cuttings.

The importance of selenium that mentioned above, become obvious in delaying or offset the degeneration processes that occurs during aging. In addition to its role in increasing rooting response of cuttings aged in modified nutrient solutions (Half strength) & lacking for B & Cu (Table 5A) & treatments lacking for Mn, B, Cu, and Fe (Table 5B) compared to complete nutrient solution.

Finally, as a conclusion oxidative processes occurs in plant body or cuttings during aging that causes a decline in rooting response of aged cuttings. However, trace elements (e. g. Se, Zn, Mn, Cu, and Fe) are anti-oxidants, acts as internal scavenges of free radicals & lowering the effects of oxidation products. These elements contains orbital-d that causes electronic exchanges in oxidation-reduction processes, and their effects differ due to differences in:
a) It's presence in plant extracts (free or bounded) (b) Area of electronic exchange (c) pH value (d) Number and position of substituting groups (e) Nutritional status of cuttings or stock plants (f) Hormonal balance (IAA content) (g) Synergistic effect between trace elements & IAA (h) Ionizing & resonance forms of elements. These factors causes decline in oxidative processes that occurs during aging there after, enhance rooting response in aged cuttings of mung bean.

Table (1): Rooting response of fresh & aged mung bean cuttings, were taken from seedlings grown in full strength of Hoagland Solutions (complete & lacking for some trace elements)for 10-days.

(A) Induced by auxin

Solution supplied to stock plants for (10) days	Aging treatment of cuttings for 3 days in:	Sub-sequent treatment for 24h . in:	Mean root No. / cutting	pH of solution
Complete nutrient solution (General control)	None	d/H_2O	22.41	5.6
Complete N. S.	None	NAA, 10^{-4}M	66.16	5.6
Complete N. S. (control)	d/H_2O	NAA, 10^{-4}M	58.33	5.6
N. S. -Zn	d/H_2O	NAA, 10^{-4}M	51.41	5.3
N. S. -Mn	d/H_2O	NAA, 10^{-4}M	53.25	5.5
N. S. -B	d/H_2O	NAA, 10^{-4}M	*46.25	5.4
N. S. -Cu	d/H_2O	NAA, 10^{-4}M	49.83	5.4
N. S. -Fe	d/H_2O	NAA, 10^{-4}M	**41.08	5.3

* A= Negative significant effect on (0.05) level. L.S. D. (0.05) =9.4871
** A= Negative Significant effect on (0.01) level. L. S. D. (0.01)=13.5698

(B) Not Induced

Solution supplied to stock plants for (10) days	Aging treatment of cuttings for 3 days in:	Sub-sequent treatment for 24h . in:	Mean root No. / cutting	pH of Hoagland solution
Complete nutrient solution (General control)	None	d/H_2O	22.41	5.6
Complete N. S.	None	NAA, 10^{-4}M	66.16	5.6
Complete N. S. (control)	d/H_2O	d/H_2O	15.5	5.6
N. S. -Zn	d/H_2O	d/H_2O	17.83	5.3
N. S. -Mn	d/H_2O	d/H_2O	18.5*	5.5
N. S. -B	d/H_2O	d/H_2O	19*	5.4
N. S. -Cu	d/H_2O	d/H_2O	17.25	5.4
N. S. -Fe	d/H_2O	d/H_2O	15.16	5.3

L. S. D. (0.05)=2.8271 L. S. D. (0.01) = 4.0436
* A= Positive Significant effect

Table(2): Influnce of SeO_2 in rooting response of fresh mung bean cuttings.

Conc. (ppm) / pH / Control d/H_2O)	0.001	0.01	0.1	1	10	30	50	100
pH	5	5	5	5	5	4.5	3.6	3.4
8.2	22.16**	23**	24**	19.91**	28.5**	13.2	0	0

L.S.D. (0.05) = 6.2756 L.S.D. (0.01) = 8.304
A** Psitive significant effect on (0.01) level

Table (3): Rooting response of fresh mung bean cuttings treated with different strength of Hoagland & modified (Hoagland +SeO_2) solutions (pH=6.5).

