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HYDROGEN BONDING OF PHENOLIC COMPOUNDS, ASCORBATE AND SUGARS AS ANTI-OXIDANT AGENTS ON INDOLE ACETIC ACID (IAA) LEVEL VIA OXIDATIVE HYPOTHESIS DURING AGING OF MUNG BEAN CUTTINGS

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ABSTRACT

Depending on number and position of hydroxyl groups of some phenolic compounds, ascorbate and sugars, and their effects on IAA levels through oxidative hypothesis that accompanied aging phenomenon of mung bean cuttings were studied. The data revealed, highly significant increase in rooting response of cuttings aged in o-coumaric acid, caffeic acid, and p-hydroquinone all at 10^{-3} M, ascorbate at 200-500 ppm and sucrose at 3%. However, significant increase was there in rooting response of cuttings aged in cinnamic acid, phenol, o-hydroxyphenol (Catechol) at concentrations (10^{-3}, 10^{-5}, and 10^{-5}) M, respectively compared to control (d/H_2O). All of these compounds caused offseting or stopping of the oxidative processes that occurs during aging as anti-oxidant agents which, acts as free radical scavengers against oxidative processes products and then promoting IAA biosynthesis. Quantitative estimation of IAA by spectrophotometric method verified a highly significant increase of IAA content in hypocotyl of cuttings aged in the above compounds. The discussion draws the attention about the electronic conjugation area of ascorbate and hydrogen bonding of phenolic compounds and the oxidation of hydrogen-oxygen bond between hydroxyl groups of sugars in terms of rooting response of mung been cuttings, which is affected primarily by auxins.

Key words: Aging, Anti-Oxidant defense mechanism, Ascorbic acid, IAA biosynthesis, Mung bean, Oxidant agents, Oxidative hypothesis, Phenolic compounds, Rooting response, Sugars.

INTRODUCTION

Generally, phenolic compounds are considered as secondary by-products of plant metabolism. These are mostly raised from phenylalanine or its close precursor shikimic acid and contain at least one hydroxyl group[1]. They mentioned that the functions of phenolic

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compounds are: controlling the activity and formation of some enzymes, regulation of growth and development of plants, IAA level via its influence on the activity of IAA – oxidase. In addition, phenolic compounds act as co-factors, thereafter, influencing rooting response of fresh cuttings[2] and aged cuttings[3] of mung bean.

The phenolic compounds differ in their ability for oxidative damage and their reactions with other phenols, amino acids, proteins, and mineral ions[4]. However, its stability does'nt depend only on pH and storage period, but also on the structural formula[5]. For example, trans-cinnamic acid can be described with no significant change in its absorption spectrum with pH, and particularly, it was stable under high pH. Mainwhile, ferulic acid having one OH group was stable at high pH, whereas caffeic acid with two OH groups was changed dramatically with changing pH between 7-11.[5] They found that the two OH groups that join benzene ring were responsible for the above changes. Notwithstanding, gallic acid having three OH groups was described as unstable under high pH.

The polycyclic phenols are more complex than monocyclic phenols, and their ionizable and resonance forms are more resistant for damage by pH than monocyclic phenols[5]. It was sugested that phenolic hydroxyl groups may be responsible for spectronic changes for three unstable phenolic compounds e. g. caffeic acid, chlorogenic acid and gallic acid. The changes in absoption spectrum of the above compounds are irreversable, which makes it ready to change under the influence of pH. These changes may be attributed to the formation of unstable intermediate "Quinone" and other resonance forms[6].

It is noteworthy, that phenolic compounds may act in different ways during metabolism that occurs in cuttings. For example phenolic compounds may effect on auxin-conjugation[7], the transpiration loss (e.g. caffeic acid) and hence, the auxin uptake, when supplied to cuttings simultaneously with phenolic compounds[3]. Alternatively, the roles of phenolic compounds (e.g. Cinnamic acid, 10^{-3}M) is in offseting or stopping the processes that leads to diminish rooting response in aged cuttings of mung bean[3]. He suggested that cinnamic acid may act as auxin-protector against IAA-oxidase and caused significant rooting respose in aged cuttings as is the case in fresh cuttings.

On the other hand, carbohydrates (CHO) of the cuttings are important as a source of energy and carbon skeletons. So, sub-dividing of CHO into soluble CHO, available CHO for metabolism and storage CHO whether soluble or insoluble, may help to determine the roles of CHO in root formation in cuttings[8]. In addition, CHO concentration in cuttings was influenced partially, when cuttings were treated with auxin. Obviously, supplied auxin promotes mobilization of CHO in leaves and upper parts of stem into root initiation zone[9].

It is noteworthy, that supplied sucrose might be converted to reducing sugars when absorbed by plants[10] or inhibit stock plants and roots development[11]. Sucrose, glucose and mannose may inhibit enzyme activities[12]. In addition, sugars have osmotic effects[13] and perhaps certain sugars may be antagonize the effects of beneficial ones[14].

Recently, it has been found that diminishing of rooting response in aged cuttings of mung bean was overcomed or stopped partially by supplying sucrose at concentration (1.5%)[15]. They mentioned that supplied sucrose was partially offset or retard the processes that occurs during aging via its preservation of CHO and protein content that was necessary for adventitious root formation (ARF). In addition, nutrational factors were supplied by cotyledons as endogenous source of CHO (using 5-day-old seedlings), which leads to complete stopping of aging phenomenon in aged cuttings compared to fresh cuttings of mung bean[16]. Finally, the progressive decline in CHO content in water extracts of detached cotyledons of mung bean taken from seedlings at different ages (4, 5, 6 and 7 days) reflected the progressive loss in rooting response of mung bean cuttings, when taken from seedlings at stage of fully expanded primary leaves[17].

EXPERIMENTAL

Materials and methods

Cultivation of stock plants: Seeds of mung bean (*Phaseolus aureus* Roxb. Var. local) were soaked overnight, sown in moistened (with distilled water) sterilized sawdust in plastic trays. Seedlings raised in growth chamber provided with a continuous light (light intensity 3000-3500 Lux), temperature 25 ± 1^{o}C and relative humidity 60-70%.

Preparation of cuttings: Cuttings were prepared according to Hess[18] from 10-day-old light grown seedlings. These cuttings are 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 hypocotyl (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 (Twelve cuttings were used per treatment). Then these were transferred to boric acid (10 μg/mL) for 6 days[19] before counting the root numbers.

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 were transferred to boric acid (10 μg/mL) for further 6 days before counting the root number per cutting.

During all these treatments, cuttings were held under the same conditions as mentioned above for raising stock seedlings. Completely randomized design (CRD) with 3 replicates was conducted in all experiments for statistical analysis[20].

Preparation of solutions

Boric acid solution: Prepared (10 µg/mL) and employed as rooting medium[19].

Phenolic compounds solutions: Two groups of phenolic compounds were used. The first group included four compounds with difference in the number of OH groups (e.g. Cinnamic acid, o-coumaric acid, caffeic acid and gallic acid). The second group included another four compounds differing in the possition of OH groups e.g. phenol, o-hydroxyphenel (catechol), m-hydroxyphenol (resorcinol) and p- hydroxyphenol (quinol). All the above compounds were prepared at four different concentrations 10^{-9}, 10^{-7}, 10^{-5}, and 10^{-3} M for each.

Ascorbic acid solutions: Six ascorbate solutions were prepared and used at following concentrations - 50, 100, 200, 300, 400 and 500 ppm.

Sugar solutions: Three sugars were used (glucose, fructose, and sucrose) at three concentrations - 1%, 2% and 3%.

Quantitative determination of IAA: Naturally occurring auxin (IAA) was measured spectrophotometrically in primary leaves, terminal bud, epicotyls and hypocotyls[21,22]. The above procedure was modified, which includes the reaction of IAA with acetic anhydride to form 2-methyl-indole-α pyrone. Synthetic IAA was used for standard curve.

