

INTERACTION BETWEEN AUXIN & HERBICIDES AND THEIR ROLES IN ROOTING RESPONSE OF FRESH AND AGED MUNG BEAN (*Phaseolus aureus* ROXB.) CUTTINGS.

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Abstract

The influence of auxins and herbicides individually or in combination on rooting response of fresh cuttings, when supplied during ageing, or as inductive auxin treatment (IAT) were studied. The results revealed:

- a) 2,4-D at 10^{-3} M or 10^{-4} M for 1/2h as (IAT), had no significant effect on rooting response, when cuttings aged in Paraquat (PQ). Whereas, 2,4-D for 24h at (10^{-4} M) (as IAT) had significant promotion ($\geq 166\%$) in cuttings aged in PQ. This confirms the role of PQ in stopping the processes that occurs during ageing, but not during rooting per see.
- b) Replacement of 2,4-D by IAA (5×10^{-4} M) as IAT for 24h doesn't change the trend of events in cuttings aged in PQ or 2,4-D. Seemingly, both herbicides had no effect, when IAT was presented by IAA exclusively.
- c) Both herbicides had no effect in fresh cutting even when supplied simultaneously whereas, PQ simultaneously with IAA induces 59% over IAA alone.

Key words: Ageing, Anti-oxidant defense mechanism, 2,4-D, IAA, Metabolic interaction, Mung bean, Oxidative stress , PQ and Rooting response.

1- INTRODUCTION

Ageing is a multi-conceptual phenomenon , was studied in different experimental systems and interpreted by several hypotheses , such as, genetic and biochemical changes that lead to the gradual senescence and death of cell or organism (Coombs, 1986). However in terms of adventitious root formation (ARF), it means the processes that lead to diminishing rooting response in mung bean cuttings by delaying the inductive auxin treatment (cuttings kept in deionized water during ageing period (Jarvis, 1986).

In addition, many attempts have been used to control ageing phenomenon (stopping the processes that occurs during ageing) was achieved by several physio – chemical ways . Such as keeping cuttings during ageing period in 1.5% sucrose (Shaheed & Salim ,2002 a), dilute auxins , such as , IAA , 10^{-8} M (Shaheed , 1987) and 400 mg/l ascorbate (Shaheed et al, 2010) to maintain the sensitivity of cuttings to inductive auxin treatment (IAT) and inducing a number of roots equal statistically to that in the control (fresh cuttings). Furthermore, using antioxidants of phenolic compounds such as o-coumaric acid, caffeic acid, p-hydroxy phenol and hydroquinone, all of these at concs. (10^{-3} M) (shaheed et, al, 2010). Obviously indole butyric acid (IBA) considered the widely used Auxin in stimulating rooting is due to 1) its ability to stimulate root initiation 2) weak toxicity and persist longer in plant tissues compared to indole acetic acid (IAA) (Hartmann et, al, 2002).

Meanwhile, the insensitivity of olive cuttings var. Domad for exogenously IBA has been interpreted on the basis of differences in metabolism and transport of IBA (Wiesman & Epstein, 1987). Whereas, using a combination of IBA and NAA at optimal concentrations increases rooting response in olive

cuttings compared to its supply individually (Isfiendiyaroglu & Ozeker, 2008). Also the ability of some secondary metabolites (growth retardant/ inhibitors) for exp: phenolics, polyamines to modulate the effect of hormones during rooting response (Hartmann et al, 2002).

In addition, hormones and other growth regulators were used for different purposes in the control of some aspects of plants development, and commonly used Auxin in weeds control is 2,4-D (Hopkins & Huner, 2009). The latter indicated that 2,4-D appear as effective Auxin at low conc.s , whereas , it is effective as herbicide at high conc.s . For not clear reasons, these herbicides are toxic, some of these are selectively for broad-leaved like 2,4-D (Chinallia, 2007) and others not selective as (PQ) , but it is more effective against narrow leaved weeds (Suntres, 2002).

Moreover, the induction of roots formation in cuttings by auxins is still not clear on the molecular level (Ludwig-Muller et al, 2005). The latter was suggested that rooting response in Arabidopsis cuttings attributed to the interaction between natural Auxin (IAA) and exogenous (IBA). However, the difference in ability for roots formation is due to the difference in metabolism of auxin (Blaskova et al, 1989) . Obviously Indol butyric acid aspartate (IBASP) is more effective than Free IBA in stimulating adventitious roots formation in mung bean cuttings due to long persistence during rooting (Wiesman, et al, 1989), As well as other differences in Uptake and Transport which must be taken in a count in rooting pattern (Esptein & Ludwig-Muller, 1993) .