Strength / Solution		Control (d/ H_2O)	Full	Half	Quarter
A	Hoagland	11.5	17.1*	16.9*	16.7*
B	Modified		22.6** (1ppm)	21.2** (0.5ppm)	17.2* (0.25ppm)
C	Modified		26** (0.1ppm)	17.7** (0.05ppm)	16.3* (0.025ppm)

Figures in parentheses refer to SeO_2 concentrations
A* = Positive Significant effect (0.05 level). L.S.D.(0.05) = 4.696
A** = Positive Significant effect (0.01 level). L.S.D.(0.01) = 6.211

Table (4): Rooting response of aged mung bean cuttings treated with different strengths of Hoagland modified (Hoagland + SeO_2) solutions (pH=6.5)

Strength / Solution		Control (d/ H_2O)	Full	Half	Quarter
A	Hoagland	12.3	11.8	17.7	15
B	Modified		15.9 (1ppm)	25.9** (0.5ppm)	20.2** (0.25ppm)
C	Modified		23.5** (0.1ppm)	20.1** (0.05ppm)	20.5** (0.025ppm)

Figures in parentheses refer to SeO_2 conc.s
A* = Significant effect (0.05 level) L.S.D. (0.05) = 5.830
A**= Significant effect (0.01 level) L.S.D. (0.01) = 7.712

Table (5): Rooting response of fresh and aged mung bean cuttings, were taken from seedlings grown is modified solutions (Half strength), both complete or lacking for some elements for 10-days.

(A): Induced by auxin (NAA, 10^{-4} M)

Solution (pH=6.5) Supplied to Stock Plants for (10) Days	Aging Treatment of cuttings for 3 Days In:	Sub-Squent Treatment for 24 h. In:	Mean Root No./ Cutting
Complete modified N.S. (general control)	None	d/ H_2O	7.33
Complete modified N.S.	None	NAA, 10^{-4}M	29.25
Complete modified N.S. (control)	d/ H_2O	NAA, 10^{-4}M	14.25
Modified S.- Se	d/ H_2O	NAA, 10^{-4}M	11.83
Modified S.- Zn	d/ H_2O	NAA, 10^{-4}M	11.33
Modified S.- Mn	d/ H_2O	NAA, 10^{-4}M	13.92
Modified S.- B	d/ H_2O	NAA, 10^{-4}M	17.08
Modified S.- Cu	d/ H_2O	NAA, 10^{-4}M	17.42
Modified S.- Fe	d/ H_2O	NAA, 10^{-4}M	13.42
Modified S.- (Se,Zn,Mn)	d/ H_2O	NAA, 10^{-4}M	18.16
Modified S.- (B, Cu, Fe)	d/ H_2O	NAA, 10^{-4}M	11.08

L.S.D. (0.05) = 4.555 L.S.D. (0.01) = 6.03

(B): Not induced.

Solution (pH=6.5) Supplied to Stock Plants for (10) Days	Aging Treatment of cuttings for 3 Days In:	Sub-Squent Treatment for 24 h. In:	Mean Root No./ Cutting
Complete modified N.S. (general control)	None	d/ H_2O	7.33
Complete modified N.S.	None	NAA, 10^{-4}M	29.25
Complete modified N.S. (control)	d/ H_2O	d/ H_2O	7.17
Modified S.- Se	d/ H_2O	d/ H_2O	6.42
Modified S.- Zn	d/ H_2O	d/ H_2O	7.83
Modified S.- Mn	d/ H_2O	d/ H_2O	9
Modified S.- B	d/ H_2O	d/ H_2O	10.17*
Modified S.- Cu	d/ H_2O	d/ H_2O	9.17
Modified S.- Fe	d/ H_2O	d/ H_2O	9.5
Modified S.- (Se,Zn,Mn)	d/ H_2O	d/ H_2O	8.83
Modified S.- (B, Cu, Fe)	d/ H_2O	d/ H_2O	7.5

L.S.D. (0.05) = 2.41 L.S.D. (0.01) = 3.191

A* = Positive significant effect

Table (6): Influence of modified nutrient solutions, both complete or lacking for some trace elements (Half strength) is rooting response of aged mung bean cuttings

Solution	(d/H_2O) general control	Complete control	-Se	-Zn	-Mn	-B	-Cu	-Fe	-Mn Zn Se	-Fe Cu B
Mean Root No./ cutting	**10.2	18.1	15.2	19.8	*12.5	14.8	14.6	13.7	*12.3	15

L.S.D. (0.05) = 5.017 L.S.D. (0.01) = 6.637

A* = Negative significant effect (0.05 level) A** = Negative significant effect (0.01 level)

