

Metabolic changes of lipids & proteins during aging in terms of rooting response in mung bean (*Phaseolus aureus* Roxb.) cuttings

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Abstract

Metabolic changes of lipids & proteins which occurs during aging of mung bean (*Phaseolus aureus*) cuttings were studied. The decline in rooting response was coincided with the decline of lipid & protein contents and verified by increasing the activity of lipoyxygenase & protease respectively. The data revealed the followings:-

1. A decline in rooting response of aged cuttings (held in distilled water for 3 days) compared to fresh cuttings.
2. A significant increases in malondialdehyde (MDA) content by (88%), activity of lipoyxygenase by (277.2%) and activity of protease by (212.1%). Whereas the proteins & antioxidants content (e.g. glutathione & ascorbate) were declined in aged cuttings compared to fresh cuttings.
3. For controlling aging phenomenon, the cuttings were kept in anise (*Pimpinella anisum* L.) seeds extract (1%), cinnamic acid (10^{-3} M) & solutions of volatile oils, which led to stop the processes that occurs during aging in terms of rooting response by stopping the increase in MDA content, lipoyxygenase activity & protease activity, and maintaining protein & lipid contents as well as glutathione & ascorbate contents.

The discussion was focused on enzymes that associated with lipid peroxidation & the free radicals that produced by this process has increased during aging in terms of its final product (MDA). In addition the role of ascorbic acid and glutathione as antioxidants that involved in antioxidant defense mechanisms in terms of adventitious root formation in cuttings were assayed.

الخلاصة

تمت دراسة التغيرات الايضية للدهون والبروتينات التي تحدث خلال ظاهرة التعمير بدلالة تكوين الجذور العرضية في عقل الماش *Phaseolus aureus* Roxb. وأستخدمت العقل بأعتبارها نظاماً تجريبياً من بادرات نامية لمدة عشرة أيام تحت ظروف قياسية (إضاءة مستمرة وبشدة ضوئية ٣٠٠٠-٣٥٠٠ لوكس، ودرجة حرارة ٢٥ ± ١ سليزي، ورطوبة نسبية ٦٠-٧٠%). حيث وجد أن الانخفاض في استجابة تجذير عقل الماش المعمرة يتزامن مع الانخفاض في كل من الدهون والبروتينات الناتجين عن زيادة نشاط أنزيمي (LOX) Lipoxygenase و Protease. وكان مجمل النتائج ما يأتي:-

- ١- قلة استجابة تجذير عقل الماش المعمرة بالماء المقطر لمدة ثلاثة أيام مقارنة بالعقل الطرية.
 - ٢- زيادة معنوية لكل من محتوى Malondialdehyde (MDA) (٨٨,٨%)، وفعالية أنزيمات (LOX) (١٢٢,٢%) و Protease (١١٢,١٤%) ، بينما أنخفض محتوى البروتين (٩٠%) ومحتوى مضادات الاكسدة مثل الكلوتاثيون (٤١,٤٣%) والاسكوربيت (٦٦,٨٥%) في العقل المعمرة مقارنة بعينة السيطرة.
 - ٣- بهدف السيطرة على ظاهرة التعمير، حفظت العقل بمحاليل اليانسون ١% وحامض سيناميك (10^{-٣} مولاري) ومحاليل الزيوت التطايرية، مما أدى الى إيقاف العمليات التي تحدث خلال التعمير بدلالة تكوين الجذور العرضية، من خلال إيقاف الزيادة الحاصلة في محتوى MDA وفعالية أنزيمات LOX و Protease والحفاظ على محتوى البروتين والكلوتاثيون والاسكوربيت.
- تمحورت المناقشة حول نشاط الانزيمات الخاصة بأكسدة الدهون والجذور الحرة الناتجة عنها والتي تزداد خلال ظاهرة التعمير بدلالة الناتج النهائي (MDA) . وكذلك الدور الذي يلعبه حامض الاسكوريك والكلوتاثيون ضمن الميكانيكيات الدفاعية المضادة للاكسدة بدلالة تكوين الجذور العرضية في العقل.