On the other hand, the decline in IAA-Oxidase activity and the high level of auxin protectors may indicate the necessity of auxin accumulation in the induction of rooting as confirmed by several previous studies, such as. (Liu et al, 1996) . While period that coincides with the highest enzyme activity and the lowest level for the auxin protectors linked to the early initiation phase, they may indicate requirements to reduce the normal level (IAA) for an appropriate hormonal balance (Moncousin, 1991). At the level of physiological events that lead to initiation of roots, Ludwig-Muller and his colleagues (2005) was found that: 1) IBA is one of the natural factors in Arabidopsis (but not IAA) induce adventitious root formation. 2) The timing of Auxin supply is important to distinguish between Callus and roots formation. 3) The system used (cuttings from Arabidopsis) is appropriate for the diagnosis of genes that correlated with root formation. 4) as well as the IAA-deficient Mutant has been used to analyze the overlap between the IBA / IAA during root formation. Moreover , research lines have shown that part of IBA effect was attributed to direct action of IBA as normal auxin (Poupart & Waddell, 2000), despite the possibility of converting IBA to IAA through β - Oxidation (Bartel , et al; 2001). For example , the drought stress and osmotic stress stimulants synthesis of IBA and thereby increase its content, while IAA is less affected (Ludwig-Muller et al; 1995).

Finally, it is common in agricultural practices, that cuttings were treated with herbicides due to the possibility that cuttings may be infected by various fungi during rooting (Thielges & Hoitink, 1972), a condition that those herbicides does not affect rooting response positively or negatively in case of induction of these cuttings by a particular chemical. Given that lack of a study on the already interaction of herbicides and Auxin in rooting response of fresh and aged cuttings of mung bean, as well as keeping cuttings during ageing period as inductive auxin treatment after ageing was considered the aim of this study.

2- MATERIALS AND METHODS

2-1: Growth of stock plants & Preparation of cuttings :-

Seeds (*Phaseolus aureus* Roxb.Var.local) germination and seedlings growth were carried out in growth cabinet at $25\pm 1^{\circ}\text{C}$ under continuous illumination supplied by warm white fluorescent tubes

(1500-1800 lux) and relative humidity of 60 -70% (Binder GmbH/ Germany). Stem cuttings were prepared according to (Hess, 1961) from 10- day – old light grown seedlings. The cutting had apical bud, a pair of fully expanded primary leaves , epicotyl and 3cm of hypocotyl under cotyledonary nodes , after removal of root system .

2-2: Preparation of solutions

2,4-D and IAA were initially dissolved in small volume of absolute ethanol (Final conc. of abs . eth . is 0.2%) . This conc. is not effective in ARF of mung been cuttings (Middleton et al ; 1978 a). PQ (27% active substance / United phosphorus Ltd India) was prepared at 1% (v/v) in d/H₂O , then diluted to the required concentrations.

2-3: Basal treatment of cuttings

Basal part (hypocotyl) of fresh cuttings were treated for 1/2h or 24h with tested solutions (d/H₂O, 2,4-D or IAA) or in combination of these solutions . Thereafter ,cuttings were transferred to boric acid (5µg /ml) for 6 days as rooting medium because of the role of boron in development of root primordial in to visible roots (Middleton et al; 1978 b).

2-4: Ageing treatments

Cuttings were aged by dipping in d/H₂O or in appropriate tested solutions of PQ an 2,4-D of different conc.s for 3-days prior to their treatment with IAA or alternatively with 2,4-D (as inductive auxin treatment = IAT). During all these treatments, cuttings were held under the same conditions as mentioned above for raising stock seedlings.

Twelve cuttings were used per treatment for rooting test were placed 4 per glass vial containing 15ml (3cm depth) of the appropriate solution . All experiments were designed as completes randomized design for statistical analysis , depending on L.S.D and T-test for treatment comparison by using analysis of variance (ANOVA) for all experiments as individual (Spiegel , 1975).

