

Effect of Mineral Acids on Rooting Response of Aging Mung Bean (*Phaseolus aureus* Roxb.) Cuttings via Indole Acetic Acid Level

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Abstract:
The influence of sulfuric acid (H_2SO_4) as a strong mineral acid on rooting response of fresh and aging mung bean cuttings has been studied on the level of (IAA) . The data revealed significant increase in rooting response of aged cuttings (for 3 days in d/H_2O) with (0.001 and 0.01%) concentrations of H_2SO_4 solution and highly significant increase in rooting response of aged cuttings (for 3 days in H_2SO_4 solution) with (0.0001 , 0.001 and 0.01%) concentration , While highly concentration (0.1 and 0.5 %) revealed highly significant decrease in rooting response compared to control (d/H_2O). Quantitative estimation of IAA by spectrophotometric method as indicators for oxidative processes that occur during aging phenomenon verified a highly significant increase of IAA content in hypocotyles of aged cuttings in optimal concentration of H_2SO_4 .
Key words: Aging, IAA biosynthesis, Rooting response, Mineral acids, Macronutrients, Stem cuttings.

1. Introduction

Acid precipitation has been identified as a major environmental concern in many countries (Galloway , *et.al.,*1978) . Formed following the oxidation and hydration of sulfur and nitrogen oxides in the atmosphere , acid precipitation is defined as rain or snow having pH values less than approximately 5.6 (likens & Bormann , 1974) . The major affect of acid rain is the result of the acidity of the soil in which the plant is located the lower the pH , the more acidic the soil (Barnhart , 1986) . The effect of most pollutants are highly variable depending on species sensitivity , the intensity and duration of exposure to the pollutants , wind , rainfull and other meteorological factors (Hopkins , 1999) .

Sulfur element is present in soils in both inorganic and organic substances . Humus also contains sulfur as constituents of various organic molecules from which they are released , mainly as sulfates , through the activity of microorganisms . Most higher plants absorb sulfur from the soil solution mainly in the oxidized forms (SO_4^{-2}) . Sulfur is absorbed to some extent as the less highly oxidized sulfite (SO^{-}_3) and thiosulfate ($S_2O_3^{-2}$) ions (James , 1981) . Sulfur is immobile nutrient in the plant which not able to move from one plant part to another and deficiency symptoms will initially occur in the younger or upper leaves and be localized (McCauley , *et.al.,*2009) .

Sulfur deficiency is not a common problem , since there are numerous microorganisms capable of oxidizing sulfides or decomposing organic sulfur compounds . Indeed it is often difficult to demonstrate sulfur deficiencies in greenhouses in industrial area because of the high concentrations of airborne sulfur (Hopkins , 1999) .

Soil pH has two major effects ; competition and injury . A low pH is believed to reduce cation uptake by competition between hydrogen ions and the other cations for sites on a carrier . At high pH , hydroxyl or bicarbonate ions might compete with other anions , thus reducing anion uptake . Acidity or alkalinity therefore has a profound influence on the relative absorption of anions and cations . At high pH where the absorption of cations is favoured the discrepancy between cation and anion absorption is balanced by greater accumulation of organic anions within the tissues . The organic acid is synthesized by utilizing carbon dioxide or bicarbonate ions taken up from the medium . At pH values outside the physiological range , the ion uptake mechanism is damaged , probably by disruption of membranes (James , 1981) .

Aging in terms of adventitious root formation (ARF) means a decline in rooting response of aged compared to fresh cuttings . This decline in rooting response occurred progressively with time when inductive auxin treatment was delayed by holding cuttings in deionzed H_2O particularly in mung bean cuttings (Jarvis , 1986) . In addition , Leshem (1981) proposed a free radical theory to explain the damage of plant and animal cells with progressing age . 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 , Ishii and his colloquies (2001) showed that free radicals and its derivatives in aged cell and organs (in Nematodes) regenerated primarily in mitochonderia as undesirable products through oxidative phosphorylation . (Davies , 1983) described aging as a phenomenon that fundamentally concerned with degenerative changes in metabolism . The later author mentioned that alteration of hormonal balances was the only molecular event leading to these changes .

2.1. Materials and Methods

2.1.1. 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 were raised in growth chamber provided with a continuous light (light intensity 3000-3500 Lux) , temperature25± 1°C and relative humidity 60-70% for ten days.

2.1.2. Preparation of Cuttings

Cuttings were prepared according to (Hess ,1961) from 10 days old light grown seedlings .These cuttings described by having small terminal bud , pair of fully expanded primary leaves, a whole epicotyls and hypocotyls (3 cm length) under cotyledonary nodes , after removal of root system.

