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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 nitric acid (HNO_3) as a strong mineral acid on rooting response of fresh and aging mung bean cuttings has been studied on the level of Indole-3-Acetic Acid (IAA). The data revealed significant increase in rooting response of fresh cuttings treated with (0.0001% and 0.1%) concentrations of HNO_3 solution, significant increase in rooting response of aged cuttings (for 3 days in d/H_2O) with (0.0001% and 0.01%) concentration of HNO_3 solution and highly significant increase in rooting response of aged cuttings (for 3 days in HNO_3 solution) with (0.0001%) concentration, while highly concentration (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 occurrence during aging phenomenon verified a highly significant increase of IAA content in hypocotyles of fresh and aged cuttings in optimal concentration of HNO_3.

Key words: Aging, IAA biosynthesis, rooting response, mineral acids, macronutrients, stem cuttings.

1. Introduction

Nitric acid, one component of acid deposition, travels through the air as gaseous nitrogen, making up 80% of the lower atmosphere, plants rely on the nitrate ion (NO_3^-) because it and the ammonium ion (NH_4^+) are the easiest ions for plants to absorb [1]. Once in the plant, NO_3^- is reduced NH_4^+ before incorporation in to amino acids, proteins, and other nitrogenous organic molecules, Nitrogen is very mobile in the plant, Thus the symptoms of nitrogen deficiency generally appear first in the older leaves, where, the older leaves will turn completely yellow or tan and fall of the plant [2].

The use of nitrogen fertilizers adds hydrogen ions to the soil, making it more acidic [3]. These ions can displace other positively-charged mineral ions, such as magnesium and calcium, from binding sites on soil particles [1]. Acidity of the soil influences the physical properties of the soil, the availability of certain minerals to plants, and the biological activity of the soil. It consequently strongly influences plant growth [4].

Soil pH has two major effects, competition and injury. A low pH is believed to reduce cation uptake by competition between hydrogen ions and 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 favored the discrepancy between cation and anion absorption is balanced by greater accumulation of organic anions within the tissue. 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 [5].

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

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treatment was delayed by holding cuttings in deionized H_2O particularly in mung bean cuttings [6]. In addition, Leshem [7] 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 antioxidant agents act internally to suppress the free radicals, hence, reducing the processes that occurs during aging in plants. However, Ishii and et al. [8] showed that free radicals and its derivatives in aged cell and organs (in Nematodes) regenerated primarily in mitochondria as undesirable products through oxidative phosphorylation, Davies [9] 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. Materials and Methods

2.1 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 3,000-3,500 Lux), temperature 25 °C ± 1 °C and relative humidity 60%-70% for ten days.

2.2 Preparation of Cuttings

Cuttings were prepared according to Hess [10] 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.

2.3 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 h with d/H_2O or tested solutions (12 cuttings/treatment), then transferred to boric acid (10 µg/mL) for 6 days, before counting the root numbers.

2.4 Aging Treatments

Cuttings were held immediately after taken from 10 days old seedlings in d/H_2O for 3 days, if the purpose was controlling of aging phenomenon. Physiologically, aged cuttings treated with tested solution for 24 h, 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 et al. [11]. Completely randomized design (CRD) was conducted in all experiments for statistical analysis according to Spiegel [12].

2.5 Preparation of Solutions

Boric acid solution: prepared at (10 µg/mL) and employed as rooting medium [13];

Synthetic auxin solution: Indole-3-Acetic Acid (IAA) was initially dissolved in small amount of absolute alcohol to prepare (5×10^{-4} M) [14];

Nitric acid solution: HNO_3 (68%) was prepared as (%) percent solution (V/V), by dissolving 1 mL of HNO_3 in 99 mL of d/H_2O to achieve 1% as stock solution, then diluted to the required concentration.

