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**SHORT COMMUNICATION**

# EFFECT OF CALCIUM ON LIPIDS OF GREEN LEAFY VEGETABLES

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**The present investigation was carried out to study the effect of calcium on the lipids and lipid peroxidation of the green leafy vegetables like *Amaranthus tricolar* L., *Rumex vesicarius* L. and *Spinacea oleracea* L. The green leafy vegetables were grown in earthern pots and different concentrations of $CaCl_2$ (10, 50 and 100 mM) were applied to the soil (in the form of ionic solution). It was observed that application of 50 mM $CaCl_2$ resulted in higher levels of calcium ($Ca^{2+}$), calmodulin (CaM), total lipids, glycolipids and phospholipids in the leaves of the three species. 50 mM $CaCl_2$ treatment was found to be very effective in controlling lipid peroxidation compared to 10 and 100 mM $CaCl_2$ treatments. *Amaranthus* responded effectively to 50 mM amended $CaCl_2$ compared to the other two species of GLVs.**

***Key words:*** **Calcium, glycolipids, lipid, lipid peroxidation, phospholipids**

Unlike other vegetables the preservation of GLVs is a problem because of its wilting nature followed by senescence after harvesting. Lipid peroxidation (LPO) is an inherent feature of senescing cells (Dhindsa *et al*. 1981, Arora *et al*. 2002). $Ca^{2+}$ & $Mg^{2+}$ stabilize the photosynthetic membranes by binding to the negatively charged groups on membrane surface (Mc Carty 1980). The importance of $Ca^{2+}$ in plant growth and development is attributed to multifunctional $Ca^{2+}$ binding protein calmodulin (CAM). It is ubiquitous and activates number of enzymes and thus plays an important role in diverse cellular functions (Anderson and Cormier 1978). CAM controls cell proliferation and a wide variety of $Ca^{2+}$ mediated cellular events (Thompson *et al*. 1989). Oxidative stress is characterized by over production of highly active oxygen species (AOS). One of the most damaging effects of these AOS in cells is the peroxidation of membrane lipids. Senescence in plant tissue is accompanied by changes in LPO with simultaneous decline in membrane lipids (Halliwell 1981). $Ca^{2+}$ signaling is required for acquisition of tolerance or resistance to the stress (Reddy 2001). During cabbage leaf senescence reduced relative levels of chlorophyll and soluble proteins were observed with decreased ratio of PUFA/SFA of phospholipids and enhanced lipoxigenase activity (Cheour *et al*. 1992). AOS attack proteins, lipids, nucleic acids and the degree of damage depends on the balance between the formation of an AOS and its removal by the antioxidative scavenging systems (Hernandez and Almanja 2002, Umar *et al*. 2007). Number of articles has been published on the role of $Ca^{2+}$ in senescence but the effect of $Ca^{2+}$ in GLVs is not adequately understood. Hence the present study was undertaken to screen the suitable $Ca^{2+}$ concentration required for three species of GLVs.

The seeds of three species were surface sterilized with mercuric chloride and washed repeatedly with sterile distilled water. The seeds of three species *Amaranthus tricolar* L., *Rumex vesicarius* L. and

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*Spinaceae oleracea* L. were sown in pots containing red loamy soil mixed with farmyard manure in the ratio 3:1. After germination, the seedlings each with main root and three lateral roots were transplanted in separate pots. One week after transplantation, 10, 50 and 100 mM of $CaCl_2$ were amended to the pots. Control plants were given distilled water. Two weeks after treatment third leaf from the top was selected for the present study.