3- RESULTS

The effect of 2,4-D on rooting response of cuttings already aged for 3-days in different conc. of PQ was revealed in table (1) . The average roots number of cuttings aged in d/H₂O for 3 – days and in the absence of supplied auxin (general control) is 6.3 roots/cutting. whereas , cuttings aged in d/H₂O for 3-days before supplying the inductive auxin treatment (IAT) (2,4-D) at 10⁻³M for 1/2 h were developed 22.3 roots . Which is significantly effective by 3.5 folds compared to control (253.9 %) . However ,cuttings were aged in PQ particularly at conc. (1×10⁻⁷%), prior to exogenous supply of 2,4-D , (10⁻³M) (IAT) developed 26 roots /cutting , which is not significant compared to 2,4-D alone (cuttings were kept in d/H₂O during ageing period instead of PQ thereafter , supplied with 2,4-D as IAT (22.3 roots) .

For controlling the processes that occurred during ageing in cuttings aged in different conc.s of PQ, the above experiment has been repeated twice: a) with a lower conc. of 2,4-D (10⁻⁴M) as IAT & at the same period (1/2h). b) with a lower conc. (10⁻⁴M) of 2,4-D as IAT but for 24h. The results of 1st experiment (Table-2) were revealed that cuttings already aged in PQ at conc. (1×10⁻⁷ %) were maintained its sensitivity for IAT represented by 2,4-D , that developed a higher number of roots equal to 18. The latter figure statistically represent a significant increase (146.5%) on 0.01 level of probability compared to cuttings aged in d/H₂O & untreated with auxin (control) whereas , such increase is not significant in comparison to cuttings supplied with 2,4-D (10⁻⁴ M). In other words, PQ does not stop the processes that occurs during ageing at this conc. Mean-while, other conc. have no significant effect on rooting response except the inhibitory conc. (1× 10⁻⁸%) at 0.05 level .

Table(1): Effect of 2,4-D, 10^{-3} M for 1/2h on rooting response of mung bean cuttings aged in paraquat

Cuttings aged for 3-days in :-	Subsequent treatment for 1/2h :-	Mean root number / cutting
d/H ₂ O	d/H ₂ O	6.3
d/H ₂ O	2,4-D , 10^{-3} M	22.3
PQ , (5 X 10^{-7})%	2,4-D , 10^{-3} M	20
PQ , (1 X 10^{-7})%	2,4-D , 10^{-3} M	26*
PQ , (5 X 10^{-8})%	2,4-D , 10^{-3} M	21
PQ , (1 X 10^{-8})%	2,4-D , 10^{-3} M	11.6

*L .S. D (0.05) = 8.874 ; L. S . D. (0.01) = 11.951.

Table(2):Effect of 2,4-D , 10^{-4} M for 1/2h on rooting response of mung bean cuttings aged in paraquat

Cuttings aged for 3-days in :-	Subsequent treatment for 1/2h :-	Mean root number / cutting
d/H ₂ O	d/H ₂ O	7.3
d/H ₂ O	2,4-D , 10^{-4} M	17.3
PQ , (5 X 10^{-7})%	2,4-D , 10^{-4} M	12.6
PQ , (1 X 10^{-7})%	2,4-D , 10^{-4} M	18*
PQ , (5 X 10^{-8})%	2,4-D , 10^{-4} M	16
PQ , (1 X 10^{-8})%	2,4-D , 10^{-4} M	9.3

*L .S. D (0.05) = 5.476 ; L. S . D. (0.01) = 7.375.

The results of 2nd experiment (Table-3) were revealed that PQ at conc. ($1 \times 10^{-7}\%$) stopped all processes that occurs in cuttings aged in PQ , that developed 31 roots with increment equal to 166.3% compared to cutting aged in d/H₂O & consequently supplied with 2,4-D as IAT (19.3 roots) . The latter case, confirm the role of PQ in stopping the processes that occurs during ageing *per sue*(but not during rooting response) particularly when 2,4-D was supplied for 24h as IAT.

Table (3): Effect of 2,4-D, 10^{-4} M for 24h on rooting response of mung bean cuttings aged in paraquat

Cuttings aged for 3-days in	Subsequent treatment for 24h :-	Mean root number / cutting
d/H ₂ O	d/H ₂ O	8.3
d/H ₂ O	2,4-D , 10^{-4} M	19.3
PQ , (5 X 10^{-7})%	2,4-D , 10^{-4} M	22
PQ , (1 X 10^{-7})%	2,4-D , 10^{-4} M	31*
PQ , (5 X 10^{-8})%	2,4-D , 10^{-4} M	24
PQ , (1 X 10^{-8})%	2,4-D , 10^{-4} M	15.3

*L .S. D (0.05) = 9.621 ; L. S . D. (0.01) = 12.957.