RESULTS AND DISCUSSION

Physiological part

Effects of phenolic compounds in rooting response of fresh and aged cuttings: Table 1 shows the effects of two groups of phenolic compounds in rooting response of fresh cuttings, when supplied to cuttings immediately. The 1st group included four compounds (e.g. cinnamic acid, o-coumaric acid, caffeic acid and gallic acid), which differ in their strucural formula in number of OH groups that join benzene ring. While, the 2nd groups included another four compounds e.g. phenol, o-hydroxyphenol (catechol), m-hydroxyphenol (resorcinol) and p-hydroxyphenol (quinol) differ in the position of OH groups on benzene ring. The results revealed that the number of roots developed in fresh, untreated cuttings (d/H_2O) is 8.9 roots per cutting. However, the number of roots developed in cuttings treated

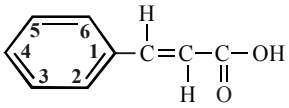
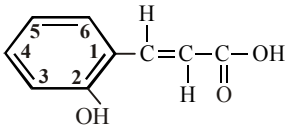
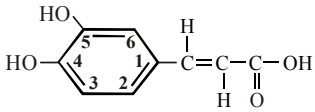
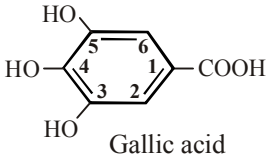
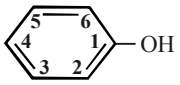
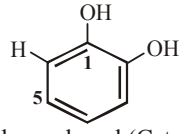
with different concentrations of cinnamic acid (10^{-9}, 10^{-7}, 10^{-5} and 10^{-3}M) at pH (6.3, 6.3, 6.2 and 3.7), (19, 13.2, 11.7 and 25.2) roots, respectively. All these figures increased to 213.5%, 148.3%, 131.5% and 283.1%, respectively as compared to control. Statistically, cuttings treated with 10^{-9} and 10^{-3}M at pH 6.3 and 3.7, respectively, were positively highly significant on probability level (0.01), while cuttings treated with 10^{-7}M at pH 6.3 was positively significant on probability level (0.05). whereas cuttings treated with 10^{-5}M at pH 6.3 has no significant effect compared to control treatment.

Generally, high concentration (10^{-3}M) promotes significantly a high rooting response in terms of root number in all treatments, except m-hydroxyphenol (resorcinol) as compared to control treatment (d/H_2O). On the other hand, all low concentrations (10^{-9}, 10^{-7} and 10^{-5}M) have no significant effect on rooting response of fresh mung bean cuttings except the following cases: (a) cinnamic acid at (10^{-9} and 10^{-7} M) have positive significant differences on (0.01) level of probability for the 1st concentration and on (0.5) level for the 2nd concentration, as compared to control; (b) o-coumaric acid at (10^{-9}M) have a positive significant difference on (0.05) level and (c) phenol at (10^{-9}M) have negative significant difference on (0.05) level, as compared to control.

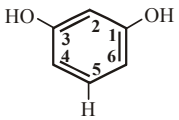
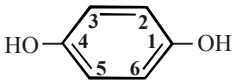
The influence of phenolic compounds on rooting response of aged mung bean cuttings has been shown in Table 2. Cuttings aged in d/H_2O for three days developed (12.2) roots per cutting. Application of different phenolic compounds at concentrations (10^{-9} to 10^{-3}) M to investigate their influences on delaying or stopping of aging processes leads to diminishing rooting response in aged cuttings had significant effects in that direction. However, mean root numbers/cutting of all concentrations tested of cinnamic acid were 13.9, 17.7, 13.7 and 19.1, respectively. All these figures increased to 113.9%, 145.1%, 112.3% and 156.6%, respectively as compared to control. Statistically, no significant differences between treatments except cuttings hold at 10^{-3}M having positive significant diference on probability level of 0.05 as compared to control.

Generally, cuttings aged at high concentration (10^{-3}M) in o-coumaric acid, caffeic acid and p-hydroxyphenol (Quinol) and at pH 3.8, 3.4 and 5.6, respectively, have doubled the responsiveness to ARF into 3.4, 2.3 and 3 folds, respectively. Statistically, these figures are having a highly positive significant difference on (0.01) level as compared to control. Meanwhile, cuttings aged in cinnamic acid, phenol and o-hydroxyphenol (catechol) at concentrations (10^{-3}, 10^{-5} and 10^{-5}) M respectively and at pH 3.7, 5.1 and 5.3, respectively, have increased the ability of rooting response to 1.6 folds in each case. Statistically, the above treatments are having a positive significant difference on (0.05) level as compared to control treatment. Whereas, other treatments in Table 2 have no significance from statistical point of view as compared to control.

Table 1: Influence of phenolic compounds on rooting response of fresh mung bean cuttings

Phenolic compounds and structural formula	Concentration (M)	Mean root Number./cutting	pH
d/H_2O	0.0	8.9	7.0
 Cinnamic acid	10^{-9}	19.0**	6.3
	10^{-7}	13.2*	6.3
	10^{-5}	11.7	6.2
	10^{-3}	25.2**	3.7
 o-Coumaric acid	10^{-9}	14.4*	6.1
	10^{-7}	12.8	5.9
	10^{-5}	12.2	5.9
	10^{-3}	19.6**	3.8
 Caffeic acid	10^{-9}	7.9	5.5
	10^{-7}	6.8	5.5
	10^{-5}	6.4	5.5
	10^{-3}	18.0**	3.4
 Gallic acid	10^{-9}	7.2	6.5
	10^{-7}	7.4	5.8
	10^{-5}	7.3	5.6
	10^{-3}	14.1*	3.2
 Phenol	10^{-9}	*4.4	5.2
	10^{-7}	6.0	5.1
	10^{-5}	5.8	5.1
	10^{-3}	22.1**	5.1
 o-Hydroxyphenol (Catechol)	10^{-9}	7.4	5.3
	10^{-7}	8.8	5.3
	10^{-5}	5.6	5.3
	10^{-3}	16.3**	5.2

Cont…

Phenolic compounds and structural formula	Concentration (M)	Mean root Number./cutting	pH
 m-Hydroxyphenol (Resorcinol)	10^{-9}	5.4	5.5
	10^{-7}	8.1	5.4
	10^{-5}	7.2	5.3
	10^{-3}	11.8	5.2
 p-Hydroxyphenol (Quinol)	10^{-9}	7.7	5.9
	10^{-7}	5.7	5.6
	10^{-5}	7.4	5.6
	10^{-3}	15.7**	5.6

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days. Then these were treated with the above concentrations of different phenolic compounds for 24 h. Thereafter, these were transferred to boric acid (10 μg/mL) for 6 d. LSD (0.05) = 4.291 LSD (0.01) = 5.653.

A* = positive significant effect; A** = positive highly significant effect; *A = Negative significant effect

Effect of ascorbic acid on rooting response of fresh and aged cuttings

Table 3 shows the effects of ascorbate in rooting response of fresh cuttings. The average umber of roots developed in fresh, untreated cuttings (d/H_2O) is 10.2 roots/cutting; whereas, the number of roots developed in cuttings treated with different concentrations of ascorbate 50, 100, 200, 300, 400 and 500 ppm were 14.4,13.5,18.8,19.8,26.4 and 27.8 roots, respectively. The percentage of increase over control was 41.2%, 32.4%, 84.3%, 94.1%158.8% and 172.5%, respectively. However, the number of roots developed in cuttings aged for 3-days in ascorbate solutions as mentioned above is 13.4, 16.2, 20.1, 29.5, 27.9 and 32.8 roots, respectively as compared to control 12.6 roots (Table 4).

These results indicated that high concentrations of ascorbate (200-500) ppm promoted the rooting response of fresh mung bean cuttings (Table 3) and stopped the processes that occurs during aging completely (Table 4).

