# The Effect of *Ortho* and *Para* -Hydroxy cinnamic acid and Caffeic acid on Adventitious Root Formation of Mung bean Cuttings in Presence or Absence of Boron

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## Abstract

The effect of *O*- and *P*- Hydroxy cinnamic acid and Caffeic acid on rooting response of mung bean cuttings with respect to auxin (IAA) & boron has been studied . The effective concentration is $10^{-3}$M for all of the above tested compounds, whether cuttings were taken from 10 –day-old light grown seedlings in deionized $H_2O$ or in boric acid (5µg\ml). The results were revealed that at this conc. the above compounds induce adventitious rooting by 267.7% ,219.1% , 202.9% over control respectively , in cuttings taken from seedlings grown in Deionized \$H_2O$; whereas, the percentage of induction was declined to 188.3%, 174.6%, 180.6% respectively in cuttings taken from seedlings grown in boric acid (5µg\ml). However, caffeic acid was significantly increase adventitious root formation in simultaneous application with IAA over control in presence or absence of boric acid compared to *o*- coumaric acid & *p*-coumaric acid, but the rooting response was declined in cuttings were taken from seedlings grown in boric acid compared to d\d $H_2O$ ( 533.9% & 498%) respectively . The role of boron in combination with the above phenolic compounds individually was suggested to affects the decarboxylation of IAA that catalyzed by IAA-Oxidase.

**Key words : Boron, Decarboxylation , IAA , IAA-Oxidase, mung bean cuttings , Phenolic compounds, Rooting Response.**

## الخلاصة

إن تأثير الأورثو – والبارا–هيدروكسي حامض السيناميك وكذلك حامض الكافيئيك في استجابة تجذير عقل ألماش وعلاقة ذلك بهرمون الأوكسين (IAA) وحامض البوريك قد درست . وكان التركيز المؤثر ايجابياً لجميع المركبات اعلاه هو $10^{-3}$M , بغض النظر عن كون العقل مأخوذة من بادرات نامية في الماء أو حامض البوريك (5 مايكروغرام/ مل ) . لقد بينت النتائج إن جميع المركبات اعلاه وعند هذا التركيز قد استحثت تكوين الجذور العرضية بنسبة ( 267.7 , 219.1 , 202.9) % على التوالي عن معاملة السيطرة في العقل النامية في الماء الخالي من الايونات , بينما انخفضت جميع النسب الى ( 188.3 , 174.6 , 180.6 ) % على

التوالي في العقل المأخوذة من بادرات نامية في حامض البوريك . هذا ومن جانب آخر , فأن Caffeic acid قد زاد معنوياً من استجابة التجذير عندما يجهز في أن واحد مع IAA بوجود / غياب حامض البوريك مقارنة مع ortho و para حامض الكيوماريك . فضلا عن إن استجابة التجذير قد انخفضت بشكل عام في العقل المأخوذة من بادرات نامية في حامض البوريك مقارنة بالماء المقطر , حيث كانت القيم 533.9% و 498 % على التوالي . إن دور البورون ضمن توليفة مع المركبات الفينولية اعلاه بشكل منفرد قد تم اقتراحه من خلال تأثيره في عملية IAA– decarboxylation بمساعدة إنزيم IAA–oxidase .

## Introduction

Rooting is a complex process, which is affected by multiple factors including phytohormones , phenolic compounds ,nutritional status and genetic characteristics[1]. Adventitious root formation comprises, generally, two phases : first is the initiation phase , and second, is the growth and development phase . Jarvis (1986)[2] subdivided the initiation phase into three phases in *Phaseolus aureus* var. Berkin:-a) Induction phase: characterized by the accumulation of IAA in root initiation zone (hypocoltyl), and declining in the activity of IAA-Oxidase (Foong & Barnes, 1981)[3]. In addition to basipetal transport of carbohydrate & auxin protectors (*O*-diphenols) as a result of wounding (Stonier, 1971)[4]. b) Early initiation phase : In which cell division take place by the accumulative IAA that subsequently, leads to the formation of root primordia. c) Late initiation phase : characterized by reaching IAA-Oxidase it's peak of the activity, that caused diminishing of IAA level and consequently promoting the development of root primordial. Second, Growth & development phase , include the converstion of root primordia into visible roots.