2.1.3. Basal Treatment of Cuttings

Dipping of the whole hypocotyls (3 cm depth) in glass vials required 15 ml of tested solutions. Fresh cuttings were treated for 24 hr. with d/H_2O or tested solutions (Twelve cuttings/treatment) , then transferred to boric acid (10 µg/ml) for 6 days , before counting the root numbers.

2.1.4. Aging Treatments

Cuttings were held immediately after taken from 10 days old seedlings in d/H_2O for 3 days , if the purpose is controlling of aging phenomenon . Physiologically , aged cuttings treated with tested solution for 24 hr. , then transferred to boric acid (10 µg/ml) for further 6 days before counting the root number per cutting . The area of 1st true tri-foliated leaf in cuttings measured according to Stickler and his colloquies (Stickler , *et.al.,* 1961) . Completely randomized design (CRD) was conducted in all experiments for statistical analysis according to (Spiegle , 1975) .

2.2. Preparation of Solutions

2.2.1. Boric Acid Solution : prepared at (10 µg/ml) and employed as rooting medium (Middleton , *et.al.,* 1978b) .

2.2.2. Synthetic Auxin Solution : Indole-3-Acetic Acid (IAA) was initially dissolved in small amount of absolute alcohol to prepare ($5x10^{-4}$M) (Middleton , *et.al.,* 1978a) .

2.2.3. Sulfuric Acid Solution : H_2SO_4 (98%) prepared as (%) percent solution (V/V) , by dissolving (1) ml of H_2SO_4 in (99) ml of d/H_2O to achieve 1% as stock solution , then diluted to the required concentrations .

2.3. Quantitative Determination of IAA

Naturally occurring auxin (IAA) was measured spectrophotometrically in hypocotyls of fresh and aged cuttings, according to (Stoessl & Venis 1970) , (Plieninger , *et.al.,*1964) . The above procedure (were modified) include the reaction of IAA with acetic anhydride to form 2-Methyl-Indole-α pyrone . Synthetic IAA was used for standard curve.

3. Results

3.1. Physiological Part

3.1.1. Effect of (H_2SO_4) in rooting response of fresh and aged cuttings:

Table 1. shows the effects of H_2SO_4 in rooting response of fresh cuttings , when supplied to cuttings immediately . The results revealed that the means of roots number , roots length (mm) and leaf area (cm^2) as the mean of one cutting developed in fresh , untreated cuttings (general control d/H_2O) are (16.3 root , 14.073 mm and 0.772 cm^2) respectively . These means in cuttings treated with auxin (special control IAA) are (39.7 root , 1.686 mm , 0.041 cm^2) respectively , while these in cuttings treated with H_2SO_4 are (17.2 , 14.3 , 12.6 , 1.1 and 0) root , (6.377 , 8.933 , 9.14 , 0.29 and 0) mm and (0 , 0.185 , 0.314 , 0 and 0) cm^2 at pH (4.15 , 3.55 , 2.85 , 1.92 and 1.16) respectively . Statistically , cuttings treated with 0.0001 % concentration of H_2SO_4 at pH (4.15) have no significant increase on rooting response , while cuttings treated with (0.001 and 0.01 %) concentration of H_2SO_4 have no significant decrease on rooting response . At the same time , cuttings treated with (IAA) pH (4.38) was positively significant at ($P \geq 0.05$) compared to control treatment (d/H_2O) . Generally , high concentration of H_2SO_4 (0.1 and 0.5%) have negative significant difference on (0.01) level , as compared to control (d/H_2O) . On the other hand , Table 1 revealed negative significant difference on ($P \geq 0.05$ and $P \leq 0.05$) in mean root length and leaf area in all treatments compared to control treatment (d/H_2O) .

The influence of H_2SO_4 on rooting response of aged mung bean cuttings has been shown in Table 2. The results revealed that means of roots number , roots length (mm) and leaf area (cm^2) as the mean of one cutting developed in cuttings aged in d/H_2O for three days are (14.875 root , 9.259 mm and 0.313 cm^2) respectively . These means in aged cuttings in (IAA) are (34.375 root , 2.583 mm and 0 cm^2) respectively . On the other hand , these means in aged cuttings in (d/H_2O) for 3 days and treated for 24 hr. with H_2SO_4 solutions are (15.375 , 29.125 , 28.25 , 0 and 0) root , (9.584 , 6.171 , 1.382 , 0 and 0) mm and (0.401 , 0.097 , 0.069 , 0 and 0) cm^2 respectively at pH (4.15 , 3.55 , 2.85 , 1.92 and 1.16) . Aged cuttings in (0.001 and 0.01%) concentration of H_2SO_4 at pH (3.55 and 2.85) , were positively highly significant in rooting response ($P \geq 0.05$ and $P \leq 0.05$) ,