2.6 Quantitative Determination of IAA

Naturally occurring auxin (IAA) was measured spectrophotometrically in hypocotyls of fresh and aged cuttings, according to Stoessl and Venis [15], Plieninge et al. [16]. The above procedure (were modified) included 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 (HNO_3) in Rooting Response of Fresh and Aged Cuttings

Table 1 shows the effects of HNO_3 in rooting response of fresh cuttings, when supplied to cuttings

Table 1 Influence of (HNO_3) 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	14.14	0.818	6.63
IAA	0.00876 (5×10^4 M)	35.4**	**1.586	**0.045	4.38
	0.0001	23.4*	**6.1	**0	5.56
	0.001	15.3	18.063*	**0.063	4.19
(HNO_3)	0.01	11.9	28.208**	**0.189	3.15
	0.1	31.3**	**5.047	**0.329	2.21
	0.5	**0	**0	**0	1.59

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days; then treated for 24h in the above concentration of (HNO_3); Thereafter, transferred to boric acid (10 μg/mL) for 6 days; Mean of root number LSD (0.01) = 9.255, LSD (0.05) = 6.471, mean of root length LSD (0.01) = 4.417, LSD (0.05) = 3.088, mean of leaf area, LSD (0.01) = 0.309, LSD (0.05) = 0.255 .

immediately. The results revealed that the means of roots number, roots length (mm), leaf area (cm^2) as the mean of one cutting developed in fresh, untreated cuttings (general control d/H_2O) are (16.3 root, 14.14 mm, 0.818 cm^2) respectively. These means in cuttings treated with auxin (special control IAA) are (35.4 root, 1.586 mm, 0.045 cm^2) respectively, while these in cuttings treated with HNO_3 are (23.4, 15.3, 11.9, 31.3 and 0) root, (6.1, 18.06, 28.208, 5.047 and 0) mm and (0, 0.063 ,0.189, 0.329 and 0) cm^2 at pH (5.56, 4.19, 3.15, 2.21, 1.59) respectively. Statistically, cuttings treated with 0.1% of HNO_3 at pH 2.21 were positively highly significant ($P \geq 0.05$). At the same time, cuttings treated with (IAA) pH (4.38) was positively significant at the same probability level compared to control treatment (d/H_2O), while cuttings treated with 0.0001% of HNO_3 at pH (5.56) was positively significant ($P \leq 0.05$). Generally, high concentration of HNO_3 (0.5%) have no significant effect on rooting response in all treatments. On the other hand, cuttings treated with (0.01% and 0.001%) concentration of HNO_3 at pH (3.15 and 4.19) respectively, were positively highly significant in mean root length ($P \geq 0.05$ and ≤ 0.05)respectively, compared to control (d/H_2O), while cuttings treated with (IAA) have negative significant difference on $P \geq 0.05$ level, as compared to control (d/H_2O). Negative significant difference on ($P \geq 0.05$) level in mean leaf area in all treatments was found as well as cuttings treated with (IAA) compared to control treatment (d/H_2O).

The influence of HNO_3 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) leaf area (cm^2) as the mean of one cutting developed in cuttings aged in d/H_2O for three days are 10 root, 7.615 mm, 0.144 cm^2, respectively, These mean in aged cuttings in IAA are 31.375 root, 3.128 mm, 0 cm^2, respectively, on the other hand, these means in aged cuttings in (d/H_2O) for 3 days and treated for 24 h. with HNO_3 solution are (15.75, 11, 22.25, 4.25 and 0) root, (6.178, 7.609, 5.043, 0.964 and 0) mm and (0.12, 0.253, 0.091, 0.03 and 0) cm^2 respectively at pH (5.56, 4.19, 3.15, 2.21 and 1.59), aged cuttings in 0.01% and 0.0001% concentration of HNO_3 at pH (3.15 and 5.56), were positively highly significant in rooting response ($P \geq 0.05$ and ≤ 0.05), compared to control (d/H_2O). At the same time, aged cuttings in IAA solution at pH (4.38) was positively significant ($P \geq 0.05$) compared to control treatment (d/H_2O).