The formation of root primordium cells depends on the endogenous auxins in the cutting and on a synergic compound such as a diphenol . It has been concluded by observations on different plant species that high concentration of free auxin are needed during the induction phase of adventitious rooting , whereas during later stages , high auxin levels obviously have an inhibitory action on differentiation and outgrowth of root primordia[5]. One well known IAA metabolic pathway consists of the oxidative decarboxylation of the side chain of IAA by IAA-Oxidase [E.C. 1.11.3.8] that oxidize IAA and supposedly destroy it's physiological activity ,leading to the formation of either indole-3-methanol or 3-methyleneoxindole[6,7].The effectiveness of phenolic compounds depends on number of OH-groups and on their position at the aromatic ring [8]. 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[9], the transpiration loss (e.g. caffeic acid) and hence, the auxin uptake, when supplied to cuttings simultaneously with phenolic compounds[10]. Alternatively, the roles of phenolic compounds (e.g.Cinnamic acid,$10^{-3}$M) is in offsetting or stopping the processes that leads to diminish rooting response in aged cuttings of mung bean[10] . The letter was suggested that cinnamic acid may act as auxin-protector against IAA-oxidase and caused significant rooting response in aged cuttings as it was the case in fresh cuttings. Boron, is required for development of root primordia on stem cuttings of mung bean light grown

seedlings[11]. Auxin initiate the regeneration of roots but boron must be supplied even during the growth of stock plant from which cuttings are derived , it is not required for primordial development until 72h after cuttings were taken[12]. In the presence of Boron in the form of borate , it is possible by complexing of borate with orthodiphenols the enhancing of IAA-Oxidase activity occur , the diminished auxin concentration resulting from this permits development and subsequent growth of root primordia [13]. The aim of study is Investigation the effect of the Phenolic compounds (Mono & Di Hydroxyl groups) On the adventitious root formation in the presence or absence of Boron .

## Materials & Methods

**Seed germination :**

Mung bean (*Phaseolus aureus* Roxb.) seeds were soaked in tap water for 12 h and then sown in moistened sterilized sawdust in plastic trays. Seedlings were grown in growth cabinet provided with a continuous light (light intensity 1500 – 1800 Lux), temperature $25 \pm 1^{\circ}C$ and relative humidity 60-70%.

**Preparation of cuttings :**

Cuttings were made from10-day-old light grown seedlings according to Hess[26]. These cuttings are consisted of a 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**:

Six out of twelve cuttings per treatment were placed in 50-ml beaker containing 31 ml of deionized water or tested solutions. The cuttings were treated with phenolic compounds (*O*-coumaric acid , *P*-coumaric acid & Caffeic acid) at ($10^{-3}$,$10^{-4}$,$10^{-5}$,$10^{-6}$ M) for 24 h, then transferred to rooting medium [ d\H2O if seedlings grown in boric acid (5µg\ml), or boric acid (5µg\ml) for seedlings grown in deionized water ]. Number of visible roots was determined 6 day after cuttings treatment.

After detection of the effective concentration for each phenolic compound , cuttings were treated in combination of [the effective Conc. of each phenolic compound with the optimal concentration of IAA ($5\times10^{-4}$M)] .

**Preparation of solutions**

**Boric acid solution:**

Prepared (5µg\ml) and employed as rooting medium[14]. Because of it's necessity in development of root primordia into visible roots (Middleton, 1978b)[15].

**Phenolic compounds solutions:** Three phenolic compounds were used, (*O*-hydroxycinnamic acid, *P*-hydrdoxycinnamic acid and Caffeic acid). All of these compounds were prepared at four different concentrations $10^{-6}$,$10^{-5}$,$10^{-4}$, and $10^{-3}$ M for each.

**Indole-3-acetic acid solution:**

IAA was prepared by dissolving in small amount of absolute alcohol (Middleton, 1978a)[16].at it's optimal concentration($5\times10^{-4}$M) (Shaheed, 1987)[17].