Effects of sugars on rooting response of fresh and aged cuttings

The influence of sugars (glucose, fructose and sucrose) on rooting response of fresh cuttings has been shown in Table 5. All sugars promote ARF in fresh cuttings of mung bean.

Statistically, positive significant effects at all concentrations were tested except glucose without significant effect at concentration 3% as compared to control.

The influence of these sugars on rooting response of aged cuttings has bean shown in Table 4. The results revealed that keeping cuttings in sucrose solution for three days (aging period) at concentration, 3% and pH 4.4 completely inhibited all processes that leads to diminishing rooting response in aged cuttings and produced the highest number of roots/ cutting (19.1 roots), with the increment of root numbers in terms of percentage over control is equal to 101.1%. In addition, all other sugars at all concentrations have no significant differences.

Biochemical part

Quantitation determination of IAA

Effect of phenolic compounds on IAA level in aged cuttings

The effect of aging on IAA level in hypocotyl of cuttings was observed from seedlings grown in d/H_2O for 10-days and aged for 3 days in different phenolic compounds (at optimal concentrations) for rooting response. The results have been shown in Fig. (1a). IAA level in 1 g hypocotyls of cuttings aged in d/H_2O (control treatment) is 11.067 m moles. whereas IAA level in 1 g hypocotyl of cuttings aged in tested phenolic compounds (e.g. cinnamic acid, 10^{-3}M (pH = 3.75), o-coumaric acid, 10^{-3} M (pH = 3.85), caffeic acid, 10^{-3}M (pH = 3.4), gallic acid, 10^{-7}M (pH = 3.85), phenol, 10^{-5} M (pH = 5.7), o-hydroxyphenol (catechol), 10^{-5}M (pH = 5.3), m-hydroxyphenol (resorcinol), 10^{-3}M (pH = 5.25), and p-hydroxyphenol (quinol), 10^{-3}M (pH = 5.7) are 12.923, 14.325, 15.592, 20.47, 14.755, 14.053, 13.013 and 15.049 m moles, respectively. Statistically, all the above figures are positive highly significant on 0.01 level of probability as compared to control.

Effect of ascorbic acid on IAA level in aged cuttings

Fig. (1b) shows that IAA contents in hypocotyls of mung bean cuttings taken from seedlings grown in d/H_2O for 10 days, and aged for 3 days in ascorbate solution at concentration 500 ppm and pH 2.2. IAA level in 1 g hypocotyls of cuttings aged in d/H_2O (control) is 11.067 m moles. whereas, IAA level in 1 g hypocotyls of cuttings aged in ascorbate solution is 17.357 m moles. The percentage of increase was 56.8% over control. Statistically, it is highly positive significant difference on (0.01) level as compared to control.

Effect of sugars on IAA level in aged cuttings

The effect of aging on IAA level in hypocotyl of cuttings was observed from seedlings grown in d/H_2O for 10 days and aged for 3 days in different sugar solutions

(glucose, fructose and sucrose) at optimal concentrations for rooting response (3%) and at pH 6.2, 5.8 and 4.4, respectively has been shown in Fig. (3b). IAA level in 1 g hypocotyl of cuttings aged in d/H_2O (control) is 11.067 m moles whereas, IAA level in 1 g hypocotyl of cuttings aged in glucose, fructose and sucrose is 17.628, 15.683 and 15.411 m moles, respectively. Statistically, positive significant differences was observed on (0.01) level for glucose, whereas, on (0.05) level for fructose and sucrose as compared to control.

The natural content and distribution of IAA in fresh and aged cuttings of mung bean were measured from seedlings grown in d/H_2O for 10 days. The results have been presented in Fig. (3c). IAA level in 1 g of primary leaves and apical buds was 16.995 m moles, epicotyls 14.777 m moles, and hypocotyls 11.0222 m moles. In contrast, with aged cuttings, IAA level in primary leaves and apical buds was 14.778 m moles, epicotyls 9.936 m moles, and hypocotyls 11.067 m moles. Obviously, the total IAA level was declined in aged cuttings into 35.781 m moles as compared to fresh cuttings 42.794 m moles.

Davies[23] described aging as a 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.

The processes that leads to diminish rooting response of mung bean cuttings during aging may be attributed to loss of co-factors[24] with age or decrease of auxin contents in hypocotyl (root initiation zone) or elsewhere in the cuttings, for example leaves[25].

The nature of oxidative processes was studied, which presumably increased during aging, depending on the availability of oxidative agent from one side and the decrease of agents that involved in antioxidant defense mechanisms from the other side. So, our spectrophotometrical measurements of total naturally occurring auxin (IAA) in aged cuttings taken from seedlings grown in d/H_2O for 10 days was declined to 35.781 m moles, compared to that in fresh cuttings 42.794 m moles Fig. (1d). These results confirm one of the hypotheses that explain aging causes (or processes that occur during aging), which is the decline of naturally occurring auxin (IAA). The above hypothesis has been verified by using the same kind of cuttings and using auxin bio-assay technique[25]. However, the decline in IAA content of aged mung bean cuttings, may be 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[26] denoted decline in auxin content in leaves during senescence. In addition, Wilkins[27] mentioned that auxin biosynthesis declines or stops in fully expanded leaves. This was confirmed by our results in Fig. (1d) about the significant decline of IAA level in leaves of aged cuttings (13.05%) as compared to fresh

cuttings. (b) Decline of basipetal transport of IAA. Obviously, this was confirmed by Shaheed[28] in aged mung bean cuttings, after foliar application of C^{14}-IAA). (c) Conversion of free IAA to conjugated auxin during rooting response[29] and (d) Occurrence of high level of oxidative processes in aged cuttings, which must be discussed later through the results obtained is this investigation.

However, cuttings kept in different phenolic compounds for three days (aging period), developed statistically significant rooting response. In other words, some phenolics stopped the processes that occurs during aging completely in terms of ARF (e.g. o-coumaric acid, caffeic acid, and p-hydroxyphenol (quinol) at higher concentrations (10^{-3}M). Meanwhile, some other phenolics e.g. Cinamic acid, phenol, and o-hydroxyphenol (catechol) at (10^{-3}, 10^{-5} and 10^{-5}M) concentrations, respectively, stopped aging partially whereas, statistically gallic acid and m-hydroxyphenol (resorcinol) have no effect in all concentrations tested.

The role of phenolic compounds in offseting, stopping, delaying or retarding the processes that leads to diminish rooting response in aged cuttings is difficult to interpret. However, the statistical analyses of IAA content in hypocotyl of cuttings aged in these substances (Fig.1a) developed an significant increase in all treatments compared to control. Consequently, IAA content was the highest in gallic acid (20.479 m moles), although the latter compound at the same concentration (10^{-5}M) has no effect on rooting response or its inhibitory effects at higher concentrations (Table 2).

However, the significant rooting response of mung bean cuttings may be attributed to the capability of phenolic compounds under investigation (as anti-oxidant) for trapping free radicals, because of presence of high electronic conjugation in these compounds comparing to other anti-oxidants. Obviously, in the structural formula of cinnamic acid, the direction of electronic conjugation in from the ring to the carboxyl group, this electronic movement depends on ionization ability of acidic hydrogen atom in carboxyl group. It might be a weak acid, so the electronic movement from ring to carboxyl group was also weak too; and thereafter, the area of electronic conjugation from ring to carboxyl group was inactive because of the weakness of hydrogen acidity of (OH) group. This leads to decline in its activity as anti-oxidant comparing to o-coumaric acid (see Table 2). Seemingly, the roots number of cuttings aged in cinnamic acid was 19.1 roots whereas, in o-coumaric acid, it was 42 roots.

o-Coumaric acid is characterized by a high acidity compared to cinnamic acid, because of the presence of (OH) group at *ortho* position. The function of this position is to

push electrons (ring activator, or electron donor) and hence, leads to increase the active electronic conjugation to include the area of OH, ring, and side group in the direction of COOH group (directed electronic conjugation). The above explanation gave strong character for o-coumaric acid as anti-oxidant as compared to cinnamic acid. It is because of having a high scavenging character of oxidizing electrons by persisting it longer inside the ring, comparing to electronic conjugation area of cinnamic acid. This was raised through rooting response in terms of 42 root/cutting compared to 19.1 root in cinnamic acid.