Generally, high concentration (0.5%) has no significant effect on rooting response in all treatments. On the other hand, statistically, aged cutting in 0.001% concentration of HNO_3 at pH (4.19) was positively significant in mean leaf area (p 0.05), compared to control (d/H_2O). Table 2 revealed negative significant difference on ($P \geq 0.05$ and ≤ 0.05) in mean root length in all treatments as well as aged cuttings in IAA solution compared to control treatment (d/H_2O).

The influence of HNO_3 on rooting response of aged mung bean cuttings in solution has been shown in

Table 2 Influence of (HNO_3) on rooting response of aged mung bean 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	10	7.615	0.144	6.63
IAA	0.00876 (5×10^4 M)	31.375**	**3.128	*0	4.38
	0.0001	15.75*	*6.178	0.12	5.56
	0.001	11	7.609	0.253*	4.19
HNO_3	0.01	22.25**	**5.043	0.091	3.15
	0.1	*4.25	**0.964	*0.03	2.21
	0.5	**0	**0	*0	1.59

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 24 h in the above concentration of (HNO_3); Thereafter, transferred to boric acid (10 μg/ml) for 6 days; Mean of root number LSD (0.01) = 7.544, LSD (0.05) = 5.243, mean of root length LSD (0.01) = 1.868, LSD (0.05) = 1.298 mean of leaf area, LSD (0.01) = 0.152, LSD (0.05) = 0.106.

Table 3 Influence of (HNO_3) on rooting response of aged mung bean cuttings for 3 days in HNO_3 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	22.4	16.955	1.02	6.63
IAA	0.00876 (5×10^4 M)	15.6	**2.773	**0	4.38
	0.0001	44.6**	**5.595	**0.285	5.56
	0.001	26.5	24.272**	1.627**	4.19
HNO_3	0.01	18.1	22.985**	1.724**	3.15
	0.1	*12.4	**5.182	**0.124	2.21
	0.5	**0	**0	**0	1.59

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days; Then aged for 3 days in the above concentration of (HNO_3); Thereafter, transferred to boric acid (10 μg/mL) for 6 day; Mean of root number LSD (0.01) = 10.588, LSD (0.05) = 7.403, mean of root length LSD (0.01) = 4.306, LSD (0.05) = 3.010, mean of leaf area, LSD (0.01) = 0.367, LSD (0.05) = 0.257.

Table 3. The results revealed that means of roots number, roots length (mm), leaf areas (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 (22.4 root, 16.955 mm,1.02 cm^2) respectively. The means in aged cuttings in IAA for three days (special control IAA) are 15.6 root, 2.773 mm, 0 cm^2, respectively. The means in aged cuttings in HNO_3 solution for three days are 44.6, 26.5 ,18.1, 12.4 and 0 root, 5.595, 24.272, 22.985, 5.182 and 0 mm and 0.285, 1.627, 1.724, 0.124 and 0 cm^2 at pH (5.56, 4.19, 3.15, 2.21 and 1.59) respectively. Aged cuttings for three days in 0.0001% concentration of HNO_3 solution at pH 5.56 was positively highly significant in rooting response ($P \geq 0.05$), compared to control (d/H_2O). This increase has doubled the responsiveness to ARF into 3 folds compared to rooting response of auxin aged cuttings. Generally, high concentration (0.5%) have no significant effect on rooting response in all treatments, aged cuttings in 0.001 and 0.01% concentration of NHO_3 at pH (4.19 and 3.15) were positively significant in mean root lengths and mean leaf areas ($P \geq 0.05$) compared to control (d/H_2O).

3.2 Biochemical Part

3.2.1 Quantization Determination of IAA

3.2.1.1 Effect of HNO_3 on IAA Level in Fresh Cuttings

Fig. 2 shows IAA level in hypocotyls of fresh mung bean cuttings treated in optimal concentration of HNO_3 and IAA. IAA level in 1 g hypocotyls of fresh cuttings (general control d/H_2O) is 11.067 mmol, whereas, IAA level in 1 g hypocotyls of cuttings treated with synthetic IAA (special control treatment) is 16.022 m molar. IAA level in 1 g hypocotyls of cutting treated with HNO_3 solution (0.1%, pH = 2.21) is 14.846 mmol. Treatment with IAA and HNO_3 revealed positive