compared to control (d/H_2O) . At the same time , Cuttings Aged in IAA solution at pH (4.38) was positively significant ($p \geq 0.05$) compared to control treatment (d/H_2O) .
Generally, high concentration (0.1 and 0.5%) have no significant effect on rooting response in all treatments . On the other hand , statistically , aged cutting in all concentration of H_2SO_4 as well as aged cuttings in IAA solution revealed negative significant difference in mean root length and mean leaf area on ($P \geq 0.05$ and $P \leq 0.05$) except (0.0001 %) concentration revealed no significant increase compared to control (d/H_2O) .
The influence of H_2SO_4 on rooting response of aged mung bean cuttings in solution for 3 days has been shown in Table 3. The results revealed that means of roots number , roots length (mm) , leaf area (cm^2) as the mean of one cutting developed in aged cuttings in (d/H_2O) for three days (General control d/H_2O) are (17 root , 17.284 mm and 1.016 cm^2) respectively . These means in aged cuttings in (IAA) for three days (special control IAA) are (8.3 root , 2.752 mm and 0 cm^2) respectively . The means in aged cuttings in H_2SO_4 solution for three days are (45.4 , 40.2 , 65.7 , 0 and 0) root , (5.436 , 9.561 , 6.801 , 0 and 0) mm and (0.536 , 0.746 , 0.665 , 0 and 0) cm^2 , at pH (4.15 , 3.55 , 2.85 , 1.92 and 1.16) respectively . Aged cuttings for three days in (0.0001 , 0.001 and 0.01 %) concentration of H_2SO_4 solution at pH (4.15 , 3.55 and 2.85) was positively highly significant in rooting response ($p \geq 0.05$) , compared to control (d/H_2O) . The increase in rooting response of mung bean cuttings at (0.01%) concentration has doubled the responsiveness to Adventitious Root Formation (ARF) in to (7.9) folds compared to rooting response of auxin aged cuttings . Generally , high concentration (0.1 and 0.5%) have no significant effect on rooting response in all treatments . Aged cuttings in all treatment were negatively significant in mean root lengths and mean leaf areas ($P \geq 0.05$ and $P \leq 0.05$) compared to control treatment (d/H_2O) .

3.2. Biochemical Part

3.2.1. Quantization determination of IAA

3.2.1.1. Effect of H_2SO_4 on IAA level in fresh cuttings

Fig. 2a. shows IAA level in hypocotyls of fresh mung bean cuttings treated in optimal concentration of H_2SO_4 and IAA . IAA level in 1g. hypocotyls of fresh cuttings (General control d/H_2O) is (11.316) m molar . whereas , IAA level in 1g. hypocotyls of cuttings treated with synthetic IAA (special control treatment) is (16.022) m molar . IAA level in 1g. hypocotyls of cutting treated with H_2SO_4 solution (0.0001% pH= 4.15) is (12.04) m molar . Treatment with IAA revealed positive highly significant ($p \geq 0.05$) as compared to control treatment (d/H_2O) . On the other hand , treatment with H_2SO_4 solution revealed no significant increase in IAA level compared to control treatment (d/H_2O) and significant decrease ($p \geq 0.05$) compared to control treatment IAA .

3.2.1.2. Effect of H_2SO_4 on IAA level in aged cuttings for 3 days in (d/H_2O)

Fig. 2b. shows IAA level in hypocotyls of mung bean cuttings taken from seedlings grown in d/H_2O for 10 days , aged for 3 days in (d/H_2O) and treated with optimal concentrations of IAA and H_2SO_4 (for 24 hr.) . IAA level in 1g. hypocotyls of aged cuttings (general control d/H_2O) is (11.09) m molar , IAA level in 1g. hypocotyls of aged cuttings in synthetic auxin (IAA) is (15.162) m molar , while , IAA level in 1g. hypocotyls of cuttings aged in H_2SO_4 solution (0.001% pH = 3.55) is (14.167) m molar . Aging treatments with IAA and H_2SO_4 revealed positive highly significant ($p \geq 0.05$) as compared to control treatment (d/H_2O) . On the other hand , treatments revealed significant decrease ($p \leq 0.05$) in content of IAA in aged cuttings in H_2SO_4 compared to control treatment of IAA.