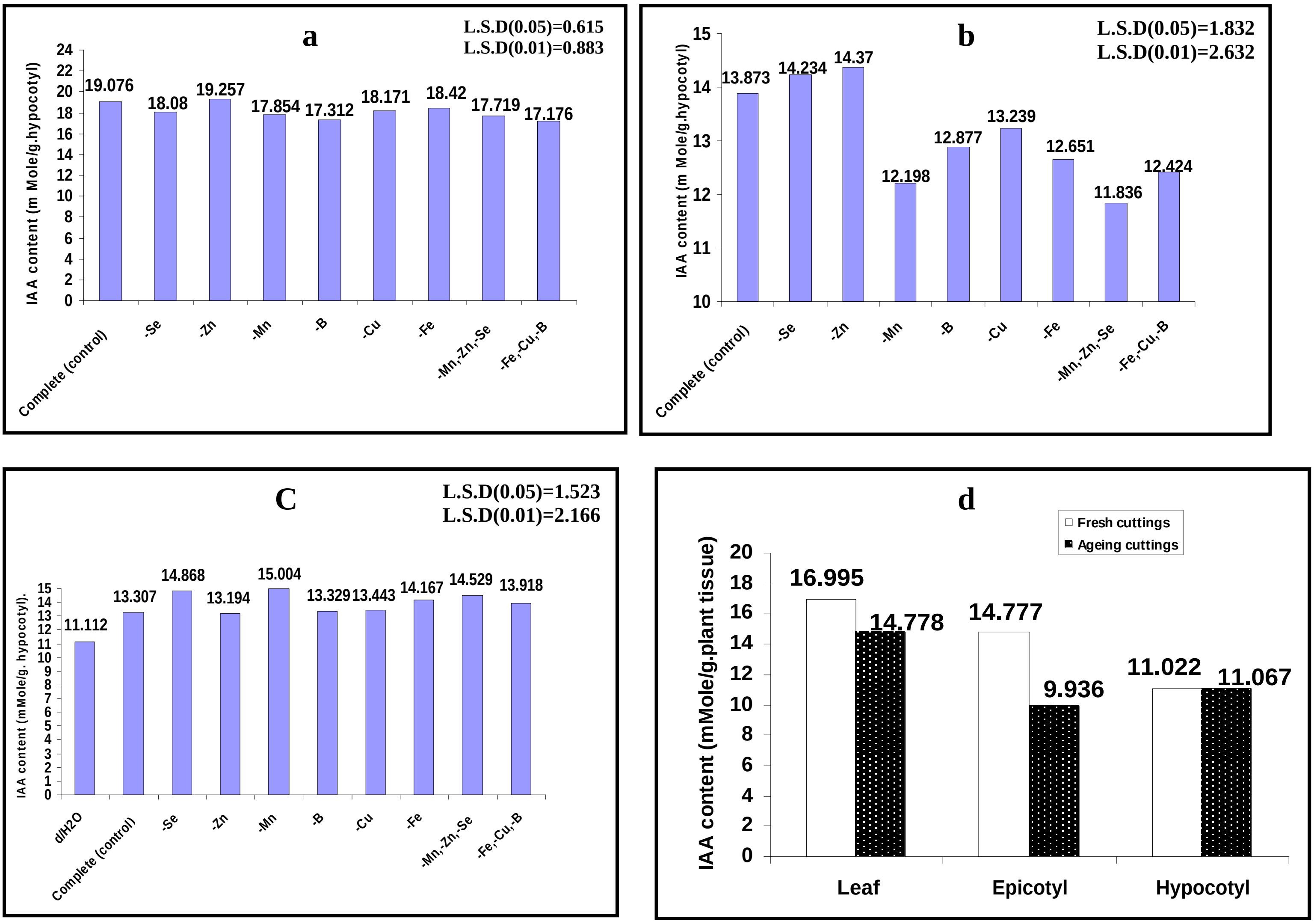

Fig. (1): IAA content (m Mole/g. plant tissue) in mung bean cuttings.

- **a- Fresh cuttings, were taken from seedlings grown in M.N.S., both complete or lacking for certain elements (Half strength).**
- **b- Aged cuttings, were taken from seedlings, grown in the above solutions, aged in d/H_2O.**
- **c- Aged cuttings, were taken from seedlings, grown in d/H_2O, aged in above solutions.**
- **d- Fresh cuttings, grown in d/H_2O (Total IAA= 42.794 mMole); Aged cuttings, grown in d/H_2O and aged in d/H_2O (Total IAA= 35.781 mMole).**

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