Calcium content was determined by the method of Piper (1957). The dry powder of the leaf material incubated overnight in triacid mixture (perchloric acid: sulphuric acid: nitric acid, 2: 1: 10) and heated to 180°C until a clear solution was obtained. The solution was diluted with distilled water and filtered through whatman filter paper. The $Ca^{2+}$ content was measured at 422.7 nm using the Atomic absorption spectrophotometer. CaM was extracted and purified using the method of LeRoux and Dubery (1989). The protein was subjected to heat treatment for 3 min at $85^0$C, the resulting precipitate removed and the protein solution was adjusted with 5 mM $Ca^{2+}$. The protein was absorbed on to a $Ca^{2+}$ induced hydrophobic interaction chromatography packed with phenyl sepharose CL-4B. CaM was desorbed from the column by the inclusion of 5 mM EGTA. CaM containing fractions were pooled dialyzed, lyophilized and UV spectra were recorded. Protein concentration was determined by the method of Bradford (1976).

Total Lipids were extracted from the leaves followed by the method of Hoppe and Heitefuss (1974). Total glycolipids were estimated by determining the total sugars in lipid samples by phenol -sulphuric acid method described by Roughan and Batt (1968). The phosphorus content in phospholipids was estimated by the method of Bartlett's (1959). The lipids dissolved in chloroform were digested by adding perchloric acid. Subsequently 1-amino -2 - naphthol - 4- sulphonic acid reagent was added and the contents were heated. After cooling the colour intensity was measured at 660 nm using Shimadzu spectrophotometer. The level of LPO in the leaf tissue was measured in terms of malondialdehyde (MDA) a product of LPO content determined by the thiobarbituric acid (TBA) reaction with minor modification of the method of Heath and Packer (1968).

Data were analysed by ANOVA. The values are the mean ± SE of five replications and means compared by the least significant difference test at the 0.05 level.

Calcium content was estimated from the leaves of three GLVs after amendment of $CaCl_2$ to the plants. Among the treatments 50 mM $CaCl_2$ treated plants maintained maximum levels of $Ca^{2+}$ and CaM than that of other two treatments (Figs.1 & 2). *Amarathus* maintained higher levels of CaM followed by *Rumex* and *Spinacea*. Lowest levels of $Ca^{2+}$and CaM were found

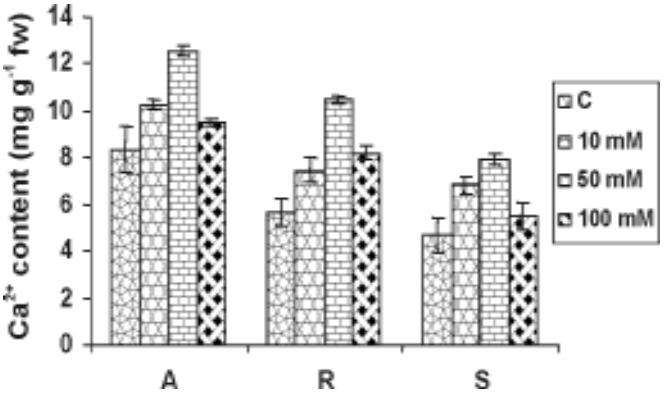


**Fig. 1. Effect of amended $CaCl_2$ on $Ca^{2+}$ content of *Amaranthus* (A), *Rumex* (R), *Spinacea* (S) green leafy vegetables. (Each value is mean ± SE of five replications P<0.05).**

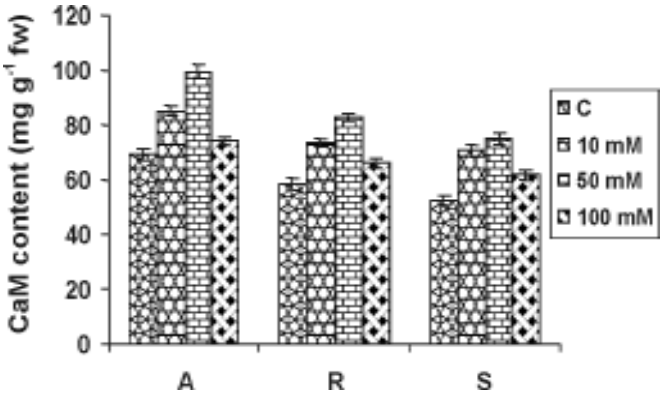


**Fig. 2. Effect of amended $CaCl_2$ on CaM content of *Amaranthus* (A), *Rumex* (R), *Spinacea* (S) green leafy vegetables. (Each value is mean ± SE of five replications P<0.05).**