Introduction

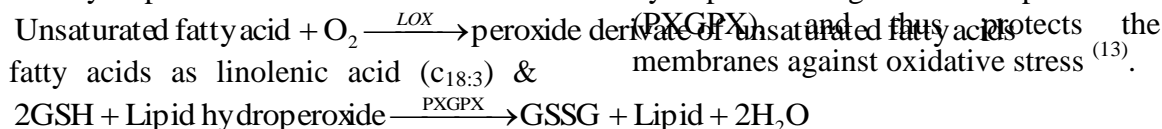
Aging has been described in different ways depending on experimental systems used in each case therefore, many hypotheses have been given to explain aging causes. These are: decline of IAA content ⁽¹⁾, blockage of xylem vessels ⁽²⁾, permeability perturbation ⁽³⁾, nutritional status ^(4,5), decline of rooting Co-factors ⁽⁶⁾, increasing of abscisic acid level ⁽⁷⁾, decline of phenolic compounds (Auxin protectors) ⁽⁸⁾, blockage of sieve tubes by callose ⁽⁹⁾, increasing of IAA-oxidase activity ⁽¹⁰⁾ & oxidative hypothesis ⁽¹¹⁾.

According to the last hypothesis, oxidative processes greatly increased during aging as a result of abundance in

oxidation agents on one side, or decreasing in antioxidant defence mechanisms agents on the other side. Because of oxidative processes that causes of both decrease in lipids content (by lipid peroxidation) & decrease of protein content (by increase of protease activity) is accompanied by permeability perturbation

of membranes, and hence, decline of rooting response of cuttings ⁽¹²⁾. The decline of lipids and proteins content is accompanied by permeability perturbation of the membranes, this leads to increase of efflux (leakage) in terms of electrolytes leakage, as well as percentage of Na, K, Mg & Ca ions leakage in aged compared to fresh cuttings. Lipid peroxidation (LP) is

process occurs in natural conditions, but it increases under stress⁽¹³⁾. Lipid peroxidation is happened in two ways:- spontaneous (autoxidation) and catalyzed by lipoxygenase⁽¹¹⁾, both kinds lead to formation of group of active substances such as free radicals, hydroperoxides and secondary products produced from aldehyde oxidation. Lipoxygenases (LOXs) are dioxygenases that catalyzed the addition of molecular oxygen to polyunsaturated fatty acids (PUFA) containing the group cis, cis-1,4-pentadiene which oxidize to fatty acid hydroperoxides



Ascorbate is vitamin soluble in water. It is able to react with free radicals, it is chain-breaking antioxidant⁽¹⁶⁾. Vitamins such nicotinamide, pyridoxine, thiamine, ascorbic acid, carotene, vitamin K & adenine showed positive effect in pea rooting response⁽¹⁷⁾.

Materials & Methods

Growth of stock plants & preparation of cuttings

Seed germination and seedling growth were carried out in growth chamber at 25±1 °C under continuous illumination supplied by warm white fluorescent tubes (3000-3500) lux and relative humidity of 60-70%.

Stem cuttings were prepared, according to⁽¹⁸⁾, from 10-day-old light grown seedlings. The cuttings had apical bud, a pair of expanded primary leaves, epicotyl and 3 cm of hypocotyl under cotyledonary nodes.

Aging treatment

Cuttings were aged by dipping in d/H₂O or in appropriate tested solutions of anise extract (1%) or cinnamic acid (10⁻³ M) or volatile oil solutions for 3-

linoleic acid (C_{18:2}) contain such group⁽¹⁴⁾.

Endoproteases isoenzymes in peroxisomes purified from senescent (50-day-old) leaves showed a much higher endo-proteolytic activity than organelles from young (15-day-old) leaves⁽¹⁵⁾.

Glutathione (GSH) is low molecular non-protein thiol present in plants (achieve thiol in plant). It plays an important role in plant defence against stress through ascorbate/GSH cycle. GSH reacts lipid hydroperoxids by lipid hydroperoxide glutathione peroxidase (PXGPX) and thus protects the

days prior to their treatment with NAA, 10⁻⁴M (inductive auxin treatment). 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 tests & were placed 4 per glass vial containing 15 ml (3 cm depth) of the appropriate solution. All experiments were designed as completely randomized design & the statistical analysis was done according to⁽¹⁹⁾.