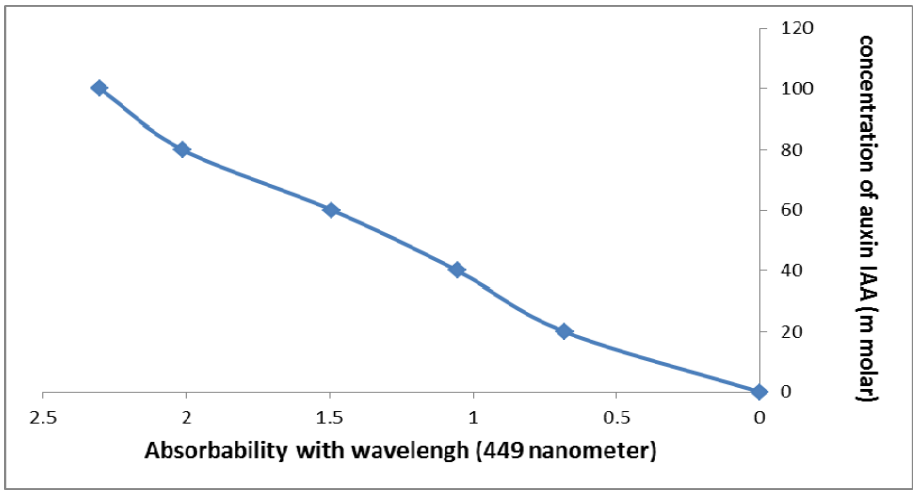

Fig. 1 Standard carve of different concentration of auxin (IAA) and absorbability with wave length (449 nanometer).

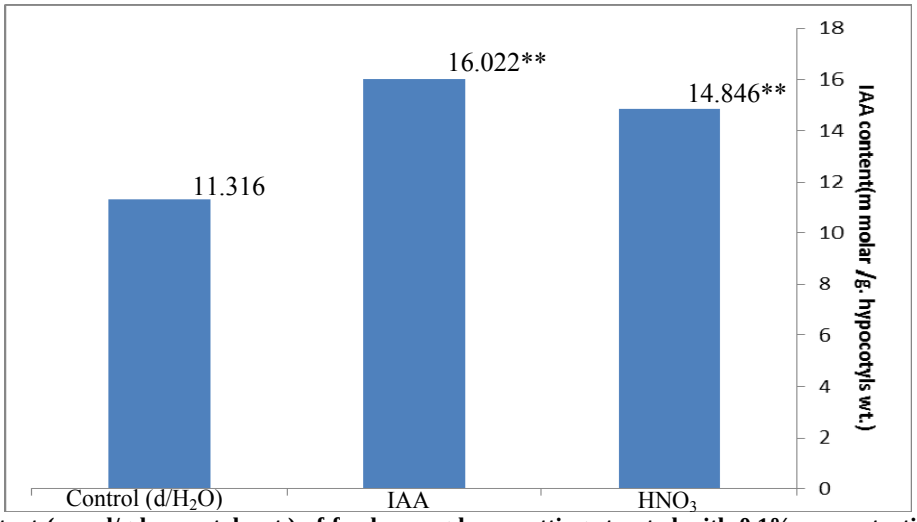

Fig. 2a IAA content (mmol/g hypocotyls wt.) of fresh mung bean cuttings treated with 0.1% concentration (pH = 2.21) of HNO_3 solution and 5×10^4 M concentration (pH = 4.38) of IAA solution, LSD (0.05) = 0.845, LSD (0.01) = 1.632.