3.2.1.3. Effect of H_2SO_4 on IAA level in aged cuttings for 3 days in solution

Fig. 2c. shows IAA level in hypocotyls of mung bean cuttings taken from seedlings grown in (d/H_2O) for 10 days and aged for 3 days in IAA and H_2SO_4 solution (at optimal concentration) for rooting response . IAA level in 1g. hypocotyls of aged cuttings for 3 days in d/H_2O (general control d/H_2O) is (11.09) m molar . whereas, IAA level in 1g. hypocotyls of aged cuttings in synthetic auxin (IAA) for 3 days (special control IAA) is (8.352) m molar , while , IAA level in 1g. hypocotyls of cuttings aged for 3 days in H_2SO_4 solution (0.01% pH = 2.85) is (15.683) m molar. Aging treatment with IAA revealed significant decrease ($p \geq 0.05$) .On the other hand , treatments revealed positive highly significant ($p \geq 0.05$) in content of IAA in aged cuttings in H_2SO_4 solutions compared to special control of IAA and general control treatment (d/H_2O).

4. Discussion

The processes that leads to diminish rooting response of mung bean cuttings during aging maybe attributed to loss of co – factors (Wally , *et.al.,* 1980) with age or decrease of auxin contents in cuttings or elsewhere in the cuttings , for example leaves or epicotyls (Delaimy , 2004) or hypocotyl (root initiation zone) (Alwani , 1998) . The nature of oxidative processes was studied, which presumably increased during aging, depending on the availability of oxidative agents from one side and the decrease of agents that involved in antioxidant defense mechanisms from the other side . So , our spectrophotometrical measurements of naturally occurring auxin (IAA) in hypocotyls of cuttings taken from seedlings grown in d/H_2O for 10 days was declined to 11.09 m molar compared to that in fresh cuttings 11.316 m molar Figs. 2a and b . These results confirm the hypothesis that explain processes that occur during aging , which shows the decline of naturally occurring IAA. The above

hypothesis has been verified by using the same kind of cuttings and IAA spectrophotometrical technique (Delaimy , 2004) .
However , the decline in IAA content of aged mung bean cuttings , maybe attributed to : **(A)** Decrease in IAA biosynthesis in primary leaves of aged cuttings , which is considered as central source for IAA biosynthesis . Hartmann and his collegues (Hartmann , *et.al.,* 1988) denoted decline in IAA content in leaves during senescence . **(B)** Decline of basipetal transport of IAA (Shaheed , 1987) . **(C)** Conversion of free IAA to conjugated IAA during rooting response (Norcini , *et.al.,* 1985) . **(D)** Occurrence of high level of oxidative processes in aged cuttings (Delaimy , 2004) .
Cuttings kept in different concentration of H_2SO_4 for three days (aging period) , developed significant rooting response . In other words , some concentration stopped the processes that occurs during aging completely in terms of (ARF) Table 3. Meanwhile , some other concentrations , stopped aging partially Table 2.
The role of (H_2SO_4) in offsetting , stopping , delaying or retarding the processes that leads to diminish rooting response in aged cuttings is difficult to interpret . However , IAA content in hypocotyls of aged cuttings in these solutions Figs. 2b and c developed significant increase compared to control treatment . However , the significant rooting response of aged mung bean cuttings, the significant decrease in mean of root length and mean of leaf area Table 2 and 3. maybe attributed to the following factors :

1) The effect of Sulfuric acid as a strong acid

The significant rooting response of mung bean cuttings maybe attributed to the capability of H_2SO_4 for trapping free radicals , because of presence of high electronic conjugation in this compound . H_2SO_4 is considered as a strong acid for giving semicompletely it's proton to water. In H_2SO_4 solution , H_2SO_4 molecule dissociate giving proton to water forming positively charged cation, Hydronium ion (H_3O^+) and negatively charged anion , sulfate ion (SO_4^{-2}) , Obviously , pH depend on Concentration of dissociated acid 100% .

$$H_2SO_4 + H_2O \rightleftharpoons H_3O^+ + HSO_4^-$$

$$HSO_4^- + H_2O \rightleftharpoons H_3O^+ + SO_4^{-2}$$

However , to confirm that studies in inorganic chemistry space mentioned that a strong acid is the acid which has a strong tendency to give the proton , and acids like ($HClO_3$, H_2SO_4 , HCl, HNO_3) are considered as a strong acids because all these acids semi completely gives their protons to water . So , they appear as they having the same power in their aqueous solutions because they ionized semi completely in their dilution aqueous solutions giving hydronium ion (H_3O^+) and their salts (Burgess , 1974) .

