Arab Gulf J. Scient. Res., Agric. Biol. Sci., **B5** (1), pp. 1-11 (1987)

Relation between Calcium Concentration, Respiration, Respiratory Control Ratio, and ADP:O Ratio of Mitochondria Isolated from Normal and Cork Spotted 'Anjou' Pear Fruit[1]

A.M. Al-Ani[2] and Daryl G. Richardson

Department of Horticulture, College of Agriculture, University of Baghdad, Baghdad, Iraq; and Horticulture Department, Oregon State University, Corvallis, Oregon 97331, USA

ABSTRACT. Calcium concentration in mitochondria isolated from 'Anjou' pear (*Pyrus communis* L.) fruit affected with cork spot (3.26 μg Ca/mg of mitochondrial protein) was significantly lower than Ca concentration in mitochondria isolated from normal fruits (7.06 μg Ca/mg of mitochondrial protein). State III respiration, respiratory control ratio, and ADP:O ratio were significantly higher in mitochondria isolated from normal fruits than in mitochondria isolated from cork spotted fruits.

The results suggest that calcium deficiency reduced the functional integrity of the mitochondria isolated from ripe 'Anjou' pear fruit.

One of the major functions of Ca in plants is the maintenance of cell membranes. Calcium deficiency in the shoot apex of barley became microscopically evident within two days from the time the plants were transferred to the water culture without calcium (Marions 1962).

The effect of Ca-deficiency on cell ultrastructure appeared as structural abnormalities-resulting from the breakup of nuclear envelope and the plasma-followed by the disorganization of the mitochondria (Marions 1962).

If Ca concentration decreased to 10^{-7}, the NADH-induced mitochondrial membrane potential is reduced. When Ca concentration is increased to 10^{-6}M, the mitochondrial membrane potential is restored (Moore and Akreman 1982). The authors suggested that Ca stimulate the mitochondrial external NADH dehydrogenase *in vivo* (Moore and Akerman 1982).

1 This manuscript is a section from Ph.D. thesis for Dr. Abdulilah M. Al-Ani.

2 The correspondence: Department of Horticulture, College of Agriculture, University of Baghdad, Baghdad, Iraq.

Florell (1956) reported fewer mitochondria in wheat roots grown in nutrient solution without Ca. Increasing Ca concentration in the nutrient solution to 10^{-4}M, increased the amount of mitochondrial by 54%, and increased the dry weight of the mitochondrial protein 47% over the control (Lindblad 1959). Calcium deficiency not only decreased the synthesis of mitochondria, but also reduced their functional capability, and decreased the ADP:O ratio (Burstrom 1968). Low concentrations of Ca in the nutrient solution, produced mitochondria with shorter lifetimes (faster turnover) from wheat roots (Lindblad 1959). Calcium deficiency caused the dissolution of the lamellar system and the breakdown of membranous structures of the mitochondria (Burstrom 1968). Electron microscopic investigation of the mitochondria from Ca starved plants showed that acute Ca starvation caused swelling of the mitochondria, loss of electron impermeability of the matrix, and separation and vesiculation of the cristae, and those changes were specific for Ca (Semikhatova 1973). It was also found that mitochondria isolated from plants at early stages of Ca deficiency before any sign of growth retardation and any deficiency symptoms could be detected by electron microscope. Those mitochondria cannot withstand stress, such as high temperature, and lost their structural and functional integrity long before those of the control plants (Semikhatova 1973). This finding suggests that mitochondria formed under low Ca conditions are defective.

Mitochondrial function is highly dependant up on the concentration of Ca within the various compartment of the organelle, and the addition of Ca to the mitochondrial suspensions was retained in the mitochondrial pellet (Happel and Simson 1982).

Isolated animal mitochondria can accumulate large amounts of Ca, up to several hundred times the initial Ca content, during electron transport *in vitro* (Lehninger 1970). It was found later, that there are both high and low affinity Ca-binding sites in the inner membrane, and only the low affinity Ca-binding sites in the outer membrane of animal mitochondria (Carafoli and Gazzotti 1973).