**The Combination solutions** :

The concentrations of each phenolic compound and IAA was doubled & the volume was reduced to half , before mixing together .

## Discussion and Result

The influence of *o*-coumaric acid on rooting response of mung bean cuttings taken from 10-day-old light

grown seedlings in deionized water (A) and Boric acid (B) was observed in table (1). Cuttings treated with deionized water for 24h developed (6.2 , 6 roots/cutting) in case of A & B respectively , this was attributed to endogenous IAA in cuttings . However, *o*-coumaric acid was developed statistically the same number of adventitious roots ( 5.8 , 5.5 , 7.7 in case of B ) at lower concentrations at ($10^{-6}$,$10^{-5}$,$10^{-4}$) whereas, (6.2, 7.3) at ($10^{-6}$M) &($10^{-5}$M). However, the no. of roots increased proportionally with increasing Concentration. The higher value (22.8, 17.3) roots correlated with the higher Conc. ($10^{-3}$), this no. equal 3.6 time (or 267.7%) compared to control (6.2) in case of A and approximately 3 folds of roots compared to control or (188.3%) over control (6) in case of B .

**Table (1): Influence of O-Coumaric acid on rooting response of Mung Bean cuttings.**

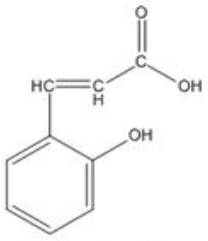
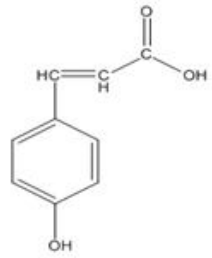
|  *O*-Coumaric acid | | 0.0 | $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M | $10^{-3}$ M |
|---|---|---|---|---|---|---|
| Mean root Number\cutting | A | 6.2 | 6.2 | 7.3 | 11 | 22.8 |
| | B | 6 | 5.8 | 5.5 | 7.7 | 17.3 |

Table (2) revealed that *p*-coumaric acid is not significantly influence the rooting response of mung bean cuttings at low concentrations except for the highest concentration used ($10^{-3}$M) which developed (21.7, 17.3 roots in case of A & B respectively ) that equal to approximately (3.5, 3) folds compared to control or (219.1% , 174.6%) in cuttings taken from seedlings grown in A & B respectively.

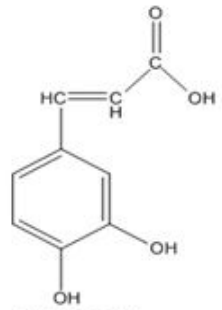
**Table (2): Influence of *P*-Coumaric acid on rooting response of Mung Bean cuttings.**

|  P-Coumaric acid | | 0.0 | $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M | $10^{-3}$ M |
|---|---|---|---|---|---|---|
| Mean root Number\cutting | A | 6.8 | 8.6 | 8.8 | 8.3 | 21.7 |
| | B | 6.3 | 6.9 | 6.3 | 7.1 | 17.3 |

**A) Stem cuttings were taken from seedlings grown in Deionized water for 10 days. Then treated with the above concentrations for 24h. Thereafter , transferred to (5µg/ml) for 6d . $LSD_{(0.05)}$ = (3.51) ; $LSD_{(0.01)}$ = (6.17).**
**B) Stem cuttings were taken from seedlings grown in Boric acid (5µg/ml) for 10 days. Then treated with the above concentrations for 24h. Thereafter , transferred to d/$H_2O$ for 6d . $LSD_{(0.05)}$ = (2.7) ; $LSD_{(0.01)}$ = (4.73).**

Table (3) pointed to the effect of caffeic acid on the rooting response of mung bean cuttings taken from seedlings grown for 10 days in (A & B) . Untreated cuttings developed (6.7 roots/cutting in each cases A & B), However, cuttings supplied with caffeic acid at Conc. between $10^{-6}$-$10^{-4}$ were promoted little increment in roots no. but statistically are not significant. Whereas , no. of roots increased to the highest value (20.3 , 18.8roots/cutting in A & B respectively ) with the highest Conc. ($10^{-3}$M). This no. equal (3 , 2.8) folds in comparison to control (6.7 roots/cutting) or by (202.9%, 180.6%) over control for A & B respectively.