Table (4) shows that rooting response of mung bean cuttings were aged in different conc. of PQ and induced by IAA . The results were revealed that fresh untreated cuttings were developed (13) roots. This was attributed to the endogenous IAA whereas , fresh cuttings treated with IAA , 5×10^{-4} M for 24h were developed 72.8 roots (increasing % is 460). However , in case of cuttings aged in d/H₂O for 3-days then transferred to boric acid (5 µg/ml) as rooting medium without supplying IAT were developed 9.2 roots , which represent a decline % in rooting response equal to 29.2 % compared to control (fresh cuttings = 13 roots) .

On the other hand , when IAT was delayed , by ageing the cuttings in d/H₂O for an intervening period (3-day), rooting response progressively declined to 46.4 roots (inhibition %=36.2) compared to fresh induced cuttings . Statistically this percent of diminishing rooting response is highly significant & attributed to the processes that occurs during ageing .

For controlling these processes (delaying) , cutting were kept for 3-days in different concs. of PQ. Generally cuttings aged in all tested conc. of PQ were developed a number of roots restricted between (34.1 -48.6 root/ cutting)after supplying IAA as IAT . The rooting response of these cuttings represented by such figures are not significant compared to cuttings aged in d/H₂O (46.4 roots). The foregoing results confirm that PQ has no effect on processes that occurs during ageing particularly when IAA was supplied for 24h on IAT.

Table(4): Effect of IAA, $5 \cdot 10^{-4}$ for 24h on rooting response of mung bean cuttings aged in paraquat .

Cuttings aged for 3-days in :-	Subsequent treatment for 24h	Mean root number / cutting
-	d/H ₂ O	13
-	10^{-4} , M× IAA,5	72.8
d/H ₂ O	d/H ₂ O	9.2
d/H ₂ O	10^{-4} , M× IAA,5	46.4
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	40.1
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	48.6*
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	35.3
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	34.1
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	34.7
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	44.3

*L .S. D (0.05) = 17.879 ; L. S . D. (0.01) = 23.780 .

In comparison with PQ , 2,4-D was supplied at different conc. during ageing of cuttings for 3-days prior to supply IAA , 5×10^{-4} M as IAT (Table-5) . Generally , cuttings aged in all tested conc.s Of 2,4-D (10^{-8} - 10^{-4} M) were developed (32.6-50.8 roots/ cutting) after supplying IAA as IAT . These figures had no significancy compared to control treatment , as it was the case with PQ . Obviously , these results confirms that 2,4-D had no effect too, on processes that occurs during ageing .

Table (5): Effect of IAA, 5×10^{-4} M for 24h on rooting response of mung bean cuttings aged in 2,4-D.

Cuttings aged for 3-days in :-	Subsequent treatment for 24h :-	Mean root number / cutting
-	d/H ₂ O	13
-	10^{-4} , M $\times$ IAA, 5	72.8
d/H ₂ O	d/H ₂ O	9.2
d/H ₂ O	10^{-4} , M $\times$ IAA, 5	46.4
2,4-D, 10^{-4} M	10^{-4} , M $\times$ IAA, 5	39.6
2,4-D, 10^{-5} M	10^{-4} , M $\times$ IAA, 5	32.7
2,4-D, 10^{-6} M	10^{-4} , M $\times$ IAA, 5	42.9
2,4-D, 10^{-7} M	10^{-4} , M $\times$ IAA, 5	44.1
2,4-D, 10^{-8} M	10^{-4} , M $\times$ IAA, 5	50.8*

*L .S. D (0.05) = 13 ; L. S . D. (0.01) = 17.293 .

Interaction between 2,4-D & PQ in rooting response of fresh mung bean cuttings has, been shown in table (6) .Fresh cuttings were treated with optimal conc. of 2,4-D were developed (31.6 roots) compared to fresh untreated cuttings (8.6 roots) . However , cuttings were supplied with different combinations of 2,4-D & PQ for 1/2 h, have no significant promotory effect compared to control (2,4-D) ,except the combination [2,4-D, 10^{-3} M + PQ, 5×10^{-6} %] that developed the highest number 22.3 roots, with increasing % equal to 159% compared to control (d/H₂O).