Measurement of Malondialdehyde content

Malondialdehyde content was determined according to⁽²⁰⁾.

Lipoxygenase activity assay

Lipoxygenase activity assayed according to⁽²⁰⁾. Sodium linoleate was used as a substrate.

Measurement of protein content

Protein content was determined according to Biuret⁽²¹⁾. Albumin used as a substrate to prepare standard curve.

Protease activity assay

Protease activity determined according to ⁽²²⁾.

Measurement of Glutathione content

Glutathione content determined according to ⁽²³⁾. Standard GSH used to prepare standard curve.

Measurement of Total Ascorbic acid content

Ascorbic acid determined according to ⁽²⁴⁾. Standard Ascorbic acid used as substrate to prepare standard curve.

Results**1. Effect of aging on rooting response of mung bean cuttings & its control by different physico-chemical ways:-**

Table (1) shows that fresh cuttings treated with auxin (Inductive auxin treatment) for 24h were developed (50.9) roots/cutting. Whereas, delaying of auxin treatment (24 h) in cuttings aged for 3-days in d/H₂O were developed (19.2) roots/cutting. In other words, the decline in rooting response is 62.3% was caused by ageing.

For controlling the aging phenomenon, cuttings were kept during aging period (3-days) in anise extract (1%), cinnamic acid solution (10⁻³M) and solutions of volatile oils. Both anise extract & cinnamic acid solution were active in stopping or delaying the process that occur during aging. It developed approximately (48) roots/cutting in both cases. Whereas, solutions of volatile oils has no influence in terms of ARF, in aged cuttings.

2. Effect of aging on lipids, proteins and antioxidants contents & its control by physico-chemical ways:-

Aging effects was observed through a significant increase in both MDA content (88%) (Fig. 1), lipoxygenase activity (277.2%) (Fig.2), protease activity (212.1%) (Fig.4), as

well as a significant decrease in protein content (90%) (Fig.3), GSH content (41.43%) (Fig.5) & Total ascorbic acid (66.85%) (Fig.6) compared to fresh cuttings. This agreed with physiological results (Table-1) which include a decline in rooting response of cuttings.

For controlling the processes that occurs during aging, cuttings kept in anise extract (1%), cinnamic acid solution (10⁻³ M) & volatile oils were solutions. The results were revealed that anise extract (1%) decreased MDA content (36%) (Fig. 1), lipoxygenase activity (39.35%) (Fig.2), protease activity (50.22%) (Fig.4), as well as it increased protein content (431.57%) (Fig.3), GSH content (155.16%) (Fig.5), total ascorbate content (19.18%) (Fig.6) compared to cuttings aged in d/H₂O. The foregoing findings were agreed with physiological results (Table-1).

On the other hand, cinnamic acid solution (10⁻³M) was successfully decreased MDA content by (19.15%) (Fig.1), LOX activity (44.27%) (Fig.2) & protease activity (43.12%) (Fig.4) compared to cuttings aged in d/H₂O, as well as it increased protein content (657.8%) (Fig.3), GSH (113.3 %) (Fig.5) compared to cuttings aged in d/H₂O, whereas it surprisingly decreased ascorbate content more than cuttings aged in d/H₂O (Fig.6), despite its effects on rooting response of mung bean cuttings (Table-1). The latter treatment developed (47.41) roots, which is not differ significantly when compared to fresh cuttings (50.9).

In addition, volatile oils solutions control aging through declining of MDA content (40.43%) (Fig.1), LOX activity (50.81 %) (Fig.2), protease activity (41.13 %) (Fig.4) compared to cuttings aged in d/H₂O, as well as increased protein content (1800 %) (Fig.3), GSH

content (113.5 %) (Fig.5) & total ascorbate content (183.4%) (Fig.6) compared to cuttings aged in d/H₂O. Despite cuttings kept in volatile oil

solutions had no effects on increasing roots number compared to cuttings kept in other solutions.