2) The effect of Sulfur element

The significant rooting response maybe attributed to the capability of sulfur element as one of sixth group and second cycle elements in periodic table . Sulfur atoms are characterized by presence of external envelope which contain individual electrons act as internal suppressors of free radicals through formation covalent bonds and lowering the effects of oxidative products that occurs during aging.
Studies mentioned that S is an essential constituent of certain amino acid and proteins , S deficiency results in the inhibition of protein and chlorophyll synthesis . S deficiency symptoms can be difficult to diagnose as effects can resemble symptoms of N and Mo deficiencies . In contrast to N , Mo deficiency , however , S deficiency symptoms initially occur in younger leaves , causing them to turn light green to yellow (chlorosis) . In later growth , the entire plant maybe pale green . Characteristic spots or stripes are generally not displayed . Additionally , plants deficient in S tend to be spindle and small and stems are often thin (Stoessl & Venis 1970) .

3) The effect of pH

The significant rooting response of mung bean cuttings maybe attributed to the acidic pH . The data revealed that (ARF) in mung bean cuttings positively affected by acidic pH. This was confirmed by prior studies by using the same kind of cuttings (Delaimy , 2004 and Delaimy , 2011) .
It has been found that roots affected by the low pH . So , Acidic Soil , like that found after acid rain has fallen , may limit plant growth simply because H^+ , the acidic part of a molecule is toxic to roots . Plant can grow in soils in a pH range of 3 to 9 . Some plant grow in more acidic soil while some grow in more alkaline soil (Salisbury & Ross , 1978) . Studies mentioned that sulfuric acid treatments commonly promoted acidification of all soils , significantly elevating cation leaching and lowering solution pH . The protons generated were responsible for more sustained acidification effects , which included elevated aluminum concentration . Short-term cation leaching for the quartzite and granite soils was attributed to an ability of the cation NH_4 and the anion SO_4 to desorbed exchangeable actions only , with little proton availability for mineral hydrolysis .
Along with the effects of pH on soil , pH also affects the plants semipermeable membrane , allowing particles to travel through the cell membrane more easily . This affects how well plants are able to absorb nutrients and how they can keep toxins out (Considine , 2002) .

4) The effect of hormonal balance

Hormonal balance has great effect in rooting response of aged mung bean cuttings . This was confirmed by Davies (1983) who deseribed aging as phenomenon that fundamentally concerned with degenerative changes in metabolism . Obviously , he mentioned that alteration of hormonal balances were the only molecular events leading to these changes . So , studies mentioned that sulfur is particularly important in the structure of proteins where disulfide bonds between neighboring cysteine and methionine residues contribute to the tertiary structure . Sulfur is also a constituent of the vitamins thiamine and biotin and of coenzyme A , an important in respiration and fatty acid metabolism . In the form of iron-sulfur proteins it is important in electron transfer reactions of photosynthesis and nitrogen fixation (Hopkins , 1999). Thereafter, explain the significant increase of IAA content in hypocotyls of aged mung bean cuttings Figs. 2b and c. which reflected the significant increase of rooting response . This was confirmed by prior studies by using the same kind of cuttings (Delaimy , 2004 , Delaimy , 2011and Al-Delaimy , 2013) .

5) Ionic balance :

In general, the rate of ion uptake affected by temperature , metabolic inhibitors , surface area , internal ionic concentration , light , pH , and salt concentration . the presence of metabolically – important anions , often stimulates the uptake of other ions , presumably through an effect on metabolism (James , 1981) . Studies mentioned that biogeochemical impacts of S and N depend upon their mobility in the canopy and soils to which they are deposited . If the anions (sulfate and nitrate) are leached , rather than being retained , they can strip the foliage and soils of valuable nutrient cations such as Ca and Mg . The leaching of sulfate and nitrate can cause acidification of soils and surface waters and mobilization of Al . Al is a natural component of soils , but in acid conditions it becomes more soluble and thus more concentrated in soil water , where it can be toxic to roots (Gough , *et.al.,* 2000) .

As more S is deposited in wetlands , the activity of S-reducing bacteria is increased , such as those that occur in wetland soils and sediments , Because these bacteria also methylate Hg , The increase in their activity increases the potential for the formation of methylmercury . This interaction between the S and Hg cycles results in increased toxicity of Hg in high-S wetlands (Heyes , *et.al.,*2000) . An example of interaction in the role of ammonia in enhancing deposition of S . As SO_2 gas is deposited to leaf surfaces , it acidifies those surfaces , which tends to slow down the SO_2 deposition process . In the presence of ammonia however , the alkaline nature of the ammonia gas counteracts the acidifying effect of the SO_2 , leading to enhanced S deposition (Fowler , *et.al.,* 2005) .