Table (6): Effect of 2,4-D & PQ supplied simultaneously in combination on rooting response of fresh mung bean cuttings.

Treatment for 24h :-	Mean root number / cutting
d/H ₂ O	8.6
2,4-D, 10^{-3} M	31.6
2,4-D, 10^{-3} M + PQ, (5 X 10^{-6}) %	22.3*
2,4-D, 10^{-3} M + PQ, (5 X 10^{-6}) %	12.3
2,4-D, 10^{-4} M + PQ, (5 X 10^{-6}) %	1.8
2,4-D, 10^{-4} M + PQ, (5 X 10^{-6}) %	7.3

*L .S. D (0.05) = 9.617 ; L. S . D. (0.01) = 12.979 .

On the other hand , the interaction between IAA & PQ in rooting response of fresh mung bean cuttings has been shown in table (7). Obviously , IAA at optimal conc. 5×10^{-4} M developed 45.3 root/cutting compared with fresh untreated cutting (8.6 roots) . However ,combination between IAA 5×10^{-4} M & PQ at two conc.(1×10^{-6} % & 5×10^{-6} %) induce 61.8 & 72.1 roots respectively . It is noteworthy , that interaction between auxins particularly IAA & herbicides particularly PQ was induced adventitious root formation in fresh mung bean cuttings which is 59% over IAA alone .

Table(7):Effect of IAA & PQ supplied simultaneously in combination on rooting response of fresh mung bean cuttings.

Treatment for 24h :-	Mean root number / cutting
d/H ₂ O	8.6
10^{-4} M $\times$ IAA ,5	45.3
10^{-4} M $\times$ IAA ,5 + PQ , (5×10^{-6}) %	72.1*
10^{-4} M $\times$ IAA ,5 + PQ , (5×10^{-6}) %	61.8

*L .S. D (0.05) = 19.035 ; L. S . D. (0.01) = 25.916 .

4- DISCUSSION

Fresh cuttings were induced by (IAA, 5×10^{-4} M) as IAT immediately when taken from 10-day old light grown seedling were developed 72.8 roots/cuttings (tables 5&4) . Whereas, any delay in supplying of IAT by keeping cuttings in d/H₂O for 3-day caused decline in rooting response by 36.3%. The above decline was considered as physiological signal for biochemical processes that occurs during ageing and lead to diminishing rooting response (shaheed,1987,Shaheed & Jabar,2009). General knowledge was pointed out 10-hypotheses that explain ageing causes in different experimental systems , which are mostly denoted the decline in rooting response , as a result of dealing auxin supply by keeping cutting in d/ H₂O for 3- days.

The results of the current study revealed that cuttings aged in d/H₂O when supplied with 2,4-D for 1/2 h at 10^{-3} M (the optimal conc. When supplied at the optimal duration for fresh cuttings , data not presented) as IAT, developed 22.3 roots/ cutting , compared to cuttings aged in d/H₂O but not induced by auxin (2,4-D) that developed 6.3 roots (Table 1) . This is occurring with other synthetic auxins that induce rooting such as IAA, IBA, NAA, so it was agreed with Grabinske and his colleagues (2003) about 2,4-D that acting as herbicide at high conc. from one side whereas , acts as endogenous plant hormones (IAA) at low conc. from other side . In addition , that all auxins can acts in the same trend by manipulating the kind of cuttings , kind of auxin , auxin conc., duration of auxin treatment , or duration between cutting preparation from stock plant & auxin treatment (ageing period) (Jarvis,1986) For controlling the processes that occurred during ageing phenomenon , cuttings were kept at different conc.s of herbicide PQ as alternative for d/ H₂O . Clearly, PQ has no significant effect on these processes whether 2,4-D was supplied as IAT (after ageing treatment for 3-days) for 1/2 h. & at conc. 10^{-3} M (Table -1) or at the same duration (1/2h) but with lower conc. 10^{-4} M (Table- 2) . Consequentially , PQ has no effect at low conc. Whereas at (1×10^{-7} %) when 2,4-D was supplied as IAT for 24h (Table -3),that developed a higher average of roots 31 with an statistically significant increase of about 166% compared to cuttings aged in d/H₂O and induced by 2,4 -D (Table -3).