Table (1): Effect of aging on rooting response of mung bean cuttings & its control by different physico-chemical ways:-

Cutting type	Subsequent treatment for 24 h.	Mean number roots cutting ⁻¹
Fresh cuttings	d/H ₂ O	10.58
Fresh cuttings	ethanol (2%)	10.08
Fresh cuttings	NAA(10 ⁻⁴ M)	50.9
Cuttings aged in d/H ₂ O (Aging)	d/H ₂ O	5.75
Cuttings aged in ethanol (2%)	ethanol (2%)	6.58
Cuttings aged in d/H ₂ O (Aging)	NAA(10 ⁻⁴ M)	19.16
Cuttings aged in anise extract (1%)	NAA(10 ⁻⁴ M)	48
Cuttings aged in cinnamic acid (10 ⁻³ M)	NAA(10 ⁻⁴ M)	47.41
Cuttings aged in volatile oil solutions	NAA(10 ⁻⁴ M)	15.16

* L.S.D (0.05)= 6.11

* L.S.D (0.01)= 8.69

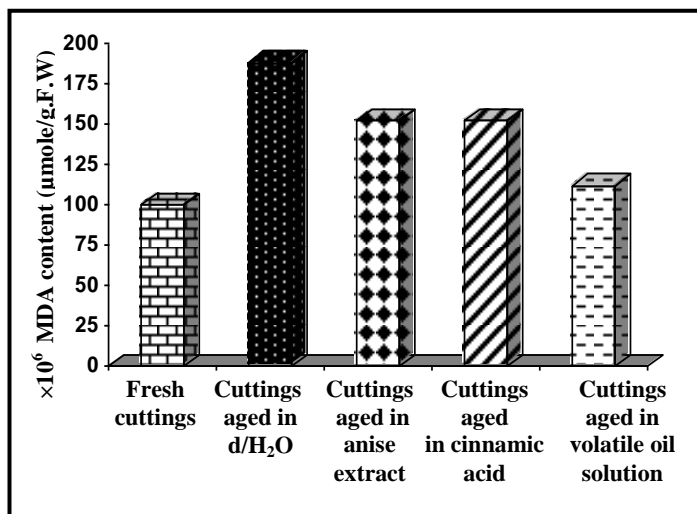


Fig. (1): Effect of aging on malondialdehyde content (μmole/g.F.W) & its control by physical-chemical ways.

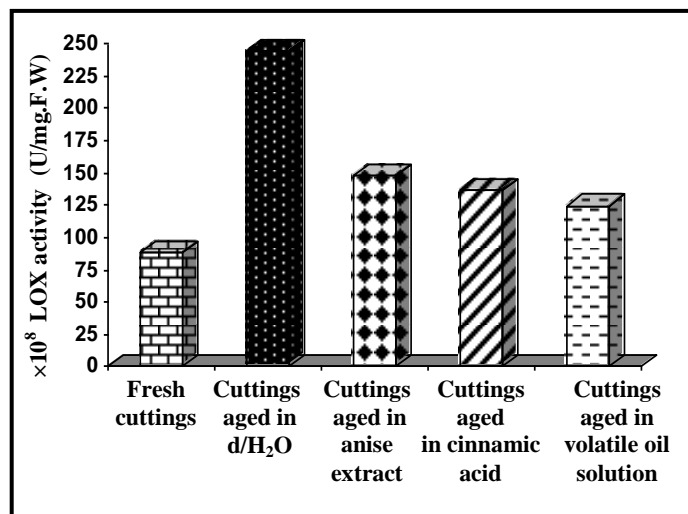


Fig. (2): Effect of aging on lipoxygenase activity (U/mg.F.W) & its control by physical-chemical ways.