6) Level of solution concentration and treatment period

Level of solution concentration and treatment period have important effect on rooting response . This confirmed by the significant increase in rooting response of aged cuttings (aged for three days in H_2SO_4 solution , then transferred to boric acid for 6 days) which revealed (65.7 root) at 0.01 % concentration Table 3. compared with rooting response of aged cuttings (aged for three days in d/ H_2O , then treated for 24 hr. in H_2SO_4 solution . Thereafter transferred to boric acid for 6 days) which revealed (28.25 roots) at the same concentrationTable 2. On the other hand , the significant increase in rooting response of aged cuttings (aged for 3 days in d/ H_2O , then treated for 24 hr. in IAA solution . Thereafter , transferred to boric acid for 6 days) which revealed (34.375 roots) at 5×10^{-4} M Table 2. compared with rooting response of aged cuttings (aged for 3 days in IAA solution , then transferred to boric acid for 6 days) which revealed (8.3 roots) Table 3 . This attributed to death the basal part of aged cuttings hsypocotyl in auxin solution because of the treatment period length (3days) at this concentration .

Generally , aged cuttings (for three days) at 0.01% concentration in H_2SO_4 at pH (2.85) , have doubled the responsiveness to ARF into relatively , 8 folds compared to IAA treatment .On the other hand , Fig. 2c revealed significant decrease in IAA content in hypocotyls of aged cuttings for three days in IAA solution (8.352) m molar compared with fresh and aged cuttings (16.022 , 15.162) m molar respectively Figs. 2a and b .

5. Conclusion

Generally and as a conclusion, aging phenomenon maybe considered as a result of oxidative processes that occurs in plant body or cuttings during aging period , that causes diminishing rooting response in aged mung bean cuttings . The role of H_2SO_4 in offsetting , stopping , delaying or retarding the processes that leads to diminish rooting response in aged cuttings maybe attributed to capability of H_2SO_4 for trapping free radicals , because of high electronic conjugation in this compound .

However , sulfuric acid tested in the current study as a strong acid has a strong tendency to giving semi completely , proton to water. In (H_2SO_4) solution, H_2SO_4 molecule ionized semi completely giving (H_3O^+) and (SO_4^{-2}) . Obviously , pH depend on concentration of dissociated acid (100%) . So , sulfur element as one of the sixth group and the second cycle elements in periodic table , it's atoms are characterized by presence of external envelope which contain individual electrons act as internal suppressors of free radicals through covalent bonds formation and lowering the effects of oxidative products that occurs during aging . However , as well as the above explanation (H_2SO_4) lowering the effects of oxidative products through the followings :**a)** Hormonal factors and IAA content (Hormonal balance). **b)**pH . c) Ionic balance**.** **d)** Level of solution concentration . **e)** Period of treatment.

Not with standing , the foregoing suitable factors may lead to decline the oxidative processes that occurs during aging and hence , causing increase of rooting response in aged mung bean cuttings.

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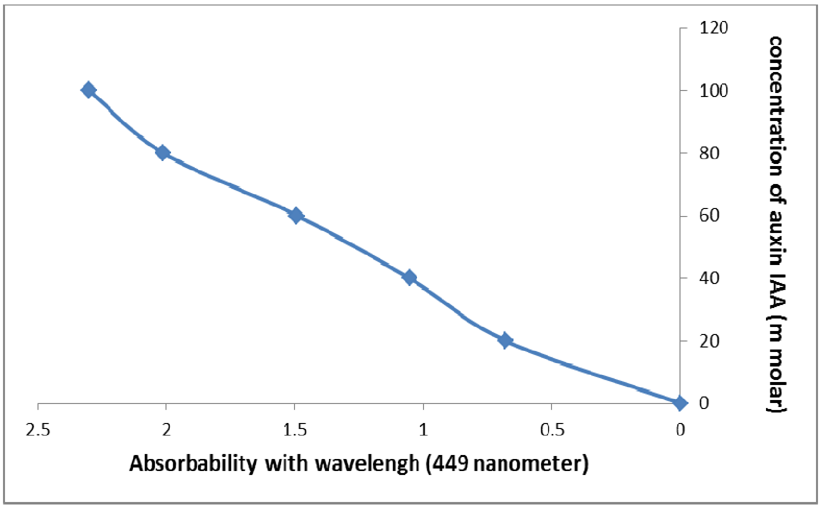