in the leaves of control plants of *Spinacea*. $Ca^{2+}$ acts as an intracellular signal to a number of fundamental cellular processes that are involved in cytoplasmic

streaming, cell division, differentiation, polarity and plant defense and stress responses (Reddy 2001). CaM is predominantly localized in cytosol and its activation depends upon the availability of internal $Ca^{2+}$. Moreover, CaM stimulates $Ca^{2+}$ transport (Hwang *et al.* 2000). $Ca^{2+}$and CaM are involved in the signal transduction mechanisms affecting plant growth and development (Kumar and Prasad 2001). Therefore, it is inferred that the amended $Ca^{2+}$ may play an important role in maintaining higher levels of extra cellular CaM in the cells for the formation of Ca-CaM complex, which is essential for modulating number of proteins.

The total lipids and two types of polar lipids, glycol and phospholipids which are important membrane constituents were estimated. Treated plants showed higher levels of lipid species than their respective controls. Maximum levels of total lipids, glyco and phospholipids were recorded in the leaves of 50 mM $CaCl_2$ treated plants than that of other two treatments (Figs. 3, 4 & 5). The glycolipids content was more than that of phospholipids in the leaves of all control and treated plants of GLVs. Since polar lipids are found almost exclusively in membranes they play vital role on the integration of the membrane, of which they are apart. Sugar phosphates serve as precursors of glycolipids synthesis. The chloroplast of higher plants is particularly rich in glycolipids (Roughan and Batt, 1968). $Ca^{2+}$ binds to certain membrane components such as phospholipids and cholesterol or specially arranged carboxylic groups and the ion can also possibly modify the pore radius or trigger conformational changes within the membrane. Association of $Ca^{2+}$ with the membrane especially phospholipids is required to maintain integrity (Smith 1978). $Ca^{2+}$ binds natural membranes and alters the permeability, excitability and adhesiveness of cell structure (Shalatz and Marinetti 1972). Phospholipids have also shown to play an important role in the binding of $Ca^{2+}$ to the membranes (Ohnishi and Ito 1974). Thus $Ca^{2+}$ plays a role in maintaining the cell membrane in functional state, in regulating the membrane and also in phase separation of phospholipids.

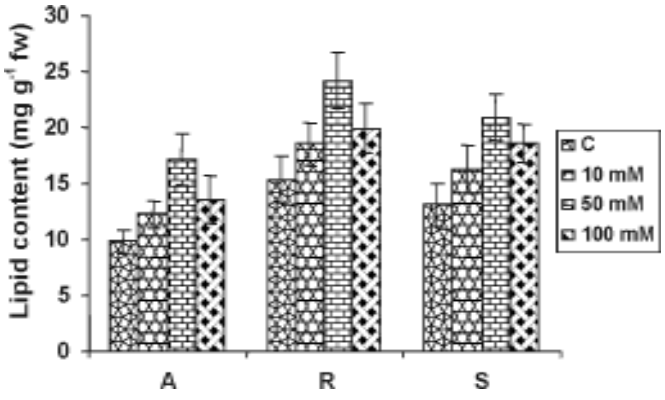


**Fig. 3. Effect of amended $CaCl_2$ on total lipid content of *Amaranthus* (A), *Rumex* (R), *Spinacea* (S) green leafy vegetables. (Each value is mean ± SE of five replications P<0.05).**