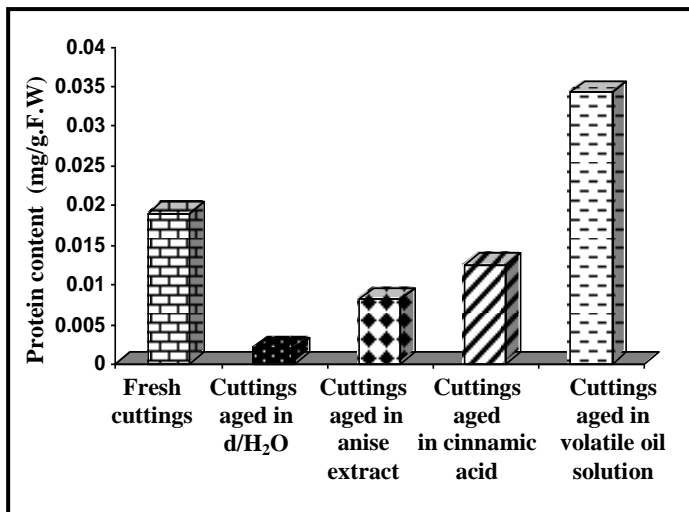


Fig. (3): Effect of aging on protein content (mg/g.F.W) & its control by physical-chemical ways.

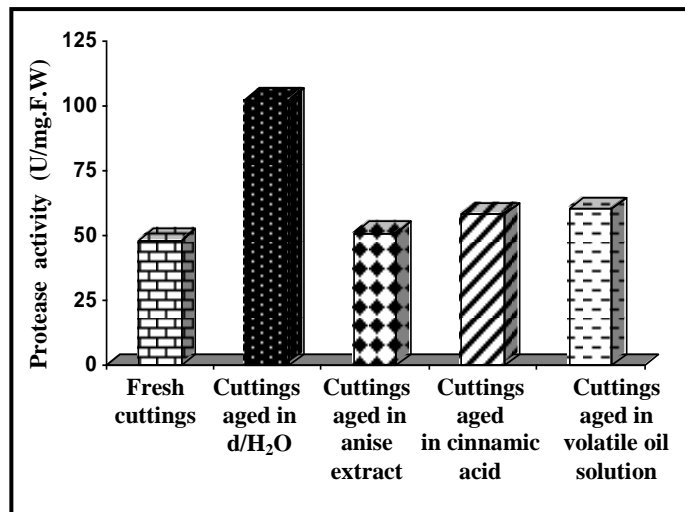


Fig. (4): Effect of aging on protease activity (U/mg.F.W) & its control by physical-chemical ways.

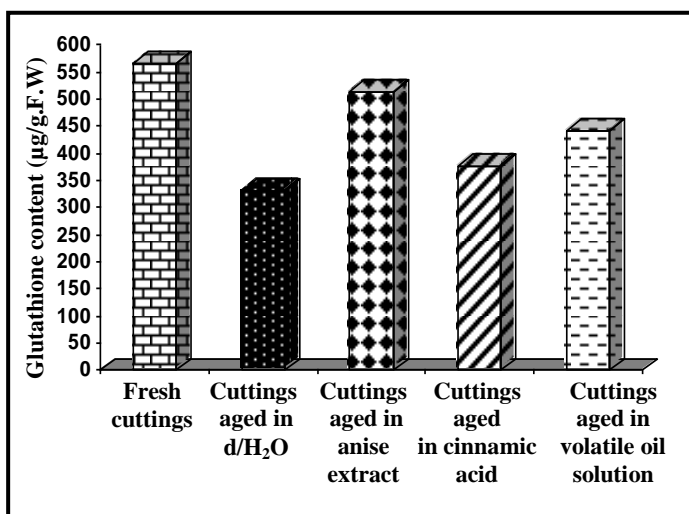


Fig. (5): Effect of aging on glutathione content (µg/g.F.W) & its control by physical-chemical ways.