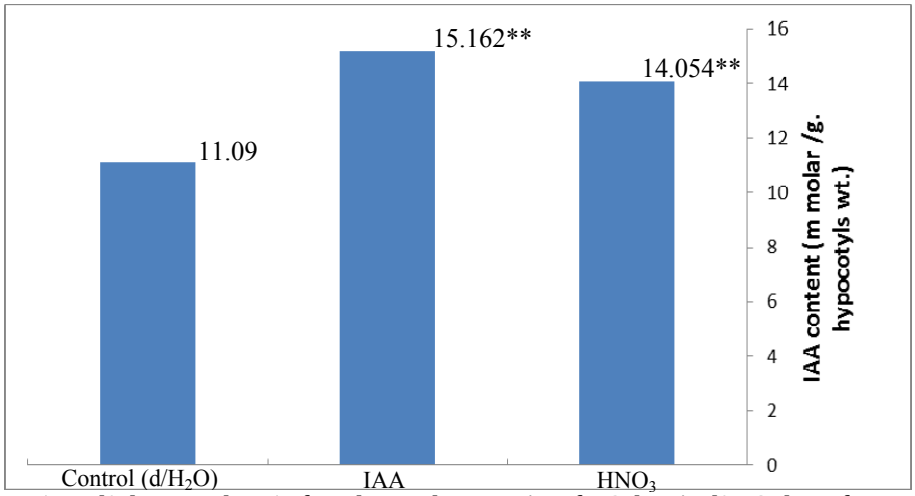

Fig. 2b IAA content (mmol/g hypocotyls wt.) of aged mung bean cuttings for 3 days in d/H_2O thereafter treated for 24 h; with 0.01% concentration of HNO_3 (pH = 3.15) and 5×10^4 M concentration (pH = 4.38) of IAA solution; LSD (0.05) = 0.818, LSD (0.01) = 1.579.

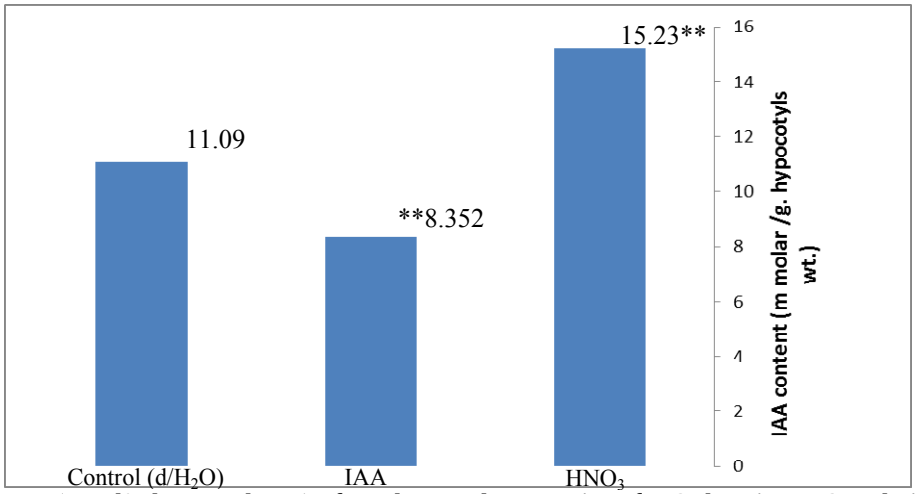

Fig. 2c IAA content (mmol/g hypocotyls wt.) of aged mung bean cuttings for 3 days in HNO_3 solution with 0.0001% concentration (pH = 5.56) and 5 × 10^4 M concentration (pH = 4.38) of IAA solution, LSD (0.05) = 0.89, LSD (0.01) = 1.730.

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 cuttings treated with HNO_3 compared to control treatment IAA.

3.2.1.2 Effect of HNO_3 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 HNO_3 for 24 h. IAA level in 1 g hypocotyls of aged cuttings (general control d/H_2O) is 11.09 m molar. IAA level in 1 g hypocotyls of aged cuttings in synthetic auxin (IAA) is 15.162 m molar, while, IAA level in 1 g hypocotyls of cuttings aged in HNO_3 solution (0.01%, pH = 3.15) is 14.054 m molar. Aging treatments with IAA and HNO_3 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 HNO_3 compared to control treatment IAA.