The presence of two (OH) groups in *meta* and *para* positions is a distinguishable feature of caffeic acid. The above positions permits *intra* hydrogen bonding, that leads to restrict the ionization of one of these (OH) groups in these positions. Thereafter, the electronic conjugation depends on ionization of one hydrogen atom of the two (OH) groups; for example, the ionization of (OH) group in *para* position with simultaneous restriction of (H) in *meta* position, gave the compound the same acidity and electronic conjugation as was the case in o-coumaric acid. Whereas, restriction of (H) of (OH) group in *para* position with simultaneous liberation of (H) of (OH) group in *meta* position from hydrogen bonding, gave a new position that carry oxygen with negative charge in *meta* position, which weakened the ring and is considered as inactivator for electronic conjugation. The above explanation reveals a weakened character for this compound as anti-oxidant. Physiological confirmation in terms of rooting response was revealed i.e. a decline in rooting response of cuttings treated with caffeic acid (28 roots), which is equal to 1/3 of rooting response with o-coumaric acid (42 roots) (see Table 2). This might prove the role of *intra* hydrogen bond between (H) in *meta* position and (O) at *para* position of (OH) group, in rooting response. This kind of bonding does not promote high electronic conjugation and hence, weaked caffeic acid as anti-oxidant as compared to o-coumaric acid

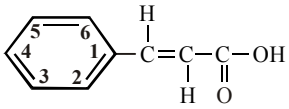
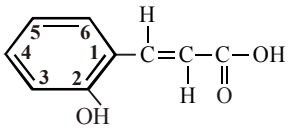
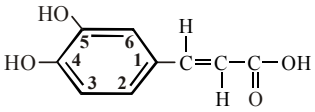
In contrast, with the above compounds, gallic acid contain three OH groups, two of them at *meta* positions (C_3 and C_5) and the third (OH) group at *para* position (C_4). These characters caused *intra* hydrogen bonding between position 3 (*meta*) and position 4 (*para*) or between position 5 (*meta*) and position 4 (*para*). This resulted in a restriction of (H) out of (OH) group at position 4(*para*) or (H) out of (OH) group at position 3 or 5 (*meta*). Consequently, restriction of position 4 resulted in twice ionization at position 3 and 5, which is acompanied with a decline in electronic conjugation because, *meta* position (3 and 5) is able to weaken the ring twice (occurrence of double substitutions). Where as, activation of the ring might occurs once via (OH) in case of ionization of (H) at *para* position (4). However, for this reason, gallic acid is characterized as a weak anti-oxidant compound, because of breaking down the electronic density around the ring and in carboxyl group

direction. Consequently, this leads to decline the ability for scavenging free radicals; thereafter, explain the diminishing rooting response in cuttings aged in this compound (Table 2).

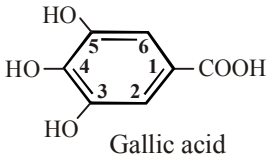
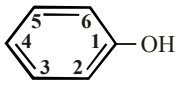
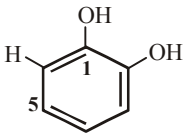
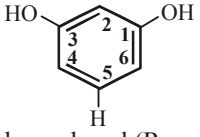
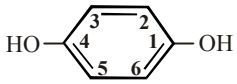
It is well known that phenol is a weak acid and having acidic dissociation constant (pk_a =11). So, the electronic conjugation between negative oxygen (after ionization of acidic hydrogen) and the ring was weak and it depends on periods of (H) ionization. Consequently, the electronic density was little, which coincides with low rooting response (19 roots) compared to caffeic acid (28 roots) and this decline is equal to 32.2% (Table 2).

In addition, o-hydroxyphenol (catechol), which contains two (OH) groups, and the second constitution of (OH) must be in position 2 (*Ortho*). This position allowed the occurrence of intra hydrogen bonding, which in turn prevents hydrogen ionization in one out of the two positions (1 or 2). For this reason, this compound was akin to phenol in electronic conjugation and reflected in their effect on rooting response (19.4 roots/cutting) at concentration 10^{-5} M.

Table 2: Influence of phenolic compounds on rooting response of aged mung bean cuttings

Phenolic compounds and structural formula	Concentration (M)	Mean root No./cutting	pH
d/H_2O	0.0	12.2	
 Cinnamic acid	10^{-9}	13.9	6.3
	10^{-7}	17.7	6.3
	10^{-5}	13.7	6.2
	10^{-3}	19.1*	3.7
 o-Coumaric acid	10^{-9}	12.9	6.1
	10^{-7}	15.2	5.9
	10^{-5}	15.3	5.9
	10^{-3}	42**	3.8
 Caffeic acid	10^{-9}	16.3	5.5
	10^{-7}	13.8	5.5
	10^{-5}	10.4	5.5
	10^{-3}	28**	3.4

Cont…

Phenolic compounds and structural formula	Concentration (M)	Mean root No./cutting	pH
 Gallic acid	10^{-9}	8.6	6.5
	10^{-7}	13.9	5.8
	10^{-5}	12	5.6
	10^{-3}	9.5	3.2
 Phenol	10^{-9}	17.4	5.2
	10^{-7}	13.4	5.1
	10^{-5}	19.4**	5.1
	10^{-3}	18	5.1
 o-Hydroxyphenol (Catechol)	10^{-9}	15.1	5.3
	10^{-7}	15.6	5.3
	10^{-5}	14.9*	5.3
	10^{-3}	14.9	5.2
 m-Hydroxyphenol (Resorcinol)	10^{-9}	13	5.5
	10^{-7}	9.5	5.4
	10^{-5}	14.1	5.3
	10^{-3}	14.6	5.2
 p-Hydroxyphenol (Quinol)	10^{-9}	14.2	5.9
	10^{-7}	15.2	5.6
	10^{-5}	17.6	5.6
	10^{-3}	37.1**	5.6

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days. Then aged for 3 days in the above concentrations of different phenolic compounds. Thereafter, transferred to boric acid (10 μg/mL) for 6 days LSD (0.05) = 5.810; LSD(0.01) = 7.654.

A* = p. s. d. A** = p. h. s.d.

On the other hand, m-hydroxyphenol (resorcinol), which again contains two (OH) groups, and the second coustitution of (OH) must be in position 3 (*meta*). This position is not allowing the intra hydrogen bonding to occur. In addition, the (OH) group in this position (*meta*) was weakened to the ring charge. Thereafter, the anti-oxidant character, which depends on electronic conjugation was weakened too, although it contains inter hydrogen bond.

However, to confirm our results that deals with -

(a) Effects of electronic conjugation area in increasing or decreasing the ability of phenolic compound in electrons scavenging and

(b) Effects of substituting groups on electronic conjugation area via the position of substituting (OH) group on the ring, and its relation to rooting response. Cuttings aged in this compound developed a number of roots which are statistically not significant compared to substituting compound at *ortho* position (Table 2). p-Hydroxyphenol (quinol) is characterized by having two (OH) groups, and the second substitution of (OH) at position 4 (*para*). This position was considered as activator for ring charge and in this position, the (OH) participating in the formation of electronic conjugation area, which involves the ring and two (OH) groups on both sides of the ring (positions 1 and 4). Thereafter, revealing a strong anti-oxidant character via big electronic conjugation area. This in turn is reflected on a high rooting response is mung bean cuttings aged with this compound as represented in developing 37.1 roots/ cutting as compared to 12.2 roots in control treatment (Table 2).