Obviously the latter case, raises that lowering conc. of 2,4-D (from 10^{-3} - 10^{-4} M) was coincided with the prolonging the duration of treatment (from 1/2h- 24h), have remarkable effect in increasing the rooting response of mung bean cuttings aged in PQ . This may be attributed to the interaction between /or action of 2,4-D with metabolic pathways of PQ (Table-6). Meanwhile, confirming the role of PQ in stopping the processes (partially/ completely according to the sp.) that occurs during ageing *per sue* , but not during rooting .

On the other hand , replacement of 2,4-D by IAA (5×10^{-4} M) as IAT for 24h doesn't change the trend of events in cuttings aged in different conc. of PQ (Table -4) or 2,4-D (Table -5) . In other words ,

both herbicides have no effect on rooting processes particularly , when IAT was presented by IAA exclusively . Seemingly , the effect of PQ through the processes that occurs during ageing of mung bean cuttings is (only positive and significant when IAT was presented as IAA.

To verify the role of PQ in ageing processes or rooting processes via interaction between herbicides & auxins using fresh cuttings , the results were revealed that both herbicides (PQ / 2,4-D) has no significantly positive effect on rooting response ,even when supplied simultaneously at optimal conc. for both (22.3 roots/table- 6) compared to 2,4-D alone (31.6 roots) whereas , supplying PQ simultaneously with IAA, significantly (on 0.01 level of probability) promotes rooting response with increment equal 59.1% over IAA alone (table-7) . According to the forgoing results, PQ is effective in aged cuttings when 2,4-D supplied as IAT. Meanwhile, PQ is effective too in fresh cuttings when supplied simultaneously only with IAA . However , as a conclusion , PQ acts synergistically with IAA in fresh cuttings but not with 2,4-D whereas , PQ acts synergistically too with 2,4-D as IAT in aged cutting but not with IAA. It is difficult to give plausible explanation for the above conclusion . However , the 1st case that involve the synergistic effect of PQ with IAA in fresh cuttings ,may be attributed to the role of PQ in stimulation of IAA-oxidase . The latter is acting for diminishing IAA to the level that required for growth & development of root primordia to visible roots (chibbar et al,1979), after its initiation under the influence of IAA at the 1st phase .

Consequently , the 2nd case that involve the synergistic effect of PQ with 2,4-d in aged cuttings , may be attributed to the role of PQ in preventing suberin biot *Babylon University*, enteses promoting or suberin hydrolysis (loosing) after its biosyntheses .this is in agreement with Clin&Neely (1983),that hypothesis that explain ageing causes (blockage of xylem vessels by suberin during ageing). This hypothesis was verified physiologically &anatomically in terms of rooting response of mung bean cuttings by (shaheed & Al-alwani, 2002) . the later conforming the above explanation by maintaining the translocation elements of xylem in active state functionally via herbicides transport (David ,1996) . seemingly , the plant tissue maintaining their integrity morphologically , through the action of herbicides on suberin loosening that lining xylem vessels org filling it with progression of ageing period.

Alternatively , the decline of SOD & CAT activity in different parts (hypocotyl , epicotyl and primary leaves) of mung bean cuttings aged in d/H₂O (Shaheed et al 2010),give clear imagination about oxidative processes that occurs during ageing (Harman, 1956 ; shaheed et al ,2009 a) , its role in degrading the biological molecules & some cellular organelles that reduce the biosynthesis of rooting response pre requisites (Scandalios ,1993) .

Such imagination may occurs via regeneration of free radicals, which by somehow influence the pathways of hormone biosynthesis , in particular that have the priority in rooting response (as auxin) . So, accordingly an increase in the activity of SOD ,CAT enzymes may abstract the way against ROS to react with the important molecules through increase the enzymatic activity of anti-oxidant defense mechanism represented by SOD /CAT in mung bean cuttings aged in 2,4-D at 10⁻³ M for 1/2 h. (Al-alwani ,et al, 2012) .

Notwithstanding ,the latter case agreed with shaaltiel and Gresssel (1986) about the role of PQ in increasing the activity of ant-oxidant defense enzyme such as SOD/CAT in *Conyza bonariensis*, because that herbicides considered as one of the factors causing the oxidative stress (scandalios, 1993). So the produced ROS (e.g. superoxide *per sue*) that converted into H₂O₂ in the presence of SOD. Consequently, H₂O₂ should be removed by catalases (peroxides) (Suntres ,2002) . In other words, the resistant was correlated with increasing SOD activity and other enzymes that capable for scavenging the regenerated free radicals .

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