3.2.1.3 Effect of HNO_3 on IAA Level in aged Cuttings for 3 days in Solution

Fig. 2c shows IAA level in hypocotyls of mung bean cuttings taken from seedings grown in d/H_2O for 10 days and aged for 3 days in IAA and HNO_3 solution at optimal concentration for rooting response. IAA level in 1 g 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 g hypocotyls of aged cuttings in synthetic auxin (IAA) for 3 days (special control IAA) is 8.352 m molar, while, IAA level in 1 g hypocotyls of cuttings aged for 3 days in HNO_3 solution (0.0001%, pH = 5.56) is 15.23 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 HNO_3 solutions compared to special control IAA and general control treatment (d/H_2O).

4. Discussion

The processes that leads to diminish rooting response of mung bean cuttings during aging may be attributed to loss of co-factors [17] with age or decrease of auxin contents in cuttings or elsewhere in the cuttings, for example leaves [18] or hypocotyl (root initiation zone) [19].

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 2b. These results confirm the hypothesis that explains 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 [18].

However, the decline in IAA content of aged mung bean cuttings, may be attributed to: (1) decrease in IAA biosynthesis in primary leaves of aged cuttings, which is considered as central source for IAA biosynthesis, Hartmann et al. [20] denoted decline in IAA content in leaves during senescence; (2) decline of basipetal transport of IAA [21]; (3) conversion of free IAA to conjugated IAA during rooting response [22]; (4) occurrence of high level of oxidative processes in aged cuttings [18].

Cuttings kept in different concentration of HNO_3 for three days (aging period), developed significant rooting response. In other words, some concentration stopped the processes that occurred during aging completely in terms of ARF Table 3. Meanwhile, some other concentration stopped aging partially Table 2.

The role of (HNO_3) in offsetting, stopping, delaying or retarding the processes that lead 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 2c) developed significant increase compared to control. However, the significant rooting response of aged mung bean cuttings, the significant increase in mean of root length and mean of leaf area Tables 2 and 3 may be attributed to the following factors:

(1) The Effect of Nitric Acid

The significant rooting response of mung bean cuttings may be attributed to the capability of HNO_3 for trapping free radicals, because of presence of high electronic conjugation in this compound. HNO_3 is considered as a strong acid for giving semicompletely it is proton to water. In HNO_3 solution, HNO_3 molecule dissociates giving proton to water forming positively charged cation, hydronium ion (H_3O^+) and negatively charged anion, nitrate ion (NO_3^-). Obviously, pH depends on concentration of dissociated acid 100%.

$$HNO_3 + H_2O \rightarrow H_3O^+ + NO_3^-$$

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 and HNO_3) are considered as strong acids because all these acids semi completely gives their protons to water. So, they appear as they have 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 [23];

(2) The Effect of Nitrogen Element

Nitrogen atoms are characterized by presence of external envelope which contains individual electrons acting as internal suppressors of free radicals through formation covalent bonds and lowering the effects of oxidative products that occur during aging.

Nitrogen and all of its counterparts (N_2, NO_2, etc.) can help and/or inhibit plant growth [3]. Nitrogen is constituent of a variety of organic compounds which are essential to the structure and metabolism of plants, nitrogen occurs, for example, in nucleic acid, proteins, chlorophyll and various co enzymes, including the nicotinamide adenine dinucleotides (NAD and NADP) [5]. On the other hand, it has been found that nitrogen stimulates abundant growth of the shoot system, favoring a high shoot/root ratio, and will often delay the onset of flowering in agricultural crops [2]. Thereafter, explain the significant increase in mean leaf area in aged cuttings at 0.001, 0.01% concentration (Tables 2 and 3);

(3) The Effect of pH

The significant rooting response of mung bean cuttings may be attributed to the acidic pH. The data revealed that ARF in mung bean cuttings positively were affected by acidic pH. This was confirmed by prior studies by using the same kind of cuttings [18].

It has been found that roots were 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 plants grow in more acidic soil while some grow in more alkaline soil [24].

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 well they can keep toxins out [1].