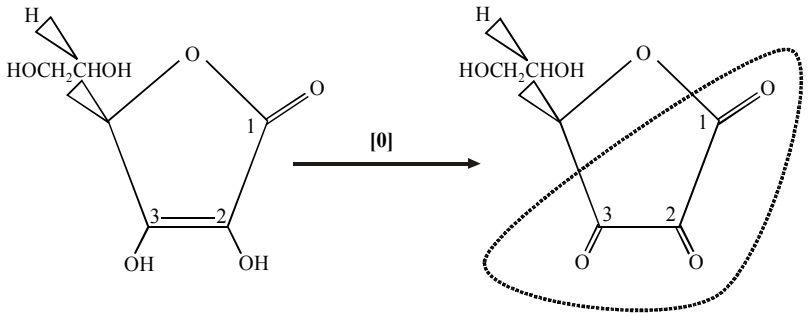
Generally, it has been well known that phenols play an inhibitory role in plant growth. Al-Saadawi et al.[30] confirms the above role by using cowpea seedlings. They mentioned that phenolic compounds inhibit the growth by reducing chlorophyll contents and reducing the uptake of Mo, Fe, K, P and N; in addition, to complete inhibition of Ca uptake. However, implication of phenolic compounds in defense mechanisms of plants, makes its biosynthesis influenced by many factors; for example, ecological factors (e.g. light, temperature, and humidity), internal factors (e.g. nutrients and plant hormones) and genetical factors. In addition, the synergistic effects between phenolic compounds in ARF occurs in presence of IBA, while in growth occurs only in presence of IAA[31].

On the other hand, statistically, the highly significant increase in auxin content as estimated in hypocotyls of cuttings aged in phenolic compounds (Fig. 1a) was attributed to the role of phenolic compounds as anti-oxidants and their effects in IAA biosynthesis. However, the evidences given by Gordon and Palego[32] confirm the above explanation. They show that phenolic compounds affected IAA biosynthesis *in vitro* from tryptophan (precursor) that mediates the formation of phenolase and quinone. Additional confirmation has been provided by Koves[33] in presence of dwarf bean enzyme and in its absence.

In addition, to the role of phenolic compounds as anti-oxidants in offseting or stopping the processes that leads to diminish rooting response in fresh and aged cuttings (Tables 1 and 2), they act as auxin-protectors against the activity of IAA-oxidase and hence,

caused significant rooting response in aged cuttings. The above presumption was confirmed by observation of Zenk and Muller[34] in which ortho-diphenols were used to control auxin level via inhibition of IAA-oxidase. Alternatively, phenolic compounds may have a role in regulating the mechanism of closing and opening of stomata in primary leaves. This is in agreement with Rai et al.[35], about phenolic compounds and their action as promoting substances in transpiration process via its counteracting the role of ABA in regulation of stomata closing. Consequently, it was found that transpiration rate in mung bean cuttings declined gradually with progressive age and hence, influence negatively the uptake of supplied auxin; thus causes diminishing of rooting response[28].

The significant increase in rooting response of fresh mung bean cuttings (Table 3) and beyond that limit in aged cuttings (Table 4) particularly at high concentrations of ascorbic acid (200-500 ppm) denoted that ascorbate is capable to stop aging processes completely. The number of roots developed in these concentrations was equal or more than fresh cuttings. In other words, cuttings aged in high concentrations of ascorbate responded as was the case in fresh cuttings, which was attributed to ascorbate as anti-oxidant having electronic conjugation system between atom No. (1) and atom No. (3) as illustrated in the equation below -



Ascorbic acid **2, 3-Dehydroascorbic acid**

and also having less electronic conjugation area compared to phenols, if characterized as anti-oxidant (three carbon atoms and three oxygen groups).

Table 3: Influence of ascorbic acid on rooting response of fresh mung bean cuttings

Conc. (ppm)	50	100	200	300	400	500
pH	3.2	2.6	2.4	2.3	2.3	2.2
Control 10.2	14.4	13.5	18.8*	19.8**	26.4**	27.8**

Table 4: Influence of ascorbic aid on rooting response of aged mung bean cuttings

Conc. (ppm)	50	100	200	300	400	500
pH	3.2	2.6	2.4	2.3	2.3	2.2
Control 12.6	13.4	16.2	20.1**	29.5**	27.9**	32.8**

Table 5: Influence of sugars (glucose, fructose and sucrose) on rooting response of aged mung bean cuttings

Conc./Solution	1%	2%	3%
d/H_2O		12.3	
Glucose	19.9* (6.2)	26.4** (6.5)	18 (6.2)
Fructose	31.2** (6.2)	34.2** (6.2)	29.1** (5.8)
Sucrose	29.6** (5.6)	26.3** (5.5)	28.4** (4.4)

Stem cuttings were taken from seedlings grown in d/H_2O for 10 days. Then treated with the above concentrations of different sugars for 24 h. Thereafter, transferred to boric acid (10 µg/mL) for 6 days. Figures in parantheses represent pH values. LSD (0.05) = 7.408; LSD (0.01) = 9.812.

A* = positive significant effect. A** = Positive highly significant effect.

Table 6: Influence of sugars (glucose, fructose and sucrose) on rooting response of aged mung bean cuttings

Conc./Solution	1%	2%	3%
d/H_2O		9.5	
Glucose	5.5 (6.2)	5.4 (6.5)	14.7 (6.2)
Fructose	4.4 (6.2)	4.5 (6.2)	5.8 (5.8)
Sucrose	6.1 (5.6)	7.9 (5.5)	19.1** (4.4)

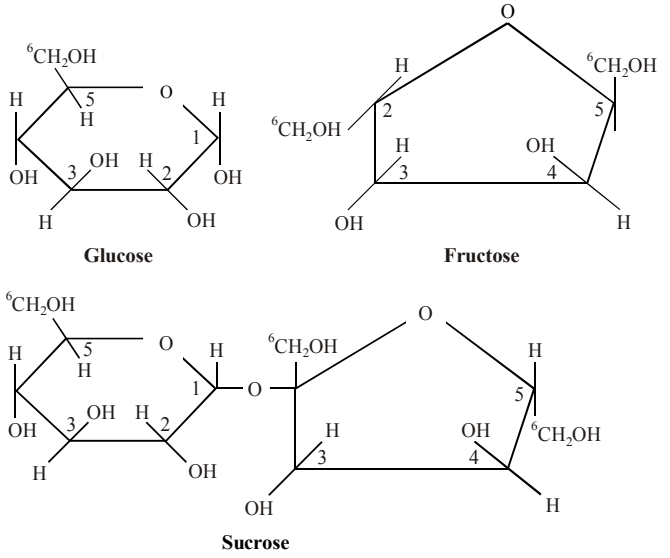
Stem cuttings were taken from seedlings grown in d/H_2O for 10 days. Then aged for 3 days in the above concentrations of different sugars. Thereafter, transferred to boric acid (10 µg/mL) for 6 days. Figures in parantheses represent pH values. LSD (0.05) = 5.533; LSD (0.01) = 7.329.

A** = Positive highly significent effect.

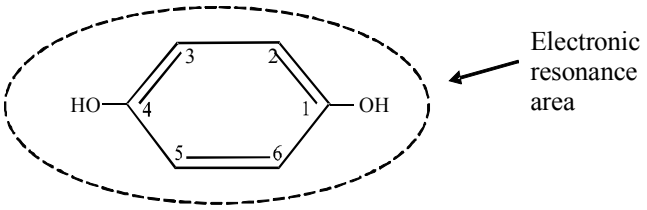
Recently, in some studies, it was found that endogenous level of ascorbate was declined (66.8%) in the same kind of cuttings during aging. which is considered as anti-oxidant and its decline was coincided with poor rooting response[36]. However, Shalata and Neumann[37] mentioned that ascorbate acts on partial inhibition of lipid peroxidation products and its accumulation in roots, stems, and leaves that formed by degenerative reactive oxygen species (ROS), which acts on damage of the fundamental members lipid, proteins, and nucleic acids. In addition, ascorbate decreases the damage of photoxidation only at high concentrations[38] and resists salt stress via its regulation of ROS concentrations[39]. Also ascorbate inhibits the main electrolytes leakage produced by peroxidative damage of plasma membranes that is induced by stress[40].