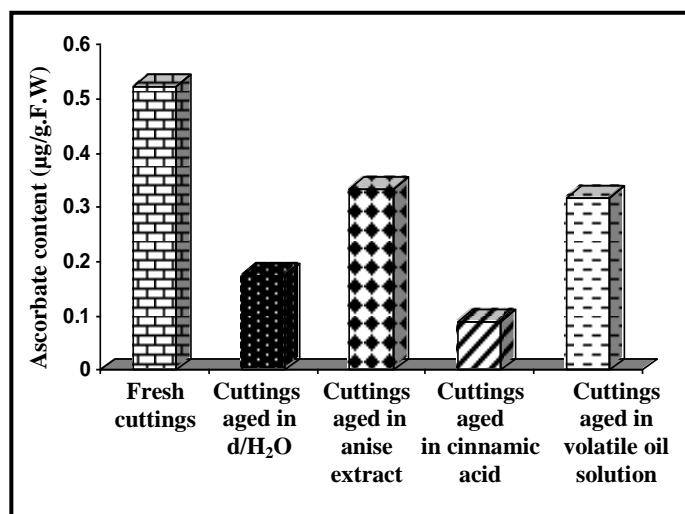


Fig. (6): Effect of aging on ascorbate content (µg/g.F.W) & its control by physical-chemical ways.

Discussion

Many metabolic changes, mostly degenerative, occurs during aging e.g. lipid peroxidation & protein breakdown enzymatically by effect of free radicals which physiologically lead to decline of rooting response in cuttings. The decline (51%) in phospholipids content & (20%) in protein content in mung bean cuttings aged in d/H₂O & which leads to

permeability perturbation in plasma membranes⁽¹²⁾. Many researchers tried to decrease the effect of these changes or its control, hence, obtaining a best rooting response. Lipid peroxidation process is occurs naturally but it increases under stress conditions⁽¹³⁾. When mung bean cuttings aged in d/H₂O for 3 days that led to decline in rooting response (Table-1), which may

attributed to decline in auxin content of aged cuttings ⁽²⁵⁾, or blockage of xylem vessels by suberin ⁽²⁶⁾, which prevent acropetal transport of supplied auxin to the base, or declining the nutritional factors (nutrition status) in aged cuttings ^(4,5), or because of permeability perturbation (result from declining of protein content & phospholipids quality ⁽¹²⁾, or decreasing rooting co-factors ⁽⁶⁾, or decreasing phenolic compounds ⁽⁸⁾, or because of oxidative processes which increase during aging as a result of abundant oxidative factors, this was confirmed by (Fig.1), that revealed increasing MDA content (an end product of lipid peroxidation) & results of (Fig.3), was devoted to decline of protein content which may attributed to the role of radicals (considered as antioxidants), or result of protease activity (Fig.4) (proteins degrading enzymes), or to decreasing factors that participates in antioxidant defense mechanism as ascorbate (Fig.5), and GSH (Fig.6). In addition, increasing the rate of this process may be attributed to increasing of LOX activity too (Fig.2). Hydrolyzing enzymes activity increases during aging such as LOXs activity, protease, RNase, DNase & chlorophyllase ⁽²⁷⁾. With the same trend, protein content decline during aging (Fig.3) & which may attributed to proteins degrading proteases (Fig.4). This agreed with ⁽²⁷⁾ about hydrolyzed enzymes activity that increased during aging, including proteases.

Additionally, antioxidants content may decline during aging such as ascorbate (Fig.6) & GSH (Fig.5) which may be attributed to oxidative processes as mentioned above because factors participates in defense mechanisms in plant which convert reduced GSH (active form in plant

defense) to oxidized GSSG as well as ascorbate from reduced form to oxidized form (dehydro ascorbic acid).

For controlling processes that occurs during aging, cuttings were kept in anise extract (1%), cinnamic acid (10^{-3} M) for 3 days (aging period) to maintain its sensitivity to inductive auxin treatment. Consequently, large number of roots developed, which significantly approaches its number in fresh cuttings (Table-1). Out of these processes which take place during aging in particular, lipid peroxidation. In other words, a decline in MDA formation (Fig.1) because of inhibition of LOXs activity (Fig.2). In addition, maintaining of protein content (declined protein degradation) (Fig.3), or maintaining of antioxidants content such as GSH (Fig.5) & ascorbate (Fig.6) which scavenges free radicals except cinnamic acid failure in stopping the decreasing in total ascorbate content (Fig.6). Alternatively, attributed to anise extract (1%) that contains (preliminary test not represented) active compounds considered as scavengers for free radicals ⁽²⁸⁾, finally protect cellular compounds. Cuttings were kept in volatile oil solutions for 3 days had no effect in processes during aging in terms of ARF, whereas, inhibited lipid peroxidation (decreasing MDA content), inhibited LOX activity (Fig.2) & maintaining protein content (Fig.3) & inhibited protease activity (Fig.4) & maintaining GSH content (Fig.6) & ascorbate content (Fig.5). This was attributed to volatile oils as terpenoid compounds which act as scavengers for free radicals & protection cellular membranes ⁽²⁸⁾, but it may not correlated with rooting response directly.