(4) The Effect of Hormonal Balance

Hormonal balance has great effect in rooting response of fresh and aged mung bean cuttings. This was confirmed by Divies [9] who deseribed aging as phenomenon that fundamentally concerned with degenerative changes in metabolism. Obviously, he mentioned that alteration of hormonal balances was the only molecular events leading to these changes. So, studies mentioned that nitrogen was a constituent of many important molecules, including amino acids, amides, alkalins, vitamins and certain hormones (e.g., indole-3-acetic acid, cytokinin) [2]. Thereafter, explain the significant increase of IAA content in hypocotyls of fresh and aged mung bean cuttings (Figs. 2a, 2b and 2c) which reflected the significant increase of rooting response. This was confirmed by prior studies by using the same kind of cuttings [18, 25, 26];

(5) Resistance of Detrimental Effect of Environmental Stress Factors

The significant increase in rooting response of aged cuttings in HNO_3 solution (Tables 2 and 3) may be attributed to the role of nitrogen to resistance of detrimental effect of environmental stress factors on crop plant, studies mentioned that it is essential to improve the mineral nutritional status of plants under marginal environmental conditions, in order to sustain their survival and to maintain high yields. Plant requirements for mineral nutrients such as nitrogen increase with increasing severity of the environmental stresses imposed by drought, heat, salinity, chilling or intense light. Nitrogen supplied at adequate levels is an essential requirement for the maintenance of photosynthesis activities and utilization of light energy in CO_2 fixation. Therefore, the improvement of mineral nutrition of plants becomes a major contributing factor in protecting them from photo-oxidative damage under marginal environmental conditions [27];

(6) Ionic Balance

In general, the rate of ion uptake was affected by temperature, metabolic inhibitors, surface area, internal ionic concentration, light, pH and salt concentration. The presence of metabolically-important anions, such as nitrate, often stimulates the uptake of other ions, presumably through an effect on metabolism [5];

(7) Level of Solution Concentration and Treatment Period

Levels of solution concentration and treatment period have important effect on rooting response. This was confirmed by the significant increase in rooting response of aged cuttings (aged for 3 days in HNO_3 solution, then transferred to boric acid for 6 days) which revealed (44.6 root) at 0.0001% concentration. Table 3 compared with rooting response of aged cuttings (aged for three days d/H_2O, then treated for 24 h in HNO_3 solution. Thereafter, transferred to boric acid for 6 days) which revealed (15.75 roots) at the same concentration (Table 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 h in IAA solution, thereafter, transferred to boric acid for 6 days) which revealed (31.375 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 (15.6 roots) at the same concentration (Table 3). However, the decline of rooting response of latter treatment attributed to death the basal part of aged cuttings hsypocotyl in auxin solution because of the treatment period length (3 days) at this concentration. Generally, aged cuttings (for 3 days) at 0.0001% concentration in HNO_3 at pH 5.56, have doubled the

responsiveness to ARF into relatively, and three 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 m and 15.162 m molar, respectively (Figs. 2a and 2b).

5. Conclusions

Generally and as a conclusion, aging phenomenon may be considered as a result of oxidative processes that occur in plant body or cuttings during aging period, which cause diminishing rooting response in aged mung bean cuttings. The role of HNO_3 in offsetting, stopping, delaying or retarding the processes that leads to diminish rooting response in aged cuttings may be attributed to capability of HNO_3 for trapping free radicals, because of high electronic conjugation in this compound.

However, nitricacid tested in the current study as a strong acid has a strong tendency to giving semi completely, proton to water. In HNO_3 solution, HNO_3 molecule ionized semi completely giving (H_3O^+) and (NO_3^-). Obviously, pH depends on concentration of dissociated acid (100%). So, nitrogen element as one of the second cycle elements in periodic table, its atoms are characterized by presence of external envelope which contains individual electrons acts as internal suppressors of free radicals through covalent bonds formation and lowering the effects of oxidative products that occur during aging. However, as well as the above explanation, HNO_3 lowers the effects of oxidative products through the followings: (1) hormonal factors and IAA content (hormonal balance); (2) pH; (3) resistance of stress; (4) ionic balance; (5) external and internal environmental effects; (6) level of solution concentration and (7) period of treatment.

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

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