The above influence was confirmed by statistically significant results of this study, which is represented by IAA content in hypocotyls of cuttings aged in ascorbate solution. Fig. (1b) revealed that IAA level is 17.357 m moles with increasing percent (56.8%) over control. The foregoing results, may be attributed to the role of ascorbate in inhibiting the activity of IAA-oxidase and hence, promoting IAA level[41]. The latter describes the last step to occur through the competition of ascorbate on auxin (IAA) or its effect on pH. Consequently, auxin was more effective at low pH, which is induced by increasing ascorbate concentration.

Mostly, sugars are considered as anti-oxidants through oxidation of oxygen-hydrogen bond in hydroxyl groups, although it is weakly ionizable due to the absence of electronic conjugation in the ring forms of sugars nature as compared to phenolic compounds and vitamin C.



For this reason, rooting response of mung bean cuttings aged in sucrose solution was high only in acidic medium (pH = 4.4), due to the posibility of hydrolysing the glycosidic bond in this kind of sugar compared to other monosaccharides (glucose and fructose), because of the difficulty for hydrolysis and oxidation. Obviously, the ability of sugars to act as anti-oxidants is less compared to ascorbate. The latter having bigger electronic conjugation area than sugars and lesser than phenolic compounds. For example, the electronic conjugation area for p-hydroxyphenol (quinol) is six carbon atoms and two hydroxyl groups. This was agreed with understanding of electronic exchange in organic chemistry field[42] and confirming the results of our study in terms of rooting response of mung bean cuttings, which become compatible with the above interpretation.



p-Hydroxyphenol (Quinol)

On the other hand, the decline in rooting response of cuttings aged in sugar solutions was tested in this study and its increase in fresh cuttings may be attributed to the nutritional status. This was verified by Shaheed and Salim[15]. They found that mung bean cuttings supplied with sucrose (1.5%) was able to control partially the processes that occur during aging via its preservation on the level of carbohydrate and protein that was essential for ARF. They also mentioned that aging process can be stopped completely in aged cuttings compared to fresh cuttings in case of supplying the cuttings with nutritional factors by cotyledons, if the latter is considered as endogenous source of carbohydrate (using 5-day-old seedlings, before cotyledons shrivels and drop-off spontaneously at day-8 of seedling age[43]. Statistically, the above results confirms the significant rooting response in cuttings aged in sucrose, 3% (pH = 4.4) compared to control treatment (Table 6). The importance of sucrose in rooting response of aged cuttings may be attributed to raise the level of soluble carbohydrate[44,45]. They obeserved that the growth of adventitious roots required high level of soluble carbohydrate. In addition, Marousky[45] mentioned that the importance of sucrose in preserving the freshness of cut-roses during aging period as was the case in fresh cuttings, is due to the role of sucrose in increasing water uptake, which is correlated to high osmotic potential of sucrose. Consequently, sucrose has importance in preservation of fresh weight of cuttings kept in these solutions via its influence on closing of stomata. In addition, sucrose has a role in decreasing the percentage of K^+ in aged cuttings, which leads to closing of stomata and preserving cuttings from deseiccation[15].

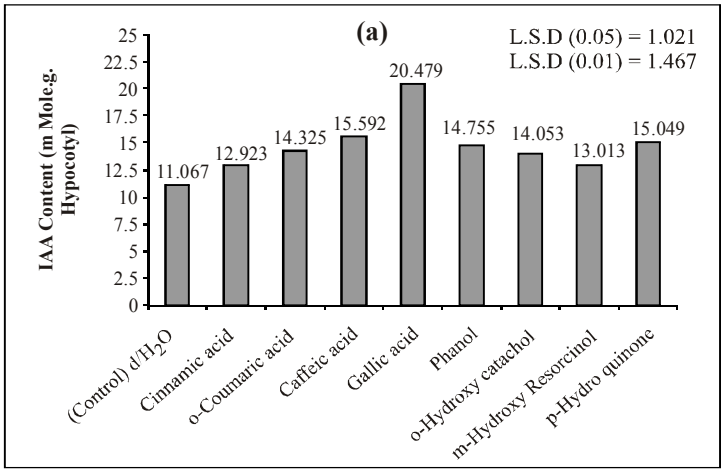
Obviously, through the delicate balance between auxin and nutritional factors, it was able to preserve root initiation precisely. The role of sucrose as anti-oxidant resides in modifying the activity of enzymes particularly that involved in auxin-metabolism via inhibiting the activity of IAA-oxidase and hence, promoting IAA level in hypocotyl (Root initiation zone). This was confirmed by quantitative measurements of IAA in the current study in hypocotyl of aged cuttings in sucrose, 3%, which developed 15.411 m moles compared to control treatment 11.067 m moles (Fig. 1d). Some studies confirmed the above results, and denoted the importance of sucrose not only as carbon source in root initiation, but acts on declining the activity of IAA-oxidase significantly in presence or absence of auxin[46].

The decline in rooting response of cuttings aged in glucose and fructose solutions at all concentrations tested and sucrose at low concentrations (1% & 2%) compared to control (Table 6), may be attributed to the un-ideal level of carbohydrate. Andus[47] showed that carbohydrate was necessary in growth and development of roots, through preservation of chlorophyll level, photosynthesis rate and cuttings content from mineral elements. Jenson[48] confirmed the above informations and showed the decline of mineral elements in aged cuttings, in addition to the decline of protein content via activity of proteases and nucleases enzymes and declining of carbohydrate content[49]. However, Shaheed and Al-Alwani[50] denoted the incapability of supplied auxin to substitute the shortage in endogenous IAA that occurs because of aging, and auxin alone was unable to control the processes that occurs during aging due to blocking of xylem vessels chemically by suberin. The sugars uptake and its translocation acropetally in cuttings, frequently occurs in xylem[51]. Perhaps, this may help in explaining the coincidence of significant increase in auxin content in hypocotyl of cuttings aged in glucose and fructose solutions (Fig 1c) and diminishing rooting response (Table 6). The auxin content was increased in terms of percentage into 59.3% and 41.7%, respectively compared to control. The conditions of carbohydrate occurrence and physiological status of stock plants affected carbohydrate metabolism in cuttings. So Reid[52] mentioned that any encharaging conditions to increase the ratio of carbohydrate to nitrogen (C/N) encharaging root formation in cuttings and the optimal rooting response of cuttings occurs in special occasion involving the presence of total soluble CHO at optimal concentration in cuttings before and during rooting[53]. In addition, the concentrations of CHO in its individual forms is having a direct relation with rooting response. For example, the concentration of reducing sugar and sucrose differ from concentration of starch in apical zone (Un-rooted) and basal zone (rooted) of stem Jack pine cuttings through vegetative propagation. Consequently, the ratio of reducing sugar to starch in cuttings was considered as sensitive-indicator and correlated with fragmentation of CHO differentially during root formation[8]. In addition, Shaheed and Salim[16] confirmed that the ratio of reducing

sugar/starch in aged mung bean cuttings is less compared to fresh cuttings whether in primary leaves, hypocotyls or in cotyledons.

Generally and as a conclusion, aging phenomenon may be considered as a result of oxidative processes that occurs in plant body or cuttings during aging period, that causes diminishing rooting response in mung bean cuttings. However, phenolic compounds and sugars tested in the current study, are anti-oxidants and act as internal suppressors of free radicals and lowering the effects of oxidative products through the followings: (a) Occurrence in plant extracts (free or bound), (b) Electronic conjugation area, (free or boand) (c) pH value, (d) Number and position of substituting OH groups, (e) Nutritional status of cuttings (f) Hormonal factors and IAA content (Hormonal balance), (g) Synergistic effect between phenolic compound and/or sugars with IAA and (h) Ionizable and resonance forms.