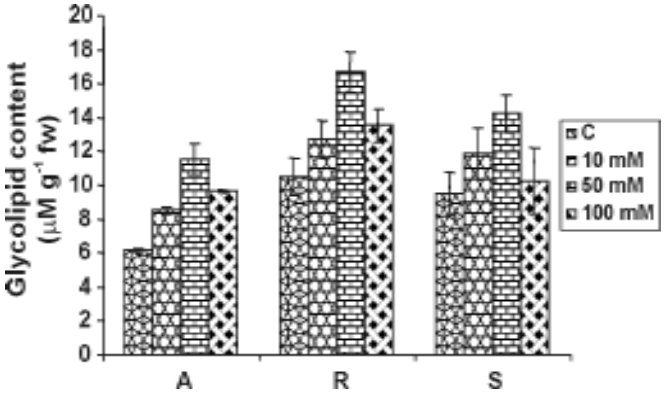


**Fig. 4. Effect of amended $CaCl_2$ on glycolipid content of *Amaranthus* (A), *Rumex* (R), *Spinacea* (S) green leafy vegetables. (Each value is mean ± SE of five replications P<0.05).**

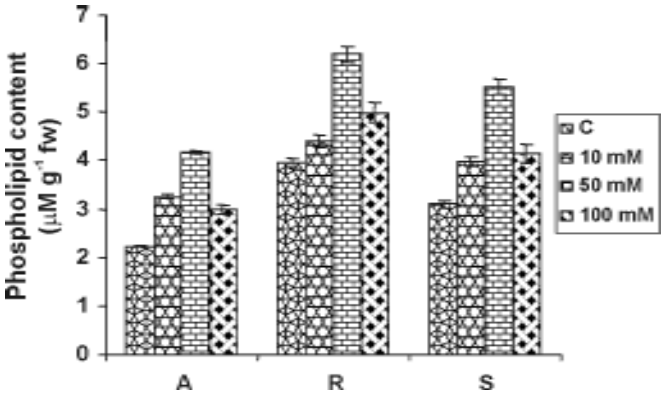


**Fig. 5. Effect of amended $CaCl_2$ on phospholipid content of *Amaranthus* (A), *Rumex* (R), *Spinacea* (S) green leafy vegetables. (Each value is mean ± SE of five replications P<0.05).**

LPO levels of three selected GLVs were observed. Among the treatments the plants treated with 50 mM $CaCl_2$ showed lower levels of LPO than the plants treated with other two treatments (Fig. 6). Higher levels

of LPO were observed in the control leaves of *Amaranthus* than that of other two plant species. However, 50 mM $CaCl_2$ treatment reduces the LPO at maximum levels in the leaves of *Rumex*. Fresh spinach leaves stored at $25^0$C causes increased TBA relative substances accompanied by yellowing and chlorophyll losses (Yamauchi and Watada 1991). LPO is a destructive chain reaction and can directly damage the structure of membranes (Dewir *et al*. 2005). The increased LPO starts as the decline in the protein and chlorophyll content (Heath and Packer 1968, Savithramma and Swamy 1989, 1995). The present study revealed that the external application of $Ca^{2+}$ (by maintaining higher levels of CaM) may be modulating the LPO of GLVs. Among the three species *Amaranthus* responded well to 50 mM $CaCl_2$ concentration than the other two species.

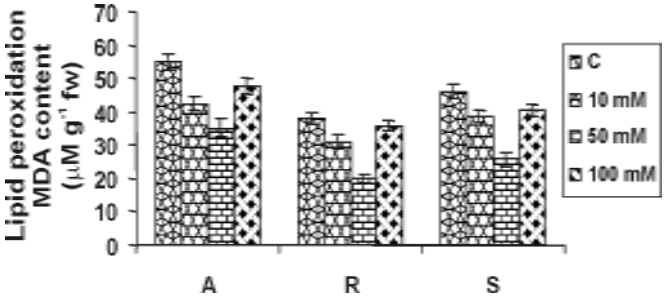


**Fig. 6. Effect of amended $CaCl_2$ on lipid peroxidation content of *Amaranthus* (A), *Rumex* (R), *Spinacea* (S) green leafy vegetables.**
**(Each value is mean ± SE of five replications P<0.05).**

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