Citrate and isocitrate exert the protective action by chelating and retaining Ca in the mitochondria, and prevent the damage due to the release of Ca from the mitochondria (Toninello *et al.* 1983). A possible participation of the enzyme NAD glycohydrolase in the mechanism of Ca release from the mitochondria (Moser *et al.* 1983).

Plant mitochondria can also accumulate Ca (Chen and Lehninger 1973). This accumulation is not specific to Ca (Bangerth 1974), since most divalent cations such as Sr^{2+} and Ba^{+2} can also be accumulated in the mitochondria (Truelove and Hanson 1966). The accumulated Ca in corn mitochondria is released from the mitochondria when respiration ceases (Earnshaw and Hanson 1973). This suggests that the accumulation of Ca and possibly other ions is only temporary accumulation

in the plant mitochondria and not permanent deposits. It was also suggested that mitochondria may control the movement and concentrations of Ca within the cells (Truelove and Hanson 1966).

Chen and Lehninger (1973) studied Ca transport in isolated mitochondria from 14 different higher plants and fungi and conclude that: Additions of Ca to isolated plant mitochondria caused little or no stimulation of state IV respiration. Uptake of Ca was inhibited by respiratory inhibitors and uncouplers. All the plants which have been studied only the mitochondria from sweet potato show both high-affinity and low-affinity Ca binding sites (as in animal mitochondria), while the mitochondria from all other plants studied showed only low-affinity Ca binding sites.

Respiratory inhibitor (ruthenium red) completely inhibited Ca uptake by corn mitochondria (Yamaya *et al.* 1984), and 65% of Ca uptake required an exogenous supply of ATP. While 35% of Ca uptake by corn mitochondria was the respiratory substrate dependant (Yamaya *et al.* 1984). Calcium uptake by plant mitochondria is energy-dependant and inhibition of Ca uptake may lead to many physiological disorders (Hertel and Dieter 1983).

The possibility of changes in mitochondrial population due to the formation of new mitochondria during fruit ripening was suggested by some workers (Lance *et al.* 1966). Since Ca is suggested to be required for the formation of new mitochondria (Burstrom 1968 and Semikhatova 1973), the newly formed mitochondria during fruit ripening may be expected to be defective in fruits with Ca-deficiency. This may explain the development of cork spot in low Ca fruits during storage and ripening.

The objectives of this study were; (a) to measure the endogenous Ca in mitochondria from 'Anjou' pear fruits affected with cork spot (Ca-deficient) in comparison to normal (no Ca-deficiency); and (b) to study the effect of Ca on the integrity of the mitochondria by measuring their activities as a function of their integrity.

Experimental

Isolation

Mature green 'anjou' pear fruits were stored at 0°C until December, 1975 when the experiment started. Mitochondria were isolated from normal fruits and fruits with cork spots by the following procedure:

Fruits were held for 4 to 5 days at 20°C to soften, then chilled at 0°C, peeled and cored, and 100 g of tissue was gently grated into 300 ml of isolation medium,

using an apparatus similar to that described by Romani *et al.* (1969). Isolation was carried out in containers surrounded by crushed ice (0 to 4°C) in a pre-cooled apparatus, and the isolation medium was cooled to 0°C before it was used. The pH was maintained at 6.7 to 6.9 during the isolation, with additions of 1N KOH. The isolation medium is similar to that used by Romani *et al.* (1974), except for the additional use of 3 mM $MgCl_2$ as suggested by some authors (Frost and Wilson 1972) to prevent aggregation of the mitochondria during isolation. The isolation medium consisted of 0.25 M sucrose, 0.05 M potassium phosphate buffer (pH 7.2), 5 mM EDTA, 0.2% polyvinylpyrollidone (PVP, 40,000 MW), 0.1% bovine serum albumin (BSA), 5 mM β-meroaptoethanol, and 3 mM $MgCl_2$. The suspension was squeezed through two layers of muslin fabric (mesh 50 strands/ cm) (Palmer 1967). The pH of the filtrate was adjusted to 6.7 to 6.9 by dropwise addition of 1N KOH. The filtrate was centrifuged in precooled, thin-walled polyallomer centrifuge tubes in a refrigerated centrifuge (Sorvall model RC2-B) held at −2 to 0°C.