**Table (3): Influence of Caffeic acid on rooting response of Mung Bean cuttings.**

|  Caffeic acid | | 0.0 | $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M | $10^{-3}$ M |
|---|---|---|---|---|---|---|
| Mean root Number\cutting | A | 6.7 | 8.6 | 8.7 | 9.3 | 20.3 |
| | B | 6.7 | 9.3 | 9.8 | 8.2 | 18.8 |

**A) Stem cuttings were taken from 10-day-old light grown seedlings in Deionized water. Then treated with the above concentrations for 24h. Thereafter , transferred to Boric acid (5µg/ml) for 6d . $LSD_{(0.05)} = (4.22)$ ; $LSD_{(0.01)} = (7.43)$ .**
**B) Stem cuttings were taken from 10-day-old light grown seedlings in Boric acid (5µg/ml) . Then treated with the above concentrations for 24h. Thereafter , transferred to $d/H_2O$ for 6d. $LSD_{(0.05)} = (4.20)$ ; $LSD_{(0.01)} = (7.413)$ .**

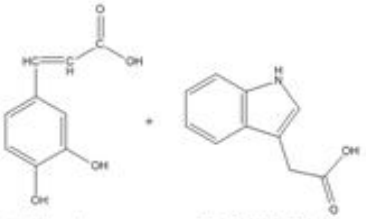
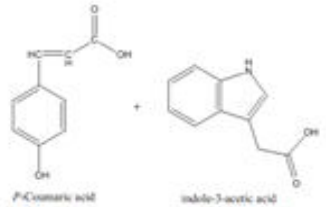
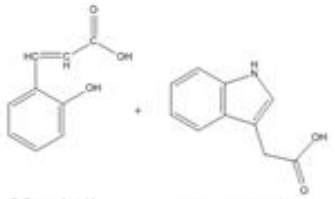
Obviously, a conclusion was raised from the comparison between tables (1,2,3) that rooting response declined in mung bean cuttings taken from seedlings grown in boric acid (5µg/ml) , and for all concentrations of *o*-coumaric acid , *p*-coumaric acid and caffeic acid. These results confirming the suggestion introduced by (Shaheed, 1987)[17]including that boric acid increase the activity of the enzyme IAA-oxidase that destroy IAA acting as it's substrate , leading to reduce rooting response . It is necessary to remind that, the priority of adventitious root formation in cuttings depends on auxins (Norcini&Heuser, 1988)[18].

The current results was in accordance with that introduced by (Shaheed *et al.*, 2010)[19] that at this concentration ($10^{-3}$M) highly significant increase in rooting response of cuttings aged in o-coumaric aid , caffeic acid and p-hydroquinone at 5% of the probability compared to control (d/H2O). . (Jarvis, 1986)[2] illustrated that phenolic compounds from many diverse compounds which are generally thought to influence auxin levels have been shown to influence root formation , where the possibility that *O*-dihydroxy compounds may influence root formation via an inhibitory influence on IAA-Oxidase. However (Fernqvist, 1966)[20] reported the dihydroxyphenols resorcinol and hydroquinone to be highly promotory in cuttings of mung bean . Furthermore *P*-coumaric, caffeic and chlorogenic acids all enhanced rooting when

supplied individually and increased their effects when supplied with auxin[20].