References

- 1-Hartmann, H.T.; Kofranek, A.M.; Rubatzky, V.E. & Flocker, W.J. (1988). Plant science, growth, development, utilization of cultivated plants; 2nd ed. Prntice Hall. Engle wood Cliffs, New Jersey pp.125.
- 2-Durkin, D. & Ruc, R. *Proc.Amer. Soc. Hort. Sci.*, 1966, **86**: 687. (Abst.)
- 3-Carlier, C. (1980). *Physiol. Veg.*, **18**: 463.
- 4-Shaheed,A.I. & Salim,S.A. *Coll. Educ. for women Univ.Baghdad.*, 2002 a, **3 (13)**: 566.
- 5-Shaheed, A.I. & Salim, S.,A. *Iraqi J. Sci.*, 2002 b, **43**: 1.
- 6-Wally, Y.A.; El-Hamady, M.M.; Boulos, S.T., & Salama, M.A. *Egypt J. Hort.*, 1980, **8 (1)**: 89.
- 7-Atkinson, C.J.; Davies, W.J. & Mansfield, T.A. *Exp. J. Bot.*, 1989, **40**: 1021.
- 8-Zenk,M.H. & Mullar,G. *Nature*, 1963, **200**: 761.
- 9-Ullrich, W. *Plantae.*, 1962, **59**: 387.
- 10-Chibbar, R.N.; Gurumurthi, K. & Nanda, N.N. *Experienta*, 1979, **15**: 202.
- 11-Siedow, J. *Ann. Rev. Plant Physiol.*, 1991, **2**: 145.
- 12-Shaheed ,A.I. & Al-Khafaji, M.A. *ATTI DELLA "FONDAZIONE GIORGIO RONCHI"*, 2008 (Accepted for pub.).
- 13-Blokhina,O.(2000). Academic Dissertation. Helsnki Univ.
- 14-Chesworth,J.M., Stuchury,T. & Scaife,J.R. (1998). An introduction to agricultural biochemistry. Champan & Hall.
- 15-Distefano,S., Palma,J.M., Gomez,M. & Rio,L.A. *Biochem.J.* 1997, **327**: 399.
- 16-Beyer R.E. *J. Biol.Membr.* 1994, **26 (4)**:349.
- 17-Aberg, B.(1961). Handb.D.Pflanzen-physiol.,14: 418.
- 18-Hess,C.E. *Plant Physiol.*, 1961, **36**: Suppl.21.
- 19-Parker, R.E. (1986). Introductory Statistics for Biology, 2nd ed. Edward Arnold, Great Britain.
- 20-Zacheo,G., Cappello,M.S., Gallo,A., Santino,A. & Cappelo,A.R. *Lebensm -Wiss u. Technol.*, 2000, **33**: 415.
- 21-Bishop, M.C. Dben-Von Laufer, J.L.; E.P. Fody and Thirty three contributors-1985. Clinical chemistry principles, procedures and correlations. Pp. 181.
- 22-Kunitz,M. *J. Gen. Physiol.*, 1947, **30**:291.
- 23-Ellman G.L.(1959).Archives of Biochemistry & Biophysics, **822**,70.
- 24-Shalata,A. & Neumann,P.M. *J. Exp. Bot.*, 2001, **52 (364)**: 2207. November 2001.
- 25-Shaheed,A.I. & AL-Alwani, B.A. *Iraqi J. Bio.*, 2001, **1(1)**: 161.
- 26-Shaheed,A.I. and AL-Alwani, B.A.2002. (Accepted for pub.)
- 27-Kar, M. & Mishra, D. *Plant Physiol.*, 1976, **57**: 315.
- 28-Harborne, J.B.(1993). Introduction to Ecological Biochemistry. Academic Press, London.