Several centrifugation procedures were evaluated (Frost and Wilson 1972, Palmer 1967, Richmond and Ozelkok 1973 and Romani *et al.* 1974), and the following procedure was found to yield active mitochondria and more mitochondrial protein than the others, from 'Anjou' pear fruit. The flow diagram for the centrifugation is shown in Fig. 1.

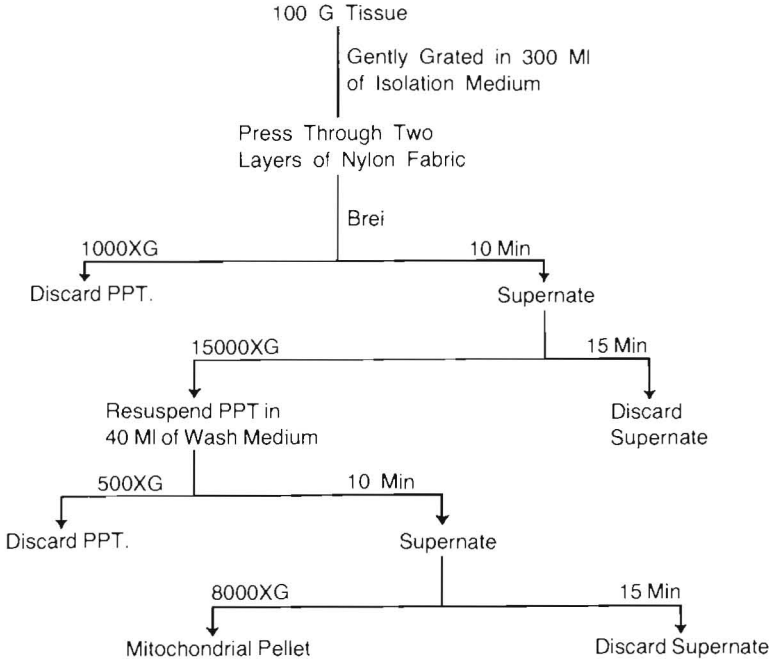

Fig. 1. Scheme for centrifugation and isolation of mitochondria from 'Anjou' pear fruit.

The wash medium is the same as that used by Romani *et al.* (1974), except β-mercaptoethanol was not used as suggested by other authors (Estabrook 1967), while 3 mM $MgCl_2$ was used as suggested earlier. The wash medium consisted of 0.25 M sucrose, 0.05 M potassium phosphate buffer (pH 7.2), 0.1% BSA, and 3 mM $MgCl_2$. The pellet was suspended in 40 ml of wash medium after the first high speed centrifugation by using a pre-cooled Teflon tissue homogenizer. After the final centrifugation step, the mitochondria were resuspended in an equal volume of wash medium and held at ice temperature before assaying. Protein was estimated by Lowry Method (Lowry *et al.* 1951), using BSA as the standard.

Mitochondria were assayed for oxygen consumption in a medium (3ml) consisting of: 0.75 mmole sucrose, 200 μmoles Pi pH 7.2), 30 μmoles a-ketoglutarate, 0.3 μmole NDA, 0.1 μmole thiamine pyrophosphate, 3 μmoles $MgCl_2$, 0.1 μmole CoA, 3 mg bovine serum albumin, 100 μg chloramphenicol and 3 to 6 mg of mitochondrial protein. This reaction medium is the same as that suggested by Romani *et al.* (1974). Oxygen consumption was measured polarographically (Estabrook 1967) at 25°C, using a Clark oxygen electrode (Yellow Springs Instruments). Rate of oxygen uptake in state III and state IV was calculated as nanoatoms of oxygen per mg of mitochondrial protein per min. Respiratory control ration (RC) was calculated by dividing the rate of oxygen uptake in state III on the rate of oxygen uptake in state IV (Chance and Williams 1955). While ADP:O ratio was calculated by dividing the amount of ADP used (μmoles) on the amount of oxygen consumed (μatoms) as described by Chance and Williams (1955), and each measurement was repeated 15 times.