Table (4) shows that treatment of cuttings were taken from seedlings grown in deionized water (A) or Boric acid (5µg/ml) (B) within a combination between the optimum concentration for both IAA ($5\times10^{-4}$M) and phenolic compounds at ($10^{-3}$M) had made the phenolic compounds to act at high levels to promote rooting response . In other word, the phenolic compounds act synergistically with auxin (IAA). In case of A , Combination of *o*-coumaric acid with IAA developed (24 roots), *p*-coumric acid with IAA (29.5 roots) and caffeic acid with IAA ( 39.3 roots) that equal (4, ≈ 5 and 6.5 ) folds compared to control (6.2 roots) respectively . Obviously, the hydroxyl group located in *Ortho* position (as in *o*-coumaric acid) was better than it's location in *Para* position (as in *p*-coumaric acid ) while two hydroxyl groups when located in *meta* and *para* positions (as in caffeic acid ) would be more effective than the formers . This confirm the role of caffeic acid in inhibition the enzyme IAA-oxidase and finally resulted in increasing the level of IAA that reflected an increasing rooting response in mung bean cuttings. While in case of B, rooting response of cuttings taken from seedlings grown in boric acid (5µg/ml) was statistically equal response when cuttings supplied within combination of *o*-coumaric acid or *p*-coumaric with IAA. These combination were developed no. of roots equal to ( 19 , 18.6 ) respectively which are not significantly different from each other's ; whereas the combination of caffeic acid with IAA developed (29.9 roots) and that equal 5 folds compared to control or (498%) over control.

**Table (4): Influence of Combination between Phenolics and the IAA on rooting response of Mung bean cutting.**

| Treatments | Mean root Number\cutting | |
|---|---|---|
| | A | B |
|  Caffeic acid ($10^{-4}$ M) indole-3-acetic acid ($5 \times 10^{-4}$ M) | 39.3 | 29.9 |
|  P-Coumaric acid ($10^{-4}$ M) indole-3-acetic acid ($5 \times 10^{-4}$ M) | 29.5 | 18.7 |
|  O-Coumaric acid ($10^{-4}$ M) indole-3-acetic acid ($5 \times 10^{-4}$ M) | 24 | 19.7 |
| The Control d\$H_2O$ | 6.2 | 5 |

**A) Stem cuttings were taken from seedlings grown in Deionized water for 10 days. Then treated with the above concentrations for 24h. Thereafter , transferred to Boric acid (5µg/ml) for 6d. $LSD_{(0.05)}$ =(8.12) . $LSD_{(0.01)}$=(15.67).**
**B) Stem cuttings were taken from seedlings grown in Boric acid (5µg/ml) for 10 days. Then treated with the above concentrations for 24h. Thereafter , transferred to d/$H_2O$ for 6d . $LSD_{(0.05)}$ =(6.52) .**

As a conclusion from the comparison between the values of A & B in tables (4) , all values of B treatments were represent diminishing of rooting response of cuttings taken from seedlings grown in boric acid (5µg/ml) compared to all values in A treatments for cuttings taken from seedlings grown in deionized water . This might confirms the formation of complexes between borate and phenolic compounds, drawing the latter from reaction field leaving IAA for the degradation via IAA-oxidase (Shaheed, 1987)[17]. The results are in agreement with (De Klerk and Guan, 2011)[21] with IAA, all tested orthodiphenols, paradiphenols and

triphenols promoted adventitious root formation from the (Malus 'Jork 9') stem slices. In addition, to The results obtained from using the concentration ($10^{-3}$M) of cinnamic acid with simultaneous application with IAA for 3 days (aging period)developed large number of roots in aged cutting ,whichsignificantly approaches its number infresh cuttings (Shaheed *et al*., 2009 a)[22] .The actions of auxin and auxin synergists on adventitious root primordium initiation and developments suggests that cellular dedifferentiation that leads to primordium initiation requires one or more enzymatically synthesized auxin-phenolic conjugates (Haissig, 1973a)[23]. Moreover, (Hess, 1969)[24] showed catechol and pyrogallol to act synergistically with IAA. The latter suggests the structural requirements for a phenolic to act as a co-factor or synergist was an *ortho*positioning of hydroxyl group and a free *para* position .

It is noteworthy, that data of the current study confirms the previous information of (Jarvis, 1986 ; Foong and Bornes, 1986 ; Stonier, 1971)[2,3,4] and revealed that the activity of IAA-oxidase immediately after cuttings were taken is 0.444 (µg IAA oxidized/30 min./ Fresh wt.) in the hypocotyls (root initiation zone ) of cuttings were taken from seedlings grown in boric acid . Moreover , the activity was declined to 0.067 (84.2% reduction) during 1 – st 24h of IAA treatment then raised after 72h to 0.435 (74% increament) in d/$H_2O$[table– 5] .