Fig.1 : Standard carve of different concentration of auxin (IAA) and Absorbability with wave length (449nanometer)

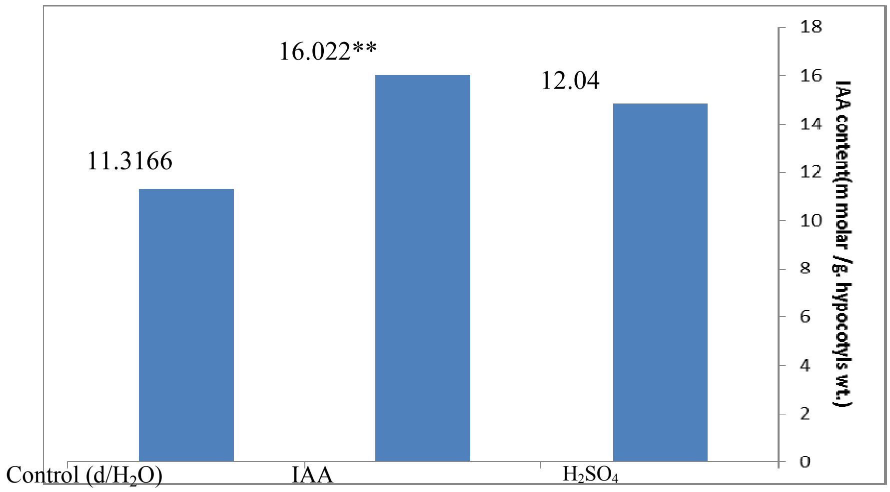

Fig.(2a): IAA content (m molar/g. hypocotyls wt.) of fresh mung bean cuttings treated with 0.0001% concentration (pH=4.15) of H_2SO_4 solution and $5x10^{-4}$ M concentration (pH=4.38) of IAA solution . LSD(0.05)=1.237 LSD(0.01)=2.388

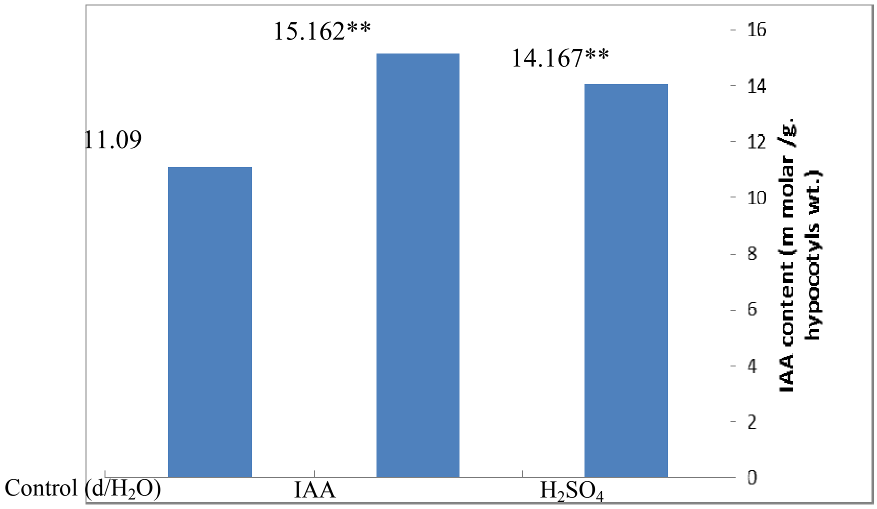

Fig.(2b): IAA content (m molar/g. hypocotyls wt.) of aged mung bean cuttings for 3 days in d/H_2O there after treated for 24h. with 0.001% concentration of H_2SO_4 (pH=3.55) and 5x10^{-4} M concentration (pH=4.38) of IAA solution LSD(0.05)=0.752 LSD(0.01)=1.451