Measurement of Calcium

Mitochondria were isolated as described above, except 300g of fruit tissue was grated in 900 ml of isolation medium. After the final centrifugation step, the mitochondria were resuspended in an equal volume of wash medium (without BSA) and referred to as the mitochondrial suspension which was used for determination of endogenous calcium.

Calcium was extracted from the mitochondrial suspension with an equal volume of 5% HCl, and 5% trichloroacetic acid (Graesser 1975). The mixture was shaken well (manually) and centrifuged at 13,000 xg for 5 min to remove mitochondrial fragments. $SrCl_2$ was added to the supernatant (final concentration 3%), and Ca was measured in a Perkin-Elmer Atomic Absorption spectrophotometer, Model 303.

Results

Isolation and Assay

The results are summarized in Table 1 and plotted on Fig. 2. The rate of O_2 uptake in "state III" of the mitochondria isolated from normal fruits is twice that

isolated from fruits with cork spot (Ca-deficient). The difference in O_2 uptake is statistically significant at the 1% level. The rate of O_2 uptake in "state IV" of mitochondria isolated from normal fruits is about 86 nanoatoms O_2/mg of mitochondrial protein/min, compared with 62 nanoatoms O_2/mg of mitochondrial protein/min, of the mitochondria isolated from cork spotted fruits. The difference in O_2 uptake in "state IV" is also statistically significant at the 1% level (Table 1). Respiratory control ratios (RC) of mitochondria from the normal fruit is about 2.2 compared with 1.4 for the mitochondria isolated from fruits with cork spot. The difference in RC is significant at the 1% level. The value of ADP:O ratio in the mitochondria isolated from normal fruit is about 2.3 while it is about 1.5 in the mitochondria from cork spotted fruits. The difference in ADP:O ratio is also significant at the 1% level (Table 1 and Fig. 2).

Calcium Concentration

Calcium concentration was significantly higher in the mitochondria isolated from normal fruits compared to the mitochondria isolated from fruits with cork spot (Table 1). Calcium concentration was about 7.1 μg/mg of mitochondrial protein in the mitochondria isolated from normal fruits compared with 3.3 μg Ca/mg of mitochondrial protein in mitochondria isolated from cork spotted fruits (Table 1).

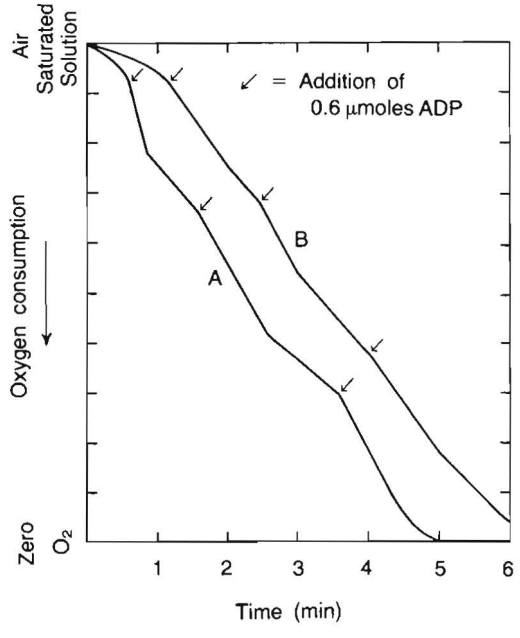

Fig. 2. Polarographic measurement of oxygen consumption by mitochondria isolated from normal (A) and cork spotted (B) 'Anjou' pear fruit.

Table 1. Respiratory activity and Ca concentration of mitochondria isolated from normal 'Anjou' pear fruit and from fruits affected with cork spot.*

Treatment	Rate of O_2 uptake**		RC ratio	ADP:O ratio	Ca Conc. μg/mg of protein
	State III	State IV			
Normal fruit	170.385	85.692	2.154	2.263	7.055
Fruit with cork spot	84.904	62.081	1.361	1.493	3.259
$LSD_{0.05}$	21.695	15.066	0.382	0.427	1.039
$LSD_{0.01}$	30.109	20.910	0.530	0.592	1.394

* For reaction medium see text.
** Nanoatoms O_2/mg of protein/min.