Notwithstanding, 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. Obviously, the current study revealed that improvement of rooting response in mung bean cuttings aged in phenolic compound was increased when the formation of *intra* hydrogen bond was verified for phenolic compounds that contains the substitutions of (OH) groups, compared to each other and to ascorbate and sugars, which has no electronic conjugation inside the ring. Thereafter, no promotion for *intra* hydrogen bonding will occur, although sugars are having more (OH) groups. Physiologically, this character reflects the decline in rooting response compared to phenolic compound. Finally, the implicated enzymes (e.g. superoxide dismulase, catalase, …….etc.) in IAA biosynthesis and its roles in improvement of anti-oxidant defense mechanisms will be our subsequent interest.



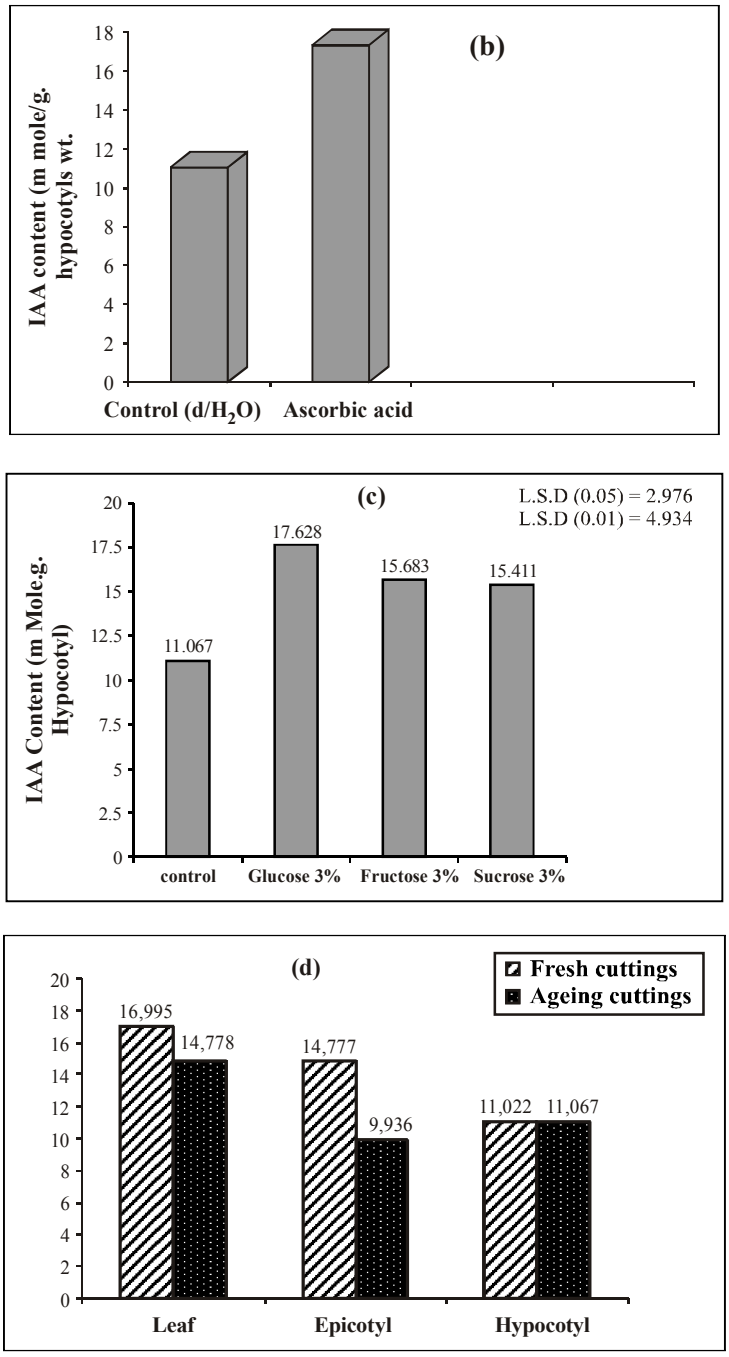


Fig. 1: IAA concentration (m mole/g plant tissue) of mung bean cuttings were taken from seedlings grown in d/H_2O for 10 days. Then, aged for 3 days in above compounds (Optimal concentration)

a- Cuttings aged in phenolic compounds.

b- Cuttings aged in ascorbic acid.

c- Cuttings aged in sugar 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).

REFERENCES

1. T. W. Goodwin and E. I. Mercer, Introduction to Plant Biochemistry, 2nd Ed. Pergamon Press Ltd. (1983) p. 667.
2. I. Fernqvist, Studies on Factors in Adventitious Root Formation, **32**, 109-244 (1966).
3. A. I. Shaheed, Effect of Secondary Metabolites on the Aging of Mung Bean Stem Cuttings, Iraqi J. Sci., **38** (1997).
4. T. Lapidot, S. Harel, B. Akiri, R. Granit and J. Kanner, pH-Dependent Forms of Red Wine Anthocynins as Antioxidants, J. Agric. Food Chem., **47**, 67-70 (1999).
5. F. Mendel and S. J. Hella. Effect of pH on the Stability of Plant Phenolic Compounds, J. Agric. Food Chem., **48** (2000).
6. M. Friedman, Chemistry, Biochemistry, and Dietary Role of Potato Polyphenols, J. Agric. Food Chem., **45**, 1523-1540 (1997).
7. M. Tomaszewski, The Mechanism of Synergistic Effects between Auxin and Some Aatural Phenolic Substences, Regulateurs uaturels de la croissance vegetal, 335-351. Nitsch. Ed., Paris (1964).
8. B. E. Haissig, Carbohydrate Accumulation and Partitioning in Pinus Banksiana Seedlings and Seedling Cutting, Physiol. Plant, **61**, 13-19 (1984).
9. B. E. Haissig, Carbohydrate and Amino Acid Concentrations during Adventitious Root Primordium Development on Pinus Banksiana Lamb. Cuttings, Forest Science. **28**, 813-821 (1982).
10. T. Welander, Influence of Nitrogen and Sucrose in the Medium and of Irradiance of the Stock Plants on Root Formation in Pelargonium Petioles Grown in vitro, Physiologia Plantarum, **43**, 136-141 (1978).
11. L. Eliasson, Effects of Nutrients and Light on Growth and Root Formation in Pisum Sativum Cuttings, Physiol. Plant, **43**, 13-18 (1978).

12. J. Rozema, Population Dynamics and Ecophysiological Adaptations of Some Coastal Members of the Juncaceae and Gramineae in Ecological Processes in Coastal Environments, R. L. Jefferies and A. J. Dary (Eds.). Blackwell. Oxford (1979) pp. 229-241.

13. S. E. Hyndman, P. M. Hasegawa and R. A. Bressan, The Role of Sucrose and Nitrogen in Adventitious Root Formation on Cultured Rose Shoots, Plants Cell Tissue Organ Culture, **1**, 229-238 (1982).

14. B. Faludi, A. F. Daniel, I. Gyurian and S. Anda, Sugar Antagonisms in Plant Tumor Cells Induced by 2, 4-Dichlorophenoxy Acetic acid, Acta Biologica Academiae Scientianum Hunganicae, **14**, 183-190 (1963).

15. A. I. Shaheed and S. A. Salim, Aging of Mung Bean (Phaseolus Aureus Roxb.) Cuttings in Relation to Exogenous Supply of Some Nutritional Factors, J. Coll. Educ. For Women, Univ. Baghdad, 13(3), 566-576 (2002).

16. A. I. Shaheed and S. A. Salim, The Role of Cotyledons as Endogenous Source of Nutritional Factors in Controlling of Aging of Mung Bean (Phaseolus Aureus Roxb.) Cuttings. Iraqi J. Sci. **43b(1)**, 1-16 (2002).