However, mung bean cuttings were revealed the same trend when taken from seedlings grown in boric acid [table – 6 ] as follows :- The activity of IAA-O immediately after cuttings were taken is 0.444 in hypocotyls . The activity was declined into 0.370 , 0.328 , 0.395 and 0.460 after 24h in d/$H_2O$ , (Caf. + IAA) , (p-C + IAA) and (o-C + IAA) respectively. . Obviously, the activity of IAA-O at its lower level with the combination of Caffeic acid + IAA . This reflects the accumulation of IAA (due to the inhibitory effect of Caffeic acid on IAA-O), thereafter it's production of adv. root formation to its maximum value in presence of Caffeic acid compared to other phenolic compounds .

The results was in accordance with (Lee *et al*., 1982)[25] in that many phenols have a profound effect on the peroxidase catalyzed oxidation of IAA , and also with their illustration that number of hydroxyl- groups and their position relative to each other as well as to other substituents affect the activity . It has been suggested that phenolics protect auxins from decarboxylation so that after application of phenolics more auxin is available to induce roots[11]. Phenolic compounds which interfere with the peroxidase-catalysed oxidation of IAA in vitro by introducing a lag prior to the onset of IAA oxidation that what was cited in (Gelinas, 1973)[26] work. However, the concept of (Stonier and Yoneda, 1967)[27] the "auxin protector" suggests that .

Table (5) IAA-Oxidase activity (µg IAA oxidized/30min/g fresh wt) in the hypocotyls of mung bean cuttings

| | Immediately after cuttings were taken | After treatments in IAA ($5\times10^{-4}$) for 24 hr | 3 days after cuttings kept in $d/H_2O$ | 6 days after cuttings kept in $d/H_2O$ |
|---|---|---|---|---|
| Hypocotyls | 0.423 | 0.067 | 0.435 | 0.085 |

Table (6): the activity of IAA-Oxidase activity(*µg IAA Oxidized/30min/g fresh tissue*) in Hypocotyls of mung bean cuttings taken from seedlings grown in (5µg/ml) Boric acid.

| | **Hypocotyls Sections** | | |
|---|---|---|---|
| **Treatments** | **Immediately After cuttings were taken** | **24 h in d/H2O** | **72 h in d/H2O** |
| **($d/H_2O$)** | 0.444 | 0.370 | 0.444 |
| ***Caffeic acid*, $10^{-3}$ *M* + *IAA*, $5\times10^{-4}$ *M*** | ..... | 0.328 | 0.356 |
| ***P*-Coumaric acid, $10^{-3}$ *M* + *IAA*, $5\times10^{-4}$ *M*** | ..... | 0.395 | 0.445 |
| ***O*-Coumaric acid, $10^{-3}$ *M* + *IAA*, $5\times10^{-4}$ *M*** | ..... | 0.460 | 0.478 |

phenolic inhibitors promote growth by preventing IAA destruction via endogenous IAA-Oxidase. As (Gelinas, 1973)[26] reported that the generally accepted theory is that phenolic inhibitors trap free radical intermediates which would otherwise contribute to the oxidation of IAA. Alternatively, phenolic compounds were acts generally as anti-oxidants. Meanwhile, the differences between their effectiveness depend on electronic conjugation area & hydrogen bonding of phenolic compounds in terms of rooting response of mung bean cuttings which

are treated primarily by IAA (Shaheed et al, 2010)[19]. .

As a conclusion a more inhibitory effect of Caffeic acid on the IAA-oxidase system may attributed to its formation of four hydrogen bonds with the amino acids residues in the active site of the enzyme system ( Bihijat-personal communication ) and as a result more restriction for the action of the IAA-oxidase upon it's substrate indole-3-acetic acid (IAA) . The interaction between the phenolic compounds used (*O*-coumaric acid, *p*-coumaric acid and Caffeic acid ) and IAA might interpreted either by their synergetic effect with IAA or by their effect via decarboxylation on the activity of IAA-Oxidase in term of adventitious root formation in cuttings, depending in both cases on their chemical structures.

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