Discussion

Isolation and Assay

The results in Table 1 and Fig. 2 show that the mitochondria isolated from normal fruits have a higher rate of O_2 uptake in both states III and IV, higher RC ratio, and higher ADP:O ratio than the mitochondria isolated from cork spotted fruits. The results presented above support the concept that the structural and functional integrity of mitochondria isolated from normal fruit is much better than that of mitochondria isolated from Ca-deficient fruits. The higher RC ratio and ADP:O ratio in mitochondria isolated from normal fruits show that the oxidation and phosphorylation are coupled, but not in the mitochondria from Ca-deficient fruits. Our results agree with some reports in the literature that Ca deficiency decreased ADP:O ratio in the mitochondria (Semikhatova 1973 and Burstrom 1968).

Calcium deficiency in the mitochondria reduced their functional capability and reduced mitochondrial membrane potential (Moore and Akerman 1982 and Happel and Simson 1982). Lower ADP:O ratio also reduced Ca uptake by the mitochondria due to the reduction in the energy required for Ca uptake (Hertel and Dieter 1983 and Yamaya *et al.* 1984).

All the attempts to isolate active mitochondria from mature green, but unripened, 'Anjou' pear fruits failed. Hence, it was not possible to compare mitochondria from normal fruits with those from cork spotted fruits at the mature green stage. The main problem likely associated with the inability to use green fruit

is that gentle grating cannot be achieved due to the nature of the fruits, and severe blending caused complete loss of activity (data not shown). The control of the pH during maceration was very important due to the acid nature of the fruits (Truelove and Hanson 1966). The pH cannot be controlled during maceration if the usual techniques (such as blender) are used unless more elaborate modification can be implemented. Another problem with the use of green fruits is the release of phenolic compounds during maceration, which tends to inhibit the activity of the mitochondrial enzymes (Loomis 1974). The use of PVP to suppress phenolics is not very effective if the pH is not maintained between 6.7 and 6.9 (Loomis 1974 and Romani *et al.* 1974). In addition to mechanical damage to the mitochondria from isolation phenolic compounds may specifically uncouple oxidative phosphorylation (Loomis 1974), which may explain the low activity of the mitochondrial isolated from green fruits which have higher levels of free phenolics than ripe fruits. Polyclar AT was reported to be very effective in binding phenolics during the isolation of enzymes (Loomis 1974). It was suggested (W.D. Loomis, personal communication) that 1 g of Polyclar AT per g of fresh weight of fruit tissue be used to be effective. Mixing this compound with the medium in the blender during maceration resulted in production of fine particles in the isolation medium, which was not possible to remove from the mitochondrial fraction by differential centrifugation.

Ripe fruits are easy to macerate in an apparatus similar to that described by Romani *et al.* (1974), which permits the control of the pH during maceration, and causes less damage to the mitochondria. Ripening may result in some changes in the metabolism of phenolics and cause some "detoxification" by changing them into inactive polymeric forms such as by glycosidation, esterification, and lignification. Ripening also may cause an increase in total mitochondrial population, due to the synthesis of new mitochondria (Lance *et al.* 1966), which may increase the apparent mitochondrial activity in the ripe fruits. The respiratory control ratio and ADP:O ratio were also increased during the ripening of avocado fruit (Lance *et al.* 1966).

Calcium Concentration

The results in Table 1 show that Ca concentration is lower in mitochondria isolated from fruits with cork spot, than the Ca concentration in mitochondria isolated from normal fruits. The evidence presented above confirms that Ca is deficient on the subcellular level in the fruits with cork spot. From the results in Table 1, we can conclude that there are some relations between Ca deficiency in the mitochondria and its functional integrity. This conclusion is supported by several reports on mitochondria from different plant sources (Semikhatova 1973, Moore and Akerman 1982, Happel and Simson 1982, and Hertel and Dieter 1983).