17. A. I. Shaheed and N. A. Abdul-Hassan, Extended Effects of Cotyledons Extracts of Mung Bean (Phaseolus Aureus Roxb.) on Rooting Response of Cuttings at Fully Expanded Primary Leaves Stage. (Sub. for Pub.) (2008).

18. C. E. Hess, The Mung Bean Bioassay for Detection of Root Promoting Substances. Plant Physiol., **36** Suppl. 21 (1961).

19. W. Middleton, B. C. Jarvis and A. Booth, The Boron Requirement for Root Development in Stem Cuttings of Phaseolus Aureus Roxb. New Phytol., **81**, 287- 297 (1978).

20. M. R. Spiegel, Theory and Problems of Probabilty Statistic Schaums Outline Series in Mathematics, McGraw Hill Book Company, New York (1975).

21. A. Stoelssl and M. A. Venis, Determination of Submicrogram Levels of Indole-3-acetic acid, New, Highly Specific Method. Anal. Biochem. **34**, 344-51 (1970).

22. H. Plieninger, W. Muller and K. Weinerth, Indole-a–Pyrone and Indole -a – Pyridone , Chemische Berichte, **97**, 667-81 (1964).

23. I. Davies, Aging. Edward Arnold, London. (1983) p. 60.

24. Y. A. Wally, M. M. El-Hamady, S. T. Boulos and M. A. Salama, Physiological and Anatomical Studies on Pecan Hard Wood Cutting, Egypt. Hort., **8(1)**, 89-100 (1980).

25. A. I. Shaheed and B. A. Al-Alwani, Aging, Causes and Control, in Relation to Adventitious Root Formation in Mung Bean (Phaseolus Aureus Roxb.) Cuttings. I. Decline of Naturally Occurring Auxin (IAA), Iraqi J. Biol., **1(1)**, 161-174 (2001).

26. H. T. Hartmann, A. M. Kofranek, V. E. Rubatzky and W. J. Flocker, Plant Science, Growth, Development, Utilization of Cultivated Plants, 2nd Ed., Printice Hall, Englewood Liffs, New Jersey, (1988) pp. 125-126.

27. M. B. Wilkins, Correlative Inhibition of Lateral Bud Growth in Phaseolus Vulgaris L. Isolation of IAA from the Inhibitory Region., J. Exp. Bot., **26(92)**, 419-424 (1975).

28. A. I. Shaheed, The Control of Adventitious Root Development in Cutting of Phaseolus Aureus Roxb. Ph. D. Thesis, University of Sheffield, U. K (1987).

29. J. G Norcini, C. W. Heuser and R. H. Hamillon, Changes in Free and Conjugated Indole-3-acetic acid during Initiation and Early Development of Adventitious Root in Mung Bean, Amer. J. Soc. Hort. Sci., **110(4)**, 528-533 (1985).

30. I. S. Al-Saadawi, S. M. Al-Hadithy and M. B. Arif. Effect of Three Phenolic Acids on Chlorophyll Content and Ions Uptake in Cowpea Seedlings, J. Chem. Ecol., **12(1)**, 221-227 (1986).

31. J. P. Nitsch and C. Nitsch, Synergistes Naturels Des Auxines Et Des Gibberellines, Bull. Soc. Bot. France, **108**, 349-362 (1961).

32. S. A. Gordon and L. G. Palego, Formation of Auxin from Tryptophan through Action of Polyphenols, Plant Physiol., **36**, 838-845 (1961).

33. E. Koves, The Effect of Phenol Carboxylic Acids Occurring in Plants on the in vitro Formation of β-Indoleacetic acid from Tryptophan, Acta Bot. Sci. Hung., **10**, 299-307 (1964).

34. M. H. Zenk and G. Mullar, In vivo Destruction of Exogenously applied Indolyl-3 acetic acid as Influenced by Naturally Occurring Phenolic Acid, Nature, **200**, 761-763 (1963).

35. V. K. Rai, S. S. Sharma and S. Sharma, Reversal of ABA-Induced Stomatal Close by Phenolic Compounds, J. Exp. Bot., **37 (174)**, 129-134 (1986).

36. A. I. Shaheed, O. M. Yasser and E. I. Merhij, Metabolic Changes of Lipids and Proteins during Aging and its Relation To Rooting Response in Mung Bean (Phaseolus Aureus Roxb.) Cuttings (Accepted for Pub. in J. Karbala Univ.) (2005).

37. A. Shalata and P. M. Neumann, Exogenous Ascorbic acid (Vit.C) increases Resistance to Salt Stress and Reduces Lipid Peroxidation, J. Exp. Bot., **52(364)**, 2207-2211 (2001).

38. R. R. Wise and A. W. Naylor, Chilling Enhanced Photooxidation. Plant Physiol., **83**, 278-282 (1987).

39. J. Zhang and M. B. Kirkham, Lipid Peroxidtion in Sorghum and Sunflower Seedling as affected by Ascorbic acid, Benzoic acid and Propylgallate, J. Plant Physiol., **149**, 489-493 (1996).

40. S. Lechno, E. Zamski and E. Tel-Or, Salt Stress-Induced Responses in Cucumber Plant. J. Plant Physiol., **150**, 206-211 (1997).

41. L. Brauner and M. Brauner, Untersuchungen Uber Die Photolyse Des Heteroauxins II-Zeitschr. Bot. **42**, 83-124 (1954).

42. R. J. Boxer, Essentials of Organic Chemistry. Wm. C. Brown Publishers, Taipei, Printed in the United States of America, 2460, Kerper Boulevard, Dubuque, IA (1997).

43. A. I. Shaheed, Correlative Influence of Seedling Age, Cotyledons and Terminal Buds on Adventitious Root Formation in Stem Cuttings of Mung Bean, J. Babylon Univ. **7(3)**, 643-650 (2002).

44. T. W. Foong and M. F. Barnes, Rooting Cofactors in Rhodendron : The Fractionation and Activity of Components from an Easy-to-root and Difficult-to-root Variety. Biochemic Physiol. Physiol. Pflanzen., **176**, 507-523 (1981).

45. F. J. Marousky, Vascular Blokage, Water Absorption, Stomatal Opening and Respiration of Cut, Better Times Roses treated with 8-Hydroxyquinoline Citrate and Sucrose, Amer. Soc. Hort. Sci., **94**, 223-226 (1969).

46. K. Gurumurti and K. K. Nanda, Phytochemistry B, 1089, 1974, Cited by Chibbar, R. N., K. Gurumurti & K. K. Nanda (1979a) (1974).

47. L. J. Audus, Plnat Growth Substances. Leonard Hill Books Ltd., London (1963).

48. P. Jenson, Effects of Interrupted K^+ Supply on Growth and Uptake of K^+, Ca^{2+}, Mg^{2+} and Na^+ in Spring Wheat, Physiol. Plant., **5b**, 259-265 (1982).

49. J. E. Dale, Studies in bryology The Growth of Leaves, Edward Arnold Ltd., London. pp.60 (1982).

50. A. I. Shaheed and B. A. Al-Alwani, Aging, Causes and Control, in Relation to Adventitious Root Formation in Mung Bean (Phaseolus Aureus Roxb.) Cuttings, II. Blockage of Xylem Vessels, Iraqi J. Sci. **43b(2)**, 58-80 (2002).

51. A. Ooishi, H. Machida, T. Hosoi and H. Komatsu, Root Formation and Respiration of the Cuttings under Different Temperatures, J. Japan Soc. Hort. Sci., **47**, 243-247 (1978).

52. R. E. Reid, The Inflence of Nutritive Conditions of Seeds and Cuttings upon the Development of Roots, Royal Hort. Soc. Rep. and Proc. IX Int. Hort. Conf., 165-169 (1930).

53. F. Champagnol, Relation Entre La Formation De Pousset Deracines Par une Bouture De Vigne Laquantite Damidon Initialement Presente. Comptus Rendus Academic Des Science (Paris), **67**, 1398-1405 (1981).

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