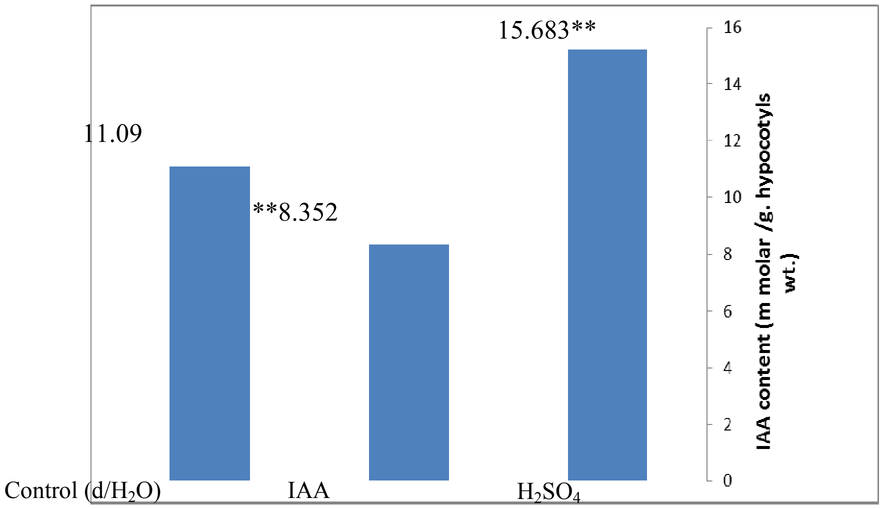

Fig.(2c): IAA content (m molar/g. hypocotyls wt.) of aged mung bean cuttings for
3 days in H_2SO_4 solution with0.01%concentration(pH=2.85) and 5x10^{-4}M concentration (pH=4.38) of IAA solution . LSD(0.05)=0.788 LSD(0.01)=1.522

Table 1:Influence of (H_2SO_4) on rooting response of fresh mung been cuttings

Solution	Concentration%	Mean root No./cutting	Mean root length /cutting (mm)	Mean of 1st true trifoliated leaf area (cm^2)	pH
d/H_2O	0	16.3	14.073	0.772	6.71
Indole acetic acid (IAA)	0.00876 (5x10^{-4}M)	39.7**	**1.686	**0.041	4.38
H_2SO_4	0.0001	17.2	**6.377	**0	4.15
	0.001	14.3	**8.933	**0.185	3.55
	0.01	12.6	**9.14	**0.314	2.85
	0.1	**1.1	**0.29	**0	1.92
	0.5	**0	**0	**0	1.16

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days .Then treated for 24h. in the above concentration of (H_2SO_4). Thereafter , transferred to boric acid (10µg/ml) for 6days.

Mean of root number LSD(0.01)= 8.79 LSD (0.05) = 6.145 , mean of root length LSD(0.01)= 1.928 LSD(0.05)= 1.348
mean of leaf area , LSD(0.01)= 0.328 LSD (0.05) = 0.229 .

Table 2:Influence of (H_2SO_4) on rooting response of aged mung bean cuttings for 3 days in d/H_2O

Solution	Concentration %	Mean root No./cutting	Mean root length/cutting(mm)	Mean of 1st true trifoliated leaf area (cm^2)	pH
d/H_2O	0	14.875	9.259	0.313	6.71
Indole acetic acid (IAA)	0.00876 ($5x10^{-4}$M)	34.375 **	**2.583	**0	4.38
H_2SO_4	0.0001	15.375	9.584	0.401	4.15
	0.001	29.125 **	**6.171	**0.097	3.55
	0.01	28.25 **	**1.382	**0.069	2.85
	0.1	**0	**0	**0	1.92
	0.5	**0	**0	**0	1.16

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days .Then aged for 3 days in d/H_2O and treated for 24h. in the above concentration of (H_2SO_4). Thereafter , transferred to boric acid (10µg/ml) for 6days
Mean of root number LSD(0.01)= 7.749 LSD (0.05) = 5.386 , mean of root length LSD(0.01)= 1.578 LSD(0.05)= 1.097
mean of leaf area , LSD(0.01)= 0.159 LSD (0.05) = 0.11 .

Table 3:Influence of (H_2SO_4) on rooting response of aged mung bean cuttings for 3days in H_2SO_4 solution

Solution	Concentration %	Mean root No./cutting	Mean root length/cutting(mm)	Mean of 1st true trifoliated leaf area (cm^2)	pH
d/H_2O	0	17	17.284	1.016	6.71
Indole actic acid (IAA)	0.00876 ($5x10^{-4}$M)	8.3	**2.752	**0	4.36
H_2SO_4	0.0001	45.4**	**5.436	**0.536	4.15
	0.001	40.2**	**9.561	0.746	3.55
	0.01	65.7**	**6.801	*0.665	2.85
	0.1	**0	**0	**0	1.92
	0.5	**0	**0	**0	1.16

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days .Then aged for 3 days in the above concentration of (H_2SO_4). Thereafter , transferred to boric acid (10µg/ml) for 6days.
Mean of root number LSD(0.01)= 13.638 LSD (0.05) = 9.535 , mean of root length LSD(0.01)= 2.04 LSD(0.05)= 1.426
mean of leaf area , LSD(0.01)= 0.409 LSD (0.05) = 0.286 .