Our data showed that Ca concentration in pear mitochondria is slightly higher than what was reported in avocado mitochondria. Calcium concentration in

avocado reportedly was 73.3 nanomoles Ca per mg of protein and in turnip root is 181 nanomoles Ca per mg of protein (Chen and Lehninger 1973). Converting the data in Table 1, we found that Ca concentration in pear mitochondria is about 81 and 176 nanomoles Ca per mg of mitochondrial protein. It was reported that endogenous Ca concentration in isolated mitochondria varies depending on the species, the type of tissue used for isolation, and the conditions of isolation (Chen and Lehninger 1973, and Lehninger *et al.* 1967). The reported endogenous Ca concentration is mostly measured in mitochondria isolated by the discontinuous sucrose gradient method, extracted by acids and measured by emission spectroscopy (Lehninger *et al.* 1967). The mitochondria in our work were not isolated by the discontinuous sucrose gradient which may have helped to maintain higher levels of mineral ions.

References

Bangerth, F. (1974) The function of calcium in the cell and in the subcellular units of apple fruit, *Acta Horticulturae* **45:** 43-47.

Burstrom, H.G. (1968) Calcium and plant growth, *Biol. Rev.* **43:** 287-316.

Carafoli, E. and **Gazzotti, P.** (1973) The reaction of Ca with the inner and outer memberane of mitochondria, *Experientia* **29:** 409-410.

Chance, B. and **Williams, G.R.** (1955) Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization, *J. Biol. Chem.* **217:** 383-393.

Chen, C.H. and **Lehninger, A.L.** (1973) Ca^{++} transport activity in mitochondria from some plant tissues, *Arch. Biochem. Biophys.* **157:** 183-196.

Earnshaw, J. and **Hanson, J.B.** (1973) Inhibition of postoxidative calcium release in corn mitochondria by inorganic phosphate, *Plant Physiol.* **52:** 403-406.

Estabrook, R.W. (1967) Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios, pp. 41-47. *in:* **Estabrook, R.W.** and **Pullman, M.E.** (ed.) *Methods in Enzymology*, Vol. 10, Academic Press Inc., New York.

Florell, C. (1956) The influence of calcium on root mitochondria, *Physiol. Plant.* **9:** 236-242.

Frost, R. and **Wilson, H.** (1972) Isolation of bean mitochondria and the influence of gibberellic acid on the metabolism, *Phyton* **29:** 79-88.

Graesser, R.J. (1975) *Intermediary metabolite regulation and control of calcium uptake by isolated mung bean mitochondria,* M.A. Thesis, in the University of Texas at Austin, 59 p.

Happel, R.D. and **Simson, J.A.V.** (1982) Distribution of mitochondrial calcium; pyronateim precipitiation and atomic absorption spectroscopy, *J. Histochem. Cytochem.* **30**(4): 305-311.

Hertel, C. and **Dieter, M.** (1983) Herbicides and fungicides inhibit calcium uptake by plant mitochondria: A possible mechanism of action, *Pestic Biochem. Physiol.* **19**(3): 282-290.

Lance, C., Hobson, G., Young, R.E. and **Biale, J.B.** (1966) Metabolic proceses in cytoplasmic particles of the avocado fruits. VII. Oxidative and phosphorylative activities throughout the climacteric cycle, *Plant Phyio.* **40:** 1116-1123.

Lehninger, A.L. (1970) Mitochondria and calcium ion transport, *Biochem. J.* **119:** 120-138.

Lehninger, A.L., Carafoli, E. and **Rossi, C.S.** (1967) Ion movement in mitochondrial systems, *Adv. Enzymol.* **29:** 259-315.

Lindblad, K.L. (1959) The influence of growth conditions on the amount and ribonucleic acid content of wheat root mitochondria, *Physiol. Plant.* **12:** 400-411.

Loomis, W.D. (1974) Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles, *Methods in Enzymology* **31:** 522-544.

Lowry, O.H., Rosebrough, N.J. Farr, A.L. and **Randall, R.J.** (1951) Protein measurement with the folin phenol reagent, *J. Biol. Chem.* **193:** 265-275.

Marinos, N.G. (1962) Studies on submicroscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley, *Amer. J. Bot.* **49:** 834-841.

Moore, A.L. and **Akerman, K.E.O.** (1982) Calcium stimulation of the external NADH dehydrogenase in Jerusalem artichocke mitochondria, *Biochem. Biophys. Res. Commun.* **109**(2): 513-517.

Moser, B., Winterhalter, K.H. and **Richter, C.** (1983) Purification and properties of a mitochondrial NAD glycohydrolase, *Arch. Biochem. Biophys.* **224:** 358-364.

Palmer, J.M. (1967) Rapid isolation of active mitochondria from plant tissue, *Nature* **216:** 1208.

Richmond, A. and **Fisher, L.K.** (1966) Decreased synthesis of mitochondrial protein in the senescent cells of pear fruit, *Life Science* **5:** 1187-1190.

Richmond, A. and **Ozelkok, S.** (1973) Survival of mitochondria *in vitro*, physical and energy parameters, *Plant Physiol.* **51:** 702-706.

Richmond, A.., Ozelkok, S. and **Fisher, L.K.** (1969) Isolation of tightly coupled mitochondria from acidic plant tissues, *Plant Physiol.* **44:** 311-312.

Romani, R.J., Tuskes, S.E. and **Ozelkok, S.** (1974) Survival of plant Mitochondria *in vitro*. Form and function after 4 days at 25°C, *Arch Biochem. Biphys.* **164:** 743-751.

Semikhatova, O.A. (1973) Studies by T.M. Bushueva on the physiological role of calcium in plants, *Sov. Plant Physiol.* **20**(5): 916-919.

Toninello, A., Lisa, F.D., Sliprandi, D. and **Siliprandi, N.** (1983) Mechanism of citrate and isocitrate protective action on rat liver mitochondria, *Biochem. Biphys. Res. Commun.* **115:** 749-755.

Truelove, B. and **Hanson, J.B.** (1966) Calcium-activated phosphate uptake in contracting corn mitochondria, *Plant Physiol.* **41:** 1004-1013.

Yamaya, T., Oaks, A. and **Matsumoto, H.** (1984) Stimulation of mitochondrial calcium uptake by light during growth of corn shoots, *Plant Physiol.* **75:** 773-777.

Received 10/07/1984;
in revised form 17/07/1985)

العلاقة بين تركيز الكالسيوم في المايتوكوندريه المعزولة من ثمار الكمثرى السليمة والمصابة بالضرر الفسلجي المسمى بالبقع الفلينية وسرعة التنفس ونسبة ADP:O ونسبة الـ (R C) [1]

[2] عبد الاله مخلف العاني و ديرل رجردسون

قسم البستنة ـ جامعة اوريكون الرسمية ـ كورفالس ـ الولايات المتحدة الأمريكية

أثبتت هذه الدراسة أن تركيز الكالسيوم في المايتوكوندريه المعزولة من ثمار الكمثرى المصابة بالضرر الفسلجي المسمى بالبقع الفلينية هو ٣,٢٦ مايكروغرام لكل ملغرام من بروتين المايتوكوندريه. أما تركيز الكالسيوم في المايتوكوندريه المعزولة من الثمار السليمة فبلغ حوالي ضعف ما هو موجود في الثمار المصابة (٧,٠٦ مايكروغرام لكل ملغرام من بروتين المايتوكوندريه). كما وجد أن سرعة التنفس في المرحلة الثالثة، ونسبة الـ RC، ونسبة ADP:O في المايتوكوندريه المعزولة من الثمار السليمة أعلى مما هو في المايتوكوندريه المعزولة من الثمار المصابة. يدل ذلك على أن نقص الكالسيوم قلل من نشاط المايتوكوندريه وقلل من تماسك أغشيتها.

١ هذا البحث يعتبر جزء من رسالة الدكتوراه للدكتور عبدالاله مخلف العاني.

٢ عنوان المراسلة: قسم البستنة كلية الزراعة ـ جامعة بغداد ـ أبو غريب ـ العراق.